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Rules of Engagement: GPCRs and G Proteins

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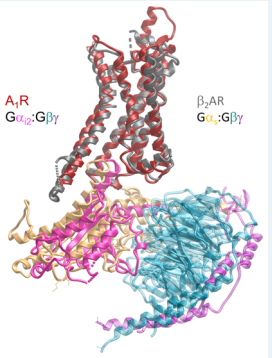
ABSTRACT: G protein-coupled receptors (GPCRs) are a key drug target class. They account for over one-third of current pharmaceuticals, and both drugs that inhibit and promote receptor function are important therapeutically; in some cases, the same GPCR can be targeted with agonists and inhibitors, depending upon disease context. There have been major breakthroughs in understanding GPCR structure and drug binding through advances in X-ray crystallography, and membrane protein stabilization. Nonetheless, these structures have predominately been of inactive receptors bound to inhibitors. Efforts to capture structures of fully active GPCRs, in particular those in complex with the canonical, physiological transducer G protein, have been limited via this approach. Very recently, advances in cryo-electron microscopy have provided access to agonist:GPCR:G protein complex structures. These promise to revolutionize our understanding of GPCR:G protein engagement and provide insight into mechanisms of efficacy and coupling selectivity and how these might be controlled by biased agonists. Here we review what we have currently learned from the new GPCR:Gs and GPCR:Gi/o complex structures.



KEYWORDS: *G protein-coupled receptor, G protein, cryo-electron microscopy, receptor-G protein coupling*

INTRODUCTION

G protein-coupled receptors (GPCRs) are transmembrane proteins that respond to a variety of stimuli including hormones, neurotransmitters, peptides, and small molecules. Signal transmission inside the cell occurs through GPCR interaction with their downstream partners, such as G proteins,[1] G protein-coupled receptor kinases (GRKs),[2] and arrestins.[3]

G proteins, the canonical coupling partner whose interaction has defined the naming of GPCRs, are heterotrimers consisting of α, β, and γ subunits. Each subunit is present in the human genome as multiple genes encoding distinct subunit subtypes, resulting in many variations in heterotrimer assembly. G proteins are primarily distinguished based on their Gα subunits, which are grouped into 4 families based on sequence similarities and functional output: G_s (Gα_s and Gα_{olf}), $G_{i/o}$ (Gα_{i1-3}, Gα_{oA}, Gα_{oB}, Gα_t, Gα_g, Gα_z), G_q (Gα_q, Gα_{11}, Gα_{14}, G$\alpha_{15/16}$) and G_{12} (Gα_{12} and Gα_{13}).[4] Both G_s and $G_{i/o}$ family members regulate activity of adenylate cyclases, either stimulating ATP to cAMP conversion (G_s) or inhibiting it, leading to a decrease in cAMP levels ($G_{i/o}$).

Despite extensive biochemical characterization, structural information on receptor complexes with G proteins remained elusive for many years until the first structure of the β_2-adrenergic receptor (β_2AR) complex with heterotrimeric G_s protein was solved in 2011 by Kobilka, Sunahara, and colleagues using X-ray crystallography.[5] Nonetheless, other GPCR-G protein complexes have remained refractive to crystallization and/or high-resolution diffraction that have limited additional structure determination by this methodology. Very recently, the "resolution revolution" in cryo-electron microscopy (cryo-EM)[6] has enabled application of this technique to structure determination of other GPCR-G_s complexes, namely of A_{2A} adenosine receptor (A_{2A}AR),[7] calcitonin receptor (CTR),[8] calcitonin-like receptor-RAMP complex (CLR-RAMP, CGRP receptor),[9] and multiples of glucagon-like peptide 1 receptor (GLP1R).[10,11] Nonetheless, GPCR complexes with other G proteins remained elusive until June 2018 when four groups, utilizing distinct approaches, independently determined structures of GPCRs with members of the $G_{i/o}$ family: A_1 adenosine receptor (A_1AR)-G_{i2},[12] μ opioid receptor (μOR)-G_{i1},[13] rhodopsin (Rho)-G_{i1},[14] and serotonin 5-HT_{1B}-mG_o[15] complexes. Comparison of these structures, together with previously solved structures of G_s complexes, gives us the first glimpse at molecular mechanisms responsible for signal transmission from GPCRs to G proteins, and structural evaluation of selectivity determinants for both interaction partners. This review will focus on all the structures of active state GPCR-heterotrimeric G protein complexes determined to date in the context of GPCR and G protein activation, complex formation, and mechanisms of discrimination between G_s and $G_{i/o}$ families.

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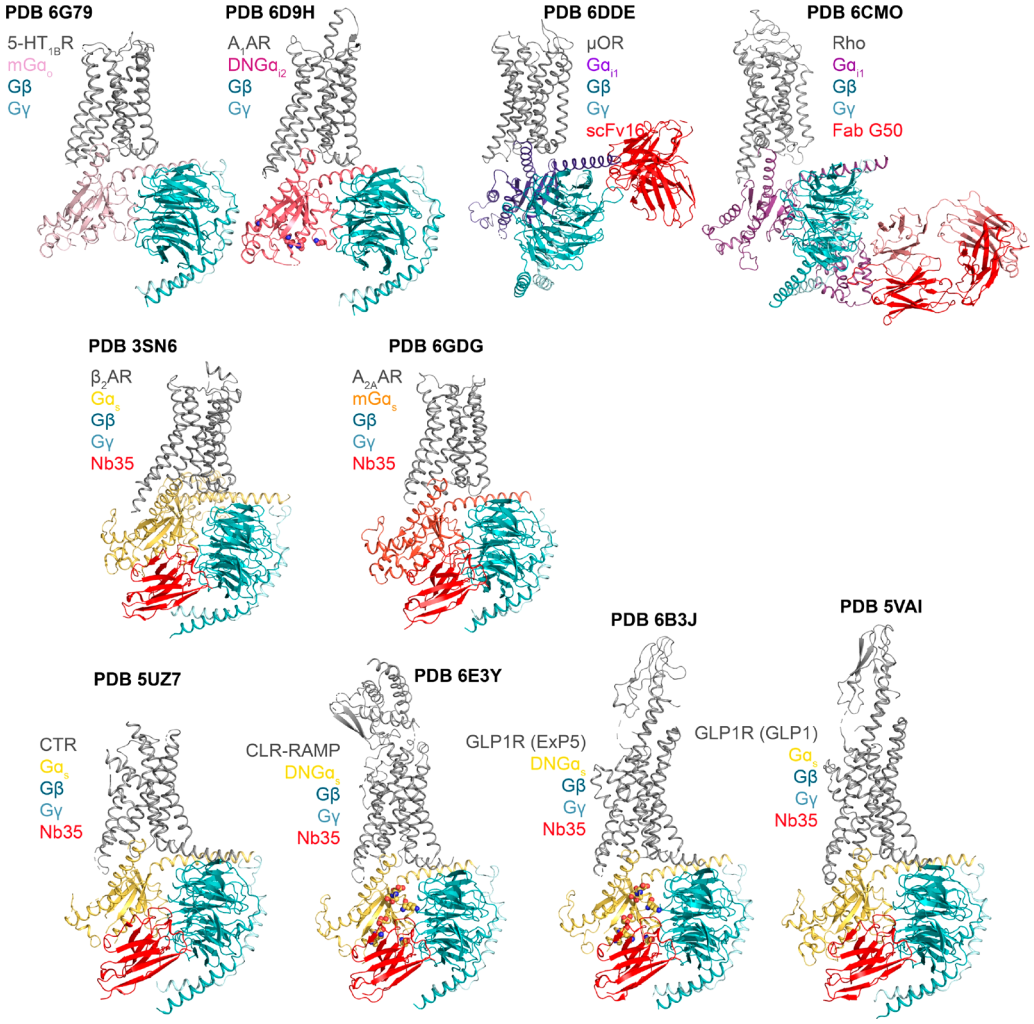

Figure 1. Methods for GPCR-G protein complex stabilization. Complexes are shown as cartoon, with receptors shown in gray, Gα_s and G$\alpha_{i/o}$ in yellow and shades of pink, Gβ in cyan, and Gγ in pale cyan. Proteins used for complex stabilization are shown in red. Stabilizing mutations in DN G proteins are shown as spheres.

APPROACHES TO GPCR-G PROTEIN COMPLEX STABILIZATION

One of the main difficulties in structure determination of GPCR-G protein complexes is the transient nature of the interaction. Capture of the very first β_2AR-G$_s$ complex was made possible through the combined application of apyrase and nanobody 35 (Nb35).[5] Apyrase, a nonselective nucleotide lyase, is capable of sequentially hydrolyzing GTP and GDP to GMP, trapping the receptor-G protein complex in a nucleotide-free state. All GPCR-G protein structures solved to date required apyrase treatment for complex formation. Selectively for Gα_sG$\beta\gamma$ heterotrimers, the nanobody (Nb35) binds and stabilizes the interface between G$\beta\gamma$ subunit and Ras Homology Domain (RHD) of Gα_s, rendering the complex insensitive to GTPγS.[16] Nb35 proved to be an invaluable tool for G$_s$ complex stabilization leading to the first full-length structure of a class B GPCR, the CTR,[8] and subsequent structures of the GLP-1R[10,11] and CGRP receptor.[9] While the availability of Nb35 for stabilization of G$_s$ complexes was important for other structures, more tools were developed to strengthen the interaction between receptor and G protein and reduce complex flexibility (Figure 1).

The Tate group developed minimal G proteins or mini-G (mG), a rationally designed RHD containing only the GTPase region of Gα proteins with additional mutations that increased thermostability and reduced nucleotide affinity.[17,18] The mG$_s$ on its own was used for crystallization and X-ray structure determination of the A$_{2A}$AR.[19] Another version of mG$_s$ protein, with an extended N-terminal helix, capable of binding the G$\beta\gamma$ subunit, was used to solve the structure of A$_{2A}$AR:mG$\alpha_s\beta\gamma$:Nb35 complex by cryo-EM.[7] Development of the mini-G versions of other Gα family members yielded mG$_o$ and made possible the determination of the serotonin 5-HT$_{1B}$:mG$\alpha_o\beta\gamma$ structure (but no additional stabilizing protein partner was required).[15]

Another approach for complex stabilization, exploited by the Sexton and Wootten laboratories, is targeted mutagenesis of the Gα subunit.[20] Following previous literature that described mutational effects on G proteins, mutations of residues involved in coordination of Mg^{2+}, and GTP's β- and γ-phosphates, plus additional residues that improved overall complex stability, led to the creation of dominant negative (DN) G proteins with reduced nucleotide affinity and enhanced interaction between Gα and G$\beta\gamma$.[21−25] This strategy was applied to Gα_s, yielding the structures of GLP1R:DNG$\alpha_s\beta\gamma$:Nb35 with the biased agonist exendin-

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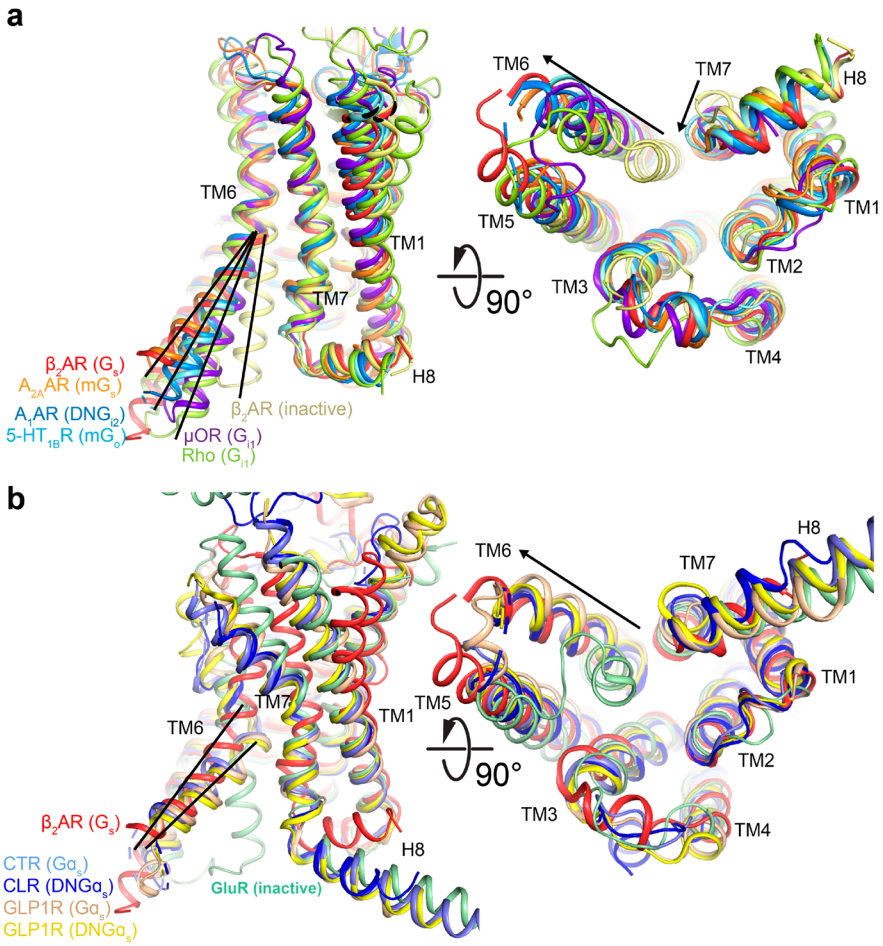

Figure 2. Receptor conformations in G_s and $G_{i/o}$ complexes. Aligned receptors are shown as cartoons and the black line depicts average TM6 displacement for Class A G_s, G_{i1}, and G_{i2}/mG_o complexes (a) or Class B G_s complexes with β_2AR shown for comparison (b). Arrows show the direction of TM6 and TM7 movement upon receptor activation.

P5[10] and the heteromeric CGRP receptor, CLR:RAMP1:DN-G$\alpha_s\beta\gamma$:Nb35 complexes,[9] and may be important for complex stabilization for lower efficacy agonists, in which the use of Nb35 alone is insufficient.[20] The DN G protein approach was also successfully applied to Gα_{i2}, resulting in the A_1AR:DNG$\alpha_{i2}\beta\gamma$ structure (without additional stabilizing protein partner).[12]

More targeted approaches for complex stabilization were also developed. In one study, an antibody single-chain variable fragment (scFv16) against a complex of Rho-G_{i1} was generated, targeting the interface of the αN helix of Gα_{i1} and the β-propeller of Gβ; this prevented GTPγS-dependent complex dissociation.[13] Consequently, this Fab was used for structure determination of the μOR:G$\alpha_{i1}\beta\gamma$:scFv16 complex. In a separate study, a phage display library was used to screen for G_{i1} heterotrimer-binding Fabs, followed by negative stain EM-guided selection of Fab_G50 that bound at the interface of the α-Helical Domain (AHD) of Gα_{i1} and the G$\beta\gamma$ subunit and stabilized a Rho:G$\alpha_{i1}\beta\gamma$:Fab_G50 complex.[14]

RECEPTOR CONFORMATIONS IN GPCR-G PROTEIN COMPLEXES

In total, there are 10 structures of active-state GPCRs in complex with heterotrimeric G proteins. The class A rhodopsin subfamily is represented by β_2AR and A_{2A}AR complexes with G_s and A_1AR, μOR, Rho, and 5-HT_{1B}-complexes with $G_{i/o}$, while class B GPCRs are represented by CTR, CLR-RAMP1, and GLP1R complexes with G_s heterotrimers. A comparison of active state receptors with corresponding inactive state structures reveals a common mechanism of activation for both G_s and G_i-coupled receptors.

In the class A GPCRs solved to date, agonist binding in itself stabilizes a small conformational rearrangement around the ligand binding site of the receptor. Closure of the orthosteric site appears to be receptor-specific and independent of the G protein binding partner. A small shift of the top of TM1 for μOR, was observed in both G_{i1}-coupled and G protein-mimetic, Nb39-bound structures.[13,26] Similarly, movements of the top of TM1 and TM2 of A_{2A}AR are initiated by agonist binding even in the absence of an intracellular binding partner.[27] These conformational changes are required to effectively accommodate agonists in the binding site. In accordance with this, only small changes in rotamer orientations were observed upon agonist binding in intermediate-active A_{2A}AR and 5-HT_{1B} structures in the absence of G proteins.[27,28]

On the other hand, reorganization of $D^{3.49}R^{3.50}Y^{3.51}$, $P^{3.40}I^{5.50}F^{6.44}$, and $N^{7.49}P^{7.50}xxY^{7.53}$ motifs in class A GPCRs is the hallmark of receptor activation and is only observed upon receptor stabilization in the active state with either a G protein or a G protein-mimicking nanobody,[29] or at least partially, in receptors with high constitutive activity.[30] Similar rotameric

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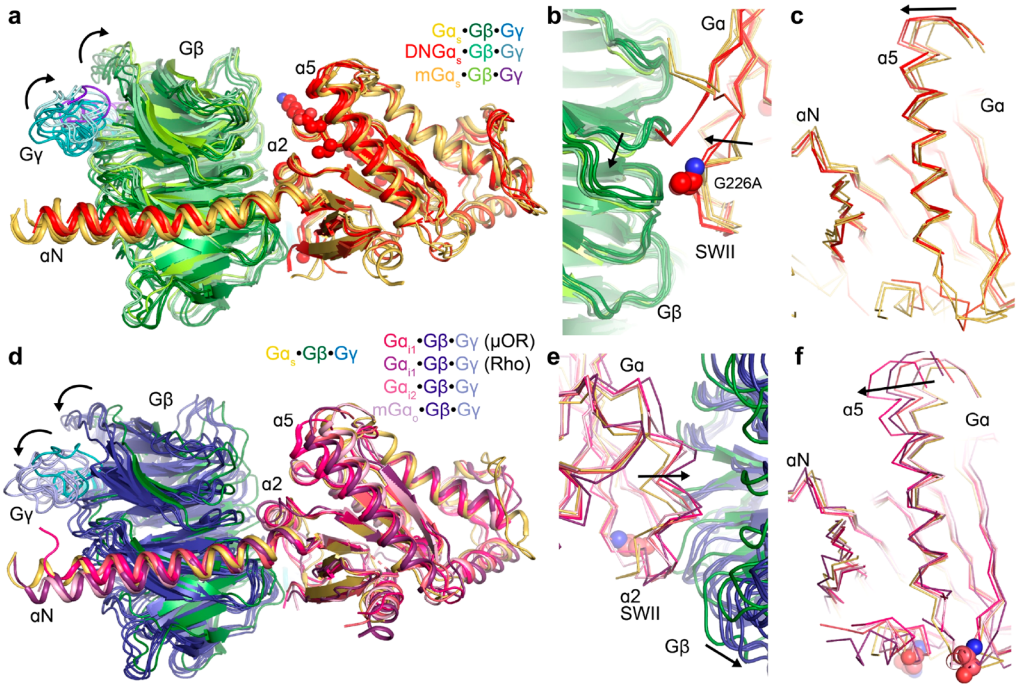

Figure 3. Heterotrimer conformations in GPCR-G protein complexes. (a–c) G_s and (d–f) $G_{i/o}$ heterotrimer conformations in receptor-G protein complexes aligned using the core of Gα subunit. (a and c) Gβ and Gγ rotate around SWII region in complexes formed with different $G_{i/o}$ family members or differently stabilized G_s complexes (wtG_s, DNG$_s$ or mG_s); (b and e) conformation of SWII region affects Gβ position relative to Gα subunit; (c and f) flexibility of the α5 helix. Dominant negative mutations in GLP1R(ExP5) are shown in spheres.

changes are observed in both G_s- and $G_{i/o}$-coupled receptor structures and are consistent with TM6 and TM7 movements that allow accommodation of the α5 helix of the Gα subunit.

The most pronounced movements during receptor activation are observed for TM6 and TM7 (Figure 2). The ~2 Å inward shift of TM7 and H8 is similar between G_s and G_i coupled complex structures. However, the extent of displacement of TM6 appears to be, at least partially, dependent on the G protein subtype. For the class A GPCRs, the largest movement is observed in G_s-coupled β_2AR and A_{2A}AR with 31.4° and 30.7° bends in TM6, relative to their inactive-state structures (PDBs 2RH1[31] and 4EIY,[32] respectively) (Figure 2a). An even larger displacement is observed for G_s-coupled class B receptor structures with 63–73° angles for CTR, CLR, and GLP1R (all measured relative to the TM6 of the inactive-state glucagon receptor, PDB 4L6R[33]) (Figure 2b). The $G_{i/o}$ coupling stabilizes conformations that exhibit smaller movements, the extent of which might also depend on the identity of the $G_{i/o}$ family member. The G_{i1} coupling leads to a 19.3° bend and an 18.3° bend in TM6 for μOR and Rho, respectively (relative to PDBs 4DKL[34] and 1U19[35]), G_{i2} to a 21° bend for A_1AR (compare to 5UEN[36]) and G_o to a 24.4° bend for 5-HT_{1B} (compare to the "intermediate-active" 4IAR[28]). Interestingly, both receptors with the smallest TM6 angle, μOR and Rho, have ordered ICL3s that make interaction with the β1-α1 interface of the G_α Ras domain. It is unclear whether the flexibility of ICL3 in other complexes is due to their longer length or increased distance from the G protein, as a consequence of the larger TM6 kink. Molecular dynamics simulations of Rho, μOR, β_2AR, and A_{2A}AR testing TM6 movement relative to the TM bundle revealed that the extent of TM6 displacement might be an intrinsic property of a particular receptor, with β_2AR and A_{2A}AR undergoing larger TM6 swings, compared to Rho and μOR.[14]

ICL1 differences are very subtle between GPCR-G protein complexes and inactive-state structures: it tends to move outward for most active-state structures, with the exception of A_{2A}AR and 5-HT_{1B}. ICL2 loops for A_1AR, 5-HT_{1B}, β_2AR, and A_{2A}AR adopt similar helical conformation. In contrast, Rho and μOR ICL2 are shifted outward. This could contribute to the very different angle of engagement that Gα adopts relative to Rho when compared to other structures (discussed below).

G PROTEIN ACTIVATION MECHANISMS

To understand conformational differences between GPCR-G protein complexes, it is important to compare structures of G proteins in the absence of receptors in an effort to distinguish changes arising from binding to different GPCRs from variations between G protein family members or complex stabilization techniques.

Previous structural studies and molecular dynamics simulations revealed that GPCR binding causes similar global conformational changes in both G_s and $G_{i/o}$ heterotrimers.[37–39] Rotation and extension of the α5 helix into the receptor core is associated with disorder in the nucleotide binding site at the interface of the RHD and AHD of the Gα subunit (P-loop and β6-α5 loop), nucleotide release and the opening of the interface between the RHD and AHD. Without stabilization by crystal contacts (β_2AR-G_s) or Fab (Rho-G_{i1}), AHDs remain very flexible, as seen in class 2D averages in negative stain and cryo-EM.[8,11–13,16] While this domain is generally masked out to maximize the resolution of EM maps, the available data indicate that there can be a preferred orientation (as seen with the ExP5 bound GLP-1R complex[10]), or multiple identifiable orientations (as seen with the sCT bound CTR[8]). The extent to which the relative mobility of the AHD of Gα-subunits is a property of the specific receptor with which it is engaged or is a component of the specific agonist-

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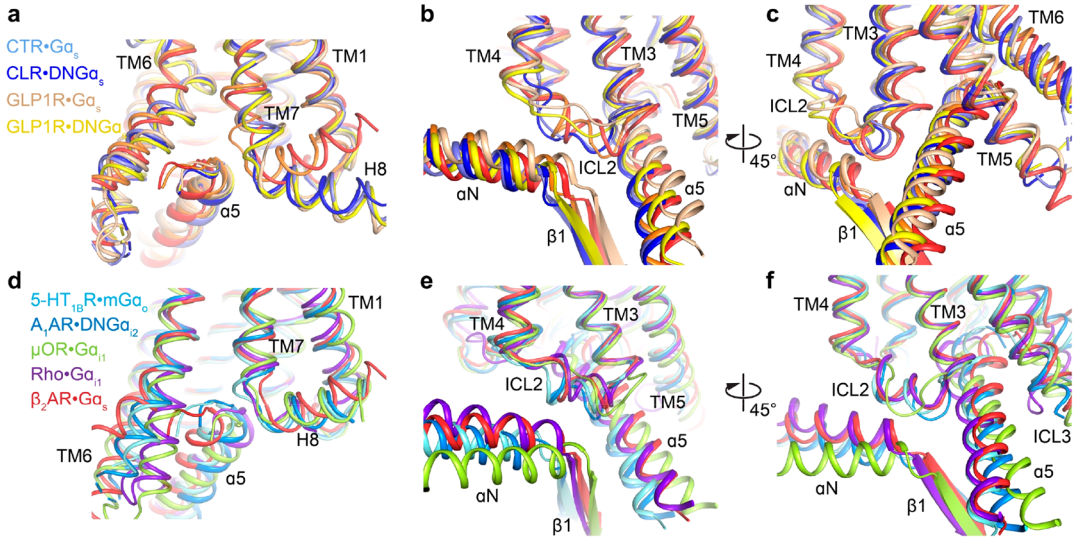

Figure 4. Receptor-Gα orientations are influenced by TM6 movement and ICL2 conformation. (a–c) $G\alpha_s$ and (d–f) $G\alpha_{i/o}$ shifts relative to the receptor in different structures. GPCR-G protein complexes were aligned using TM bundles. Gβ, Gγ, and the majority of Gα (with the exception of αN, α5 helixes and the β1 sheet) are not shown for clarity. Structures a and d show α5 helix displacement; structures b, c, e, and f show αN and α5 helix displacement caused by ICL2 and TM5/ICL3 of the receptor. Receptors are displayed in thinner cartoon, compare to Gα.

GPCR complex, is unclear. However, biophysical studies using bioluminescence energy transfer have demonstrated that conformational sampling of the G protein, linked to the position of the α-helical domain, can be influenced by individual agonists and may contribute to observed efficacy.[10,40]

Superposition of G_s complexes, on the core of the RHD of $G\alpha_s$ (excluding α5 and αN helices), revealed that stabilizing mutations have subtle effects on heterotrimer conformation leading to reduced flexibility of DN-G_s heterotrimers compared to WT-G_s (Figure 3). Notably, Gβ and Gγ subunits of WT-G_s heterotrimers appear to adopt multiple positions relative to Gα, which is not observed for complexes formed with DN-G_s. This is likely the effect of G226A mutation in DNG_s that causes a different backbone conformation of the β3-α2 loop in the switch II (SWII) region of Gα, leading to SWII stabilization and reduced affinity for GTP.[24] The stabilized SWII region makes stronger interactions with Gβ leading to reduced flexibility of the Gα–Gβ subunit orientations in DN-G_s. In contrast, in complexes formed with WT-G_s, Gβ rotates around SWII leading to an ∼4 Å displacement; measured at T34 Cα of Gβ (β_2AR and GLP1R(GLP1) complexes). Because of the extensive interactions of the Gβ, their displacement, in turn, affects the orientation of the αN helix relative to the core of the Gα subunit, leading to different degrees of αN rotation away from the α4 helix of Gα in different WT-G_s complexes. Increased flexibility of WT-G_s heterotrimers extends to the α5 helix, resulting in maximal differences of 3.2 Å at E390 Cα for WT-G_s (β_2AR and CTR complexes) compared to only 1.3 Å for DN-G_s (GLP1R(ExP5) and CLR-RAMP complexes). The flexibility in the α5 helix could potentially be the result of other DN mutations, crystallographic artifacts for the β_2AR complex, or a strain induced by interactions with individual agonist:GPCR complexes. While these data are intriguing, we still have only a very limited number of solved structures, and no direct comparisons of the same agonist:GPCR in complex with WT-G versus DN-G, which will be required to more definitively classify the distinct effects of stabilizing technologies.

A comparison of activated $G_{i/o}$ and G_s heterotrimers reveals G protein family specific variations. Different amino acid sequences and, thus, the conformation of the SWII region lead to a rotation of the Gβ subunit relative to Gα for $G_{i/o}$ versus G_s proteins, though in an opposite direction to that seen with DN-G_s. The Gβ rotation is followed by an αN tilt away from the α4 helix of Gα with the largest displacement observed for 5-HT_{1B}R. The α5 helix also adopts multiple conformations, with variations of tilt angles relative to the Gα core. The degree of Gβ, αN, and α5 displacement is different between different $G_{i/o}$ complexes and it is unclear whether it is the result of the specific Gα subunit, complex stabilization method, or bound receptor. Other G protein-specific variations include much longer αG-α4 loops in $G\alpha_s$ that extend toward ICL3 of the receptor, however, no interactions with ICL3 are apparent. Interestingly, the αG and α1 loops are displaced in Rho-G_{i1} complex structure, compared to other $G_{i/o}$ complexes, this could be a result of Fab_G50 binding to both the Gβ and AHD of Gα and stabilization of a particular lower energy conformation.

G PROTEIN ENGAGEMENT OF ACTIVATED GPCRS

Comparison of all 10 available GPCR-G protein heterotrimer complex structures allowed us, for the first time, to propose general rules for G protein engagement and to identify distinct aspects in the mode of engagement for different G protein families. Class A and class B GPCRs share low sequence identity and are quite different in their tertiary structure. Subsequently, we only aligned receptors within each receptor family for detailed G protein binding comparison, with the exception of a general analysis of G_s engagement with class A and class B GPCRs where we aligned the lower halves of the β_2AR and A_{2A}AR TM bundles with the GLP-1R (ExP5) structure.

The most extensive interactions between GPCRs and G proteins occur via the α5 helix of the RHDs: this extends into the receptor core and principally interacts with the bottom

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halves of TM3, TM5, TM6, along with ICL2 and ICL3. However, different complexes have other, less pronounced, interfaces that may be receptor and/or G protein-specific.

Comparison of G_s complexes reveals that the Gα C-terminal loop ("wavy hook") adopts a similar overall conformation within the receptor core of Class B GPCRs (Figure 4a). When compared to the position in class A receptors, the C-terminal loop exhibits lateral displacement of 3.7 Å (measured between Q390 Cα), which is likely due to the distinct TM6 conformations between the major subclasses (Figure 2b). Interestingly, the α5 angle is fixed relative to TM3 of the receptor in all G_s structures (147−149°). However, the Gα subunits (and thus, G$\beta\gamma$) in different Gs complexes rotate in the plane parallel to the membrane relative to the receptor (up to 12° rotation in GLP-1R(GLP-1)) using the β_2AR structure as the reference. This is likely a consequence of either a difference in ICL2, H8, or both between class A and class B receptors; however, the lack of lipid environment in a detergent micelle could also play a role. It is possible that the ICL2 size and conformation could determine Gα_s orientation relative to the receptor, with longer ICL2s creating steric hindrance with the middle part of the α5 helix, potentially leading to α5 tilting away from ICL2, followed by a rotation in the entire Gα subunit, and consequently also G$\beta\gamma$. In particular, β_2AR and A_{2A}AR display a small two-turn helix in ICL2 that packs against the interface of the αN-β1 junction, β2−β3 turn and α5 helix (Figure 4b,c) "pushing" the α5 helix toward TM5. The hydrophobic amino acid, F139 in ICL2, is important for a G_s-mediated response from β_2AR and for G_q-mediated responses for M1 and M3 muscarinic acetylcholine receptors (mAChRs),[41] indicating that ICL2 is important for G protein interaction for at least some of class A GPCRs. In contrast, α5 interactions with ICL2 of class B GPCRs that lack an equivalent helical structure and are shorter in length (5 residues in GLP-1R and 8 residues in CTR and CLR), likely lead to its rotation away from TM5. Alternatively, the different angle of heterotrimer interaction between class A and class B receptors could result from differences in the orientation of helix 8 (H8). Compared to solved class A complexes, the longer H8 in class B GPCRs is tilted away from the plane of the membrane (~20° tilt compared to class A GPCRs) that might, through distinct interactions with the Gβ subunit, lead to the repositioning of the entire heterotrimer.

Comparison of the class B CLR:RAMP1- and CTR- G_s complexes, solved using DN or WT Gα_s subunit, respectively, revealed that the DN mutations had little effect on Gα orientation relative to the receptor when compared to the effect caused by amino acid divergence across the receptors and local conformational changes between the GPCR structures. The GLP-1R has a shorter ICL2 compared to either CTR or CLR and this likely causes the ~5° difference in angle in the engagement of Gα_s between these receptors. There is evidence that the C-terminus of RAMP can potentially influence G protein binding;[42,43] however, it was unresolved in CLR:RAMP1:Gs density,[9] and it is likely that this interaction is either transient or requires additional accessory proteins, such as RCP.[44]

Compared to G_s complexes, the "wavy hook" at the extreme end of the α5 helix in G$\alpha_{i/o}$ subunits is translated toward ICL1 and the TM7−H8 junction (Figure 4c). The degree of this shift is directly proportional to the extent that TM6 kinks upon receptor activation, with the largest movement observed for G_{i1}-bound Rho and μOR, followed by G_{i2}-bound A_1AR, mG_o-bound 5-HT_{1B}R, and Gs complexes of β_2AR and A_{2A}AR.

Similar to class B GPCRs, the angle of the α5 helix insertion (and, thus, αN position and the tilt of the entire G protein) appears to depend on ICL2 and TM5 interactions with the Gα subunit. More extensive interactions with the middle part of the Gα protein α5 helix rigidify its position in G_s complexes (via conserved H-bonds with $Q384^{G.H5.16}$ and $R385^{G.H5.17}$ or a salt bridge with $D381^{G.H5.13}$). In contrast, the receptor-G$\alpha_{i/o}$ subunit interactions primarily depend on weak van der Waals interactions, with the exception of a salt bridge through $D342^{G.H5.13}$ in G_{i2} and mG_o complexes, and H-bonds ($K345^{G.H5.17}$ for G_{i1}-Rho and $N347^{G.H5.19}$ for G_{i1}-μOR) leading to an increased α5 helix-TM3 angle (152−162°) and larger spreads in Gα rotation angles relative to the receptor (8−22°) (Figure 4d,e). Most notably, the ICL2 of Rho lacks any secondary structure and adopts a unique conformation by extending away from the receptor. As a result, the Gα_{i1} rotates, relative to the receptor, preserving potential contacts between ICL2 and the αN helix, leading to a substantially different receptor-G protein organization compared to other complexes.[14]

Interactions with ICL3 of receptors might also influence G protein orientation relative to the receptor. Unfortunately, ICL3s are not well resolved in all available structures though this likely reflects the extent of conformational dynamics for individual GPCR:G protein complexes. For example, the density of the A_1AR-G_{i2} complex map suggests likely interactions between ICL3 and the Gα subunit; however, the map quality was insufficient for modeling, indicative of conformational fluidity of this receptor segment in the active structure. Note that this loop is better resolved for Rho and μOR suggesting that receptors with smaller TM6 kinks possibly have less flexibility due to proximity to interacting Gα. The length of ICL3 also varies dramatically between receptors. Thus, the unresolved ICL3 in A_1AR, 5-HT_{1B}R β_2AR, and A_{2A}AR might simply reflect flexibility and/or lack of secondary structure elements. Nonetheless, for the class B GLP-1R, for which structures have been solved with multiple peptide agonists, it has been speculated that stability of the interactions between ICL3 and G_s, reflected in the ICL3 map density, may be linked to G protein turnover and thus agonist efficacy.[5]

Taking all available structures into consideration, it appears that the TM6 movement and the G protein α5 C-terminal loop position might depend on the G protein family member in complex with the receptor, while interactions of ICL2 with the α5 helix might be receptor specific.

BASIS OF SELECTIVITY BETWEEN G_S AND $G_{I/O}$ COMPLEXES

GPCRs frequently couple to multiple G protein families. This includes the β_2AR and A_1AR that couple to G_s and $G_{i/o}$, making it very difficult to identify receptor residues responsible for the selective engagement of a particular binding partner. Even the $G_{i/o}$ exclusive coupler μOR can activate multiple Gα subunits from that family. Multiple investigators have attempted to identify consensus motifs for G protein binding, with limited success. This is likely due to a large divergence in the amino acid sequences of GPCRs that can couple to particular G proteins. As such, the determinants of G protein binding are likely formed by a three-dimensional epitope during receptor activation; this could differ from one receptor

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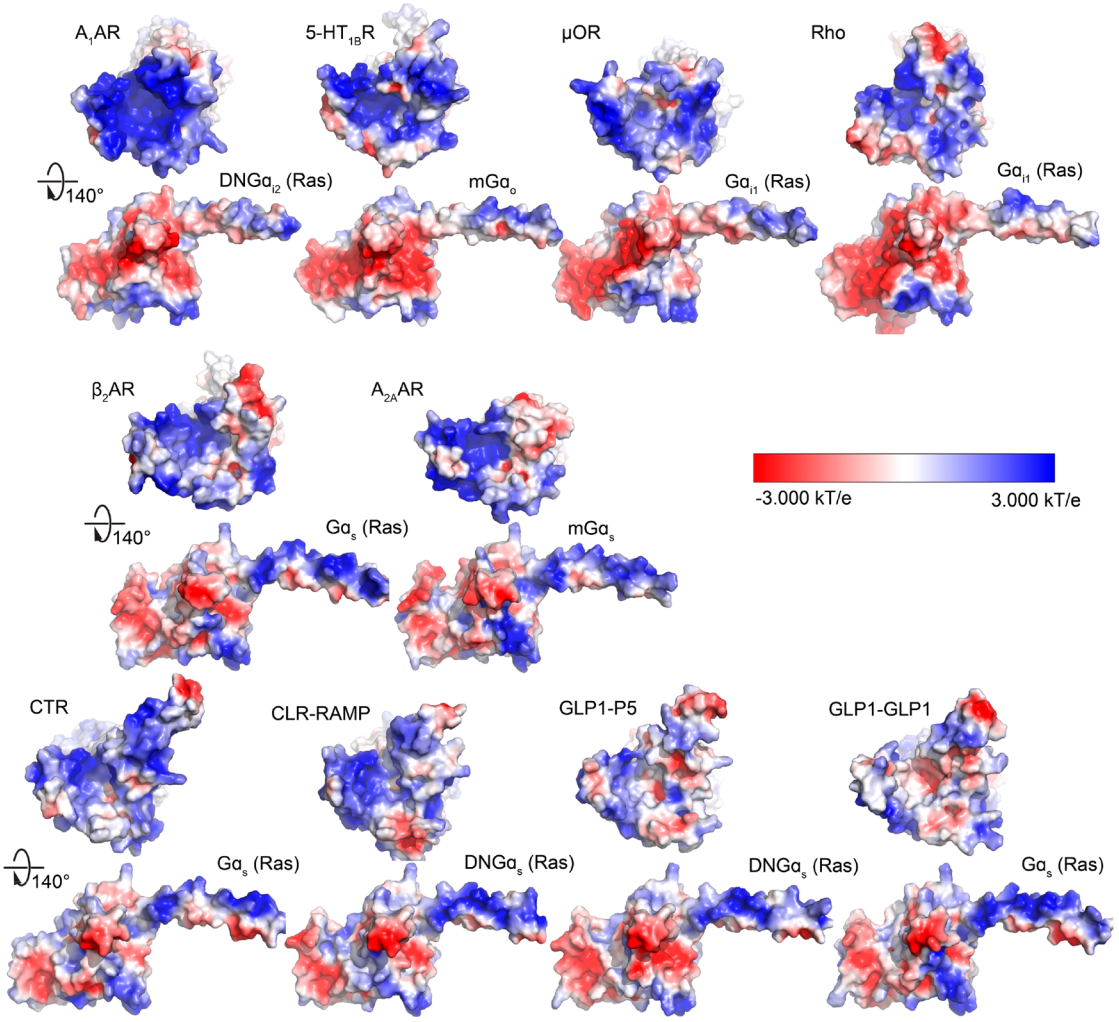

Figure 5. Electrostatic charge distribution in G protein complexes. Surface representation of receptors and Gα subunits colored according to their electrostatic charge. Charge distribution was calculated using APBS plugin in Pymol[59] following reinstatement of full side-chains where these had been stubbed in the deposited structures.

to another and, at least in part, depend on the extent of TM6 movement.[45] Nevertheless, some generalizations can be made from the comparison of the available active-state structures.

A concentration of positive charge on the intracellular side of GPCRs has been noted previously and is proposed to be important for interactions with arrestins.[46] Indeed, there is a concentration of positively charged amino acids at the G protein-binding interface that is more prominent for the class A receptors solved to date (relative to class B GPCRs); nonetheless, the charge distribution appears to vary between receptors (Figure 5). In most class A and class B receptors, the TM6−TM7 surface that faces the G protein binding site appears to be positively charged; however, the μOR and GLP-1R appear to be exceptions. While the end of the G protein α5 helix is negatively charged in all Gα subunits, Gα_{i1}, Gα_{i2}, and mGα_o have a complementary large negative charged surface formed by the α5, α4, and β6 interface. It is tempting to speculate that this charge−charge interaction could be important for receptors engaging with $G_{i/o}$ proteins. In addition, flexibility in ICL3 that is enriched in positively charged amino acids for most class A receptors could contribute to dynamic interactions between the receptor and G protein. Though speculative, changes in ICL3 conformations induced by different biased agonists could alter the strength of G protein coupling and alter relative signaling efficacy. ICL3 is poorly resolved in the class A A_1AR, 5-HT_{1B}R, β_2AR, and A_{2A}AR consistent with the potential for agonist-dependent changes to conformational sampling to contribute to the strength and specificity of G protein binding via with this interface. It is important to note that, physiologically, GPCR:G protein interactions occur within a lipid bilayer enriched in polar lipid headgroups, and interactions between both G proteins and lipids and between receptor and lipids are functionally relevant. These play an important role in GPCR stability, GPCR:G protein interactions, and G protein activation,[47−50] and are likely to provide limitations on orientations of G protein engagement with receptors that do not occur in detergents. To date, only the β_2-AR:Gs and A_2AR:miniGα_s solved by lipidic cubic phase crystallography are in a lipidic environment,[5,19] though it is encouraging that the orientation of miniGα_s alone and miniGα_s-G$\beta\gamma$ are equivalent in complexes with agonist-bound A_2AR, solved by crystallography and cryoEM, respectively.[7,19]

Most fully active GPCR complexes solved to date have been determined at a resolution sufficient for reasonably confident side chain placement (<4 Å), particularly for the GPCR:G protein interface, and thus informative comparisons between structures can be made. While there is no obvious consensus

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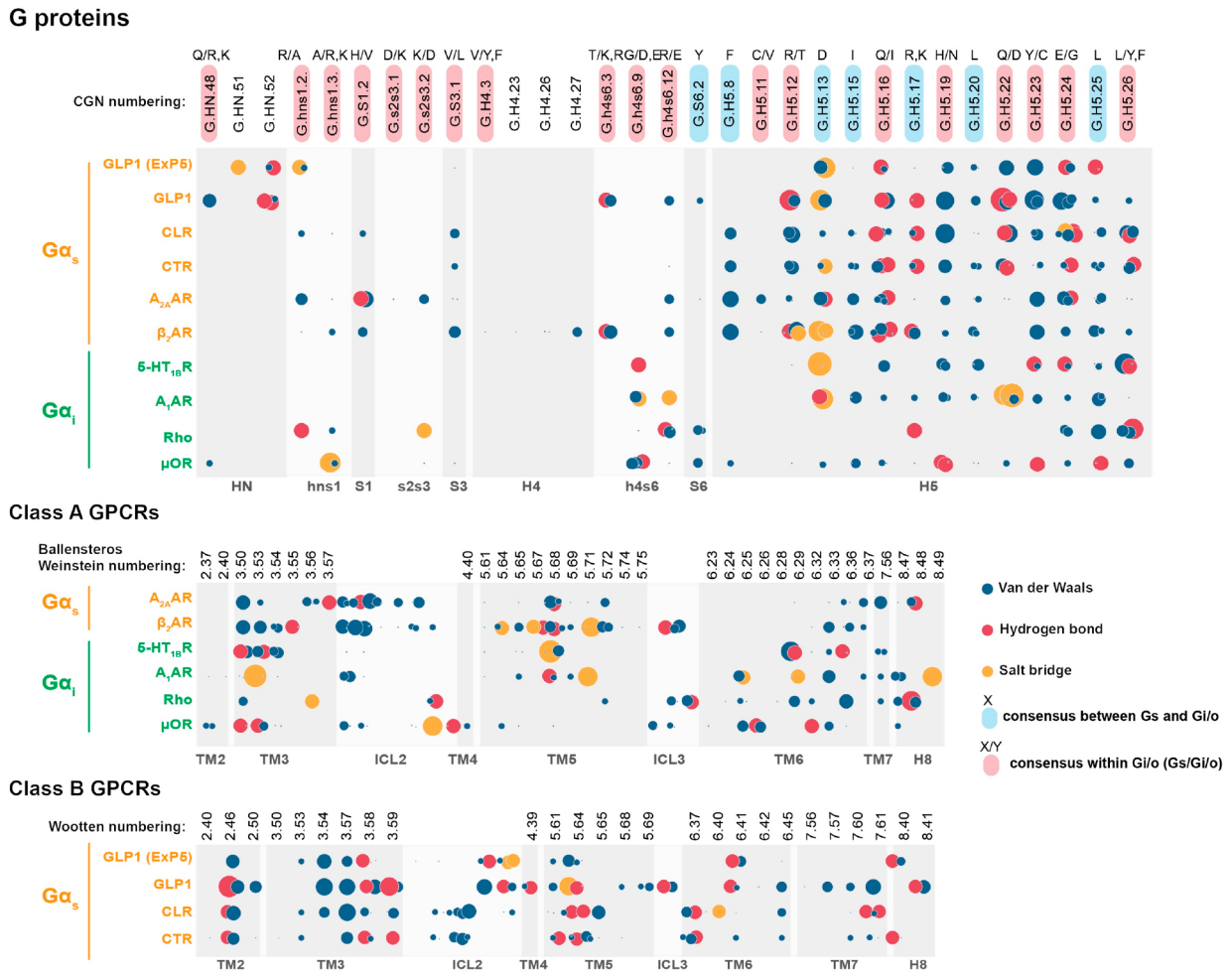

Figure 6. Receptor-Gα subunit interaction map in determined complex structures. Panels represent van der Waals (blue), H-bonds (red), and salt bridge (yellow) interactions observed in X-ray or cryo-EM structures, as calculated by 2P2I Inspector[60] using 3.5 Å, 4 Å, and 4.5 Å cut-offs for H-bond, van der Waals, and salt bridge interactions, respectively. The relative size of the depicted interaction is proportional to bond energy (proportional to the number of formed interactions for van der Waals bonds).

sequence(s) that discriminate between G_i and G_s binding, there are some receptor regions that seem to be selectively engaged: receptors in G_s complexes appear to have more interactions through their ICL2 and TM5, while TM6 and H8 are more engaged in $G_{i/o}$ complexes; this is in good correlation with the overall difference in G protein orientation relative to receptors (see above) (Figure 6). The importance of binding domains, rather than amino acid sequence for G protein selectivity have been proposed previously[51] and is an attractive hypothesis considering the plethora of GPCRs that couple to relatively few Gα subunits.

Specific for G_s binding, residue 5.64 (Wootten numbering is used for class B GPCRs) and the equivalent class A residue 5.68 (Ballesteros−Weinstein numbering) make strong interactions with the Gα protein α5 helix either via H-bond to $Q384^{G.H5.16}$ (CGN numbering,[52] Ile in $G_{i/o}$ family) or a salt bridge with $D381^{G.H5.13}$ (conserved in both G protein families). Other class B-specific interactions include a H-bond between $Q384^{G.H5.16}$ and the backbone of residue 3.58, and a H-bond between $D380^{G.H5.22}$ and the absolutely conserved class B GPCR residue, $R^{2.46}$.

In addition, receptor-specific interactions also contribute to G protein binding. Thus, ICL2 of μOR makes strong interactions with the αN-β1 junction, via a $D177^{34.55}$-$R32^{G.hns1.3}$ salt bridge, and the G protein α5 helix, via $R179^{34.57}$-$D350^{G.H5.22}$ H-bond. In GLP-1R, ICL2 also contributes to G protein binding via a $S261$-$Q35^{.HN.51}$ H-bond, and $E262^{4.38}$-$K34^{G.HN.51}$ and $R38^{G.hns1.2}$ salt bridges.

G protein residues responsible for receptor discrimination are also more likely to follow general trends as opposed to hard rules, given the different angle of α5 insertion into the receptor core. G$\alpha_{i/o}$ subunits primarily engage the receptor via the C-terminal part of the α5 helix and the "wavy hook" residues, in the same way as Gα_s. This is consistent with prior studies that identified the last five amino acid residues of the α5 helix as sufficient for switching G protein selectivity.[53,54] Interestingly, the last three amino acids of Gα_{i2} are all that is required for gaining coupling of a Gα_q chimera to the A_1AR and D_2 dopamine receptor[55] that preferentially couples to $G_{i/o}$ proteins. This is likely explained by steric hindrance between the TM7-H8 junction and larger side chains at the −3 positions of the α5 C-terminus in G_s (Glu) and G_q (Asn) families compared to the absolutely conserved Gly in the $G_{i/o}$ family. The larger TM6 movements observed in G_s complexes positions the α5 helix further away from TM7, allowing for accommodation of bulkier side chains.

While the C-terminus of α5 is also important for G_s binding, the middle of α5 appears to contribute strong interactions for

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receptor engagement. In particular, residue Q384$^{G.H5.16}$ makes a H-bond in all G_s complexes. In contrast, $G_{i/o}$ complexes compensate for fewer α5 contacts with additional α4-β6 loop interactions, and this may possibly be linked to a smaller TM6 movement. In agreement with this, interactions with the α4 helix and/or α4-β6 loop were found to be important for $G_{i/o}$ coupling for a number of receptors (5-HT_{1A}R, 5-HT_{1B}R and M_2mAChR,[51] Rho[56]) and could also be involved as determinants of selectivity within the $G_{i/o}$ family, for example, for discrimination between Gα_t and Gα_{i1} subunits.[51] In contrast, the α4-β6 loop is not essential for G_s coupling to the β_2AR,[57] consistent with the hypothesis that, in different G protein families, distinct domains of the Gα subunit could be responsible for receptor selectivity.

It is expected that with the wide adaptation of cryo-EM the number of GPCR-G protein structures will continue to rise rapidly, providing many more GPCR-G protein complexes that will advance our understanding of molecular mechanisms of cellular signaling. An important caveat to the interpretation of available (and likely future) structural data is that they are determined in non-native environments. All cryo-EM structures to date have been solved in detergent micelles, and it is well-known that G proteins form subtype-, and subunit-, specific interactions with lipids (reviewed in ref 58) that may spatially restrict the relative orientations that G proteins can adopt when bound to activated receptor. Crystal structures require distinct stabilization strategies and very specific conditions to allow productive crystal packing. Comparisons of individual agonist:GPCR complexes, solved by alternate stabilization technologies, and solved in both detergent and more native lipid environments, will be required for a more nuanced understanding of what the new structures can tell us about G protein selectivity and activation; behaviors that are critical to mechanistic understanding of efficacy and biased agonism. For example, contact differences occur between the GLP-1 receptor and Gs protein in structures solved with agonists of different efficacy (Figure 6, lower panel),[10,11] indicating that the nature of the ligand will contribute to the transducer interface. Nonetheless, we have entered into an exciting new era of GPCR structural biology that promises to answer many long-held questions on how GPCRs work.

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