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Conformational flexibility and structural dynamics in GPCR-mediated G protein activation: a perspective

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Abstract

Structure and dynamics of G proteins and their cognate receptors, both alone and in complex, are becoming increasingly accessible to experimental techniques. Understanding the conformational changes and timelines which govern these changes can lead to new insights into the processes of ligand binding and associated G protein activation. Experimental systems may involve the use of, or otherwise stabilize, non-native environments. This can complicate our understanding of structural and dynamical features of processes such as the ionic lock, Tryptophan toggle, and G protein flexibility. While elements in the receptor's transmembrane helices and the C-terminal α5 helix of Gα undergo well defined structural changes, regions subject to conformational flexibility may be important in fine-tuning the interactions between activated receptors and G proteins. The pairing of computational and experimental approaches will continue to provide powerful tools to probe the conformation and dynamics of receptor-mediated G protein activation.

Introduction

Early structures of G protein coupled receptors (GPCRs) and G proteins reveal much of what we know about the conformations associated with distinct signaling states, but not the pathways that link these states or the dynamics associated with each of these states. Agonist binding to receptors and binding of cognate G proteins to activated receptors leads to the high-affinity state of the receptor, while catalyzing GDP release from the G protein. These events are accompanied by dynamic conformational changes in both receptors and G proteins on a time scale associated with receptor-mediated G protein activation. Each state is likely represented by an ensemble of conformations, however the experimental methods used to study these states may themselves perturb the system. While molecular dynamics (MD) simulations examine dynamics, there are challenges inherent with these approaches as well, such as convergence and under-sampling, especially as protein size increases. Conversion is generally thought to occur if system has sampled all possible states, and if the timescale is sufficiently long for a reliable prediction to be made[1]. While each approach has its own drawbacks, the combination of experimental data, molecular dynamics simulations, and crystallographic determinations together can be used in a complementary fashion to

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reveal protein dynamics and conformational flexibility associated with receptor-mediated G protein activation.

Conformational dynamics associated with GPCR activation

Dynamics of ligand binding

Rhodopsin, a prototypical class A GPCR, was the GPCR for which a structure was first determined[2]. Crystal structures of rhodopsin reveal distinctly different orientations for the retinal ligand[2–4], resulting in some lack of certainty as to the orientation *in vivo*. Shedding light on this issue, Mertz et al.[5] combined ^{2}H NMR data with MD simulations to reveal that activation of rhodopsin (Rho) results in an ensemble of activated conformational states, which may help account for the divergent orientations of the ligand in crystal structures. Similarly, MD dynamics of dark Rho revealed that the beta-ionone ring of 11-cis-retinal is mobile in the binding pocket[6]. Results from experiments which examine protein structural dynamics, combined with molecular dynamics simulations and structural determinations together indicate that receptors are capable of adopting multiple conformations, depending on the nature of the bound ligand. Thus, conformational flexibility may combine with an induced fit mechanism to help stabilize a subset of conformations. Similarly, microsecond MD simulations of the A_{2A} adenosine receptor demonstrate that a large degree of dynamics accompanies binding of adenosine, and reveal more than one binding orientation for ligand[7]. Only one of these orientations is reflected in the A_{2A} receptor crystal structure[7–9]. On the other hand, binding to a synthetic agonist which is two to three orders of magnitude greater in efficacy than adenosine markedly reduces conformational variability in the receptor[7; 10]. This suggests that the difference in efficacy is due to the synthetic agonist's ability to stabilize a smaller subset of active conformations, increasing the likelihood of G protein activation.

Ionic lock variability

The initial structure of dark rhodopsin[2] lead to early hypotheses that an inactive-state ionic lock between residues in transmembrane (TM) helices 3 and 6, Arg 3.49 and Glu 6.30, respectively, would be broken in the process of GPCR activation. In the case of rhodopsin, breakage of this ionic lock exposes transducin binding elements[11], and biochemical studies suggest that breakage of the lock accompanies agonist activation of β_2AR[12; 13]. Somewhat surprisingly, the structures of activated β_1AR[14], β_2AR[15–17] and opsin[18] were all seen with the ionic lock in the locked orientation, despite earlier predictions. Using microsecond MD simulations, Dror et al.[19] demonstrate that the ionic lock forms and breaks spontaneously in the β_2AR, suggesting that the lock is a dynamic process. Hints as to how this might occur in Rho was revealed by the NMR study cited above[5], which suggests that destabilization of the ionic lock involves rotation of the C=NH$^+$ group of the protonated Schiff base during retinal isomerization. Proton transfer from the protonated Schiff base during retinal isomerization results in a key rearrangement of E/DRY residues involved in the ionic lock. Taken together, these studies suggest that the ensemble of activated Rho conformations may be triggered by retinal isomerization[5].

The ionic lock, its relation to the activation state of the receptor, and factors governing the equilibrium between the open and closed states may be receptor- and context-specific. However, since the simulations which observed the dynamic nature of the ionic lock were performed without the T4-lysozyme (T4L) used to stabilize the crystal structure of the β_2AR[19], it may be that the presence of T4L modulates the equilibrium between locked and unlocked states in the structural determination. A microsecond MD simulation of the β_2AR performed by Romo et al. in 2010 in the absence of ligands or stabilizing proteins confirms the dynamic state of the ionic lock[20]. In addition to the open and locked conformation, this

simulation reveals the presence of an intermediate, semi-open state containing a bridging water molecule. This is accompanied by changes in the orientation of TM helices, which remain hydrated throughout the simulation. However, these data are not meant to imply that the lock is unimportant for function. While the mutation of R in the E/DRY motif of rhodopsin type GPCRs abrogates G protein function[21; 22], mutation of the conserved Glu in the ERY motif of the bradykinin B2 receptor to either R or A turns agonists into functional antagonists, decreasing phosphoinositol signaling and increasing constitutive internalization of receptors[23]. These types of studies help increase our understanding of processes such as biased agonism and functional selectivity that result in ligand-dependent differences in signaling pathways, through either arrestin binding or through differential signaling to G proteins[24]. These studies also point to a potential role for the E/DRY motif in signaling. It is interesting to note that in muscarinic as well as opioid receptor structures, the acidic residue in the DRY motif is linked thorugh a salt bridge to a conserved Arg in IC2[25]. Ligands which alter the structural dynamics of this region may play a role in functional selectivity, given the ability of the agonists to act as antagonists in the bradykinin B2 system.

Energetics of ligand binding

MD simulations on the nanosecond timescale provide valuable information regarding structural dynamics of extracellular and intracellular loops[26–28] and TM helices associated with ligand binding to GPCRs[1]. More recently, a long-timescale MD study in 2011 by Dror et al. was used to investigate the energetics of ligand binding to β_2AR[29]. The authors observed that the ligand pauses in an entryway, or vestibule region before moving through a spatially restricted path to the site seen in crystallographic structures. Surprisingly, the highest energy barrier is associated with entry into the vestibule. This study suggests that the ligand is desolvated as it moves into the vestibule, and the remainder of its hydration shell is lost as it moves into the binding pocket seen in crystallographic studies. In contrast to small conformational changes seen on the ligand binding side, the intracellular side of the receptor exhibits changes in conformation of an even greater magnitude than that seen on the ligand binding side. Furthermore, a distinct intermediate state of the receptor was identified, and the authors propose that this state may facilitate G protein binding, offering new options to design therapies which stabilize or perturb specific receptor conformations.

Tryptophan conformation and receptor hydration

A combination of computational approaches can be used to address questions regarding receptor conformations associated with activation. Increasingly, normal mode analysis (NMA) is being paired with nanosecond and even microsecond MD simulations. With this approach, Louet and colleagues[30] observed features of another Group A GPCR, ghrelin, which matches those of the activated β_2AR and opsin structures. This includes a movement of TM 6 and 7 that opens a pocket for G protein binding. Furthermore, while early crystallographic studies of GPCRs suggested the presence of a Trp toggle switch, this too appeared to be questionable, in the light of later structures. Helping to reconcile these divergent observations, the combination of NMA and MD simulations by Louet et al.[30] reveals that this highly conserved Trp in the C**W**LP motif of GPCRs is able to flip conformation. Furthermore, this flip is observed without applying any constraint to the simulation. An unbiased MD simulation by Hurst et al.[31] demonstrates that the entrance of *sn*-2-arachidonylglycerol (2-AG) into the binding pocket of the cannabinoid receptor is sufficient to break the ionic lock, and full binding of 2-AG into the ligand binding site results in a reorientation of the conserved Trp in the C**W**LP motif of this class A GPCR. This reorientation is accompanied by influx of water upon receptor activation[31], consistent with radiolytic footprinting of rhodopsin[32], as well as in MD simulations of rhodopsin activation[33].

A crystal structure of the A_{2A} adenosine receptor bound to an antagonist contained three distinct water clusters which were visible at 1.8 Å[34]; on the extracellular face, in the TM core, and at the intracellular face, near the E/DRY motif. The waters in the central TM region are coordinated to a Na^+ ion that may play a role in receptor activation. In the agonist bound A_{2A} receptor, the ligand induced change in helix III prevents water binding[9; 10]. Thus, the presence of water and activation-induced changes in conformation which alter hydration of the receptor may be common features in GPCRs[6; 31–33].

Conformational flexibility in the receptor core

Studies employing dynamic single-molecule force spectroscopy have also been used to investigate membrane-bound proteins[35; 36]. This approach allows the measurement of kinetic responses such that conformational variability during receptor activation can be quantified, along with other parameters such as unfolding free energy and mechanical flexibility[35]. Using this technique, Zocher and colleagues found that the basal activity of the β_2AR is due to a high level of conformational variability in the core of the receptor, and that ligands alter the receptor's energy landscape by modifying the receptor's core[36]. Both agonists and inverse agonists increase the flexibility of the core, thus increasing the overall number of possible conformations, as well as enhancing the probability of the receptor adopting an activated conformation. However, this would not necessarily cause all receptor molecules to adopt an activated conformation. Binding of a G protein (or a molecule which mimics it) is predicted to further increase the number of receptor molecules in the active conformation. The ability to quantify the conformational variability of the receptor core may lead to a better understanding of how ligand binding stabilizes specific conformations through stabilization of structural segments within the core of the β_2AR[36].

Role of lipids in conformational flexibility and structural dynamics of receptors

However, we cannot consider the receptor in isolation. In addition to the myriad of membrane-bound and peripheral proteins in close proximity to receptors, receptors are surrounded by lipids in the membrane. To determine if lipids alter the dynamic state of receptors, Zocher et al. extended their 2012 study to include a lipid which mimics cholesterol[37]. Using dynamic single-molecule force spectroscopy, they found that cholesterol increases the kinetic stability of the β_2AR, increasing the free energy barriers that stabilize each segment of the receptor against unfolding. These results suggest that the forces governing the structural dynamics of the receptor, and the energetics that stabilize receptor conformation, are influenced by lipids. This was not entirely unexpected, as early studies with rhodopsin demonstrated that cholesterol alters the metarhodopsin (Meta) I and Meta II equilibrium towards the inactive, Meta I state[38]. MD studies also suggest that more than one binding site exists for cholesterol in the $A2_A$ receptor[39], and one of these sites was subsequently confirmed by structural determination of this receptor[34]. Since lipid rafts are thought to exhibit distinct lipid composition and subcellular localizations within the cell, rafts may play roles in the spatial regulation of signaling downstream of receptor activation[37]. However, the ability to isolate such membrane subdomains remains challenging, particularly because the methods used to isolate rafts may themselves influence a non-physiologic lipid composition.

Ligand binding alters dynamics on the intracellular face of the receptor

Since biased MD simulations can reveal trajectories that may or may not be relevant to biological signaling, despite well-defined endpoints[40], the pairing of experimental evidence with simulation can enhance our understanding and increase confidence in the results of such studies. NMR has long been used as a tool for studying protein dynamics in solution. The propensity of ligands to alter the environment of both the extracellular and intracellular sides of the β_2AR was demonstrated by a recent study combining NMR experiments with

MD simulations by Nygaard and colleagues[41]. By examining the environment of a distinct set of residues in the receptor in the agonist-bound state, as well as bound to both an agonist and a G-protein mimicking nanobody, they found that ligand binding stabilizes the orientation of the extracellular side of the receptor, while increasing protein conformational variability at the intracellular side. Binding of both the agonist and G-protein mimic are required to reduce the dynamics at the intracellular side and fully stabilize the activated state of the receptor. Likewise, West and colleagues used hydrogen-deuterium exchange to identify changes in receptor conformation[42]. This study demonstrated that agonists increase conformational flexibility in the β_2AR, while inverse agonists have a stabilizing effect. Activation of Rho also resulted in enhanced hydrogen-deuterium exchange, consistent with an activation-dependent increase in the conformational dynamics of the receptor[43]. The propensity for agonists to increase conformational variability in receptors may be responsible for the relatively fewer receptor structures determined in the activated state. However, as agonists which preferentially stabilize a specific active state are identified, such as in the structure of the agonist-bound A_{2A} receptor[10], more active state structures are likely to be determined.

Conformational variability in the nucleotide-free, receptor-bound G protein

Flexibility of the helical domain

The receptor-bound G_s complex[16] is the first structural determination of an activated receptor bound to a G protein. This study confirms numerous previous structural and biochemical studies which indicated that activation of a GPCR is accompanied by the outward movement of TM6 away from TM3, exposing a pocket for G protein binding. Not surprisingly, the structure confirms the interaction of the C terminus (CT) of the Gα protein with a pocket on the receptor opened by receptor activation. The structure also identifies a number of additional and less extensive interactions between the receptor and G protein, such as the interaction of intracellular loop 2 (IL2) of the receptor with αN/β1 hinge, β2/β3 loop, and TM5 of the receptor with α4 and β6 residues. Furthermore, this structure of the nucleotide-free receptor-G protein complex exhibits a loss of interdomain contacts, originally predicted in[44] to accompany receptor-mediated G protein activation. Interestingly, an earlier computational study using MD simulations of isolated, nucleotide-bound $G\alpha_t$ proteins performed by Ceruso and colleagues[45] hints at the interdomain reorientation that is now known to be a feature of receptor-bound G proteins. A more recent double electron electron resonance (DEER) study demonstrates that receptor activation is accompanied by a separation between the helical and GTPase domains in a rhodopsin-G_i model system[46], an observation qualitatively confirmed shortly thereafter by the β_2AR-G_s structural determination[16]. However, the exact placement of the helical domain in this crystal structure[16] diverges from that in the DEER study (Fig. 1A), which may be due to the different conformations stabilized by the different techniques, or more likely due to an inherent flexibility of the helical domain upon GDP release.

The distribution of distances between pairs of residues spanning the helical and GTPase domains in this original DEER study[46] indicated that there is a wide variability in the location of the helical domain in the receptor-bound Gα. Using a Rosetta-based approach to incorporating DEER distance distributions into a model of the receptor-bound G protein complex, we obtained an ensemble of structures which exhibited a highly flexible helical domain (in preparation). In this model, the helical domain was highly dynamic in the activated, receptor-bound, nucleotide-free state, in contrast to the GTPase domain, which remains in an orientation defined by the insertion of the CT of Gα into the receptor, as seen in the β_2AR-G_s structure[16; 47] and a previous model[46]. Importantly, the conformational variability associated with the nucleotide-free state is not simply due to the loss of nucleotide. Ridge and colleagues demonstrated in an NMR study in 2006[48] that receptor

activation results in an increase in protein dynamics in the Gα subunit that are beyond the increases in dynamics observed in an isolated, nucleotide-free Gα protein[49].

Communicating receptor activation to GDP release

Interaction of a G protein with an activated receptor results in a marked conformational change in the CT of Gα and a highly flexible helical domain[16; 50]. Using a combination of MD simulation and NMA, Louet et al.[30] proposed that receptor-mediated nucleotide release occurs by a concerted mechanism that opens the GDP pocket as the receptor induces conformational changes in the C-terminal α5 helix, along with motions of α5, αG, α4, and the αN/β1 hinge. This study suggests that egress of the GDP may occur through either the base or phosphate side of the nucleotide. This study also predicts an important role for stabilization of the kink in the αA helix, necessary for a rigid body rotation of the helical domain away from the GTPase domain.

A hydrophobic triad links IC2 to αN/β1 hinge, β2/β3 loop and α5 helix of Gα

The CT of αG and residues in the α4 helix and α4/β6 loop have long been known from functional studies to be important for receptor-mediated G protein activation[51–57]. The CT of Gα plays well-established roles in receptor coupling, and both the crystal structure of the receptor-bound Gα complex and associated deuterium exchange studies demonstrate that this region is highly immobilized by interaction with activated receptors[16; 32; 58]. The β_2AR-G_s structure also implicates regions other than the CT in receptor-G protein coupling, such as the α4 and α4/β6 loop, the β2/β3 loop and αN/β1 hinge of Gα[16], as well as the IC2 of the receptor (Fig. 1B-C). Residues linked to the E/DRY motif in the IC2 loop of Rho also display reduced hydrogen-deuterium exchange in the activated Rho-Gt complex[32], consistent with its role in coupling to Gα proteins. Loops and hinges are regions of high conformational variability that may enable fine-tuning of interactions between receptor and G protein. In Gα proteins, the β2/β3 loop is located in a critical region between Switches (Sw) I and II, and this loop contacts activated receptor in the β_2AR-G_s complex[16]. In a recent study, site-specific labeling was used to demonstrate that receptor activation is communicated from the β2/β3 loop to Switches (Sw) I and II, resulting in enhanced packing of individual residues throughout Sw I and II of G_i proteins[59].

In the β_2AR-G_s complex, a hydrophobic triad of residues links receptor to G protein through a hydrophobic pocket[59]. This triad consists of F139 in IC2 of the β_2AR, together with conserved residues in the β2/β3 loop (V217) and C-terminal α5 helix of $G\alpha_s$ (F376, Fig. 1B-C). In the deuterium exchange study by Palczewski and colleagues, the peptide that encompasses the residue homologous to V217 in $G\alpha_t$ displayed a low solvent accessibility when in complex with activated rhodopsin, roughly equivalent to the solvent accessibility of the CT, and the αN/β1 hinge also displayed a relatively low degree of solvent accessibility, in comparison to the remainder of the $G\alpha_t$ protein in the activated complex[32]. The αN/β1 hinge implicated in receptor coupling in the β_2AR-G_s complex[16] is allosterically linked to residues in the hydrophobic triad[59] (Fig. 1C). In the cannabinoid receptor system, mutation of the homologous IC2 residue, L222 to either A or P, eliminates any coupling to G_s[60], but does not perturb coupling to G_i, suggesting a role for the IC2 in G protein selectivity[61]. Furthermore, mutation of a nearby β_2AR IC2 loop residue, Y141, eliminates potentiation of adenylyl cyclase activity by insulin. These results (and others) suggest a role for IC2 in modulating G protein signaling[62–69], with some studies also implicating this region in the selectivity of receptor-G protein coupling[70–72].

IC2 conformational flexibility

A study by Burstein et al.[69] in the 1990's implicates the IC2 in coupling of muscarinic receptors to $G\alpha_i$ proteins[62–69]. Based on mutational results alone, they predicted a helical

conformation for the IC2 region, with one face containing residues important for receptor activation, and another other face involved in coupling to G proteins. Indeed, the crystal structure of the activated β_2AR-G_s complex confirms not only the helical structure for IC2 when bound to the activated G protein, but also the linkage of residues on the intracellular side of IC2 to the DRY motif, with the opposing side of the helix in contact with G protein[17]. In the antagonist and inverse agonist bound β_2AR, F139 in IC2 is angled away from the hydrophobic pocket formed by the juxtaposition with residues from the β2/β3 loop and the α5 helix (Fig. 1B-C)[73; 74]. Other receptor systems which exhibit a helical conformation for IL2 include β_1AR, M2R and M3R, μ-OP and δ-OR, as well as the A_{2A} adrenergic receptor[25]. This particular IC2 loop residue has been shown to play an important role in physiology, as a L to S mutation in the residue that is homologous to F139 in the GPCR, GPR54, causes idiopathic hypogonadotropic hypogonadism, a disorder associated with delayed puberty and infertility[64].

Conformational flexibility of the hydrophobic triad and αN/β1 hinge

In $G\alpha_t$, mutation of the Phe homologous to F376 in $G\alpha_s$ enhances receptor-mediated nucleotide exchange[75], while mutation of the residue homologous to $G\alpha_s$ V217 in the β2/β3 loop of $G\alpha_i$ significantly reduces receptor-mediated nucleotide exchange[59]. Several studies have also implicated the αN and αN/β1 hinge in receptor activation, consistent with observations from the β_2AR-G_s structure[55; 76–78]. An all-atom MD simulation of the rhodopsin-transducin complex also identified the β2/β3 loop, the αN/β1 hinge, and the α5 helix in the interactions of the Gα protein interactions with activated receptor[79]. This simulation indicates that the complex is dynamic, and samples many conformations during this microsecond simulation. These studies support a very dynamic receptor-G protein interface that includes contributions from regions far removed from the CT of Gα, in contrast to the low degree of solvent accessibility and dynamics in the CT of Gα itself. This is evident in deuterium exchange experiments of G_s and G_t with activated receptors[32; 58], consistent with the well-established role of the CT in binding to activated receptors[56; 57; 80; 81].

On the other hand, residues in the αN/β1 hinge region of $G\alpha_s$, when incubated with activated receptors, exhibited increased exchange over the time course of the experiment, indicative of enhanced dynamics in this region in the receptor-G protein complex[58]. Interestingly, F139 in IC2, part of the hydrophobic triad linking receptor to the Gα protein, exhibits a distinctly altered conformation in the antagonist-bound and inverse-agonist bound β_2AR structures (Fig. 1C), as compared to the G-protein bound structure. The helical conformation adopted by IC2 in the β_2AR-G_s protein complex is absent without the bound G protein. Studies have shown that phosphorylation of Tyr 141 in the IC2 of β_2AR shifts the receptor equilibrium towards the active conformation[62], while mutation of Tyr 149 in the β_1AR decreases stability of this receptor. In β_2AR-G_s[82], interaction of F139 of the receptor with residues 217 and 376 of $G\alpha_s$ would be expected to decrease packing surrounding the αN/β1 hinge region (Fig. 1C). In fact, deuterium exchange shows a time dependent increase in solvent exposure and the structural dynamics of αN/β1 hinge upon interaction with activated receptor[58]. More studies are needed to determine the functional importance of the increased structural dynamics in αN/β1 hinge in receptor-mediated G protein activation.

α5 α1 and αG Conformational variability in the receptor-bound complex

There is a marked increase in protein dynamics in αG of the Gα subunit when bound to β_2AR, evidenced by the increase in the time-dependence of deuterium exchange in this region[58]. The activated Rho-G_t complex also exhibits enhanced deuterium exchange in the αG region of the Gα subunit[32]. Computational studies suggest that αG undergoes conformational changes upon receptor activation[83], consistent with these deuterium

exchange studies. The αG helix of Gα is in close proximity to bound GDP and the α5 helix, as well as proximity to residues in the helical domain (Fig. 2A), and thus may be a critical point linking the two domains. Another important allosteric linkage between the domains is likely mediated by interactions between the α1 and α5 helices of the G subunit. The α5 helix contacts the α1 helix (overview, Fig 2A), and α1 links the GTPase to the helical domain through the αA helix. At the bottom of the α1 helix is the P loop (Fig. 2B), so named due to its interaction with the phosphate of bound nucleotide (Fig. 2B, phosphates of GDP in orange and red). Thus, conformational changes at the CT of Gα may be communicated to the bound nucleotide, both directly and indirectly, leading to the observed increase in conformational flexibility of the helical domain (Fig. 3A-C)[46; 47; 84]. The receptor induces a large conformational change in the CT, which alters interaction with the guanine ring of the bound nucleotide[51; 85; 86] through a rotation and translation of the C-terminal α5 helix[50]. Receptor-mediated changes in the CT may be communicated to the α1 helix and phosphate binding P-loop, as suggested by a study by Sakmar and colleagues[86]. In that study, mutations in the α5 and α1 helix result in perturbation of receptor-mediated nucleotide exchange. This is consistent with MD simulation by Weinstein and colleagues[45] which reveals a role for the linkage between α5 and α1, as well as with the β2/β3 loop in interdomain flexibility associated with G protein activation.

Nucleotide binding reduces G protein conformational flexibility

Nucleotide binding restores contacts between the domains, as seen in crystal structures of GTPγS-bound Gα proteins[44; 87; 88]. This is also seen in the reduction of line widths of spin-labeled Gα proteins upon GTPγS binding in EPR studies[89]. It is likely that nucleotide binding mediates decreased conformational flexibility, which stabilizes conformations that favor interaction with binding partners. Although the excess of GTP present within the cell overwhelmingly favors GTP binding to activated G proteins in the receptor-bound complex, a recent study indicates that the environment of individually labeled Sw I residues in the activated complex mimic that of the same residues in the GTPγS-bound state, suggesting that receptor activation may pre-organize these regions for subsequent GTP binding[59]. In the case of G_i proteins, N-terminal myristoylation (myr), a permanent co-translational modification of G_i family proteins, including G_t, reduces the already low degree of structural dynamics at the base of the α5 helix in the AlF_4-activated protein[90]. This is consistent with a myr-dependent stabilization of bound nucleotide. Structural dynamics of the activated G protein are also influenced by myr in regions distal from the NT and in regions of Gα known to be involved nucleotide binding[90]. Thus, myristoylation may play a role in modulation G protein conformational flexibility in the GTP-bound protein.

Conclusion

The studies described here reveal potential pathways for activation and the activation dynamics implicated in receptor-mediated G protein activation. Taken together, these studies demonstrate that there is more than one conformation associated with activated receptors, as well as for activated, nucleotide-free Gα bound to these receptors. The inter-conversion between distinct activated states, and the timescale for inter-conversion between these states is still largely unknown. Furthermore, the ensemble of conformations that are associated with activation, and the relative energy of each state is still to be determined. In the receptor-G protein complex, these studies paint a picture of a highly dynamic Gα helical domain, with limited structural dynamics at the CT of Gα. In addition, receptor activation may alter dynamics in conformationally variable regions of the receptor and G protein that are known to participate in receptor G protein coupling, including the IC2 loop of the receptor, and the αN/β1 hinge and β2/β3 loop of Gα[16]. These structural dynamics may modulate effects of conformational changes that are mediated by the CT of Gα binding to activated receptors. These changes are likely propagated from the extreme Gα CT that binds

to receptor to the base of the α5 helix of the G protein[50; 85; 86] and throughout the GTPase domain, as well as across the nucleotide binding cleft to the helical domain. Together these result in a conformationally flexible helical domain in the receptor-bound, nucleotide-free state[46; 47; 84]. This may occur as a concerted mechanism, or step wise, and time-resolved experiments will be required in order to fully elucidate the order and pathway of the conformational changes that are induced by receptor activation to result in a fully activated Gα protein. Investigation of these questions will increase our understanding of conformation and dynamics that regulate G protein signaling *in vivo*.

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ABBREVIATIONS

GPCR	G protein coupled receptor
MD	molecular dynamics
NMR	nuclear magnetic resonance
DEER	double electron electron resonance
DEER	double-electron electromagnetic resonance
Rho	rhodopsin
TM	transmembrane
T4L	T4-lysozyme
NMA	normal mode analysis
2-AG	*sn*-2-arachidonylglycerol
Meta	metarhodopsin
CT	C terminus
IL2	intracellular loop 2
P-loop	loop which binds phosphate in Gα
myr	myristoylation
EC	extracellular
IL	intracellular
OR	opioid receptor

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Highlights

- Crystal structures represent lowest-energy conformations, while GPCRs and G proteins are dynamic.
- Experimental and computational approaches can be used to conformation and dynamics.
- The receptor-G protein complex exhibits a flexible and dynamic helical domain.
- A hydrophobic triad of residues links IC2 of receptor to β2/β3 loop and C terminus of Gα
- Flexible loops in receptor and G protein may fine tune interaction between receptor and G protein

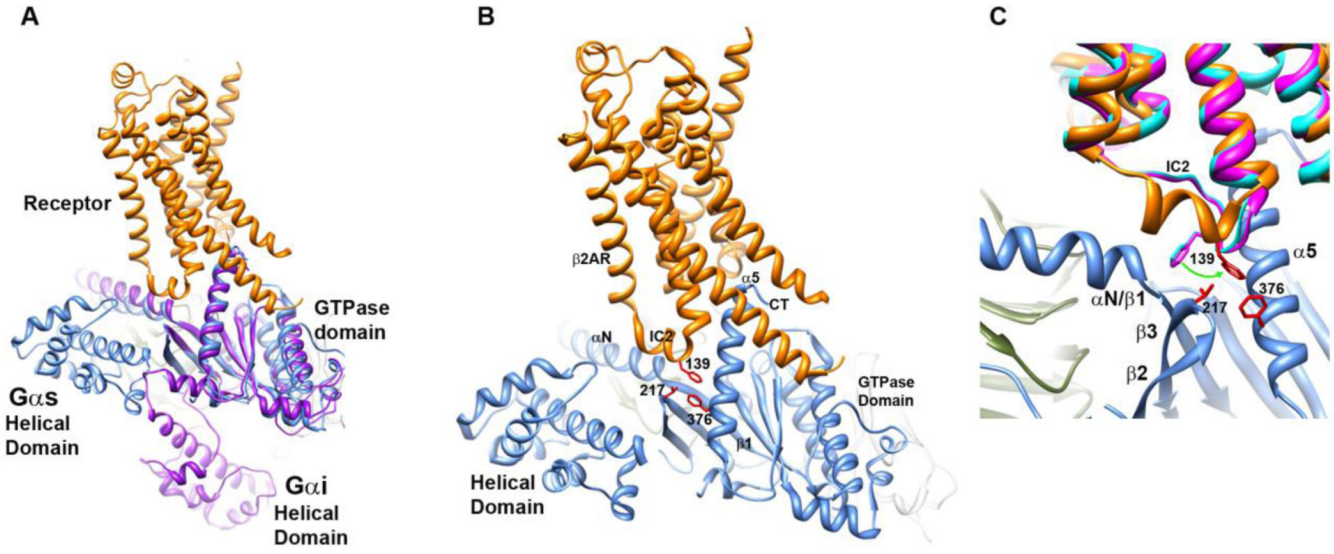

Fig. 1.

The receptor-G protein complex. **A.** Comparison of the positions of the helical domains of Gα in β_2AR-G_s (β_2AR in orange, G_s in blue) vs. model derived from[32], shown in purple. **B.** Hydrophobic triad of residues links IC2 of the β_2AR to the β2/β3 loop and CT α5 helix of $G\alpha_s$ in the receptor-bound complex, side chains from hydrophobic triad shown in red. **C.** Overlay of β_2AR receptor (teal, bound to antagonist, no G protein, PDB 3NYA; magenta, bound to inverse agonist, no G protein, PDB 3D4S) with that of activated complex, PDB 3SN6 (as in B).

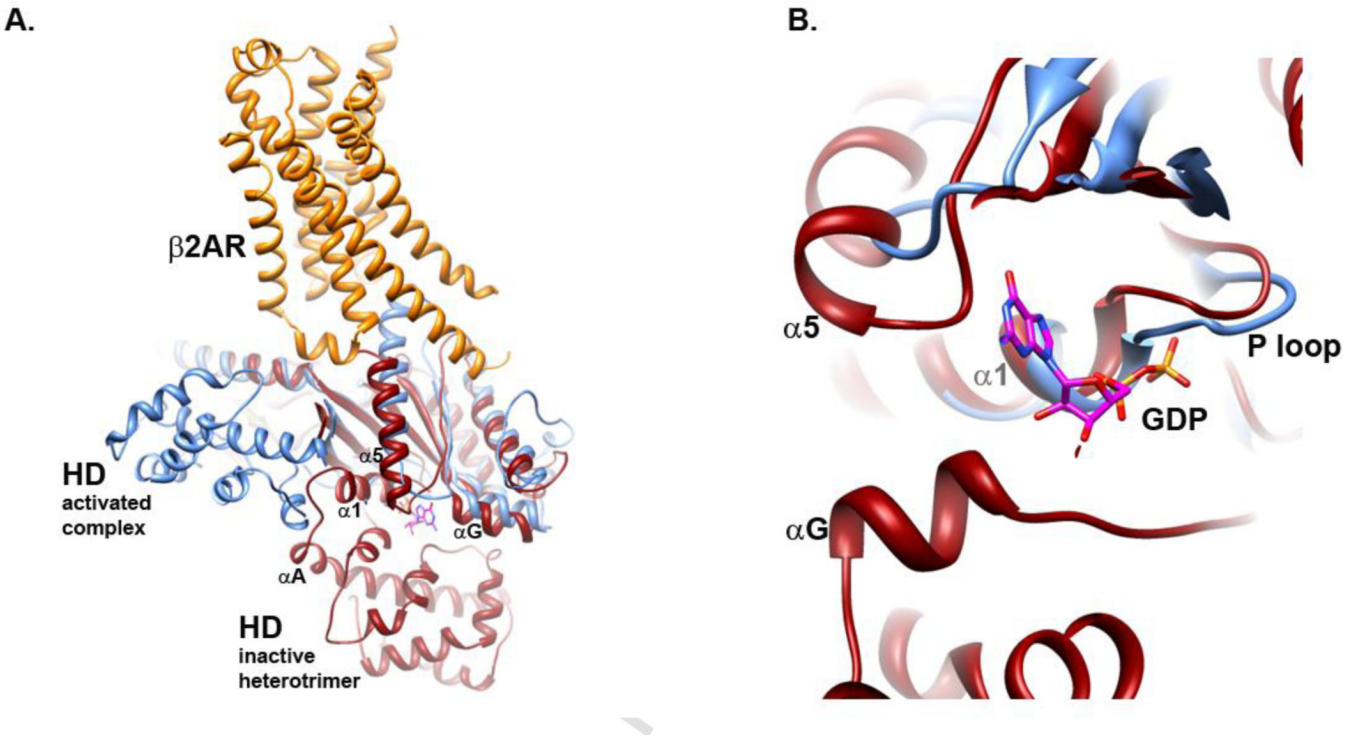

Fig. 2.

Overlay of β_2AR-G_s complex with GDP-bound heterotrimeric G protein $G\alpha\beta\gamma_i$ (PDB files 3SN6 and 1GP2, respectively). $G\alpha_s$ shown in blue, β_2AR in orange, and $G\alpha_i$ in red. Note that there is no high resolution of $G\alpha_s$GDP available for this comparison. **A**. Overview showing proximity of α5, αA and αG helices to bound GDP (sticks). **B**. Close up, rotated and slab view, showing proximity of P loop, α5, αG and αA to bound nucleotide.

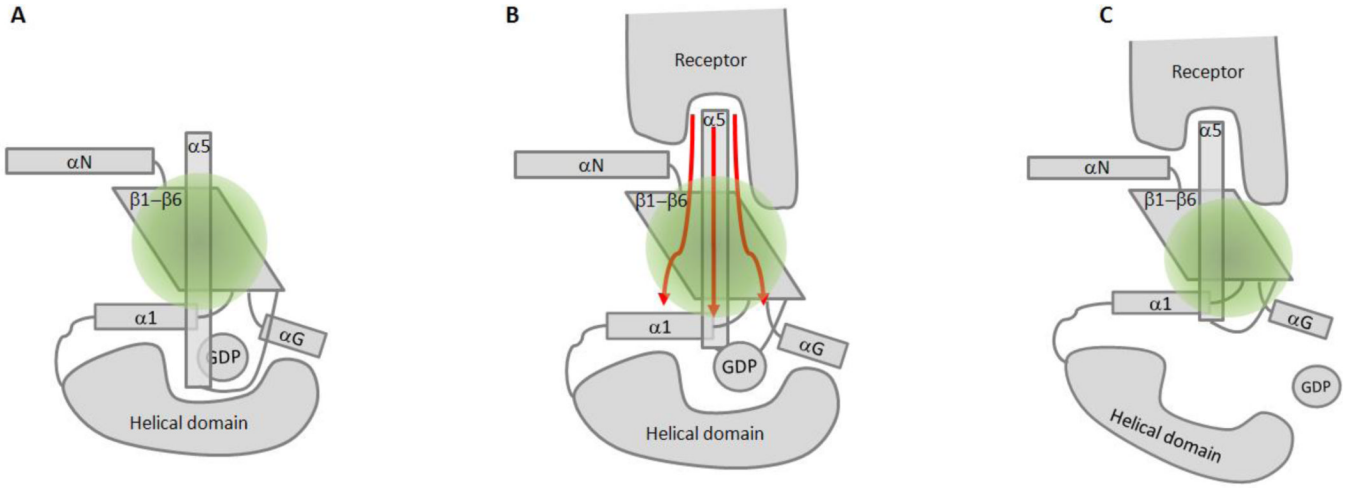

Fig. 3.

Receptor-mediated G protein activation schematic. **A.** Gα protein (Gβγ not shown), with specific elements in the GTPase domain labeled. GDP is held in the cleft between the GTPase and helical domains. **B**. Receptor activation impinges on the C-terminal α5 helix, and interactions of IC2 with secondary sites such as the αN/β1 hinge and α4/β6 loop dynamically alter interactions at the base of the α5 helix with surrounding regions. **C**. Receptor-mediated G protein activation results in the nucleotide-free, empty pocket state of the Gα protein, and a conformationally dynamic helical domain.