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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 that 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 dynamic 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.
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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 lead 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 timescale 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 the 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, MD simulations, and crystallographic determinations together can be used in a complementary fashion to 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

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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 β-ionone ring of 11-*cis*-retinal is mobile in the binding pocket.[6] Results from experiments that examine protein structural dynamics combined with MD 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 that is 2–3 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] led 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 that observed the dynamic nature of the ionic lock were performed without the T4-lysozyme used to stabilize the crystal structure of the β_2AR,[19] it may be that the presence of T4-lysozyme 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 through a salt bridge to a conserved Arg in IC2.[25] Ligands that 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

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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 that 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 *et al.*[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 TM6 and TM7 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 into the binding pocket of the cannabinoid receptor is sufficient to break the ionic lock, and full binding of *sn*-2-arachidonylglycerol 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 that 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 that 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 *et al.* 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 that 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 that 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

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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 *et al.*[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 the G protein mimic is required to reduce the dynamics at the intracellular side and fully stabilize the activated state of the receptor. Likewise, West *et al.* 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 that 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 that 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 of the receptor with the αN/β1 hinge, the 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 Ref. 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 *et al.*[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 that exhibited a highly flexible helical domain (unpublished results). 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 *et al.* 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

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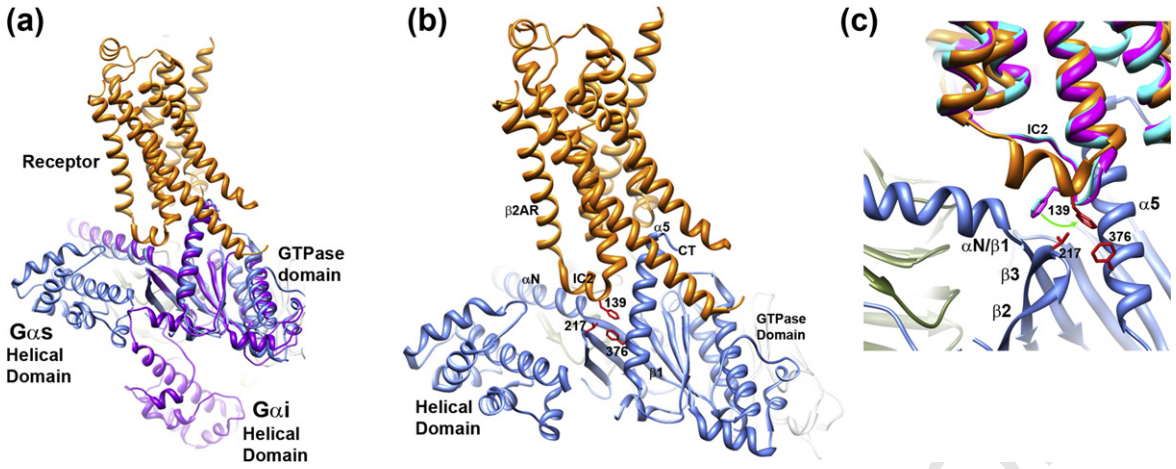

Fig. 1. The receptor–G protein complex. (a) Comparison of the positions of the helical domains of Gα in β_2AR–G_s (β_2AR in orange, G_s in blue) *versus* the model derived from Ref. 32 shown in purple. (b) Hydrophobic triad of residues links IC2 of the β_2AR to the β2/β3 loop and CT α5 helix of $G\alpha_s$ in the receptor-bound complex; side chains from the hydrophobic triad are shown in red. (c) Overlay of the β_2AR receptor [teal, bound to antagonist, no G protein, Protein Data Bank (PDB) ID: 3NYA; magenta, bound to inverse agonist, no G protein, PDB ID: 3D4S] with that of the activated complex [PDB ID: 3SN6, as in (b)].

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 the αN/β1 hinge, the β2/β3 loop, and the α5 helix of Gα

The CT of Gα and residues in the α4 helix and the 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 the αN/β1 hinge of Gα,[16] as well as the IC2 of the receptor (Fig. 1b and 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 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 the C-terminal α5 helix of $G\alpha_s$ (F376, Fig. 1b and c). In the deuterium exchange study by Palczewski *et al.*, 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.

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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 1990s 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 $\beta_2AR–G_s$ 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 and c).[73,74] Other receptor systems that exhibit a helical conformation for intracellular loop 2 include β_1AR, M2R and M3R, μ-OP and δ-OR, and the A_{2A} adrenergic receptor.[25] This particular IC2 loop residue has been shown to play an important role in physiology, as an 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 the 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 $\beta_2AR–G_s$ 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 $\beta_2AR–G_s$ 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 the stability of this receptor. In $\beta_2AR–G_s$,[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 the αN/β1 hinge upon interaction with activated receptor.[58] More studies are needed to determine the functional importance of the increased structural dynamics in the 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 Gα 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

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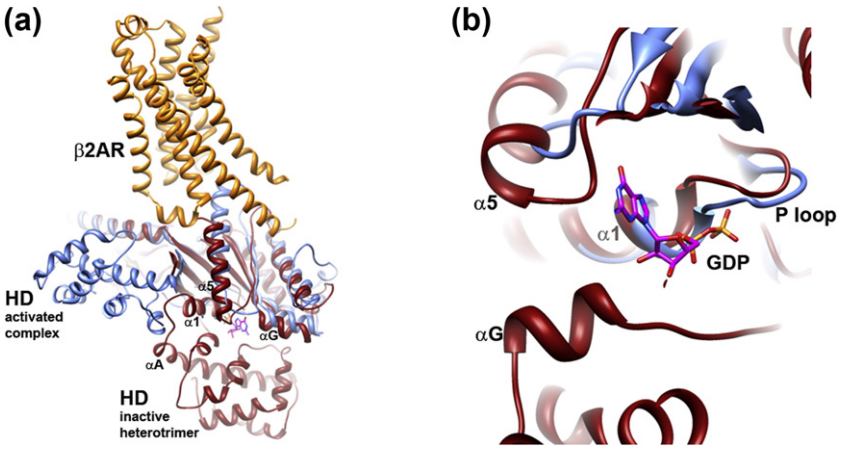

Fig. 2. Overlay of the β_2AR–G_s complex with GDP-bound heterotrimeric G protein $G\alpha\beta\gamma_i$ (PDB IDs: 3SN6 and 1GP2, respectively). $G\alpha_s$ is shown in blue, β_2AR is in orange, and $G\alpha_i$ is in red. Note that there is no high resolution of $G\alpha_s$GDP available for this comparison. (a) Overview showing proximity of α5, αA, and αG helices to bound GDP (sticks). (b) Close-up, rotated, and slab view, showing proximity of the P loop, α5, αG, and αA to bound nucleotide.

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 Kapoor *et al.*[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 *et al.*,[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

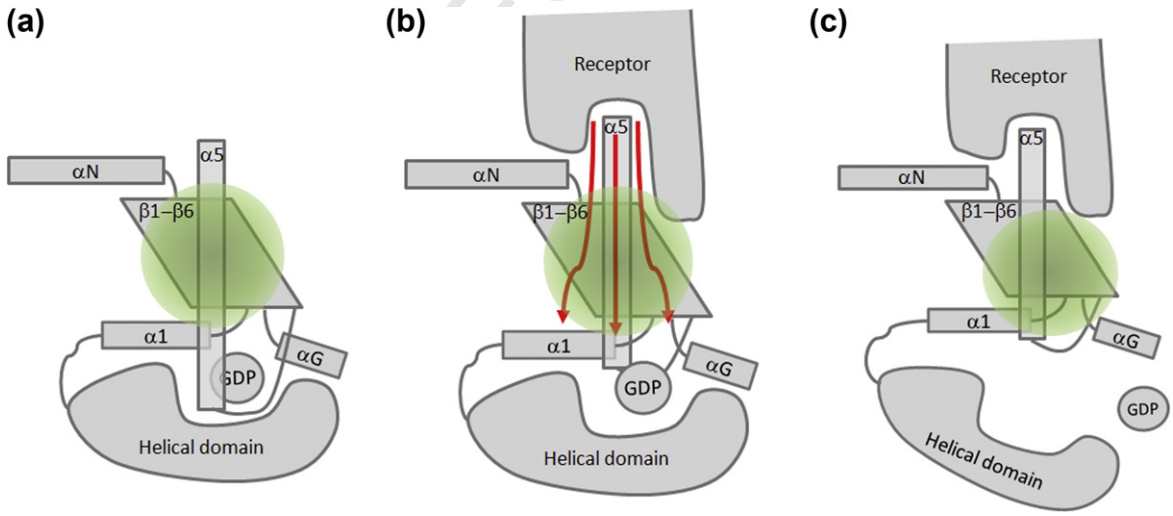

Fig. 3. Receptor-mediated G protein activation schematic. (a) Gα protein (Gβγ not shown), with specific elements in the GTPase domain labeled. GDP is held in the cleft between the GTPase and helical domains. (b) Receptor activation impinges on the C-terminal α5 helix, and interactions of IC2 with secondary sites such as the αN/β1 hinge and the 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.

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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 mimics 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 in nucleotide binding.[90] Thus, myr may play a role in the modulation of 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 are still largely unknown. Furthermore, the ensemble of conformations that are associated with activation and the relative energy of each state are 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 the 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 used:
GPCR, G protein coupled receptor; MD, molecular dynamics; DEER, double electron–electron resonance; TM, transmembrane; NMA, normal mode analysis; Meta, metarhodopsin; CT, C terminus; myr, myristoylation.

References

1. Ng, H. W., Laughton, C. A. & Doughty, S. (2013). Molecular dynamics simulations of the adenosine A2a receptor: structural stability, sampling and convergence. *J. Chem. Inf. Model.* In press.
2. Palczewski, K., Kumasaka, T., Hori, T., Behnke, C. A., Motoshima, H., Fox, B. A. *et al.* (2000). Crystal structure of rhodopsin: a G protein-coupled receptor. *Science*, **289**, 739–745.
3. Nakamichi, H. & Okada, T. (2006). Local peptide movement in the photoreaction intermediate of rhodopsin. *Proc. Natl Acad. Sci. USA*, **103**, 12729–12734.
4. Standfuss, J., Edwards, P. C., D'Antona, A., Fransen, M., Xie, G., Oprian, D. D. & Schertler, G. F. (2011). The structural basis of agonist-induced activation in constitutively active rhodopsin. *Nature*, **471**, 656–660.
5. Mertz, B., Struts, A. V., Feller, S. E. & Brown, M. F. (2012). Molecular simulations and solid-state NMR investigate dynamical structure in rhodopsin activation. *Biochim. Biophys. Acta*, **1818**, 241–251.
6. Lau, P. W., Grossfield, A., Feller, S. E., Pitman, M. C. & Brown, M. F. (2007). Dynamic structure of retinylidene ligand of rhodopsin probed by molecular simulations. *J. Mol. Biol.* **372**, 906–917.
7. Lee, J. Y. & Lyman, E. (2012). Agonist dynamics and conformational selection during microsecond simulations of the A(2A) adenosine receptor. *Biophys. J.* **102**, 2114–2120.
8. Jaakola, V. P., Griffith, M. T., Hanson, M. A., Cherezov, V., Chien, E. Y., Lane, J. R. *et al.* (2008). The 2.6 angstrom crystal structure of a human A2A adenosine receptor bound to an antagonist. *Science*, **322**, 1211–1217.
9. Lebon, G., Warne, T., Edwards, P. C., Bennett, K., Langmead, C. J., Leslie, A. G. & Tate, C. G. (2011).

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ARTICLE IN PRESS

Agonist-bound adenosine A2A receptor structures reveal common features of GPCR activation. *Nature*, **474**, 521–525.
10. Xu, F., Wu, H., Katritch, V., Han, G. W., Jacobson, K. A., Gao, Z. G. *et al.* (2011). Structure of an agonist-bound human A2A adenosine receptor. *Science*, **332**, 322–327.
11. Janz, J. M. & Farrens, D. L. (2004). Rhodopsin activation exposes a key hydrophobic binding site for the transducin α-subunit C terminus. *J. Biol. Chem.* **279**, 29767–29773.
12. Kobilka, B. K. & Deupi, X. (2007). Conformational complexity of G-protein-coupled receptors. *Trends Pharmacol. Sci.* **28**, 397–406.
13. Ballesteros, J. A., Jensen, A. D., Liapakis, G., Rasmussen, S. G., Shi, L., Gether, U. & Javitch, J. A. (2001). Activation of the beta 2-adrenergic receptor involves disruption of an ionic lock between the cytoplasmic ends of transmembrane segments 3 and 6. *J. Biol. Chem.* **276**, 29171–29177.
14. Warne, T., Moukhametzianov, R., Baker, J. G., Nehme, R., Edwards, P. C., Leslie, A. G. *et al.* (2011). The structural basis for agonist and partial agonist action on a beta(1)-adrenergic receptor. *Nature*, **469**, 241–244.
15. Rosenbaum, D. M., Zhang, C., Lyons, J. A., Holl, R., Aragao, D., Arlow, D. H. *et al.* (2011). Structure and function of an irreversible agonist-beta(2) adrenoceptor complex. *Nature*, **469**, 236–240.
16. Rasmussen, S. G., Devree, B. T., Zou, Y., Kruse, A. C., Chung, K. Y., Kobilka, T. S. *et al.* (2011). Crystal structure of the beta(2) adrenergic receptor-Gs protein complex. *Nature*, **477**, 549–555.
17. Rasmussen, S. G., Choi, H. J., Fung, J. J., Pardon, E., Casarosa, P., Chae, P. S. *et al.* (2011). Structure of a nanobody-stabilized active state of the beta(2) adrenoceptor. *Nature*, **469**, 175–180.
18. Park, J. H., Scheerer, P., Hofmann, K. P., Choe, H. W. & Ernst, O. P. (2008). Crystal structure of the ligand-free G-protein-coupled receptor opsin. *Nature*, **454**, 183–187.
19. Dror, R. O., Arlow, D. H., Borhani, D. W., Jensen, M. O., Piana, S. & Shaw, D. E. (2009). Identification of two distinct inactive conformations of the beta2-adrenergic receptor reconciles structural and biochemical observations. *Proc. Natl Acad. Sci. USA*, **106**, 4689–4694.
20. Romo, T. D., Grossfield, A. & Pitman, M. C. (2010). Concerted interconversion between ionic lock substates of the beta(2) adrenergic receptor revealed by microsecond timescale molecular dynamics. *Biophys. J.* **98**, 76–84.
21. Rovati, G. E., Capra, V. & Neubig, R. R. (2007). The highly conserved DRY motif of class A G protein-coupled receptors: beyond the ground state. *Mol. Pharmacol.* **71**, 959–964.
22. Schneider, E. H., Schnell, D., Strasser, A., Dove, S. & Seifert, R. (2010). Impact of the DRY motif and the missing "ionic lock" on constitutive activity and G-protein coupling of the human histamine H4 receptor. *J. Pharmacol. Exp. Ther.* **333**, 382–392.
23. Leschner, J., Wennerberg, G., Feierler, J., Bermudez, M., Welte, B., Kalatskaya, I. *et al.* (2013). Interruption of the ionic lock in the bradykinin B2 receptor results in constitutive internalization and turns several antagonists into strong agonists. *J. Pharmacol. Exp. Ther.* **344**, 85–95.
24. Liu, J. J., Horst, R., Katritch, V., Stevens, R. C. & Wuthrich, K. (2012). Biased signaling pathways in beta2-adrenergic receptor characterized by 19F-NMR. *Science*, **335**, 1106–1110.
25. Venkatakrishnan, A. J., Deupi, X., Lebon, G., Tate, C. G., Schertler, G. F. & Babu, M. M. (2013). Molecular signatures of G-protein-coupled receptors. *Nature*, **494**, 185–194.
26. Huber, T., Menon, S. & Sakmar, T. P. (2008). Structural basis for ligand binding and specificity in adrenergic receptors: implications for GPCR-targeted drug discovery. *Biochemistry*, **47**, 11013–11023.
27. Moro, S., Hoffmann, C. & Jacobson, K. A. (1999). Role of the extracellular loops of G protein-coupled receptors in ligand recognition: a molecular modeling study of the human P2Y1 receptor. *Biochemistry*, **38**, 3498–3507.
28. Avlani, V. A., Gregory, K. J., Morton, C. J., Parker, M. W., Sexton, P. M. & Christopoulos, A. (2007). Critical role for the second extracellular loop in the binding of both orthosteric and allosteric G protein-coupled receptor ligands. *J. Biol. Chem.* **282**, 25677–25686.
29. Dror, R. O., Arlow, D. H., Maragakis, P., Mildorf, T. J., Pan, A. C., Xu, H. *et al.* (2011). Activation mechanism of the beta2-adrenergic receptor. *Proc. Natl Acad. Sci. USA*, **108**, 18684–18689.
30. Louet, M., Perahia, D., Martinez, J. & Floquet, N. (2011). A concerted mechanism for opening the GDP binding pocket and release of the nucleotide in heterotrimeric G-proteins. *J. Mol. Biol.* **411**, 298–312.
31. Hurst, D. P., Grossfield, A., Lynch, D. L., Feller, S., Romo, T. D., Gawrisch, K. *et al.* (2010). A lipid pathway for ligand binding is necessary for a cannabinoid G protein-coupled receptor. *J. Biol. Chem.* **285**, 17954–17964.
32. Orban, T., Jastrzebska, B., Gupta, S., Wang, B., Miyagi, M., Chance, M. R. & Palczewski, K. (2012). Conformational dynamics of activation for the pentameric complex of dimeric G protein-coupled receptor and heterotrimeric G protein. *Structure*, **20**, 826–840.
33. Grossfield, A., Pitman, M. C., Feller, S. E., Soubias, O. & Gawrisch, K. (2008). Internal hydration increases during activation of the G-protein-coupled receptor rhodopsin. *J. Mol. Biol.* **381**, 478–486.
34. Liu, W., Chun, E., Thompson, A. A., Chubukov, P., Xu, F., Katritch, V. *et al.* (2012). Structural basis for allosteric regulation of GPCRs by sodium ions. *Science*, **337**, 232–236.
35. Janovjak, H., Sapra, K. T., Kedrov, A. & Muller, D. J. (2008). From valleys to ridges: exploring the dynamic energy landscape of single membrane proteins. *ChemPhysChem*, **9**, 954–966.
36. Zocher, M., Fung, J. J., Kobilka, B. K. & Muller, D. J. (2012). Ligand-specific interactions modulate kinetic, energetic, and mechanical properties of the human beta2 adrenergic receptor. *Structure*, **20**, 1391–1402.
37. Zocher, M., Zhang, C., Rasmussen, S. G., Kobilka, B. K. & Muller, D. J. (2012). Cholesterol increases kinetic, energetic, and mechanical stability of the human beta2-

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adrenergic receptor. *Proc. Natl Acad. Sci. USA*, **109**, E3463–E3472.
38. Boesze-Battaglia, K., Hennessey, T. & Albert, A. D. (1989). Cholesterol heterogeneity in bovine rod outer segment disk membranes. *J. Biol. Chem.* **264**, 8151–8155.
39. Lee, J. Y. & Lyman, E. (2012). Predictions for cholesterol interaction sites on the A2A adenosine receptor. *J. Am. Chem. Soc.* **134**, 16512–16515.
40. Grossfield, A. (2011). Recent progress in the study of G protein-coupled receptors with molecular dynamics computer simulations. *Biochim. Biophys. Acta*, **1808**, 1868–1878.
41. Nygaard, R., Zou, Y., Dror, R. O., Mildorf, T. J., Arlow, D. H., Manglik, A. *et al.* (2013). The dynamic process of beta(2)-adrenergic receptor activation. *Cell*, **152**, 532–542.
42. West, G. M., Chien, E. Y., Katritch, V., Gatchalian, J., Chalmers, M. J., Stevens, R. C. & Griffin, P. R. (2011). Ligand-dependent perturbation of the conformational ensemble for the GPCR beta2 adrenergic receptor revealed by HDX. *Structure*, **19**, 1424–1432.
43. Orban, T., Jastrzebska, B., Gupta, S., Wang, B., Miyagi, M., Chance, M. R. & Palczewski, K. (2013). Conformational dynamics of activation for the pentameric complex of dimeric G protein-coupled receptor and heterotrimeric G protein. *Structure*, **20**, 826–840.
44. Noel, J. P., Hamm, H. E. & Sigler, P. B. (1993). The 2.2 Å crystal structure of transducin-α complexed with GTPγS. *Nature*, **366**, 654–663.
45. Ceruso, M. A., Periole, X. & Weinstein, H. (2004). Molecular dynamics simulations of transducin: interdomain and front to back communication in activation and nucleotide exchange. *J. Mol. Biol.* **338**, 469–481.
46. Van Eps, N., Preininger, A. M., Alexander, N., Kaya, A. I., Meier, S., Meiler, J. *et al.* (2011). Interaction of a G protein with an activated receptor opens the interdomain interface in the alpha subunit. *Proc. Natl Acad. Sci. USA*, **108**, 9420–9424.
47. Westfield, G. H., Rasmussen, S. G., Su, M., Dutta, S., DeVree, B. T., Chung, K. Y. *et al.* (2011). Structural flexibility of the G alpha s alpha-helical domain in the beta2-adrenoceptor Gs complex. *Proc. Natl Acad. Sci. USA*, **108**, 16086–16091.
48. Ridge, K. D., Abdulaev, N. G., Zhang, C., Ngo, T., Brabazon, D. M. & Marino, J. P. (2006). Conformational changes associated with receptor stimulated guanine nucleotide exchange in a heterotrimeric G-protein α-subunit: NMR analysis of GTPgS-bound states. *J. Biol. Chem.* **281**, 7635–7648.
49. Thomas, C. J., Briknarova, K., Hilmer, J. K., Movahed, N., Bothner, B., Sumida, J. P. *et al.* (2012). The nucleotide exchange factor Ric-8A is a chaperone for the conformationally dynamic nucleotide-free state of Gαi1. *PLoS One*, **6**, e23197.
50. Oldham, W. M., Van Eps, N., Preininger, A. M., Hubbell, W. L. & Hamm, H. E. (2006). Mechanism of the receptor-catalyzed activation of heterotrimeric G proteins. *Nat. Struct. Mol. Biol.* **13**, 772–777.
51. Marin, E. P., Krishna, A. G. & Sakmar, T. P. (2002). Disruption of the α5 helix of transducin impairs rhodopsin-catalyzed nucleotide exchange. *Biochemistry*, **41**, 6988–6994.
52. Gilchrist, A., Vanhauwe, J. F., Li, A., Thomas, T. O., Voyno-Yasenetskaya, T. & Hamm, H. E. (2001). G alpha minigenes expressing C-terminal peptides serve as specific inhibitors of thrombin-mediated endothelial activation. *J. Biol. Chem.* **276**, 25672–25679.
53. Bae, H., Cabrera-Vera, T. M., Depree, K. M., Graber, S. G. & Hamm, H. E. (1999). Two amino acids within the α4 helix of $G\alpha_{i1}$ mediate coupling with 5-hydroxytryptamine$_{1B}$ receptors. *J. Biol. Chem.* **274**, 14963–14971.
54. Natochin, M., Granovsky, A. E., Muradov, K. G. & Artemyev, N. O. (1999). Roles of the transducin alpha-subunit alpha4-helix/alpha4-beta6 loop in the receptor and effector interactions. *J. Biol. Chem.* **274**, 7865–7869.
55. Blahos, J., Fischer, T., Brabet, I., Stauffer, D., Rovelli, G., Bockaert, J. & Pin, J. P. (2001). A novel site on the Gα-protein that recognizes heptahelical receptors. *J. Biol. Chem.* **276**, 3262–3269.
56. Hamm, H. E., Deretic, D., Arendt, A., Hargrave, P. A., Koenig, B. & Hofmann, K. P. (1988). Site of G protein binding to rhodopsin mapped with synthetic peptides from the α subunit. *Science*, **241**, 832–835.
57. Martin, E. L., Rens-Domiano, S., Schatz, P. J. & Hamm, H. E. (1996). Potent peptide analogues of a G protein receptor-binding region obtained with a combinatorial library. *J. Biol. Chem.* **271**, 361–366.
58. Chung, K. Y., Rasmussen, S. G., Liu, T., Li, S., DeVree, B. T., Chae, P. S. *et al.* (2011). Conformational changes in the G protein Gs induced by the beta2 adrenergic receptor. *Nature*, **477**, 611–615.
59. Hamm, H. E., Kaya, A. I., Gilbert, J. A., 3rd & Preininger, A. M. (2013). Linking receptor activation to changes in Sw I and II of Galpha proteins. *J. Struct. Biol.* In press. Q5
60. Peters, M. F. & Scott, C. W. (2009). Evaluating cellular impedance assays for detection of GPCR pleiotropic signaling and functional selectivity. *J. Biomol. Screen.* **14**, 246–255.
61. Chen, X. P., Yang, W., Fan, Y., Luo, J. S., Hong, K., Wang, Z. *et al.* (2010). Structural determinants in the second intracellular loop of the human cannabinoid CB1 receptor mediate selective coupling to G(s) and G(i). *Br. J. Pharmacol.* **161**, 1817–1834.
62. Valiquette, M., Parent, S., Loisel, T. P. & Bouvier, M. (1995). Mutation of tyrosine-141 inhibits insulin-promoted tyrosine phosphorylation and increased responsiveness of the human beta 2-adrenergic receptor. *EMBO J.* **14**, 5542–5549.
63. Moro, O., Lameh, J., Hogger, P. & Sadee, W. (1993). Hydrophobic amino acid in the i2 loop plays a key role in receptor–G protein coupling. *J. Biol. Chem.* **268**, 22273–22276.
64. Wacker, J. L., Feller, D. B., Tang, X. B., Defino, M. C., Namkung, Y., Lyssand, J. S. *et al.* (2008). Disease-causing mutation in GPR54 reveals the importance of the second intracellular loop for class A G-protein-coupled receptor function. *J. Biol. Chem.* **283**, 31068–31078.
65. McClue, S. J., Baron, B. M. & Harris, B. A. (1994). Activation of Gi protein by peptide structures of the muscarinic M2 receptor second intracellular loop. *Eur. J. Pharmacol.* **267**, 185–193.
66. Nussenzveig, D. R., Thaw, C. N. & Gershengorn, M. C. (1994). Inhibition of inositol phosphate second

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Please cite this article as: Anita M. Preininger, et al., Conformational Flexibility and Structural Dynamics in GPCR-Mediated G Protein Activation: A Perspective, *J. Mol. Biol.* (2013), http://dx.doi.org/10.1016/j.jmb.2013.04.011

ARTICLE IN PRESS

messenger formation by intracellular loop one of a human calcitonin receptor. Expression and mutational analysis of synthetic receptor genes. *J. Biol. Chem.* **269**, 28123–28129.
67. Pin, J. P., Gomeza, J., Joly, C. & Bockaert, J. (1995). The metabotropic glutamate receptors: their second intracellular loop plays a critical role in the G-protein coupling specificity. *Biochem. Soc. Trans.* **23**, 91–96.
68. Zhou, H., Yan, F., Yamamoto, S. & Tai, H. H. (1999). Phenylalanine 138 in the second intracellular loop of human thromboxane receptor is critical for receptor–G-protein coupling. *Biochem. Biophys. Res. Commun.* **264**, 171–175.
69. Burstein, E. S., Spalding, T. A. & Brann, M. R. (1998). The second intracellular loop of the m5 muscarinic receptor is the switch which enables G-protein coupling. *J. Biol. Chem.* **273**, 24322–24327.
70. Erlenbach, I., Kostenis, E., Schmidt, C., Serradeil-Le Gal, C., Raufaste, D., Dumont, M. E. *et al.* (2001). Single amino acid substitutions and deletions that alter the G protein coupling properties of the V2 vasopressin receptor identified in yeast by receptor random mutagenesis. *J. Biol. Chem.* **276**, 29382–29392.
71. Schoneberg, T., Kostenis, E., Liu, J., Gudermann, T. & Wess, J. (1998). Molecular aspects of vasopressin receptor function. *Adv. Exp. Med. Biol.* **449**, 347–358.
72. Blin, N., Yun, J. & Wess, J. (1995). Mapping of single amino acid residues required for selective activation of Gq/11 by the m3 muscarinic acetylcholine receptor. *J. Biol. Chem.* **270**, 17741–17748.
73. Rasmussen, S. G., Choi, H. J., Rosenbaum, D. M., Kobilka, T. S., Thian, F. S., Edwards, P. C. *et al.* (2007). Crystal structure of the human beta2 adrenergic G-protein-coupled receptor. *Nature*, **450**, 383–387.
74. Wacker, D., Fenalti, G., Brown, M. A., Katritch, V., Abagyan, R., Cherezov, V. & Stevens, R. C. (2010). Conserved binding mode of human beta2 adrenergic receptor inverse agonists and antagonist revealed by X-ray crystallography. *J. Am. Chem. Soc.* **132**, 11443–11445.
75. Marin, E. P., Krishna, A. G. & Sakmar, T. P. (2001). Rapid activation of transducin by mutations distant from the nucleotide-binding site. Evidence for a mechanistic model of receptor-catalyzed nucleotide exchange by G proteins. *J. Biol. Chem.* **276**, 27400–27405.
76. Kostenis, E., Degtyarev, M. Y., Conklin, B. R. & Wess, J. (1997). The N-terminal extension of Ga_q is critical for constraining the selectivity of receptor coupling. *J. Biol. Chem.* **272**, 19107–19110.
77. Slessareva, J. E. & Graber, S. G. (2003). Reconstitution reveals additional roles for N- and C-terminal domains of g(alpha) in muscarinic receptor coupling. *Biochemistry*, **42**, 7552–7560.
78. Preininger, A. M., Parello, J., Meier, S. M., Liao, G. & Hamm, H. E. (2008). Receptor-mediated changes at the myristoylated amino terminus of Galpha(il) proteins. *Biochemistry*, **47**, 10281–10293.
79. Sgourakis, N. G. & Garcia, A. E. (2010). The membrane complex between transducin and dark-state rhodopsin exhibits large-amplitude interface dynamics on the submicrosecond timescale: insights from all-atom MD simulations. *J. Mol. Biol.* **398**, 161–173.
80. Choe, H. W., Kim, Y. J., Park, J. H., Morizumi, T., Pai, E. F., Krauss, N. *et al.* (2011). Crystal structure of metarhodopsin II. *Nature*, **471**, 651–655.
81. Scheerer, P., Park, J. H., Hildebrand, P. W., Kim, Y. J., Krauss, N., Choe, H. W. *et al.* (2008). Crystal structure of opsin in its G-protein-interacting conformation. *Nature*, **455**, 497–502.
82. Warne, T., Serrano-Vega, M. J., Baker, J. G., Moukhametzianov, R., Edwards, P. C., Henderson, R. *et al.* (2008). Structure of a beta1-adrenergic G-protein-coupled receptor. *Nature*, **454**, 486–491.
83. Louet, M., Martinez, J. & Floquet, N. (2012). GDP release preferentially occurs on the phosphate side in heterotrimeric G-proteins. *PLoS Comput. Biol.* **8**, e1002595.
84. Abdulaev, N. G., Ngo, T., Ramon, E., Brabazon, D. M., Marino, J. P. & Ridge, K. D. (2006). The receptor-bound "empty pocket" state of the heterotrimeric G-protein alpha-subunit is conformationally dynamic. *Biochemistry*, **45**, 12986–12997.
85. Preininger, A., Funk, M., Meier, S., Oldham, W., Johnston, C., Adhikary, S. *et al.* (2009). Helix dipole movement and conformational variability contribute to allosteric GDP release in Gi subunits. *Biochemistry*, **48**, 2630–2642.
86. Kapoor, N., Menon, S. T., Chauhan, R., Sachdev, P. & Sakmar, T. P. (2009). Structural evidence for a sequential release mechanism for activation of heterotrimeric G proteins. *J. Mol. Biol.* **393**, 882–897.
87. Tesmer, J. J., Sunahara, R. K., Gilman, A. G. & Sprang, S. R. (1997). Crystal structure of the catalytic domains of adenylyl cyclase in a complex with G_{sa}·GTPgS. *Science*, **278**, 1907–1916.
88. Coleman, D. E., Berghuis, A. M., Lee, E., Linder, M. E., Gilman, A. G. & Sprang, S. R. (1994). Structures of active conformations of G_{ia1} and the mechanism of GTP hydrolysis. *Science*, **265**, 1405–1412.
89. Van Eps, N., Oldham, W. M., Hamm, H. E. & Hubbell, W. L. (2006). Structural and dynamical changes in an alpha-subunit of a heterotrimeric G protein along the activation pathway. *Proc. Natl Acad. Sci. USA*, **103**, 16194–16199.
90. Preininger, A. M., Kaya, A. I., Gilbert, J. A., III, Busenlehner, L. S., Armstrong, R. N. & Hamm, H. E. (2012). Myristoylation exerts direct and allosteric effects on Galpha conformation and dynamics in solution. *Biochemistry*, **51**, 1911–1924.

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