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Structure and dynamics of GPCR signaling complexes

Daniel Hilger[#], **Matthieu Masureel**[#], and **Brian K. Kobilka**[*]

Department of Molecular and Cellular Physiology, Stanford University School of Medicine, Stanford, CA, USA.

[#] These authors contributed equally to this work.

Abstract

G-protein-coupled receptors (GPCRs) relay numerous extracellular signals by triggering intracellular signaling through coupling with G proteins and arrestins. Recent breakthroughs in the structural determination of GPCRs and GPCR-transducer complexes represent important steps toward deciphering GPCR signal transduction at a molecular level. A full understanding of the molecular basis of GPCR-mediated signaling requires elucidation of the dynamics of receptors and their transducer complexes as well as their energy landscapes and conformational transition rates. Here, we summarize current insights into the structural plasticity of GPCR-G-protein and GPCR-arrestin complexes that underlies the regulation of the receptor's intracellular signaling profile.

GPCRs are versatile, seven-transmembrane-domain proteins that regulate a diverse array of intracellular signaling cascades in response to hormones, neurotransmitters, ions, photons, odorants and other stimuli. As such, they play an essential role in physiology and disease and represent attractive drug targets. Activation of GPCRs initiates signaling through heterotrimeric G proteins, as well as through G-protein-independent pathways by G-protein-coupled receptor kinase (GRK)-mediated phosphorylation and arrestin coupling (Fig. 1). Many GPCRs show basal activity that can be modulated by ligands of different efficacy. Full agonists are able to induce the maximal signaling response, whereas partial agonists and inverse agonists promote submaximal signaling or decrease basal activity, respectively. Furthermore, some ligands are known as biased ligands because they selectively activate certain receptor-associated pathways at the expense of others[1]. A surge in crystal structures of active and inactive receptors in the last decade has provided a detailed molecular framework underlying ligand binding and receptor activation[2–5]. The most common feature of receptor activation entails a major reorganization of the cytoplasmic side, where a large outward movement of transmembrane helix 6 (TM6), combined with rearrangements of other helices, exposes an intracellular pocket that can effectively engage G proteins, GRKs

[*]**Correspondence and requests for materials** should be addressed to B.K.K. kobilka@stanford.edu.

Daniel Hilger and Matthieu Masureel contributed equally to this work.

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and arrestins to form functional signaling complexes. Biophysical characterization of GPCRs has extended this picture and shown that receptors cannot be described as simple bimodal ‘on–off ‘ switches, but should rather be viewed as highly dynamic systems that exist in a multitude of functionally distinct conformations (reviewed in refs. [6,7]). Ligands can regulate the receptor activity through conformational selection of distinct states, thereby altering the degree of the overall signaling response[8] or resulting in signaling bias[9,10]. As only weak allosteric coupling exists between the ligand-binding pocket and the G-protein- and arrestin-coupling interface, engagement of the transducer is required to fully stabilize the active receptor conformation[11–13].

Recent breakthroughs in the structural determination of GPCRs in complex with G protein or arrestin have greatly advanced the deciphering of GPCR signal transduction at a molecular level[14–19]. A full understanding, however, requires an investigation of the conformational dynamics of receptor–transducer complexes that will elucidate factors affecting the energy landscapes and conformational transition rates of these complexes. In the sections below, we focus on recently gained insights on receptor–G-protein and receptor–arrestin complexes (for a recent review on receptor–GRK complexes, see ref. [20]).

GPCR-mediated activation of G proteins

The G-protein activation cycle.

The classical signal transduction through GPCRs is dependent on receptor-mediated activation of heterotrimeric G proteins, which are composed of three subunits, Gα, Gβ and Gγ. In contrast to the large repertoire of different GPCR genes found in the human genome, only four major G-protein families (G_s, $G_{i/o}$, $G_{q/11}$ and $G_{12/13}$) have been classified based on sequence homology between more than 21 identified human Gα isotypes encoded by 16 individual genes[21,22]. The ability of this limited number of G proteins to couple to a diverse set of receptors suggests a conserved mechanism for receptor-catalyzed G-protein activation that involves changes in the nucleotide-bound state of the Gα subunit (Fig. 1). When bound to GDP, Gα associates with the Gβγ dimer to form the inactive heterotrimer. Receptor activation promotes the engagement of the GDP-bound heterotrimer that accelerates GDP dissociation from Gα, a process that represents the rate-limiting step in G-protein activation[23]. The resulting nucleotide-free receptor–G-protein complex exhibits a very short lifetime, owing to the high GTP concentration in cells that facilitates rapid GTP binding to the nucleotide-binding site of the G protein. Subsequently, the Gα subunit undergoes conformational changes that result in the dissociation of the Gα and Gβγ subunits. Both subunits have been shown to modulate the activity of different downstream effector proteins. Gα subunits target effectors including adenylyl cyclases, cGMP phosphodiesterase, phospholipase C and RhoGEFs[24,25], while Gβγ can recruit GRKs to the membrane and regulate G-protein-coupled, inwardly rectifying potassium channels, voltage-dependent Ca^{2+} channels, adenylyl cyclases, phospholipase C, phosphoinosite 3 kinase and mitogen-activated protein kinases[26,27]. The cellular response is terminated when the Gα subunit hydrolyzes GTP to GDP, owing to its intrinsic GTPase activity, and then reassociates with Gβγ, completing the G-protein activation circle. GTPase-activating proteins (GAPs), including regulators of G-protein signaling (RGS) proteins, can interact with activated Gα

subunits to increase their intrinsic GTP hydrolysis rate up to more than 2,000-fold and thus represent important modulators of the intensity and duration of GPCR-mediated signaling (see refs. [28,29] for reviews).

Structural insights into receptor-mediated nucleotide release.

One of the most fundamental questions in understanding GPCR signaling is how receptors catalyze nucleotide exchange in G proteins. Structural studies have revealed that the nucleotide-binding site in G proteins is located between the two domains of Gα: the α-helical domain (AHD) and the Ras-like (Ras) GTPase domain (for reviews see refs. [30,31]) (Fig. 2a). The majority of contacts involved in the binding of GDP are provided by the Ras domain, which is structurally homologous to monomeric G proteins and elongation factors like $p21^{ras}$ and EF-Tu. It is composed of a six-stranded β-sheet (β1–β6) and five α-helices (α1–α5). Several loops within the Ras domain play important roles in nucleotide binding. In particular, the β1–α1 loop that links the β1 strand and the α1 helix—also known as the diphosphate-binding or P loop—coordinates the nucleotide phosphates together with the adjacent α1 helix, while the β5–α4 and the β6–α5 loops engage the guanine ring of GDP. Another part of the nucleotide-binding pocket is formed by the AHD, which is unique to heterotrimeric G proteins and contains six α-helices (αA–αF) that are inserted between α1 and β2 of the Ras domain. By packing against the Ras domain, the AHD buries the bound nucleotide in the interface between the two domains.

The tight apposition of the AHD and the Ras domain first observed in the crystal structure of the Gα subunit of transducing ($G\alpha_t$) led to the hypothesis that G-protein coupling to receptors may result in domain separation followed by GDP dissociation through the created exit pathway[32]. This was later confirmed in the crystal structure of the β_2-adrenergic receptor (β_2AR) in complex with the G_s heterotrimer that shows a large displacement of the AHD relative to the Ras domain upon nucleotide release[14](Fig. 2a). Furthermore, double electron–electron resonance (DEER) spectroscopy[33], hydrogen/deuterium exchange (HDX) measurements[34], single-particle EM[35], mutagenesis[36–38] and molecular dynamics (MD) simulations[39,40] on multiple G-protein isotypes have provided evidence that domain separation is of general importance for nucleotide release in all heterotrimeric G proteins. Although the AHD in the complex structure of β_2AR–G_s is stabilized in one position, owing to its involvement in crystal-lattice contacts, single-particle EM analysis of β_2AR–G_s as well as recent cryo-EM structures of the calcitonin and glucagon-like peptide 1 receptor in complex with G_s demonstrate that the delocalized AHD is highly dynamic and adopts variable conformations with respect to the Ras domain. Because the AHD is known to promote tight binding of the nucleotide to the Ras domain[41], it is possible that modulation of the AHD dynamics might influence GTP loading to the nucleotide-free complex. Therefore, we speculate that interactions between the AHD and other signaling proteins could stabilize the open conformation of the AHD and might slow down GTP-mediated dissociation and activation of the G protein.

Although domain separation seems essential for rapid nucleotide release, outward movement of AHD alone appears to be insufficient to induce GDP dissociation from the Ras domain. This has been shown by deletions of the AHD resulting in an isolated Ras domain that still

retained nucleotide-binding ability[41]. Furthermore, recent long-timescale MD simulations of GDP-bound G-protein heterotrimers, supported by intramolecular DEER distance measurements, have revealed that the nucleotide remains bound to the Ras domain upon separation of the two domains or even when the entire AHD has been deleted[40]. This suggests that nucleotide release needs to be triggered through other receptor-mediated conformational changes within the Ras domain that are transmitted to the nucleotide-binding site to promote GDP dissociation. Indeed, numerous studies have provided evidence for multiple allosteric connections between the receptor–G-protein interface and the GDP-binding site that probably play important roles in the nucleotide release of the G protein. Below, we describe these allosteric connections and discuss their roles in receptor-mediated destabilization of the GDP-binding site.

One of the best-studied regions in G proteins is the C-terminal α5 helix of Gα, which plays an essential role for receptor coupling and G-protein activation[42–47]. In the currently available crystal and cryo-EM structures of receptors in complex with G-protein heterotrimers[14,15,17] or an engineered Gα subunit[16], the C-terminal α5 helix of Gα forms the most extensive interface between the G protein and mainly TM3, TM5, TM6, and intracellular loops ICL2 and ICL3 of the receptor. In these structures, G-protein coupling and the generation of the nucleotide-free state resulted in displacement of α5 by a rotational translation of the helix into the receptor core (Fig. 2b). The movement of α5 appears to be associated with changes in other regions of the Ras domain that are involved in the formation of the nucleotide-binding pocket. One of the regions that undergo α5-mediated structural rearrangements is the β6–α5 loop. This loop is located at the N terminus of the α5 helix and contains the conserved TCAT motif, which is important for the coordination of the purine ring of the bound nucleotide. The displacement of α5 in the GPCR–G-protein complex has been shown to disrupt the nucleotide-bound conformation and increase the flexibility of the β6–α5 loop[34,40]. Furthermore, mutations within the β6–α5 loop were found to accelerate spontaneous GDP dissociation from Gα in the absence of a receptor, thus demonstrating the importance of this region for nucleotide binding[48–50].

Another route for transmitting receptor-mediated conformational changes of α5 to the nucleotide-binding site has been proposed based on mutagenesis[51–53] and computational studies[39,54,55]. In the GDP-bound state of the G protein, α5 interacts with α1 and the two β-strands β2 and β3 through highly conserved hydrophobic contacts. Rotation and translation of α5 in the receptor-bound state disrupt these hydrophobic core interactions, resulting in the destabilization of α1, as shown by HDX measurements[34] and the poor electron and EM density obtained for this helix in receptor–G-protein complex structures[14,15,17] (Fig. 2b). The α1 helix is involved in binding of both the diphosphate of GDP via the Walker A motif (GXXXXGK(S/T)) and the αF helix of the AHD. Thus, structural perturbation of α1 has been proposed to accelerate GDP release and destabilize the AHD–Ras domain interface.

Aside from the α5 helix, the αN–β1 hinge region has been shown to be important for receptor-mediated nucleotide release[56–58]. In the available complex structures, the αN–β1 junction interacts with ICL2 of the receptor and connects it with the P loop of the nucleotide-binding site through the adjacent β1 strand[14–17] (Fig. 2b). HDX measurements revealed that this region becomes more dynamic upon coupling of the G protein to the

activated receptor[34]. Furthermore, mutations in ICL2 of rhodopsin have been reported to preserve coupling to the canonical G protein transducin but to impair the receptor's ability to induce GDP release[57]. Together, these studies provide evidence for an allosteric connection between ICL2 and the P loop of the G protein. Coupling of the G protein to the receptor might induce ICL2-dependent conformational changes in the αN–β1 hinge region that will be transmitted through β1 to the P loop. Disruption of the P-loop conformation probably results in GDP release, owing to its role in the coordination of the β-phosphate of the nucleotide.

In summary, the described allosteric connections between the receptor and the nucleotide-binding site have been shown to play important roles in the activation of the G protein. Most likely, they represent cooperative pathways that allow simultaneous destabilization of both the purine- and phosphate-coordination site of the bound nucleotide upon receptor coupling, ensuring an efficient GDP release from the G protein. Furthermore, the allosteric interactions between the receptor and the nucleotide-binding site could also play an important role for GTP binding and destabilization of the receptor–G-protein complex, as discussed below.

Ligand effects on the dynamics of GPCR–G-protein complexes.

GPCR-mediated signaling through heterotrimeric G proteins can be modulated by agonists of different efficacies. At the most basic level, the molecular basis of ligand efficacy can be explained by the ligand's ability to increase the population of active receptor conformations that are able to engage and activate G proteins. Therefore, differences in ligand efficacy are normally interpreted as changes in the efficiency and kinetics of G-protein coupling, owing to variations in the conformation and dynamics of the receptor. More recently, additional determinants of ligand efficacy have been described that include the effect of orthosteric ligands on the nucleotide-binding affinities of the receptor-engaged G protein. Measurements of the apparent GDP affinity of receptor–G-protein complexes bound to ligands of different efficacies demonstrated that full-agonist-stabilized complexes exhibit lower affinities for GDP in comparison to partial agonists[59–63]. Furthermore, GDP is known to increase the relative efficacy differences between full and partial agonists in classic GTPγS binding experiments, providing additional evidence that partial-agonist-occupied receptor–G-protein complexes are more sensitive to GDP binding than complexes bound to full agonists[64]. In the context of ligand efficacy, the observed tighter binding of GDP in the presence of partial agonists probably reduces the number of successful nucleotide-exchange events, which lowers the magnitude of the cellular response.

On a structural level, the influence of orthosteric ligands on the nucleotide-binding affinity of receptor-coupled G proteins can be explained by ligand-dependent modulations of the allosteric connections between the receptor and the nucleotide-binding site described above. Evidence for this has been recently provided by a single-molecule FRET study on the dynamics of the β_2AR–G_s complex[63]. Here, examination of ligand-dependent TM6 movements in the β_2 adrenergic receptor in the presence of the G_s heterotrimer and different nucleotides revealed the existence of transient nucleotide-bound β_2AR–G_s species that are in equilibrium with the nucleotide-free state reported in the crystal structure of the β_2AR–G_s

complex[14]. In the presence of GDP, more efficacious agonists were shown to increase the probability of GDP release, thereby shifting the equilibrium toward the nucleotide-free state. Because of the observed ligand-dependent dynamics of TM6 in G-protein-bound complexes in the presence of nucleotides, it is possible that the efficacy of ligands bound to the orthosteric ligand-binding site determines how efficiently the C-terminal α5 helix of the G protein stably engages the intracellular cavity of the receptor. Thus, ligand-dependent dynamics of TM6 might be transmitted through α5 to the nucleotide-binding site, as described above, in order to modulate the nucleotide affinity. This notion is supported by NMR studies on G proteins, which show that G proteins can sample different nucleotide-bound and nucleotide-free states[65,66] and that receptor coupling is responsible for increases in the conformational dynamics of the G-protein heterotrimer[67].

Interestingly, another study on the structure and dynamics of the calcitonin receptor–G_s complex provided evidence for a different mechanism in which ligands with distinct potency and efficacy induce distinct G-protein conformations[68]. Based on bioluminescent resonance energy transfer (BRET) and native-gel fluorescence resonance energy transfer (FRET) measurements between the α and γ subunits of the G protein, the authors suggested that the ligand-induced conformational changes are transmitted from the receptor to the coupled transducer. The resulting, distinct G-protein conformations showed differences in their sensitivity for GTP disruption that lead to changes in the G-protein turnover in cells.

Although these studies have provided important insights into the ligand-dependent regulation of nucleotide exchange in receptor-coupled G proteins, more work is required to fully understand the molecular details of receptor-catalyzed nucleotide exchange in G proteins and how it can be modulated by ligands of different efficacy.

Arrestin activation and dynamics

Since their discovery in the 1970s and 1980s, arrestins have evolved from terminators of G-protein signaling by mere steric hindrance at the receptor-coupling interface to multifunctional adaptor proteins that form a central node in multiple G-protein-independent signaling pathways. While their functional roles and their numerous protein-interaction partners constitute an exciting and expanding field of research[69–72], much remains to be learned about how receptor activation triggers conformational changes in arrestin and about the ligand-dependent function of a receptor-coupled arrestin. This section aims to focus on the role of arrestin's structural features and conformational dynamics, especially when coupled to receptors.

Structural features of arrestins.

In contrast with the known diversity of G proteins, only four arrestin isoforms exist. Arrestin-1 and arrestin-4, also known as rod and cone arrestin, respectively, are mainly found in the eye, whereas arrestin-2 and arrestin-3, also known as β-arrestin1 and β-arrestin2, are distributed ubiquitously. Only the double knockout of arrestin-2 and arrestin-3 is embryonically lethal in mice[73], which suggests some functional redundancy between β-arrestin isoforms. However, differences in expression level[74,75], cellular localization[76] and binding affinity for receptors[77] suggest partially distinct roles for both nonvisual

isoforms[73,78]. Structures of all four isoforms have been solved in the inactive state[79–82], as reviewed in ref. [83]. For arrestin-1, the pre-active R175E mutant was crystallized in what is probably an intermediate activation state[84], whereas active-state structures have been obtained individually for the constitutively active splice variant p44 (ref. [85]) and for a preactivated mutant fused to phosphorylated, constitutively active rhodopsin[19]. Active arrestin-2 has been crystallized bound to the phosphorylated C tail of the vasopressin receptor, stabilized by an active-state-specific antibody fragment[86], and its coupling to a chimeric β_2V_2R receptor has been visualized by single-particle negative-stain EM[87]. Most recently, the structure of inositol hexakisphosphate (IP_6)-activated arrestin-3 was solved[88]. From these structures and the biophysical studies detailed below, global arrestin features, basal-state interactions and activation hallmarks appear conserved across isoforms.

Arrestins are ~45-kDa proteins organized in an N and C lobe, each forming a β-stranded sandwich structure connected by a hinge region (Fig. 3a). At this N- and C-domain interface, the C loop, finger loop and middle loop form the central crest, flanked by the lariat or 17–18 loop, part of which is often referred to as the gate loop. Two loops on the outer side of the C-terminal lobe compose the C edge, which interacts with the membrane bilayer upon arrestin activation[89,90]. Two major interaction networks maintain arrestin in its basal, inactive conformation. First, the three-element interaction, is mediated by hydrophobic interactions between the proximal part of the C tail and the N-terminal β-strand I and α-helix I (Fig. 3b). Second, the polar core, is a hydrogen-bond network between five buried solvent-excluded charged residues on the N-terminal β-strands III and X, the gate loop and the distal part of the C tail (Fig. 3c). Together, these interactions keep the central crest regions closely packed and form an intramolecular N–C lock, preventing access to a positively charged groove on the N lobe.

Upon activation by receptor binding, arrestin undergoes several major conformational changes, namely disruption of the three-element interaction, breakage of the polar core, a ~20° interdomain rotation, release of the C tail and substantial rearrangements of the finger, middle and gate loops, recently reviewed in ref. [91] (Fig. 3). These changes expose the concave surface of the arrestin N lobe and its positively charged phosphointeraction sites, such as lysines and arginines that engage GRK-phosphorylated serines and threonines of the receptor C tail. At present, it is unclear which aspects of receptor binding induce each change and what sequence of events leads to arrestin activation, and it is unknown which exact changes are required for different downstream signaling responses. However, much has recently been learned on the GPCR–arrestin binding interface from the recent rhodopsin–arrestin-1 complex structure[19] (Fig. 4). The interactions are essentially mediated by two interfaces: the receptor C tail with the arrestin N-lobe groove and the receptor core with the arrestin central crest. The core engagement of the receptor with arrestin's central crest is mainly mediated by three interactions: (i) the arrestin finger loop with the receptor intracellular binding pocket, (ii) the C-terminal base of the finger loop and the back loop in arrestin with the intracellular ends of TM5 and TM6, connected by ICL3 in rhodopsin and (iii) the cleft formed between the middle, lariat and C loops in arrestin, with the intracellular end of TM3 and ICL2 in rhodopsin. Earlier mutational and functional mapping studies of arrestin have provided additional validation of these interaction interfaces[89] and identified residues involved in receptor coupling specificity[92–94].

Arrestin conformational dynamics.

Several studies have investigated how receptors, ligands and phosphorylation patterns modulate the conformational states and dynamics of engaged arrestin, increasing our understanding of the molecular underpinnings behind distinct functional outcomes. In the following sections, we consider the experimental evidence for multiple arrestin conformations and the high conformational flexibility and heterogeneity of arrestin.

The effect of receptor coupling was first investigated in the rhodopsin–arrestin-1 complex by electron paramagnetic resonance (EPR), providing early biophysical evidence that the arrestin C tail becomes disordered upon receptor coupling, indicative of its release[95]. Similarly for nonvisual arrestins, BRET measurements between an N-terminal *Renilla* luciferase and a C-terminal yellow fluorescent protein fused to arrestin-3 provided initial low-resolution measurements of receptor- and ligand-specific arrestin conformational changes probably affecting C-tail release[96,97]. More recent BRET- and FRET-based investigations of arrestin-3 have indicated that regions both on the periphery and within the N and C domains of arrestin also show receptor- and ligand-specific conformational signatures[98,99].

The ability of arrestin to specifically trigger multiple signaling pathways suggests that it is capable of adopting multiple conformations to effectively engage different downstream effectors, even when bound to a receptor. The plasticity of its surface probably plays an important role in this process. EPR studies have shown that binding to inactive dark phosphorylated rhodopsin (P-Rh) decreases the mobility of the arrestin-1 finger, middle and C loops, whereas binding to light-activated phosphorylated rhodopsin (P-Rh*) further decreases finger-loop mobility but restores middle-loop mobility[95,100]. A highly flexible middle loop, although located in the central crest, which interacts with the receptor, was also observed for arrestin-2 and arrestin-3 bound to rhodopsin[101]. Interestingly however, when coupled to β_2AR, both the finger and middle loops of arrestin-2 showed decreased hydrogen–deuterium exchange[87]. At least two sequential conformational changes were observed in fluorescently labeled arrestin-1 upon interaction with P-Rh* (ref. [102]), and it was found that the finger loop only engages the receptor upon agonist binding, whereas one of the C-edge loops can engage unliganded receptor[103]. Engagement of distinct arrestin-1 elements to unphosphorylated light-activated and dark-phosphorylated rhodopsin, inducing conformational changes distinct to those triggered by binding of P-Rh*, was further evidenced by solution NMR of labeled arrestin-1 (ref. [104]).

Another layer of signaling complexity, and hence, potential conformational variability, is added by GRK-mediated phosphorylation of the receptor C terminus and/or intracellular loops. Distinct GRKs imprint specific phosphoserine–phosphothreonine patterns, so-called ‘phosphorylation (bar)codes’, first suggested in ref. [105], onto the receptor[106–108], which are then differentially ‘read out’ by arrestin, thereby modulating interaction affinity and triggering specific signaling outcomes[107,109–111]. A recently solved crystal structure of the rhodopsin–visual arrestin complex together with receptor–arrestin proximity assays[19] as well as earlier rhodopsin studies[112,113] suggest that the presence of three distinctly spaced patches of receptor-attached phosphates or negative charges is critical for high-affinity binding between receptor and arrestin, which may explain why some receptors interact

weakly or transiently with arrestin and others bind strongly[77]. Using the same biosensor that evidenced receptor- and ligand-specific conformational signatures in arrestin-3, it has been shown that distinct phosphorylation patterns on β_2AR also induce distinct BRET signatures[107]. An elegant ^{19}F-NMR study combining unnatural amino acid incorporation in arrestin-2 with phosphopeptides mimicking distinct receptor C-terminal phosphorylation patterns evidenced that not only the direct phosphosensing sites but also remote sites in the C loop, lariat loop and one of the C-edge loops showed peptide-specific chemical-shift signatures[114]. Moreover, the same study showed two types of conformastional states, either slow- or fast-exchanging for arrestin-2 bound to the phosphorylated C tail of the vasopressin receptor.

Although arrestin binding and activation is often described as a two-step process in which phosphorylated receptor C-tail engagement necessarily precedes its core engagement, the picture is probably more complex. Early[115] as well as more recent studies[116–118] (Latorraca N. R., personal communication) indicate that receptor-core and C-tail engagement can each independently mediate arrestin activation and signaling events. As such, multiple types of receptor–arrestin complexes probably exist, with distinct or complementary functional outcomes. Moreover, binding of arrestin to both unphosphorylated and phosphorylated receptor, but with distinct binding modes and functional outcomes, as observed for the M1 muscarinic acetylcholine receptor[119], rhodopsin[120] and other receptors[121,122], further expands the spectrum of arrestin-mediated GPCR signaling.

Taken together, these biophysical studies have revealed multiple interaction modes between arrestin and receptor, as well as substantial conformational variability within arrestin while coupled to a GPCR. Most likely, phospho-barcoding of the receptor C tail can act independently or in concert with ligand-dependent core conformations to achieve specific arrestin conformations. The receptor–arrestin binding interface appears very dynamic, with the high conformational flexibility of arrestin probably intimately linked to its multiple signaling functions. Arrestins thus show a high degree of conformational plasticity and loose allosteric coupling between regions that can be stabilized by specific receptor-interaction surfaces. This plasticity is illustrated by the fact that a C-tail truncation of arrestin-2 induces a pre-activated, phosphorylation-independent phenotype in cellular assays, but still crystallizes in the inactive form[80]. Similarly, the constitutively active splice variant p44 crystallizes in different forms[85,123]. On the other hand, transient interactions with receptors seem sufficient to elicit activation responses after receptor dissociation, suggesting that some active conformational states can be maintained in the absence of a receptor[98,119,124].

Conclusion and outlook

GPCRs are very versatile proteins that exist in multiple conformations, in which loose allosteric coupling between the orthosteric ligand-binding site and the intracellular transducer-binding site is probably responsible for the ability of activated receptors to regulate multiple intracellular signaling pathways. Currently available functional, structural, spectroscopic and computational studies suggest that receptor–transducer complexes are also highly dynamic and that the coupled transducers can undergo conformational transitions (independently or in response to receptor binding, or both). Moreover, they indicate that the

conformational dynamics of G proteins and arrestins can be modulated by the ligand- and/or phosphorylation-dependent states of the receptor, providing evidence for an allosteric regulation that can influence transducer activation and downstream signaling events. However, more studies will be required to fully understand how the interplay of receptor and effector conformational states and dynamics tune the functional outcomes in the cell and how this is regulated by different ligands. The multitude of conformational states of GPCRs and their signaling complexes have been proven difficult to capture crystallographically. As such, investigations by spectroscopic methods like fluorescence, EPR and NMR spectroscopy and structural approaches using cryo-EM are required to fully understand GPCR signaling at the molecular level. Furthermore, time-resolved measurements such as HDX measurements and radiolytic footprinting are needed to delineate the sequence of events that are important for the association and dissociation of GPCR–transducer complexes. Together, these studies will also help us to identify the factors that determine the coupling selectivity of receptors for different G proteins and arrestins.

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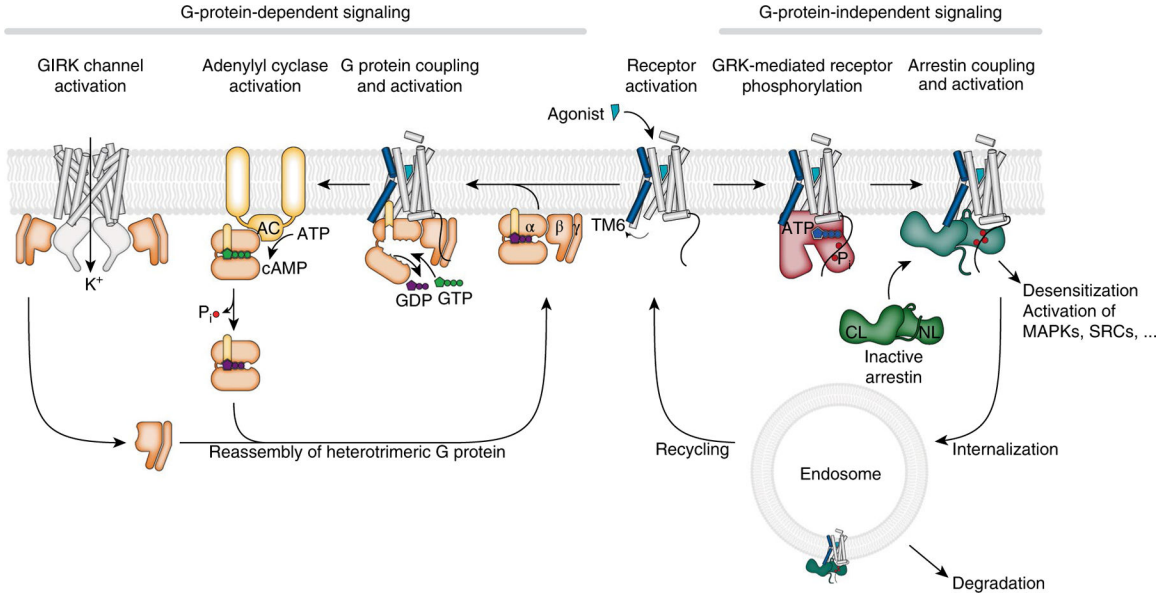

Fig. 1. G-protein-coupled receptor signal transduction.

Receptors regulate multiple intracellular signaling cascades including G-protein-dependent and G-protein-independent pathways. Agonist binding activates the receptor by inducing conformational changes that involve an outward shift of the transmembrane domain (TM6, blue). The activated receptor can bind to a diverse set of intracellular signaling proteins including G proteins (orange), GRKs (red) and arrestins (green, inactive arrestin; blue-green, active arrestin). Coupling of heterotrimeric G proteins to the receptor triggers nucleotide exchange followed by dissociation of the G protein into the Gα and Gβγ subunits. Both subunits can regulate different downstream effector proteins. GTP-bound Gα subunits can modulate the activity of adenylyl cyclase (AC, yellow), while Gβγ can interact with G-protein-coupled inwardly rectifying potassium channels (GIRK, cylindrical TM representation, gray). G-protein-mediated signaling is terminated by hydrolysis of GTP and reassociation of Gα with Gβγ to form the inactive heterotrimer. Activation of the receptor can also lead to phosphorylation by GRKs and subsequent coupling to arrestin. Arrestin coupling to the receptor leads to desensitization and arrestin-mediated activation of downstream effector proteins like mitogen-activated protein kinases (MAPKs) or SRC kinases. Arrestin activation also promotes the internalization of the receptor into endosomes followed by degradation or recycling of the receptor to the plasma membrane. NL, N lobe of arrestin; CL, C lobe of arrestin.

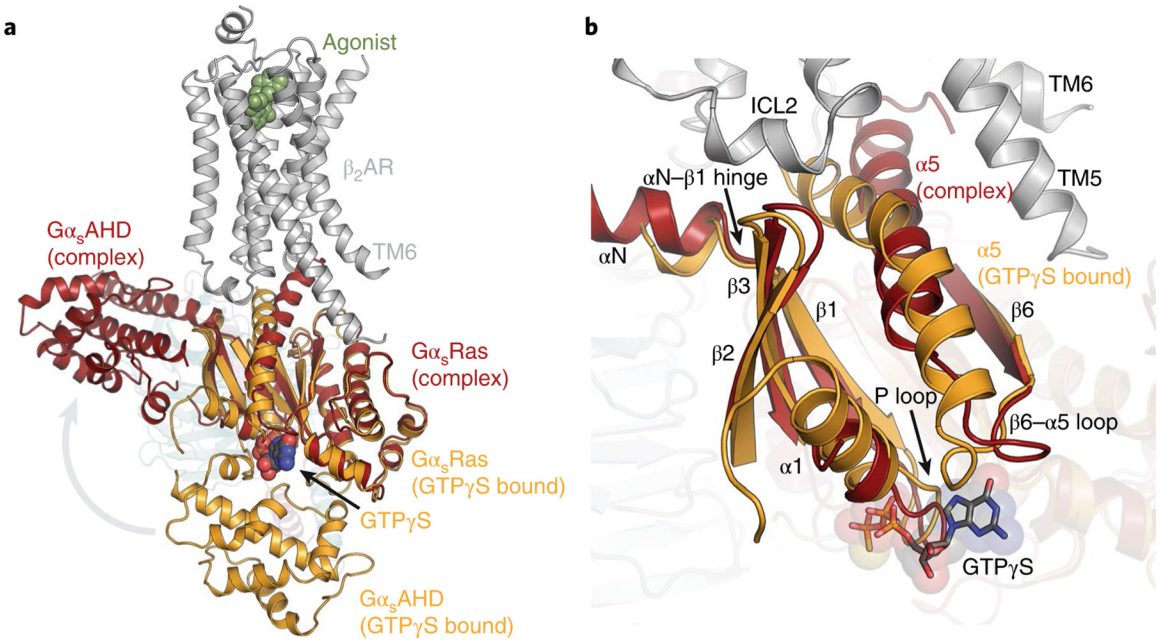

Fig. 2. Receptor-mediated conformational changes in Gα.

a, Structural comparison of nucleotide-free Gαs (red) coupled to β2AR (gray, PDB 3SN6) and GTPγS-bound Gαs (orange, PDB 1AZT). GTPγS is shown as spheres. Receptor coupling of Gs induces an outward movement of the α-helical domain of Gαs (GαsAHD) relative to its position in the GTPγS-bound state. Gβ (cyan) and Gγ (magenta) have been made transparent for clarity. **b**, The α5 helix undergoes a rotational translation into an intracellular cavity of the receptor that is formed by outward movement of transmembrane helices TM5 and TM6 upon receptor activation. Displacement of α5 leads to perturbation of the β6–α5 loop and the hydrophobic core interaction between α5, β2 and β3 and α1, which results in a rearrangement of the β6–α5 loop and destabilization of α1, which are important for the binding of the purine ring and the phosphates of the nucleotide, respectively. Interaction between the intracellular loop 2 (ICL2) of the receptor and the αN–β1 hinge region of Gαs may also lead to conformational changes in β1 and the adjacent P loop that forms part of the phosphate-binding site in the GTPγS-bound Gαs structure.

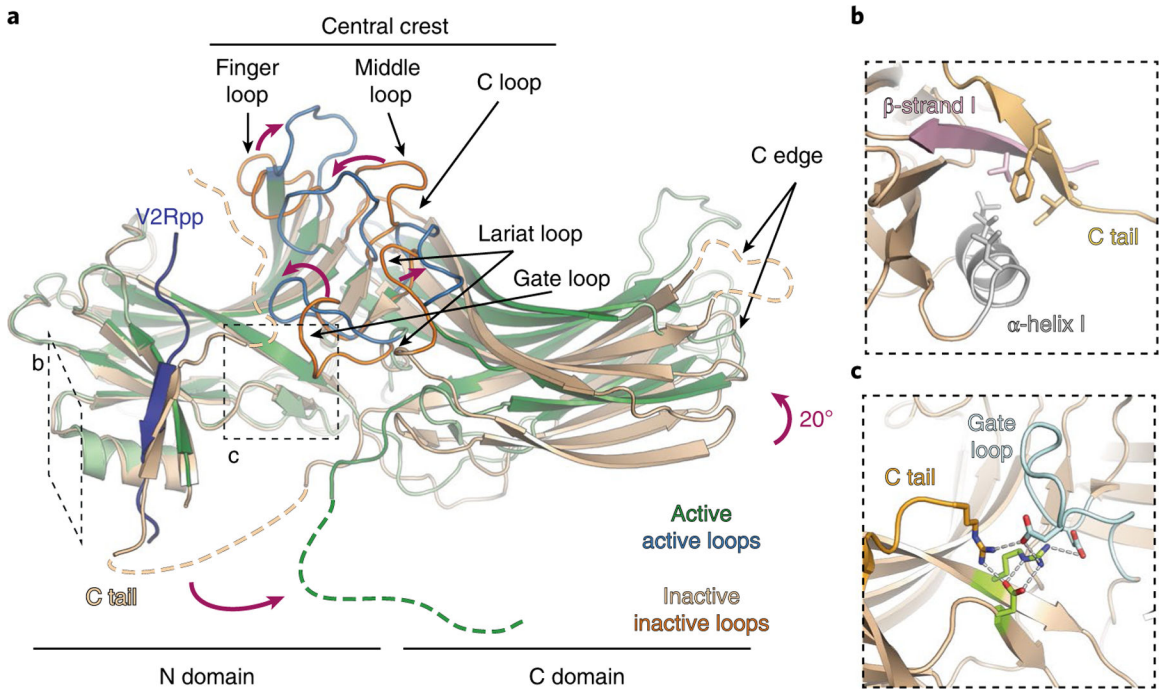

Fig. 3. Conformational changes in arrestin-2 upon activation.

a, Overlay of arrestin-2 in its inactive (wheat, PDB 1G4M) and active (green, PDB 4JQI) states. In the inactive conformation, the arrestin C tail (dashed lines represent unresolved residues) docks onto the arrestin N lobe. The active state was obtained by crystallizing arrestin in the presence of a peptide corresponding to the fully phosphorylated vasopressin receptor 2 C terminus (V2Rpp, dark blue) and an active-state-stabilizing antibody fragment (not shown). Activation induces major conformational changes (indicated by purple arrows): rearrangements of the loops at the N–C-domain interface, displacement of the arrestin C tail and an ~20° interdomain rotation. Two major interaction networks maintain arrestin in its basal, inactive conformation. **b**, The three-element interaction is mediated by bulky hydrophobic residues (shown in stick representation) between the C tail, the β-strand I and the α-helix I. **c**, The polar core is a conserved network of charged residues (shown in stick representation) forming ionic interactions (dashed gray lines) between the N-terminal β-strands III and X, the gate loop and the C tail.

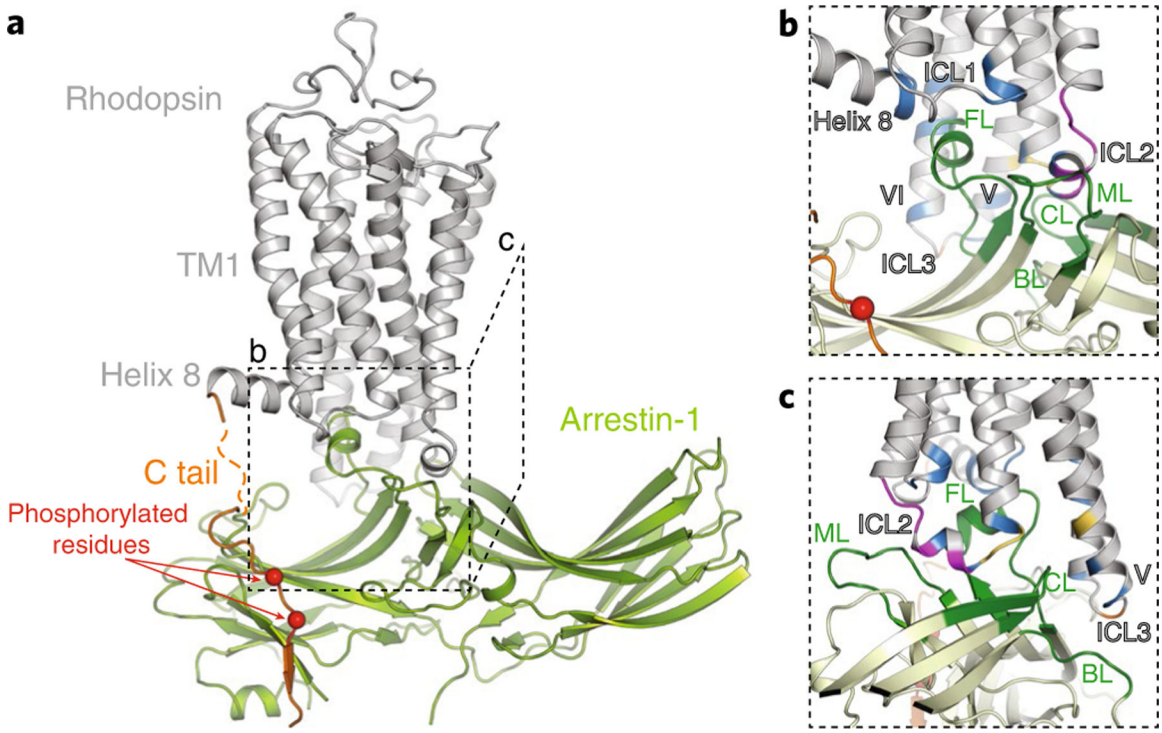

Fig. 4. Structure and interaction interface of the rhodopsin–arrestin-1 complex.

a, Overview of the rhodopsin–arrestin-1 structure (PDB 5W0P), obtained by fusing a preactivated arrestin mutant (shown in green, a triple alanine mutation disrupting the three-element interaction) to a constitutively active rhodopsin mutant (gray). Two of the three known phosphorylation sites (red) on the rhodopsin C tail (orange) are resolved in the structure. **b**,**c**, Front view (**b**) and side view (**c**) of the rhodopsin–arrestin interface, with the structural elements of arrestin that interact with the receptor highlighted in dark green and the interacting receptor residues colored according to the arrestin loops that they are interacting with (blue, finger loop (FL); magenta, middle loop (ML); yellow, C loop (CL); orange, back loop (BL)).