Published in final edited form as:

Angew Chem Int Ed Engl. 2013 June 17; 52(25): 6380–6388. doi:10.1002/anie.201302116.

The Structural Basis of G-Protein-Coupled Receptor Signaling (Nobel Lecture)**

Brian Kobilka*

*Stanford University School of Medicine, Department of Molecular and Cellular Physiology, 279 Campus Drive, Stanford, CA 94305 (USA)

Keywords

β_2 adrenergic receptor; G protein coupled receptors; Nobel lecture; protein structures; signaling

Introduction

Complex organisms require a sophisticated communication network to maintain homeostasis. Cells from different parts of our bodies communicate with each other using chemical messengers in the form of hormones and neurotransmitters. Cells process information encoded in these chemical messages using G-protein-coupled receptors (GPCRs) located in the plasma membrane. GPCRs also mediate communication with the outside world. The senses of sight, smell and taste are mediated by GPCRs. GPCRs are nature's most versatile chemical sensors. There are over 800 GPCRs in the human genome and they respond to a broad spectrum of chemical entities ranging from photons, protons and calcium ions, small organic molecules (including odorants and neurotransmitters), to peptides and glycoproteins.

The classical role of a GPCR is to detect the presence of an extracellular agonist, transmit the information across the plasma membrane, and activate a cytoplasmic heterotrimeric G protein, leading to modulation of downstream effector proteins. Taking the human β_2 adrenergic receptor (β_2 AR) as an example, binding of adrenaline leads to activation of G_{α_s} , stimulation of adenylyl cyclase, cAMP accumulation, PKA activation, and phosphorylation of proteins involved in cell metabolism (Figure 1). However, a wealth of research has now demonstrated that many GPCRs have more complex signaling repertoires. For example, the β_2 AR couples to both G_{α_s} and G_{α_i} in cardiac myocytes,^[1] and can also signal through MAP kinase pathways in a G-protein-independent manner via arrestin.^[2, 3] Similarly, the process of GPCR desensitization involves multiple pathways, including receptor phosphorylation events, arrestin-mediated internalization into endosomes, receptor recycling, and lysosomal degradation. These activities are further complicated by the possibility of GPCR oligomerization,^[4] and the localization of receptors to specific membrane compartments

** Copyright© The Nobel Foundation 2012. We thank the Nobel Foundation, Stockholm, for permission to print this lecture.

having different complements of signaling proteins and different lipid bilayer compositions. Such multifaceted functional behavior has been observed for many different GPCRs.

How does this complexity of functional behavior reconcile with the biochemical and biophysical properties of GPCRs? The effect of a ligand on the structure and biophysical properties of a receptor, and thereby the biological response, is known as the ligand efficacy. Natural and synthetic ligands can be grouped into different efficacy classes (Figure 1 inset): 1) full agonists are capable of maximal receptor stimulation; 2) partial agonists are unable to proffer full activity even at saturating concentrations; 3) neutral antagonists have no effect on signaling activity, but can prevent other ligands from binding to the receptor; 4) inverse agonists reduce the level of basal or constitutive activity below that of the unliganded receptor. For GPCRs capable of coupling to multiple signaling systems, specific ligands can have differential relative efficacies towards the different pathways. In the extreme case, even opposite activities towards different signaling pathways are observed: for the β_2 AR, agonists toward the arrestin/MAP kinase pathway are also inverse agonists for the classical $G_{\alpha s}$ /cAMP/PKA pathway.^[2,5]

Given the central role played by GPCRs in nearly all physiologic processes, they represent the largest group of targets for drug discovery for a broad spectrum of diseases. A better understanding of the structural basis for the complex signaling behavior of GPCRs should lead to more efficient and economical approaches to drug discovery.

Early Insights into GPCR structure

First insights into GPCR structure came from the sequencing of rhodopsin and the cloning of the β_2 AR and other GPCRs in the 1980s. My research career in this field began in 1984 when I joined the laboratory of Dr. Robert Lefkowitz. As a postdoctoral fellow in the Lefkowitz laboratory, I was involved in the cloning of the β_2 AR.^[6] This was the first look at the primary amino acid sequence of GPCR activated by a diffusible ligand (hormone or neurotransmitter). At the time we were surprised by the sequence homology with rhodopsin. However, this comparison and insights from the cloning of other GPCRs that soon followed confirmed the seven-transmembrane topology to be a signature of GPCRs.

My first efforts to understand the structural basis of β_2 AR function took advantage of having access to other adrenergic receptors that we had cloned. Generating chimeric receptors composed of different combinations of sequence from β_2 AR and α_2 AR allowed us to identify domains involved in ligand binding and G-protein-coupling specificity.^[7] After I started my lab at Stanford University at the end of 1989 we continued to refine the map of functional domains through a series of studies using chimeric receptors and site-directed mutants.^[8–10] These studies also identified intramolecular contacts that helped define the arrangement of transmembrane segments in the lipid bilayer.^[11]

Realizing the limitations of mutagenesis to define protein structure, I began to explore methods for the large-scale production and purification of the β_2 AR to enable the use of biophysical methods to study receptor structure and the conformational changes involved in receptor activation. The β_2 AR was an ideal model system for these studies because of the existing wealth of structural information from mutagenesis studies, and the rich diversity of

commercially available ligands for this receptor (agonists, partial agonists, neutral antagonists, and inverse agonists). Nevertheless, this effort was particularly challenging because of the inherent problems associated with expression and purification of these relatively unstable membrane proteins.

Initial work focused on understanding β_2 AR biosynthesis in an effort to identify factors that might facilitate large-scale production.^[12] The β_2 AR is a type IIIb membrane protein and lacks a cleavable signal sequence. We found that insertion of a cleavable signal sequence from influenza hemagglutinin improved insertion of the amino terminus and transmembrane segment (TM) 1, and enhanced functional expression in insect cells by two-fold.^[13] Using this modification, together with affinity tags at the amino and carboxyl terminus we established a protocol to express and purify sufficient quantities of β_2 AR for biophysical studies.^[14]

In 1993, Ulrik Gether and Sansan Lin joined the lab and began applying fluorescence spectroscopy and other biochemical and biophysical approaches to characterize β_2 AR structure and conformational changes in response to binding of various ligands. Using relatively simple techniques such as circular dichroism and intrinsic tryptophan fluorescence gave us insights into the biochemical behavior of the β_2 AR that would ultimately be important for crystallography, such as the stabilizing effect of ligands, particularly antagonists and inverse agonists.^[15] By labeling the β_2 AR with small, environmentally sensitive fluorescence probes we were able to observe structural changes in response to agonist binding.^[16–18]

These initial studies led to a series of experiments using fluorescence spectroscopy to characterize the mechanism by agonist binding and activation. These experiments focused primarily on transmembrane segment (TM) 6 which earlier experiments suggested underwent the largest structural changes upon agonist binding. Purified β_2 AR was labeled at the cytoplasmic end of TM6 with a small environmentally sensitive fluorescent probe. By monitoring changes in the fluorescence as a function of time, we observed that the agonist binding and activation occurred through at least one conformational intermediate, and that agonists and partial agonists stabilize distinct states.^[19–21] We also observed that agonists alone do not stabilize a single active conformation.^[19] As a result of these findings, together with a growing body of evidence for ligand-specific signaling behavior in cells, GPCRs were no longer thought to behave as simple two-state switches. Rather, they are more properly thought of as molecular “rheostats”, able to sample a continuum of conformations with relatively closely spaced energies.^[22, 23] These biophysical and functional experiments suggested that chemical interactions between a ligand and a receptor led to the stabilization of a ligand-specific conformation or ensemble of conformations that interact with specific cytoplasmic signaling and regulatory proteins.

Crystallography

The first insights into the three-dimensional structure of GPCRs came from rhodopsin, which differs from most other GPCRs in its relatively high biochemical stability and its natural abundance in a native tissue, bovine retina. Gebhard Schertler's lab provided the

first structures of rhodopsin from two-dimensional crystals generated in lipids from rod outer segment membranes.^[24] This structure revealed the general architecture of the seven transmembrane (TM) helices, and was the basis for most GPCR homology models until Okada and Palczewski^[25] obtained the first high-resolution three-dimensional structure of rhodopsin in 2000. The elegantly simple approach developed by Okada for purifying rhodopsin from rod outer segments using only detergent extraction suggested that lipids extracted with rhodopsin might be important for protein stability and/or crystallogenesis.^[26] More recently, Ernst and Hofmann produced the first active-state structures of opsin from native rhodopsin.^[27, 28]

In contrast to rhodopsin, GPCRs for hormones and neurotransmitters are not expressed in tissues at sufficient levels for biophysical studies and are much less stable when extracted from membranes with detergents. Nevertheless, through incremental improvements in insect cell expression and the efficiency of the purification procedure we were able to produce sufficient quantities of β_2 AR (1–10 mg) to start crystallography trials around 1998. However, it was not until 2004 that we obtained the first crystals of the β_2 AR large enough to examine by X-ray diffraction. These crystals were still very small (< 50 μ m) and we were not able to see diffraction at conventional synchrotron beamlines. I showed a picture of these crystals to Gebhard Schertler who was working with Christian Riek and Manfred Burghammer at the European Synchrotron Radiation Facility (ESRF) in Grenoble to develop a microfocus beamline. Gebhard invited me to bring some of our β_2 AR crystals to the ESRF. Using a high-intensity 5 μ m beam we were able to see diffraction compatible with a protein crystal at a resolution of approximately 20 Å. While we were disappointed in the poor quality of the diffraction, we were encouraged by the fact that we were able to form crystals of the β_2 AR. This was an important milestone in the effort and suggested that a crystal structure of the β_2 AR was not an impossible goal.

In 2005 Dan Rosenbaum and Søren Rasmussen, two very talented and intrepid postdoctoral fellows, joined the crystallography effort. Based on our experience at that time we speculated that there were two impediments to crystallography: the dynamic character of the protein, and the very small polar surface area available for crystal lattice contacts. Our biophysical and biochemical studies had suggested that the β_2 AR was a flexible, dynamic protein with the cytoplasmic ends of TM5 and TM6, and the intervening third intracellular loop as being the most flexible. We speculated that the dynamic character of this region of the receptor led to conformational heterogeneity that prevented the formation of well-ordered crystals. At the same time biochemical studies showed that the largest stretches of polar amino acids were largely unstructured and not suitable for forming crystal lattice contacts. Søren and Dan took two different approaches to address these problems and to generate better quality crystals of the β_2 AR. Søren identified a monoclonal antibody fragment (Fab) that bound to the cytoplasmic ends of TM5 and TM6. This antibody came out of a collaboration I initiated in 2003 with Dan Rohr at Medarex, a company specializing in therapeutic antigodies. The goal of the collaboration was to generate antibodies that recognized a three-dimensional epitope on native β_2 AR for use in crystallography. As immunogen, I prepared purified β_2 AR reconstituted into phospholipid vesicles to maintain its native conformation. We obtained 13 different monoclonal antibodies from Medarex, and

Søren and colleagues in the lab subsequently identified one that bound only to native β_2 AR and localized its binding site to a region between TM5 and TM6.^[29]

As an alternative strategy, Dan used protein engineering to replace the same flexible, dynamic region of the β_2 AR between TM5 and TM6 of the β_2 AR with T4 lysozyme (T4L).^[30] T4L was chosen because it is a very stable and highly crystallizable soluble protein with amino and carboxyl termini well positioned to fit between TM5 and TM6.

Both approaches were designed to minimize conformational flexibility, or at a minimum, mask the most dynamic surface of the receptor and at the same time increase the amount of polar surface area available for forming crystal lattice contacts. During 2006 we obtained crystals using both approaches combined with a lipid-based media known as bicelles (consisting of a mixture of lipid and detergent) that had been shown to be suitable for membrane protein crystallization.^[31] Initial crystals of the β_2 AR–Fab and the β_2 AR–T4L fusion protein complex both diffracted to below 4 Å. We subsequently obtained a 3.4 Å structure of the β_2 AR–Fab complex grown in bicelles.^[32] This was our first look at the three-dimensional structure of the β_2 AR, but a higher resolution structure would soon follow (Figure 2A).

In the fall of 2006 we sent purified β_2 AR–T4L complex to Vadim Cherezov in the lab of Raymond Stevens at Scripps. Vadim had trained with Martin Caffrey at The Ohio State University. Martin's lab had recently developed miniaturized, high-throughput methods for lipidic cubic phase (LCP) crystallography.^[33,34] We previously explored the use of LCP methods to crystallize the β_2 AR in 1999 in collaboration with Peter Nollert; however, at that time the methods were very labor intensive and used relatively large amounts of protein to screen very few conditions. The methods developed in Martin's lab together with the robot built by his team enabled screening of thousands of conditions with a few milligrams of protein.^[35] Vadim had recently joined the Stevens' lab bringing with him a LCP robot on loan from Martin Caffrey. This collaboration led to a 2.4 Å structure of the β_2 AR–T4L complex (Figure 2B).^[30,36] The fusion protein strategy developed for the β_2 AR has since been successfully applied to a growing number of other GPCRs. Through collaborative efforts with several other groups, my lab recently used the same fusion protein approach to determine structures of the M2 and M3 muscarinic receptors,^[37,38] the μ and δ opioid receptors,^[39,40] and the protease-activated receptor PAR1.^[41] More recently we have found that fusing T4L to the amino terminus of the β_2 AR and simply deleting most of the third intracellular loop can also facilitate crystallization.^[42]

Another approach that has succeeded in obtaining GPCR crystal structures involves scanning mutagenesis to identify thermostabilizing mutations. Chris Tate and Gebhard Schertler and their colleagues pioneered this approach to obtain the structure of the avian β_1 AR.^[43] These stabilizing mutations may reduce structural flexibility and permit the use of detergents having a smaller micelle size. This approach has also been used to obtain the structure of the adenosine A2A receptor^[44] and, in combination with a T4L fusion protein strategy, the neurotensin receptor.^[45]

Capturing Active States by Crystallography

Immediately after obtaining these initial inactive-state structures of the β_2 AR we initiated efforts to capture active states by crystallography. Using the methods that were successful in obtaining inactive-state structures, we were not able to obtain crystals of a β_2 AR bound to an agonist. Our concern was that due to the relatively low affinity of agonists (when compared to the very high affinity inverse-agonist carazolol used to obtain inactive-state structures), we had incomplete occupancy of the receptor under crystallography conditions. This would lead to conformational heterogeneity. To overcome this problem, Dan Rosenbaum worked with Ralph Holl and Peter Gmeiner (University of Erlangen) to develop a covalent agonist for the β_2 AR. Using this approach they were able to obtain crystals of the covalent agonist-bound β_2 AR; however, the cytoplasmic face of the receptor was indistinguishable from the inactive-state structure.^[46]

These disappointing results were consistent with what we had learned from earlier biophysical studies, that agonists alone do not fully stabilize the active state of the β_2 AR. This was first observed using fluorescence spectroscopy^[19, 47] and confirmed in more recent studies using NMR spectroscopy.^[48] Figure 3 is a cartoon illustrating the dynamic character of the receptor showing that the receptor exists as an ensemble of conformations. Due to the flexibility of the unliganded β_2 AR, a small population can be in an active conformation, accounting the phenomenon of basal activity. On binding agonist the cytoplasmic interface becomes even more dynamic, sampling a broader spectrum of conformations. This contributes to the challenges in crystallizing agonist-bound receptor. Fluorescence and NMR experiments showed us that stabilization of the active state required that the receptor must form a complex with its G protein, or some other binding protein that binds to the active conformation.^[47, 48]

Our efforts to crystallize the β_2 AR–G_s complex were in progress, but not yet successful. As an alternative we were exploring antibodies and other binding proteins. In May of 2009 I had the good fortune to meet Jan Steyaert (Free University of Brussels) at a Gordon Conference in Italy. Jan was pioneering the application of single-chain camelid antibody fragments, known as nanobodies, as facilitators of protein crystallogenesis. Shortly after the conference I sent Jan purified, agonist-bound β_2 AR reconstituted into phospholipid vesicles for immunizing llamas. By November 2009 we had our first nanobodies and Søren Rasmussen identified one that exhibited G-protein-like properties. This nanobody (Nb80) bound to purified β_2 AR and allosterically enhanced agonist binding affinity by 100-fold, similar to what is observed in a β_2 AR–G_s complex. This β_2 AR–Nb80 complex gave us the first picture of the active-state conformation of the β_2 AR (Figure 4A).^[49]

The β_2 AR–G_s Complex

In 2005 I met Roger Sunahara (University of Michigan) at a Gordon Conference and we began working together to understand the structural basis of cooperative interactions between the β_2 AR and its G protein G_s. Our long-term goal was to crystallize the β_2 AR–G_s complex. The ultimate success of this effort would require an extensive network of collaborations with investigators from diverse disciplines.

One of the most important contributions to this effort was the use of single-particle electron microscopy (EM) to provide structural insights that guided our crystallization strategy. By 2009 Søren Rasmussen in my lab and Brian Devree in Roger's lab were making considerable progress on the biochemistry of the complex. They were able to form a relatively stable $\beta_2\text{AR}-\text{G}_s$ complex that migrated as a single peak by size exclusion chromatography, however we were not able to grow crystals. We sent protein to Georgios Skiniotis, and expert in single-particle EM methods at the University of Michigan. Our first view of the $\beta_2\text{AR}-\text{G}_s$ complex came from a low-resolution structure generated from negative stained EM images. This structure revealed an unexpected feature of the complex that was one of the biggest obstacles to crystal growth. The $\text{G}_{\alpha s}$ subunit is composed of an α -helical domain and a Ras-like domain with the GDP binding pocket at the interface. The EM structure revealed that the α -helical domain of the $\text{G}_{\alpha s}$ subunit was conformationally heterogeneous. Subsequent EM studies helped to identify chemical additives that minimized the conformational heterogeneity, as well as a nanobody (Nb35) that stabilized the complex.

Other contributions to the success of the $\beta_2\text{AR}-\text{G}_s$ crystallography include the identification of an ultra-high affinity agonist (BI-167107) from Boehringer Ingelheim. This agonist has a dissociation half-life of more than 30 h ensuring that the $\beta_2\text{AR}$ would be occupied by an agonist at all times. Another important reagent was a new detergent MNG-3 provided by Pil Seok Chae and Sam Gellman at the University of Wisconsin, Madison.^[50] This detergent stabilized the $\beta_2\text{AR}-\text{G}_s$ complex during incorporation into the mesophase lipid used for crystallography. Martin Caffrey provided a special mesophase lipid (7.7 MAG) that enabled the application of lipidic cubic phase methods to a large protein complex.^[34] To further stabilize the $\beta_2\text{AR}-\text{G}_s$ complex we worked with Jan Steyaert to develop a nanobody (Nb35) that bound to the interface between the α and β subunits of G_s . Finally, replacement of the amino terminus of the $\beta_2\text{AR}$ with T4L facilitated packing interactions with the extracellular surface.

The first crystals of the $\beta_2\text{AR}-\text{G}_s$ complex were obtained in April 2011 and we were ultimately able to solve the structure to 3.2 Å (Figure 4B).^[51] The structure revealed how the binding of a small agonist at the extracellular side of the receptor propagates structural changes across the lipid bilayer to effect activation of a cytosolic G protein. In this process, small structural changes around the binding pocket are amplified to very large structural changes in the G protein.

Figures 5–8 follow the process of activation from agonist-stabilized changes in the $\beta_2\text{AR}$ to receptor-mediated changes in G_s . As shown in Figure 5, structural differences between the inactive and active state $\beta_2\text{AR}$ structures are relatively small, particularly around the ligand binding pocket. The largest changes are observed at the cytoplasmic surface including a 14 Å outward movement of TM6.

Figure 6 compares the ligand binding pockets for active and inactive structures. Amino acids within 4 Å of the agonist BI167107 are shown. Changes in the binding pocket are relatively subtle, with the agonist pocket being smaller than that of the inverse agonist-bound receptor. The largest difference is a 2 Å inward movement around Ser207 in TM5. Ser 203, 204 and 207 have previously been shown to be important for agonist binding and activation.

To understand how these small changes in the binding pocket contribute to the larger 14 Å movement at the cytoplasmic end of the receptor we looked for the changes in packing interactions between TM segments below the ligand binding pocket. As shown in Figure 7, a set of conserved amino acids pack together to stabilize the inactive conformation (Figure 7A). These include the highly conserved Pro 211 in TM5 and Phe282 in TM6 as well as Ile 121 in TM3 and Asn318 in TM7. This packing arrangement is not compatible with the small inward movement of TM5 and requires a rearrangement to accommodate the agonist-bound active state (Figure 7B). This rearrangement involves a rotation of TM6 around Phe282 that is largely responsible for the large outward movement at its cytoplasmic end (Figure 7C).

Figure 8 shows the structural changes in G_s upon forming a complex with agonist-bound β₂AR. The inactive state of G_s heterotrimer is modeled from the crystal structure of the G_i heterotrimer. The G_{αs} subunit is composed of a Ras-like GTPase domain and an α-helical domain. The GDP (guanosine diphosphate) binding site occupies the interface between these two domains. On coupling to the β₂AR, the G_{αs} subunit undergoes large structural changes with the α-helical domain being displaced by approximately 130°. The carboxyl terminal α5-helix of the Ras-like domain is displaced 5 Å into the core of the active receptor, stabilizing the active state of the receptor. This displacement of the α5-helix as well as more subtle changes transmitted from the receptor to the GDP binding pocket through the (β1-strand of the Ras domain are responsible for dissociation of GDP. The empty-state β₂AR–G_s complex is poised for activation by GTP (guanosine triphosphate). The very large displacement of α-helical domain was not expected from prior studies; however, as noted above, low-resolution single-particle EM studies by Georgios Skiniotis revealed that the α-helical domain is highly dynamic.

Conclusions

The β₂AR–G_s complex crystal structure provides the first high-resolution view of transmembrane signaling for a GPCR. We now have a framework to design experiments to investigate the mechanism of complex formation, GTP binding and complex dissociation. Of particular interest will be studies designed to determine the functional significance of the large movement of the G_{αs} α-helical domain relative to Ras-like domain that is observed in the β₂AR–G_s complex. Nevertheless, the β₂AR–G_s structure leaves an important question unanswered. It does not explain why the β₂AR preferentially couples to G_s. While some of the β₂AR sequences involved in the β₂AR–G_s interface have been shown to have a role in G protein coupling; there is no clear consensus sequence for G_s-coupling specificity when these segments are aligned with other G_s-coupled GPCRs. Coupling specificity may be dictated by interactions between the β₂AR and G_s that precede the formation of the nucleotide-free complex. While the studies outlined in this lecture have advanced the field, much work remains to be done before we can fully understand and pharmacologically control signaling by these fascinating membrane proteins.

Acknowledgments

This lecture represents work done since 1984 and reflects contributions of many colleagues, postdoctoral fellows and graduate students, as well as inspiration provided by the work of other investigators in the field. I want to single out a few colleagues for special thanks. My wife Tong Sun Kobilka has been my colleague, advisor and strongest

advocate for over thirty years. We began working together at Duke, and continue to this day. I want to thank Bob Lefkowitz for his mentorship during my fellowship and beyond. Bill Weis has been my colleague and advisor on all matters having to do with interpreting diffraction data and solving difficult crystal structures. I want to thank Roger Sunahara for initiating our very enjoyable and fruitful collaboration on the β_2 AR– G_s structure. Finally, I want to thank the other members of the team of scientists that contributed to the β_2 AR– G_s structural effort. At Stanford: Søren Rasmussen, Foon Sun Thian, Yaozhong Zou, Andrew Kruse, Ka Young Chung, and Jesper Mathiesen. At the University of Michigan: Brian DeVree, Diane Calinski, Gerwin Westfield, and Georgios Skiniotis. At The University of Wisconsin, Madison: Pil Seok Chae and Sam Gellman. At the Free University of Brussels: Els Pardon and Jan Steyaert. At Trinity College Dublin: Joseph Lyons, Syed Shah, and Martin Caffrey. This work has been supported by grants from the National Institutes of Health (NINDS and NIGMS), and gifts from the Mathers Foundation and Lundbeck.

References

1. Xiao RP, Avdonin P, Zhou YY, Cheng H, Akhter SA, Eschenhagen T, Lefkowitz RJ, Koch WJ, Lakatta EG. Coupling of β_2 -adrenoceptor to G_i proteins and its physiological relevance in murine cardiac myocytes. *Circ Res*. 1999; 84:43–52. [PubMed: 9915773]
2. Azzi M, Charest PG, Angers S, Rousseau G, Kohout T, Bouvier M, Pineyro G. β -Arrestin-mediated activation of MAPK by inverse agonists reveals distinct active conformations for G protein-coupled receptors. *Proc Natl Acad Sci USA*. 2003; 100:11406–11411. [PubMed: 13679574]
3. Shenoy SK, Drake MT, Nelson CD, Houtz DA, Xiao K, Madabushi S, Reiter E, Premont RT, Lichtarge O, Lefkowitz RJ. β -Arrestin-dependent, G protein-independent ERK1/2 activation by the β_2 adrenergic receptor. *J Biol Chem*. 2006; 281:1261–1273. [PubMed: 16280323]
4. Terrillon S, Bouvier M. Roles of G-protein-coupled receptor dimerization. *EMBO Rep*. 2004; 5:30–34. [PubMed: 14710183]
5. Wisler JW, DeWire SM, Whalen EJ, Violin JD, Drake MT, Ahn S, Shenoy SK, Lefkowitz RJ. A unique mechanism of β -blocker action: Carvedilol stimulates β -arrestin signaling. *Proc Natl Acad Sci USA*. 2007; 104:16657–16662. [PubMed: 17925438]
6. Dixon RA, Kobilka BK, Strader DJ, Benovic JL, Dohlman HG, Frielle T, Bolanowski MA, Bennett CD, Rands E, Diehl RE, Mumford RA, Slater EE, Sigal IS, Caron MG, Lefkowitz RJ, Strader CD. Cloning of the gene and cDNA for mammalian β -adrenergic receptor and homology with rhodopsin. *Nature*. 1986; 321:75–79. [PubMed: 3010132]
7. Kobilka BK, Kobilka TS, Daniel K, Regan JW, Caron MG, Lefkowitz RJ. Chimeric α_2 - β_2 -adrenergic receptors: delineation of domains involved in effector coupling and ligand binding specificity. *Science*. 1988; 240:1310–1316. [PubMed: 2836950]
8. Suryanarayana S, Daunt DA, Von Zastrow M, Kobilka BK. A point mutation in the seventh hydrophobic domain of the α_2 adrenergic receptor increases its affinity for a family of β receptor antagonists. *J Biol Chem*. 1991; 266:15488–15492. [PubMed: 1678390]
9. Guan XM, Kobilka TS, Kobilka BK. Enhancement of membrane insertion and function in a type IIIb membrane protein following introduction of a cleavable signal peptide. *J Biol Chem*. 1992; 267:21995–21998. [PubMed: 1331042]
10. Suryanarayana S, Kobilka BK. Amino acid substitutions at position 312 in the seventh hydrophobic segment of the β_2 -adrenergic receptor modify ligand-binding specificity. *Mol Pharmacol*. 1993; 44:111–114. [PubMed: 8101966]
11. Mizobe T, Maze M, Lam V, Suryanarayana S, Kobilka BK. Arrangement of transmembrane domains in adrenergic receptors. Similarity to bacteriorhodopsin. *J Biol Chem*. 1996; 271:2387–2389. [PubMed: 8576196]
12. Kobilka BK. The role of cytosolic and membrane factors in processing of the human β_2 adrenergic receptor following translocation and glycosylation in a cell-free system. *J Biol Chem*. 1990; 265:7610–7618. [PubMed: 1692024]
13. Guan XM, Peroutka SJ, Kobilka BK. Identification of a single amino acid residue responsible for the binding of a class of β -adrenergic receptor antagonists to 5-hydroxytryptamine 1A receptors. *Mol Pharmacol*. 1992; 41:695–698. [PubMed: 1349154]
14. Kobilka BK. Amino and carboxyl terminal modifications to facilitate the production and purification of a G protein-coupled receptor. *Anal Biochem*. 1995; 231:269–271. [PubMed: 8678314]

15. Lin S, Gether U, Kobilka BK. Ligand stabilization of the β_2 adrenergic receptor: effect of DTT on receptor conformation monitored by circular dichroism and fluorescence spectroscopy. *Biochemistry*. 1996; 35:14445–14451. [PubMed: 8931540]
16. Gether U, Lin S, Kobilka BK. Fluorescent labeling of purified β_2 adrenergic receptor. Evidence for ligand-specific conformational changes. *J Biol Chem*. 1995; 270:28268–28275. [PubMed: 7499324]
17. Gether U, Ballesteros JA, Seifert R, Sanders-Bush E, Weinstein H, Kobilka BK. Structural instability of a constitutively active G protein-coupled receptor. Agonist-independent activation due to conformational flexibility. *J Biol Chem*. 1997; 272:2587–2590. [PubMed: 9006889]
18. Gether U, Lin S, Ghanouni P, Ballesteros JA, Weinstein H, Kobilka BK. Agonists induce conformational changes in transmembrane domains III and VI of the β_2 adrenoceptor. *EMBO J*. 1997; 16:6737–6747. [PubMed: 9362488]
19. Ghanouni P, Gryczynski Z, Steenhuis JJ, Lee TW, Farrens DL, Lakowicz JR, Kobilka BK. Functionally different agonists induce distinct conformations in the G protein coupling domain of the β_2 adrenergic receptor. *J Biol Chem*. 2001; 276:24433–24436. [PubMed: 11320077]
20. Swaminath G, Xiang Y, Lee TW, Steenhuis J, Parnot C, Kobilka BK. Sequential binding of agonists to the β_2 adrenoceptor: Kinetic evidence for intermediate conformational states. *J Biol Chem*. 2004; 279:686–691. [PubMed: 14559905]
21. Swaminath G, Deupi X, Lee TW, Zhu W, Thian FS, Kobilka TS, Kobilka B. Probing the β_2 adrenoceptor binding site with catechol reveals differences in binding and activation by agonists and partial agonists. *J Biol Chem*. 2005; 280:22165–22171. [PubMed: 15817484]
22. Deupi X, Kobilka B. Activation of G protein-coupled receptors. *Adv Protein Chem*. 2007; 74:137–166. [PubMed: 17854657]
23. Deupi X, Kobilka BK. Energy landscapes as a tool to integrate GPCR structure, dynamics, and function. *Physiology*. 2010; 25:293–303. [PubMed: 20940434]
24. Unger VM, Hargrave PA, Baldwin JM, Schertler GF. Arrangement of rhodopsin transmembrane α -helices. *Nature*. 1997; 389:203–206. [PubMed: 9296501]
25. Palczewski K, Kumasaka T, Hori T, Behnke CA, Motoshima H, Fox BA, Le Trong I, Teller DC, Okada T, Stenkamp RE, Yamamoto M, Miyano M. Crystal structure of rhodopsin: A G protein-coupled receptor. *Science*. 2000; 289:739–745. [PubMed: 10926528]
26. Okada T, Le Trong I, Fox BA, Behnke CA, Stenkamp RE, Palczewski K. X-Ray diffraction analysis of three-dimensional crystals of bovine rhodopsin obtained from mixed micelles. *J Struct Biol*. 2000; 130:73–80. [PubMed: 10806093]
27. Park JH, Scheerer P, Hofmann KP, Choe HW, Ernst OP. Crystal structure of the ligand-free G-protein-coupled receptor opsin. *Nature*. 2008; 454:183–187. [PubMed: 18563085]
28. Scheerer P, Park JH, Hildebrand PW, Kim YJ, Krauss N, Choe HW, Hofmann KP, Ernst OP. Crystal structure of opsin in its G-protein-interacting conformation. *Nature*. 2008; 455:497–502. [PubMed: 18818650]
29. Day PW, Rasmussen SG, Parnot C, Fung JJ, Masood A, Kobilka TS, Yao XJ, Choi HJ, Weis WI, Rohrer DK, Kobilka BK. A monoclonal antibody for G protein-coupled receptor crystallography. *Nat Methods*. 2007; 4:927–929. [PubMed: 17952087]
30. Rosenbaum DM, Cherezov V, Hanson MA, Rasmussen SG, Thian FS, Kobilka TS, Choi HJ, Yao XJ, Weis WI, Stevens RC, Kobilka BK. GPCR engineering yields high-resolution structural insights into β_2 -adrenergic receptor function. *Science*. 2007; 318:1266–1273. [PubMed: 17962519]
31. Faham S, Bowie JU. Bicelle crystallization: a new method for crystallizing membrane proteins yields a monomeric bacteriorhodopsin structure. *J Mol Biol*. 2002; 316:1–6. [PubMed: 11829498]
32. Rasmussen SG, Choi HJ, Rosenbaum DM, Kobilka TS, Thian FS, Edwards PC, Burghammer M, Ratnala VR, Sanishvili R, Fischetti RF, Schertler GF, Weis WI, Kobilka BK. Crystal structure of the human β_2 adrenergic G-protein-coupled receptor. *Nature*. 2007; 450:383–387. [PubMed: 17952055]
33. Cheng A, Hummel B, Qiu H, Caffrey M. A simple mechanical mixer for small viscous lipid-containing samples. *Chem Phys Lipids*. 1998; 95:11–21. [PubMed: 9807807]

34. Misquitta LV, Misquitta Y, Cherezov V, Slattery O, Mohan JM, Hart D, Zhaltina M, Cramer WA, Caffrey M. Membrane protein crystallization in lipidic mesophases with tailored bilayers. *Structure*. 2004; 12:2113–2124. [PubMed: 15576026]
35. Cherezov V, Peddi A, Muthusubramaniam L, Zheng YF, Caffrey M. A robotic system for crystallizing membrane and soluble proteins in lipidic mesophases. *Acta Crystallogr Sect D*. 2004; 60:1795–1807. [PubMed: 15388926]
36. Cherezov V, Rosenbaum DM, Hanson MA, Rasmussen SG, Thian FS, Kobilka TS, Choi HJ, Kuhn P, Weis WI, Kobilka BK, Stevens RC. High-resolution crystal structure of an engineered human β_2 -adrenergic G protein-coupled receptor. *Science*. 2007; 318:1258–1265. [PubMed: 17962520]
37. Haga K, Kruse AC, Asada H, Yurugi-Kobayashi T, Shiroishi M, Zhang C, Weis WI, Okada T, Kobilka BK, Haga T, Kobayashi T. Structure of the human M2 muscarinic acetylcholine receptor bound to an antagonist. *Nature*. 2012; 482:547–551. [PubMed: 22278061]
38. Kruse AC, Hu J, Pan AC, Arlow DH, Rosenbaum DM, Rosemond E, Green HF, Liu T, Chae PS, Dror RO, Shaw DE, Weis WI, Wess J, Kobilka BK. Structure and dynamics of the M3 muscarinic acetylcholine receptor. *Nature*. 2012; 482:552–556. [PubMed: 22358844]
39. Granier S, Manglik A, Kruse AC, Kobilka TS, Thian FS, Weis WI, Kobilka BK. Structure of the δ -opioid receptor bound to naltrindole. *Nature*. 2012; 485:400–404. [PubMed: 22596164]
40. Manglik A, Kruse AC, Kobilka TS, Thian FS, Mathiesen JM, Sunahara RK, Pardo L, Weis WI, Kobilka BK, Granier S. Crystal structure of the μ -opioid receptor bound to a morphinan antagonist. *Nature*. 2012; 485:321–326. [PubMed: 22437502]
41. Chung KY, Day PW, Velez-Ruiz G, Sunahara RK, Kobilka BK. Identification of GPCR-interacting cytosolic proteins using HDL particles and mass spectrometry-based proteomic approach. *PLoS One*. 2013; 8:e54942. [PubMed: 23372797]
42. Zou Y, Weis WI, Kobilka BK. N-terminal T4 lysozyme fusion facilitates crystallization of a G protein coupled receptor. *PLoS One*. 2012; 7:e46039. [PubMed: 23056231]
43. Warne T, Serrano-Vega MJ, Baker JG, Moukhametzianov R, Edwards PC, Henderson R, Leslie AG, Tate CG, Schertler GF. Structure of a β_1 -adrenergic G-protein-coupled receptor. *Nature*. 2008; 454:486–491. [PubMed: 18594507]
44. Lebon G, Warne T, Edwards PC, Bennett K, Langmead CJ, Leslie AG, Tate CG. Agonist-bound adenosine A2A receptor structures reveal common features of GPCR activation. *Nature*. 2011; 474:521–525. [PubMed: 21593763]
45. White JF, Noinaj N, Shibata Y, Love J, Kloss B, Xu F, Gvozdenovic-Jeremic J, Shah P, Shiloach J, Tate CG, Grisshammer R. Structure of the agonist-bound neurotensin receptor. *Nature*. 2012; 490:508–513. [PubMed: 23051748]
46. Rosenbaum DM, Zhang C, Lyons JA, Holl R, Aragao D, Arlow DH, Rasmussen SG, Choi HJ, Devree BT, Sunahara RK, Chae PS, Gellman SH, Dror RO, Shaw DE, Weis WI, Caffrey M, Gmeiner P, Kobilka BK. Structure and function of an irreversible agonist- β_2 adrenoceptor complex. *Nature*. 2011; 469:236–240. [PubMed: 21228876]
47. Yao XJ, Velez Ruiz G, Whorton MR, Rasmussen SG, DeVree BT, Deupi X, Sunahara RK, Kobilka B. The effect of ligand efficacy on the formation and stability of a GPCR–G protein complex. *Proc Natl Acad Sci USA*. 2009; 106:9501–9506. [PubMed: 19470481]
48. Nygaard R, Zou Y, Dror RO, Mildorf TJ, Arlow DH, Manglik A, Pan AC, Liu CW, Fung JJ, Bokoch MP, Thian FS, Kobilka TS, Shaw DE, Mueller L, Prosser RS, Kobilka BK. The dynamic process of β_2 -adrenergic receptor activation. *Cell*. 2013; 152:532–542. [PubMed: 23374348]
49. Rasmussen SG, Choi HJ, Fung JJ, Pardon E, Casarosa P, Chae PS, Devree BT, Rosenbaum DM, Thian FS, Kobilka TS, Schnapp A, Konetzki I, Sunahara RK, Gellman SH, Pautsch A, Steyaert J, Weis WI, Kobilka BK. Structure of a nanobody-stabilized active state of the β_2 adrenoceptor. *Nature*. 2011; 469:175–180. [PubMed: 21228869]
50. Chae PS, Rasmussen SG, Rana RR, Gotfryd K, Chandra R, Goren MA, Kruse AC, Nurva S, Loland CJ, Pierre Y, Drew D, Popot JL, Picot D, Fox BG, Guan L, Gether U, Byrne B, Kobilka B, Gellman SH. Maltose-neopentyl glycol (MNG) amphiphiles for solubilization, stabilization and crystallization of membrane proteins. *Nat Methods*. 2010; 7:1003–1008. [PubMed: 21037590]
51. Rasmussen SG, DeVree BT, Zou Y, Kruse AC, Chung KY, Kobilka TS, Thian FS, Chae PS, Pardon E, Calinski D, Mathiesen JM, Shah ST, Lyons JA, Caffrey M, Gellman SH, Steyaert J,

Skiniotis G, Weis WI, Sunahara RK, Kobilka BK. Crystal structure of the β_2 adrenergic receptor– G_s protein complex. *Nature*. 2011; 477:549–555. [PubMed: 21772288]

The β_2 AR modulates the activity of multiple signaling pathways

Figure 1.

The complex signaling and regulatory behavior of the β_2 AR. The inset illustrates the concept of ligand efficacy. PKA=protein kinase A, PKC=protein kinase C, PDE=phosphodiesterase, cAMP=cyclic adenosin monophosphate, G_s =stimulative regulatory G protein, G_i =inhibitory regulatory G protein, GRK=G-protein-coupled receptor kinase, ERK=extracellular signal-regulated kinase.

Figure 2.

First crystal structures of β_2 AR in the inactive states. A) The β_2 AR-Fab complex. B) The β_2 AR-T4L fusion protein.

Figure 3.

Cartoon illustrating the dynamic character of the β_2 AR. In the absence of a ligand, the G protein coupling interface of the receptor exists in an ensemble of predominantly low-energy conformations. Rare active-state conformations are responsible for basal activity. Agonist binding increases the dynamic properties of the β_2 AR, increasing the probability of active-state conformations. Only G protein binding can fully stabilize the active state.

Figure 4.
Active-state structures of the human β_2 AR.

Figure 5.

A comparison of the carazolol-bound, inactive-state structure of the $\beta_2\text{AR}$ (gray) and the active-state structure of the $\beta_2\text{AR}$ (green) from the $\beta_2\text{AR-G}_s$ complex.

Figure 6.

A comparison of the binding pocket for the inverse agonist carazolol in the inactive-state structure of the β_2 AR (gray) and the BI167107 binding pocket for the active-state structure of the β_2 AR (green).

Figure 7.

Packing interactions of conserved amino acids in inactive and active states of the β_2 AR.

Only TM3, TM5, TM6, and TM7 are shown. A) A set of conserved amino acids (represented as spheres) pack together to stabilize the inactive conformation. These include the highly conserved Pro211 in TM5 and Phe282 in TM6 as well as Ile 121 in TM3 and Asn318 in TM7. B) Active state. The packing arrangement observed in the inactive state is not compatible with the small inward movement of TM5 and requires a rearrangement to accommodate the agonist-bound active state. C) Active and inactive structures are superimposed. Pro211, Phe282, Ile 121, and Asn318 are represented as sticks to more easily compare differences in position.

Figure 8.

Comparison of inactive GDP-bound G_s (left) with the β_2AR - G_s complex (middle). The two structures are superimposed in the right panel. The GDP-bound G_s heterotrimer was modeled from the crystal structure of the G_i heterotrimer.