



The Structural Basis of G Protein Coupled Receptor Signaling

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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. G protein coupled receptors (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, and 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 AR as an example, binding of adrenaline leads to activation of *Gas*, stimulation of adenylyl cyclase, cAMP accumulation, PKA activation, and phosphorylation of proteins involved in cell metabolism (Fig. 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 *Gas* and *Gai* in cardiac myocytes (Xiao et al. 1999), and can also signal through MAP kinase pathways in a G protein-independent manner via arrestin (Azzi et al. 2003;

Shenoy et al. 2006). 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 factors such as GPCR oligomerization (Terrillon and Bouvier 2004), localization to specific membrane compartments, and resulting differences in lipid bilayer composition. 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 (Fig. 1 inset): full agonists are capable of maximal receptor stimulation; partial agonists are unable to promote full activity even at saturating concentrations; neutral antagonists have no effect on signaling activity, but can prevent other ligands from binding to the receptor; 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,

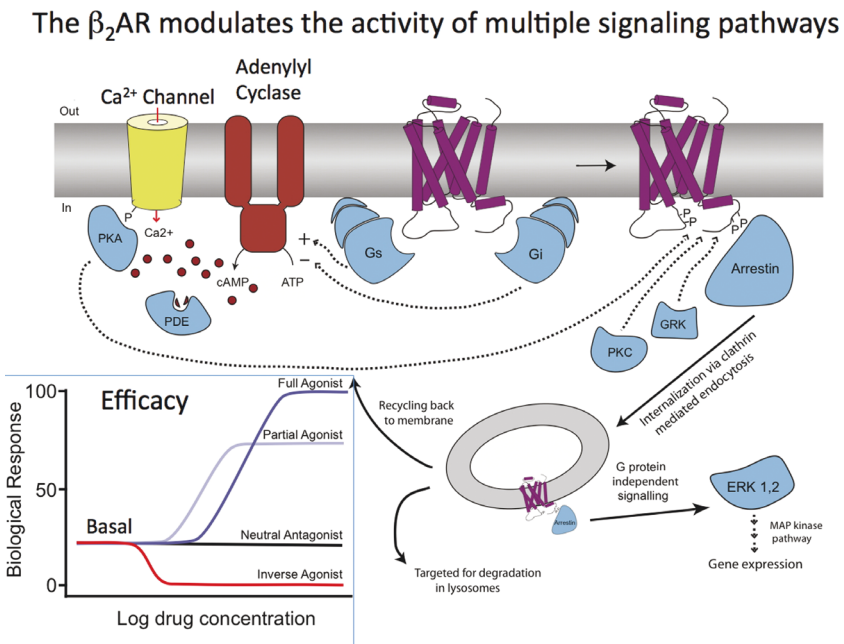


FIGURE 1. The complex signaling and regulatory behavior of the β_2 AR. The inset illustrates the concept of ligand efficacy.

specific ligands can have different relative efficacies towards the different pathways. In the extreme case, even opposite activities towards different signaling pathways are observed: for the β_2 adrenergic receptor (β_2 AR), a partial agonist toward the arrestin/MAP kinase pathway are also inverse agonists for the classical G α phas/cAMP/PKA pathway (Azzi et al. 2003; Wisler et al. 2007).

Given the central role played by GPCRs in nearly all physiological 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 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 (Dixon et al. 1986). This was the first look at the primary amino acid sequence of a 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_2A AR allowed us to identify domains involved in ligand binding and G protein coupling specificity (Kobilka et al. 1988). 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 (Suryanarayana et al. 1991; Guan et al. 1992; Suryanarayana and Kobilka 1993) these studies also identified intramolecular contacts that helped define the arrangement of transmembrane segments in the lipid bilayer (Mizobe et al. 1996).

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 (Kobilka 1990). 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 (Guan et al. 1992). 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 (Kobilka 1995).

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 (Lin et al. 1996). By labeling the β_2 AR with small, environmentally sensitive fluorescence probes we were able to observe structural changes in response to agonist binding (Gether et al. 1995; Gether et al. 1997; Gether et al. 1997).

These initial studies led to a series of experiments using fluorescence spectroscopy to characterize the mechanism of 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 (Ghanouni et al. 2001; Swaminath et al. 2004; Swaminath et al. 2005). We also observed that agonists alone do not stabilize a single active conformation (Ghanouni et al. 2001). 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 (Deupi and Kobilka 2007; Deupi and Kobilka 2010). 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 (Unger et al. 1997). 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 (Palczewski et al. 2000) 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 (Okada et al. 2000). More recently, Ernst and Hoffman produced the first active state structures of opsin from native rhodopsin (Park et al. 2008; Scheerer et al. 2008).

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 milligrams) to start crystallography trials around 1998. However, it wasn't 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 microns) 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 micron beam, we were able to see diffraction compatible with a protein crystal at a resolution of approximately 20Å. 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 antibodies. 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 an 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 (Day et al. 2007).

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) (Rosenbaum et al. 2007). T4 lysozyme 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 (Faham and Bowie 2002). 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 (Rasmussen

et al. 2007). This was our first look at the three dimensional structure of the β_2 AR, but a higher resolution structure would soon follow (Fig. 1A).

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 (Cheng et al. 1998; Misquitta et al. 2004). 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 (Cherezov et al. 2004). Vadim had recently joined the Stevens lab, bringing with him an LCP robot on loan from Martin Caffrey. This collaboration led to a 2.4 Å structure of the β_2 AR-T4L complex (Cherezov et al. 2007; Rosenbaum et al. 2007) (Fig.2B). 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

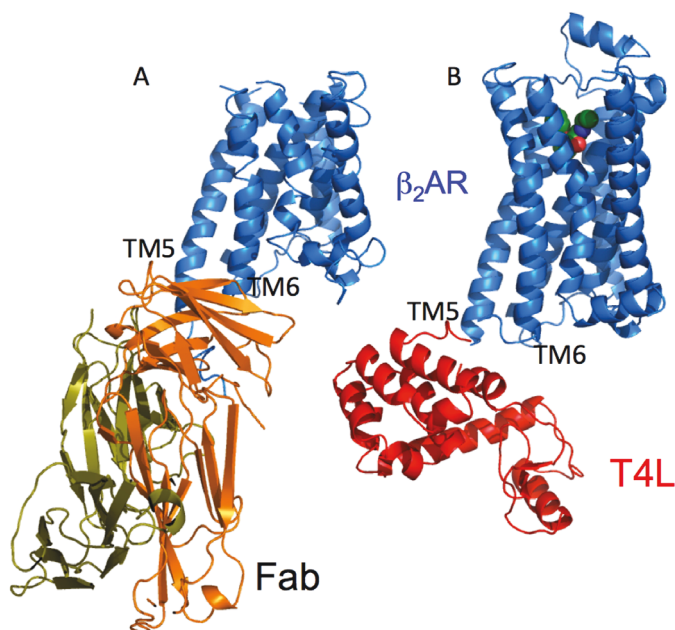


FIGURE 2. First crystal structures of β_2 AR in the inactive states. A. The β_2 AR-Fab complex. B. The β_2 AR-T4 Lysozyme (T4L) fusion protein.

determine structures of the M2 and M3 muscarinic receptors (Haga et al. 2012; Kruse et al. 2012), the mu and delta opioid receptors (Granier et al. 2012; Manglik et al. 2012), and the protease activated receptor PAR1 (Chung et al. 2013). 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 (Zou et al. 2012).

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 (Warne et al. 2008). 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 (Lebon et al. 2011) and, in combination with a T4 lysozyme fusion protein strategy, the neurotensin receptor (White et al. 2012).

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 (Rosenbaum et al. 2011).

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 (Ghanouni et al. 2001; Yao et al. 2009) and confirmed in more recent studies using NMR spectroscopy (Nygaard et al. 2013). 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 for the phenomenon of basal activity. On binding the agonist, the cytoplasmic interface becomes

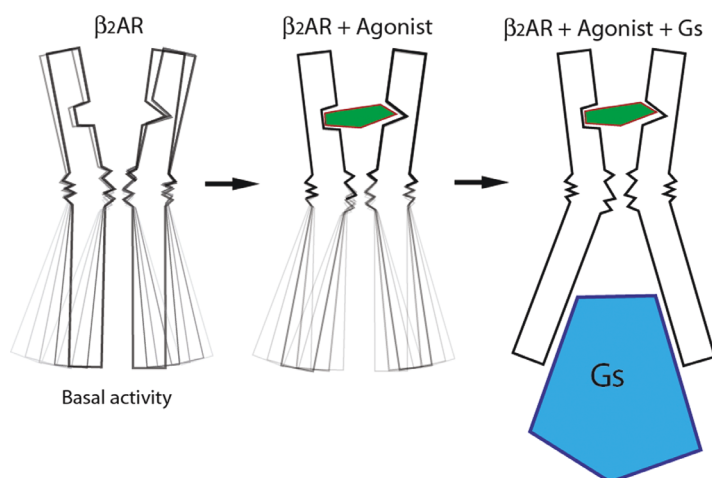


FIGURE 3. Cartoon illustrating the dynamic character of the $\beta_2\text{AR}$. In the absence of a ligand, the G protein coupling interface of the receptor exists in an ensemble of predominantly low energy conformations. Rare active-state conformations are responsible for basal activity. Agonist binding increases the dynamic properties of the $\beta_2\text{AR}$, increasing the probability of active-state conformations. Only G protein binding can fully stabilize the active state.

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 protein that binds to and stabilizes the active conformation (Yao et al. 2009; Nygaard et al. 2013).

Our efforts to crystallize the $\beta_2\text{AR}$ -Gs 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 crystallogensis. Shortly after the conference I sent Jan purified, agonist-bound $\beta_2\text{AR}$ 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 $\beta_2\text{AR}$ and allosterically enhanced agonist binding affinity by 100-fold, similar to what is observed in a $\beta_2\text{AR}$ -Gs complex. This $\beta_2\text{AR}$ -Nb80 complex gave us the first picture of the active-state conformation of the $\beta_2\text{AR}$ (Rasmussen et al. 2011) (Fig. 4A).

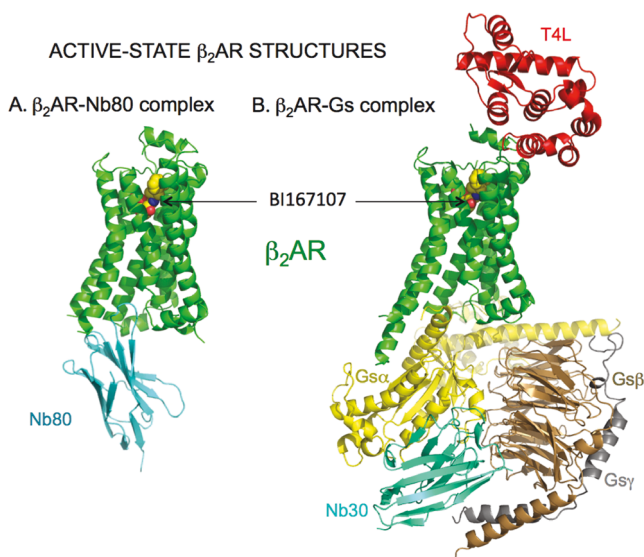


FIGURE 4. Active-state structures of the human β_2 AR.

THE β_2 AR-GS 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 Gs. Our long-term goal was to crystallize the β_2 AR-Gs 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 β_2 AR-Gs 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, an expert in single particle EM methods at the University of Michigan. Our first view of the β_2 AR-Gs 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 G α s subunit is composed of an alpha helical domain and a Ras-like domain with the GCP binding pocket at the interface. The EM structure revealed that the alpha helical domain of the

Gs alpha 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 β_2 AR-Gs 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 hours, ensuring that the β_2 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 (Chae et al. 2010). This detergent stabilized the β_2 AR-Gs 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 (Misquitta et al. 2004). To further stabilize the β_2 AR-Gs complex, we worked with Jan Steyaert to develop a nanobody (Nb35) that bound to the interface between the alpha and beta subunits of Gs. Finally, replacement of the amino terminus of the β_2 AR with T4 lysozyme facilitated packing interactions with the extracellular surface.

The first crystals of the β_2 AR-Gs complex were obtained in April 2011 and we were ultimately able to solve the structure to 3.2Å (Fig. 4B) (Rasmussen et al. 2011). 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 β_2 AR to receptor mediated changes in Gs. As shown in Figure 5, structural differences between the inactive and active state β_2 AR structures are relatively small, particularly around the ligand binding pocket. The largest changes are observed at the cytoplasmic surface including a 14Å outward movement of TM6.

Fig. 6 compares the ligand binding pockets for active and inactive structures. Amino acids within 4Å 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 2Å inward movement around Ser207 in TM5. Ser203, 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 14Å 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

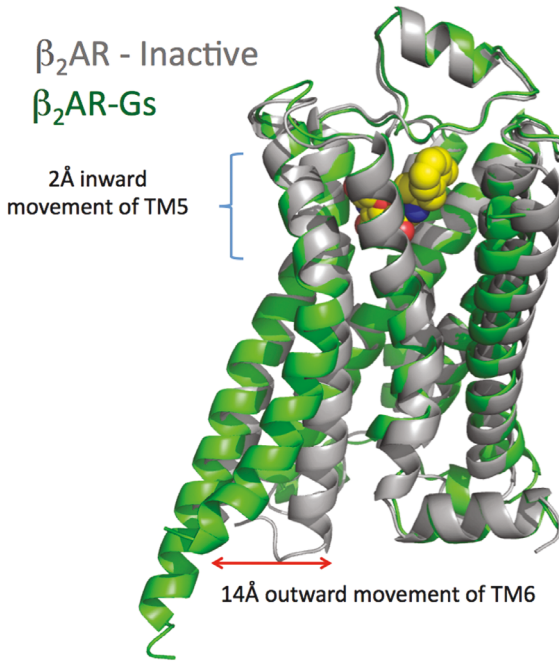


FIGURE 5. A comparison of the carazolol-bound, inactive-state structure of the $\beta_2\text{AR}$ (gray) and the active-state structure of the $\beta_2\text{AR}$ (green) from the $\beta_2\text{AR-Gs}$ complex.

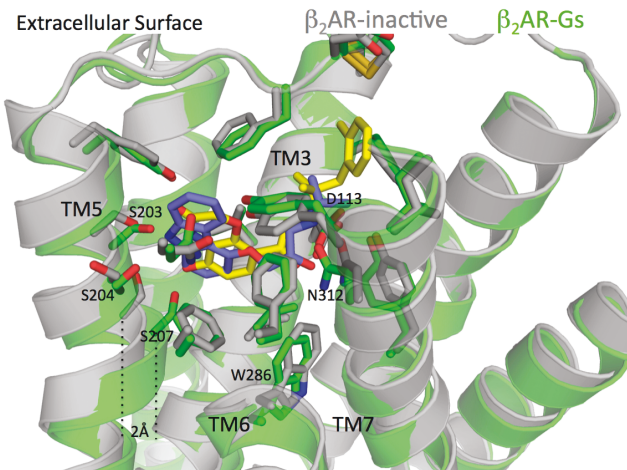


FIGURE 6. A comparison of the binding pocket for the inverse agonist carazolol in the inactive-state structure of the $\beta_2\text{AR}$ (gray) and the B167107 binding pocket for the active-state structure of the $\beta_2\text{AR}$ (green).

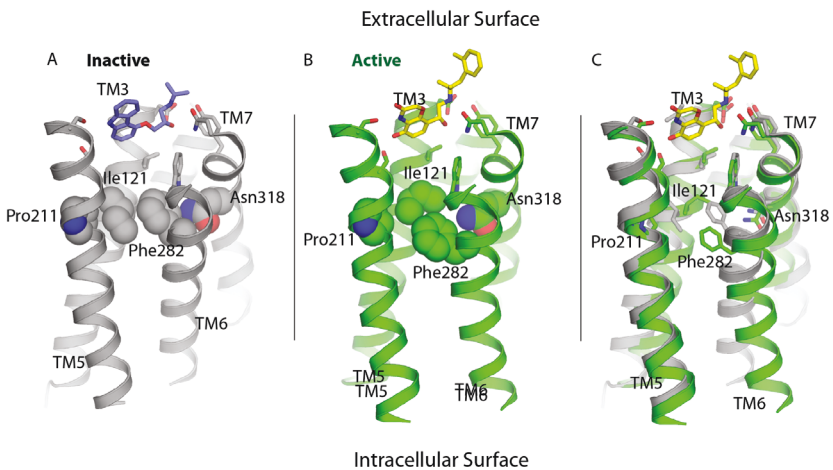


FIGURE 7. Packing interactions of conserved amino acids in inactive and active states of the β_2 AR. Only TM3, TM5, TM6 and TM7 are shown. **A.** A set of conserved amino acids (represented as spheres) pack together to stabilize the inactive conformation (Fig. 7A). These include the highly conserved Pro211 in TM5 and Phe282 in TM6 as well as Ile121 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. Pro 211, Phe282, Ile121 and Asn318 are represented as sticks to more easily compare differences in position.

pack together to stabilize the inactive conformation (Fig. 7A). These include the highly conserved Pro 211 in TM5 and Phe 282 in TM6 as well as Ile 121 in TM3 and Asn 318 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 (Fig. 7B). This rearrangement involves a rotation of TM6 around Phe282 that is largely responsible for the large outward movement at its cytoplasmic end (Fig. 7C).

Figure 8 shows the structural changes in Gs upon forming a complex with agonist-bound β_2 AR. The inactive state of the Gs heterotrimer is modeled from the crystal structure of the Gi heterotrimer. The Gas subunit is composed of a Ras-like GTPase domain and an α -helical domain. The GDP binding site occupies the interface between these two domains. On coupling to the β_2 AR, the Gas subunit undergoes large structural changes, with the α -helical domain being displaced by approximately 130° . The carboxyl terminal $\alpha 5$ -helix of the Ras-like domain is displaced 5\AA into the core of the active receptor, stabilizing the active state of the receptor. This displacement of the $\alpha 5$ -helix as well as more subtle changes transmitted from the receptor to the GDP binding pocket through the

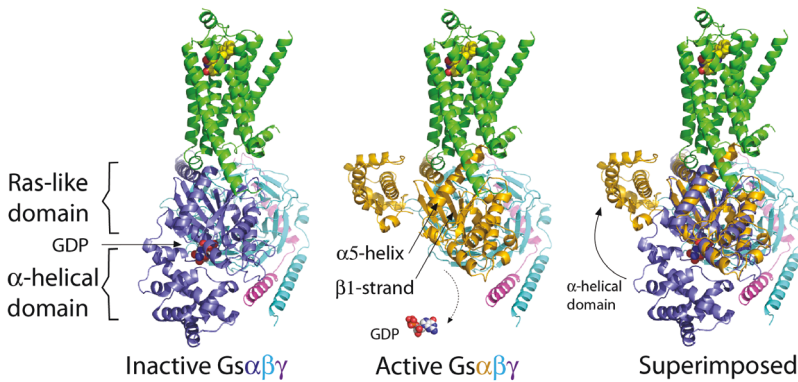


FIGURE 8. Comparison of inactive GDP bound Gs (left panel) with the β_2 AR-Gs complex (middle panel). The two structures are superimposed in the right panel. The GDP-bound Gs heterotrimer was modeled from the crystal structure of the G_i heterotrimer.

$\beta 1$ -strand of the Ras domain are responsible for dissociation of GDP. The empty-state β_2 AR-Gs complex is poised for activation by GTP. The very large displacement of alpha helical domain was not expected from prior studies; however, as noted above, low-resolution single particle EM studies by Georgios Skiniotis revealed that the alpha helical domain is highly dynamic.

CONCLUSIONS

The β_2 AR-Gs 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 Gs α -helical domain relative to the Ras-like domain that is observed in the β_2 AR-Gs complex. Nevertheless, the β_2 AR-Gs structure leaves an important question unanswered. It does not explain why the β_2 AR preferentially couples to Gs. While some of the β_2 AR sequences involved in the β_2 AR-Gs interface have been shown to have a role in G protein coupling; there is no clear consensus sequence for Gs-coupling specificity when these segments are aligned with other Gs-coupled GPCRs. Coupling specificity may be dictated by interactions between the β_2 AR and Gs 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.

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Portrait photo of professor Kobilka by photographer Ulla Montan.