Journal of Medicinal Chemistry
Miniperspective

J. Med. Chem. **2010,** *53,* 923–932 **923**
DOI: 10.1021/jm900419a

Scope and Difficulty in Generating Theoretical Insights Regarding Ligand Recognition and Activation of the β_2 Adrenergic Receptor

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Received April 1, 2009

Introduction

The seven transmembrane G-protein-coupled receptors (7TMs[a]) that make up the largest group of membrane proteins involved in signal transduction are directly implicated in several physiological and pathological events.[1] As a result, there has been an increased interest in understanding the essentials of their ligand recognition and activation processes. The β_2 adrenoceptor (β_2AR) is one of the few 7TMs whose 3-D structure is available in the Protein Data Bank (PDB). Currently, the 3-D structures of only four 7TMs have been reported from crystallographic studies. These 7TMs are rhodopsin, the β_2 and β_1 adrenoceptors, and the A_2A adenosine receptor. Some other 7TMs have been studied by the same methodology, but only poor results have been obtained.[1]

The crystal structures of the β_2AR allow one to reexamine and reassess the existing data obtained from mutagenesis and biochemical studies. In fact, much of the data correlating receptor mutations and ligand binding can be explained from a structural perspective.[1,2] We have used the I-TASSER server,[3−5] which uses multiple-threading alignment by LOMETS and iterative TASSER simulations, to build 3-D models or "maps" of the complete sequence of the β_2AR (see Figure 1) in order to obtain structural insights into the spatial arrangement of β_2AR components. It is also possible to use docking methods to study the interactions between a set of ligands and the β_2AR and to find binding pockets with suggested interactions for each ligand. Additionally, it is possible to study the structural consequences of β_2AR mutations using ligand and/or protein−β_2AR complex energy affinity values, which can be computed using docking methods and molecular dynamic (MD) simulations. MD simulations allow one to study the insertion of the β_2AR into the lipid bilayer, as well as the effect of the adjacent fatty acids and cholesterol, which are normally present.[6] Also, trace elements that can modulate the action of the β_2AR (e.g., Zn^{2+}),[7] as well as other molecules (see the section Study of the Allosteric Modulation of the β_2AR) that can disrupt its expression (by glycosylation or phosphorylation processes),[8] can be studied with current methodologies. Furthermore, by the coupling of modeling with protein−protein docking procedures, it is now possible to study the interactions between the β_2AR and other similar/dissimilar proteins in structural detail. These studies may include proteins that are involved with intracellular signaling events that are activated by ligand recognition at the β_2AR.[1] These investigations are both enriched and challenged by the large amount of in vitro data. Therefore, a key point in studying the behavior of the β_2AR is identifying conformational states of the receptor.[2] Additionally, differences have been reported in the ligand affinities from in vitro studies on 7TM populations, which contain homo- and hetero-oligomers of the β_2AR, and from studies using the "isolated-β_2AR".[9,10] In contrast, there have been many studies on the influence of specific β_2AR moieties on ligands and the effects of stereoisomeric ligands on the recognition of, and interaction with, the β_2AR binding site.[1,2] However, some structural details remain unclear. Elucidating these phenomena could help in the design of new and improved antagonist/agonist ligands. This could be achieved using computational tools that help to clarify electronic, atomic, and dynamic details. However, some limitations have been well established. For example, it is not feasible for electronic structure methods to study the whole macromolecules such as the β_2AR using quantum chemistry alone or coupled to MD simulations to analyze β_2AR behavior as well as the amino acid side chain and backbone flexibility; consequently, these theoretical studies are excluded in studies of ligand−protein complexes. Also, MD simulations on large systems, such as those including several proteins, will require advanced computational resources that are not yet available; this technique cannot produce trajectories longer than a few microseconds, and it has other computational limitations. However, with the current computational resources, approximate details of the recognition between the β_2AR and well-known ligands are available from theoretical studies.[1,2] Data obtained in silico, which have been analyzed and compared with in vitro data, provide confidence in the insights gained using theoretical tools.

In this Miniperspective, a wide scope of β_2AR ligand recognition and activation studies are discussed. Selected examples are presented from the field, as well as opportunities for future studies and structure-based ligand design using advances in molecular modeling.

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[a]Abbreviations: 7TM, seven transmembrane domain; β_2AR, β_2 adrenoceptor; PDB, Brookhaven Protein Data Bank; 3-D, three-dimensional; ICL3, third intracellular loop; MD, molecular dynamics; TM, transmembrane domain; QSAR, quantitative structure−activity relationship.

Published on Web 10/05/2009

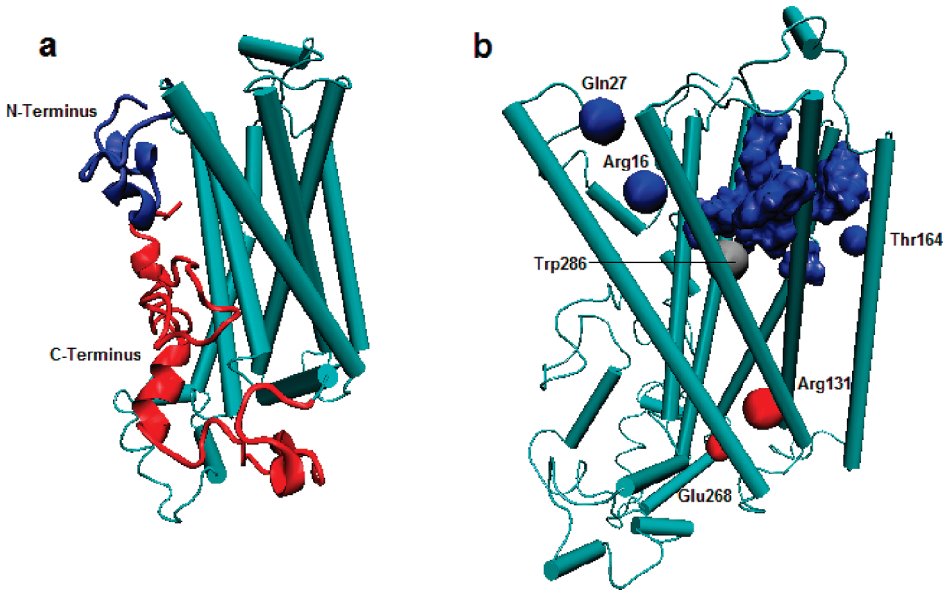

Figure 1. Model of the 3-D structure of the full sequence of β_2AR. (a) The structure reported by Cherezov is in cyan (including amino acids 29−342). The amino (in blue) and carboxy (in red) termini were modeled by ab initio methods and added to the crystal structure by the I-Tasser server.[3−5] (b) Some interesting sites of the β_2AR involved in activation. The amino acids in the binding site are represented by the blue surface (from ref 7). The "ionic lock" (Arg131 and Glu268) is shown as red spheres, while Trp286 at the "toggle switch" is shown as a gray sphere. Also, the amino acids involved in human β_2AR polymorphisms (Arg16, Gln27, and Thr164) are depicted as blue spheres.

Structural Insights from Visualization of the Three-Dimensional Structure of the β_2AR

Because of the great of interest in the β_2AR as a pharmacological target, several researchers have focused on obtaining a 3-D model of the receptor. Thus, some models have been defined using primary sequence comparisons, secondary structure predictions, and three-dimensional homology building, using bacteriorhodopsin[11] and bovine rhodopsin as templates.[12] The β_2AR models obtained were used to study structural and ligand-recognition properties.[13,14]

Characterization of the human β_2AR by 3-D crystallography was a laborious and long-anticipated task that has recently been brought to fruition by the Kobilka group.[15,16] The main difficulties encountered in this project, after purification of the protein, were the low levels of membrane expression and a particular instability in the third intracellular loop (ICL3), which plays an essential role in interactions with G proteins. This problem was solved by either fusion with T4 lysozyme or cocrystallization with an antibody fab fragment. As a result, three structures were recently obtained by X-ray crystallography (PDB codes 2r4r,[16] 2r4s,[16] and 2rh1,[15] as well as a more recent cholesterol-bound structure 3d4s).[17] Although each structure is distinct, all were very similar conformationally when they were superimposed.[18] Furthermore, crystal structures for other 7TMs have been obtained (PDB codes 2vt4 for β_1AR,[19] and 3eml for the A_{2A} adenosine receptor).[20] Comparisons between these 3-D structures (by superposition) provide insights into the molecular arrangements and for rational drug design.[21,22] These 3-D structures allow for an investigation into the roles of some of the amino acids in the ligand recognition process. This type of study is of increasing interest, since it permits an objective visualization of the interaction of the ligand. There have been many studies that try to elucidate the key factors in the structure of ligand−target complexes using theoretical computational methods.[1,14,23,24]

Currently, we have adequate evidence to support a broad understanding of the interactions between ligands and key receptor amino acids that are important for obtaining a biological effect.[1] However, it is difficult to study the fine details (e.g., conformations, local energies, and charges) of specific amino acids in a protein under experimental conditions because they may undergo several conformational changes. In recent years, an increasing volume of evidence, including that from experiments and molecular dynamics simulations, has pointed toward the existence of multiple β_2AR active conformational states[1,2,22] and has described several effectors that lead to different signaling outputs that selectively activate binding to specific ligands.[25,26] This is consistent with the concept of a "receptosome" complex[26] to explain the divergence of 7TMs from the classical representation of a transduction unit consisting of the receptor, a G protein, and a protein effector.

There have been several studies that have used computational tools to observe dynamic behavior in the β_2AR and to identify key amino acids involved in the ligand recognition process.[1,2,23,27] These studies have produced some relevant results, such as the implication of hydrogen bonds to water with the ligand−receptor,[27−29] specifically between the agonist and the β_2AR residues allocated in TM5. Other key findings were the existence of an energy barrier separating the different conformational states generated in antagonist- and agonist-bound states[29] and the existence of distinct β_2AR inactive conformations.[30] These studies have also allowed for the identification of key changes in specific residues (Figure 1).[18] These studies are consistent with experimental evidence, and the conformational changes can be visualized as a multistep process that involves various states. The specific amino acid side chains implicated in ligand binding are not arranged to simultaneously coordinate the ligand but occur in a sequence between the receptor and the agonist such that each interaction increases the probability of the subsequent interaction.[23,29−32] However, these studies were carried out on models built from the previously disclosed rhodopsin structure. These homology models have been used to study the

roles of particular highly conserved amino acids, including those identified as "7TMs microswitches (several highly conserved residues)".[33] So "toggle switch" from Trp286 to Phe290, the "ionic lock (electrostatic interaction)" consisting of Arg131 and Glu268, and other features implicated in G protein activation, such as the DRY motif, other "rotamers switches", Leu272 and Glu122, have been studied.[33−35]

The previous homology modeling and the recent X-ray crystal structures of the β_2AR allow for an elucidation of the interactions between the ligands and the amino acids that are implicated in ligand recognition. In this sense, Lefkowitz shows correlations between the interactions with carazolol observed in the crystallized receptor and previous in vitro evidence from experimental mutations.[2] There are extensive interactions observed between the receptor and the carazolol ligand. For example, Asp113 and Asn312 were previously found to be crucial for ligand binding to the β_2AR. Correspondingly, the interactions of both Asp113 and Asn312 with O(17) and N(19) of carazolol were verified, which explained why mutations at these positions are detrimental to ligand binding.[15,16] Also, Val114 and Phe290 interact with the C(8) to C(13) ring of the carbazole moiety of carazolol and form a hydrophobic sandwich with this aryl moiety. These interactions could explain the loss of affinity for aryl-containing antagonists and agonists upon mutation of Val114 to Ala.[2]

A key reason for studying the behavior of the β_2AR is to identify conformational states of the receptor. Thus, MD simulations with different objectives have been carried out using the recently obtained 3-D structures. Bhattacharya et al.[29] proposed a universally applicable computational method for studying ligand efficacy and 7TM activation. They used simulations of at least 15 ns in length to show that ligands induced conformational changes that were relevant for the development of functionally specific drugs that stabilize a particular receptor conformation. Also, it has been proposed that particular movements (particularly in TM5) are markers for the active/inactive β_2AR states. Han et al.[35] identified conformational rearrangements in the dynamics of the TM7−8 segment that relate to the properties of the conserved NP*xx*Y(*x*)5,6F motif, and showed that they can be used to identify active-state-like conformational elements in the corresponding regions of the new structures of rhodopsin and the β_2AR (using 45 ns simulation trajectories). A recent study reports 600 ns MD simulations that quantified the mobility of the β_2AR in a membrane bilayer environment and showed how the binding of adrenaline, an agonist, caused conformational changes to the ligand-binding pocket and neighboring helices.[36] All of these simulations were well supported and produced results that could be correlated with the results of experimental studies. Recently Dror et al. performed microsecond-time scale MD simulations and found two distinct inactive conformations, which reconciled structural and biochemical observations.[30]

A 3-D Structural Perspective on the Central Role That Mutated Residues Play in Ligand Recognition and Receptor Activation

As previously discussed, the solved X-ray crystal structures for some 7TMs have allowed for the identification of potential binding sites for known ligands. Furthermore, these 3-D models have corroborated the experimental data explaining ligand recognition by the use of single and multiple mutations. Several results have demonstrated or suggested a role for specific amino acid residues. In the β_2AR, it was proposed that conformational states are able to activate the coupled G-protein depending on key movements in the TM3, TM5, TM6, and TM7 regions.[1] These movements can be determined by specific interactions. Figure 2 provides a summary of point mutation studies involving the β_2AR, depicted on a 3-D model showing the key protein surfaces. Ligand interactions with the Tyr199, Ser203, Ser204, and Ser207 residues of TM5 have been implicated in the generation of a conformational active state,[32,37] and mutations of these sites suggest that the agonist-binding pocket is not rigid but is dynamically formed as the ligand builds an increasing number of contacts with the receptor.[38] In addition, Tyr199 can participate in antagonist binding and can block agonist access to the critical serine residues in TM5.[37] Also, Thr118 in TM3 has been implicated as an important interaction site for full agonists.[39]

The pharmacological effects of agonists and antagonists, as well as their β_2AR affinity, have been well described. Mutations of Asp79, Cys106, Cys184, Ser204, Ser207, Asn293, Tyr308, and Asn318 selectively disrupt agonist but not antagonist binding.[39−43] Receptors with mutations at Asp113, Val114, Ile164, Ser203, and Asn312 disrupt the binding of both classes of molecules (Figure 2a).[40,43−46] Receptors with mutations in the segment between residues 266 and 272 show an agonist-independent activation of adenylyl cyclase.[47] In addition, this constitutively active mutant receptor exhibits (a) an increased affinity for agonists (even in the absence of guanine nucleotide-binding regulatory protein, G protein) but not antagonists, with a correlation between the extent of the affinity increase and the intrinsic activity of the ligand, (b) an increased potency to agonists stimulating adenylyl cyclase, and (c) an increased intrinsic activity to partial agonists.[47] Chimeric receptors that contain a mixture of β_1 and β_2 adrenergic receptor segments suggest that the TM4 segment determines the agonist specificity for the subtype.[48] In some mutagenesis studies, stereoselectivity in the binding of ligands has been associated with either the Ser165 or Asn293 residues.[49] Mutation of the Asn293 residue abolishes agonist-induced, but not constitutive, activity.[40,50] In this sense, there has been evidence that ligand interactions with Tyr308 in TM7 and the conformation of a second stereogenic center found in some ligands are very important for ligand affinity.[51]

On the other hand, some mutations have been shown not to modify the binding of ligands but to disrupt the receptor expression and/or agonist-independent activity, such as mutations at Arg15, Gly16, Glu27, Ala76, Cys77, Ala78, Cys116, Ser120, Ala128, Ser161, Ser165, and Cys285.[48,52] Mutations of Asp130, Cys327, and Cys341 reduce the ability of the receptor to activate adenylate cyclase.[39,53] Deletion of some residues in ICL3, the C-terminus, or at positions Tyr350 and Tyr354 affect phosphorylation of the β_2AR and its down-regulation (Figure 2b).[40]

In addition, polymorphisms in Arg16Gly, Gln27Glu, and Thr164Ile in human β_2AR have been related to the β agonist responses (see Figures 1 and 2).[54,55]

Furthermore, some point and segment mutations have been shown to leave the binding of ligands[40] unmodified, and some research has suggested an essential role for the disulfide bonds in the receptor,[56] which could be very important in maintaining the 3-D structure of the β_2AR and its ligand recognition processes.

Each of these conformational changes can be visualized (Figure 1) and analyzed from a structural perspective. Studies on the consequences of the conformational changes caused by

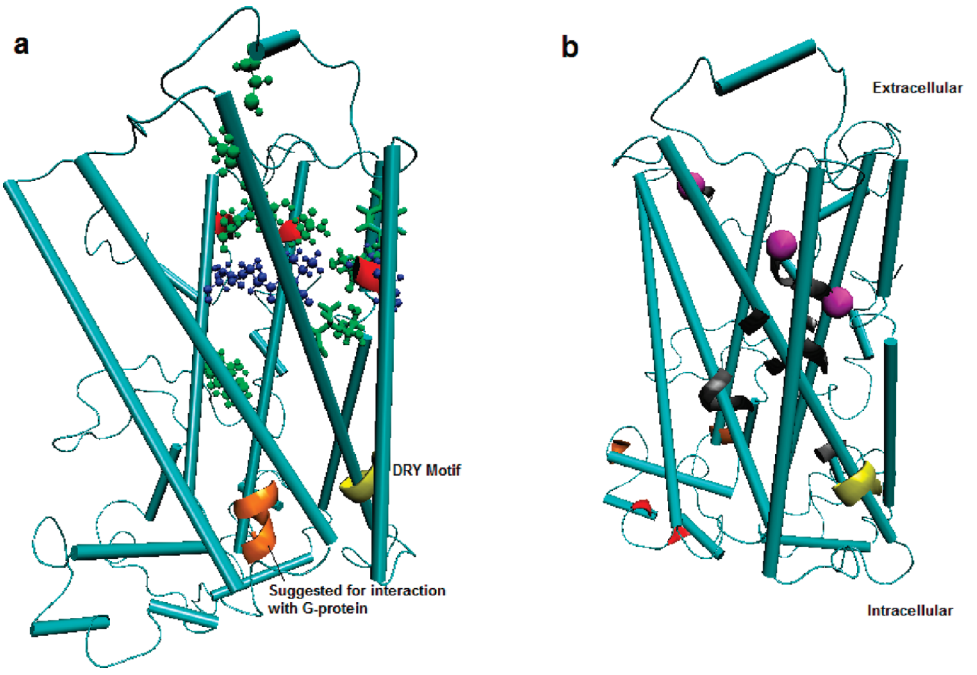

Figure 2. A 3-D perspective of some of the amino acids mutated in order to study β_2AR behavior. (a) The amino acids implicated in forming the β_2AR active state are shown in a stick representation (Thr118, Tyr199, Ser 203, Ser204, and Ser207). Amino acids whose mutation disrupts only agonist binding are shown in a green ball and stick representation (Asp79, Cys106, Cys184, Ser204, Ser207, Asn293, Tyr308, and Asn318). Amino acids whose mutation disrupts both agonist and antagonist binding are shown in blue (Asp113, Val114, Ile164, Ser203, and Asn312). Also, the DRY motif is noted (amino acids 130−132 in yellow, amino acids 266−272 in orange, and the amino acids whose mutation disrupts stereoselectivity (Ser165, Asn293, and Tyr308 in red)). (b) Mutation sites that disrupt β_2AR expression or basal activity are in gray. The mutation of amino acids represented in orange provoked a reduction in ability to activate adenylate cyclase, and the mutation of amino acids in red affected phosphorylation and β_2AR expression down-regulation. Amino acids involved in human β_2AR polymorphism are depicted as violet spheres.

mutations can be carried out by means of homology modeling techniques. Additionally, the impact of a mutation on ligand recognition can be predicted using docking methods and MD simulations.

Study of the Allosteric Modulation of the β_2AR

There is increasing interest in studies of the allosteric modulation of the β_2AR and other 7TMs.[7] Several groups, such as the Canadian Team in GPCR Allosteric Regulation (CTiGAR), have combined their efforts and expertise to address these phenomena. This team of researchers has been conscious of the strengths of computational methods in this endeavor.[2,26]

In the case of the β_2AR, allosteric modulation by zinc[7] is well-known and modulations due to interactions with cholesterol,[6] histamine,[57] and particular interactions with the G protein have been mentioned.[58] No extensive studies of these modulators have been completed using theoretical approaches, despite the fact that capable tools exist. In the case of small molecules, rigid and flexible docking simulations (or combinations of both) provide a way to study ligand recognition and to search for allosteric sites, mirroring work on other 7TMs.[59] MD simulations of β_2AR−modulator complexes should yield information about changes in the behavior of the receptor.[7]

Data on the interactions of 7TMs with crystal structures of the G protein have been obtained that can be used to guide protein−protein docking simulations and MD studies.[60,61] Moreover, there have been recent predictions on β_2AR−G interactions.[62]

Also, it should be noted that β_2AR interactions can be modulated with the same or distinct 7TMs when the β_2AR is found in homo- or heterodimeric (or higher order oligomeric) forms.[5,6] This issue remains controversial. It has been suggested that these β_2AR complexes may regulate export of the receptor to the plasma membrane.[63] However, receptor dimerization appears not to be essential for G protein activation. Differences in ligand affinities have been observed in vitro when G-protein-coupled or homo- and hetero-oligomers of the β_2AR are compared with "isolated β_2AR".[64] Furthermore, differential pharmacologies and the function and regulation of β_2AR dimers (and oligomers) have suggested other ways to selectively target 7TMs in different tissues. These studies have hinted that the mechanism of action of several pharmacological agents may be different in vivo than was anticipated from simple ligand-screening programs that rely on heterogeneous expression of a single 7TM.[6] These phenomena can also be studied by theoretical means. This area is potentially a further application of protein−protein docking and the analysis of a ligand's effects on protein (β_2AR) complexes using MD simulations. Reggio et al. have demonstrated this in a review of the available methodologies for modeling 7TM dimers and oligomers.[65] Additionally, recent experimental studies have been published on 7TM heterodimerization.[66]

Achievements in the Design of β_2AR Ligands

Mathematical inferences from structure−activity relationship (SAR) studies are theoretical methods that have been broadly applied by pharmacologists to elucidate the importance of specific chemical moieties of a ligand in recognizing the binding site on a receptor. This technique has led to the discovery of some essential components of

specific β_2AR ligands and has been key for recognizing the interactions and characteristic effects of adrenergic drugs.[22,67]

Theoretical approaches to aid drug design and development and the application of these methods to the β_2AR are common. In this field, Topiol et al. have focused on obtaining data for drug discovery, first for antagonists[67] and later for active compounds,[68] using high throughput docking procedures. Additionally, they have generated meticulous representations of key interactions between ligands on the β_2AR and other 7TMs.[22] Recently obtained models require careful testing to obtain the best and most accurate ligand affinities on the experimental β_2AR structure. Homology models of 7TMs, especially those supported by experimental data and molecular docking experiments, have been widely used in computational medicinal chemistry for drug discovery purposes (e.g., virtual screening and the generation of docking-based quantitative structure−activity relationship, QSAR, models).[69] The 3-D visualization of interactions allows one to use 3-D QSAR techniques (e.g., CoMFA, CoMSIA, MaP) to infer the effects of specific moieties in the recognition process. Encouraging results have led to the general acceptance of these models. The recent publication of crystal structures for some 7TMs has convincingly proven that these receptors share a structurally conserved 7TM core, which strongly supports the hypotheses that 7TMs were built on the basis of homology modeling; however, some residues had not been not included in the 7TM core model. In this sense, Costanzi recently found a high degree of similarity between *S*-carazolol docked in β_2AR models with the second extracellular loop built de novo and Phe290 in gauche+ and the β_2AR crystal structure reported.[69]

The technology exists to test a great number of ligands with 3-D models.[69−71] These tests have been aimed at elucidating the importance of specific interactions with predetermined amino acids on the receptor. For example, a structure-based virtual screening of full and partial agonists of the β_2AR was recently reported.[70] In this study, 13 known β_2AR antagonist/inverse agonists, 13 known partial/full agonists, and 980 chemically similar but randomly selected compounds were tested. This study suggested that the inactive state of the β_2AR is suitable not only to retrieve inverse agonists/antagonists but also to identify partial/full agonists by docking-based in silico screening methods. Moreover, this study showed that specific, experimentally driven changes in the "rigid" inverse agonist-bound state can be sufficient to obtain a partial or full agonist-selective receptor structure.[70] In this sense, recent studies by Topiol et al. and Kolb et al. have shown complementary data from theoretical and experimental assays for well-known β_2AR ligands.[71] There has also been a remarkable ability to test nonexistent (putative) compounds for their function as viable ligands for the β_2AR.[70] From these studies, it is possible to propose new compounds for in vitro studies. In this sense, we have reported a set of boron-containing compounds with probable activity on the β_2AR. In the case of one of these compounds, activity has been demonstrated in vitro.[72] As seen in these examples, these computational tools are a viable means to analyze the characteristics of both ligands and targets to obtain an "optimal ligand". Such a ligand should have the ideal physicochemical and structural characteristics for a specific application. Computational tools offer significant help in the development of drugs that interact with the β_2AR.

Stereochemistry Ligand Recognition by the β_2AR

The stereospecificity of biological responses have been clearly shown, and theories modeling the stereochemical preferences of the main pharmacological receptors are being developed.[73] There are chiral biomolecules whose synthesis, recognition, activation of signaling pathways, and metabolism could be dependent on their stereochemistry properties.[73−75] Thus, the endogenous catecholamines, adrenaline and noradrenaline, which have only one stereogenic center, are synthesized only as the *R* enantiomer.[74] This enantiomer has been shown to be more potent than the *S* form.[75] However, the role of the chiral center is not completely understood, since even though it is clearly linked to selectivity and receptor activation, it is not the sole determinant. For example, catechol and dopamine, which do not have a carbon stereocenter, successfully activate signaling pathways by interaction with the β_2AR.[33]

Enantiospecificity has been increasingly understood at the molecular and atomic levels.[76] Several computational tools and experimental methods have been applied to this purpose, and as a result, the active enantiomeric forms (eutomers) of some drugs (including salbutamol a well-known β_2AR agonist) are commercially available.[77] Thus, through site-directed mutagenesis, the amino acids responsible for the stereospecific recognition of ligands have been proposed in a great number of cases, in particular, for the β_2AR.[49,51]

The properties of single enantiomers should be studied because the recognition properties and effects of each enantiomer are different and could be the reason some treatments fail when the molecules are administrated in a racemic mixture.[77,78]

In the case of β_2AR activation for the treatment of asthma, it is desirable to use a single enantiomer of salbutamol.[78] The *R* enantiomers of most adrenergic agonists have been reported as the eutomers for producing a relaxant effect in smooth muscle. However, there are only a few studies on the recognition of these eutomers by the β_2AR.[29,45]

Only a few theoretical studies have been published on this topic. Recently, it was reported that *S*-carazolol has the same conformation about its ethanolamine moiety as the classical receptor agonists *R*-adrenaline[36] and *R*-isoprenaline.[70] Our group has also contributed to this topic.[79] Docking simulations were performed on the 3-D structure of the human receptor obtained by Cherezov et al.[15] (PDB code 2rh1) and a set of known ligands. In these computational experiments, we obtained a refined model by means of a straightforward optimization procedure (10 000 steps at 0 K using the steepest descendent protocol employing the CHARMM27), which, although representing a conformational state of the β_2AR, allowed for the prediction of ligand interactions in the binding site and affinity values for the majority of β_2AR *R* and *S* agonists and *R*-antagonists tested.

In these docking simulations, no interactions were found between Ser165 or Asn293 and the hydroxyl group at the chiral center common to the ligands tested. In the crystal structures of the β_2AR with *S*-carazolol or *S*-pindolol (PDB codes 2rh1 and 3d4s) and in a model obtained with *R*-adrenaline,[36] no interactions were found between an atom bonded to the chiral center with Ser165, Asn293, or Tyr308. This observation is in disagreement with the suggestion by Wieland et al. However, the loss of stereoselectivity with mutation of Asn293 was observed.[49]

We agree with the idea that Asn293 and Tyr 308 residues are implicated in β_2AR ligand stereoselectivity,[49−51] but we

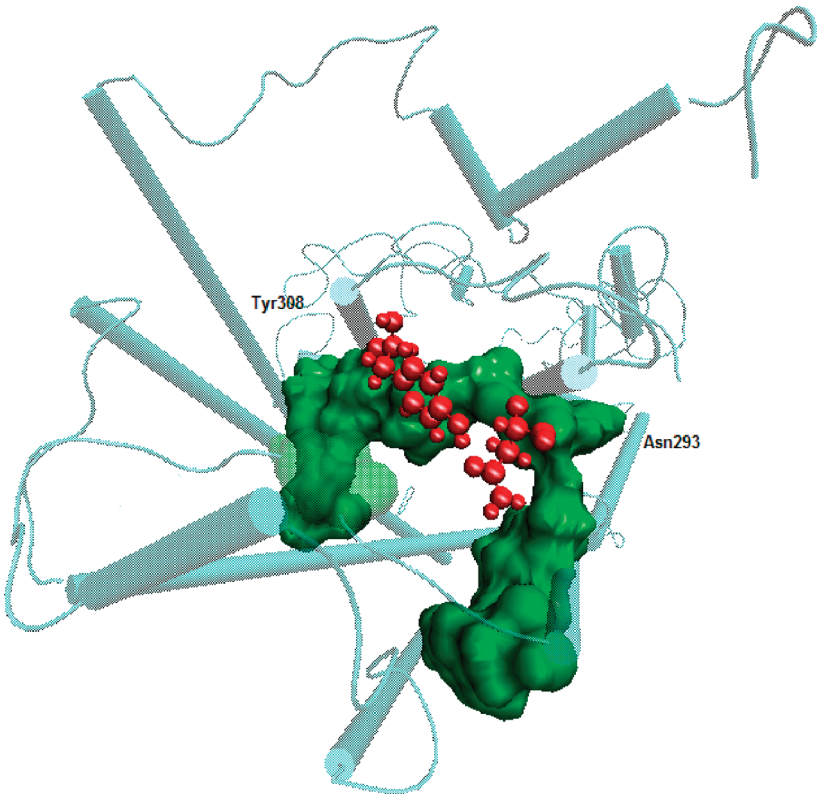

Figure 3. Some amino acids involved in stereoselectivity (shown in a red ball and stick representation) and their disposition in relation to the binding site[18] (shown as a green surface representation).

propose that this can occur in a previous step to the ligand-coupling at the binding pocket[79] and we suggest that selectivity appears to be a property of β_2AR that is determined by a specific distribution of amino acids that limits access to the binding site, such as Asn293 and Tyr308 (Figure 3). These residues could also participate in the binding site for each *R* or *S* ligand and the effectors involved in the biological response after the ligand−receptor complex is formed. This can be comparable to the recent work by Mustafi et al., which describes the different entrance−exits of the retinal binding site in opsin.[21] More theoretical and experimental evidence is necessary to study the access to the β_2AR-binding site as a factor and support of or discarding of this assumption.

Other Applications of Available Theoretical Tools To Study β_2AR Ligand Recognition and Behavior

One feature of the β_2AR is that it can provide a weak signal even in the absence of ligand, a property referred to as constitutive activity. This has been presumed to result from spontaneous, albeit scant, isomerization of the inactive receptor to an active conformation. Agonists stabilize the active conformations (*R**) of the receptor, thus promoting cellular signaling. Mutations of certain residues in the β_2AR that greatly augment the constitutive activity of the receptor have been described by Samama et al.[47] and can be simulated by the model built using either ab initio or homology methods. These models would demonstrate the structural and dynamic consequences of these mutations.

As previously discussed, a model of the β_2AR with the G protein can be built and analyzed to delineate the differences between wild-type and mutant receptors that mostly have mutations in the third intracellular loop. Also, it is possible to use the models constructed for testing differences in ligand affinity for comparisons with the uncoupled receptor structure. Some differences should be expected, but a detailed analysis of the disruption observed in in vitro experiments should be performed.[43]

Similarly, in silico studies of mutations may generate insight into the structural consequences of polymorphisms. In the human β_2AR, at least two polymorphisms are widely implicated in ligand recognition and pathophysiological processes (polymorphisms Arg16Gly and Ser27Gly).[54,55] In the first approach, we built 3-D models of the complete sequence of β_2AR, with different residues at positions 16 and 27. Molecular modeling was done using the 2rh1 and 2vt4 crystal structures as templates by I-TASSER server.[3−5] In Figure 4 are shown the structural differences between a wild-type receptor and one with a mutation at position 16.

An alternative task is homology modeling (based on the X-ray crystal structure of the human β_2AR) to build β_2ARs from different animal species. These models may help rationalize the similarities and differences in ligand binding reported between species.[36] We have reported a 3-D model of the structure of the guinea pig β_2AR, which can aid in vitro guinea pig models to develop β_2AR drugs.[80]

Finally, there is notable interest in studying the role of the carboxy and amino termini, which are not included in the experimental 3-D structures (Figures 1 and 4). Since it is difficult to build a de novo 3-D representation of these amino acid sequences and add them to the crystal structure, validation from experimental results is necessary. These β_2AR termini are important in glycosylation and phosphorylation processes[8] or inclusive to the different responses of the β_1AR.[81] A 3-D representation of the β_2AR may allow one to infer the structural and functional consequences of these events. This task is possible and has been completed with other

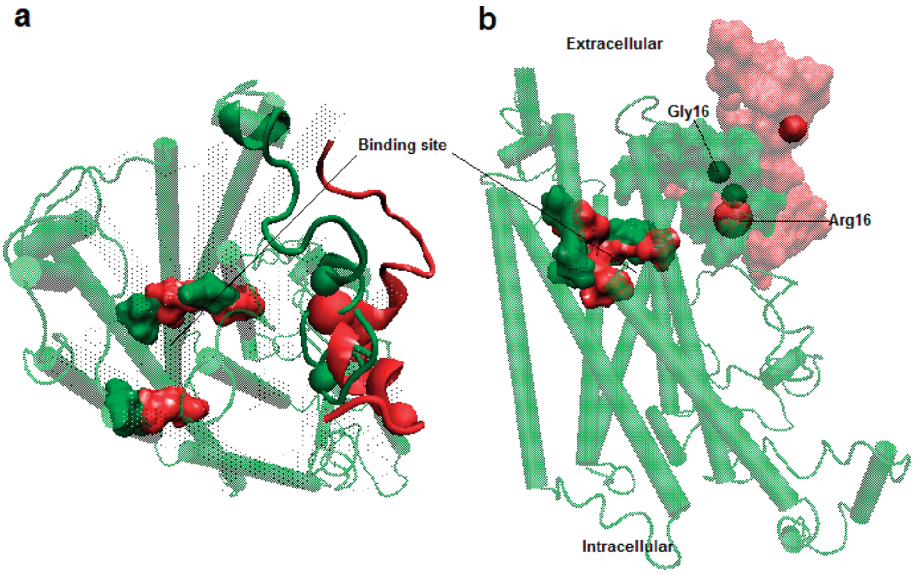

Figure 4. Probable structural consequences of Arg16Gly polymorphisms of the human β_2AR. The wild-type (in red) and polymorphic allele with glycine in position 16 (in green) are represent in 3-D homology models. As seen in the figure, the binding sites (represented as surfaces) are similar in both. However, the modeled N-terminus is different between these 3-D models. (a) In an extracellular view, amino acids 1−28 are shown in a cartoon representation. (b) In a lateral view, amino acids 1−28 are shown in a surface representation. In both views amino acids 5 and 16 are represented as spheres.

proteins.[82] Currently, several programs such as Glyprot[83] and Net Phos[84] are available for this purpose.

Concluding Remarks and Perspectives

The recently disclosed crystal structures of the β_2AR require a reassessment of the existing mutagenesis, biochemical, and theoretical data. It is necessary to synthesize these studies such that the effects of key residues can be understood, singly or in groups, regarding the stereoselectivity of activation of the β_2AR and the implications of specific effectors. These results can be visualized in order to build insights regarding ligand recognition and activation of the β_2 adrenergic receptor.

Because of the 3-D models obtained by X-ray crystallographic studies of some 7TMs, it is possible to identify the specific distribution of amino acids involved in ligand access to the binding site. These structures also allow for the contribution of particular residues in each specific ligand binding interaction to be understood in several β_2AR−ligand complex conformations, which can be involved in different biological responses. It is believed that some specific and common characteristics can be identified in each ligand−7TM complex. Mustafi et al. have reported a comparison of the most recently characterized receptors with the opsin receptor.[21] Greater understanding of this point could allow for the development of drugs with more selective actions than those currently available.

The available in vivo, in vitro, and in silico tools currently in place allow for the very precise results that are necessary, given that conformational and potentially constitutional changes can be determined by interactions at the amino acid, atomic, or even subatomic level. These changes are difficult to study using the conventional tools of molecular biology, such as mutational studies, because of the sheer number of possible variations generated by each amino acid mutation induced in the receptors.

Computational simulations that allow for flexibility of, at least, the amino acid side chains can consider the effects of lipid bilayer membranes, hydration, carbohydrates, and nearby proteins innate to the β_2AR's function to provide successful, general results on the behavior and drug-induced modulation of the β_2AR. However, there are several limitations due to the "high-level" calculations needed for studying large multiatomic/multistructure systems and the capacity of current computational resources. However, this capacity is increasing at an amazing rate, which has been demonstrated in some of the cited works.[25,26,30,31,62]

At present, considerable basic structural information has been obtained, but more data are needed to describe the molecular mechanism of ligand recognition at 7TMs, using the β_2AR as a model. In future, the selectivity of ligands judged by their affinity and effects should be examined along with the key residues for recognition and activation processes.

More MD simulations should be done to support our knowledge of activation mechanisms of 7TMs that go beyond the classically described coupling or decoupling to G proteins. Other mechanisms of activation warranting further study include those due to alternative pathways of cellular signaling, such as the β-arrestin- or phosphodiesterase-dependent effects, which have been recently reported,[81,85,86] and the behavior of conformations adopted by the receptor that trigger these pathways.

Acknowledgment. The authors were supported by grants from the CONACYT (Grant 62488) and COFAA-SIP/IPN (Grant 20090618). All of the figures in this work were generated with the VMD 1.8.6 software.[87]

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References

(1) Weis, W.; Kobilka, B. Structural insights in G-protein coupled receptors activation. *Curr. Opin. Struct. Biol.* **2008**, *18*, 734–740.
(2) Shukla, A. K.; Sun, J.; Lefkowitz, R. J. Crystallizing thinking about the β2-adrenergic receptor. *Mol. Pharmacol.* **2008**, *73*, 1333–1338.
(3) Zhang, Y. I-TASSER server for protein 3D structure prediction. *BMC Bioinf.* **2008**, *9*, 40–47.
(4) Zhang, Y. Template-based modeling and free modeling by I-TASSER in CASP7. *Proteins* **2007**, *8*, 108–117.
(5) Wu, S.; Skolnick, J.; Zhang, Y. Ab initio modeling of small proteins by iterative TASSER simulations. *BMC Biol.* **2007**, *5*, 17.
(6) Pontier, S.; Percherancier, Y.; Galandrin, S.; Breit, A.; Gales, C.; Bouvier, M. Cholesterol-dependent separation of the β_2 adrenergic receptor from its partners determines signaling efficacy. *J. Biol. Chem.* **2008**, *283*, 24659–24672.
(7) Conn, P. J.; Christopoulos, A.; Lindsley, C. W. Allosteric modulators of 7TMs: a novel approach for the treatment of CNS disorders. *Nat. Rev. Drug Discovery* **2009**, *8*, 41–54.
(8) Dohlman, H. G.; Bouvier, M.; Benovic, J. L.; Caron, M. G.; Lefkowitz, R. J. The multiple membrane spanning topography of the beta 2-adrenergic receptor. Localization of the sites of binding, glycosylation, and regulatory phosphorylation by limited proteolysis. *J. Biol. Chem.* **1987**, *15* (262), 14282–14288.
(9) Sartania, N.; Appelbe, S.; Pediani, J. D.; Milligan, G. Agonist occupancy of a single monomeric element is sufficient to cause internalization of the dimeric beta2 adrenoceptor. *Cell. Signalling* **2007**, *19*, 1928–1938.
(10) Milligan, G. G-Protein-coupled receptor heterodimers: pharmacology, function and relevance to drug discovery. *Drug Discovery Today* **2006**, *11*, 541–549.
(11) Hibert, M. F.; Trumpp-Kallmeyer, S.; Bruinvels, A.; Hoflack, J. Three-dimensional models of neurotransmitter G-binding protein-coupled receptors. *Mol. Pharmacol.* **1991**, *40*, 8–15.
(12) Furse, K. E.; Lybrand, T. P. Three-dimensional models for beta-adrenergic receptor complexes with agonists and antagonists. *J. Med. Chem.* **2003**, *46*, 4450–4462.
(13) Kobilka, B. K.; Deupi, X. Conformational complexity of G-protein-coupled receptors. *Trends Pharmacol. Sci.* **2007**, *28*, 397–406.
(14) Goddard, W. A., III; Abrol, R. 3-Dimensional structures of G protein-coupled receptors and binding sites of agonists and antagonists. *J. Nutr.* **2007**, *137*, 1528S–1538S.
(15) Cherezov, V.; Rosenbaum, D. M.; Hanson, M. A.; Rasmussen, S. G.; Thian, F. S.; Kobilka, T. S.; Choi, H. J.; Kuhn, P.; Weis, W. I.; Kobilka, B. K.; Stevens, R. C. High-resolution crystal structure of an engineered human beta2-adrenergic G protein-coupled receptor. *Science* **2007**, *318*, 1258–1265.
(16) Rasmussen, S. G.; Choi, H. J.; Rosenbaum, D. M.; Kobilka, T. S.; Thian, F. S.; Edwards, P. C.; Burghammer, M.; Ratnala, V. R.; Sanishvili, R.; Fischetti, R. F.; Schertler, G. F.; Weis, W. I.; Kobilka, B. K. Crystal structure of the human beta2 adrenergic G-protein-coupled receptor. *Nature* **2007**, *450*, 383–387.
(17) Hanson, M. A.; Cherezov, V.; Griffith, M. T.; Roth, C. B.; Jaakola, V. P.; Chien, E. Y.; Velasquez, J.; Kuhn, P.; Stevens, R. C. A specific cholesterol binding site is established by the 2.8 Å structure of the human beta2-adrenergic receptor. *Structure* **2008**, *16*, 897–905.
(18) Rosenbaum, D. M.; Cherezov, V.; Hanson, M. A.; Rasmussen, S. G.; Thian, F. S.; Kobilka, T. S.; Choi, H. J.; Yao, X. J.; Weis, W. I.; Stevens, R. C.; Kobilka, B. K. 7TM engineering yields high-resolution structural insights into beta2-adrenergic receptor function. *Science* **2007**, *318*, 1266–1273.
(19) Warne, T.; Serrano-Vega, M. J.; Baker, J. G.; Moukhametzianov, R.; Edwards, P. C.; Henderson, R.; Leslie, A. G.; Tate, C. G.; Schertler, G. F. Structure of a beta1-adrenergic G-protein-coupled receptor. *Nature* **2008**, *454*, 486–491.
(20) Jaakola, V. P.; Griffith, M. T.; Hanson, M. A.; Cherezov, V.; Chien, E. Y.; Lane, J. R.; Ijzerman, A. P.; Stevens, R. C. The 2.6 angstrom crystal structure of a human A2A adenosine receptor bound to an antagonist. *Science* **2008**, *322*, 1211–1217.
(21) Mustafi, D.; Palczewski, K. Topoloy of class A G protein-coupled receptors: insights gained from crystal structures of rhodopsins, adrenergic and adenosine receptors. *Mol. Pharmacol.* **2009**, *75*, 1–12.
(22) Topiol, S.; Sabio, M. X-ray structure breakthroughs in the 7TM transmembrane region. *Biochem. Pharmacol.* **2009**, *78*, 11–20.
(23) Rubenstein, L. A.; Zauhar, R. J.; Lanzara, R. G. Molecular dynamics of a biophysical model for beta2-adrenergic and G protein-coupled receptor activation. *J. Mol. Graphics Modell.* **2006**, *25*, 396–409.
(24) Kneissl, B.; Leonhardt, B.; Hildebrandt, A.; Tautermann, C. S. Revisiting automated G-protein coupled receptor modeling: the benefit of additional template structures for a neurokinin-1 receptor model. *J. Med. Chem.* **2009**, *52*, 3166–3173.
(25) Bockaert, J.; Fagni, L.; Dumuis, A.; Marin, P. 7TM interacting proteins (GIP). *Pharmacol. Ther.* **2004**, *103*, 203–221.
(26) Audet, M.; Bouvier, M. Insights into signaling from the β2-adrenergic receptor structure. *Nat. Chem. Biol.* **2008**, *4*, 397–403.
(27) Spijker, P.; Vaidehi, N.; Freddolino, L. P.; Hilbers, P. A. J.; Goddard, W. A. Dynamic behavior of fully solvated β_2-adrenergic receptor, embedded in the membrane with bound agonist or antagonist. *Proc. Natl. Acad. Sci.U.S.A.* **2006**, *103*, 4882–4887.
(28) Angel, T. E.; Chance, M. R.; Palczewski, K. Conserved waters mediate structural and functional activation of family A (rhodopsin-like) G protein-coupled receptors. *Proc. Natl. Acad. Sci.U.S.A.* **2009**, *106*, 8555–8560.
(29) Bhattacharya, S.; Hall, S. E.; Li, H.; Vaidehi, N. Ligand stabilized conformational states of human beta 2 adrenergic receptor: insight into G protein coupled receptor activation. *Biophys. J.* **2008**, *94*, 2027–2042.
(30) Dror, R. O.; Arlow, D. H.; Borhani, D. W.; Jensen, M. Ø.; Piana, S.; Shaw, D. E. Identification of two distinct inactive conformations of the beta2-adrenergic receptor reconciles structural and biochemical observations. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 4689–4694.
(31) Swaminath, G.; Xiang, Y.; Lee, T. W.; Steenhuis, J.; Parnot, C.; Kobilka, B. K. Sequential binding of agonists to the β2 adrenoceptor. *J. Biol. Chem.* **2004**, *279*, 686–691.
(32) Ambrosio, C.; Molinari, P.; Cotecchia, S.; Costa, T. Catechol-binding serines of beta(2)-adrenergic receptors control the equilibrium between active and inactive receptor states. *Mol. Pharmacol.* **2000**, *57*, 198–210.
(33) Nygaard, R.; Frimurer, T. M.; Holst, B.; Rosenkilde, M. M.; Schwartz, T. W. Ligand binding and micro-switches in 7TM receptor structures. *Trends Pharmacol. Sci.* **2009**, *30*, 249–259.
(34) Roth, C. B.; Hanson, M.; Stevens, R. Stabilization of the human β2-adrenergic receptor TM4−TM3−TM5 helix interface by mutagenesis of Glu122 3.41, a critical residue in 7TM structure. *J. Mol. Biol.* **2008**, *376*, 1305–1319.
(35) Han, D. S.; Wang, S. X.; Weinstien, H. Active state-like conformational elements in the b2-AR and a photoactivated intermédiate of rhodopsin identified by dynamic propierties of 7TMs. *Biochemistry* **2008**, *47*, 7317–7321.
(36) Huber, T.; Menon, S.; Sakmar, T. P. Structural basis for ligand binding and specificity in adrenergic receptors: implications for 7TM-targeted drug discovery. *Biochemistry* **2009**, *47*, 11013–11023.
(37) Wu, Z.; Thiriot, D.; Ruoho, A. E. Tyr199 in transmembrane domain 5 of the b2-adrenergic receptor interacts directly with the pharmacophore of a unique fluorenone-based antagonist. *Biochem. J.* **2001**, *354*, 485–491.
(38) Carmine, R.; Molinari, P.; Sbraccia, M.; Ambrosio, C.; Costa, T. "Induced-fit" mechanism for catecholamine binding to the β2-adrenergic receptor. *Mol. Pharmacol.* **2004**, *66*, 356–366.
(39) Chelikani, P.; Hornak, V.; Eilers, M; Reeves, P. J.; Smith, S. O.; RajBhandary, U. L.; Khorana, H. G. Role of group-conserved residues in the helical core of beta2-adrenergic receptor. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 7027–7032.

(40) Savarese, T. M.; Fraser, C. M. In vitro mutagenesis and the search for structure−function relationships among G protein-coupled receptors. *Biochem. J.* **1992**, *283*, 1–19.
(41) Wu, Z.; Thiriot, D.; Ruoho, A. E. Tyr199 in transmembrane domain 5 of the b2-adrenergic receptor interacts directly with the pharmacophore of a unique fluorenone-based antagonist. *Biochem. J.* **2001**, *354*, 485–491.
(42) Carmine, R.; Molinari, P.; Sbraccia, M.; Ambrosio, C.; Costa, T. "Induced-fit" mechanism for catecholamine binding to the β2-adrenergic receptor. *Mol. Pharmacol.* **2004**, *66*, 356–366.
(43) Strader, C. D.; Sigal, I. S.; Candelore, M. R.; Rands, E.; Hill, W. S.; Dixon, R. A. Conserved aspartic acid residues 79 and 113 of the beta-adrenergic receptor have different roles in receptor function. *J. Biol. Chem.* **1988**, *263*, 10267–10271.
(44) Green, S. A.; Cole, G.; Jacinto, M.; Innis, S.; Stephe, B.; Liggett, S. A polymorphism of the human beta 2-adrenergic receptor within the fourth transmembrane domain alters ligand binding and functional properties of the receptor. *J. Biol. Chem.* **1993**, *268*, 23116–23121.
(45) Ambrosio, C; Molinari, P; Fanelli, F; Chuman, Y; Sbraccia, M; Ugur, O.; Costa, T. Different structural requirements for the constitutive and the agonist-induced activities of the β2-adrenergic receptor. *J. Biol. Chem.* **2005**, *124*, 23464–23474.
(46) Strader, C. D. A single amino acid substitution in β-adrenergic receptor promotes partial agonist activity by antagonist. *J. Biol. Chem.* **1989**, *264*, 16470–16477.
(47) Samama, P.; Cotecchia, S.; Costa, T.; Lefkowitz, R. J. A mutation-induced activated state of the beta 2-adrenergic receptor. Extending the ternary complex model. *J. Biol. Chem.* **1993**, *268*, 4625–4636.
(48) Frielle, T.; Daniel, K. W.; Caron, M. G.; Lefkowitz, R. J. Structural basis of beta-adrenergic receptor subtype specificity studied with chimeric beta 1/beta 2-adrenergic receptors. *Proc. Nat. Acad. Sci. U.S.A.* **1988**, *85*, 9494–9498.
(49) Wieland, K.; Zuurmond, H. M.; Krasel, C.; Ijzerman, A. P.; Lohse, M. J. Involvement of Asn-293 in stereospecific agonist recognition and in activation of the beta 2-adrenergic receptor. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 9276–9281.
(50) Hannawacker, A; Krasel, C.; Lohse, M. J. Mutation of Asn293 to Asp in transmembrane helix VI abolishes agonist-induced but not constitutive activity of the 2-adrenergic receptor. *Mol. Pharmacol.* **2002**, *62*, 1431–1437.
(51) Kikkawa, H.; Isogaya, M.; Nagao, T.; Kurose, H. The role of the seventh transmembrane region in high affinity binding of a beta 2-selective agonist TA-2005. *Mol. Pharmacol.* **1998**, *53*, 128–134.
(52) Green, S. A.; Turki, J.; Innis, M.; Liggett, S. B. Amino-terminal polymorphisms of the human beta 2-adrenergic receptor impart distinct agonist-promoted regulatory properties. *Biochemistry* **1994**, *33*, 9414–9419.
(53) O'Dowd, B. F.; Hnatowich, M.; Regan, J. W.; Leader, W. M.; Caron, M. G.; Lefkowitz, R. J. Site-directed mutagenesis of the cytoplasmic domains of the human beta 2-adrenergic receptor. Localization of regions involved in G protein-receptor coupling. *J. Biol. Chem.* **1998**, *263*, 15985–15992.
(54) Ahles, A.; Engelhardt, S. Polymorphisms determine beta-adrenoceptor conformation: implications for cardiovascular disease and therapy. *Trends Pharmacol. Sci.* **2009**, *30*, 188–193.
(55) Turki, J.; Pak, J.; Green, S. A.; Martin, R. J.; Liggett, S. B. Genetic polymorphisms of the beta 2-adrenergic receptor in nocturnal and non nocturnal asthma. Evidence that Gly16 correlates with the nocturnal phenotype. *J. Clin. Invest.* **1995**, *195*, 1635–1641.
(56) Fraser, C. M. Site-directed mutagenesis of β-adrenergic receptor. Identification of conserved cysteine residues that independently affect ligand binding and receptor activation. *J. Biol. Chem.* **1989**, *264*, 9266–9270.
(57) Ramos-Jimenez, J.; Soria-Jasso, L. E.; López-Colombo, A.; Reyes-Esparza, J. A.; Camacho, J.; Arias-Montaño, J. A. Histamine augments b2-adrenoceptor-induced cyclic AMP accumulation in human prostate cáncer cells DU-145 independently of known histamine receptors. *Biochem. Pharmacol.* **2007**, *73*, 814–823.
(58) Kaya, A. I.; Ugur, O.; Oner, S. S.; Bastepe, M.; Onaran, H. O. Coupling of beta2 adrenoceptor to XL-alpha-s and G-alpha-s: a new insight into ligand-induced G protein activation. *J. Pharmacol. Exp. Ther.* **2009**, *329*, 350–359.
(59) Espinoza-Fonseca, L. M.; Trujillo-Ferrara, J. G. Identification of multiple allosteric sites on the M1 muscarinic acetylcholine receptor. *FEBS Lett.* **2005**, *579*, 6726–6732.
(60) Johnston, C. A.; Kimple, A. J.; Guiguere, P. M.; Siderovski, D. P. Structure of the parathyroid hormone receptor C terminus bound to the G-protein dimer beta1gamma2. *Structure* **2008**, *16*, 1086–1094.
(61) Scheerer, P.; Park, J. H.; Hildebrand, P. W.; Kim, Y. J.; Krauss, N.; Choe, H. W.; Hofmann, K. P.; Ernst, O. P. Crystal structure of opsin in its G-protein-interacting conformation. *Nature* **2008**, *455*, 497–502.
(62) Yao, X. J.; Vélez, R. G.; Whorton, M. R.; Rasmussen, S. G.; DeVree, B. T.; Deupi, X.; Sunahara, R. K.; Kobilka, B. The effect of ligand efficacy on the formation and stability of a 7TM-G protein complex. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 9501–9506.
(63) Salahpour, A.; Angers, S.; Mercier, J. F.; Lagacé, M.; Marullo, S.; Bouvier, M. Homodimeriztion of the beta2-adrenergic receptor as a prerequisite for cell surface targeting. *J. Biol. Chem.* **2004**, *279*, 33390–33397.
(64) Whorton, M. R.; Bokoch, M. P.; Rasmussen, S. G.; Huang, B.; Zare, R. N.; Kobilka, B.; Sunahara, R. K. A monomeric G protein-coupled receptor isolated in a high-density lipoprotein particle efficiently activates its G protein. *Proc. Natl. Acad. Sci U.S.A.* **2007**, *104*, 7682–7687.
(65) Reggio, P. H. Computational methods in drug design: modeling G protein-coupled receptor monomers, dimers and oligomers. *AAPS J.* **2006**, *8*, E322–E336.
(66) González-Maeso, J.; Ang, R. L.; Yuen, T.; Chan, P.; Weisstaub, N. V.; López-Giménez, J. F.; Zhou, M.; Okawa, Y.; Callado, L. F.; Milligan, G.; Gingrich, J. A.; Filizola, M.; Meana, J. J.; Sealfon, S. C. Identification of a serotonin/glutamate receptor complex implicated in psychosis. *Nature* **2008**, *452*, 93–97.
(67) Topiol, S.; Sabio, M. Use of the X-ray structure of the beta2-adrenergic receptor for drug discovery. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1598–1602.
(68) Sabio, M.; Jones, K.; Topiol, S. Use of the X-ray structure of the beta2-adrenergic receptor for drug discovery. Part 2: Identification of active compounds. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5391–5395.
(69) Costanzi, S. On the applicability of 7TM homology models to computer-aided drug discovery: a comparison between in silico and crystal structures of the b2-adrenergic receptor. *J. Med. Chem.* **2008**, *51*, 2907–2914.
(70) De Graaf, C.; Rognan, D. Selective structure-based virtual screening for full and partial agonists of the b2 adrenergic receptor. *J. Med. Chem.* **2008**, *51*, 4978–4985.
(71) Kolb, P.; Rosenbaum, D. M.; Irwin, J. J.; Fung, J. J.; Kobilka, B. K.; Shoichet, B. K. Structure-based discovery of beta2-adrenergic receptor ligands. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 6843–6848.
(72) Soriano-Ursúa, M. A.; Valencia-Hernández, I.; Arellano-Mendoza, M. G.; Correa-Basurto, J.; Trujillo-Ferrara, J. Synthesis, pharmacological and in silico evaluation of 1-(4-di-hydroxy-3,5-dioxa-4-borabicyclo[4.4.0]deca-7,9,11-trien-9-yl)-2-(*tert*-butylamino)-ethanol, a compound designed to act as a beta2 adrenoceptor agonist. *Eur. J. Med. Chem.* **2009**, *44*, 2840–2846.
(73) Soudjin, W.; van Wijngaarden, I.; IJzerman, A. P. Stereoselectivity of drug−receptor interactions. *IDrugs* **2003**, *6*, 43–56.
(74) Abderhalden, E.; Muller, F. Uber das verhalten des blutdruckes nach intravenoser einfuhrung von 1-, d und dl-suprarenin. *Hoppe-Seyler's Z. Physiol. Chem.* **1908**, *58*, 185–188.
(75) Cushny, A. R. The action of optical isomers. III. Adrenalin. *J. Physiol.* **1908**, *37*, 130–138.
(76) Coupez, B.; Lewis, R. A. Docking and scoring: theoretically easy, practically imposible? *Curr. Med. Chem.* **2006**, *13*, 2995–3003.
(77) Page, C. P.; Morley, J. Contrasting properties of albuterol stereoisomers. *J. Allergy Clin. Immunol.* **1999**, *104*, S31–S41.
(78) Quinn, C. The cost effectiveness of levalbuterol versus racemic albuterol. *Am. J. Managed Care* **2007**, *10*, S153–S157.
(79) Soriano-Ursúa, M. A.; Trujillo-Ferrara, J. G.; Correa-Basurto, J. Docking studies on a refined human β_2 adrenoceptor model yield theoretical affinity values in function with experimental values for *R*-ligands, but not for *S*-antagonists. *J. Mol. Model.* **2009**, DOI 10.1007/s00894-009-0563-5.
(80) Soriano-Ursúa, M. A.; Trujillo-Ferrara, J.; Correa-Basurto, J. Homology modeling and flex-ligand docking studies on the guinea pig β2 adrenoceptor: structural and experimental similarities/differences with the human β2. *J. Mol. Model.* **2009**, *15*, 1203–1211.
(81) Richter, W.; Day, P.; Agrawal, R.; Bruss, M. D.; Granier, S.; Wang, Y. L.; Rasmussen, S. G.; Horner, K.; Wang, P.; Lei, T.; Patterson, A. J.; Kobilka, B.; Conti, M. Signaling from β1- and β2-adrenergic receptors is defined by differential interactions with PDE4. *EMBO J.* **2008**, *27*, 384–393.
(82) Ishtiaq, A.; Hoessli, D.; Gupta, R.; Walker-Nasir, E.; Rafik, S. M.; Choudhary, M. I.; Shakoori, A. R.; Nasir-ud-Din In silico determination of intracellular glycosylation and phosphorylation sites in human selectins: implications for biological function. *J. Cell. Biochem.* **2007**, *100*, 1558–1572.
(83) Bohne-Lang, A.; von der Lieth, C. W. GlyProt: in silico glycosylation of proteins. *Nucleic Acids Res.* **2005**, *33*, W214–W219.

(84) Xue, Y.; Zhou, F.; Zhu, M.; Ahmed, K.; Chen, G.; Yao, X. GPS: a comprehensive www server for phosporylation sites prediction. *Nucleic Acids Res*. **2005**, *33*, W184–W187.
(85) Luttrell, L. M.; Lefkowitz, R. J. The role of beta-arrestins in the termination and transduction of G-protein-coupled receptor signals. *J. Cell Sci*. **2002**, *115*, 455–465.
(86) Azzi, M.; Charest, P. G.; Angers, S.; Rousseau, G.; Kohout, T.; Bouvier, M.; Piñeyro, G. Beta-arrestin-mediated activation of MAPK by inverse agonists reveals distinct active conformations for 7TM. *Proc. Natl. Acad. Sci. U.S.A*. **2003**, *100*, 11406–11411.
(87) Humphrey, W.; Dalke, A.; Schulten, K. VMD: visual molecular dynamics. *J. Mol. Graphics* **1996**, *14*, 33–38.