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Analysis of Full and Partial Agonists Binding to β_2-Adrenergic Receptor Suggests a Role of Transmembrane Helix V in Agonist-Specific Conformational Changes

Vsevolod Katritch[1], **Kimberly A. Reynolds**[1], **Vadim Cherezov**[1], **Michael A. Hanson**[1], **Christopher B. Roth**[1], **Mark Yeager**[2,3], and **Ruben Abagyan**[1]

1*Department of Molecular Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037 USA*

2*Department of Cell Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037 USA*

3*Department of Molecular Physiology and Biological Physics, University of Virginia Health System, PO Box 800736, Charlottesville, VA 22908-0736*

Abstract

The 2.4 Å crystal structure of the β_2-adrenergic receptor (β_2AR) in complex with the high-affinity inverse agonist (-)-carazolol provides a detailed structural framework for the analysis of ligand recognition by adrenergic receptors. Insights into agonist binding and the corresponding conformational changes triggering GPCR activation mechanism are of special interest. Here we show that while the carazolol pocket captured in the β_2AR crystal structure accommodates (-)-isoproterenol and other agonists without steric clashes, a finite movement of the flexible extracellular part of TM-V helix (TM-Ve) obtained by receptor optimization in the presence of docked ligand can further improve the calculated binding affinities for agonist compounds. Tilting of TM-Ve towards the receptor axis provides a more complete description of polar receptor/ligand interactions for full and partial agonists, by enabling optimal engagement of agonists with two experimentally identified anchor sites, formed by Asp113/Asn312 and Ser203/Ser204/Ser207 side chains. Further, receptor models incorporating a flexible TM-V backbone allow reliable prediction of binding affinities for a set of diverse ligands, suggesting potential utility of this approach to design of effective and subtype-specific agonists for adrenergic receptors. Systematic differences in capacity of partial, full and inverse agonists to induce TM-V helix tilt in the β_2AR model suggest potential role of TM-V as a conformational "rheostat" involved in the whole spectrum of β_2AR responses to small molecule signals.

Keywords

Adrenergic; GPCR; G-protein; agonist; antagonist; activation; flexible docking; binding energy; conformational change

Corresponding authors: Vsevolod Katritch, Department of Molecular Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037 USA; Tel: +1 858-784-8591; Fax: +1 858-784-8299; E-mail: katritch@scripps.edu & Ruben Abagyan, Department of Molecular Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037 USA; Tel: +1 858-784-8595; Fax: +1 858-784-8299; E-mail: abagyan@scripps.edu.

Supplementary Materials

Supplementary materials include PDB coordinates of energy optimized β_2AR-lignad models, listed in Table 2.

Introduction

A diverse family of more than 800 heptahelical G-protein coupled receptors (GPCRs) plays a critical role in recognition of neurotransmitters, cytokines, hormones, light and other extracellular signals and comprises targets for about a half of existing drugs (Lagerstrom and Schioth 2008; Tyndall and Sandilya 2005). While detailed knowledge of ligand-receptor interactions would be instrumental in design of new and improved clinical candidates, the insight into spatial structure of GPCR has been limited to *ab initio* models (Goddard and Abrol 2007) or models based on rhodopsin crystal structure (Palczewski and others 2000). The first high resolution crystal structure of a GPCR with diffusible ligand has been determined recently for β_2-adrenergic receptor (β_2AR) (Cherezov and others 2007; Rosenbaum and others 2007) revealing in atomic detail the binding interactions for an inverse agonist (-)-carazolol (PDB code 2RH1). This structure, followed by other co-crystals of β_2AR (Hanson and others 2008b) and β_1AR (Warne and others 2008) with the antagonists (-)-timolol (PDB code 3D4S) and (-)-cyanopindolol (PDB code 2VT4) respectively, represents a leap forward in the understanding of the inactive state of adrenergic GPCRs. At the same time, its potential utility as a structural template for the prediction of agonist binding conformations and relative affinities requires careful assessment (Kobilka and Schertler 2008). Indeed, the binding of agonists, especially full agonists, is expected to be accompanied by some conformational changes in the β_2AR, which are reflected in highly synergistic contributions of the agonist functional groups in the receptor binding and activation (Del Carmine and others 2004; Liapakis and others 2000; Liapakis and others 2004). Kinetics studies (Kobilka 2007; Swaminath and others 2004) also support existence of early intermediate states in the β_2AR induced by agonist recognition and binding. Fast initial adjustments of the receptor to an agonists binding may be followed by slower downstream changes potentially involving a rotamer “toggle switch” (Shi and others 2002) and movements of TM domains (Schwartz and others 2006)), leading to GPCR activation.

Extensive mutation analysis has established that a full β_2AR agonist isoproterenol and related catecholamine compounds engage specific amino acid side chains of transmembrane (TM) helical domains III, V, VI and VII (Hannawacker and others 2002; Kobilka 2007; Liapakis and others 2000; Sato and others 1999; Strader and others 1989; Xhaard and others 2006) in a pocket that largely overlaps with the binding pocket of carazolol in the β_2AR co-crystal (Cherezov and others 2007). Some of these receptor interactions are common for agonists and antagonists alike, while the others are specific to agonists only. Thus, most β-adrenergic agonists and antagonists have a positively charged amine or ethanolamine groups (“tails”) which has been shown to interact with the receptor anchor site formed by Asp113$^{3.32}$ and Asn312$^{7.39}$ side chains (Strader and others 1988; Suryanarayana and Kobilka 1993) (superscript numbering according to ref.(Ballesteros and Weinstein 1995)). However, a strong polar interaction network between the catechol functional group (“head”) and another anchor site formed by serines Ser203$^{5.42}$, Ser204$^{5.43}$, Ser207$^{5.46}$ on TM-V is specific for agonists only; this network is most extensive for “full” agonists, conferring maximal activity of the β_2AR. Both “tail” and “head” anchor interactions are critical for agonist specific β_2AR activation, as removal of any of the corresponding polar moieties in the agonists or in the receptor has been shown to dramatically reduce agonist activity (Ambrosio and others 2000; Liapakis and others 2004; Strader and others 1989). Initial analysis of agonist binding geometry in ref. (Rosenbaum and others 2007) however, shows that when ethanolamine tail of isoproterenol is superimposed onto the corresponding atoms of carazolol in the β_2AR crystal structure, the cathechol hydroxyls of the agonist are too distant from the TM-V serines to form the anchor hydrogen bonds. These simple geometry considerations suggest that some adjustments in the binding pocket, and specifically in the TM5 domain, may be required to achieve optimal agonist binding (V.K., V.C, M.A.H., C.B.R and R.A. unpublished)(Warne and others 2008).

Herein we report the results of more rigorous, energy-based conformational modeling of representative antagonists and agonists (Figure 1), which supports agonist-specific changes in the β_2AR binding pocket. The fully flexible ligands were docked into the pocket using three distinct approaches, in which β_2AR was represented as 1) a rigid receptor derived from the pdb-deposited coordinates of the heavy atoms (2RH1), 2) a receptor with flexible side chains in the binding pocket, and 3) a receptor with flexibility in side chain conformation and limited flexibility in the protein backbone of the TM-V helix. Using the first and the second approaches we demonstrate that the binding pocket conformation of the carazolol-β_2AR complex is sterically compatible with isoptorerenol and other full agonists, and that binding can be somewhat improved through rotamer changes in Ser203$^{5.42}$, Ser204$^{5.43}$ and Ser207$^{5.46}$ side chains. However, optimal engagement of both experimentally determined ligand/receptor anchor interactions cannot be achieved without additional flexibility in the β_2AR backbone, as in the third approach. The predicted backbone movements comprise a finite (~2Å) "inward" tilt of the mobile extracellular segment of TM-V α-helix (TM-Ve), which improves the calculated binding affinity for full agonists as much as ~1000 fold. Further, incorporation of a flexible TM-V backbone allows consistent prediction of experimental binding affinities for a diverse set of β_2AR ligands from full agonists to inverse agonists. Interestingly, the optimal TM-Ve tilt and the accompanying improvement in calculated binding affinity are less pronounced for partial agonists; the movement is completely abolished for the antagonists and inverse agonists studied here. This strongly differentiating response to agonist and antagonist binding suggests a potential role of TM-Ve tilt as a regulator in the initial stages of the signal transduction mechanism in β_2AR and closely related aminergic receptors.

Results

Energy based refinement of the β_2AR model for antagonists/inverse agonist binding

To establish the suitability of the β_2AR crystal structure for the prediction of ligand conformations, we started with docking of (-)-carazolol and several other antagonists/inverse agonists into "rigid" and "flexible side chain" models of the receptor. As shown in Figure 2A, redocking of (-)-carazolol into the *rigid model* of β_2AR (PDB code 2rh1) consistently reproduced the X-ray coordinates of the ligand heavy atoms with RMSD ~ 0.3 Å. Similarly, docking into the β_2AR model with flexible side chains resulted in (-)-carazolol geometry nearly identical to the crystal structure (RMSD ~ 0.25 Å). The β_2AR binding pocket conformation was also preserved, with the exception of two serine side chains in the TM-V domain (Figure 2B). Most notably, the energy-optimized rotamer of Ser203$^{5.42}$ side chain significantly improved hydrogen bonding to carbazole with N-O distance reduced from 3.3 Å to 2.7 Å, and also allowed formation of a hydrogen bond to Tyr199$^{5.38}$ backbone carbonyl. The latter type of Ser/Thr side chain to main chain H-bonds (Oγ(i)--O(i-4)), frequently found in the middle of α-helices is considered as an important protein stabilizing interaction (Ballesteros and others 2000; Eswar and Ramakrishnan 2000). The predicted rotamer of another, Ser204$^{5.43}$ side chain supported an improved geometry of hydrogen bonding with Ala200$^{5.39}$ backbone. Thus, this model suggests a possibility of hydrogen bond pairing between Ser204$^{5.43}$ hydroxyl and Asn293$^{6.55}$ side chain nitrogen, albeit with a marginal O-N distance (3.3.Å). The optimized rotamers for both Ser203$^{5.42}$ and Ser204$^{5.43}$ side chains are consistent with the electron densities observed for the β_2AR-carazolo crystal structure (Cherezov and others 2007). Moreover, the predicted configuration of Ser204$^{5.43}$ side chain and its interaction network can be found in the β_2AR crystal structure with timolol (PDB code 3D4S)..

Docking of other antagonists/inverse agonists in the β_2AR flexible side chain model also resulted in ligand conformations similar to (-)-carazolol, with some notable variations. Thus, for the (+)-carazolol stereoisomer model, the switch from (-) to (+) stereoisomer of the ethanolamine "tail" resulted in partial loss of hydrogen bonding network with Asp113$^{3.32}$/

Asn312$^{7.39}$ anchor site, which is in line with a substantial (~20 fold) stereo-selectivity towards (-)-carazolol. (All-atom models for these and other ligands can be found in Supplementary Materials in pdb format)

Full agonist (-)-isoproterenol binding to the β_2AR receptor

β_2AR agonists are expected to bind to different conformational states of receptor than antagonists or inverse agonists. Therefore, one of the questions we need to answer is whether binding of catecholamine agonists such as (-)-isoproterenol is sterically compatible with the carazolol-bound conformational state of the β_2AR captured in the crystal structure (Cherezov and others 2007). The second question is whether specific changes in β_2AR model side chain or backbone conformation can be identified that substantially improve agonist binding affinity and its interactions with known anchor sites.

(-)-Isoproterenol in the rigid and flexible side chain β_2AR models—Docking into the *rigid model* of the β_2AR consistently yielded a single low energy conformation of (-)-isoproterenol (Figure 3A), where the ligand fitted into the carazolol-binding pocket without any apparent steric clashes. Moreover, a detailed analysis of (-)-isoproterenol interactions shows energetically favorable contribution to the ligand binding energy for all 14 contact residues in the "rigid" β_2AR pocket. At the same time the model in Figure 3A shows that when the ethanolamine "tail" of (-)-isoproterenol is anchored at the Asp113$^{3.32}$/Asn312$^{7.39}$ anchor site, its catechol "head" is unable to reach hydroxyls of Ser203$^{5.42}$, Ser204$^{5.43}$, and Ser207$^{5.46}$ in TM-V. This is also consistent with the observation by Rosenbaum et al based on a simple superposition of ethanolamine "tails" of carazolol and isoproterenol in the β_2AR binding pocket (Rosenbaum and others 2007).

Docking of (-)-isoproterenol in the *flexible side chain model* of β_2AR provides more rigorous evaluation of agonist interactions with the receptor anchor sites (Figures 3B and 3C). The results show that although rotameric adjustments in the TM-V serines and other residues in the vicinity of the binding site substantially improve binding of (-)-isoproterenol, the formation of optimal hydrogen bonding interactions between the ligand and both interaction sites remains physically impossible. Interestingly, instead of one global energy minimum, the docking into the flexible side chain model of the β_2AR resulted in two clusters of (-)-isoproterenol conformations. The majority of docking runs (15 out of 20) yielded a low energy pose shown in Figure 3B, where (-)-isoproterenol is shifted towards TM-V, allowing the catechol "head" to form a strong hydrogen bond network with the TM-V serines. However, this position of the ligand forced a rearrangement and partial loss of the ethanolamine group interactions with the Asp113$^{3.32}$-Asn312$^{7.39}$ site. In contrast, in the remaining five runs of the docking procedure the complex fully retained the interaction network for the isoproterenol ethanolamine group with the Asp113$^{3.32}$/Asn312$^{7.39}$ anchor, while stretching the ligand catechol group toward the TM-V serines (Figure 3C). Despite the agonist being almost perfectly aligned between the anchor sites, the distances between the catechol and the hydroxyls of TM-V serines in this conformation were still too great to allow formation of a hydrogen bond network. This analysis demonstrates that side chain flexibility alone does not provide enough adjustment in the β_2AR model to satisfy the experimental constraints associated with the binding of full agonists to the two anchor sites simultaneously. Therefore, some changes in the protein backbone conformation, as compared to β_2AR-carazolol complex, are required to engage both functional groups of the full agonist.

TM-V helix tilt upon (-)-isoproterenol binding—Because one of the two critical anchor sites for agonist binding is located on the extracellular part of TM-V, the optimal binding arrangement may be achieved by movement of the TM-V domain towards Asp113$^{3.32}$/Asn312$^{7.39}$ on TM-III/TM-VII. The extracellular α-helical segment of TM-V (residues

197-204, further referred to as "TM-Ve") is flanked by a relatively flexible loop EL2 and a helical "bulge" above the highly conserved Pro211$^{5.50}$ residue, which suggests an enhanced mobility of TM-Ve. The possibility of an inward movement of TM-Ve is further supported by previous studies reporting conformational flexibility in this region (Javitch and others 2002; Javitch and others 1995) (Chelikani and others 2007). In contrast, a significant movement of helices TM-III/TM-VII towards TM-V appears to be less likely, as TM-III is known to form a tight folding core of β_2AR together with helices TM-I/TM-II/TM-IV (Chelikani and others 2007).

To analyze a possibility of TM-Ve movement, we developed a conformational *model of the β_2AR with backbone flexibility* introduced in a portion of the extracellular loop EL2 (residues 191-196) and in the proline-induced bulge of TM-V (residues 205-210), as described in Methods. The backbone flexibility, combined with flexibility of side chains in the proximity of the binding site, allow for substantial motility of the TM-Ve segment. Docking of (-)-isoproterenol into this β_2AR model consistently resulted in a receptor conformation with an inward tilt of the TM-Ve helix, as shown in Figure 4. The TM-Ve tilt corresponds to an approximately 2 Å shift of the TM-V anchor site toward helices TM-III/TM-VII, as measured for Ser203$^{5.42}$ Cα atom. Analysis of H-bonding distances in this model shows that such TM-Ve tilt is sufficient to bring the two anchor sites of the binding pocket within optimal distance for interactions with both the catechol and ethanolamine groups of isoproterenol simultaneously **(see** Table 1). The move of the TM-V extracellular domain toward the ligand binding pocket does not result in any serious steric overlaps with other helices of the protein, and is accompanied by only minor adjustment of the flexible side chains on the helix-helix interfaces. The resulting improvement in (-)-isoproterenol binding affinity upon TM-Ve tilt was predicted to be as high as 3 pK_d, i.e. about 1000-fold.

The conformational model of the β_2AR-isoproterenol complex consistently reproduced H-bonding of catechol meta-OH with Ser203$^{5.42}$ and para-OH with Ser207$^{5.46}$ side chains, as previously derived from mutation studies (Liapakis and others 2000; Strader and others 1989). At the same time, the model predicts an alternative interaction network for Ser204$^{5.43}$ side chain, where instead of directly engaging (-)-isoproterenol meta-OH (Strader and others 1989), the Ser204$^{5.43}$ hydroxyl is involved in two intramolecular hydrogen bonds. This intramolecular network, also involving the Tyr308$^{7.35}$ and Asn293$^{6.55}$ side chains and Ala200$^{5.39}$ main chain (e.g. Figure 2C) can be found in the crystal structure of the β_2AR-timolol complex (Hanson and others 2008a). The modeling results also suggested that (-)-isoproterenol binding and the corresponding TM-Ve tilt can significantly improve this interaction network by reducing the donor-acceptor distance in Asn293$^{6.55}$-Ser204$^{5.43}$ hydrogen bond from 3.3 to 2.6 Å (compare Figures 2C and 4).

TM-V movement and binding affinity predictions for a diverse set of β_2AR agonists

The β_2AR conformational models, both with rigid and with flexible TM-V backbone, were applied to predict binding poses and affinities for a set of diverse β_2AR agonists. Interestingly, TM-Ve flexibility produced a marked improvement in the calculated binding affinities for agonist compounds while having little effect on antagonist binding affinities (results summarized in Table 2). Full agonists were all predicted to have 2 - 3 pK_d (100-1000 fold) improved binding affinities upon a significant (1.6 - 2.2 Å) inward shift of the TM-Ve anchor site. In contrast, for the antagonist and the inverse agonist complexes the predicted position of TM-Ve remains close to the β_2AR-carazolol crystal structure with little change in the calculated binding affinity. For partial agonists the amount of TM-Ve tilt and its effect on binding affinities are intermediate, with the smallest affinity difference predicted for MAPE at 0.5 pK_d.

The correlation of predicted and experimental binding affinities for the diverse set of full, partial and inverse agonists/antagonists is illustrated in Figure 5A. Note, that while the model

with the *rigid TM-V backbone* has rather poor accuracy of the binding affinity predictions (R^2~0.75 and RMSD~ 1.3 pK_d), the *flexible TM-V model* improves accuracy to R^2=0.89 and RMSD ~ 0.7 pK_d.

For carazolol and a few representative agonists we also performed a more detailed conformational analysis, by evaluating ligand binding in β_2AR models with a range of fixed positions of the TM-Ve helix. The resulting dependencies of the binding affinity as a function of the TM-Ve shift in Figure 5B are in good agreement with the "single point" data n Figure 5A. While calculated affinity of (-)-carazolol steadily decreases with the TM-V anchor shifting inward, the affinity curves for agonists show a range of improved affinity values. Note, that full agonists (-)-isoproterenol and (-)-epinephrine have similar shapes of the affinity curve with maxima at about 1.8-2.2 Å shift of the TM-Ve anchor, this distinct shape may reflect engagement of individual anchor residues in the binding site. Partial agonist dopamine has a relatively flat affinity profile between 0.9 and 1.4 Å shifts of the TM-Ve, while MAPE affinity is almost independent from the TM-Ve movements in the whole range studied. Further details of the 3D conformations and affinities for several representative full and partial agonists are presented in the following sections (the 3D models of these ligand-β_2AR complexes can also be found in Supplementary Materials).

Full agonist binding—The endogenous adrenergic agonists (-)-epinephrine and (-)-norepinephrine are close chemical analogues of isoproterenol, with either a methyl group or a proton, respectively, at the tip of the ethanolamine "tail". Both compounds promote full activation of the receptor, although with higher EC_{50} values than isoproterenol. Based on biochemical data, both ligands are expected to interact with the same anchor sites of β_2AR as isoproterenol (Liapakis and others 2004). Indeed, docking of (-)-epinephrine and (-)-norepinephrine in the β_2AR model with flexible TM-V backbone consistently predicts binding poses and interaction patterns very similar to those of (-)-isoproterenol, with the corresponding heavy atom RMSDs of 0.2 Å and 0.5 Å respectively. A slightly smaller shift of the TM-V anchor site found for (-)-norepinephrine complex, ~1.6 Å, can be attributed to its smaller "tail" which allows more adjustment in the position of the anchor side chains Asp113$^{3.32}$ and Asn312$^{7.39}$. Also, lack of hydrophobic interaction with the aromatic system of Trp109$^{3.28}$ is apparently responsible for reduced affinity of these ligands as compared to isoproterenol; this effect was quantitatively predicted by the corresponding models, as shown in Table 2.

Partial agonist binding—Dopamine, salbutamol and MAPE (halostachine) are partial agonists of the β_2AR, and may interact differently with the receptor, perhaps stabilizing different conformational states as compared to the full agonists (Baker 2005; Kikkawa and others 1997; Seifert and others 2001; Swaminath and others 2005). As illustrated in Figure 6A, *dopamine* is predicted to bind to the same binding pocket as isoproterenol, forming hydrogen bond networks with TM-V serines through its catechol head. In the absence of the alkyl-hydroxyl moiety, the ligand's tail binds only through the amino group interactions with the Asp113$^{3.32}$/Asn312$^{7.39}$ anchor. The spatial position of dopamine amino tail in the anchor site is not as well defined as for ethanolamine tail common for other ligands. This difference is reflected in a wider peak of the affinity profile in Figure 5B, smaller TM-Ve movement and smaller energy gain, as compared to (-)-isoproterenol (see Table 2).

Salbutamol docking to the flexible TM-V model of the β_2AR (Figure 6B) indicates that the replacement of the ligand's meta-OH with hydroxymethyl group can dramatically modify its interaction pattern with TM-V serines. Thus, the model suggests that in contrast to catechol meta-OH, the meta-hydroxymethyl group of salbutamol may serve as a hydrogen bond acceptor for both Ser203$^{5.42}$ and Ser204$^{5.43}$. The formation of these two H-bonds, though, is offset by the loss of two intramolecular side-chain to main-chain H-bonds, and therefore it is unlikely to contribute significantly to the overall ligand binding affinity. The modified aromatic

head of salbutamol also allows an adjustment of the molecule and its ethanolamine tail position toward the TM-III/TM-VII anchor. This, in turn results in a smaller optimal shift of TM-Ve anchor site (0.7 Å) as compared to the value predicted for isoproterenol-bound β_2AR. A close similarity of salbutamol to isoproterenol and the observed consistency of the docking poses suggest that salbutamol can occupy the same pocket as the other agonists in our study. The previously observed non-competitive binding of catechol molecules to salbutamol-β_2AR complex (Kobilka 2007) can possibly be explained by an alternative binding pose of the catechol itself, which is a small and non-specific compound.

MAPE is a partial β_2AR agonist lacking both hydroxyls in the aromatic "head" of the molecule, and thus unable to form any hydrogen bonds with TM-V serines. Though the MAPE binding pose in the model in Figure 6C leaves a space for TM-V inward tilt, the corresponding gain in ligand binding affinity was predicted to be small (>0.5 pK_d), as compared to ~2.7 pK_d for (-)-isoproterenol (see Figure 5B and Table 2).

Binding and selectivity of a non-catechol full agonist TA-2005—The above analysis was performed for a representative set of full and partial agonists of the β_2AR with relatively broad specificity across the β-adrenergic family. However, the molecular basis of agonist selectivity for the β-adrenergic receptors is of special interest for clinical applications, where β_1AR antagonists ("β-blockers") are widely used for treatment of heart disease and β_2AR agonists are indicated for asthma (Baker 2005). A comparison of residues in the β_2AR binding pocket with the equivalent positions in the other members of the β-adrenergic family reveals that the residues predicted to interact with the endogenous agonists are highly conserved. The one non-conserved residue, Tyr308$^{7.35}$ (Phe359 in β_1AR), has been previously implicated in the high binding selectivity of the non-catechol full agonist TA-2005 for the β_2AR (Kikkawa and others 1997; Kikkawa and others 1998).

To analyze the basis for TA-2005 specificity at the structural level, we performed docking of the ligand to β_2AR in the framework of the flexible TM-V backbone model described above. The lowest energy conformation of the complex (Figure 7) has a hydrogen bond between the oxygen of the *p*-methoxyphenyl group of TA-2005 and Tyr308$^{7.35}$, in addition to the anchor interactions with Asp113$^{3.32}$/Asn312$^{7.39}$ and Ser203$^{5.42}$/Ser207$^{5.46}$/Ser204$^{5.43}$ sites similar to those predicted for isoproterenol. The model suggests that 8-hydroxy-carbostyril functional "head" can be as effective as the catechol moiety in its interaction with TM-V serines, and that optimal binding of TA-2005 requires a similar shift of the TM-V anchor site (2.2 Å) as for (-)-isoproterenol in the Figure 4 model.

The described model also provides a reasonable explanation of TA-2005 selectivity to β_2AR, predicting approximately 1 pK_d affinity drop for Y308F and Y308A mutations, as shown in Table 2 (Kikkawa and others 1998). Although other mechanisms can be also involved in selectivity for this and other β-adrenergic agonists, this example suggests a potential utility of the model in structure-based discovery of new subtype specific β-adrenergic ligands.

Discussion

The high resolution crystal structure of the β_2AR complex with inverse agonist (-)-carazolol (Cherezov and others 2007) provides a solid template for analysis of the β_2AR interactions with different classes of ligands. Although largely consistent with previous knowledge, the results of structure-based modeling of (-)-isoproterenol and other agonists suggests some new details of binding to the β_2AR and associated conformational changes in the receptor.

Ethanolamine tail interactions with Asp113$^{3.32}$ and Asn312$^{7.39}$ anchor site

The crystal structure of the β_2AR-carazolol complex (Cherezov and others 2007) reveals the geometry of the ethanolamine tail anchor site (see Figure 2 and Table 1), which involves both N+ and β-OH groups of the ligand and Asp113$^{3.32}$ and Asn312$^{7.39}$ side chains of the β_2AR, and also a stabilizing Asp113$^{3.32}$-Tyr316$^{7.43}$ intramolecular H-bond. An exceptional strength of this polar interaction network suggests that it may be preserved for agonists as well, and indeed, we consistently found a very similar ethanolamine tail conformation for agonists in all rigid and flexible types of β_2AR models (Figures 3AC, 4, 6B,C, 7).

While the salt bridge between Asp113$^{3.32}$ and N+ is well established as a key anchor for both agonists and antagonists binding (Strader and others 1988), the details of β-OH - Asn312$^{7.39}$ interaction were poorly understood. Some models suggested that another asparagine side chain, Asn293$^{6.55}$ forms a hydrogen bond with the β-OH group of agonists, probably resulting in a β-OH "up" orientation towards the extracellular end of the receptor (Hannawacker and others 2002; Wieland and others 1996). The rationale for such models was based on mutation data (Del Carmine and others 2004; Del Carmine and others 2002), which implicated Asn293$^{6.55}$ in binding affinity of catecholamine agonists.

At the same time, biochemical data from ref (Suryanarayana and Kobilka 1993), support involvement of Asn312$^{7.39}$ side chain in β_2AR H-bonding to both agonists and antagonists, because N312A mutation was found to be responsible for a ~100-fold (~2pK_d) reduction of the binding affinity for both types of ligands. The β_2AR crystal structure with carazolol and our β_2AR models with agonists suggest a fully buried β-OH "down" conformation, involved in at least two hydrogen bonds with both Asp113$^{3.32}$ and Asn312$^{7.39}$ side chains, but not Asn293$^{6.55}$ side chain located more than 10Å away. A switch of β-OH from "down" to "up" position for agonist would result in a loss of two strong buried H-bonds in exchange for a single solvent exposed H-bond to Asn293$^{6.55}$, which is very unfavorable.

The results of agonist-β_2AR modeling here support alternative mechanisms of Asn293$^{6.55}$ side chain contribution to agonist binding affinity and stereospecificity. One of these mechanisms is illustrated by the model in Figure 4, which shows formation of a hydrogen bond between Asn293$^{6.55}$ and the catechol meta-OH of (-)-isoproterenol. Another indirect contribution to agonist affinity may come from participation of the Asn293$^{6.55}$ side chain in a stabilizing intramolecular H-bonding network with Ser204$^{5.43}$ and Tyr308$^{7.35}$ side chains (see Figure 4 and discussion of TM-V serines below).

Aromatic ring interactions with the hydrophobic patch of the β_2AR pocket

Interaction of the ligand aromatic "head" with the hydrophobic patch in the middle part of the β_2AR binding pocket is another interaction contributing to β_2AR agonists, antagonists and inverse agonist binding. In the β_2AR crystal structure with carazolol (Cherezov and others 2007), the hydrophobic contacts include Trp286$^{6.48}$, Phe290$^{6.52}$, Val114$^{3.33}$, Val117$^{3.36}$ - mostly with the "bottom" ring of the carbazole aromatic system, and also Phe193$^{5.32}$, Tyr199$^{5.38}$, Phe289$^{6.51}$ - mostly with the "upper" ring of the carbazole. In our β_2AR models with (-)-isoproterenol and other agonists, the hydrophobic contacts of the catechol ring are similar to those contacts of the "bottom" carbazole ring, with the only exception that a somewhat shifted position of the catechol rings allows an additional Van der Waals contact with Phe289$^{6.51}$ side chain (Figure 4).

The (-)-isoproterenol molecule can adopt the same axial orientation of its aromatic "head", as the one found for (-)-carazolol, despite its shorter linker to the ethanolamine "tail". This makes (-)-isoproterenol sterically compatible with the carazolol-bound pocket in β_2AR, and our modeling results confirm the absence of van der Waals clashes or torsional stress in (-)-

isoproterenol even when it is docked into β_2AR crystallographic coordinates (Figure 3A). A ready accommodation of (-)-isoproterenol within the carazolol-defined β_2AR crystal structure is somewhat surprising, because significant rearrangements in the hydrophobic part of the binding pocket were expected between antagonist-bound and agonist-bound states. According to the "rotamer toggle switch" hypothesis (Shi and others 2002), for example agonist binding is expected to trigger rotation in Trp$^{6.48}$ and Phe290$^{6.52}$ in the binding pocket. Favorable contacts of these two aromatic side chains with both (-)-carazolol and (-)-isoproterenol in β_2AR models do not support a direct mechanistic connection between agonist binding and conformational changes in Trp$^{6.48}$/Phe290$^{6.52}$ side chains. On the other hand, our results do not exclude "toggle switch" in these residues as a part of large scale downstream conformational changes in β_2AR.

Role of TM-V serines in agonist binding

The most important anchor interaction specific for β_2AR agonists is represented by a hydrogen bond network between catechol "head" moiety and serine side chains of the TM-V helical domain (Ambrosio and others 2000; Del Carmine and others 2002; Sato and others 1999; Strader and others 1989). Our modeling consistently predicts specific configurations of this interaction network for several catecholamine agonists (e.g. Figure 4). This extensive network combines not only three H-bonds involving both catechol hydroxyls, but also four intramolecular H-bonds involving Ser203$^{5.42}$, Ser204$^{5.43}$ and Ser207$^{5.43}$ side chains.

Simple distance measurements in ref (Rosenbaum and others 2007) indicate that the "tail" (ethanolamine) and the "head" (catechol) anchor sites are too far apart in the β_2AR-carazolol crystal structure to afford simultaneous contacts with isoproterenol. The conformational modeling in this study suggests that a finite tilt of the TM-V extracellular helix (TM-Ve) is required to resolve this discrepancy and bring the anchor sites closer together for optimal binding of isoproterenol and other full agonists. The tilt of TM-Ve helix and engagement of both anchors is associated with a major (~1000 fold) improvement in the predicted binding affinity for isoproterenol and other full agonists to the β_2AR. Such significant energetic coupling between binding of full agonists (but not antagonists) and TM-V tilt suggests important role of this movement in the conformational changes leading to β_2AR activation.

A coordinated inward shift of all three TM-Ve serines is also in a good agreement with observed synergistic contribution of individual functional groups into affinity and efficacy of β_2AR agonists (Del Carmine and others 2004; Liapakis and others 2000; Liapakis and others 2004). For example, results in ref. (Liapakis and others 2004) show that addition of both hydroxyl groups to the phenol ring of halostachine (HAL) increases its affinity to β_2AR about 120-fold, while individual pOH and mOH additions yield only marginal (~1 fold and ~3.5 fold) contributions. Our conformational model provides a simple structural basis for such synergy: both catechol hydroxyls together can stabilize an optimal TM-Ve tilt to assure full engagement of corresponding H-bonds, whereas individual hydroxyls are not sufficient to shift TM-V and would make only suboptimal contact with TM-V serines, if any.

Our models consistently reproduce the well characterized H-bonding pattern of the catechol pOH with Ser207$^{5.43}$ and mOH with Ser203$^{5.42}$ (Liapakis and others 2000; Strader and others 1989). At the same time Ser204$^{5.43}$ hydroxyl is predicted to participate in strong intramolecular bonding network in the β_2AR but not in direct H-bonding with mOH of agonists, as proposed by early mutagenesis studies (Strader and others 1989). It is possible that the optimized conformation in our models reflects only one static snapshot of the dynamic polar interaction network in the β_2AR-agonist complex. However, hydrogen bonding of Ser204$^{5.43}$ with Asn293$^{6.55}$ side chain and Ala200$^{5.39}$ main chain has been also observed in the β_2AR crystal structure with antagonist timolol (Hanson and others 2008a). According to our models (Figure

2C and 4) further stabilization of this interaction network, particularly improvement of Ser204$^{5.43}$ - Asn293$^{6.55}$ H-bond distance from 3.3 to 2.6 Å, can be achieved upon binding of a full agonist and inward tilt of TM-Ve domain. This stabilizing effect of Ser204$^{5.43}$ - Asn293$^{6.55}$ H-bond suggests a significant indirect contribution of Ser204$^{5.43}$ side chain into agonist binding affinity, and also predicts significant impact of the S204A mutation on the basal activity of β_2AR. Indeed, experimental results in ref.(Ambrosio and others 2000) provide some initial evidence in support of this effect by demonstrating decrease in β_2AR basal activity by as much as 50%-60% upon the S204A/S207A double mutation. More specific measurements of individual effects of Ser204$^{5.43}$ mutation on β_2AR basal activity and ligand binding will be needed to resolve the direct and/or indirect contributions of Ser204$^{5.43}$ to agonist binding.

Potential role of TM-V movements in the activation mechanism—Conformational changes required for the optimal accommodation of agonists in the β_2AR binding pocket should be considered in the broader context of agonist-induced receptor activation. Previous biophysical studies have pointed to existence of several intermediate steps in the conformational changes associated with binding of full agonists to the β_2AR (Del Carmine and others 2004; Liapakis and others 2004; Swaminath and others 2004). The multistep ligand binding hypothesis ((Kobilka and Deupi 2007) and (Kobilka 2007)) describes two fast steps, followed by a distinct slow step in the conformational changes. In the *first step*, an agonist is expected to fit into the relatively loose binding pocket and engage in polar interactions with either the ethanolamine anchor site (Asp113$^{3.32}$/ Asn312$^{7.39}$) or the catechol anchor site (TM-V serines). In the *second step*, specific for catecholamine-like agonists, conformational changes facilitate full engagement of the β_2AR with both “head” and “tail” of the full agonist (Figure 4). Finally, the *third, slow step* may involve substantial movements/deformations in the TM helices and probably a rotamer “toggle switch” in aromatic side chains, leading to significant changes on the receptor cytoplasmic side and to G-protein binding/activation.

The results presented in the current study suggest that binding affinities of agonists and ligand/receptor interactions can be reliably predicted using an intermediate conformation of the receptor, which corresponds to the first and second (fast) steps described above. This is in line with recent studies from our group (Reynolds and others 2008) and others (de Graaf and Rognan 2008), where models based on minor conformational changes in the TM-V domain of β_2AR-carazolol crystal structure were shown to effectively select for agonists in virtual ligand screening (VLS). In the ref (de Graaf and Rognan 2008) though, the adjustments in the agonist-binding model were limited to rotamer changes in Ser203$^{5.42}$, Ser204$^{5.43}$ and Ser207$^{5.43}$ side chains only, similar to those observed in our models with rigid backbone. Screening with this rigid backbone model was not selective for β_2AR agonists when used with the standard scoring functions. To gain selectivity, the authors introduced protein-ligand interaction fingerprints (IFP), which explicitly boosts scoring term for user-specified “anchor” hydrogen bonds.

The current study and our VLS model (Reynolds and others 2008) demonstrate that agonist selectivity can be achieved by a minor backbone shift in TM-V helix, without incorporating knowledge-based terms in the scoring function. Interestingly, our model suggests that optimal binding of full agonists requires the largest shift of the TM-Ve anchor site (~1.6÷2.2 Å), which is also accompanied by a largest gain in binding affinity (~1000-fold). Partial agonists have weaker interactions with either (or both) anchor sites, which results in a whole spectrum of optimal TM-Ve positions, as shown in Figure 5B and Table 2. On the other end of the spectrum, inverse agonists like carazolol can stabilize the “inactive” receptor conformation by preventing the inward movement of TM-Ve, and thereby suppress spontaneous activation. Such direct ligand control over direction and magnitude of TM-V movement may play an important role in the “rheostat” behavior (Kobilka and Deupi 2007) of the β_2AR and some other aminergic

GPCRs, where ligands can 'dial in' different levels of receptor activity ranging from full activation to full blockage.

The exact mechanism by which the ligand-dependent movements in the extracellular part of TM-V affect signal propagation into the cytoplasmic portion of the β_2AR is not clear yet. One plausible explanation would be that the tilting of TM-Ve can result in a "seesaw" movement of the TM-V around Pro211$^{5.50}$ kink, dislocating the cytoplasmic ends of TM-V and TM-VI away from the receptor axis and changing topology of the G-protein binding site (Schwartz and others 2006). A relatively high flexibility of proline-induced kinks in TM-V and other TM helices though, may hamper such direct seesaw movements. In the recently published ligand-free activated opsin (Ops*) structures {Park, 2008 #374; Scheerer, 2008 #488}, for example, the TM-IV kink angle is sharply increased as compared to dark-state rhodopsin, so that extracellular and cytoplasmic ends of TM-VI are *both* tilted outward in the Ops*. Therefore, further experimental and theoretical inquiries are needed to grasp a complex interplay between movements of TM helices, their parts and individual side chains that leads to β_2AR activation.

Conclusions

A critical role of Ser203$^{5.42}$/Ser204$^{5.43}$/Ser207$^{5.43}$ ("head") and Asp113$^{3.32}$/Asn312$^{7.39}$ ("tail") anchor sites in agonist binding and receptor activation is well documented for the β_2AR (Ambrosio and others 2000; Del Carmine and others 2002; Strader and others 1989) and several other closely related GPCRs in adrenergic, dopamine and serotonin families (Coley and others 2000; Hwa and Perez 1996; Mansour and others 1992; Wang and others 1991). Our study suggests a structural mechanism for direct contribution of these interactions in the conformational changes of the ligand binding pocket, and their role in differentiation between agonistic and antagonistic effect of β_2AR ligands. The β_2AR models with TM-V hinge flexibility afford prediction of conformational preferences and binding affinities for the whole spectrum of β_2AR ligands from full agonists to inverse agonists, which are consistent with existing experimental data. At the same time, note that the polar TM-V anchor site is not universally conserved in GPCRs. Therefore the proposed here role of TM-V in regulation of receptor activation may be specific only to this therapeutically important group of receptors. This suggests that along with some "universal" features similar across different GPCRs classes (Schwartz and others 2006), some family-specific and/or function-specific (Hoffmann and others 2008) mechanisms should be considered in analysis of GPCR activation.

Computational Methods

All-atom molecular models and 3D graphics in this work were generated with the ICM-Pro software package, version 3.5-1 (Molsoft LLC). The molecular objects were described in terms of internal coordinate variables (Abagyan and others 1994), and modified ECEPP/3 potentials (Nemethy and others 1992), as implemented in the ICM program (Abagyan and others 2007). Charges for ligands were taken from the MMFF description (Halgren 1995).

Rigid and flexible models of β2ARprotein

The *rigid* ICM model of β_2AR protein was prepared from the PDB coordinates (PDB ID: 2rh1) (Cherezov and others 2007) using ICM conversion procedure. This includes addition of hydrogen atoms to the receptor structure, selection of the energetically favorable His, Asn and Gln side chains, and local minimization of hydrogens in the internal coordinates space. Water molecules and carazolol ligand were subsequently removed from the model. No coordinates of β_2AR heavy atoms were changed from those in the crystal structure.

The β_2AR *model with flexible side chains* was obtained by unfixing specific sets of torsion angles in the rigid model. All χ torsion angles for residues with atoms in 8 Å radius from carazolol atoms were set free, and participate in Monte Carlo optimization.

For the β_2AR *model with flexible TM-V backbone*, in addition to flexible side chains in the binding pocket, all torsion angles (χ ω, ψ and φ) were unfixed in a portion of the extracellular loop EL2 (residues 191-196) and around the proline-induced kink of TM-V (residues 205-210). Free torsion variables in these residues allow for motility of the TM-V extracellular helix (residues 197-204) as a rigid body, while keeping the rest of the protein backbone fixed. Standard disulfide bridging constraints were imposed between thiol groups of Cys191 and Cys106 side chains.

Flexible ligand docking

For all β_2AR receptor models, docking was performed by placing ligand in a random position within 10 Å from the binding pocket and global optimization of the complex conformational energy. Stochastic global energy optimization of the complex was performed using the ICM Monte Carlo (MC) procedure with minimization (Abagyan and Totrov 1994; Abagyan and others 1994).

To facilitate side chain rotamer switches in flexible β_2AR models, the first 10^6 steps of the MC procedure used “soft” vdW potentials and high MC temperature, followed by another 10^6 steps with “exact” vdW method and gradually decreasing temperature. In the models with TM-V backbone flexibility, a minor external force was applied to the top portion of TM5 in the direction of TM3-TM7 helices. The force was adjusted using a ligand-free β_2AR model, so that the best conformational energy of the model with TM-Ve helix tilted by 2 Å inward is equal to the best conformational energy of the model with original position of TM-V (no shift), thus providing a mean to evenly sample the whole range of TM-Ve positions in between. Specifically we used an ICM harmonic “distance restraint” between Cα atoms of Ser203 and Gly37 in TM-I, with weight W=0.41 and “upper wall” D_u=1.0, as described in ICM manual. The inward shift of the Ser203 Cα atom in angstroms was used as a measure of the tilt for the whole TM-Ve helix backbone.

At least ten independent runs of the docking procedure were performed for each ligand-β_2AR complex. The docking results were considered “consistent” when at least 70% of the individual runs resulted in conformations clustered within a root mean square deviation (RMSD) of <0.5Å to the overall best energy pose of the ligand.

Binding affinity predictions

Ligand-receptor binding energy ΔG^{pred} was calculated as the conformational energy of the ligand bound into the β_2AR receptor minus optimized conformational energy of the free ligand. Changes in the receptor conformational energy between apo- and ligand-bound forms cannot be predicted with a high accuracy and were not accounted for in ΔG^{pred} calculations. The energy functions included the following ICM terms with the corresponding default weights: van der Waals (“vw” +“14”), hydrogen bonding (“hb”), distant dependent electrostatics (“el”), torsion (“to”) and desolvation term (“sf”, surfaceTension=0.004). The ICM entropy term for ligands was not included in the final affinity calculations, since it did not improve the prediction accuracy in the initial tests. A more accurate treatment of the entropy that accounts for both ligand and receptor changes may be beneficial in the future studies. The predicted binding affinities were calculated by linear transformation of the binding energy (pK_d^{pred} = $0.313*\Delta G^{pred}$ - 5.8) to match the scale of the experimentally measured pK_d for this set.

Most experimental ligand binding data in Table 2 we obtained from ref. (Del Carmine and others). Though the pK_d measurements for carazolol (Manalan and others 1981) and TA-2005 (Kikkawa and others 1998) were performed at different assay conditions, the values of pK_d for common ligands in these studies were consistent with those in (Del Carmine and others) with 0.4 pK_d, maximum deviation.

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Abbreviations

β_2AR, β_2-adrenergic receptor; GPCR, G protein-coupled receptor; RMSD, root mean square deviation; TM, transmembrane; Max_n, maximum atom deviation; EL2, extracellular loop 2.

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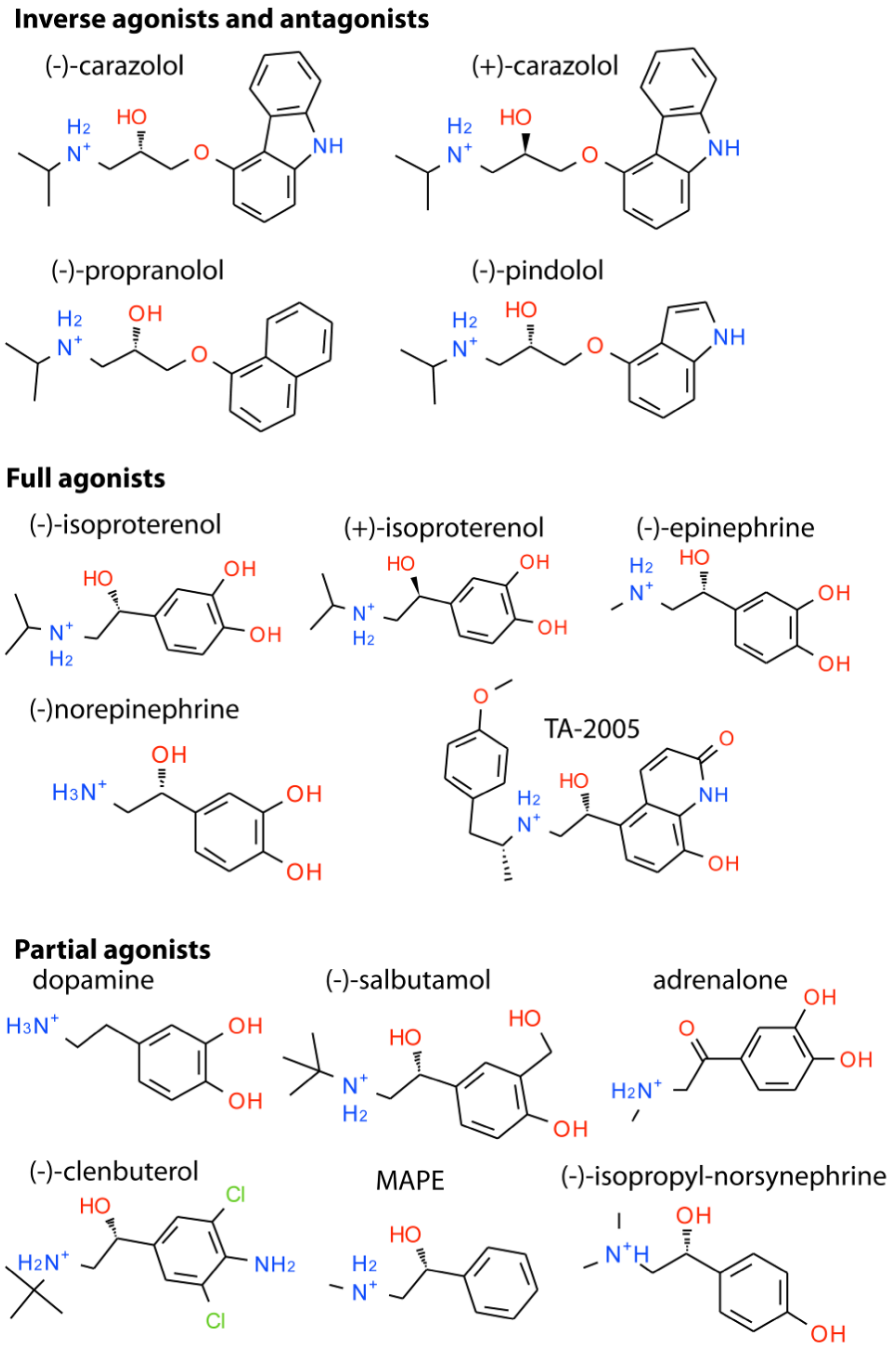

Fig 1.

Chemical structures of β_2AR ligands used in this study. Note that (S)-β-OH chiral center of (-)-carazolol is sterically equivalent to (R)-β-OH of (-)-isoproterenol and other chiral agonists shown. To avoid confusion we will use only (-) or (+) notion to indicate chirality of ligands in this paper.

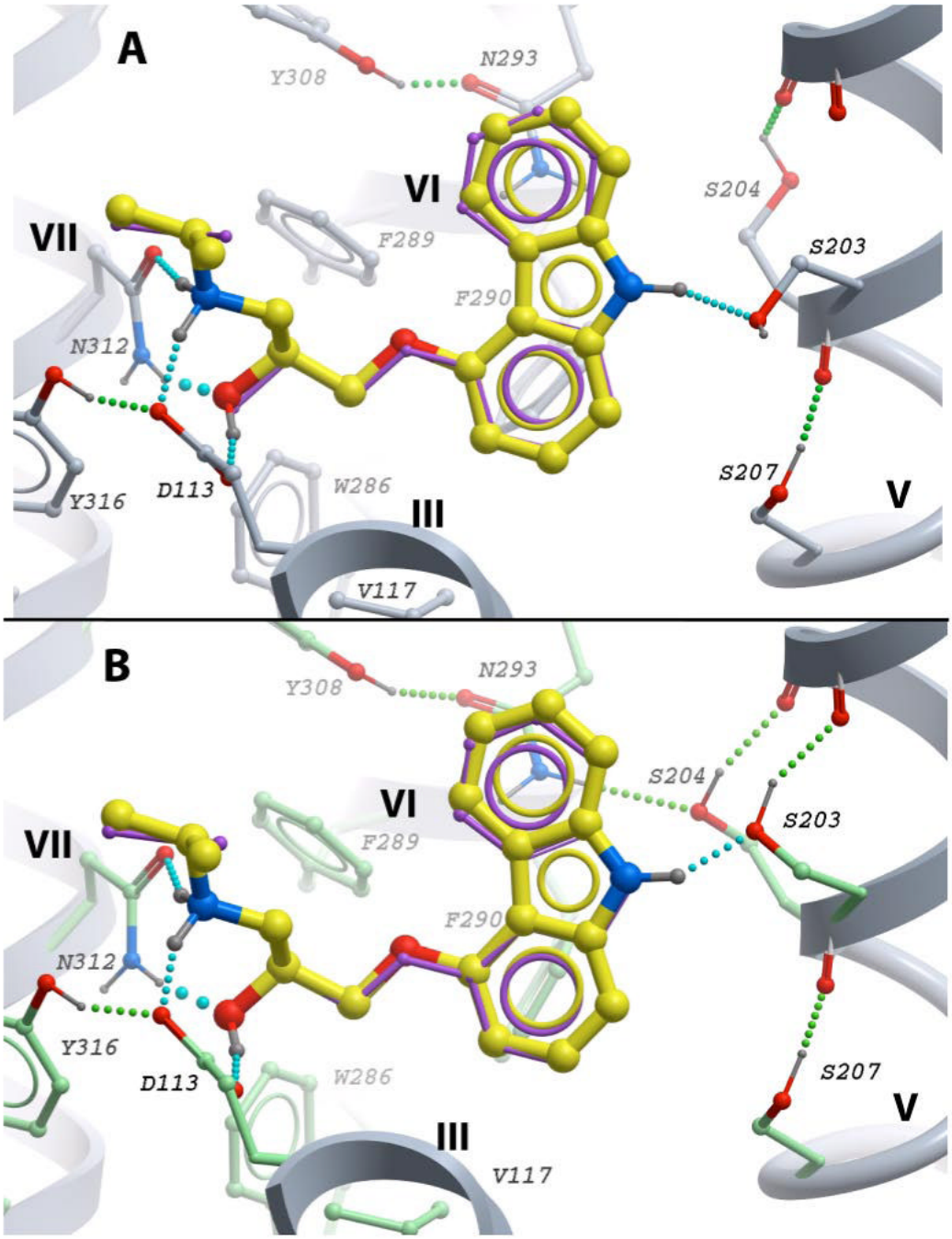

Fig 2.

Predicted conformations of (-)-carazolol in **(A)** rigid and **(B)** flexible side chain β_2AR models. The protein models are shown as grey backbone ribbon; β_2AR side chains in direct contact with carazolol are shown as sticks, except for Tyr199$^{5.38}$, Val114$^{3.33}$ and Phe193$^{5.32}$ in front of the ligand, which are omitted for clarity. Rigid side chains are shown as sticks with grey carbon atoms, flexible side chains with green carbon atoms. The exact position of (-)-carazolol in the crystal structure is shown by thin purple sticks, the predicted ligand poses are shown by thicker sticks with yellow carbon atoms. Ligand-receptor hydrogen bonds are shown by chains of cyan balls; intracellular hydrogen bonds for the binding pocket side chains are shown as

chains of green balls. The models and graphics in **Fig 2** and other figures in this study are generated with ICM-Pro software (Molsoft LLC).

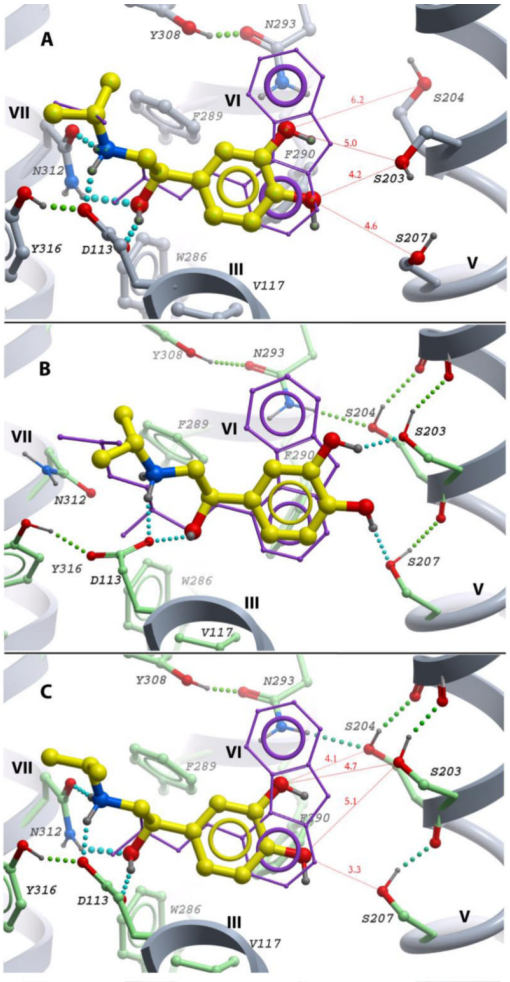

Fig 3.

Structural modeling of agonist (-)-isoproterenol in the β_2AR binding site. View point and color scheme as in Figure 2. **A)** The best energy conformation of (-)-isoproterenol, optimized in the *rigid* β_2AR model. Distances from catechol hydroxyls to TM-V serines are shown as red dashed lines. **B)** One of the two local minima conformations for (-)-isoproterenol in β_2AR model with *flexible side chains*; optimal hydrogen bond network with TM-V serines shown **C)** An alternative local minimum conformation of (-)-isoproterenol with optimal interaction network between ethanolamine tail and Asp113$^{3.32}$/ Asn312$^{7.39}$ anchor site. Distances to TM-V serines are shown as red dashed lines.

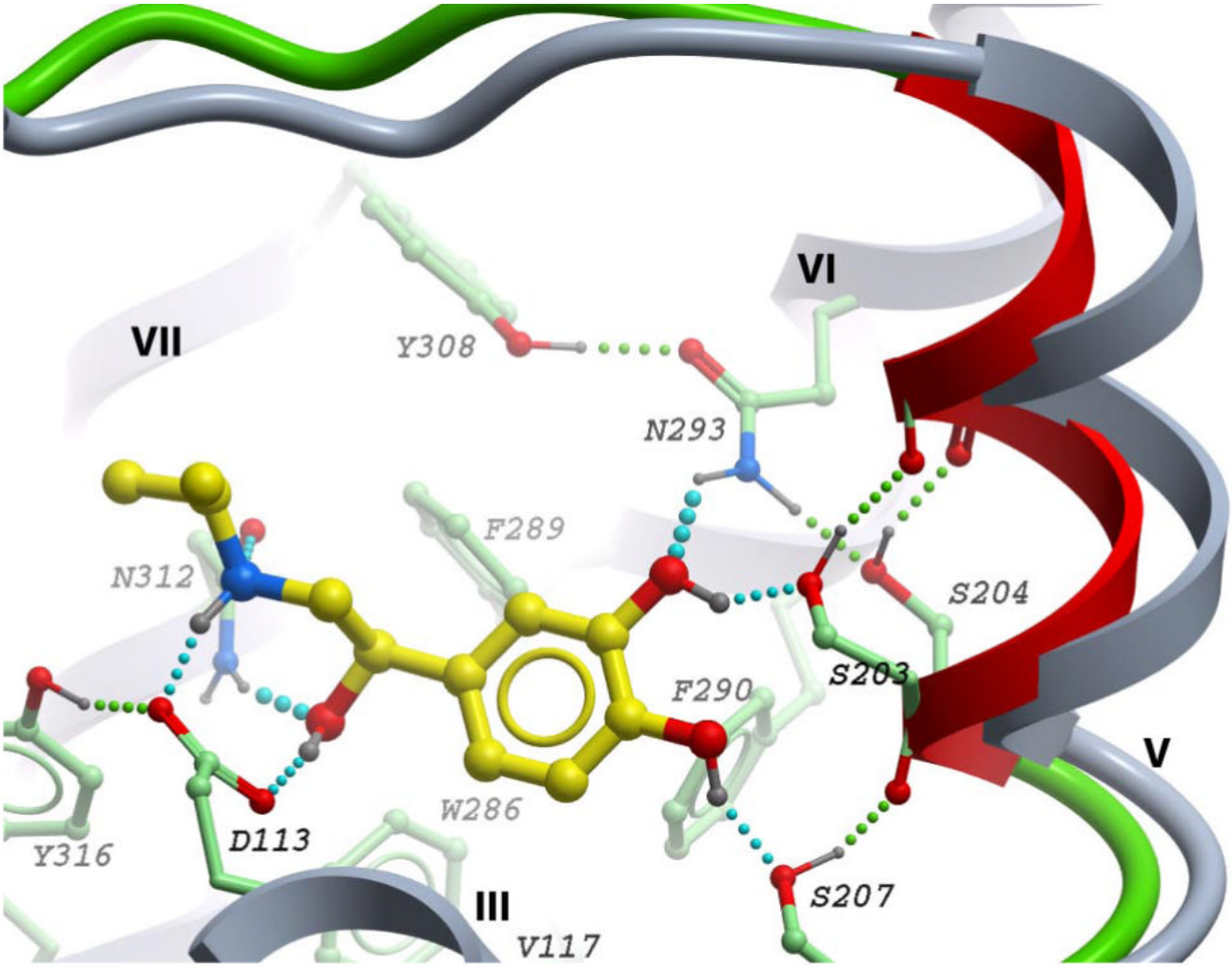

Fig 4.

The best energy conformation of (-)-isoproterenol in β_2AR model with backbone flexibility (colored green) in the TM-V proline-induced kink (residues 205-210) and a portion of EL2 loop (191-196). The extracellular portion of TM-V (TM-Ve) is shown as red ribbon. The original position of TM-Ve and other static helices are shown as grey ribbon, flexible side chains in proximity of the binding site have carbon atoms colored green. Supplementary materials contain 3D atomic coordinates for this model, as well as for β_2AR models with other ligands studied in this work.

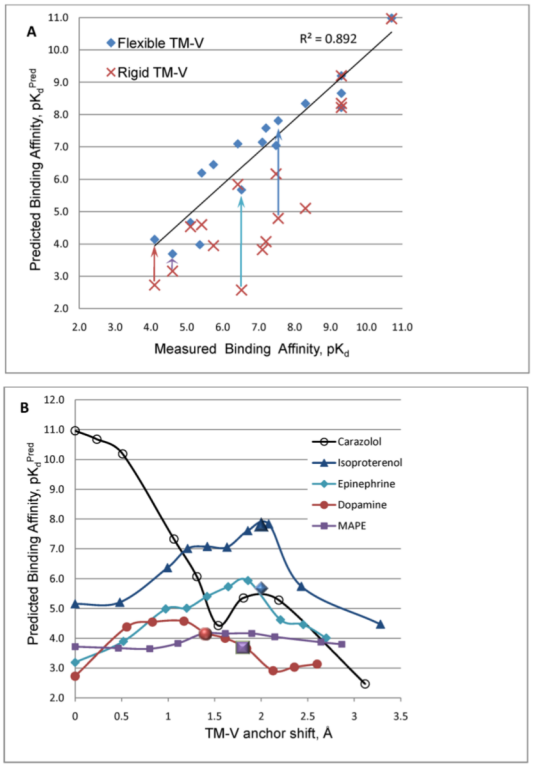

Fig 5.

A) Comparison of the predicted and measured ligand binding affinities (pK_d), as listed in Table 3 of the paper. Affinities are shown for β_2AR conformational models with rigid (brown crosses) and flexible (blue diamonds) TM-V backbone. The arrows illustrate improvements in the ligand binding affinity from the rigid TM-V model to the flexible TM-V model for agonists isoproterenol (dark blue), epinephrine (light blue), dopamine (brown) and MAPE (plum). Accuracy of affinity predictions estimated as R^2=0.75, RMSE=1.3 pK_d for the rigid backbone model, and R^2=0.89, RMSE=0.7 pK_d for the flexible TM-V model.

B) Binding affinities for inverse agonist carazolol, full agonists (-)-isoproterenol and (-)-epinephrine, as well as partial agonists dopamine and MAPE, calculated as a function of TM-V anchor site shift. Results of single-point analysis for agonists from Table 3 are shown by bigger shapes.

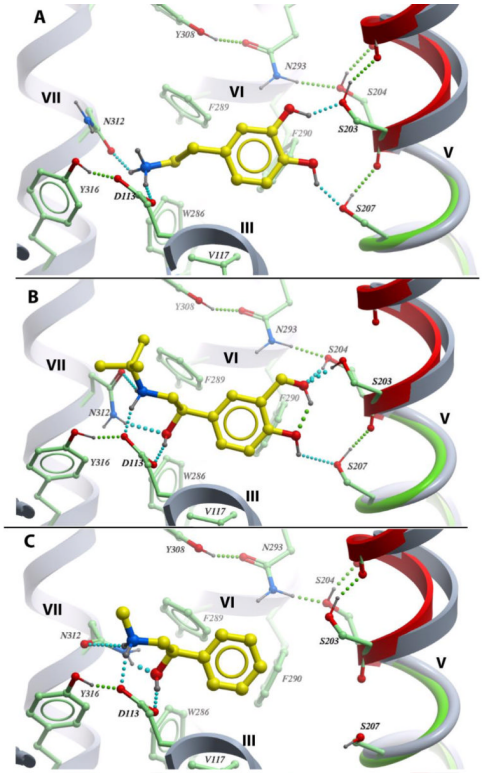

Fig 6.

Predicted dopamine **(A),** salbutamol **(B)** and MAPE **(C)** binding into β_2AR model with flexible side chains. Color scheme as in Fig 4.

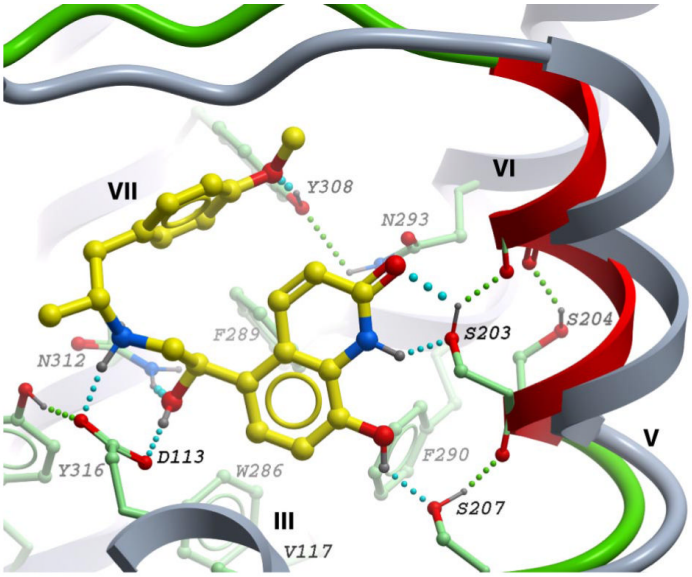

Fig 7.
Predicted binding of TA-2005 into β_2AR model with flexible side chains and TM-V backbone shift. Color scheme as in Fig 4.

Table 1

Receptor-ligand hydrogen bond distances for different conformations of the β_2AR- (-)-isoproterenol complex. The columns correspond to models in Figure 3B, Figure 3C and Figure 4 respectively. Distances, not compatible with hydrogen bonding are highlighted by **bold** type

Hydrogen bonds (β2AR : Isoproterenol)	Donor-Acceptor Distance, Å		
	Catechol-anchored	Amino-anchored	Flexible TM-V model
OD1 (Asp113) - N^+ (amino)	2.7	2.7	2.9
OD2(Asp113) - O (β-OH)	2.5	2.7	2.7
OD1 (Asn312) - N^+ (amino)	**4.4**	2.9	3.1
ND2 (Asn312) - O (β-OH)	**6.0**	3.2	3.2
OG(Ser207) - O (para)	2.7	3.3	2.6
OG(Ser203) - O (para)	3.0	**4.5**	3.0
OG(Ser203) - O (meta)	2.7	**5.1**	2.6
OG(Ser204) - O (meta)	3.5	**4.1**	3.9

Table 2

Predicted ligand binding affinities for β_2AR conformational models with/without TM-V backbone flexibility and corresponding shift of TM-V anchor site. Calculations for TA-2005 performed with the wild-type β_2AR and two Tyr308$^{7.35}$ mutants

Ligand	Predicted affinity[a] pK_d^{Pred}		Predicted TM-V anchor shift, Å	Predicted pK_d gain due to TM-V shift	Measured Affinity, pK_d
	Rigid TM-V	Flexible TM-V			
Inverse agonists and antagonists					
(-)-Carazolol	11.0	11.0	0.0	0.0	10.7[b]
(+)-Carazolol	9.2	9.2	0.0	0.0	9.3[b]
(-)-Propranolol	8.2	8.2	0.0	0.0	9.3[c]
(-)-Pindolol	8.3	8.7	0.2	0.3	9.3[c]
Full agonists					
(-)-Isoproterenol	4.8	7.8	2.0	3.0	7.5[c]
(+)-Isoproterenol	3.9	6.5	1.6	2.5	5.7[c]
(-)-Epinephrine	2.6	5.7	2.0	3.1	6.5[c]
(-)-Norepinephrine	1.7	4.0	1.6	2.3	5.4[c]
(R,R)-TA-2005	5.1	8.3	2.2	3.2	8.3[d]
(R,R)-TA-2005 (Y308F)	4.1	7.6	1.9	3.5	7.2[d]
(R,R)-TA-2005 (Y308A)	3.8	7.1	2.1	3.3	7.1[d]
Partial agonists					
(-)-Salbutamol	5.8	7.1	1.1	1.3	6.4[c]
Dopamine	2.7	4.1	1.4	1.4	4.1[c]
MAPE	3.2	3.7	1.8	0.5	4.6[c]
(-)-Clenbuterol	6.2	7.0	0.9	0.9	7.5[c]
(-)-Isopropyl-norsynephrine	4.6	6.2	1.7	1.6	5.4[c]
Adrenalone	4.5	4.7	1.6	0.1	5.1[c]

[a] Calculated as predicted binding energy normalized to the measured affinity scale: $pK_d^{pred} = 0.313*\Delta G^{pred} - 5.8$

[b] data in ref. (Manalan and others 1981)

[c] data in ref. (Del Carmine and others)

[d] data in ref. (Kikkawa and others 1998)