ACS Medicinal Chemistry Letters

LETTER

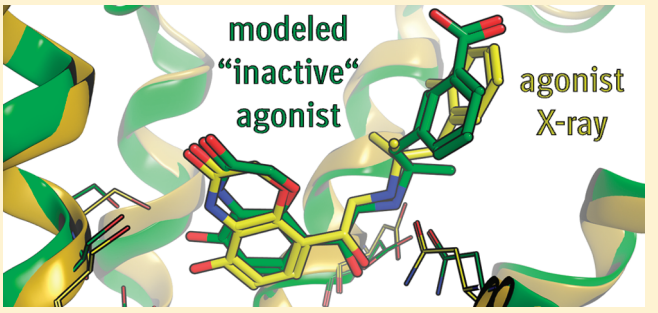
pubs.acs.org/acsmedchemlett

# The Implication of the First Agonist Bound Activated GPCR X-ray Structure on GPCR in Silico Modeling

Christofer S. Tautermann* and Alexander Pautsch

Lead Identification and Optimization Support, Boehringer Ingelheim Pharma GmbH & Co. KG, 88397 Biberach an der Riss, Germany

**ABSTRACT:** The very recently published first X-ray structure of the $\beta$2 adrenergic receptor in its active state hosting a small molecule (PDB ID: 3P0G) reveals a lot of information about the G-protein-coupled receptor (GPCR) activation process from a structural point of view. When compared to the inactive state crystal structure of $\beta$2, large differences are seen in the GPCR helical structure at the cytoplasmatic side, whereas very subtle changes occur at the ligand binding site. The observation that there are hardly any differences in the binding site of agonists and inverse agonists implies that in silico predictions of the efficacy of ligands will be very hard. This is illustrated by the example of an already published binding mode of a $\beta$2 agonist, which has been modeled into the inactive state X-ray structure of the $\beta$2 receptor. When comparing the modeled structure to the new activated X-ray structure, quantitative agreement of the binding mode is found, implying that the subtle changes between agonist binding to the activated state and inverse agonist binding to the inactive state can currently not be captured by standard in silico modeling methods.




Structural information for G-protein-coupled receptors (GPCRs) is still limited. Starting with the X-ray structure of dark state bovine rhodopsin in 2000,[1] the modeling community had a template at atomic resolution at hand, which was used for modeling all GPCRs. Only few approaches have been developed, which did not directly rely on this structure.[2] It took another 7 years until the first crystal structure of a GPCR of larger pharmaceutical interest was solved; the $\beta$2 adrenergic receptor bound to the inverse agonist carazolol, crystallized with either an antibody[3] or as a T4-lysozyme fusion.[4] Although the sequence identity of $\beta$2 and rhodopsin is below 20%, the 3D structure of the transmembrane (TM) regions was shown to be quite similar. Large structural differences are found in the loop regions, where the location and the secondary structure strongly deviate. The largest difference close to the ligand binding site is found in the extracellular loop 2 (EL2). The EL2 forms a 2-stranded $\beta$-sheet in rhodopsin and dips down into the binding site of retinal, whereas a short α-helix far above the ligand binding site is found in $\beta$2.

The next receptor from a different family of class A GPCRs was solved in 2008, when a X-ray structure of the $A_{2A}$ adenosine receptor ($A_{2A}$AR) T4-lysozyme fusion in its inactive state was published.[5] Again, the TM regions are structurally similar to rhodopsin and $\beta$2 although the sequence similarity is very low. The Cα RMSD of the TM regions of $A_{2A}$AR to $\beta$2 and rhodopsin was found to be smaller than 2 Å, indicating a high structural homology.

The big difference to the already known structures has again been found in the loop regions, which are strongly involved in ligand binding in $A_{2A}$AR, which is in sharp contrast to $\beta$2, where only F193 on EL2 is interacting with the ligand. Before the release of the $A_{2A}$AR structure, a competition was held for research groups involved in GPCR modeling. In this so-called "GPCR Dock 2008" event,[6] the GPCR modeling community was asked to propose a receptor model for the $A_{2A}$A receptor and the binding mode for a given inverse agonist. In July 2010, the next event has been scheduled to predict the CXCR4 chemokine receptor and dopamine D3 receptor structures. Both structures have been released very recently.[7,8] The structures are in their inactive state, and the EL2 regions strongly deviate from all EL2 structures known so far. Nonetheless, the TM regions are again similar to the known folds. The results of the 2008 competition clearly showed that the prediction of the helical parts of the receptor was not the major problem, as most models were found within 3 Å Cα RMSD to the crystal structure, when only regarding the TM regions. However, loop structure and ligand binding mode predictions proved to be very challenging. Especially, the correct loop structure was only predicted at a Cα RMSD of about 4 Å by one participant, and all other predictions were above 7 Å in RMSD, thus far away from the actual structure.[6] The detailed results of the "GPCR Dock 2010" assessment will be published in due course, and the assessment of the in silico models confirms the observations from the 2008 contest. The prediction of the TM regions works quite well, especially for the D3 receptor, where close templates for homology modeling are available. However, in the prediction of EL2 geometries, there is still much

**Received:** October 21, 2010
**Accepted:** March 31, 2011
**Published:** March 31, 2011

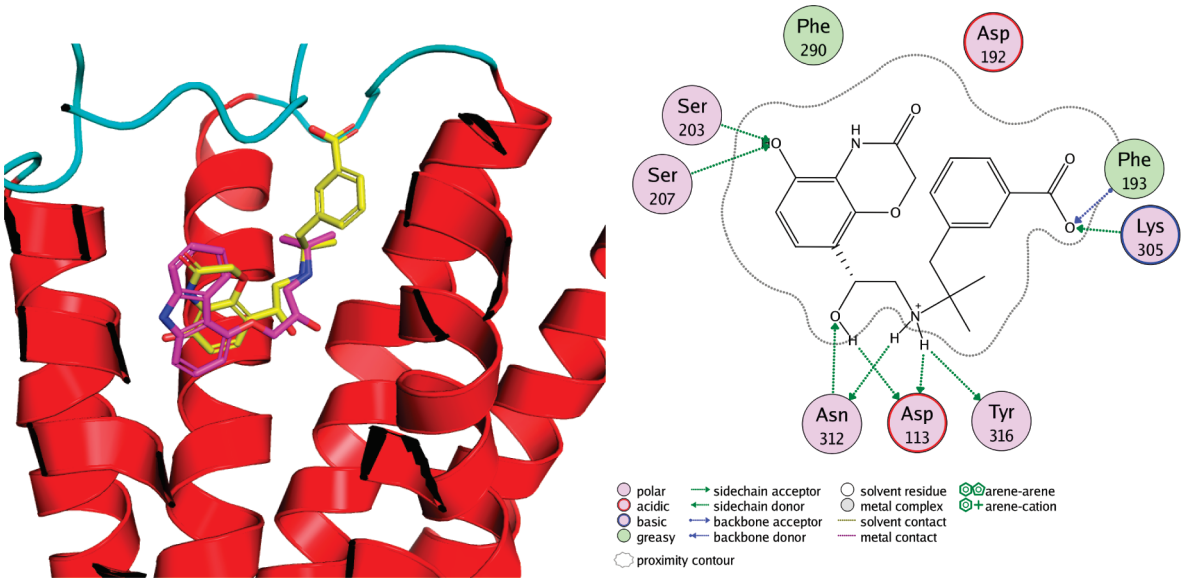



**Figure 1.** Left: Recently published binding mode of compound **6m** from ref 13 (yellow) in comparison to carazolol (magenta) in the inactive state $\beta$2 crystal structure (2RH1). Right: Ligand interactions as predicted in ref 13. Reprinted with permission from the supporting material of ref 13. Copyright 2009 Elsevier.

room for improvement (personal communication with Prof. Raymond Stevens).

These competitions realistically reflect the current state of the art in GPCR modeling, as TM regions of class A receptors can apparently be predicted in a satisfying way. However, this holds only true for receptors in the inactive state because all reported ligand bound X-ray structures, which are used as modeling templates, are crystallized in an inactive state so far. A retinal-free structure of opsin with a small polypeptide, which mimics the G-protein, has been reported,[9] but this structure is not the fully activated all-trans retinal bound state of rhodopsin.

Thus, no reliable template for direct homology modeling of the active state has been available. Various modes of activation have been suggested, and the modeling problem was solved either by starting at the inactive state and subsequent modification according to an activation hypothesis[10] or automated ligand induced helical movement.[11] In many cases, it was decided to stick to the inactive state structure, because there is no validated "method" to activate a receptor in silico. Therefore, there was strong need for the first crystal structure of a fully activated ligand bound GPCR.

The very recently elucidated crystal structure of $\beta$2 bound to the long acting $\beta$2 agonist (LABA) BI-167107 from the benzoxazinone class of compounds is now the first example of such an activated ligand−receptor complex.[12] The G-protein is substituted by a camelid nanobody and fluorescence spectroscopy, and agonist binding affinity experiments indicate that the nanobody stabilizes a conformation in wild type $\beta$2 that is very similar to that stabilized by the corresponding G-protein. Therefore, the authors suggest that the structure resembles an agonist-bound GPCR in its active state.[12] Quite surprisingly, the ligand binding site and the EL2 are very similar to the inactive structure. Only at the cytoplasmatic face of the receptor, large helical movements are seen (for a detailed structural discussion, the reader is asked to refer to the original paper[12]). Unfortunately, no competition as in the case of $A_{2A}$AR was held; thus, it is not clear how well modeling would have had predicted the structure in advance.

A year before the active state X-ray structure was solved, a modeled binding mode of a related benzoxazinone (compound **6m** in ref 13) in $\beta$2 has been published.[13] Lacking active state GPCR templates at that point, the modeled binding mode (Figure 1) relied on the inactive carazolol bound $\beta$2 structure. For comparison, Figure 2 shows the binding mode in the activated agonist bound X-ray structure of $\beta$2.[12]

An overlay of the actual crystal structure and the modeled binding mode (Figure 3) shows that the predicted binding mode of compound **6m** quantitatively overlaps with the crystal structure for the identical parts of the ligands (heavy atoms RMSD 0.5 Å). The receptor model itself shows an overall Cα RMSD of 2.0 Å to the crystal structure. However, when only considering residues within the binding site (<4.5 Å distance to the ligand, also including loop residues), the Cα RMSD and the all atom RMSD account to 0.7 and 0.9 Å, respectively. This again emphasizes the small variability in the binding site upon $\beta$2 activation.

The implication of this good prospective prediction is manifold. First, one may propose that the known inactive structures of GPCRs are sufficiently good templates also for activated state modeling. This proposition will be subject to debate as this structure is the first ligand-bound activated state structure published so far. It will need the elucidation of some more activated GPCR structures to see if the small movements in the binding site upon activation are typical for $\beta$2 (or even just for this selected agonist) only or if small changes in the binding site are a general feature upon activation. The latter hypothesis is supported by several very recently solved crystal structures of $\beta$1 and $\beta$2 in their inactive state with bound agonists.[14,15] The structures lack a G-protein mimicking entity and thus do not possess the cytosolic cavity between TM3 and TM6 as seen in the activated GPCR structures of $\beta$2[12] and opsin.[16] Although the structures are bound to an agonist, they are in an inactive state, which corresponds to a low affinity agonist bound state.[17] Trapping the structures in the low affinity state could only be achieved by either covalently linking the ligand to the receptor[15] or by stabilizing receptor mutants constraining the receptor to its

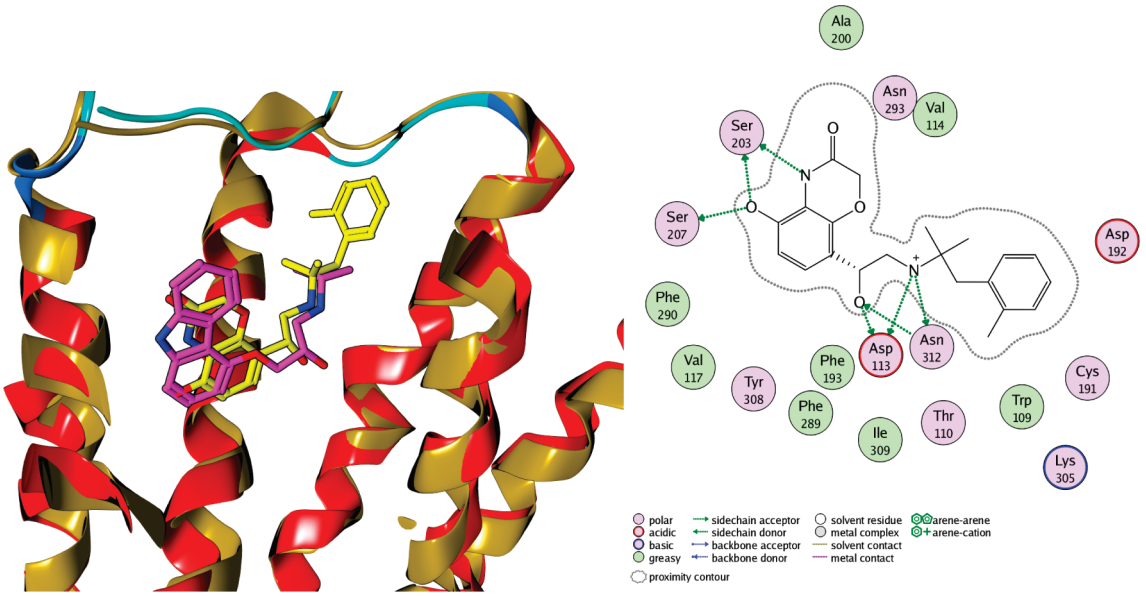



**Figure 2.** Left: Binding mode of the agonist BI-167107 in the active state $\beta$2 crystal structure (3P0G, yellow structure and brown backbone) in comparison to carazolol in the inactive state $\beta$2 crystal structure (2RH1, magenta sticks and red backbone). Right: Ligand interactions of BI-167107 in 3P0G.

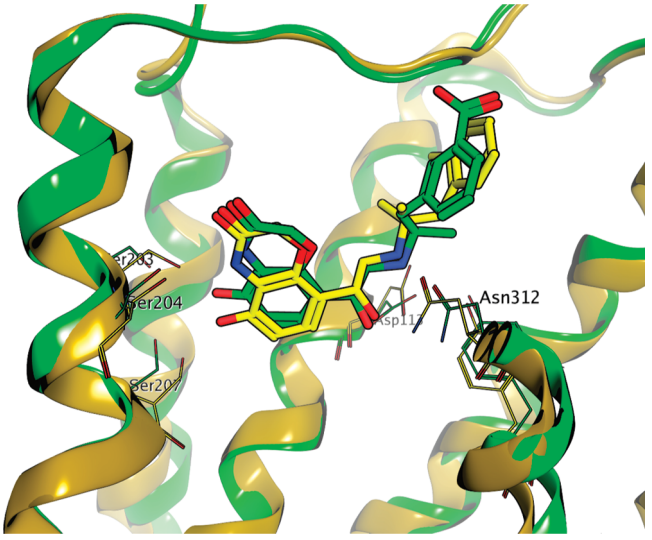



**Figure 3.** Superposition of the modeled binding mode of compound **6m** from ref 13 (green) and the activated $\beta$2 crystal structure bound to BI-167107 (yellow).[12]

inactive state.[14] However, for all agonist-bound structures (inactive or activated), the ligand binding site is very similar to the inverse agonist bound cavity, supporting the hypothesis that agonist binding and activation by agonists do not induce large changes in the ligand binding site. Comparison of the side chain positions of the activated and inactive receptors revealed a strong movement of F282$^{6.44}$ (superscripts indicate Weinstein−Ballesteros nomenclature[18]) in $\beta$2, a residue that is one turn below W286$^{6.48}$, which is known as the "toggle switch". The toggling (i.e., changing the rotameric position) of W6.48 is postulated to be the first step in the GPCR activation process,[10] but the rotamer of W6.48 is found to be the same in all ligand-bound structures (activated and inactive) as well as in opsin. Therefore, the molecular switch seems to be one turn below W286$^{6.48}$, where an apolar network of I121$^{3.40}$, P211$^{5.50}$, and F282$^{6.44}$ exists in $\beta$2, which changes its interactions upon activation.[12] It will have to be shown if this mechanism is conserved in all GPCRs, but for the various $\beta$2 structures, it seems to be very plausible. The inactive agonist bound $\beta$2 structure[15] does not show any significant displacement of the mentioned side chains, but the activated $\beta$2 structure does. This implies a function of the network in the GPCR activation procedure, and this information might be used for modeling the active state of GPCRs, starting from inactive structures. The movement of F6.44 is also observed in the activation of rhodopsin, as both retinal-free opsin structures[9,16] possess a cavity for transducin at the cytoplasmatic receptor surface and a different location of F6.44 as compared to dark state rhodopsin.[1]

Very recently, also an agonist-bound structure of $A_{2A}$AR was reported[19] (PDB ID: 3QAK), and although no G-protein mimicking pepdide was used for crystallization, an outward movement of TM6 at the cytosolic face is seen. In addition to that, a sliding movement of TM3 along its axis and a rotameric switch of tyrosin in the NPxxY motif are observed. These structural changes are in line with the changes taking place upon activation from rhodopsin to opsin. Therefore, it is likely that this new structure of $A_{2A}$AR is in an (at least partially) activated state. The structural changes in the binding site as compared to the inactive $A_{2A}$AR are relatively small, given the large structural differences of the ligands present in the inactive and active state X-ray structures. Comparison of the receptor regions directly below the binding site reveals a significant movement of F242$^{6.44}$ and a rotameric switch of I92$^{3.40}$. This observation confirms the hypothesis mentioned above, as the hydrophobic network consisting of residues 3.40, 5.50, and 6.44 changes its interaction upon activation.

Also for $A_{2A}$AR, the question arises if the agonist-bound structure would have been predicted by in silico methods. A study by Ivanov et al.[20] shows that by the use of the inactive

crystal structure, very reasonable binding modes for agonists can be predicted. Especially the location of the ribose ring and the adenine core correspond quite well to the observations of the agonist-bound $A_{2A}$AR crystal structure.

The second implication of the good prospective prediction of agonist binding modes based on inactive receptor structures might be very worrying for the GPCR modeling community. Because of the high similarity of binding sites, the in silico discrimination between agonists and inverse agonists in receptor models will be very difficult, if not impossible. In the present case, a LABA has been modeled into an inactive receptor structure, and the binding mode from the crystal structure of the active state has been captured very well. This, in turn, means that the ligand fits both, activated and inactivated receptor structures. Therefore, modeling of binding modes will work well, if any more or less reliable structure (activated or inactive) of the GPCR is available. The major drawback of this implication is that from a modeling point of view, agonists and inverse agonists fit the same pocket, and the ligand efficacy cannot be predicted in silico by target-based methods. As a consequence, this also applies to the inability to predict any ligand-biased signaling by conventional modeling, which requires even more subtle differentiation than between agonists and inverse agonists.

This finding, however, emphasizes the large importance of the first activated agonist-bound GPCR crystal structure to the modeling community. In addition, these reported observations stress the need for the elucidation of additional new structures of various (different) activated GPCRs to obtain a basis for a systematic in silico modeling of active GPCRs. Agonist-bound inactive GPCR structures are extremely useful to get a deeper understanding of the GPCR activation process, however, for homology modeling structures of fully activated GPCRs will be the preferred templates. The additional need for new computational approaches for the prediction of the activity of GPCR ligands is also strongly emphasized by the new agonist-bound X-ray structures. Conventional modeling methods are having a hard time to predict the efficacy of a small molecule GPCR ligand—if it is an agonist or an inverse agonist. It will be even more difficult to predict partial (inverse) agonists as well as positive or negative allosteric modulators to GPCRs.

From the recent agonist-bound structures, it is implicated that a molecular switch in the center of the GPCR one turn below the postulated W6.48 "toggle switch" might be necessary for activation. This knowledge might help modelers to enable the transition from inactive to active state structures in silico. The future will show if methods are sufficiently sensitive to predict ligand action correctly.

## AUTHOR INFORMATION

### Corresponding Author

*Tel: 0049(0)7351 54-94030. E-mail: christofer.tautermann@boehringer-ingelheim.com.

## ACKNOWLEDGMENT

We thank Prof. Raymond Stevens for communicating the results of the GPCR Dock 2010 assessment and for useful discussions.

## REFERENCES

(1) Palczewski, K.; Kumasaka, T.; Hori, T.; Behnke, C. A.; Motoshima, H.; Fox, B. A.; Le, T. I.; Teller, D. C.; Okada, T.; Stenkamp, R. E.; Yamamoto, M.; Miyano, M. Crystal structure of rhodopsin: A G protein-coupled receptor. *Science* **2000**, *289*, 739–745.

(2) Shacham, S.; Marantz, Y.; Bar-Haim, S.; Kalid, O.; Warshaviak, D.; Avisar, N.; Inbal, B.; Heifetz, A.; Fichman, M.; Topf, M.; Naor, Z.; Noiman, S.; Becker, O. M. PREDICT modeling and in-silico screening for G-protein coupled receptors. *Proteins* **2004**, *57*, 51–86.

(3) Rasmussen, S. G. F.; Choi, H. J.; Rosenbaum, D. M.; Kobilka, T. S.; Thian, F. S.; Edwards, P. C.; Burghammer, M.; Ratnala, V. R. P.; Sanishvili, R.; Fischetti, R. F.; Schertler, G. F. X.; Weis, W. I.; Kobilka, B. K. Crystal structure of the human beta2 adrenergic G-protein-coupled receptor. *Nature* **2007**, *450*, 383–387.

(4) Cherezov, V.; Rosenbaum, D. M.; Hanson, M. A.; Rasmussen, S. G. F.; 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 2-Adrenergic G Protein Coupled Receptor. *Science* **2007**, *318*, 1258–1265.

(5) Jaakola, V. P.; Griffith, M. T.; Hanson, M. A.; Cherezov, V.; Chien, E. Y. T.; 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.

(6) Michino, M.; Abola, E.; participantsGPCR, D.; Brooks, C. L.; Dixon, J. S.; Moult, J.; Stevens, R. C. Community-wide assessment of GPCR structure modelling and ligand docking: GPCR Dock 2008. *Nat. Rev. Drug Discovery* **2009**, *8*, 455–463.

(7) Chien, E. Y. T.; Liu, W.; Zhao, Q.; Katritch, V.; Won Han, G.; Hanson, M. A.; Shi, L.; Newman, A. H.; Javitch, J. A.; Cherezov, V.; Stevens, R. C. Structure of the Human Dopamine D3 Receptor in Complex with a D2/D3 Selective Antagonist. *Science* **2010**, *330*, 1091–1095.

(8) Wu, B.; Chien, E. Y.; Mol, C. D.; Fenalti, G.; Liu, W.; Katritch, V.; Abagyan, R.; Brooun, A.; Wells, P.; Bi, F. C.; Hamel, D. J.; Kuhn, P.; Handel, T. M.; Cherezov, V.; Stevens, R. C. Structures of the CXCR4 Chemokine GPCR with Small-Molecule and Cyclic Peptide Antagonists. *Science* **2010**, *330*, 1066–1071.

(9) Park, J. H.; Scheerer, P.; Hofmann, K. P.; Choe, H. W.; Ernst, O. P. Crystal structure of the ligand-free G-protein-coupled receptor opsin. *Nature* **2008**, *454*, 183–187.

(10) Schwartz, T. W.; Frimurer, T. M.; Holst, B.; Rosenkilde, M. M.; Elling, C. E. Molecular mechanism of 7TM receptor activation--a global toggle switch model. *Annu. Rev. Pharmacol. Toxicol.* **2006**, *46*, 481–519.

(11) Bhattacharya, S.; Vaidehi, N. Computational mapping of the conformational transitions in agonist selective pathways of a G-protein coupled receptor. *J. Am. Chem. Soc.* **2010**, *132*, 5205–5214.

(12) Rasmussen, S. G. F.; Choi, H. J.; Fung, J. J.; Pardon, E.; Casarosa, P.; Chae, P. S.; DeVree, B. T.; Rosenbaum, D. M.; Thian, F. S.; Kobilka, T. S.; Schnapp, A.; Konetzki, I.; Sunahara, R. K.; Gellman, S. H.; Pautsch, A.; Steyaert, J.; Weis, W. I.; Kobilka, B. K. Structure of a nanobody-stabilized active state of the beta2 adrenoceptor. *Nature* **2011**, *469*, 175–180.

(13) Hoenke, C.; Bouyssou, T.; Tautermann, C. S.; Rudolf, K.; Schnapp, A.; Konetzki, I. Use of 5-hydroxy-4H-benzo[1,4]oxazin-3-ones as beta(2)-adrenoceptor agonists. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6640–6644.

(14) Warne, T.; Moukhametzianov, R.; Baker, J. G.; Nehme, R.; Edwards, P. C.; Leslie, A. G. W.; Schertler, G. F. X.; Tate, C. G. The structural basis for agonist and partial agonist action on a beta1-adrenergic receptor. *Nature* **2011**, *469*, 241–244.

(15) Rosenbaum, D. M.; Zhang, C.; Lyons, J. A.; Holl, R.; Aragao, D.; Arlow, D. H.; Rasmussen, S. G. F.; Choi, H. J.; DeVree, B. T.; Sunahara, R. K.; Chae, P. S.; Gellman, S. H.; Dror, R. O.; Shaw, D. E.; Weis, W. I.; Caffrey, M.; Gmeiner, P.; Kobilka, B. K. Structure and function of an irreversible agonist-beta2 adrenoceptor complex. *Nature* **2011**, *469*, 236–240.

(16) Scheerer, P.; Park, J. H.; Hildebrand, P. W.; Kim, Y. J.; Krausz, 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.

(17) De Lean, A.; Stadel, J. M.; Lefkowitz, R. J. A ternary complex model explains the agonist-specific binding properties of the adenylate

cyclase-coupled beta-adrenergic receptor. *J. Biol. Chem.* **1980**, *255*, 7108–7117.

(18) Ballesteros, J. A.; Weinstein, H. Integrated methods for the construction of three-dimensional models and computational probing of structure-function relations in G protein-coupled receptors. *Methods Neurosci.* **1995**, *25*, 366–428.

(19) Xu, F.; Wu, H.; Katritch, V.; Han, G. W.; Jacobson, K. A.; Gao, Z. G.; Cherezov, V.; Stevens, R. C. Structure of an Agonist-Bound Human A2A Adenosine Receptor. *Science*, DOI: 10.1126/science.1202793.

(20) Ivanov, A. A.; Barak, D.; Jacobson, K. A. Evaluation of homology modeling of G-protein-coupled receptors in light of the A(2A) adenosine receptor crystallographic structure. *J. Med. Chem.* **2009**, *52*, 3284–3292.