

GPCR agonist binding revealed by modeling and crystallography

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Despite recent progress in structural coverage of the G-protein-coupled receptor (GPCR) family, high plasticity of these membrane proteins poses additional challenges for crystallographic studies of their complexes with different classes of ligands, especially agonists. The ability to predict computationally the binding of natural and clinically relevant agonists and corresponding changes in the receptor pocket, starting from inactive GPCR structures, is therefore of great interest for understanding GPCR biology and drug action. Comparison of computational models published in 2009 and 2010 with recently determined agonist-bound structures of β -adrenergic and adenosine A_{2A} receptors reveals high accuracy of the predicted agonist binding poses (0.8 Å and 1.7 Å respectively) and receptor interactions. In the case of the β_2 AR, energy-based models with limited backbone flexibility have also allowed characterization of side-chain rotations and a finite backbone shift in the pocket region as determinants of full, partial or inverse agonism. Development of accurate models of agonist binding for other GPCRs will be instrumental for functional and pharmacological studies, complementing biochemical and crystallographic techniques.

GPCRs

As the key players in recognition of extracellular signals, GPCRs represent the largest (>800) and most important superfamily of clinical targets in disorders of neural, immune, cardiovascular, endocrine systems and cancer [1–3]. GPCRs can be activated by endogenous or synthetic agonists, inhibited by antagonists and inverse agonists, or affected by allosteric modulators [4], and each of these classes of ligands is therapeutically relevant. The crystal structures of β -adrenergic (β_2 AR and β_1 AR) [5–8], adenosine A_{2A} (A_{2A} AR) [9], chemokine CXCR4 [10], dopamine D3 (D3R) [11] and most recently histamine H1 (H1R) [12] receptors in complex with stabilizing antagonists provide a long-sought 3D structural framework for studies of GPCR function and future drug discovery efforts [13,14]. Over the past few years the applicability of docking and virtual ligand-screening (VLS) technologies to GPCR crystal structures [15–18] has been validated by co-crystallization of some of these ligands [8] and prospective identifications of novel β_2 AR and A_{2A} AR antagonist

chemotypes [19–23]. Similar VLS studies are underway for other therapeutically relevant GPCRs whose structures have appeared over the last year [10–12].

At the same time, the intrinsic conformational plasticity of GPCRs was thought to be a major obstacle to using inactive-state receptor structures for analysis of agonist binding (e.g. [24]). Indeed, plasticity is an innate part of GPCR function in signal transduction across the cellular membrane, and large-scale changes have been observed in the intracellular G-protein interaction site [25,26]. Substantial rearrangements were also expected in the ligand-binding pocket, where rotations of the transmembrane 6 (TM6) and TM7 helices, and aromatic side-chain rotamer switches (e.g. the W6.48 ‘toggle switch’) were proposed [27]. The complexity and magnitude of the proposed changes, and discrepancies between ligand binding poses, implied low accuracy of those early models based on distant homology with rhodopsin.

The new platform for modeling of ligand binding was established in 2007 by crystal structures of the inactive β_2 AR–carazolol complex [5]. Studies, based on this high-resolution platform, suggested that agonist-dependent changes in the pocket could be rather limited, yet specific and predictable [17,28]. Moreover, these structure-based models with backbone flexibility suggested specific triggers for receptor activation, explaining the structural basis of full, partial and inverse agonism in the β_2 AR [17]. Agonist binding to the A_{2A} AR was also modeled [18,29] based on its crystal structure in inactive form [9], although in this case flexibility was limited to the pocket side-chains. Here we compare these blindly predicted models with the recently determined crystal structures of agonist-bound complexes of β_2 AR [30,31], β_1 AR [32], and A_{2A} AR [33,34]. The results demonstrate the utility of structure-based conformational modeling in the analysis of ligand-dependent functional plasticity of the GPCR binding pocket, as well as in providing a 3D framework for rational drug discovery.

Details of β_2 AR agonist binding revealed by modeling and crystallography

Early biochemical and mutagenesis studies of the β_2 AR established two major anchor interactions for full agonists, in which common amino groups form a salt bridge with the Asp113^{3.32} carboxyl [35], whereas the polar groups of catechol or similar moiety interact with serine side-chains in TM5 [36,37]. Other details of agonist interactions and agonist-induced changes in the β_2 AR pocket were also proposed based on modeling interpretation of experimental data, such

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Opinion

as (i) a rotamer switch in Trp6.48 and other aromatic residues ('rotamer toggle switch' [38], (ii) 'up' orientation and polar interaction with Asn293^{6.55} for the β -OH group in agonists [39], (iii) direct interaction of the catechol group with Ser204^{5.43} in TM5; all of these specific changes require large rearrangements of TM6 and TM7 backbone in the binding pocket (e.g. reviewed in [27,40]).

Availability of the high-resolution inactive β_2 AR-carazolol structure in 2007 (PDB:2rh1) [5] provided a new structural platform and a new wave of interest in deciphering the mechanisms of agonist binding and activation in GPCRs (e.g. reviewed in [41]). Some of the new models published as recently as 2011 reproduced the early concepts, including rotamer toggle switch and β -OH contact with Asn293^{6.55} [42,43], and/or introduced other large-scale motions in the pockets such as 60° axial rotation of TM5 [44]).

Conversely, other docking and modeling studies supported much smaller changes in the inactive crystal structure of the β_2 AR upon agonist binding [45]. Thus, the initial agonist-induced fit can be modeled by changing only rotameric states of serine side-chains in TM5 [16,17,28], although these changes were thought to represent a low-affinity binding mode of agonists. More comprehensive analysis of β_2 AR ligand binding in flexible β_2 AR models performed in 2008 also identified a finite ~ 2 Å inward shift of TM5 as a requirement for optimal ('high-affinity') binding of full agonists [17]. This study used energy-based docking [46,47] in an atomistic model of the β_2 AR with fully-flexible side-chains and limited backbone flexibility in TM5 domain. A physics-based energy function and highly efficient biased-probability Monte Carlo (BPMC) procedure [48] in internal coordinates [49] allowed global convergence of the conformational sampling. Importantly, the procedure did not use ligand-receptor restraints, allowing the model to naturally sample possible ligand-receptor contacts. A similar TM5 shift was also obtained later by Vilar *et al.* [50] in a 2011 study that used induced-fit docking and molecular dynamics with backbone flexibility expanded to all TM helices. Although supporting the two anchor interactions for full agonists (with Asp113^{3.32} and TM5 serines), modeling results in [16,17,50] questioned the other important details of agonist binding and proposed alternative interpretations of experimental data (Figure 1). Thus, the β -OH moiety of the ethanolamine 'tail' of agonists was found consistently docked into the same anchor site between Asp113^{3.32} and Asn312^{7.39} as carazolol and other inverse agonists and antagonists [8]; the role of Asn293^{6.55} in agonist stereoselectivity [39] was explained by interactions with the catechol ring instead of direct contact with β -OH. Also, optimal conformations of agonists did not require rotamer 'toggle switch' [38] in Trp286^{6.48} or other aromatic residues in the pocket, suggesting similar contacts of the ligand aromatic 'core' across all classes of high-affinity β_2 AR ligands.

At the same time, the key difference between the binding of inverse agonists and full agonists was explained in these models by a finite inward shift and positional restraint of the TM5 extracellular portion. The TM5 shift in the model occurred as a consequence of relatively high

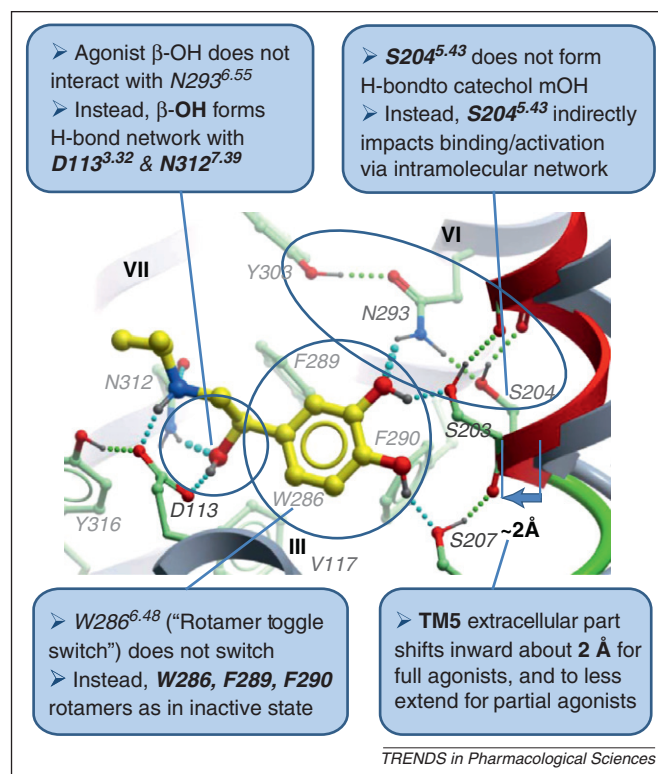


Figure 1. Key new features of full agonist isoproterenol binding and conformational changes in the β_2 AR ligand-binding pocket predicted in 2008 by conformational modeling based on crystal structure of inactive structure of the β_2 AR-carazolol complex (PDB:2rh1). In this published model (supplementary information in [17]) the ligand is shown with yellow carbons; the contact residues of the β_2 AR are shown with green carbons. The shifted TM5 helix (red and green ribbon) is compared to the original backbone in the crystal structure (grey). Predicted hydrogen bonds are shown by cyan (ligand-receptor) and green (intramolecular) spheres.

conformational freedom in this domain and strong hydrogen bonding between the ligand catechol 'head' and reoriented Ser203^{5.42} and Ser207^{5.46} side-chains. Unlike the other two serines in TM5, the Ser204^{5.43} side-chain did not make contact with catecholamine agonists, whereas its role in binding and activation was explained by polar interaction with Asn293^{6.55}, which helped to stabilize inward shift of TM5 helix (Figure 1). In the model the resulting pocket contraction between helices TM5 and TM3/TM7 allowed optimal engagement of both agonist ethanolamine tail and polar groups on the aromatic system with anchor sites of polar interactions, characteristic of the high-affinity binding state of β_2 AR-agonist complexes. Based on this modeling and existing structure-activity relationship (SAR) and mutagenesis data [36,37,51–55], the magnitude of the ligand-induced shift in TM5 was predicted to be one of the key determinants that distinguished between full and partial activation of the β_2 AR, whereas blocking of the inward TM5 movement provided an explanation for the reduction of basal activity by inverse agonists.

We were very pleased to find these modeling predictions to be largely validated by the recently published structures of β_2 AR and β_1 AR complexes with agonists [30,32]. Both β_2 AR and β_1 AR co-crystals show high precision of our binding-pocket models and a number of non-trivial details of ligand polar interactions with TM5 and TM6 side-chains (Figure 2). The accuracy of the predictions for agonist

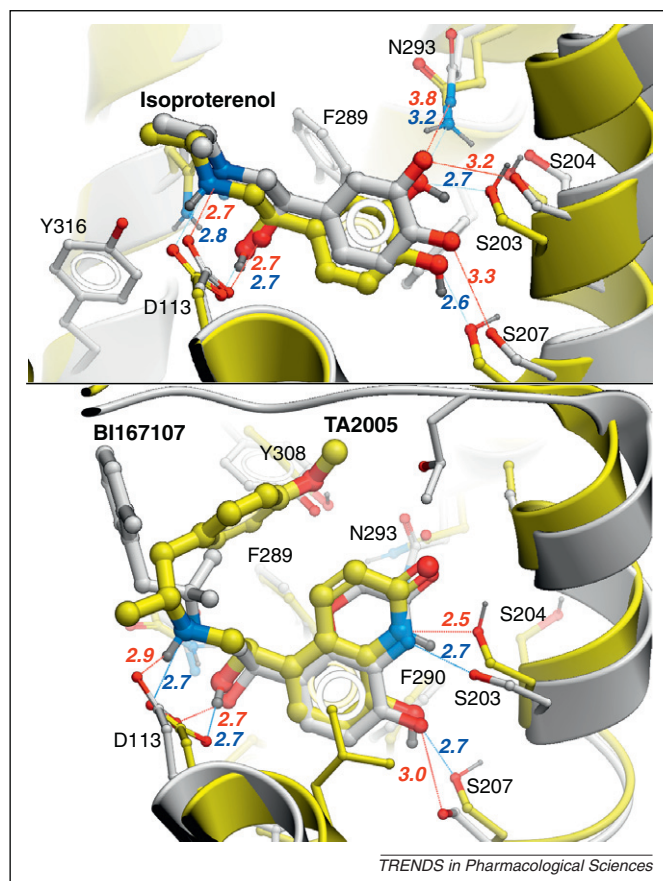


Figure 2. Accuracy of agonist- β_2 AR binding predictions. Models predicted in [17] are shown with yellow carbons and blue polar-interaction distances, whereas crystal structures are shown with grey carbon atoms and red interaction distances. (a) Comparison of agonist-receptor interactions in the energy-optimized isoproterenol- β_2 AR model and the crystal structure of isoproterenol complex with the closely related β_1 AR (PDB: 2Y03) which has identical residues of the binding pocket. The model correctly predicted all ligand-receptor polar interactions and hydrophobic contacts (100% identical contacts at 4 Å cutoff). Agonist poses have RMSD = 0.8 Å, whereas contact side-chains RMSD = 1.1 Å between predicted and crystal structure. Note that in the crystal structure the β_1 AR is in the inactive state, with hydrogen bonds still having slightly suboptimal length. Further minor inward adjustment of TM5 position and a corresponding slight shift of ligand that are likely to occur upon activation of the receptor should improve model/structure RMSD even further. (b) Comparison of ligand-receptor interactions between the energy-optimized model of TA2005- β_2 AR and the crystal structure of the BI-167107- β_2 AR complex in nanobody-stabilized active state. Similar bicyclic and ethanolamine moieties of these full agonists have RMSD = 0.5 Å, and binding pocket contact side-chains RMSD = 0.9 Å. The phenol-containing 'tail' of BI-167107 does not have polar groups as in TA2005, and this could explain the differences in this part of the complex.

binding poses RMSD (root mean square deviation) = 0.8 Å and 0.5 Å for two full agonist models) and contact side-chains (RMSD = 1.1 Å and 0.9 Å respectively) is comparable with those expected for crystal structures in the Protein Databank (PDB) [56]. Moreover, the crystal structure of activated state agonist- β_2 AR complex [30] (PDB code 3P0Q) reveals contraction of the pocket via 2.1 Å inward shift of TM5 top part above conserved Pro211^{5,50}, similar to the 2.0 Å TM5 shift in our blind predictions (compare Figure 4 in ref [17] and Figure 4a in ref [30]). Although the activated β_2 AR structure show that movements in TM3 (Asp113^{3,32}) and TM7 (Asn312^{7,3}) also contribute to this compaction, both do not exceed 0.4 Å [30].

Note that our flexible docking study [17] did not attempt to model the downstream activation-related changes in the

receptor intracellular half, which for modeling purposes were considered to be decoupled from immediate ligand-induced changes in the pocket. Such decoupling is not only a practical trick to focus modeling on the binding pocket itself, but it may partially reflect the crystallographic behavior of some GPCR-agonist systems [32], as discussed below.

Adenosine A_{2A} agonist binding predictions

Adenosine A_{2A} receptor (A_{2A}AR) modeling studies [18,29] provide another example of successful modeling of agonist binding based on an inactive GPCR structure [9]. Our study [18] combined mutagenesis experiments with theoretical predictions, where the agonist NECA (5'-N-ethyl-carboxamido adenosine) and its C2 substitute CGS21680 were docked into the crystal structure with subsequent refinement of the pocket side-chains. As in the case of the β_2 AR, the predicted A_{2A}AR agonist docking poses suggested some interactions that are common for agonist and antagonist binding, including aromatic ring and exocyclic amine core contacts, as well as distinct new interactions specific for ribose moiety of agonists. Although initial rigid docking gave an ambiguous binding pose with suboptimal polar interactions, the energy-based ligand-guided refinement of the binding pocket made possible selection of one optimal pose of the ribose ring, as shown in Figure 3. The refinement also made possible prediction of the binding-energy changes for several mutations in the pocket, and this turned out to be in qualitative agreement with experimental assays. Similar binding poses and orientation of the ribose ring were predicted independently by Ivanov *et al.* for NECA and the endogenous ligand adenosine [29].

The first agonist-bound A_{2A}AR crystal structures have been determined very recently, thanks to identification of a conformationally selective agonist UK-432097 in one case [33] and extensive mutagenesis screening in the other [34], both helping to stabilize A_{2A}AR in activated form. Despite the differences in ligands and receptor stabilization technologies, the position and receptor contacts for the common adenosine scaffold for UK-432097 (PDB:3QAK), NECA (PDB:2YDO) and adenosine (2YDV) in these three structures is essentially identical (RMSD < 0.5 Å). Comparison of this common scaffold with the A_{2A}AR-NECA models shows close similarity of the predicted poses overall and in the ribose ring specifically, with RMSD = 1.7 Å for [18], 1.9 for [29], and 0.9 Å between the two independent NECA models in these studies. Models in both studies predicted all major polar interactions of the ribose rings, including H-bonds with His278^{7,43}, Ser277^{7,42} and Thr88^{3,36}, although the latter interaction had an apparently suboptimal 4.5–4.7 Å donor-acceptor distance [33].

The difference in agonist position between the crystal structure of the activated A_{2A}AR and the models can largely be explained by a systematic ligand shift by about 1.4–1.5 Å. Unlike β_2 AR-agonist modeling described above [17], the preliminary modeling of A_{2A}AR-agonist binding [18,29] did not attempt to include backbone changes in conformational refinement of the complex. As is now apparent from the crystal structures, more accurate positioning of the ligand and formation of optimal H-bond with

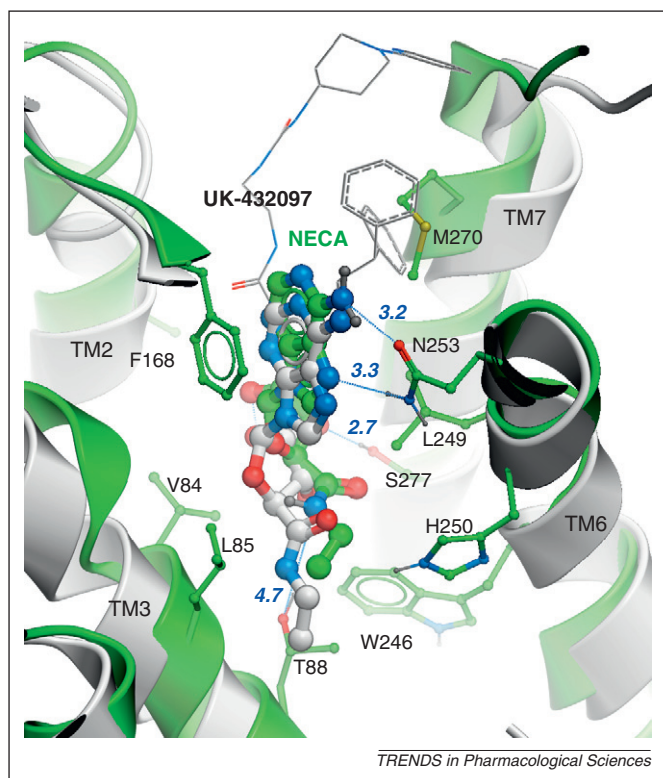


Figure 3. Agonist binding to the adenosine A_{2A} receptor. Binding predicted in the model of the NECA- A_{2A} AR complex [18] is shown with green carbons; the UK-432097 agonist and protein backbone of crystal structure [33] (PDB: 3QAK) are shown in grey. For clarity, bulky C2 and N6 substitutions UK-432097 are shown by thin lines. Key polar interaction are shown by blue lines and distances in Å. Comparison of common substructures of the two agonists shows predictions of NECA binding pose with RMSD 1.7 Å, which reproduces most polar interactions for the ribose ring of the agonists. At the same time, the NECA molecule in the model is systematically shifted upwards, and this precludes optimal interaction of its amide group with the Thr88^{3,36} side-chain. Adjustment of the ligand into its optimal position and engagement of all polar interactions requires a shift of the conserved Trp246^{6,48} side-chain, which is a part of the activation mechanism in the A_{2A} AR and some other GPCRs.

Thr88^{3,36} would be impossible without specific changes in the receptor backbone. These changes involve shifting of the Trp246^{6,48} residue that allow agonists to settle into position slightly deeper in the binding pocket, and also the upward shift of TM3 helix along its axis that brings the Thr88^{3,36} hydroxyl into optimal contact with the amino substituent in the ribose ring.

What can we learn about activation mechanisms from modeling?

As illustrated by the β_2 AR [16,17,28,50] and A_{2A} AR [18,29] examples, conformational modeling based on inactive GPCR structures and available biochemical data can predict agonist binding poses with accuracies in some cases comparable to crystal structures. In both receptors, initial docking of agonists into inactive receptor conformations was accommodated by only minor changes in the pocket side-chains; such models may correspond to a low-affinity ligand-binding state of the receptor. In the β_2 AR case [17,50], modeling was also successful in predicting contraction of the pocket via movement of TM5 helix (1–2 Å), which corresponds to a high-affinity binding state, very similar to those found later in the activated crystal structure of the β_2 AR [30]. Of course, the accuracy of the

conformational modeling depends on the range of agonist-induced movements in the pocket, especially in the protein backbone. Although both currently available structure-validated examples support a limited range of agonist-induced pocket changes and predictability of agonist binding, further theoretical and structural studies will be required to test this hypothesis for other GPCR subfamilies.

Whereas the above modeling studies focused on ligand binding and changes in the extracellular pocket, they did not attempt to model coupling of the ligand-induced changes with activation-related downstream rearrangements in G-protein binding site. Such downstream changes, including dynamics of ionic lock and other ‘microswitches’, have been the subject of several other modeling studies (e.g. [41,57]). The nature of the downstream changes has been also revealed by active-like ligand-free opsin structures [26,58] and all-*trans* retinal rhodopsin [59], and most recently by structures of activated A_{2A} AR [33] and β_2 AR in complex with G protein [60] or its surrogate [30]. The consensus changes for all three activation models on the intracellular side include inward shift of TM7 and a concerted movement of TM5 and TM6, where the latter swings outward to open a crevice for insertion of $G\alpha$ terminus. Interestingly, soft coupling between (1) initial ligand binding, (2) small extracellular and (3) large downstream changes is not only a convenient approximation useful in modeling of agonist-receptor interactions described here [17,18,28], but also appears to reflect the allosteric nature of the activation mechanism in GPCRs [61]. Thus, recent crystal structures captured a conformation of covalently-bound full agonist FAUC50 in complex with β_2 AR that is indistinguishable from the inactive conformation [31], suggesting decoupling of stages 1 and 2. Another structure, that of thermostabilized β_1 AR complex with full agonist isoproterenol, shows a receptor with at least partial changes in the binding pocket (1 Å shift of TM5), but lacking any downstream changes in the intracellular part, showing partial decoupling of stages 2 and 3 [32].

In addition to these general trends, both modeling and crystallography suggest that immediate triggers of agonist-dependent activation can vary substantially between GPCRs; this would also be expected from the diversity of GPCR ligands and binding pockets. For example, direct polar interactions of TM5 serines with agonists and corresponding movements in the TM5 helix that were observed in the β_2 AR [31] were not found in the A_{2A} AR, where pocket contraction primarily results from agonist-mediated shift of TM7 towards TM3. Moreover, even within the adrenergic family, TM5 movement is unlikely to play such an important role, because many of these GPCRs lack polar groups in the corresponding positions of TM5 or TM7. Another type of trigger involves ligand steric ‘push’ on specific side-chains in the pocket as a result of the ligand adjusting its position to optimize interactions in the pocket. Comparison of inactive and activated A_{2A} AR, for example [33], shows that the ribose ring of agonists cannot fit optimally into the pocket without an approximately 2 Å movement of the conserved Trp6.48 side-chain in the ‘cWxP’ motif. By contrast, β_2 AR agonists do not make direct contact with the Trp6.48 side-chain, and its smaller movement may be indirectly promoted by ligand

TM5 shift via side-chain changes in Ile121^{3,40} and Phe282^{6,44}, as suggested in [30]. Note that the movements of Trp6.48 observed in all crystal structures, including the fully activated β_2 AR-G $\alpha\beta\gamma$ complex [60], do not actually involve ‘rotamer toggle switch’ in this residue [38]. Instead, the Trp6.48 side-chain moves together with the protein backbone, promoting partial unwinding of TM6 in the proline-induced kink, and a corresponding swing of the TM6 intracellular tip.

Some other movements of TM helices may depend on ligand choice for both active and inactive crystal forms, reflecting conformational (and potentially functional [62]) selectivity. Such an example can be found in the A_{2A}AR complex with agonist UK-432097, where the bulky C2 and N6 adenosine substituents of this compound apparently push on extracellular loop 3 (ECL3) and the top of the TM7 helix, promoting a see-saw movement of TM7. In some cases such consequences of ligand-specific motions may be possible to predict computationally, giving insight into the conformational and functional selectivity of ligands. In other cases, exemplified by concerted movement in TM5 and TM6 recently described in detail for the adrenergic receptor [30], molecular mechanisms connecting extracellular and intracellular changes can be surprisingly complex and less predictable. Moreover, better understanding of such details of functional selectivity may require further structural and biochemical studies of GPCR complexes with G-proteins [60] and other downstream effectors.

Applications to GPCR drug discovery

As the pace of GPCR structure determination improves, several highly encouraging examples demonstrate the utility of the crystal structures in the discovery of new high-affinity compounds as potential drug leads [20,21]. However, it is not clear if a standard structure-based drug-discovery paradigm, which requires routine co-crystallization of receptors with novel lead molecules, would work for GPCRs. Because of their intrinsic flexibility, GPCRs are preferentially crystallized with selected high-affinity stabilizing compounds, whereas crystallization with suboptimal leads requires elaborate point-mutation technologies to achieve sufficient stability [32,34,63]. Moreover, even within the same subfamily, some GPCR subtypes will be less amenable to crystallization, leaving desirable targets without crystal structures.

Conformational modeling is perfectly positioned to fill these significant gaps in structural knowledge. As the computational studies here suggest, energy-based conformational modeling can accurately predict binding poses and certain levels of ligand-induced fit in the binding pocket, including small shifts in protein backbone. Such refined models of GPCR-lead complexes will be of great value for rationalizing existing SAR data and can direct structure-guided design of substitutions for lead optimization.

Another potential application of GPCR binding-pocket models is virtual ligand screening (VLS) for novel chemotypes. This tool is relevant for many GPCR targets, given the somewhat limited chemical space explored to date by traditional drug-discovery methods such as HTS and ligand-based scaffold hopping. We show that models optimized for the binding of a specific ligand class, for example

agonist-optimized models, can be highly selective for this class in a large-scale virtual screening (VLS) benchmark [16]. This approach was recently developed into an automated iterative ligand-guided receptor optimization algorithm (LiBERO) [64,65] that generates an optimal VLS model conformation based on a defined set of known ligands. This tool was also used to develop optimal VLS models for all four adenosine receptor subtypes [23], suggesting the primary structural determinants of subtype selectivity in this family. In prospective screening the optimized A_{2A}AR VLS model demonstrated an exceptionally high hit-rate (40%), allowing discovery of a diverse and novel set of lead-like antagonists with as high as 60 nM affinity [21].

Note, that optimal VLS models can differ somewhat from individual ligand-receptor models – whereas the latter are optimized for binding of a specific ligand, the best VLS models represent a compromise between multiple ligand complexes. In some cases, different ligand-dependent receptor states can be clustered into two or more alternative VLS models, which can be used separately or in 4D screening procedures [66].

Concluding remarks

Firmly grounded in high-resolution crystal structures of inactive GPCRs and supported by biochemical data, conformational modeling can be a practical tool helping to shape the emerging concept of activation mechanisms in adrenergic and potentially other GPCRs. The modeling studies of agonist-GPCR complexes and later crystal structures have detailed small but functionally important changes in the binding pocket allosterically coupled with larger downstream changes in the intracellular side of the receptor. Understanding the remarkable diversity of ligand-dependent activation triggers can further benefit from comprehensive modeling of different ligand classes, which takes into account side-chain and small backbone deviations in the binding pocket. As crystallography is yielding more structures of GPCRs, including first examples of active state GPCRs, this type of modeling will become even more accurate and reliable.

Although important insights into agonist binding and induced changes can be gained without explicit modeling of downstream changes, understanding GPCR signaling requires establishing the whole path from ligand binding to downstream activation of G-protein or other effectors. Recent crystal structures of the activated β_2 AR- and A_{2A}AR-agonist complexes provide some details of such connections as a first step towards establishing a reliable 3D framework for computational analysis of ligand structural and functional selectivity in these and other GPCRs.

Other extensions of computational approaches involve a combination of agonist modeling with GPCR subtype modeling [23], helping to generate a comprehensive picture of subtype and functional selectivity for whole GPCR subfamilies. Moreover, accumulation of experimental data on allosteric modulators and crystal structures of GPCRs will help to expand accurate conformational modeling to this important class of candidate drugs and their binding sites [67]. Of course in each case it will be vital to establish validation of the models wherever

possible through extensive ligand-based benchmarking [21], biochemical studies and crystallography.

References

- Tyndall, J.D. and Sandilya, R. (2005) GPCR agonists and antagonists in the clinic. *Med. Chem.* 1, 405–421
- Overington, J.P. *et al.* (2006) How many drug targets are there? *Nat. Rev. Drug Discov.* 5, 993–996
- Lagerstrom, M.C. and Schioth, H.B. (2008) Structural diversity of G protein-coupled receptors and significance for drug discovery. *Nat. Rev. Drug Discov.* 7, 339–357
- Smith, N.J. *et al.* (2011) When simple agonism is not enough: emerging modalities of GPCR ligands. *Mol. Cell. Endocrinol.* 331, 241–247
- Cherezov, V. *et al.* (2007) High-resolution crystal structure of an engineered human beta2-adrenergic G protein-coupled receptor. *Science* 318, 1258–1265
- Rosenbaum, D.M. *et al.* (2007) GPCR engineering yields high-resolution structural insights into beta2-adrenergic receptor function. *Science* 318, 1266–1273
- Warne, T. *et al.* (2008) Structure of a beta-adrenergic G-protein-coupled receptor. *Nature* 454, 486–491
- Wacker, D. *et al.* (2010) Conserved binding mode of human beta2 adrenergic receptor inverse agonists and antagonist revealed by X-ray crystallography. *J. Am. Chem. Soc.* 132, 11443–11445
- Jaakola, V.P. *et al.* (2008) The 2.6 angstrom crystal structure of a human A2A adenosine receptor bound to an antagonist. *Science* 322, 1211–1217
- Wu, B. *et al.* (2010) Structures of the CXCR4 chemokine GPCR with small-molecule and cyclic peptide antagonists. *Science* 330, 1066–1071
- Chien, E.Y. *et al.* (2010) Structure of the human dopamine D3 receptor in complex with a D2/D3 selective antagonist. *Science* 330, 1091–1095
- Shimamura, T. *et al.* (2011) Molecular basis of antihistamine specificity against human histamine H1 receptor. *Nature* 475, 65–70
- Reynolds, K. *et al.* (2010) Structure and modeling of GPCRs: implications for drug discovery. In *GPCR Molecular Pharmacology and Drug Targeting: Shifting Paradigms and New Directions* (Gilchrist, A., ed.), pp. 385–433, Wiley & Sons
- Congreve, M. *et al.* (2011) Progress in structure based drug design for G protein-coupled receptors. *J. Med. Chem.* 54, 4283–4311
- Topiol, S. and Sabio, M. (2008) Use of the X-ray structure of the Beta2-adrenergic receptor for drug discovery. *Bioorg. Med. Chem. Lett.* 18, 1598–1602
- Reynolds, K.A. *et al.* (2009) Identifying conformational changes of the beta adrenoceptor that enable accurate prediction of ligand/receptor interactions and screening for GPCR modulators. *J. Comput. Aided Mol. Des.* 23, 273–288
- Katritch, V. *et al.* (2009) Analysis of full and partial agonists binding to beta2-adrenergic receptor suggests a role of transmembrane helix V in agonist-specific conformational changes. *J. Mol. Recognit.* 22, 307–318
- Jaakola, V.P. *et al.* (2010) Ligand binding and subtype selectivity of the human A(2A) adenosine receptor: identification and characterization of essential amino acid residues. *J. Biol. Chem.* 285, 13032–13044
- Sabio, M. *et al.* (2008) Use of the X-ray structure of the beta2-adrenergic receptor for drug discovery. Part 2: identification of active compounds. *Bioorg. Med. Chem. Lett.* 18, 5391–5395
- Kolb, P. *et al.* (2009) Structure-based discovery of beta2-adrenergic receptor ligands. *Proc. Natl. Acad. Sci. U.S.A.* 106, 6843–6848
- Katritch, V. *et al.* (2010) Structure-based discovery of novel chemotypes for adenosine A(2A) receptor antagonists. *J. Med. Chem.* 53, 1799–1809
- Carlsson, J. *et al.* (2010) Structure-based discovery of A2A adenosine receptor ligands. *J. Med. Chem.* 53, 3748–3755
- Katritch, V. *et al.* (2011) Structure based prediction of subtype-selectivity for adenosine receptor antagonists. *Neuropharmacology* 60, 108–115
- Kobilka, B. and Schertler, G.F. (2008) New G-protein-coupled receptor crystal structures: insights and limitations. *Trends Pharmacol. Sci.* 29, 79–83
- Altenbach, C. *et al.* (2008) High-resolution distance mapping in rhodopsin reveals the pattern of helix movement due to activation. *Proc. Natl. Acad. Sci. U.S.A.* 105, 7439–7444
- Scheerer, P. *et al.* (2008) Crystal structure of opsin in its G-protein-interacting conformation. *Nature* 455, 497–502
- Schwartz, T.W. *et al.* (2006) Molecular mechanism of 7TM receptor activation – a global toggle switch model. *Annu. Rev. Pharmacol. Toxicol.* 46, 481–519
- de Graaf, C. and Rognan, D. (2008) Selective structure-based virtual screening for full and partial agonists of the beta2 adrenergic receptor. *J. Med. Chem.* 51, 4978–4985
- Ivanov, A.A. *et al.* (2009) Evaluation of homology modeling of G-protein-coupled receptors in light of the A(2A) adenosine receptor crystallographic structure. *J. Med. Chem.* 52, 3284–3292
- Rasmussen, S.G. *et al.* (2011) Structure of a nanobody-stabilized active state of the beta adrenoceptor. *Nature* 469, 175–180
- Rosenbaum, D.M. *et al.* (2011) Structure and function of an irreversible agonist-beta adrenoceptor complex. *Nature* 469, 236–240
- Warne, T. *et al.* (2011) The structural basis for agonist and partial agonist action on a beta-adrenergic receptor. *Nature* 469, 241–244
- Xu, F. *et al.* (2011) Structure of an agonist-bound human A2A adenosine receptor. *Science* 332, 322–327
- Lebon, G. *et al.* (2011) Agonist-bound adenosine A(2A) receptor structures reveal common features of GPCR activation. *Nature* 474, 521–525
- Strader, C.D. *et al.* (1988) Conserved aspartic acid residues 79 and 113 of the beta-adrenergic receptor have different roles in receptor function. *J. Biol. Chem.* 263, 10267–10271
- Strader, C.D. *et al.* (1989) Identification of two serine residues involved in agonist activation of the beta-adrenergic receptor. *J. Biol. Chem.* 264, 13572–13578
- Liapakis, G. *et al.* (2000) The forgotten serine. A critical role for Ser-203S.42 in ligand binding to and activation of the beta2-adrenergic receptor. *J. Biol. Chem.* 275, 37779–37788
- Shi, L. *et al.* (2002) Beta2 adrenergic receptor activation. Modulation of the proline kink in transmembrane 6 by a rotamer toggle switch. *J. Biol. Chem.* 277, 40989–40996
- Wieland, K. *et al.* (1996) Involvement of Asn-293 in stereospecific agonist recognition and in activation of the beta 2-adrenergic receptor. *Proc. Natl. Acad. Sci. U.S.A.* 93, 9276–9281
- Kobilka, B.K. *et al.* (2007) G protein coupled receptor structure and activation. *Biochim. Biophys. Acta* 1768, 794–807
- Nygaard, R. *et al.* (2009) Ligand binding and micro-switches in 7TM receptor structures. *Trends Pharmacol. Sci.* 30, 249–259
- Simpson, L.M. *et al.* (2011) Modeling GPCR active state conformations: the beta-adrenergic receptor. *Proteins* 79, 1441–1457
- Vanni, S. *et al.* (2011) Predicting novel binding modes of agonists to beta adrenergic receptors using all-atom molecular dynamics simulations. *PLoS Comput. Biol.* 7, e1001053
- Bhattacharya, S. *et al.* (2008) Ligand-stabilized conformational states of human beta adrenergic receptor: insight into G-protein-coupled receptor activation. *Biophys. J.* 94, 2027–2042
- Poopot, C. *et al.* (2009) Structural basis of the selectivity of the beta-adrenergic receptor for fluorinated catecholamines. *Bioorg. Med. Chem.* 17, 7987–7992
- Totrov, M. and Abagyan, R. (1997) Flexible protein-ligand docking by global energy optimization in internal coordinates. *Proteins (Suppl. 1)*, 215–220
- Abagyan, R.A. *et al.* (2011) In *ICM Manual (V. 3.6)* MolSoft LLC
- Abagyan, R. and Totrov, M. (1994) Biased probability Monte Carlo conformational searches and electrostatic calculations for peptides and proteins. *J. Mol. Biol.* 235, 983–1002
- Abagyan, R.A. *et al.* (1994) Icm: a new method for protein modeling and design: applications to docking and structure prediction from the distorted native conformation. *J. Comp. Chem.* 15, 488–506
- Vilar, S. *et al.* (2011) *In silico* analysis of the binding of agonists and blockers to the beta2-adrenergic receptor. *J. Mol. Graph. Model.* 29, 809–817
- Ambrosio, C. *et al.* (2000) Catechol-binding serines of beta-adrenergic receptors control the equilibrium between active and inactive receptor states. *Mol. Pharmacol.* 57, 198–210
- Liapakis, G. *et al.* (2004) Synergistic contributions of the functional groups of epinephrine to its affinity and efficacy at the beta2 adrenergic receptor. *Mol. Pharmacol.* 65, 1181–1190
- Sato, T. *et al.* (1999) Ser203 as well as Ser204 and Ser207 in fifth transmembrane domain of the human beta2-adrenoceptor contributes to agonist binding and receptor activation. *Br. J. Pharmacol.* 128, 272–274

- 54 Hannawacker, A. *et al.* (2002) Mutation of Asn293 to Asp in transmembrane helix VI abolishes agonist-induced but not constitutive activity of the beta-adrenergic receptor. *Mol. Pharmacol.* 62, 1431–1437
- 55 Xhaard, H. *et al.* (2006) Molecular evolution of adrenoceptors and dopamine receptors: implications for the binding of catecholamines. *J. Med. Chem.* 49, 1706–1719
- 56 Kufareva, I. *et al.* (2011) Status of GPCR modeling and docking as reflected by community wide GPCR Dock 2010 assessment. *Structure* 19, 1108–1126
- 57 Dror, R.O. *et al.* (2009) Identification of two distinct inactive conformations of the beta2-adrenergic receptor reconciles structural and biochemical observations. *Proc. Natl. Acad. Sci. U.S.A.* 106, 4689–4694
- 58 Park, J.H. *et al.* (2008) Crystal structure of the ligand-free G-protein-coupled receptor opsin. *Nature* 454, 183–187
- 59 Standfuss, J. *et al.* (2011) The structural basis of agonist-induced activation in constitutively active rhodopsin. *Nature* 471, 656–660
- 60 Rasmussen, S.G. *et al.* (2011) Crystal structure of the beta adrenergic receptor-Gs protein complex. *Nature* DOI: 10.1038/nature10361 [Epub ahead of print]
- 61 Changeux, J.P. and Edelstein, S.J. (2005) Allosteric mechanisms of signal transduction. *Science* 308, 1424–1428
- 62 Kenakin, T. *et al.* (2010) Seven transmembrane receptors as shapeshifting proteins: the impact of allosteric modulation and functional selectivity on new drug discovery. *Pharmacol. Rev.* 62, 265–304
- 63 Warne, T. *et al.* (2009) Development and crystallization of a minimal thermostabilised G protein-coupled receptor. *Protein Expr. Purif.* 65, 204–213
- 64 Katritch, V. *et al.* (2010) GPCR 3D homology models for ligand screening: lessons learned from blind predictions of adenosine A2a receptor complex. *Proteins* 78, 197–211
- 65 Katritch, V. *et al.* Ligand Guided Receptor Optimization. In *Homology Modeling: Methods and Protocols*. (Orry A. and Abagyan, R. eds), Springer (in press).
- 66 Bottegoni, G. *et al.* (2009) Four-dimensional docking: a fast and accurate account of discrete receptor flexibility in ligand docking. *J. Med. Chem.* 52, 397–406
- 67 Keov, P. *et al.* (2011) Allosteric modulation of G protein-coupled receptors: a pharmacological perspective. *Neuropharmacology* 60, 24–35