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GPCR structures in drug design, emerging opportunities with new structures

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ABSTRACT

In recent years, GPCR targets from diverse regions of phylogenetic space have been determined. This effort has culminated this year in the determination of representatives of all major classes of GPCRs (A, B, C, and F). Although much of the now well established knowledge on GPCR structures has been known for some years, the new high-resolution structures allow structural insight into the causes of ligand efficacy, biased signaling, and allosteric modulation. In this digest the structural basis for GPCR signaling in the light of the new structures is reviewed and the use of the new non-class A GPCRs for drug design is discussed.

Cellular signal transduction pathways are canonical points for drug interaction, where whole downstream signaling cascades can be manipulated by interfering with a single target. Signal transduction interfaces between two compartments, such as the cell exterior and interior, provide an especially well suited point for signal regulation due to the spatial separation of ligand interaction site and effect. With about 850 members G-protein coupled receptors (GPCRs) form the largest human superfamily of receptors,[1] being responsible for signal transduction from the cytosol to the cell interior. Out of this number about 350 receptors are potentially druggable[2] although for about 100 so called orphan receptors[3] neither the endogenous ligand nor the physiological function is yet known. The opportunity to have a molecular switch at hand, which triggers a signaling cascade in the cell interior, and in addition the heterogeneous distribution of GPCRs in different tissues, makes them a highly interesting pharmaceutical class of targets.[4] It has been estimated, that about 50% of all modern drugs act on GPCRs. Thereby documenting that this class of receptors has been a successful target for the pharmaceutical industry.[5]

As their function implicates, GPCRs are transmembrane proteins, which makes structure elucidation much more difficult than for globular proteins. Until the structure of bovine rhodopsin was solved[6] in 2000 no X-ray structure of any GPCR was available. Although being a huge step forward in terms of structural understanding of GPCRs, the rhodopsin structure itself was only of limited help for conventional drug design on pharmaceutically more relevant GPCRs. It took more than seven years until β2 and β1 adrenergic receptors (β2R and β1R) were solved employing new receptor stabilization and crystallization techniques,[7–9] opening the door to a strongly accelerated elucidation frequency of new receptors. To date (June 2014) X-ray structures of 20 different class A, two class B, one class C, and one frizzled GPCR, spanning large sections of the phylogenetic tree, have been published.[10] This now gives pharmaceutical researchers the opportunity to use these structures for drug design purposes. Most especially, the very recent structures of non-class A GPCRs may serve as invaluable templates for ligand design for these very difficult target classes.

Although both classes are attractive drug target families, very few small molecule drugs targeting class B or C GPCRs are on the market. This reflects the impact of their apparently highly demanding binding sites, in terms of druggability. For class A receptors some structures with approved drugs are available. These include the complexes of: maraviroc in CCR5, tiotropium in M3 receptor, adenosine in A_{2A} receptor ($A_{2A}R$), carvedilol in β1R, doxepin in H1 receptor (H1R), ergotamine in 5-HT_{1B} as well as 5-HT_{2B} receptors, and voraxapar in the protease-activated receptor PAR-1. In addition to the new X-ray structures from classes B and C, a first structure of an agonist bound M2 receptor structure with allosteric modulator as well as two very high-resolution structures of $A_{2A}R$ and δ-opioid receptors (δ-OR) were recently reported, providing first insight into the allosteric regulation, biased signaling and solvent networks in GPCRs. These recent advances and their implication for drug design will be discussed in the following sections. Several recent reviews cover the use of GPCR structures in structure based drug design. For a non-exhaustive list please see references 11–13. Structure based drug design for GPCRs is currently in most cases limited to virtual screening[14] or to modeling a ligand of interest into a public domain X-ray structure of a GPCR

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(or homology model relying thereon). In very rare cases (co-)crystal structures with the compound of interest are generated. These studies correspond to the most preferred scenario for ligand optimization, because no uncertainties regarding the ligand binding pose and induced fit effects are envisioned. One of the few reported studies is the structure guided optimization of $A_{2A}R$ antagonists reported by Congreve et al.[15] In that study compounds discovered by virtual screening were optimized by use of their corresponding X-ray structures. At this time, most pharmaceutical companies do not have the facilities to crystallize GPCRs, therefore the emphasis of this digest will be on the use of public domain structures for drug design.

Insight from high-resolution structures: Recently the structures of $A_{2A}R$ and δ-OR[16,17] have been solved with a resolution of less than 2 Å. Although X-ray structures of both receptors have been reported before, only very high-resolution structures clarify the location of solvent molecules, as well the binding site of sodium ions. Although the two receptors stem from completely different subfamilies of GPCRs, the internal water network and sodium binding site are highly conserved. The metal binding site involves some of the known conserved motifs of class A GPCRs (such as the NPxxY motif on TM7, the WxP motif on TM6 as well as the N2.50 motif on TM2—TM stands for transmembrane helix), as shown in the overlay of the structures in Figure 1.

The current understanding is that sodium is an allosteric modulator of GPCRs[18] influencing the ability of β-arresting signaling, as discussed in the next chapter. The water network connecting the ligand binding site with the intracellular interface is very much conserved in the two high-resolution structures. In addition, also many other GPCR X-ray structures show a direct involvement of water molecules in ligand binding to the receptor. For example in $A_{2A}R$, β1R, CXCR4, δ-OR, κ-OR, PAR-1, and sphingosine-1-phosphate receptor 1 (S1P1) X-ray structures show water mediated ligand contacts to the receptor.[13] The high degree of involvement of water molecules in GPCR ligand binding makes rational ligand design without corresponding X-ray structure very difficult. This is because the structure activity relationships are greatly changed when a bridging water entity is replaced. The knowledge that water location is crucial in GPCRs has led to studies which explicitly investigate the thermodynamic properties of water molecules in GPCR X-ray structures to answer the question: Can specific water molecules can be replaced by ligand atoms?[19]

Ligand bias: In recent years, the awareness of the importance of different signaling capabilities of various ligands through GPCRs has steadily grown. One GPCR can signal through multiple different downstream pathways. Beyond the name-giving signaling through G-proteins, signaling through β-arrestin or direct signaling through receptor associated kinases is known[20,21] Ligands can activate different signaling pathways to differing extents ('ligand biased signaling'), where current insight suggests that the different pathways are decoupled.[22] The understanding of the consequences of different signaling behavior is crucial for drug design, especially as it has been assumed that ligand bias can be exploited to design superior next-generation drugs, for example, for heart failure[23] or antipsychotics.[24]

Although the different signaling pathways have been known for some time, ligand features or receptor behavior leading to signaling-biased receptor states have not been fully elucidated. The current understanding of the differences of G-protein and β-arrestin signaling is that activation of either pathway results from different conformational changes to the receptor.[22] The signaling through G-proteins works via association of a GTP-loaded G-protein at the TM3–TM5–TM6 interface of the respective GPCR, while β-arrestin is proposed to dock at the spatially distinct TM1–TM2–TM7

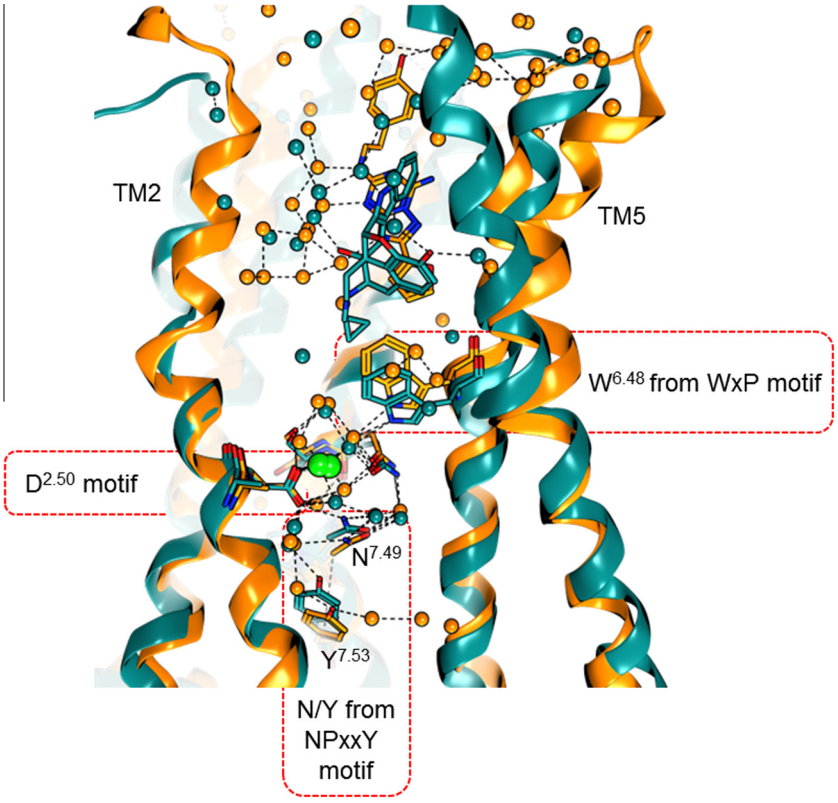

Figure 1. Overlay of the two available very high-resolution GPCR structures of $A_{2A}R$ (orange, PDB code: 4EIY) and δ-OR (cyan, PDB code: 4N6H). The water network around the conserved sodium (green spheres) binding site involves three of the well-known conserved motifs in class A GPCRs. A large water network is also seen in the binding pocket of both ligands, mediating ligand interactions with the receptor. (The residue numbering corresponds to the Weinstein-Ballesteros nomenclature for class A GPCRs.)

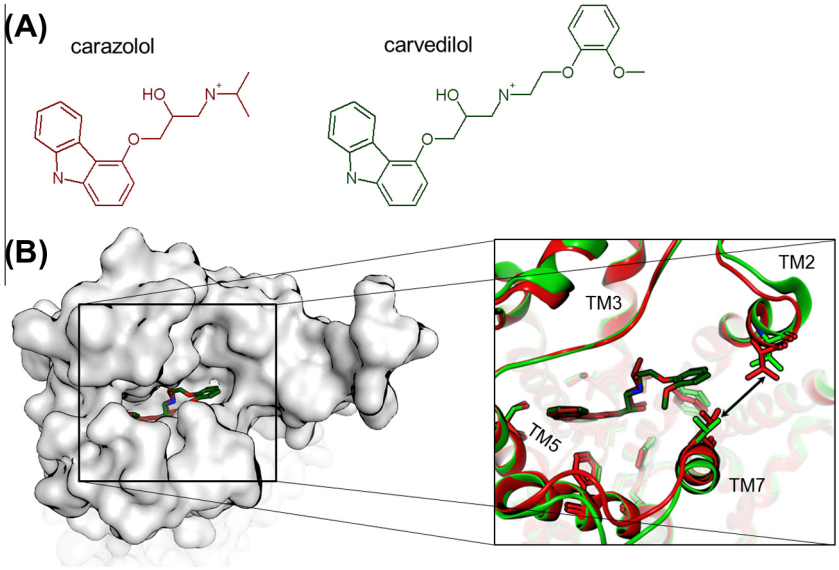

Figure 2. (A) structures of carazolol (inverse agonist) and carvedilol (biased ligand, agonist for β-arrestin, antagonist for G-protein pathway). (B) Extracellular view of the overlay of X-ray structures of β1 bound with carazolol (red, 2YCW) and carvedilol (green, 4AMJ). The biased ligand carvedilol (dark green) induces a significantly increased distance (2 Å) between TM2 and TM7.

interface. From a drug design point of view the more important question is the interaction of biased ligands with the respective receptors. For class A receptors it has been shown that ligands which activate the G-protein pathway trigger the conformational switch of two interacting side chains on TM3 (3.40) and TM6 (6.44) just below the generic binding site.[25] This results in a straightening of TM3, a kinking of TM6 and the opening of the G-protein binding site at the intracellular surface of the receptor. For β-arrestin signaling no β-arrestin bound activated structures are available yet, but the collected evidence points towards the involvement of the TM1–TM2–TM7 water network/sodium binding site in the activation process. Disrupting the sodium network in δ-OR induces high levels of constitutive activity in β-arrestin signaling, while completely shutting down basal G-protein signaling.[16] Interaction of the ligand with TM7 is postulated to modulate β-arrestin signaling.[22] Structures of β1R with biased ligands support this view,[26] as some small structural variations in ligand binding are seen close to TM2 and TM7. A supportive example for the ability to modulate the signaling efficacy of β1R ligands is shown in Figure 2. The size extension of the inverse agonist carazolol towards TM7 results in carvedilol, which is an antagonist for G-protein signaling, but an agonist for the β-arrestin

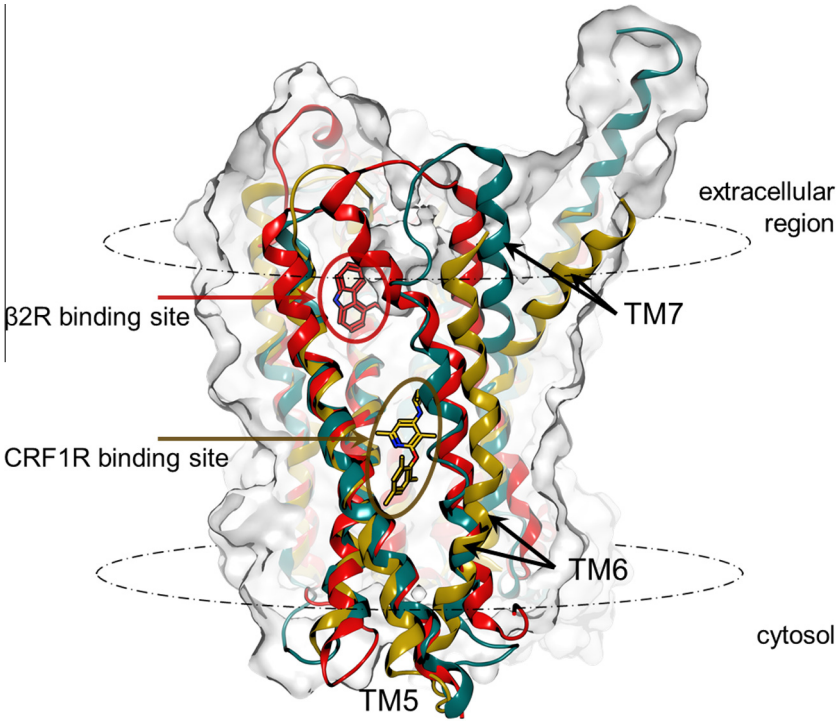

Figure 3. Overlay of the class B structures (CRF1R in gold and GCGR in cyan) with β2R (2RH1 in red).

pathway.[27] The overlay of the β1R crystal structures bound to these two ligands shows distinct differences in the position of TM2 and TM7. This example shows how ligand bias can be introduced by quite simple chemical modifications. To investigate the problem from a receptor point of view, the two serotonin receptors 5-HT_{1B} and 5-HT_{2B} have been crystallized both with the same ligand bound.[28,29] Ergotamine is a 'conventional' full agonist on the 5-HT_{1B} receptor, activating both signaling pathways, but it is a biased agonist on the 5-HT_{2B} receptor strongly favoring the β-arrestin pathway. Comparison of the structures confirms the current hypothesis of the conformational switch of residues 3.40 and 6.44 for G-protein activation and a conformational change in TM7 for β-arrestin activation.[28]

However, it is currently not possible to predict the efficacy of ligands a priori,[30] therefore only compound synthesis and biological testing will definitely shed light on the actual signaling behavior of each ligand. Several examples have now shown that very minor modifications can turn antagonists into activators,[31] even allowing antagonist design starting from known agonists.[32] To wrap up, through the latest class A GPCR structures a deeper understanding of GPCR activation and biased signaling has been achieved, giving medicinal chemists ideas to develop SAR in either pathway direction. The prerequisite for biased drug discovery is a clear biological rationale about which pathway of a GPCR has to be (de)activated in order to gain the optimal effect in the targeted disease.[33]

Class B and class C GPCRs: Since the solution of β2 in 2007[9] nearly 20 different class A GPCRs in various activation states have been solved before the first non-class A structures emerged with the smoothened receptor (class F),[34] glucagon receptor (GCGR, class B),[35] the corticotropin releasing factor 1 receptor (CRF1R, class B),[36] and very recently the metabotropic glutamate receptor 1 (mGluR1, class C).[37] Receptors from these classes are very attractive drug-targets, but it has proven to be very hard to find small molecule drugs which can make it to the market. For class B, the secretin receptors, only peptide drugs are on the market, although strong efforts were directed in finding small molecules.[38] The endogenous ligands for class B GPCRs are mid-size peptide hormones, interacting with the GPCR over a large protein-protein interaction surface. Finding drugs to interrupt such interactions often proves to be quite demanding, especially the identification of the actual interaction site of the ligand with the receptor is hard to determine without X-ray crystallography. For class C with baclofen (competitively targeting the $GABA_B$ receptor) and cinacalcet (allosterically targeting the calcium sensing receptor) two drugs are registered, however for the family of metabotropic glutamate receptors no drugs are approved so far, although these targets are identified as highly rewarding drug targets for allosteric modulation.[39]

Class B. Although the non-class A GPCRs do not share the well-known conserved class A motifs, the overall fold and the interhelical interaction networks are strongly conserved.[38] TM helices 1–5 of the solved class B receptors are in better structural agreement with the class A counterparts than TMs 6 and 7. The largest difference of class B structures in comparison to class A is the very deep and open V-shaped crevice. Figure 3 shows a comparison of a prototypical class A receptor (β2R) with the two class B receptors.

In addition to the larger crevice, the location of the antagonist binding site in CRF1R is very surprising, being much deeper buried in the helical bundle than in all known class A cases (shifted more than 10 Å further towards the cytosol). As already mentioned, the arrangements of the transmembrane helices is well conserved

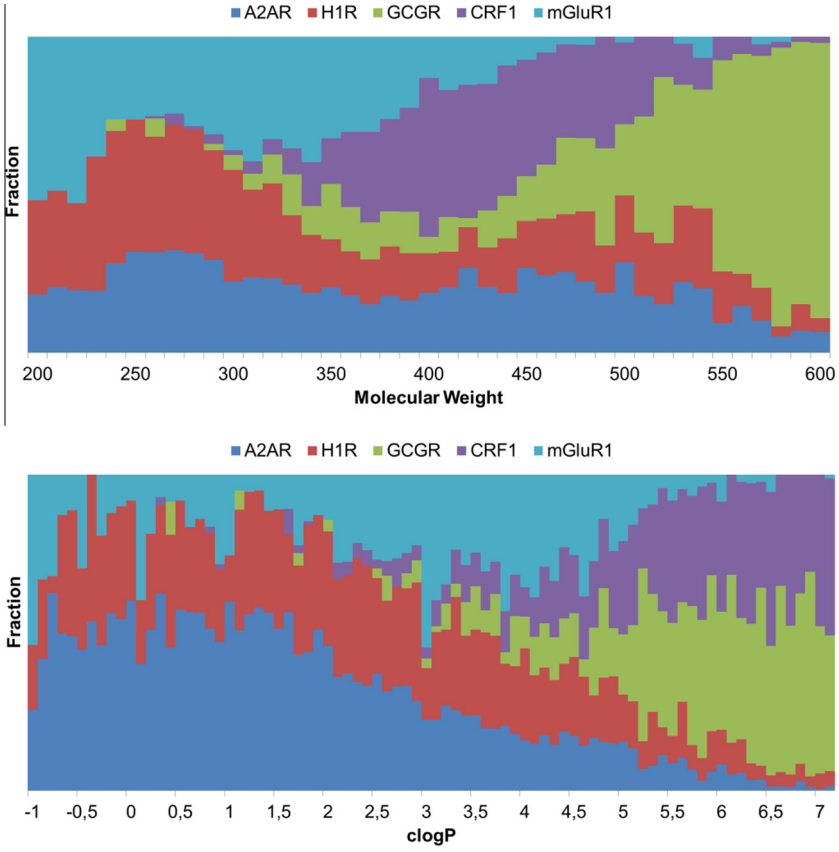

Figure 4. Distribution of molecular weight and *c*log*P* for all compounds reported to be active in ChEMBL for $A_{2A}R$, H1R, GCGR, CRF1R, and mGluR1. The overall pictures shows that ligands for GCGR are heavy and lipophilic, ligands for CRF1R are medium sized but also very lipophilic. Also ligands for mGluR1 are generally small in size, the lipophilicity is fairly high. As examples for druggable targets the distributions for $A_{2A}R$ and H1R reveal low lipophilicity and a large fraction of small ligands.

between class A and class B GPCRs, but TM6 and TM7 show strong deviations, also within class B structures. Within CRF1R these helices are positioned much further away from the center of the helical bundle, where TM6 has a different tilt and TM7 shows a strong outward kink compared to GCGR. It is speculated that this difference may be the consequence of ligand binding, because CRF1R has been solved with a small molecule antagonist bound, whereas in GCGR the ligand density could not be recovered. Therefore, the binding crevice of CRF1R is the largest one in all GPCRs solved so far. In depth investigation of class B binding sites reveals they have a low druggability[38] characterized by an open pocket which is mostly filled with bulk-like water and the presence of only one hot spot residue. In this respect the class B pockets are similar to the CXCR4 small molecule ligand binding site. With the low druggability in mind, it is not surprising that no small molecule drugs are marketed for class B GPCRs.

Examination of all published GCGR and CFR1R active ligands from ChEMBL,[40] reveals that their calculated physicochemical properties are predominantly in an unfavorable region. This is anticipated to cause safety problems and unacceptable PK properties, as is also reported in the literature.[41] Druggable targets, such as H1R or $A_{2A}R$, show a completely different profile for their ligands with low molecular weight and low *c*log*P* distributions, as shown in Figure 4. The optimization of initial screening hits or lead classes is much more demanding for heavy and/or lipophilic compounds as found for class B receptors. All these reasons make the first class B X-ray structures invaluable tools for medicinal chemists because they may enable structure based drug design efforts. With these structures, and homology models derived therefrom, a focused effort to reduce the ligand's liabilities can be guided by in silico modeling support. To demonstrate the usability of the CRF1R structure we take the ligand conformation from the X-ray structure and overlay it with the 300 currently (status April 14) most potent ligands (potency range 0.2–3 nM) reported in ChEMBL[40] coming from 49 different publications. The ligand based superposition is done in a purely automated fashion[42] and the result is scientifically not actually surprising, but striking—the overall shape of the set of ligands matches exactly the shape of the binding pocket (see Fig. 5), although various core modifications (bicyclic cores) are observed. Thus, all highly potent compounds are apparently binding to the same binding pocket in a similar orientation. This means in turn that the availability of the X-ray structure early on would have triggered much more focused exploration of the lead classes leading to a faster progression during optimization and probably more drug-like compounds.

For GCGR the small molecule antagonist cannot be found in the X-ray structure, and no obvious binding mode can be proposed. However, with a large set of single point mutagenesis experiments at least a conclusive binding hypothesis for glucagon could be established.[36] The direct use of this structure for drug design will not be straightforward, but rather some modeling steps will be needed for a proper description of a lead class binding mode. Powerful techniques, such as molecular dynamics simulations, which are currently applied to GPCRs on a regular basis,[43] will help to investigate the dynamics of the receptors and to look for induced binding pockets.

Class C. Class C has been the last class of GPCRs to be elucidated by X-ray crystallography. Very recently the heptahelical transmembrane region (abbreviated as 7-TM region) of mGluR1 bound to a negative allosteric modulator (NAM) has been reported.[37] Class C is highly relevant for drug design because class C receptors play important roles in synaptic transmission and calcium homeostasis, comprising the metabotropic glutamate receptors (mGluRs), the γ-aminobutyric acid B receptors ($GABA_B$), the calcium sensing

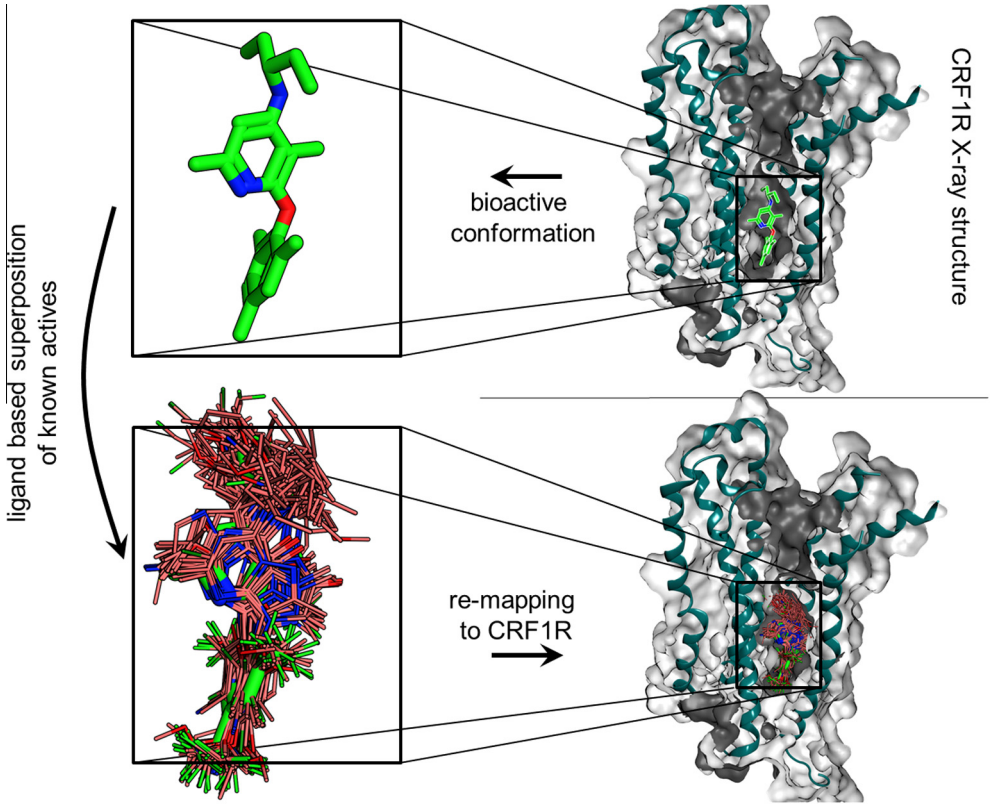

Figure 5. When superposing CRF1R active compounds from ChEMBL with the bioactive conformation retrieved from the CRFR1 X-ray structure, the resulting ligand set neatly fits into the receptor binding site.

receptor and taste receptors. Class C GPCRs are characterized by a large N-terminal domain (the so-called venus flytrap domain) which is the recognition site for the small endogenous agonists, such as glutamate, GABA or Ca^{2+}. Upon agonist binding in the N-terminal region, the 7-TM-bundle undergoes conformational change by a currently unknown mechanism. This eventually enables G-protein association, involving a cysteine-rich domain, which links the venus-flytrap domain with the 7-TM-domain of the receptor. Due to the highly conserved nature of the orthosteric binding site of the individual class C subclasses, drug selectivity can essentially only be achieved by allosteric modulation. There is a long history for the development of allosteric modulators of class C receptors and eventually most modulators have been shown to bind in the 7-TM-region, close to the generic binding site in class A receptors.[44] However, for the mGluRs and $GABA_B$ receptors currently no marketed small molecule allosteric modulator is available, although these receptors have been identified as very attractive drug targets for CNS disorders.[45] The known ligands for mGluRs are mostly linear, flat and often quite small, but hydrophobic molecules (see Fig. 4) with non drug-like properties. In addition to that, often only very subtle structural differentiation between molecules working as positive or negative allosteric modulators (PAMs or NAMs) are found.[46] Small structural changes can convert NAMs into PAMs or even SAMs, which stands for 'silent allosteric modulator' and is a binding ligand, which acts functionally neutral.[47] In return small changes in mGluRs binding sites, introduced by single point mutations, can invert the efficacy of ligands.[48] To understand these decisive differences, structurally knowledge of the allosteric binding site is essential.

The X-ray structure of the mGluR1 receptor[37] confirms the hypothesis that the class C allosteric binding site is overlapping with the class A generic (or orthosteric) small molecule binding site. The accessibility to the binding site is obstructed by loop regions, and only a very narrow entry channel to the receptor is visible in the structure, as shown in Figure 6. This receptor peculiarity as well as the topology of the binding site itself explains the preference for mostly small, linear and flat ligands by mGluR1. The binding site is lipophilic and restricted in size, corresponding to the ligand weight and *c* log*P* distribution shown in Figure 4. The large hope is that this structure will help now to guide lead optimization campaigns to yield drugs that are more successful. However, due to the quite featureless mGluR1 binding site, in silico approaches for ligand placement, such as ligand docking, are very challenging. Especially, the subtle dependence of ligand efficacy on small structural changes requires a very accurate binding mode prediction to be of use for compound optimization. It remains to be clarified if the structural differences between NAM and PAM bound class C GPCRs are somehow related to the differences between agonist and inverse agonist binding in class A receptors. Beyond that, it is not known how the venus flytrap domain communicates with the TM region and why these receptors only work as covalently linked dimers. All these additional layers of complexity render the class C receptors currently to the least understood class of GPCRs. However, the solution of the first class C GPCR structure was one of the major milestones for a proper understanding of class C structure and function.

Conclusions: The recent solution of class B and C GPCRs structures as well as very high-resolution structures of two class A GPCRs has opened new views on GPCR structure and function. The difference between G-protein and β-arrestin signaling can to some extent be explained by interpretation of the corresponding X-ray structures. It is now only a matter of time until the first β-arrestin bound GPCR will be solved to test the validity of the current view. This understanding is extremely important, but the application to biased ligand design is not straightforward, as long as we are at the stage of explaining rather than predicting effects.

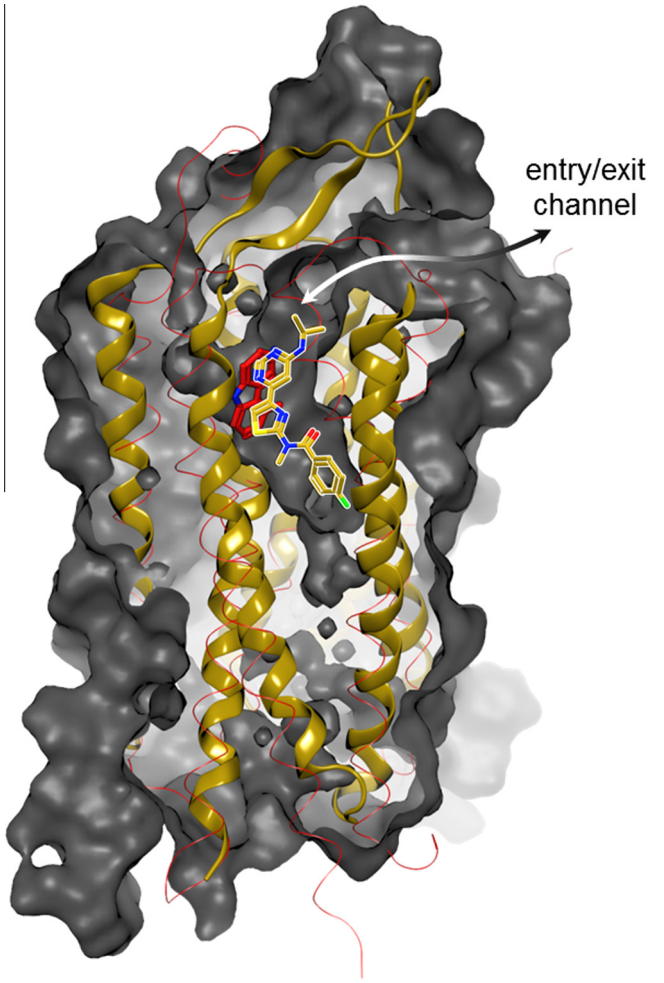

Figure 6. X-ray structure of mGluR1 bound to a negative allosteric modulator (gold). β2R bound to carazolol is shown as overlay (red). The ligand binding sites overlap strongly.

The structures from class B and C are milestones. However the use of these structures in drug design is also not straightforward because the ligand binding sites are either very large, or very lipophilic, thus being of low druggability. For class B ligand binding may lead to strong induced structural changes, however the ligand is not seen in all class B receptor X-ray structures. Still, the said structures are invaluable tools for the interpretation of ligand SAR, and with the combination of modeling efforts, supported by mutagenesis information, these structures will prove to be powerful tools in lead optimization programs.

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