Receptor Structures

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The Golden Age of GPCR Structural Biology: Any Impact on Drug Design?**

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GPCRs (guanine nucleotide binding protein coupled receptors) are one of the most universal ways chosen by nature to transmit signals into cells. These receptors are localized in the cell membrane and, through conformational changes induced by extracellular binding of transmitter molecules, relay information from the outside to the inside. Upon activation, the intracellular part binds the heterotrimeric G proteins. The activated G proteins then regulate effector proteins, a process fuelled by the hydrolysis of guanosine triphosphate (GTP). Despite the exclusivity suggested by the name, it has become apparent in recent years that the activation of downstream pathways can also be mediated by β-arrestin or by direct interaction with kinases.[1] The key role played by GPCRs is also manifested by the fact that about 30% of drugs on the market at present target one of them.[2] Yet, despite their importance, crystal structures of these receptors have been rare. The scientific community has tried insistently, but efforts have been hampered by the fact that GPCRs must be embedded in a membrane-like environment to retain structural integrity, which is not easily achieved experimentally. Moreover, all GPCRs feature flexible intra- and extracellular loops, and can only be expressed at low yield. Even more aggravating is the fact that many GPCRs (with the notable exception of rhodopsin, which was also the first receptor to be crystallized[3]) show basal signaling activity, and thus greater conformational flexibility, even in the absence of a ligand. GPCR ligands are classified based on their effect on this basal signaling activity: if they increase activity, they are called agonists (from Greek αγωνιστής: rival); a decrease makes a molecule an inverse agonist; and no change is characteristic of an antagonist. It is important to note here that the classification of a ligand can be different for different downstream interaction partners of the GPCR.[4]

The recent crystal structures that have been determined since 2007 have circumvented the aforementioned problems in one of three ways: through 1) mutations that bestowed greater thermostability and expression levels on the receptors; 2) replacement of the particularly mobile intracellular loop 3 with T4 lysozyme; or 3) stabilization with anti- and nanobodies. Table 1 shows an overview of the available structures to date and the crystallization technique used.

Table 1: GPCR structures solved to date.

Receptor	PDB ID
β_2-adrenergic	2RH1,[a] 2R4R,[b] 2R4S,[b] 3D4S,[a] 3KJ6,[b] 3NY8,[a] 3NY9,[a] 3NYA,[a] 3SN6[b]
β_1-adrenergic	2VT4,[c] 2Y00,[c] 2Y01,[c] 2Y02,[c] 2Y03,[c] 2Y04[c]
adenosine A_{2A}	2YDO,[c] 2YDV,[c] 3EML,[a] 3QAK[a]
dopamine D_3	3PBL[a,c]
CXCR4	3ODU,[a,c] 3OE0,[a,c] 3OE6,[a,c] 3OE8,[a,c] 3OE9[a,c]
histamine H_1	3RZE[a]
rhodopsin	1F88, 1GZM, 1HZX, 1LN6, 1L9H, 1U19, 2I35, 2Z73
opsin	2J4Y,[c] 2I36, 2I37, 3DQB, 3CAP

[a] T4-lysozyme insertion. [b] Anti- or nanobody. [c] Thermostabilized mutant.

All of the structures solved so far share the same overall architecture: the membrane is crossed seven times by α helices, starting with the N terminus on the extracellular side. The helices are connected by three intracellular and three extracellular loops (Figure 1). This confirms what was suspected to be the universal layout when the rhodopsin structures were obtained. Where it becomes really interesting are the binding sites. Despite the conservation of the helices, the location of the binding sites (both in sequence and in space) was less obvious. Perhaps the biggest surprise was that the locations of the ligands differ quite a bit between the receptors (Figure 1), especially in the cases of the small-molecule antagonist IT1t and the cyclic 15-residue peptide CVX15 bound to CXCR4.[5] Consequently, when one looks at homology models of GPCRs from before 2007, one finds that the helices are well reproduced whereas the binding sites and ligand orientations are sometimes off, even in sequence space.

In terms of the conformational changes upon receptor activation, the crystal structures provide support for the notion that there is not only one inactive and one active state, but multiple states between the inactive and active confor-

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mations. Rhodopsin in particular was crystallized in several stages of activation. Very recently the structure of β_2AR in complex with the stimulatory type of heteromeric G protein (Gs) and an agonist in the orthosteric site was published.[6]

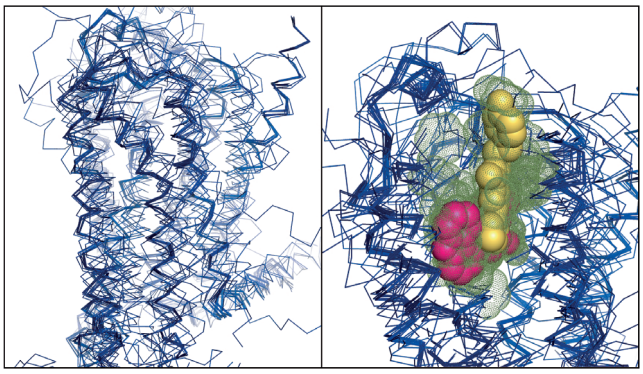

Figure 1. Left: Overlay of the twenty non-rhodopsin GPCR structures with a ligand in the orthosteric site. Structures are shown as ribbons with the extracellular side pointing up. Note the almost perfect overlay of the helices. Right: Detail of merged binding sites of several ligands, shown as green dotted surfaces, highlighting the different extent to which they utilize the sites. Two inverse agonists, carazolol (2RH1, β_2AR) and ZM241385 (3EML, A_{2A}R), are shown as magenta and yellow spheres, respectively, as points of reference. Some residues have been removed for clarity.

This structure presumably corresponds to the highest degree of activation, as exemplified by the largest movement of helix 6 ever observed. Outward movements of helices 5 and 6 on the intracellular side are thought to be required for activation as they open up the G-protein-binding site. Surprisingly, these movements are hardly echoed in the ligand binding site—although some side-chain conformations adjust, the overall backbone geometry remains rather similar, even in the latest G-protein-bound structure. In β_2AR, the only differences are a contraction of the binding site and modifications of the angles of the side chains of three serine residues in helix 5.[7] At present, this small change is best observed for β_1AR with a sole move of about 1 Å between the agonist- and inverse-agonist-bound states. It is interesting to compare these structures with the thermostabilized structure of A_{2A}R bound to two agonists.[8] While the overall changes in the transmembrane helices are similar (movement of helices 5 and 6; formation of a bulge in helix 5), the changes in the binding site are larger in the latter, including rotation of a valine side chain. Moreover, whereas the key interactions of agonists are with helix 5 in the β-adrenergic receptors, they are with helix 7 in the A_{2A}R.[8]

Another interesting facet is the conformation of the loops, particularly the extracellular ones. Bioinformatic analysis had shown that length and sequence is highly conserved for extracellular loop and least for extracellular loop 2 (EL2). The new structures now add different secondary structures and interactions to that picture. EL2 in β_1AR and β_2AR features a small α helix, whereas it forms β sheets or hairpins in most other GPCRs. Moreover, the extent to which EL2 closes off the binding site varies widely, from completely occluded in rhodopsin to relatively open in A_{2A}R and CXCR4. This topic has been reviewed recently by Peeters et al.[9]

Importantly, the structures now emerging enable more reliable protein-structure-based ligand discovery. Computational screens can utilize the crystal structures directly, and such screens have yielded not only high hit rates but also, and potentially more importantly, novel chemotypes.[10] Second, in silico techniques can benefit from significantly more relevant homology models based on structural data of closer-related receptor structures.[11] The latter have been assessed by the community in blind predictions in 2008 and 2010.[12] Not entirely surprisingly, homology modeling worked better when the template was close in sequence space. The main challenge still is to get the alignment right—an error of one residue translates to an offset of a side chain that points away by roughly 120° from the original position because of the spiral geometry of a helix. Moreover, as the binding modes of the ligands vary widely, it can be very difficult to predict them, even if the underlying protein homology model is close to the crystal structure.

In conclusion, our understanding of GPCR structure and mechanism has advanced considerably with the recent structures. Interestingly, despite very similar overall architecture, all receptors crystallized so far differ in key aspects of ligand binding and receptor activation. However, the conformational differences between agonist- and inverse-agonist-bound structures are much smaller than expected, making them difficult to exploit with structure-based methods. We can thus look forward to learning more and seeing yet more differences with every new structure published.

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