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Allosteric binding: structures reveal new ways to tame G protein-coupled receptors

“...the wisest prediction is that more surprises and opportunities are yet to be uncovered.”

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G protein-coupled receptors (GPCRs) comprise an important class of integral membrane proteins, and are the targets of many drugs in common use. GPCRs are activated by a diverse range of signaling molecules including photons, protons, metal ions, small-molecule neurotransmitters, nucleotides, lipids, peptide hormones and proteins such as chemokines, engaging intracellular G proteins and arrestins to transmit the signal. All GPCRs contain a core domain of seven transmembrane (TM) helices, and several have an additional domain located on the extracellular side of the receptor. The signaling molecule that normally activates the GPCR (the natural agonist) typically binds to a site within the bundle of TM helices, although some also engage an extracellular domain (ECD), and some bind solely to the ECD. Therapeutic agents that mimic the effect of the natural ligand in activating the receptor are also agonists, while antagonists prevent activation by the natural ligand. In this simple model, it was generally accepted that most therapeutic agents should bind in the same region of the receptor as the natural agonist, the orthosteric-binding site, although the existence of allosteric modulators of GPCRs, acting through a range of mechanisms, is well established [1]. Now, with the advent of a new age in GPCR structural biology, with published x-ray structures for >30 different GPCRs complexed with a wide range of ligands, high resolution

views of the diversity of ways in which compounds can bind to and modulate GPCRs are emerging.

The x-ray structures of Class A GPCR (rhodopsin-like) β_2 and β_1 adrenergic receptors complexed with antagonists were published in 2007 and 2008 [2,3]. Subsequently structures with other ligands have been reported, including both antagonists and agonists, and the culmination of this area was the publication of the β_2 receptor in complex with the G protein in the full agonist signaling conformation [4]. This set of structures and further data from other systems in which both agonists and antagonist co-complexes have been solved has given us a good understanding of the conformational changes that occur on receptor activation. Typically, the activated form of the receptor with an agonist bound has a slightly contracted orthosteric site, with one or more of the TM helices moving into the site. These changes are transmitted to the intracellular surface, where the most dramatic difference is a shift of TM helix 6 (TM6) away from the core of the receptor to allow the G protein to engage.

Mechanistic insights have also emerged from structures solved of GPCRs complexed with molecules other than orthosteric agonists and antagonists. The active structure of the human M_2 muscarinic acetylcholine receptor, stabilized by a G protein mimetic camelid antibody fragment, was solved in complex with the posi-

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tive allosteric modulator (PAM) LY2119620 and the orthosteric agonist iperoxo simultaneously bound [5]. Comparison of this structure with that of the complex without the PAM shows the PAM engages with a largely preformed binding site in the ‘extracellular vestibule’ of the iperoxo-bound receptor, inducing a slight contraction of this outer binding pocket. This structure illustrates one mode of PAM binding in which the agonist protein conformation is further stabilized by the PAM binding to an essentially contiguous pocket and is likely to slow the off-rate of the agonist, thereby boosting its effective potency. This mode of binding is likely to occur in other receptors, particularly Class A monoamine receptors with small molecule natural agonists where vestibules are thought to exist.

Other mechanisms of allosteric regulation can make use of the fact that GPCRs undergo significant conformational change upon activation. Hence an agent that stabilizes one conformation preferentially and/or prevents the formation of an alternative conformation, can be an allosteric modulator. Such a mechanism was revealed in a high-resolution structure of the adenosine A_{2A} receptor, in which occupation by sodium of a pocket within the helical bundle appears to prevent the movement of helices required for activation of the receptor [6]. This is in line with the well-known negative effect of sodium on the binding of agonists.

“...a structure of the Class F smoothened receptor, with the extracellular domain present, revealed two binding sites for allosteric ligands.”

Our own work resulted in the x-ray structure of the Class B (secretin) corticotropin releasing factor receptor 1 in complex with the well-studied antagonist CP-376395 [7]. Mutational screening in the presence of CP-376395 resulted in the identification of a construct with 12 point mutations that exhibited significantly increased thermal stability in a range of detergents, facilitating crystallization. The natural ligand, corticotropin-releasing factor (CRF), is a 41 amino-acid peptide hormone that interacts with the receptor through both the TM helical bundle and a large amino-terminal ECD. However, the small lipophilic antagonist CP-376395 does not bind in the orthosteric site as had been anticipated, but instead occupies an allosteric site deeper within the receptor. The mechanism of allosteric antagonism appears to rely on preventing the movement of the helices required for activation, analogous to that of sodium in the A_{2A} receptor. Molecular dynamics simulations suggest the allosteric site is collapsed in the absence of the ligand, implying an induced fit mode of bind-

ing, consistent with the extremely slow binding kinetics of this compound.

While the corticotropin-releasing factor antagonist exerts its effect from within the helical bundle, a different type of allosteric site was revealed by the 2.5 Å x-ray structure of another Class B GPCR, the human glucagon receptor (GCGR) [8]. This was complexed with the clinically-studied antagonist MK-0893, which, remarkably, binds outside the TM helical bundle, wedged between TM6 and TM7, and extending into the lipid bilayer. This unexpected binding site was validated by mutagenesis of key residues identified in the structure, confirming their role in the binding of MK-0893 to the receptor. The extra-helical binding site for MK-0893, which is structurally similar to other GCGR antagonists, suggested that it prevents activation of the receptor by the peptide hormone glucagon by restricting the outward movement of the TM6 helix, which is required for G-protein coupling. Interestingly, consideration of the sequences of other Class B receptors indicates that equivalent binding sites may exist in some or all of them. Hence, the structure of GCGR with MK-0893 has provided insights into the mechanism of activation of Class B receptors and gives new opportunities for structure-based drug design.

An extra-helical binding mode was also observed in the structure of the Class A $P2Y_1$ receptor bound to the antagonist BPTU [9]. In this case the allosteric antagonist appears to act by restricting the movement of TM3 required for activation, and comparison of the sequences of related receptors shows that residues key to binding the antagonist are unique to $P2Y_1$, suggesting in this case a unique allosteric site.

Yet another allosteric mechanism exists in Class C receptors, where the natural agonist bind to an ECD and requires the receptor to be dimerized to induce conformational changes in the TM domain to trigger signaling. X-ray structures of $mGlu_1$ and $mGlu_5$ have revealed how negative allosteric modulators seem to bind to and stabilize inactive forms of the TM domains of these receptors [10,11]. Intriguingly, within this class it is well established that small alterations to the chemical structures of ligands can change them from being negative to positive allosteric modulators, and the x-ray structures give clues as to how these changes could shift from stabilizing inactive forms to stabilizing active forms of the receptors.

More recently, a structure of the Class F smoothened receptor, with the ECDs present, revealed two binding sites for allosteric ligands [12]. One, in the extracellular region, was occupied by cholesterol, and appears to promote binding of the natural agonist, the Hedgehog

protein. The other, in the TM region, is the site for the clinically used antagonist vismodegib, which appears to cause loss of cholesterol binding and inhibit signaling.

GPCR signaling requires significant conformational changes within the TM domain, triggered by agonist binding, and is sometimes coupled to interactions with ECDs. In hindsight it perhaps may have been expected that many binding sites and mechanisms for positive and negative allosteric regulation might therefore exist in greater numbers than are seen in other systems, for example, enzymes. The structures are now unveiling the mechanistic details of this diverse range of allosteric modulators, and the high resolution views of ligand–protein interactions can be used for the structure-based design of new

agents. We can expect further fascinating details of allosteric mechanisms to be revealed by subsequent x-ray structures and the wisest prediction is that more surprises and opportunities are yet to be uncovered.

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The authors work at Heptares Therapeutics, which is an organization involved in structure-based drug design for G protein-coupled receptor targets. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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