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Why we need many more G protein-coupled receptor structures

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G protein-coupled receptors (GPCRs) are integral membrane proteins that reside in the plasma membrane of eukaryotic cells. They are central to transmitting signals from the extracellular milieu to the inside of the cell. Ligand binding on the outer surface of GPCRs leads to conformational changes within the receptor, triggering the activation of G proteins and the interaction with kinases and arrestin molecules in the cytosol [1]. The realization that some ligands can activate only a subset of the intracellular effectors that contact a given receptor led to the concept of ligand-biased signaling [2]. In addition, receptor dimerization and oligomerization is thought to allow further fine-tuning of the signaling response [3]. With more than 800 members in the human genome, GPCRs constitute the largest protein family involved in signal transduction, and are major targets for the development of drugs. The importance of GPCRs has been recognized in the 2012 Nobel Prize in Chemistry to Brian K Kobilka and Robert J Lefkowitz.

In the last three to four decades, many elegant biochemical and biophysical experiments have provided a framework of how GPCRs function. However, these studies did not offer insight into the molecular events from ligand binding to receptor activation [4]. In the past few years, we started to see an explosion in the field of GPCR structure determination. In the year 2000, the first crystal structure of a GPCR, the visual pigment rhodopsin, was determined [5]; the second crystal structure, the β_2 -adrenergic receptor, was published only in 2007 [6–8]. By December 2012, crystal structures of 16 unique GPCRs [101] and one solid-state NMR structure [9] have been reported. This exciting progress required a tremendous amount of methods development: optimization of the overproduction of GPCRs in heterologous systems; increasing the probability of crystal formation by identifying high-affinity ligands for cocrystallization, thus stabilizing receptors into one conformation; the

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use of the T4 lysozyme technology replacing the third inner loop [8], and other fusion partners [10], to promote crystal contacts; advancing crystallization methods including detergent and bicelle-based [11] systems, and in particular the miniaturization and automation of the lipidic cubic phase crystallization method [12,13]; the development and implementation of the concept of conformational thermostabilization of GPCRs [14]; the development of microfocus x-ray synchrotron technologies such as the mini-beam [15] combined with raster capabilities to analyze the small GPCR crystals; and progress in NMR spectroscopy methods [9].

We have now over 60 Protein Data Bank entries with 16 unique GPCR structures. These are receptors in complex with antagonists or inverse agonists, with agonists, and with G protein or a G protein-mimicking antibody. These structures are examples of key intermediates in the GPCR activation mechanism, such as inactive receptor states bound to antagonists and inverse agonists, inactive low-affinity agonist-bound conformations, activated states characterized by the rearrangement of helices and key side-chain residues on the intracellular receptor side, and a distinct G protein signaling conformation of a receptor in complex with a heterotrimeric G protein.

The GPCR sequences of the human genome have been classified into five main families (rhodopsin family or class A, secretin receptor family or class B, glutamate receptor family or class C, the adhesion receptors and frizzled/taste receptors) [16]. All receptors for which structures have been published belong to the class A of GPCRs, although the structure of a class B family member has recently been solved but not yet made public. Most of the solved structures are for members of the α group of class A GPCRs. Beyond the α group, the structures of several peptide receptors from the γ group of class A GPCRs have been solved, including the chemokine receptor CXCR4 and all four opioid receptors (these structures have been solved with small antagonists). Most recently, we have solved the structure of an engineered neurotensin receptor from the β group of class A GPCRs in an active-like conformation with the peptide neurotensin bound, the first ever structure of a peptide receptor in complex with a peptide agonist [17]. The structure of a protease-activated receptor from the δ group of class A GPCRs was also recently reported [18].

Given the accumulating wealth of structural information on GPCRs in various conformational states, in complex with inverse agonists, antagonists and agonists, and even a heterotrimeric G protein, one might be tempted to conclude at a general mechanistic concept for GPCR-mediated signaling. The author will argue along several lines as to why we need to generate many more GPCR structures to begin to understand the fine details of subtype selectivity of ligand binding including allosteric modulators and the conformational dynamics of GPCRs, especially in view of downstream signaling toward specific G proteins or arrestin-activated pathways. Only when harnessed with such an advanced degree of knowledge, will the design of modern pharmaceuticals for highly targeted therapeutic intervention become reality.

Expanding the structural repertoire beyond the rhodopsin family

Non-rhodopsin family members have elaborate N-termini involved in ligand binding; those N-terminal domains range in size from 80 amino acid residues (class B) to several thousand residues (adhesion receptors). For example, metabotropic glutamate receptors (class C) have so-called Venus fly trap domains binding the amino acid glutamate. Receptor activation is triggered by the interaction of the ligand-occupied N-terminal domain with the extracellular surface of the receptor transmembrane domain. Although structural information of isolated ligand-binding N-terminal domains is available, only structures of the entire receptor will provide insight into the signaling mechanism of non-rhodopsin GPCRs.

Diversity of ligands

GPCRs bind a breathtaking variety of ligands, ranging from odor molecules to small ligands such as dopamine or epinephrine, to neuropeptides and large glycoprotein hormones, to name a few. This striking variety of ligands is reflected in the structural diversity of the extracellular regions of the receptors. For example, variations such as nonproline kinks and bulges are found at the extracellular ends of the transmembrane helices [19]. An example of a new paradigm for peptide agonist binding was revealed with the most recent structure of the neurotensin receptor NTS1 in complex with its peptide neurotensin [17]. In contrast to the agonist-specific interactions made by isoprenaline in the β_1 -adrenergic receptor, adenosine in the adenosine A_{2A} receptor and all-trans-retinal in rhodopsin, occurring at a similar depth in the receptor within the transmembrane bundle, the neurotensin peptide does not penetrate the receptor as deeply, with the C-terminus of neurotensin being over 5 Å closer to the extracellular surface than the chemical groups in isoprenaline, adenosine and all-trans-retinal that make agonist-specific contacts to their respective receptors. This striking difference between the binding mode of neurotensin in NTS1 and the binding of agonists in the β_1 -adrenergic receptor, adenosine A_{2A} receptor and rhodopsin indicates that the mode of activation of the neurotensin receptor is subtly different from these receptors. Many more agonist-bound GPCR structures are required to understand in detail how the binding of structurally diverse ligands leads to conformational changes within the receptor thought to be more conserved.

GPCR complexes

It is thought that the agonist binding event leads to conformational changes within the receptor that are more conserved. This is also reflected by the fact that there is a much smaller number of G proteins, kinases and arrestin molecules present inside the cell. To date, there is only one structure of a GPCR in complex with a heterotrimeric G protein available, the β_2 -adrenergic receptor bound to the G_s -type G protein [20]. Immediate questions as to the specificity of G_i and G_q protein activation by their respective receptors or the role of receptor dimers await high resolution structures of more GPCR–G protein complexes. Structures of receptors with kinases and arrestin molecules will provide details of the mechanism of biased signaling.

Computational biology

Computational approaches to identify new ligands have greatly benefitted from the availability of GPCR crystal structures. Modeling exercises [21] indicate that homology models based on 35–40% sequence identity are useful for ligand profiling [3]. This sequence identity cutoff for homology modeling suggests that the current crystal structures will cover only a limited number of GPCRs, and that many more receptor structures will be required to cover all GPCRs [3].

The availability and further development of sophisticated experimental tools allows us now to address and answer questions about mechanistic events of GPCR-mediated signaling in unprecedented detail. The cell signaling community is eagerly awaiting many more GPCR structures to come.

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