Beyond on or off

Structural studies of GPCRs defining conformational states en route to activation and clarifying the mechanisms of activation, ligand bias, and signaling will be critical for discovering new drugs that target a range of diseases.

G-protein-coupled receptors (GPCRs) constitute the largest class of eukaryotic membrane proteins and are involved in numerous and varied biological functions including sensory transduction, hormone response, and neurotransmission. Over 100 GPCRs are the targets of ~34% of the US Food and Drug Administration–approved drugs. Directly targeting GPCRs with drugs generally involves binding to the sites used by natural ligands, making knowledge of the natural binding pockets critically important for drug development. Such insights can be obtained from determining the atomic structures in ligand-bound states, as has been achieved for receptors in each of the mammalian GPCR classes with diverse ligands, from among the nearly 300 total solved GPCR structures. Over the past few years, there has been a huge increase in available GPCR structural information, providing a molecular understanding of ligand recognition, receptor dynamics and activation, coupling to downstream effectors (G protein and arrestin), and biased signaling. The database of GPCR structures is rich, yet includes fewer than 60 of the over 800 known GPCRs. The findings have been largely generalizable, and the structures show distinct similarities in their limited large-scale conformational changes (as inactive GPCRs constitute a majority of the structures).

Understanding at a molecular level how ligands engage with their GPCRs and how drugs can selectively target these receptors is critical for the discovery of novel treatments of conditions ranging from inflammation to cancer, hypertension, and cardiovascular disease. In this issue, we present four additional ligand-bound structures of GPCRs in the disease-relevant prostanoid receptor family determined by X-ray crystallography. Prostanoids, including prostaglandins and thromboxanes, are bioactive lipids that are released in response to various stimuli to mediate inflammatory and anaphylactic reactions and vasoconstriction via activation of nine GPCR subtypes including prostaglandin E_2 receptor 3 (EP3), prostaglandin E_2 receptor 4 (EP4), and thromboxane A_2 receptor (TP). Toyoda et al. determined the structure of the antagonist-bound EP4 in complex with an inhibitory antibody, while Fan et al. determined TP structures bound to two nonprostanoid antagonists. Morimoto et al. and Audet et al. solved two structures of EP3 in active conformations bound to the endogenous agonist PGE_2 and the related drug misoprostol, respectively.

The architectures of EP3, EP4, and TP are similar, featuring, of course, the canonical seven-transmembrane helical fold of GPCRs, as well as occluded ligand-binding pockets that are structurally similar among the three receptors, suggesting common mechanisms for ligand recognition and downstream signaling, as noted in a News and Views piece by Hollenstein. Hollenstein also argues that a better understanding of GPCR signaling phenomena such as partial agonism and the newly appreciated biased signaling will require more structural and biophysical studies, including structures of GPCRs in complex with downstream effectors, G proteins and arrestins, as well as biased ligands (that invoke either G proteins or arrestin, but not both) with different efficacies.

Indeed, the wave of GPCR structures that have appeared over the past few years among the varied GPCR families has begun to include structures with these downstream effectors (e.g., *Nature* **536**, 104–107, 2018) and biased ligands (e.g., *Nat. Struct. Mol. Biol.* **25**, 787–796, 2018), with an aim toward a greater atomic-level understanding of activation and signaling through GPCRs. As well, a greater emphasis on structures of full-length, wild-type receptors closer to their physiologically relevant state than an inhibitor-bound inactive state is becoming more common. This has provided insights that expand our view of GPCR states beyond simply being on or off, to a range of conformational states with differing degrees of activity. Beyond X-ray crystallography, other techniques such as NMR and EPR can be used alongside to gain new insights into dynamics, as well as to identify the individual conformational states a receptor visits en route to activation to inform on conformational bias, polypharmacology, and biased signaling, which can be used to design more specific and efficacious drugs with fewer side effects.

Cryo-electron microscopy (cryo-EM) has also entered the realm of GPCR structural studies, with a handful of structures now available (http://gpcrdb.org/). Cryo-EM has a distinct advantage, particularly important for membrane proteins such as GPCRs, in not requiring crystallization of the sample and needing relatively small sample sizes. As cryo-EM becomes more commonplace, its limitations such as low resolution, size limit, and throughput will be mitigated, allowing greater access to structural information (particularly for inactive GPCRs that do not have effector bound, making them currently inaccessible to cryo-EM). For now, however, complexes that can inform on signaling, such as GPCRs complexed with G proteins, arrestins, and GPCR kinases (GRKs) that are important for GPCR desensitization and resensitization, are within reach. Further prioritization of active structures will be critical for gaining new insights into GPCR activation and to fully appreciate the likely larger diversity of GPCR conformations that should help to explain why there are 800+ GPCRs.

With the estimated cost of determining a crystal structure in excess of one million US dollars, and the time to do so averaging a few years, it is important to prioritize which new structures should be solved. Beyond practical considerations, receptor families in which there is little structural information represent receptors ripe for experimentation. In the case of the four papers presenting prostanoid receptor structures in this issue, the authors were keenly interested in disease-relevant GPCRs, as outlined by the GPCR Consortium (http://gpcrconsortium.org), having a long-standing interest in the biology of these receptors and their characterization as targets for cardiovascular disease (as for TP), post-partum hemorrhaging, and labor induction (as for EP3). The fact that they accomplished solving the structures nearly simultaneously attests to the often collegial spirit of the GPCR community that should continue collaboration to define the areas of most unmet need (*Nat. Rev. Drug Disc.* **16**, 829–842, 2017).

Despite recent progress made in structural studies of GPCRs, support of these fundamental insights and continued drug development will require focused efforts to understand the biology of GPCR signaling through technology improvements and collaboration. ❐

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