Shining a light on GPCR complexes

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The G protein–coupled receptor (GPCR) signaling pathways mediating information exchange across the cell membrane are central to a variety of biological processes and therapeutic strategies, but visualizing the molecular-level details of this exchange has been difficult for all but a few GPCR–G protein complexes. A study by Gao et al. now reports new strategies and tools to obtain receptor complexes in a near-native state, revealing insights into the gross conformational features of rhodopsin-transducin interactions and setting the stage for future studies.

G protein–coupled receptors (GPCRs) transmit information (conveyed in the form of hormones, cytokines, neurotransmitters, and light) into cells to regulate nearly every aspect of human physiology. GPCRs are both the largest family of transmembrane proteins and the target of ~50% of all therapeutics on the market, making studies of their structure and function essential for deciphering cell signaling mechanisms and informing drug design. However, GPCRs are also integral membrane proteins and contain several flexible regions, making structural studies enormously challenging. In addition, their function depends on their signaling partners, the heterotrimeric G proteins, meaning that the most informative structures would be of GPCR–G protein complexes.

Early attempts to gain structural information on GPCR–G protein complexes demanded new methods to express and purify sufficient quantities of the receptors, the identification of detergents appropriate for purification and crystallization, and inhibition of complex dynamics to create homogenous structural information. The groundbreaking and elegant solutions to meet these challenges led to the first X-ray crystallographic study of a class A GPCR β2-adrenergic receptor (β2AR) with the G protein Gαi, first reported in 2011 by Kobilka, Sunahara, Skiniotis, Steyaert, and co-workers (1). Another structure of a class A GPCR–G protein complex, of rhodopsin with the G protein transducin (Gγt), was obtained in late 2011 (2). Although rhodopsin was the first GPCR crystal structure to be solved in 2000 (3), issues with expression of the receptor in heterologous systems and stabilization of rhodopsin–Gαi hampered efforts to crystallize the complex, and the 2011 report was at relatively low resolution. It was not until June 2017 that additional higher resolution structures of GPCR–G protein complexes of two class B GPCRs emerged (4, 5). The structures of calcitonin receptor (CTR) and glucagon-like peptide 1 receptor (GLP-1R) with Gαs were solved by cryo-electron microscopy (cryoEM) using tagged and/or truncated receptors expressed and purified from insect cells. Of note, the β2AR, CTR, and GLP-1R complex structures with Gαs were all stabilized by the use of a camelid antibody Nb35, which binds with high affinity to the interface of two G protein subunits: Gβ and the Ras-like domain of Gαs. The use of these modified constructs and additional biomolecules, although critical for obtaining these first structural insights, left open the question of whether they stabilized only one of a possible subset of native conformations. Additionally, the inability of newer structures to visualize all domains of the G protein made it difficult to develop general principles regarding GPCR–G protein coupling.

The new strategies and analysis from Gao et al. (6) are different from previous X-ray and cryoEM studies in several ways. First, the authors isolated native bovine rhodopsin and the obligate heterodimer β1γ1 complex from photoreceptors, which they paired with a chimeric Gαi subunit. Second, the authors induced formation of the GPCR–G protein complex via light activation in bovine rod outer segment membranes prior to any solubilization or purification of the receptor complex. Third, the authors identified a new detergent that stabilized the system sufficiently to allow purification of the intact complex, in contrast to previous studies that isolated the components separately and then formed the complex in vitro. Finally, the small angle X-ray scattering and negative-stained EM used to create a model of the rhodopsin–Gαi complex (Fig. 1) were carried out without the use of the stabilizing camelid antibody. Therefore, this structure likely represents a close approximation of the GPCR–G protein activation complex formed in its native lipid environment.

The structural model by Gao et al. (6) provides new information relevant to two outstanding questions: One concerns the large movement of the Gα helical domain, thought to be necessary for release of GDP from the deeply buried active site. This movement is one of the unique features of the GPCR–G protein complex that was first revealed in spectroscopic experiments of Gαi activation by rhodopsin (7) and then visualized upon crystallization of β2AR–Gαi (1). However, the degree of this interdomain opening, when it can be observed, varies for

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2 The abbreviations used are: GPCR, G protein–coupled receptor; β2AR, β2-adrenergic receptor; CTR, calcitonin receptor; cryoEM, cryo-electron microscopy; GLP-1R, glucagon-like peptide 1 receptor.
different complexes from relatively small movements or rotations up to a maximum of a 127° rotation in the crystal structure of β2AR–G<sub>S</sub>. CryoEM studies of the β<sub>2</sub>AR–G<sub>S</sub> complex and low resolution images of the CTR–G<sub>S</sub> complex further indicate the helical domain can occupy multiple conformations and has a high degree of mobility (4, 8). Gao et al. (6) provide additional support for the existence of an ensemble of open positions, observing variable orientations for the helical domain of G<sub>T</sub>, between that of the fully closed state in the inactive GDP-bound heterotrimer and the open and nucleotide-free state observed for β<sub>2</sub>AR–G<sub>S</sub> (6). It is possible that the fully open G protein is a rare state that is stabilized by the Nb35 nanobody within the confines of a crystal lattice. More likely, it may simply represent the most open state of a range of conformations that are sampled by the Ga helical domain during receptor-mediated nucleotide exchange.

The data from Gao et al. (6) also shed light on another issue, that of the stoichiometry between GPCR and G protein. All of the GPCR–G<sub>S</sub> complexes solved to date have a 1:1 stoichiometry of GPCR:G<sub>S</sub> protein (1, 4, 5). However, early low resolution structures of the rhodopsin–G<sub>T</sub> complex indicated a 2:1 stoichiometry, suggesting the dimeric receptor was the minimal functional unit for G<sub>T</sub> activation (3). This idea was challenged by later reconstitutions of G proteins with either β<sub>2</sub>AR or rhodopsin (9, 10), which suggested that monomeric receptors can efficiently activate their respective G proteins while maintaining the unique allosteric and kinetic properties expected for each receptor. Gao et al. (6) now use UV/visible spectroscopy, nucleotide binding, small angle X-ray scattering, and EM to confirm that a minimal unit of one rhodopsin and one G<sub>T</sub> heterotrimer is sufficient for full activation.

**Editors’ Pick Highlight:** Visualization of rhodopsin-G<sub>T</sub>

There are now >40 structures for different GPCRs available in a variety of ligand-bound states. These display remarkable structural similarity on the cytoplasmic side, including seemingly preserved G protein activation mechanisms, while providing similarly remarkable diversity in their extracellular binding sites. This new work from Cerione and colleagues (6) adds to our ever-expanding appreciation for this versatile family of receptors and should set the stage for additional structural work in the future.

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![Figure 1. The organization of the light-activated rhodopsin-G<sub>T</sub> complex as determined by Gao et al. (6). A cartoon model of rhodopsin–G<sub>T</sub> complex](image-url)