



Structural Aspects of GPCR-G Protein Coupling

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G protein-coupled receptors (GPCRs) are membrane receptors; approximately 40% of drugs on the market target GPCRs. A precise understanding of the activation mechanism of GPCRs would facilitate the development of more effective and less toxic drugs. Heterotrimeric G proteins are important molecular switches in GPCR-mediated signal transduction. An agonist-activated receptor interacts with specific sites on G proteins and promotes the release of GDP from the $G\alpha$ subunit. Because of the important biological role of the GPCR-G protein coupling, conformational changes in the G protein upon receptor coupling have been of great interest. One of the most important questions was the interface between the GPCR and G proteins and the structural mechanism of GPCR-induced G protein activation. A number of biochemical and biophysical studies have been performed since the late 80s to address these questions; there was a significant breakthrough in 2011 when the crystal structure of a GPCR-G protein complex was solved. This review discusses the structural aspects of GPCR-G protein coupling by comparing the results of previous biochemical and biophysical studies to the GPCR-G protein crystal structure.

Key words: GPCR, G protein, Structure

INTRODUCTION

In 1994, Alfred G. Gilman and Martin Rodbell were awarded the Nobel Prize in Physiology or Medicine for "their discovery of G-proteins and the role of these proteins in signal transduction in cells." Eighteen years later, Brian K Kobilka and Robert J Lefkowitz won the Nobel Prize in Chemistry for "studies of G-protein-coupled receptors (GPCRs)". GPCRs are plasma membrane receptors that perform vital signaling functions in vision, olfactory perception, metabolism, endocrine system, neuromuscular regulation and CNS system (1). Approximately 800 GPCRs are identified in the human genome, and many are involved in diseases such as cardiovascular, metabolic, neurodegenerative, psychiatric, cancer, and infectious diseases. Thus, 40% of drugs in current use targets GPCRs for the treatment of various diseases including heart failure (e.g β -adrenoceptors), peptic ulcer (histamine receptors), prostatic carcinoma (gonadorelin receptors), hypertension (adren-

gic and angiotensin receptors), pain (opioid receptors) and bronchial asthma (β 2-adrenoceptors). All GPCRs share a seven-transmembrane (TM) α -helical structure with an extracellular N-terminus and an intracellular C-terminus (2). Upon agonist binding on the extracellular side of GPCRs, TM segments and intracellular side of the receptor undergo conformational changes, which induce the coupling and activation of the heterotrimeric G proteins (3).

The heterotrimeric G protein is composed of $G\alpha$, $G\beta$ and $G\gamma$ subunits, and $G\alpha$ subunit contains GDP in its inactive resting state (4). Upon activation by GPCR, GDP is released from $G\alpha$ subunit and is replaced by GTP, which dissociates $G\beta\gamma$ subunits from $G\alpha$ and activates G proteins (4). The activated G proteins go back to the resting state by hydrolyzing a phosphate group from GTP converting it to GDP (4). There are 21 $G\alpha$, 6 $G\beta$ and 12 $G\gamma$ subunits identified in human, and G proteins are typically grouped into four main classes (Gs, Gi/o, Gq/11, and G12/13) depending on the similarity of $G\alpha$ subunits (5). The combination of 21 $G\alpha$, 6 $G\beta$ and 12 $G\gamma$ subunits creates various distinct heterotrimeric complexes, which contributes the specificity with regards to both GPCRs and effect or systems (4).

Characterization of the structure and the dynamics of proteins are critical for a better understanding of the molecular basis of normal and abnormal physiological processes and drug development. Structural studies of GPCRs and their interaction with corresponding G proteins would provide

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important information on the biochemistry, biophysics and medicinal chemistry of these important therapeutic targets (6). Therefore, precise understanding of the structural mechanisms of GPCR-G protein coupling will lead to develop more effective and less toxic drugs with fewer side effects. Consequently, enormous effort has been put into the characterization of structures of GPCRs and G proteins. The crystal structures of various G proteins including $G\alpha_s$, $G\alpha_t$, $G\alpha_i$, $G\beta\gamma$ dimer and $G\alpha\beta\gamma$ heterotrimer have been successfully obtained mostly in 90's (7-16). Due to the technical difficulties of obtaining crystals of membrane proteins, however, a few mammalian GPCR structures have been obtained mostly during past 6 years (17), and there is only one crystal structure of the receptor-G protein complex (18). This review will summarize the recent advance in the understanding of the structural aspect of G protein activation by GPCRs.

GPCR-G PROTEIN INTERFACE

High resolution crystal structures of various G proteins have been determined in their inactive (GDP-bound), transition (GDP·AlF-bound) and active (GTP γ S-bound) states (7-16). These structural studies and other biochemical studies revealed the location of the nucleotide-binding pocket and the interface between $G\alpha$ and $G\beta\gamma$ subunits (Fig. 1). The nucleotide-binding pocket is located between Ras-like domain and α -helical domain of $G\alpha$ subunit surrounded by four flexible regions (p-loop, switch I, switch II, and switch III) (Fig. 1B). Ras-like domain hydrolyze GTP and provide binding sites for $G\beta$ subunit (Fig. 1A). The N-terminus of $G\alpha$ subunit is reported to be critical for the structure and function of $G\alpha$ subunit (19-21) and is myristoylated or palmitoylated suggesting the role of this region in the

attachment to the plasma membrane.

In 1988, Hamm *et al.* first identified that the C-terminus of $G\alpha$ subunit is the critical binding site to the GPCR (22). This result, with the finding that the N-terminus of $G\alpha$ subunit is myristoylated, allowed scientists to orient G proteins relative to plasma membrane and GPCRs (Fig. 2A). Since late 80's, numerous efforts have been put to find the G protein-GPCR interface. The involvement of C-terminus of $G\alpha$ subunit has been extensively investigated by various biochemical and biophysical studies including peptide competition assays (22,23), mutation studies (24-26), and crosslinking experiments (3,27,28). The crosslinking experiments provided that TM6 or intracellular loop (ICL) 3 of a GPCR is the major contact sites for the C-terminus of $G\alpha$ subunit (27,28). Two different crystal structures of a GPCR with a $G\alpha$ subunit C-terminal peptide confirmed the interaction of C-terminus of G protein with a GPCR (29,30). These two crystal structures agreed that C-terminus of $G\alpha$ subunit is inserted into the cytoplasmic pocket surrounded by TM domains of a GPCR (29,30).

Besides the C-terminus, there are other regions that have been reported to interact with GPCRs. Crosslinking experiments suggested that α N of $G\alpha$ subunit contacts with GPCRs (27,31,32). However, these studies are conflicting each other in terms of which regions of GPCR interact with α N of $G\alpha$ subunit. α 2-adrenergic receptor interacts with α N of $G\alpha$ subunit with its ICL3 (31); Rhodopsin with its ICL1 (32); M3 muscarinic acetylcholine receptor with its ICL2 (27). α 4/ β 6 loop of $G\alpha$ subunit is also numerous reported to interact with GPCRs by mutation studies (33-35) and crosslinking experiments (27). A crystal structure of $G\alpha$ subunit with ICL3 peptide showed that ICL3 interacts with α 4/ β 6 loop (35), whereas the crosslinking experiment suggested that helix 8 of GPCR interacts with α 4/ β 6

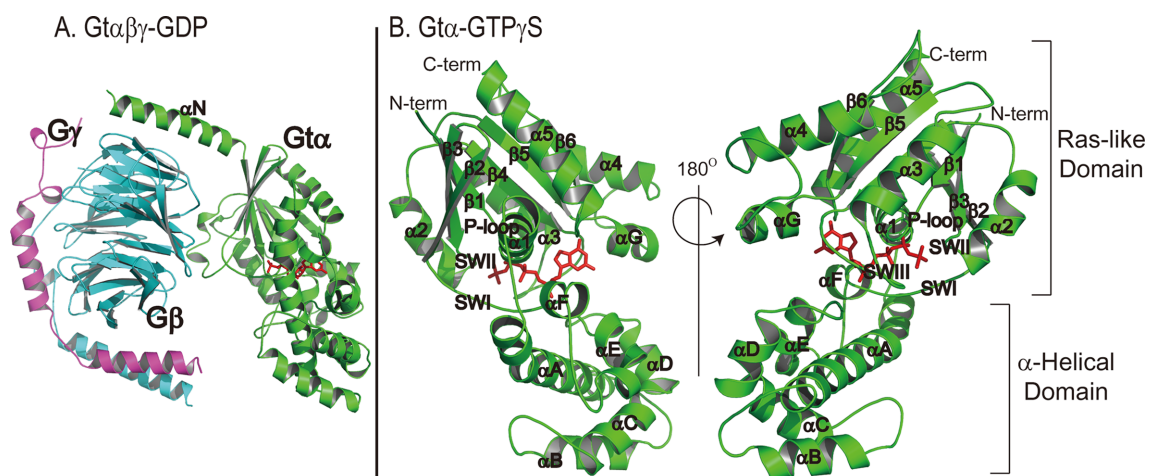


Fig. 1. Crystal structures of $G\alpha\beta\gamma$ heterotrimer with GDP (A) and $G\alpha$ subunit with GTP γ S (B). A. The crystal structure of inactive resting state $G\alpha\beta\gamma$ heterotrimer with GDP is adapted from PDB file (PDB: 1GOT). Green: $G\alpha$, Cyan: $G\beta$, Magenta: $G\gamma$, red: GDP. B. The crystal structure of active $G\alpha$ subunit with GTP γ S is adapted from PDB file (PDB: 1TND). Green: $G\alpha$, Red: GTP β S.

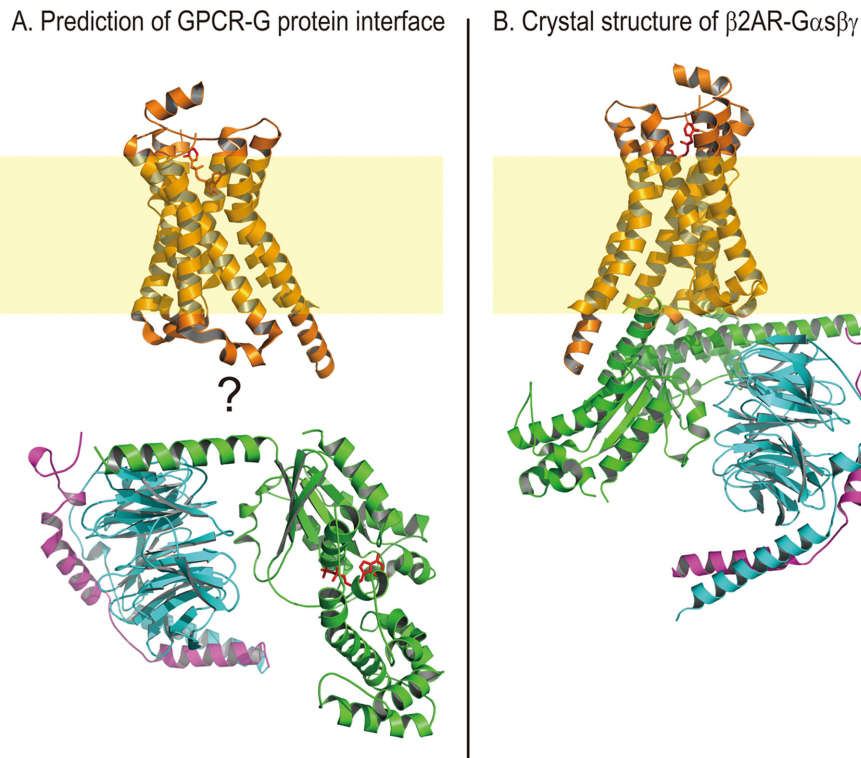


Fig. 2. Interaction between GPCR and G protein heterotrimer. A. Predicted model of GPCR-G protein coupling. Orange: GPCR, Green: $G\alpha$, Cyan: $G\beta$, Magenta: $G\gamma$, red: GDP. B. The crystal structure of $\beta 2AR$ - $G\alpha\beta\gamma$ protein complex (PDB: 3SN6). Orange: $\beta 2AR$, Green: $G\alpha$, Cyan: $G\beta$, Magenta: $G\gamma$.

loop (27). Few studies suggested that $\alpha 3/\beta 5$ loop and $\beta 2/\beta 3$ loop also interact with GPCRs (5,27,36,37). In spite of these extensive efforts to identify the GPCR-G protein interface, it has been debating, until recently, what are the exact interface and the orientation of GPCR-G protein coupling.

The breakthrough in understanding the nature of GPCR-G protein interaction was made by the first crystal structure of the $\beta 2$ adrenergic receptor ($\beta 2AR$)- $G\alpha\beta\gamma$ complex (Fig. 2) (18). In the structure, $G\alpha$ subunit is in its nucleotide-free empty intermediate state. The C-terminus of $G\alpha$ subunit is inserted into the cytoplasmic pocket of $\beta 2AR$, which is consistent with previous biochemical studies and GPCR- $G\alpha$ C-terminal peptide crystal structures. The crystal structure shows two other major contact sites; αN - $\beta 1$ hinge and $\alpha 4/\beta 6$ regions of $G\alpha$ subunit, which are consistent with previous biochemical and biophysical studies. However, $\alpha 3/\beta 5$ loop and $\beta 2/\beta 3$ loop of $G\alpha$ subunit, which were reported to contact with a GPCR, did not interact with GPCR in the crystal structure. This inconsistency implies that there might be more than one conformation of the GPCR-G protein complex or different types of G protein interact with GPCR at different orientations. More studies are needed to determine the interface at different conformational stages or in different GPCR-G protein pairs.

CONFORMATIONAL CHANGES OF G PROTEIN BY GPCR

The long un-resolving question was how a GPCR activates G proteins and release GDP from a $G\alpha$ subunit. Since the nucleotide-binding pocket is about 30 Å away from GPCR-interfacing sites of $G\alpha$ subunit, the possible conformational shifts of $G\alpha$ subunit during its activation by GPCR has been suggested. The recent crystal structure of $\beta 2AR$ - $G\alpha\beta\gamma$ complex gave profound information on how a GPCR activates G proteins (Fig. 2B) (18).

The most striking feature of the crystal structure is the large movement of the α -helical domain of $G\alpha$ subunit (Fig 2B). When compared to the GDP-bound inactive state of $G\alpha$ subunit, the α -helical domain in receptor-bound nucleotide-free state is moved away from its original position, which opens up the nucleotide-binding pocket like a clamshell shape. Electron microscopy experiments also visualized the $\beta 2AR$ - $G\alpha\beta\gamma$ complex, and this approach suggested that helical domain occupies various positions relative to the Ras-like domain, receptor and $G\beta\gamma$ (38). Earlier study with NMR showed that the receptor-bound empty pocket state of the heteromeric $G\alpha$ subunit is conformationally dynamic (39). This feature is also predicted by Van Eps *et al.* shortly before the crystal structure came out (40). Van

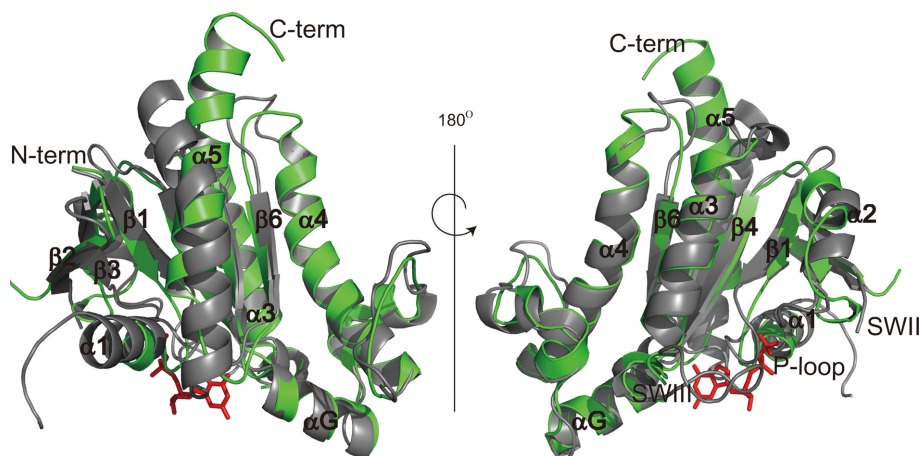


Fig. 3. Comparison of Ras-like domains of inactive-GDP-bound $G\alpha$ subunit (Grey) (PDB:1GP2) and intermediate-receptor-bound nucleotide-free $G\alpha$ subunit (Green) (PDB: 3SN6). Red: GDP.

Eps *et al.* measured the distance between chemical probes positioned on the Ras-like and α -helical domains of $G\alpha$ subunit using double electron-electron resonance spectroscopy, and they observed a large increase in the distance between the domains upon receptor binding. Despite of this big movement, the crystal structure suggested that core of α -helical domain did not undergo conformational changes.

Other major sites of conformational changes are C-terminus and its adjunct $\alpha 5$ helix of $G\alpha$ subunit (Fig. 3). In crystal structures of $G\alpha$ subunit, the C-terminus is disordered and consequently not present in the deposited crystal structures (7-16). Many biophysical studies including NMR (41-44) or X-ray crystallography (29,30) suggested that the C-terminus forms helix upon coupling to the receptor. The crystal structure of $\beta 2AR-G\alpha s\beta\gamma$ complex also showed the C-terminus of $G\alpha$ subunit as an α -helix confirming the previous findings (Fig. 3) (18). What is interesting about the C-terminus is that it is connected to $\alpha 5$ helix (Fig. 1B and Fig. 3). The $\alpha 5$ helix links the C-terminus to the $\beta 6$ - $\alpha 5$ loop that contains the conserved nucleotide-binding TCAT motif (Fig. 1B). In the crystal structure of $\beta 2AR-G\alpha s\beta\gamma$, $\alpha 5$ helix is pull-up towards to the receptor resulting in about 6 Å-movement away from the nucleotide-binding pocket (Fig. 3) (18). Previously, Oldham *et al.* proposed the receptor-induced rigid-body movement of $\alpha 5$ helix upon by using electron paramagnetic resonance spectroscopy (45). Recently two hydrogen-deuterium exchange mass spectrometry (HDX-MS) studies showed conformational changes in the

N-terminus of $\alpha 5$ helix near the $\beta 6$ - $\alpha 5$ loop (46,47). By changing the solvent from H_2O to D_2O , hydrogens in the amide bonds are switched to deuterons. The pattern of deuterium labeling provides information about the solvent accessibility and thus protein conformation. With this method, it was established that receptor binding increases the solvent accessibility of the N-terminus of $\alpha 5$ helix, which implies that this region becomes more flexible or dynamic. Increased flexibility and movement of $\alpha 5$ helix would affect the stability of nearby nucleotide-binding pocket and promote the release of GDP. The crucial role of $\alpha 5$ helix in transferring the structural changes of receptor-binding region to the nucleotide-binding pocket has also been well-described in previous mutation studies (48-51) in which the insertion of poly glycine (48) or proline (50) inhibited the signal transduction of activated receptors.

Similar to $\alpha 5$ helix, the strand of αN helix- $\beta 1$ sheet region of $G\alpha$ subunit may translate receptor-induced conformational changes to the nucleotide-binding pocket. $\alpha N/\beta 1$ hinge region is another major GPCR-G protein contact site in the crystal structure, and $\beta 1$ sheet is connected to the p-loop that interacts with the phosphate group of GDP (Fig. 3) (18). The crystal structure does not show significant conformational changes in either αN helix or $\beta 1$ sheet. However, fluorescence labeling study detected conformational changes of αN helix (52), and HDX-MS study detected increased solvent accessibility of $\beta 1$ sheet suggesting the increased flexibility or dynamics in this region (47). This discrepancy

Table 1. X-ray crystal structures used in the Figures

Structure	PDB number	State	Reference
$G\alpha\beta\gamma$ heterotrimer with GDP	1GOT	Inactive	8
$G\alpha$ subunit with $GTP\gamma S$	1TND	Active	13
$\beta 2AR-Gs$	3SN6	Receptor-coupled intermediate	18
$G\alpha\beta\gamma$ heterotrimer with GDP	1GP2	Inactive	10

may be due to the nature of crystallization of a protein which leads the protein in the energetically most stable state. In the solution environment, and α N helix- β 1 sheet may be flexible or dynamic.

In the crystal structure and HDX-MS experiment, G $\beta\gamma$ subunits do not contact with β 2AR and do not undergo conformational changes upon receptor binding (18,47). However, G $\beta\gamma$ subunits have been implicated to interact with the receptor or modulate the activation of G proteins. Several studies suggested that GPCR interacts with C-terminus of G γ subunit (53), and GPCR uses G $\beta\gamma$ subunits to distort the G α subunit nucleotide-binding pocket resulting in the release of GDP (54,55). The crystal structure is a snapshot of stable nucleotide-free β 2AR-G $\alpha\beta\gamma$ complex, and it does not show the initial coupling modes. The involvement of G $\beta\gamma$ subunits in G protein activation might be the early stage of receptor coupling and needs more investigation.

PERSPECTIVES

Last twenty five years, the continuous efforts in biochemistry, biophysics, molecular and cell biology provided the basic information of structural mechanism of GPCR-mediated G protein activation. Numerous studies support the model that C-terminus and α N- β 1 hinge region of G α subunit are the major contact sites, and interaction of the receptor to these sites transmit conformational changes to nucleotide-binding pocket of G α subunit. However, there are still more needed to be investigated. One of the great interests in this field is how GPCRs recognize specific G proteins. Biochemical, biophysical and mutation studies suggested several regions of G α subunit or GPCR as factors that determines the selectivity, but without solid conclusion. More crystal structures with various GPCR-G protein pairs will help to understand the specificity of GPCR-G protein coupling. Second, GPCRs do not only act as a monomer but also as oligomers (56-58). What would be the coupling mode between G proteins and oligomerized GPCRs? Would GPCR oligomerization affect the G protein selectivity? Third, recent studies suggested that G proteins can interact with inactive GPCRs as a pre-assembled complex (27,59-62). The preassembly of GPCR-G protein can speed-up the signal transduction and provide G protein selectivity of GPCRs (63). Therefore, understanding the conformation of preassembled GPCR-G protein complex will provide important aspect of GPCR-G protein coupling. Finally, although a number of studies suggested models for the conformational shift from receptor binding site to the nucleotide-binding pocket to explain the structural mechanism of GPCR-mediated G protein activation, we do not have a solid answer yet. A crystal structure is a snapshot of one conformation of the molecules. Therefore, more biochemical or biophysical studies are needed to understand this fundamental event of G protein activation.

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REFERENCES

1. Lundstrom, K. (2009) An overview on GPCRs and drug discovery: structure-based drug design and structural biology on GPCRs. *Methods Mol Biol.*, **552**, 51-66.
2. Nikiforovich, G.V., Taylor, C.M. and Marshall, G.R. (2007) Modeling of the complex between transducin and photoactivated rhodopsin, a prototypical G-protein-coupled receptor. *Biochemistry*, **46**, 4734-4744.
3. Wang, X., Kim, S.H., Ablonczy, Z., Crouch, R.K. and Knapp, D.R. (2004) Probing rhodopsin-transducin interactions by surface modification and mass spectrometry. *Biochemistry*, **43**, 11153-11162.
4. Oldham, W.M. and Hamm, H.E. (2008) Heterotrimeric G protein activation by G-protein-coupled receptors. *Nat. Rev. Mol. Cell Biol.*, **9**, 60-71.
5. Baltoumas, F.A., Theodoropoulou, M.C. and Hamdrakas, S.J. (2013) Interactions of the α -subunits of heterotrimeric G-proteins with GPCRs, effectors and RGS proteins: a critical review and analysis of interacting surfaces, conformational shifts, structural diversity and electrostatic potentials. *J. Struct. Biol.*, **182**, 209-218.
6. Tesmer, J.J. (2010) The quest to understand heterotrimeric G protein signaling. *Nat. Struct. Mol. Biol.*, **17**, 650-652.
7. Jones, J.C., Duffy, J.W., Machius, M., Temple, B.R., Dohman, H.G. and Jones, A.M. (2011) The crystal structure of a self-activating G protein alpha subunit reveals its distinct mechanism of signal initiation. *Sci. Signaling*, **4**, ra8.
8. Lambright, D.G., Sondek, J., Bohm, A., Skiba, N.P., Hamm, H.E. and Sigler, P.B. (1996) The 2.0A crystal structure of a heterotrimeric G protein. *Nature*, **379**, 311-319.
9. Sondek, J., Bohm, A., Lambright, D.G., Hamm, H.E. and Sigler, P.B. (1996) Crystal structure of a G-protein beta gamma dimer at 2.1A resolution. *Nature*, **379**, 369-374.
10. Wall, M.A., Coleman, D.E., Lee, E., Iiguez-Lluhi, J.A., Posner, B.A., Gilman, A.G. and Sprang, S.R. (1995) The structure of the G protein heterotrimer Gi alpha 1 beta 1 gamma 2. *Cell*, **83**, 1047-1058.
11. Lambright, D.G., Noel, J.P., Hamm, H.E. and Sigler, P.B. (1994) Structural determinants for activation of the alpha-subunit of a heterotrimeric G protein. *Nature*, **369**, 621-628.
12. Sondek, J., Lambright, D.G., Noel, J.P., Hamm, H.E. and Sigler, P.B. (1994) GTPase mechanism of Gproteins from the 1.7-A crystal structure of transducin alpha-GDP-AIF-4. *Nature*, **372**, 276-279.
13. Noel, J.P., Hamm, H.E. and Sigler, P.B. (1993) The 2.2A crystal structure of transducin-alpha complexed with GTP gamma S. *Nature*, **366**, 654-663.
14. Sunahara, R.K., Tesmer, J.J., Gilman, A.G. and Sprang, S.R.

- (1997) Crystal structure of the adenylyl cyclase activator G α . *Science*, **278**, 1943-1947.
15. Mixon, M.B., Lee, E., Coleman, D.E., Berghuis, A.M., Gilman, A.G. and Sprang, S.R. (1995) Tertiary and quaternary structural changes in G α 1 induced by GTP hydrolysis. *Science*, **270**, 954-960.
 16. Coleman, D.E., Berghuis, A.M., Lee, E., Linder, M.E., Gilman, A.G. and Sprang, S.R. (1994) Structures of active conformations of G α 1 and the mechanism of GTP hydrolysis. *Science*, **265**, 1405-1412.
 17. Venkatakrishnan, A.J., Deupi, X., Lebon, G., Tate, C.G., Schertler, G.F. and Babu, M.M. (2013) Molecular signatures of G-protein-coupled receptors. *Nature*, **494**, 185-194.
 18. Rasmussen, S.G., DeVree, B.T., Zou, Y., Kruse, A.C., Chung, K.Y., Kobilka, T.S., Thian, F.S., Chae, P.S., Pardon, E., Calinski, D., Mathiesen, J.M., Shah, S.T., Lyons, J.A., Caffrey, M., Gellman, S.H., Steyaert, J., Skiniotis, G., Weis, W.I., Sunahara, R.K. and Kobilka, B.K. (2011) Grystal structure of the β 2 adrenergic receptor-Gs protein complex. *Nature*, **477**, 549-555.
 19. Preininger, A.M., Van Eps, N., Yu, N.J., Medkova, M., Hubbell, W.L. and Hamm, H.E. (2003) The myristoylated amino terminus of Galpha(i)(1) plays a critical role in the structure and function of Galpha(i)(1) subunits in solution. *Biochemistry*, **42**, 7931-7941.
 20. Franco, M., Chardin, P., Chabre, M. and Paris, S. (1996) Myristoylation-facilitated binding of the G protein ARF1GDP to membrane phospholipids is required for its activation by a soluble nucleotide exchange factor. *J. Biol. Chem.*, **271**, 1573-1578.
 21. Degtyarev, M.Y., Spiegel, A.M. and Jones, T.L. (1994) Palmitoylation of a G protein alpha i subunit requires membrane localization not myristoylation. *J. Biol. Chem.*, **269**, 30898-30903.
 22. Hamm, H.E., Deretic, D., Arendt, A., Hargrave, P.A., Koenig, B. and Hofmann, K.P. (1988) Site of G protein binding to rhodopsin mapped with synthetic peptides from the alpha subunit. *Science*, **241**, 832-835.
 23. Feldman, D.S., Zamah, A.M., Pierce, K.L., Miller, W.E., Kelly, F., Rapacciuolo, A., Rockman, H.A., Koch, W.J. and Luttrell, L.M. (2002) Selective inhibition of heterotrimeric Gs signaling. Targeting the receptor-G protein interface using a peptide minigene encoding the Galpha(s) carboxyl terminus. *J. Biol. Chem.*, **277**, 28631-28640.
 24. Aris, L., Gilchrist, A., Rens-Domiano, S., Meyer, C., Schatz, P.J., Dratz, E.A. and Hamm, H.E. (2001) Structural requirements for the stabilization of metarhodopsin II by the C terminus of the alpha subunit of transducin. *J. Biol. Chem.*, **276**, 2333-2339.
 25. Schwindinger, W.F., Miric, A., Zimmerman, D. and Levine, M.A. (1994) A novel Gs alpha mutant in a patient with Albright hereditary osteodystrophy uncouples cell surface receptors from adenylyl cyclase. *J. Biol. Chem.*, **269**, 25387-25391.
 26. Natochin, M., Muradov, K.G., McEntaffer, R.L. and Artyemyev, N.O. (2000) Rhodopsin recognition by mutant G(s)alpha containing C-terminal residues of transducin. *J. Biol. Chem.*, **275**, 2669-2675.
 27. Hu, J., Wang, Y., Zhang, X., Lloyd, J.R., Li, J.H., Karpiak, J., Costanzi, S. and Wess, J. (2010) Structural basis of G protein-coupled receptor-G protein interactions. *Nat. Chem. Biol.*, **6**, 541-548.
 28. Cai, K., Itoh, Y. and Khorana, H.G. (2001) Mapping of contact sites in complex formation between transducin and light-activated rhodopsin by covalent crosslinking: use of a photo-activatable reagent. *Proc. Natl. Acad. Sci. U. S. A.*, **98**, 4877-4882.
 29. Scheerer, P., Park, J.H., Hildebrand, P.W., Kim, Y.J., Krauss, N., Choe, H.W., Hofmann, K.P. and Ernst, O.P. (2008) Crystal structure of opsin in its G-protein-interacting conformation. *Nature*, **455**, 497-502.
 30. Choe, H.W., Kim, Y.J., Park, J.H., Morizumi, T., Pai, E.F., Krauss, N., Hofmann, K.P., Scheerer, P. and Ernst, O.P. (2011) Crystal structure of metarhodopsin II. *Nature*, **471**, 651-655.
 31. Taylor, J.M., Jacob-Mosier, G.G., Lawton, R.G., Remmers, A.E. and Neubig, R.R. (1994) Binding of an alpha 2 adrenergic receptor third intracellular loop peptide to G beta and the amino terminus of G alpha. *J. Biol. Chem.*, **269**, 27618-27624.
 32. Itoh, Y., Cai, K. and Khorana, H.G. (2001) Mapping of contact sites in complex formation between light-activated rhodopsin and transducin by covalent crosslinking: use of a chemically preactivated reagent. *Proc. Natl. Acad. Sci. U. S. A.*, **98**, 4883-4887.
 33. Natochin, M., Granovsky, A.E., Muradov, K.G. and Artyemyev, N.O. (1999) Roles of the transducin alpha-subunit alpha4-helix/alpha4-beta6 loop in the receptor and effector interactions. *J. Biol. Chem.*, **274**, 7865-7869.
 34. Bae, H., Cabrera-Vera, T.M., Depree, K.M., Graber, S.G. and Hamm, H.E. (1999) Two amino acids within the alpha4 helix of Galpha1 mediate coupling with 5-hydroxytryptamine1B receptors. *J. Biol. Chem.*, **274**, 14963-14971.
 35. Johnston, C.A. and Siderovski, D.P. (2007) Structural basis for nucleotide exchange on G alpha i subunits and receptor coupling specificity. *Proc. Natl. Acad. Sci. U. S. A.*, **104**, 2001-2006.
 36. Grishina, G. and Berlot, C.H. (2000) A surface-exposed region of G(s)alpha in which substitutions decrease receptor-mediated activation and increase receptor affinity. *Mol. Pharmacol.*, **57**, 1081-1092.
 37. Yu, M.Y., Ho, M.K., Liu, A.M. and Wong, Y.H. (2008) Mutations on the Switch III region and the alpha3 helix of Galpha16 differentially affect receptor coupling and regulation of downstream effectors. *J. Mol. Signaling*, **3**, 17.
 38. Westfield, G.H., Rasmussen, S.G., Su, M., Dutta, S., DeVree, B.T., Chung, K.Y., Calinski, D., Velez-Ruiz, G., Oleskie, A.N., Pardon, E., Chae, P.S., Liu, T., Li, S., Woods, V.L. Jr., Steyaert, J., Kobilka, B.K., Sunahara, R.K. and Skiniotis, G. (2011) Structural flexibility of the G alpha s alpha-helical domain in the beta2-adrenoceptor Gs complex. *Proc. Natl. Acad. Sci. U. S. A.*, **108**, 16086-16091.
 39. Abdulaev, N.G., Ngo, T., Ramon, E., Brabazon, D.M., Marino, J.P. and Ridge, K.D. (2006) The receptor-bound "empty pocket" state of the heterotrimeric G-protein alpha-subunit is conformationally dynamic. *Biochemistry*, **45**, 12986-12997.
 40. Van Eps, N., Preininger, A.M., Alexander, N., Kaya, A.I., Meier, S., Meiler, J., Hamm, H.E. and Hubbell, W.L. (2011) Interaction of a G protein with an activated receptor opens the interdomain interface in the alpha subunit. *Proc. Natl. Acad.*

- Sci. U. S. A.*, **108**, 9420-9424.
41. Dratz, E.A., Furstenuau, J.E., Lambert, C.G., Thireault, D.L., Rarick, H., Schepers, T., Pakhlevanians, S. and Hamm, H.E. (1993) NMR structure of a receptor-bound G-protein peptide. *Nature*, **363**, 276-281.
 42. Kisselev, O.G., Kao, J., Ponder, J.W., Fann, Y.C., Gautam, N. and Marshall, G.R. (1998) Light-activated rhodopsin induces structural binding motif in G protein alpha subunit. *Proc. Natl. Acad. Sci. U. S. A.*, **95**, 4270-4275.
 43. Koenig, B.W., Kontaxis, G., Mitchell, D.C., Louis, J.M., Litman, B.J. and Bax, A. (2002) Structure and orientation of a G protein fragment in the receptor bound state from residual dipolar couplings. *J. Mol. Biol.*, **322**, 441-461.
 44. Brabazon, D.M., Abdulaev, N.G., Marino, J.P. and Ridge, K.D. (2003) Evidence for structural changes in carboxyl-terminal peptides of transducin alpha-subunit upon binding a soluble mimic of light-activated rhodopsin. *Biochemistry*, **42**, 302-311.
 45. Oldham, W.M., Van Eps, N., Preininger, A.M., Hubbell, W.L. and Hamm, H.E. (2006) Mechanism of the receptor-catalyzed activation of heterotrimeric G proteins. *Nat. Struct. Mol. Biol.*, **13**, 772-777.
 46. Orban, T., Jastrzebska, B., Gupta, S., Wang, B., Miyagi, M., Chance, M.R. and Palczewski, K. (2012) Conformational dynamics of activation for the pentameric complex of dimeric G protein-coupled receptor and heterotrimeric G protein. *Structure*, **20**, 826-840.
 47. Chung, K.Y., Rasmussen, S.G., Liu, T., Li, S., DeVree, B.T., Chae, P.S., Calinski, D., Kobilka, B.K., Woods, V.L. Jr. and Sunahara, R.K. (2011) Conformational changes in the G protein Gs induced by the β_2 adrenergic receptor. *Nature*, **477**, 611-615.
 48. Natochin, M., Moussaif, M. and Artemyev, N.O. (2001) Probing the mechanism of rhodopsin-catalyzed transducin activation. *J. Neurochem.*, **77**, 202-210.
 49. Marin, E.P., Krishna, A.G. and Sakmar, T.P. (2001) Rapid activation of transducin by mutations distant from the nucleotide-binding site: evidence for a mechanistic model of receptor-catalyzed nucleotide exchange by G proteins. *J. Biol. Chem.*, **276**, 27400-27405.
 50. Marin, E.P., Krishna, A.G. and Sakmar, T.P. (2002) Disruption of the alpha5 helix of transducin impairs rhodopsin-catalyzed nucleotide exchange. *Biochemistry*, **41**, 6988-6994.
 51. Kapoor, N., Menon, S.T., Chauhan, R., Sachdev, P. and Sakmar, T.P. (2009) Structural evidence for a sequential release mechanism for activation of heterotrimeric G proteins. *J. Mol. Biol.*, **393**, 882-897.
 52. Preininger, A.M., Parello, J., Meier, S.M., Liao, G. and Hamm, H.E. (2008) Receptor-mediated changes at the myristoylated amino terminus of Galpha(il) proteins. *Biochemistry*, **47**, 10281-10293.
 53. Kisselev, O.G. and Downs, M.A. (2003) Rhodopsin controls a conformational switch on the transducin gamma subunit. *Structure*, **11**, 367-373.
 54. Cherfils, J. and Chabre, M. (2003) Activation of G-protein Galpha subunits by receptors through Galpha-Gbeta and Galpha-Ggamma interactions. *Trends Biochem. Sci.*, **28**, 13-17.
 55. Rondard, P., Iiri, T., Srinivasan, S., Meng, E., Fujita, T. and Bourne, H.R. (2001) Mutant G protein alpha subunit activated by Gbeta gamma: a model for receptor activation? *Proc. Natl. Acad. Sci. U. S. A.*, **98**, 6150-6155.
 56. Patowary, S., Alvarez-Curto, E., Xu, T.R., Holz, J.D., Oliver, J.A., Milligan, G. and Raicu, V. (2013) The muscarinic M3 acetylcholine receptor exists as two differently sized complexes at the plasma membrane. *Biochem. J.*, **452**, 303-312.
 57. Watts, A.O., van Lipzig, M.M., Jaeger, W.C., Seeber, R.M., van Zwam, M., Vinet, J., van der Lee, M.M., Siderius, M., Zaman, G.J., Boddeke, H.W., Smit, M.J., Pflieger, K.D., Leurs, R. and Vischer, H.F. (2013) Identification and profiling of CXCR3-CXCR4 chemokine receptor heteromer complexes. *Br. J. Pharmacol.*, **168**, 1662-1674.
 58. Teitler, M. and Klein, M.T. (2012) A new approach for studying GPCR dimers: drug-induced inactivation and reactivation to reveal GPCR dimer function in vitro, in primary culture, and in vivo. *Pharmacol. Ther.*, **133**, 205-217.
 59. Ayoub, M.A., Al-Senaidy, A. and Pin, J.P. (2012) Receptor-G protein interaction studied by bioluminescence resonance energy transfer: lessons from protease-activated receptor 1. *Front. Endocrinol. (Lausanne)*, **3**, 82.
 60. Qin, K., Dong, C., Wu, G. and Lambert, N.A. (2011) Inactive-state preassembly of G(q)-coupled receptors and G(q) heterotrimers. *Nat. Chem. Biol.*, **7**, 740-747.
 61. Qin, K., Sethi, P.R. and Lambert, N.A. (2008) Abundance and stability of complexes containing inactive G protein-coupled receptors and G proteins. *FASEB J.*, **22**, 2920-2927.
 62. Gales, C., Van Durm, J.J., Schaak, S., Pontier, S., Percherancier, Y., Audet, M., Paris, H. and Bouvier, M. (2006) Probing the activation-promoted structural rearrangements in preassembled receptor-G protein complexes. *Nat. Struct. Mol. Biol.*, **13**, 778-786.
 63. Challiss, R.A. and Wess, J. (2011) Receptors: GPCR-G protein preassembly? *Nat. Chem. Biol.*, **7**, 657-658.