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G protein βγ subunits: Central mediators of G protein-coupled receptor signaling

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Abstract

G protein βγ subunits are central participants in G protein-coupled receptor signaling pathways. They interact with receptors, G protein α subunits and downstream targets to coordinate multiple, different GPCR functions. Much is known about the biology of Gβγ subunits but mysteries remain. Here, we will review what is known about general aspects of structure and function of Gβγ as well as discuss emerging mechanisms for regulation of Gβγ signaling. Recent data suggest that Gβγ is a potential therapeutic drug target. Thus, a thorough understanding of the molecular and physiological functions of Gβγ has significant implications.

Keywords

G protein-coupled receptor; G protein βγ subunits; signal transduction; protein-protein interactions

Heterotrimeric G proteins and signaling by Gβγ subunits

G protein β subunits were first discovered as components of G proteins almost 30 years ago. Despite enormous advances since then, there remain multiple emerging and unanswered questions about the fundamental details of the biochemical roles for Gβγ in GPCR-dependent G protein activation, as well as questions about broader roles in novel signaling mechanisms, physiology and pathophysiology.

Heterotrimeric G proteins consisting of multiple isoforms of distinct Gα, β and γ subunits mediate the actions of a wide variety of cell surface receptors [1–3]. Receptors catalyze exchange of tightly bound GDP for GTP on the α subunit in a process that requires the complete heterotrimer. In the classical model for G protein signaling, binding of GTP results in activation of the G protein and dissociation of the Gα subunit from the Gβγ subunits (Fig. 1A). In recent years, a variety of reports have suggested additional modes of activation that could either add complexity to the classical model or represent entirely independent mechanisms for heterotrimeric G protein regulation [4–10]. Whatever the mode of G protein activation, the Gα and Gβγ subunits both interact with effector molecules, such as phospholipases and ion channels, in a manner that leads to their activation. Gβγ does not have a catalytic site and thus acts as a modulator of G protein signaling through regulated protein-protein interactions. The list of molecules that have been reported to bind to Gβγ continues to grow. While great progress has been made in the understanding of Gβγ structure and function, fundamental mechanisms for molecular recognition and effector regulation by Gβγ have yet to be fully elucidated. Additionally, how all these interactions are coordinated to mediate various G protein signaling processes in cells and tissues is not entirely clear. In this review we will discuss general background concerning Gβγ structure and function with an emphasis on new and emerging mechanisms and approaches for studying Gβγ signaling. These new data are leading to a greater

understanding of how Gβγ functions at a mechanistic level and at a coordinated physiological level in cells and tissues. Together, this information could establish a basis for development of future therapeutic interventions.

General structure of the Gβγ subunits

The first high resolution structures of the Gβγ subunits arose from X-ray crystal structures of G protein heterotrimers elucidated by the Sprang group [11], and independently by Paul Sigler's group [12]. Subsequently, the structure of the Gβγ dimer alone was solved [13]. The Gβ subunits fold into a prototypical β-propeller comprised of four-stranded β sheets forming each of the seven blades of the propeller (Fig. 1B). The first 57–70 amino acids N-terminal to the β-propeller comprise an α-helical domain that forms a tight coiled-coil interaction with the γ subunit. The most highly conserved regions of the protein are the β sheets of the propeller and variable loops connect the β strands. Two independent structural elements of the Gα subunits interact with different regions of Gβγ. The Gα N-terminal α helix (yellow, Fig. 1C) interacts with the side of the β propeller at blade 1. The Gα switch II region that undergoes conformational changes upon GTP binding interacts with the top of the β propeller (dark blue helix, Fig. 1C). In the structures of Gβγ that have been solved there is very little difference in overall structure, with and without bound Gα or other binding partners [13–15]. An exception is a structure of Gβγ bound to phosducin showing a movement of blades 6 and 7 of the β propeller creating a cavity between these two blades [16]. These movements have not been seen in other structures of Gβγ [15] and their significance remains to be determined. The apparent lack of significant conformation changes of the Gβγ subunit upon G protein activation has led to the concept that Gβγ activity, with respect to downstream signaling, is regulated by the mode of Gα subunit binding, with activation-dependent changes in Gα subunit conformation leading to uncovering a signaling surface on Gβγ [1]. A current debate concerns whether this involves subunit dissociation or subunit rearrangement.

Synthesis and Trafficking of Gβγ

G protein βγ subunits are membrane bound proteins that had been suggested to exist almost exclusively on the plasma membrane (PM) tethered to the membrane through post-translational modification. Recent data has led to significant insight into how G protein subunits are synthesized, assembled, processed, and targeted to membranes. A comprehensive review of G protein subunit assembly and trafficking has recently been published [17], but key points relevant to Gβγ assembly and trafficking will be summarized here. Gβ and γ subunits have no transmembrane hydrophobic domains and are synthesized in the cytoplasm. This process has recently been shown to require specific chaperone proteins. Two chaperones are involved in proper folding and stabilization of the newly synthesized Gβ subunit. The CCT1 (also known as TRiC) chaperonin complex binds newly synthesized Gβ but not Gγ and is required for folding into the seven bladed propeller structure [18]. Phosducin like protein (PhLP) appears to act as a co-chaperone with CCT, regulating CCT mediated folding of Gβ [19]. Once folded, CCT dissociates and PhLP remains bound until assembly with Gγ [20]. A potential chaperone specific for Gγ subunits has also been recently identified as Dopamine Receptor-interacting Protein 78 (DRiP78). DRiP78 binds to Gγ and PhLP and may be required for Gβγ assembly [21]. DRiP78 is localized to the Endoplasmic Reticulum (ER), suggesting that some aspects of the initial assembly process of Gγ with Gβ could occur on the ER.

Once assembled, the Gγ subunit is processed at the C-terminus to attach an isoprenoid moiety. Gγ has signal sequences that direct prenylation with either a farnesyl or geranyl-geranyl moiety. The first step in the C-terminal processing reaction is covalent attachment of the isoprenoid group to a cysteine that is four amino acids from the C-terminus by either geranyl-geranyl or farnesyl transferase. Once modified with lipid the Gβγ complex is targeted to the ER, where a

protease, rasconverting enzyme (Rce1) removes the C-terminal three amino acids from the Gγ subunit. Isoprenyl cysteine carboxy methyl transferase (Icmt) catalyzes carboxy-methylation of the C-terminus of Gγ to yield the fully modified subunit. Assembly with Gα may also occur on the cytoplasmic surface of the ER prior to trafficking to the PM through an unknown mechanism.

Assembly with Gα is important for PM targeting of Gβγ. It was previously thought that geranyl-geranylation of Gγ would be sufficient for PM targeting, but expression of Gβγ alone leads to localization to intracellular membranous structures, primarily ER. However, when coexpressed with Gα, Gβγ localizes primarily to the PM. It has been proposed that Gα lipid modification provides an additional signal that is required for efficient PM targeting of both Gα and Gβγ [22].

Recent data indicates that once Gβγ is fully processed it can translocate to intracellular membranes. Initial work by Berlot and colleagues examined trafficking of $G\alpha_s\beta_1\gamma_7$ complexes after activation by the β2AR [23]. Here, the receptor and the G proteins internalize but segregate to different intracellular compartments upon stimulation with isoproterenol. The G protein Gα and Gβγ subunits initially show diffuse cytoplasmic distribution followed by colocalization on intracellular vesicles distinct from βAR containing vesicles. Subsequent work by others has suggested GPCR- activation results in translocation of fluorescently tagged G protein γ subunits or tagged β subunits to intracellular membranes such as the Golgi or ER [24]. Distinct families of Gγ subunits were found that translocate, whereas other families do not [25]. For example it was found that $\gamma_{1,\ 5,\ 9,\ 10,\ 11\ \text{and}\ 13}$ support M2 receptor-dependent translocation, whereas $\gamma_{2,\ 3,\ 4,\ 7,\ 8\ \text{and}\ 12}$ do not [26]. Differences in translocation appeared to correlate with specific sequences in the C-terminus of Gγ. These findings are intriguing and suggest specific functional roles for Gγ subunit subtypes although the role of the translocation remains to be defined.

G protein β and γ subunit subtypes

Five different Gβ subunit and 12 different Gγ subunit genes have been identified in the human and mouse genomes [27]. The $G\beta_{1-4}$ subunits share greater than 80% amino acid sequence identity compared to 50% identity for $G\beta_5$. There is significantly lower identity amongst the γ subunit subtypes. These different subunit subtypes can pair to form unique $G\beta_x\gamma_x$ combinations. The functional significance of the diversity of individual Gβγ subunit combinations is not well understood. Interpretation of phenotypes resulting from knockout of individual Gβγ subunits is complicated by the fact that Gβγ participates in multiple, integrated functional interactions with receptors, Gα subunits, and effectors. Thus, the resulting phenotypes can be difficult to attribute to a specific functional interaction. Nevertheless, there is evidence from knockout studies to suggest that specific Gβγ subtypes interact with particular GPCRs. Ribozyme-directed $G\gamma_7$ subunit depletion impaired β-adrenergic receptor signaling but not signaling by another Gs coupled receptor, PGE_1 [28]. Genetic deletion of specific γ subunits in mice results in specific phenotypes. For example, deletion of $G\gamma_7$ resulted in distinct behavioral changes associated with specific loss of cAMP production in the striatum [29], and deletion of $G\gamma_3$ results in changes in metabolism resulting in resistance to a high fat diet [30]. In both of these cases loss of the specific Gγ subunits also resulted in a loss in specific Gα expression, indicating roles for specific Gαβγ combinations in these phenotypes.

One instructive example comes from a recent study of Gγ functions in the *Arabidopsis thaliana* plant system. In this plant and other species there is one Gα subunit, one Gβ subunit, and two Gγ subunits that share approximately 50% amino acid identity [31–34]. Knockout and overexpression of these two Gγ subunits allowed for a relatively simple dissection of the functional significance of the two Gγ subunit isoforms. In this system, many of the functional

effects appear to be mediated by Gβγ. For example, Gβ plays a specific role in plant pathogen resistance and knockout of A. thaliana $G\gamma_1$ increased susceptibility to infection with a pathogenic fungus, while $G\gamma_2$ deletion had no significant effect on this function [35]. Similar specificity was seen with other processes involved in seed germination and root development. Thus in this relatively simple G protein system, clear, distinct roles are observed for different Gγ subunits. The mammalian system is obviously much more complex with many different Gγ subunits, where many detailed experiments will be required to sort out their individual specific functions.

To date, there is no specific, satisfactory mechanistic explanation for the selectivity for different Gβγ subunits that is observed in intact cells. Although some selectivity has been observed in some *in vitro* reconstitution systems, the difference between subtypes is generally not dramatic. An exception is Gβγ complexes containing the $G\gamma_1$ subunit, which is strictly localized to the retina as part of the transducin heterotrimer. These complexes are generally less potent for activation of effectors such as adenylyl cyclase (AC) and phospholipase C (PLC) [36,37] and couple less strongly to GPCRs other than rhodopsin. $G\gamma_1$ is modified with a 15 carbon farnesyl rather than a 20 carbon geranylgeranyl lipid moiety and some of the difference may result from this, but there is also evidence that there are sequence determinants on $G\gamma_1$ that may be partially responsible for differences in efficacy and potency of this subunit [38]. Some other examples of biochemical selectivity for effectors exist but, in general, the differences are not enough to explain the striking differences observed in intact cell systems or *in vivo*.

$G\beta_5$

$G\beta_5$ is clearly an outlier with respect to sequence homology with other Gβ subunits, with 53% identity to the most closely related Gβ subunit. Initial evidence that $G\beta_5$ was a bona fide Gβ subunit was its ability to assemble with Gγ subunits in transfected cells to activate PLCβ2 [39,40]. Further analysis of purified $G\beta_5\gamma_2$ complexes revealed that Gγ was loosely bound and could be separated from the $G\beta_5$ subunit under non-denaturing detergent conditions where other Gβγ combinations are not separable [41,42]. This $G\beta_5\gamma_2$ complex was initially proposed to be only capable of binding to $G\alpha_q$ [43] but other workers demonstrated interactions with $G\alpha_{i/o}$-GDP [42,44].

This latter data indicates that $G\beta_5$ has the determinants to direct $G\beta_5$ containing complexes to GDP-bound Gα subunits. Siderovski and colleagues were the first to recognize that members of the RGS7 (R7) subfamily of regulators of G protein signaling (RGS proteins) contained regions with significant homology to Gγ subunits (ggl domains) and predicted that they could potentially assemble with Gβ subunits [45] (Fig. 2A and B). Biochemical studies demonstrated that R7 family members could form stable complexes with $G\beta_5$ but not other Gβ subunits. In parallel, Slepak and colleagues purified native $G\beta_5$ from bovine retina and identified R7 family members as tightly associated proteins that co-purified [46]. In neither of these studies was Gγ found to co-purify or form stable complexes with $G\beta_5$ [47]. This leads to a currently unresolved debate as to whether $G\beta_5\gamma_x$ is present in native cells or tissues. It has been suggested that the difficulty in finding $G\beta_5\gamma_x$ in native tissues is due to its inherent instability in detergent that is required to extract Gβγ subunits from native tissue. A recent study examining $G\beta_5$ complex formation with different Gγ subunits in cells using bifluorescence complementation (BiFC) suggests that $G\beta_5$ slightly prefers $G\gamma_2$ as a binding partner relative to RGS7. These data suggest that in native tissues, if $G\gamma_2$ is present in significant quantity, it would assemble with $G\beta_5$ [44]. On the other hand, other factors such as molecular chaperones may add a level of control to assembly that is not observed in the transfected HEK293 cells.

While the occurrence of this $G\beta_5$ RGS complex is very novel and exciting it is not clear how this complex is regulated and what the functional and physiological role of the complex is in

GPCR signaling. Some exciting clues have come with the discovery of a protein, R7BP, that binds to R7 family members [48,49]. This protein binds to the DEP (for Disheveled, EGL-10, Pleckstrin) homology domain of R7 family members and can regulate the distribution of $G\beta_5$R7 complexes in cells [48,49]. R7BP can be palmitoylated near its carboxy-terminus, and regulated palmitoylation depalmitoylation at this site determines the subcellular localization of the R7BP/$G\beta_5$/R7 complex [49]. Palmitoylated R7BP targets the complex to the PM where it can efficiently inhibit GPCR mediated processes through its RGS domain, while the depalmitoylated form undergoes nuclear-cytoplasmic shuttling that could be involved in regulation of transcription [49,50]. Interestingly, the DEP domain of R7 is involved in an intra molecular interaction with the $G\beta_5$ subunit in the $G\beta_5$/R7 complex [51,52]. In the recently solved crystal structure of $G\beta_5$-RGS9 complex, the DEP domain, in conjunction with the ggl-DEP linker, occludes the Gα binding site on $G\beta_5$ [52].

Regulatory mechanisms may exist that "uncap" the Gα binding site on $G\beta_5$ to allow productive interactions with Gα subunits for receptor catalyzed nucleotide exchange reactions [53,54]. For example, R7BP could affect this interaction which could, in turn, affect the activity of the $G\beta_5$/R7 complex (Fig. 2B and C). Binding of R7BP to $G\beta_5$/R7 complex improves the activity of the complex as a GAP through PM targeting, but additional mechanisms must exist that involve reversible interactions of the DEP domain with $G\beta_5$ to allow receptor-G protein coupling.

While these regulatory mechanisms are emerging for the $G\beta_5$/R7 complexes, the functional purpose of $G\beta_5$ association with this complex is still unclear. While $\beta_5\gamma_x$ complexes may not be biologically relevant signaling complexes, analysis of the complexes provides information on the molecular determinants that $G\beta_5$ itself may bring to the $G\beta_5$/R7 complex. For example, studies with $G\beta_5\gamma_2$ indicate $G\beta_5$ is capable of binding Gα subunits, interacting with Gβγ regulated effectors, and participating in receptor mediated G protein nucleotide exchange. That these functionalities exist in $G\beta_5$ suggests that $G\beta_5$ could bring some of these functions to the $G\beta_5$/R7 complex.

Central functional role in G protein coupled receptor signaling

G protein βγ subunits are required for GPCR signaling

Early reconstitution studies with receptors and purified G proteins indicate that Gβγ is required for GPCR catalyzed nucleotide exchange [55,56]. Studies examining muscarinic receptor coupling to $G\alpha_o$ initially suggested that a possible reason for the requirement for Gβγ was that Gβγ was simply required to target $G\alpha_o$ to the membrane and that, once properly oriented at the plasma membrane, Gα could productively engage the receptor [57]. While this may be part of the requirement for Gβγ function in receptor coupling, it does not exclude other mechanisms for promoting coupling and cannot, in itself, explain receptor selectivity for particular Gβγ isoforms. Another possibility that is supported in part by structural data is that binding of Gα to Gβγ organizes the structure of the Gα subunit such that it is a substrate for receptor interactions. Free $G\alpha_i$ subunits are in a distinct conformation relative to the structures in the G protein heterotrimer [11,12,58,59]. For example, in the structure of $G\alpha_i$-GDP the amino terminus forms a distinct globular domain that adopts an extended helical conformation in direct contact with Gβγ in the $G\alpha_i$-GDPβg complex [58]. The amino terminus of the Gα subunit is important for engagement of phospholipid membranes through lipid modifications at the N-terminus. Additionally, amino acids in the Gα N-terminus are important for receptor-G protein coupling, suggesting that Gβγ may help present Gα in the appropriate conformation to the receptor [60].

GPCRs also interact directly with G protein βγ subunits. A peptide mimic of the third loop of the α_{2A} adrenergic receptor catalyzed nucleotide exchange on $G\alpha_o$ in a purified system only

in the presence of the Gβγ subunits [61]. The peptide was directly crosslinked to the G protein β subunit, and the crosslinking site was mapped to the C-terminus [62]. Other studies have demonstrated direct binding of receptor fragments to Gβγ subunits primarily using glutathione S-transferase (GST) fusion protein binding assays. For example, a portion of the C-terminal tail of the parathyroid hormone receptor bound directly to Gβγ subunits [63]. The specific Gβγ binding site was disrupted in the full length PTH receptor by site directed mutagenesis leading to a loss of downstream signaling to PLC and AC. In other studies with the third intracellular loop of the M3 muscarinic receptor, disruption of the Gβγ binding site did not affect downstream signaling but inhibited receptor desensitization [64]. The data suggest that maintenance of Gβγ binding to this loop facilitated recruitment of G protein coupled receptor kinase (GRK) to the receptor.

Another point of contact between Gβγ and receptors is the C-terminus of the Gγ subunit. As discussed earlier, Gγ is prenylated at a C-terminal cysteine. This prenyl modification is required for receptor-G protein coupling but, since it is also required for membrane targeting, it is difficult to determine if the G protein coupling requirement simply reflects the need for Gβγ to be at the membrane or if it is a direct physical coupling between the receptor and Gγ. Evidence for direct physical interactions comes from experiments utilizing prenylated C-terminal peptides from $G\gamma_1$. These peptides inhibit receptor-G protein coupling and alter the activation state of rhodopsin, indicating a direct physical interaction [65]. NMR studies show a specific conformational alteration of the peptide upon receptor activation [66,67]. In many of the structures of Gβγ this region of Gγ is disordered, suggesting this region may be conformationally flexible.

Still unanswered is how direct GPCR-Gβγ interactions are mechanistically involved in the G protein activation process. This, in part, comes from our current lack of general understanding of the mechanistic details of GPCR stimulated nucleotide exchange. As discussed, some models of receptor activation portray the Gβγ subunit as a passive participant that scaffolds Gα at the membrane, while others indicate an active role. Two models that include Gβγ as an active participant include the lever hypothesis and the gearshift model. In the lever hypothesis, the receptor has been proposed to engage both Gα and Gβγ and pry the two molecules apart by pulling on the N-terminus of Gα, acting as a lever to open up the interface between the switch II region of the Gα subunit contacting the Gβ subunit (Fig. 3A). As the subunits rearrange, the Gβ subunit pulls open the nucleotide binding pocket on Gα, enhancing the off-rate of GDP [68]. One line of evidence in support of this hypothesis is that mutation of amino acids on Gβ that directly interact with Gα switch II prevents Gβ-dependent Gα activation. In contrast, other data indicates that the Switch II Gβ interface can rapidly “breathe” without full subunit dissociation. This model, depicted in figure 4A, is based on evidence that Gα N-terminal interactions and Gα Switch II interactions with Gβ are individually weak but the combined bivalent interaction is strong [69,70]. Peptides and proteins appear to be able to bind to Switch II binding surface on Gβ during breathing and cause G protein βγ subunit activation without nucleotide exchange on Gα, as discussed in section 6 (Fig. 4B and C). Since the Gα/βγ interactions at these surfaces are weak, they may not be sufficiently stable to pry open the nucleotide binding pocket as the subunits reorient relative to one another in the lever model.

The gear shift hypothesis suggests that the receptor increases interactions between the coiled-coil amino terminus of the Gβγ complex and the helical domain of Gα, pushing the helical and Ras-like domains of Gα apart to provide an exit route for GDP (Fig. 3B) [71]. Recent BRET data examining Gα-βγ interactions in intact cells demonstrate that some parts of the helical domain of Gα move away from the N-terminus of Gγ subunits upon activation, but the region linking the helical and GTPase domains move closer to Gγ-N. The authors suggest that these movements, where parts of Gβγ and Gα move closer together during the activation process, are most consistent with the gearshift model for G protein activation [6]. In either mechanism,

Gβγ is intimately involved in the process of nucleotide exchange. For a detailed discussion of these models see Oldham and Hamm [60].

If, in fact, there are direct interactions between receptors and Gβγ that are important for the mechanisms of nucleotide exchange and subunit dissociation, it has significant implications for the specificity with which receptors recognize specific Gβγ isoforms. If the sole function of Gβγ were to serve as a scaffold for Gα, the reported selectivity of receptors for particular Gβγ subtypes would be unlikely to have been observed.

Mechanisms for activation of Gβγ signaling

In addition to its supporting role in GPCR-dependent Gα interactions, Gβγ acts to directly regulate down-stream signaling in its own right. The first effector found to be activated by Gβγ was the acetylcholine-regulated inwardly-rectifying K^+ channel in atrial myocytes [72]. A key observation in isolated inside-out patches from atrial myocytes was that the acetyl choline driven channel activation was independent of soluble second messengers, suggesting that the subunits of the Gi protein could directly activate the channel. This led to attempts to directly activate the channel in excised atrial membrane patches with Gβγ and Gα subunits [72,73]. After considerable controversy a consensus emerged that Gβγ subunits are the primary mediators of channel activation through direct binding to the channel [74,75]. At first, recognition of Gβγ as the primary mediator of channel activation was controversial, in part, because Gβγ mediating direct downstream functions had not been previously demonstrated, and because precedent with adenylyl cyclase demonstrated that $G\alpha_s$ was responsible for direct stimulation of adenylyl cyclase activity. Additional support for the idea that Gβγ is a signal mediator in its own right came from genetic analysis of the pheromone signaling pathway in yeast, indicating that Gβγ is the key activator of downstream signaling from the G protein coupled pheromone receptor [76]. Today, the list of proteins that interact with Gβγ has expanded to encompass a large number of targets (Table 1). In some cases, these are enzymes or channels and it is clear that Gβγ-binding has a functional effect on the activity of the target. In the case of ERK, Gβγ expression in cells leads to ERK activation, but the direct binding target and the exact mechanism for this activation has not been defined. In many instances the regulation of the target has a clearly documented physiological correlate whereas, in others, the physiological significance of the Gβγ-target interaction is not clear. Overall, however, it is now well established that the Gβγ subunits play major roles in mediating downstream signaling from GPCRs and may be as prevalent as those mediated by Gα subunits.

When considering how G proteins are activated by GPCRs, it is the Gα subunit that undergoes significant conformational changes upon binding of GTP, suggesting an obvious mechanism for Gα activation. So how can Gβγ be “turned on” to propagate a down-stream signal? The current model, as discussed earlier, is based on the subunit dissociation model where Gα subunits occlude effector binding surfaces on G protein βγ subunits until activated by binding of GTP. The conformational changes in Gα lead to dissociation from Gβγ to expose effector interaction surfaces on Gβγ (Fig. 1). Some evidence for this idea stems from the observation that addition of purified GαGDP to *in vitro* assays of Gβγ-dependent effector activation inhibits effector regulation [72,77]. Since there is no apparently critical difference in Gβγ subunit structure in either the free or heterotrimeric structure, it suggests that G protein activation does not cause alteration of Gβγ subunit conformation [11–13]. A direct test of the hypothesis that a signaling surface on Gβγ is covered by Gα involved alanine substitution of amino acids at the Gα subunit-binding surface of the Gβ subunit and testing the purified mutant protein for activation of effectors in various *in vitro* assay systems [78,79]. Many of these purified Gβγ mutants were unable to efficiently regulate effectors. An important conclusion from this analysis was that each effector utilized this surface with both overlapping and unique subsets of amino acids within the Gα subunit binding surface. Complementary studies used a similar

mutational approach to map effector binding sites in the blade regions of the Gβ propeller and identified amino acids outside the Gα subunit interface important for effector regulation, with some amino acids involved in unique effector interactions [80]. Thus, G protein α subunit activation likely exposes surfaces on Gβ that form a core site for effector binding but multiple other Gβγ surfaces also participate in effector binding and activation.

Pertussis toxin-sensitive signaling by G protein βγ subunits

As discussed above, acetyl choline-dependent regulation of the atrial K^+ channel is now known to be through Gβγ binding to the channel. This acetylcholine regulation of the potassium current is inhibited in isolated atrial myocytes by pretreatment with pertussis toxin (PTX) which selectively modifies $G\alpha_i$ family G proteins [81]. Many other processes dependent on Gβγ downstream signaling are also inhibited by PTX. For example, GPCR-dependent PLC activation is mediated by pertussis toxin-sensitive and -insensitive mechanisms [82,83]. The PTX-insensitive pathways are primarily mediated by $G\alpha_q$-dependent activation of PLCβ or pathways involving Rho and PLCε [83–85]. PTX-sensitive pathways were presumed to be mediated by members of the $G\alpha_i$ family yet purified PTX- sensitive $G\alpha_i$ family subunits were unable to reconstitute activation of PLC. On the other hand purified Gβγ subunits were able to activate PLCβ isoforms *in vitro*, albeit at relatively high concentrations compared to typical activation by G protein α_q subunits [86–88]. This suggested that Gβγ subunits released from Gi heterotrimers were responsible for PTX-sensitive GPCR-dependent PIP_2 hydrolysis. The model for these and other related systems is that PTX-dependent ADP ribosylation of the Gα subunits prevents productive interactions between the heterotrimer and receptors preventing nucleotide exchange and activation of $G\alpha_i$, keeping Gβγ sequestered in an inactive state (Fig. 1A). Many of the GPCR-dependent physiological processes inhibited by PTX are mediated by Gβγ subunits rather than Gα [72,89–91]. Fewer examples of PTX-insensitive processes being mediated by the Gβγ subunits have been reported but likely exist [92]. Thus, most Gβγ-dependent signaling appears to arise from Gi proteins.

The apparent specificity of Gβγ-dependent signaling for Gi-coupled receptors presents a conundrum in terms of what regulates the selectivity of Gβγ-dependent processes. In theory GPCR-dependent activation of any G protein subtype would, upon nucleotide exchange-dependent activation of the Gα subunit, lead to free Gβγ that could activate any of the Gβγ effectors. Several hypotheses have been proposed to explain the observation that not all GPCRs activate Gβγ-dependent signaling processes. 1) Gβγ subtypes are specifically associated with particular receptors and G protein α subunits and confer effector activation selectivity. The problem with this hypothesis is that specific Gβγ subtypes have not been shown to be selective for particular effectors, so while specific subtypes may be associated with particular receptors; it is not clear how these subtypes would confer selectivity for particular effector pathways. 2) The potency for Gβγ subunit dependent activation of effectors is 10–100 fold lower than for Gα subunit mediated effects (see [86] for example). This suggests that activation of receptors that activate $G\alpha_s$ or $G\alpha_q$ would cause activation of Gα subunit-dependent effectors at levels of G protein activation that would be significantly lower than that required to release enough Gβγ to activate a Gβγ-dependent effector. So, for example, under conditions required to achieve maximal inositol phosphate release through a G_q-coupled receptor, Gβγ-dependent processes would not be activated. Since $G\alpha_i$ has a relatively low affinity for AC and G_i proteins are relatively abundant, activation of G_i coupled receptors could release enough Gβγ to achieve significant effector activation. Based on the ideas discussed in the above section, one would still predict that G_i coupled receptor-activation would lead to stimulation of many of the effectors listed in Table 1. Some other ideas that could account for the fact that not all Gβγ-regulated effectors are activated by Gi coupled receptors, or other receptor types, include: 1) Tissue specific expression of some of the components provides some constraint, with some effectors expressed relatively specifically in certain tissues. For example, PLCβ2 and PI3-

kinase γ are relatively restricted in expression to monocytic cells, and so would only be activated by GPCRs in these cells, but for other effectors an additional mechanism must exist in cells with multiple GPCRs, 2) Restricting the subcellular location of specific effectors and receptors could impart specificity, 3) Precoupling of receptors G proteins and effectors could confer specificity. For example, GIRK channels have been shown to preferentially form complexes with hetero-trimers containing specific $G\alpha_i$ subunits and, while Gα subunits do not regulate channel activation, they do bind directly to the channel [93,94]. If particular Gα subunits provide a docking surface for Gβγ targets this could control specificity for specific Gα subunit subtypes [95], 4) Simultaneous activation of GPCRs with other receptors could lead to availability of Gβγ in concert with other cellular signals such as phosphorylation, providing a coincidence detection mechanism for activation of specific effectors.

Effector recognition by Gβγ

The targets listed in Table 1 that are recognized by Gβγ comprise a diverse array of molecules, many of which are unrelated in terms of structure and sequence. A key question is: What is the nature of molecular recognition that allows Gβγ to interact specifically and productively with this diverse array of targets? We will discuss here what is known about the nature of recognition of targets by Gβγ based on both direct structural visualization of complexes as well as other biochemical analyses.

Three dimensional crystal structures of Gβγ effector complexes: G protein coupled-receptor kinase 2 (GRK2) and phosducin

The structure of Gβγ has been solved in complexes with Gα subunits, GRK2, and phosducin. Detailed examination of the nature of the interactions supports the general hypothesis that there are common and unique interactions amongst various Gβγ targets. This is exemplified best in the co-crystal structure of Gβγ and phosducin [14,16]. Phosducin, a protein first identified as a regulator in the visual signaling system, binds to Gβγ and is composed of distinct N and C-terminal domains. Both of these domains are required for productive interactions with Gβγ. In the three dimensional structure of the complex, the N-terminus of phosducin associates with an area that overlaps with the GαGDP binding site on the top of the β-propeller, while the C-terminus interacts with the sides of the propeller at blades 1 and 7, a region that does not overlap with GαGDP binding [14,16]. On the other hand the GRK2-$G\beta_1\gamma_2$ interface is dominated by interactions at the GαGDP binding site on the top surface of Gβ [15,96]. Interestingly while both phosducin and GRK2 have interactions on the top of the β-propeller at the Gα subunit interface, the modes of interaction are quite different when compared to Gα. For example the GRK2 C-terminus is an extended α helix followed by a short C-terminal loop and it is the loop that interacts with amino acids that also contact the Gα subunit [97]. In the Gα subunit the major contacts with these same amino acids on Gβ are from the switch II α helix region and it is the side chains extending from this helix that interact with Gβ. Thus, completely structurally distinct motifs from Gα and GRK2 interact with a very similar contact surface on Gβ.

Peptide mapping approaches

While crystallography is invaluable in determining protein interaction surfaces, thus far only a limited number of complexes of Gβγ with binding partners have been solved by this method. As an alternative, biochemical methods have been used to map effector binding surfaces. A particularly fruitful approach has been to use synthetic peptides from Gβγ target molecules. Initial studies in this area identified a peptide from type II adenylyl cyclase that binds to Gβγ and blocks Gβγ-dependent regulation of multiple, Gβγ-regulated effectors [98]. The authors used a molecular modeling and chemical crosslinking approach to identify the binding site for this peptide on Gβγ subunits [99,100]. This binding site mapped to a surface near the Gα switch

II-binding site on Gβγ subunits and correlates well with the mutagenic mapping analysis of ACII contacts on Gβ.

A similar approach was used to map interaction sites between PLCβ2 and Gβγ. Initial analysis indicated that a protein fragment containing a region of the catalytic domain could block Gβγ-dependent PLCβ2 activation in transfected COS cells and bound to purified Gβγ *in vitro* [101]. Further analysis with peptides and chemical crosslinking mapped the binding site for this peptide to two sites on G protein βγ subunits, one in the switch II binding region and another at the amino terminus of the Gβ subunit near cysteine 25 (Fig. 1B, spacefilled CPK) [42,102,103]. Crosslinking to both of these sites was blocked by preincubation by intact PLCβ2 or PLCβ3 [102]. This indicated that the amino terminus of Gβ may function as an effector binding site. Mutagenesis of this site in the Gβ subunit to disrupt PLC interactions actually potentiated Gβγ-dependent activation of PLCβ2, suggesting that binding of this site to PLCβ2 inhibits PLC activity [103]. Interestingly, this site plays a role in activation of PLCβ2 in the presence of AGS8, as will be described in a later section. The observation that the amino terminus of Gβ is an important interaction site in mammals awaits confirmation by other laboratories, but it correlates directly with an effector binding site identified in yeast Gβγ subunits [104,105].

Another approach to examining effector binding surfaces on Gβγ and regulation of target molecules has been to use peptides from Gβ subunits and test them in effector regulation assays. Peptides from different blades of the Gβ propeller were shown to inhibit Gβγ dependent regulation of type II adenylyl cyclase [99] or PLCβ2 while others stimulated PLCβ2 independent of Gβγ [106]. This led to the concept that there are distinct effector-binding and signal-transfer surfaces on Gβγ. This is based on the hypothesis that blocking peptides correspond to binding surfaces that contribute to the energetics of the Gβγ-target binding, but are not involved in altering target activity. On the other hand, activating peptides from Gβγ have been proposed to represent signal transfer surfaces that mediate the activation of the effector. This interesting concept remains to be developed further with mutagenic analysis of intact Gβγ subunits.

Protein Interaction "Hot Spot" on Gβγ

Identification of the specific amino acids in Gβγ involved in individual target recognition does not explain the molecular basis for Gβγ-dependent recognition of diverse effector structures. Various Gβγ binding motifs within effectors have been proposed [98] but it has become clear that there is no single consensus sequence or structural motif that mediates binding to Gβγ. As an approach to understanding this, Gβγ subunits were used as targets inarandom peptide phage display screen in an attempt to identify consensus sequences for binding to distinct surfaces on Gβγ [107]. Multiple, distinct peptides were identified that apparently bound to the same surface on Gβγ based on competition and mutational analysis. This result, where large protein surfaces are subjected to selection in naïve random peptide-binding screens, and only a small portion of the overall surface mediates binding of diverse sets of peptide sequences, is indicative of a preferred protein binding surface [108,109]. Combining these data with alanine scanning mutagenesis and structural analysis has led to the concept of energetic "hot spots" that provide key energetic residues for binding at a protein-protein interface, but also have intrinsic physical-chemical characteristics that are optimal for mediating multiple protein-protein interactions [109]. Some characteristics of these surfaces are flexibility and the opportunity for mediating multiple types of chemical interactions (ionic, hydrophobic) without strict geometric requirements for binding [110]. In this way a single binding site can accommodate multiple structural and chemical motifs.

Crystallographic determination of the structure of a phage display selected peptide (SIGK) bound to Gβγ identified the preferred binding surface as a site corresponding to the Gα subunit

switch II binding region on Gβ [111]. Alanine substitution of all of the amino acids within 6Å of this peptide binding site defined amino acids required for peptide binding. Each of these alanine substituted mutants was then tested for ability to affect binding of other peptides identified in the original phage display screen. Each of the peptides had a unique pattern of requirements for interactions with specific amino acids within the binding site. This demonstrated that the "hot spot" has the inherent ability to bind multiple binding sequences with unique sets of interactions that can be exploited by natural binding partners and suggests a mechanism for Gβγ interaction with multiple different sequences and structures.

Mechanisms for effector regulation by Gβγ

In the previous section, modes of binding and recognition of targets by Gβγ were discussed, but how Gβγ-binding translates into alterations in functional activity of downstream targets has also been investigated by multiple laboratories. Two general mechanisms for effector regulation by Gβγ depend on whether the target is cytosolic or membrane bound. In the case of cytosolic proteins such as PLCβ2 or GRK2, whose substrates are localized to the plasma membrane, a potential mechanism for activation is recruitment to the plasma membrane by membrane-bound Gβγ. For other targets, such as adenylyl cyclases or GIRK channels, that are transmembrane proteins, regulation must occur through conformational alteration. While many effectors are activated by Gβγ, the potential mechanisms for regulation of each of these are too numerous to be discussed here. GRK2 and GIRK regulation will be discussed briefly because mechanisms of activation of GRK2 and GIRK have been well studied and represent examples of either translocation-based or allosteric regulation. The mechanism for activation of PLCβ by Gβγ is less clear and may, in fact, be regulated by both translocation and allosteric regulation, as will be discussed in greater detail.

Activation of GRK2 by Gβγ

An example of an enzyme whose activity is regulated by Gβγ-dependent translocation is GRK2. GRK2 is normally cytoplasmic and there is strong evidence that, during receptor activation, free Gβγ subunits are released that provide a binding site for GRK2. In cooperation with phosphatidylinositol 4,5 bisphosphate (PIP_2), Gβγ subunits recruit GRK2 to the membrane, where it can interact with and phosphorylate activated GPCRs [112]. In addition to its role in membrane recruitment, Gβγ could allosterically modulate GRK2 function. Complexes between Gβγ and GRK2 have been crystallized and the structures solved [15,96]. Since the GRK2 structure in the absence of Gβγ was not solved, it could not be determined whether Gβγ-binding alters the structure of GRK2. Biochemical analyses suggest subtle rearrangements of the GRK2 structure upon Gβγ-binding, but the functional significance of these alterations are not clear [97].

Activation of GIRK channels by Gβγ

In the case of all membrane bound proteins such as ion channels and adenylyl cyclases the activation mechanism requires structural alterations rather than translocation. No direct structural data yet exists that demonstrates specific alterations of effector conformation upon Gβγ binding. For Gβγ-dependent regulation of GIRK, a combination of mutagenic analysis, biophysical studies of channel properties, and homology modeling based on a bacterial voltage-dependent K^+ channel, have been used to develop a proposed mechanism for Gβγ-dependent activation. The model suggests that Gβγ binding to an intracellular soluble domain of the channel strengthens interactions between the channel and PIP_2 and alters the position of a helix at the mouth of the conductance pore to increase the activity of the channel [113,114]. For this and other targets the details of conformational changes that occur upon Gβγ binding are unknown and await detailed atomic level structural determination of an effector with and without bound Gβγ.

Activation of PLCβ by G protein βγ subunits

Two independent analyses examined whether translocation is necessary for Gβγ-dependent activation of PLC activity. Both found that PLCβ has an intrinsic capacity to bind to membrane surfaces that is independent of interactions with Gβγ subunits [115,116]. In these experiments, Gβγ subunits did not alter the proportion of PLC associated with membrane surface but, at the same time, increased PLC activity. This indicates that one mechanism for activation of PLC isoforms is to alter its enzymatic activity either through conformational alteration of the active site or modulating the orientation of PLC with respect to the membrane surface.

Key to understanding how Gβγ activates PLC that is bound at the membrane is to understand the mode of interaction of Gβγ with PLC. Structures of PLCδ1 and PLCβ2 have been solved that provide a detailed picture of the domain organization of these enzymes (Fig. 5A and B) [117, 118]. PLCδ and PLCβ2 share very similar domain structures. Both contain an N-terminal pleckstrin homology domain followed by an EF hand domain, conserved X and Y domains that comprise the catalytic domain and a C2 lipid binding domain. In PLCβ2 the C2 domain is followed by an extended C-terminal domain that interacts with $G\alpha_q$ GTP. The PH domain of PLCδ was deleted in the expressed protein used to solve the PLCδ structure and the C-terminal extension beyond the C2 domain was removed in the protein used to solve the PLCβ2 structure. Early biochemical studies indicated that deletion of the PLCβ2 C-terminus eliminates regulation by $G\alpha_q$ without affecting Gβγ-dependent regulation [119]. While a structure has been solved for a Rac-PLCβ2 complex, no structural data exists as yet for the Gβγ/PLCβ complex. On the other hand, biochemical approaches have yielded information about the nature of Gβγ-PLC interactions. Two sites for interaction of Gβγ on PLCβ2 have been proposed, one on the catalytic domain and one on the PH domain. Here, the data supporting these two sites will be presented and the implications with respect to regulation of PLC enzymatic activity by Gβγ will be discussed.

The first evidence that the catalytic domain could interact with Gβγ came from a screen of fragments of PLCβ2 for their ability to compete for PLCβ2 activation by Gβγ in transfected tissue culture cells [101]. Two overlapping fragments from the catalytic Y domain of PLC blocked activation by Gβγ or a G_i coupled C5A receptor but not the G_q coupled α_1-adrenergic receptor. A GST fusion protein comprising a portion of one of these fragments, L580-V641 within the conserved Y domain, bound directly to purified Gβγ *in vitro*, demonstrating a direct interaction between the catalytic domain of PLCβ2 and Gβγ. To further narrow down the interaction region, examination of a homology model of PLCβ2 based on the structure of PLCδ identified surface exposed regions likely to be accessible to Gβγ [102]. Overlapping peptide fragments corresponding to these exposed regions were synthesized and shown to inhibit Gβγ-dependent activation of PLCβ2 in a purified system leading to identification of E574-K583 as a Gβγ binding region on PLCβ2 (Fig. 5B, light blue helix). Direct interaction of these peptides from the PLCβ2 catalytic domain with Gβγ was confirmed by chemical crosslinking to both Gβ and Gγ in a manner that was competed with excess PLCβ2 or PLCβ3 holoenzyme [102]. To confirm that this region was important for PLCβ2 activation in the context of the PLCβ2 holoenzyme, triple alanine substitutions in the PLCβ2 E574-K583 helix inhibited activation of PLCβ2 by Gβγ subunits with minimal effects on PLC basal enzymatic activity [103]. Finally, triple alanine mutation of E574, L575 and K576 disrupted direct binding of purified PLCβ2 to Gβγ [120]. Together, these data strongly suggest that this region of the catalytic domain is involved in direct interactions with the Gβγ subunit and that interaction of Gβγ with these amino acids regulates PLCβ2 activity.

In support of the idea that the pleckstrin homology domain confers binding and activation by Gβγ is the observation that the isolated PH domain from PLCβ2 interacts with Gβγ on membrane surfaces as detected by fluorescence resonance energy transfer [121]. A second key observation is that splicing of the PLCβ2 PH domain onto PLCδ confers the ability of PLCδ

to be activated by G protein βγ subunits [122]. This chimeric PLC bound to lipid membranes with properties similar to PLCβ2, suggesting the activation involved conformational activation rather than membrane translocation. Point mutations in the PH domain of the chimera inhibited Gβγ-dependent activation. On the other hand, in chimeras of PLCβ2 with the PH domain of PLCβ1, there is no substantial lossin activation of the enzymeby Gβγ despite the fact that PLCβ1 is not activated by Gβγ [123]. This suggests that domains other than the PH domain are required for activation of PLC by Gβγ, consistent with the observed binding of Gβγ to the catalytic domain. How might Gβγ binding to the PH domain or the catalytic domain of PLC alter enzymatic activity, since Gβγ can cause enzyme activation without enzyme translocation? Recent studies of Rac-dependent activation of PLC show that Rac can activate PLCβ2 but not PLCβ1 and that this requires interaction with the PH domain [123]. Structures of PLCβ2, with and without bound Rac2, have recently been solved [118]. Rac2 interacts with the PH domain of PLCβ2 but there is no significant conformational difference between the structures of free- and Rac2-bound PLCβ2. This indicates Rac2 may activate PLCβ2 by causing either translocation of PLCβ2 or alterations in interactions of the enzyme with the membrane. In the structures of PLCβ2 solved by Sondek's group, a linker region between the X and Y domains of the catalytic domain is folded back to occlude access of substrate to the enzyme active site (Fig. 5B). This suggests a potential mechanism for activation that involves removal of this inhibitory linker from the active site [124]. Since the linker still occludes the active site in the RacGTP-bound PLCβ2 co-structure, the investigators propose that Rac causes alterations in interactions of the active site with charged lipids in the membrane that lead to displacement from the active site. Overall, the inhibitory linker model could allow for multiple modes of protein binding to achieve increases in enzyme activity. These could involve reorientation of PLC at the membrane that would allow negatively charged lipid head groups to pull this domain from the active site, for proteins to bind directly to this region, or for proteins to bind at a distance to cause conformational alterations that relieve this constraint.

Scarlata and colleagues propose that binding of Gβγ to the PH domain alters the orientation of the PH domain relative to the catalytic domain, allowing the catalytic domain to productively interact with the substrate at the membrane surface [125,126]. In favor of this hypothesis, measurements of interdomain movements of a PLCδ/PLCβ chimera by FRET indicate that Gβγ causes alterations in interactions between the catalytic domain and the PH domain. This mechanism could be operating as an independent mechanism for PLC activation or it could work in concert with direct binding of Gβγ to the catalytic domain. Binding of Gβγ at the catalytic domain, or the PH domain, could alter interactions of the catalytic domain with the membrane that would relieve autoinhibition or could cause displacement of the linker through conformational alterations in the protein. Further biochemical and structural analysis will be required to determine the validity of these proposed mechanisms.

Receptor-independent mechanisms for activation of G protein signaling through Gβγ

An emerging area is non-receptor and nucleotide exchange-independent mechanisms for G protein activation [7,127]. Some of these mechanisms involve binding of proteins to Gα subunits leading to release of free Gβγ subunits, but other proteins and peptides have been recently found that activate G protein βγ subunit signaling through direct binding to Gβγ. Since Gβγ is not thought to undergo conformational changes that could lead to nucleotide exchange on Gα or result in subunit dissociation, the mechanisms for action of these molecules that bind directly to Gβγ are not obvious. In most cases, detailed studies of these mechanism have not been done, but some examples are discussed that shed new light on potential roles of Gβγ in G protein activation are discussed below.

Activation of Gβγ signaling by Gβγ binding peptides SIRK/SIGK

An instructive study is based on the observation that some of the peptides identified through phage display screening that bind to the Gβγ "hot spot" cause activation of G protein dependent signaling pathways in cells. SIRK peptide was discovered in a the phage display screen using G protein βγ subunits as a target for binding [107]. Despite being discovered in a naive random peptide screen, the peptide bound to a biologically relevant signaling surface, as demonstrated by its ability to block Gβγ-dependent PLCβ2 and PI3Kγ activation *in vitro*. It did not affect Gβγ-dependent inhibition of adenylyl cyclase *in vitro* or inhibition of N-type Ca^{2+} channels in SCG neurons, demonstrating selectivity for inhibition of some Gβγ targets. A surprise came when studying the effects of cell-permeable versions (either tat-modified or myristoylated versions) of SIRK (mSIRK or tatSIRK) and a related peptide SIGK in intact cells. These peptides, predicted to inhibit G protein signaling, rapidly, potently and effectively activated the ERK/MAP kinase pathway in intact cells in a Gβγ-dependent manner [128]. To confirm that Gβγ was the target of these peptides in intact cells, the effects of mSIRK on CHO cells, transfected with mutant Gβ(βW332A), which does not bind the peptide, were examined. In these cells, with strong constitutive expression of Gβ(W332A) and $G\gamma_2$, the expressed subunits appear to substitute for a significant proportion of the endogenous Gβγ complexes, and substantially inhibit mSIRK dependent ERK activation [129]. This strongly supports the idea that mSIRK activates Gβγ subunit signaling in intact cells by binding directly to Gβγ subunits. To explain this observation it was proposed that the peptide must be binding to Gβγ in a way that leads to exposure of Gβγ signaling surfaces involved in ERK activation that are not themselves blocked by binding of the peptide. The direct Gβγ-binding effector responsible for ERK activation is not known, but the effector-binding surface required for activation of the ERK pathway must be different from the peptide binding site and the PLCβ2 binding site. To understand the mechanism of action of these peptides their effects on Gα/βγ interactions were examined. Kinetic and equilibrium analysis indicated that SIRK and SIGK enhanced the rate of G protein subunit dissociation in the presence of excess GDP and the absence of GTP [10, 128]. Additionally, other peptides known to bind to Gβγ and compete for Gα subunit interactions did not influence Gα subunit dissociation kinetics. This argues that the basis for the effect is not a strict competition for Gαβγ subunit interactions, since this would be expected to alter equilibrium binding without affecting dissociation kinetics of a preformed complex. The solved structure of SIGK peptide/Gβγ complex showed SIGK bound to the Gα subunit switch II-binding region on Gβγ [111] (See Fig. 1C and Fig. 6). This suggests that SIGK/SIRK should directly compete for Gα binding to Gβγ. A proposed model for how Gβγ could enhance subunit dissociation that can explain most of the data is depicted in figure 4B. As described previously, the Gα/βγ switch II interface is in a dynamic state of association and dissociation ("breathing") while overall Gβγ/α interactions are maintained by the Gα N-terminal helix. We propose that SIRK/SIGK can insert into this interface during this transient breathing and block this part of the Gβγ/α interaction. The resulting dissociation rate would then only be limited by the off rate for the weakly interacting N-terminal α helix, leading to rapid subunit dissociation. This model fits much of the data and provides supporting evidence that this surface "breathes". On the other hand, the model predicts that any peptide that binds at this interface and competes for Gα/βγ interactions in an equilibrium experiment should enhance subunit dissociation, which is not what is observed. This discrepancy suggests the effects of the peptide involve a mechanism other than simple competition [10,111]. Such a mechanism remains to be established.

Overall, these studies highlight a novel potential mechanism for G protein activation that could be exploited physiologically by receptors, by Activators of G protein signaling (AGS proteins), or pharmacologically.

AGS proteins—Activators of G protein signaling (AGS proteins) are a group of structurally distinct proteins discovered in a yeast-based screen for activation of the Gβγ dependent pheromone response pathway [4,7,127]. The mechanisms for G protein activation by proteins that bind Gα subunits (Class I and Class II AGS proteins) are simple to understand. For example, Class I AGS proteins include DexRas and promote nucleotide exchange on Gα subunits, releasing free Gβγ through a mechanism similar to receptors. Class II AGS proteins contain a GPR or Goloco motif that binds to $G\alpha_{i/o}$ family subunits and promotes Gβγ subunit dissociation through a nucleotide exchange-independent mechanism leading to accumulation of free Gβγ subunits that can activate downstream targets. The GPR/Goloco motif in these proteins binds to the switch II region of the Gα subunit near the interface between Gα and Gβγ subunits [130]. This results in a conformational change in switch II at the Gα/βγ interface, disrupting Gα/βγ interactions and leading to subunit dissociation.

Class III AGS proteins that bind directly to Gβγ are less well investigated or understood. Since Gβγ is not thought to undergo significant conformational alterations, it is difficult to imagine a mechanism that does not involve binding of the AGS protein to the Gα/βγ interface. But if the AGS protein bound to the region on Gβγ at the Gα/βγ interface, it would obscure this critical signaling surface on Gβγ required for activation of target proteins. Thus a conundrum is presented where somehow these activating proteins that bind the Gβγ subunits must relieve the constraints of the GDP bound heterotrimer yet still allow Gβγ to signal downstream.

Some insight into the mechanism of action of these proteins comes from a recent analysis of AGS8. AGS8 was found in the yeast-based screen using a cDNA library derived from a rat model of transient cardiac ischemia. AGS8 binds to Gβγ subunits but does not significantly affect Gβγ-dependent PLCβ2 activation when Gβγ is transfected into COS cells in the absence of Gα subunits [131]. However, AGS8 relieves the inhibition of PLC seen when Gα subunits are transfected with Gβγ subunits and Gβγ-dependent PLC activation is inhibited due to formation of the heterotrimer. The AGS8 binding site on the G protein βγ subunit appears to reside at the Gα/Gβγ interface at a site that overlaps with the SIGK binding site. This observation was puzzling since AGS8 did not block PLC activation, yet amino acids at the SIGK binding surface are required for PLC activation. Another surprise is that AGS8 does not promote subunit dissociation or block $G\alpha_{i1}$ subunit binding to Gβγ. These observations are difficult to reconcile with the SIGK data demonstrating that peptide binding at this site led to dissociation of Gα from Gβγ, until it was found that AGS8 could also bind to the $G\alpha_{i1}$ subunit in a nucleotide-independent manner. SIGK promotes subunit dissociation by binding at the Gα/Gβγ interface but binds only to Gβγ, so Gα subunits are released. AGS8 binds both Gα and Gβγ resulting in retention of Gα subunit binding in the complex (Fig. 4C). Thus, AGS8 binds to the G protein heterotrimer by binding the Gβγ and Gα subunits simultaneously and does not cause dissociation of these subunits, yet it activates PLCβ2 signaling by a GaGDPbg heterotrimer.

In this AGS8/Gα/Gβγ complex, the mechanism by which Gβγ could activate PLCβ2 is not easily explained based on our current understanding of regulation of Gβγ-dependent signal transduction. A critical Gβγ surface for signaling to PLCβ2 activation is bound to AGS8. In our model, when AGS8 binds to the “hot spot” and forms a signaling complex with Gα and Gβγ subunits, the PLCβ2 inhibitory site at the amino terminus becomes a stimulatory binding site. This PLCβ2 binding site was previously identified as an inhibitory site by chemical crosslinking and mutagenesis [103] (discussed in section 7). This implies that AGS8 alters Gβγ conformation or orientation at the membrane to make the bound complex competent for downstream signaling. An alternative model is that AGS8 itself provides binding determinants for PLC binding in conjunction with amino acids at the Gβγ N-terminus that participate in PLC activation. More direct evidence to address these ideas awaits further structural investigation.

These two examples (SIGK and AGS8) of Gβγ-dependent, nucleotide exchange-independent, signaling mechanisms suggest additional modes of G protein activation outside of the well defined classical paradigm for G protein activation. How these biochemically characterized mechanisms operate in a physiological context remains to be determined. With emerging evidence that receptors bind directly to G protein βγ subunits these observations may also be relevant to GPCR signaling. It is possible that some receptors, in addition to causing nucleotide exchange, can also promote subunit dissociation that is mechanistically independent of the nucleotide exchange process on Gα subunits. On the other hand there is increasing evidence that under some GPCR-dependent G protein activation conditions the subunits may not dissociate [5,6,132]. The molecular model described for the action of AGS8 suggests potential mechanisms for non-dissociated G protein signaling complexes to promote downstream signaling. Overall, it is clear that the current simple picture of Gβγ as a passive participant in the G protein activation and signaling process needs revision.

NDPK phosphorylation of Gβγ

G protein β subunits have been found to be substrates for phosphorylation in a variety of tissues [133,134]. A model has been developed where transient high-energy phosphorylation of a histidine residue serves as a phosphate donor involved in transfer of phosphate from the Gβ subunit to GDP associated with Gα subunits leading to activation of the Gα subunit and subsequent signaling in a GPCR-independent manner. The amino acid phosphorylated in Gβ is His 266, and requires nucleotide diphospho (NDP) kinase. Direct reconstitution of phosphorylation with purified NDP kinase has not been achieved, suggesting a requirement for an additional cofactor in the reaction. The significance of this process was unclear until a recent study in cardiac myocytes suggested a role in regulation of cAMP levels [135]. In these studies a $G\beta_1$ His 266 Leu mutant was transduced into neonatal or adult cardiac myocytes where the mutant is functionally incorporated into endogenous heterotrimers replacing the endogenous subunits. Basal cAMP levels were reduced in both neonatal and adult cardiac myocytes in cells transduced with $G\beta_1$ His 266 L compared to cells transduced with wild type $G\beta_1$. Interestingly, baseline contractility was reduced by this mutant in adult myocytes without any affect on stimulation by a β-adrenergic receptor agonist. These data suggest that, in a physiological system, this receptor-independent signaling mechanism that relies on transient phosphorylation of Gβ, regulates baseline cAMP levels and contractility in the heart.

Physiological significance of Gβγ activation

G protein βγ subunit-mediated activation of effectors has diverse roles in regulating of cell physiology. In excitable cells, Gβγ subunits released from G_i modulate membrane potential through activation of K^+ channels and inhibition of voltage gated Ca^{2+} channels. In neurons this suppresses excitability and inhibits neurotransmitter release. In atrial myocytes vagal release of acetylcholine suppresses heart rate through Gβγ-dependent activation of K^+ channels [74]. In migrating immune cells, chemokine receptors, such as the IL-8 receptor or CXCR4, are coupled to the release of Gβγ subunits from G_i [136,137] that is critical for mediating directional chemotaxis as well as release of superoxide and other inflammatory mediators. Several mouse knockout studies implicate Gβγ-regulated effectors in various physiological functions; For example, in mice lacking Gβγ-regulated PLCβ3, morphine acting at G_i linked opioid receptors produced painkilling effects at much lower doses [138]. Genetic deletion of Gβγ-regulated PI3Kg resulted in decreased neutrophil migration and a reduction in inflammation [139,140].

Activation of multiple G_i and G_q-coupled receptors, including thrombin, lysophosphatidic acid (LPA), and acetylcholine receptors, results in a mitogenic response in several cell types. MAP kinases are critical components in the growth-promoting pathways regulated by these receptors. Gβγ subunits indirectly activate MAP kinase, suggesting that Gβγ subunits may

mediate the growth-promoting effects of many G protein-coupled receptors [141,142]. Sequestering Gβγ in smooth muscle cells inhibits serum stimulated growth and vascular restenosis [143].

G Protein βγ subunits as a target for therapeutic development

The diverse functionality of Gβγ signaling in cellular physiology suggests that manipulating Gβγ function could have significant therapeutic potential. On the other hand Gβγ is known to be required for G protein activation by all G protein coupled receptors, so blocking **all** Gβγ functions would be predicted to have side effects. The potential therapeutic usefulness of targeting Gβγ signaling has been investigated extensively using the carboxy terminus of GRK2 (GRK2ct) [143–147] and, to a lesser extent, with other Gβγ binding peptides such as QEHA [148]. GRK2ct, despite binding at the Gα/βγ "hot spot" interface, interferes with Gβγ signaling to downstream targets without disrupting GPCR dependent G protein activation in general. The basis for this selectivity is unclear. This has strong implications for small molecule development, indicating that a strategy that targets the Gα/βγ interface "hot spot" could successfully block downstream Gβγ signaling without disrupting G protein signaling in general.

Gβγ and heart failure—One well studied example where GRK2ct has been used to demonstrate the therapeutic potential of targeting Gβγ is in cardiac function and failure. One of the characteristics of heart failure is the loss of β-adrenergic receptor (βAR)-dependent cardiac reserve. A prominent hypothesis is that the underlying mechanism involves an increase in the activity of GRK2, a kinase that phosphorylates and desensitizes the βAR as well as other GPCRs. During progression to heart failure, chronically elevated catecholamine levels lead to chronic stimulation of βAR resulting in chronic desensitization of the receptor by GRK2. GRK2 activity is controlled by Gβγ which, upon GPCR activation, is released and recruits GRK2 to the receptor, leading to its phosphorylation and desensitization. GRK2ct blocks this recruitment and enhances βAR function. A seminal study indicating successful application of this strategy was the demonstration that transgenic cardiac over-expression of GRK2ct in mice increased cardiac performance in response to βAR stimulation [147]. Later, it was demonstrated that cardiac over-expression of GRK2ct in murine models of heart failure dramatically rescued cardiac function [146] and expression of GRK2ct in cardiac myocytes isolated from biopsies of human heart failure patients significantly improved contractile function [149]. These and a plethora of other studies have shown the value of blocking Gβγ signaling function in improving cardiac functions in disease [145].

Gβγ and inflammation—Chemokines and chemokine receptors have been the subject of anti-inflammatory pharmaceutical development [150–156]. A potential problem is the overwhelming complexity of these signaling molecules (multiple chemokines, chemokine receptors, and redundancy) making it difficult to know which specific receptors to target for conditions such as arthritis. Polychemokine [157] or combinations of different chemokine [158] antagonists have been suggested, but there may be chemokines that act as an agonist at one receptor and an antagonist at another [159]. Of recent interest is the demonstration that deletion of PI3Kγ in mice inhibits neutrophil migration in response to chemoattractants and inhibits inflammation. PI3Kγ activity is directly regulated by Gβγ released from chemokine and chemotactic peptide receptors and is relatively selectively expressed in monocytic cells, suggesting that blocking Gβγ-regulation of PI3Kγ could be an effective strategy for treating inflammatory diseases that may overcome the necessity to target mutliple chemokine receptors [139]. In a related study it was demonstrated that deletion of PI3Kγ protected apoE$^{-/-}$ mice from development of atherosclerosis, potentially through disabling macrophage migration and inflammatory functions [160]. An alternate approach that is currently being investigated is specific pharmacological targeting of PI3K catalytic activity with inhibitors that are relatively

selective for PI3Kγ relative to other PI3K isoforms [161]. In this approach blocking PI3Kγ would circumvent the problems associated with chemokine receptor redundancy by blocking a common signaling target of chemokines. An alternate approach may be to inhibit Gβγ-dependent activation of PI3Kγ, which would selectively block PI3Kγ relative to other PI3K isoforms since these isoforms are not regulated primarily by Gβγ.

These are just two of multiple examples where Gβγ binding proteins or peptides have been used to demonstrate the involvement of Gβγ in pathology and disease and where inhibition of Gβγ with these agents has ameliorated the pathology. Other examples include vascular restenosis [143], drug addiction [162] and prostate cancer [144].

Small Molecule Targeting of Gβγ

Screening the NCI diversity library against the "hot spot"

Given that Gβγ may be a suitable target for therapeutic development, our laboratory screened for small molecules that could be used *in vivo* to inhibit Gβγ signaling. The "hot spot" was targeted because this is a major site of protein-protein interactions and our studies with peptides suggested that differential modulation of G protein signaling functions could be accomplished by binding to this site. In this screen a number of molecules that bound to the "hot spot" were identified based on the ability to compete with SIGK binding and bound with IC_{50} values ranging from 0.2 to 50 μM [107]. More recently direct binding of M119 and a related molecule, gallein, to Gβγ was examined by surface plasmon resonance [163]. In the SPR assay, gallein bound to immobilized Gβγ with an apparent K_d that was similar to the IC_{50} value obtained for M119- or gallein-dependent inhibition of SIGK binding. Structurally related molecules that did not compete for SIGK peptide binding did not bind in the SPR assay, confirming the specificity of the SPR assay for active compound binding.

Protein-protein interactions

While the compounds identified in the screen inhibited interactions between $G\beta_1\gamma_2$ and the peptide SIGK, it is thought to be relatively difficult for small compounds to disrupt true protein-protein interactions. Thus, selected compounds were tested for their ability to disrupt protein interactions with *bona fide* Gβγ binding partners: $G\alpha_{i1}$ and effectors. The overall $G\alpha_{i1}$-βγ interaction surface spans 1800 $Å^2$ [11,12] and the dissociation constant (K_d) for $G\alpha_{i1}$ binding to Gβγ is approximately 1 nM [164]. One compound, M119, potently inhibited $G\alpha_{i1}$ binding to $G\beta_1\gamma_2$. M119 and other compounds inhibited binding of effector molecules to Gβγ both in direct binding assays and in functional reconstitution experiments.

Based on the selectivity of phage displayed peptides that bound to the "hot spot", and the idea that each target has a unique "foot print" on the Gβγ surface, it was predicted that different small molecules, binding in different ways to the "hot spot", would have distinct effects on individual Gβγ-target interactions. Initial support for this idea came from comparative analysis of M119 and M201 with respect to target interactions. While both compounds were able to compete for Gβγ-GRK2 interactions with similar potency, M119, and not M201, blocked Gβγ-dependent activation of PLCβ2 *in vitro*. This indicates that both compounds can bind to Gβγ but have differential effects on Gβγ protein-protein interactions. Other compounds also have similar selectivity characteristics (unpublished data).

Analysis of compound efficacy and selectivity in intact cells

Based on the biochemical selectivities described in the previous section, it would be predicted that the compounds should be able to differentially modulate Gβγ-dependent signaling processes downstream of GPCRs. This was tested in neutrophils where Gβγ mediates signaling responses to chemoattractants and chemokines that are responsible for directing chemotactic

migration and superoxide production involved in inflammatory responses. The pathways regulated in these cells include activation of PI3-kinase γ, PLC activation, ERK1/2 activation and GRK2 regulation. Compounds that inhibited Gβγ-dependent PLC and PI3-kinase activation *in vitro* were able to inhibit these pathways in neutrophils in response to chemoattractants. These compounds did not block activation of ERK1/2 by fMLP, indicating that GPCR signaling was intact and demonstrating a level of selectivity of the compounds for G protein βγ subunit signaling in intact cells. Compounds shown to be selective for particular pathways *in vitro* displayed similar characteristics in cells. M201, for example, blocked GRK2 recruitment but did not affect Gβγ-dependent PLC activation, while M119 was able to inhibit both, consistent with their *in vitro* properties.

In vivo evaluation of small molecules

Based on the discussion of therapeutic relevance it would be predicted that compounds that inhibit Gβγ signaling would have predictable and potentially beneficial effects *in vivo*. Some areas with clear potential include heart failure and inflammation. As discussed earlier, knockout of PLCβ3 leads to increased potency of morpine-dependent analgesia. Since PLCβ3 is regulated by Gβγ, one would predict that Gβγ-blocking compounds, if introduced into analgesic centers in the brain, would have similar effects.

PLCβ3 and Opioid-dependent antinociception—Co-administration of M119 with morphine intracerebro-ventricularly (i.c.v) resulted in an 11-fold increase in the analgesic potency of morphine, whereas administration of M119 alone had no effect on antinociception. Importantly, M119 also had no effect on morphine-dependent antinociception in PLCβ3$^{-/-}$ mice. Gβγ may block interactions with PLCβ3 but not Gα or other effectors such as K^+ or Ca^{2+} channels critical for the actions of opioid agonists [165]. If M119 were globally blocking Gβγ subunit functions, morphine-induced antinociception would have been attenuated rather than potentiated with M119 co-administration. These data highlight the specificity of M119 actions and the selective nature of M119 both *in vitro* and *in vivo*.

Neutrophil Chemotaxis and inflammation—As discussed, Gβγ-dependent activation of PI3kγ in neutrophils is important in directing neutrophil migration in response to chemoattractants. Activation of this receptor system leads to a gradient of PIP_3 production with enhanced accumulation at the leading edge of the cell that is important for polarizing the cells in the direction of the chemo-attractant [166,167]. In animal models of neutrophil chemotaxis, deletion of PI3Kγ results in defects in neutrophil accumulation and reduced inflammation [139,140]. Since PI3Kγ and other molecules important for chemoattractant-dependent chemotaxis are activated by Gβγ, M119 and the related molecule, gallein were tested for their ability to inhibit chemoattractant-dependent neutrophil migration [163]. M119 and gallein significantly blunted fMLP-, but not Gβγ-independent GM-CSF-dependent, neutrophil migration, supporting the idea that blocking Gβγ-dependent signaling in neutrophils inhibits migration. Consistent with this data, gallein inhibited inflammation in a whole animal model of inflammatory processes. In a carrageenan-induced footpad inflammation assay, intraperitoneal and oral administration of gallein inhibited inflammatory responses with a potency similar to a cyclo-oxygenase inhibitor, indomethacin. Thus, inhibiting Gβγ signaling with small molecules could be a novel approach to treat inflammation.

Basis for Gβγ targeting and selectivity by small molecules

Molecules were found that bound to Gβγ and selectively inhibited Gβγ protein-protein interactions in a limited screen of a small set of organic molecules. Two readily apparent questions that arise are: 1) What is the molecular basis for small molecule selectivity and, 2) what are the properties of the Gβγ “hot spot” that allow it to bind to small molecules with relatively high affinity?

With regard to the first question, one hypothesis is that small molecule selectivity is based on differential spatial occupancy of the "hot spot". The basic premise as discussed in section 7 is that different Gβγ targets interact with the "hot spot" utilizing different subsets of amino acids on Gβ for binding. If the small molecules occupy different spatial regions of the hot spot and the basis for their effects is steric occlusion of effector interactions, the prediction is that the compounds would have distinct effector inhibition profiles based on where they bound in the "hot spot". An alternate hypothesis is that the chemistries of the compounds, rather than steric effects, alter target binding. For example, a compound containing a carboxylic acid moiety could introduce a negative charge at the surface that could differentially alter effector binding. Currently, direct evidence in support of either of these hypotheses is lacking, but identification of the binding modes for each compound, either by mutagenesis or structural methods, should provide some illumination.

A prevalent idea is that finding small molecules that bind at protein interaction surfaces to disrupt protein-protein interactions is difficult. In contrast either to active sites of enzymes or cell surface receptors, protein-protein interaction surfaces have been thought to be generally flat and may not have a clearly defined three dimensional binding pocket that can support the multiple interactions in three dimensions that are likely required for high affinity binding of a small molecule to a protein [168,169]. A second issue is that protein interfaces are generally large, often greater than 1500 $Å^2$, suggesting that occupation of a small portion of this surface with a small molecule might not disrupt enough of the binding energy to disrupt the interaction. Increasingly, however, examples of small molecules that bind to crevasses in protein interaction surfaces and disrupt protein-protein interactions are emerging [170]. In the case of Gβγ, because of the hole in the middle of the β-propeller, the protein-protein interaction surface is concave rather than flat, providing 3-dimensionality to the surface that may provide more binding interactions for small molecules. This interaction surface is also a "hot spot", as previously discussed, that contributes a large portion of the binding energy for Gβγ-target interactions. Thus, binding of small molecules to this surface would be predicted to disrupt this critical binding site and inhibit interactions between Gβγ and its effectors. This combination of having a good binding site for small molecules overlapping with a critical protein interaction surface may not be coincidental and could reflect the inherent "binding" capability of this site.

Concluding remarks

G protein βγ subunits are central participants in G protein signaling, scaffolding receptors, G protein α subunits, and effectors. As investigations of this protein continue to move forward, its importance in a myriad of physiological functions is increasingly appreciated. Despite years of investigations by many investigators, novel and interesting properties, mechanisms and functions for these proteins continue to emerge, and this will likely continue. Some of the major questions still remaining concern how signaling specificity is maintained with such a promiscuous signaling protein and what is the molecular significance of the very large isoform diversity of these Gβγ combinations. Given the biological potential of these proteins as therapeutic targets, answering these questions could contribute significantly to development of novel pharmacologic approaches to therapeutics for a number of important diseases.

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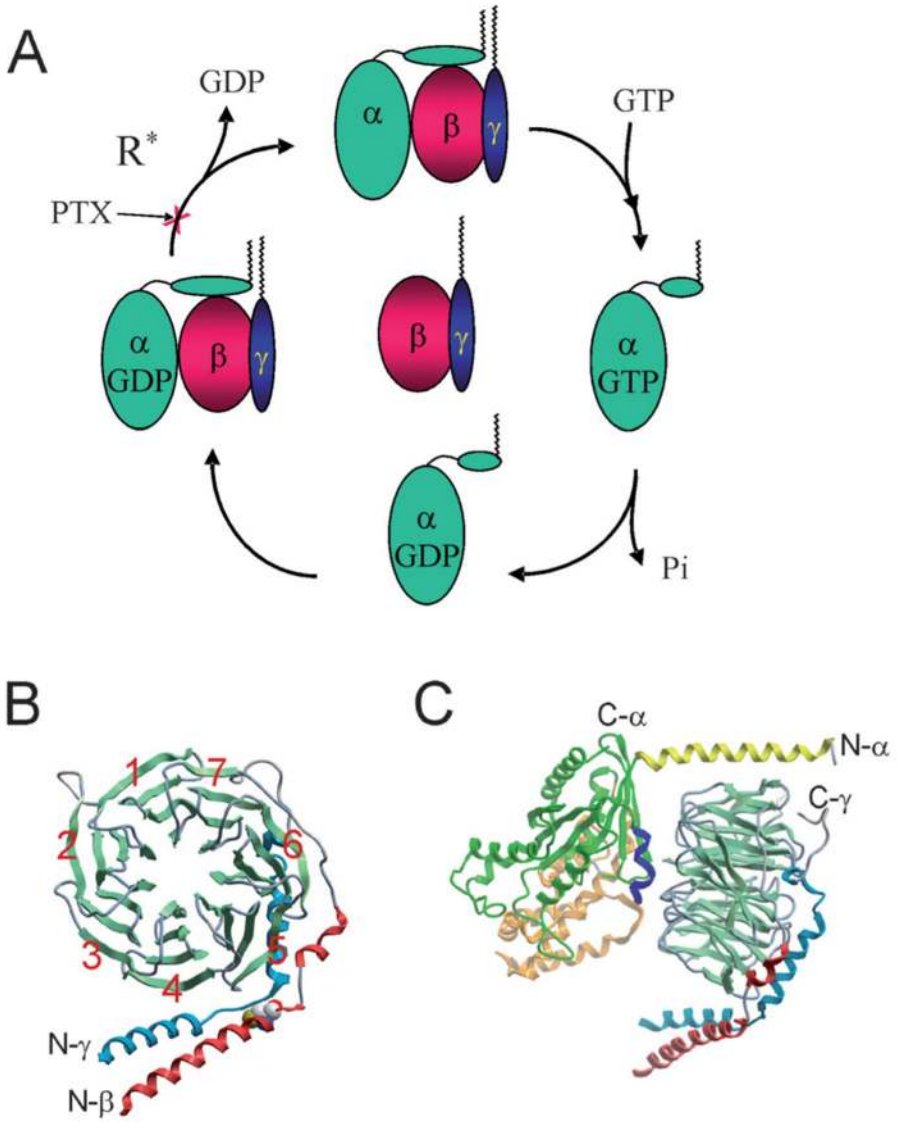

Figure 1.

(*A*) The G protein cycle. The Gα subunit bound to GDP interacts with the Gβ subunit with two contacts involving the N-terminal domain and the GTP binding domain. This is the inactive resting state. This complex is a substrate for the activated GPCR (R*) which catalyzes an increase in the dissociation rate of GDP from the Gα subunit leading to the nucleotide free Gαβγ complex. This complex is very short lived in the cell where high concentrations of GTP in the cell bind to the empty nucleotide binding site to drive a conformational change in the Gα subunit. This conformation change leads to the active GαGTP subunit, perhaps separated from signaling competent free Gβγ subunits. The Gα subunit has the intrinsic capacity to hydrolyze GTP to GDP, allowing reassembly with Gβγ to return to the resting state. Regulators of G protein signaling (RGS proteins) can bind to Gα and enhance the rate of GTP hydrolysis. Pertussis Toxin (PTX) modifies the G protein α subunit and prevents interactions of the GαGDPβγ heterotrimer with R*. Also shown are myristoyl and isoprenoid lipid groups at the Gα N-terminus and Gγ C-terminus respectively. (*B*) Ribbon diagram representing the three dimensional crystal structure of the Gβγ subunits. The blades of the propeller are numbered as

in Wall et al. [11]. The Gβ N-terminal helix is in red and forms a coiled coil interaction with the Gγ subunit in blue. (*C*) Ribbon diagram of the G protein heterotrimer with the Gβγ subunit rotated 90° relative to B. The Gα N-terminus is represented as a yellow helix and the Gα Switch II region is in dark blue.

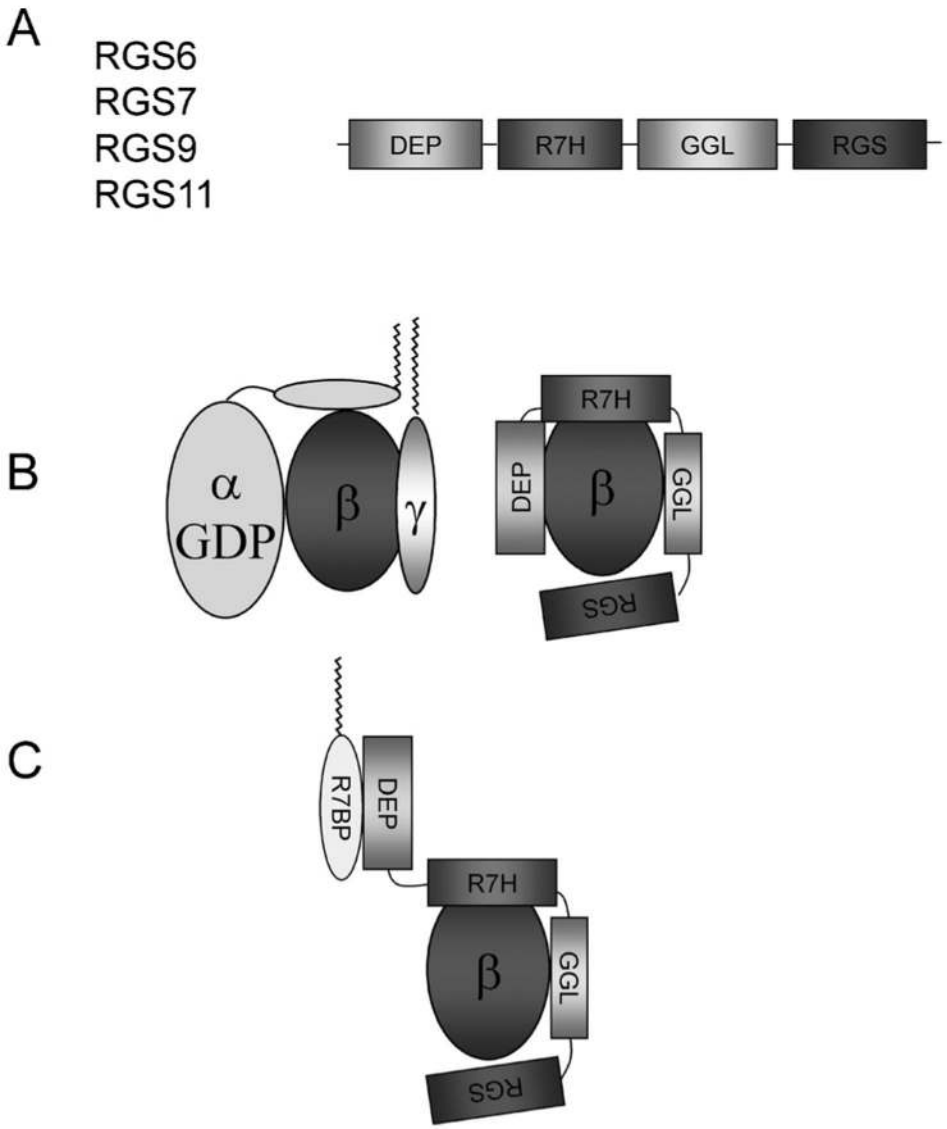

Figure 2.

(*A*) Domain organization of the R7 family of RGS proteins. DEP is the Disheveled, EGL-10, Pleckstrin homology domain, R7H is the R7 homology domain, ggl is the G protein γ like domain, and RGS is the RGS homology domain. (*B*) Depiction of the interactions of an R7 protein with Gβ with the ggl domain interacting with $G\beta_5$ instead of Gγ and the DEP domain potentially interacting with the Gα subunit binding site on Gβ. (*C*) Model depicting palmitoylated R7 binding protein bound to the DEP domain of R7. Evidence suggests that this binding could alter DEP domain interactions with $G\beta_5$ to expose binding surfaces on $G\beta_5$. The palmitoylated R7BP could then direct binding to the plasma membrane.

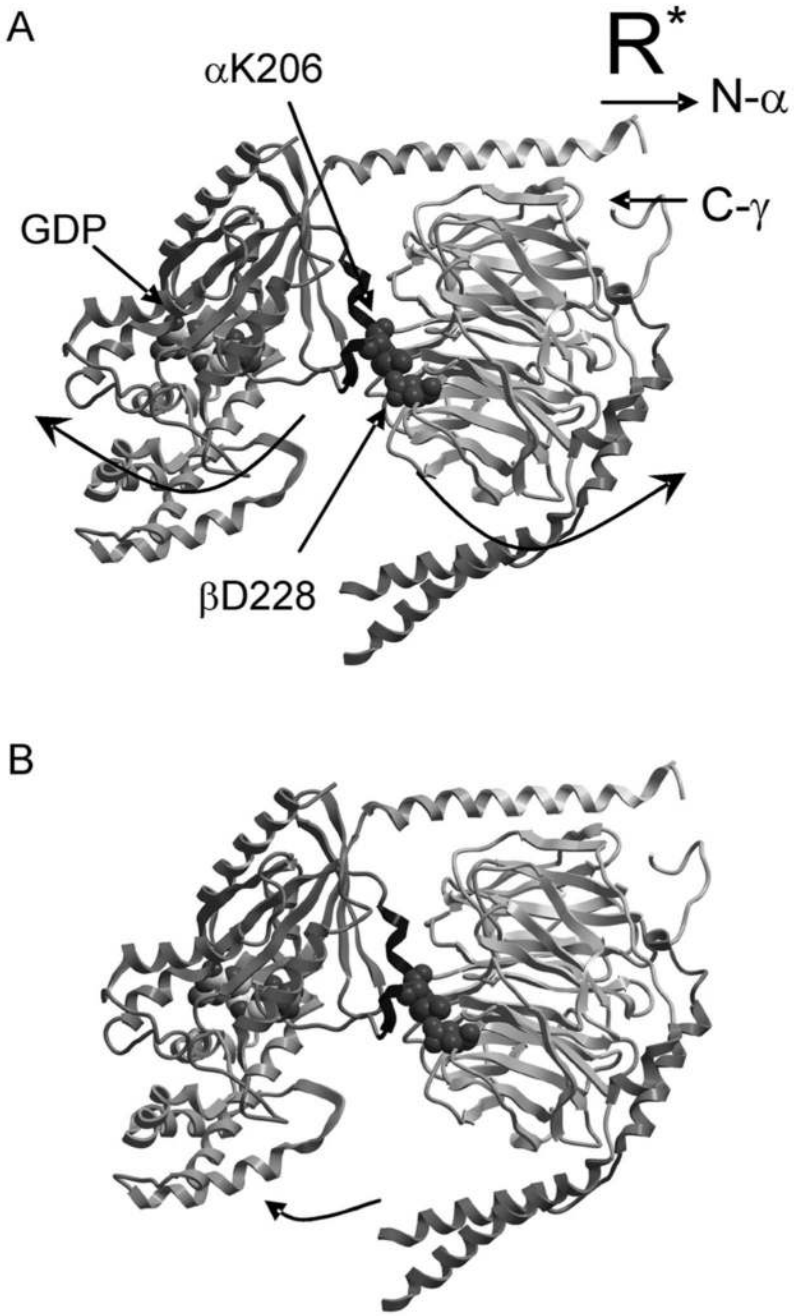

Figure 3.

Models for participation of Gβγ subunits in regulation of nucleotide exchange. (*A*) Lever arm model for Gβγ dependent activation of Gα subunit nucleotide exchange. In this model R* interaction with the N-terminus of the Gα subunit and the C-terminus of Gγ results in Gβγ acting as a lever to pull open the nucleotide binding site on Gα to enhance the rate of GDP release. Critical interactions at the Gα switch II/Gβγ interface (GβD228 and GαK206) are required for Gβ to pull on Gα switch II to open the nucleotide binding pocket. (*B*) Gear shift model for Gβγ-dependent activation of nucleotide exchange. Here the receptor causes the Gβγ subunits to move closer to the Gα subunit. This results in a potential physical interaction

between the N-terminal helical Gβγ coiled-coil extension and the helical domain of Gα, leading to opening of the nucleotide binding pocket and release of GDP.

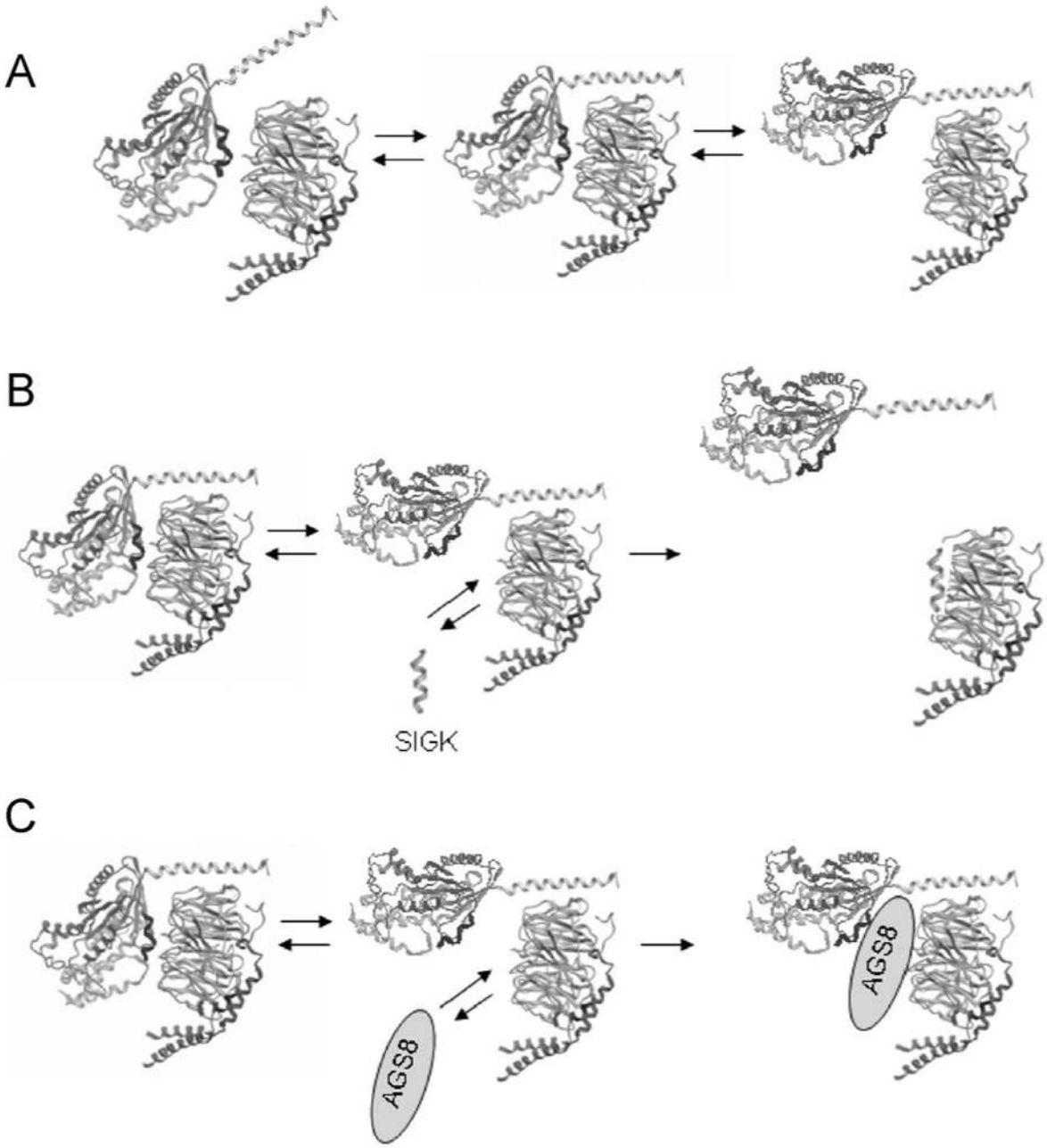

Figure 4.

Models for "breathing" of Gα/βγ interfaces and non-receptor dependent activation mechanisms. (*A*) In the center is a ribbon diagram depicting Gα binding to Gβγ showing the two components of a bivalent interaction of Gα with Gβγ, the Gα N-terminal helix interaction with the side of blade one of the Gβ β-propeller and the Gα switch II interaction with the top of the Gβ β-propeller (see Fig 1.). In this model either of these two interfaces can open and close in rapid equilibrium without subunit dissociation. Only when both contacts are broken simultaneously can subunit dissociation occur. (*B*) SIGK-dependent subunit dissociation. In this model when the Gα switch II Gβ interfaces open up, SIGK can bind and prevent closure of this interface, resulting in an enhanced rate of subunit dissociation. (*C*) AGS8-dependent G

protein activation. Similar to, B., when the Gα switch II Gβ interface opens AGS8 binds, but since AGS8 can bind to Gα and Gβ, the bivalent interaction of Gα with the complex is maintained and subunit dissociation does not occur.

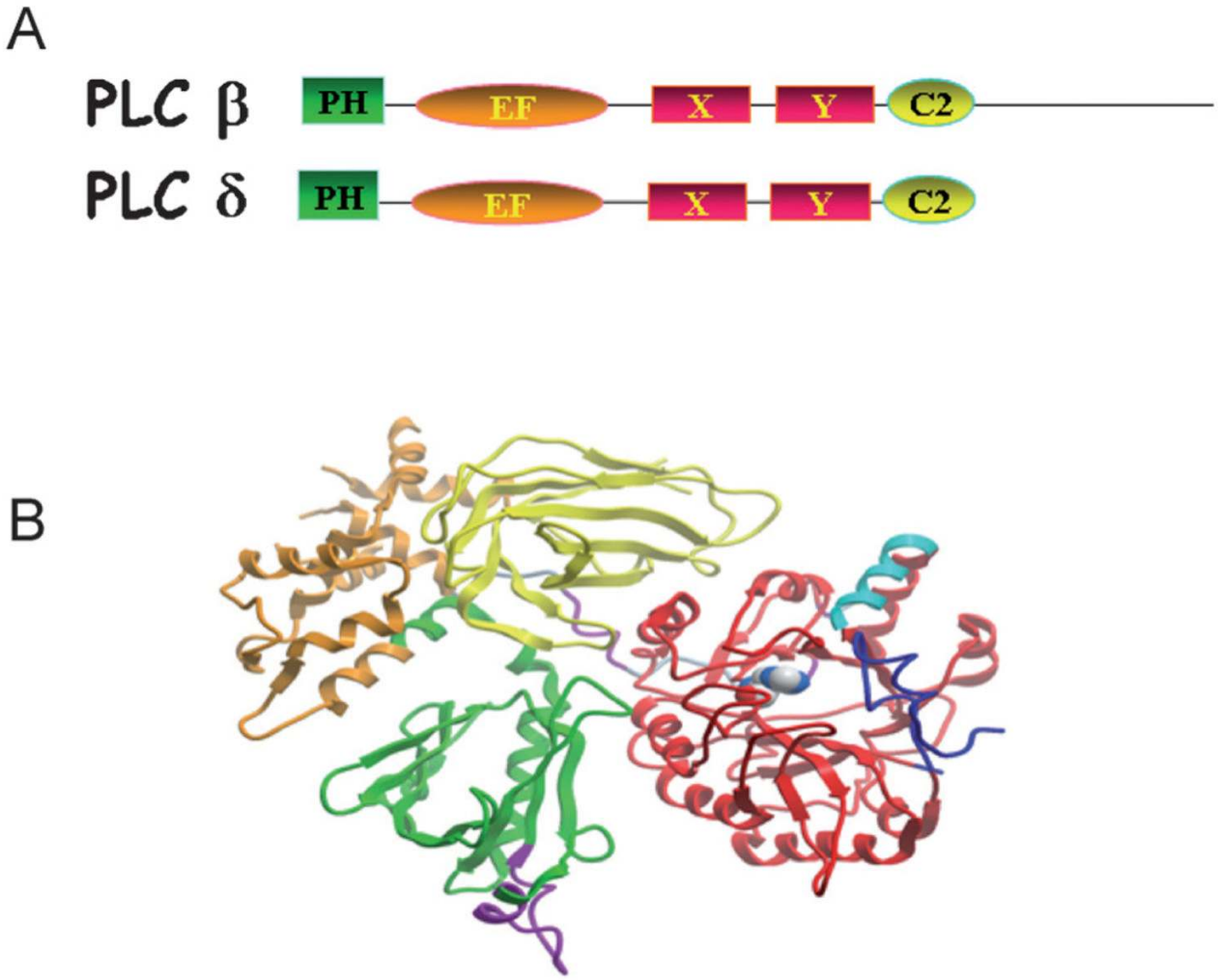

Figure 5.

(*A*) Domain organization of PLCδ and PLCβ. Pleckstrin homology domain (PH), EF hand domain (EF), catalytic domain (X and Y), C2 domain (C2). (*B*) Ribbon representation of PLCβ2 (from coordinates 2FJU) with domains color coded as for A: in turquoise, the helical region of the PLCβ2 catalytic domain (574–583) found to interact with Gβγ; in dark blue, the X-Y linker domain that caps the enzyme active site; in purple, a region of the PH domain important for Gβγ-dependent PLC activation; in CPK spacefill, the catalytic histidine required for PIP_2 hydrolysis.

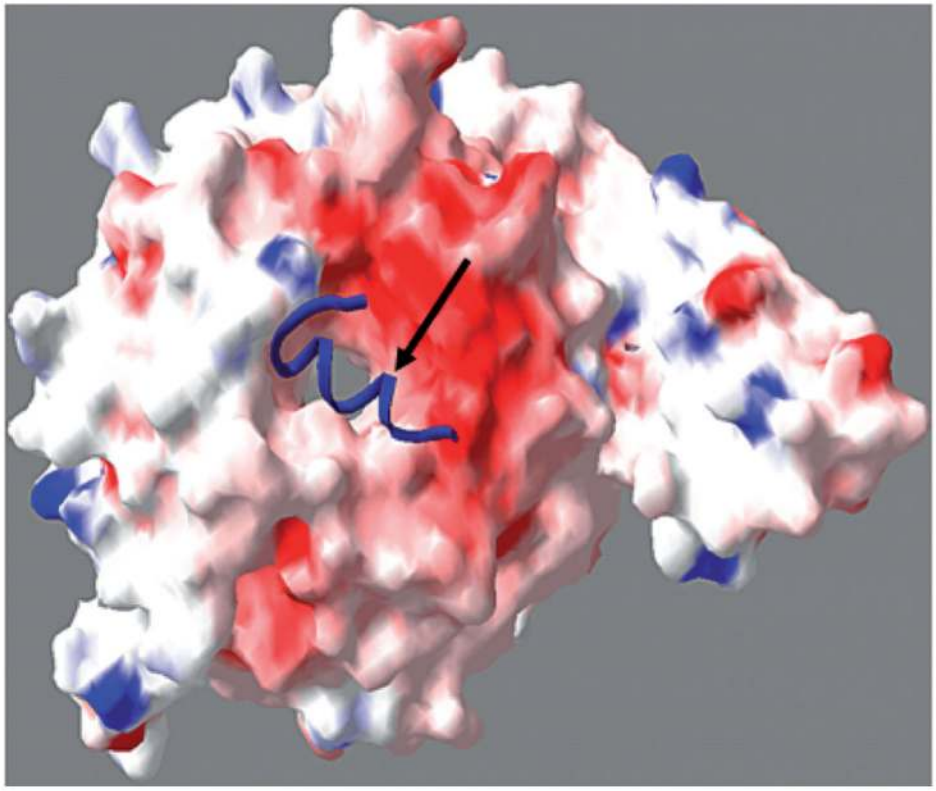

Figure 6.
Surface potential representation of Gβγ with SIGK (blue ribbon) bound at the "hot spot". Blue areas are positively charged, red areas are negatively charged and white areas are neutral.

Table 1

Gβγ subunit targets[1].

Physiological Gβγ effectors (direct)
inwardly rectifying K^+ channel (GIRK1/GIRK2, GIRK1/GIRK4) [72,75,171]
GPCR kinase 2 and 3 [172]
PLC β1, β2 and β3 [77,86–88,173]
Adenylyl cyclase (activation), II, IV, VII [174,175]
Adenylyl cyclase (inhibition), I, III, V, VI [174–177]
N type Ca^{2+} channels [91]
P/Q type Ca^{2+} channels [178]
Phosphoinositide 3 kinase γ [89,179]
SNAP-25 [180,181]
P-Rex1 Rac GEF [90]
Proteins regulated by Gβγ
PAK (p21 activated kinase) [182]
Raf-1[183]
Dynamin [184]
Ras GRF [185]
Dbl [186]
Btk kinase [187]
Tubulin/microtubules [188,189]
Histone deacetylase 5 (HDAC5) [190]
P114 RhoGEF [191]
RGS4 [192]
RGS3 [193]
ATP sensitive K^+ channel [194]
Phospholipase D1 [195]
IP_3 receptor 1 [196]
T type Ca^{2+} channels [197,198]
FLJ008 Rac/cdc42 GEF [199]
Gβγ binding proteins
RACK I [200]
Group III AGS proteins [4,131]
AGS2 TcTex1 [201]
AGS7 Thyroid receptor Interacting Protein (TRIP13)
AGS8 KIAA1866
AGS9 Rpn10
Gβγ effectors (indirect)
MAP kinase [202]
PLA_2 [203]

[1] Physiological Gβγ effectors are those proteins that directly bind Gβγ and for which a clear physiologic role for Gβγ interaction has been established. Proteins regulated by Gβγ are proteins known to bind and have activity regulated by Gβγ but for which the physiological role for the interaction has not been established. Gβγ-binding proteins are proteins that bind to Gβγ but where regulation of an activity has not been demonstrated.