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Understanding Molecular Recognition by G protein βγ Subunits on the Path to Pharmacological Targeting

Yuan Lin and Alan V. Smrcka*

Departments of Pharmacology and Physiology, and Biochemistry and Biophysics, The University of Rochester School of Medicine and Dentistry, 601 Elmwood Ave, Rochester, NY, 14642

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Running Title: Gβγ molecular recognition and targeting

* Correspondence should be addressed to:

Alan V. Smrcka

Departments of Pharmacology and Physiology, and

Biochemistry and Biophysics

University of Rochester School of Medicine and Dentistry

601 Elmwood Avenue

Rochester, NY 14642

Email: (Alan_Smrcka@urmc.rochester.edu)

Telephone: (585)-275-0892

Fax: (585)-273-2652

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Abbreviations:

GPCR- G protein-coupled receptor

GRK2- G protein-coupled receptor kinase-2

PLC- Phospholipase C

AC- Adenylyl cyclase

SIGK-SIGKAFKILGYPDYD

SIRK-SIRKALNILGYPDYD

GIRK- G proteind-coupled inwardly rectifying potassium channel

PI3K- phosphatidylinositol 3-kinase

GEF- guanine nucleotide exchange factor

NMR- nuclear magnetic resonance

ELISA- enzyme linked immunosorbent assay

ERK- extracellular regulated kinase

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Abstract

Heterotrimeric G proteins, composed of Gα and Gβγ subunits, transduce extracellular signals via G-protein-coupled receptors (GPCRs) to modulate many important intracellular responses. The Gβγ subunits hold a central position in this signaling system and have been implicated in multiple aspects of physiology and the pathophysiology of disease. The Gβ subunit belongs to a large family of WD40 repeat proteins with a circular β-bladed propeller structure. This structure allows Gβγ to interact with a broad range of proteins to play diverse roles. How Gβγ interacts with and regulates such a wide variety of partners, yet maintains specificity, is an interesting problem in protein-protein molecular recognition in signal transduction, where signal transfer by proteins is often driven by modular conserved recognition motifs. Evidence has accumulated that one mechanism for Gβγ multitarget recognition is through an intrinsically flexible protein surface or "hot spot" that accommodates multiple modes of binding. Since each target has a unique recognition mode for Gβγ subunits it suggests that these interactions could be selectively manipulated with small molecules which could have significant therapeutic potential.

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Introduction

G protein-coupled receptors (GPCRs) mediate multiple physiological processes and represent the largest single family of cell surface receptors (Lagerstrom and Schioth, 2008). GPCRs respond to a wide array of ligands, including hormones, peptides, proteins, lipids, neurotransmitters, nucleotides, ions and photons (Lagerstrom and Schioth, 2008). Because of their central role in biology and physiology, they are major targets of current pharmaceuticals and continue to be very important drug targets (Flower, 1999; Ma and Zemmel, 2002). Recently there has been an explosion of structural information about the nature of these receptors that promises to move drug discovery targeted at these receptors at an increasingly rapid pace (Rosenbaum et al., 2009).

GPCRs transduce extracellular information through a number of mechanisms, but classical GPCR signaling is through direct coupling to heterotrimeric G proteins consisting of an α subunit that binds GDP and GTP and a constitutive dimer of β and γ subunits (Gilman, 1987; Hamm, 1998). In the classical model for GPCR-dependent G protein activation, GPCRs undergo ligand binding-dependent conformational changes to catalyze GDP release and subsequent binding of GTP to the G protein α subunit, leading to dissociation of Gα-GTP from Gβγ (Gilman, 1987). This dissociation event releases two signaling proteins, Gα and Gβγ that drive downstream signaling through direct protein-protein interactions (Milligan and Kostenis, 2006; Oldham and Hamm, 2008). Gα subunit signaling is terminated by hydrolysis of GTP and Gβγ signaling is terminated by

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reassociation with Gα subunits in a way that sequesters the protein recognition surface on both subunits.

The Gβγ subunits are involved in multiple aspects of GPCR-mediated signaling and regulation (Dupre et al., 2009; Smrcka, 2008). In addition to their role in downstream signaling, Gβγ subunits interact with GPCRs and Gα subunits and are critical for GPCR-dependent G protein activation. The diverse and expanding roles for Gβγ in cell signaling are numerous and have been recently reviewed (Dupre et al., 2009; Smrcka, 2008). Rather, we will focus on new concepts relating to the nature of molecular recognition by Gβγ and how pharmacological targeting of Gβγ capitalizes on these ideas.

Gβγ interaction with effectors

As discussed above, Gβγ interacts directly with a wide range of effectors and regulators to modulate diverse downstream cellular responses. The first example was discovered in 1987 when purified Gβγ was shown to activate a cardiac potassium channel normally activated by a muscarinic cholinergic receptor following stimulation by acetylcholine (Logothetis et al., 1987), although at the time this was controversial. Additional evidence for Gβγ-dependent downstream pathway activation came from genetic analysis of the pheromone signaling pathway in yeast, indicating that Gβγ is the key activator of the pheromone response downstream from the G protein coupled pheromone receptor (Whiteway et al., 1989). Since then, many Gβγ-effectors have been identified, including adenylate cyclase isoforms (AC) (Sunahara and Taussig, 2002; Tang and Gilman, 1992), G protein-coupled receptor kinase 2 (GRK2) (Pitcher et al., 1992),

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phospholipase C (PLC) β2 and β3 isoforms (Camps et al., 1992; Park et al., 1993; Smrcka and Sternweis, 1993), the aforementioned inward rectifying potassium channels (GIRK) (Logothetis et al., 1987; Nakajima et al., 1996), phosphoinositide 3 kinase γ (PI3Kγ) (Stephens et al., 1994; Stephens et al., 1997) and N-type calcium channels (Ikeda, 1996). Proteomic methods and yeast two-hybrid screening have revealed multiple novel Gβγ binding proteins. These include PDZ domain containing proteins (Li et al., 2006); guanine exchange factors (GEFs) for small G proteins such as P-Rex1 (Mayeenuddin et al., 2006), FLJ0018 (a Gβγ-activated Rac and Cdc42 GEF) (Ueda et al., 2008), and p114-RhoGEF (Niu et al., 2003); protein kinase D (PKD) (Jamora et al., 1999); receptor for activated C kinase 1 (RACK1) (Dell et al., 2002); soluble NSF attachment protein (SNAP) receptor (SNARE) complex (Yoon et al., 2007); and a Radil-Rap1A complex (Ahmed et al., 2010). A striking observation for all of these Gβγ binders is that there is not a readily apparent consensus sequence or structure that mediates binding of these proteins to Gβγ. In the next sections we will discuss ideas for how these binding partners can be accommodated by Gβγ,

Molecular recognition by Gβγ

Two major approaches have been used to understand the nature of molecular recognition by Gβγ: X-ray crystal structure determination of free and complexed Gβγ, and mutagenic analysis of effector binding sites on Gβγ. The first and only Gβγ structure free of any binding partner was the crystal structure of the $G\beta_1\gamma_1$ dimer of transducin published by Sondek et al. in 1996 (Sondek et al., 1996) (Fig. 1A). As in all the X-ray

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structures of Gβγ, the Gβ subunit folds with an N-terminal α-helix that makes extensive contacts to $G\gamma_2$ and a seven bladed β propeller domain in which each blade comprises a four-stranded β sheet. In addition to the free $G\beta_1\gamma_1$ structure, crystal structures of Gβγ associated Gα subunits (Fig. 1C) (Lambright et al., 1996; Wall et al., 1995), GRK2 (Fig. 1E) (Lodowski et al., 2003), phosducin (Fig. 1B) (Gaudet et al., 1996), and two peptides SIGK (Fig. 1D) (Davis et al., 2005) and a fragment of the C-terminus of the parathyroid hormone receptor PTH1R (Fig. 1F) (Johnston et al., 2008) have been solved with the associated Protein Data Bank (PDB) codes compiled in Table 1. The overall structure of Gβγ is unperturbed in all of the crystal structures (Fig. 1). An exception is the structural change observed in the $G\beta_1\gamma_1$-phosducin structures (Gaudet et al., 1996; Loew et al., 1998), where a cavity is introduced between blades 6 and 7 of the Gβ propeller by a movement of these two blades (Loew et al., 1998). Notably, this structural change has not been observed in other complexes thus far, but the number of Gβγ-co-complexes remains limited. Based on the apparently unchanging nature of Gβγ in the various solved co-crystal structures and thermal denaturation studies (Thomas et al., 1993), Gβγ has been thought of as a relatively rigid scaffold for protein binding that can general undergo only limited conformational changes.

The co-crystal structures reveal that effector/binding proteins share a critical interaction interface on the top of the torus of Gβ created by the β propeller fold that binds to switch II helix of the Gα subunits (Fig. 1). Alanine-scanning mutagenesis of Gβ confirms the notion that effectors such as PLCβ2, ACII, GRK2 and GIRK channels share a common binding surface on Gβγ but also reveals that Gβγ-interacting proteins use

unique combinations of residues within this common binding surface to mediate binding (Ford et al., 1998; Li et al., 1998; Panchenko et al., 1998). An interesting exception is the PTH1R C-terminus which binds to the side of the β-propeller (Fig.1F) which provides a mechanism for scaffolding Gβγ the G protein αβγ heterotrimer to this GPCR (Mahon et al., 2006). Overall, despite a common surface being used for α subunits and effectors, the unique nature of binding for each partner suggested that approaches could be developed that would allow for selective manipulation of Gβγ protein-protein interactions.

As an alternative approach to understanding the nature of molecular recognition by Gβγ, we conducted a random peptide phage display screen with Gβγ as the target (Scott et al., 2001). Interestingly, the peptides appeared to "select" the common effector interaction surface on Gβγ suggesting that the binding site had intrinsic physicochemical properties as a preferred protein-protein interaction surface or "hot spot". One peptide, SIGK, was co-crystallized with Gβγ, identifying the peptide binding site and the "hot spot" as the Gα subunit switch II binding site and the major effector binding surface. (Davis et al., 2005). Protein interaction hot spots tend to be targeted in random peptide phage display screens (Fairbrother et al., 1998) and are generally thought to contain various types of amino acids that can participate in multiple types of binding interactions (Ma et al., 2001). Hot spots are also thought to be structurally flexible to be able to accommodate different structures (Delano, 2002). Both of these characteristics would make sense in terms of molecular recognition by Gβγ where multiple proteins with diverse sequence and structure are accommodated in a single binding site.

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As discussed above, Gβγ is thought to be relatively rigid based on comparison of X-ray structures between different Gβγ-target complexes. However, X-ray crystallography is not an ideal approach for studies involving molecular flexibility since X-ray structures represent space and time averaged structure and are subject to lattice constraints. It is possible to compare the temperature factors for different regions but this gives limited information. An additional drawback of comparing different structures is that due to different conditions for crystallization it is difficult to determine to what extent subtle differences in structure represent relevant differences in solution.

NMR spectroscopy is more ideally suited to measurement of protein flexibility and dynamics in solution than is X-ray crystallography. An NMR method was developed for monitoring Gβγ conformational alterations and dynamics (Smrcka et al., 2010). In part due to protein size limitations in NMR, a specific labeling protocol was adopted in which all of the Trp positions in Gβγ were labeled with ^{15}N both at indole and amide positions. The labeled protein was then analyzed by 2D TROSY-HSQC NMR. Peaks in the spectra were assigned by site direct mutagenesis and could then be mapped to specific positions in the three dimensional structure of Gβ. Thus changes in dynamics and chemical shift position upon protein/ligand binding could be interpreted in the context of specific regions of the Gβγ dimer. Supporting the concept of a Gβγ "hot spot", the Trp residues in the Gβγ "hot spot" were unusually dynamic. Two tryptophan residues in the "hot spot" appeared to be in motion in different time scales. W99 moves in a very rapid time scale and W332 moves in an intermediate time scale with backbone and indole

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amides moving in different time regimes. It should be cautioned that this interpretation was made based on peak intensities and awaits rigorous confirmation by NMR relaxation methods to accurately determine dynamics. Nevertheless, these data together create a picture where amino acids in the "hot spot" are in motion in the absence of binding partners. Only Trp residues are monitored by this method, but it is likely that all of the amino acids in the "hot spot" are unusually dynamic. A hypothesis that arises from these measurements is that the Gβγ subunit "hot spot" surface explores a range of conformations in the uncomplexed state. This range of conformations could then present a range of structures that can accommodate different partners and provides evidence that one of the properties that allow the "hot spot" to be a preferred protein-protein interaction surface is an inherent flexibility.

Further NMR analyses of Gβγ dynamics in the presence of three different binding partners revealed different alterations at the Gβγ "hot spot" surface and supports the idea that the Gβγ "hot spot" and perhaps all of Gβγ is more conformationally flexible than is generally presumed. These molecules, $G\alpha_{i1}$-GDP, a phage display derived Gβγ-binding peptide SIGK, and phosducin, have all been co-crystallized with Gβγ. They share a binding surface at the "hot spot" and but have significantly different effects on Gβγ structure and dynamics as assessed by NMR, that is not reflected in the co-crystal structures with these molecules. $G\alpha_{i1}$-GDP subunit binding to Gβγ did not significantly alter Gβγ surface dynamics at the "hot spot". This was unexpected because Gα binding to Gβγ buries much of the "hot spot" surface including W99 and W332 residues (Park et al., 2011; Wall et al., 1995). In contrast to binding of $G\alpha_{i1}$-GDP, binding of SIGK to

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much of the same surface as Gα largely suppressed W99 and W332 dynamics. SIGK seems to select, and lock in, a particular conformation of the Gβγ "hot spot", supporting the idea that the "hot spot" can flexibly adapt to accommodate different binding partners. In addition, there were chemical shift changes of Trp residue signals at some distance from the Gβγ-SIGK interface. These changes were subtle and their biological significance unclear, but indicate that conformational information can be transmitted allosterically throughout the Gβγ molecule. Finally, binding of phosducin altered both the dynamics and induced large chemical shift changes throughout Gβ. Interestingly much, but not all, of this alteration can be accounted for by binding of the N-terminal domain phosducin at the "hot spot". These data highlight the fact that many of the assumptions about Gβγ structural flexibility and conformational alteration is based on co-crystal structures with relatively few binding partners and interpretations can be hampered by the inherent limitations of X-ray crystallography in defining physiologically relevant yet subtle alterations in structure and dynamics. It also highlights the idea that three different binding partners that interact with the same surface on Gβ have very different effects on the overall dynamics of Gβγ.

Small molecule targeting of the Gβγ "hotspot"

The random peptide phage display screen with Gβγ as the target led to identification of Gβγ-binding peptides that were selective blockers of effector regulation (Scott et al., 2001). One peptide, SIRK, blocked Gβγ-dependent activation of PLCβ and PI3Kγ *in vitro*, but not Gβγ-mediated inhibition of voltage-gated calcium channels. This

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selectivity of SIRK suggests that small molecules might be found that bind to the "hot spot" and also display effector selectivity.

On the basis of this data demonstrating selective modulation of signaling downstream of Gβγ by peptides and studies on the nature of the molecular recognition surface of Gβγ, we initiated a screen to identify small molecules that would bind to the "hot spot" on Gβγ and block downstream signaling (Bonacci et al., 2006). Compounds that bind Gβγ were identified through a combination of computational virtual screening and testing of the National Cancer Institute (NCI) chemical diversity set in a competition ELISA for the SIGK peptide. This NCI diversity is a collection of compounds that represent the chemical diversity present in the larger 250,000 compound NCI chemical library. Nine candidate compounds that inhibited SIGK binding with IC_{50}'s ranging from 100 nM to 60 μM were identified. These compounds selectively blocked effector interactions *in vitro* and in intact cells. The Gβγ inhibitory compounds could be divided into two general classes based on binding mechanism. One class, that included M119 (NSC119910, IUPAC: 2-(3,4,5-trihydroxy-6-oxoxanthen-9-yl)cyclohexane-1-carboxylic acid) and the highly related molecule gallein (IUPAC: 3',4',5',6'-tetrahydroxyspiro[2-benzofuran-3,9'-xanthene]-1-one) (Lehmann et al., 2008), referred to together as M119/gallein, bound via a reversible non-covalent mechanism (Seneviratne et al., 2011), while another class, represented by selenocystamine, formed redox-reversible covalent adducts with Gβγ (Dessal et al., 2010). Many of these redox-dependent compounds targeted a cysteine residue (C204) in the Gβ "hot spot" to form reversible mixed disulfides. The M119/gallein class of compound has been analyzed extensively for

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selective blocking of Gβγ-target interactions, the results of which are compiled in Table 2.

To understand the detailed nature of compound binding, and how specificity is generated, we applied a combination of structure activity relationship (SAR) analysis, site directed mutagenesis and X-ray structure determination to identify specific binding modes for compound interactions with Gβγ. Recently the structure of a complex between Gβγ and a reversibly binding compound, M201 (N-deacetyl colchicine, IUPAC: 7-amino-1,2,3,10-tetramethoxy-6,7-dihydro-5H-benzo[a]heptalen-9-one) was determined (Seneviratne, et al., submitted) (Fig. 2). This structure and associated mutagenic analysis show that M201 binds to the "hot spot" with contacts primarily along the sides of the central pore in the Gβγ propeller with a portion of the compound extending beyond the protein surface. Notably it appears that an important part of the binding mechanism may involve hydrogen bonding of the compound to tightly bound water in the core of the molecule. Since the binding contacts for M201 are within the core of the propeller, many of the contacts for binding are below the direct protein interaction surface of the "hot spot". This has the potential to allow for high affinity protein binding while only occluding a small subset of the surface amino acids in the "hot spot". For example, only two amino acids within the "hot spot", Y145 and L117 are occluded by M201. Site directed mutagenesis studies indicate that these amino acids are not required for PLC activation. Thus, as might be expected, M201 binding to Y145 and L117 does not inhibit, but rather potentiates PLC activation. On the other hand, these amino acids are required for GRK2 binding activation, and M201 inhibits Gβγ interaction with GRK2.

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These data support the idea that individual compounds selectively interfere with effectors because they interact with amino acids critical for activation of specific effectors. While a structure of bound M119/gallein was not determined, mutating amino acids at the binding site observed in the structure eliminates binding and functional effects of M201 but does not alter gallein binding indicating that these compounds have different binding sites on the Gβγ surface and may form the basis for the differences in effector selectivity for these two compounds.

Therapeutic potential of selective targeting Gβγ-effector interface

Studies on the nature of molecular recognition of targets by Gβγ subunits are physiologically important because many of the Gβγ-target couplings are involved in diseases and disruption of these interactions has been shown to be of potential therapeutic benefit. For example, various studies have shown that blocking Gβγ protein-protein interactions is an effective approach to preventing heart failure (Rockman et al., 1998), arterial restenosis (Iaccarino et al., 1999), hypertension (Koch et al., 1995), drug addiction (Yao et al., 2003), cancer metastasis (Müller et al., 2001) and prostate cancer (Bookout et al., 2003) in animal models. Most of these studies used GRK2ct (C-terminus of GRK2), and the Gβγ binding peptide QEHA (sequence derived from ACII) for pharmacological targeting of Gβγ. The details of these studies have been the subject of previous reviews (Smrcka, 2008; Smrcka et al., 2008). Small molecules that bind to

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Gβγ (M119/gallein) are effective in animal models of inflammation, analgesia and heart failure (Fig. 3). In addition to the direct potential benefits in the specific indications discussed above, there are other theoretical advantages to targeting Gβγ as discussed below.

Multitarget inhibition may be more therapeutically efficacious.

A major advantage of targeting GPCRs directly is pharmacological specificity. There are many GPCRs and subtypes involved in a variety of physiologies that have the potential to be selectively targeted, thus limiting side effects of a more broadly based pharmacological strategy. A downside to this approach is that high specificity can limit therapeutic efficacy in complex diseases. If multiple GPCRs are involved in the development of disease, targeting a single GPCR may not be effective; rather, inhibiting the therapeutically relevant signaling pathway(s) downstream of a group of receptors could achieve this goal. An example is chemokine receptors in rheumatoid arthritis where common Gβγ signaling systems are downstream of multiple chemokine receptor subtypes (Johnson, 2004). Thus inhibiting Gβγ signaling may be more efficacious than targeting a single GPCR. While Gβγ binding compounds are somewhat selective for downstream signaling pathways, it is unlikely that compounds will be found that bind to Gβγ and only inhibit single effector due to the overlapping nature of the binding surface. As things currently stand, compounds tend to inhibit groups of Gβγ targets. This will likely to some extent limit the specificity of this approach therapeutically, but on the other hand could provide some benefits in terms of efficacy.

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Biased agonist signaling.

Biased agonist signaling by GPCRs is a property of GPCRs that is currently an important research direction that has possible therapeutic applications (Kenakin, 2011). The overall idea is that GPCRs sample multiple conformations that signal downstream to different signaling pathways. Agonists that select particular conformations of the receptor direct the receptor to favor activation of select pathways downstream. This has important therapeutic implications because GPCRs are major drug targets and selectively modulating pathways that are therapeutically relevant could improve pharmacological specificity and efficacy. An alternate approach to biasing GPCR signaling down a particular pathway is to identify compounds that selectively interfere with pathways downstream from the receptors. Compounds identified in this way could be combined with existing GPCR agonists to alter signaling specificity and would obviate the need for identifying biased agonists for individual receptors. In this regard small molecule Gβγ inhibitors that selectively modify signaling downstream could act to bias GPCR signaling. Such molecules only inhibit a portion of the Gβγ-dependent component (See Table 2 for example) of the GPCR signal leaving the remainder of the GPCR signaling pathway intact.

An example of such a pathway where it has been proposed that M119/gallein biases GPCR signaling is in μ-opioid receptor dependent analgesia (Figure 4). Administration of M119/gallein by either intracerebroventriclular or intraperitoneal injections into mice potentiates the action of μ-opioid receptor agonists (Bonacci et al., 2006; Mathews et al., 2008). Since opioid receptor efficacy is largely dependent on Gβγ

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signaling, we propose that M119/gallein selectively blocks a Gβγ-dependent inhibitory pathway downstream of the μ-opioid receptor (PLCβ3) (Bianchi et al., 2009), while leaving other signaling pathways required for analgesia intact (N-type Ca^{2+} and GIRK channels). Thus M119/gallein apparently biases signaling downstream of the μ-opioid receptor. Further confirmation of this hypothesis requires more detailed testing of these signaling pathways, but these data support the idea that Gβγ inhibitors could be used to bias signaling pathways downstream of GPCRs.

Critical role of Gβγ in the G protein cycle and target specificity

To consider Gβγ as a feasible therapeutic target, several issues associated with its central role in the GPCR signaling cascade must be considered. Gβγ is required for interaction of the G protein heterotrimer with GPCRs. Therefore a potential therapeutic strategy must target Gβγ without disruption of this G protein cycling. Another major problem is that Gβγ expression is nearly ubiquitous so blocking all Gβγ functions might have unwanted side effects. While Gβγ subunits are universally expressed, individual effectors, and Gβγ-effector couples, have tissue specific expression and/or restricted subcellular location. With small molecule inhibitors that selectively target specific Gβγ-effector coupling, without ablating general Gβγ function, selectivity issues associated with ubiquitous Gβγ expression may be overcome.

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As discussed, small molecule Gβγ inhibitors (M119/gallein) have been used extensively to investigate Gβγ functions in cell biological and animal models of diseases. In the course of these studies, many questions concerning off-target effects have been addressed. For example, in the presence of M119/gallein, the following were observed: unimpaired isoproterenol-, $G\alpha_s$-dependent cAMP production (Casey et al., 2010); unimpaired [D-Ala^2, N-MePhe^4, Gly-ol]-enkephalin (DAMGO, a μ-opioid receptor-specific agonist), $G\alpha_i$- dependent decrease in cAMP and unimpaired μ-opioid receptor, Gβγ-dependent analgesia as discussed above (Mathews et al., 2008); no effects of compounds on δ and κ opioid receptor signaling (Mathews et al., 2008); unimpaired fMLP- Gβγ-dependent ERK activation in HL60 neutrophil-like cells (Bonacci et al., 2006), unpaired M3 muscarinic acetylcholine receptor, $G\alpha_q$- dependent Ca^{2+} regulation (Bonacci et al., 2006) and unimpaired SDF-1, $G\alpha_i$-dependent inhibition of cAMP levels (Kirui et al., 2010). All these data indicate that GPCRs function normally in the presence of Gβγ inhibitors and that there is selectivity for different Gβγ targets. These experiments do not address unanticipated off-target effects unrelated to the G protein signaling machinery that could complicate interpretation of cellular and *in vivo* experiments. Thus far, results from published *in vivo* experiments are consistent with a Gβγ-dependent mechanism of action and other experiments have not revealed significant off-target effects of gallein. For example, daily intraperitoneal injections of gallein in mice for three months were without significant observable physiological effects other than expected for inhibition of Gβγ in cardiac function. Thus if there are off-target effects they are not major. Nevertheless a more thorough investigation is wanted.

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Summary

Studies of the protein recognition properties of Gβγ have led to insights into the mechanisms by which Gβγ recognizes multiple different protein targets through a flexible binding surface that presents multiple types of potential bonding interactions. These insights have led to the development of a novel strategy for targeting Gβγ signaling that may have therapeutic potential for treatment of specific diseases but may also open up a new pharmacological approach to manipulating GPCR signaling in a more general sense.

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Figure Legends

Figure 1. Gβγ crystal structures. **A,** $G\beta_1\gamma_1$ (PDB code: 1TBG) (Sondek et al., 1996). **B,** $G\beta_1\gamma_1$•phosducin (PDB code: 2TRC) (Gaudet et al., 1996). **C,** $G\alpha_{t/i}\beta_1\gamma_1$ heterotrimer (PDB code: 1GOT) (Lambright et al., 1996). **D,** $G\beta_1\gamma_2$•SIGK peptide (PDB code: 1XHM) (Davis et al., 2005). **E,** $G\beta_1\gamma_2$•GRK2 (PDB code: 1OMW) (Lodowski et al., 2003). **F,** $G\beta_1\gamma_2$•PTH1 peptide (PDB code: 3KJ5) (Johnston et al., 2008). Gβ subunits are all colored in aquamarine; Gγ subunits are all colored in green; $G\alpha_{t/i}$ is colored in magenta with bound GDP shown as space fill in blue; phosducin is colored in marine; GRK2 is colored in yellow; SIGK peptide is colored in salmon; and PTH1 peptide is colored in orange and shown as lines.

Figure 2. Binding of M201 to the Gβγ hotspot. M201 (NSC201400) is depicted in yellow. Gβ is in blue with some of the key amino acids in the "hot spot" shown in CPK and labeled.

Figure 3. Therapeutic targets for Gβγ inhibitors.

Figure 4. Gβγ inhibitors bias the action of μ opioid receptor agonists. M119/gallein potentiates the analgesic potency of morphine in vivo. It blocks PLC activation but not calcium channel regulation in vitro. We propose that Gβγ inhibitors bias the action of morphine by blocking a hyperalgesic pathway, dependent on PLC activation, downstream of the μ-opioid receptor without blocking Gα subunit signaling or Gβγ-dependent Ca^{2+} channel or K^+ channel regulation thus potentiating opioid analgesia.

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Table 1. Gβγ structures

PDB#	Complex	Resolution (Å)	Reference
1TBG	$G\beta_1\gamma_1$	2.1	(Sondek et al., 1996)
1GOT	$G\alpha_{t/i}\beta_1\gamma_1$ heterotrimer	2.0	(Lambright et al., 1996)
1GP2	$G\alpha_{i1}\beta_1\gamma_2$ heterotrimer	2.4	(Wall et al., 1995)
1GG2	G203A-$G\alpha_{i1}\beta_1\gamma_2$ heterotrimer	2.7	(Wall et al., 1995)
2BCJ	$G\alpha_{i/q}$(GDP•AlF4-)•GRK2•$G\beta_1\gamma_2$	3.1	(Tesmer et al., 2005)
2TRC	$G\beta_1\gamma_1$•phosducin	2.4	(Gaudet et al., 1996)
1A0R	$G\beta_1\gamma_1$•phosducin	2.8	(Loew et al., 1998)
1B9X	$G\beta_1\gamma_1$S73E•phosducin	3.0	(Gaudet et al., 1999)
1B9Y	$G\beta_1\gamma_1$•phosducin	3.0	(Gaudet et al., 1999)
1OMW	$G\beta_1\gamma_2$•GRK2	2.5	(Lodowski et al., 2003)
3CIK	$G\beta_1\gamma_2$•GRK2[a]	2.75	(Tesmer et al., 2010)
3KRW	$G\beta_1\gamma_2$•GRK2•balanol	2.9	(Tesmer et al., 2010)
1XHM	$G\beta_1\gamma_2$•SIGK peptide	2.7	(Davis et al., 2005)
3KJ5	$G\beta_1\gamma_2$•PTH1 peptide	3.0	(Johnston et al., 2008)
2QNS	$G\beta_1\gamma_2$•PTH1 peptide	3.0	(Johnston et al., 2008)

[a]: Here GRK2 is from human to distinguish it from 1OMW where GRK2 is from bovine.

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Table 2. Selectivity of M119/gallein in blocking downstream effector interactions

Blocked by M119/gallein	
Phospholipase C β2, β3	(Bonacci et al., 2006; Mathews et al., 2008)
pREX guanine nucleotide exchange factor	(Lehmann et al., 2008; Qin et al., 2009; Zhao et al., 2007)
PI3Kinase γ	(Bonacci et al., 2006; Lehmann et al., 2008)
G protein-coupled receptor kinase 2	(Bonacci et al., 2006; Casey et al., 2010)
Not Blocked by M119/gallein	
N-type Ca^{2+} channel	P. Kammermeier (Unpublished observations)
Inwardly rectifying K^{+}-channel	P. Kammermeier (Unpublished observations)
ERK1/2	(Bonacci et al., 2006)
ACII, IV, VI	C. Dessauer and V. Watts (unpublished observations)

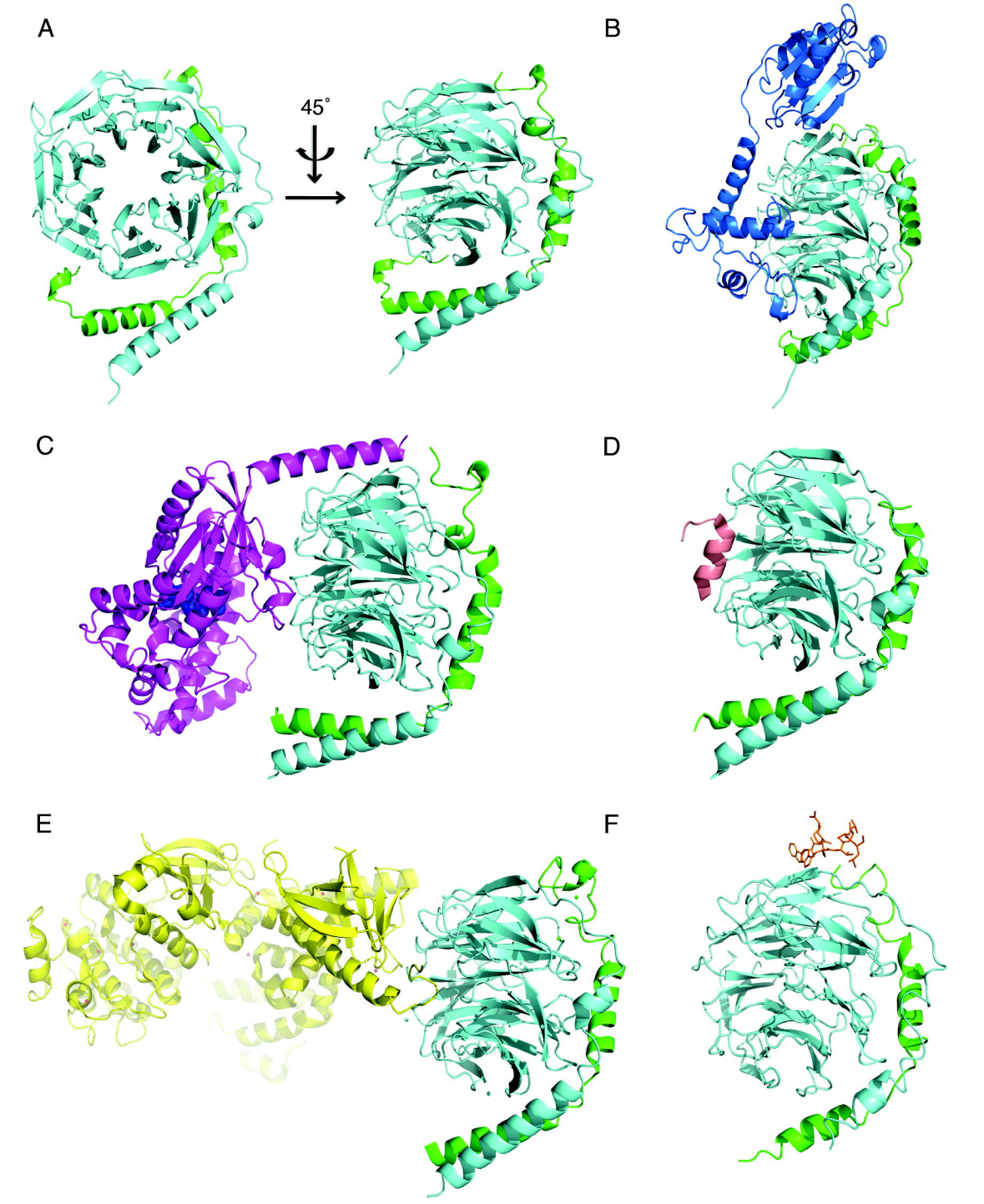


Figure 1

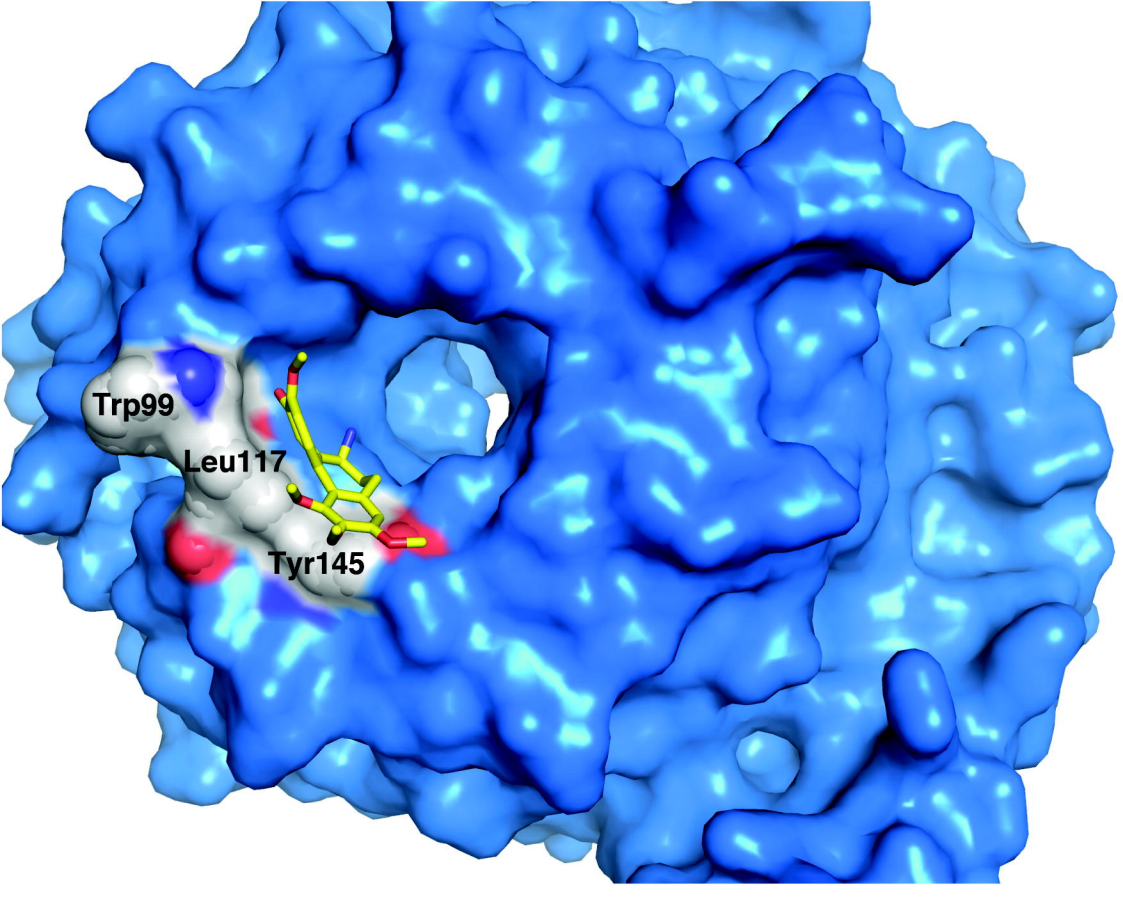


Figure 2

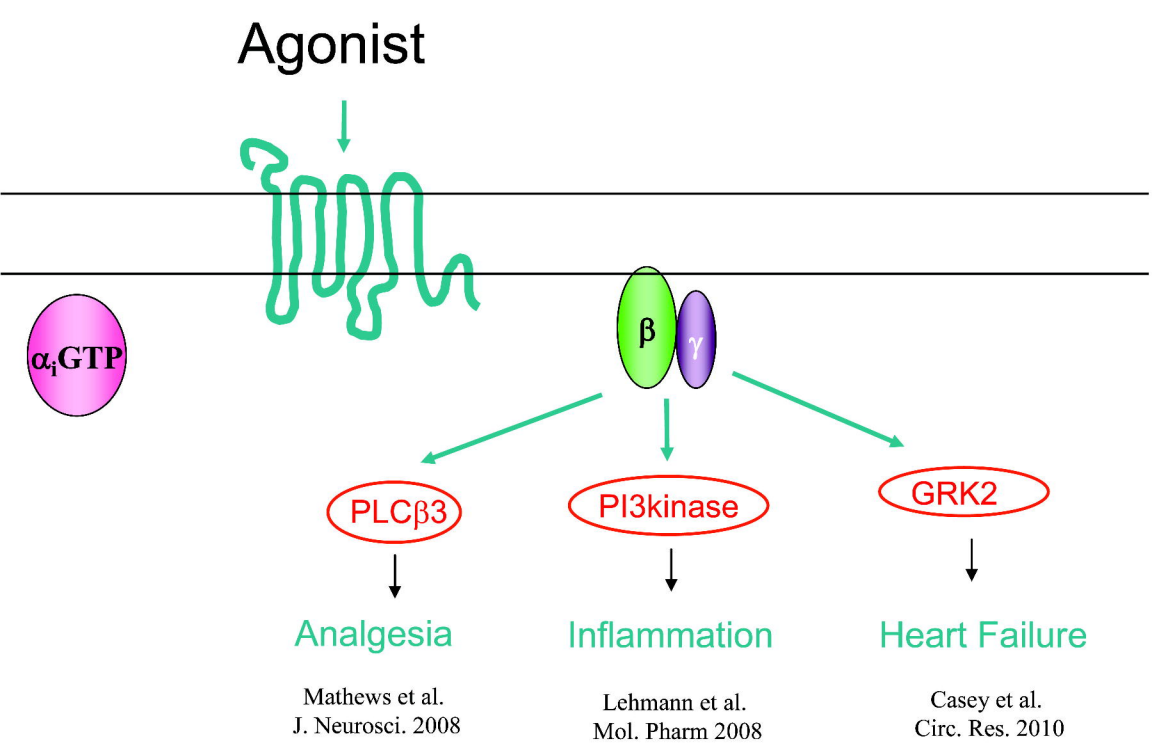
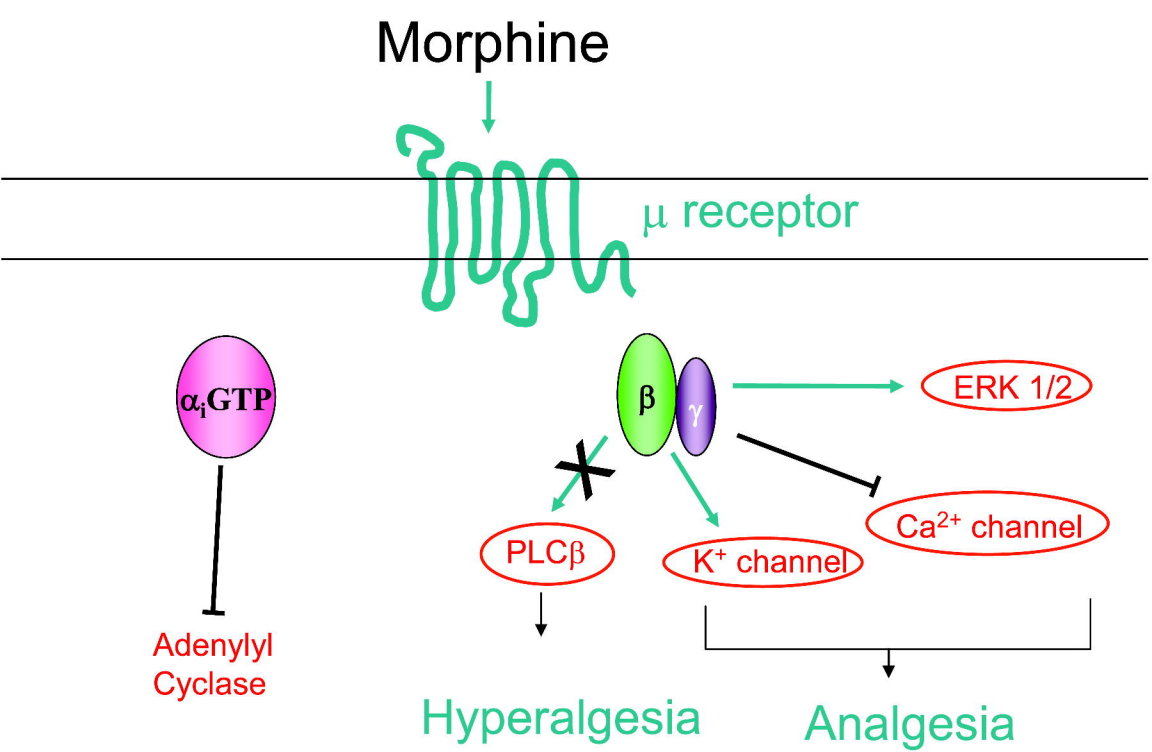

Agonist
α_iGTP
β
γ
PLCβ3
PI3kinase
GRK2
Analgesia
Inflammation
Heart Failure
Mathews et al.
J. Neurosci. 2008
Lehmann et al.
Mol. Pharm 2008
Casey et al.
Circ. Res. 2010

Figure 3



Bianchi et al. 2008

Figure 4