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Strike a Pose: $G\alpha_q$ Complexes at the Membrane

Angeline M. Lyon, Veronica G. Taylor, and John J. G. Tesmer

Life Sciences Institute and the Departments of Pharmacology and Biological Chemistry,
University of Michigan, Ann Arbor, MI, United States

Abstract

The heterotrimeric G protein $G\alpha_q$ is a central player in signal transduction, relaying signals from activated G protein-coupled receptors (GPCRs) to effectors and other proteins to elicit changes in intracellular Ca^{2+} , the actin cytoskeleton, and gene transcription. $G\alpha_q$ functions at the surface of the plasma membrane, as do its best characterized targets phospholipase C- β , p63RhoGEF, and GPCR kinase 2 (GRK2). Recent insights into the structure and function of these signaling complexes reveals several recurring themes, including complex multivalent interactions between $G\alpha_q$, its protein target, and the membrane, that are likely essential for allosteric control and maximum efficiency in signal transduction. Thus, the plasma membrane is not only a source of substrates, but also a key player in the scaffolding of $G\alpha_q$ -dependent signaling pathways.

Keywords

Phospholipase C β ; p63RhoGEF; ARHGEF25; GRK2; G protein-coupled receptor; heterotrimeric G protein signaling; $G\alpha_q$; second messengers; phospholipids; membrane; protein structure; crystallography

Signaling by heterotrimeric G protein $G\alpha$ subunits

The proteins involved in GPCR signaling pathways are essential for vision, blood pressure regulation, cardiac contractility, and numerous other processes¹. Activation of a GPCR by an extracellular signal, such as a photon, hormone, or drug, induces conformational changes that allow the GPCR to function as a guanine nucleotide exchange factor (GEF) for heterotrimeric G protein $G\alpha$ subunits², promoting the binding of GTP by $G\alpha$. Once activated, $G\alpha$ -GTP dissociates from the heterodimeric $G\beta\gamma$ subunits, and both components can bind to and activate downstream effectors at the plasma membrane to elicit changes in the concentration of second messengers such as inositol-1,4,5-triphosphate (IP₃) and diacylglycerol (DAG)³ (Figure 1).

The $G\alpha$ subunit consists of a Ras-like domain with a large α helical domain insertion³. The guanine nucleotide binding pocket is formed at the interface of the Ras-like and α helical domains, where three “switch” regions (SwI-III) adopt unique conformations dependent on the identity of the bound nucleotide. In the GTP-bound state, the switches help coordinate the catalytic Mg^{2+} ion, participate in catalysis, and contribute to the effector binding site (Figure 2a, b). In the GDP-bound state, SwII contributes instead to the $G\beta\gamma$ binding site³.

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Corresponding author: John J. G. Tesmer (tesmerjj@umich.edu).

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The N-terminus of $G\alpha$ is lipid-modified (*e.g.* myristoylated and/or palmitoylated) and can form an extended helix that interacts with GPCRs ², $G\beta\gamma$ subunits ^{4,5}, and some effectors ⁶.

The focus of this review is on the interactions formed by activated $G\alpha_q$, which represents one of the four canonical subfamilies of $G\alpha$ subunits ($G\alpha_s$, $G\alpha_{i/o}$, $G\alpha_{q/11}$ and $G\alpha_{12/13}$). The other members of this subfamily include $G\alpha_{11}$, $G\alpha_{14}$, and $G\alpha_{15/16}$. $G\alpha_q$ subfamily members are activated by GPCRs that respond to hormones and neurotransmitters such as norepinephrine, endothelin, and glutamate ⁷, and play important roles throughout the cardiovascular and nervous systems. Classically, $G\alpha_q$ activates phospholipase C- β (PLC β), which hydrolyzes phosphatidylinositol-4,5-bisphosphate (PIP₂) to generate the second messengers inositol-1,4,5-triphosphate (IP₃) and diacylglycerol (DAG), leading to increases in intracellular Ca²⁺ and activation of protein kinase C ^{8,9}. More recently, it was discovered that $G\alpha_q$ also activates the guanine nucleotide exchange factor p63RhoGEF (ARHGEF25) and its homologs Trio and Duet ^{10–13}, thereby linking GPCRs to the activation of RhoA and control of the actin cytoskeleton. In addition, $G\alpha_q$ binds to GPCR kinase 2 (GRK2) ¹⁴, an enzyme that initiates homologous desensitization by phosphorylating activated GPCRs ¹⁵. However, GRK2 is not activated via binding $G\alpha_q$. Instead, it seems to sequester $G\alpha_q$ from its other targets in a form of phosphorylation-independent desensitization ^{16–19} (Figure 1). Not all members of the $G\alpha_q$ subfamily interact with these targets in the same manner. $G\alpha_{16}$ does not bind appreciably to GRK2 ²⁰, and binds to but does not activate p63RhoGEF ²¹.

Structural and functional studies of activated $G\alpha_q$ in complex with PLC β ^{6,22}, p63RhoGEF ¹¹, and GRK2 ¹⁴ reveal some general themes. PLC β , p63RhoGEF, and GRK2 all must interact with the membrane and, in some cases, with other peripheral membrane proteins for optimal catalytic efficiency. A specific orientation of these enzymes with respect to the membrane plane is essential for their function. All three enzymes engage the effector binding site of $G\alpha_q$ using exposed hydrophobic residues on helical elements. Unexpectedly, comparison of the likely membrane-bound orientation of $G\alpha_q$ in complex with PLC β , p63RhoGEF, and GRK2 reveals that $G\alpha_q$ adopts a similar orientation with respect to the membrane plane in each assembly (Figure 3). It is also clear that the membrane itself plays one or more active roles in the function of these complexes: it can enhance the affinity of signaling components by co-localization, serve as a scaffold for the assembly of high order assemblies, and/or optimize the orientation of these proteins for maximum signaling efficiency.

Scaffolding interactions in the activation of PLC β by $G\alpha_q$

PLC β enzymes comprise two major regions: a catalytic core responsible for PIP₂ hydrolysis and a ~400 residue C-terminal extension required for $G\alpha_q$ regulation and membrane association (Figure 3a, b). The catalytic core is conserved across all PLC isozymes ²³ and contains an autoinhibitory element called the X–Y linker ²⁴, which is immediately adjacent to the active site. This linker is poorly conserved but contains highly acidic stretches of amino acids in human PLC β isoforms. In all PLC β crystal structures reported to date, the C-terminal residues of the linker are ordered and occlude the active site ⁹. It has been proposed this linker is dislodged by electrostatic repulsion when the PLC β catalytic core is brought in close proximity to the negatively charged inner leaflet of the plasma membrane ²⁴. Also adjacent to the active site is the so-called hydrophobic ridge, a series of loops with exposed hydrophobic residues believed to insert into the membrane during catalysis ²⁵ (Figure 3a, b).

The C-terminal extension can be subdivided into two domains. The first 45 amino acids form the proximal C-terminal domain (CTD), comprising the primary $G\alpha_q$ binding site and an autoinhibitory helix (H α 2'). In the absence of $G\alpha_q$, the first 25 amino acids of the proximal CTD are disordered ²⁶. Upon $G\alpha_q$ binding, these residues form a helix-turn-helix

(H α 1/H α 2) that docks into the effector binding site of G α_q via a conserved motif, ALXXPI (where X represents any amino acid) ^{6, 22}. An additional contact between G α_q and PLC β is made by an asparagine residue within the catalytic core that interacts with the active site of G α_q to increase the rate of GTP hydrolysis. Thus, PLC β functions as its own GTPase activating protein (GAP), regulating the duration of its own signal ²². Under basal conditions, the H α 2' helix of the proximal CTD interacts with a conserved cleft within the PLC β catalytic core. Disruption of the H α 2'-core interaction in human PLC β 3 increases basal activity up to ~50-fold over wild-type and decreases the efficacy of G α_q activation, suggesting that G α_q activation of PLC β includes an allosteric component ²⁶.

The last ~300 residues of the C-terminal extension form the distal CTD, a long coiled-coil domain ^{6, 27} required for maximum basal and G α_q -stimulated activity and for membrane association ^{22, 26, 28–31} (Figure 3a, b). The distal CTD features two conserved surfaces. The first is an extended positively charged region formed by clusters of basic residues along one face of the domain. Mutation of these residues decreases G α_q -stimulated activity and particulate association, suggesting they represent the primary membrane binding surface ^{28, 31, 32}. The second surface contains a cluster of conserved hydrophobic residues that binds the N-terminal helix of G α_q in the crystal structure of the G α_q -PLC β 3 complex ⁶. Abrogation of this interaction diminished maximal G α_q -stimulated activity, as did loss of G α_q palmitoylation.

Direct interactions between PLC β and G α_q increases the rate of PIP₂ hydrolysis by up to ~80-fold over basal. However, although G α_q is palmitoylated at its N-terminus and localized at the membrane ³³, there are no quantitative studies demonstrating that interactions with G α_q increase PLC β membrane association ^{34, 35}, and thus membrane recruitment on its own does not provide a holistic model for activation. Instead, it has been proposed that in the absence of G α_q , PLC β enzymes are in equilibrium between cytosolic and membrane-associated populations ^{6, 35}, which may be regulated in part through direct interactions between the distal CTD and the catalytic core, as observed in crystals and in solution by cryo-electron microscopy ⁶. G α_q binds to the H α 1/H α 2 helix-turn-helix and displaces the auto-inhibitory H α 2' helix from the catalytic core, allosterically activating PLC β . Meanwhile, the distal CTD simultaneously interacts with the membrane and with the N-terminal helix of G α_q in a manner that helps the G α_q -catalytic core complex adopt a specific orientation with respect to the membrane surface ⁶. The resulting electrostatic repulsion between the membrane and acidic regions in the X–Y linker helps to open the active site, allowing insertion of the hydrophobic ridge into the membrane and binding of the substrate PIP₂ (Figure 3a). Thus, the distal CTD and the membrane can be thought of as scaffolds that not only help optimize the orientation of the G α_q -catalytic core complex, but also allosterically activate it. Higher order scaffolding interactions are known to occur. Complexes that include GPCRs can be mediated by proteins such as WD40 repeat protein 36 (WDR36), which has been reported to interact with G α_q , PLC β and the thromboxane A₂ receptor ³⁶, and well-characterized scaffolding proteins such as InaD and Na⁺-H⁺ exchange regulatory factor (NHERF) ³⁷.

Despite recent progress in understanding the molecular mechanisms of G α_q -dependent activation, many questions remain. It is unclear whether membrane binding induces conformational changes within the catalytic core of PLC β and whether displacement of the segment of the X–Y linker that occludes the active site also contributes to structural rearrangements. The mechanism by which H α 2' mediates autoinhibition is currently unknown. Finally, given its similarity to Bin–Amphiphysin–Rvs domains, the distal CTD may play other roles in regulating G α_q -stimulated activity, such as by altering the local structure of the membrane itself ⁶.

Membrane scaffolding of the $G\alpha_q$ -p63RhoGEF-RhoA signaling complex

Although $G\alpha_q$ has been reported to interact with other RhoGEFs^{38–40}, p63RhoGEF is the best characterized direct link between G_q -coupled receptors and RhoA¹⁰. p63RhoGEF plays a role in neurite outgrowth^{41, 42}, and in smooth muscle cells it contributes to muscle contraction by promoting GTP binding by RhoA in response to activation of angiotensin II, endothelin-1 and phenylephrine-responsive GPCRs^{43–45}. Recent studies also suggest that p63RhoGEF plays an important role in breast carcinoma cell chemotaxis^{46, 47} and osteoblast differentiation⁴⁸. Two related RhoGEFs, Trio and Duet, have also been found to activate RhoA in response to binding $G\alpha_q$ ^{11, 13}. In *C. elegans*, the Trio homolog is required for proper acetylcholine-stimulated locomotion and egg-laying¹². Interestingly, $G\alpha_{16}$ binds to, but does not activate p63RhoGEF, which can lead to inhibition of $G\alpha_{16}$ -mediated activation of PLC β ²¹. Conversely, $G\alpha_{16}$ could also inhibit the activation of p63RhoGEF mediated by other $G\alpha_q$ subfamily members, although this remains to be demonstrated experimentally.

As a member of the Dbl family of RhoGEFs, the catalytic core of p63RhoGEF contains the characteristic Dbl homology (DH) domain, which harbors the principal binding site for RhoA, immediately followed by a pleckstrin homology (PH) domain that often plays a regulatory role in these enzymes. In the case of p63RhoGEF, the PH domain inhibits the catalytic activity of the DH domain, but the molecular mechanism for this is not fully understood. Binding of activated $G\alpha_q$ leads to allosteric activation of p63RhoGEF, the most obvious manifestation of which is an increase in the affinity of p63RhoGEF for apo-RhoA⁴⁹.

The crystal structure of the $G\alpha_q$ -p63RhoGEF-RhoA complex reveals that $G\alpha_q$ binds in a manner that bridges the DH and PH domains of p63RhoGEF¹¹ (Figure 3c, d). Like PLC β , the DH/PH catalytic core of p63RhoGEF features a C-terminal extension, also conserved in Trio and Duet¹³, which forms a helix-turn-helix element that docks into the effector-binding site of $G\alpha_q$. Strikingly, this extension contains the same ALXXPI motif used in PLC β isozymes to bind the effector binding site on $G\alpha_q$. Mutation of the conserved leucine in this motif (L859 in human PLC β 3, L475 in human p63RhoGEF) is sufficient to eliminate $G\alpha_q$ binding^{11, 13, 22, 31}. Consequently, PLC β and p63RhoGEF cannot simultaneously interact with a single $G\alpha_q$ molecule¹⁰, and would therefore appear to represent competitive pathways in cells where both enzymes are expressed. However, in *C. elegans*, the activity of both enzymes is required for proper function of the neuromuscular junction, suggesting there may be distinct pools of $G\alpha_q$ scaffolded in close proximity to each effector enzyme at the cell membrane¹².

The interactions between the C-terminal extension of p63RhoGEF and $G\alpha_q$ are further stabilized by bridging interactions of the DH domain with the Ras-like domain of $G\alpha_q$. Mutation of residues involved in this latter interface decreases $G\alpha_q$ -mediated activation but had little effect on affinity¹¹, including those converting positions at the C-terminus of $G\alpha_q$ to their equivalents in $G\alpha_{16}$ ⁴⁹. These results suggest that the bridging interaction of $G\alpha_q$ serves to constrain the relative orientation of the DH and PH domains⁴⁹. Indeed, comparison with structures of closely related DH/PH domains indicates that the relative orientation of the DH and PH domains in the $G\alpha_q$ -p63RhoGEF complex is atypical. Thus, in the basal state, either the p63RhoGEF PH domain occludes the RhoA binding site, or the linker helix connecting the DH and PH domains, which forms an important portion of the interface with apo-RhoA (Figure 3d), is not in an optimal configuration. When the $G\alpha_q$ -p63RhoGEF-RhoA complex is positioned such that all the lipid modifications (geranylgeranyl in RhoA, and palmitoyl in $G\alpha_q$ and p63RhoGEF) are positioned to engage the membrane (Figure 3c), the orientation of $G\alpha_q$ is similar to that observed in the $G\alpha_q$ -

PLC β complex^{6,22}. However, unlike PLC β , the entire complex is likely offset from the membrane due to the length of disordered elements bearing the lipid modifications (Figure 3c, d). Such an offset is consistent with the lack of an obvious basic electrostatic surface on the G α_q -p63RhoGEF-RhoA complex that would interact with the negatively charged inner leaflet of the membrane.

In cells, p63RhoGEF is constitutively localized to the cell membrane by virtue of three palmitoylated cysteines at its N-terminus. Mutation or deletion of these cysteines abrogates membrane association and activity of p63RhoGEF in cells, but not *in vitro*⁵⁰. This result indicates the interaction between p63RhoGEF and RhoA is not of sufficient affinity *in vivo* to drive activation in the absence of co-localization mediated by the membrane. A recent study showed that GEFT, a splice variant of p63RhoGEF that lacks N-terminal palmitoylation sites, is not membrane associated and does not efficiently couple to G α_q in cells unless it is artificially targeted to the plasma membrane. This study further concluded that the membrane plays an important role in increasing local concentration, but not encounter times⁵¹. Lipid modifications in the p63RhoGEF complex might also drive these proteins into raft-like domains, where their effective affinity would be even higher and result in highly localized signal amplification⁵².

GRK2: a non-canonical G α_q interaction partner and G protein scaffold

The catalytic core of GRK2 consists of an N-terminal RGS homology (RH) domain and a kinase domain⁵³. The GRK2 RH domain binds activated G α_q with high affinity^{14, 20, 54, 55}, but this ability seems unique to the GRK2 subfamily of GRKs. GRK2 also has a C-terminal PH domain that binds to G $\beta\gamma$ subunits and PIP₂ (Figure 3e, f), which are essential for GRK2 membrane recruitment⁵⁶ and activity *in vivo*⁵⁷, but not *in vitro*⁵⁸, echoing the role of N-terminal palmitoylation in p63RhoGEF. Crystal structures of GRK2 alone and in complex with G $\beta\gamma$, sequence conservation, and biochemical studies identified an extended basic surface along one face of GRK2 that likely interacts with the membrane^{53, 57, 59}, which includes the prenyl modification of G $\beta\gamma$, the lipid binding surface of the GRK2 PH domain, and the N-terminal helix of GRK2, which has been proposed to either interact directly with activated GPCRs⁶⁰ or phospholipid bilayers⁶¹ (Figure 3e, f). Recently, sum frequency generation (SFG) spectroscopy has provided experimental support for the proposed orientation of the GRK2-G $\beta\gamma$ complex on model phospholipid bilayers⁶².

The G α_q interaction with GRK2 shares several structural similarities with those formed with PLC β and p63RhoGEF. The RH domain of GRK2 binds the effector binding site of G α_q using a helix analogous to the H α 1 helix of PLC β 3 and the first helix of the p63RhoGEF C-terminal extension¹⁴. Although it lacks an ALXXPI motif, a conserved leucine (human GRK2 L118) plays a structurally equivalent role to the leucine found in this motif and is likewise essential for G α_q binding⁵⁴. There are significant differences in the GRK2 RH domain interface that confer its specificity among G α_q subfamily members²⁰. G α_q -Tyr261 forms multiple hydrogen bonds with GRK2 that cannot be formed by the leucine at this position in G α_{16} , and mutation of G α_q -Tyr261 to leucine abrogates activation-dependent binding to GRK2¹⁴. Thus, the GRK2 RH domain can be thought of as a scaffolding domain that presents the associated protein kinase and PH domains to the membrane in a manner that allows simultaneous binding of activated receptors, G $\beta\gamma$ subunits, and G α_q (Figure 3e, f). For its part, the membrane co-localizes these proteins, thereby enhancing their effective affinity, and allosterically activates GRK2 via the binding of PIP₂ to the PH domain. When the G α_q -GRK2 complex is positioned in its expected membrane binding orientation, G α_q once again adopts a similar orientation to that observed in the PLC β and p63RhoGEF complexes.

A conserved pose in Gα_q signaling

In all published structures of its protein complexes, the predicted orientation of activated Gα_q at the membrane is similar (Figure 3). Its position in the p63RhoGEF complex is the most distinct, but in this case there is a greater degree of uncertainty about the membrane orientation of the complex. Is this common pose a coincidence, or is there a functional basis? All the substrates of known Gα_q targets are integral membrane components (e.g. PIP₂, RhoA, or GPCRs). The N-terminus of Gα_q is disordered in the absence of a protein interaction partner and palmitoylated, which allows the Ras-like domain of the Gα_q core to remain tethered to the membrane following activation and provides flexibility for Gα to engage its targets in a manner that does not interfere with their ability to interact with the membrane, their substrates, or other peripheral or integral membrane protein partners. Indeed, PLCβ, p63RhoGEF, GRK2 and their complexes must all engage the membrane via multiple sites for efficient signal transduction, and having Gα_q offset from the membrane and on the periphery of the complex likely facilitates these interactions. It is also possible that the pose of Gα_q also serves to accommodate higher order scaffolding interactions.

PLCβ is unique among the Gα_q binding partners in that it has significant intrinsic GAP activity towards Gα_q. However, the interactions of p63RhoGEF and GRK2 with Gα_q do not preclude the binding of RGS proteins like RGS2, which are also membrane localized⁶³ and can readily fit in the space between the membrane surface and these complexes when docked on Gα_q⁶⁴ (Figure 2c, d). Thus, the orientation of Gα_q in these latter complexes may also permit the cell to regulate the duration of these interactions, analogous to how RGS9 modulates the duration of the Gα_t-phosphodiesterase γ (PDEγ) complex during phototransduction⁶⁵ (Figure 2e). In the case of the Gα_q-GRK2 complex, the binding of RGS proteins could also contribute to phosphorylation-independent inhibition of Gα_q signaling, wherein GRK2 not only sequesters activated Gα_q, but also presents it to RGS proteins for rapid deactivation. In the case of PLCβ, RGS proteins such as RGS2 likely regulate signaling by serving as an effector antagonist⁶⁶ because the PLCβ and RGS2 binding sites on Gα_q extensively overlap⁶⁷.

Concluding remarks

As the Gα_q complexes described above reveal, the interactions between G protein, effector, and membrane can be quite complex, as befitting pathways of central importance to cell physiology. The importance of the membrane in these interactions is evident, whether it simply supplies the enzyme substrate or regulatory lipids for the target enzyme, enhances the local concentration of the interacting groups, or helps to orchestrate an allosteric change in the Gα_q target. Yet the membrane is clearly not the only player. In the future it will be important to describe the molecular basis for how scaffolding proteins such as WDR36 organize the Gα_q signaling pathway in a manner that also promotes efficient interactions with GPCRs and/or downstream second messenger targets such as protein kinase C^{36,37}. It is possible that such higher order scaffolding complexes also help to segregate the activation of distinct pools of Gα_q effectors, as suggested by the requirement for both p63RhoGEF and PLCβ in proper locomotion and egg laying in *C. elegans*¹² (Figure 1). The complexity of these interactions, however, allows for many layers of regulation and many opportunities for selective therapeutic intervention. For example, the binding site for the Hα2' helix on the PLC catalytic core may represent an allosteric regulatory pocket unique to the PLCβ isoforms⁹, and compounds that inhibit the palmitoylation of p63RhoGEF will likely inhibit chemotaxis in cancer cells⁵¹.

The membrane orientation of other Gα subfamily complexes, and consequently the role of the membrane in regulating their activity, remains poorly understood. This gap in our

knowledge occurs because the membrane binding determinants in crystal structures of their complexes are typically either truncated and/or not resolved. For example, the role of the transmembrane domains of mammalian adenylyl cyclase (AC) remains controversial⁶⁸ despite examples of accessory domains in ACs playing a critical role in dictating the conformation of its catalytic core⁶⁹. The orientation of the G α_s -adenylyl cyclase catalytic core structure⁷⁰ relative to its integral membrane domains and the functional consequence of this orientation is not known. Such knowledge will be essential for a comprehensive understanding of how scaffolding interactions, mediated by the membrane and/or other proteins, help dictate the strength, duration, and fidelity of GPCR signaling.

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- $G\alpha_q$ and its target proteins signal in a highly membrane-dependent manner
- The membrane plays a key role in organizing and regulating high order $G\alpha_q$ assemblies
- $G\alpha_q$ adopts a similar pose with respect to the membrane when bound to structurally diverse proteins
- The orientation of $G\alpha_q$ is enforced by multivalent interactions with its targets

Figure 1. Interplay of G_{αq}-mediated signaling processes

GPCR activation catalyzes the binding of GTP to the G_{αq} subunit, leading to its activation and dissociation from the receptor and the G_{βγ} heterodimer. G_{αq}-GTP then interacts with PLCβ or p63RhoGEF, leading to production of the second messengers IP₃ and DAG or activation of RhoA, respectively. GRK2 phosphorylation of G_q-coupled receptors initiates homologous desensitization, and GRK2 binding to G_{αq}-GTP sequesters it from its downstream targets, resulting in phosphorylation-independent desensitization. Meanwhile, RGS proteins catalyze the hydrolysis of GTP on G_{αq}, leading to its deactivation, and can act on G_{αq} while it is in complex with p63RhoGEF or GRK2⁵⁰, or block its interactions with PLCβ. In addition to a helix-turn-helix motif (orange elbow), G_{αq} must interact with multiple domains of PLCβ and p63RhoGEF for full functionality. In the case of PLCβ, the N-terminal helix (blue rectangle) and the Ras-like domain of G_{αq} contact the distal CTD (orange), and the C2 and EF hands domains of the catalytic core, respectively. In the case of p63RhoGEF, the Ras-like domain and C-terminal helix of G_{αq} bridge the DH and PH domains of p63RhoGEF. G_{αq} must also simultaneously interact with the membrane via palmitoylation sites at its N-terminus. Disordered regions within the proteins, primarily those bearing the lipid modifications of G_{αq}, G_{βγ}, p63RhoGEF, and RhoA to the membrane are shown as black lines. Palmitoyl and geranylgeranyl groups are modeled as orange sticks embedded within the membrane.

Figure 2. Structural and functional features of activated $G\alpha_q$

(a) Structure of $G\alpha_q$ in its activated state. $G\alpha_q$ (shown as a gray cartoon with green β strands and red switch regions) is trapped in a GTP hydrolysis transition-like state upon addition of GDP and AlF_4^- (blue ball and sticks). Mg^{2+} is shown as a black sphere. The effector binding site is formed in part by the cleft between SwII and the $\alpha 3$ helix. In most structures of activated $G\alpha_q$, ~30 N-terminal and ~5 C-terminal residues are disordered (not shown). (b) Accessible surface of activated $G\alpha_q$, in the same orientation as in (a). Residues that primarily contribute to the effector binding site, as defined by the contacts made by the helix-turn-helix motif found in PLC β and p63RhoGEF, are shown in orange. Note that in each case interactions are also formed with regions of $G\alpha_q$ outside this cleft, which are likely critical for high affinity binding. Residues that primarily contribute to regulator of G

protein signaling (RGS) binding, as defined by the structure of the RGS2-G α_q complex ⁵², are shown in purple. Some residues, particularly in SwII, contribute to both interaction sites. The asterisk demarks the position of a hydrophobic pocket between the SwII and $\alpha 3$ helices that is exploited by the leucine in the ALXXPI motifs of PLC β and p63RhoGEF. (**c**, **d**, **e**) RGS proteins (green) and effectors (cyan) can often bind their G α targets (surface representation as in panel **b**) simultaneously, such that they allosterically regulate the interaction and function of each other. Ternary complexes formed between p63RhoGEF (**c**) or GRK2 (**d**) with the RGS2-G α_q complex have been confirmed biochemically ⁶⁴. The RGS9-G $\alpha_{i/t}$ -PDE γ complex (**e**) is the only ternary complex that has been structurally characterized ⁶⁵. To create the models shown in panels **c** and **d**, the RGS2-G α_q complex ⁶⁷ was superimposed on the p63RhoGEF ¹¹ and GRK2 ¹⁴ complexes, respectively, using the G α subunit for alignment. Only the helical bundle subdomain of the GRK2 RH domain is shown for clarity. Yellow regions correspond to helical elements that engage the effector binding site as defined in panel **b**.

Figure 3. $G\alpha_q$ complexes modeled at the cell membrane

(a) The $G\alpha_q$ -PLC β_3 complex. PLC β_3 (cyan) is oriented such that its active site is in close proximity to the lipid bilayer. The catalytic Ca^{2+} (black sphere) defines the location of the active site. Residues that constitute the hydrophobic ridge, which may anchor the active site in the membrane, are shown in green spheres, and the conserved basic region of the distal CTD, thought to form the primary membrane binding site, is shown in blue spheres. The helix-turn-helix (H α_1 /H α_2) in the proximal CTD of PLC β (yellow) docks to the effector binding site of $G\alpha_q$ (shown as in Figure 2b). The end of the proximal CTD and start of the distal CTD are denoted by blue asterisks. The palmitoyl groups at the N-terminus of $G\alpha_q$ are shown as red sticks inserted within the membrane (grey rectangle). All disordered regions, including the X-Y linker (black) proposed to be displaced upon interaction with the membrane, are shown as dashed lines. To generate this model, the distal CTD in complex with the $G\alpha_q$ N-terminal helix and the $G\alpha_q$ -catalytic core complex from PDB entry 4GNK were each independently docked at a shared surface. The $G\alpha_q$ N-terminus-distal CTD complex was then rotated in the plane of this surface to allow the N-terminal helix to join the Ras-like domain of $G\alpha_q$. (b) The $G\alpha_q$ -PLC β_3 complex as viewed from the perspective of the putative membrane surface. (c) The $G\alpha_q$ -p63RhoGEF RhoA-complex. The DH (light green) and PH (cyan) domains form the primary binding sites for RhoA (yellow accessible surface with pink switch regions) and activated $G\alpha_q$ (shown as in Figure 2b). As in PLC β , the p63RhoGEF catalytic core has an extension that forms a helix-turn-helix (yellow) which docks in the $G\alpha_q$ effector binding site. The orientation of $G\alpha_q$ -p63RhoGEF-RhoA complex at the membrane is less well determined, as the membrane binding determinants are all found in disordered elements: the C-terminus of RhoA (13 disordered residues containing a polybasic region ending in a geranylgeranyl group), the N-terminus of p63RhoGEF (three palmitoylation sites positioned ~125 residues N-terminal to the DH domain), and the N-terminus of $G\alpha_q$ (two palmitoylation sites ~25 residues N-terminal to the beginning of the Ras-like domain). (d) The $G\alpha_q$ -p63RhoGEF-RhoA complex viewed from the perspective of the membrane. Red asterisks mark the last ordered residues at the C-terminus of RhoA

and N-terminus of $G\alpha_q$, which are attached to flexible sequences that are lipid modified. (e) The $G\alpha_q$ -GRK2- $G\beta\gamma$ complex. The RH domain of GRK2 (cyan) binds to the effector binding site of $G\alpha_q$ using a helix (yellow) analogous to the first helix of helix-turn-helix motif found in PLC β and p63RhoGEF. GRK2 binds to the membrane primarily via its PH domain, which binds to both PIP₂ and heterotrimeric $G\beta\gamma$ subunits (blue and green), which are prenylated. GRK2 is modeled in an active configuration with an ordered N- terminal helix (demarcated by “N”) based on a recent structure of GRK6⁴⁶. This helix has amphipathic character and is believed to interact with either activated GPCRs in the membrane or with the phospholipid bilayer itself⁴⁵. (f) The $G\alpha_q$ -GRK2- $G\beta\gamma$ complex viewed from the perspective of the membrane surface.