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Front Cover: Electrostatic surface potential for Gα subunits, from −5 kT(red) to +5kT(blue). Potential maps were calculated with APBS 1.3 and displayed using PyMol. Members of different Gα families and subfamilies diverge significantly in electrostatic properties. This diversity can be an important factor in the regulation of Gα interactions with various partners. See the article by Baltoumas et al. in this issue.

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Interactions of the α-subunits of heterotrimeric G-proteins with GPCRs, effectors and RGS proteins: A critical review and analysis of interacting surfaces, conformational shifts, structural diversity and electrostatic potentials

Fotis A. Baltoumas, Margarita C. Theodoropoulou, Stavros J. Hamodrakas *

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1. Introduction

G-protein coupled receptors (GPCRs) are one of the largest and most diverse groups of cell membrane receptors in eukaryotic organisms. Nearly 800 human genes encode GPCRs that mediate most cellular responses to hormones and neurotransmitters, as well as sensory stimuli (Bjarnadottir et al., 2006). Furthermore, these receptors have been identified as key elements in a number of diseases, including various metabolism and nervous system disorders, some types of cancer and HIV infection. As a result, today, GPCRs are the targets for ~30% of pharmaceuticals on the market (Oldham and Hamm, 2008). Several classification systems have been proposed for this superfamily categorization, each focusing on different GPCR aspects. The most popular system (Kolakowski, 1994) classifies GPCRs into six families based on their sequence homology and functional similarity.

All GPCRs are characterized by the presence of seven transmembrane α-helical segments, an extracellular N-terminus, an intracellular C-terminus and three interhelical loop regions on each side of the membrane (Kristiansen, 2004; Rosenbaum et al., 2009). This widely accepted common GPCR topology has been confirmed by crystal structures which include Rhodopsin, β2 and β1 adrenergic receptors, δ2 adenosine, CXR4 chemokine, D3 dopamine and H1 histamine, M2 and M3 muscarinic, S1P1 sphingosin, and the recently solved K, μ, δ, and nocicepin/orphanin FQ opioid receptors (Chien et al., 2010; Granier et al., 2012; Haga et al., 2012; Hanson et al., 2012; Jaakola et al., 2008; Kruse et al., 2012; Manglik et al., 2012; Palczewski et al., 2000; Rasmussen et al., 2007; Shimamura et al., 2011; Thompson et al., 2012; Warne et al., 2008; Wu et al., 2010, 2012). Furthermore, a recent NMR study revealed the three-dimensional structure of another human chemokine receptor, CXCR1 (Park et al., 2012).

Keywords: G-proteins
G-protein-coupled receptors (GPCRs)
RGS
Effectors
Signal transduction

Abbreviations: GPCR, G-protein-coupled receptor; Go, α subunit of heterotrimeric G-proteins; Gβ, β subunit of heterotrimeric G-proteins; Gγ, γ subunit of heterotrimeric G-proteins; β2AR, β2 adrenergic receptor; RGS, regulator of G-protein signaling; GAP, GTPase accelerating protein; GRK2, G-protein coupled receptor kinase 2; PDE, phosphodiesterase γ; PLC, phospholipase C; RhoGEF, rho guanine nucleotide exchange factor; LARG, leukemia associated rho guanine nucleotide exchange protein.

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Heterotrimeric G-proteins are molecular switches that regulate intracellular signaling cascades in response to GPCR activation. They are composed by α, β, and γ subunits, and possess a binding site for GTP (active conformation) or GDP (inactive), located in the Gα subunit (McCudden et al., 2005). In mammals there are 21 Gα subunits encoded by 16 genes, 6 Gβ subunits encoded by 5 genes and 12 Gγ subunits (Downes and Gautam, 1999). G-proteins are typically grouped depending on Gα similarity into four main classes, Gα1, Gα12, Gα13 and Gα15 (Cabrera-Vera et al., 2003) (Table 1). Stimulation of GPCRs by agonists leads to the activation of G-proteins, which dissociate to GTP-Gαs and GDP-Gαi3,Gαq,Gα12 (Wall et al., 1995). This step is often catalyzed by proteins known as regulators of G-protein signaling (RGS) that bind Gαs and Gαq (RZ, R12) contain members that regulate the GTPase activity of Gαs, composed by nine antiparallel strands (Lambright et al., 1994) (Fig. 1A). The GTPase domain formed by five α-helices that bind to Gαs, Gαq, Gα12, 1ZCA and Gα13, 1ZCB, 3CX8 etc. (Cutler et al., 2021).

The Gαs subunit families and subfamilies.

<table>
<thead>
<tr>
<th>Families</th>
<th>Subfamilies</th>
<th>Subunits with available structures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gα11</td>
<td>Gα1</td>
<td>Gα1β1: 1G2P, 1GA etc.</td>
</tr>
<tr>
<td></td>
<td>Gα11</td>
<td>Gα1β1: 2V4Z, 2IH8 etc.</td>
</tr>
<tr>
<td>Gα12</td>
<td>Gα12</td>
<td>Gα12: 1TAG, 2FQJ etc.</td>
</tr>
<tr>
<td>Gα13</td>
<td>Gα13</td>
<td>Gα13: 3CT K</td>
</tr>
<tr>
<td>Gα14</td>
<td>Gα14</td>
<td>Gα14: 1AZT, 1AZS etc.</td>
</tr>
<tr>
<td>Gα15</td>
<td>Gα15</td>
<td>Gα15: 3AH, 2RGN etc.</td>
</tr>
<tr>
<td>Gα16</td>
<td>Gα16</td>
<td>Gα16: 1AZS, 1AZT etc.</td>
</tr>
<tr>
<td>Gα21</td>
<td>Gα21</td>
<td>Gα21: 1ZCA</td>
</tr>
<tr>
<td>Gα21</td>
<td>Gα21</td>
<td>Gα21: 1ZCB, 3CX8 etc.</td>
</tr>
</tbody>
</table>

In this work, our goal was to study the interactions between Gα subunits, GPCRs and effectors, and to arrive at structural implications for these interactions, using both information from crystal structures and from experimental and computational studies.

2. Methods

2.1. Data collection and interface identification

Initially, we performed an extensive literature search on the interactions of G-proteins with GPCRs, effectors and RGS proteins, gathering information concerning solved structures, mutagenesis experiments and computational studies. We then compiled a dataset of solved structures of Gα subunits, as well as several structures of various RGS proteins from the Protein Data Bank (Berman et al., 2000). All G-protein structures were examined in terms of resolution, number of Gα chains, Guanine nucleotide binding state and the presence of mutations, disordered or truncated regions and interacting partners (Supplementary Table S1). Our initial dataset was updated each time a new Gα structure was published.

In several occasions, the literature accompanying solved structures did not provide enough information regarding specific residues participating in interactions. Therefore, we conducted our own interface identification of Gα complexes. Residues participating in interactions of Gα subunits with their binding partners were identified by analyzing the structures of Gα complexes with SPPIIDER (Porollo and Meller, 2007). SPPIIDER calculates the difference in Relative Solvent Accessible (RSA) surface values between the unbound and bound protein chains of a complex for each residue, and applies a user defined cut-off for the identification of interacting residues. In our analysis we applied the default cut-off value of 4% RSA. A sum of interactions between Gα subunits and their partners is presented in Fig. 1C and Table 2. Sequences for all Gα subunits and RGS domains with solved structures were obtained from UniProt (UniProt Consortium, 2012) and aligned with Clustal X 2.1 (Larkin et al., 2007). Further editing of alignment results was performed with JalView 2.7 (Clamp et al., 2004; Waterhouse et al., 2009).

2.2. Structural comparison and electrostatics

Having located interacting sites and residues on Gα sequences and structures, the next step was to examine how these areas behave during G-protein activation, by comparing structures of inactive and active or transition state Gα subunits through structural alignments for Gα11, Gα6, Gα12, Gα13, Gαq and Gα12. We also compared Gα families and subfamilies, superimposing structures of Gα11, Gα13, Gα6, Gα12, Gα13 and Gα12 subunits. Criteria applied in the selection of Gα structures was resolution and resemblance of sequence and structure to wild type G-proteins. When available, the alignments were performed using structures of similar resolution (2.2–2.9 Å) and with the minimum number of mutations and truncated or disordered regions possible. Structural alignments and RMSD calculations were performed with Dalilite v.3 on the Dali Server (Holm and Rosenstrom, 2010), as well as with PyMol v.1.2 (DeLano, 2002). Distance measurements and modeling were prepared with PyMol. Calculated RMSD values are presented in Table 3 and Supplementary Tables S2 and S3.

Finally, we calculated the electrostatic potential of members from the four Gα families using the Poisson–Boltzmann equation. The electrostatic potential was also calculated for effectors appearing in structures of complexes with Gα subunits, as well as specific RGS proteins. Calculations for atomic radii, charges and hydrogen atoms were prepared with PDB2PQR 1.7, (Dolinsky et al., 2004, 2007; Unni et al., 2011), using the PARSE force field. Potential maps
were calculated with APBS 1.3 (Baker et al., 2001; Unni et al., 2011). Temperature was set to 298.15 K, and biomolecular and solvent dielectric constants were set to 2 and 78.54, respectively. Modeling and presentation of results were prepared with PyMol.

### 2.3. Calculation of intermolecular energies

In order to evaluate the importance of electrostatics in $G_{\alpha}$ interactions, we performed a series of energy analyses in known $G_{\alpha}$–RGS and $G_{\alpha}$–effector complexes. Structures were colored gray, while contact sites of effectors and GPCRs are colored green and blue, respectively. The structure of $G_{\alpha}$ in A and B is inactive Cyp-4–bound $G_{\alpha}$ (PDB: 1GP2). C. Sequence alignment of $G_{\alpha}$ subunits with solved crystal structures. Secondary structure is represented by red cylinders for helices and green arrows for strands. The Switch regions are identified by cyan boxes. $G_{\alpha}$ residues participating in interactions, as are identified in the crystal structures of complexes, are orange for RGS proteins, green for effectors, purple for both and blue for GPCRs. Additional interactions suggested by complexes of receptors or G-proteins with peptides, mutagenesis and computational studies are colored grey for GPCRs and black for effectors. The sequences used are rat $G_{\alpha}$i1 (UniProt: P10824), human $G_{\alpha}$i3 (UniProt: P08754), bovine $G_{\alpha}$t (UniProt: P04695) and $G_{\alpha}$s (UniProt: P04896), and mouse $G_{\alpha}$o (UniProt: P18872), $G_{\alpha}$12 (UniProt: P27600), $G_{\alpha}$13 (UniProt: P27601) and $G_{\alpha}$q (UniProt: P21279).

**Fig. 1.** A. Structural elements of $G_{\alpha}$ subunits. B. Effector and GPCR interacting regions on the surface of $G_{\alpha}$, according to crystal structures, mutagenesis and computational studies. Structures are colored gray, while contact sites of effectors and GPCRs are colored green and blue, respectively.

**Notes:**
- Experimental and/or computational methods, to drive the procedure (Dominguez et al., 2003). The HADDOCK docking protocol consists of three stages, namely, a rigid body energy minimization, a semi flexible refinement in torsion angle space and a final refinement in explicit solvent (de Vries et al., 2010).
- For the calculation of intermolecular energies the last stage was utilized. The structures of the complexes were subjected to a gentle refinement in explicit water (van Dijk and Bonvin, 2006), using the “Refinement Interface” of the web server (Kastritis et al., 2012).
- The sequences used are rat $G_{\alpha}$i1 (UniProt: P10824), human $G_{\alpha}$i3 (UniProt: P08754), bovine $G_{\alpha}$t (UniProt: P04695) and $G_{\alpha}$s (UniProt: P04896), and mouse $G_{\alpha}$o (UniProt: P18872), $G_{\alpha}$12 (UniProt: P27600), $G_{\alpha}$13 (UniProt: P27601) and $G_{\alpha}$q (UniProt: P21279).
3.1. The GPCR–G-protein complex

Until recently little was known concerning GPCR–G-protein interactions. Mutagenesis studies and trials with G-protein chimeric subunits suggested that Gx subunits use primarily their C-terminus and a4–b6 loop to interact with the cytoplasmic pocket opened by the receptor activation (Aris et al., 2001; Bae et al., 1999; Cai et al., 2009). Furthermore, a recent computational study proposed interactions between the acetylholine receptor M3R and residues in the N- and C-terminus, as well as the b2–j3 loop of inactive Gx (Hu et al., 2010).

The first breakthrough in unveiling the nature of GPCR–G-protein interactions was the solved structure of the b2AR–Gxq complex (Rasmussen et al., 2011). In the deposited structure, b2AR was crystallized in its activated form, adapting a conformation similar to that found in previous activated GPCR structures (Rasmussen et al., 2011b; Scheerer et al., 2008). Gxq appears in an intermediate, nucleotide–empty state, the most striking feature of which is the extensive movement of the a-helical domain. The complex is stabilized by the insertion of the Nb35 nanobody to the open Gxq subunit and Gb2, which binds to the extracellular a-loop of b2AR.

Interactions between the receptor and Gx include the cytoplasmic ends of the 5th and 6th transmembrane helices and the 2nd loop of b2AR, and residues at the C-terminus, a4–b6 loop and N-terminal aN-b1 loop of Gxq. Additional interactions include the C-terminus of b2AR and residues in the b2–j3 loop and a-helical region of Gxq. There are no direct contacts between b2AR and Gb2, although it is possible that the latter could interact with a second receptor in cases of GPCR oligomerization. However, the lack of coordinates for the 3rd intracellular loop of b2AR, a region that has been indicated to be pivotal in the formation of the GPCR–Gx complex could mean the presence of more, unobserved interactions between the receptor and Gx, perhaps with elements like the a3–j5 loop. Furthermore, this structure is a snapshot of the complex of b2AR with the intermediate state of Gxq, but reveals little information regarding the early interactions of the receptor with the GDP-bound subunit.

3.2. RGS proteins use conserved residues to interact with Gx subunits

A number of structures of complexes between Gx subunits and various RGS proteins or RGS fragments have been deposited on the PDB, shedding light on the nature of Gx–RGS interactions. Most subunits in these structures are from the Gx4a family, including Gx4a, Gx6a, Gx2a, and Gx6b (Kimple et al., 2002, 2009; Sammon et al., 2007; Slep et al., 2001, 2008; Soundararajan et al., 2008; Terness et al., 1997b). Furthermore, a recently solved structure of the Gx4–RGS2 complex has revealed the nature of interactions between RGS proteins and other Gx families (Nance et al., 2013). The binding of RGS proteins to Gx subunits allows the stabilization and study of the transition state for GTP hydrolysis (Kimple et al., 2009).

Table 2

<table>
<thead>
<tr>
<th>Structures</th>
<th>Gx</th>
<th>PDB ID</th>
<th>N-term.</th>
<th>Sw. I</th>
<th>Sw. II</th>
<th>Sw. III</th>
<th>a3</th>
<th>a3–j5</th>
<th>a4–j6</th>
<th>C-term.</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gx(14)–b2AR</td>
<td>Gx</td>
<td>3SN6</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>*(b2–j3, g–a4, helical domain)</td>
</tr>
<tr>
<td><strong>Effectors complexes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Gx2–Adenyl Cyclase</strong></td>
<td>Gx</td>
<td>1AZT</td>
<td>etc.</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>*(helical domain)</td>
</tr>
<tr>
<td><strong>PDE–Gx2–RS3</strong></td>
<td>Gx</td>
<td>1FQJ</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><strong>Gx3–p115RhoGEF–RhoA</strong></td>
<td>Gx</td>
<td>1HZ</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>*(helical domain)</td>
</tr>
<tr>
<td><strong>Gx4–PLCβ3</strong></td>
<td>Gx</td>
<td>2BCJ</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><strong>Gx5–p63RhoGEF–RhoA</strong></td>
<td>Gx</td>
<td>3C8X</td>
<td>etc.</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>*(helical domain)</td>
</tr>
<tr>
<td><strong>Gx6–i1,2–1753 Plaque display peptide</strong></td>
<td>Gx</td>
<td>3C83</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>*(helical domain)</td>
</tr>
</tbody>
</table>

a Chimeric subunits with Switch regions replaced with Gx3.
b The plus (+) sign indicates the sites on Gx, which interact in each respective complex.
c The minus (–) sign indicates the sites on Gx that do not interact.

d The RMSD is calculated by using ELE (de Vries et al., 2000).
Many RGS proteins can regulate the activity of more than one Gα subunits. For example, RGS4 interacts with Gαq as well as Gα16 and RGS16 can regulate both Gαi and Gαo subfamilies. On the other hand, some RGS domains display specificity towards their interacting partners. For example, RGS2 is normally Gαq exclusive, and is the only known member of the R4 subfamily to express such selectivity. However, mutations in three specific residues enable interactions with Gαq11 without affecting its GAP activity exclusive towards its original partner (Kimple et al., 2009). Other RGS domains contact Gαi/o members exclusively, and some display an even greater selectivity towards their partners. RGS6 is selective towards Gαi1, Gα12 and Gα14 cannot interact with Gαi/o subunits other than Gαi1 and Gαi2, and Gαi9 shows higher affinity towards Gαi1 rather than Gαi5 and Gαi3 (Slep et al., 2001; Soundararajan et al., 2008), even though most of the interface residues of these three subunits are identical or similar (Soundararajan et al., 2008). As far as Gαi1 only selective RGS domains are concerned, RGS12 and RGS14 specificity towards Gαi1 has been attributed to the GoLoco motif located in their C-terminus. However, other RGS domains, such as RGS6 and RGS10, also display similar specificity, without having this motif in their sequence. In fact, the structure of the RGS10–Gαi3 complex shows that this selective towards Gαi GAP uses identical or similar residues with other RGS proteins that regulate both Gαi1 and Gαq (Supplementary Fig. S1). It is worth noting that Gαq only selective RGS domains interact with residues in the helical domain of Gαq as well as the Switch regions of Gαi1 and Gαi3, and cannot regulate Gαo.

Structural data demonstrate that RGS proteins contact specific residues in all three Switch regions, thus regulating GTPase activity, and in certain cases they also contact parts of the helical domain of Gαq/o proteins, specifically residues in the αA helix. Interacting residues in Switch I and Switch II of RGS interacting Gαq/o subunits are highly conserved, with Gαq being the most diverse. Switch III displays most differences between Gαq/o subunits (Fig. 1C). Several RGS proteins also contact the helical domain. Most interactions include the αA helix. Furthermore, our analysis with SPPIDER showed additional interactions with residues in the αB–αC loop in certain cases. The recently solved Gαq–RGS2 structure also displays interactions of this kind, which are believed to contribute to the selectivity of RGS2. Different RGS domains, even though they adopt a similar fold, show distinct diversity in their sequences. However, most interacting residues are identical or similar (Supplementary Fig. S1).

3.3. The nature of Gα–effector interactions

Our current understanding of the structural basis of interactions between G-proteins and their effectors depends heavily on the crystal structures of Gα–effector complexes deposited in PDB. These structures include Gαq bound to the catalytic C1/C2 domains of adenylyl cyclase, Gαi binding the phosphodiesterase γ (PDEγ) subunit in the presence of RGS9, Gαq in complex with GRK2 and Gβγ, p63RhoGEF and phospholipase Cβ3, and Gα13 in complex with the N-terminal rgRGS domains of p115RhoGEF and PDZishopGEF (Chen et al., 2005, 2008; Hajicek et al., 2011; Lutz et al., 2007; Slep et al., 2001; Tesmer et al., 1997a, 2005; Waldo et al., 2010). An additional structure of Gα11 and an active state selective peptide demonstrated similar interfaces (Johnston et al., 2006). The structural data indicate that all effectors bind to a common recognition surface of Gαq, comprised of Switch II, the α3 helix and the α3–β5 loop (Sprang et al., 2007). Switch II and α3 interacting residues are highly conserved among different Gα subunits, with Gαq and Gα12/13 being the most diverse. However, the α3–β5 loop differs both in sequence and in structure between families, and has been suggested to be a key in the selectivity of G-proteins towards their effectors (Sprang et al., 2007).

Apart from the effector binding site of Switch II, α3 and α3–β5, certain effectors demonstrate additional interactions with other Gα surfaces (Table 2). The p63RhoGEF–Gαq complex displays interactions of the effector with the C-terminus and the α4–β6 loop, PLCδ3, and p115RhoGEF, which have been shown to act as GTase accelerating proteins (GAPS) towards Gαq and Gα13, respectively, contact residues in Switch I, III and the N-terminal part of Switch II, which form the binding surface of RGS proteins (Fig. 1). The p115RhoGEF rgRGS domain and N-terminus additionally contact certain parts of the α helical domain of Gα13, though not the same as most RGS proteins. PDZishopGEF, highly homologous to p115RhoGEF, displays similar interactions, although it lacks GAP activity towards Gα13 (Chen et al., 2008).

Several interactions were also identified by mutagenesis, as well as in silico studies. Gαq/o subunits have been suggested to use the α4–β6 loop to bind to a site of adenylyl cyclase distinct from the one of Gαi1 (Dessauer et al., 1998; Grishina and Berlot, 1997). Gαq has been shown to use residues in the N-terminus to bind PDEY, a fact not observed in the structure of the complex because Gα had a truncated N-terminus (Grant et al., 2006). Studies using chimeric proteins identified Gαq and Gαi1 binding to β-tubulin using residues from the GTase domain, and a protein–protein docking trial between Gαi and β-tubulin suggested interactions with the common effector site, as well as the N- and C-terminus and α4–β6 (Chen et al., 2003; Dave et al., 2009; Layden et al., 2008). A recent study, combining NMR, mutagenesis and protein–protein docking trials, indicates a new interaction site on the helical domain of Gαi13 for p115RhoGEF (Chen et al., 2012) formed by the DH/PH domains of the effector and the β–αC loop of Gα13. This particular loop also contacts RGS domains in some Gα–RGS complexes. Interface residues of Gα that contact various binding partners are shown in Fig. 1C, displaying information both from crystal structures and from mutagenesis or computational studies.

Our analysis of several different structures of the Gαq–adenylyl cyclase complex shows additional interactions with some residues in Switch I and the N-terminus of Gαq. Similarly, the Gαq–PLCδ3 complex displays interfaces with the N-terminal β1 strand. Additional contacts are observed between p63RhoGEF and residues in the α4–β6 loop and C-terminus of Gαq. Interactions with α4–β6 are also observed in a more recent structure of the complex between Gα13–p115RhoGEF (Hajicek et al., 2011), as well as the complexes with PDZishopGEF (Chen et al., 2008). In these structures Gα13 is mostly native type, while the chimeric subunit in the original Gα13–p115RhoGEF complex (Chen et al., 2005) has a number of effector binding residues replaced with the ones from Gα11.

Unlike Gαq/o subunits, which follow a conserved structural motif, G–protein effectors are a large and diverse group of various enzymes, ion channels, regulators and cytoskeleton components, with very different structural and functional characteristics, thus making it more difficult for researchers to establish a set of common features. Gαq activates adenylyl cyclase by interacting with elements in both of its cytoplasmic domains, C1 and C2 (Tesmer et al., 1997a), while mutagenesis experiments indicate that the adenylyl cyclase binding site for inhibitory Gαq proteins is located in its C1 domain only (Dessauer et al., 1998). Effectors such as p63RhoGEF and Phospholipase Cβ3 use helix-loop-helix domains to contact the Gαq Switch II and α3 regions with residues both in the two helices and in the loop, and a similar structural feature is used by GRK2 in its complex with Gαq (Lutz et al., 2007; Tesmer et al., 2005; Waldo et al., 2010), however that is not the case with adenylyl cyclase, which uses residues both from multiple α-helices from the C2 domain as well as a loop and a β-strand from C1 to contact Gαq. Mutagenesis experiments suggest that β-tubulin uses its nucleotide binding domain, formed by a β-strand, a helix and the loop connecting them, to contact Gαq, which also differs from...
the structural feature used by the three Gαq effectors (Chen et al., 2003; Layden et al., 2008).

The Guanine nucleotide exchange factor (GEF) activity of RhoGEFs is located in their DH/PH domains, and p63RhoGEF contacts or RGS-box domains to interact with Gαrs (Lutz et al., 2007). On the other hand, RGS containing p115RhoGEF and PDZ-RhoGEF use their rgRGS or RGS-box domains to interact with Gαq in an effector like fashion, but it is not clear how this interface leads to the activation of their GEF activity, since the DH/PH domains are missing from the structures of their complexes with Gαq (Chen et al., 2005; Chen et al., 2008; Hajicek et al., 2011). However, a recent combination of biochemical and protein–protein docking studies suggests that the DH/PH domains of p115RhoGEF interact with residues in the α-helical domain of Gαq (Chen et al., 2012).

Effectors containing RGS homologous domains, such as the RH domain of GRK2 and the rgRGS of p115RhoGEF and PDZ-RhoGEF use these domains to interact with the common effector surfaces of Gαq and Gα13, respectively, but these interactions do not affect the Gαq subunits’ GT-Pase activity in any way. On the contrary, p115 acts as a GAP towards Gα13 using residues outside its RGS-box, namely the N-terminal EDEF motif (Chen et al., 2005). Mutation of this sequence to its PDZ-RhoGEF equivalent, EEDY, abolishes GAP activity towards Gα13 (Chen et al., 2008). Similarly, phospholipase Cβ3, while it does not have any structural similarities with RGS proteins, it displays GAP activity when bound to Gαq, by contacting residues in the three Switch regions and the α-helical domain with its third and fourth EF hands, as well as residues in the linker between the TIM barrel and the C2 domain (Waldo et al., 2010).

3.4. Overlapping interacting sites on Gαq surfaces

The study of the literature, as well as our own observations reveal that certain surfaces of Gαq subunits can often participate in binding both effectors and GPCRs (Fig. 1, Table 2). These include the N- and C-terminus and the α4–β6 loop. The α3–β5 loop, a part of the common effector surface, has also been implicated in the regulation of Gα activation and binding to receptors by many biochemical and mutagenesis studies, and therefore can be considered among the receptor interacting sites of Gαq, even though there are no definitive structural data supporting this interface. It should be noted that certain residues in the C-terminus and α4–β6, and perhaps the N-terminus and α3–β5 sites, have been implicated in forming contacts both with effectors and with GPCRs. For instance, the residues of the LRIST peptide in the α4–β6 loop of Gαq have been shown to contact adenyl cyclase β3AR, and, possibly, β-tubulin. Finally, specific sites in the α-helical domain, namely the αA helix and the αB–αC loop, have been shown to contact several effectors and RGS proteins, and the βAR-GαqGβγ complex also displays an interaction between the receptor and the helical domain of Gαq.

3.5. Structural shifts during Gαq activation

A series of conformational shifts occur during Gαq activation. Binding of Gαq–Gβγ to a receptor will result in the opening of the nucleotide cleft for nucleotide exchange, by vast movement of the α-helical domain (Rasmussen et al., 2011a). Gαq activation also causes rearrangements in distinct sites of the GT-Pase domain. Comparison of active and inactive structures of Gαi1, Gαs and Gα12 subunits through structure superposition shows Switch II moving about 4–8 Å from its position in the inactive subunit, as well as a 3–6 Å movement of Switch III (Supplementary Fig. S2). In the case of inactive Gα13, most of the Switch II region is disordered, but it can be assumed to move in a similar fashion. Superposition of the GT-Pase domains of active and empty Gαq also displays shifts of the Switch regions (Supplementary Fig. S3). On the other hand, the α3 helix and its adjacent loop show little or no movement. The RMSD values of the alignments between active and inactive Gαq subunits are shown in Table 3.

Alignments of Gαq and Gα13 active forms to inactive and empty – state subunits, respectively, presents displacement of the α4–β6 loop (3–5 Å) and C-terminus (2–10 Å), however these sites show little movement in Gα13 (Supplementary Figs. S2 & S3). Most Gαq structures are truncated in their N-terminus, preventing full observation of its behavior during activation or binding to effectors. This also affects most structural alignments between Gαq subunits, resulting in relatively low RMSD values. However, alignment of inactive and RGS4–bound Gαq subunits, as well as subunits expressed in the presence of ions such as SO4−, all of which have coordinates for the N-terminus, display vast structural changes of the N-terminal α-helix (Supplementary Fig. S4). This flexibility of the N-terminus could account for its participation in various Gαq interactions, including binding to effectors or RGS proteins.

3.6. Structural diversity of Gαq interaction sites between different subunits

Structure superpositions of different Gαq subunits show that the GT-Pase domain structure is conserved among the four Gαq families; still, a few deviations are observed (Supplementary Tables S2 & S3). The α3–β5 and especially the α4–β6 loops of Gαi1 and Gαs differentiate not only in sequence but also in structural conformation, as shown by superposition of their active subunits; with the Gαq α4–β6 loop located ~5–6 Å away from the Gαi1 loop. Similar deviations are observed in the alignments of Gαq with Gα21 and Gα212 (Supplementary Fig. S5). It is possible that this feature may differentiate Gαq binding to GPCRs and certain effectors. On the other hand, members of the same Gα family show little difference in most of their sequence and structure features. Comparison of Gα212/13 structures shows differences in the α-helical region, specifically the αB–αC loop, and the α4–β6 loop of Gα13 appears to be one residue longer than the one of Gα212. However most of the α-helical and GT-Pase domains show no differences between Gα212/13 subunits, and almost all effector-contacting residues of Gα13 are present in Gα212. Similarly, Gα13 subfamilies are highly conserved in sequence and structure, with minor changes mostly located in the helical domain.

3.7. Electrostatic diversity of Gαq surfaces

Many effectors can be contacted by different G-proteins, with their Gαq interacting surfaces tolerating substantial variation. This allows the use of chimeric Gαq subunits in structural studies of G-protein–effector complexes (Sprang et al., 2007). Still, a number of binding partners display specificity in their interactions with G-proteins, even in the level of Gα family. A component of microtubules, β-tubulin, can interact with Gα21, but not Gαq, or Gα12 (Chen et al., 2003), even though these members of the Gαq family are highly conserved in both sequence and structure. One additional example is p115RhoGEF, which binds and expresses GAP activity to both members of the Gα21/12 family, but its GEF activity is activated only by Gα13 (Hart et al., 1998). Another RhoGEF, EF, LARG, can be stimulated by Gαq and Gα13, but not Gα12, unless it is tyrosine – phosphorylated (Booden et al., 2002; Suzuki et al., 2003), even though Gα12 can still contact its unphosphorylated form. Binding specificity is also observed in several Gαq–RGS interactions, as mentioned in Section 3.2. Many of these cases cannot be fully explained by differences in amino acid sequence or secondary structure.
Comparison of the electrostatic properties of the four different families, as well as their subfamilies, indicates a potential factor in determining Gα contacts (Fig. 2, Supplementary Fig. S6). The electrostatic potential of the otherwise highly conserved common effector site is significantly diverse among different Gα families and, in specific cases, among members of the same family, as shown in Fig. 2. Striking examples of this diversity are Gα12 and Gα13, which differ greatly, in this regard, both from other Gα subunits and from each other. The electrostatic surface potential of both subunits is generally more positive compared to other Gα families; however the Switch II/α3 binding pocket differentiates between Gα12 and Gα13 as well. Diversity among subfamilies is also observed in subunits from the Gαi/o family, though not as radical as in the case of Gα12/13. Gαq, compared to other Gα subunits, has a mostly non-polar effector site. Deviations are also observed in the α3–β5 loop as well as the Switch I and III regions. Differences of electrostatic potential in surfaces formed by the α-helical domain, the α3–β5 and α4–β6 loops and C-terminus (Supplementary Fig. S6) are expected, due to the sequence diversity of these sites.

3.8. The electrostatics of RGS domains

In addition to Gα subunits, the electrostatic properties of several RGS domains were calculated and compared. Furthermore, calculation and comparison of the intermolecular energies of known Gα–RGS structures reveals the participation of different types of interactions in the intermolecular energy between Gα and RGS domains (Supplementary Table S4). In almost all Gα–RGS complexes, the values of desolvation (EDES), and van der Waals (EVdw) energies are similar among different structures. Diversity is observed, in specific cases, mainly in the values of electrostatic energy (EELE). Since almost all structures contain complexes of GRS domains with Gαi/o subunits, it is not possible to compare binding energies between complexes of the same GRS with different Gα families. However, the present data is sufficient in order to make some observations.

RGS domains with the ability to regulate both Gαi/o and Gαq subunits generally show very negatively charged Gα interacting surfaces (Supplementary Fig. S7), and interact mainly with residues in the three Switch regions of the GTPase domain of Gα. These domains belong mostly to the R4 subfamily of RGS proteins. On the other hand, comparison of electrostatic properties between Gαi selective RGS proteins shows that the structures possess slightly less negative surfaces compared to GRS domains with no interacting specificity (Supplementary Fig. S7). It seems that, while almost all interacting residues are identical or conserved among GRS domains, the diversity of the surrounding regions leads to difference in surface potential.

An interesting case is RGS9, which displays a highly positive surface (Supplementary Fig. S7), despite its sequence similarity with other RGS domains (Supplementary Fig. S1). RGS9 interacts with Gαt exclusively, and also contacts the γ subunit of phosphodiesterase, as shown in the Gαt–PDEγ–RGS9 complex. Despite sequence and structure similarity between Gαi/o members, RGS9 lacks GAP ability towards other members of the family.
As mentioned in Section 3.2, wild type RGS2 normally regulates only Gαq. However, a triple mutant of the domain (C106S, N184D, E191K) gains the ability to contact and regulate Gαi3 members. Comparison of the potential between the wild type and the mutant domain reveals that the three mutations alter the potential of the C-terminal surface of RGS2, which contacts the helical domain of Gαi3. Specifically, the substitution of E191 to K results in a surface less charged, compared to that of the wild type (Supplementary Fig. S7). Comparison of energies also reveals a significant diversity in Electrostatic energy values (Supplementary Table S4), with a difference greater than 200 kcal/mol.

It is known from literature that mutant RGS2, while capable of regulating Gαq, displays lower affinity towards it (K_D = 1.25 μM) compared to that of Gαq and RGS2 (K_D = 22 ± 9 nM) (Kimple et al., 2009; Nance et al., 2013). Through our calculations we see that the Gαq–RGS2 WT complex displays a more favorable E_ELE value (−500 kcal/mol) as opposed to Gαi3–RGS2 mutant (−300 kcal/mol). The difference in E_ELE values suggests that electrostatic diversity of RGS2 surfaces, caused by the three mutations, is a factor in RGS2 affinity towards Gαx subunits.

3.9. Complementarity of effectors and Gx interacting surfaces

Since effectors show high diversity in sequence, structure and function, it is difficult to establish a set of common features. However, calculation and comparison of their electrostatic properties show that, at least in some cases, similarities can be observed. On the other hand, the diversity of effector sizes, which range from small chains such as PDEδ to large proteins such as adenylyl cyclase or PLCβ3, leads to vast differences in Buried Surface Area (BSA) of Gα–effector complexes. Therefore, attempts of comparison of intermolecular energies (Supplementary Table S5) can only be made in cases with similar BSA values.

Effector interacting sites can be grouped into two categories: sites that interact with the conserved effector binding pocket, formed by Switch II and α3, and sites that interact with other parts of Gαx, namely the N- and C-termini, the α2–β5 and α4–β6 loops and parts of the helical domain. The sites that contact the Switch II/α3 pocket generally complement its electrostatic properties. The effector pocket of both Gαq and Gαi3 subunits is positively charged. The known Gαq site and the proposed Gαi3 site on the surface of adenylyl cyclase are negatively charged, complementing their G-protein partners (Supplementary Fig. S8A).

The negative surface of phosphodiesterase γ comes in contact with the effector pocket of Gαq as well as the RGS9 domain, which are both relatively positively charged (Supplementary Fig. S8B). The Gαq–PDEγ interface is driven by hydrophobic contacts of W70 from PDEγ. However, the overall structure of PDEγ has a negatively charged surface, while the switch II/α3 pocket of Gαq has a relatively positive contour. Therefore, the charge of the surrounding regions could affect the nature of interactions, and could be a factor in the reasons why PDEγ cannot interact with other Gαij/o members. Also, the presence of RGS9 in the complex should not be neglected. It is an interesting fact that, according to HADDOCK's calculations, the E_ELE value of the RGS9–Gαq interface is more favorable in the presence of PDEγ (Supplementary Table S5).

In the case of Gαq regulated effectors, namely phospholipase Cβ, GRK2 and p63RhoGEF, the pathophysiologic sites that contact the effector pocket are mostly unchanged, and complement the generally non polar surface of the Gαq pocket (Supplementary Fig. S9A). Similarly, the RGS5 domains of p115RhoGEF and PDZRh-oGEF come in contact with the positively charged pocket of Gα13 (Supplementary Fig. S9B). This complementarity probably allows p115RhoGEF to bind to and regulate the GTase activity of Gα13 as well. Interestingly, the complexes of Gα13 with p115RhoGEF and PDZRh-oGEF display similar values of E_DW but diverse in E_ELE and, more importantly, in E_ELE (Supplementary Table S5). The displayed diversity in E_ELE values could suggest difference in the affinity of the two effectors towards Gα13. Indeed, measurements with isothermal titration calorimetry show differences in the K_D values for the p115RhoGEF–Gα13 (K_D = 3–5 μM) and PDZRh-oGEF–Gα13 (K_D ~300–500 nM) complexes (Chen et al., 2005; Chen et al., 2008).

Sites that contact other surfaces of Gαx also display interesting electrostatic properties. In the case of p63RhoGEF, all sites that contact the negatively charged C-terminus and α4–β6 loop are highly positive. The EF hands 3 and 4 of PLCβ3, which regulate the GTase activity of Gαq, are slightly positive as well, complementing the negatively charged surface of Switch III, while the TIM barrel–C2 linker, which contacts the positive surface of Switch I, is negatively charged (Supplementary Fig. S9A). HADDOCK’s calculations show that Gαq – p63RhoGEF and Gαq–PLCβ3 complexes also display differences in E_ELE values (Supplementary Table S5). This difference can be attributed to the p63RhoGEF’s positive potential in DH/PH surfaces that contact the negative surfaces of α4–β6 and the C-terminus in Gαq.

The most interesting case, however, is p115RhoGEF. While it is the γ1RGS domain that makes effector-like interactions with Gα13, the GEF activity is believed to be regulated by elements outside this region, specifically the DH and PH domains. As mentioned above, the γ1RGS domain complements the effector surfaces of both Gα12/13 proteins, however the effector can only be activated by Gα13. A recent combination of biochemical, NMR and computational studies suggests that regulation of GEF ability by G-proteins is performed in distinct sites of p115RhoGEF. One is the MGMT sequence following the RGS box, which interacts with the α3–β5 loop of Gα12. Mutations in this site prevent GEF activation of the effector from G-proteins, thus showing its importance in p115–Gαx interactions (Chen et al., 2012). Furthermore, the α3–β5 loop diverges between Gα12 and Gα13 in terms of electrostatic properties despite sequence similarity. Another proposed site is the surface of the DH domain, not present in the Gα13–p115 RGRS complexes. This surface is suggested to contact the γb–αc site of the helical domain in Gα13. This site diverges greatly between the two members of Gα12/13, however its significance seems to be less important, since mutations of this area in Gα13 do not abolish GEF activation of p115RhoGEF (Chen et al., 2012).

3.10. Electrostatic diversity as a potential factor in Gαx interactions

Diversity observed in the Switch regions of Gαij/o proteins could account for RGS coupling specificity, while the small but significant changes in the effector binding pocket between Gαq, Gαi3, Gα12 and Gα13 could explain affinity towards β-tubulin. The electrostatic properties of the α-helical domain sites that contact RGS proteins also vary between Gα12/Gα13, Gαq and Gα12. This observation suggests that these sites also play a part in the selectivity of Gα–RGS interactions. Considering the fact that the α-helical domain of Gα subunits also contacts several effectors using residues in this site, it could be assumed that its electrostatic properties can affect interactions with effectors as well.

The potential of the effector pocket is generally positive in Gα subunits with the exception of Gαq. This could be one of the reasons that, certain effectors can be regulated by Gαq proteins, exclusively. Electrostatic complementarity could also be a fact in the activation of p115RhoGEF by Gα13 rather than Gα12. The potential of α3–β5 loop, which contacts the MGMT motif, diverges between the two Gα12/13 subunits and could, therefore, be a factor in the effector’s selectivity towards them. It should be noted that Gα12, on the whole, displays a positively charged solvent accessible surface, whereas Gα13 in some aspects resembles other Gαx subunits. This diversity could also affect Gα13’s coupling to effectors, since electrostatic properties have been shown to regulate protein–pro-
tein interactions from distances up to 10 Å (van Dijk and Bonvin, 2006).

Furthermore, we have observed that in several cases the inter-
face area extends to the surface of the α-helical domain, specifi-
cally in regions that diverge electrostatically between different
subunits. Overall, we see that what is most interacting surfaces of Gx subunits are in many cases complementary, in terms of elec-
 trostatic properties, to the protein surfaces they come in contact
with. Moreover, in certain cases, diversity in electrostatic energy
values suggests that electrostatic complementarity can influence
affinity. It is therefore possible that the electrostatic potential of
protein surfaces may play a significant role in Gx interacting with
various effectors, RGS proteins and perhaps Gβγ heterodimers or
GPCRs.

4. Conclusions

Heterotrimeric G-proteins are the mediators in most GPCR –
mediated signaling pathways, acting as molecular switches for
the regulation of a large number of cell responses, and are conse-
quently a key element in the study of signal transduction, as well
as the treatment of various related diseases. We have identified
certain surfaces of Gx subunits that can, in many cases, participate
in binding both receptors and effectors. These surfaces include the
N- and C-terminal regions, the α4–β6 loop and the α3–β5 loop. The
differences displayed in the sequence and structure of these sites
can perhaps account for Gx specificity towards their binding part-
ners. Furthermore, the diversity in the electrostatic potential of Gx
surfaces, combined with observed electrostatic properties of vari-
ous effectors and RGS structures, suggests that electrostatic com-
plementarity can be an additional factor in the regulation of
effectors by G-proteins, as well as Gx interactions with RGS pro-
teins. Finally, our observations concerning the α-helical domain
reveal features that strengthen the hypothesis of an occasionally
important role in interactions for this part of Gx subunits, which has
been mostly neglected in the past. Information provided by
this study could find applications, in future, more detailed studies
of the structural basis of G-protein interactions with GPCRs and
nov-el effectors.

Author contributions

F.A.B. and M.C.T. collected the data, performed structural com-
parses and evaluated the results. F.A.B. calculated and compared
the maps of the electrostatic potential. M.C.T. performed the en-
ergy calculations, analysis and comparisons. S.J.H. supervised the
whole project and evaluated the results. All authors contributed
to the writing of the manuscript. All authors have read and approve
the final version of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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References

requirements for the stabilization of metarhodopsin II by the C terminus of the alpha
acids within the αs54 helix of Galphai1 mediate coupling with 5-
Sci. USA 98, 10037–10041.
Bjarnadottir, T.K., Gloriam, D.E., Hellstrand, S.H., Kristiansson, H., Fredriksson, R.,
et al., 2006. Comprehensive repertoire and phylogenetic analysis of G
nucleotide exchange factor promotes G alpha q-coupled activation of RhoA.
24, 765–781.
between transducin and light-activated rhodopsin by covalent crosslinking: use of
of Galphai required for the transactivation of Galphai by tubulin is implicated in
p115RhoGEF rGKS domain-Galphai3/11 chimera complex suggests convergent
of the activated states of Galphai3 by the rGKS domain of PDBZRhGK.
Structure 16, 1532–1543.
Chen, Z., Guo, L., Hadas, J., Gutfowski, S., Sprang, S.R., et al., 2012. Activation of p115-
RhoGEF requires direct association of Galphai3 and the D3I homology domain.
J. Biol. Chem. 287, 25490–25500.
Chien, E.Y., Liu, W., Zhao, Q., Katritch, V., Han, G.W., et al., 2010. Structure of the
dominant dopamine D3 receptor in complex with a D2/D3 selective antagonist.
Science 330, 1091–1095.
Bioinformatics 20, 426–427.
G-proteins interact directly with cytoskeletal components to modify microtubule-dependent cellular processes. Neurosignals 17, 100–108.
de Vries, S.J., van Dijk, M., Bonvin, A.M., 2010. The HADDOCK web server for data-
HADDOCK versus HADDOCK: new features and performance of HADDOCR2.0
on the CAPRI targets. Proteins 69, 726–733.
Carlos, CA, USA.
Galphai binding site on type V adenyl cyclase. J. Biol. Chem. 273, 25831–
25839.
automated pipeline for the setup of Poisson–Boltzmann electrostatics
Dolinsky, T.J., Czodrowski, P., Li, H., Nielsen, J.E., Jensen, J.H., et al., 2007. PD2BPQR:
expanding and upgraded automated preparation of biomolecular structures
docking approach based on biochemical or biophysical information. J. Am.
Chem. Soc. 125, 1731–1737.
544–552.
the delta-opioid receptor bound to naltrindole. Nature 485, 400–404.
Grant, J.E., Guo, L.W., Westling, M.M., Martemyanov, K.A., Arshavsky, V.Y., et al.,
2006. The N terminus of CTP gamma S-activated transducin alpha-subunit
interacts with the C terminus of the cGMP phosphodiesterase gamma-subunit.
J. Biol. Chem. 281, 6194–6202.


