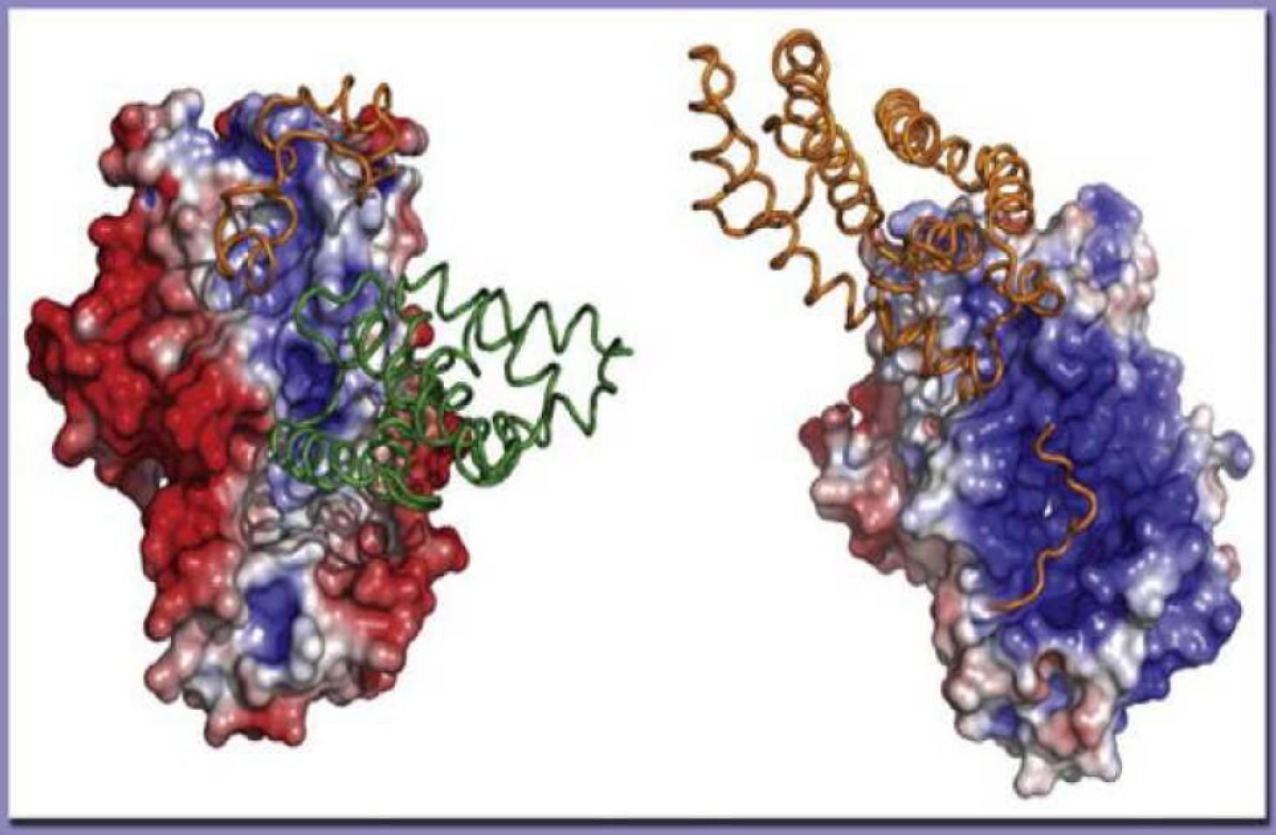
Volume 182, Issue 3, June 2013
Completing Volume 182
ISSN 1047-8477

Journal of Structural Biology



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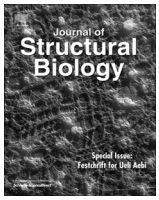

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Front Cover: Electrostatic surface potential for Gα subunits, from –5 Kt(red) to +5kT(blue). Potential maps were calculated with APBS 1.3 and displayed using PyMol. Members of different Gα families and subfamilies diverge significantly in electrostatic 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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Abstracted/indexed in Biological Abstracts, Chemical Abstracts, Current Contents/Life Sciences, EMBASE, EMBiology, Excerpta Medica, MEDLINE® Research Alert, SCISEARCH, Science Citation Index. Also covered in the citation database SciVerse Scopus®. Full text available on SciVerse ScienceDirect®

1047-8477(201306)182:3;1-8

Journal of Structural Biology 182 (2013) 209–218

Contents lists available at SciVerse ScienceDirect

Journal of Structural Biology

journal homepage: www.elsevier.com/locate/yjsbi

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

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ARTICLE INFO

Article history:
Received 4 December 2012
Received in revised form 6 March 2013
Accepted 11 March 2013
Available online 21 March 2013

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

ABSTRACT

G-protein coupled receptors (GPCRs) are one of the largest families of membrane receptors in eukaryotes. Heterotrimeric G-proteins, composed of α, β and γ subunits, are important molecular switches in the mediation of GPCR signaling. Receptor stimulation after the binding of a suitable ligand leads to G-protein heterotrimer activation and dissociation into the Gα subunit and Gβγ heterodimer. These subunits then interact with a large number of effectors, leading to several cell responses. We studied the interactions between Gα subunits and their binding partners, using information from structural, mutagenesis and Bioinformatics studies, and conducted a series of comparisons of sequence, structure, electrostatic properties and intermolecular energies among different Gα families and subfamilies. We identified a number of Gα surfaces that may, in several occasions, participate in interactions with receptors as well as effectors. The study of Gα interacting surfaces in terms of sequence, structure and electrostatic potential reveals features that may account for the Gα subunit's behavior towards its interacting partners. The electrostatic properties of the Gα subunits, which in some cases differ greatly not only between families but also between subfamilies, as well as the G-protein interacting surfaces of effectors and regulators of G-protein signaling (RGS) suggest that electrostatic complementarity may be an important factor in G-protein interactions. Energy calculations also support this notion. This information may be useful in future studies of G-protein interactions with GPCRs and effectors.

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, β_2 and β_1 adrenergic receptors, $\alpha_2\alpha$ adenosine, CXR_4 chemokine, D_3 dopamine and H_1 histamine, M_2 and M_3 muscarinic, $S1P_1$ sphingosin, and the recently solved κ, 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, $CXCR_1$ (Park et al., 2012).

Abbreviations: GPCR, G-protein-coupled receptor; Gα, α subunit of heterotrimeric G-proteins; Gβ, β subunit of heterotrimeric G-proteins; Gγ, γ subunit of heterotrimeric G-proteins; β_2AR, β2 adrenergic receptor; RGS, regulator of G-protein signaling; GAP, GTPase accelerating protein; GRK2, G-protein coupled receptor kinase 2; PDEγ, phosphodiesterase γ; PLCβ_3, phospholipase Cβ_3; RhoGEF, rho guanine nucleotide exchange factor; LARG, leukemia associated rho guanine nucleotide exchange protein.

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http://dx.doi.org/10.1016/j.jsb.2013.03.004

Table 1
The Gα subunit families and subfamilies.

Families	Subfamilies	Subunits with available structures
$G\alpha_{i/o}$	$G\alpha_i$	$G\alpha_{i1}$: 1GP2, 1GIA etc. $G\alpha_{i3}$: 2V4Z, 2IHB etc.
	$G\alpha_t$	$G\alpha_t$: 1TAG, 2FQJ etc.
	$G\alpha_o$	$G\alpha_o$: 3C7 K
	$G\alpha_z$	
$G\alpha_s$	$G\alpha_s$	$G\alpha_s$: 1AZT, 1AZS etc.
	$G\alpha_{olf}$	
$G\alpha_{q/11}$	$G\alpha_q$	$G\alpha_q$: 3AH8, 2RGN etc.
	$G\alpha_{11}$	
	$G\alpha_{15/16}$	
$G\alpha_{12/13}$	$G\alpha_{12}$	$G\alpha_{12}$: 1ZCA
	$G\alpha_{13}$	$G\alpha_{13}$: 1ZCB, 3CX8 etc.

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, $G\alpha_s$, $G\alpha_{i/o}$, $G\alpha_{q/11}$ and $G\alpha_{12/13}$ (Cabrera-Vera et al., 2003) (Table 1). Stimulation of GPCRs by agonists leads to the activation of G-proteins, which dissociate to Gα and Gβγ. These subunits then interact with several effector molecules, leading to a wide range of cellular and physiological responses. The signal is terminated when the intrinsic GTPase activity of Gα hydrolyzes GTP to GDP, thus shifting Gα to the inactive state.

This step is often catalyzed by proteins known as regulators of G-protein signaling (RGS) that bind Gα and accelerate GTP hydrolysis (Oldham and Hamm, 2008). The RGS domain, a conserved fold composed of nine α-helices, regulates this activity. RGS-containing proteins are grouped into eight subfamilies, four of which (R4, R7, RZ, R12) contain members that regulate the GTPase activity of Gα subunits (Soundararajan et al., 2008).

A number of crystal structures of different Gα subunits from all four families, as well as Gβγ heterodimers, are available and have provided the framework for understanding the basis of G-protein signaling (Table 1). Gα subunits adopt a conserved fold composed of an α-helical N-terminus, a helical domain of six α-helices and a GTPase domain formed by five α-helices surrounding a β-sheet of six antiparallel strands (Lambright et al., 1994) (Fig. 1A). The GTPase domain hydrolyzes GTP and provides most of Gα's binding surfaces for Gβγ, receptors, effectors and G-protein regulators such as RGS proteins. Three flexible sites in this domain, called Switches I, II and III may adopt different conformations during Gα activation. The Gβ subunit adopts a seven bladed propeller structure composed by seven WD40 sequence repeats and an α-helical N-terminus. Gγ is a small subunit composed by two α-helices that bind to the N-terminus and the 5th and 6th blade of Gβ. Gβ binds to Gα by contacting a hydrophobic pocket made by Switches I and II, as well as a part of the N-terminus. There is no structural evidence for direct interactions between Gα and Gγ (Wall et al., 1995).

Effectors form a diverse group of proteins that, through their interactions with Gα and Gβγ, either act as second messengers or lead to direct physiological responses (Kristiansen, 2004). Many proteins can act as effectors, including enzymes such as adenylyl cyclases and phospholipases, ion channels, adhesion proteins and tubulins, and various studies over the years have identified a large number of novel G-protein effectors (Hewavitharana and Wedegaertner, 2012; Woehler and Ponimaskin, 2009). Each Gα family and Gβγ dimer can bind to a number of different effectors, and many effectors can be regulated by more than one G-proteins.

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 information both 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 SPPIDER (Porollo and Meller, 2007). SPPIDER 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 $G\alpha_{i1}$, $G\alpha_t$, $G\alpha_s$, $G\alpha_q$ and $G\alpha_{13}$. We also compared Gα families and subfamilies, superimposing structures of $G\alpha_{i1}$, $G\alpha_{i3}$, $G\alpha_t$, $G\alpha_o$, $G\alpha_s$, $G\alpha_q$, $G\alpha_{12}$ and $G\alpha_{13}$ 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 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

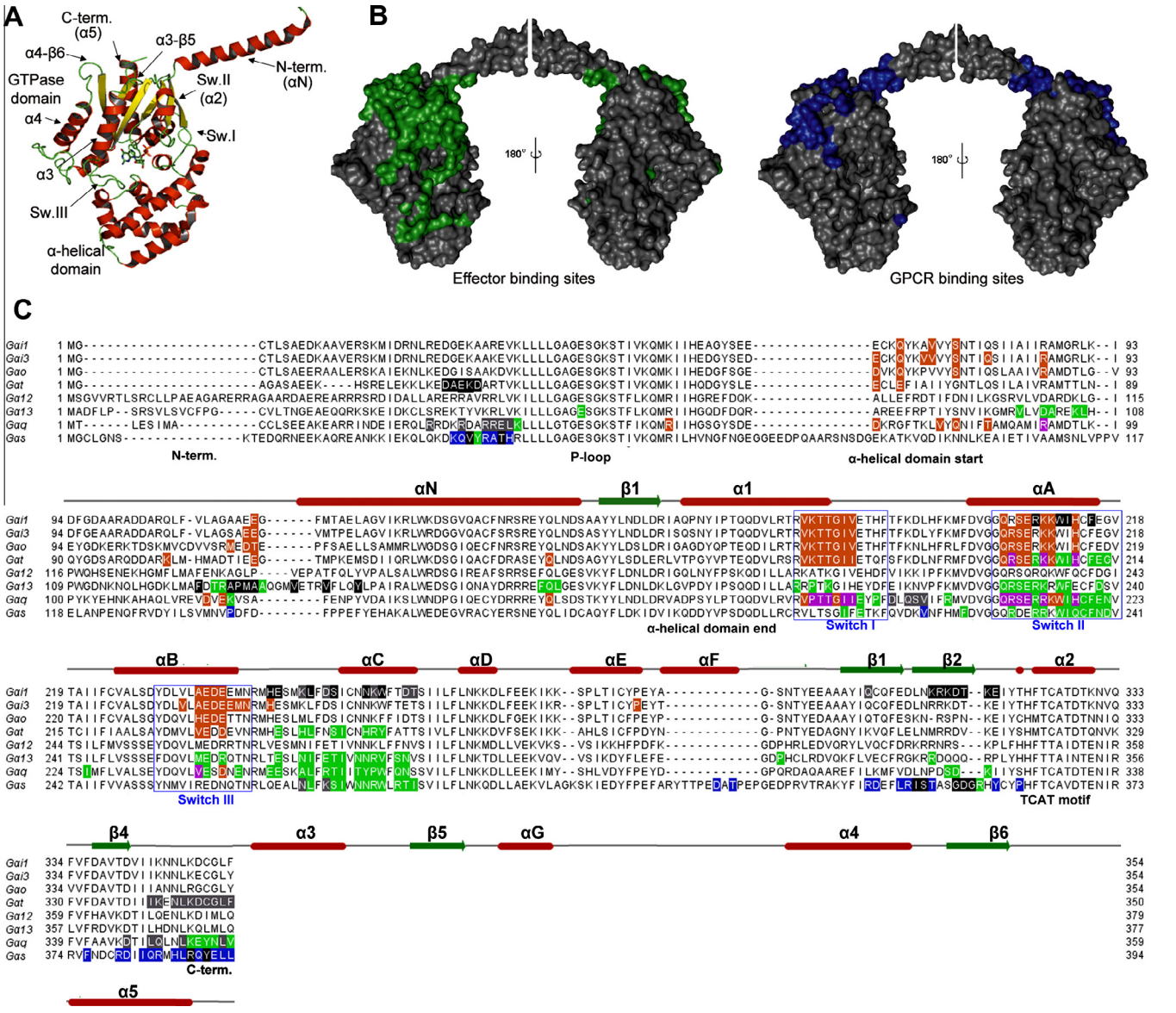

Fig.1. A. Structural elements of Gα subunits. B. Effector and GPCR interacting regions on the surface of Gα, according to crystal structures, mutagenesis and computational studies. Structures are colored gray, while contact sites of effectors and GPCRs are colored green and blue, respectively. The structure of Gα in A and B is inactive Gβγ–bound $G\alpha_{i1}$ (PDB: 1GP2). C. Sequence alignment of Gα subunits with solved crystal structures. Secondary structure is represented by red cylinders for helices and green arrows for strands. The Switch regions are identified with cyan boxes. Gα 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).

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 Gα interactions, we performed a series of energy analyses in known Gα–RGS and Gα–effector complexes. Structures of the complexes were subjected to calculation of intermolecular energies using HADDOCK v. 2.1 (de Vries et al., 2007) through the HADDOCK web server (de Vries et al., 2010). HADDOCK is a data-driven protein–protein docking approach that incorporates structural knowledge (ambiguous interaction restraints, AIRs), derived from various 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 coordinates of ions such as Mg^{2+} and Ca^{2+} were retained, since they often play a vital part in Gα functions. The results generated by HADDOCK include van der Waals (E_{VDW}), electrostatic (E_{ELE}), and desolvation energies (E_{DES}), buried surface area (BSA) and the HADDOCK score, a weighted sum of the above in combination with AIRs restraint energy, which can be used as criteria in the selection

Table 2
GPCR and effector interaction sites on the Gα subunit, identified from crystal structures.

Structures											
	Gα	PDB ID	N-term.	Sw. I	Sw. II	Sw. III	α3	α3–β5	α4–β6	C-term.	Other
GPCR Complex											
Gαβγ–β_2AR	$G\alpha_s$	3SN6	+[b]	–[c]	–	–	–	–	+	+	+(β2–β3, αG–α4, helical domain)
Effector complexes											
$G\alpha_s$-Adenylyl Cyclase	$G\alpha_s$	1AZT etc.	+	+	+	–	+	+	–	–	+(helical domain)
PDEγ–$G\alpha_t$–RGS9	$G\alpha_{t/i}$[a]	1FQJ	–	–	+	–	+	+	–	–	–
$G\alpha_{13/i}$–p115RhoGEF rgRGS	$G\alpha_{13/i}$[a]	1SHZ	–	+	+	+	+	+	–	–	+(helical domain)
$G\alpha_q$–GRK2–Gβγ	$G\alpha_q$	2BCJ	–	–	+	–	+	+	–	–	–
$G\alpha_q$–p63RhoGEF– RhoA	$G\alpha_q$	2RGN	–	–	+	–	+	+	+	+	–
$G\alpha_{13}$–PDZRhoGEF rgRGS	$G\alpha_{13}$	3CX8 etc.	–	+	+	+	+	+	+	–	+(helical domain)
$G\alpha_q$–PLCβ3	$G\alpha_q$	3OHM	+	+	+	+	+	+	+	–	+(helical domain)
$G\alpha_{13}$–p115RhoGEF rgRGS	$G\alpha_{13}$	1SHZ	–	+	+	+	+	+	+	–	+(helical domain)
$G\alpha_{i1}$–KB-1753 phage display peptide	$G\alpha_{i1}$	2G83	–	–	+	–	+	+	–	–	–

[a] Chimeric subunits with Switch regions replaced with $G\alpha_{i1}$.
[b] The plus (+) sign indicates the sites on Gα, which interact in each respective complex.
[c] The minus (–) sign indicates the sites on Gα that do not interact.

Table 3
RMSD (Å) values of aligned active and inactive Gα subunits.

Gα	PDB[a]	RMSD (Å)
$G\alpha_{i1}$	1GP2 (A)–1GIA (A)	1.5
$G\alpha_t$	1TAG (A)–1TND (A)	1.1
$G\alpha_q$[b]	3AH8 (A)–3OHM (A)	1.9
$G\alpha_{13}$	1ZCB (A)–3CX8 (A)	1.4
$G\alpha_s$ GTPase domain[c]	3SN6 (A)–1AZT (A) GTPase domains	2.1

[a] The PDB IDs and chains (in parentheses) of structures used.
[b] In the case of $G\alpha_q$, the GDP–AlF_4^- bound subunit is used as an active state.
[c] RMSD (Å) value of superimposed GTPase domains of active and empty–state $G\alpha_s$. Alignment of complete subunits gives a higher RMSD value (18.1 Å) due to the vast movement of the helical domain.

of the best docking solution. Since our goal was energy calculation of known structures of complexes, the score is omitted from presentation. CNS 2.1 is utilized for performing structural calculations (Brunger et al., 1998). Non bonded interactions are calculated with the OPLS force field with a cut-off of 8.5 Å (Jorgensen and Tirado-Rives, 1988). The electrostatic potential (E_{ELE}) is calculated by using a shift function, while a switching function (between 6.5 and 8.5 Å) is used to define the van der Waals potential (E_{VDW}) (de Vries et al., 2010; Dominguez et al., 2003). The results of the calculations are presented in Supplementary Tables S4 and S5.

3. Results and discussion

3.1. The GPCR–Gα complex

Until recently little was known concerning GPCR–G-protein interactions. Mutagenesis studies and trials with G-protein chimeras suggested that Gα subunits use primarily their C-terminus and α4–β6 loop to interact with the cytoplasmic pocket opened by receptor activation (Aris et al., 2001; Bae et al., 1999; Cai et al., 2001; Hamm et al., 1988). Crystal structures of complexes between Rhodopsin active intermediates and the C-terminus of $G\alpha_t$ supported this assumption (Choe et al., 2011; Scheerer et al., 2008). Additional studies included residues in the N-terminus (Ho and Wong, 2000; Taylor et al., 1994), as well as more sites on the surface of the GTPase domain. Mutagenesis studies with members of the $G\alpha_s$ family showed that specific mutations in the sequence of the α3–β5 loop altered the Gα subunit's binding affinity towards adrenergic receptors (Grishina and Berlot, 2000). Subsequent biochemical and computational studies also advocated the role of α3–β5 as a GPCR interacting site on Gα (Yu et al., 2008). Finally, a recent computational study proposed interactions between the acetylholine receptor M3R and residues in the N-and C-termini, as well as the β2–β3 loop of inactive $G\alpha_q$ (Hu et al., 2010).

The first breakthrough in unveiling the nature of GPCR–Gα interactions was the solved structure of the β_2AR–$G\alpha_s$Gβγ complex (Rasmussen et al., 2011a). In the deposited structure, β_2AR 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). $G\alpha_s$ appears in an intermediate, nucleotide – empty state, the most striking feature of which is the extensive movement of the α-helical domain. The complex is stabilized by the insertion of the Nb35 nanobody between the open Gα subunit and Gβγ, and T4 Lysozyme, which binds at the extracellular area of β_2AR.

Interactions between the receptor and Gα include the cytoplasmic ends of the 5th and 6th transmembrane helices and the 2nd loop of β_2AR, and residues at the C-terminus, α4–β6 loop and N-terminal αN-β1 loop of Gα. Additional interactions include the C-terminus of β_2AR and residues in the β2–β3 loop and α-helical region of $G\alpha_s$ (Table 2). There are no direct contacts between β_2AR and Gβγ, 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 β_2AR, a region that has been indicated to be pivotal in the formation of the GPCR–Gα complex could mean the presence of more, unobserved interactions between the receptor and Gα, perhaps with elements like the α3–β5 loop. Furthermore, this structure is a snapshot of the complex of β_2AR with the intermediate state of $G\alpha_s$, 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 Gα subunits

A number of structures of complexes between Gα subunits and various RGS proteins or RGS fragments have been deposited on the PDB, shedding light on the nature of Gα–RGS interactions. Most subunits in these structures are from the $G\alpha_{i/o}$ family, including $G\alpha_{i1}$, $G\alpha_{i3}$, $G\alpha_t$ and $G\alpha_o$ (Kimple et al., 2002, 2009; Sammond et al., 2007; Slep et al., 2001, 2008; Soundararajan et al., 2008; Tesmer et al., 1997b). Furthermore, a recently solved structure of the $G\alpha_q$–RGS2 complex has revealed the nature of interactions between RGS proteins and other Gα families (Nance et al., 2013). The binding of RGS proteins to Gα subunits allows the stabilization and study of the transition state for GTP hydrolysis (Kimple et al., 2009).

Many RGS proteins can regulate the activity of more than one Gα subunits. For example, RGS4 interacts with $G\alpha_q$ as well as $G\alpha_i$, and RGS16 can regulate both $G\alpha_i$ and $G\alpha_o$ subfamilies. On the other hand, some RGS domains display specificity towards their interacting partners. For example, RGS2 is normally $G\alpha_q$ exclusive, and is the only known member of the R4 subfamily to express such selectivity. However, mutations in three specific residues enable interactions with $G\alpha_{i3}$ without affecting its GAP activity towards its original partner (Kimple et al., 2009). Other RGS domains contact $G\alpha_{i/o}$ members exclusively, and some display an even greater selectivity towards their partners. RGS6 is selective towards $G\alpha_{i1}$. RGS12 and RGS14 cannot interact with $G\alpha_{i/o}$ subunits other than $G\alpha_{i1}$ and $G\alpha_{i3}$, and RGS9 shows higher affinity towards $G\alpha_t$ rather than $G\alpha_{i1}$ and $G\alpha_{i3}$ (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 $G\alpha_i$ only selective RGS domains are concerned, RGS12 and RGS14 specificity towards $G\alpha_{i1}$ 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–$G\alpha_{i3}$ complex shows that this selective towards $G\alpha_i$ GAP uses identical or similar residues with other RGS proteins that regulate both $G\alpha_{i/o}$ and $G\alpha_q$ (Supplementary Fig. S1). It is worth noting that $G\alpha_i$ only selective RGS domains interact with residues in the helical domain of Gα as well as the Switch regions of $G\alpha_{i1}$ and $G\alpha_{i3}$, and cannot regulate $G\alpha_t$.

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 $G\alpha_{i/o}$ proteins, specifically residues in the αA helix. Interacting residues in Switch I and Switch II of RGS interacting Gα subunits are highly conserved, with $G\alpha_q$ being the most diverse. Switch III displays most differences between Gα 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 $G\alpha_q$–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 $G\alpha_s$ bound to the catalytic C1/C2 domains of adenylyl cyclase, $G\alpha_t$ binding the phosphodiesterase γ (PDEγ) subunit in the presence of RGS9, $G\alpha_q$ in complex with GRK2 and Gβγ, p63RhoGEF and phospholipase $C\beta_3$, and $G\alpha_{13}$ in complex with the N-terminal rgRGS domains of p115RhoGEF and PDZRhoGEF (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 $G\alpha_{i1}$ 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 Gα, 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 $G\alpha_s$ and $G\alpha_{12/13}$ 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–$G\alpha_q$ complex displays interactions of the effector with the C-terminus and the α4–β6 loop. PLCβ3, and p115RhoGEF, which have been shown to act as GTPase accelerating proteins (GAPs) towards $G\alpha_q$ and $G\alpha_{13}$, 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 $G\alpha_{13}$, though not the same as most RGS proteins. PDZRhoGEF, highly homologous to p115RhoGEF, displays similar interactions, although it lacks GAP activity towards $G\alpha_{13}$ (Chen et al., 2008).

Several interactions were also identified by mutagenesis, as well as *in silico* studies. $G\alpha_i$ subunits have been suggested to use the α4–β6 loop to bind to a site of adenylyl cyclase distinct from the one of $G\alpha_s$ (Dessauer et al., 1998; Grishina and Berlot, 1997). $G\alpha_t$ has been shown to use residues in the N-terminus to bind PDEγ, 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 $G\alpha_s$ and $G\alpha_{i1}$ binding to β-tubulin using residues from the GTPase domain, and a protein–protein docking trial between $G\alpha_s$ 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 $G\alpha_{13}$ for p115RhoGEF (Chen et al., 2012) formed by the DH/PH domains of the effector and the αB–αC loop of $G\alpha_{13}$. 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 $G\alpha_s$–adenylyl cyclase complex shows additional interactions with some residues in Switch I and the N-terminus of Gα. Similarly, the $G\alpha_q$–PLCβ3 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 $G\alpha_q$. Interactions with α4–β6 are also observed in a more recent structure of the complex between $G\alpha_{13}$–p115RhoGEF (Hajicek et al., 2011), as well as the complexes with PDZRhoGEF (Chen et al., 2008). In these structures $G\alpha_{13}$ is mostly native type, while the chimeric subunit in the original $G\alpha_{13/i}$–p115RhoGEF complex (Chen et al., 2005) has a number of effector binding residues replaced with the ones from $G\alpha_{i1}$.

Unlike Gα 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. $G\alpha_s$ 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 $G\alpha_i$ proteins is located in its C1 domain only (Dessauer et al., 1998). Effectors such as p63RhoGEF and Phospholipase $C\beta_3$ use helix-loop-helix domains to contact the $G\alpha_q$ 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 $G\alpha_q$ (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 $G\alpha_s$. 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 $G\alpha_s$, which also differs from

the structural feature used by the three $G\alpha_q$ 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 $G\alpha_q$ with residues in these domains (Lutz et al., 2007). On the other hand, RGS containing p115RhoGEF and PDZRhoGEF use their rgRGS or RGS-box domains to interact with $G\alpha_{13}$ in an effector like fashion, but it is not clear how this interface leads to the activation of their GEF ability, since the DH/PH domains are missing from the structures of their complexes with $G\alpha_{13}$ (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 $G\alpha_{13}$ (Chen et al., 2012).

Effectors containing RGS homologous domains, such as the RH domain of GRK2 and the rgRGS of p115RhoGEF and PDZRhoGEF use these domains to interact with the common effector surfaces of $G\alpha_q$ and $G\alpha_{13}$, respectively, but these interactions do not affect the Gα subunits' GTPase activity in any way. On the contrary, p115 acts as a GAP towards $G\alpha_{13}$ using residues outside its RGS-box, namely the N-terminal EDEDF motif (Chen et al., 2005). Mutation of this sequence to its PDZRhoGEF equivalent, EEDY, abolishes GAP activity towards $G\alpha_{13}$ (Chen et al., 2008). Similarly, phospholipase $C\beta_3$, while it does not have any structural similarities with RGS proteins, it displays GAP activity when bound to $G\alpha_q$, 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 Gα surfaces

The study of the literature, as well as our own observations reveal that certain surfaces of Gα 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 Gα, 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 istance, the residues of the LRIST peptide in the α4–β6 loop of $G\alpha_s$ have been shown to contact adenylyl cyclase β_2A.R. 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 $\beta_2AR–G\alpha_sG\beta\gamma$ complex also displays an interaction between the receptor and the helical domain of $G\alpha_s$.

3.5. Structural shifts during Gα activation

A series of conformational shifts occur during Gα activation. Binding of Gα–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). Gα activation also causes rearrangements in distinct sites of the GTPase domain. Comparison of active and inactive structures of $G\alpha_{i1}$, $G\alpha_t$ and $G\alpha_q$ 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 $G\alpha_{13}$ most of the Switch II region is disordered, but it can be assumed to move in a similar fashion. Superposition of the GTPase domains of active and empty $G\alpha_s$ 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 Gα subunits are shown in Table 3.

Alignments of $G\alpha_q$ and $G\alpha_s$ 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 $G\alpha_{i/o}$ and $G\alpha_{13}$ (Supplementary Figs. S2 & S3). Most Gα 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 Gα subunits, resulting in relatively low RMSD values. However, alignment of inactive and RGS4–bound $G\alpha_{i1}$ subunits, as well as subunits expressed in the presence of ions such as SO_4^-, 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 Gα interactions, including binding to effectors or RGS proteins.

3.6. Structural diversity of Gα interaction sites between different subunits

Structure superpositions of different Gα subunits show that the GTPase domain structure is conserved among the four Gα families; still, a few deviations are observed (Supplementary Tables S2 & S3). The α3-β5 and especially the α4–β6 loops of $G\alpha_{i1}$ and $G\alpha_s$ differentiate not only in sequence but also in structural conformation, as shown by superposition of their active subunits; with the $G\alpha_s$ α4–β6 loop located ~5–6 Å away from the $G\alpha_{i1}$ loop. Similar deviations are observed in the alignments of $G\alpha_s$ with $G\alpha_q$ and $G\alpha\alpha_{12}$ (Supplementary Fig. S5). It is possible that this feature may differentiate Gα 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 $G\alpha_{12/13}$ structures shows differences in the α-helical region, specifically the αB–αC loop, and the α4–β6 loop of $G\alpha_{13}$ appears to be one residue longer than the one of $G\alpha_{12}$. However most of the α-helical and GTPase domains show no differences between $G\alpha_{12/13}$ subunits, and almost all effector-contacting residues of $G\alpha_{13}$ are present in $G\alpha_{12}$. Similarly, $G\alpha_{i/o}$ subfamilies are highly conserved in sequence and structure, with minor changes mostly located in the helical domain.

3.7. Electrostatic diversity of Gα surfaces

Many effectors can be contacted by different G-proteins, with their Gα interacting surfaces tolerating substantial variation. This allows the use of chimeric Gα 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 subfamily. A component of microtubules, β-tubulin, can interact with $G\alpha_{i1}$, but not $G\alpha_t$ or $G\alpha_o$ (Chen et al., 2003), even though these members of the $G\alpha_{i/o}$ 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 $G\alpha_{12/13}$ family, but its GEF activity is activated only by $G\alpha_{13}$ (Hart et al., 1998). Another RhoGEF, LARG, can be stimulated by $G\alpha_q$ and $G\alpha_{13}$ but not $G\alpha_{12}$, unless it is tyrosine – phosphorylated (Booden et al., 2002; Suzuki et al., 2003), even though $G\alpha_{12}$ can still contact its unphosphorylated form. Binding specificity is also observed in several Gα–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 $G\alpha_{12}$ and $G\alpha_{13}$, 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 $G\alpha_{12}$ and $G\alpha_{13}$ as well. Diversity among subfamilies is also observed in subunits from the $G\alpha_{i/o}$ family, though not as radical as in the case of $G\alpha_{12/13}$. $G\alpha_q$, 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 (E_{DES}), and van der Waals (E_{VDW}) energies are similar among different structures. Diversity is observed, in specific cases, mainly in the values of electrostatic energy (E_{ELE}). Since almost all structures contain complexes of RGS domains with $G\alpha_{i/o}$ subunits, it is not possible to compare binding energies between complexes of the same RGS with different Gα families. However, the present data is sufficient in order to make some observations.

RGS domains with the ability to regulate both $G\alpha_{i/o}$ and $G\alpha_q$ 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 $G\alpha_i$ selective RGS proteins shows that the structures possess slightly less negative surfaces compared to RGS domains with no interacting specificity (Supplementary Fig. S7). It seems that, while almost all interacting residues are identical or conserved among RGS 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 $G\alpha_t$ exclusively, and also contacts the γ subunit of phosphodiesterase, as shown in the $G\alpha_t$–PDEγ–RGS9 complex. Despite sequence and structure similarity between $G\alpha_{i/o}$ members, RGS9 lacks GAP ability towards other members of the family.

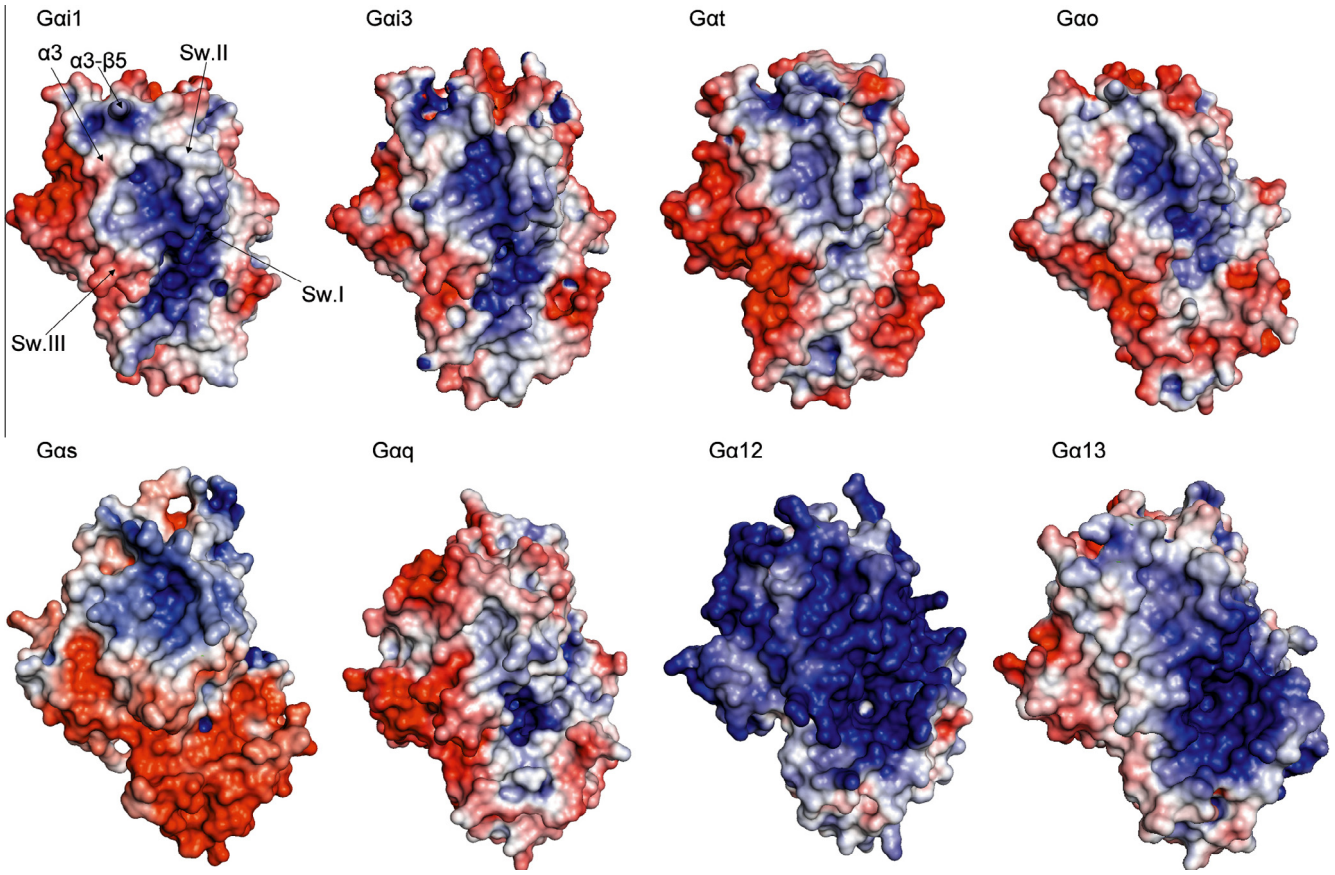

Fig.2. Electrostatic molecular surfaces of Gα subunits. All subunits are oriented in the same way as in Fig.1. Charged surfaces are colored in shades of blue for positive and red for negative charges, while uncharged surfaces are colored white. Subunit surfaces are contoured from −5 (red) to +5 (blue) kT/e^- based on the potential of the solvent accessible surface. All subunits are in their activated state, with the exception of $G\alpha_{i3}$ and $G\alpha_o$, which are in their RGS-bound state. There is a conserved, effector-binding pocket between Switch II and α3. A visualization of the potential of the opposite side of the Gα subunits is shown in Supplementary Fig. S6. Crystal structures used are $G\alpha_{i1}$ (PDB: 1GIA), $G\alpha_{i3}$ (PDB: 2V4Z), $G\alpha_t$ (PDB: 1TND), $G\alpha_o$ (PDB: 3C7K), $G\alpha_s$ (PDB: 1AZT), $G\alpha_q$ (PDB: 3AH8), $G\alpha_{12}$ (PDB: 1ZCA) and $G\alpha_{13}$ (PDB: 3CX8).

As mentioned in Section 3.2, wild type RGS2 normally regulates only $G\alpha_q$. However, a triple mutant of the domain (C106S, N184D, E191K) gains the ability to contact and regulate $G\alpha_i$ 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 $G\alpha_{i3}$. 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 $G\alpha_i$, displays lower affinity towards it (K_D = 1.25 μM) compared to that of $G\alpha_q$ and RGS2 (K_D = 22 ± 9 nM) (Kimple et al., 2009; Nance et al., 2013). Through our calculations we see that the $G\alpha_q$–RGS2 W.T. complex displays a more favorable E_{ELE} value (∼−500 kcal/mol) as opposed to $G\alpha_{i3}$–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 Gα subunits.

3.9. Complementarity of effectors and Gα 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 PDEγ to large proteins such as adenylyl cyclase or $PLC\beta_3$, 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 Gα, namely the N- and C-termini, the α3–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 $G\alpha_s$ and $G\alpha_i$ subunits is positively charged. The known $G\alpha_s$ site and the proposed $G\alpha_i$ 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 $G\alpha_t$ as well as the RGS9 domain, which are both relatively positively charged (Supplementary Fig. S8B). The $G\alpha_t$–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 $G\alpha_t$ 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 $G\alpha_{i/o}$ 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–$G\alpha_t$ interface is more favorable in the presence of PDEγ (Supplementary Table S5).

In the case of $G\alpha_q$ regulated effectors, namely phospholipase $C\beta_3$, GRK2 and p63RhoGEF, the amphipathic sites that contact the effector pocket are mostly uncharged, and complement the generally non polar surface of the $G\alpha_q$ pocket (Supplementary Fig. S9A). Similarly, the rgRGS domains of p115RhoGEF and PDZRhoGEF come in contact with the positively charged pocket of $G\alpha_{13}$ (Supplementary Fig. S9B). This complementarity probably allows p115RhoGEF to bind to and regulate the GTPase activity of $G\alpha_{12}$ as well. Interestingly, the complexes of $G\alpha_{13}$ with p115RhoGEF and PDZRhoGEF display similar values of E_{VDW} but diverse in E_{DES} 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 $G\alpha_{13}$. Indeed, measurements with isothermal titration calorimetry show differences in the K_D values for the p115RhoGEF–$G\alpha_{13/i}$ (K_D = 3–5 μM) and PDZRhoGEF–$G\alpha_{13}$ (K_D ∼300–500 nM) complexes (Chen et al., 2005; Chen et al., 2008).

Sites that contact other surfaces of Gα 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 $PLC\beta_3$, which regulate the GTPase activity of $G\alpha_q$, 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 $G\alpha_q$ - p63RhoGEF and $G\alpha_q$–$PLC\beta_3$ 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 $G\alpha_q$.

The most interesting case, however, is p115RhoGEF. While it is the rgRGS domain that makes effector-like interactions with $G\alpha_{13}$, the GEF activity is believed to be regulated by elements outside this region, specifically the DH and PH domains. As mentioned above, the rgRGS domain complements the effector surfaces of both $G\alpha_{12/13}$ proteins, however the effector can only be activated by $G\alpha_{13}$. 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 $G\alpha_{13}$. Mutations in this site prevent GEF activation of the effector from G-proteins, thus showing its importance in p115–Gα interactions (Chen et al., 2012). Furthermore, the α3–β5 loop diverges between $G\alpha_{12}$ and $G\alpha_{13}$ in terms of electrostatic properties despite sequence similarity. Another proposed site is the surface of the DH domain, not present in the $G\alpha_{13}$–p115 rgRGS complexes. This surface is suggested to contact the αB–αC site of the helical domain in $G\alpha_{13}$. This site diverges greatly between the two members of $G\alpha_{12/13}$, however its significance seems to be less important, since mutations of this area in $G\alpha_{13}$ do not abolish GEF activation of p115RhoGEF (Chen et al., 2012).

3.10. Electrostatic diversity as a potential factor in Gα interactions

Diversity observed in the Switch regions of $G\alpha_{i/o}$ proteins could account for RGS coupling specificity, while the small but significant changes in the effector binding pocket between $G\alpha_{i1}$ and $G\alpha_{i3}$, $G\alpha_t$ and $G\alpha_o$ could explain affinity towards β-tubulin. The electrostatic properties of the α-helical domain sites that contact RGS proteins also vary between $G\alpha_{i1}$/$G\alpha_{i3}$, $G\alpha_t$ and $G\alpha_q$. 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 $G\alpha_q$. This could be one of the reasons that, certain effectors can be regulated by $G\alpha_q$ proteins, exclusively. Electrostatic complementarity could also be a fact in the activation of p115RhoGEF by $G\alpha_{13}$ rather than $G\alpha_{12}$. The potential of α3–β5 loop, which contacts the MGMT motif, diverges between the two $G\alpha_{12/13}$ subunits and could, therefore, be a factor in the effector's selectivity towards them. It should be noted that $G\alpha_{12}$, on the whole, displays a positively charged solvent accessible surface, whereas $G\alpha_{13}$ in some aspects resembles other Gαα subunits. This diversity could also affect $G\alpha_{12}$'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 interface area extends to the surface of the α-helical domain, specifically in regions that diverge electrostatically between different subunits. Overall, what we see is that most interacting surfaces of Gα subunits are in many cases complementary, in terms of electrostatic 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 Gα 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 consequently a key element in the study of signal transduction, as well as the treatment of various related diseases. We have identified certain surfaces of Gα 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 Gα specificity towards their binding partners. Furthermore, the diversity in the electrostatic potential of Gα surfaces, combined with observed electrostatic properties of various effectors and RGS structures, suggests that electrostatic complementarity can be an additional factor in the regulation of effectors by G-proteins, as well as Gα interactions with RGS proteins. 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 Gα 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 novel effectors.

Author contributions

F.A.B. and M.C.T. collected the data, performed structural comparisons and evaluated the results. F.A.B. calculated and compared the maps of the electrostatic potential. M.C.T. performed the energy 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.

Acknowledgments

We thank the University of Athens for support. The present work was funded by the SYNERGASIA 2009 PROGRAMME. This Programme is co-funded by the European Regional Development Fund and National resources (Project Code 09SYN-13-999), General Secretariat for Research and Technology of the Greek Ministry of Education and Religious Affairs, Culture and Sports. We should like to thank the handling editor and the reviewers of this manuscript for their very useful and constructive criticism.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jsb.2013.03.004.

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