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ORIGINAL PAPER

GoLoco motif proteins binding to $G\alpha_{i1}$: insights from molecular simulations

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Abstract Molecular dynamics simulations, computational alanine scanning and sequence analysis were used to investigate the structural properties of the $G\alpha_{i1}$/GoLoco peptide complex. Using these methodologies, binding of the GoLoco motif peptide to the $G\alpha_{i1}$ subunit was found to restrict the relative movement of the helical and catalytic domains in the $G\alpha_{i1}$ subunit, which is in agreement with a proposed mechanism of GDP dissociation inhibition by GoLoco motif proteins. In addition, the results provide further insights into the role of the "Switch IV" region located within the helical domain of Gα, the conformation of which might be important for interactions with various Gα partners.

Keywords MD simulations · G protein · GoLoco motif · Computational alanine scanning

Abbreviations

MD	molecular dynamics
GPCR	G protein-coupled receptor
GDP	Guanine diphosphate
GTP	Guanine triphosphate
HD	Helical domain
CD	Catalytic domain
RGS	Regulators of G protein signalling
RMSD	Root mean square deviation
RMSF	Root mean square fluctuation
SD	Standard deviation

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Introduction

Heterotrimeric G proteins play a crucial role in fundamental signal transduction events [1]. In the inactive state, G proteins exist as stable heterotrimers consisting of the α-subunit (Gα) in complex with guanine diphosphate (GDP) and a dimer formed by the tightly bound β- and γ-subunits (Gβ and Gγ). In humans, more than two dozen different Gα subunits are encoded by 16 genes, 6 Gβ subunits by 5 genes, and Gγ subunits by 12 genes [2, 3]. Based on sequence homology and their differential biological activities, Gαs are divided into four classes: $G\alpha_{i/o}$, $G\alpha_{s}$, $G\alpha_{q}$ and $G\alpha_{12/13}$ [4–6].

Gα consists of a catalytic (the Ras-like or GTPase) domain (CD) connected by two flexible linkers (linkers 1 and 2) to the six-helix bundle domain (helical domain, HD), and their interface serves as a GDP binding pocket (Fig. 1). Gβ is made of an N-terminal α-helix followed by a β-propeller domain, formed by seven so-called WD repeat motifs, each comprised of approximately 43 amino acids. Its overall fold is completed by the interactions of strands from WD1 and WD7. Gγ, which is significantly smaller than the other two subunits (it normally consists of ~70 residues), is comprised of two helices connected by a loop.

Activation of the G protein-coupled receptor (GPCR) leads to conformational changes resulting in increased affinity of the GPCR for the inactive-state G protein heterotrimer. Binding of the activated GPCR to the G protein trimer causes further changes in the latter; the main structural changes in $G\alpha_{i1}$ may involve a shift of the entire HD [7] and movement of the α5 helix [8]. These rearrangements lead to GDP release, with the formation of an "empty" state, whose experimental structural model has not yet been solved and which was shown to be transient and conformationally dynamic [9]. The active state is then

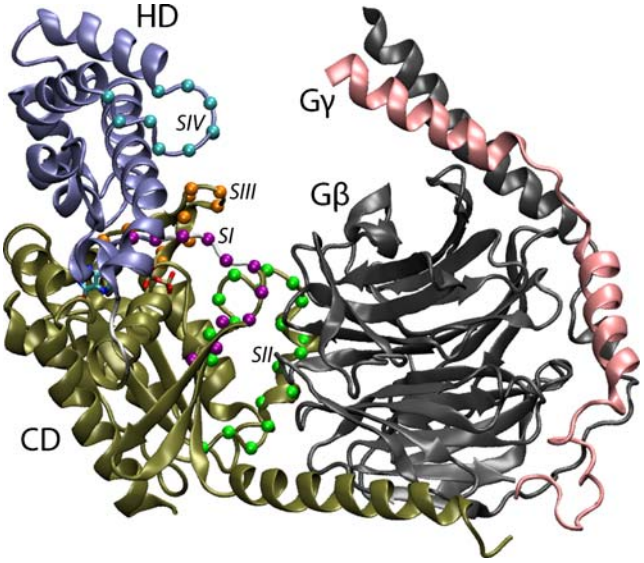

Fig. 1 X-ray structure of $G\alpha_{i1}\beta_1\gamma_2$ in the trimeric inactive state [18]. The $G\alpha_{i1}$ subunit consists of the catalytic (*CD*, *tan*) and the helical (*HD*, *ice-blue*) domains. The nucleotide-binding pocket is located at the interface of the domains: the GDP molecule is bound between the HD and CD and shown as *licorice*. Switches I (*SI*, *purple*), II (*SII*, *green*), III (*SIII*, *orange*) and IV (*SIV*, the α_B–α_C loop, *cyan*) are represented as *spheres* located at the positions of the Cα atoms. The $G\beta_1$ subunit is coloured *silver* and $G\gamma_2$ is *pink*. The figure was prepared using VMD [57]

achieved by binding to GTP·Mg^{2+}, upon which, depending on the type of Gα subunit, it either dissociates from G$\beta\gamma$ or a subunit rearrangement takes place [7, 10–15].

Comparison of the structures of the inactive and active states of the Gα subunits for which experimental structural models are available ($G\alpha_{i1}$ and $G\alpha_t$ [16–19]) show that activation is associated with conformational changes and a decrease in the flexibility of the so called Switches I–III of Gα (*SI–SIII*, Fig. 1). An important role for another region—the α_B–α_C loop [also often referred to as Switch IV (*SIV*); Fig. 1]—has been suggested [13, 14, 20]. SIV also changes its conformation upon cycling between different states in some Gα subunits [16, 21]. For example, in $G\alpha_{i1}$, SIV is less ordered in the inactive state—the α_B helix unfolds by approximately one turn and the B-factors in this region are significantly higher [17, 18]. In contrast, in $G\alpha_t$ this region does not change its conformation and has similar B-factors in both states [16, 21]. Other changes in the Gα subunit in the solid-state are very small; in particular, the angle between the helical and catalytic domains in different states differs by less than 5° in crystal structures of Gαs [19].

GTP-bound Gα then activates or inhibits downstream effectors [22]. These effectors or the so-called regulators of G proteins signalling (RGS) proteins [23, 24], which may also bind to Gα·GTP, catalyse GTP to GDP hydrolysis [25–29]. The GDP-bound Gα subunit has significantly greater affinity for the G$\beta\gamma$ subunits than Gα in the active state, and it readily re-associates with G$\beta\gamma$, restoring the initial trimeric inactive state [30].

The widely accepted model of GPCR signalling assumes that the lifetime of Gα in its GTP-bound state determines the duration of signalling from both Gα·GTP and the free G$\beta\gamma$ dimer. However, this assumption has been challenged with the discovery of an additional class of Gα-regulatory proteins, which contains the characteristic "GoLoco"[1] motif [31, 32]. The GoLoco motif, which consists of 19 residues ending in a highly conserved D[E]QR triad [33, 34], it is found in several diverse proteins, e.g. mammalian RGS12 and RGS14, Purkinje cell protein-2, Rap1GAP, GPSM2/LGN and others [31, 35]. GoLoco motif proteins bind specifically to a certain-type of Gα subunit in their monomeric inactive (GDP-bound) state, preventing GDP dissociation, and thus serving as GDP dissociation inhibitors—GDI [31, 34, 36–39]. Some GoLoco motif proteins (e.g. AGS3, RGS12, RGS14 and Pins) exhibit activity solely on $G\alpha_i$ subunits, whereas some others (e.g. Pcp2, Rap1GAP, LGN) also bind to and act on $G\alpha_o$ subunits, but do not interact with Gαs from other families ($G\alpha_s$, $G\alpha_q$, $G\alpha_{12/13}$) [39–42]. The GoLoco motif containing RGS12 and RGS14 proteins show sub-selectivity within the $G\alpha_{i/o}$-subunit family, interacting with $G\alpha_{i1}$ and $G\alpha_{i3}$, but not with $G\alpha_{i2}$ and $G\alpha_o$ [38, 39, 43]. The subunit selectivity of Gα towards GoLoco motifs has been shown to correlate strongly with the HD[2]; replacement of the HD of $G\alpha_o$ with that of $G\alpha_{i1}$ results in potent GDI activity towards this chimeric Gα subunit. The reverse chimeric protein, with the HD of $G\alpha_{i1}$ substituted by that of $G\alpha_o$, exhibited decreased GDI activity [32]. A more recent study using chimeric $G\alpha_{i1}$ and $G\alpha_{i2}$ subunits identified the α_A–α_B and α_B–α_C (SIV) loops within the HD as the key regions involved in GoLoco motif selectivity [43]. Skiba et al. [44] also found that SIV determines Gα selectivity for RGS proteins. Finally, a residue within the HD of $G\alpha_{i1}$, N149, was identified experimentally as being crucial for the binding of $G\alpha_{i1}$ to GoLoco motif proteins [45].

Two atomic-resolution structures of $G\alpha_{i1}$·GDP bound to the GoLoco motif peptide from RGS14 protein [32, 46] indicate that the peptide makes extensive contacts with both the helical and catalytic domains of $G\alpha_{i1}$, forming a sort of "bridge" between these domains (Fig. 2)[3]. Comparison of the structures of $G\alpha_{i1}$ in the trimeric inactive state [18] and in the $G\alpha_{i1}$/GoLoco complex [46] clearly shows conformational differences in several regions of $G\alpha_{i1}$, restricted mostly to Switches I–IV (Fig. S1), which suggests that structural changes within these important regions of Gα are needed to discriminate between GoLoco motif proteins and

[1] The "GoLoco" term arises from "$G\alpha_{i/o}$-Loco" interaction.
[2] HD is the most variable region among Gαs.
[3] For a comprehensive analysis of $G\alpha_{i1}$/GoLoco motif interactions, the reader is referred to references [31, 45].

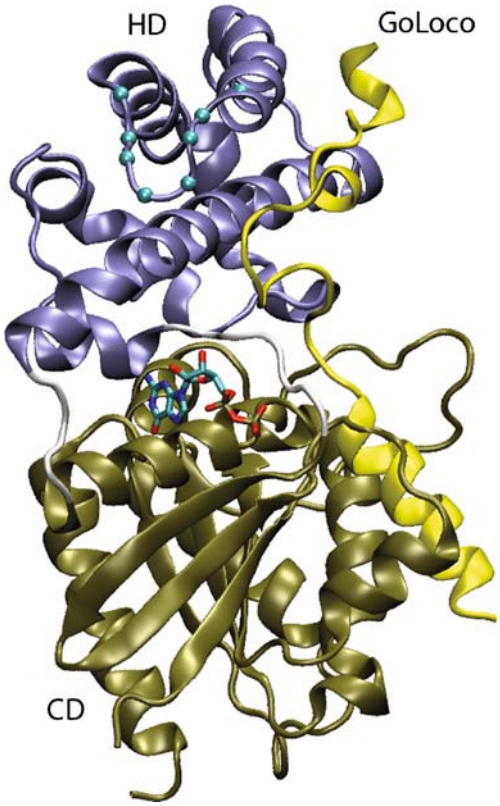

Fig. 2 X-ray structure of the $G\alpha_{i1}$/GoLoco motif peptide in the RGS14 protein complex [46]. CD and HD domains are shown in *tan* and *ice-blue*, respectively. The GoLoco (GoLoco) peptide of RGS14 protein is shown in *yellow*

Gβγ. These structures also show that Gα·GDP/GoLoco interactions would exclude Gβγ binding, as binding areas on Gα for GoLoco proteins and Gβγ overlap [34, 40]. Thus, formation of a Gα·GDP/GoLoco complex may allow for continued Gβγ-effector signalling in the absence of receptor-catalysed Gα·GTP formation. In addition, there is evidence that the $G\alpha_i$·GDP/GoLoco complex, rather than $G\alpha_i$·GTP, may represent the active specie in some cases [47–50], which is consistent with a model of receptor-independent activation of G protein signalling by GoLoco proteins [51]. Also, such a complex might be required for the nucleotide exchange (GEF) activity of RIC8 [47, 49, 52].

A mechanism for GoLoco-mediated GDI activity has been proposed [31, 32]: GPCR activation would change the orientation of the HD with respect to the CD, thus loosening Gα's grip on the nucleotide and allowing it to dissociate [7]. I would like to remark that, in agreement with previous studies [53, 54], a large-scale motion of the two Gα domains in the inactive trimeric state of $G\alpha_{i1}\beta_1\gamma_2$ has been observed recently in molecular dynamics (MD) simulations, while no significant motion in the simulation of the active state of $G\alpha_{i1}$ has been found [20]. This suggests that the relative movement of domains might indeed be required for GDP release, while GTP-bound Gα is more conformationally stable [20]. Thus, to inhibit GDP release, the GoLoco peptide may act as a Gα clamp to restrict the movement of the two Gα domains relative to each other in the monomeric GDP-bound state [31]. In addition, the R516 residue within the conserved "D[E]QR" triad of the GoLoco motif makes direct contact with the α- and β-phosphate groups of GDP, thus stabilising the bound nucleotide, which also contributes to GDI activity [45].

In this work, computational alanine scanning, sequence analysis and MD simulations are applied to address several issues related to the binding of GoLoco motif proteins to Gα subunits. A possible rationale for the differential behaviour (subunit dissociation/rearrangement) of various Gαs during activation/deactivation processes is discussed.

Methods

Model structures

The X-ray structure of human $G\alpha_{i1}$ bound to the GoLoco motif-containing peptide from the Gα regulator, RGS14 protein, at 2.2 Å resolution (*PDB ID*: 2OM2 [46]) was used (Fig. 2). Residues from the N- and C-termini (1–29 and 350–354) of $G\alpha_{i1}$ are not resolved in the crystal structure and were not included in the simulations. The latter is not expected to affect significantly the electrostatics and structural properties of the complex in MD simulations as both termini are relatively far from both the $G\alpha_{i1}$/RGS14 and HD/CD interfaces. In addition, the minimal effect of the truncation of terminal residues on G protein complex stability has been demonstrated recently in electrostatic calculations [20]. For the GoLoco motif peptide, only the 36 residues present in the X-ray structure (496–531) were used. For the MD simulations of isolated $G\alpha_{i1}$ the same X-ray structure [46] was used but the GoLoco motif peptide was removed manually.

Force field and setup for MD simulations

The all-atom Charmm27 force field [55, 56] was used for proteins, the GDP molecule and ions. To neutralise the overall charge of the systems and to keep the salt concentration equal to ~0.1 M, 25 sodium and 17 chloride ions were added for the $G\alpha_{i1}$·GDP/GoLoco peptide system using VMD plugin [57], and 25 sodium and 20 chloride ions for the $G\alpha_{i1}$·GDP system. The systems were solvated with ~23,000 TIP3P water molecules [58]. The water box dimensions after the equilibration stages were ~86×100×87 Å and ~86×98×89 for the GoLoco-bound and the empty states of $G\alpha_{i1}$, respectively.

Residues H57, H188, H322 at $G\alpha_{i1}$ and H513 at RGS14 were protonated at their δ_{ε} atoms, while H195, H213 and H244 at $G\alpha_{i1}$ were protonated at their δ_{δ} atoms based on the p*K*a calculations performed on the X-ray model with the program MCCE2.0 [59] and on visual inspection of the crystal structure. Based on the same calculations, residues E58 and E245 on $G\alpha_{i1}$ were assumed to be neutral.

All the MD calculations were performed using the program NAMD [60]. Periodic boundary conditions were employed. A real-space cutoff of 10 Å was used for both van der Waals and long-range electrostatics, and the distance at which the switching function begins to take effect was 8 Å. The time step was 2 fs. The SHAKE algorithm [61] was used to fix all bond lengths. Constant temperature (300 K) was set with a Langevin thermostat [62], with a coupling coefficient of 0.2 ps^{-1}. A Nosé-Hoover Langevin barostat [63] was used to maintain constant pressure with an oscillation period of 200 fs and the damping time scale set to 50 fs.

Two steepest descent energy minimisation steps were performed: (1) solvent, ions and all hydrogen atoms of the protein and GDP molecules; (2) the whole system). This was followed by (3) 1 ns of restrained MD in which all non-hydrogen atoms of the protein and ligand molecules were constrained to their initial position using springs with force constants 1 kcal mol^{-1} $Å^{-2}$, (4) 1 ns of restrained MD in which all Cα-atoms of protein molecules as well as all non-hydrogen atoms of GDP were constrained using the same force constant as in (3). The systems were then released to 35 ns of unrestrained MD in which coordinates were saved every 1 ps.

Analysis of MD trajectories

MD trajectories were analysed with Gromacs [64, 65], VMD [57] and small scripts. The average values and standard deviations (SD) for the root mean square deviation (RMSD; Table 2), as well as the root mean square fluctuation (RMSF) values, were calculated by excluding the first 10 ns of MD trajectories. RMSFs were calculated separately for each domain of $G\alpha_{i1}$, except for calculations for the SI region (located partially on linker 1) for which the entire $G\alpha_{i1}$ was used. The large scale (essential) modes were obtained by diagonalising the Cα atom's covariance matrix [66, 67] and analysed with the program DynDom [68, 69]. As the RMSD of $G\alpha_{i1}$ increased during the first ~10 ns in the simulation where the GoLoco peptide was removed (see Model structures), this time-interval of MD trajectories for the essential dynamics analysis was excluded in both cases.

Computational alanine scanning

Mutations destabilising $G\alpha_{i1}$/RGS14 protein interactions were identified by computational alanine scanning using the Robetta server [70, 71]. This procedure allows for a quick (albeit very approximate) estimate of the changes in non-covalent interaction free energies between two proteins upon mutation of residues at the interface to alanines (see [71] for details of the computational alanine scanning procedure and the free energy function used for calculating the effects of the alanine mutation on the binding free energy of a protein–protein complex). Hot spots are identified as those residues at the interface whose mutation to alanine causes a free energy loss greater than 1 kcal mol^{-1}. Calculations were performed on the X-ray structure [46] along with ten snapshots extracted from the MD trajectory at equal (3.5 ns) time-intervals. Only residues for which $\Delta\Delta G_{bind}$ values were larger than 1 kcal mol^{-1} are reported.

Sequence alignment

The sequences of representative human Gα subunits were aligned using the program T-Coffee [72]. The multiple sequence alignment was visualised with Jalview2.3 [73].[4]

Results and discussion

Computational alanine scanning and sequence analysis

Computational alanine scanning of the $G\alpha_{i1}$·GDP/GoLoco peptide complex was performed to identify the residues that are most important for stabilising $G\alpha_{i1}$/GoLoco interactions (Table 1). Because of the aforementioned importance of the Gα HD for the specificity for GoLoco motif proteins [32], conservation/substitution of hot-spot residues within the HD is likely to be one of the key factors determining selectivity. Alignment of sequences of Gα subunits (Fig. S2) revealed that all the hot-spot residues within the CD are conserved or conservatively mutated, while the degree of conservation of these residues within the HD is much lower (Table 1; Fig. S2). In the HD, only residue N76 is present in all Gα subunits. Other residues are usually conserved only within the $G\alpha_{i/o}$ family, except for some positions in $G\alpha_{z}$. Residue F108, located near SIV, and which is present in $G\alpha_{i1/2/3}$ subunits but not in $G\alpha_{o}$, might be of particular interest as it may help to rationalise differences in specificities of $G\alpha_{i}$ and $G\alpha_{o}$ subunits to some GoLoco motif proteins [32, 40]. However, direct experimental validation is definitely needed before drawing any final conclusion from this observation. Residues within the HD that are not conserved among Gαs and show very

[4] A multiple sequence alignment of the region within the HD contatining most of the hot-spot residues is available in the electronic supplementary material (Fig. S2).

Table 1 Results of computational alanine scanning obtained using the Robetta server [70, 71] on the X-ray structure of the $G\alpha_{i1}$/RGS14 complex (X-ray [46]) as well as on ten snapshots extracted from molecular dynamics (MD) simulations every 3.5 ns. Conservation of hot-spot residues among different Gαs is also indicated. Residues of $G\alpha_{i1}$ located within the helical domain (HD) are in italics. Only residues with ΔΔG values above 1 kcal mol^{-1} are shown

Residue on $G\alpha_{i1}$	$\Delta\Delta G_{bind}$ (kcal/mol), X-ray	$\Delta\Delta G_{bind}$ (kcal/mol), MD	Conservation among Gαs	Residue on RGS14	$\Delta\Delta G_{bind}$ (kcal/mol) X-ray	$\Delta\Delta G_{bind}$ (kcal/mol) MD
E43	4.2	3.1±2.0	N in $G\alpha_z$	I497	0.7	1.1±0.2
N76	4.1	2.6±0.6	Conserved	L500	1.6	1.7±0.3
Q79	5.5	5.2±0.7	Not conserved	V501	1.4	1.0±0.2
R86	1.5	1.1±0.2	Not conserved	L503	1.8	1.8±0.4
R105	1.9	0.8±0.4	Not conserved	L504	2.2	2.3±0.1
F108	2.8	2.5±0.2	Not conserved	N505	3.0	2.8±0.9
N149	4.4	3.8±0.8	Not conserved	V507	1.2	1.7±0.1
R178	3.4	3.0±1.1	Conserved	Q508	1.9	3.0±0.5
R208	1.4	0.5±0.4	Conserved	S510	0.0	2.6±2.4
W211	3.0	3.6±0.5	Conserved	Q515	5.6	4.2±0.9
R242	0.9	1.5±1.2	I in $G\alpha_{12/13}$	R516	3.2	3.1±1.3
L249	2.1	2.0±0.2		L518	1.2	1.6±0.4
				L519	2.0	2.4±0.4
				L524	1.4	1.7±0.2
				L526	1.3	1.3±0.5
				F529	2.5	2.6±1.1
				L530	2.1	2.1±0.2

large changes in $\Delta\Delta G_{bind}$ (i.e. more than 4 kcal mol^{-1})—Q79 and N149—are also of special interest as they might be the main determinants of selectivity of GoLoco motifs for specific Gα subunits. Consistently, as mentioned in the Introduction, mutation of N149 to isoleucine did indeed reveal the crucial role played by this residue in selectivity of GoLoco motif proteins towards $G\alpha_i$ [45]. Thus, it would be interesting to investigate how mutation of Q79 would affect binding of GoLoco peptides.

R86 in $G\alpha_{i1}$ is another interesting residue: in recent MD simulations of the inactive state of the $G\alpha_{i1}\beta_1\gamma_2$ trimer, residues E115 and E116 within SIV were attracted by R86 (causing motion of SIV along the α_A helix), while no significant changes were observed in the empty state trimer, despite the same initial conformation of the simulated system [20]. In addition, a very different conformation of this region in the inactive vs active states in $G\alpha_{i1}$ also accounted for the lower mobility of SIV in the latter [20]. It is also interesting to note that residues R86 and E116 in $G\alpha_{i1}$ have been identified recently (using Rosetta [74]) as residues whose mutation to F and L residues, respectively, may enhance binding of the GoLoco motif-containing RGS14 protein [46]. The E116L mutation has also been tested experimentally, indeed yielding an increase in the affinity of $G\alpha_{i1}$ towards RGS14 [46]. The latter result is not surprising because (1) E116 does not seem to form specific interactions with residues of RGS14[5], and (2) the arginine residues at the position of R86 are also present in some Gαs that do not bind GoLoco motif proteins, e.g. $G\alpha_{q/11/14}$. Thus, differences in sequence, conformation and mobility of SIV in various Gαs might be responsible for differences in the binding to GoLoco motif-containing proteins.

It has already been noted [75] that the N-termini of several Gγ subunits share some sequence similarity with the GoLoco motif; specifically, most of them possess "D[E]Q" residues. Interestingly, the glutamine residue (Q515) in the conserved "D[E]QR" GoLoco triad is the most important one for binding to $G\alpha_{i1}$ ($\Delta\Delta G_{bind}$=5.6 kcal mol^{-1}; see Table 1). FRET and BRET experiments [7, 13, 14] indicated that the N-terminus of Gγ approaches, and may bind to, the HD of some Gα subunits upon activation. Residues within the HD were also shown to be responsible for different structural changes (subunit rearrangements/dissociations) occurring during the activation of various Gα proteins [13]. All these factors suggest that residues in the HD, and in particular within SIV, might also be responsible for binding of the N-termini of Gγ subunits, explaining the differential behaviour of various Gαs during the activation process (subunit dissociations/rearrangements) [13–15]. In

[5] In the X-ray structure [46], the distance between atoms OD1@E116 on $G\alpha_{i1}$ and N@L519 on RGS14 is ~3.6 Å.

this context, it would be very interesting to check experimentally how mutations of R86 and E116, e.g. to alanines, would affect the binding of Gα to GoLoco motif proteins, as well as the conformational changes in the heterotrimeric G protein complex upon activation by GPCR.

MD simulations of $G\alpha_{i1}$·GDP/GoLoco motif complex

MD simulations of the $G\alpha_{i1}$·GDP/GoLoco motif peptide in solution were performed to investigate the structural properties of this quaternary complex. The structure of the complex appears to be equilibrated after ~5 ns (Fig. S3). All average RMSD values and SDs are summarised in Table 2. The RMSD of the $G\alpha_{i1}$·GDP/GoLoco peptide complex is similar to that of the RMSD of $G\alpha_{i1}$ alone, indicating the stability of the quaternary structure of the complex (Fig. S3). Virtually all important interactions between $G\alpha_{i1}$ and the GoLoco peptide are retained, as shown by the computational alanine scanning performed on ten snapshot-structures extracted at equal (3.5 ns) time-intervals from the MD trajectory (Table 1). The GoLoco peptide, however, was rather mobile, showing the highest SD among all the structural segments analysed, which is not surprising as its regular secondary structure content is significantly lower than that of $G\alpha_{i1}$. From Table 2 it is also clear that individual domains of $G\alpha_{i1}$ are more stable than the whole subunit, leading to the conclusion that there might be a relative domain motion. The GDP molecule was very stable, maintaining most of the interactions with the protein seen in the X-ray structure (Table S1).

To check if there is indeed any significant motion between the domains of $G\alpha_{i1}$, an essential dynamics analysis [66, 67] was performed on the MD trajectory of $G\alpha_{i1}$, and two snapshots of $G\alpha_{i1}$ were extracted that are separated maximally in the direction of the first eigenvector. The program DynDom [68, 69] was used to analyse domain motion in $G\alpha_{i1}$. Consistent with previous computational studies [20, 54], large-scale motion of $G\alpha_{i1}$ involves the relative movement of HD and CD: these domains bend around residues 62–64 and 175–177, located very close to linkers 1 and 2, and the bending angle is ~13°. As already discussed [7, 54], such a motion may play a role in opening the route for GDP release since the HD might be displaced after activation by GPCR.

MD simulations of $G\alpha_{i1}$·GDP

To test the hypothesis that GoLoco motif proteins may restrict the relative motion of two Gα domains, inhibiting GDP release [31], we performed MD simulations of the $G\alpha_{i1}$·GDP complex in the absence of the GoLoco peptide (see Methods). A longer simulation time (~10 ns) was needed to get the complex equilibrated, and in this case the RMSD values for all structural segments were higher (Table 2; Fig. S3).

Functionally important regions SII–IV were more mobile here and underwent significant structural changes (Figs. 3, S4). In particular, within SII, the largest difference in RMSF values between two simulations was found for residue Q204. This residue has been shown to be very important both for the intrinsic GTPase activity of Gα [76, 77] and for binding to Gβγ [20]. Another large difference is found for SIV (Figs. 3, S4): the N-terminal part of the α_A helix became partially unwound by ~1 turn (residues 111–114) and moved along the α_A helix, leading to a conformation similar to that found in previous MD simulations of the $G\alpha_{i1}\beta_1\gamma_2$ trimer [20]. As in the previous simulation, the GDP molecule was also stable here (Table S1). However, some local changes were observed in the binding pocket due to the removal of the GoLoco peptide. In particular, the side chain of the catalytically important R178 residue lost its interaction with the side chain of residue E43 near the beginning of the simulation and moved towards a region previously occupied by R516 of the GoLoco protein (Fig. S5). This results in a stable interaction of R178 with the phosphate groups of the GDP molecule (Table S1; Fig. S5).

The aim here was also to identify the domain motion of Gα in order to compare the results of the two simulations: a similar large-scale motion was identified for the domains of $G\alpha_{i1}$, with bending residues being 59–62 and 178–183, which are located on linkers 1 and 2. The bending angle is

Table 2 The average root mean square deviation (RMSD) and standard deviation (SD) values calculated on the MD trajectories of the $G\alpha_{i1}$/GoLoco complex and those of isolated $G\alpha_{i1}$. The first 10 ns of the trajectories were excluded from the analysis. *CD* Catalytic domain

	$G\alpha_{i1}$/GoLoco complex	Isolated $G\alpha_{i1}$
$G\alpha_{i1}$/GoLoco complex	2.2±0.1	–
$G\alpha_{i1}$	2.2±0.1	3.2±0.2
GoLoco peptide	2.2±0.3	–
CD	1.7±0.1	2.2±0.1
HD	1.8±0.1	2.3±0.3
HD excluding SIV	1.1±0.1	1.6±0.2
HD excluding SIV upon superposition of CD	2.8±0.4	4.8±0.6

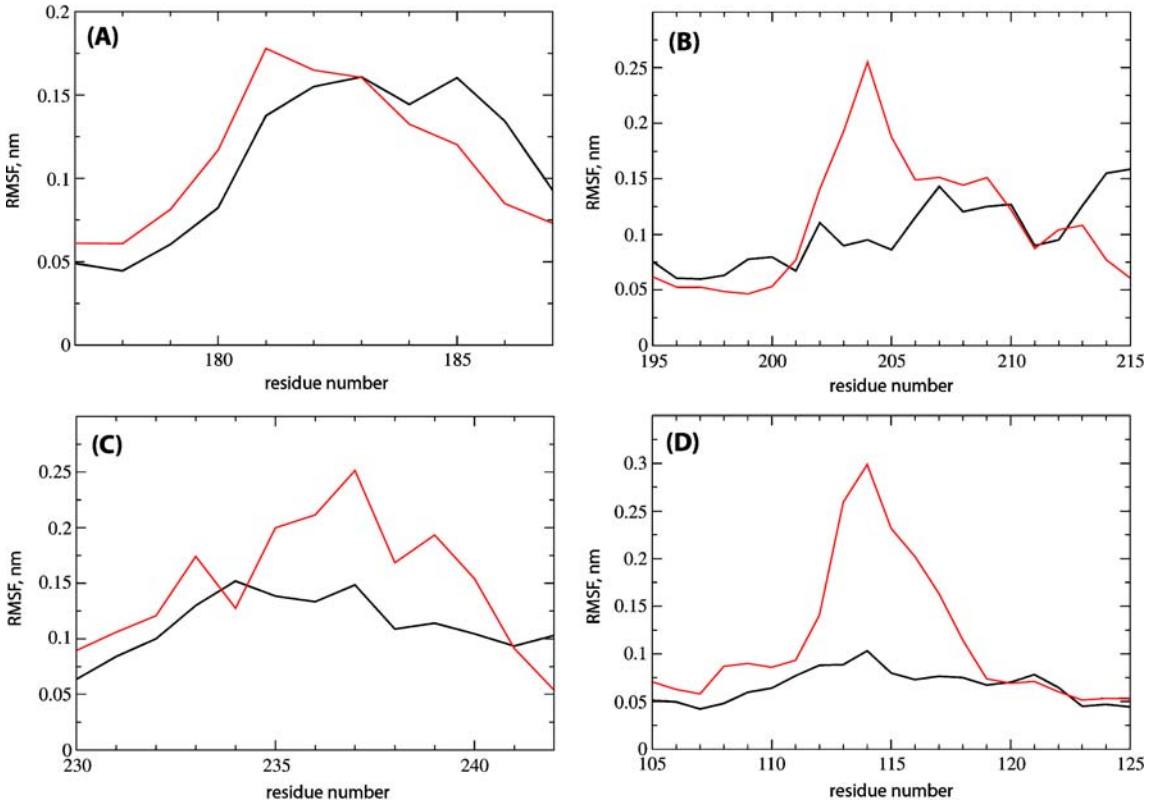

Fig. 3a–d Root mean square fluctuation (RMSF) values for Switches I–IV of $G\alpha_{i1}$ discussed throughout the text. *Black lines* Simulation of $G\alpha_{i1}$ in complex with GoLoco motif peptide; *red lines* simulation of isolated $G\alpha_{i1}$. **a** SI, **b** SII, **c** SIII, **d** SIV

~21°, larger than in the previous case. This clearly indicates that binding of the GoLoco peptide may indeed restrict the movement of domains in $G\alpha_{i1}$, which helps confer a GDI activity.

Conclusions

Sequence analysis, computational alanine scanning and MD simulations of the $G\alpha_{i1}$·GDP/GoLoco peptide complex and $G\alpha_{i1}$·GDP alone were performed to investigate molecular aspects of GoLoco motif proteins binding to $G\alpha_{i1}$. It was found that the GoLoco motif peptide, when bound to $G\alpha_{i1}$·GDP, restricts the relative domain motion of $G\alpha_{i1}$. As domain motion has been proposed to be required for the release of GDP after binding to activated GPCR [7, 78], our computational results confirm a proposed mechanism of GDI activity of GoLoco-motif-containing proteins via stabilisation of the relative positions of two Gα domains [31]. Clearly, our results do not contradict the notion that GoLoco proteins prevent GDP dissociation also by direct interaction with a bound nucleotide and/or by stabilising the positions of several side chains of $G\alpha_{i1}$ that are involved directly in the binding of GDP [32, 45]. Instead, both mechanisms might complement each other, as already pointed out by Kimple et al. [31]. Further computational studies are needed to investigate the effect of removal of the GoLoco peptide on the strength of GDP binding. In addition, upon removal of the GoLoco motif peptide, significant structural changes in functionally important regions (Switches II–IV) were observed. SIV, whose conformation differs in various states only in some Gαs [16–18, 21], and which has been proposed to be responsible for different structural changes in the G protein heterotrimers (Gα/Gβγ subunit dissociations/rearrangements [13, 14]), was very mobile in the simulation of isolated Gα, while it was restricted in its ability to move when the GoLoco peptide was bound. Since it has already been observed that the N-termini of several Gγ subunits share some homology with the GoLoco motif and the N-termini of Gγ were proposed to bind within the interface between SIV and the α_A helix [75], it may be concluded that several residues within the HD (Table 1), especially those located close to SIV, which are important for binding of the GoLoco peptide, might also be responsible for the differential conformational changes in the trimer upon activation. The latter, of course, must be verified experimentally.

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