

OPEN ACCESS

Mode of interaction of the $G\alpha_o$ subunit of heterotrimeric G proteins with the GoLoco1 motif of *Drosophila* Pins is determined by guanine nucleotides

Anne-Marie Luchtenborg*, Vladimir Purvanov†, Bogdan S. Melnik‡, Simon Becker§ and Vladimir L. Katanaev*||¹

*Department of Pharmacology and Toxicology, University of Lausanne, CH-1011, Switzerland

†Biotechnology Institute Thurgau, Kreuzlingen, CH-8280, Switzerland

‡Institute of Protein Research, Russian Academy of Sciences, 142290 Pushchino, Russian Federation

§Department of Biology, University of Konstanz, 78457 Konstanz, Germany

||School of Biomedicine, Far Eastern Federal University, Vladivostok 690922, Russian Federation

Synopsis

Drosophila GoLoco motif-containing protein Pins is unusual in its highly efficient interaction with both GDP- and the GTP-loaded forms of the α -subunit of the heterotrimeric Go protein. We analysed the interactions of $G\alpha_o$ in its two nucleotide forms with GoLoco1 – the first of the three GoLoco domains of Pins – and the possible structures of the resulting complexes, through combination of conventional fluorescence and FRET measurements as well as through molecular modelling. Our data suggest that the orientation of the GoLoco1 motif on $G\alpha_o$ significantly differs between the two nucleotide states of the latter. In other words, a rotation of the GoLoco1 peptide in respect with $G\alpha_o$ must accompany the nucleotide exchange in $G\alpha_o$. The sterical hindrance requiring such a rotation probably contributes to the guanine nucleotide exchange inhibitor activity of GoLoco1 and Pins as a whole. Our data have important implications for the mechanisms of Pins regulation in the process of asymmetric cell divisions.

Key words: asymmetric cell division, FRET, GoLoco, heterotrimeric G proteins, nucleotide exchange.

Cite this article as: *Bioscience Reports* (2015) **35**, e00271, doi:10.1042/BSR20150201

INTRODUCTION

Heterotrimeric G proteins mediate signalling by G protein-coupled receptors (GPCRs), the biggest receptor family in the animal kingdom [1]. The α -subunits of heterotrimeric G proteins determine the specificity in GPCR signal transduction and interact with various effectors [2]. GoLoco motifs present in many animal proteins specifically bind to α -subunits of the Gi/o subclass of heterotrimeric G proteins [3]. We have demonstrated that in *Drosophila*, the Go protein links the polarizing information provided by the GPCR Frizzled with the GoLoco-containing protein Pins to regulate the process of asymmetric cell divisions in the sensory organ lineage [4,5]. Atypically for GoLoco- $G\alpha$ interactions, Pins efficiently interacts with both the GDP-loaded (inactive) and the GTP-loaded (activated) forms of $G\alpha_o$ [4–6]; in

other proteins, the exclusive interaction of $G\alpha$ -GDP with GoLoco motifs has been described [3]. Our findings have identified Pins as a target of the $G\alpha_o$ -mediated GPCR signalling [5], as opposed to other GoLoco motif-containing proteins which are believed to act as modulators of the $G\alpha$ -subunits [3].

We have narrowed down the $G\alpha_o$ -GTP-interacting region of Pins to the GoLoco1 motif (the first of three GoLoco motifs present in Pins) [5]. Through a combination of biophysical approaches, we now characterize the interactions between GoLoco1 and the two nucleotide forms of $G\alpha_o$. Our data shed light on the possible mechanisms of formation of these unusual complexes and suggest that the orientation of GoLoco1 on $G\alpha_o$ is determined by the nucleotide state of the latter. These findings have important implications for the molecular mechanisms regulating activity of the multi-domain Pins protein in the process of cell polarization downstream from GPCR- $G\alpha_o$ signalling.

Abbreviations: GPCR; G protein-coupled receptor.

¹ To whom correspondence should be addressed (email vladimir.katanaev@unil.ch).

MATERIALS AND METHODS

Site-specific mutagenesis

Site-specific mutagenesis of *Drosophila* $G\alpha o$ (class II isoform) was performed through high-fidelity amplification (with Pfu DNA-polymerase, Fermentas) of the pQE32- $G\alpha o$ plasmid [5] with the following oligonucleotides introducing the point mutations: for W132F, forward 5'-ccatgaaacgcctct'tc'caggccagg-agtgc-3', reverse 5'-cctggcctg'ga'agaggcgttccatggcggcc-3'; for W212F, forward 5'-cgtaagaaat'tc'atacactgctcgaagatg-3', reverse 5'-cagtgtat'ga'attcttacctccgagcgc-3'; for W259F, forward 5'-gtaacaacaaat'tc'ttcacggacacctcg-3', reverse 5'-ccgtgaa'ga'attgtgttaccagatcag-3'. PCR products were treated with DpnI to remove the methylated template, purified by a gel-extraction kit (Peqlab) and used for bacterial transformation. The resulting plasmids were sequence-verified.

Preparation of $G\alpha o$ proteins and GoLoco peptides

Hexahistidine-tagged *Drosophila* $G\alpha o$, $G\alpha o$ [W132F; W212F] and $G\alpha o$ [W132F; W259F] were purified following [5]. In brief, Top10 bacteria transformed with the corresponding construct were grown in LB media until an $OD_{600} = 0.6$, expression was induced with 0.1 mM IPTG and cells further grown overnight at room temperature. Cells were harvested, resuspended in PBS supplemented with Complete Protease Inhibitor cocktail (Roche) and lysed with Lysozyme and sonication. Binding of the His₆ tagged proteins to the Ni-NTA agarose was conducted in 10 mM Imidazole in PBS. After washing in 20 mM Imidazole/PBS the proteins were eluted in 200–250 mM Imidazole/PBS. Their specific activities varied from 20 to 60%, and were determined as described [5]. Preloading of purified $G\alpha o$ with GDP or GTP γ S was performed for 30 min as described [5]; from parallel BODIPY-GTP γ S binding experiments the loading efficiency of $G\alpha o$, measured in percentage of B_{max} , was determined as >97%. The 36-mer GoLoco1 peptide and the GoLoco1 peptide with the dansyl (5-(dimethylamino)naphthalene-1-sulfonyl) group attached to the side chain of the N-terminal lysine were synthesized by Pepscan Presto BV. Non-dansylated GoLoco1 was dissolved in water, dansyl-GoLoco1 was dissolved at 10 mM in 50% propan-2-ol and stored at -20°C.

Fluorescence measurements

Fluorescence measurements were performed at RT using the Biotek SynergyMx plate reader. For all experiments, the $G\alpha o$ subunits were pre-incubated with 1 mM GDP or GTP γ S for >30 min at RT. For FRET measurements, preloaded $G\alpha o$ was added in a final concentration of 10 μ M to increasing concentration of dansylated GoLoco1 in HKB (50 mM Hepes-KOH, 100 mM KCl, 10 mM NaCl, 2 mM EGTA, 5 mM MgCl₂, 1 mM DTT) and incubated for 30 min before measurement. Trp-to-dansyl FRET was measured at the wavelength of 535 nm after excitation at 280 nm. Similarly, for the tryptophan fluorescence measurement, 5 μ M preloaded $G\alpha o$ were incubated for 30 min with unlabelled

or dansylated GoLoco1. Tryptophan fluorescence was measured at 330 nm after excitation at 280 nm. For the competition experiment, preloaded $G\alpha o$ was added at the 1:1 ratio (final concentration of 5–10 μ M) to dansylated GoLoco1 and pre-incubated for 10 min. Subsequently, increasing concentrations of the unlabelled GoLoco1 peptide in HKB were added and incubated for 30 min to allow establishment of the equilibrium. Trp-to-dansyl FRET was measured as above.

K_d and K_i values were calculated using the Prism program. To calculate the E values (FRET efficiency), background fluorescence in the absence of added $G\alpha o$ was subtracted from the absolute data of tryptophan fluorescence and E calculated as $(1 - F_{D-A}/F_D)$, where F_{D-A} was the measured fluorescence of 5 μ M $G\alpha o$ -dansyl-GoLoco1 complex, and F_D that of 5 μ M $G\alpha o$ -GoLoco1 complex. The distance r between the donor and acceptor fluorophores was calculated using the Förster equation $1/E = 1 + (r/R_0)^6$. R_0 depends on the orientation factor κ^2 of the dipole-dipole interaction which is a function of the specific dipole orientation of donor and acceptor. In case of dynamic isotropic movement of the labels, κ^2 is 2/3, which is the case for tryptophan and dansyl that are covalently linked with a single atom bond [7]. With this assumption, R_0 for the tryptophan/dansyl pair is 21 Å (1 Å = 0.1 nm) [8].

Structure modelling

Structure modelling was performed using the PDB entry 1KJY describing the GDP form of human $G\alpha i$ in complex with the GoLoco motif of RGS14 [9,10]. As human $G\alpha i$ and *Drosophila* $G\alpha o$ sequences share 71% identical residues, modelling of $G\alpha o$ could be performed using SwissModel [11]. The Coot package [12] was used for introducing the sequence of the Pins GoLoco motif, and for changing GDP into GTP, guided by the GDP:AlF₄⁺ coordinates of the mouse $G\alpha o$ [13]. To optimize the resulting interactions, energy minimization calculations were performed using CNS 1.2 [14] on a Linux workstation using a force field with explicit hydrogens, corresponding to the files protein-allhdg.top and protein-allhdg.param. The images (Figures 3A and 3B) were produced using Maestro (Version 9.0.211, Schrödinger Inc).

RESULTS AND DISCUSSION

Previously we have demonstrated that the GoLoco1 domain of *Drosophila* Pins efficiently interacts with both the GDP-loaded and the GTP-loaded form of $G\alpha o$ [5]. In order to investigate the unusual interaction of the GoLoco1 domain with $G\alpha o$ -GTP in detail, we performed a series of FRET experiments. To this end, we synthesized the extended 35 amino acid long Pins GoLoco1 peptide and its fluorescent analogue, where the side chain of the N-terminal lysine was dansylated (*d*GoLoco1, Figure 1A). The dansyl group added on proteins/peptides has been extensively used as an acceptor of energy from excited tryptophans in FRET experiments [15,16]. We first confirmed the binding

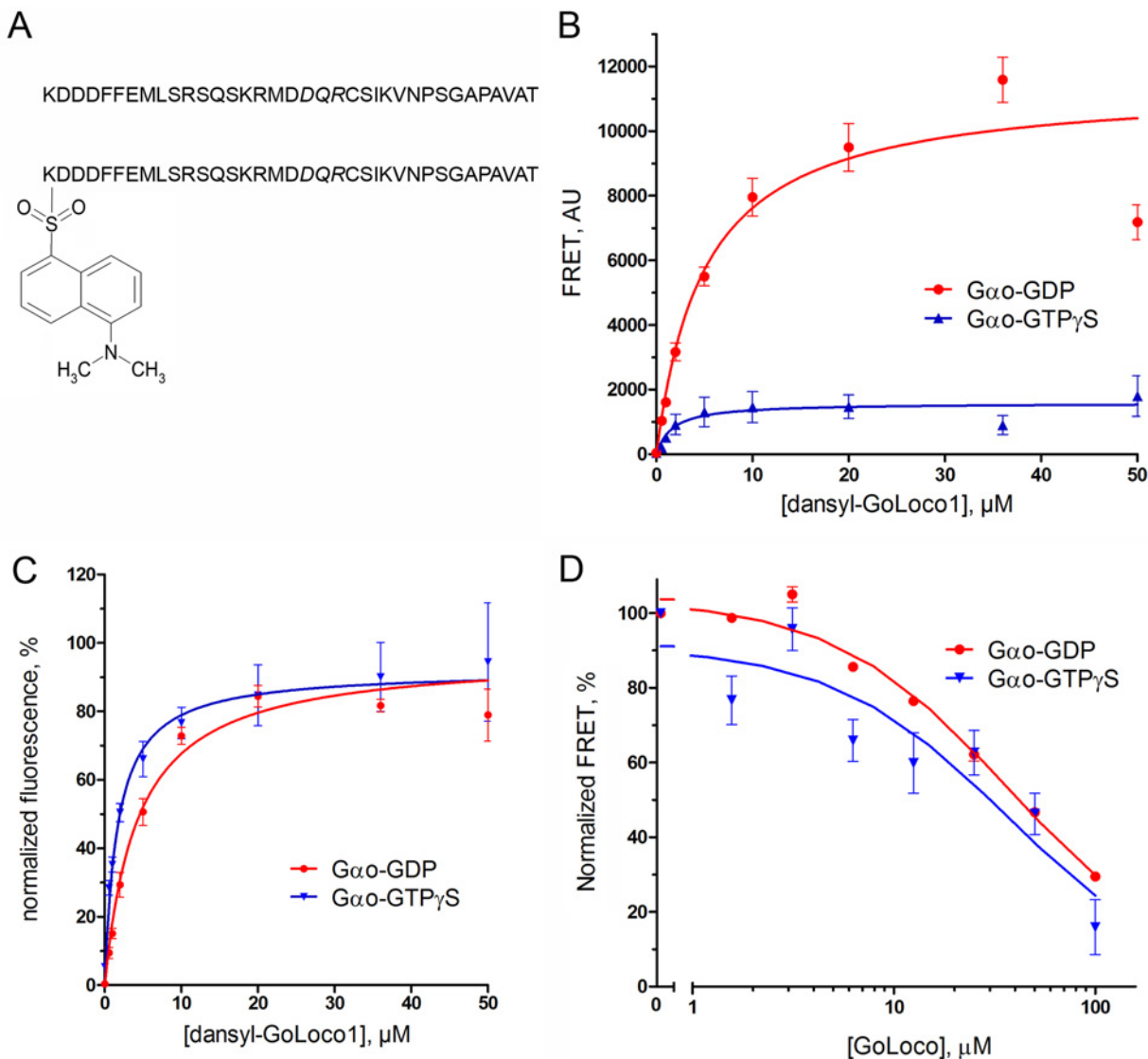


Figure 1 GoLoco1 binds with similar affinity but different orientation to $G\alpha_o$ -GTP and $G\alpha_o$ -GDP

(A) Sequence of the GoLoco1 peptide of Pins and schematic representation of its dansylated variant, with the dansyl group attached to the side chain of Lys¹ of this sequence. The DQR triad conserved among GoLoco domains and mediating the interaction with the guanine nucleotide within $G\alpha$ is shown in italics. (B and C) Saturation curves of binding of increasing concentrations of dansyl-GoLoco1 to $G\alpha_o$ preloaded with GDP or GTP γ S as measured by FRET of tryptophan to dansyl indicate comparable binding of $G\alpha_o$ -GDP and $G\alpha_o$ -GTP γ S to the peptide. The data is presented as arbitrary fluorescence units (B) and normalized fluorescence units (C). (D) Competition experiment using increasing concentrations of the unlabelled GoLoco1 peptide decreasing the normalized FRET signal from $G\alpha_o$ -GDP and $G\alpha_o$ -GTP γ S complexes with the dansylated peptide. All the data are presented as mean \pm S.E.M., $n = 7$.

of GoLoco1 with $G\alpha_o$, taking advantage of the fact that FRET occurs between the donor tryptophan and the acceptor dansyl in case donor and acceptor are in close proximity. Upon excitation of tryptophan, a FRET signal should therefore only be seen upon binding of *d*GoLoco1 to $G\alpha_o$, and the strength of the signal should be proportional to the distance between the tryptophan donor and the dansyl acceptor. Indeed, we detect a robust FRET signal to *d*GoLoco1 from both nucleotide

states of $G\alpha_o$, although the signal from $G\alpha_o$ -GTP γ S was several folds lower than from $G\alpha_o$ -GDP (Figure 1B). However, analysis of the saturation curves shows that the affinity of *d*GoLoco1 to the two nucleotide forms of $G\alpha_o$ is similar (Figure 1C): $K_d = 4.6 \mu\text{M}$ for $G\alpha_o$ -GDP-*d*GoLoco1 and $K_d = 2.1 \mu\text{M}$ for $G\alpha_o$ -GTP γ S-*d*GoLoco1. Further, *d*GoLoco1 from both complexes can be similarly outcompeted by the non-dansylated GoLoco1 peptide (Figure 1D). The K_i values resulting from these

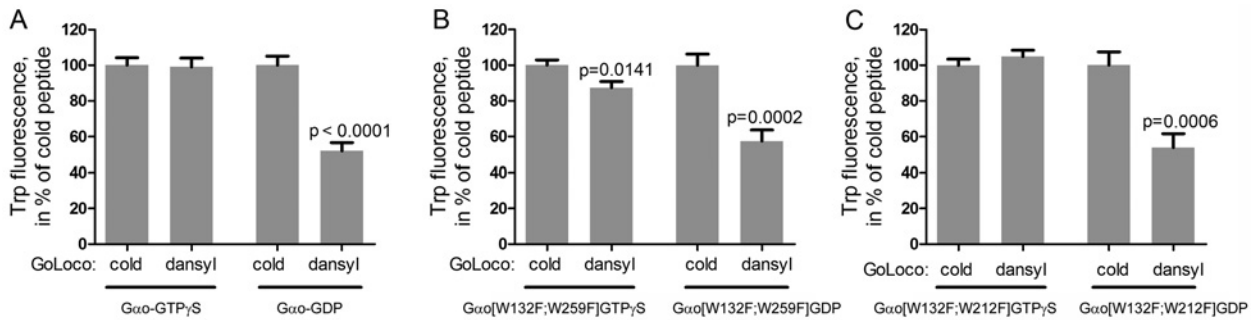


Figure 2 The N-terminal lysine is more distant when binding to G α -GTP

(A) Tryptophan fluorescence of wild-type G α o preloaded with either GDP or GTP γ S in presence of the unlabelled or dansylated GoLoco1 peptide. (B) Tryptophan fluorescence of the mutant G α o[W132F;W259F] preloaded with the guanine nucleotides as indicated. (C) Tryptophan fluorescence of the mutant G α o[W132F;W212F] preloaded with the guanine nucleotides as indicated. Data are normalized to the fluorescence in presence of the unlabelled GoLoco1 peptide and present as mean \pm S.E.M., $n = 5$. P -values calculated by the Student t -test are given for the statistically significant differences.

competition experiments are $17.6 \mu\text{M}$ for G α o-GDP and $10.6 \mu\text{M}$ for G α o-GTP γ S. The finding that K_i for unlabelled GoLoco1 is somewhat higher than K_d for d GoLoco1 may indicate that the hydrophobic dansyl group increases the affinity of the GoLoco1 peptide to G α o. In any regard, we conclude that the binding affinity of the GoLoco1 peptide of Pins to G α o is similar for the two nucleotide states of the G protein, but that the proximity of the N-terminus of GoLoco1 to G α o differs in the two nucleotide states.

The Förster critical transfer distance R_0 for the tryptophan/dansyl pair, the distance at which 50% quenching of the tryptophan fluorescence is induced due to the proximity of the dansyl acceptor, is 21 \AA assuming a value of $2/3$ for the orientation factor κ^2 [8]. By the decrease in tryptophan fluorescence due to energy transfer to the acceptor dansyl in proximity, the distance between the donor and acceptor molecules can be calculated using the Förster equation $1/E = 1 + (r/R_0)^6$, where E is the FRET efficiency and r is the distance between donor and acceptor (see Materials and Methods section) [15,17].

No tryptophan residues are present in the GoLoco1 peptide (Figure 1A). *Drosophila* G α o contains three tryptophan residues: Trp¹³² located in the helical domain far from the GoLoco1-interacting region, Trp²¹² in the switch II region and Trp²⁵⁹ in the α 3-helix. The latter two regions of G α o mediate the binding of GoLoco1 (see later), and thus their tryptophan residues might be involved in the FRET. To investigate this issue in more detail, we substituted tryptophan residues with phenylalanines, producing two double mutant G α o versions with Trp²¹² or Trp²⁵⁹ as the only remaining tryptophan. Such mutations on Trp¹³² and Trp²¹² have been performed previously on other G α -subunits and shown not to affect the overall activity of the G proteins [18,19]. The W259F substitution was also expected to produce no significant functional differences, as this substitution naturally occurs in the class I isoform of mammalian G α o.

We next measured tryptophan fluorescence of wild-type and the two double mutant forms of G α o, G α o[W132F; W212F]

and G α o[W132F; W259F], preloaded with GDP or GTP γ S, in complexes with the unlabelled GoLoco1 and d GoLoco1 peptides.

We see a robust decrease in tryptophan fluorescence of all three G α o-GDP forms in the presence of d GoLoco1 but not unlabelled GoLoco1 (Figure 2). In contrast, no or low decrease can be detected for the G α o-GTP γ S- d GoLoco1 complexes (Figure 2), confirming the presented above (see Figure 1B) and more sensitive FRET measurements which suggest the large distance between tryptophan residues and dansyl for the GTP-loaded forms. A detectable decrease can only be seen for the GTP-loaded form with Trp²¹² as the only remaining tryptophan (Figure 2B), suggesting the closest proximity of this residue of G α o-GTP γ S to the dansyl. We think that no decrease in tryptophan fluorescence could be detected for the G α o-GTP γ S- d GoLoco1 complexes using wild-type G α o due to the masking of the effect of Trp²¹² by the other two, non-participating tryptophan residues.

The FRET efficiency for the Trp²¹²-only mutant form (= G α o[W132F;W259F] mutant form) is calculated as $E_{\text{GTP}\gamma\text{S}} = 0.13$ for G α o-GTP γ S, and as $E_{\text{GDP}} = 0.54$ for G α o-GDP. Using the Förster equation, we then calculate the distance between Trp²¹² of G α o-GTP γ S and the dansyl group of GoLoco1 as $r_{\text{GTP}\gamma\text{S}} \sim 30 \text{ \AA}$. Similar calculation for G α o-GDP yields $r_{\text{GDP}} \sim 21 \text{ \AA}$.

Similar analysis of the data for the Trp²⁵⁹-only mutant (= G α o[W132F;W212F] mutant form) (Figure 3C) produce for G α o-GDP $E_{\text{GDP}} = 0.48$ and $r_{\text{GDP}} \sim 21 \text{ \AA}$. In case of G α o-GTP γ S, no reduction in tryptophan fluorescence is observed. Given the relationship of R_0 and the distance of acceptor and donor r with the power of 6, one can assume a separation of tryptophan and dansyl of $>31.5 \text{ \AA}$ [7]. These data are summarized in Table 1.

Thus, we conclude that, for G α o-GDP, the distance between Trp²¹² and the N-terminus of GoLoco1 is $\sim 21 \text{ \AA}$ and equals the distance from Trp²⁵⁹ and the N-terminus of GoLoco1. In contrast, for the GTP-loaded form of G α o, this distance for Trp²¹² is $\sim 30 \text{ \AA}$, and for Trp²⁵⁹ it is larger than 31.5 \AA . These findings argue for a substantially distinct conformation of the G α o-GoLoco1 complexes depending on the nucleotide state of the G protein.

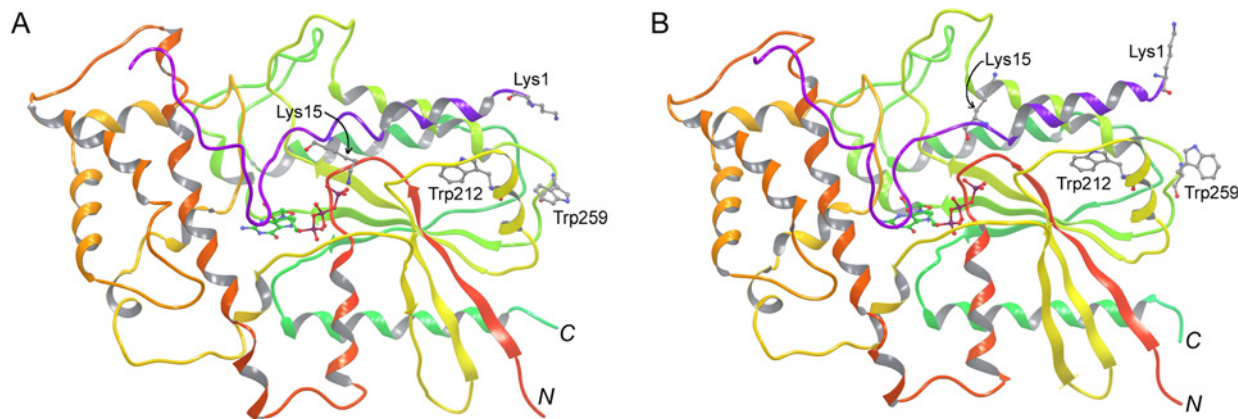


Figure 3 Molecular modelling and analysis of the $G\alpha_o$ -GoLoco1 complex

(A) Model of the $G\alpha_o$ -GTP-GoLoco1 complex built using the 1KJY structure of the homologous mammalian complex of $G\alpha_{i1}$ -GDP with the GoLoco region of RGS14 as the basis. (B) The model built using the 2OM2 structure of the same complex. In both models GoLoco1 is shown in blue-magenta, N-terminus to the right. The helical domain of $G\alpha_o$ is left, the catalytic domain is right, with the GTP placed in the centre of the cleft between the two domains. Side chains of four amino acids are shown: Lys¹ and Lys¹⁵ of GoLoco1, Trp²¹² of switch II of $G\alpha_o$ and Trp²⁵⁹ of α_3 -helix of $G\alpha_o$. Lys¹⁵ is highlighted to illustrate that the whole α -helix of GoLoco1 (and not just Lys¹) rotates from one conformation to the other.

Table 1 FRET efficiency and distance Data are given as mean \pm S.E.M., $n = 5$. N.P. – not possible to calculate.

| G-protein | E | R |
|-------------------------|--------------------|------------------------------|
| $G\alpha_o$ | | |
| GTP γ S-bound | -0.022 ± 0.072 | N.P. |
| GDP-bound | 0.474 ± 0.028 | N.P. |
| $G\alpha_o$ W132F-W212F | | |
| GTP γ S-bound | -0.017 ± 0.048 | $>31.5 \text{ \AA}$ |
| GDP-bound | 0.478 ± 0.054 | $21.38 \pm 0.77 \text{ \AA}$ |
| $G\alpha_o$ W132F-W259F | | |
| GTP γ S-bound | 0.127 ± 0.029 | $29.66 \pm 1.41 \text{ \AA}$ |
| GDP-bound | 0.544 ± 0.100 | $20.64 \pm 1.64 \text{ \AA}$ |

To visualize the possible differences in the conformation of the $G\alpha_o$ -GoLoco1 complexes in the presence of GDP compared with GTP, we performed molecular modelling of the complexes. The structure of a homologous human complex of $G\alpha_{i1}$ -GDP with the GoLoco region of RGS14 [9] was used as the basis for our model of the *Drosophila* $G\alpha_o$ -GTP-GoLoco1 complex; the model was further optimized using the structure of mouse $G\alpha_o$ -GDP:AIF₄⁺ [13]. The resulting structure of the $G\alpha_o$ -GTP-GoLoco1 interaction shows the side chain of N-terminal lysine of GoLoco1 in close proximity to Trp²¹² and Trp²⁵⁹ of $G\alpha_o$ (Figure 3A), with the distances from the side chain of GoLoco's Lys¹ to $G\alpha_o$ ' Trp²¹² and Trp²⁵⁹ being 4.5 and 9.2 \AA , respectively. However, when a different structure of the same $G\alpha_{i1}$ -GoLoco complex [10] was used as the basis for modelling, the side chain of Lys¹ was found to point away from the protein (Figure 3B), resulting in the distances to Trp²¹² and Trp²⁵⁹ being 11.7 and 13.6 \AA , respectively. In fact, quite significant differences in the orientation of the first α -helix of RGS14' GoLoco could be seen between the two structures [9,10] explaining the different rota-

tion of the modelled α -helix of Pins' GoLoco1 motif (Figures 3A and 3B).

We do not know the reason for the difference between the two $G\alpha_{i1}$ -GoLoco structures [9,10]. However, we note that the two predicted rotations of the *Drosophila* Pins' GoLoco1 in the complex with $G\alpha_o$ (Figures 3A and 3B) reflect the two possible conformations of the complex which we predicted from our FRET experiments. Taking into consideration the dimension of the dansyl group which is $\sim 4.7 \text{ \AA}$, we suggest that the model of Figure 3(A) being close to the GDP data, and Figure 3(B) being close to the GTP γ S data (Table 1).

These two conformations of GoLoco1 require quite a substantial rotation of the α -helix (Figures 3A and 3B). The existence of such a rotation currently remains a possibility, which must be verified by direct structural analysis. In any case, our data demonstrate that the conformation of the GoLoco1 region of Pins on $G\alpha_o$ is substantially different depending on the nucleotide state of $G\alpha_o$. These findings have quite important implications for the biochemistry and physiology of the GPCR- $G\alpha$ -Pins signalling. Indeed, Pins and other GoLoco domain-containing proteins possess an activity inhibiting the nucleotide exchange on $G\alpha$ -subunits [3,5]. Stabilization of the β -phosphate of GDP by a conserved arginine within the DQR triad (see Figure 1A) of the GoLoco sequence has been proposed as the mechanism of this inhibition [9]. We here wish to propose the steric hindrance, required to perform the rotation (or another structural reorganization) of the GoLoco peptide on $G\alpha$ for the nucleotide exchange, as an additional leverage to this inhibition, relevant at least for the *Drosophila* $G\alpha_o$ -Pins interaction.

Further, the different rotation/organization of the GoLoco1 peptide in respect with $G\alpha_o$, observed in the two nucleotide states of the G protein, is likely to have significant consequences to the signal transduction mediated by the $G\alpha_o$ -Pins interaction. Indeed,

the multidomain Pins binds a number of other proteins, including the microtubule anchoring protein NuMA [20]. Coordinated binding of Pins to NuMA and $G\alpha$ has been reported [21,22]. We can predict that the ability of Pins to coordinate asymmetric cell divisions is regulated differently by the GDP- and GTP-bound forms of $G\alpha$. This is likely to provide important regulatory mechanisms for different GPCR- $G\alpha$ -Pins signalling schemes, such as Frizzled- $G\alpha$ -Pins cascade in case of asymmetric cell divisions in the sensory organ lineage [5] and in *Tre1*- $G\alpha$ -Pins cascade in asymmetric neuroblast divisions [6].

AUTHOR CONTRIBUTION

Anne-Marie Luchtenborg performed most of the experiments and wrote the paper, Vladimir Purvanov performed site-directed mutagenesis and performed the initial set of some experiments, Bogdan Melnik contributed to the design of the FRET experiments, Simon Becker performed molecular modelling, and Vladimir Katanaev conceived and designed the experiments, interpreted the data and wrote the paper.

ACKNOWLEDGEMENTS

We thank members of the Katanaev lab for fruitful discussions.

FUNDING

This work was supported by the Russian Foundation for Basic Research [grant number 13-04-00923 (to B.S.M.)]; the Swiss National Science Foundation [grant number 31003A_138350 (to V.L.K.)]; grant Alzheimer research Switzerland ARS (to V.L.K.) and the Synapsis Foundation (to V.L.K.).

REFERENCES

- Pierce, K.L., Premont, R.T. and Lefkowitz, R.J. (2002) Seven-transmembrane receptors. *Nat. Rev. Mol. Cell Biol.* **3**, 639–650 [CrossRef PubMed](#)
- Sprang, S.R., Chen, Z. and Du, X. (2007) Structural basis of effector regulation and signal termination in heterotrimeric G α proteins. *Adv. Protein Chem.* **74**, 1–65 [CrossRef PubMed](#)
- Willard, F.S., Kimple, R.J. and Siderovski, D.P. (2004) Return of the GDI: the GoLoco motif in cell division. *Annu. Rev. Biochem.* **73**, 925–951 [CrossRef PubMed](#)
- Katanaev, V.L. and Tomlinson, A. (2006) Dual roles for the trimeric G protein Go in asymmetric cell division in *Drosophila*. *Proc. Natl. Acad. Sci. U.S.A.* **103**, 6524–6529 [CrossRef PubMed](#)
- Kopein, D. and Katanaev, V.L. (2009) *Drosophila* GoLoco-protein pins is a target of G α (o)-mediated G protein-coupled receptor signaling. *Mol. Biol. Cell* **20**, 3865–3877 [CrossRef PubMed](#)
- Yoshiura, S., Ohta, N. and Matsuzaki, F. (2012) *Tre1* GPCR signaling orients stem cell divisions in the *Drosophila* central nervous system. *Dev. Cell* **22**, 79–91 [CrossRef PubMed](#)
- dos Remedios, C.G. and Moens, P.D. (1995) Fluorescence resonance energy transfer spectroscopy is a reliable “ruler” for measuring structural changes in proteins. Dispelling the problem of the unknown orientation factor. *J. Struct. Biol.* **115**, 175–185 [CrossRef PubMed](#)
- Stryer, L. (1959) Intramolecular resonance transfer of energy in proteins. *Biochim. Biophys. Acta* **35**, 242–244 [CrossRef PubMed](#)
- Kimple, R.J., Kimple, M.E., Betts, L., Sondek, J. and Siderovski, D.P. (2002) Structural determinants for GoLoco-induced inhibition of nucleotide release by Galpha subunits. *Nature* **416**, 878–881 [CrossRef PubMed](#)
- Sammond, D.W., Eletr, Z.M., Purbeck, C., Kimple, R.J., Siderovski, D.P. and Kuhlman, B. (2007) Structure-based protocol for identifying mutations that enhance protein-protein binding affinities. *J. Mol. Biol.* **371**, 1392–1404 [CrossRef PubMed](#)
- Schwede, T., Kopp, J., Guex, N. and Peitsch, M.C. (2003) SWISS-MODEL: an automated protein homology-modeling server. *Nucleic Acids Res* **31**, 3381–3385 [CrossRef PubMed](#)
- Emsley, P. and Cowtan, K. (2004) Coot: model-building tools for molecular graphics. *Acta Crystallogr. D Biol. Crystallogr.* **60**, 2126–2132 [CrossRef PubMed](#)
- Slep, K.C., Kercher, M.A., Wieland, T., Chen, C.K., Simon, M.I. and Sigler, P.B. (2008) Molecular architecture of Galphao and the structural basis for RGS16-mediated deactivation. *Proc. Natl. Acad. Sci. U.S.A.* **105**, 6243–6248 [CrossRef PubMed](#)
- Brunger, A.T., Adams, P.D., Clore, G.M., DeLano, W.L., Gros, P., Grosse-Kunstleve, R.W., Jiang, J.S., Kuszewski, J., Nilges, M., Pannu, N.S. et al. (1998) Crystallography & NMR system: a new software suite for macromolecular structure determination. *Acta Crystallogr. D Biol. Crystallogr.* **54**, 905–921 [CrossRef PubMed](#)
- Wu, P. and Brand, L. (1994) Resonance energy transfer: methods and applications. *Anal. Biochem.* **218**, 1–13 [CrossRef PubMed](#)
- Steinberg, I.Z. (1971) Long-range nonradiative transfer of electronic excitation energy in proteins and polypeptides. *Annu. Rev. Biochem.* **40**, 83–114 [CrossRef PubMed](#)
- Michalet, X., Weiss, S. and Jager, M. (2006) Single-molecule fluorescence studies of protein folding and conformational dynamics. *Chem. Rev.* **106**, 1785–1813 [CrossRef PubMed](#)
- Faurobert, E., Otto-Bruc, A., Chardin, P. and Chabre, M. (1993) Tryptophan W207 in transducin T alpha is the fluorescence sensor of the G protein activation switch and is involved in the effector binding. *EMBO J.* **12**, 4191–4198 [PubMed](#)
- Lan, K.L., Remmers, A.E. and Neubig, R.R. (1998) Roles of G(o)alpha tryptophans in GTP hydrolysis, GDP release, and fluorescence signals. *Biochemistry* **37**, 837–843 [CrossRef PubMed](#)
- Du, Q., Stukenberg, P.T. and Macara, I.G. (2001) A mammalian partner of inscuteable binds NuMA and regulates mitotic spindle organization. *Nat. Cell Biol.* **3**, 1069–1075 [CrossRef PubMed](#)
- Du, Q. and Macara, I.G. (2004) Mammalian Pins is a conformational switch that links NuMA to heterotrimeric G proteins. *Cell* **119**, 503–516 [CrossRef PubMed](#)
- Nipper, R.W., Siller, K.H., Smith, N.R., Doe, C.Q. and Prehoda, K.E. (2007) Galphai generates multiple Pins activation states to link cortical polarity and spindle orientation in *Drosophila* neuroblasts. *Proc. Natl. Acad. Sci. U.S.A.* **104**, 14306–14311 [CrossRef PubMed](#)

Received 31 July 2015/10 September 2015; accepted 28 September 2015

Accepted Manuscript online 16 October 2015, doi 10.1042/BSR20150201
