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Interaction of class A G protein-coupled receptors with G proteins★✪

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A model for interaction of class A G protein-coupled receptor with the G protein G_α subunit is proposed using the rhodopsin-transducin (RD/Gt) prototype. The model combines the resolved interactions/distances, essential in the active RD*/Gt system, with the structure of Gt_α C-terminal peptide bound to RD* while stabilizing it. Assuming the interactions involve conserved parts of the partners, the model specifies the conserved Helix 2 non-polar X- - -X, Helix 3 DRY and Helix 7/8 NP- -Y- - F RD* motifs interacting with the Gt_α C-terminal peptide, in compliance with the structure of the latter. A concomitant role of Gt_α and Gt_γ C-termini in stabilizing RD* could possibly be resolved assuming a receptor dimer as requisite for G protein activation.

GDP-binding (G) protein-coupled receptors (GPCRs) are single-chain integral membrane proteins, composed of a heptahelical transmembrane (7TM) domain and the exo- and endo-domains, made of inter-helical loops, with the N- and C-termini, respectively. GPCRs, occupying about 3% of the human genome, mediate actions of about 50% used drugs making about 25% of the top-100-selling list (Klabunde & Hessler, 2002). Activation of a GPCR upon binding an agonist consists of in allosteric structure rearrangement, transduced through the 7TM domain from the exo-side to the GPCR-G protein in-

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Abbreviations: CL1-CL3, cytosolic(endo) loops 1-3; EL1-EL3, exo loops 1-3; G protein, GDP-binding protein; GPCR, G protein-coupled receptor; MD, molecular dynamics; MII or RD*, Meta II (activated) form of RD: rhodopsin; PDB, Protein Data Bank; 7TM, heptahelical transmembrane domain; TM1-TM7, transmembrane helices 1-7.

terface on the cytosolic (endo-) side. Its molecular mechanism is unknown, in contrast to the reasonably well established activation mechanism of $G_{\alpha\beta\gamma}$ subtypes (Noel *et al.*, 1993; Lambright *et al.*, 1994; 1996). The GPCR→G protein activation seems to be a common mode in otherwise diverse cascades of signal transduction from miscellaneous extracellular media to the cytosol. A great impetus to the GPCR research was added by the solving of the first historical structure of their representative, that of dark-adapted (inactive) rhodopsin (RD) (Palczewski *et al.*, 2000; Teller *et al.*, 2001; Okada *et al.*, 2002), triggering a number of new comments and reviews on possible signal transduction mechanism and GPCR activation (e.g.: Okada & Palczewski, 2001; Okada *et al.*, 2001; Hamm, 2001; Sakmar, 2002; Meng & Bourne, 2001; Ballesteros & Palczewski, 2001; Lu *et al.*, 2002; Stenkamp *et al.*, 2002; Koenig, 2002; Hunyady *et al.*, 2003; Filipek *et al.*, 2003a; 2003b; Cherfils & Chabre, 2003; Mirzadegan *et al.*, 2003) and stimulating work to elucidate this mechanism (e.g.: Altenbach *et al.*, 2001; Ballesteros *et al.*, 2001; Arimoto *et al.*, 2001; Aris *et al.*, 2001; Koenig *et al.*, 2002; Bissantz *et al.*, 2003; Fritze *et al.*, 2003; Kisselev & Downs, 2003; Liang *et al.*, 2003).

Despite some doubts a few aspects regarding details of GPCR activation seem widely agreed upon. Firstly, there is an agreement as to the common activation mechanism for Family A members, highlighting a role of conservative residues, accompanied with a strong confidence that the RD structure makes a good template for the whole Family A GPCRs (Okada & Palczewski, 2001; Okada *et al.*, 2001; Hamm, 2001; Meng & Bourne, 2001; Ballesteros & Palczewski, 2001; Ballesteros *et al.*, 2001; Sakmar, 2002; Lu *et al.*, 2002; Stenkamp *et al.*, 2002; Koenig, 2002, Hunyady *et al.*, 2003; Filipek *et al.*, 2003a; 2003b; Mirzadegan *et al.*, 2003; Fritze *et al.*, 2003; Bissantz *et al.*, 2003), see, however, a disagreement with the above view (Archer *et al.*, 2003). Secondly, an agreement as to the opening, upon agonist-triggered activation, of the cytosolic GPCR domain into a cavity, capable of accommodating selected interacting fragments of the G protein at the receptor–G protein interface. It is proven that the cavity results from an outward movement of the TM6 cytosolic side by about 8 Å, and those of TM7 and TM2, with accompanying loops, by about 2–3 Å (Farrens *et al.*, 1996; Altenbach *et al.*, 2001). Thirdly, it is known from two independent NMR studies (Kisselev *et al.*, 1998; Koenig *et al.*, 2002) that the Gt_{α} C-terminal undecapeptide $Gt_{\alpha}(340–350)$ – essential for stabilization of activated RD (RD*, Meta II, MII) – converts into an α-helix in the presence of RD*. Thus, the structure of this peptide, unresolved in solid-state Gt (Noel *et al.*, 1993; Lambright *et al.*, 1994; Lambright *et al.*, 1996), makes a natural extension of α5 in Gt_{α} forming a tip, potentially capable of fitting into a cavity in RD* at the receptor–Gt interface. It is known that this undecapeptide, likewise a C-terminal Gt_{γ}-farnesyl peptide, stabilizes RD* (Hamm *et al.*, 1988; Martin *et al.*, 1996; Aris *et al.*, 2001). Finally, the structure of this C-terminal $Gt_{\gamma}(60–71)$farnesyl peptide in a complex with RD* has just been elucidated to be an amphipathic helix, likely placed on the bilayer surface in its interfacial region (Kisselev & Downs, 2003). Thus, in this work, assuming a possible complement between the Gt_{α} tip and the RD* cavity, we attempt to prove that in any receptor–G_{α} system an arrangement is possible, involving conservative residues of both parties in equivalent sets of interactions. To this aim, commonly approved general arrangements between G_{α} and receptor are assumed (Onrust *et al.*, 1997; Bourne, 1997; Iiri *et al.*, 1998; Hamm, 2001; Koenig *et al.*, 2002; Sakmar, 2002; Lu *et al.*, 2002). It is also shown that a parallel, simultaneous or sequential accommodation of both Gt_{α} (Aris *et al.*, 2001; Martin *et al.*, 1996) and Gt_{γ} (Kisselev *et al.*, 1999; Ernst *et al.*, 2000) C-terminal peptides interacting with the same RD*

molecule is hardly possible, unless an RD*-dimer (oligomer) is assumed.

METHODS

Starting coordinates for all heavy atoms of dark-adapted RD were obtained from the X-ray data (Palczewski *et al.*, 2000), entry 1f88 in the Protein Data Bank (PDB) (Berman *et al.*, 2000). Having sealed the first gap in the structure (236–239; using SYBYL (1999) program) we ignored the C-terminal end of the sequence (beyond the second unresolved gap) so that in this work we used the unbroken RD sequence 1–327, terminating 6 and 4 residues C-terminal to the peripheral cytosolic Helix 8 and C323-palmitoyl anchor of putative cytosolic loop 4 (CL4), respectively. The resulting structure is free of most of surface obstacles (e.g. the S334–A348 C-terminal plug, seen in the dark RD) for a smooth RD*–Gt interface and conditioned for a potential cavity, capable of receiving the C-terminal helical $\alpha 5$ extension. Subsequently, the retinylidene attached through a Schiff-base nitrogen at K296 in TM7, was set all-*trans* while the charges at D83 (TM2), E113, E122, E134 (TM3) and K296 (TM7) were set neutral, typical of MII state (Fahmy *et al.*, 1993; Okada *et al.*, 2001; Saam *et al.*, 2002; Röhrig *et al.*, 2002). The retinal was parameterized as recommended in the AMBER manual (Case *et al.*, 1997). Using target-driven MD, the cytosolic side of RD, beginning from helix-breaking hinges (G89–G90 in TM2, P267 in TM6 and retylidene–K296 in TM7), was forcefully modified, so that the set of ultimate distances between the cytosolic ends of TM2–TM3, TM7–TM3 and TM6–TM3 approximately met those agreed upon and recommended in the literature (Altenbach *et al.*, 2001; Arimoto *et al.*, 2001). The specific constraints and procedures were elaborated in trial-and-error repeats and resulted in an algorithm consisting of a 30 ps severely constrained MD *in vacuo* at room temperature, with the constraints listed in Table 1. After this target-driven MD, the structure was energy-minimized to remove close van der Waals contacts and considered as a crude RD* model ready for further use. Its molecular electrostatic potential surface at the RD*–$Gt_{\alpha\beta\gamma}$·GDP interface is shown in Fig. 1, bottom.

The complete Gt_{α} subunit was modeled through a fusion of the $Gt_{\alpha\beta\gamma}$·GDP structure (PDB entry 1got) with the Gt_{α} C-terminal 340–350 undecapeptide (PDB entry 1aqg) in a complex with RD* (Kisselev *et al.*, 1998), *via* their overlapping region I340-K-E342 and subsequent elimination of double residues from this region. The complete $Gt_{\alpha\beta\gamma}$·GDP was energy-minimized to remove van der Waals clashes, and as such ready for further use. Its molecular potential surface at the

Table 1. Constraints elaborated for 30 ps MD in vacuo to bring the starting RD* structure to meet structural features drawn in the literature (Altenbach *et al.*, 2001; Arimoto *et al.*, 2001)

Backbone torsional harmonic, k (kcal·mol^{-1}·deg^{-2})	loops $k_\varphi = k_\psi = 1$, $k_\omega = 50$; TM6 and TM7 $k_\varphi = k_\psi = k_\omega = 9000$, other TM $k_\varphi = k_\psi = k_\omega = 1000$; residues at helical kinks $k_\varphi = k_\psi = 50$, $k_\omega = 500$			
Cα positional	TM1 whole, E33-Q64; TM2 none; TM3 whole, G106-V139; TM4 whole, E150-V173; TM5 whole, N200-L226; TM6 exo half, P267-H278; TM7 exo half P285-K296			
List of distances [a] (Å)	TM1 V61–TM2 Y74	13.5	TM3 V139–TM6 K248	15.6
	TM1 V61–CL4 N310	15.0	TM3 V139–TM6 E249	11.5
	TM3 V135–TM2 Y74	16.0	TM3 V139–TM6 Y250	8.5
harmonic, k[b]	TM3 V135–CL4 N310	17.5	TM3 V139–TM6 T251	15.6
			TM3 V139–TM6 R252	15.6

[a] Cα-Cα based; [b] (kcal·mol^{-1}·Å^{-2}) linearly increasing during 30 ps simulation from 900 (start) to 3000 (end).

RD*–$Gt_{\alpha\beta\gamma}$·GDP interface is shown in Fig. 1, top.

The docking of both structures was guided by the established view on how they would mutually complement at their interface (Bourne, 1997; Onrust *et al.*, 1997; Iiri *et al.*, 1998; Oliveira *et al.*, 1999; Hamm, 2001; Koenig *et al.*, 2002; Sakmar, 2002; Lu *et al.*, 2002; Cherfils & Chabre, 2003). Manual-visual docking was done using the SwissPDBViever package (Guex *et al.*, 1999). Apart from the experimentally determined residues at the interface (Onrust *et al.*, 1997; Iiri *et al.*, 1998; Koenig *et al.*, 2002) the mutual complement of electrostatic potential surface was assumed as a key factor (Oliveira *et al.*, 1999; Hamm, 2001). Using manual trial and error docking with visual feedback, a reasonable mutual arrangement was reached, meeting both the specific interactions between selected amino-acid residues and a more general complementarity between the RD* endo-cavity and the $Gt_{\alpha\beta\gamma}$·GDP tip as well as the recommended mutual overall interface, including extended specific interactions and surface electrostatic potential complement (Onrust *et al.*, 1997; Hamm, 2001; Koenig *et al.*, 2002), see Fig. 1. Finally, the Gt_{α} C-terminal peptide 338–350 was left in the cavity, the complex was energy-minimized, immersed in a fully hydrated well equilibrated lipid bilayer model, the system heated and submitted to a long-term MD at room temperature. The united-atom OPLS (Jorgensen & Tirado-Rives, 1988) force field as implemented in AMBER 5.0 was used. Currently, the MD has reached about 10 000 ps (Ślusarz & Ciarkowski, unpublished). Simultaneously, three other activated receptor-G_{α} C-terminal peptide systems were modeled,

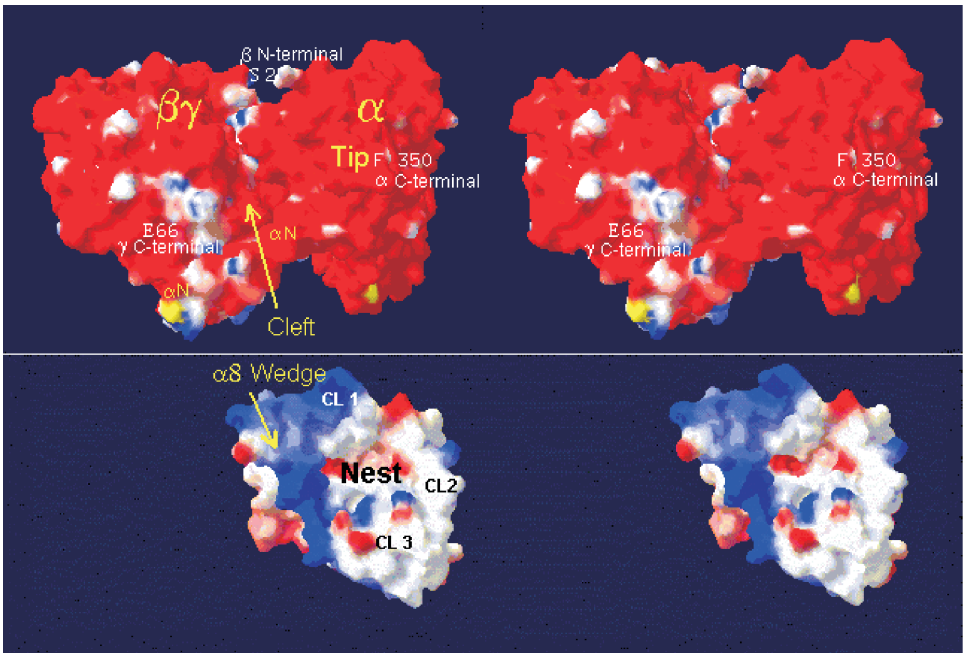

Figure 1. Electrostatic potential in stereo, calculated using protonation/dissociation states typical of pH 7 for basic/acidic amino acids respectively, and the resulting GROMOS96 point atom charges (van Gunsteren & Berendsen, 1996) for all amino acids, as implemented in SPDBViewer program.

The potential is mapped on the molecular surface: red –1.8, white 0, blue 1.8 (in kT/e units). Top: $Gt_{\alpha\beta\gamma}$·GDP interface; bottom: RD* interface. The interface clearly indicates the vast excess negative potential on the $Gt_{\alpha\beta\gamma}$ surface complementary to an excess positive potential at the RD* surface. For reference, the **visible** N- and C-terminal residues of the Gt_{α}, Gt_{β} and Gt_{γ} subunits are labeled accordingly. So are other distinct features in the both proteins. Folding top-to-bottom along the white line hinge roughly satisfies the Cleft-Wedge and the Tip-Nest fits and produces the structure given in Fig. 2.

viz. V1aR-Gq/11, OTR-Gq/11 and V2R-Gs, for examination of their mutual complement and consistency.

RESULTS AND DISCUSSION

The manual docking of Gt to the RD* model resulted in a complex shown in Fig. 2. Under the docking conditions described in Methods, a rather narrow margin has resulted for our trial-and-error. This is reasonable, given that complementary in terms of electrostatic potentials, see Fig. 1. Consequently, this interface exhausts most of both rotational and translational mutual freedom, leaving only some rotation of RD* relative to Gt around a common vertical axis, see Fig. 2, if the suggested mutual interactions, e.g. those between Gt_{α} N-terminus and RD* Helix 8 C-terminus, and others (Onrust *et al.*, 1997; Bourne, 1997; Iiri *et al.*, 1998; Hamm, 2001; Koenig *et al.*, 2002; Lu *et al.*, 2002) are to be preserved. It has become immediately clear

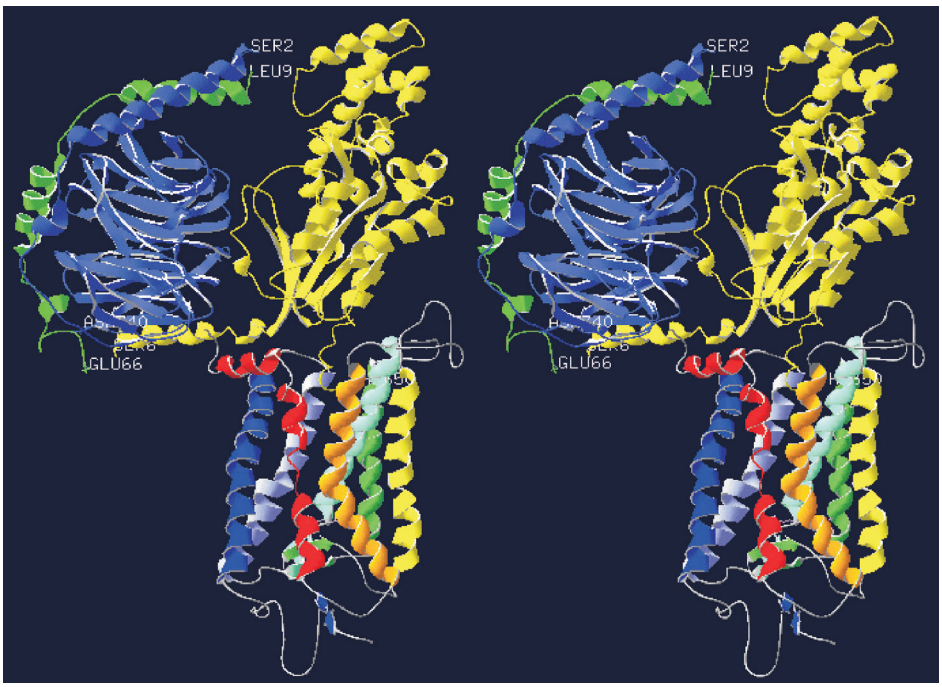

Figure 2. An outlook of the raw RD*–$Gt_{\alpha\beta\gamma}$·GDP complex, in stereo, meeting docking criteria discussed in the text.

The complex results from matching the interfacing surfaces, given in Fig. 1, by folding them at the white horizontal line, taken as a hinge, in Fig. 1. RD* (bottom) is colored according to the sequence progression, notice the break of TM7 (orange-to-red) at its kink near K296, the site of attachment of all-*trans*-retinal (not shown). Gt_{α}·GDP is yellow, Gt_{β} blue and Gt_{γ} green. The N- and C-termini of the $Gt_{\alpha\beta\gamma}$ subunits are labeled. For instance, Gt_{α} starts with S6 at αN in far left and ends with F350 nested in the RD* cavity at the receptor–Gt interface. Notice that for concomitant interactions of both αN-CL3 and RD*–Gt_{γ} C-terminus, a second receptor molecule is needed, parallel to and at the rear-left position to the first one. The current 1:1 stoichiometry can at most be compatible with simultaneous interaction: RD* with Gt_{α} C-terminus and CL3 with Gt_{α} α4–β6-loop, see text.

the rotational freedom of RD* *versus* $Gt_{\alpha\beta\gamma}$·GDP is quite limited in view of the generally approved mutual position. This arrangement compromises the fit of the Gt_{α} tip to the RD* cavity with putative planes of interface, relatively smooth in both parties and that under these restraints the fit of the Gt_{α} tip to the RD* cavity is roughly compatible with three clusters of conservative interactions shown in Fig. 3: (i) a set of polar ionic interactions involving Gt_{α}(340–350) K341, K345, D346 and F350 C-terminal carboxylate

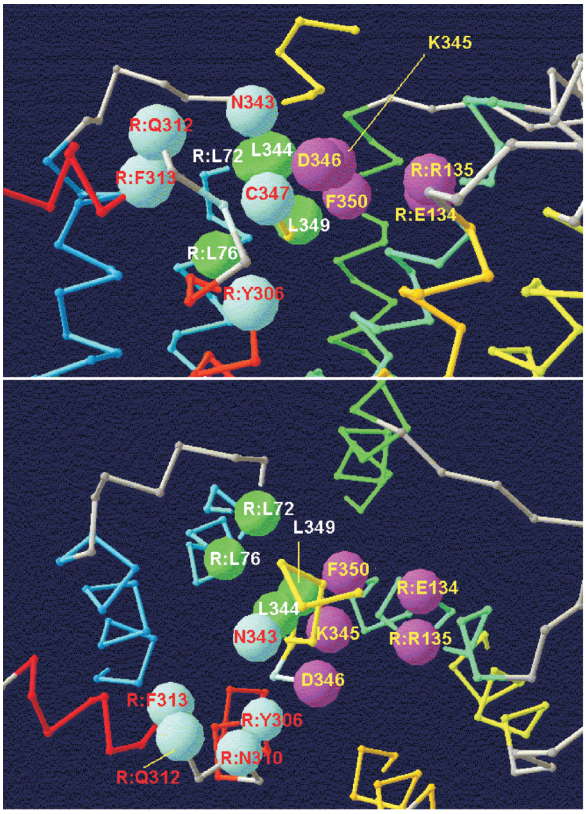

Figure 3. A blow-up of the putative conservative interactions between Gt_α(338–350) peptide and its RD* putative nest.

Top: a side view, orientation as in Fig. 2; bottom: a top view, orientation as in Fig. 1, bottom. The clusters of conservative, potentially interacting residues are symbolically shown as balls: (i) polar ionic interactions are shown violet; (ii) strictly conservative non-polar ones are shown green; and (iii) conservative polar-π aromatic are shown cyan. The labels of the receptor residues begin with "R".

and RD* TM3 E134 and R135 from the ERY motif; (ii) those involving strictly conservative Gt_α L344 and L349 and RD* L72 and L76 in TM2; and finally (iii) Gt_α C347 and Y306-N310-F314 from the TM7-Helix 8 boundary in RD*.

This fit involves mainly conserved residues in the both parties. Thus, the proposed set of interactions at the cavity-tip interface appears to constitute an interaction core common to the whole Class A GPCR family. This hypothesis goes at least two steps further than a recent one (Oliveira *et al.*, 1999) proposed, when the dark-adapted RD structure was unknown while the general RD* architecture at the Gt interface was less distinctly articulated. First, we doubtless are able to exploit the complete atomic-resolution structure of the Gt_α subunit in a complex with RD*, including the formerly unresolved C-terminus of established structural-functional significance (Martin *et al.*, 1996; Kisselev *et al.*, 1998; Kisselev *et al.*, 1999; Aris *et al.*, 2001; Hamm, 2001; Koenig *et al.*, 2002). Secondly, we are able to hypothesize on the structure of the putative RD* opening at the RD*–Gt interface to more detail than ever so far. It is very fortunate that the coupling of these two new features, modeled on the RD*–Gt interface as described above, seems to be compatible with the coupling of Family A GPCR to its respective G protein. This seemingly general prospect strengthens the present hypothesis. Currently, we are carrying out the unconstrained molecular dynamics (MD) of the raw RD*–Gt_α(338–350) complex, embedded in a fully hydrated lipid bilayer. At the time of this writing the MD has advanced to about 10 ns. MD of this time-scale would possibly allow for the appearance of tendencies in dynamical structure changes, likely enabling an assessment whether the RD*–Gt_α(338–350) complex approaches a stable state or is structurally inconsistent. Which would bear on the answer whether this model could suffice to explain the stabilization of RD* by Gt_α(338–350) or a more advanced one would be required, see below.

On the other hand, it is also clearly seen that this model (actually, any model meeting the 1:1 RD* to Gt stoichiometry) can hardly accommodate the Gt_α and Gt_γ(50–71)farnesyl C-termini for a two-site activation of Gt, see Fig. 2. Were these parallel or sequential interactions (Kisselev *et al.*, 1999), they would require an RD* oligomer, at least a dimer. Indeed, in recent two papers, extensive oligomerization of RD (dark-adapted) and its apoprotein, opsin, in rod outer segments (ROS) of mice has been demonstrated (Fotiadis *et al.*, 2003; Liang *et al.*, 2003) using atomic force microscopy (AFM). There are

also numerous indications that other Family A GPCRs may function as dimers; for a recent review, see (George *et al.*, 2002). As demonstrated by AFM, the RD and opsin molecules alike make extensive and quasi-regular rows of dimers filling most of the membrane surface of ROS (Liang *et al.*, 2003). The authors propose the best fit of the experimental RD structure in this network and simultaneously suggest a putative RD*–Gt complex meeting the 2 : 1 stoichiometry. The RD units interface each other utilizing their TM4 and TM5 in a head-to-head interaction of C_2 symmetry and in this disposition admit a Gt heterotrimer. This imposes on Gt an orientation rotated by ~180° around its vertical axis, relative to that given in Fig. 2. Simultaneously, the dimers form rows utilizing side-to-side interactions between CL3 and CL1, also including respective helices from adjacent dimers. And, finally, the rows run densely enough relative to one another to allow weaker tail-to-tail interactions utilizing TM1 again in the C_2 symmetry, from yet different adjacent RD pairs. It is conceivable, given only a minor rearrangements of this assembly network upon activation of RD to RD*, that the latter tail-to-tail dimer rather than a head-to-head one, could make an operationally active unit meeting the RD*–Gt 2 : 1 stoichiometry and simultaneously being in agreement with Fig. 2. Clearly, both models while compatible with important experimental aspects, need more experimental proof to be confirmed, improved or rejected.

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