Interaction of a G protein with an activated receptor opens the interdomain interface in the alpha subunit

Ned Van Eps¹,², Anita M. Preininger²,³, Nathan Alexander⁴,¹, Ali I. Kaya⁵, Scott Meier⁶, Jens Meiler²,², Heidi E. Hammb,² and Wayne L. Hubbell²,²

¹Jules Stein Eye Institute and Department of Chemistry and Biochemistry, University of California, Los Angeles, CA 90095-7008; ²Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN 37232-6600; ³Department of Chemistry, Vanderbilt University, Nashville, TN 37232-6600

Contributed by Wayne L. Hubbell, April 14, 2011 (sent for review March 12, 2011)

In G-protein signaling, an activated receptor catalyzes GDP/GTP exchange on the Gα subunit of a heterotrimeric G protein. In an initial step, receptor interaction with Gαi allosterically triggers GDP release from a binding site located between the nucleotide binding domain and a helical domain, but the molecular mechanism is unknown. In this study, site-directed spin labeling and double electron–electron resonance spectroscopy are employed to reveal a large-scale separation of the domains that provides a direct pathway for nucleotide escape. Cross-linking studies show that the domain separation is required for receptor enhancement of nucleotide exchange rates. The interdomain opening is coupled to receptor binding via the C-terminal helix of Gαi, the extension of which is a high-affinity receptor binding element.

signal transduction | structural polymorphism

The α-subunit (Gα) of heterotrimeric G proteins (Gαβγ) mediates signal transduction in a variety of cell signaling pathways (1). Multiple conformational states of Gα are involved in the signal transduction pathway shown in Fig. 1A. In the inactive state, the Gα subunit contains a bound GDP [Gαi(GDP)] and has a high affinity for Gβγ. When activated by an appropriate signal, a membrane-bound G-protein coupled receptor (GPCR) binds the heterotrimer in a quaternary complex, leading to the dissociation of GDP and formation of an “empty complex” [Gαi(0)], which subsequently binds GTP. The affinity of Gαi(GTP) for Gβγ is dramatically reduced relative to Gαi(GDP), resulting in functional dissociation of active Gαi(GTP) from the membrane-bound complex. The active Gαi(GTP) subsequently binds downstream effectors to trigger a variety of regulatory events, depending on the particular system. Thus, the GPCR acts to catalyze GDP/GTP exchange via an empty complex. Crystallographic (2–7), biochemical (8), and biophysical (9–11) studies have elucidated details of the conformational states of Gα that correspond to the discrete steps indicated in Fig. 1A, but the mechanism by which receptor interaction leads to release of the bound GDP from Gα and the structure of the empty complex remain a major target of research in this field.

The Gαi subunit has two structural domains, namely a nucleotide binding domain and a helical domain that partially occludes the bound nucleotide (Fig. 1B). From the initial Gαi crystal structure in 1993, Noel et al. (2) recognized that nucleotide release would probably require an opening between the two domains in the empty complex, but in the intervening 18 years there has been little compelling experimental support for this idea. Nevertheless, some constraints on the general topology of the complex are known. For example, numerous studies indicate that the C terminus of Gαi is bound tightly to the receptor in the empty complex (9). In addition, the N-terminal helix of Gαi is associated with Gβγ and with the membrane via N-terminal myristoylation (12, 13). Together, these constraints fix the position of the nucleotide domain with respect to the membrane. The helical domain is connected to the nucleotide domain through two flexible linkers, and linker 1 (switch I) undergoes conformational changes upon receptor binding (10). These observations provided the motivation to look for relative motion of the two Gα domains during formation of the empty complex.

For this purpose, site-directed spin labeling (SDSL) and double electron–electron resonance (DEER) spectroscopy were employed to measure distances between pairs of spin labels, with one label in each domain. Distances were measured for each state in A. (C) Distance distributions for the indicated doubly spin-labeled mutants. (Top) Compares Gαi(GDP) and Gαi(GTP); (Middle) compares Gαi(GDP) and R*; (Lower) compares Gαi(GDP) and Gαi(GTP); traces are color coded to match states in A.

---

Fig. 1. Receptor activation of G proteins leads to a separation between domains. (A) The pathway of Gα activation via activated rhodopsin (R*). The alpha subunit is color coded to denote the four different states investigated by SDSL/DEER spectroscopy. (B) Ribbon model of Gαi(GDP) (PDB ID code 1G2P). The helical and nucleotide binding domains are colored green and light blue, respectively, and GDP is shown as magenta spheres. Relevant secondary structural elements are noted for reference. The C-terminal helix of Gαi is colored yellow; six disordered residues at the C terminus are not displayed. The N-terminal helix is truncated for convenience. Sites from which R1 nitroxide side chains were selected pair wise for distance measurements are indicated by spheres; dotted traces indicated specific distances measured for each state in A. (C) Distance distributions for the indicated doubly spin-labeled mutants. (Top) Compares Gαi(GDP) and Gαi(GTP); (Middle) compares Gαi(GDP) and R*; (Lower) compares Gαi(GDP) and Gαi(GTP); traces are color coded to match states in A.

---


The authors declare no conflict of interest.

N.V.E., A.M.P., and N.A. contributed equally to this work.

1To whom correspondence should be addressed. E-mail: jens.meiler@vanderbilt.edu, heidi.hammb@vanderbilt.edu, or hubbellv@jpeiu.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1105810108/-/DCSupplemental.

www.pnas.org/cgi/doi/10.1073/pnas.1105810108

PNAS Early Edition | 1 of 5
Results and Discussion

Using SDSL and DEER spectroscopy, distances were measured for each state of \(G_{\alpha i}\) along the activation pathway using activated rhodopsin (R*) as the GPCR. In these experiments, the R1 nitroxide side chain (Fig. S1) was introduced via cysteine substitution mutagenesis into the background of \(G_{\alpha i}\) with reactive cysteines removed, HexaI (\(G_{\alpha i}\) HI) (14). Fig. 1B shows the set of sites from which pairs were selected and the five specific interdomain distances investigated.

All doubly spin-labeled proteins bind to R* to an extent similar to the \(G_{\alpha i}\) HI parent protein as shown in direct endpoint binding assays (Fig. S2). In addition, they are all functional with respect to receptor-mediated nucleotide exchange, although mutants 138R1/276R1 and 157R1/333R1 have, respectively, about 40% and 55% of the receptor-catalyzed nucleotide exchange rate of the parent \(G_{\alpha i}\) HI protein (Fig. S2). The reduced rates suggest that the residues involved are important in modulating receptor-mediated nucleotide exchange. In crystal structures of the inactive protein, residues Asn157 and Glu276 are involved in side chain (17) and are involved in side chain H bonding and electrostatic interactions, respectively, and mutation of these may influence local conformation.

DEER spectroscopy relies on magnetic dipolar interactions between spin labels to measure interspin distances in the range of \(\approx 17-60\) Å (15, 16). Of particular importance is the ability to resolve multiple distances and the widths of the distributions. Fig. 1C compares the distance probability distributions for the five transdomain R1 pairs in each of the four states of \(G_{\alpha i}\), i.e., \(G_{\alpha i}(\text{GDP}), G_{\alpha i}(\text{GDP})_\alpha, G_{\alpha i}(\text{GDP})_\gamma,\) and \(G_{\alpha i}(\text{GTP})\). For each pair, the measured most probable distances for \(G_{\alpha i}(\text{GDP})\) and \(G_{\alpha i}(\text{GDP})_\gamma\) agree well with expectations from the crystal structures (5–7) and models of the R1 side chain (17). In all cases there is little difference between \(G_{\alpha i}(\text{GDP})\) and \(G_{\alpha i}(\text{GDP})_\beta\).

Upon photoactivation of rhodopsin and formation of the \(R^*\) • \(G_{\alpha i}(0)_\beta\) complex, there is a remarkable increase in each interspin distance, with increases being as large as 20 Å (at 90/238) (for details, see SI Text and Figs. S3 and S4). Moreover, there is a dramatic increase in width of each distribution as well as multiple distances in most cases. It is of interest that distances present in the \(G_{\alpha i}(0)_\beta\) distributions correspond approximately to minor populations already present in \(G_{\alpha i}(\text{GDP})\) and \(G_{\alpha i}(\text{GDP})_\gamma\), suggesting that activation may shift an existing equilibrium. Although the exact widths of the distributions in \(G_{\alpha i}(0)_\beta\) may not be well determined in each case, they are clearly broader than possible from multiple rotamers of R1, suggesting spatial disorder of the \(G_{\alpha i}\) protein in the empty-pocket state of the activated complex (see SI Text). Finally, addition of GTP/S restores a state with a most probable distance and width of distribution similar to the GDP bound state. This is in agreement with expectations from GTP/S bound crystal structures (6).

The EPR spectra of R1 residues at the sites shown in Fig. 1B have little or no changes upon receptor activation (Fig. 2). This result, taken together with the very large distance changes observed, ensure that the detected distance increases reflect global domain movement rather than simple R1 side chain rearrangements due to changes in local environment. Collectively, the data strongly support a model for a \(G_{\alpha i}(0)_\beta\) in which the helical domain is displaced relative to the nucleotide domain in the heterotrimer, and in which the structure is highly flexible with respect to the relative domain orientations.

To visualize the domain opening, a model of the empty complex on the receptor was constructed that is consistent with the DEER and other available experimental data (see SI Text). To generate the model, the heterotrimeric Gq was docked with the photocoreceptor using crystal structures of \(G_{\alpha i}(\text{GDP})_\beta\) (4, 7) and opsin in complex with the high-affinity \(G_{\alpha i}\) C-terminal peptide (18). The \(G_{\alpha i}\) C-terminal helix was fused with the high-affinity \(G_{\alpha i}\) C-terminal peptide bound to opsin (for details, see SI Text and Figs. S3 and S6), which provided a convenient starting point for the model (19). The myristoylated N-terminal amphipathic helix was placed parallel to the membrane surface and the heterotrimer oriented such that both the myristoyl group and the nearby farnesylated C terminus of the Gy-subunit can be inserted into the membrane; together these hydrophobic interactions cooperatively drive membrane binding of the intact heterotrimer (20).

The procedure required chain breaks within the linker regions of the \(\alpha\)-subunit (between residues 59–60 and 184–185) and resulted in clashes in loop regions within the heterotrimer that were then resolved through loop reconstruction and model relaxation in Rosetta (21, 22). A rigid body docking protocol was executed to find placements of the helical domain consistent with the DEER distance restraints (SI Text, Fig. S7, and Table S1). An ensemble of models was found to be in agreement with the experimental distances from DEER data, consistent with the increase in width of the distance distributions (Fig. S8). The model that agrees best with the most probable distances from DEER data (Fig. 3B) fulfills all distance restraints within the error of the experiment and involves an approximately 8-Å motion of the helical domain away from the nucleotide binding domain (Movie S1).
domain as well as an approximately 29° rotation relative to its starting position (Fig. S9).

The model shown in Fig. 3 incorporates a constraint gleaned from an interesting feature of the \( G_\alpha \) structure. In the structure, helix \( \alpha A \) has a pronounced kink (dotted circle, Fig. 1A) that is not due to proline or glycine residues in the sequence. Rather, the strained kink may be stabilized by a three-element network of packing interactions between the \( \alpha 5/\beta 6 \) turn, the \( \alpha F \) helix, and the helix \( \alpha A \). Previous results showed that receptor interaction with \( G_\alpha \) moves the \( \alpha 5/\beta 6 \) turn, a change that could weaken the three-element interaction and trigger kink relaxation, thus moving the body of the helical domain relative to the nucleotide domain. Coupling between \( \alpha 5 \) and \( \alpha F \) was suggested by several \( G_\alpha \) proteins that act as functional mimetics of the receptor-bound state (23). Kink relaxation is incorporated into the preliminary model of Fig. 3, but the actual relative movement of the helical domain shown in the figure does not depend on this mechanism, which will be examined in future studies. An animation showing the features of this model and the avenue for nucleotide escape can be found in SI Text.

The C terminus of \( G_\alpha \) is a critical interaction site between the G protein and the receptor (9, 24–26) as illustrated in the model of Fig. 3. Previous studies demonstrate that the C terminus undergoes a disorder-to-order transition upon binding to activated receptors, inducing structural changes that are important for efficient GDP release (27–29). \( G_\alpha \) with a flexible 5-glycine linker inserted at the base of the \( \alpha 5 \) helix (at residue 343, Fig. 4A) binds to \( R^* \) but eliminates a receptor-mediated movement of this helix, increases basal exchange, and uncouples nucleotide exchange from binding (9, 30). We have introduced the same 5-glycine insertion into the interdomain pair, R90R1/E238R1. Fig. 4 shows the distance distribution for the various states of \( G_\alpha \), to be compared with those of the parent protein shown in Fig. 1C. Remarkably, the 5-Gly insertion results in a bimodal distance distribution in all states, the components of which correspond approximately to the open and closed positions of the helical domain. However, the distribution for the population at longer distances (approximately 40 Å) is substantially sharper than that in Fig. 1C. Apparently, the perturbation of \( \alpha 5 \) by the insertion uncouples movement of the helical domain from receptor interaction. Although additional studies would be required to characterize the states of the insertion mutant, the result suggests a critical role of the C terminus in allosteric communication from the receptor to helical domain opening and the nucleotide binding pocket.

Is the domain rearrangement required for GDP release? To address this question, the two domains were cross-linked, disallowing the domain opening. For this purpose, a bifunctional, thiol-directed bis-maleimide was selected to cross-link cysteine

**Fig. 4.** A 5-Gly insertion in \( \alpha 5 \) of \( G_\alpha \) uncouples domain opening from receptor binding. (A) Ribbon model of \( G_\alpha \) (GDP) showing the location of the 5-Gly insertion between residues 343–344; additional residues (345–354, blue ribbon) from the opsinpeptide crystal structure (3DQ8.pdb) were added after the insert to suggest the subunit bound to activated rhodopsin. (B) Distance distributions of 90R1/238R1 compared for \( G_\alpha \) (GDP) and \( G_\alpha \) (GDP) \( \beta \gamma \) (Top), \( G_\alpha \) (GDP) \( \beta \gamma \) and \( R^* \) (Middle), and \( G_\alpha \) (GDP) and \( G_\alpha \) (GTP) (Lower). The 5-Gly insert bearing the 90R1/238R1 double mutation binds to \( R^* \) in native disc membranes to approximately the same extent as the \( G_\alpha \)HI parent.

**Fig. 5.** Cross-linking of the helical and nucleotide domains of a R90C-E238C \( G_\alpha \) double mutant. (A) Model of the bis-maleimido interdomain cross-linker; the color code is as in Fig. 1. (B) Binding of the cross-linked mutant to rhodopsin in disc membranes. (C) Basal and receptor-stimulated nucleotide exchange rates for the bis-maleimido cross-linked (XL) \( G_\alpha \). For comparison, the \( G_\alpha \)HI and R90R1/E238R1 nucleotide exchange rates are shown. (Inset) Tryptophan fluorescence changes of the XL \( G_\alpha \) subunit upon aluminum fluoride addition.
residues in the R90C-E238C protein, based on the predicted proximity between these thiols in the Gα1(GDP) protein (Fig. 5A). Cross-linking resulted in a Gα1(GDP)βγ-protein competent to bind activated receptors to approximately the same extent as the parent protein (Fig. 5B). Moreover, the cross-linked protein undergoes aluminum fluoride-dependent conformational changes (Fig. 5C, Inset) consistent with an active, properly folded protein. On the other hand, this protein exhibited severely impaired rates of basal and receptor-mediated nucleotide exchange (Fig. 5C), demonstrating the essential nature of the domain separation in receptor-mediated G-protein activation. The basal nucleotide exchange rate was only slightly reduced (Fig. 5C), suggesting an effect specific to receptor-mediated nucleotide release, the slow step in G-protein activation.

Conclusions

This study demonstrates that the result of G-protein interaction with an activated receptor is propagated allosterically to reorient the distant helical domain of Gαi, opening the domain interface in formation of a flexible ternary receptor–G-protein complex. Preventing the large interdomain movement through cross-linking markedly reduces the rate of catalyzed nucleotide exchange, demonstrating the crucial role of the interdomain opening in receptor-mediated G-protein activation. Although the detailed mechanism is currently under further investigation, this domain opening would be predicted to reduce the GDP binding energy as interactions are lost upon opening of the domain interface. Together these changes help broaden our understanding of the conformational changes in the G protein that lead to GDP release, the slow step in G-protein activation.

Methods

Membrane Binding Assays. The ability of wild-type and Gαi proteins containing the side chain R1 (Fig. 5A) to bind rhodopsin was tested as described previously (31). For additional details, see SI Methods.

Cross-Linking. The bifunctional cross-linking reagent, 1,1-biss(maleimido) triethylene glycol (Pierce Biotechnology) was incubated in a 2:1 molar ratio with Gαi Hexa-labeled at 4 °C for 2 h in 50 mM Tris, 130 mM NaCl, 10 mM MgCl2, 5 mM EDTA, 100 μM GDP, at pH 7.0. After 2 h, reaction was quenched with chromatography buffer (50 mM Tris, 130 mM NaCl, 2 mM MgCl2, 10 μM GDP, 1 μM EDTA, 1 mM DTT, pH 7.5) and concentrated in this buffer. The concentrated, cross-linked monomeric protein was then purified by gel filtration FPLC on a calibrated SW2000 column (Sigma). Calibration was performed under the same conditions as purification, using a broad range of molecular weight standards (Biorad).

Nucleotide Exchange Assays. G proteins and rod outer segment (ROS) membranes were prepared essentially as previously described (10). The rates of basal and receptor-mediated nucleotide exchange of the spin-labeled Gαi proteins were measured at excitation/emission wavelengths of 290/340 nm in buffer containing 50 mM Tris, 130 mM NaCl, 2 mM MgCl2, 1 mM DTT, 1 μM EDTA, pH 7.5, for 40 min at 18 °C after addition of 10 μM GTPγS. For receptor-stimulated exchange, proteins were reconstituted with an equimolar amount of Gαi/βγ (200 nM each) prior to measurement of exchange; the experiments were performed in the presence of light activated rhodopsin (100 nM) obtained from urea washed ROS membranes. Basal exchange was carried out in the absence of rhodopsin and Gαi. The data were normalized to the baseline and the fluorescence maximum, and rate of exchange was determined by fitting the data to an exponential association curve. Rates shown in Fig. S2B are from a minimum of four independent experiments (± SEM).

Spin Labeling and Electron Paramagnetic Resonance Measurements. Spin labeling was carried out in buffer containing 20 mM 3-(N-norbornyl)propane-sulfonic acid (pH 6.8), 100 mM NaCl, 2 mM MgCl2, 50 μM GTP, and 10% glycerol (vol/vol). The Gαi double mutants were incubated with the sulfhydryl spin-label 5-(1-oxy-2,2,5,5-tetramethylpyrroline-3-methyl)-methanethiosulfonate in a 2:1 molar ratio at room temperature for 5 min. Noncovalently bound nitroxide was removed by extensive washing with labeling buffer using a 30-kDa molecular weight concentration. A series of EPR spectra were recorded for each spin-labeled mutant. Continuous wave (CW) EPR spectra were recorded at room temperature on a Bruker E580 spectrometer using a high-sensitivity resonator (HSO118) at X-band, microwave frequencies. Each spectrum was collected using a 100-G field scan at a microwave power of 19.92 mW. Optimal field-modulation amplitudes were selected to give maximal signal intensity without line-shape distortion. The data were typically averages of approximately 20 scans.

Four-Pulse DEER Measurements. The spin-labeled proteins were flash frozen within quartz capillaries in a liquid nitrogen bath. After freezing, they were loaded into a 2-mm split-ring resonator, and DEER measurements were performed at 80 K on a Bruker Elexys E580 spectrometer. Four-pulse DEER was carried out as previously described (32), with the x-pulse pump (16 ns) positioned at the absorption maximum of the field swept nitroxide center line and the observer τ (16 ns) and τ/2 (8 ns) pulses at the absorption maximum of the low-field line. The buffer used for DEER measurements was similar to the CW EPR experiments. Four different states of each double-labeled mutant were measured to determine conformational changes along the G-protein activation pathway. All DEER data were analyzed with the DEER Analysis 2011 software package freely available at the Web site http://www.epr.ethz.ch/, and with a Labview software package provided by Christian Altenbach (Jules Stein Eye Institute, Los Angeles, CA). Details for utilization of the DEER Analysis 2011 software package were previously described (33). Background correction of the primary dipolar evolution data was performed as described (33). For distance distributions below 20 Å, excitation bandwidth corrections were applied (34). These corrections had very little effect on the computed distributions. Tikhonov regularization techniques were used for fitting the data using L-curve methods for determining the regularization parameter (35). In some instances, Gaussian fitting was also employed where distribution widths of the Gaussian fits were guided by Tikhonov results. Figs. 53 and 54 show the background-corrected dipolar evolution data, the dipolar spectra, and the normalized integral representations of the distance distributions. For the distances between the nucleotide and helical domains in the receptor-bound empty complex, the width of the distribution may not be well determined due to the limited collection time of the dipolar evolution. Nevertheless, the fact that the distribution is indeed broad is revealed by the lack of well-defined oscillations in the dipolar evolution.

Modeling of the Complex Based on Available Information, Including DEER Distances. For details about the complex, see SI Methods.

ACKNOWLEDGMENTS. The authors gratefully acknowledge helpful discussions with C. Altenbach and thank C. Hubbell, C. J. Lopez, V. V. Gurevich, and C. R. Sanders for carefully reading the manuscript. Research reported here was supported by National Institutes of Health Grants GM080403 (to J.M.), EY006062 (to H.E.H.), EY005216 (to W.L.H.) and the Jules Stein Professorship endowment (to W.L.H.). N.A. was supported by National Research Service Award MH086222.


Supporting Information

Van Eps et al. 10.1073/pnas.1105810108

SI Methods.

Membrane Binding Assays. $G_{ia}$ (5 μM) subunits were preincubated with $G_{βγ}$ (10 μM) subunits on ice for 10 min. Then, in the dark, rhodopsin (50 μM) within native membranes was added to the heterotrimeric G protein in a buffer containing 50 mM Tris (pH 8.0), 100 mM NaCl, 1 mM MgCl₂, and incubated on ice for 5 min. For dark measurements, reaction mixtures were protected from light for the rest of the procedure. Light activated samples, as well as light activated samples with GTPγS (100 μM), were incubated on ice for 30 min. The membranes in each treatment (dark, light, and light plus GTPγS) were pelleted by centrifugation at 20,000 × g for 1 h at 4°C, and supernatants were removed from pellets. For the dark samples, supernatants were removed under dim red light. The supernatants and pellets of each treatment were boiled and resolved by SDS-PAGE. The protein samples were visualized with tants and pellets of each treatment were boiled and resolved via the Rosetta loop building protocol.

α-Helical Domain Docking. EPR distance measurements display a reorientation of the helical domain of the α-subunit when the heterotrimer binds to the receptor (Fig. 1). In order to capture this conformational motion, the α-helical domain was detached from the rest of the α-subunit by introduction of chain breaks between residues 59/60 and 184/185 of chain A of the $G_{αiγ}$ structure. Next, a rigid body docking protocol was executed to sample possible placements of the helical domain with respect to the α-subunit. A total of 140,000 structures were created using Rosetta (3). The starting position of the α-helical domain was initially perturbed by up to 1.5 Å and 4° rotation. During docking trajectories translations of up to 0.05 Å and rotations of up to 2.5° were performed in a stepwise procedure. The command line flags used follow:

```
docking_protocol.linuxgccrelease -in:file:s start.pdb -out:struct 100 -docking:dock_per 1.5 4 -docking:dock_mcm_trans_magnitude 0.05 -docking:dock_mcm_rot_magnitude 2.5 -out:overwrite
```

Filtering of α-Helical Domain Docking Models. Docking models were filtered for agreement with EPR distance data after docking. Agreement with the EPR distance restraints is calculated according to the knowledge-based potential given by Hirst et al. (4). Agreement can be expressed with a value between 0 (no agreement) and -1 (perfect agreement, Fig. S7A). In addition to the EPR distances, a filter was applied to ensure the chain break created at the cut points can be resolved through remodeling a minimal number of residues around the cut points. This filter minimizes the distances between residues 59/60 and 184/185 of the α-subunit of $G_{αiγ}$ (Fig. S7B). The 1,000 models that pass both filters undergo a clustering analysis (Fig. S8), and the cluster center that agrees best with the experimental data is used for all further analysis (Table S1). This model shows a translation of approximately 8 Å and a rotation of 29° of the α-helical domain compared to its starting position.

The increased width in the distance distributions obtained from EPR spectroscopy (Fig. 1C) suggests a flexible relative orientation of the helical domain with respect to the heterotrimer in the receptor-bound state. The ensemble of 1,000 models in agreement with the EPR data might reflect part of this spatial disorder. A single model was selected to facilitate discussion of the general movement of the α-helical domain, as it is consistent between all models (Figs. S8 and S9). We conclude that this movement is well defined by the experimental data. Additional experimental measurements will be necessary to determine the parameters of the spatial disorder.
Rosetta loop building (5) and relaxation protocols (6) were utilized in order to reconnect the helical domain back to the rest of the α-subunit and refine the complex within the Rosetta energy functions. In addition, the αA helix (α-subunit residues 63–90) is unkinked in the model of the activated heterotrimer–receptor complex solely for demonstrative purposes of a possible mechanism of leverage for generating the helical domain movement (see main article).


Fig. S1. The nitroxide R1 side chain.

(A) Binding of doubly spin-labeled mutants to rhodopsin in disc membranes. (B) Basal and receptor catalyzed nucleotide exchange rates for the doubly spin-labeled mutants. Assays were performed as described in Methods.
Fig. S3. (A) Background corrected dipolar evolution data for each double-labeled mutant along the activation pathway. Gray traces show fits to each individual dipolar evolution. (B) Fourier transformation of the dipolar evolution data given in A yields the dipolar spectra in B. The data are shown for each spin-labeled double mutant along the activation pathway.
Fig. S4. Normalized integral representations of the distance distributions shown in Fig. 1C of the main text. Such representations are particularly useful for visually estimating the relative populations of the distances. This is illustrated, for example, in the top panel of the 90–238 mutant; the major population is about 80%. The most probable distance for a population is estimated from the midpoint of the transition.

Fig. S5. A BLAST (Basic Local Alignment Search Tool) sequence alignment of Gt and the Gt/Gt chimera of 1GOT, which was used in comparative modeling. The sequence alignment features a single gap (red) within the N-terminal \( \alpha \)-helix of the protein. The Gt region (residues 216–294 of the 1GOT sequence) is shown in orange. The \( \alpha \)-helical domain is shown in green. The C-terminal helix and 11 residues of the opsin-bound peptide are shown in yellow and blue, respectively.
Fig. S6. Superposition of transducin’s C-terminal helix with the opsin-bound peptide ligand. (A) The opsin structure is shown as orange ribbon with the 11 residue C-terminal peptide of transducin as blue sticks (PDB ID code 3DQB). The C terminus of the α-subunit of G-1GOT in yellow has been superimposed so that residues 344–347 overlap with the first four residues of the peptide. (B) The residues from the peptide are merged with G-1GOT by replacing residues 344–347 of the α-subunit with the first four residues of the peptide.

Fig. S7. The 1,000 models with repositioned helical domain filtered by EPR score and chain break distance. (A) The models were scored for agreement with the distance measurements according to the knowledge-based potential of Hirst et al. (4). The potential provides a score between −1 (perfect agreement) to zero (no agreement). Shown is the fraction of models for which a given score is observed for each EPR measurement. (B) It is important that the docking protocol does not introduce too large of a chain break between the helical domain and the rest of the α-subunit. Shown is the fraction of models with which a given Cα–Cα distance is observed for the two cut points. The distances were calculated before the chain breaks were removed, which was accomplished by reconstructing the linker regions between the helical domain and the rest of the α-subunit (5, 6). In both A and B, gray areas have counts of zero.
Fig. S8. The 1,000 models resulting from repositioning the helical domain were hierarchically clustered. A distance cutoff between clusters of 2.0 Å results in five cluster centers. Residue 90 is shown as alpha carbon spheres to guide the eye in distinguishing the different orientations of the helical domain. The cluster centers show relatively similar placements of the helical domain. The color coding shown above is similar to Fig. 3 in the main text. The C-terminal helix of the nucleotide binding domain is shown in yellow with its last 11 amino acids colored blue.

Fig. S9. Shown is the position of the helical domain in the unbound heterotrimer as determined from crystallography (PDB ID code 1GOT) (magenta ribbon) compared to the receptor-bound $G_\alpha_i$-1GOT model (green ribbon). The relative positions of the two helical domains (model versus 1GOT structure) were determined by aligning the nucleotide binding domains of the $\alpha$-subunit (light blue ribbon) in the two structures. (Top) The $C_\alpha-C_\alpha$ distances at opposite ends of the helical domain were calculated in order to demonstrate the extent of the movement captured by the docking protocol. The distances were calculated between residues 51 and 66 (Top, dashed lines), and between residues 90 and 277 (Bottom, dashed lines). Coordinates of residues 51 and 277 outside the helical domain are used for reference. The helical domain is shown to rotate 29°. (Middle) The change in distance of residue 66 from residue 51 is 8.1 Å. (Bottom) The change in distance of residue 90 from residue 277 is 7.1 Å.
Movie S1. Animation showing the hypothesized conformational changes leading to GDP release. The crystal structure of rhodopsin before activation [red, PDB ID code 1U19 (1)] transitions to the activated state [orange, R*, PDB ID code 3DQB (2)]. The GDP-bound heterotrimer binds to R* and the helical domain of Gα(GDP) opens away from the nucleotide binding domain. The opening movement allows GDP release leading to Gα(GDP)βγ. Color scheme is Gβ, tan; Gγ, black; Gα(GDP)helical domain, green; Gα(GDP)nucleotide binding domain, gray; GDP, spheres. The animation was created using Pymol RigiMOL (Schrodinger, LLC).

Table S1. Agreement of the receptor-bound Gαi-1GOT model with experimentally measured EPR distances

<table>
<thead>
<tr>
<th>Mutant:</th>
<th>90/238</th>
<th>157/333</th>
<th>171/276</th>
<th>141/333</th>
<th>138/276</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPR experiment:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free heterotrimer</td>
<td>18 Å</td>
<td>28 Å</td>
<td>26 Å</td>
<td>33 Å</td>
<td>20 Å</td>
</tr>
<tr>
<td>Bound to activated receptor</td>
<td>38 Å</td>
<td>45 Å</td>
<td>34 Å</td>
<td>46 Å</td>
<td>34 Å</td>
</tr>
<tr>
<td>Distance change</td>
<td>20 Å</td>
<td>17 Å</td>
<td>8 Å</td>
<td>13 Å</td>
<td>14 Å</td>
</tr>
<tr>
<td>Structures:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free heterotrimer</td>
<td>11 Å</td>
<td>25 Å</td>
<td>23 Å</td>
<td>32 Å</td>
<td>16 Å</td>
</tr>
<tr>
<td>Bound to activated receptor</td>
<td>32 Å</td>
<td>40 Å</td>
<td>25 Å</td>
<td>41 Å</td>
<td>29 Å</td>
</tr>
<tr>
<td>Distance change</td>
<td>21 Å</td>
<td>15 Å</td>
<td>2 Å</td>
<td>9 Å</td>
<td>13 Å</td>
</tr>
<tr>
<td>Agreement between experiment and model according to KBP</td>
<td>–0.96</td>
<td>–0.96</td>
<td>–0.71</td>
<td>–0.96</td>
<td>–0.97</td>
</tr>
</tbody>
</table>

The EPR distances in the table are determined from the most probable distances in each distribution. The distances measured in models are measured between Cβ atoms. Distances for the free heterotrimer were calculated using the experimental crystal structure (PDB ID code 1GOT). Distances for the receptor-bound state were calculated using the Gαi-1GOT model. Distance agreement between the receptor-bound model and the EPR measurements were calculated according to the knowledge-based scoring potential (KBP) (4). Perfect agreement would be –1.0 and no agreement would be 0.0.