

RESEARCH ARTICLE

Computational Simulation of the Activation Cycle of G α Subunit in the G Protein Cycle Using an Elastic Network Model

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Abstract

Agonist-activated G protein-coupled receptors (GPCRs) interact with GDP-bound G protein heterotrimers (G $\alpha\beta\gamma$) promoting GDP/GTP exchange, which results in dissociation of G α from the receptor and G $\beta\gamma$. The GTPase activity of G α hydrolyzes GTP to GDP, and the GDP-bound G α interacts with G $\beta\gamma$, forming a GDP-bound G protein heterotrimer. The G protein cycle is allosterically modulated by conformational changes of the G α subunit. Although biochemical and biophysical methods have elucidated the structure and dynamics of G α , the precise conformational mechanisms underlying the G protein cycle are not fully understood yet. Simulation methods could help to provide additional details to gain further insight into G protein signal transduction mechanisms. In this study, using the available X-ray crystal structures of G α , we simulated the entire G protein cycle and described not only the steric features of the G α structure, but also conformational changes at each step. Each reference structure in the G protein cycle was modeled as an elastic network model and subjected to normal mode analysis. Our simulation data suggests that activated receptors trigger conformational changes of the G α subunit that are thermodynamically favorable for opening of the nucleotide-binding pocket and GDP release. Furthermore, the effects of GTP binding and hydrolysis on mobility changes of the C and N termini and switch regions are elucidated. In summary, our simulation results enabled us to provide detailed descriptions of the structural and dynamic features of the G protein cycle.

Introduction

G protein-coupled receptors (GPCRs) are transmembrane receptors that have critical roles in normal physiology and pathologies of vision, olfactory perception, metabolism, the endocrine system, neuromuscular regulation, and central nervous system (CNS) [1]. G protein heterotrimers (G $\alpha\beta\gamma$) are the canonical downstream molecules of GPCRs. Agonist binding to GPCRs induces conformational changes in the transmembrane and intracellular domains, which

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triggers interaction with G proteins [2]. Gα subunit mediates signal transduction from activated GPCRs, and is regulated by the exchange between GDP and GTP molecules in the nucleotide-binding pocket. High resolution X-ray crystal structures of GDP-or GTP-bound G proteins have revealed that the nucleotide-binding pocket is located between the Ras and the helical domains of the Gα subunit [3–12]. Various biochemical and biophysical studies have also delineated the interface between GPCRs and G proteins; this interface includes the αN helix and the C-terminus of the Gα subunit (Fig 1)[13–16].

The different conformational states of the Gα subunit during its activation cycle are illustrated in Fig 2. The Gα subunit forms a heterotrimer with the Gβγ subunit when GDP is bound, while the GDP-bound Gαβγ heterotrimer is in an inactive state [17]. Agonist-activated GPCR binds to the inactive GDP-bound Gαβγ heterotrimer [Gαβγ(GDP)], which leads to the release of GDP, thereby forming a nucleotide-free GPCR/Gαβγ complex [R-Gαβγ(0)]. In the nucleotide-free conformation, the helical domain undergoes large movement and the nucleotide-binding pocket opens [18,19]. Subsequently, GTP enters the empty nucleotide-binding pocket of the Gα subunit, which results in a closed Gα conformation and simultaneous dissociation of Gβγ and GPCR [17]. Eventually, the GTP-bound Gα subunit is converted into the active form [Gα(GTP)], which activates downstream effector proteins. Because of the GTPase activity of the Ras domain, GTP is hydrolyzed to GDP, thereby transforming the structure of Gα and increasing its affinity for Gβγ; this structure forms the initial, inactive GDP-bound Gαβγ [Gαβγ(GDP)][17].

While the conformational states of the Gα subunit in the G protein cycle have been studied in detail by X-ray crystallography, electron microscopy, electron paramagnetic resonance, hydrogen-deuterium exchange mass spectrometry, and bioluminescence resonance energy transfer [18–20], the mechanisms relating signal transduction to the conformational changes of the Gα subunit between states remain elusive. For example, the mechanism of how receptors catalyze nucleotide exchanges remains to be determined [3]. A few computational studies analyzed the G protein activation process that cannot be easily addressed by experimental study [21–27]. However, these studies on the dynamic features of G protein were limited to either an individual step within the entire G protein cycle or a specific region of the Gα subunit.

In the present study, we simulated the series of structural transitions of the Gα subunit that occur during the G protein cycle. Several critical issues were addressed: how the interaction between the receptor and the G protein leads to the release of GDP; and what the effect of GTP binding to the empty receptor/G protein complex is. We used β₂-adrenergic receptor (β₂AR) and the Gs protein as a model GPCR and G protein pair because the X-ray crystal structure of

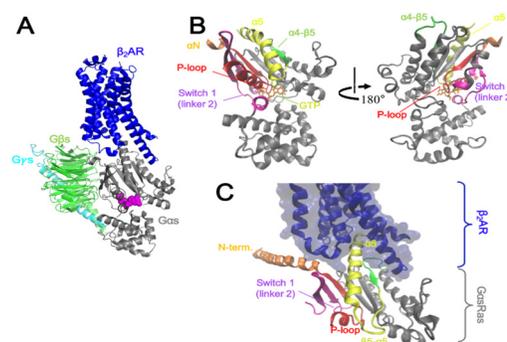


Fig 1. Ribbon diagrams of various components of the β₂AR-Gs heterodimer complex. (A) β₂AR is shown in blue, Gas in gray, Gβs in green, Gγs in cyan and GDP in magenta. (B) Gas structure represented by its major components for G protein activation. (C) β₂AR-binding interface of GasRas.

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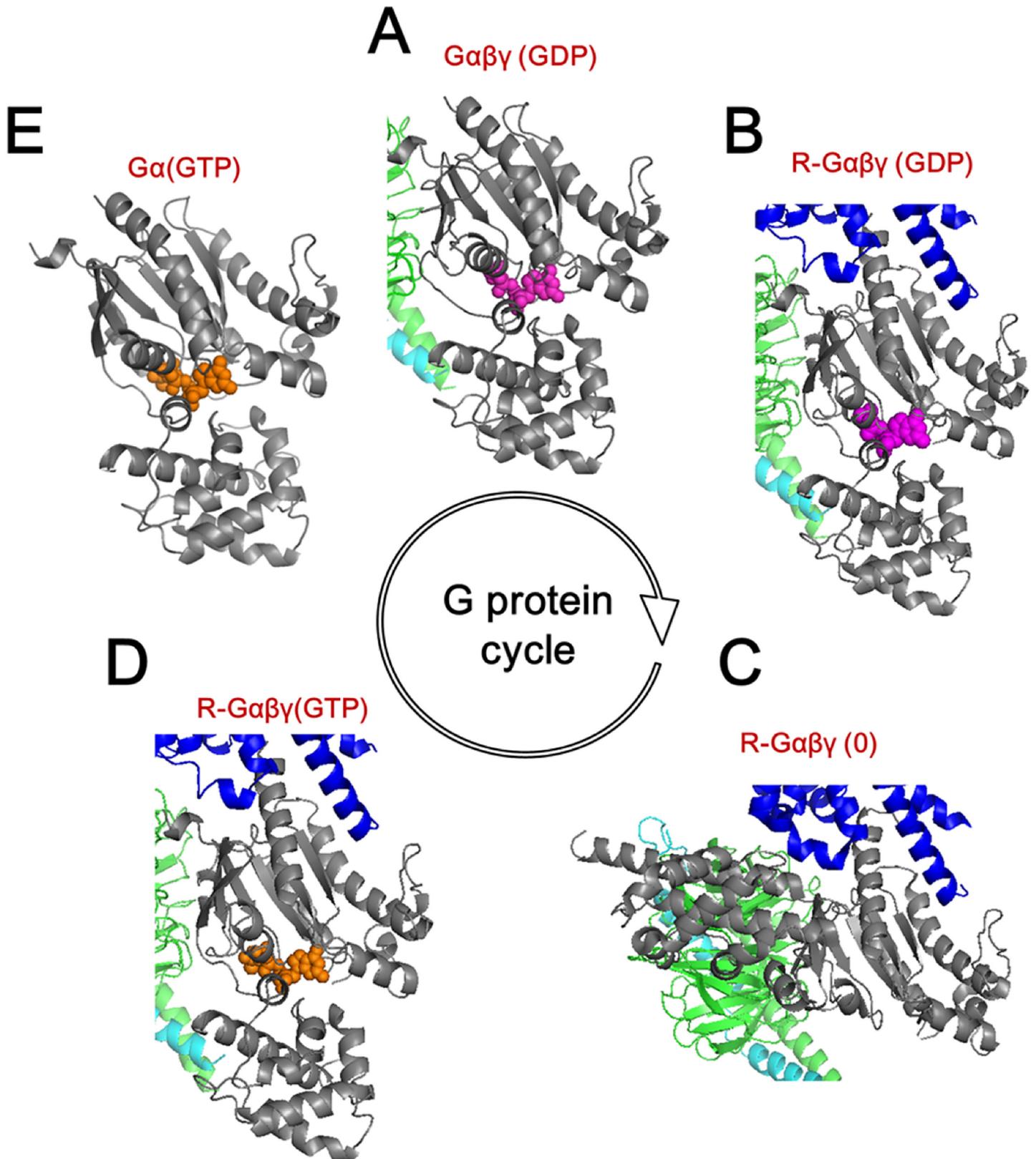


Fig 2. Overview of the G protein cycle. (A) Inactive GDP (purple)-bound Gs protein heterotrimer with α (grey), β (green), and γ (cyan) subunits. (B) Activated receptor (blue)-bound temporal state of G $\alpha\beta\gamma$ (GDP). (C) Receptor-bound fully opened G $\alpha\beta\gamma$. (D) The receptor-bound temporal state of G $\alpha\beta\gamma$ with GTP (orange). (E) Active GTP-bound G α . Receptor and G $\beta\gamma$ subunits were truncated for convenience.

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the β_2 AR-Gs complex is the only reported high-resolution structure of the GPCR-G protein complex. We divided the G protein cycle into five conformational steps, based on two existing crystal structures (Fig 2C and 2E) [10,18] and three engineered models (Fig 2A, 2B and 2D). To describe large conformational changes during the G protein cycle, normal mode analysis (NMA) was performed based on the recently developed Mass-Weighted Chemical Elastic Network Model (MWCENM) [28,29]. Mean squared fluctuation comparison between two consecutive steps and NMA for each step clarified the role of GPCR and GTP binding in the conformational changes of G α and the effects on each subsequent event in the G protein cycle.

Materials and Methods

Selection and modeling of conformational steps

Of the four types of G proteins (Gs, Gi, Gq, and G12)[30], we chose to use Gs coupled with β_2 AR as a model system. For convenience, we defined all Gs-related structures as “G” and the β_2 AR as “receptor” or “R.”

Even though only three X-ray structures of Gs are available in the Protein Data Bank (PDB) (G α (GTP γ S) [1azt.pdb], R-G $\alpha\beta\gamma$ (0) [3sn6.pdb], and adenylyl cyclase-bound G α [1azs.pdb]), we were able to model the three possible intermediate states by combining two structures (1azt.pdb and 3sn6.pdb) from other classes of G proteins (Gt and Gi).

The major geometrical differences between the inactive G $\alpha\beta\gamma$ (GDP) model and the G α (GTP γ S) model are the type of nucleotides bound (GDP/GTP) and the presence of G $\beta\gamma$. Because the structure of Gi has been defined for both forms, inactive and active [13], we adopted inactive and active Gi structure to model the structure of GDP-bound G $\alpha\beta\gamma$ heterotrimer. By applying these topological differences, we established an engineered G $\alpha\beta\gamma$ (GDP) model (Fig 2A). The sequence of G α s was maintained; only the topological information from G α i was adopted. This engineered structure was refined by the energy minimization package in Swiss-PDB Viewer [31]. A detailed description of how the engineered G $\alpha\beta\gamma$ (GDP) model was made is provided in supplementary information (See S1 Fig).

For the engineered R-G $\alpha\beta\gamma$ (GDP) model, the G α model was constructed by combining the G α sRas domain from R-G $\alpha\beta\gamma$ (0) (PDB ID: 3SN6) and the G α sAH domain from G $\alpha\beta\gamma$ (GDP). We made two assumptions. First, when the receptor is bound to G α , the G α sRas domain undergoes a conformational change by forming an interface with the receptor while the G α sAH domain maintains its structure. Second, during the subsequent opening motion of G α , G α sRas becomes firmly attached to the activated β_2 AR and has no degree of mobility [19]. Moreover, G α sAH, except in the linker 2 region, also undergoes a rigid body movement. Therefore, the intermediate R-G $\alpha\beta\gamma$ (GDP) model was generated based on the G $\alpha\beta\gamma$ (GDP) model with the replacement of G α sRas by the corresponding region of R-G $\alpha\beta\gamma$ (0) (See S2 Fig). This engineered structure was stabilized by energy minimization as described above.

The R-G $\alpha\beta\gamma$ (GTP) model (Fig 2D) is an energetically unfavorable structure but is a plausible intermediate state between R-G $\alpha\beta\gamma$ (0) (Fig 2C) and G α (GTP) (Fig 2E). The basic concept for rendering this model is the same as described for the previous case, i.e., the two subdomains of G α show rigid body motion with uniform conformation during the closing motion of G α . Therefore, this engineered complex, R-G $\alpha\beta\gamma$ (GTP), is very similar to R-G $\alpha\beta\gamma$ (GDP) except for the type of nucleotides. Energy minimization is subsequently conducted to obtain the refined structure.

MWCENM with NMA

As an elastic network models (ENM), MWCENM has several advantages with regard to simulation accuracy and computational efficiency because not only the elastic network optimized with various stiffness values according to the types of chemical interaction as listed in [S1 Table](#), but the inertial effect of each amino acid as a concentrated mass at the representative α-carbon is taken into account [28]. [S3 Fig](#) compares traditional distance-cutoff based ENM with MWCENM. In our simulation model, nucleotides in Gα model (GDP/GTP) were coarse-grained with mass-weighted representative atoms tightly connected to each other by covalent bonds, and to surrounding Gα atoms with hydrogen bonds and van der Waals (vdW) forces (see [S4 Fig](#)). Moreover, all the constraints from the receptor and Gβγ subunit are applied to the Gα model in the same manner as listed in [S1 Table](#), which lower the computation burden as same level of single Gα model. This method can generate more plausible conformational changes than traditional ENMs due to use of a realistic connection matrix, especially in the closed form of proteins such as Gα in the B structure model of Gα protein. Moreover, higher b-factor correlation and its invariance to cutoff distance (see [S5 Fig](#)) supports our choice of MWCENM as our simulation model [28]. In MWCENM, the total kinetic energy in a network of n point masses is given by

$$T = \frac{1}{2} \sum_{i=1}^n m_i \|\dot{x}_i(t)\|^2 \quad (1)$$

where m_i corresponds to a specific concentrated mass value according to the amino acid type, $x_i(t)$ is the position of the i th atom at time t , and dot represents a time derivative. In addition, the total potential energy is given by:

$$V = \frac{1}{2} \sum_{i=1}^{n-1} \sum_{j=i+1}^n k_{i,j} \{ \|x_i(t) - x_j(t)\| - \|x_i(0) - x_j(0)\| \}^2 \quad (2)$$

where $k_{i,j}$ is a spring constant between the i th and j th atom based on [S1 Table](#). After the construction of MWCENM, we used NMA to study the dynamics of the target proteins. In NMA, the equation of motion is derived from Lagrangian mechanics as follows:

$$\frac{d}{dt} \left(\frac{\partial L}{\partial \dot{\delta}_i} \right) - \frac{\partial L}{\partial \delta} = 0 \quad (3)$$

where $L = T - V$ and δ_i is the i th component of the generalized deviation vector $\delta \in \mathbb{R}^{3N}$. This physically represents a small fluctuation from the initial position of the i th atom $x_i(0)$ so that $x_i(t) = x_i(0) + \delta_i(t)$. From Eqs 1 to 3, we can derive the following equation of motion (Its full derivation is available at Ref. [32]):

$$M\ddot{\delta} + K\delta = 0 \quad (4)$$

where M is the mass matrix (diagonal matrix) and K is the stiffness (Hessian) matrix defined by $K_{i,j} = \partial^2 V / \partial x_i \partial x_j$. From this equation of motion, one can calculate eigenvectors and eigenvalues by diagonalization of the mass-weighted Hessian matrix, $M^{-1/2} K M^{-1/2}$, for a given protein system, which describe the vibrational frequencies and corresponding vibration modes, respectively [33].

Elastic Network Interpolation

We used the ENI method to generate the anharmonic pathways for conformational transitions between two metastable conformations (closed R-Gαβγ(GDP) and open R-Gαβγ(0)) [34,35]. In this study, we generated intermediate conformations by solving the following potential-like

cost function, in which the initial conformation (closed R-Gαβγ(GDP)) is deformed toward the target conformation (open R-Gαβγ(0)) to minimize this function:

$$C(\delta) = \frac{1}{2} \sum_{i=1}^{n-1} \sum_{j=i+1}^n \gamma_{ij} \{ \|x_i + \delta_i - x_j - \delta_j\| - l_{ij} \}^2 \quad (5)$$

Here, l_{ij} is the ideal length at a certain intermediate state determined by distance interpolation. l_{ij} can be expressed as

$$l_{ij} = (1 - \alpha) \|x_i - x_j\| + \alpha \|\chi_i - \chi_j\| \quad (6)$$

where α is the coefficient that specifies the extent to which a given state is along the pathway from $\{x_i\}$ towards the target conformation $\{\chi_i\}$. The linking matrix γ is similar to that of MWCENM, but different because it is defined as a union matrix between two linking matrices for $\{x_i\}$ and $\{\chi_i\}$ in the sense that γ_{ij} has a value of 1 when residues i and j are within the cutoff range of 12 Å in either conformation. For more details, refer to reference [32]. Energetically, intermediate states show the higher energy values than those of the given two conformations. The pathway generated from ENI was validated using overlap values that were obtained by comparison with the results from NMA.

Matlab codes for both MWCENM and ENI are available for download on the online morph server KOSMOS (<http://bioengineering.skku.ac.kr/kosmos>) [36].

Overlap Value

The overlap value is widely used as a measure of similarity between the direction of conformational change and the direction given by normal mode. The general overlap value [37] is defined as

$$O_j = \frac{|\sum_{i=1}^{3N} a_{ij} \Delta r_i|}{|\sum_{i=1}^{3N} a_{ij}^2 \sum_{i=1}^{3N} \Delta r_i^2|^{1/2}} \quad (7)$$

where O_j is the overlap value between the conformational change vector and the j^{th} normal mode vector. a_{ij} is the j^{th} eigenvector of the i^{th} representative atom, and r_i is the displacement vector of the i^{th} atom after two given structures are superimposed. A higher overlap value indicates higher similarity and modeling accuracy. An overlap value of 1 indicates that the computed normal mode vectors are perfectly matched to the direction of conformational change. However, in a large deformation such as the global hinge motion of Gα, the conformational change vector is not comparable with the normal mode vector because it does not represent the instantaneous direction any more [38]. Alternatively, one can compare it with normal mode vectors obtained from each intermediate conformation between two endpoint structures.

Results and Discussion

1. Conformational change of Gαs upon β₂AR binding (A→B→C in Fig 2)

1-1. Possible binding sequence of Gαs to β₂AR. When the Gs subunit interacts with activated β₂AR, several parts of Gαs undergo structural changes in an energetically favorable direction. In particular, the αN helix (the N-terminus) and the C-terminus of Gα are well-mapped GPCR-binding sites that act as two conduits between the interface of the activated GPCR and the nucleotide-binding pocket [16,39–42]. Previous studies also highlighted these two regions as the key determinants of conformational changes in Gα when a GPCR-binding event occurs [18,43]. However, the binding sequence is still under debate. Herrmann and his coworkers suggested that rhodopsin first interacts with lipid moieties on the N-terminus of transducin and

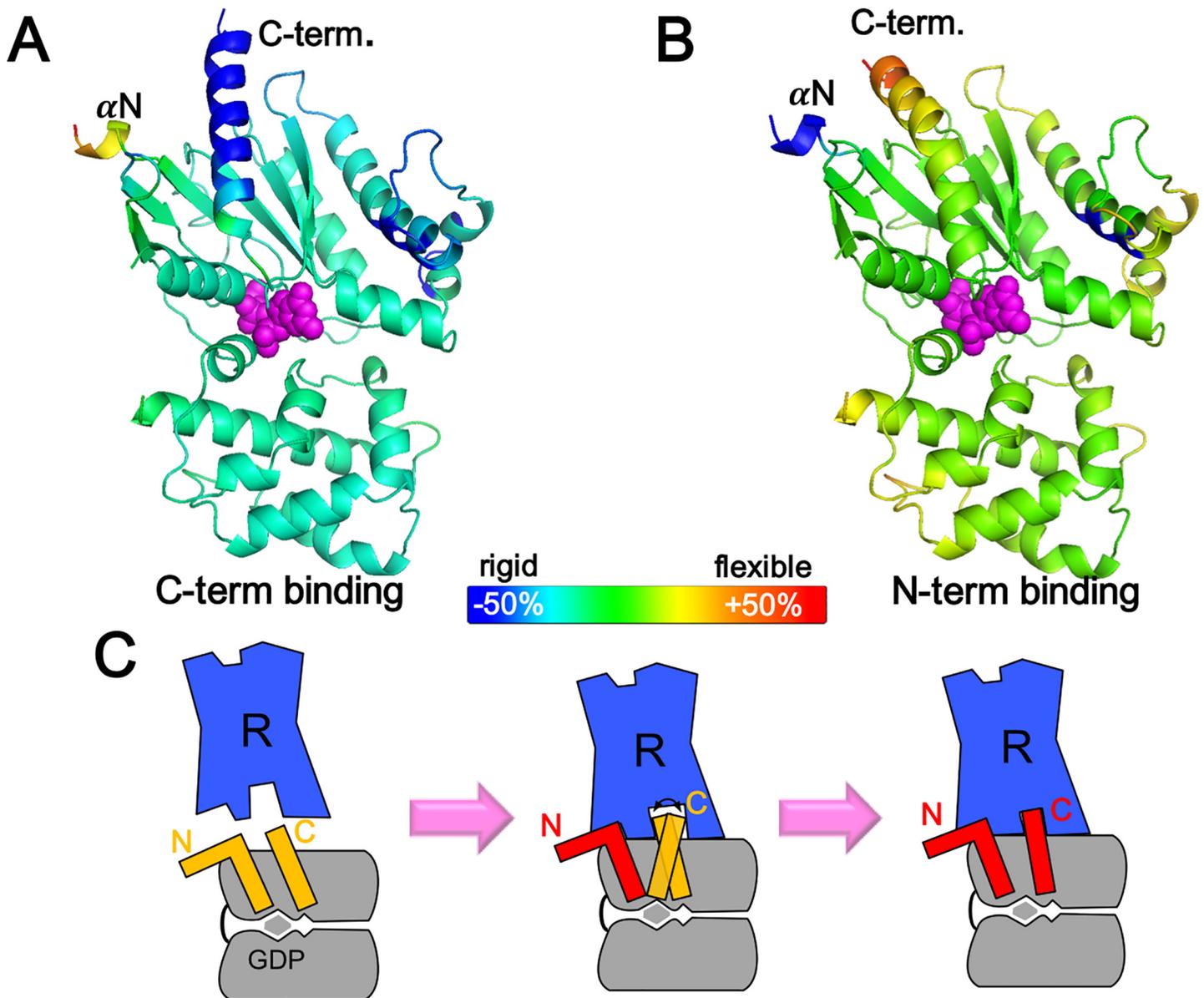


Fig 3. Mobility change of Gαs after the initial binding to β₂AR either at the C-terminus (A) or the N-terminus (B). The theoretical value at each residue was calculated by mean squared fluctuation between Gαβγ(GDP) and pre-R-Gαβγ(GDP) in percentile and is displayed according to the indicated color map. The residue in blue (red) is considered to become rigid (flexible). (C) Schematic of the proposed binding event between Gαs (gray) and β₂AR (blue). Activated β₂AR may initially be bound to the N terminus of Gα, leading to a relatively flexible C terminus, which increases the probability that it will find its proper binding position to the receptor. Eventually, this binding event is completed by the C terminal binding.

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subsequently with the αN/β1 hinge of Gα, thereby making the C-terminus of Gα available for rhodopsin binding [44,45]. However, sequential interactions may differ according to the type of G protein and the experimental systems.

In this study, we conjectured the binding sequence of the initial interaction between Gαs and β₂AR based on the change in mean squared fluctuation. As shown in Fig 3, we assumed two possible pre-R-Gαβγ(GDP) structures, one in which the N-terminus was bound to the activated GPCR while the C-terminus remained unbound, and vice versa. It should be noted that the majority of αN is missing from existing high-resolution structures of nucleotide-

bound Gs proteins. However, we conducted our analysis as described below because the β_2 AR binding interface at the N-terminus is the α N/ β 1 hinge, which is defined in the high-resolution structures. Each pre-R-G $\alpha\beta\gamma$ (GDP) was constructed based on the G $\alpha\beta\gamma$ (GDP) structure with replacement of one terminus by the corresponding part from the modeled R-G $\alpha\beta\gamma$ (GDP) structure (the modeling process is described in the Materials and Methods). Only the replaced terminus was assumed to be connected to the receptor. Our basic premise is that if the mean squared fluctuation value of the target region increases, a binding event is very likely to occur in that region. Under the condition that one part of a protein is already bound to the target, higher mobility of the other part would better facilitate completion of the next binding event to the fixed target. In the conformational selection model, which is one of the hypotheses to explain protein-protein docking mechanisms like an induced fit, a flexible structure has a high likelihood of accessing target conformations due to wide ensemble of conformations [46]. It also makes sense in that a highly flexible structure usually has a low energy barrier in an energy landscape, which facilitates conformational changes [47].

In both cases, binding to the first terminus leads to high mobility of the other terminus, which indicates that once either terminus binds to the receptor, sequential binding of the other terminus occurs more easily. Even though this does not clearly indicate the most favorable binding sequence, we assume that initial binding involves the N-terminus, because initial binding at the N-terminus leads to more flexibility in the C-terminus than vice versa (Fig 3).

The average decrements in mean squared fluctuation upon initial binding of the C-terminus and N-terminus was 12.9% and 7.6%, respectively. Even though the initial bindings of either terminus decreased the flexibility of the overall structure, the decrease in mean squared fluctuation was much higher for initial C-terminus binding. This result implies that initial binding of C-terminus would impose more constraints on the mobility of G α sRas, so that it would be much harder to bind to the receptor or to release GDP compared to the case of initial N-terminus binding. Based on this, we hypothesize that the N-terminus interacts first with the receptor and then the C-terminus binds. This proposed binding event is summarized in Fig 3.

1–2. Structural change of G α s upon receptor binding–GDP release mechanism 1. It is essential to understand the structural changes of G α upon coupling to the receptor. In fact, the region with the highest structural change is considered the greatest contributor to mutual binding effects. Direct structural comparison of G $\alpha\beta\gamma$ (GDP) and R-G $\alpha\beta\gamma$ (GDP) revealed specific regions known to be important for G α s and β_2 AR coupling. As described above, G α s underwent a dramatic conformational change at the C and N-termini of G α sRas, the interfaces for the interaction with β_2 AR (Fig 4A and 4B). Moreover, the α 5 helix, which undergoes the most remarkable change [48], rotated and translated into the proximity of the α 4- β 6 under the uniform intrinsic conformation shown in a previous study (Fig 4B and 4C)[2]. As a result, it formed a new strong interaction with the α 4- β 6 loop (Fig 4C, red dotted lines).

Other perturbed regions, including the G α sAH domain, are quantitatively defined in Fig 4B. Interestingly, the nucleotide-binding pocket consisting of β 6- α 5, the P-loop, and switch 2 also showed notable structural changes related to the release of GDP (Fig 4B and 4D)[49]. Because of the distortion within the β 6- α 5 loop induced by the upward movement of the α 5 helix away from the GDP molecule, the P-loop moved very close to GDP, i.e., acted like a push button in the range of repulsive vdW forces. Another possible scenario of GDP release in this pocket region is that a concerted motion is triggered by the P-loop movement and then the subsequent distortion of the α 1 helix leads to disconnection with β 6- α 5 and GDP [50]. It is likely that these concerted motions may be the first step of GDP release from the nucleotide-binding pocket. These hypotheses provide some support to a previous study in which receptor binding was shown to disrupt the interaction between β 6- α 5, the α F helix, and the α A helix [19].

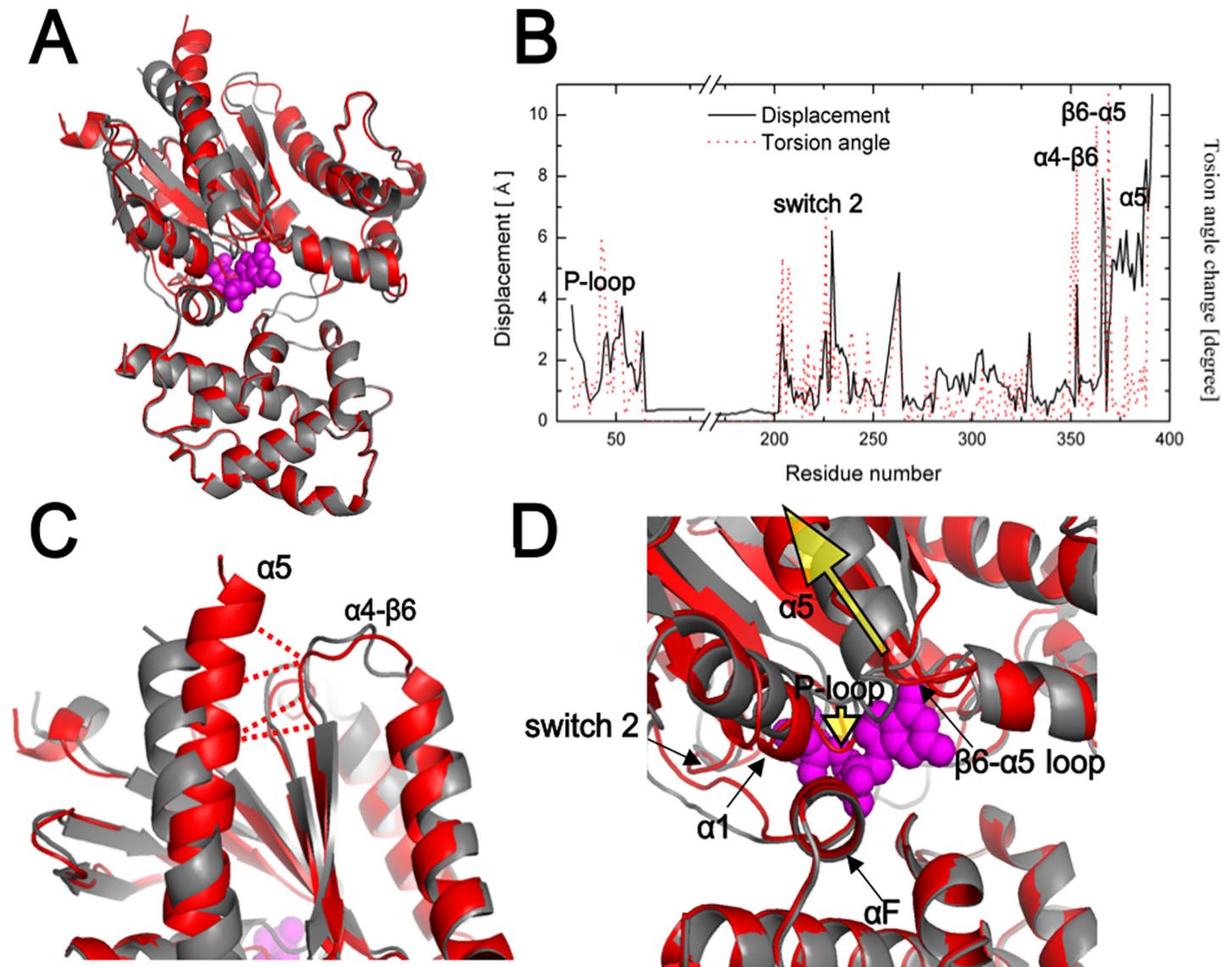


Fig 4. Topological changes of the Gas protein upon β_2 AR binding. The Gas structures in the $G\alpha\beta\gamma$ (GDP) and R- $G\alpha\beta\gamma$ (GDP) states are colored in gray and red, respectively. (A) Comparison of the structures of G α with and without β_2 AR. Compared to the GasAH domain, the GasRas domain shows marked displacement. (B) Topological change (displacement and torsional angle) of Gas upon β_2 AR binding. (C) The receptor-binding interface of Gas. The distance between the $\alpha 5$ helix and $\alpha 4$ - $\beta 6$ decreases to 7 Å, which is within the interaction range. Red dotted lines represent new interactions between $\alpha 5$ helix and $\alpha 4$ - $\beta 6$ (D) Nucleotide-binding pocket (switch 2, P-loop, $\beta 6$ - $\alpha 5$ loop, and $\alpha 5$ helix). A significant change in the topology occurs near the nucleotide-binding pocket. The directions of movement of the $\alpha 5$ helix and P-loop are indicated by the yellow arrows.

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1–3. Dynamic features of G α s at R-G $\alpha\beta\gamma$ (0)–GDP release mechanism 2. The above-mentioned β_2 AR coupling to G α s caused geometrical changes and perturbation of relevant connection states. On the basis of this force field difference and according to the topological changes, we analyzed the dynamic features of G α s coupled to β_2 AR to determine the sequential GDP releasing mechanism. Comparison of the mean squared fluctuation values of $G\alpha\beta\gamma$ (GDP) and R- $G\alpha\beta\gamma$ (GDP) clearly demonstrated mobility changes at the atomic level (Fig 5). A mobility change was defined as the difference between two mean squared fluctuation values in percentile. It is not surprising that the major receptor binding regions, which include the C-

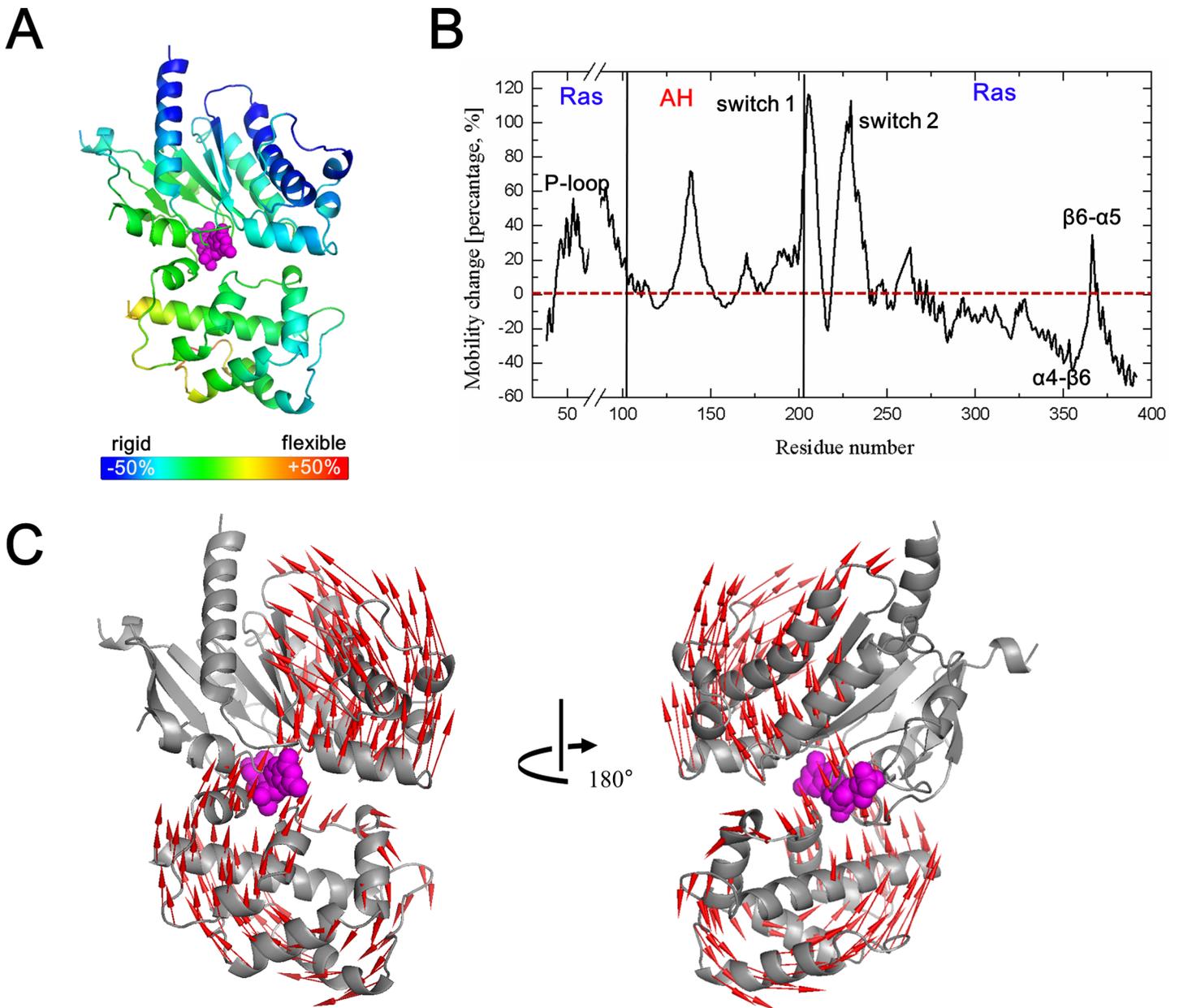


Fig 5. Dynamic features of the R-Gαβγ(GDP) complex. (A and B) Change in the mean squared fluctuation of Gas upon β₂AR binding. Mobility changes of switches 1 and 2, P-loop, β₆-α₅ loop, α₄-β₆ loop, and α₅ helix are marked here. Overall, the GasRas domain loses its intrinsic flexibility due to direct binding to the receptor. (C) Shape of R-Gαβγ(GDP) in the fourth normal mode. The open motion corresponding to the conformational change direction in Gα is shown.

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terminus, α_N, and α₄-β₆, showed decreased mean squared fluctuation values due to newly introduced constraints upon receptor interaction (Fig 5A and 5B)[2,10,51,52]. The most constrained regions with the lowest flexibility would be the most sustainable for the sequential reaction and interactions among all residues in Gα. These results are in good agreement with a previous study where structural mass spectrometry was employed to demonstrate that the C-terminus is immobilized by interaction with activated receptors [53]. In contrast, highly mobile regions were concentrated at the nucleotide-binding pocket and surrounding regions (Fig 5A

and 5B). Not only the remarkable structural changes, as described in the previous section, but also the high mobility of the P-loop, $\beta 6$ - $\alpha 5$ loop, and switch 2 demonstrates that β_2 AR binding ultimately leads to the release of GDP through perturbation of a specific region in the nucleotide-binding pocket [54].

Another interesting region with a high mean squared fluctuation value was the switch 1 (linker 2) region (Fig 5A and 5B), which connects two G α subdomains. The high mobility of the linker in G α provides a basis for the sequential opening motion [55–57], which is another mechanism for receptor-mediated release of GDP. NMA of R-G $\alpha\beta\gamma$ (GDP) yielded a precise opening motion of G α in the fourth mode (Fig 5C). In this dominant mode, as expected, the G α sAH and G α sRas domains had moved in opposite directions to each other with a hinge point in the linker region, which is favorable for the release of GDP. In addition, the exit route caused by this opening motion showed good agreement with previous studies that reported that GDP release occurs on the phosphate-binding side [49,58]. In the case of G $\alpha\beta\gamma$ (GDP), bending and twist modes with unchanged nucleotide-binding pocket dominantly occur at fourth and fifth modes. Because of the very tight binding between the sub-domains, the nucleotide-binding pocket was completely blocked (“like a pearl in the shell”), making it difficult for GDP to exit. Similar results were observed in the G α structure without GDP (see S6 Fig).

An open question is whether the dissociation of subdomains is the cause or effect of GDP release [59]. Because the solvent accessible area at the nucleotide pocket of R-G $\alpha\beta\gamma$ (GDP) was slightly increased in dominant opening mode (see S2 Table), in contrast to the twist and bending modes of G $\alpha\beta\gamma$ (GDP) and G $\alpha\beta\gamma$ (0), the opening mode was topologically more favorable than the other modes for the release of GDP. In addition, normal mode results of the R-G $\alpha\beta\gamma$ (0) complex were very similar to those of R-G $\alpha\beta\gamma$ (GDP) (see S6 Fig). This strongly suggests that the necessary condition for the opening motion of G α is not the absence of GDP, but the structural change caused by β_2 AR binding. Therefore, the release of GDP may not trigger the dissociation of G α but it just requires the receptor binding first to increase the GDP solvent-accessible surface area.

A recent simulation study also suggested that domain opening occurs spontaneously even in the absence of GDP and that domain opening is necessary but not sufficient for rapid GDP release because the receptor-induced structural rearrangement in the Ras domain of G α is also needed for GDP release [60]. Another study reported that water molecules could enter the nucleotide-binding pocket and interact with GDP or surrounding residues during opening of the nucleotide-binding pocket [49]. In conclusion, multiple mechanisms are likely to contribute to receptor-induced GDP release. Structural and dynamical changes in the nucleotide-binding pocket may cooperate with the flexible linker region, which would weaken the interaction between G α and GDP and also expose the bound GDP to the solvent.

2. A feasible pathway for G α conformational transition (B \rightarrow C in Fig 2)

As reported previously, R-G $\alpha\beta\gamma$ (0) shows a large conformational change of about 130° rotation with respect to R-G $\alpha\beta\gamma$ (GDP) [58]. This observation contributed to our decision to study the conformational transitions between the two states to understand the underlying mechanism. We defined the states of R-G $\alpha\beta\gamma$ (GDP) and R-G $\alpha\beta\gamma$ (0) as closed and open forms of G α , respectively, and generated a series of intermediate conformations using elastic network interpolation (ENI) (see Materials and Methods section).

Simulation results for the conformational transition from the closed to the open G α structure are shown in Fig 6A. Despite the large conformational change of G α , the ENI method reliably generated a pathway of feasible and continuous intermediates showing smooth changes in root-mean-square deviation and cost function values (Fig 6B). Fig 6C shows the mobility changes of each intermediate conformation relative to the closed G α conformation. The

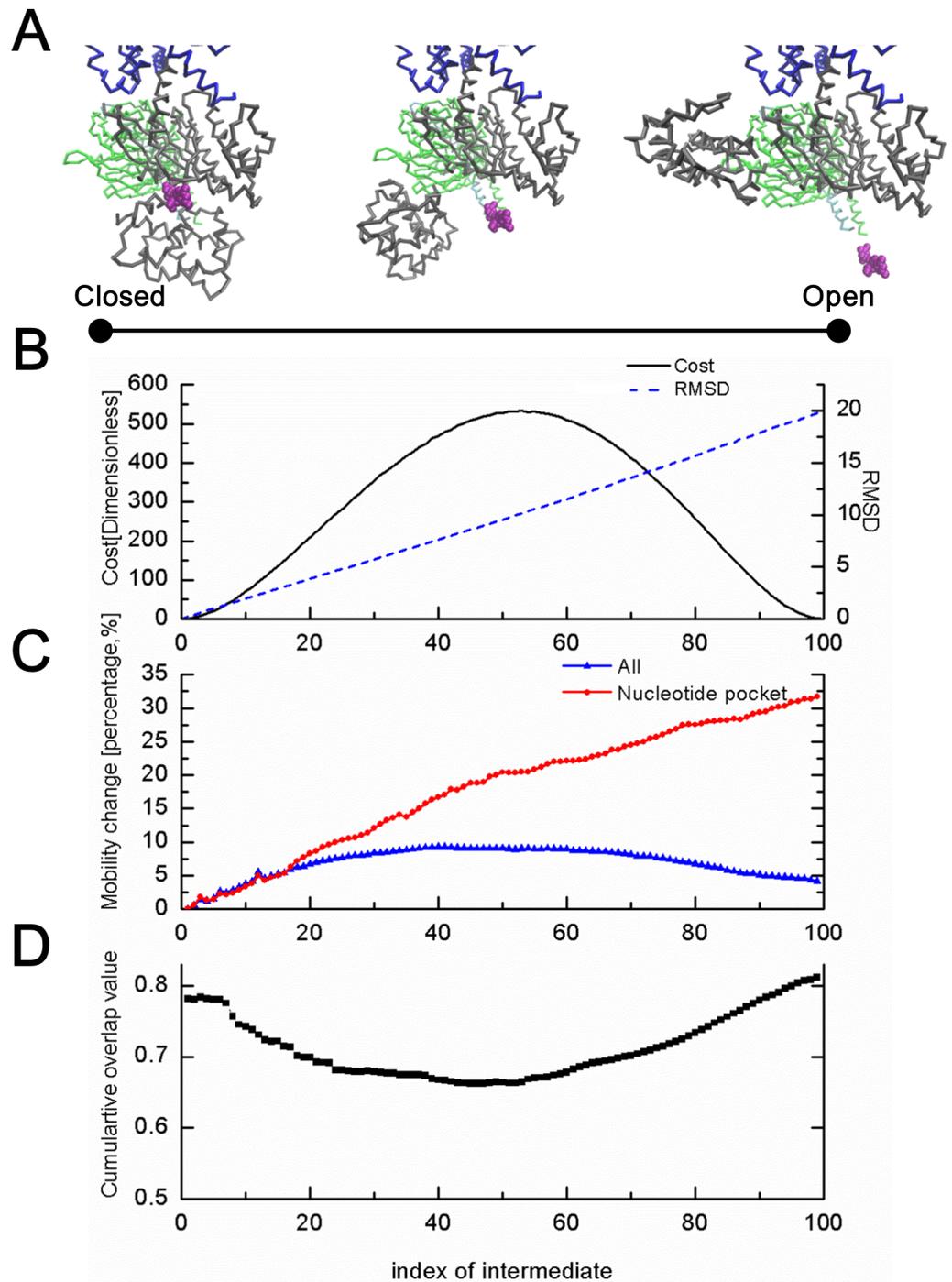


Fig 6. Simulation results of intermediate conformational states between the closed and open Gas structures obtained using elastic network interpolation (ENI). (A) Possible states of Gas. A large conformational change was observed around the GasAH domain showing the swing motion with respect to the fixed GasRas domain. (B) Root-mean-square deviation and cost function values of all intermediate conformational states with respect to the initial, closed Gas structure. The smooth changes in both values represent the conformational changes and fluctuations in energy between the two reference structures of Gas well. (C) Mobility changes during the transition. Intermediate conformations were more flexible than the two endpoint structures. Particularly, the mobility of the nucleotide-binding pocket region kept increasing during the transition. (D) Verification of the ENI pathway of the Gas structure using the cumulative overlap values between the set of first 5% lowest normal modes and the directional vector to the target structure from each intermediate.

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intermediate conformations always show higher mobility and the highest one is observed near the 40th intermediate conformation, which is a quantitative indication of being more flexible and excited intermediates to overcome the ground state barrier composed of the two end conformations. In particular, the nucleotide-binding pocket became very flexible (approximately 33% increase in mobility), compared to the overall intermediate structure, providing space for easy release of GDP (Fig 6C). In addition, the high overlap values between normal modes and the directional vector to the target conformation from each intermediate conformation gave quantitative support to our simulation results. In this comparison, the first 5% lowest normal modes of each intermediate were used to compute the cumulative square overlap (CSO). Fig 6D shows that the overlap value, which quantifies the ability of the proposed transition pathway to follow the target direction to the R-G $\alpha\beta\gamma(0)$ conformation, was higher than 0.65 over the entire pathway.

In addition, the virtual torsion angle difference between R-G $\alpha\beta\gamma(\text{GDP})$ and R-G $\alpha\beta\gamma(0)$ was calculated (S7 Fig). Significant angle changes were observed between residues 58 and 88 and around residue 200, both of which are known as linker regions that connect the G α sAH and G α sRas domains (yellow dotted and ribbon in S7 Fig respectively). In contrast, the other parts of the G α s, including all secondary structures, changed only slightly. These results indicate that the flexible linker regions induced the large conformational changes of G α s without any intra-conformational change in the two subdomains.

3. Conformational change of G α s upon GTP binding (C \rightarrow D \rightarrow E in Fig 2)

It is apparent that GTP allows the G protein cycle to continue by dissociating the receptor and G $\beta\gamma$ from G α ; however, the GTP-induced conformational changes in G α are not yet clearly understood. In this section, we focus on the changes in the mobility of G α upon GTP binding. Comparison of the calculated mean squared fluctuation values between R-G $\alpha\beta\gamma(0)$ and R-G $\alpha\beta\gamma(\text{GTP})$ (Fig 7A) specifically demonstrated the changes in mobility of G α , which can explain the mechanism of dissociation of the receptor and G $\beta\gamma$. First, major regions of the GPCR-G protein interface, such as α N, the C terminus, and the α 4- β 6 loop were disordered to enable decoupling of β_2 AR. Second, not large but relatively significant increases in mean squared fluctuation values in the switch 2 region contributed to the high mobility required for the dissociation of G $\beta\gamma$, which resembles the environment of residues in switch 2 illuminated by site-specific fluorescence [61]. Interestingly, the mean squared fluctuation values decreased in two major regions, the P loop and switch 1 (linker 2) corresponding to the nucleotide-binding pocket and the critical connection loop for the two G α subdomains, respectively. To retain the closed form of G α , these regions need to be stabilized by binding of GTP [54]. In conclusion, mobility changes of G α caused by GTP binding enabled subsequent conformational changes to the closed form of G α resulting in dissociation of the receptor and G $\beta\gamma$.

4. GTP hydrolysis and the effect of G $\beta\gamma$ binding on G α s (E \rightarrow A in Fig 2)

After dissociation of G $\beta\gamma$ and β_2 AR from G α s(GTP), the fully active GTP-bound G α can interact with downstream effector proteins. Activation is terminated by the hydrolysis of GTP to GDP through the intrinsic GTPase activity of G α . Moreover, the following G $\alpha(\text{GDP})$ structure is known to show a conformational change which subsequently facilitates the binding of G $\beta\gamma$. Structural comparison between G $\alpha\beta\gamma$ heterotrimer [4,6] and G $\alpha(\text{GTP}\gamma\text{s})$ [9,12] revealed that the G $\beta\gamma$ binding region in G α forms a different structure from GDP-bound state to GTP-bound state; with GTP, the G $\beta\gamma$ binding region in G α alters so that G $\beta\gamma$ cannot interact with G α . To determine the effects of GTP hydrolysis and sequential G $\beta\gamma$ binding to G α , the mean squared fluctuation values of G $\alpha(\text{GTP})$ and G $\alpha\beta\gamma(\text{GDP})$ were compared. As expected, mobility (Fig 7B) decreased in switches 1 and 2 because of the generation of an additional constraint

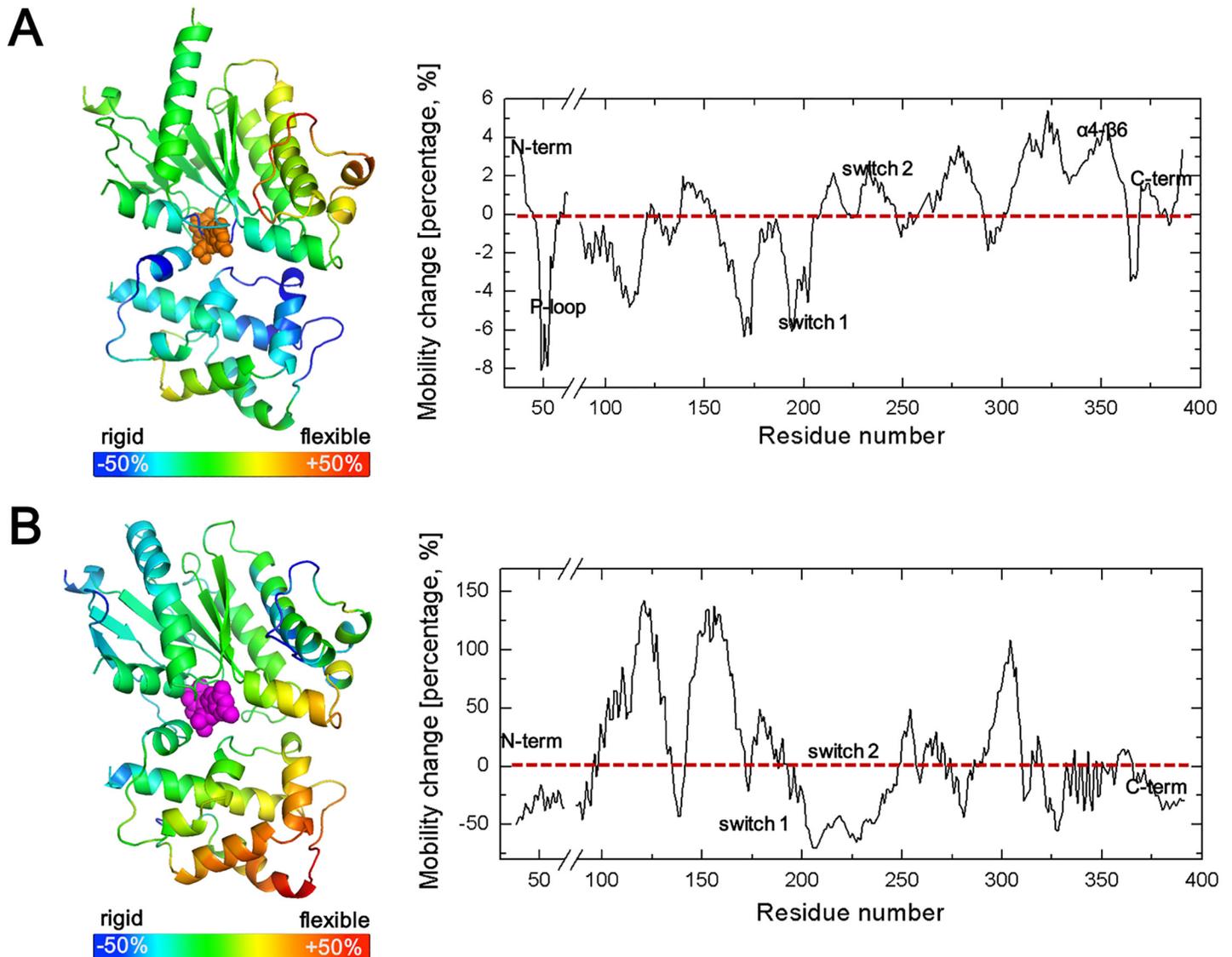


Fig 7. Role of GTP and its hydrolysis in Gα mobility. (A) Change in mean squared fluctuation of Gα in R-Gαβγ(0) following the addition of GTP. Receptor binding regions (N terminus, C terminus, α4-β6 loop) and Gβγ binding regions (switch 2) became highly mobile for easy dissociation of bound structures. In contrast, the mean squared fluctuation values of nucleotide-binding pocket regions (P-loop, switch 1) decreased, thereby blocking the opening motion between the two sub-domains of Gα. (B) Change in mean squared fluctuation of Gα following formation of Gαβγ(GDP) relative to Gα in Gα(GTP). The change in mobility reflects the effects that GTP hydrolysis and Gβγ binding have on Gα.

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with Gβγ. Interestingly, the mobility of the C and N termini decreased, which contributed to sequential β₂AR binding. Suppose that two uncoupled proteins randomly vibrate in solution. In this case, less fluctuation (i.e., low mobility) would be much more favorable for protein-protein interaction. As a result, GTP hydrolysis and Gβγ binding both play important roles in Gα (GDP) stabilization, which is required for the next signaling cycle.

Conclusions

We determined various static conformations of Gα during the G protein signaling cycle based on analysis of X-ray crystallography data. In particular, the recently published crystal structure

of an active state ternary complex, composed of agonist-occupied monomeric β_2 AR and nucleotide-free Gs heterotrimer, contributed to a better understanding of the G α cycle [18]. In our simulation, based on the elastic network model (ENM), we analyzed these critical intermediate structures and connected them with dynamic processes. To gain insight into the role of protein and nucleotide binding in the conformational changes of G α , we hypothesized the existence of the intermediate complexes of R-G $\alpha\beta\gamma$ (GDP) and R-G $\alpha\beta\gamma$ (GTP), which are virtual but plausible states in the G protein cycle. These intermediate structures might exist in nature, but they have not yet been reported [62].

Comparison of the mobility and structure of G $\alpha\beta\gamma$ (GDP) and R-G $\alpha\beta\gamma$ (GDP) showed that both N and C termini of G α act as binding sites for β_2 AR and conformational changes in the nucleotide-binding pocket of G α induce the release of GDP. Furthermore, our data indicated that, in terms of mutual dynamic mobility, the GPCR-G protein binding event preferentially occurs at the N terminus first, and then the C terminus. Topological changes, including perturbation in the nucleotide-binding pocket, provide a mechanism for how the receptor alters G α to release GDP. Concerted motions of distortion within the β_6 - α_5 loop and P loop movement are the first steps for opening the tightly closed pocket to allow nucleotide exchange.

Another interesting observation is collective motion between G α sAH and G α sRas. The opening motion at the fourth mode shape of R-G $\alpha\beta\gamma$ (GDP) indicated that the substantial decrease in the linking numbers around the nucleotide-binding pocket results in a large conformational change and also determined its opening direction at the phosphate-binding side, which was proposed in a recent study using targeted molecular dynamics [58]. The high overlap values between the NM and ENI results of G α validate our simulation results. However, our finding that GDP affects the dynamic features of G α is contradictory to previous research [55]. Regardless of the presence of GDP, the G α structure bound to β_2 AR would be likely to show almost identical inter-domain dynamics, which indicates that GDP does not only stabilize sub-domain interactions but is consequentially released from G α after opening of the nucleotide-binding pocket, which exposes its solvent-accessible surface. Regardless of whether β_2 AR binding event triggers the release of GDP directly or indirectly, it is unlikely that GDP causes large structural or dynamical changes in G α .

Our ENI simulations also provided a possible pathway from the closed R-G $\alpha\beta\gamma$ (GDP) complex to the fully opened R-G $\alpha\beta\gamma$ (0) complex. In this process, the nucleotide-binding pocket is continuously and linearly exposed to the GDP-solvent area, increasing the possibility of GDP release. In addition, only the linker region between the two sub-domains of G α changes in conformation, similar to a hinge point in the middle of two rigid bodies. Based on this result, we predict that the G protein signal cycle can potentially be regulated or inhibited by rendering this region flexible (S8 Fig).

GDP/GTP exchange was also studied by mean squared fluctuation calculations. Despite the small size of the GTP molecule, additional contacts between G α and GTP could contribute to closing of the nucleotide-binding pocket and simultaneously dissociating of β_2 AR and G $\beta\gamma$ because of the high flexibility at the receptor- and G $\beta\gamma$ -binding interfaces of G α .

As the last step in the G protein signaling cycle, GTP hydrolysis and G $\beta\gamma$ binding to G α are thought to contribute to subsequent β_2 AR binding by decreasing the mobility of the N and C termini.

In conclusion, by studying the static and dynamic characteristics of the G α structure, we gained novel structural insights into the mechanisms of G protein signaling. Even though some sequential events, such as the structural consequences of GTP/GDP binding to R-G $\alpha\beta\gamma$ (0), are still under debate, we described for the first time the entire G protein signaling cycle (see [S1 Video](#)) with our ENM-based simulation model.

Supporting Information

S1 Fig. Process of making an engineered G $\alpha\beta\gamma$ (GDP) model. (A) Comparison of the structures of G α proteins from the Gs (1azt.pdb) and Gi (1gia.pdb) class. Despite the remarkable structural differences at loops region, the rmsd value between these two structures is very low, about 1.56 Å. (B) Comparison of the structures of active (1gia.pdb) and inactive (1gp2.pdb) G α i. Switch 2 and linker 2 regions, which are G β and nucleotide binding regions, respectively, show marked topological differences except for the loop regions. (C) The sequence and topology of the engineered G $\alpha\beta\gamma$ (GDP) model. The engineered model was constructed by combining residues from G α s (residues 35–102 and 246–385) and in G α i (residues 180–222, switch 2 and linker 2 regions).

(TIF)

S2 Fig. Concept underlying the engineered R-G $\alpha\beta\gamma$ (GDP) model. G α in the engineered R-G $\alpha\beta\gamma$ (GDP) model was constructed by combining the G α sRas domain from R-G $\alpha\beta\gamma$ (0) and the G α sAH domain from engineered G $\alpha\beta\gamma$ (GDP), respectively.

(TIF)

S3 Fig. Schematic of the traditional ENM (left) and MWCENM (right). In the traditional ENM, the representative alpha carbons are colored in orange and their interactions within the cutoff distance of 12 Å are shown as blue solid lines. In MWCENM, each representative atom is colored according to the types of amino acid. Chemical interactions are depicted by various types of lines. Black, blue, green solid and blue dashed lines represent backbone, ionic bonds, hydrogen bonds and van der Waals interactions within a cutoff distance of 8 Å, respectively.

(TIF)

S4 Fig. Coarse-grained model of the nucleotide (GTP). Each representative atom defined in a dotted circle is weighted by the total mass of its surrounding atoms (shown in the same color) and connected to each other by covalent bonds. The coarse-grained model for GDP was exactly the same as that of GTP without the five atoms colored in blue.

(TIF)

S5 Fig. B-factor correlation comparison of G α in R-G $\alpha\beta\gamma$ (0) [3sn6.pdb] between the traditional ENM (white bar) and MWCENM (black bar). Using various cutoff values from 8 to 16 Å, the traditional ENM showed cutoff dependency and relatively low b-factor correlation values under the 0.4, whereas the MWCENM had much more robust b-factor correlations, regardless of the distance cutoff values. The lowest correlation value with the cutoff of 8 Å in the traditional ENM was caused by an underconstrained system with sparse spring connections.

(TIF)

S6 Fig. Effect of GDP on the dynamic behaviors of G α . (A) Comparison of mean squared fluctuation values according to the presence or absence of GDP at R-G $\alpha\beta\gamma$ and G $\alpha\beta\gamma$. In both structures, the correlation coefficients for G $\alpha\beta\gamma$ (0.99) and R-G $\alpha\beta\gamma$ (0.99) clearly indicated that GDP has little effect on the B-factor. (B) Comparison of NMA results of target structures based on cumulative square overlap (CSO) distributions over the first ten lowest modes. The CSO values of R-G $\alpha\beta\gamma$ was almost 0.80, whereas that of G $\alpha\beta\gamma$ was only about 0.60, regardless of the presence of GDP. Additionally, the opening motion of G α occurred at the 4th and the 5th modes only from R-G $\alpha\beta\gamma$ (GDP) and R-G $\alpha\beta\gamma$, respectively; not from G $\alpha\beta\gamma$ structures.

(TIF)

S7 Fig. Virtual torsion angle change of the G α structure from R-G $\alpha\beta\gamma$ (GDP) to R-G $\alpha\beta\gamma$ (0). The two peaks represent the residues for which the torsion angles varied significantly. Two

linker parts presented in yellow in the G α structure correspond to these regions. The dotted line, which represents the first linker but which is missing in our structures, is drawn on the basis of the existing structure of G α (1gp2.pdb)

(TIF)

S8 Fig. CSO comparison of R-G $\alpha\beta\gamma$ (GDP) with a normal (blue) and a strong (red) constraint at linker regions. CSO over the first twenty lowest modes drops from 0.9 to 0.8 with a 100 times stronger spring constant under a two times wider range of cutoff around the linker residues.

(TIFF)

S1 Table. Stiffness values in MWCENM.

(PDF)

S2 Table. Change in solvent-accessible surface area at the nucleotide pocket region and GDP in R-G $\alpha\beta\gamma$ (GDP) through the open motion. Solvent-accessible surface area is calculated with PyMOL with 1 Å solvent radius.

(PDF)

S1 Video. Animation showing the hypothesized conformational changes of the G protein throughout the G protein cycle. The GDP-bound heterotrimer (*gray*) binds to activated receptor (*blue*), and the nucleotide-binding pocket region consisting of $\beta 6$ - $\alpha 5$, the P-loop and switch 2 are perturbation, resulting in GDP (*purple*) release. Subsequently, the helical domain of G α (GDP) opens away from the receptor-anchored Ras domain, which allows GDP release and GTP (*orange*) to enter. Reorientation of G α caused by GTP binding leads to dissociation of the receptor and G $\beta\gamma$ (G β ; *green* and G γ ; *cyan*). GTP hydrolysis leads to rebinding of G $\beta\gamma$ to G α in order to restart the G protein signaling cycle.

(AVI)

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Author Contributions

Conceived and designed the experiments: MHK YJK MKK. Performed the experiments: MHK YJK. Analyzed the data: MHK KYC MKK. Contributed reagents/materials/analysis tools: MHK YJK TJJ JBC. Wrote the paper: MHK KYC MKK HRK.

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