Folding-based molecular simulations reveal mechanisms of the rotary motor F$_1$–ATPase

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Biomolecular machines fulfill their function through large conformational changes that typically occur on the millisecond time scale or longer. Conventional atomistic simulations can only reach microseconds at the moment. Here, extending the minimalist model developed for protein folding, we propose the “switching Go model” and use it to simulate the rotary motion of ATP-driven molecular motor F$_1$–ATPase. The simulation recovers the unidirectional 120° rotation of the γ-subunit, the rotor. The rotation was induced solely by steric repulsion from the αβ$_3$γ subunits, the stator, which undergoes conformation change during ATP hydrolysis. In silico alanine mutagenesis further elucidated which residues play specific roles in the rotation. Finally, regarding the mechanochemical coupling scheme, we found that the tri-site model does not lead to successful rotation but that the always-bi-site model (7), can reproduce low-frequency harmonic fluctuation described computational framework, which we call a “switching Go model,” for simulating large-amplitude motion of biomolecular machines. We then apply it to the rotary motion of F$_1$–ATPase.

F$_1$–ATPase, the catalytic part of the energy conversion enzyme F$_0$F$_1$–ATP synthase, is known to act as a rotary motor upon ATP hydrolysis (19–24). Single-molecule experiments have visualized the rotation and elucidated some of the details; the γ-subunit rotates unidirectionally in discrete 120° steps with each ATP hydrolysis (25), and the 120° step can be divided into 90° and 30° substeps at lower ATP concentration (26). The dwell time before the 90° substep depends on ATP concentration, and the one before the 30° substep includes two rates of an ~1-ms time scale. These findings suggest that the 90° substep involves ATP binding, and the 30° substep may correspond to ATP hydrolysis and release of products (ADP, phosphate, or both). These experiments have given much insight into the rotational mechanisms as described above; however, the resolution of those studies is limited to ~40 nm, which cannot provide a complete structural view of the F$_1$ dynamics.

The minimal catalytic complex of F$_1$ contains seven subunits αβ$_3$γ, whose x-ray structure (27) is depicted in Fig. 1a. The central stalk γ is a rotor, whereas the alternately arranged α$_3$ and β$_3$ subunits form a ring acting as the stator of the rotary motor. There are three catalytic nucleotide-binding sites, which are at the interfaces of β-subunits with the neighboring α-subunits. In the crystal structure determined in 1994 (27), one catalytic site is filled with ATP analog and another with ADP, and the last is empty. The structures of the ATP-bound subunits are denoted as β$_{TP}$ and α$_{TP}$, where β$_{TP}$ takes on the “closed form” in its C terminus (Fig. 1b Right Lower) (28) and the catalytic interface is loosely packed. The ADP-bound β-subunit, β$_{DP}$, also takes the closed form, and the interface to α$_{DP}$ is tightly packed. The structures of nucleotide-free subunits are denoted as β$_1$ and α$_1$, where β$_1$ has “open form” in its C terminus (Fig. 1b Left Lower) (28). From the static structure, we can deduce that the catalysis-induced binding motion of β between closed and open forms produce torque on γ leading to rotation (27, 29). Given the static x-ray structures at high resolution and the dynamic single

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molecule observations at low resolution, currently missing is the time-dependent structural insight that connects the two.

In this work, by using our proposed switching Go model, we realize structure-based molecular simulations of F$_{1}$-ATPase rotation upon hydrolysis, providing us with time-dependent structural insights on F$_{1}$ rotation. We address which parts of the molecule play what roles in rotation in a site-specific manner. Furthermore, combining all available experimental data with simulation results, we identify the mechanochanical coupling mechanism, which has long been under debate in the field. This work opens an avenue of simulating large-scale motion involved in dynamical function of large biomolecular complexes by folding-based model.

Results

Switching Go Model. There are many versions of Go models, which concisely realize perfect funnel energy landscape (13) of proteins (13–18). Among them, we use the version of Clementi et al. (18), where amino acids are represented by single spheres centered at C$_{α}$ atoms, which are sequentially connected by virtual bonds, and interactions are specified so that structures closer to the native structure are more stable (30). See Materials and Methods for details.

The switching Go model is based on the idea that the shape of the funnel depends on chemical composition of the system and thus is altered upon ligand binding (31). We illustrate this notion for the conformational transition in β-subunit of F$_{1}$-ATPase from the nucleotide-free state “E” to the ATP-bound state “TP” upon binding to ATP (Fig. 1b). In this case, we consider two funnels; one for E state, at the bottom of which is the βE structure (Fig. 1b, cyan funnel), and another one for the TP state, at the bottom of which is the βTP structure (Fig. 1b, green funnel). Go potentials of the β-subunit for the states E and TP are denoted as $V_{β}(R|Eβ)$ and $V_{β}(R|TPβ)$, respectively, where $Rβ$ collectively represents the coordinates of amino acids in the β-subunit, and E and TP indicate the nucleotide state.

We start simulations from the βE structure using the Go potential $V_{β}(R|Eβ)$ appropriate for the nucleotide-free state E$_β$ (blue funnel in Fig. 1b). Upon binding ATP, we then switch the energy to another Go potential $V_{β}(R|TPβ)$, appropriate for the ATP-bound state TP$_β$ (green funnel in Fig. 1b). Fig. 1c plots degree of conformational change as a function of time. The amount of conformational change is defined by a set of amino acid pairs that are in the native contact at the final structure, βTP, and that are not in the native contact at the initial structure, βE (for the definition of the native contact, see Materials and Methods). Within this set, the fraction that is formed as the measure of conformational change. We performed molecular dynamics simulations (32, 33) at a temperature $T_1$ and switched the Go potential at the 10,000th time step, observing the structural relaxation of the β from βE to βTP in 200 steps (Fig. 1c). Simulation of relatively large-scale conformational changes was achieved within a very short time by switching Go model.

Modeling the $αββγ$ Complex of F$_{1}$-ATPase. Next, we move on to the model of the F$_{1}$ complex. The model of the $αββγ$ complex of F$_{1}$ has several important features. (i) A Go potential $V_{β}$ funnels the simulated γ-subunit into its structure of the 1994 complex so that the γ only fluctuates around its native structure. (ii) For the $αββγ$ ring, we used the switching Go potential $V_{αβγ}(R|aβbγ|X_{aβbγ})$, where the ring structure is funneled to the conformation characterized by nucleotide states of the three αβ-subunits $X_{aβbγ}$. Each of the three αβ-subunits may have nucleotide states of either TP, DP + P$_i$, DP, or E states (here, we omit the possibility that P$_i$ alone is bound, but the ADP is released based on structural observation). Thus, $X_{aβbγ}$ varies among $4^3 = 64$ types of nucleotide states. Here, there is some room of uncertainty in mapping the nucleotide states to the reference structures, which will be described thoroughly later. For example, when $X_{aβbγ}$, the nucleotide state (DP, E, TP)$_{aβbγ}$, the stable conformation of three αβ-subunits are $αβ_{DP}^{1994}$, $αβ_{E}^{1994}$, and $αβ_{TP}^{1994}$. [The superscript 1994 means that structures are taken from the x-ray structure determined in 1994 (27).] To induce conformational changes of the $αβγ$ ring, this Go potential is switched, e.g., for the simulation of conformational change upon ATP binding at the catalytic site of the αβ$_2$, we switch the Go potential from $V_{αβγ}(R|aβbγ)$ to

hydrolysis at site 3, which are conducted all at once. (Here, the potential is the sum of four terms: $V_{\alpha,\beta}(R_{\alpha,\beta}(\alpha_1,\alpha_2,\alpha_3)\{DP, TP, TP\})_{\alpha_1^*,\alpha_2^*,\alpha_3^*}$). (iii) For the interaction between the $\gamma$ and the $\alpha_3\beta_3$ ring $V_{\alpha_3\beta_3-\gamma}$ we used a simple, short-ranged steric repulsion between amino acids. Very importantly, the switching $G_0$ potential was imposed only for the intra-$\alpha_3\beta_3$ ring interactions, but not the $\gamma$ rotation, and thus the rotation is not biased by design. (iv) The N termini of the three $\beta$-subunits and the C termini of $\gamma$ are constrained to their initial positions by springs $V_{\text{spring}}$ which mimics the His-tag used to fix $F_1$ in the single molecule experiments (19). The total potential is the sum of four terms: $V = V_\gamma + V_{\alpha,\beta} + V_{\alpha_3\beta_3-\gamma} + V_{\text{spring}}$. See Materials and Methods and Table 1, which is published as supporting information on the PNAS web site, for details.

$F_1$ Simulation of All-at-Once Catalysis. The first set of $F_1$ simulations started with the 1994 structure and the potential $V_{\alpha,\beta}(R_{\alpha,\beta}(\alpha_1,\alpha_2,\alpha_3)\{DP, TP, TP\})_{\alpha_1^*,\alpha_2^*,\alpha_3^*}$ for which the lowest energy state corresponds to the 1994 structure, $(\alpha^{1994}, \alpha^{1994}, \alpha^{1994})$ (27). After the 30,000 time steps, the potential is switched to $V_{\alpha,\beta}(R_{\alpha,\beta}(\alpha_1,\alpha_2,\alpha_3)\{E, TP, DP\})_{\alpha_1^*,\alpha_2^*,\alpha_3^*}$, in which the simulated structures are funneled to the structure, $(\alpha^{1994}, \alpha^{1994}, \alpha^{1994})$. The switching corresponds to a single cycle of the catalysis consisting of ADP release from the catalytic site of the $\alpha\beta_1$ (termed site 1 hereafter), ATP binding at site 2, and ATP hydrolysis at site 3, which are conducted all at once. (Here, the phosphate, $P_i$, release may occur before or after reaching the nucleotide state of the 1994 structure.) Repeating the simulations 20 times, we monitored the rotary angle of $\gamma$ as a function of time. In all trajectories, the $\gamma$ angle weakly fluctuated around $-15.5^\circ$ before the switching, and after the switching, the $\gamma$ rotated $\sim 120^\circ$ in a counterclockwise direction, consistent with the observations of the single-molecule experiment (19, 25) (Fig. 2a; see also Movie 1, which is published as supporting information on the PNAS web site). Fig. 2b plots the $\gamma$ angle and the degree of structural relaxation in the $\alpha_3\beta_3$ ring, indicating that structural relaxation in the $\alpha_3\beta_3$ ring is followed by the $\gamma$ rotation. The $\gamma$ rotation was induced solely by steric repulsion upon the conformational change in the $\alpha_3\beta_3$ ring. Although factors not included in this simulation, such as hydrophobic and side-chain interactions, may perturb the results quantitatively, structural complementarities are of primary importance for the rotation.

Critical Residues for Producing Torque. To decipher which parts of the $\gamma$ and $\alpha_3\beta_3$ are essential for transferring the torque, we performed a series of simulations analogous to Ala-scanning mutagenesis (34). In silico mutagenesis removed the steric interactions of five consecutive residues in $\gamma$, from $\gamma(i-2)$ to $\gamma(i+2)$, and for each $i$ we repeated 40 simulations of all-at-once catalysis just as described in the previous subsection. The number of trajectories that successfully completed the $120^\circ$ rotation is shown at two different temperatures ($T_1$ and $T_2 = 5T_1$) in Fig. 3 as a function of central residue number $i$. At the lower temperature $T_1$, we found three critical regions required for the $\gamma$ rotation: $\gamma$Ala-1–Asp-5, $\gamma$Ile-13–Met-25, and $\gamma$Ala-231–Ala-235. We highlighted these three portions in white and the portion of the $\beta$-subunit that contact them in yellow in Fig. 4. Both $\gamma$Ile-13–Met-25, and $\gamma$Ala-231–Ala-235 form contacts with the portion of $\beta$ that shows the major structural change (the yellow portion in the C-terminal of the $\beta$-subunit colored by cyan in Fig. 4b and c). This portion of $\beta$ is not present in the highly conserved DELSEED region but is localized nearby. This result is consistent with the experimental inference that the DELSEED region does not play a critical role in torque transfer (35). The portions around the $\gamma$Ile-13–Met-25 and $\gamma$Ala-231–Ala-235 regions are likely the sites of action of the $\gamma$ rotation. The other important site, $\gamma$Ala-1–Asp-5, keeps contacts with $\alpha$- and $\beta$-subunits at their middle portion, the switch II region that does not show significant structural change (Fig. 4b and c). Thus, $\gamma$Ala-1–Asp-5 probably works as a fulcrum. Interestingly, any trajectories that did not achieve a $120^\circ$ rotation of $\gamma$ in these mutagenesis simulations still showed a $\gamma$ rotation up to $30^\circ$ from

![Fig. 3. Simulations analogous to Ala-scanning mutagenesis (34). The number of completely rotated trajectories, out of 40 trials, is plotted against the central residue $i$ of $\gamma$, around which five consecutive residues are “mutated.” All-at-once catalysis simulations were conducted at two temperatures, $T_1$ (red) and $T_2 = 5 \times T_1$ (blue).](image)

![Fig. 4. Residues that transfer the torque. According to the results represented in Fig. 3, the three critical parts in $\gamma$ are plotted in white: $\gamma$Ala-1–Asp-5, $\gamma$Ile-13–Met-25, and $\gamma$Ala-231–Ala-235. a-c are different views of the identical structure taken from a simulated snapshot after $30^\circ$ rotation. The $\beta$ subunits in green, cyan, and magenta are in the nucleotide states $TP$, $E$, and $DP$, respectively, before all-at-once catalysis switch. Shown are views from $\alpha\beta$ (a), $\alpha\beta$ (b), and $\alpha\beta$ (c). The subunit in pink (only in b) is the $\alpha$-subunit in DP state. In b, only $\gamma$1–44 is represented. The residues in $\beta$ that are within 11 Å from the three critical parts of $\gamma$ are in yellow.](image)
the initial 1994 structure (27). (We show an example of the simulations in Fig. 7, which is published as supporting information on the PNAS web site.) This result implies that the initial 30° rotation is less sensitive to steric interactions. At higher temperatures, the results were less clear, although a similar tendency was found.

Mechanochemical Coupling: Tri-Site Model vs. Always-Bi-Site Model.

Next, we address how the catalytic reactions are related to conformational changes in αβ subunits and γ rotation substeps. Experiments showed that the 90° substeps are driven by ATP binding (26), and two, and only two, β-subunits are occupied by nucleotides before the ATP binding (36). We first assumed that the 1994 structure and its corresponding nucleotide state {DP, E, TP} must open its C-terminal domain upon phosphate release from site 2 and the ADP release at site 1. Finally, ATP hydrolysis occurs at site 3 (Fig. 6a).

Simulations consistent with this mechanism were conducted starting from the 1994 structure with {DP, E, TP}αβ,αβ,αβ. Those failed to reproduce results consistent with experiments. As Abrahams et al. (27) suggested, the nucleotide state of {DP, E, TP}αβ,αβ,αβ with the 1994 structure may correspond to the MgADP-inhibition state and is not on the pathway.
(Fig. 6 a and b). We first simulated with $V_{\alpha\beta}(R_{\alpha\beta1},a_{\alpha\beta1}) \{DP + P_\gamma, E, TP\}_{a_{\alpha\beta1},a_{\gamma1}}$, where site 1 is funnelled to $a_{\beta1OP}$ of the 1994 structure; the reference structure is $(\alpha_{\beta1}, \alpha_{\beta1E}, \alpha_{\beta1P})$. At the 30,000th step, this potential is switched to $V_{\alpha\beta}(R_{\alpha\beta2},a_{\alpha\beta2}) \{DP, E, TP\}_{a_{\alpha\beta2},a_{\gamma2}}$, where site 1 has to go through the funnel centered at the $\alpha_{\beta2P01}$ of the half-closed $\beta$ structure in the 2001 complex (40); the reference structure is $(\alpha_{\beta2}, \alpha_{\beta2E}, \alpha_{\beta2P})$. Next, we switched the potential to $V_{\alpha\beta}(R_{\alpha\beta3},a_{\alpha\beta3}) \{E, TP, TP\}_{a_{\alpha\beta3},a_{\gamma3}}$ at the 60,000th step, and $V_{\alpha\beta}(R_{\alpha\beta4},a_{\alpha\beta4}) \{E, TP, DP\}_{a_{\alpha\beta4},a_{\gamma4}}$ at the 90,000th step. The former potential funnelled the simulated structures to $(\alpha_{\beta4}), \alpha_{\beta4P}, \alpha_{\beta4E}$ and the latter to $(\alpha_{\beta4}), \alpha_{\beta4TP}$, $\alpha_{\beta4P}$. The resulting trajectories and histogram are shown in Fig. 6 b and c (see Movie 2, which is published as supporting information on the PNAS web site) where we found the $\sim 30^\circ$ step upon phosphate release and the $\sim 80^\circ$ step upon the coupled reactions of the ATP binding and ADP release, which are perfectly consistent with experiments. Interestingly, the $\gamma$-subunit rotated $\sim 10^\circ$ upon ATP hydrolysis. This finding might be related to the discrepancy between $90^\circ$ and $30^\circ$ substeps with ATP as a substrate and $80^\circ$ and $40^\circ$ substeps with slowly hydrolysable substrate ATP($\gamma$S) (42).

Discussion and Conclusion

The results from the experiment and simulation led us to the following rotational mechanisms. Starting from the 1994 structure and the nucleotide state $\{DP + P_\gamma, E, TP\} \{a_{\alpha\beta1},a_{\gamma1}\}$ remodeling of the site 1 $\beta$-subunits from closed to half-closed form upon phosphate release makes space between the $\alpha_{\beta1}$ and the $\gamma$, leading to $30^\circ$ rotation of $\gamma$. Next, ATP-binding at site 2 induces the $\beta$-subunits closing motion, which pushes the $\gamma$-subunit primarily through contacts near $\gamma$ile-13–Met-25, and $\gamma$ala-231–Ala-235 leading to $\gamma$ rotation. This motion further induces $\beta$ opening at site 1, thus accelerating the ADP release. The latter $\beta$ opening motion at site 1 is crucial for making space for $\gamma$ to complete $\sim 80^\circ$ rotation (see Movie 2). The ATP-binding, the $80^\circ$ rotation, and ADP release are so tightly coupled that they are in the single kinetic process. Hydrolysis might induce $\gamma$ rotation of $\sim 10^\circ$. We note that current simulations do not rule out the so-called bi-site model (43) at very low ATP concentration.

It is surprising that steric repulsion between the $\gamma$ and the $\alpha_{\beta1}$ ring along a particular $120^\circ$ rotation, but also $30^\circ$ and $90^\circ$ substeps. Here, the shape complementarities between the $\gamma$ and $\alpha_{\beta1}$ play crucial roles in functional dynamics of $\gamma$. It may be visualized as the “dynamic version of lock-and-key” mechanism: the shape of the keyhole, the $\alpha_{\beta1}$ ring, changes in the catalytic cycle, which rotates the key, the $\gamma$-subunit, by structural complementarities. It may be one reason for $\gamma$ to exhibit more deterministic motion (25) than that of linear molecular motors such as myosin (44).

As in the F$_1$–ATPase rotary motion, biomolecular functional motion takes milliseconds, a time scale that is orders of magnitude longer that the current reach of atomistic molecular dynamics simulations (3, 4). A folding-based simulation model provides a powerful tool of deducing functional dynamics of large biomolecular complexes from static structural information, once structures in multiple states are experimentally supplied. Here, we reported an initial application of this approach to F$_1$–ATPase.

There are many ways to further improve the current model of F$_1$. First of all, a remarkable feature of F$_1$–ATPase is its reversibility. By enforcing the rotation of $\gamma$, F$_1$–ATPase can synthesize ATP from ADP and P$_\gamma$ (45). For simulating it, we need to take into consideration the feedback mechanism from the $\alpha_{\beta1}$ ring structure to the chemical reaction of nucleotide. Second, the current switching Gō model introduced the “vertical excitation” for jumping from the initial funnel to the final funnel (see Fig. 1b). Physically, this way of simulation corresponds to “photoexcitation,” whereas the real process studied is of course thermally activated. The present approach may be viewed as a rough approximation of the real process for addressing structure–function relationship. For pursuing more physical aspects, however, the present approach needs to be modified. Recently, methods in this direction were investigated (8, 9, 46). Third, we did not explicitly include nucleotide molecules, such as ATP, here. Explicit inclusion of ligand molecules is desired. Improvement toward these directions is needed.

Materials and Methods

Molecular Simulation with Gō Models. Clementi’s version of the C$^\alpha$ Gō model has the effective energy function

$$V(R|X) = \sum_{\text{bonds}} K_{\alpha\beta}(r_{ij} - r_{i\alpha})^2 + \sum_{\text{angles}} K_{\alpha\beta}(\theta_{ij} - \theta_{i\beta})^2$$

$$+ \sum_{\text{dihedral}} \{K_\gamma[1 - \cos(\phi_{ij} - \phi_{i\gamma})]$$

$$+ K_\gamma[1 - \cos(3(\phi_{ij} - \phi_{i\gamma}))]$$

$$+ \sum_{\text{native contact}} \left[ \left(\frac{r_{ij}}{r_{ij}}\right)_{12}^2 - \left(\frac{r_{ij}}{r_{ij}}\right)_{10}^2 \right]$$

$$+ \sum_{\text{nonnative contact}} \left[ \left(\frac{D_{ij}}{r_{ij}}\right)_{12}^2 \right] \right].$$

Here, $R$ collectively represents the Cartesian coordinates of C$^\alpha$ atoms. $X$ signifies the native structure of the simulated protein. In this equation, the first term keeps the chain connectivity; $r_{ij}$ is the length of the $i$th virtual bond between the $i$th and $i$th amino acids. The second and the third terms represent the local torsional interactions; $\theta_{ij}$ and $\phi_{ij}$ stand for the virtual bond angle between the $i$–$j$th and $i$th bonds and the virtual dihedral angle around the $i$–$j$th bond, respectively. The fourth and fifth terms are nonlocal interactions. The former includes interactions between “native contact” pairs of amino acids that are spatially close in the native structure (see below for details), and the last term is a nonspecific repulsion between amino acid pairs. The $r_{ij}$ is the distance between the C$^\alpha$ atoms of the $i$th and $j$th amino acids. Structural information on $X$ is used for setting the parameters; all of the constant parameters with the subscript $X$ indicate the values of the corresponding variables at the structure $X$. For example, because the variable $r_{ij}$ is the distance between the $i$th and $j$th residues, the parameter $r_{X_{\alpha\beta1}}$ has the value of $r_{ij}$ in the $X_{\alpha\beta1}$ structure. Native contact in the fifth term is defined as below. If one of the nonhydrogen atoms in the $i$th amino acid is within a distance of 6.5 Å from any nonhydrogen atom in the $j$th amino acid, we define the pair of the $i$th and $j$th amino acids as being native contact (amino acid pairs that are not in the native contact are called nonnative pairs). We note that all of the terms except the last one are set up so that each term has the lowest energy when the conformation $R$ coincides with the structure $X$; these terms realize the funnel-like energy landscape. Parameters $K_{\gamma} = 100.0$, $K_\gamma = 20.0$, $K_\gamma^{(1)} = 1.0$, $K_\gamma^{(3)} = 0.5$, $e = 0.36$, and $D_{ij} = 4.0$ are used throughout the present work. In the switching Gō model proposed here, the reference structure $X$ is switched upon change in the nucleotide state.

The energy function for the F$_1$ complex consists of four terms: $V = V_{\gamma} + V_{\alpha\beta1} + V_{\alpha\beta1,\gamma} + V_{\text{spring}}$. $V_\gamma$ is a Gō model for $\gamma$ subunit, in which the bottom structure $X_{\alpha\beta1}$ corresponds to the $\gamma$ structure in the 1994 complex, $V_{\alpha\beta1}(R_{\alpha\beta1},a_{\alpha\beta1},a_{\gamma1})_{X_{\alpha\beta1},a_{\gamma1}}$ is the switching Gō model for the $\alpha_{\beta1}$ ring, where the reference structure $X$ depends on the nucleotide states. For example, when $X = \{DP, E, TP\}$, the stable conformations of three $\alpha_{\beta1}$-subunits
are $\alpha_{TP}^{1994}$ and $\alpha_{DP}^{1994}$, and $\alpha_{TP}^{1994}$ (see Crystal Structures Used in Materials and Methods). $V_{\alpha\beta \gamma}$ is the short-range steric repulsion defined as

$$V_{\alpha\beta \gamma} = \sum_{i \in \alpha, j \in \beta, k \in \gamma} e(D_2/r_{ij})^{12},$$

where $D_2 = 6.0 \text{ Å}$. $V'_{\text{spring}}$ is introduced to keep the F1 complex on a plane and is defined as

$$V'_{\text{spring}} = \sum_{i \in \beta, j \in \beta, k \gamma} \left\{ k(L_i - L_{i,0})^2, \begin{array}{ll} 0, & (L_i \geq L_{i,0}), \\ (L_i < L_{i,0}), & \end{array} \right.$$  

where $k = 0.18$, $L_i$ is the distance of the $i$th residue from its initial position, and $L_{i,0} = 1.5 \text{ Å}$ ($i = \beta, 9, \beta, 9$, $\beta, 9$, and $L_{i,0} = 2.0 \text{ Å}$ for $i = \gamma$). See Supporting Text, which is published as supporting information on the PNAS website, for more details.

Molecular dynamics simulations were performed by integrating Newton equation with “velocity Verlet” algorithm (32), and the velocity rescaling proposed by Berendsen et al. (33) was used to realize constant temperature simulation. Temperatures used were $T_1 = 4 \times 10^{-3}$ and $5T_1$. The mass for all amino acids was identical.

**Crystal Structures Used.** We used the reference structures of the $\alpha_2\beta_2\gamma$ complex that correspond to the bottoms of the funnels in G0 potentials. All of the reference structures were taken from two crystal structures determined in 1994 and 2001 by Walker and coworkers, which we refer as the 1994 (27) and the 2001 (40) structures, respectively. As for the $\gamma$ stalk, we used the conformation in the 1994 structure as the reference structure throughout the work. Conversely, the reference structure of the $\alpha_2\beta_2$ ring varied depending on the states of three catalytic binding sites. Because the catalytic nucleotide binding sites are located at the interfaces between the $\alpha$- and $\beta$-subunits, we treated the $\alpha$- and $\beta$-subunits that sandwich the catalytic sites grouped together, which are colored by analogous hue in Fig. 1a. From the 1994 and 2001 structures, we took four different reference structures of grouped $\alpha$-$\beta$-subunits. We denoted each of them as $\alpha_{DP}^{1994}$, $\alpha_{DP}^{1994}$, $\alpha_{TP}^{1994}$, or $\alpha_{DP}^{2001}$. The superscript $y$ represents the parent structure, from which the structure of $\alpha_{DP}$ is taken; $y$ is either 1994 or 2001. In the 1994 structure, A is TP, DP, or E. $\alpha_{TP}^{1994}$ has ATP-analog in the catalytic site; the structure of the $\beta$-subunit is in the closed form in C-terminal region, and the interface is loosely packed. $\alpha_{DP}^{1994}$ does not have nucleotides at the catalytic site, and the $\beta$-subunit takes the open form in C-terminal region. In the 2001 structure, we picked up $\alpha$-$\beta$-structure as the reference structure, which the $\beta$-subunit takes as a half-closed form (28): its C-terminal is between the closed form in $\alpha_{TP}^{1994}$ and the open form in $\alpha_{DP}^{1994}$. We call it $\alpha_{DP}^{2001}$.

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