Supplementary Information

Insights into the Origin of the High Energy Conversion Efficiency of F_1-ATPase

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SI1. Origin of the difference between the present conclusions and those of Mukherjee, 2011 (1)

The difference between the conclusions of the two groups is due to the difference in the assignment of the step involving the $\beta_E \rightarrow \beta_{TP}$ transition. We have assigned this to occur during the 80° rotation, whereas Mukherjee and Warshel have assigned this to occur with the P_i release before 40° rotation (1). Consequently, there is an agreement that the $\beta_E \rightarrow \beta_{TP}$ transition is one of the major steps producing large free energy (FE) change. In addition, the FE associated with ATP hydrolysis in β_{DP}* is zero, which is achieved by a higher binding affinity of ATP relative to those of ADP and P_i (*SI Appendix*, Table S1) compensating the large FE of ATP hydrolysis in water (-11.5 kcal/mol). Finally, we note that Pu and Karplus (2) have shown "how conformational changes of the catalytic β–subunits act on the γ-subunit through repulsive van der Waals interactions to generate a torque that drives unidirectional rotation, as observed experimentally." This contrasts with the proposal of Mukherjee and Warshel that the rotation is due to electrostatic interactions (1, 3).

SI2. Update of Gao and Karplus model, 2003 (4)

The Gao and Karplus model (4) of the sequence of events has to be modified to make it consistent with the present results, as shown in (Figure 1A), including the state where ATP hydrolysis takes place versus ATP binding, and the separation of $\alpha_3\beta_3$ conformational change from the γ rotation both in the ATP binding and catalytic dwells. In particular, as discussed in the main text, we conclude that some parts of the γ subunit, such as the coiled-coil region, change their conformation together with the $\alpha_3\beta_3$ conformational change, while the rotation of the globular portion of γ occurs in response to the $\alpha_3\beta_3$ conformational change.

SI3. Revision of the Volkán-Kacsó and Marcus model

Based on the description of the ATP/ADP exchange events in Figure 2A and *SI Appendix,* Figure S1 and the model of Volkán-Kacsó and Marcus (V-K&M) (5), $\Delta G^o(\theta)$ (i.e., the standard free energy of the ATP/ADP exchange within the $\alpha_3\beta_3$ hexamer) can be defined as

$$\Delta G^o(\theta) = \Delta G_s^o(\theta) - [W_{ATP}^r - W_{ADP}^p] - k_B T ln[Z_{ADP}/Z_{ATP}] \quad \text{(S1)}$$

where $\Delta G_s^o(\theta)$ is the standard free energy of the overall ATP/ADP exchange process, W_{ATP}^r and W_{ADP}^p are the work terms to bring ATP and ADP to the binding site of the enzyme in the reactant and product states, respectively, and Z_{ATP} and Z_{ADP} are the collision frequencies (i.e., the pre-exponential factors) of ATP and ADP with the enzyme, respectively. This equation differs from the corresponding equation (eq. (10)) in Ref. (5). Specifically, only the ATP binding in the forward direction (as the sum of the ATP collision frequency with the enzyme and the binding of it to β_E (i.e., $W_{ATP}^r - k_B T ln[(h/k_B T)Z_{ATP}]$)) is considered in the V-K&M model. By contrast, *SI Appendix*, eq. (S1) includes the ADP binding in the reverse direction ($W_{ADP}^r - k_B T ln[(h/k_B T)Z_{ADP}]$), as well as the ATP binding in the forward direction. Based on the very similar binding affinities of ATP and ADP to β_E (25 μM for ATP vs. 20 μM for ADP (6)), we can assume that

the two W terms in *SI Appendix*, eq. (S1) cancel within the uncertainty of the experimental values; the same also applies to the two Z terms.

Then, $\Delta G^o(\theta)$ as a function of the rotation angle θ can be written as

$$\Delta G^o(\theta) = \Delta G_s^o(\theta) = -k_B T ln[k_{off}/k_{on}]. \quad \text{(S2)}$$

We assume that the k_{on} and k_{off} rates for ATP determined at different rotation angle by Watanabe *et al*. (7) are for the ATP/ADP exchange process because they are determined for the forward and backward rotation probabilities during the stalling experiment. Using these k_{on} and k_{off} values, we have $\Delta G^o(\theta)$ = -10.7 kcal/mol at $\theta = \theta_i$. Combining the $\Delta G^o(\theta_i)$ value with the rotational contribution by eq. (3) and introducing $\Delta G^o_{\gamma-rot}(\theta_i \rightarrow \theta_f)$ from eq. (4) yields ΔG_0^o =-12.9 kcal/mol with κ =16 pN·nm and -13.5 kcal/mol with κ =20 pN·nm, respectively. These values are similar to ΔG_{S0}^o = -12.3 kcal/mol in Ref. (5); it would be equal to ΔG_0^o by introduction of the same modification to ΔG_0^o as that given in *SI Appendix*, eq. (S1). We note that these values for the reaction binding step are substantially larger than the ΔG_0^o value of -6.0 kcal/mol reported by V-K&M (5), due to the explicit inclusion of the ADP binding contribution in the reverse process. The larger value is consistent with the free energy estimation of -12.5 kcal/mol based on a recent umbrella sampling MD simulation (8) and also an earlier theoretical estimate by Gao *et al.* (4).

SI4. Reorganization energy (λ) of the stalled rotation system.

Following V-K&M (5), the reorganization energy (λ) can be determined at each rotation angle using eq. (1). We first obtain the activation energy $\Delta G^{\dagger}(\theta)$ of the ATP/ADP exchange process at each angle from eq.(7) of (5) by use of the transition state theory expression (i.e., $\Delta G^{\dagger}(\theta) = -k_B T ln[k_{on}(\theta)/Z_{ATP}]$), the $k_{on}(\theta)$ values of Ref. (7), and the Z=10^{11} $M^{-1}s^{-1}$ (5). As expected, $\Delta G^{\dagger}(\theta)$ value decreases with the increase of θ, from 5.5 kcal/mol at θ = 0° (θ_i) to 3.4 kcal/mol at θ = 80° (θ_f). These values are then used together with the $\Delta G^o(\theta)$ values from *SI Appendix,* eq. (S1) and W_{ATP}^r = -9.1

kcal/mol (5) to determine the λ value at each stalling angle. Interestingly, the resultant λ values change very little for different θ values. For example, the λ value is 78.3 kcal/mol at θ = 0° and 76.9 kcal/mol at θ = 80°. The relative independence of λ on the stalling angle is due to the cancellation of the θ-dependence between $\Delta G^{\dagger}(\theta)$ and $\Delta G^{o}(\theta)$; more precisely, the θ-dependence of $\Delta G^{o}(\theta)$ counterbalances the angle dependence of $\Delta G^{\dagger}(\theta)$ when solving eq. (1). However, use of the exact solution of eq. (1) to determine the reorganization energy, instead of the approximation of eq. (18) of V-K&M (5), yields a somewhat larger angle dependence of λ due to the additional ΔG^{o} dependence. Whereas it changes by only 0.1 kcal/mol between 0° and 80° using eq. (18) of V-K&M (5), with the exact solution of eq. (1), it changes by 1.4 kcal/mol.

The relative *angle independence* of λ has two implications for understanding the *angle dependence* of the ATP/ADP exchange rates. First, the ATP/ADP exchange in the stalled rotation system involves the same conformational change of the enzyme at all rotational angles; e.g., the same closure of β_E with ATP binding and the same opening of β_{HO} with ADP release. This results in the small variation of the reorganization energy (i.e., ≤ 1.4 kcal/mol). Second, because of the approximate invariance of λ, the elastic deformation work terms, which are the remaining terms, must cause the change of the ATP/ADP exchange barrier heights. This result is illustrated in Figure 2. In the figure, the two energy surfaces are

$$G_R^o(\theta) = G_R^o(\theta_i) + w^r(\theta), \text{ and } G_P^o(\theta) = G_P^o(\theta_i) - w^p(\theta) \quad \text{(S3)}$$

where $G_R^o(\theta)$ and $G_P^o(\theta)$, respectively, are the free energies of the reactant and product states at θ relative to those in the initial θ_i = 0° state. Figure 2 shows that the free energy minimum of the reactant state increases by the elastic deformation work term with the increase in θ (i.e., the γ rotation angle), and the opposite happens in the product state free energy surface (*SI Appendix*, Figure S2). This results in the decrease of the overall free energy of the reaction (i.e., $\Delta G^{o}(\theta)$) with the increase of θ. Then, in accord with eq. (1), $\Delta G^{\dagger}(\theta)$ decreases, with the leading term changing linearly with the change of the

reaction free energy (i.e., by the deformation work terms). As noted by V-K&M (5), this dependence of barrier heights on the deformation work terms provides an explanation of the exponential dependence of the exchange rates on the γ rotation angle in both the stalled (7) and controlled rotation systems (9). This is different from the results of Czub et al. (8), who suggested that the angle dependence is due to a progressive closure of β_E in response to the rotation of γ, implying that the two events occur simultaneously. Given the angle independence of λ, the closure of β_E is induced by ATP binding and does not have to be simultaneous with the γ rotation. As discussed in the *Reorganization energy (λ) of the freely rotating system* subsection (see *SI Appendix,* SI5), γ rotation occurs in response to the change in the free energy minima between the two $\alpha_3\beta_3$ conformations after the ATP/ADP exchange (Figure 2A).

Figure 2B can also be interpreted as the two-dimensional free energy surface of the freely rotating system, in which the two reaction coordinates are the ATP/ADP exchange with associated change of the enzyme conformation and the γ rotation. Then, the lowest free energy barrier relative to the free energy of the reactant state at θ_i (i.e., the effective barrier, $\Delta G^{\dagger}_{eff}(\theta)$) occurs θ = 38°, the value is 5.0 kcal/mol for κ = 16 pN·nm (*SI Appendix,* Figure S2) and 5.1 kcal/mol for κ = 20 pN·nm. These values are essentially the same as the value of $\Delta G_0^{\dagger}$= 5.2 kcal/mol determined for the freely rotating system with k_{on}^{ATP}= 1.5×10^7 M^{-1}s^{-1}, as well as the barrier of 5.0 kcal/mol given in Ref. (5). It is important to note that because the free energy barrier differs by less than 0.5 kcal/mol for angles between 0° and 40°, the barrier crossing can occur at any angle in this range, followed by γ rotation to 80°.

SI5. Reorganization energy (λ) of the freely rotating system; comparison with stalled system

Applying the same procedure to the freely rotating system, the λ value obtained is 80.3 kcal/mol using ΔG_0^o = -12.9 kcal/mol (κ=16 pN·nm) and 81.4 kcal/mol using ΔG_0^o = -13.5 kcal/mol (κ=20 pN·nm), respectively. These values are larger than the λ value of 68 kcal/mol for ATP binding of V-K&M (10). The difference is due to the fact that we have included the contribution of ADP binding explicitly in *SI*

Appendix, eq. (S1), whilst it was ignored in the V-K&M model (5). The larger value obtained by us suggests that there is a larger protein and solvent reorganization energy. It is presumably associated with the interaction difference of ADP between β_E and β_{HO} in the reactant state free energy surface (G_R in *SI Appendix*, Figure S1)

The λ values for the freely rotation system are also larger than the values estimated for the stalled rotation system: for example, 80.3 kcal/mol (κ=16 pN·nm) versus 78.3 kcal/mol at θ = 0°. As noted above, the independence of λ of the rotation angle suggests that the ATP/ADP exchange involves the ($\alpha_3\beta_3$) conformational change of the enzyme which is the same for all angles. Given that, the difference between the λ values of the freely rotating and the stalled rotation systems is due to the 80° γ rotation that occurs during the exchange process. As illustrated in Figure 3, the γ rotation in the freely rotating system results in slightly different free energy (FE) surfaces of the reactant and product states from those of the stalled rotation system. More specifically, the FE surface of the reactant state of the freely rotating system (G_{R-FR}; blue curve in Figure 3) is gradually shifted from that of the stalled rotation system (black solid curve; $G_R(0°)$) at 0° to that (black dashed curve; $G_R(80°)$) at 80° by the deformation work term (eq. (1)) during the ATP/ADP exchange, because of the rotation of γ during the exchange process (see Fig. 2A). The same shift occurs in the product state FE surface in the backward direction (G_{P-FR}; red curve in Figure 2B; i.e., from the green dashed curve ($G_P(80°)$) at 80° to green solid curve ($G_P(0°)$) at 0°). These changes result in the increase of λ by the deformational work term. Indeed, the subtraction of the $\Delta G^o_{\gamma-rot}(\theta_i \rightarrow \theta_f)$ value from the λ value of the freely rotating system gives 78.1 kcal/mol for κ = 16 pN·nm and 78.6 kcal/mol for κ = 20 pN·nm, respectively, which are essentially the same as the λ value at θ = 0°.

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Table S1. Binding free energies (kcal/mol) and standard free energies of ATP hydrolysis at binding sites of different β subunit conformations.[a] The values in italic are for the estimated values in the present analysis based on the assumption on their structures and ligand binding affinities. Details are provided in the main text.

	β_E	β_{HC}	β_{HO}[b]	β_{DP}	β_{DP}*[c]	β_{TP}
ATP	-6.3	-3.1	*-5.5*	-7.8	*-17.6*	-13.2
ADP	-6.2	-6.3	*-8.1*	-10.0	*-10.0*	-6.2
P_i	-0.4	-4.1	*-2.2*	-0.4	*-0.4*	-0.4
ΔG^o_{rxn} [d]	-7.8	-14.3	*-12.1*	-9.8	*0.0*	-0.63 (1.4)

[a]Most binding and reaction free energy values are adopted from Ref. (4).
[b]The binding affinities and reaction free energy for β_{HO} are estimated as an average between the corresponding values of β_{HC} and β_{DP}.
[c]ADP and P_i binding data for β_{DP}* are assumed to be the same to β_{DP}, and the value for ATP is determined based on the assumption that the reaction free energy for ATP hydrolysis in β_{DP}* is zero, which requires the shift of the value by -7.3 kcal/mol, i.e., ΔG^o for ATP hydrolysis in water.
[d]The reaction free energy for ATP hydrolysis in each β conformation (4). The value in parentheses is the estimated value from experiments (11).

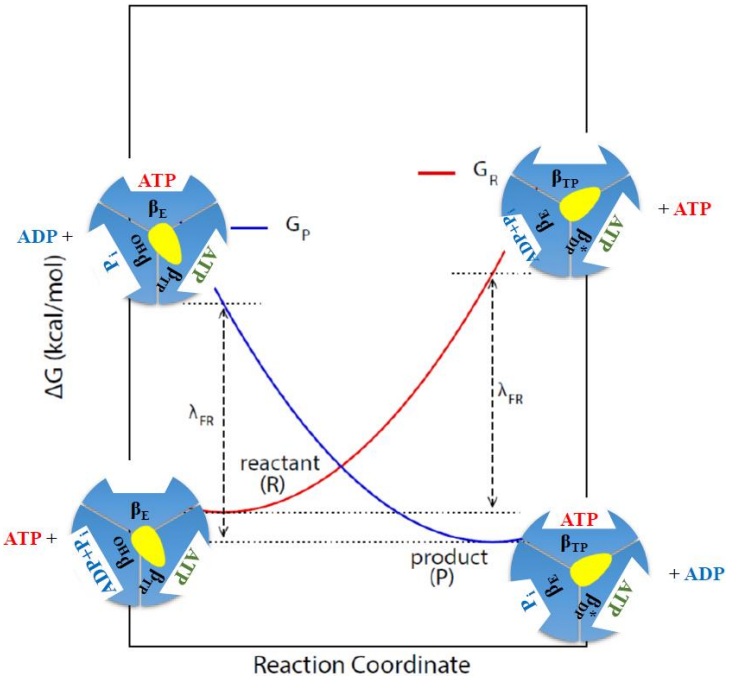

Figure S1. Schematic diagram of the ATP/ADP exchange process for the freely rotating system described by the revised Volkán-Kacsó and Marcus (V-K&M) model proposed in this work. For the free energy surfaces of the reactant (G_R) and product states (G_P), the representative $\alpha_3\beta_3\gamma$ conformations are indicated for the states before and after ATP/ADP exchange. At each reaction coordinate value, the vertical transition from G_R to G_P represents the ATP/ADP exchange reaction. The change along each free energy surface (i.e., G_R or G_P) represents the conformational change of $\alpha_3\beta_3\gamma$ between that in the reactant and product states shown in Figure 2A. Therefore, the reorganization energy λ_{FR} on the reactant state free energy surface (G_R) is the free energy of the $\alpha_3\beta_3\gamma$ conformational change plus the free energy difference of the ADP interaction between β_E and β_{HO}. On the product state free energy surface (G_P), λ_{FR} is the free energy of the $\alpha_3\beta_3\gamma$ conformational change plus the free energy change for ATP interaction between β_E and β_{TP}.

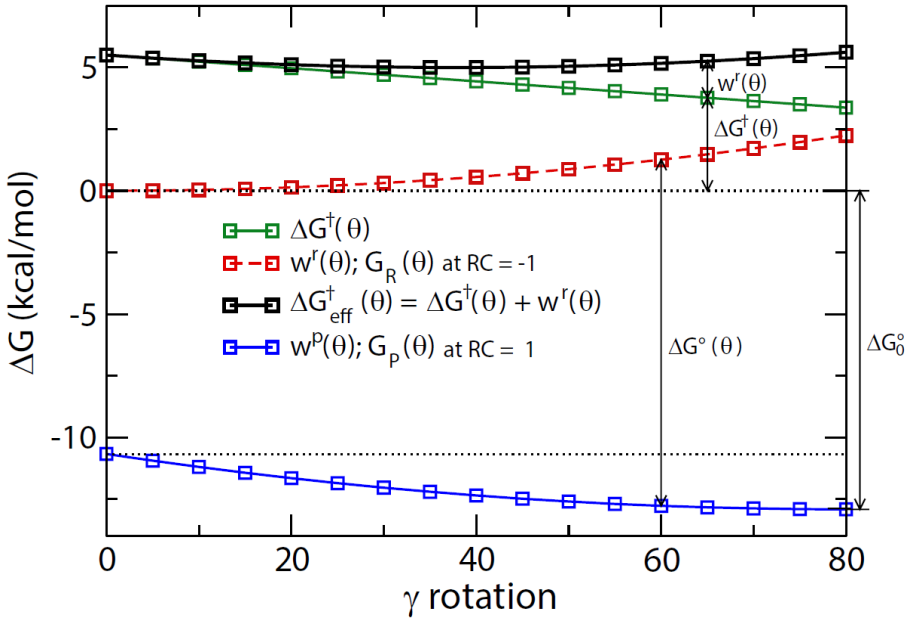

Figure S2. The change of the (effective) free energy barrier ($\Delta G^{\dagger}_{eff}$) and reaction free energy (ΔG^{o}) in Figure 2B as a function of γ rotation angle. $\Delta G^{\dagger}(\theta)$ is the free energy barrier for the ATP/ADP exchange process, $w^{r}(\theta)$ is the work term in the reactant state surface, and $\Delta G^{\dagger}_{eff}(\theta)$ is the free energy barrier relative to the free energy of the reactant state at θ_i (i.e., the sum of the two first terms) presented in Figure 2B. For κ = 16 pN·nm, the lowest $\Delta G^{\dagger}_{eff}(\theta)$ value occurs at 38°. In the figure, $w^{p}(\theta)$ is shown relative to $G_P(80°)$ at the reaction coordinate (RC) = 1.0, i.e., at the product state (Figure 2B), which is -12.9 kcal/mol (Figure 3). Therefore, the $w^{p}(\theta)$ curve represents the $G_P(\theta)$ values at RC = 1.0 for each θ. For $w^{r}(\theta)$, it follows the $G_R(\theta)$ value at RC = -1.0, i.e., the reactant state. Then, the reaction free energy, $\Delta G^{o}(\theta)$ is the gap between the two lines ($w^{r}(\theta)$ and $w^{p}(\theta)$), while ΔG^{o}_{0} is the difference of $G_P(80°)$ at RC=1.0 from $G_P(0°)$ at RC=-1.0, which is zero by definition.