

Optimal Rectification without Forward-Current Suppression by Biological Molecular Motor

Yohei Nakayama and Shoichi Toyabe*

Department of Applied Physics, Graduate School of Engineering, Tohoku University,
Aoba 6-6-05, Sendai 980-8579, Japan

(Received 29 October 2020; accepted 23 April 2021; published 20 May 2021)

We experimentally show that biological molecular motor F_1 -ATPase (F_1) implements an optimal rectification mechanism. The rectification mechanism hardly suppresses the synthesis of adenosine triphosphate by F_1 , which is F_1 's physiological role, while inhibiting the unfavorable hydrolysis of adenosine triphosphate. This optimal rectification contrasts highly with that of a simple ratchet model, where the inhibition of the backward current is inevitably accompanied by the suppression of the forward current. Our detailed analysis of single-molecule trajectories demonstrates a novel but simple rectification mechanism of F_1 with parallel landscapes and asymmetric transition rates.

DOI: 10.1103/PhysRevLett.126.208101

Introduction.—Energy transduction is essential for living systems. In particular, adenosine triphosphate (ATP) plays a central role in biological energy transduction as a source of free energy for diverse processes. F_0F_1 is a cellular ATP factory [1] composed of two coupled motors: F_0 and F_1 [2–4] [Fig. 1(a)]. F_0 , driven by the flow of hydrogen ion (H^+) through it, rotates the γ shaft of F_1 forcefully. Then, F_1 synthesizes ATP from adenosine diphosphate (ADP) and inorganic phosphate (P_i) by converting the mechanical work to chemical free energy [5,6] with high efficiency [7–9]. However, there might be certain freely diffusing F_1 molecules that are not involved in the F_0F_1 complex. Since F_1 is a reversible motor, these isolated F_1 molecules rotate in the opposite direction and hydrolyze ATP to ADP and P_i . Such futile ATP hydrolysis may also take place in the F_0F_1 complex when the driving force of F_0 becomes insufficient for synthesizing ATP.

The suppression of futile ATP consumption may be regarded as a rectification that blocks the unfavorable current (ATP hydrolysis) while sustaining the favorable current (ATP synthesis). The rectification of current in small fluctuating systems such as biological molecular motors is not straightforward since they work at an energy scale comparable to that of thermal energy [23]. For example, let us consider a Brownian particle in an asymmetric ratchet potential [Fig. 1(b)], where the current responds asymmetrically to the reversal of the external field. However, this simple ratchet model inherits a trade-off: the barrier needs to be high to block the backward current activated by thermal fluctuation, while the high barrier reduces the forward current as well. We emphasize that the rectification discussed here is the asymmetry of the response to the reversal of the stationary driving force and is not that in the Brownian ratchet, which induces unidirectional current by rectifying periodic or stochastic

variation of the mechanical potential, driving force, or temperature [24,25].

According to the literature, previous biochemical experiments imply that the phenomenon called inhibition serves as the rectification mechanism of F_1 for suppressing the futile ATP consumption [26–28]. Besides, single-molecule experiments have shown that the inhibition causes long pauses of the rotation and can be activated mechanically [29–31]. However, how the inhibition causes such a rectification, which has not been observed at the

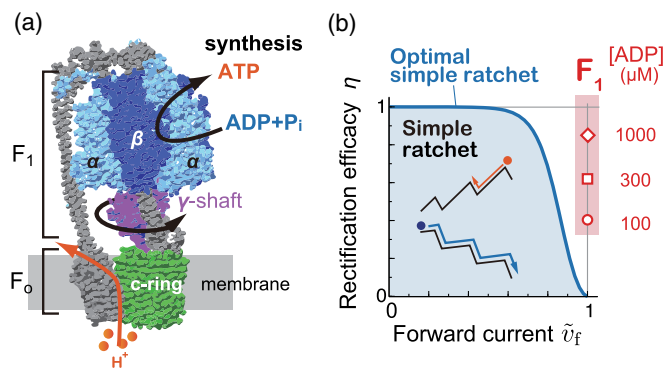


FIG. 1. F_0F_1 -ATP synthase and current rectification. (a) F_0F_1 -ATP synthase converts the electrochemical potential of H^+ to the chemical free energy of ATP via rotary machinery [4]. (b) The forward current vs the rectification efficacy of F_1 and simple ratchet model. The symbols correspond to the experimental results of F_1 under different concentrations of ADP. $[ATP] = 100 \mu M$ and $[P_i] = 1 mM$. For $[ADP] = 1 mM$, we measured $\tilde{v}$ only in $N_{ex} = 0$ and assumed $\tilde{v}_f = 1$ as in the other cases. The range of the current and rectification efficacy numerically obtained for the simple ratchet model with a sawtooth potential is shown by the shaded area under the curve. (See the Supplemental Material [10] and Fig. S1 for details.)

single-molecule level, remains elusive. In this Letter, we aim to demonstrate the rectification by inhibition and reveal its mechanism. We have used single-molecule experiments of the isolated F_1 under external torque as a model system of F_1 working in the F_0F_1 complex.

Results.—We observed the rotation of a single F_1 molecule under an external torque, which is induced by the electrorotation method to the probe fixed on the γ shaft [7,32,33] [Fig. 2(a)]. (See the Supplemental Material [10] for details of the experimental setup.) The rotation rate changes depending on the value of the external torque N_{ex} .

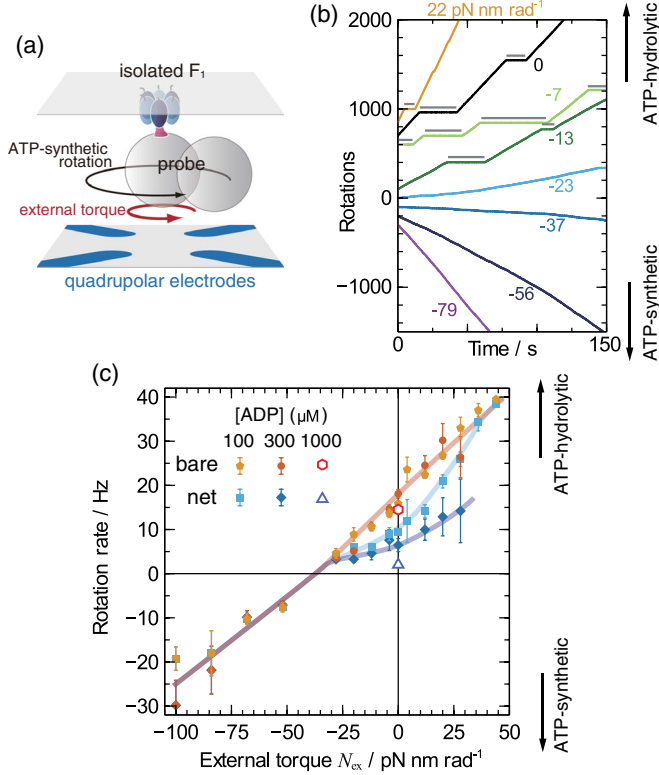


FIG. 2. Rotation of a single molecule of F_1 ($\alpha_3\beta_3\gamma$ subcomplex) under an external torque as a model system of F_0F_1 . (a) The schematics of the experimental setup. The rotation is probed by a 300 nm dimeric probe particle attached to the γ shaft. The electrorotation method using a 10 MHz rotating electric field induces external torque on the probe. (b) Single-molecule trajectories in the absence or presence of external torque with indicated values. The gray lines indicate the pauses corresponding to the inhibited state. The direction of the ATP-hydrolytic rotation is set as positive. (c) Torque dependence of v_{bare} and v_{net} for different concentrations of ADP. $[\text{ATP}] = 100 \mu\text{M}$ and $[\text{P}_i] = 1 \text{ mM}$. The rotation rates were averaged within bins with widths of 8 and 16 pN nm/rad for the ATP-hydrolytic and synthetic rotations, respectively. The data at $N_{\text{ex}} = 0$ were averaged separately. Data points are lacking near the stalled state because it was difficult to identify the inhibition pauses in this region where the rotation rate almost vanishes. The fitting of the solid curves is a guide for the eye. Error bars denote standard errors of the mean. (See Figs. S2 and S3 [10] for the other condition: $[\text{ATP}] = 100 \mu\text{M}$, $[\text{ADP}] = 100 \mu\text{M}$, and $[\text{P}_i] = 100 \mu\text{M}$.)

When we applied a strong torque, the rotational direction was inverted to the ATP-synthetic direction [Fig. 2(b)].

We observed intermittent long pauses of the F_1 rotation, as indicated by the gray lines in Fig. 2(b). The pauses occurred stochastically at three angular positions separated by 120° , which is a characteristic of the inhibition [30]. We found that the inhibitory pauses took place only in the ATP-hydrolytic rotations and not in the ATP-synthetic rotations. This selective inhibition of the ATP hydrolysis is consistent with the previous bulk experiments [26–28], supporting the idea that the inhibition serves as the rectification mechanism.

To characterize the inhibition, the rotational trajectories were divided into the rotating and inhibited states based on the instantaneous rotation rates (see the Supplemental Material [10] for details). Figure 2(c) and Fig. S2 in [10] show the bare and the net rotation rates, v_{bare} and v_{net} , corresponding to the rotation excluding and including the inhibition, respectively. As already implied by the trajectories in Fig. 2(b), the inhibition-mediated suppression of the net rotation rate was only seen in the ATP-hydrolytic rotation. The increase in the ADP concentration further suppressed v_{net} . On the other hand, v_{bare} was not significantly affected.

We here introduce the mean normalized current $\tilde{v}$ and the rectification efficacy η for evaluating the performance of the rectification. $\tilde{v}$ is the ratio between the mean currents with and without the rectification mechanism and is given as $\tilde{v} = v_{\text{net}}/v_{\text{bare}}$ for F_1 . η is defined as $\eta = 1 - |\tilde{v}(N)|/|\tilde{v}(-N)|$ based on the response asymmetry where N is the driving force. Since F_1 hydrolyzes three ATP molecules per rotation, the driving force of F_1 is given as the free energy change associated with an ATP hydrolysis $\Delta\mu$ divided by 120° , that is, $N_{\text{motor}} = \Delta\mu/120^\circ$. Then, $N = N_{\text{ex}} + N_{\text{motor}}$. The values of $\Delta\mu$ are calculated by a method developed in [34] and are in the range of 63 to 82 pN nm in our experimental conditions. The relation between the forward normalized current $\tilde{v}_f = |\tilde{v}(-N)|$ and η at $N = N_{\text{motor}}$ of F_1 is compared to that of a simple ratchet model in Fig. 1(b) (see the Supplemental Material [10] for details and Fig. S1 for the other values of N). We observed that F_1 achieved large η values without suppressing the ATP-synthetic current ($\tilde{v}_f \simeq 1$), whereas the simple ratchet model could not reach $\eta > 0$ and $\tilde{v}_f = 1$ simultaneously. Thus, F_1 implements a rectification mechanism that circumvents the rectification trade-off between $\tilde{v}_f$ and η inherited by the simple ratchet.

Next, we examined the mean duration of the rotating and inhibited states to evaluate the inhibition dynamics. The mean duration of the inhibited state, τ_{inh} , exhibited a peak at $N_{\text{ex}} \simeq 0$ [Fig. 3(a)]. On the other hand, the mean duration of the rotating state, τ_{rot} , had no significant dependence on N_{ex} . Accordingly, the time fraction of the inhibited state $F = \tau_{\text{inh}}/(\tau_{\text{inh}} + \tau_{\text{rot}})$ has a peak at $N_{\text{ex}} \simeq 0$ [Fig. 3(b)]. Hence, we concluded that the suppression of the rotation is

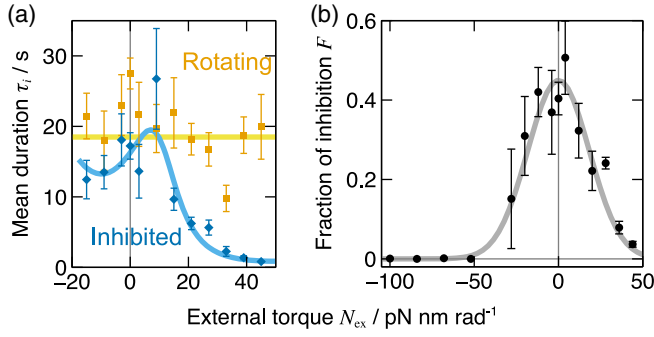


FIG. 3. Torque dependence of τ_{rot} (yellow square) and τ_{inh} (blue diamond) (a), and F (b). $[ATP] = 100 \mu M$, $[ADP] = 100 \mu M$, and $[P_i] = 1 mM$. The blue curve in (a) is the fitting curve to τ_{inh} by Eqs. (1) and (2). The obtained values of the fitting parameters are $\theta^* - L = 12^\circ$ and $S - \theta^* = 59^\circ$. The yellow line is the mean of τ_{rot} . The gray curve in (b) shows the fitting by a Gaussian function. The widths of the bins for averaging are 6 pN nm/rad for τ_{rot} and τ_{inh} , and the same as Fig. 2(c) for F . The plots of τ_{rot} and τ_{inh} are limited for $N_{ex} > -20$ pN nm/rad because no pause is observed otherwise. Error bars are standard errors of the mean. See Fig. S4 for the other conditions and Fig. S5 for the distributions of the duration of each state [10].

mainly regulated by τ_{inh} . An important feature is that the external torque in both directions reduced the value of τ_{inh} . This observation implied the existence of two activation paths from the inhibited state, given the previous observations that the F_1 elementary reactions typically depend on the angle monotonically [35].

The activation dynamics observed in the rotational trajectories indicated the involvement of two such activation paths (Fig. 4). We found that F_1 is stochastically activated in two ways: activation with or without a transient rewinding in the ATP-synthetic direction [Fig. 4(a)]. The magnitude of rewinding reached up to $\sim 120^\circ$. The probability of the rewinding activation, p , increased when an external torque was applied in the ATP-synthetic direction [Fig. 4(b)]. This result is consistent with the picture that there are two elementary reactions whose rates monotonically depend on the angle. The qualitative tendency was the same for different concentrations of ADP and P_i . Note that p may be underestimated since it is not always possible to identify the short rewinding rotations by discriminating them from the thermal fluctuations.

Finally, we recovered the free energy landscapes corresponding to the three inhibited states separated by 120° [Fig. 5(a) and Fig. S6 in [10]] to model the rectification mechanism. The i th landscape $U_i(\theta)$ was recovered from the angular distributions in the corresponding inhibited state for multiple values of the external torques (see the Supplemental Material [10] for details). In addition, we roughly estimated the free energy landscape of the rotating state $U_R(\theta)$ as a straight line with a slope of N_{motor} . We found that the left side of $U_i(\theta)$ has a slope close to N_{motor} and is nearly parallel to $U_R(\theta)$. Since the reversal of the

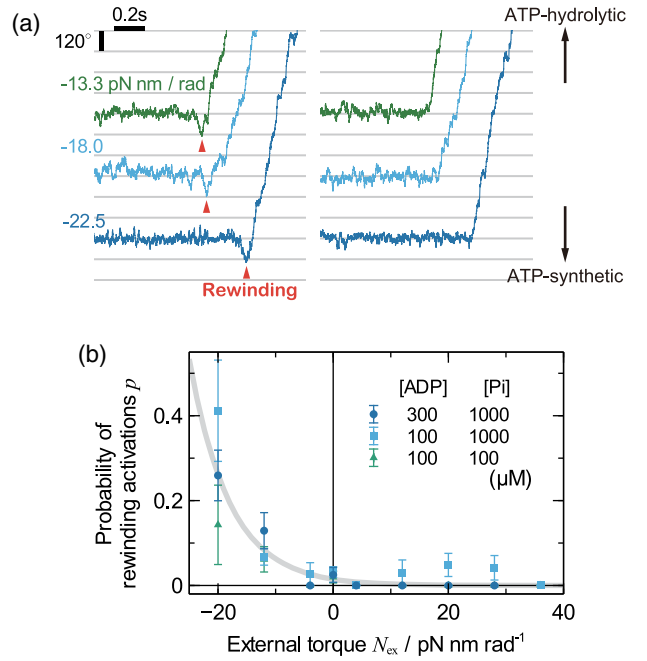


FIG. 4. Dynamics of activation from the inhibited state. (a) Trajectories of activation with (left) or without (right) the transient rewinding in the ATP-synthetic direction (red triangles) under indicated values of external torque. $[ATP] = 100 \mu M$, $[ADP] = 100 \mu M$, and $[P_i] = 1 mM$ for all trajectories. (b) Probability of activation with rewinding p at different values of torque for various concentrations of ADP and P_i . $[ATP] = 100 \mu M$. We did not estimate p for $N_{ex} < -20$ pN nm/rad as inhibition was rarely observed in that region. The widths of bins are 8 pN nm/rad. The solid curve is an exponential function as a guide to the eye. See [10] for error bars.

driving force changes these slopes from N_{motor} to $-N_{motor}$, the free energy landscape has a mirror symmetry between the ATP-hydrolytic and ATP-synthetic rotations.

Model.—Based on the experimental results, we propose a model for describing the rectification mechanism [Fig. 5(b)]. The rectification is the asymmetric response to the reversal of the driving force. The presence of the symmetry of the free energy landscape indicates that the rectification does not arise from the energetic asymmetry but from the kinetic asymmetry between the activation rates. We here validate this kinetic asymmetry.

The experimental results suggested two paths for the activation from the inhibited state to the rotating state. Let L and S be the locations of the transition from the inhibited state to the rotating state [Fig. 5(b)]. In this model, τ_{inh} is given as

$$\tau_{inh} = [\alpha_L \rho_i(L) + \alpha_S \rho_i(S)]^{-1}, \quad (1)$$

where $\rho_i(\theta)$ is the probability density function of θ in the i th inhibited state, and α_L and α_S are the constant parameters. The result that τ_{inh} has a peak at $N_{ex} \simeq 0$ [Fig. 3(a)] implies that the activation rates at L and S , that

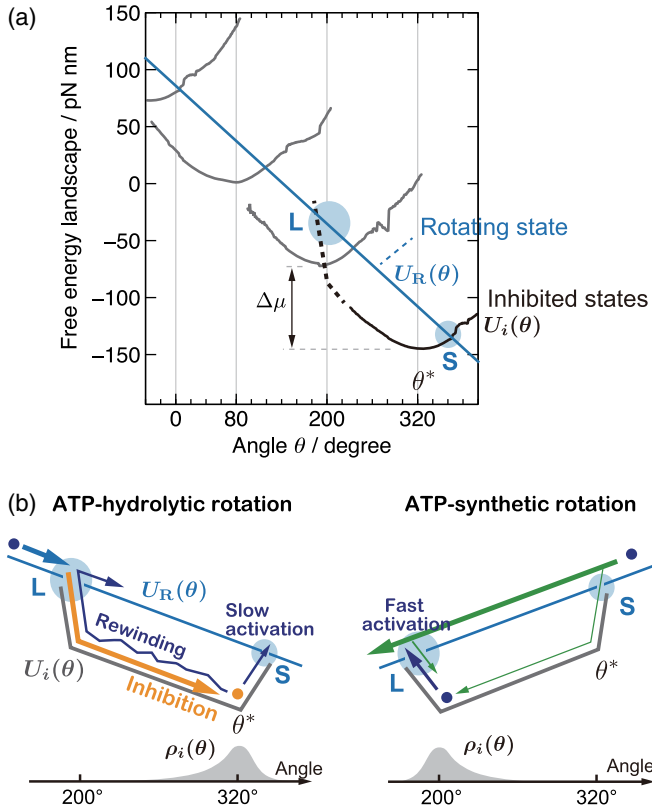


FIG. 5. Model depicting the rectification by F_1 . (a) Free energy landscapes of the inhibited states $U_i(\theta)$ recovered from the trajectories of a single F_1 molecule. The dashed part is a guide for the eye to indicate the correspondence with Fig. 5(b). Three free energy landscapes correspond to three inhibited states separated by 120° . The landscape minima are vertically shifted by $\Delta\mu = 73$ pN nm from each other. The roughly estimated free energy landscape of the rotating state $U_R(\theta)$ is shown by the blue line. [ATP] = 100 μ M, [ADP] = 100 μ M, and [P_i] = 1 mM. See [10] for the definition of the angle origin and the relative height between $U_R(\theta)$ and $U_i(\theta)$. (b) Our proposed schematic model of rectification by F_1 . In the ATP-hydrolytic rotation, the inhibited state serves as a trap (“Inhibition”). The torque to the ATP-synthetic direction enhances the activation with the rewinding to L (“Rewinding”). In contrast, the inhibited state is quickly activated in the ATP-synthetic rotation (“Fast activation”).

is, $\alpha_L \rho_i(L)$ and $\alpha_S \rho_i(S)$, have similar values at $N_{\text{ex}} \simeq 0$. On the other hand, it is natural to expect $\rho_i(L) \ll \rho_i(S)$ since $U_i(L) > U_i(S)$ follows from the locations of L and S , which are 120° backward [36] and $\sim 40^\circ$ forward [30] from $\theta^* = \text{argmin}_\theta U_i(\theta)$, respectively. Thus, a large kinetic asymmetry, $\alpha_L/\alpha_S \gg 1$, is deduced. See [10] for the validation of the above argument based on the reaction scheme.

We next quantitatively evaluate the value α_L/α_S from the dependence of τ_{inh} on N_{ex} (Fig. 3). Assuming that the angular distribution is sufficiently equilibrated in the inhibited state, $\rho_i(\theta) = e^{-\beta[U_i(\theta) - N_{\text{ex}}\theta]}/Z$ holds, where β is the ambient inverse temperature, and $Z = \int e^{-\beta[U_i(\theta) - N_{\text{ex}}\theta]} d\theta$ is

the partition function. By evaluating Z using the saddle point approximation, $\rho_i(\theta)$ is rewritten as

$$\rho_i(\theta) \propto \exp \left\{ -\beta \left[U_i(\theta) - U_i(\theta^*) - N_{\text{ex}}(\theta - \theta^*) + \frac{N_{\text{ex}}^2}{2\kappa} \right] \right\}, \quad (2)$$

where the curvature $\kappa = U_i''(\theta)$ is regarded as a constant. Equation (1) with Eq. (2) fitted τ_{inh} well within the experimental errors [Fig. 3(a)]. The fitting parameters gives a ratio $\alpha_L e^{-\beta U_i(L)} / [\alpha_S e^{-\beta U_i(S)}] = 19$. Given that $U_i(L) - U_i(S) \sim \Delta\mu$, we obtain a rough estimation of the kinetic asymmetry as $\alpha_L/\alpha_S \sim 10^8$. The angular equilibration assumed here might not apply to L because of its fast kinetics. However, α_L/α_S becomes larger in this diffusion-limited situation. Thus, the above evaluation provides the lower bound of the asymmetry.

The enormous kinetic asymmetry between L and S brings about the rectification in the following manner. If the free energy landscape is completely symmetric as illustrated in Fig. 5(b), the local detailed balance condition [37] imposes that the ratio of the transition rates between the rotating and inhibited states at L is the same as that at S . Thus, the result $\alpha_L/\alpha_S \gg 1$ indicates a significantly greater transition rate from the rotating to the inhibited state at L than that at S . Therefore, in the absence of external torque, the ATP-hydrolytic rotation is easily trapped by the inhibited state through L and is hardly activated since the activation rate at S is small and the thermal diffusion to L is rare. On the other hand, in the ATP-synthetic rotation, a rapid activation takes place through L , which is close to the free energy minimum of the inhibited state. Hence, we conclude that the rectification arises from the kinetic asymmetry. Note that this rectification mechanism itself does not consume free energy.

Concluding remarks.—We showed that the core mechanism of rectification by F_1 is derived from the kinetic asymmetry between the two activation paths from the inhibited state [Fig. 5(b)]. This mechanism circumvents the trade-off inherited by the simple ratchet; a high barrier for the brake of the backward current inevitably suppresses the forward current as well. A minimal modification to circumvent this trade-off is to add a landscape that serves as a brake. The large efficacy without the suppression of the forward current is achieved by changing the probability of being in the additional landscape. Our striking finding is that F_1 employs kinetic asymmetry instead of the asymmetry of the free energy landscape to change the probability. Current control is ubiquitous across biological systems, including voltage-gated ion channels. An intriguing question is whether the above mechanism is common to biological systems or is a characteristic of F_1 .

The development of a theoretical aspect may help elucidate the design principle of the rectification mechanism. Current is a fundamental quantity in nonequilibrium

physics. General frameworks such as the fluctuation theorem [37] and thermodynamic uncertainty relation [38,39] have been developed to characterize current. However, to the best of our knowledge, the general framework of current rectification has not yet been investigated. The development of a theoretical framework would provide a unified viewpoint for these processes.

The refinement of the model is also an essential task for better quantitative evaluation. For example, Eqs. (1) and (2) with fixing the angular distance $\theta^* - L = 120^\circ$ cannot fit τ_{inh} in the range of $N_{\text{ex}} < 0$ (Fig. S7 in [10]), whereas $\theta^* - L$ is expected to be equal to the maximum magnitude of the transient rewinding, 120° [Fig. 4(a)]. In addition, such a large transient rewinding may not be expected to be driven by rotational Brownian motion on the recovered free energy landscape [Fig. 5(a)]. These may imply that there are multiple chemical states in the inhibited state. In such a case, it would be necessary to analyze the chemical state-specific landscapes as done in [40] and reexamine the values of the angular distances, $\theta^* - L$ and $S - \theta^*$. Therefore, we expect our results to be the first step toward a detailed analysis of the inhibited state.

We thank Eiro Muneyuki for technical assistance with the sample preparation. We appreciate the helpful discussions with Yuki Izumida and Tomoaki Okaniwa. This work was supported by JSPS KAKENHI (No. JP18H05427, No. JP19H01864).

*Corresponding author.

toyabe@tohoku.ac.jp

- [1] P. D. Boyer, *Annu. Rev. Biochem.* **66**, 717 (1997).
- [2] J. P. Abrahams, A. G. W. Leslie, R. Lutter, and J. E. Walker, *Nature (London)* **370**, 621 (1994).
- [3] P. D. Boyer, *Biochim. Biophys. Acta* **1140**, 215 (1993).
- [4] H. Guo, T. Suzuki, and J. L. Rubinstein, *eLife* **8**, e43128 (2019).
- [5] H. Itoh, A. Takahashi, K. Adachi, H. Noji, R. Yasuda, M. Yoshida, and K. Kinosita, Jr., *Nature (London)* **427**, 465 (2004).
- [6] Y. Rondelez, G. Tresset, T. Nakashima, Y. Kato-Yamada, H. Fujita, S. Takeuchi, and H. Noji, *Nature (London)* **433**, 773 (2005).
- [7] S. Toyabe, T. Watanabe-Nakayama, T. Okamoto, S. Kudo, and E. Muneyuki, *Proc. Natl. Acad. Sci. U.S.A.* **108**, 17951 (2011).
- [8] E. Saita, T. Suzuki, K. Kinosita, and M. Yoshida, *Proc. Natl. Acad. Sci. U.S.A.* **112**, 9626 (2015).
- [9] N. Soga, K. Kimura, K. Kinosita, M. Yoshida, and T. Suzuki, *Proc. Natl. Acad. Sci. U.S.A.* **114**, 4960 (2017).
- [10] See Supplemental Material, which includes Refs. [11–22], at <http://link.aps.org/supplemental/10.1103/PhysRevLett.126.208101> for materials and methods, additional discussions, and Figs. S1–S9.
- [11] S. Toyabe and E. Muneyuki, *New J. Phys.* **17**, 015008 (2015).
- [12] M. Washizu, Y. Kurahashi, H. Iochi, O. Kurosawa, S. Aizawa, S. Kudo, Y. Magariyama, and H. Hotani, *IEEE Trans. Ind. Appl.* **29**, 286 (1991).
- [13] H. C. Berg and L. Turner, *Biophys. J.* **65**, 2201 (1993).
- [14] C. M. Bishop, *Pattern Recognition and Machine Learning*, Information Science and Statistics (Springer, New York, 2006).
- [15] H. Risken, *The Fokker-Planck Equation: Methods of Solution and Applications*, 2nd ed., Springer Series in Synergetics, Vol. 18 (Springer-Verlag, New York, 1996).
- [16] Y. Shirakihara, A. Shiratori, H. Tanikawa, M. Nakasako, M. Yoshida, and T. Suzuki, *FEBS J.* **282**, 2895 (2015).
- [17] R. Yasuda, H. Noji, K. Kinosita, Jr., and M. Yoshida, *Cell* **93**, 1117 (1998).
- [18] T. Nishizaka, K. Oiwa, H. Noji, S. Kimura, E. Muneyuki, M. Yoshida, and K. Kinosita, Jr., *Nat. Struct. Mol. Biol.* **11**, 142 (2004).
- [19] K. Shimabukuro, R. Yasuda, E. Muneyuki, K. Y. Hara, K. Kinosita, and M. Yoshida, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 14731 (2003).
- [20] T. Ariga, E. Muneyuki, and M. Yoshida, *Nat. Struct. Mol. Biol.* **14**, 841 (2007).
- [21] K. Adachi, K. Oiwa, T. Nishizaka, S. Furuie, H. Noji, H. Itoh, M. Yoshida, and K. Kinosita, Jr., *Cell* **130**, 309 (2007).
- [22] R. Watanabe and H. Noji, *Nat. Commun.* **5**, 3486 (2014).
- [23] C. Bustamante, J. Liphardt, and F. Ritort, *Phys. Today* **58**, No. 7, 43 (2005).
- [24] R. D. Astumian, *Science* **276**, 917 (1997).
- [25] P. Reimann, *Phys. Rep.* **361**, 57 (2002).
- [26] A. Syroeshkin, E. Vasilyeva, and A. Vinogradov, *FEBS Lett.* **366**, 29 (1995).
- [27] D. Bald, T. Amano, E. Muneyuki, B. Pitard, J.-L. Rigaud, J. Kruij, T. Hisabori, M. Yoshida, and M. Shibata, *J. Biol. Chem.* **273**, 865 (1998).
- [28] M. Galkin and A. Vinogradov, *FEBS Lett.* **448**, 123 (1999).
- [29] H. Noji, R. Yasuda, M. Yoshida, and K. Kinosita, *Nature (London)* **386**, 299 (1997).
- [30] Y. Hirono-Hara, H. Noji, M. Nishiura, E. Muneyuki, K. Y. Hara, R. Yasuda, K. Kinosita, and M. Yoshida, *Proc. Natl. Acad. Sci. U.S.A.* **98**, 13649 (2001).
- [31] Y. Hirono-Hara, K. Ishizuka, K. Kinosita, Jr., M. Yoshida, and H. Noji, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 4288 (2005).
- [32] T. Watanabe-Nakayama, S. Toyabe, S. Kudo, S. Sugiyama, M. Yoshida, and E. Muneyuki, *Biochem. Biophys. Res. Commun.* **366**, 951 (2008).
- [33] S. Toyabe, T. Okamoto, T. Watanabe-Nakayama, H. Taketani, S. Kudo, and E. Muneyuki, *Phys. Rev. Lett.* **104**, 198103 (2010).
- [34] K. Krab and J. van Wezel, *Biochim. Biophys. Acta* **1098**, 172 (1992).
- [35] R. Watanabe, D. Okuno, S. Sakakihara, K. Shimabukuro, R. Iino, M. Yoshida, and H. Noji, *Nat. Chem. Biol.* **8**, 86 (2012).
- [36] R. Watanabe, R. Iino, and H. Noji, *Nat. Chem. Biol.* **6**, 814 (2010).
- [37] U. Seifert, *Rep. Prog. Phys.* **75**, 126001 (2012).
- [38] A. C. Barato and U. Seifert, *Phys. Rev. Lett.* **114**, 158101 (2015).
- [39] A. Dechant and S.-i. Sasa, *Proc. Natl. Acad. Sci. U.S.A.* **117**, 6430 (2020).
- [40] S. Toyabe, H. Ueno, and E. Muneyuki, *Europhys. Lett.* **97**, 40004 (2012).