

Efficiently Driving F_1 Molecular Motor in Experiment by Suppressing Nonequilibrium Variation

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F_1 -ATPase (F_1) is central to cellular energy transduction. Forcibly rotated by another motor F_o , F_1 catalyzes adenosine triphosphate (ATP) synthesis by converting mechanical work into chemical free energy stored in the molecule ATP. The details of how F_o drives F_1 are not fully understood; however, evaluating efficient ways to rotate F_1 could provide fruitful insights into this driving since there is a selective pressure to improve efficiency. Here, we show that rotating F_1 with an angle clamp is significantly more efficient than a constant torque. Our experiments, combined with theory and simulation, indicate that the angle clamp significantly suppresses the nonequilibrium variation that contributes to the futile dissipation of input work.

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Nanomachines, such as biological molecular motors and artificially synthesized machines, are thought to utilize fluctuations to improve their performance in environments where fluctuations are dominant [1]. Hence, it is natural to think that efficient control of nanomachines differs from that of macroscopic engines. The F_oF_1 -adenosine triphosphate (ATP) synthase is a celebrated example of a biological nanomachine [Fig. 1(a), inset]. A prominent characteristic of this enzyme is that it consists of two mechanically coupled motors, F_o and F_1 . The c ring of F_o rotates the connected γ shaft in F_1 , driven by an electrochemical gradient of H^+ ions across the membrane in which it is embedded. F_1 then synthesizes an ATP molecule from adenosine diphosphate (ADP) and phosphate every 120° rotation of the γ shaft by converting part of the induced mechanical work W into chemical free energy $\Delta\mu$ embodied in an ATP molecule [2–5].

Even in such microscopic systems, the second law of thermodynamics still places fundamental limits on control [8–10]. If F_o rotates the γ shaft 120° quasistatically (infinitely slowly), the average work by F_o per 120° rotation is $W = \Delta\mu$ [3–5], but at finite speed $W > \Delta\mu$. The mean

excess work $W_d = W - \Delta\mu \geq 0$ results in entropy production and, therefore, quantifies the cost for driving the system out of equilibrium. Importantly, W_d depends on the history of how the γ shaft is rotated, prompting interest in understanding optimal ways to rotate the γ shaft that minimize W_d [11,12].

Although the structure of ATP synthase has been solved at high resolution [13], the details of how F_o exerts a torque on F_1 remain to be understood. Considering that ATP synthase is a primary energy converter in the cell, it would be natural to expect that evolution has improved its efficiency. Therefore, exploring efficient control to rotate the γ shaft should help us understand the mechanical coupling between F_o and F_1 .

To quantify how driving affects the work, here we experimentally rotate the γ shaft of isolated F_1 (Supplemental Material, Sec. S1 [14]) in the ATP-synthetic direction as a model system of F_oF_1 free-energy transduction and compare the work during two distinct driving modes that represent extremes of control: the *constant-torque* mode dictates the driving strength, whereas the *angle-clamp* mode controls the mean rotation speed [Fig. 1(b)]. The torque is induced on the γ shaft through a dielectric probe attached to the shaft [Fig. 1(a)]. With the relatively large size of the probe, the mechanochemical coupling can be effectively modeled so that ATP hydrolysis and synthesis respectively correspond to 120° shifts of the potential in opposite directions [16,17] [Fig. 1(c), Supplemental Material, Sec. S2 [14]].

Thermodynamics—Constant-torque mode induces a constant torque, independent of the probe angle, via a rotating

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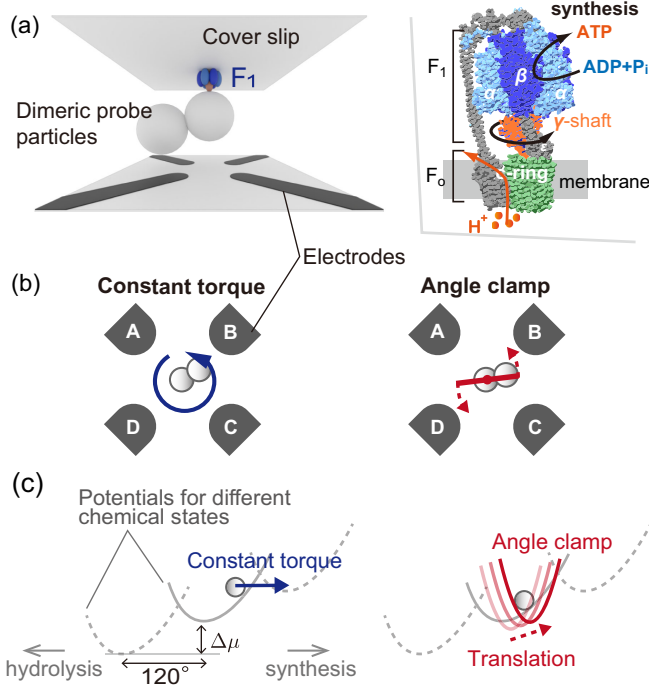


FIG. 1. Single-molecule control of F_1 as a model system of ATP synthase. (a) We externally rotate F_1 derived from thermophilic *Bacillus* PS3 [6] by applying torque on the probe attached to its γ shaft. We observe the rotation by video microscopy at 4 kHz. Inset: F_0F_1 -ATP synthase. (b) We realize constant-torque and angle-clamp driving modes by varying the amplitudes and phases of the alternating-current voltages induced on the four electrodes (A–D) [7]. (c) The free-energy profile results from the interaction between the γ shaft and stator of F_1 and the chemical free-energy change $\Delta\mu$ of ATP hydrolysis. We rotate the probe attached to the γ shaft in the ATP-synthetic direction by a constant torque (left) or by a trapping torque with the trap center rotated at a constant rate (right).

electric field [3,18,19]. In this case, the average rotation rate is not an independent variable but is determined by frictional drag and the net torque on the probe, which is the sum of the induced external torque and the torque exerted by F_1 . The work done on F_1 per 120° rotation by a constant torque N_{ext} is simply

$$W_{\text{trq}} = N_{\text{ext}} \times 120^\circ. \quad (1)$$

For the alternative angle-clamp mode, an electric field oscillating at high frequency induces a sinusoidal external potential $U_{\text{trap}}(\theta - \theta_{\text{trap}}) = -\frac{1}{4}k \cos 2(\theta - \theta_{\text{trap}})$ [7]. The potential has a period of 180° and hence two energetic minima at θ_{trap} and $\theta_{\text{trap}} + 180^\circ$, corresponding to the orientation of the oscillating electric field. Near minima, $U_{\text{trap}}(\theta - \theta_{\text{trap}}) \simeq k(\theta - \theta_{\text{trap}})^2/2$, so k corresponds to the trap's spring constant. We rotate θ_{trap} at a constant rate v_{trap} , thereby dictating the probe's rotation rate (throughout, we exclude the rare trajectories that escape from the local

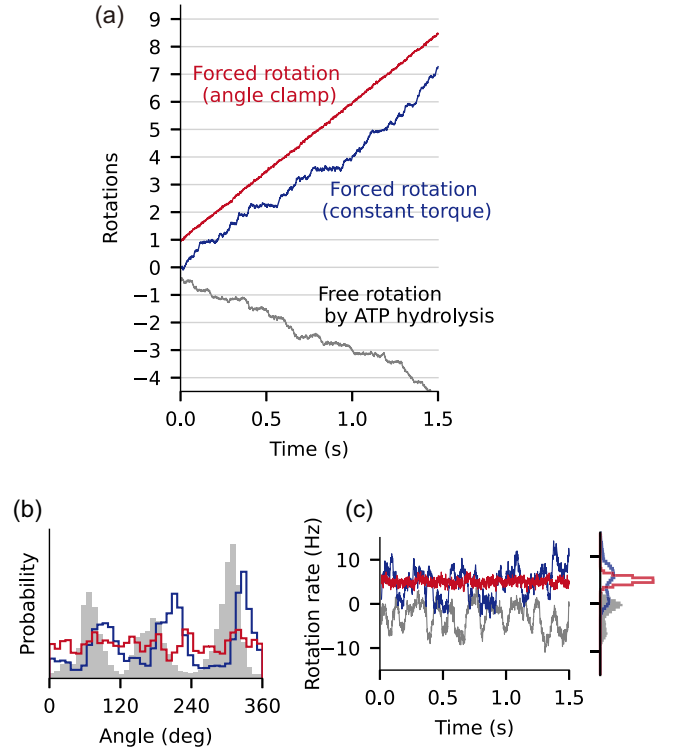


FIG. 2. Rotational trajectories and angular distributions. (a) Rotational trajectories, (b) angular distributions, and (c) rotational rates averaged over a 0.05 s time window, for forced ATP-synthetic rotations under constant torque (blue) and angle clamp (red) and free ATP-hydrolytic rotations (gray). $[\text{ATP}] = [\text{ADP}] = 0.4 \mu\text{M}$.

potential well) [2,4]. The mean work per 120° rotation of the potential is

$$W_{\text{ang}} = \left\langle \int_0^\tau \frac{\partial U_{\text{trap}}(\theta - \theta_{\text{trap}})}{\partial \theta_{\text{trap}}} v_{\text{trap}} dt \right\rangle. \quad (2)$$

Here, $\langle \cdot \rangle$ is an ensemble average over stochastic fluctuations of $\theta(t)$, and $\tau = 120^\circ/v$ is the duration of the 120° rotation, for mean angular velocity v .

Forced rotation—The motor rotates in the ATP synthetic direction when subjected to a sufficiently strong constant torque or rotating angle clamp [Fig. 2(a)]; however, the rotational behavior differs significantly between the two modes. On one hand, under constant torque, for low $[\text{ATP}]$ and $[\text{ADP}]$ F_1 takes three relatively discrete steps [Fig. 2(b)], as previously reported [3]. On the other hand, under the angle clamp the rotation is smooth, without distinct steps; accordingly, the velocity fluctuations are smaller [Fig. 2(c)].

Work—We measure W_{trq} [Eq. (1)] and W_{ang} [Eq. (2)] under different rotation rates and $[\text{ATP}] = [\text{ADP}]$ concentrations [Fig. 3(a)]. Phosphate concentration $[\text{P}_i] = 1 \text{ mM}$ is fixed, so $\Delta\mu = \Delta\mu^\circ + k_B T \ln([\text{ATP}]/[\text{ADP}][\text{P}_i])$ remains fixed at $\approx 18.3 k_B T$, for Boltzmann constant k_B and absolute temperature T . ATP and ADP are mixed in the

forms of MgATP and MgADP; hence the concentrations of these chemical species influence the ionic strength, but this does not significantly affect the standard free energy $\Delta\mu^\circ$. Both W_{trq} and W_{ang} increase with the average rotation rate [Fig. 3(a)]. Under all tested conditions, $W_{\text{trq}} > W_{\text{ang}}$ for a given rotation rate, with the difference being higher for lower [ATP] and [ADP].

The Stokes efficiency

$$\eta_{\text{Stokes}} \equiv \frac{\Gamma v \times 120^\circ}{W_d} \quad (3)$$

quantifies the efficiency of driving against viscous drag [20,21]. Here, Γ is the rotational friction coefficient of the probe-shaft complex, $\Gamma v \times 120^\circ$ is the output work against viscous drag per 120° rotation if the probe realized a constant angular velocity v , and W_d is the input work consumed per 120° rotation. In contrast to the thermodynamic efficiency $\eta_{\text{TD}} \equiv \Delta\mu/W$, the Stokes efficiency compares the performance of the different driving methods while accounting for the inevitable costs of driving at a particular mean rate.

η_{Stokes} is evaluated by calculating the mean of W_d across rotation frequencies between 4 Hz and 6 Hz. The angle clamp has larger η_{Stokes} than the constant torque [Fig. 3(b)] and reaches a value similar to 1, reflecting the small velocity variation [Fig. 2(c)]. Under constant torque, greater [ATP] = [ADP] concentrations give larger η_{Stokes} : at low [ATP] = [ADP], stepwise trajectories are observed, where in the dwell between steps the molecule awaits substrate binding, whereas at high [ATP] = [ADP], such dwells are short, and the trajectories are smoother. Under the angle clamp, η_{Stokes} does not significantly depend on substrate concentrations.

To understand these results, we simulated forced rotation using an established model [17] parametrized from single-molecule experimental data [Fig. S5 [14]]. The simulation qualitatively reproduces the experimental work measurements, validating the model [Fig. 3(a)]. Specifically, the simulations systematically show that the angle clamp requires less work across the explored range of rotation rates and substrate concentrations [Fig. S5(a) [14]]. The fits of experimental data by simulation [Fig. 3(a), solid curves] again show the systematic agreement of simulation and experiment in ranking the work, and hence η_{Stokes} [Fig. 3(b)], of the two driving methods.

Dependence on the trap stiffness—The spring constant k of the angle clamp varies from molecule to molecule because k depends on multiple factors, including the geometry of the dimeric probe. We evaluated η_{Stokes} in the angle clamp separately for molecules with large or small k values [Fig. 3(c)]. To eliminate k -dependent calibration errors in θ_{trap} [Supplemental Material, Sec. S1.3 [14]], η_{Stokes} was evaluated from the linear-fit slopes α of W vs v as $\eta_{\text{Stokes}} = \Gamma \times 120^\circ / \alpha$. η_{Stokes} is not

seen to significantly depend on k . This insensitivity to k is consistent with our simulations [Fig. S5(c) [14]], which found only a slight decrease in W (i.e., an increase in η_{Stokes}) with k . We cannot access a broad range of k values in both experiments and simulations because, for weak k , the probe easily slips out of the local minimum of $U_{\text{trap}}(\theta)$; however, for harmonic $U_{\text{trap}}(\theta)$ with small k , the external torque is only weakly dependent on θ . Hence, an angle clamp with small k may be similar to a constant torque, and it is expected that W_{ang} is larger for smaller k .

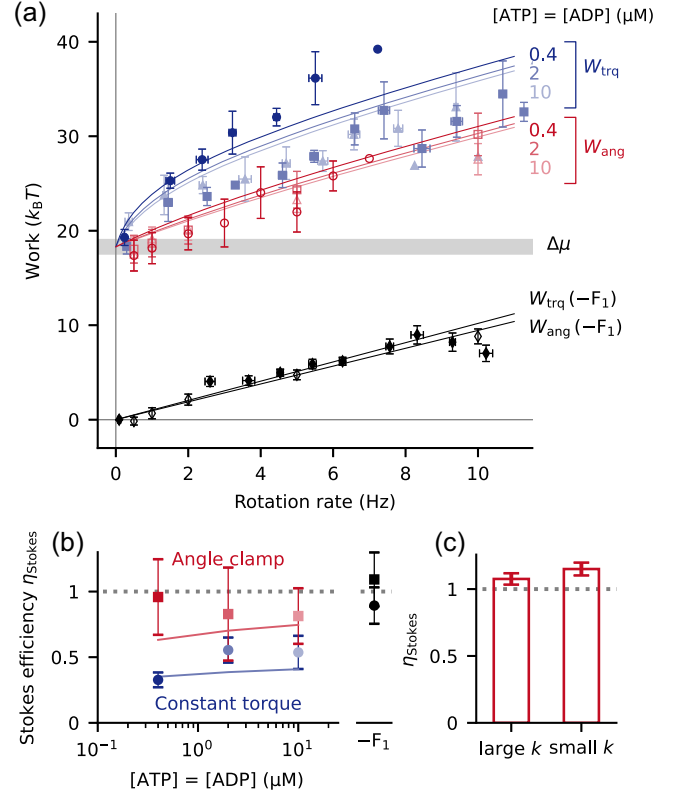


FIG. 3. (a) Average work to rotate the γ shaft 120° , for [ATP] = [ADP] = 0.4 μM (circles), 2 μM (squares), and 10 μM (triangles) by constant torque (blue filled symbols) and angle clamp (red open symbols). Solid curves show fits of simulations (Supplemental Material, Sec. S2 [14]). The negative control $-F_1$ corresponds to a freely rotating Brownian dimeric probe pinned on a glass surface without F_1 . 159 trajectories (32 molecules) and 278 trajectories (34 molecules) are measured for the angle-clamp and constant-torque modes, respectively. W_{trq} is averaged across bins with 1-Hz width. Error bars indicate standard errors (SE) in each bin. (b) The Stokes efficiency η_{Stokes} [Eq. (3)] for different [ATP] = [ADP], calculated using the mean of W_d for rotational rates between 4 Hz and 6 Hz. Solid curves are simulations for 5 Hz. Error bars indicate SE obtained by error propagation from the SE of Γ , v , and W_d . (c) η_{Stokes} in angle clamp was separately evaluated (using linear fit slope of work vs global mean velocity) for molecules with large or small k values (threshold is 70 $k_B T/\text{rad}^2$, and different [ATP] = [ADP] concentrations are mixed). Error bars indicate SE.

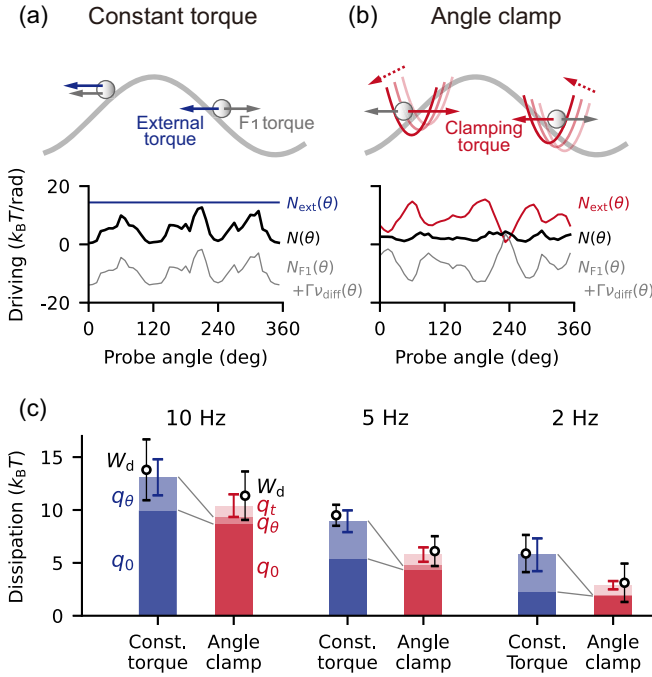


FIG. 4. The mechanism for reduced work under angle clamp. Schematic of the mechanism (top) and representative examples of torque balance (bottom) under constant torque (a) and angle clamp (b), for average rotation rate ≈ 5 Hz. The net torque is obtained from the local mean velocity as $N(\theta) = \Gamma \nu(\theta)$. Subtracting the external torque $N_{\text{ext}}(\theta)$ gives the composite torque by F_1 and diffusional flow: $N_{F_1}(\theta) + N_{\text{diff}}(\theta) = N(\theta) - N_{\text{ext}}(\theta)$. (c) Dissipation estimates q [Eq. (5)] (bars) and $W_d = W - \Delta\mu$ with W calculated by Eq. (2) (circles) per 120° rotation, for indicated average rotation rate and $[\text{ATP}] = [\text{ADP}] = 10 \mu\text{M}$. q and its decompositions are obtained by integrating the terms in Eq. (5) over 120° rotation. Error bars indicate standard errors.

Nonequilibrium variation—To understand the mechanism behind the angle clamp’s advantage, we consider a Brownian particle on a one-dimensional potential for simplicity. We define the local mean angular velocity $\nu(\theta, t)$ as the ensemble average of the angular velocity $d\theta(t)/dt$ at angle θ and time t [Supplemental Material, Sec. S1.4, Fig. S3 [14]]. Constant torque produces a steady state, leading to $\nu(\theta, t) = \nu(\theta)$ independent of t . In the angle-clamp mode, $\nu(\theta, t)$ is periodic in t with a period of 3τ (the duration of a 360° rotation of the angle clamp).

In this setup, $\dot{W}_d$ coincides with the mean heat rate determined by the squared average of $\nu(\theta, t)$ [8]:

$$\dot{q} \equiv \Gamma \langle \overline{\nu(\theta, t)^2} \rangle. \quad (4)$$

Throughout, the dot on variables indicates a time-averaged rate. For an arbitrary quantity $A(\theta, t)$, $A(\theta) = \overline{A(\theta, t)} \equiv [3\tau p(\theta)]^{-1} \int_0^{3\tau} dt A(\theta, t) p(\theta, t)$ is the local mean at θ over a full rotation of the angle clamp. The probability density $p(\theta, t)$ over θ at given t has normalization

$\int_0^{360^\circ} d\theta p(\theta, t) = 1$, and the time-averaged probability density over θ is $p(\theta) \equiv (3\tau)^{-1} \int_0^{3\tau} dt p(\theta, t)$. $\langle \overline{A(\theta)} \rangle \equiv \int_0^{360^\circ} d\theta A(\theta) p(\theta)$ is the average over θ of an arbitrary quantity $A(\theta)$. The local mean net torque exerted on the probe is $N(\theta, t) = \Gamma \nu(\theta, t)$, the sum of the torque by external driving (N_{ext}) and F_1 (N_{F_1}), and the diffusional driving by the gradient of the probability density ($\Gamma \nu_{\text{diff}}$). Here, $\nu_{\text{diff}} = -(k_B T / \Gamma) \partial \ln p(\theta, t) / \partial \theta$ [8].

The mean heat rate in Eq. (4) is a (double) expectation of the convex function $\nu(\theta, t)^2$, which admits the decomposition $\langle \overline{\nu(\theta, t)^2} \rangle = v^2 + \langle \overline{[\nu(\theta, t) - v]^2} \rangle + \langle \overline{[\nu(\theta, t) - \nu(\theta)]^2} \rangle$, where $\nu(\theta) \equiv \overline{\nu(\theta, t)}$ and $v \equiv \langle \overline{\nu(\theta)} \rangle$. This is analogous to the standard identity $\mathbb{E}[x^2] = \mathbb{E}[x]^2 + \sigma^2[x]$ (for expectation $\mathbb{E}$ and variance σ^2), and it more precisely pinpoints the different sources of dissipation in each driving method.

The decomposition of the mean heat rate is equivalently

$$\dot{q} = \underbrace{N_0^2 / \Gamma}_{\dot{q}_0} + \underbrace{\langle \overline{[N(\theta) - N_0]^2} \rangle / \Gamma}_{\dot{q}_\theta} + \underbrace{\langle \overline{[N(\theta, t) - N(\theta)]^2} \rangle / \Gamma}_{\dot{q}_t}. \quad (5)$$

Here, $N(\theta) = \overline{N(\theta, t)}$ is the local mean torque at θ , and $N_0 = \langle \overline{N(\theta)} \rangle = \Gamma v$ is the global mean torque. The right-hand side terms respectively quantify the dissipation originating from global mean angular velocity v , angular variation of $\nu(\theta)$ around v , and temporal variation of $\nu(\theta, t)$ around $\nu(\theta)$ (respectively denoted as $\dot{q}_0$, $\dot{q}_\theta$, and $\dot{q}_t$).

Under constant-torque and angle-clamp modes, we independently evaluated $N(\theta)$ and $N_{\text{ext}}(\theta)$, thereby quantifying the composite internal driving $N_{F_1}(\theta) + \Gamma \nu_{\text{diff}}(\theta) = N(\theta) - N_{\text{ext}}(\theta)$ [Figs. 4(a) and 4(b)]. The angular variation of $N(\theta)$ under the angle clamp is significantly less than that under constant torque. This can be intuitively understood: under constant (externally applied) torque, the net torque and hence the rotation rate is lower when facing resistive torque from F_1 and higher when receiving assistive torque (and these systematically vary across angles); however, under the angle clamp, the external trapping potential increases torque when the γ shaft lags (at the ensemble level, due to resistive torque from F_1) and decreases torque when the γ shaft rotates particularly rapidly (generally when F_1 provides assistive torque), thus acting as a suspension to suppress variation of the rotation rate. By contrast, constant-torque driving acts without any “shocks.”

Accordingly, the angle clamp has significantly smaller dissipation q_θ due to angular variation of $\nu(\theta)$ [Fig. 4(c)]. By contrast, dissipation q_t owing to temporal variation vanishes under constant torque since the ensemble is at steady state: $N(\theta, t) = N(\theta)$. At high substrate concentrations such as $10 \mu\text{M}$ (larger than the Michaelis constant $K_M \approx 2 \mu\text{M}$ under experimental conditions [22]), substrate binding does not significantly slow the chemical rates, and F_1 ’s mechanical dynamics is well approximated using a

one-dimensional potential. Consistently, in this regime q is similar to W_d [Fig. 4(c)]. While the angle clamp has significant temporal variation q_t , the total variation $q_\theta + q_t$ was greater for constant torque than for angle clamp. The results demonstrate that the angle clamp reduces nonequilibrium variation by a suspension mechanism and, therefore, reduces the dissipation.

At high substrate concentrations, chemical reactions are sufficiently fast compared to rotational diffusion such that dissipation by chemical switching is negligible. Then, $\nu(\theta, t)$ encompasses all the dissipation by Eq. (4). At low substrate concentrations, switching between distinct mechanical potentials has an important effect. Indeed, for 0.4 and 2 μM we find a significant difference between W_d and apparent heat q , due to the unobserved chemical degree of freedom [23] [Fig. S4 [14]].

Many attempts have been made to relate the magnitude of the nonequilibrium fluctuations to work or entropy production [24], based on, e.g., fluctuation theorems [25], the fluctuation-dissipation theorem [26], or thermodynamic uncertainty relations (TURs) [27,28]; however, it is not straightforward to apply these relations to the present setup. For instance, the TUR provides an upper bound (determined by the diffusion coefficient) on the Stokes efficiency [29], but the TUR is generally valid only in steady state, and its application to the angle-clamping mode is not straightforward.

Discussion—In this paper, we explored efficient control of F_1 using single-molecule experiments and simulations. We demonstrated that angle-clamp driving requires significantly less work than constant-torque driving. Further analysis revealed the crucial importance of suppressing variation in achieving efficient control.

We rotated the angle clamp at a constant rate. However, optimal control theory [30,31] indicates that the work could be further reduced by modulating the clamp's instantaneous rotation rate and stiffness depending on F_1 's potential profile. Guided by work-minimizing protocols in the linear-response regime [32], uniform excess power over t has been suggested as a general design principle for the optimal protocol. This framework has been applied to DNA-pulling experiments [33] and simulations of F_1 [11,12] and active particles [34]. The forms of Eqs. (4) and (5) suggest that uniform entropy production (instead of uniform excess power) is a natural guiding principle for minimizing work; the linear-response limit is a special case where all excess work is immediately dissipated as heat. In contrast, constant torque drives the system to a nonequilibrium steady state. In this case, work is minimized when power $N_{\text{ext}}\nu(\theta)$ is uniform over θ , given that the dynamics are approximated by a one-dimensional Langevin equation. Future studies should more broadly test the validity of these guiding principles.

The torque generated by F_0 using the electrochemical gradient of H^+ ions is transmitted to the γ shaft of F_1 via an

elastic coupling; this coupling naturally suggests an angle-clamp driving mechanism. The smaller work by angle clamp also suggests a selective advantage for this driving mechanism. However, F_0 (itself a strongly fluctuating object) can only implement stochastic driving [35], and in the intact F_0F_1 holoenzyme F_0 's driving is not independent of F_1 . Thus, the true *in vivo* situation is likely more complicated than our angle-clamp experiments. Future experiments, including the simultaneous observation of the rotations of F_0 and F_1 , should help clarify the intervening torque-transmission mechanism.

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Data availability—The data that support the findings of this Letter are not publicly available upon publication because it is not technically feasible and/or the cost of preparing, depositing, and hosting the data would be prohibitive within the terms of this research project. The data are available from the authors upon reasonable request.

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