Supporting information

Table 1 Parameters used in the model calculations

Quantity		Value	Comments
Potential periodicity, δ		$2\pi/26$	(40, 41)
Rotor drag coefficient, ζ_θ		2×10^{-4} pN.nm.s/rad^2	Estimated (2)
Bead diffusion constant ,		0.01 – 200 pN.nm.s/rad^2	Calculated from Stokes' Law
Linkage spring constant,		150-500 pN.nm/rad^2	Estimated from experimental measurements (2, 12)
Saw-tooth potential height, U		10 k$_B$T	Ad hoc
Ratio of the two potential branches, L$_{left}$/L$_{right}$		1/9	
Potential bumps	height	15 k$_B$T	
	width	0.2δ	
	centers	0.1δ (State1), 0.6δ (State 2)	
Transition windows	θ_α,	$0.1\delta, 0.6\delta$	
	θ_β	$0.58\delta, (0.58+0.5)\delta$	Fitting data
	k$_m$	0.9×10^5 (T = 22.7^{o}C) 0.5×10^5 (T = 17.7^{o}C) 0.45×10^5 (T = 15.8^{o}C)	

Figure 4 (a) The variables defined in the experimental arrangement where the rotation is measured via a polystyrene bead attached to the flagellum. (b) The effect of a soft linkage in the BFM system. Calculations were done with eight stators. The black line and points are the motor torque-speed relation under a conservative load (in this case, a constant torque applied directly to the rotor); the red line and points are calculated from the model where the viscous load is attached to the motor via a compliant linkage. To the motor, this appears as a purely conservative load, as in the experiments. The plateau region extends to backward rotations, also in agreement with experiments . If the linkage is rigid, so that the motor sees only the viscous load, the motor torque-speed curve is concave rather than convex (blue line and points).

Figure 5 Schematic illustration of the computational grid for solving the 2-dimensional Fokker-Planck equations.

Figure 6 Schematic illustration of the chemical cycle of transporting ions from the periplasm side to the cytoplasm side. where 'O' and 'E' represent occupied and empty states, and the subscripts (P, C) refer to the periplasm and cytoplasm; i.e. 'O_P' means that the complex is in the occupied periplasm half channel conformation.

Figure 7 Hypothetical stator conformational transitions driving the power stroke. (a) Top view of a rotor-stator arrangement and a possbile power generation mechanism due to relative rotation between MotA and MotB. The stators form two tracks interacting with the rotor corresponding to counter-clockwise and clockwise rotations. Change in the occupation states results in relative rotation between MotA and MotB for both of the two half stators driving cyclic motions of the cytoplasmic loops on MotA. The two cytoplasmic loops engage the rotor alternately during the first and second half of the cycle to push the rotor. (b) Switching rotation direction can be achived by a conformational change in the FliG C-terminal domain controlled by binding CheY onto the rotor . The identified charged residues at the rotor-stator interface may help bias one of the two conformations.

Figure 8 Schematic illustration of the effect of external torque on the effective chemical transition rates. Here we show three stators working together. Each ball represents the angular distance between a reference point on the rotor and a stator. Neglecting stator springs, the three balls are rigidly coupled by the rotor. (a) Under a small or no load, the potentials are less tilted, so that the first two stators may push the rotor out of the chemical transition window of the third stator before chemical transition can take place in the third stator. The system then is trapped in the composite potential well of the three stators. In this situation, the forward rotation of the rotor is resisted by the third stator, and so it has to wait until thermal fluctuations bring it back into the transition window of the third stator, whereupon a chemical transition to the next potential, initiates a new power stroke by the third stator, permitting forward rotation. Consequently, the effective chemical transition rate is low since the peak of the distribution function ρ in Eq. (5) is away from the transition window. (b) Under high load, the potentials are tilted so the above mentioned situation is less likely to happen, which results in a higher effective chemical transition rate than that in case a, and time-scale separation between the motor and the load is held. (c) While treated explicitly, stator spring fluctuations may help the system to 'search' for the transition region. Consequently a smaller transition window is necessary to make the stator-stator inteference large enough to reproduce the observed motor torque-speed relation.

Figure 9 The current model uses thermally excited transitions to describe both ion hopping onto and off the stator, and thermally excited triggering of the stator conformational changes (power stroke).This simplified treatment gives nonlinear pmf dependence at high speed (panel a and b). In panel c, Equation (17) is used to fit the experimental data at high speed shown in Figure 2b of Gabel and Berg (8) with $\alpha = 20$, $\beta = 2$. The theoretical curve and the experimental data are given by the solid line and circles, respectively. Given the experimental uncertainties and the simple theoretical analysis to obtain Equation (17), the agreement is remarkable.

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Figure 10 (a) Kinetic networks for proton hopping. (b) Free energy profiles within the MotA/MotB complex. We have omitted the intermediate steps with single occupancy.

Figure 11 Torque-speed curves with different numbers of stators predicted by the model. At low speeds, the motor torque increases linearly with the number of stators. At high speed, the motor torque decreases to zero more quickly on adding stators due to stator mutual inteference, the same reason resulting in the sharp transition of the motor-torque-speed curve.

Supporting Text

The role of elasticity in the flagellar motor

Here we show that, if elasticity in the system is neglected, the motor torque-speed relation can be quite different under a conservative load and under a viscous load. Consider the general case where a motor moves along spatial potentials, $V_i(\theta)$, corresponding to $i = 1,...,m$ chemical states. The governing equations for the motor motion are,

$$\partial_t \rho_i(\theta,t) = D\partial_\theta \left[\frac{\rho_i(\theta,t)}{k_B T} \frac{d}{d\theta}(V_i(\theta) + \tau_{ext}\theta) + \partial_\theta \rho_i(\theta,t) \right] + \sum_{j \neq i} \left[K_{ij}(\theta)\rho_j(\theta,t) - K_{ji}(\theta)\rho_i(\theta,t) \right],$$

(9)

where $\partial_\theta \equiv \partial/\partial\theta$. $\rho_i(\theta,t)$ is the probability density for the motor to be at position θ, time t, and chemical state i. D is the diffusion constant, k_B is the Boltzman's constant, T is the temperature, $V_i(\theta)$ is the potential function in chemical state i, τ_{ext} is the external torque, and K_{ij} is the chemical transition rate from state j to i. The steady state solution is obtained by setting the left side of (9) to zero. The motor torque τ_M is given by

$$\tau_M = \zeta_M \nu_M + \tau_{ext} = -\sum_{i=1}^{m} \int_0^\delta d\theta \rho_i(\theta) \cdot \partial_\theta V_i(\theta)$$

(10)

where the motor drag coefficient $\zeta_M = k_B T/D$. Thus the motor torque depends on the probability distributions, $\rho_i(\theta)$. Equation (9) shows that the probability distributions depend linearly on the diffusion constant, but exponentially on the external load, since the latter modifies the effective potentials directly and thus has exponential effects on the probability distributions. Thus one would expect different motor torque-speed relations for purely viscous or conservative loading, as illustrated in Figure 4b. However, this is not what is experimentally observed for the flagellar motor.

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This apparent discrepancy can be resolved if the motor and load are linked by an elastic (not necessarily linear) linkage. At steady state, a motor pulling a cargo through an elastic linkage is described by

$$-\frac{\partial J_\theta(s,\theta,\theta_L)}{\partial \theta} - \frac{\partial J_{\theta_L}(s,\theta,\theta_L)}{\partial \theta_L} + K\rho = 0, \tag{11}$$

where

$$J_\theta(s,\theta,\theta_L) = -D_\theta\left[\frac{1}{k_BT}\frac{\partial V_s}{\partial \theta}\rho_s + \frac{\partial \rho_s}{\partial \theta}\right], \quad J_{\theta_L}(s,\theta,\theta_L) = -D_{\theta_L}\left[\frac{1}{k_BT}\frac{\partial V_s}{\partial \theta_L}\rho_s + \frac{\partial \rho_s}{\partial \theta_L}\right], \tag{12}$$

where θ and θ_L describe motor and load positions, respectively, K is the chemical transition matrix, and ρ is the probability density of finding the system at the state (s, θ, θ_L). For simplicity, we assume the linkage is harmonic $V_s = V_0(\theta) + \frac{1}{2}\kappa(\theta - \theta_L)^2$ with a spring constant κ, but the conclusion holds for more general linkage potentials.

If the system is *tightly coupled,* i.e. the chemical transitions are, on average, always associated with a fixed mechanical translation distance, θ, then the motor can be viewed approximately as diffusion along an *effective* 1-D path (the minimum energy path, or MEP) on the reaction-rotation surface (42). The potential along the effective 1D path in the large scale can be approximated by a linear potential with slope $\Delta G/\delta$, but with local features given by $V(\theta)$. ΔG and δ refer to the free energy drop and translocation distance along the mechanical degree of freedom associated with one motor cycle. Then the system can be reduced to a load elastically linked to a motor driven by a 1-D tilted potential, which is similar to the problem studied by Elston and Peskin (32). Their main conclusion is that the load experiences a linear effective potential created by the motor in the limit $D_{\theta_L}/D_\theta \to 0$, and $\kappa \to 0$. Rather than reproducing their analysis, we give a heuristic discussion.

With a stiff spring, local features of the potential are important. However, with a very soft spring,

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the potential can be well approximated by a harmonic potential plus the linear potential $(\Delta G/\delta)\cdot\theta$. Consequently the *effective* potential experienced by the load is

$$\bar{V}(\theta_L) = -k_B T \ln \int_{-\infty}^{\infty} d\theta \exp\left(-\frac{1}{k_B T}\left[V(\theta) + \tau_{ext}\theta_L + \frac{1}{2}\kappa(\theta - \theta_L)^2\right]\right)$$

$$= \tau_{ext}\theta_L - k_B T \ln \int_{-\infty}^{\infty} d\theta \exp\left(-\frac{1}{k_B T}\left[V(\theta) + \frac{1}{2}\kappa(\theta - \theta_L)^2\right]\right)$$

$$\approx (\tau_{ext} - \Delta G / \delta)\theta_L + \text{constant}$$

Therefore, the effect of the viscous load on the motor is to exert a torque with an average value of $\kappa(\theta - \theta_L)$, which is balanced by the motor torque in the steady state. The apparent motor torque depends on the relative dynamics between the motor and the load. The motor dynamics is affected by the motor diffusion constant, chemical transition rates, as well as the potential shapes, while the load dynamics is affected only by its diffusion constant and the spring constant.

The effective rate for stator transitions is defined as: $\bar{k} \sim \int k(\theta_M)\tilde{\rho}(\theta_M)d\theta_M$,

where $\tilde{\rho}(\theta_M) = \int \rho(\theta_M, \theta_L)d\theta_L$. Therefore, $\tilde{\rho}$, and so $\bar{k}$ are affected by the torque from the load through the spring. When several kinetic processes are involved, the slowest one is rate limiting. We are interested in the case that motor relaxation within the potential well of each chemical state is much faster than chemical transitions and the load relaxation. Then the two relevant time scales in the system are $1/\bar{k}$, and ζ/κ, where κ is the spring constant, and ζ is the load drag coefficient. Define the dimensionless ratio $r = \bar{k}\zeta/\kappa$. In the limit when (i) the time scale of the load is much longer than that of the motor ($r \gg 1$), (ii) tight coupling holds, and (iii) the linkage is sufficiently soft (*i.e.*, it allows the motor to explore several units of δ),

$$\zeta_L\omega \approx \Delta G / \delta = (\Delta H - T\Delta S) / \delta. \tag{13}$$

From this it is clear that, in this limit, (i) the speed scales linearly with temperature, and (ii) the calculated motor torque-speed curves under conservative load and under viscous load are identical (see Figure 4b). Therefore, for most of the results reported in this paper, we used the 1-

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D calculations under a conservative load.

In the case that the system is not perfectly tightly coupled between chemical transitions and mechanical motion (which is likely in the case of mycoplasma (38)), Eq. (13) can be generalized to

$$\zeta_L \omega \approx \Delta G / \delta = f(\Delta H - T\Delta S) / \delta \tag{14}$$

where f takes the value between 0 and 1.

Computational details

The chemical transitions are modeled as,

$$k_0 = fk_m, \tag{15}$$

Where

$$f = \begin{cases} \dfrac{\theta - \theta_\alpha}{\frac{1}{2}\left(\theta_\beta - \theta_\alpha\right)}, & \text{for } \theta_\alpha < \theta < \frac{1}{2}\left(\theta_\alpha + \theta_\beta\right) \\[3em] \left(1 - \dfrac{\theta - \frac{1}{2}\left(\theta_\alpha + \theta_\beta\right)}{\frac{1}{2}\left(\theta_\beta - \theta_\alpha\right)}\right), & \text{for } \frac{1}{2}\left(\theta_\alpha + \theta_\beta\right) < \theta < \theta_\beta \\[2em] 0 & \text{otherwise} \end{cases}$$

is the transition window related to the rotor-stator interactions, and θ is the angular position of the rotor. The expression for the transitions happened in the second window is obtained by shifting all the angular coordinates in the above expressions by half of the rotor cycle $2\pi/26$.

We solved the steady-state Fokker-Planck equations for the 1D case (with the rotor subject to a conservative load or rigidly linked to a viscous load), and for the 2D case (with the rotor elastically linked to a viscous load). For the 1D calculations, and the 2D calculations with ≤ 4 stators, the algorithm developed by Xing *et al.* allows solving the coupled Fokker-Planck

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equations directly (17). In our calculations, we found that results converged with 16 subregions within one rotor period $\delta = 2\pi/26$ for each degree of freedom. The steady-state probability density function can be defined within the range $\theta \in [0, \delta]$, and $\theta_L \in [\theta - \theta_a, \theta + \theta_b]$. The range of θ_L is chosen to be large enough so that for a given θ the density at the boundary is negligible (due to the elastic linkage between the motor and the load). Two types of boundary conditions are used for transitions out of the working region. At a given θ, absorbing boundary conditions are used for transitions out of the range of θ_L, thus the transition rates are zero. For transitions that move the system out of the range $\theta \in [0, \delta]$, the following symmetry holds: $\rho_s(\theta, \theta_L) = \rho_s(\theta + \delta, \theta_L + \delta)$. The computational grid is illustrated in Figure 5. The steady-state solutions are obtained by solving the linear equations with the MATLAB built-in solver, and the steady state rotation rates ω are then calculated from the steady-state solution. The motor torque is calculated from the relation $(\zeta_\theta + \zeta_{\theta_L})\omega = \tau_M$.

Noticing the equivalent motor torque-speed relations for the motor system under a conservative load and a viscous load elastically linked to the motor, the pmf dependence results were obtained in the following way. First the motor torque-speed relations with various pmf were calculated for the motor system under conservative loads. Then the rotation speed for a given load with drag coefficient ζ was the intersection between the load line $\zeta\omega$ and the motor torque speed curve with certain pmf.

All the Fokker-Planck results were also tested against results obtained by Langevin trajectory calculations. Stochastic simulations were done for the Langevin trajectories (Wiener Process) in the following way:

$$\theta(t + \Delta t) = \theta(t) - \frac{D_\theta}{k_B T} \frac{\partial V_s}{\partial \theta} \Delta t + \sqrt{2D_\theta \Delta t} \xi_\theta(t), \tag{16}$$

where $\xi(t)$ is a normal random variable with zero mean and unit variance (26, 27), and the angular coordinate θ is actually a two-component vector (θ, θ_L) in the case of motor elastically linked to a viscous load. The chemical state of each stator is updated simultaneously by the

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following rule. In chemical state s at time t, the probability of a transition to chemical state s′ is determined by the corresponding chemical rates $k_{s \rightarrow s'} \Delta t$ for s′ ≠ s, and $1 - \sum_{s \neq s'} k_{s \rightarrow s'} \Delta t$ for s′ = s.

The destination state is chosen using a Monte Carlo scheme (28). In all of calculations, the step size is $\Delta t = 10^{-11}$ s. The rotation rate is determined by the difference between the final and initial motor positions within the given time interval. The final results are obtained by averaging over 10 trajectories, each 0.06s long. Results with the two methods agree within numerical errors.

All the model parameters used in this work are given in Table 1. To fit the data shown in Figure 2, we tuned the rate constant k_m and the right boundary of the transition window θ_β in Equation (15).

Details not resolved by the model

Here we discuss some details that cannot be resolved by our current model and propose several competing schemes. Further experimental studies must be performed to resolve these issues.

For an ion-driven motor, the ion-transport complex ($MotA_4MotB_2$ for proton BFM, and rotor/stator for the F_o motor (43)) must exist in at least two conformations, which open alternately to the cytoplasm or periplasm. When the ion-transport complex is in the conformation where the half channel is open to the periplasm (p), an ion can hop onto a channel site. A protein reorganization transforms to the conformation where the cytoplasm half-channel is open (c) whereupon the ion can dissociate into the cytoplasm. To utilize the free energy associated with the transporting ion for mechanical work, the two half channels must be dislocated or the connection between them blocked so that an ion cannot access the cytoplasm directly from the periplasm; the transformation between the two conformations can be used to generate mechanical work. Figure 6 gives the minimal kinetic path that a complex must follow. Experimentally, it has been shown that the interface between MotA and MotB contains the ion pathway of the BFM. Blair and coworkers showed that the MotA/MotB interface is solvent accessible for about one third of the way from either end (30). This is consistent with a model structure proposed by Zhai et al (44). Residues D32 are the only requisite binding sites for ions, and may expose to the two sides of the membrane alternatively (45). The $MotA_4MotB_2$ probably

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behaves analogously to a transporter; indeed, genetic studies show homology between the stator and transporters (44). If the energy of ion binding drives the conformational change, then we expect that for the occupied state, conformation C is preferred, and for the empty state, conformation P is preferred. Thus a half stator switches between its two conformations triggered by ions hopping on and off D32. Reorganization of the complex makes possible hydrogen bond breaking and reforming between the twin D32s and the transported ions (protons or sodiums) and the two hydrophilic regions on MotAs that extend to the cytoplasm and periplasm, respectively. This is analogous to the electron-transfer process described by classical Marcus theory, where solvent reorganization makes electron hopping energetically allowed (46, 47). For protons, significant tunneling effects may occur in this step, which may explain the observed isotope effect (6).

Motions of the trans-membrane helices result in motions of the cytoplasmic loop between MotA helix 2 and 3 which contacts the rotor electrostatically and/or sterically. The loop motion can be up-and-down (which would require an inclined rotor-stator contact surface as shown in the accompanying movie), rotational as proposed by Schmitt (23) or, most likely, a combination of the two motions (as illustrated in Figure 7a). Residues like Proline 173 and 222 may function on transforming the helix motion to the loop motion (21).

The actual situation is more complicated since there are two half stators (which compose two ion channels). Berg has shown that the duty ratio of the flagellar motor must be close to one even if only one stator works (2). Thus the two half stators must work cooperatively so that the stator is always engaged. There are two possible schemes for the two half stators.

- *Scheme 1*: The two half channels both assume either conformation P or C. Binding of one ion from the periplasm enhances binding of the second ion. Then the $MotA_4MotB_2$ complex with conformation O_pO_p becomes mechanically unstable, and transforms to conformation O_cO_c. Two ions are released to the cytoplasm, which leaves the $MotA_4MotB_2$ complex with conformation E_cE_c mechanically unstable, and it transforms back to conformation E_pE_p. Two cytoplasmic loops from the two half stators become engaged with the rotor alternatively corresponding to the two conformational changes to generate the two power strokes. A schematic cartoon movie with a rotor-stator arrangement consistent to the stator model

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proposed by Blair and coworkers is included.

- *Scheme 2*: mutual steric constraints within the $MotA_4MOtB_2$ complex require that if one half channel assumes conformation P, then the other one must assume conformation C, and vice versa. Conformational changes happen between O_pE_c and O_cE_p, and between E_cO_p and E_pO_c.

A schematic illustration of a rotor-stator arrangement consistent with the stator model proposed by Blair and coworkers is shown in Figure 7a.

Our proposed schemes are closely related to the kinematic models proposed by Lauger (48), Blair (3, 22), and Schmitt (23). The 2-state model used in this work is a consequence of assuming that the MotA/MotB conformational change is much slower than other steps, and that co-operative proton binding reduces the four possible ion-binding states to two. Both of the above two schemes are consistent with the high duty ratio requirement, but further experimental studies are needed for conclusive distinctions. In the current work we have used Scheme 1; however, all the conclusions hold as well for Scheme 2.

Finally, the flagellar motor can rotate in both clockwise (CW) and counter-clockwise (CCW) directions. A possible switching mechanism is shown in Figure 7b.

Issues that need to be addressed in future studies are:

- MotB is attached to the peptidoglycan layer by a long loop, and there is a long cytoplasmic loop between Helix 2 and 3 of the MotA. A certain degree of elasticity may be associated with these loops that may affect motor function.

- Stators may interact with one another (e.g., mutual exclusion due to steric constraints). This may affect the stator dynamics by affecting synchronization.

- We did not explore how stator distributions affect the motor dynamics, e.g. Vernier mismatch effects (49). An especially interesting question is the recovery pattern during resurrection experiments may not be unique. For example, if two stators recover, they can be either

neighbors or separated by one or more stators. Will this produce different torque-speed curves?

- A more detailed treatment of the stator chemical states than we have used is probably necessary. For this purpose mechano-chemical measurements at different pH and membrane potentials are needed.

More on pmf dependence at high speed

Here we will show that the weak pmf dependence at high speed can be explained if conformational changes much slower than ion hopping rates are involved within the stator. The crude theoretical analysis given below gives a general pmf dependence of the rotation rate at very small external load as:

$$\omega \propto f = \frac{(1 - e^{-2pmf})}{1 + \alpha e^{-pmf} + \beta e^{-2pmf}} \tag{17}$$

As expected, the linear dependence on pmf at small pmf can be achieved by Taylor expanding the above expression in the pmf. Figure 9 shows that the above formula can be used to fit the observed 'linear' pmf dependence at high speed (8)).

To go beyond the analysis discussed here, we need to construct a more detailed model that treats the concentration gradient, and the membrane potential explicitly. However, these require more systematic and reliable experimental measurement, which is under the way.

Here we use the scheme shown in Figure 10 to show the derivation of equation (17). The free energy of each state can be given approximately by

$$G_2 = G_3 \sim G_1 - G_{Ep} + G_{Op} - \ln(C_p / C_0)$$

$$G_4 \sim G_1 - 2G_{Ep} + 2G_{Op} - \ln(C_p^2 / C_0^2)$$

$$G_5 \sim G_1 - 2G_{Ep} + 2G_{Oc} - \ln(C_p^2 / C_0^2)$$

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$$G_6 = G_7 \sim G_1 - 2G_{Ep} + G_{Oc} + G_{Ec} - \ln(C_p^2 / C_c C_0)$$

$$G_8 \sim G_1 - 2G_{Ep} + 2G_{Ec} - \ln(C_p^2 / C_c^2)$$

Here the energy is in units of $k_B T$. Then

$$p_1 \sim \frac{\exp(-G_1)}{\sum\limits_{i=1}^{4} \exp(-G_i)} p_I \sim \frac{1}{\left(1 + e^{(G_{Ep} - G_{Op}) + \ln(C_p / C_0)}\right)^2} p_I$$

$$p_4 \sim \frac{\exp(-G_4)}{\sum\limits_{i=1}^{4} \exp(-G_i)} p_I$$

$$\sim \frac{e^{2\left(G_{Ep} - G_{Op} + \ln(C_p / C_0)\right)}}{\left(1 + e^{(G_{Ep} - G_{Op}) + \ln(C_p / C_0)}\right)^2} p_I$$

$$p_5 \sim \frac{\exp(-G_5)}{\sum\limits_{i=5}^{8} \exp(-G_i)} p_{II}$$

$$\sim \frac{e^{2(G_{Ec} - G_{Oc}) + 2\ln(C_c / C_0)}}{\left(1 + e^{(G_{Ec} - G_{Oc}) + \ln(C_c / C_0)}\right)^2} p_{II}$$

$$p_8 \sim \frac{\exp(-G_8)}{\sum\limits_{i=5}^{8} \exp(-G_i)} p_{II}$$

$$\sim \frac{1}{\left(1 + e^{(G_{Ec} - G_{Oc}) + \ln(C_c / C_0)}\right)^2} p_{II}$$

and

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$$\frac{k_a}{k_a^{-1}} = e^{2(G_{Ec} - G_{Ep})}, \qquad\qquad \frac{k_b}{k_b^{-1}} = e^{-2(G_{Oc} - G_{Op})}$$

Since,

$$\frac{dp_I}{dt} = p_8 k_a + p_5 k_b^{-1} - p_4 k_b - p_1 k_a^{-1}, \quad p_I + p_{II} = 1,$$

one has,

$$\frac{dp_I}{dt} = p_8 k_a + p_5 k_b^{-1} - p_4 k_b - p_1 k_a^{-1}$$

$$= \frac{k_a + e^{2(G_{Ec} - G_{Oc}) + 2\ln(C_c/C_0)} k_b^{-1}}{\left(1 + e^{(G_{Ec} - G_{Oc}) + \ln(C_c/C_0)}\right)^2} p_{II} - \frac{e^{2(G_{Ep} - G_{Op}) + 2\ln(C_p/C_0)} e^{-2(G_{Oc} - G_{Op})} k_b^{-1} + e^{-2(G_{Ec} - G_{Ep})} k_a}{\left(1 + e^{(G_{Ep} - G_{Op}) + \ln(C_p/C_0)}\right)^2} p_I$$

$$= \frac{k_a + e^{2(G_{Ec} - G_{Oc}) + 2\ln(C_c/C_0)} k_b^{-1}}{\left(1 + e^{(G_{Ec} - G_{Oc}) + \ln(C_c/C_0)}\right)^2} - \left(\frac{k_a + e^{2(G_{Ec} - G_{Oc}) + 2\ln(C_c/C_0)} k_b^{-1}}{\left(1 + e^{(G_{Ec} - G_{Oc}) + \ln(C_c/C_0)}\right)^2} + \frac{e^{2(G_{Ep} - G_{Oc}) + 2\ln(C_p/C_0)} k_b^{-1} + e^{-2(G_{Ec} - G_{Ep})} k_a}{\left(1 + e^{(G_{Ep} - G_{Op}) + \ln(C_p/C_0)}\right)^2} \right) p_I$$

$$= \frac{k_a + e^{2(G_{Ec} - G_{Oc}) + 2\ln(C_c/C_0)} k_b^{-1}}{\left(1 + e^{(G_{Ec} - G_{Oc}) + \ln(C_c/C_0)}\right)^2}$$

$$- \left(\frac{\left(k_a + e^{2(G_{Ec} - G_{Oc}) + 2\ln(C_c/C_0)} k_b^{-1}\right)\left(1 + e^{(G_{Ep} - G_{Op}) + \ln(C_p/C_0)}\right)^2}{\left(1 + e^{(G_{Ec} - G_{Oc}) + \ln(C_c/C_0)}\right)^2 \left(1 + e^{(G_{Ep} - G_{Op}) + \ln(C_p/C_0)}\right)^2} \right) p_I$$

$$- \left(\frac{\left(1 + e^{(G_{Ec} - G_{Oc}) + \ln(C_c/C_0)}\right)^2 \left(e^{2(G_{Ep} - G_{Oc}) + 2\ln(C_p/C_0)} k_b^{-1} + e^{-2(G_{Ec} - G_{Ep})} k_a\right)}{\left(1 + e^{(G_{Ec} - G_{Oc}) + \ln(C_c/C_0)}\right)^2 \left(1 + e^{(G_{Ep} - G_{Op}) + \ln(C_p/C_0)}\right)^2} \right) p_I$$

$$= 0$$

So,

$$p_I = \frac{A}{A + B},$$

where

$$A = \left(k_a + e^{2(G_{Ec}-G_{Oc})+2\ln(C_c/C_0)}k_b^{-1}\right)\left(1+e^{(G_{Ep}-G_{Op})+\ln(C_p/C_0)}\right)^2$$

$$= \left(k_a + e^{2(G_{Ec}-G_{Oc})+2\ln(C_c/C_0)}k_b^{-1}\right)\left(1+e^{(G_{Ep}-G_{Op})+\ln(C_p/C_0)}\right)^2$$

$$= e^{2(G_{Ec}-G_{Oc})+2\ln(C_p/C_0)}\left(e^{-2(G_{Ec}-G_{Oc})-2\ln(C_p/C_0)}k_a + e^{2\ln(C_c/C_p)}k_b^{-1}\right)\left(1+e^{(G_{Ep}-G_{Op})+\ln(C_p/C_0)}\right)^2,$$

$$B = \left(1+e^{(G_{Ec}-G_{Oc})+\ln(C_c/C_0)}\right)^2\left(e^{2\left(G_{Ep}-G_{Oc}+\ln(C_P/C_0)\right)}k_b^{-1} + e^{-2(G_{Ec}-G_{Ep})}k_a\right)$$

$$= e^{2(G_{Ep}-G_{Oc})+2\ln(C_p/C_0)}\left(e^{-(G_{Ec}-G_{Oc})-2\ln(C_p/C_0)} + e^{\ln(C_c/C_p)}\right)^2\left(e^{2\left(G_{EC}-G_{Oc}+\ln(C_P/C_0)\right)}k_b^{-1} + k_a\right).$$

Thus

$$\omega \propto p_4 k_b - p_5 k_b^{-1}$$

$$= \frac{e^{2\left(G_{Ep}-G_{Op}+\ln(C_p/C_0)\right)}}{\left(1+e^{(G_{Ep}-G_{Op})+\ln(C_p/C_0)}\right)^2}p_I e^{-2(G_{Oc}-G_{Op})}k_b^{-1} - \frac{e^{2(G_{Ec}-G_{Oc})+2\ln(C_c/C_0)}}{\left(1+e^{(G_{Ec}-G_{Oc})+\ln(C_c/C_0)}\right)^2}p_{II}k_b^{-1}$$

$$= \frac{e^{2\left(G_{Ep}-G_{Oc}+\ln(C_p/C_0)\right)}}{\left(1+e^{(G_{Ep}-G_{Op})+\ln(C_p/C_0)}\right)^2}p_I k_b^{-1} - \frac{e^{2(G_{Ec}-G_{Oc})+2\ln(C_c/C_0)}}{\left(1+e^{(G_{Ec}-G_{Oc})+\ln(C_c/C_0)}\right)^2}p_{II}k_b^{-1}$$

$$= \left(\frac{e^{2\left(G_{Ep}-G_{Oc}+\ln(C_p/C_0)\right)}A}{\left(1+e^{(G_{Ep}-G_{Op})+\ln(C_p/C_0)}\right)^2} - \frac{e^{2(G_{Ec}-G_{Oc})+2\ln(C_c/C_0)}B}{\left(1+e^{(G_{Ec}-G_{Oc})+\ln(C_c/C_0)}\right)^2}\right)\frac{1}{A+B}k_b^{-1}$$

$$= \frac{\left(e^{2\left(G_{Ep}-G_{Oc}+\ln(C_p/C_0)\right)}k_a + e^{2\left(G_{Ep}+G_{Ec}-2G_{Oc}+\ln(C_pC_c/C_0C_0)\right)}k_b^{-1}\right) - \left(e^{2\left(G_{Ec}+G_{Ep}-2G_{Oc}+\ln(C_pC_c/C_0C_0)\right)}k_b^{-1} + e^{2(G_{Ep}-G_{Oc}+\ln(C_c/C_0))}k_a\right)}{A+B}k$$

$$= \frac{e^{2\left(G_{Ep}-G_{Oc}+\ln(C_p/C_0)\right)}k_a k_b^{-1}\left(1-e^{2\ln(C_c/C_p)}\right)}{A+B}$$

The above result shows that the rotation rate versus pmf can be written in a form given by Equation (17) (notice in the experiments of Gabel *et al.*, the ion concentration outside the cell−C_p in the above expressions−can be treated as a constant). A similar analysis using the scheme with two protons being translated alternately gives the same expression as Equation (17).

More on the torque-speed relation

The prediction of the model is shown in Figure 11. In the experiments of Ryu *et. al.*, no measurement was made in the very high-speed region (15). While the torque-speed curves with different number of stators were extrapolated so they cross at the same position, this trend is not supported by their data. Yet the curve of one stator looks much more like the model developed here predicts. Thus the predicted trend illustrated in Figure 11 can be checked experimentally.

A possible exception is if the power stroke is shorter so there is a flat region in the potentials. A stator (or half stator) engages to the rotor, exerts a power stroke to push the rotor rotating. At the end of the power stroke, the stator stops, the rotor is free to move forward (under the power stroke of other stators) without the necessity to escape a potential well due to interactions with the stator. In this case, mutual destructive interference of stators may not be observed. Therefore, either proof or disproof of the predictions shown in Figure 11 gives us some information about the potential forms of the rotor-stator interactions.

Stator springs

Each stator is attached to the cell body via an elastic linkage between MotB and the polypeptideglycan. Consequently, a stator may fluctuate around its equilibrium position. To model rotor rotation and the stator fluctuation explicitly, the equations of motion for one stator become:

$$\textbf{Rotor:} \quad \underbrace{\zeta_R \frac{d\theta}{dt}}_{\substack{\text{Viscous drag torque} \\ \text{on the rotor}}} = \underbrace{-\frac{\partial}{\partial\theta} V_{RS}\left(s, \theta - \theta_S\right)}_{\substack{\text{Rotor-Stator} \\ \text{interaction force}}} - \underbrace{\kappa\left(\theta - \theta_L\right)}_{\substack{\text{Elastic coupling} \\ \text{force}}} + \underbrace{\sqrt{2k_B T \zeta_R}\, f_R(t)}_{\substack{\text{Brownian torque} \\ \text{on the rotor}}} \tag{18}$$

$$\textbf{Load:} \quad \underbrace{\zeta_L \frac{d\theta_L}{dt}}_{\substack{\text{Viscous drag} \\ \text{force on the Load}}} = \underbrace{\kappa\left(\theta - \theta_L\right)}_{\substack{\text{Elastic coupling} \\ \text{force}}} + \underbrace{\sqrt{2k_B T \zeta_L}\, f_L(t)}_{\substack{\text{Brownian force} \\ \text{on the load}}} \tag{19}$$

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Stator:
$$\underbrace{\zeta_S \frac{d\theta_S}{dt}}_{\substack{\text{Viscous drag torque}\\\text{on the stator}}} = \underbrace{\frac{\partial}{\partial\theta_S} V_{RS}(s,\theta-\theta_S)}_{\substack{\text{Rotor-Stator}\\\text{interaction force}}} - \underbrace{\kappa_S(\theta_S-\theta_0)}_{\substack{\text{Elastic coupling}\\\text{force}}} + \underbrace{\sqrt{2k_BT\zeta_S}\,f_S(t)}_{\substack{\text{Brownian torque}\\\text{on the stator}}} \quad (20)$$

In equation (20), ζ_S is the drag coefficient, θ_S, and θ_0 are, the angular position equilibrium position of the stator, and κ_S is spring constant of the stator spring. By averaging Equation (20), one can show that

$$\left\langle \frac{\partial}{\partial\theta_S} V_{RS}(s,\theta-\theta_S) \right\rangle = \left\langle \kappa_S(\theta_S-\theta_0) \right\rangle \quad (21)$$

Thus, the stator, on average, tilts opposite to the rotation direction, in accodance with Newton's laws. In the text, the minimum energy path (MEP) is defined by the force balance relation,

$$\frac{\partial}{\partial\theta_S} V_{RS}(s,\theta-\theta_S) = \kappa_S(\theta_S-\theta_0) \quad (22).$$

This constraint reduces the dimension by one. The remaining degrees of freedom are orthogonal to the MEP and are high frequency modes, so that they contribute to the effective drag coefficient and the random force.

Assume that the radii of the rotor and stator are ~ 20 and 4 nm, respectively, and the rotor rotates within cytosol of viscosity ~ 0.1 Poise, and the stator moves within the membrane of viscosity about 10 Poise. The rotational drag coefficient of the rotor is given by $\zeta_r^{Rot} \sim 8\pi\eta_w R_r^3$. Assume that the stator has an ellipsoid shape with vertical axis half the horizontal axis R_s. Then the translational drag coefficient of the stator is approximately $\zeta_s^{Trans} \sim 8\pi\eta_m R_s$. To compare the two terms, we need to transform the latter into a rotational drag coefficient along a circle with radius

R_r: $\underbrace{\tau_s}_{\substack{\text{drag torque}\\\text{on the stator}}} = \underbrace{F}_{\substack{\text{drag torque}\\\text{on the stator}}} \cdot R_r = \zeta_s^{Trans}\frac{dx}{dt}R_r = \zeta_s^{Trans}\frac{d\theta_s}{dt}R_r^2 \sim 8\pi\eta_m R_s R_r^2 \frac{d\theta_s}{dt} = \zeta_s^{Rot}\frac{d\theta_s}{dt}$. The ratio of the

rotor/stator drag coefficients is $\dfrac{\zeta_r^{Rot}}{\zeta_s^{Rot}} \sim \dfrac{R_r^3\eta_w}{R_s R_r^2\eta_m} \sim \dfrac{1}{20}$. Given all the uncertainties in our estimation,

and the fact that viscosity contribution may also come from the portion of the connection between the rotor and the flagellum passing through the membrane, the stators move roughly on the same time scale as the rotor does [*].

Explicit treatments of the stator springs does not change the physics revealed by the motor torque-speed relations computed in the text. Indeed, including stator springs makes fitting the data on the rotation speed dependence on pmf easier, since the stator fluctuation characteristic time introduces an extra time scale into the system. Stator fluctuations help the system escape the situation illustrated in Figure 8a, since the rotor-stator interaction surfaces are no longer rigidly coupled. These fluctuations increase the effective rate constants by broadening the effective transition windows (see Figure 8c). Modeling the stator springs explicitly dramatically increases the number of degrees of freedom in the model, and so the computational load. Since we focus here on the main physics underlying the motor torque-speed relations, we leave more realistic treatment of the stator springs for a future study. The model system shown in this work should be understood as renormalized system with the stator spring effects implicitly embedded in.

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[*] Uncertanty of the exact values of the rotor and stator diffusion constants does not affect our ability to fit the motor torque-speed relations, which are mainly determined by the much slower load diffusion constant and effective chemical transition rates.

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