

Reconstruction of the Bacterial Flagellar Motor's Energy Landscape, Viscous Load, and Torque Generation Across Diffusion Regimes

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The bacterial flagellar motor (BFM) converts transmembrane ion flux into directed mechanical rotation, driving bacterial motility. Despite extensive study, the frictional forces and energetics governing its torque generation remain poorly understood. Here we combine single-molecule rotation measurements with stochastic thermodynamics to quantitatively estimate its effective torque, viscous drag, and activation energy barriers. We present three complementary methods based on solutions to the Smoluchowski equation for overdamped diffusion in a tilted periodic potential, which use as input the steady-state angular velocity and rotational diffusion data from individual *Escherichia coli* motors spanning different dynamical regimes. Crucially, these three methods require neither active external torque control, nor prior knowledge of the system's viscous drag or the motor's torque output. The first method assumes as input a model-dependent sinusoidal effective potential (single Fourier mode), albeit with unknown periodicity, yielding closed-form results in the low- and high-tilt limits, whereas the last two methods use a full Fourier-based reconstruction to output the now model-independent effective potential landscape. These approaches yield consistent estimates of the potential periodicity (≈ 26 -fold symmetry), energy barrier height ($\approx 2\text{--}4 k_B T$), and internal friction coefficient ($\approx 0.1 \text{ pN nm s/rad}^2$). These findings suggest that the reconstructed effective energy landscape is dominated by interactions associated with the LP-ring bearing. Our results reveal that the BFM's torque-velocity relationship deviates significantly from the linear approximation near the critical tilt, where angular diffusion is maximized. More broadly, our framework provides a coherent strategy for reconstructing nanoscale energy landscapes from single-molecule data and is generalizable to other stepping molecular motors operating in cyclic conditions.

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I. INTRODUCTION

Molecular motors are self-assembling biological machines composed of proteins that convert (electro-)chemical energy into mechanical work. They play essential roles in processes such as intracellular transport, cellular motility, muscle contraction, and ATP synthesis [1,2]. Their operation is governed by energy landscapes that depend on mechanical coordinates as well as internal chemical and conformational states. In a full mechanochemical description, motion results from transitions among these state-dependent landscapes. In some systems, however, the unresolved internal dynamics can be coarse-grained into an effective drive acting on a measured mechanical coordinate (for rotary motors such as ATP synthase and the bacterial flagellar motor this is one periodic angular coordinate), which may then be described

phenomenologically by overdamped Brownian motion in a tilted periodic potential [3,4].

The bacterial flagellar motor (BFM) is the rotary molecular motor embedded in the bacterial cell envelope that drives the rotation of the extracellular helical flagellum, enabling swimming, chemotaxis, and infection [5,6]. Structurally [see Figs. 1(a) and 1(b)], the motor comprises multiple protein rings embedded in the membranes and cytoplasm, with the cytoplasmic portion forming the rotor [6,7]. A central rod extends from the rotor through the peptidoglycan (cell wall) and outer membrane, where its high-speed rotation is supported by the LP ring, a pair of protein rings embedded in the peptidoglycan and outer membrane that function together as a molecular bearing [7,8]. The motor is powered by the flow of ions down an electrochemical gradient across the inner membrane; the ions pass through stator units anchored to the peptidoglycan at the rotor's periphery, which convert electrochemical energy into mechanical torque through ion-coupled conformational changes and rotor-stator interactions [9].

Single-molecule experiments have shown that the BFM rotates in approximately 26 discrete steps per revolution [10–13]. These steps were initially interpreted as elementary torque-generating events, attributed to periodic engagement between stator units and the rotor protein FliG [10]. However, subsequent structural studies revealed that the rotor contains 34 FliG subunits [14], while the LP ring

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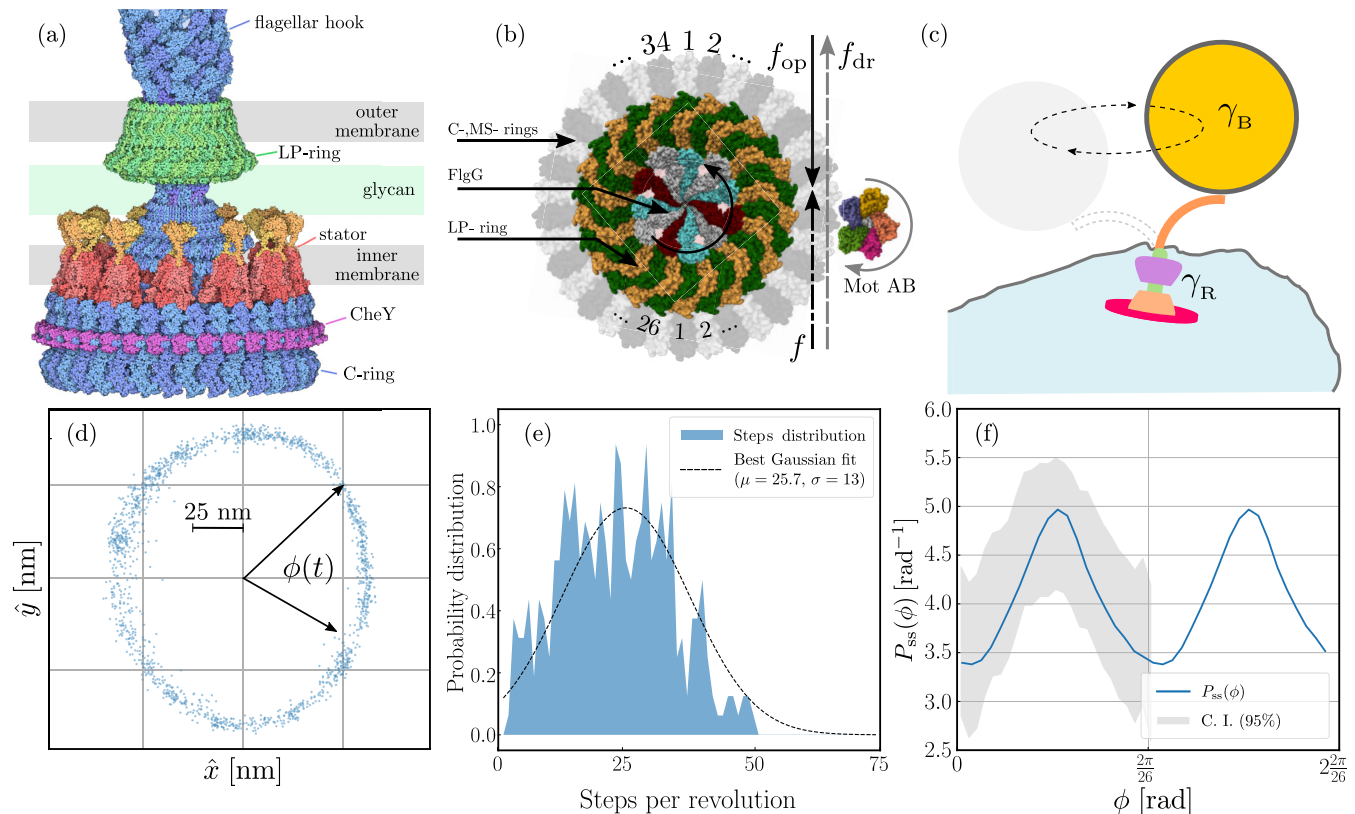


FIG. 1. (a) Side view of the BFM's protein structure (by David Goodsell under CC-BY-4.0 license [26]). (b) Composed top view displaying key rotational symmetries of the BFM's components and a schematic representation of the torques involved (f_{dr} , f_{op} , f) in its motion. (c) Schematic representation of the BFM bead assay and the relevant viscous loads present, γ_B and γ_R , for the bead and internal ring, respectively. (d) Example of the angular position time series obtained from single-molecule experiments tracking the center of mass of the attached bead. (e) Ensemble Empirical Mode Decomposition (EEMD) analysis of the stepping size for a set of 95 individual traces showing a mean stepping very close to 26 steps per revolution. (f) Normalized probability distribution of the angular position folded over the main periodicity found by the EEMD analysis (C.I. denotes confidence interval).

is the only component of the motor with 26-fold symmetry [see Figs. 1(a) and 1(b)] [12]. This prompted a reinterpretation: the observed stepping arises not from torque generation, but from interactions between the rotating rod and the LP ring, which imposes an external periodic energy landscape on its motion. In the model developed below, we reconstruct this effective bearing potential. This reconstruction is performed on a mesoscopic timescale over which the load is fixed, ion-flux fluctuations are fast compared with the observed stepping time, and stator unit recruitment events remain rare [15]. These conditions allow us to treat the stator mechanochemical cycle as a coarse-grained, effective stationary drive.

The LP ring represents a unique mechanical solution in biology, enabling stable high-speed rotation while likely minimizing mechanical resistance. Its function relies on electrostatic repulsion and steric alignment between the rod and the bearing [8,16], plausibly producing a 26-fold periodic bearing potential that influences the measured rotational dynamics [17,18]. In addition to stabilizing the rod, interactions at the rod-LP-ring interface may introduce periodic energy barriers and contribute to the internal mechanical resistance along the rod's angular coordinate. Despite its central mechanical role, the energy landscape imposed by the LP ring

remains largely unquantified [18], with only recent preliminary estimates available [19].

In recent work, Hayashi *et al.* [20] used electrorotation to apply a controlled external torque to F_1 -ATPase while measuring changes in rotational velocity and diffusion. They observed that diffusion peaked near the critical tilt condition, where the applied torque balances the energy barriers in the motor's periodic potential. This enabled direct reconstruction of the underlying energy landscape without assumptions about internal friction or kinetic details. Such experiments are possible for soluble motors like F_1 -ATPase, where torque can be directly applied in reconstituted *in vitro* assays. For the BFM, however, these measurements are more challenging. Although optical [21], magnetic [22], or electrorotation techniques [23] can in principle be used to impose torque, most experimental studies rely on passive observation of rotation, where neither the torque generated by the motor nor the rotational drag coefficient (including internal and probe contributions) are directly measurable. In typical assays, the only available observables are the steady-state angular velocity and the effective rotational diffusion coefficient, both extracted from the motion of a tethered bead. Given that the LP ring is expected to impose the dominant periodic component of the energy landscape in

this regime, we ask whether these observables can be used to quantitatively reconstruct its energy profile.

Here we develop a stochastic thermodynamics framework for rotational motion in a tilted periodic potential. Motivated by typical BFM experiments, we ask whether a simple combination of the measured angular velocity and diffusion can serve as a reliable proxy for the underlying energy landscape. We show that this is possible across three distinct regimes: low-tilt, where motion is barrier-limited; high-tilt, where free drift dominates; and an intermediate (critical-tilt) regime, where diffusion is maximized. These correspond to torque times periodicity [see Eq. (10)] values that are small, large, or comparable to the height of the underlying energy barriers. Our approach enables the quantitative reconstruction of the LP ring's energy landscape and the internal drag coefficient of the motor, even when torque and drag are unknown. More broadly, the same framework applies to any molecular motor operating in a periodic energy landscape, particularly in systems where active control is limited, forces cannot be measured directly, or potentially when the landscape is heterogeneous or time-dependent [19]. By providing a unified description of motor dynamics across physical regimes, this work offers a new theoretical tool for probing energy transduction at the nanoscale.

II. SINGLE MOTOR MEASUREMENTS

We measured the steady-state rotation of 95 individual *Escherichia coli* BFMs via a bead assay [24], tracking the rotation of 100 nm diameter gold beads attached to the flagellar hook [see Fig. 1(c)], as described in Materials and Methods. The data were acquired at an acquisition rate of 109 500 frames per second. The bead center was tracked as it rotated slightly off-axis, yielding its circular $x(t)$, $y(t)$ trajectory [see Fig. 1(d)], the BFM rotation angle, $\phi(t) = \text{atan2}[y(t), x(t)]$, and the instantaneous angular speed, $\omega(t) = \dot{\phi}(t)$. From each motor, we extracted the steady-state angular speed, $\omega_{ss} \simeq \langle \Delta\phi \rangle / \Delta t$, and the rotational diffusion coefficient, $D_{ss} \simeq (\langle (\Delta\phi)^2 \rangle - \langle \Delta\phi \rangle^2) / (2\Delta t)$, from the longest portion of the trace exhibiting sustained constant speed. This segment corresponds to the mesoscopic nonequilibrium steady state of interest in this work (see Materials and Methods). Therefore, the diffusion coefficient D_{ss} is reported only for the normal-diffusion regime, $\alpha = 1$, where D_{ss} has units of $\text{rad}^2 \text{s}^{-1}$. For anomalous diffusion, the corresponding generalized coefficient D_α would have units $\text{rad}^2 \text{s}^{-\alpha}$.

We analyzed the steady-state angular probability distribution $P_{ss}(\phi)$ for each motor using Ensemble Empirical Mode Decomposition (see Sec. VI) to extract the dominant periodic components [25]. As shown in Fig. 1(e), we found a global dominant periodicity of $25.7 (\pm 13)$ preferred positions per revolution, in agreement with previous studies that reported approximately 26 [10–13], and consistent with the 26-fold symmetry of the LP ring.

III. BACKGROUND THEORY OF DIFFUSION IN A PERIODIC TILTED POTENTIAL

Motivated by the periodicity observed in the angle $\phi(t)$, we aimed to develop analytical methods to extract features

of the underlying energy landscape from the experimentally accessible quantities $P_{ss}(\phi)$, ω_{ss} , and D_{ss} . Our approach builds on the established theoretical framework for one-dimensional diffusion in tilted periodic potentials, a class of systems described by different formulations [27,28]. In this work, we focus on the Smoluchowski formulation and present its key analytical results.

We write the potential as $V(\phi) = V_{eq}(\phi) - f\phi$, where ϕ is the azimuthal angle of rotation, f is the effective constant tilt torque (resulting from the difference between driving and opposing torques on the system $f = f_{dr} - f_{op}$, as seen in Fig. 1(b)). Given the observed quasi single-mode periodicity, the equilibrium potential is chosen as $V_{eq}(\phi) = \frac{E_a}{2} \cos(2\pi\phi/L)$, presenting energy barriers of height E_a , and periodicity L (corresponding to a $\mathcal{P}$ -fold symmetry with $\mathcal{P} = 2\pi/L$). The evolution of the system is described by its probability density function $P(\phi, t)$, which can be computed using the Smoluchowski equation:

$$\frac{\partial}{\partial t} P(\phi, t) = \mathcal{L} P(\phi, t), \quad (1)$$

where $\mathcal{L}$ is the evolution operator defined by

$$\gamma \mathcal{L} = \frac{\partial}{\partial \phi} \left[\frac{\partial V(\phi)}{\partial \phi} + \Theta \frac{\partial}{\partial \phi} \right], \quad (2)$$

with γ the viscous drag coefficient and $\Theta = k_B T$ the thermal energy [28]. We are interested in the steady state of the system where closed solutions for P_{ss} , ω_{ss} , and D_{ss} are known [29] and can be written as

$$P_{ss}(\phi) = \frac{I_+(\phi)}{\langle I_+(\phi) \rangle_L}, \quad (3)$$

$$\omega_{ss} = \lim_{\Delta t \rightarrow \infty} \frac{\langle \Delta\phi \rangle}{\Delta t} = \frac{L\Theta}{\gamma} \frac{(1 - e^{-fL/\Theta})}{\langle I_+(\phi) \rangle_L}, \quad (4)$$

$$D_{ss} = \lim_{\Delta t \rightarrow \infty} \frac{\langle (\Delta\phi)^2 \rangle - \langle \Delta\phi \rangle^2}{2\Delta t} = \frac{L^2\Theta}{\gamma} \frac{\langle I_+(\phi)^2 \rangle_L - \langle I_+(\phi) \rangle_L^2}{\langle I_+(\phi) \rangle_L^3} \quad (5)$$

with

$$I_\pm(\phi) = \int_0^L d\varphi e^{\pm[V(\phi) - V(\phi \mp \varphi)]/\Theta} \quad (6)$$

and

$$\langle g(\phi) \rangle_L = \int_0^L d\phi g(\phi). \quad (7)$$

In addition, the system is also characterized by its rate of entropy production, σ , for which $T\sigma \equiv \omega_{ss}f$ can be bounded below by the thermodynamic uncertainty relation (TUR) [30]:

$$T\varsigma_{\text{TUR}} = \frac{\langle \Delta\phi \rangle}{\Delta t} \left(2\Theta \frac{\langle \Delta\phi \rangle}{\langle (\Delta\phi)^2 \rangle - \langle \Delta\phi \rangle^2} \right) \leq T\sigma = \omega_{ss}f. \quad (8)$$

The bound of the entropy rate ς_{TUR} is based on the analysis of the system's fluctuations, requiring no direct knowledge of the tilt f , and was initially applied in the study of Brownian motors experiencing a tilted periodic potential. From the inequality stated by Eq. (8) and the definitions in Eqs. (4) and (5), we define an effective tilt f_0 as

$$2\Theta \frac{\langle \Delta\phi \rangle}{\langle (\Delta\phi)^2 \rangle - \langle \Delta\phi \rangle^2} = \Theta \frac{\omega_{ss}}{D_{ss}} \equiv f_0. \quad (9)$$

The significance of f_0 comes from the feature that it can be constructed directly from observable quantities and, depending on the operating parameter regime, can serve as a good approximation for the actual tilt f , as will be discussed below.

IV. RECONSTRUCTION OF THE ENERGY LANDSCAPE

Our objective is to derive analytical expressions that allow the determination of the energy landscape, by estimating the energy barrier E_a , the periodicity L , and the tilting torque f from experimental data.

The viscous load γ , which governs the dynamics within the potential, requires careful consideration. Since the geometry and size of the bead tethered to the motor are known, the Stokes expression for the drag on a sphere can be applied. However, two complications arise. First, the bead's proximity to the cell surface requires hydrodynamic corrections [31], which depend on the bead-surface gap, which is not experimentally accessible. Second, internal friction contributions from motor components add a hidden drag component, which has been estimated [32] from a fluctuation analysis (power spectrum measurements), but remains to be verified. Crucially, two of the three methods we describe below yield γ directly, removing the need for prior estimation.

In the following analysis, we divide the parameter space into three regimes based on the relative strength of the tilting torque, using the dimensionless parameter

$$\epsilon \equiv \frac{fL}{\pi E_a}. \quad (10)$$

The three regimes are defined as follows: (1) *Low-tilt* regime, $\epsilon < 1$, corresponding to near-equilibrium conditions; (2) *High-tilt* regime, $\epsilon > 1$, characterizing far-from-equilibrium dynamics; and (3) *Critical-tilt* regime, $\epsilon \approx 1$, marking the crossover between these two extremes. The parameter ϵ is derived via dimensional analysis in such a way that $\epsilon = 1$ corresponds to the critical tilt introduced by Reimann *et al.* [29].

A. Method 1

For our first method, we adopt a formulation of the Smoluchowski equation (1) based on its spectral decomposition, using techniques inspired by solid-state physics. This eigenspectrum-based approach enables robust analysis and allows for the reconstruction of the unknown energy landscape from dynamic observables [27,28,33–37]. Due to the periodicity of the term $\frac{\partial}{\partial \phi} V(\phi)$ in the operator $\mathcal{L}$ in Eq. (1), the distribution P_{ss} is also periodic. Equation (1) can thus be expanded in a Fourier series, enabling the use of Bloch's theorem as an analytical framework [28,38]. Within this formalism, the evolution operator $\mathcal{L}$ satisfies the eigenvalue equation [27,28,33]

$$\mathcal{L}\Phi_{\alpha,k}(\phi) = -\lambda_{\alpha,k}\Phi_{\alpha,k}(\phi), \quad (11)$$

where k is the wave number defined within the Brillouin zone, and $\alpha \in \mathbb{N}$ is the band index. The eigenfunctions $\Phi_{\alpha,k}(\phi) = \exp(ik\phi)u_{\alpha,k}(\phi)$ take the Bloch form, where $u_{\alpha,k}(\phi + L) = u_{\alpha,k}(\phi)$ has the same periodicity of $V_{eq}(\phi)$, and the corresponding eigenvalues $\lambda_{\alpha,k}$ are generally complex for $f \neq 0$.

The velocity and diffusion coefficient in this formalism are determined by [39]

$$\omega_{ss} = \left. \frac{\partial \text{Im}\{\lambda_{0,k}\}}{\partial k} \right|_{k=0}, \quad (12)$$

$$D_{ss} = \left. \frac{1}{2} \frac{\partial^2 \text{Re}\{\lambda_{0,k}\}}{\partial k^2} \right|_{k=0}. \quad (13)$$

This establishes a direct connection between the observables and the underlying eigenvalue band structure. To proceed, we focus on the three different parameter regions defined by ϵ to find explicit expressions of ω_{ss} , D_{ss} , and f .

Low-tilt regime ($\epsilon < 1$). When $\epsilon < 1$ and $E_a \gg \Theta$, the eigenstates in higher bands decay rapidly and can be neglected at long times, corresponding to the Kramers regime. This approximation allows Eq. (11) to be reduced to a master equation with well-localized states, and well defined transition rates ($h_{\pm}$) to nearest neighbors only [33,34]. In this regime the steady-state speed and the rotational diffusion coefficient are given by, $\omega_{ss}^{\text{lo}} = L(h_+ - h_-)$ and $D_{ss}^{\text{lo}} = L^2(h_+ + h_-)/2$ [Eqs. (12) and (13)], where

$$h_{\pm} = \left(\frac{\pi E_a}{L^2 \gamma} \right) e^{-E_a/\Theta \pm fL/(2\Theta)}, \quad (14)$$

leading to

$$\omega_{ss}^{\text{lo}} = 2 \sinh(fL/2\Theta) \left(\frac{\pi E_a}{L\gamma} \right) e^{-E_a/\Theta} \quad (\epsilon < 1), \quad (15)$$

$$D_{ss}^{\text{lo}} = \cosh(fL/2\Theta) \left(\frac{\pi E_a}{\gamma} \right) e^{-E_a/\Theta} \quad (\epsilon < 1). \quad (16)$$

Using Eqs. (15) and (16), we can further write D_{ss} as a function of ω_{ss} as

$$D_{ss}^{\text{lo}}(\omega_{ss}^{\text{lo}}) = D_K \sqrt{1 + \left(\frac{L\omega_{ss}^{\text{lo}}}{2D_K} \right)^2} \quad (\epsilon < 1), \quad (17)$$

where we have defined

$$D_K = (\pi E_a/\gamma) e^{-E_a/\Theta}, \quad (18)$$

the Kramers expression for the equilibrium diffusion coefficient, resulting from the suppression of diffusion by the potential landscape barriers. This approximation can only be justified for energy barriers that are sufficiently large compared with Θ [see the Supplemental Material [40]]. As we will see, this relationship, together with the corresponding expression derived below for the case $\epsilon > 1$ [Eq. (28)], will allow us to fit the experimental results for D_{ss} as a function of ω_{ss} to estimate the parameters E_a , L , and γ . To estimate the tilt torque f , we write the ratio $f_0 = \Theta\omega_{ss}/D_{ss}$ from Eq. (9) [41] as

$$f_0 = \Theta \frac{\omega_{ss}^{\text{lo}}}{D_{ss}^{\text{lo}}} = \frac{2\Theta}{L} \tanh\left(\frac{fL}{2\Theta}\right). \quad (19)$$

The tilt torque f can thus be expressed as

$$f = \frac{2\Theta}{L} \tanh^{-1}\left(\frac{Lf_0}{2\Theta}\right), \quad (20)$$

whose series expansion is given by

$$f \approx f_0 \left[1 + \frac{1}{12} \left(\frac{Lf_0}{\Theta} \right)^2 \right] \equiv f^{\text{lo}}(f_0), \quad (21)$$

demonstrating that $f_0 = \Theta\omega_{ss}/D_{ss}$ is a good approximation for f in this regime, as long as the first correction in Eq. (21) is small, which is true for $\epsilon \ll 2\Theta/(\pi E_a) < 1$.

High-tilt regime ($\epsilon > 1$). In the regime $\epsilon > 1$, we will employ perturbation theory to the Smoluchowski equation to obtain the corresponding expressions of ω_{ss} , D_{ss} , and f . We consider the evolution operator Eq. (2) as $\mathcal{L} = \mathcal{L}_0 + \mathcal{L}_1$, where

$$\gamma\mathcal{L}_0 = \left\{ -f \frac{\partial}{\partial\phi} + \Theta \frac{\partial^2}{\partial\phi^2} \right\} \quad (22)$$

and

$$\gamma\mathcal{L}_1 = \frac{\partial}{\partial\phi} \left[\frac{\partial V_{eq}(\phi)}{\partial\phi} \right]. \quad (23)$$

Due to the single-mode periodicity, we expand $V_{eq}(\phi)$ and $u_{\alpha,k}(\phi)|_{\alpha=0}$ in Fourier series [36], obtaining

$$\bar{k} \left\{ [\bar{k} + i\bar{f}] \hat{u}_n^{0,\bar{k}} + \frac{\bar{E}_a}{4} [\hat{u}_{n+1}^{0,\bar{k}} - \hat{u}_{n-1}^{0,\bar{k}}] \right\} = \bar{\lambda}_{\alpha,\bar{k}} \hat{u}_n^{0,\bar{k}}, \quad (24)$$

where $\hat{u}_n^{0,k}$ is the n th Fourier coefficient of $u_{0,k}(\phi)$, and where we define the dimensionless quantities $\bar{k} = kL/2\pi$, $\bar{f} = fL/2\pi\Theta$, $\bar{E}_a = E_a/\Theta$, and $\bar{\lambda} = \gamma\lambda L^2/\Theta(2\pi)^2$. Noting that $\bar{E}_a/\bar{f} = 2/\epsilon$, the condition $\epsilon > 1$ implies that $\bar{E}_a/\bar{f} \ll 1$, and therefore perturbation theory is applicable. Via nondegenerate perturbation theory up to second order in $\bar{E}_a$, we find that the lowest eigenvalue is

$$\bar{\lambda}_{0,\bar{k}} \approx \bar{k}^2 + i\bar{f}\bar{k} + \frac{\bar{E}_a^2}{16} \left[\frac{\bar{k}(\bar{k}+1)}{1+i\bar{f}+2\bar{k}} + \frac{\bar{k}(\bar{k}-1)}{1-i\bar{f}-2\bar{k}} \right]. \quad (25)$$

By inserting Eq. (25) in Eqs. (12) and (13), we can derive the explicit approximate expressions [36]

$$\omega_{ss}^{hi} \approx \frac{f}{\gamma} - \frac{f}{2\gamma} \left(\frac{\pi E_a}{2fL} \right)^2 \quad (\epsilon > 1), \quad (26)$$

$$D_{ss}^{hi} \approx \frac{\Theta}{\gamma} + \frac{3\Theta}{2\gamma} \left(\frac{\pi E_a}{2fL} \right)^2 \quad (\epsilon > 1). \quad (27)$$

As done in Eq. (17) for the case $\epsilon < 1$, from Eqs. (26) and (27) we can write D_{ss} explicitly as a function of ω_{ss} ,

$$\frac{D_{ss}^{hi}(\omega_{ss}^{hi})}{D_E} = 1 + 6 \left(\frac{L\omega_{ss}^{hi}}{2\beta} + \sqrt{1 + 8 \left(\frac{L\omega_{ss}^{hi}}{2\beta} \right)^2} \right)^{-2} \quad (\epsilon > 1), \quad (28)$$

where $D_E = \Theta/\gamma$ is the barrier-free Einstein diffusion coefficient, and we have defined $\beta = (\pi E_a/\gamma)$ [noting that $D_K = \beta \exp(-E_a/\Theta)$]. As mentioned above, the two expressions for $D_{ss}(\omega_{ss})$ in Eqs. (17) and (28), for the case $\epsilon < 1$ and $\epsilon > 1$ respectively, will allow us to fit the experimental results for $D_{ss}(\omega_{ss})$ to estimate the parameters E_a , L , and γ . In order to evaluate the tilt f from the observables, without assuming a value for γ , we use Eqs. (26) and (27) to write

$$f_0 = \Theta \frac{\omega_{ss}^{hi}}{D_{ss}^{hi}} \approx f \left[1 - \frac{1}{8} \left(\frac{2\pi E_a}{fL} \right)^2 \right] + \mathcal{O}^4. \quad (29)$$

Ignoring the higher-order terms, we can rearrange this expression to write f as an explicit function of f_0 :

$$f \approx f_0 \left[\frac{1}{2} + \frac{1}{2} \sqrt{1 + 2 \left(\frac{\pi E_a}{f_0 L} \right)^2} \right] \equiv f^{hi}(f_0). \quad (30)$$

Since in this regime $\pi E_a/(f_0 L) \approx \pi E_a/(fL) = 1/\epsilon \ll 1$, the dominant term in Eq. (30) is $f_0 = \Theta\omega_{ss}/D_{ss}$. We thus see that the value of the tilt torque f can be approximated by f_0 for both $\epsilon \ll 1$ [Eq. (21)] and $\epsilon \gg 1$ [Eq. (30)]. In both these limits ω_{ss} is proportional to f , and the coefficient of proportionality is given by the corresponding Einstein relation: D_E/Θ for $\epsilon \gg 1$ (free diffusion regime) and D_K/Θ for $\epsilon \ll 1$ (linear response regime where the fluctuation-dissipation theorem holds).

Critical-tilt regime ($\epsilon \simeq 1$). Unlike for the previous two regimes, in the intermediate regime $\epsilon \simeq 1$, it is not easy to obtain simple separate expressions for ω_{ss} and D_{ss} . Ideally, for a set of $D_{ss}(\omega_{ss})$ experimental results with enough values in the $\epsilon < 1$ and $\epsilon > 1$ limits, one can estimate E_a , γ , L by fitting the high and low ϵ data separately using Eqs. (17) and (28). This would then enable us to numerically evaluate Eqs. (4)-(5) in the intermediate regime, $\epsilon \simeq 1$. However, accurate evaluation for very large barriers ($E_a > 15\Theta$) can be challenging. If such is the case, or in the absence of sufficient experimental data in the $\epsilon < 1$ and/or $\epsilon > 1$ limits, interpolation techniques that connect the low-tilt and high-tilt regimes in a continuous way can be used. For the case of diffusion, we note that D_{ss}^{lo} increases as ω_{ss} increases, D_{ss}^{hi} increases as ω_{ss} decreases, and the two limiting forms cross near the critical tilt ($\epsilon \simeq 1$). In the Kramers regime (i.e., large enough barrier height, E_a) for which $D_K \ll D_E$, we can therefore smoothly interpolate between low and high ω_{ss} by treating the two limiting expressions for the diffusion coefficients, D_{ss}^{lo} and D_{ss}^{hi} , as two electrical resistors in parallel:

$$\frac{1}{D_{ss}} \approx \frac{1}{D_{ss}^{lo}} + \frac{1}{D_{ss}^{hi}} \equiv \frac{1}{D_{ss}^{cr}}. \quad (31)$$

This interpolation leads to an easily implemented analytical expression that is in good agreement with the exact (numerical) results [see Fig. 2(a)].

The intermediate linear behavior observed in Fig. 2(a), where $D_{ss} \approx L\omega_{ss}/2$, is obtained in the strong-asymmetric, high-tilt, limit of the Kramers regime, reached when $[L\omega_{ss}/(2D_K)]^2 \gg 1$. The physical interpretation in terms of nearest-neighbor Kramers transition rates is simple: when the tilt is high enough, backward transitions are negligible ($h_- \ll h_+$), yielding $\omega_{ss} \approx Lh_+$ and $D_{ss} \approx L^2 h_+/2 \approx L\omega_{ss}/2$. This linear behavior leads directly to an estimate of the motor periodicity, L , because no other system parameters are involved.

In the case of the torque, we can interpolate between the low-tilt Eq. (21) and high-tilt Eq. (30) limits using the following Padé approximant:

$$\Theta \frac{\omega_{ss}}{D_{ss}} \approx f \frac{\left(\frac{\Theta}{\pi E_a} \right)^2 + \frac{2}{3} \left(\frac{fL}{2\pi E_a} \right)^4}{\left(\frac{\Theta}{\pi E_a} \right)^2 + \frac{1}{6} \left(\frac{fL}{2\pi E_a} \right)^2 + \frac{2}{3} \left(\frac{fL}{2\pi E_a} \right)^4}. \quad (32)$$

This expression shows that in the $\epsilon \simeq 1$ regime the effect of the energy barriers cannot be neglected and is outside the scope of the Kramers approximation. In this case, the roots of

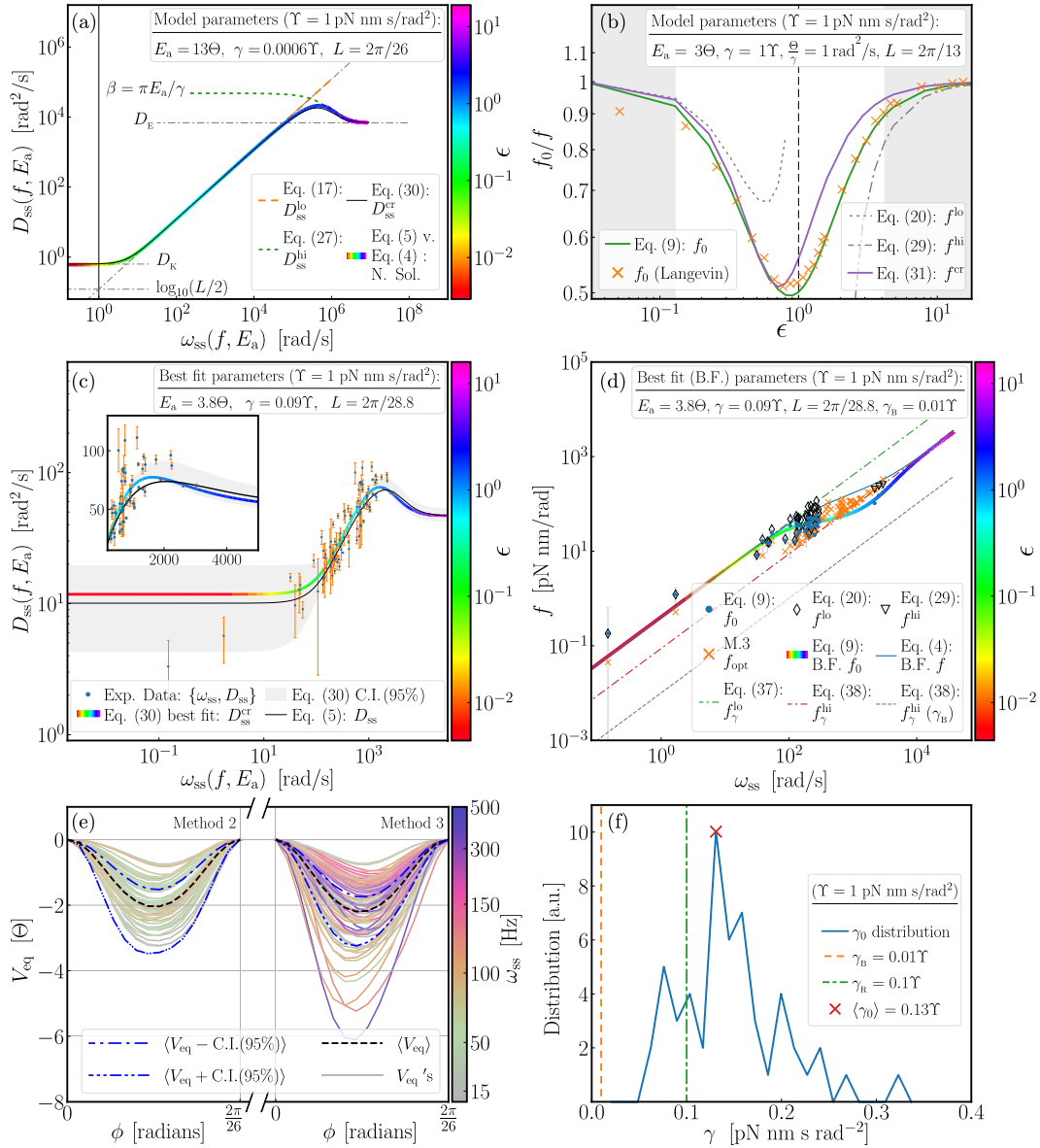


FIG. 2. Illustrative theoretical results for Method 1 and application to experimental data to reconstruct: E_a (Methods 1–3), γ (Methods 1 and 2), effective torque (Methods 1 and 3), and V_{eq} (Methods 2 and 3). (a) Theoretical parametric plot D_{ss} vs. ω_{ss} along with the Method 1 approximations, Eqs. (17), (28), and (31), used to determine D_{ss} for a model potential. (b) Relative error between f and the Method 1 torque estimates, Eqs. (21), (30), and (32), as a function of ϵ for a model potential. (c) Parametric plot D_{ss} vs. ω_{ss} for the experimental data and best fit model (rainbow curve parametrized by ϵ) using the interpolation (31). The shaded gray area shows the 95% confidence interval; the black line is the exact numerical result for the same parameters used in the fit. (d) Corresponding torque values obtained from the experimental ω_{ss} values [f_0 , corrected models, f_{opt} , Eqs. (4) and (9) best fit and asymptotic behavior included]. (e) Resulting energy landscapes from individual traces using (left) Method 2 and (right) Method 3, color bar represents the corresponding experimental ω_{ss} values. (f) Distribution of γ_0 values calculated from f_0 and Method 2 barriers. In all panels, the model parameters and best fit parameters are listed in their respective legends.

Eq. (32) can be used to find f , if $\Theta\omega_{ss}/D_{ss}$ and E_a are known (see the Supplemental Material (SM) [40]). We will call such a solution f^{cr} .

Finally, in the total absence of a tilt torque ($f = 0$), we can estimate the typical time τ_L to diffuse one step L over the energy barrier E_a :

$$\tau_L = \frac{L^2}{D_K} = \frac{L^2\gamma}{\pi E_a} e^{E_a/\Theta}, \quad (33)$$

In Figs. 2(a) and 2(b), we show the behavior of the theoretical expressions found above for the diffusion coefficient, D_{ss} , as a function of the speed, ω_{ss} , and for the approximate tilt torque f , respectively compared with their true values, spanning the regions from $\epsilon < 1$ (low speed) to $\epsilon > 1$ (high speed). Figure 2(b) shows the effective tilt, f_0 , normalized by f , as a function of the parameter ϵ , quantifying the accuracy of f_0 as an approximation for the true tilt f in the limiting cases of low-tilt ($\epsilon \ll 1$) and high-tilt ($\epsilon \gg 1$), respectively.

Figure 2(b) highlights the breakdown of the linear approximation in the intermediate regime $\epsilon \simeq 1$.

Experimental data fitting. In Fig. 2(c) we plot the experimentally measured values of D_{ss} and corresponding ω_{ss} for each motor in our dataset, together with the best (nonlinear) fit obtained by fitting the approximate interpolation formula, D_{ss}^{cr} [Eq. (31)], to the data (with the periodicity L , friction coefficient γ , and barrier height E_a as fitting parameters). The best-fit parameters are $L = 0.218 \pm 0.014$ rad, $\gamma = (0.087 \pm 0.005)$ pN nm s/rad², and $E_a/\Theta = 3.8 \pm 0.4$ (the use of the Kramers approximation in D_{ss}^{cr} , Eq. (31), leads to an approximately 5% overestimate of E_a with respect to the value obtained using the exact expression, Eq. SI.2; see Fig. S3(a) in SM [40]). The best fit is plotted using a color code for ϵ that illustrates clearly the low ω_{ss} plateau at D_K for $\epsilon < 0.1$, the maximum near the critical tilt at $\epsilon = 1$, and the high ω_{ss} plateau at D_E for $\epsilon > 10$. The fitted periodicity of the system, L , leads to 28.8 ± 1.8 steps per turn, in semiquantitative agreement with the already discussed and expected 26-fold periodicity of the LP ring. More data for both slow motors ($\omega_{ss} < 10$ rad/s) and fast motors ($\omega_{ss} > 1000$ rad/s) would allow us to refine our fits.

In Fig. 2(d) we show the experimental estimates of the tilt f as a function of the measured speed, obtained from the measured values of ω_{ss} and D_{ss} using Eq. (21) (valid for $\epsilon < 1$) and Eq. (30) (valid for $\epsilon > 1$). A linear fit of the form $f = \gamma \omega_{ss}$ applied to the data yields an effective drag coefficient $\gamma = 0.12$ pN nm s/rad², in reasonable agreement with the value obtained independently in Fig. 2(c) given the difference in methods used. It is worth noting that the determined viscous drag is approximately one order of magnitude larger than the expected value based on the hydrodynamic drag γ_B of a rotating 100 nm sphere, even after accounting for proximity corrections near a surface [42]. This discrepancy likely reflects the contribution of internal drag, γ_R , within the motor, which has been largely neglected until now. Internal drag arises from interactions between the rotor and static structural elements of the motor, such as the LP ring. Under the low external load conditions of our experiments, we conclude that internal friction is the dominant contributor to the total viscous drag.

B. Method 2

In our second method, similar to the approach used to obtain Eq. (24), we expand the Smoluchowski equation (1) as a Fourier series in the case of a general equilibrium potential with an arbitrary number of periodic modes [35,43,44]. This method of reconstruction is robust and has been widely tested for different scenarios, including high-dimensional systems, outside the Kramers regime, and systems with hidden degrees of freedom [43,44]. In this way we obtain a set of matrix equations

$$-\left(\Theta k - \frac{ifL}{2\pi}\right)\hat{P}_k^{ss} = \sum_q q \hat{V}_q^{eq} \hat{P}_{k-q}^{ss} \quad k \neq 0, \quad (34)$$

$$\omega_{ss} = \frac{f}{\gamma} - \frac{i2\pi}{\gamma} \sum_q q \hat{V}_q^{eq} \hat{P}_{-q}^{ss}, \quad (35)$$

where k and q are the wave numbers, and $\hat{\cdot}$ denotes the Fourier coefficients. These equations can be used to reconstruct V_{eq} by

rearranging Eq. (34) as

$$\hat{V}_q^{eq} = -\left[\sum_q q \hat{P}_{k-q}^{ss}\right]^{-1} \left(\Theta k - \frac{ifL}{2\pi}\right) \hat{P}_k^{ss} \quad k \neq 0, \quad (36)$$

an expression that requires the experimental estimate of $P_{ss}(\phi)$ and f . As discussed in Sec. II, $P_{ss}(\phi)$ can be obtained for every experimental single-motor trace [see Fig. 1(f)]. To estimate the tilt f , we first make the simplifying assumption that each trace falls within either the $\epsilon \ll 1$ or $\epsilon \gg 1$ regime and then approximate f by f_0 as defined in Eq. (9). Interestingly, this method allows us to estimate the drag γ from the experimental observables, instead of assuming a value for it. Once V_{eq} is reconstructed, Eq. (35) can be used to estimate γ from

$$\gamma \approx \frac{f_0}{\omega_{ss}} - \frac{i2\pi}{\omega_{ss}} \sum_q q \hat{V}_q^{eq} \hat{P}_{-q}^{ss} \equiv \gamma_0. \quad (37)$$

Experimental data fitting. In Fig. 2(e) (left panel) we plot the resulting $V_{eq}(\phi)$ for every motor measured, together with the mean of all the reconstructed potentials. The resulting energy barriers are distributed from 1 to 3Θ , with an average of 2Θ , a value smaller than the $(3.80 \pm 0.40)\Theta$ found in Method 1 (but in agreement with the result of Method 3 below). The possible reasons for this discrepancy are (1) Method 1 assumes that the system is in the Kramers regime making barriers by default larger than 3Θ [34]. (2) The short traces may limit the accuracy of the P_{ss} estimation required by Methods 2 and 3 [43]. (3) Method 1 naturally gives greater weight to the left and right tails of the experimental D_{ss} vs ω_{ss} curve, which are directly governed by E_a , unlike Methods 2 and 3, which rely on a simple arithmetic mean. In Fig. 2(f) we show the experimental distribution of the values of γ , calculated for each motor. The mean value of the distribution is $\gamma = 0.13$ pN nm s/rad², consistent with the estimates of Method 1, highlighting again the dominant role of the motor's internal drag, γ_R , over the hydrodynamical drag of the attached bead, γ_B , for these experiments [see Fig. 1(c)].

C. Method 3

Originally developed as a variant of Method 2 in the absence of information for f [43], this method involves an iterative self-consistent solution of Eqs. (35) and (36), and requires as inputs the experimental values for the angular distribution $P_{ss}(\phi)$, angular velocity ω_{ss} , and γ . The optimization algorithm works as follows:

- (1) We obtain P_{ss} , ω_{ss} , γ .
- (2) $f^{(i)} \equiv \gamma \omega_{ss}$ serves as initial guess.
- (3) Eq. (36) with P_{ss} and $f^{(i)}$ returns $V_{eq}^{(i)}$.
- (4) Eq. (35) with $V_{eq}^{(i)}$, $f^{(i)}$, and γ returns $\omega^{(i)}$.
- (5) We check $|\omega_{ss} - \omega^{(i)}| \leq \delta$, with $0 \leq \delta \ll 1$ a set tolerance.

(a) True: $f^{(i)} \equiv f_{opt}$, $V_{eq}^{(i)} \equiv V_{eq}^{opt}$

(b) False: we update the input $f^{(i+1)} = f^{(i)} + \Delta f$, iterating steps 3–5 until $|\omega_{ss} - \omega^{(i+1)}| \leq \delta$.

Δf is chosen according to the Nelder-Mead algorithm.

Because this method is not limited to a specific ϵ regime, it therefore allows us to analyze all measured motors. Based on the results from Methods 1 and 2 above, we take $\gamma \approx 0.12$ pN nm s/rad² to account for both the internal motor

TABLE I. Comparison of the three methods developed for extracting torque, viscous drag, and the potential landscape from bacterial flagellar motor bead assay data.

Methodology	Method 1: Spectral decomposition of Smoluchowski equation via Bloch's theorem	Method 2: Fourier expansion of the Smoluchowski equation in the case of a general equilibrium potential	Method 3: Iterative self-consistent landscape inversion
Inputs and relevant equations	ω_{ss} , D_{ss} ; Eqs. (17), (21), (28) and (30)	$P_{ss}(\phi)$, L , ω_{ss} , D_{ss} ; Eqs. (36) and (37)	$P_{ss}(\phi)$, L , ω_{ss} , γ ; Eqs. (35) and (36)
Outputs	$f(\omega_{ss})$, γ , E_a , L	$V_{eq}(\phi)$, γ_0 , E_a , f_0	$V_{eq}(\phi)$, E_a , f
Assumptions and notes	Valid for $\epsilon < 1$ or $\epsilon > 1$; $f \approx f_0$; Single-mode sinusoidal potential	Valid for $\epsilon < 1$ or $\epsilon > 1$; $f \approx f_0$; Valid for arbitrary periodic potentials	Valid for all ϵ ; $\gamma = 0.12$ pN nm s/rad ² ; Valid for arbitrary periodic potentials
Experimental results	$\gamma = (0.09 \pm 0.005)$ pN nm s/rad ² $E_a = (3.8 \pm 0.4) \Theta$ $2\pi/L = (28.8 \pm 1.8)$ steps	$\langle \gamma_0 \rangle = (0.13 \pm 0.07)$ pN nm s/rad ² $E_a \approx (2 \pm 1.2) \Theta$	$E_a = (2 \pm 1.2) \Theta$
Pros	Traces analyzed can be relatively short. Does not require knowledge of γ	A single trace is enough for applicability. Does not require knowledge of γ	A single trace is enough for applicability. Does not require knowledge of f
Cons	Requires multiple traces spanning across different ϵ regimes Imposes the shape of the potential	Accurate estimation of P_{ss} requires motor's trace with sustained constant ω_{ss} , D_{ss} during large recording times Not applicable in the $\epsilon \approx 1$ case	Accurate estimation of P_{ss} requires motor's trace with sustained constant ω_{ss} , D_{ss} during large recording times Requires knowledge of γ

drag and the external load of the attached bead. This choice of γ yields predicted D_{ss} values that are consistent with the experimental measurements. In SM [40], we show that using substantially different values of γ leads to predicted D_{ss} values that deviate from the experimental data.

Experimental data fitting. In Fig. 2(e) (right panel) we plot the results of the reconstruction of the potential V_{eq} , obtained for each motor. The energy barriers and their average ($E_a \sim 2.0 \pm 1.2 \Theta$), despite a larger spread, are in good agreement with the results of Method 2 [Fig. 2(e), left panel]. Since Methods 2 and 3 share the same procedure to estimate P_{ss} , it is likely that this common step is the main source of the comparatively low-energy barriers obtained relative to Method 1.

V. DISCUSSION

Understanding how molecular motors transduce (electro-) chemical energy into directed mechanical motion requires not only identifying the molecular components involved and their interactions, but also characterizing the effective energy landscape in which they operate, determined by periodic structural constraints, and internal mechanical resistance at the atomic scale. In this work we developed and applied three complementary methods of analysis to quantitatively characterize the energy landscape that shapes the rotational dynamics of molecular motors operating in the presence of periodic potentials. We applied these methods to the BFM, where the LP ring acts as a molecular bearing imposing a 26-fold periodic potential landscape on the rotor, which dominates the BFM's dynamics at low loads. Crucially, our approach requires neither the application of external torque, nor prior knowledge of the system's viscous drag or the motor's torque output.

The three methods developed in this work, along with the results they provide when applied to BFM rotation data, are summarized in Table I. Method 1 assumes a single Fourier

mode (cosine potential) and uses analytical solutions to the Smoluchowski equation in the limits of weak and strong tilt [Eqs. (17), (28), and (31)] to relate the measured rotational velocity and diffusion to the viscous drag, periodicity, and energy barrier. This method can also be used to relate the measured rotational velocity and diffusion to the underlying torque via Eqs. (20), (30), and (32). Methods 2 and 3 relax the single-cosine assumption by allowing arbitrary static periodic bearing landscapes, but they still assume that an effective stationary landscape can be defined over the analyzed time window. In this sense, Method 2 employs a model-independent, Fourier-based reconstruction of the Smoluchowski equation, by fitting the full steady-state angular distribution P_{ss} , along with velocity and diffusion, to extract the underlying potential and drag. Method 3 provides a direct reconstruction of the energy landscape by integrating P_{ss} under the assumption of known viscous drag.

The viscous drag coefficient experienced by the motor was estimated as $\gamma \approx 0.1$ pN nm s/rad², with consistent values obtained from Methods 1 and 2. This result, which is in quantitative agreement with a previous estimate [32] of internal drag, suggests that internal structural interactions, including those at the rod-LP ring interface, can contribute significantly to mechanical resistance and rotational dissipation (see SM [40], Effective drag: short and long time dynamics). We conclude that, in the low-load regime, the hydrodynamic drag of the bead, often estimated using the Stokes formulation with surface proximity corrections [42], contributes only a small portion of the total measured resistance. The corresponding drag coefficient (~ 0.008 – 0.01 pN nm s/rad²) implies that the internal viscous friction, one order of magnitude higher, is the primary source of dissipation in the far-from-equilibrium regime. In the close-to-equilibrium regime, on the other hand, a large enough potential landscape energy barrier plays a major role in determining the diffusion coefficient. Therefore,

in both limiting scenarios, very-low (very-high) tilts, two different linear asymptotic limits for the torque ($f \propto \omega_{ss}$) can be deduced. The corresponding proportionality constants are related to the barrier-suppressed diffusion, D_K , and the barrier-free diffusion, D_E , respectively:

$$f_{\gamma}^{\text{lo}} \approx \frac{\Theta}{D_K} \omega_{ss} = \gamma \frac{\Theta}{\pi E_a} e^{E_a/\Theta} \omega_{ss}, \quad (38)$$

$$f_{\gamma}^{\text{hi}} \approx \frac{\Theta}{D_E} \omega_{ss} = \gamma \omega_{ss}. \quad (39)$$

These asymptotic limits are displayed in Fig. 2(d) using the best-fit parameters found by Method 1. For contrast, this plot also included the case where f_{γ}^{hi} only accounts for the bead's viscous drag γ_B , highlighting again the need to include the internal drag contribution at low loads.

Cryo-EM structures reveal a tight interface between the rod and LP ring, involving both electrostatic and steric interactions [8,12]. Opposing charges and potential salt bridges likely stabilize the rotor and support high-speed rotation, while also introducing periodic energy barriers that contribute to the internal resistance and shape motor dynamics. All three of our methods enabled estimates of the height of the LP ring-rod energy barrier. Method 1 gave a barrier height of $E_a = 3.8 \Theta$. Method 2 yielded a distribution of E_a values between 1 and 3Θ , with an average around 2Θ , and Method 3 produced a similar average of $E_a \sim 2 \Theta$. Our estimates for E_a and γ are in qualitative agreement with those of a previous modeling study [18] in which, contrary to our own study, a 26-fold periodicity was assumed as input to investigate the stepping of the BFM. Complementary, if single-molecule trajectories with sufficient angular and temporal resolution could be obtained from BFM variants in which the LP-ring contribution is removed, weakened, or otherwise modified, our methodology could be used to probe the dominant remaining periodic landscape. The only other experimental measurement of this barrier height was recently reported by Rieu *et al.* [19], who used high-speed back-scattering darkfield polarization microscopy to track with exceptional angular and temporal resolution the equilibrium diffusion of gold nanorods (i.e., in the absence of applied torque) attached to the bacterial hook [19]. By analyzing angular hopping events, they inferred energy barriers on the order of 13Θ . We offer a few speculative explanations for why our estimates differ from those of Rieu *et al.* In their study, the authors estimated E_a by applying Kramers' theory to measured dwell times between angular states, a method that relies on assumptions about the shape of the energy landscape. They assumed a periodic parabolic barrier shape, which leads to a cusp at the peak and a harmonic form at the potential minimum. This choice modifies the prefactor appearing in Kramers' rate and leads to an overestimate of the barrier height with respect to the one obtained for a cosine potential (we estimate this overestimate to be less than 20% for barrier heights between 4 and 13Θ). Secondly, and probably principally, their calculation includes only the hydrodynamic drag of the rotating rod, neglecting internal viscous drag of the motor itself. As mentioned above, our results indicate that this internal drag dominates under low-load conditions, and omitting it would lead to an overestimate for E_a . In Fig. S.2 in SM [40] we explore how the different choices of γ for a fixed equilibrium diffusion value

lead to different energy barriers. Finally, the passive bearing measurements of Rieu *et al.* were performed in the absence of stator units, whereas our measurements probe actively rotating motors operating in nonequilibrium steady states with one or two engaged stators. For the long-time steady-state observables considered here, fine-scale roughness in the equilibrium free-energy landscape of the bearing is expected to be less important than the dominant periodicity and effective barrier height. Thus, our coarse-grained landscape provides a baseline description that captures the relevant nonequilibrium dynamical system's observables and is complementary to the study conducted by Rieu *et al.* Whether dynamic coupling with stator units modulates the effective potential during active rotation, thereby reducing the observed barrier height, remains an open question. Importantly, Rieu *et al.* also observed anomalous intrawell diffusion, non-Poissonian transition kinetics, rough 26-fold landscapes, and slow shifts in preferred angular positions, highlighting that the bearing dynamics are richer than what can be captured by a smooth, static one-dimensional potential.

Despite these complications, the tilted periodic potential provides a useful coarse-grained description of the long-time steady-state dynamics measured here. In this description, $V_{\text{eq}}(\phi)$ denotes the effective rod-LP-ring bearing potential, while the constant tilt f represents the coarse-grained drive produced by stator mechanochemical cycling. The model does not attempt to reconstruct the multiple internal motor potentials associated with different stator states, ion-coupled transitions, or rotor-stator conformational changes. Rather, it reconstructs the effective energy landscape governing the experimentally observed angular coordinate after coarse graining over unresolved mechanochemical degrees of freedom. This distinction is particularly important in the low-load regime analyzed here, where motors are likely driven by one, or very few, stator units. Accordingly, f should be interpreted as a time-averaged effective drive inferred from ω_{ss} and D_{ss} over trace segments with approximately stationary dynamics, rather than as the instantaneous torque generated by an individual stator. Elastic compliance in the hook, rotor, bearing, and bead linkage may further filter fast microscopic torque fluctuations. Thus, the model should be viewed as a baseline thermodynamic description of steady-state drift and diffusion in the measured angular coordinate, rather than as a microscopic model of BFM torque generation. The same mesoscopic formalism could therefore be adapted to linear motors, such as microtubule-based motors, actin-based motors, by replacing the angular coordinate and torque with a linear coordinate and effective force, provided that the measured motion can be treated as stationary drift and diffusion in an effective periodic landscape over the analyzed time window.

The energy landscape of this molecular bearing raises interesting questions about its evolutionary tuning and biological function. One may imagine that evolution has tuned the barrier height to balance structural stability, maintaining alignment of the rod during high-speed rotation, with minimal mechanical resistance, thus maximizing efficiency. Depending on the number and duty ratio of engaged stator units, we could imagine that this landscape may also act as a ratcheting mechanism, providing resistance to backward rotation when torque output is low or when stator units transiently disengage.

This may be especially important in species like *E. coli*, where stator assembly is dynamic [15,45–47], compared to motors like *Campylobacter jejuni*, where stators seem stably assembled [13,48]. In systems that experience mechanical strain, such as during flagellar (un-)wrapping [49], one could imagine that larger energy barriers may be advantageous, if they enhance the structural rigidity of the rod-bearing interface. Motility strategy may also shape barrier height, as peritrichously flagellated bacteria and polar-flagellated species face distinct mechanical challenges that may select for different optimal landscapes. Finally, even subtle variations in rod-LP ring alignment, post-translational modifications, or local symmetry could generate cell-to-cell variability in the energy landscape, leading to cell-to-cell diversity in motor behavior and motility. We believe that the methods introduced here could provide a potential landscape model-independent means of quantitatively probing some of these questions, as well as offer a general framework for probing energy transduction in other molecular motors.

VI. MATERIALS AND METHODS

Data set. We measured the steady-state rotation of 95 individual BFMs of a nonswitching strain of *Escherichia coli* via a bead assay [24], tracking the rotation of 100 nm diameter gold beads attached to the flagellar hook, as previously described [50]. The BFMs were driven by either wildtype *E. coli* stator units or chimeric Na^+ stator units under different proton and sodium motive force conditions to generate a broad range of rotation speeds. Part of this dataset was reported in [50], while the remaining traces are newly analyzed here. The plasmon-enhanced scattered light from the gold bead was imaged using backscattering dark-field microscopy onto a high-speed camera at 109 500 frames per second. The bead center was tracked as it rotated slightly off-axis, yielding its circular $x(t), y(t)$ trajectory [Fig. 3(a) inset]. Once centered at the origin, the trajectory yielded the time trace of the BFM rotation angle, $\phi(t) = \text{atan2}[y(t), x(t)]$, from which we computed the angular speed and diffusion. The positional noise of the dark-field microscope was <1 nm per axis [51], corresponding to an angular uncertainty of $\sim 1^\circ$ per frame for typical orbital radii of ~ 50 nm; we conservatively take 3° as an upper bound.

Calculation of ω_{ss}, D_{ss} . Our analysis focuses on the stationary dynamics of motors rotating at sustained constant speed. Therefore, for each trajectory analyzed, we extracted the steady-state angular velocity, ω_{ss} , and the rotational diffusion coefficient, D_{ss} , from the longest uninterrupted portion of the trace displaying approximately constant speed. These intervals were identified by visual inspection and typically lasted between 0.3 s and 2 s [see, for example, Fig. 3(a)]. To estimate the steady-state angular velocity, we performed a linear regression of the mean angular displacement, $\langle \Delta\phi \rangle = \omega_{ss}\Delta t + b$, as a function of the lag time Δt over the selected interval [cf. Fig. 3(a)]. For the diffusion analysis, we first computed the centered mean-squared angular displacement, $\overline{\text{MSD}} = \langle (\Delta\phi)^2 \rangle - \langle \Delta\phi \rangle^2$, as a function of the lag time Δt ; in all cases, 90 logarithmically spaced lags were used. The steady-state normal diffusion regime is reached when $\overline{\text{MSD}} = D_{ss}2\Delta t$. We therefore considered the lag-dependent

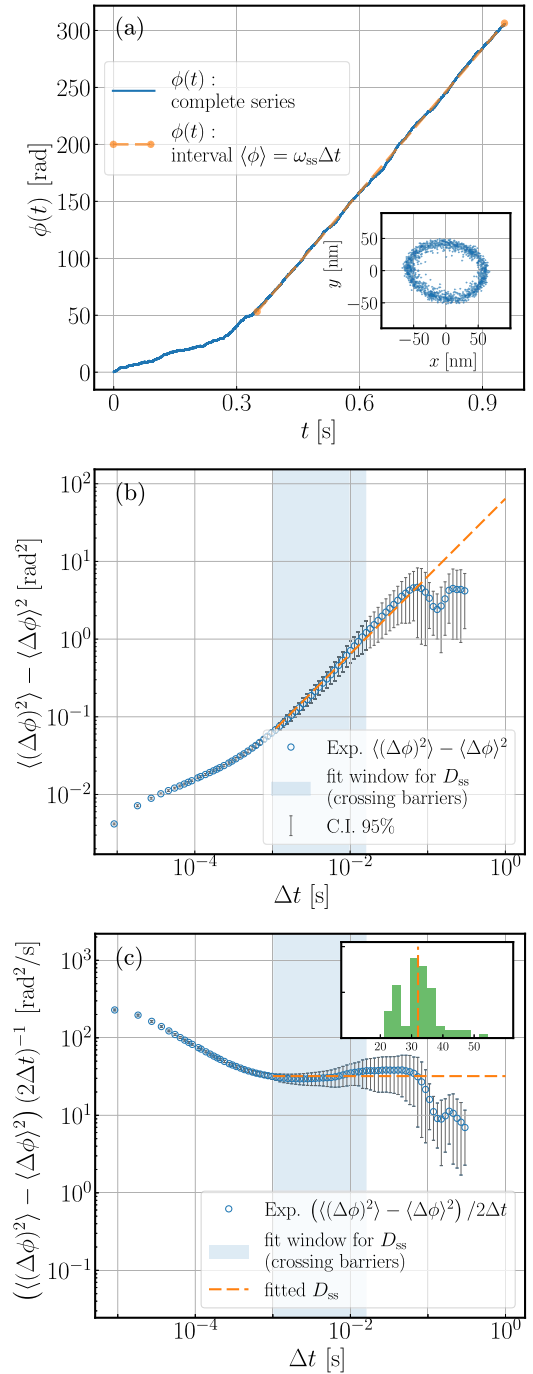


FIG. 3. Example trace showing: (a) (solid) Accumulated angle versus time plot, (dashed) longest uninterrupted portion used to calculate ω_{ss} , and inset $\{x(t), y(t)\}$ coordinates every 60 points. (b) Centered mean-squared angular displacement ($\overline{\text{MSD}} = \langle (\Delta\phi)^2 \rangle - \langle \Delta\phi \rangle^2$) analysis for increasing lag Δt used to determine D_{ss} , including (bars) 95% confidence intervals. (c) Relaxation towards steady state of $\langle (\Delta\phi)^2 \rangle - \langle \Delta\phi \rangle^2 / 2\Delta t$ for increasing lag Δt , determined (orange dashed) D_{ss} mean value and inset (green bars) dispersion within fitting window.

diffusion estimate, $\overline{\text{MSD}}/(2\Delta t)$, and identified the steady-state diffusion regime as the range of consecutive lag times over which $\overline{\text{MSD}}/\Delta t$ displayed a plateau. Equivalently, defining $X = \log_{10}(\Delta t)$ and $Y = \log_{10}(\overline{\text{MSD}}/(2\Delta t))$, we selected

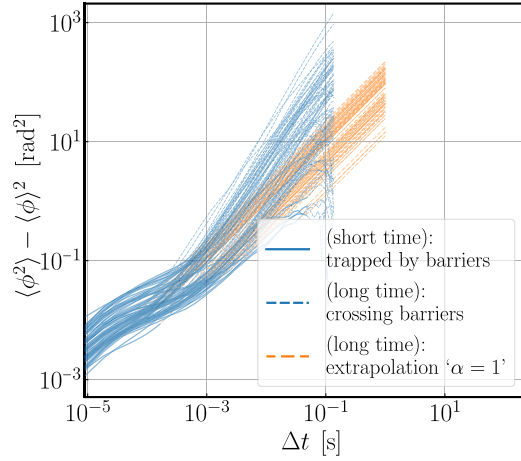


FIG. 4. Set of traces showing: centered mean-squared angular displacement ($\text{MSD} = \langle (\Delta\phi)^2 \rangle - \langle \Delta\phi \rangle^2$) analysis for increasing lag Δt used to determine D_{ss} .

lag intervals for which $dY/dX \sim 0$, within a 10% tolerance. Then we extracted the value of D_{ss} by averaging $\overline{\text{MSD}}/(2\Delta t)$ over this plateau region, and the corresponding standard deviation was reported as the uncertainty in Fig. 3(c). Because the analyzed trajectories are relatively short, the selected lag times typically lie in the range $\Delta t \in (0.1 \text{ ms}, 0.1 \text{ s})$. At longer lag times, deviations from normal diffusion are observed, but these points have low statistical significance and are therefore not representative of the steady-state dynamics considered here, as discussed in Ref. [52].

To assess the robustness of the selected lag intervals used to estimate D_{ss} , we computed confidence intervals for the MSD. These intervals account for three main sources of uncertainty: instrumental angular noise, $\sigma_\phi \leq 0.06 \text{ rad}$ ($\sim 3^\circ$), finite sample size (relevant for short trajectories), and correlations arising from overlapping increments at short lag times. The error propagation of the former gives an uncertainty to the MSD of $\sigma_M^2 = 2\sigma_\phi^2 \leq 0.0072 \text{ rad}^2$. The standard error was estimated as $\text{SE} \approx \overline{\text{MSD}}\sqrt{2/N_{\text{eff}}}$, where $N_{\text{eff}} \sim N\delta t/\Delta t$ is the effective number of statistically independent increments at lag time Δt , with N the number of data points in the analyzed segment and δt the acquisition time step. Therefore, the 95% confidence interval was estimated as $\text{C.I.} = 1.96 \times \text{MSD} \sqrt{2/N_{\text{eff}}}$. In Figs. 3(b) and 3(c) the $\overline{\text{MSD}}$ with 95% confidence intervals and D_{ss} are shown respectively, while Fig. 4 summarizes the D_{ss} calculation for the entire dataset.

Calculation of the fundamental periodicity using Methods 2 and 3. To determine the fundamental periodicity of the motor and identify the angular step size from experimental traces in Methods 2 and 3, we first computed the histogram of the angular position $\phi(t)$, which we call $H(\phi)|_{2\pi} \equiv H(\phi \bmod 2\pi)$. Because $\phi(t)$ is derived from experimental position tracking, it reflects both biological variability (e.g., fluctuations in motor dynamics) and experimental noise (e.g., imaging and tracking uncertainty), which together make it harder to identify any underlying periodicity in the histogram. To reduce this noise and extract underlying physical periodic features, we applied Ensemble Empirical Mode Decomposition (EEMD) to $H(\phi)$ [25]. EEMD is an adaptive filtering technique designed to decompose non-stationary and nonlinear signals into a sum

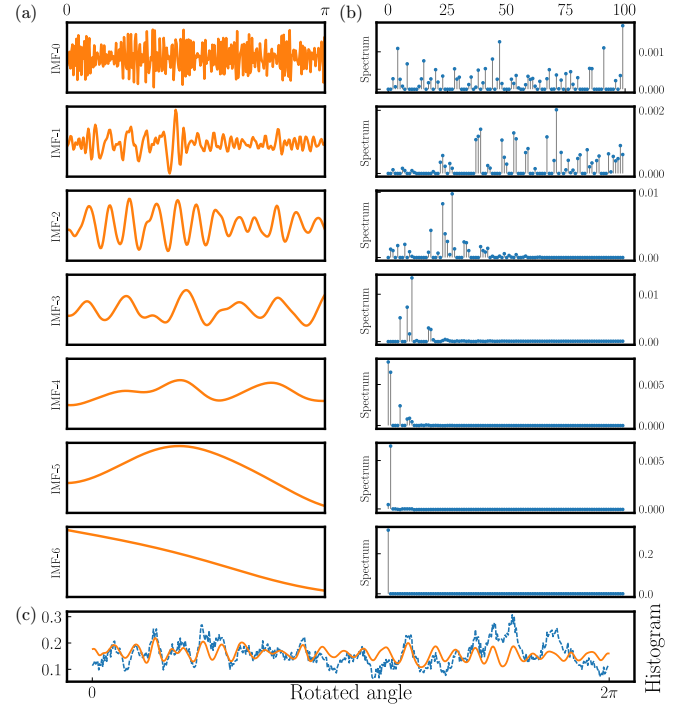


FIG. 5. EEMD analysis of the trace shown in Fig. 3(a). (a) The IMFs ordered in decreasing frequency, (b) the corresponding spectra as function of the steps per rotation, and (c) angular histogram of the original signal $H(\phi)|_{2\pi}$ (dashed line) and the filtered signal $H(\phi)^\dagger|_{2\pi}$ (solid line).

of intrinsic mode functions (IMFs). Each IMF represents oscillatory components at different timescales, allowing us to identify dominant periodicities in the signal without requiring prior assumptions about the signal's frequency content.

In Fig. 5 we show how the original angular histogram $H(\phi)|_{2\pi}$ is decomposed into a set of IMFs using EEMD, ordered from high- to low-frequency content. Across all traces, the second mode (IMF-2) exhibits a clear 26-fold periodicity [see Fig. 1(e)]. The sixth mode (IMF-6) captures the slow baseline variation associated with the overall shape of the original distribution (i.e., the normalization envelope). Other IMFs were excluded from further analysis. We reconstructed a denoised version of the angular histogram, $H(\phi)^\dagger|_{2\pi}$, using only IMF-2 and IMF-6. From this, we identified the fundamental periodicity as $L = 2\pi/25.7 \approx 2\pi/26$, which we then used to compute an approximate steady-state angular probability distribution, $P_{ss}(\phi) \approx H(\phi)^\dagger|_L$, shown in Fig. 1(f).

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DATA AVAILABILITY

The data that support the findings of this article 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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