Power strokes in molecular motors: Predictive, irrelevant, or somewhere in between?

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For several decades, molecular motor directionality has been rationalized in terms of the free energy of molecular conformations visited before and after the motor takes a step, a so-called power stroke mechanism with analogues in macroscopic engines. Despite theoretical and experimental demonstrations of its flaws, the power stroke language is quite ingrained, and some communities still value power stroke intuition. By building a catalysis-driven motor into simulated numerical experiments, we here systematically report on how directionality responds when the motor is modified accordingly to power stroke intuition. We confirm that the power stroke mechanism generally does not predict motor directionality. Nevertheless, the simulations illustrate that the relative stability of molecular conformations should be included as a potential design element to adjust the motor directional bias. Though power strokes are formally unimportant for determining directionality, we show that practical attempts to alter a power stroke have side effects that can in fact alter the bias. The change in the bias can align with what power stroke intuition would have suggested, offering a potential explanation for why the flawed power stroke mechanism can retain apparent utility when engineering specific systems.

INTRODUCTION

Giving a preferred direction to the stochastic motion of molecules challenges our physical intuition, which is strongly informed by the macroscopic, deterministic regime [1]. At the nanoscale, inertial dynamics gives way to the random dance of Brownian motion, a process that can be subtly biased to drift in one direction or another [2]. Living systems achieve directionality using molecular machinery [3] like kinesins [4], dyneins [5], and myosins [6], motor proteins that move along microtubules in a preferred orientation. These autonomous motors are chemically driven by the hydrolysis of adenosine triphosphate (ATP) into adenosine diphosphate (ADP) and inorganic phosphate (P). Under physiological conditions, a thermodynamic driving force favors a net decomposition of ATP, causing the motors to experience an environment with nonequilibrium concentrations of ATP, ADP, and P. By catalyzing ATP decomposition, the molecular motors couple their motion to the chemical driving force, transducing the free energy from the environment into directed motion [7, 8]. Extensive experimental [9–19] and theoretical [7, 20–23] studies have dissected the mechanistic aspects of these processive motor proteins, leading to a comprehensive characterization of the chemistry that underlies their stochastic stride.

One feature that is quite commonly observed (but by no means universal [24]) across different architectures of motor proteins is that the conformational changes that allow a motor to take a step are free energetically downhill in the direction of motion [17, 25, 26]. Consider, for

instance, the six-state kinetic model of kinesin in Figure 1. The motor's function relies upon executing complete cycles, but only the highlighted step corresponds to the motor's directional motion. Furthermore, that forward step is accompanied by a drop in free energy of about 36 kJ/mol, a drop so large that the steps appear to be essentially irreversible. These thermodynamically favored stepping reactions are often named "power strokes" and described as free-energy releasing, large-amplitude conformational changes [27]. Early work conceived of a power stroke in terms of macroscopic concepts like ballistic motion and elastic strain [27–29], but these macroscopic ideas are misleading on the nanoscale. [2, 30] The modern meaning of the phrase power stroke, which accommodates the nonelastic, nonballistic reality of microscopic motion, quantifies the power stroke in three equivalent ways: as the free energy drop due to the forward step, as a measure of how much more stable the pre-step conformation is than the post-step one, and as a logarithm of the equilibrium constant, K_{eq}, for partitioning between those two conformations if the process were not fueled. [24, 31, 32]

Since the initial discussions of power strokes [35, 36], the downhill power stroke step has been proposed as a feature necessary to generate a directional bias in molecular motors and perform work, dictating their directionality (see Figure 1) [13, 29, 37–41]. Pictorially, the power stroke mechanism has been described as "the molecular analogue of an inclined plane" [42], reflecting the idea that the direction of the thermodynamic tilt determines the direction of the flow. Power stroke intuition has guided successful experimental efforts to reverse the direction of biological motors [43–47] and to realize artificial light-driven motors [48–51].

However, power strokes are known to be an incomplete and even problematic proxy for the directionality of

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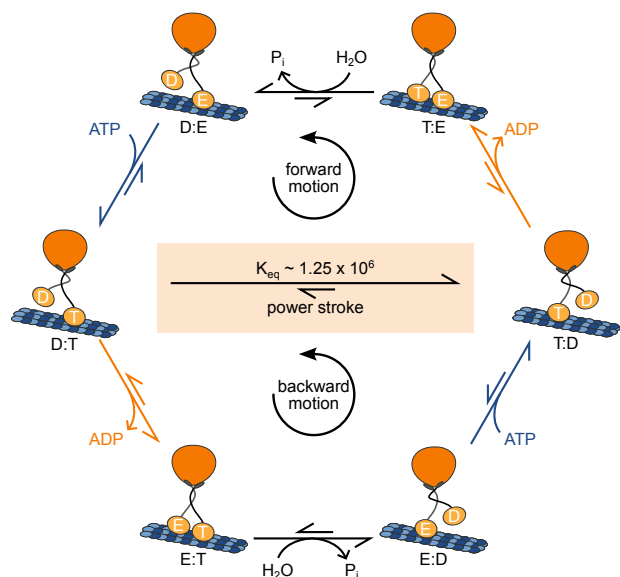† todd.gingrich@northwestern.edu.

FIG. 1.   **Kinesin's "power stroke".** Sketch of a minimal chemical reaction network model for kinesin motion [21] validated in single-molecule experiments [33, 34]. Different kinesin conformations are distinguished by the type of nucleotide attached to each head (T, ATP; D, ADP; E, empty). The outer reactions change the chemical state of one head without leading to horizontal motion. The inner reaction corresponds to a large-amplitude conformational change, swapping the two heads through a horizontal step. Experimental data [21, 33, 34] show that this stepping reaction has an equilibrium constant of 1.25×10^6, corresponding to a free energy difference of 36 kJ/mol in favor of the T:D conformation under physiological conditions. Under continuous ATP-to-ADP turnover, forward movement (left-to-right in the cartoons) is preferred. The strong thermodynamic preference for the D:T transition compared to T:D is commonly denoted as a "power stroke" due to its free-energy-releasing character. Note that the power stroke, as used here, does not mean a process that releases elastic strain stored in the molecular structure leading to the ballistic motion of the moving head [28, 29]. Attempts to explain directionality exclusively in terms of this power stroke are fraught because they do not account for the need to regenerate the high-free-energy D:T state.

chemically fueled motors [31, 52–54]. In short, theoretical arguments clarify that conformational changes yielding directed motion can be free energetically uphill, downhill, or even flat, thus providing no information on directionality. It is not enough to analyze the free energy drop between two conformations because an operating motor cannot simply relax from a high-free-energy state; it must subsequently regenerate that state to complete a cycle. Prior theoretical work explaining why power strokes cannot determine directionality is sound, yet some communities have continued rationalizing motor directionality in terms of the power stroke language. The persistence of power stroke explanations owes, at least partially, to how that conceptual framework has guided many groups to successfully engineer motors. By altering a molecular motor so as to change the power stroke, the motor often

responds in the way that the power stroke logic would have suggested. [43, 47] Here, we set out to bring new clarity to why the power stroke logic appears to work as a design tool for engineering catalysis-driven motor performance even though the power strokes themselves do not determine directionality [32].

Our approach leverages explicit coarse-grained simulations of a minimal-model motor [55] that was inspired by an experimentally realized motor [56]. In the last decade, chemists have designed such catalysis-driven motors as synthetic model systems [57–60] capable of probing the same fundamental principles of physics and chemistry that govern more complex protein motors [32, 52, 61, 62]. The model systems [56, 58] were designed without a "downhill" step. In other words, they specifically lack a power stroke, yet they realize directional motion via a mechanism known as a Brownian information ratchet [63–65]. By building that fundamental mechanism into simulated numerical experiments, we here report on how directionality responds when a power stroke feature, with variable strength, is added. In particular, we focus on the motor's bias, a measure of directionality quantifying the fraction of steps a motor takes in a specific direction.

Our work complements previous efforts [31, 32, 52, 66] to understand the relationship (or lack thereof) between power strokes and directionality in molecular motors and related systems. Kinetic models have already clarified that directionality only emerges when different motor conformations have different catalytic properties, a condition known as kinetic asymmetry [67–70]. Based on these models, power strokes are *irrelevant* [31] for directionality, in that the kinetic asymmetry and hence the motor bias can run counter to the power stroke's orientation, a fact our simulations explicitly confirm. One might, however, naively misinterpret the irrelevance as a stronger claim: that introducing a power stroke into the motor chemistry would not affect the motor's bias. Our simulations clearly caution against that interpretation; adjusting the strength of the power stroke does alter the motor's bias. The conflict with kinetic models reflects a limitation of the kinetic models, which imagine that a power stroke can be adjusted as an isolated parameter. Our simulations more faithfully mimic the experimental situation, whereby one can adjust interactions between moieties so as to change the relative stability of two conformations. The altered interactions indeed tune the power stroke, but as a side effect they can impact the kinetics of transformations between additional conformations. For example, one might engineer a kinesin with the goal of altering the relative stability of the D:T and T:D states in Fig. 1, but the mutations one introduces to alter the power stroke will not affect the kinetics of the horizontal power stroke step in isolation. Those mutations also change the kinetics of the "outer reactions" of Fig. 1, and these kinetic side effects (not the change in the power stroke itself) can change the motor's bias.

Having established that turning up the power stroke

can indeed change the bias, we numerically probe whether that change is in the direction that power stroke intuition would suggest. In other words, if one tilts the inclined plane more strongly, does the bias increase in the direction of the tilt? Our simulations show regimes within the same system in which larger power strokes yield more bias and regimes in which they yield less bias. Interestingly, however, we find that *within the regimes where the motor generates the most current*, turning up the power stroke typically turns up the bias. This observation could explain why power strokes appear common throughout operational biological motors and why they have already been effectively used to engineer molecular motors.

RESULTS AND DISCUSSION

A. Context and central question

The central question of this work is whether a molecular motor's bias can be engineered by tuning a power stroke. Addressing that question requires some precision about *how* one would envision tuning it. It is therefore attractive to specialize in synthetic molecular motors, a setting in which one can be explicit about how to adjust the strength of attraction and repulsion between a small number of chemical moieties. We focus on the first experimental example of a catalysis-driven molecular motor, shown in Figure 2. [56] The motor is a catenane consisting of a benzylic amide ring (green) that executes a random walk in which it dwells at one fumaramide binding site (orange) before taking a rare hop to the other degenerate binding site. This hop, initiated by a thermal fluctuation, can proceed clockwise or counterclockwise around a track. By coupling to a catalytic reaction, that random walk can be biased to prefer clockwise rotations.

The biased motion has been understood using the kinetic model of Figure 2a, which reduces the many-body dynamics to a stochastic process in which the whole system transitions between six metastable conformations. The green ring can be at either binding site, while the white catalytic hydroxy residues along the track can be blocked or unblocked by red fluorenylmethoxycarbonyl (Fmoc) protecting groups. Note that the chemical reaction network models of the system tend to neglect low-probability motor configurations with no Fmoc groups on the track because blocking groups form faster than they are removed during experimental motor operation [56]. If both catalytic sites are blocked, the ring cannot hop from one binding site to the other. If none are blocked, there can be no preferred direction by symmetry. Motion with a directed bias requires a blocking Fmoc on one side of the ring but not on the other, which makes the four singly blocked conformations particularly important. Two of those conformations have the green shuttling ring proximal to the red blocking groups, and the other conformations have those two far enough apart that

they do not interact. Due to structural asymmetry (or anisotropy [60]) in the chemical design, a transition from distal to proximal state corresponds to clockwise rotation of the shuttling ring (see Figure 2a). Akin to kinesin's downhill step, the power stroke mechanism would suggest that the shuttling ring will move clockwise if the free energy of the proximal conformation is lowered relative to that of the distal conformation. Experimental measurements convincingly put to rest the idea that a power stroke determines directionality; directed motion results even in the case of degenerate proximal and distal conformations ($K_{eq} = 1$) [56, 58].

In fact, it is now well established that molecular motors operate via the Brownian information ratchet mechanism, where kinetic asymmetry arises from the interplay between structural asymmetry and catalytic fuel consumption. [68, 69] The rate of catalysis, and therefore the rate at which Fmoc groups block the catalytic sites, depends on the position of the ring. In particular, when the ring sits on a binding site, it hinders the proximal catalytic site and thus disfavors the addition of a blocking group. This kinetic asymmetry promotes the distal conformation over the proximal one, disfavoring addition of blocking groups at the catalytic site close to the ring. The resulting population imbalance is what ultimately creates directionality by making distal-to-proximal jumps more frequent than proximal-to-distal (see SI Section 1.1 for a detailed illustration of state-of-the-art kinetic modeling).

The six-state network picture of Figure 2a is frequently accompanied by the free energetic picture of Figure 2b, showing relative free energies of the six states, as well as the barrier heights that separate these states. [32, 60, 69] This picture offers a graphical view of both the power stroke and the kinetic asymmetry. Whereas the power stroke is uniquely determined by conformational free energies, kinetic asymmetry is uniquely determined by the barrier absolute heights, as derived in SI Section 1.2. Consequently, one should expect that altering the free energy of some of the metastable conformations cannot change the motor's directionality, as it was recently verified in closely related rotaxanes. [66] Important theoretical work has considered the kinetic consequences of adjusting one or more energies and barriers, treating those energy levels as controllable parameters in the theory. [31, 71] For example, the no pumping theorem states that it is impossible to generate directed motion by temporally adjusting conformational energies if the barriers are held fixed. [72, 73] Similar no-go results have also been proven to be quite universal in chemical reaction network models and extended to a class of properties broader than directionality. [74] The apparent conclusion is that power strokes are simply irrelevant to kinetic asymmetry and motor directionality.

Power stroke irrelevance stands out in stark contrast to experimental observations of biological motors and the effectiveness of power stroke engineering. People have tried to resolve the apparent contradiction by introducing hybrid models where the power stroke mechanism
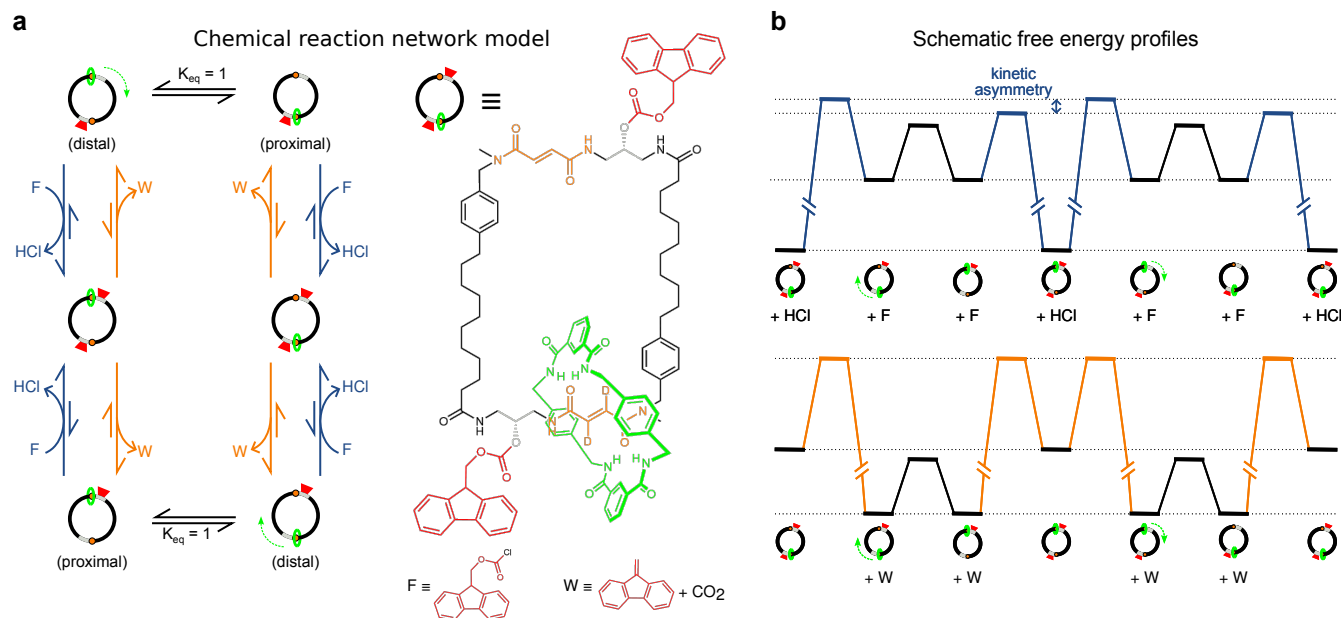
FIG. 2. **Chemical reaction network picture of an experimentally realized catenane motor. a**, Chemical reaction network and molecular structure of catenane motor. Processes shown in blue and orange indicate the reactions directly involved in the catalysis of the fuel-to-waste turnover F $\rightleftharpoons$ W + HCl. **b**, The system is characterized by free energies of the motor configurations and barriers that govern the rates of transitions between these configurations when mediated by Fmoc attachment reactions (blue) or by dibenzofulvene formation reactions (orange). Kinetic models have analyzed the kinetic asymmetry of the shuttling-ring motion arising from the difference between the barriers for the proximal and distal Fmoc-attachment reactions. That analysis suggests the free energies of the states are irrelevant, but our work highlights that physical strategies to change the free energies of states alter the barriers in coupled manners. Free energies and barriers are not parameters that can be adjusted independently.

and the Brownian information ratchet mechanism coexist, quantifying the contributions from each mechanism to the overall directionality. [8, 27, 40, 41, 75] For example, a class of models distinguishes between the two mechanisms based on the value of a load-sharing factor that dictates how a load would perturb the forward and reverse stepping rates. [41] These models account for power stroke engineering, but they have been criticized [50] in that they rely on a tight coupling assumption (backward motion must happen against fuel-to-waste gradient) that is not verified in the artificial systems realized to date. [60, 65]

We claim that there is no contradiction between the Brownian information ratchet mechanism and successful power stroke engineering experiments. Furthermore, successful power stroke engineering does not imply that the power stroke mechanism explains directionality. Our central hypothesis is that the design modifications one introduces to stabilize or destabilize a specific conformation will typically also affect the barrier heights. [32] The kinetic model no-go results are valid, but they assume a level of control that is uncommon in molecular systems, namely that the free energy of metastable states can be adjusted without impacting the free energy of the transition state barriers (see SI Section 1.3 for an example of how such no-go results are derived). Explicitly capturing how changes to the state stability will also change

barrier heights is challenging. Experiments would require extreme system control, while kinetic models intrinsically treat conformation and transition state free energies as independent variables. In other words, the framework in which the Brownian information ratchet mechanism is usually introduced, that of chemical reaction networks and schematic free energy profiles in Figure 2, is poorly equipped to predict if and how a design modification aimed at stabilizing a given conformation will also change the kinetic asymmetry via the new barrier heights. In the following, we rely on a molecular dynamics model that is uniquely positioned to explore our hypothesis explicitly, modeling the catenane motor beyond chemical reaction networks.

The simulation model

We sought an explicit molecular dynamics model that captures the key features of the catenane motor [56] using the minimal set of physical ingredients leveraged by all chemically driven molecular machines in general, namely short and long-range interactions and Brownian motion [2, 52]. As detailed in SI Section 2.1, to build such a model, we coarse-grain moieties into volume-excluding spherical particles whose "chemical identity" is entirely determined by how they interact with each other. In

essence, one can think of this model as a collection of particles which reciprocally attract and repel each other according to fixed potentials. Those particles move in space with overdamped, non-ballistic Langevin dynamics that incorporate (1) forces between the particles, (2) drag forces with an implicit solvent, and (3) random stochastic forces to mimic the thermal environment (see SI Section 2.2 for the explicit equations of motion and SI Section 3.1 for the assessment of their non-ballistic character and the time scales involved). Due to its coarse-grained character, our model allows one to collect statistics over numerous ring cycles, a time scale that is challenging to achieve with current classical all-atom molecular dynamics simulations, which typically require enhanced sampling techniques to capture molecular machine conformational dynamics. [76, 77] For illustrative purposes, we will color the particles according to their functional roles and use those colors to keep track of the different particle types.

As shown in Figure 3a and described previously [55], our classical model can explicitly simulate catalyzed coarse-grained chemical reactions. Consider a cluster of four blue particles bound along the edges of a tetrahedron encapsulating a single red particle, whose presence strains the blue tetrahedron. As a result, the filled tetrahedral cluster (FTC) is a metastable species; the stochastic dynamics eventually leads the red central particle (C) to escape, leaving an empty tetrahedral cluster (ETC) [78]. These uncatalyzed decomposition events are rare, as they require a large thermal fluctuation, but a patch of three white particles can catalyze this FTC $\rightleftharpoons$ ETC + C reaction. As shown in Figure 3a (bottom), when an FTC gets sufficiently close, interactions with the white particles stretch the blue particles enough for the red C to escape and bind to the white particles. The typical mechanism for the catalyzed FTC decomposition thus proceeds via a long-lived intermediate where a C particle binds to the catalytic unit and eventually leaves. This mechanism can be thought of as a continuous, microscopically reversible version of the Michaelis-Menten scheme executed explicitly within a molecular dynamics simulation.

Akin to how the catenane motor shown in Figure 2a is coupled to the fuel-to-waste turnover, the FTC $\rightleftharpoons$ ETC + C reaction couples to our coarse-grained motor model. Briefly, we choose interparticle potentials (see SI Section 2.1 for details) so that black particles form a stable circular track around which moves a shuttling ring built from green particles. That green ring feels an attraction to two different orange particles, modeling binding sites, located on opposing sides of the black track. Finally, two catalytic units are placed next to the binding sites, mimicking the structural asymmetry of the original experimental design. Similarly to the experimental catenane motor, kinetic asymmetry emerges in our simulation due to the steric hindrance of the green ring shielding the proximal catalytic site from FTC species.

A functional motor emerges when the fueling reaction is kept out of equilibrium by injecting FTC and removing ETC and C, achieved in our case by chemostats that preserve a chemical potential difference between the FTC, ETC, and C species, namely $\mu_{\text{FTC}} - \mu_{\text{ETC}} - \mu_{\text{C}} > 0$, as illustrated in Figure 3c. Our prior work has shown that the model explicitly couples the FTC $\rightleftharpoons$ ETC + C reaction to the shuttling ring motion, with shuttling much faster than catalysis as in the experimental motor [55]. We have also shown that the mean current can be flipped between the clockwise and counterclockwise orientations with structural changes [79], and statistical fluctuations in the current decrease with increasing FTC consumption [80].

Introducing a power stroke

The simulations allow us to measure how nonequilibrium steady-state dynamical behavior, most notably the motor's directional bias (the fraction of ring's cycles in a specific direction) and current (the net cycling rate), depends on the interactions between motor moieties. In the present work, we focus on how the motor's performance depends on the introduction of a power stroke, achieved by setting the strength of interactions between red blocking groups and green ring particles. Within our simulation model, it is straightforward to adjust those interactions, thereby stabilizing or destabilizing the proximal state as sketched in Figure 4a. More precisely, the model includes a set of Lennard-Jones pair potentials determining how one specific kind of particle attracts or repels the others:

$$U_{\text{Lennard-Jones}}(\boldsymbol{r}) = 4\epsilon_{\text{R}} \left(\frac{\sigma}{|\boldsymbol{r}|} \right)^{12} - 4\epsilon_{\text{A}} \left(\frac{\sigma}{|\boldsymbol{r}|} \right)^6, \quad (1)$$

where $|\boldsymbol{r}|$ is the distance between the particles, σ is their volume-exclusion radius, and ϵ_{R} and ϵ_{A} are respectively the strengths of steric (short-ranged) and long-ranged interactions between red and green particles. In our model, the particles are always bulky ($\epsilon_{\text{R}} > 0$) and long-range interactions can be either zero or attractive ($\epsilon_{\text{A}} \geq 0$). We can translate from the interaction strengths (ϵ_{R} and ϵ_{A}) into the chemical language of an equilibrium constant since

$$K_{\text{eq}}(\epsilon_{\text{A}}, \epsilon_{\text{R}}) = \frac{[\text{proximal}]_{\text{eq}}}{[\text{distal}]_{\text{eq}}}, \quad (2)$$

which is an effective quantification of the power stroke. Though we directly tune both ϵ_{R} and ϵ_{A} in our numerical experiments, we can extract the resulting $K_{\text{eq}}(\epsilon_{\text{A}}, \epsilon_{\text{R}})$ and thereafter focus our attention on how the motor performance depends on it. $K_{\text{eq}} = 1$ corresponds to degenerate proximal and distal states—no power stroke as in the experimental catenane motor. [56] —while $K_{\text{eq}} > 1$ corresponds to a power stroke in the clockwise direction. By making the green and red particles strongly repulsive, we can also induce a power stroke in the counterclockwise direction with $K_{\text{eq}} < 1$. In synthetic terms, varying
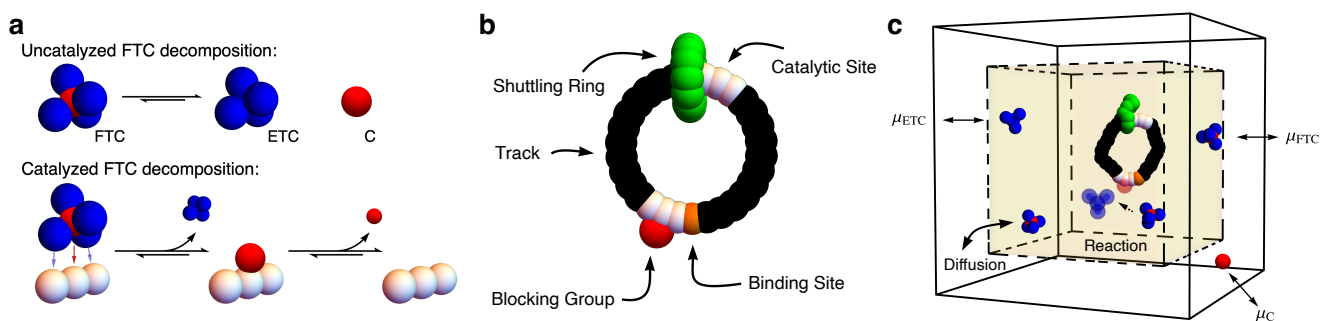
FIG. 3. **A catalysis-driven motor simulated explicitly with coarse-grained molecular dynamics. a**, Coarse-grained catalysis. A bulky red particle (C) can escape from a full tetrahedral cluster (FTC) to leave behind an empty tetrahedral cluster (ETC). The uncatalyzed decomposition (top) is thermodynamically favored, but it requires harmonic bond interactions connecting the blue particles (see SI Section 2.1) to fluctuate enough to accommodate the escape. The process is faster in the presence of a catalytic unit (bottom), a patch of three white particles whose interactions with blue and red particles can both stretch the FTC and withdraw the C. **b**, Structure of the coarse-grained motor. A ring of particles (green) can freely diffuse along a track (black) incorporating two binding sites (orange) when its path is not hindered by a blocking group (red). The latter can form as long-lived intermediates during FTC decomposition catalyzed by either catalytic unit. **c**, Simulation box. As detailed in SI Section 2.2.1, a periodic boundary condition simulation box is divided into a motor-containing region (yellow), where particles move according to Langevin dynamics, and an exterior region (white), where the Langevin dynamics are supplemented with grand canonical Monte Carlo chemostats that hold FTC, ETC, and C species at fixed chemical potentials μ_{FTC}, μ_{ETC}, and μ_{C}, respectively, such that $\mu_{\text{FTC}} - \mu_{\text{ETC}} - \mu_{\text{C}} > 0$. This setup allows the motor to be simulated under nonequilibrium conditions associated with a surplus of FTC (see SI for movies depicting representative simulations).
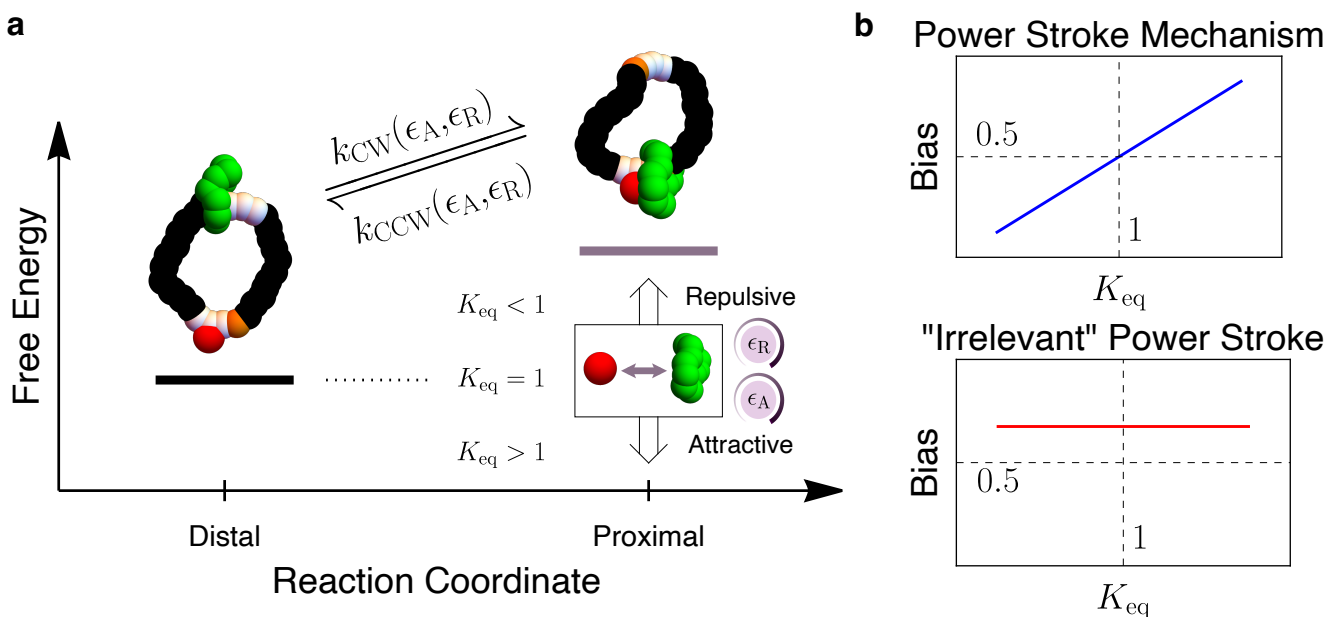

FIG. 4. **Modeling the power stroke. a**, Sketch of how the power stroke can be tuned in our simulation model. By varying the steric repulsion (ϵ_{R}) and the long-range attraction (ϵ_{A}) parameters between the green ring and the red barrier in Equation 1, the thermodynamic stability of the proximal conformation with respect to the distal one can be arbitrarily varied, thus tuning the magnitude of the power stroke, quantified by K_{eq}. The average rate for transitioning between the two conformations (k_{CW} and k_{CCW}) will vary accordingly and can be directly extracted from steady-state simulations. **b**, Hypothesized responses to varying proximal conformation free energy. According to the power stroke mechanism (top), the free energy difference between the distal and proximal conformations dictates directionality, and the motor's bias (as defined in Equation (3)) increases monotonically with K_{eq}, being 0.5 when $K_{\text{eq}} = 1$ as neither conformation is thermodynamically favored. Kinetic models [31, 32] (bottom) have clarified that the motor bias would not change if the free energy of the motor conformations were varied in isolation, i.e., without affecting transition state free energies. In this scenario (discussed as "Case 2" in Ref. [32]), varying the power stroke is irrelevant to the bias value. Consequently, deviations from this behavior are signatures that the free energy of motor conformations are not varied in isolation.

ϵ_R can be qualitatively thought of as varying the steric bulk of the blocking group, while ϵ_A allows that blocking group to associate with the green macrocycle to form a stabilized complex. Such strategies to experimentally introduce a power stroke in the motor have been previously proposed [71, 81] and are here explicitly built into simulated numerical experiments. Note that introducing power strokes in this way does not imply the presence of a mechanical strain in the molecular structure.

Motor bias as a function of K_{eq}

For each green-red interaction strength, we simulate motors under nonequilibrium conditions and count the number of clockwise (n_{CW}) and counterclockwise (n_{CCW}) cycles performed by the ring once the system has reached the steady state. The clockwise bias,

$$\text{bias} = \frac{n_{CW}}{n_{CW} + n_{CCW}} \qquad (3)$$

is a measure of the fraction of completed cycles in the clockwise direction, so a bias of 0.5 implies no directionality. If the power stroke were a generic determinant of the motor direction, the bias would be expected to be 0.5 when $K_{eq} = 1$. Furthermore, one would anticipate that the bias climbs above 0.5 when $K_{eq} > 1$ (favors proximal) and drops below 0.5 when $K_{eq} < 1$ (favors distal), as in Figure 4b (top). In other words, one would expect a positive differential response—turn up the power stroke and the bias will grow accordingly. We did not expect the power stroke arguments to be determinative of directionality. Prior kinetic models and experiments have already made this point clear, but those arguments do not anticipate the differential response to an altered power stroke. We highlight that this differential response is particularly important for engineering because it is desirable to anticipate if a modification will more strongly bias the motor. Crucially, the differential response depends on *how* one varies K_{eq}.

A first guess comes from considering a kinetic model in which *only* the free energy of the proximal state is affected by the introduction of the power stroke and the free energies of all other states and transition states remain unaffected (see SI Figure S3a for an illustration in terms of the free energy profiles in Figure 2a). In that scenario, the bias would not be affected, as it is assumed that barrier heights will remain fixed [31, 71]. In graphical terms, the bias would be flat as a function of K_{eq}, as in Figure 4b (bottom). In other words, the power stroke is irrelevant. However, it is not particularly physical to independently vary the free energy of a single state. A feature of our *simulation* model as compared to the *kinetic* chemical reaction network models is that we introduce energetic interactions between the moieties, we do not just change the energies of isolated states in a network model. By directly controlling interaction energies, we change the motor in the same sort of way one might

in a real experiment (e.g., making a moiety bulkier, more charged, etc.). One might adjust those interactions with the explicit goal of changing, say, the proximal state's free energy, but other energies could also shift as a side effect. For instance, when the attraction between the ring and the barrier is tuned up in our simulation model, it has the effect of increasing the power stroke as visualized in Figure 4a, but it also decreases the rate at which a red C particle detaches from the catalytic site in the proximal conformation, altering the kinetic asymmetry and thus the bias as a side effect. Our model naturally captures those side effects, effects which can allow power stroke engineering to alter the motor's bias.

Indeed, Figure 5a-b (top) shows that the bias responds non-trivially to changes in the power stroke. In agreement with the theoretical arguments [31], the data illustrate that the direction of the power stroke does not generically align with the bias. Figure 5a (top), for example, shows clockwise bias whether K_{eq} is greater than or less than 1. At the same time, the data show that altering the power stroke is not irrelevant in that the bias changes when K_{eq} is tuned. The particular way the bias varies with K_{eq} depends on how K_{eq} is adjusted, either through ϵ_R or ϵ_A. For three different fixed strengths of blocking group repulsion (i.e. bulkiness), we adjusted the attractions from weakly attractive to strongly attractive to drive from $K_{eq} \ll 1$ to $K_{eq} \gg 1$, revealing the bias to be essentially insensitive to the repulsion strength (see Figure 5a (top)). However, for a fixed attraction strength, there is a threshold at which the repulsion becomes so weak that the ring can pass over a barrier, thus causing the motor bias to collapse and even go negative (Figure 5b (top)).

Power strokes as an engineering tool

We have shown simulation results that explicitly illustrate a situation in which the power stroke does not align with the directional bias; the bias exceeds 0.5 even when K_{eq} is less than one. One may, nevertheless, ask if the differential version of power stroke intuition can hold, namely if making the power stroke more strongly downhill to the right actually makes the bias push more strongly to the right. Such a differential response would be useful for engineering because one could then use K_{eq}, the quantity that regulates how probability is divided between only two states, to anticipate the direction of the changing bias. Since a change in interaction energies between pairs of moieties simultaneously shifts energies of many states, it is a tremendous simplification if one can reason about the motor by focusing only on those two states involved in a power stroke. The nonmonotonic dependence of the bias on K_{eq} in Figure 5 reflects that simplification cannot hold broadly because there are regimes in which increasing K_{eq} further decreases the bias. Nevertheless, simplification appears to hold within the monotonically increasing regimes observed in the top plots of
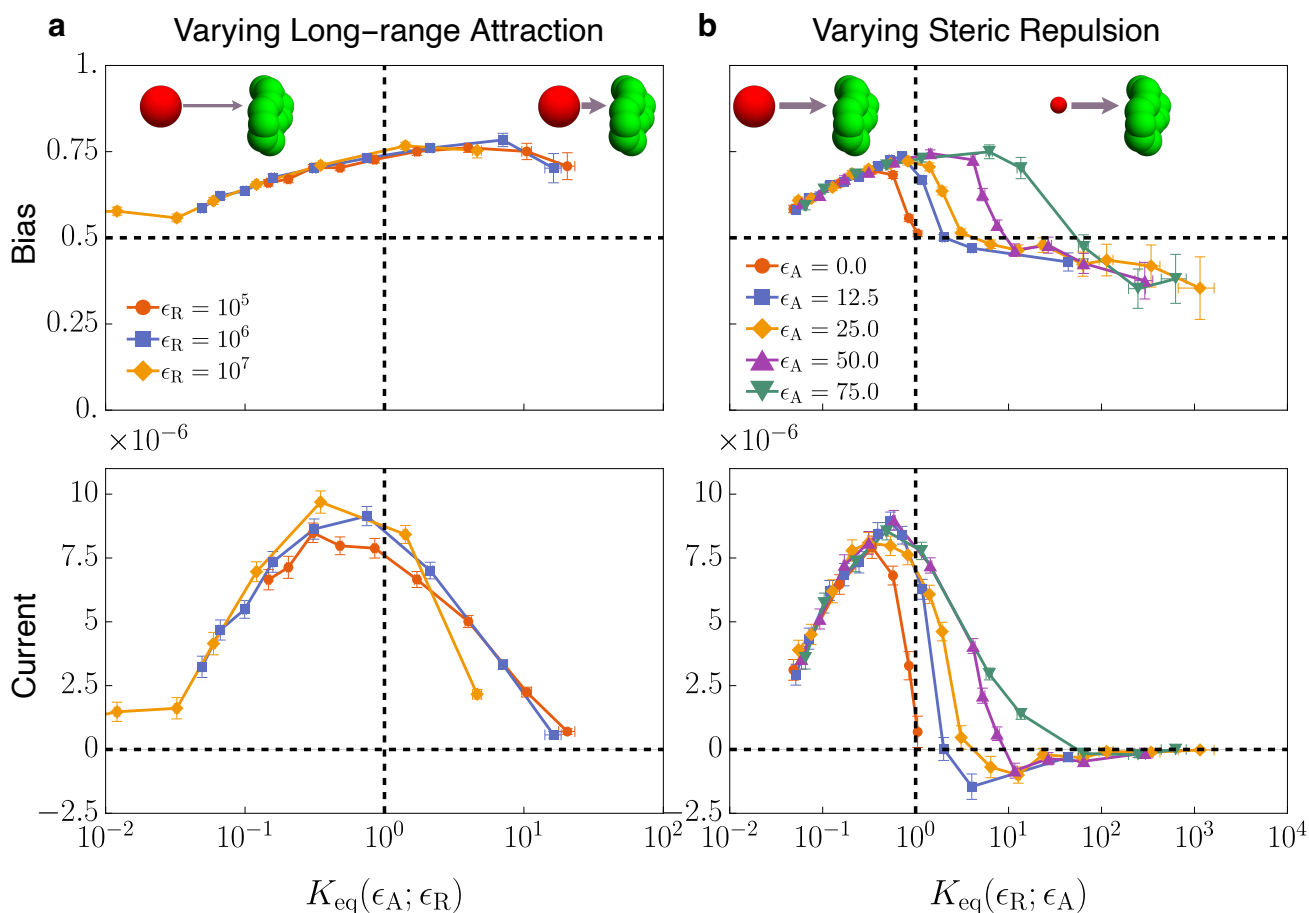
FIG. 5. **The effect of altering the power stroke.** Each data point was obtained by averaging over 100 independent simulations of motor designs with different values of the attraction (ϵ_A) and repulsion (ϵ_R) parameters defining the interaction between green ring and red barrier particles in Equation (1) (see SI Section 3.3 for values). All other parameters are set to their default values as in Ref. [55] (reference parameters correspond to "Motor II" in that study). The bias and current are computed from the simulation data according to Equations (3) and (4), respectively. As indicated in the plot legends, colored lines connect motor designs with the same ϵ_A or ϵ_R, with the other parameter being varied along the horizontal axis, thus tuning K_{eq}. SI Sections 3.2 and 3.3, respectively, provide a further explanation of the K_{eq} calculation and a detailed rationalization of the plots based on the physical ingredients of the model. **a,** Motor's directional bias (top) and current (bottom) obtained by varying ϵ_A for fixed values of ϵ_R. **b,** Motor's directional bias (top) and current (bottom) obtained by varying ϵ_R for fixed values of ϵ_A.

Figure 5a-b, and we observe that these regimes align with the parameter regimes that produce high-current motors.

To make this claim, we compute

$$\text{current} = \frac{n_{CW} - n_{CCW}}{t_{obs}}, \qquad (4)$$

where t_{obs} is the observed simulation time. Figure 5a-b (bottom) shows the current versus K_{eq}, demonstrating the alignment qualitatively; the values of K_{eq} that give high-current motors are precisely the same values that give a monotonically increasing bias-K_{eq} relation (see SI Section 3.4 for a quantitative data analysis). Within this fast-motor regime, turning up the power stroke indeed turns up the bias. The observed correlation between high-current and differential power stroke can be understood in two steps. First, why would the differential

power stroke be anticipated for "good" motors? Second, why would it break for the "poor" ones?

Kinetic models offer a quite generic suggestion for the emergence of the differential power stroke logic if one assumes that shifting the energy of a state will also shift the barrier of a nearby transition state. In particular, the Hammond postulate (or Leffler's assumption) suggests that the transition state will most resemble either the reactant or product, whichever is closest in free energy. [82, 83] Consequently, if a change to a pair potential is designed to change the energy of the proximal state, the Hammond postulate predicts whether the barrier to exit that proximal state will also shift. In the SI Section 1.3, we illustrate how Hammond's argument, combined with the kinetic model of Figure 2, predicts that bias will increase with increasing K_{eq}. Note, however, that

this logic is dependent upon a particular kinetic model, including the identification of the relevant metastable states, the allowable transitions between states, and the barriers of those transitions. Such a model is specifically built around the mechanism of the functional motor in Figure 2. If energy levels shift too dramatically, it is easy to imagine that that mechanism can break down such that the original kinetic model is missing important states and transitions. For example, the blocking groups could be made so small that they no longer block the passage of the shuttling ring, as it happens for some designs in Figure 5b. In that case, Figure 2's kinetic model is no longer suitable. It is reasonable that the parameters which break the fundamental ratcheting mechanism will not only disrupt the Hammond postulate argument but will also degrade the current, as our simulation shows.

These observations could provide an explanation for why power stroke engineering can sometimes work even though power strokes do not generically determine directionality. Given the starting point of a functional motor, found by evolution or designed cleverly, our model suggests that regimes might exist where the change in that motor's bias may well be anticipated by adjusting the power stroke. On a side note, the bottom plots in Figure 5a-b are consistent with experimental observations that currents in enzyme catalysis are optimized when all the states in a catalytic mechanism have more or less the same free energy ($K_{eq} \approx 1$) [84]. This is no surprise, as the motor is ultimately a catalyst for FTC decomposition, and reiterates that our minimal model well reproduces features observed in real chemical systems.

Metastability, coarse graining, and the quantification of power strokes

The power stroke idea centers around an identification of only two important conformations, one visited before and one after a motor's processive step. It was introduced to explain the nonequilibrium dynamics in terms of K_{eq}, which expresses how probability partitions between those two states in a dynamic equilibrium (see Equation (2)). The quantification of that K_{eq} involves some nuance because it presupposes that the two macrostates, corresponding, say, to distal and proximal conformations, can be cleanly defined. That definition involves averaging over many microscopic states, each of which is classified as one of the two macrostates. In general, it is challenging to precisely group many microstates into macrostates, but we are focused on situations with extreme timescale separation—a ring metastably stays on a binding site for long times before transiting to the other site relatively quickly. That timescale separation makes classification more clear cut. Experimentally, the distal conformation may contribute a particular NMR peak and the proximal another peak, such that K_{eq} is extracted as the ratio of peaks in equilibrium [56–58, 66]. In the simulations, we must introduce a grouping proce-

dure for sorting microstates into the distal and proximal macrostates. While different reasonable choices can subtly alter the quantification of K_{eq}, our results are practically insensitive to minor ambiguities defining the boundary of the macrostates. This insensitivity emerges for two reasons: Shifting all points in Figure 5 left or right by small amounts does not alter the essential shape of the curves, and besides, the small variation in the choice of coarse graining impacts K_{eq} by a small (order one) multiplicative factor while the power stroke engineering we discuss causes K_{eq} to vary by orders of magnitude.

While Equation 2 defines K_{eq} in terms of equilibrium populations of the distal and proximal macrostates, because equilibrium obeys detailed balance, K_{eq} can equivalently be cast as a ratio of kinetic rate constants $K_{eq} = k_{CW}/k_{CCW}$. Because our simulations involve chemostats which hold the system away from equilibrium, the latter formulation is particularly convenient, as those rate constants can be extracted directly from the nonequilibrium simulations. In practice, as detailed in Section 3.2 of the SI, we extract the rate constants by counting how many transitions per unit time are observed between the states shown in Figure 4a.

As we have discussed, a shortcoming of the power stroke framing is that it focuses only on two coarse-grained states. Our results confirm that the mechanism of the motor and its directionality simply cannot be deduced from only those two states. Rather, the motor's mechanism is better reflected by coarse graining the kinetics into a more complete Markov model that introduces more states to account for barrier addition and removal events. In SI Section 3.3 and 3.5, we discuss how a Markov model with only 16 states is sufficient to rationalize the nonmonotonic changes to motor performance plotted in Figure 5.

B. Conclusion

We have provided a resolution to a molecular motor conundrum. On the one hand, power strokes are observed in nature, have been used by many to explain directionality in molecular motors, and have provided practical intuition for engineering attempts. On the other hand, kinetic models clearly show that power strokes cannot determine directionality, and synthetic motors lacking power strokes have been successfully designed. Kinetic models also suggest that one should expect the motor's directionality to be unaltered by changing the thermodynamic stability of its conformations, overlooking power stroke engineering. Our simulation-based approach confirms that the power stroke mechanism cannot predict the directionality of a catalysis-driven motor, which is, in fact, determined by structural and kinetic asymmetry through a Brownian information ratchet mechanism. At the same time, our explicit model displays regimes where changes to the motor's bias correlate with changes to the power stroke magnitude. It is, therefore, possible that

similar correlations are present in biological motors that have been optimized throughout evolution. This might justify the apparent effectiveness of the power stroke intuition in its differential version, which is the basis of successful engineering experiments on biological motors.

The work suggests that introducing power strokes in chemically-driven synthetic molecular motors might affect their directional bias, thus providing a practical way to alter kinetic asymmetry in Brownian information ratchets. This suggestion is somewhat in contrast with the understanding provided by kinetic models [81], which accounts for a similar behavior only in those light-driven motors that implement a power stroke mechanism [50, 51] or in energy ratchets [65, 85]. We come to a different conclusion because our simulation model captures additional aspects of the physics which are hard to a priori build into kinetic models. We could extract a kinetic model from the simulations that captures those effects, but this requires knowledge of how design modifications impact all the kinetic rate constants in the model. By mimicking experiments more directly, our simulation model naturally captures these side effects and could be used to assess the effect of those design modifications. We conclude that experimental data aligning with power stroke intuition are possible and accounted for by the Brownian information ratchet mechanism and the concept of kinetic asymmetry. However, power stroke intuition is not generally valid in catalysis-driven systems and predicting when and how introducing power strokes might help engineering motors requires system-specific studies. Generalizing our molecular dynamics approach to explicitly simulate far from equilibrium chemical systems, even beyond molecular motors, can be of great help for the field, complementing kinetic models.

Supporting Information Available: The Supporting Information is available free of charge.

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