Irrelevance of the Power Stroke for the Directionality, Stopping Force, and Optimal Efficiency of Chemically Driven Molecular Machines

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ABSTRACT A simple model for a chemically driven molecular walker shows that the elastic energy stored by the molecule and released during the conformational change known as the power-stroke (i.e., the free-energy difference between the pre- and post-power-stroke states) is irrelevant for determining the directionality, stopping force, and efficiency of the motor. Further, the apportionment of the dependence on the externally applied force between the forward and reverse rate constants of the power-stroke (or indeed among all rate constants) is irrelevant for determining the directionality, stopping force, and efficiency of the motor. Arguments based on the principle of microscopic reversibility demonstrate that this result is general for all chemically driven molecular machines, and even more broadly that the relative energies of the states of the motor have no role in determining the directionality, stopping force, or optimal efficiency of the machine. Instead, the directionality, stopping force, and optimal efficiency are determined solely by the relative heights of the energy barriers between the states. Molecular recognition—the ability of a molecular machine to discriminate between substrate and product depending on the state of the machine—is far more important for determining the intrinsic directionality and thermodynamics of chemo-mechanical coupling than are the details of the internal mechanical conformational motions of the machine. In contrast to the conclusions for chemical driving, a power-stroke is very important for the directionality and efficiency of light-driven molecular machines and for molecular machines driven by external modulation of thermodynamic parameters.

INTRODUCTION

The concept of a power-stroke—a viscoelastic, free-energy releasing, large-amplitude conformational change (1) in which a molecular machine undergoes a transition from a high-energy, pre-power-stroke state to a lower-energy, post-power-stroke state while moving directionally relative to a polymeric track—has dominated the discussion of the mechanism of biomolecular motors for much of the last half century (2,3), and is often speculated to be an important element for designing synthetic molecular walkers (4,5). In the 1990s, an alternate Brownian motor mechanism (6–9) was proposed. The essence of the Brownian mechanism is that backward motion is hindered by barriers on the free-energy landscape and that forward motion occurs because of thermal noise combined with mass action, with no need for a special free-energy–releasing conformational change. Subsequently, many articles have discussed whether adenosine triphosphate (ATP)-driven molecular motors operate by a power-stroke mechanism, by a Brownian motor mechanism, or by a combination of the two (1,10).

As experimental techniques advanced it became clear that much, if not most, of the motion of many molecular motors is in fact due to conformational changes that can now be visualized directly (11). Thus, it would seem clear that the power-stroke must play a significant role in the conversion of chemical free-energy to mechanical work. However, neither experiment nor rigorous theory has been brought to bear on a straightforward question—is the difference in the energies of the pre- and post-power-stroke states important for determining the direction of motion and the steady-state thermodynamics of mechano-chemical energy transduction by biological (12) or synthetic (5) molecular walkers and other molecular machines? In this article, I describe a simple model of a molecular walker for which the kinetics and thermodynamics can be calculated (Fig. 1). Surprisingly, the exact solution of the kinetic equations shows that neither the difference in the energies of the pre- and post-power-stroke states, nor the position of the transition state relative to the pre- and post-power-stroke states, influences the direction of motion, the stopping force, or the optimal steady-state thermodynamics of the machine. The principle of microscopic reversibility is used to show that this result is totally general—the relative energies of the states of any chemically driven molecular machine have no role whatsoever in determining the directionality of the machine, the stopping force, or the optimal thermodynamic operation. Instead, the directionality, stopping force, and optimal thermodynamic behavior are specified entirely by the heights of the energy barriers for the transitions involving the interconversion between substrate and product.

In contrast to this result for internal chemical driving, the power-stroke does play an essential role in determining the direction of motion and thermodynamics of light-driven
motors (13) and motors driven by external modulation of thermodynamic parameters (14). The difference between chemistry- and light-driven motors can be understood in terms of microscopic reversibility (15,16). Mechanisms for converting chemical free-energy to mechanical motion must obey microscopic reversibility, whereas photochemically driven mechanisms are constrained instead by the Einstein relations between absorption and stimulated and spontaneous emission (17). For an elaboration of the differences between light-driven (18,19) and chemically driven mechanisms see the Supporting Material. The importance of microscopic reversibility for chemically driven molecular motors, see the Supporting Material. The constraints of microscopic reversibility, have led many workers in the field of biomolecular motors to the incorrect conclusion that a power-stroke is an important element for determining the direction and thermodynamic properties of chemically driven molecular motors.

RESULTS

The Scheme in Fig. 1 can be written as a simple triangle kinetic diagram, much like that used by Onsager (20) to illustrate the importance of microscopic reversibility for chemical processes (Figure 2).

Borrowing notation from Huxley (21), “f_{on}” is the rate of binding to, and “g_{off}” is the rate of detachment from, site i = L,R. These processes are not the microscopic reverses of one another, and each cycle of binding by f_{on} and detachment by g_{off} involves the catalysis of the process S → P (ATP hydrolysis, i.e., ATP → ADP + P_{i}, in the case of Huxley’s theory for muscle), driven by the free-energy change Δμ = μ_{S} − μ_{R}. In many models, including Huxley’s original treatment of muscle contraction (21), the driving reaction (e.g., ATP hydrolysis) is treated as an irreversible process—i.e., the rates f_{on} and f_{off} (the microscopic reverses of f_{on} and g_{off}) are often assumed to be negligibly slow and ignored in kinetic models of motor function. The energy profiles for a step via f_{on} and f_{off} and via g_{on} and g_{off}, are shown above and below the cartoon, respectively, where the state energies U_L, U_R, and U_E and transition state energies E_{f,}, E_{g,}, E_{f,}, and E_{g,} are indicated. The spacing between monomeric subunits A-B on the track is the step length l, d is the distance covered by the power-stroke (reasonably assumed to be a relatively large fraction of l), and ad is the distance covered in the transition from state L to the activation barrier E_{LR}.

FIGURE 1 Schematic diagram for a chemically driven molecular walker with two a priori identical heads, the mirror symmetry of which is broken by binding to a polar track with an A-terminus and a B-terminus. A detached head can reside either near the binding site on the left of the attached head (i.e., on the A-terminus side) in a configuration labeled L, or near the binding site on the right of the attached head (i.e., on the B-terminus side) in a configuration labeled R. The rate at which a head binds to the track is denoted f_{on} and the rate at which the head dissociates from the track is denoted g_{off}. The process g_{off} is not the microscopic reverse of f_{on} and each cycle of attaching to the track by f_{on} and detaching by g_{off} involves catalysis of a reaction S → P, where i = L,R. The processes f_{on} and g_{off} are often assumed to be negligibly slow and ignored in kinetic models of motor function. The energy profiles for a step via f_{on} and f_{off} and via g_{on} and g_{off}, are shown above and below the cartoon, respectively, where the state energies U_L, U_R, and U_E and transition state energies E_{f,}, E_{g,}, E_{f,}, and E_{g,} are indicated. The spacing between monomeric subunits A-B on the track is the step length l, d is the distance covered by the power-stroke (reasonably assumed to be a relatively large fraction of l), and ad is the distance covered in the transition from state L to the activation barrier E_{LR}.

Constraints of microscopic reversibility

Microscopic reversibility constrains the rate constants to obey the relations

\[
\frac{f_{on}^R}{f_{on}^L} = \frac{f_{on}^L}{f_{on}^R} = e^{\Delta\mu_T},
\]

\[
\frac{f_{on}^L k_{LR}^R}{f_{off}^L k_{LR}^R} = \frac{g_{off}^R k_{RL}^L}{g_{off}^R k_{RL}^L} = e^{\Delta\mu_T},
\]

(1)
where $F$ is the component of the force along the backbone of the track, and hence $FL$ is the mechanical work done in completing one step to the right along the track. Other relations can also be derived by considering the different stepping paths available to the motor. The motor steps to the right (toward the B-terminus) if dissociation of a head occurs most probably at the rear by process \( g_L^{\text{off}} \), and reassociation of the head occurs most probably at the front by process \( f_L^{\text{on}} \). These two possibilities can be illustrated schematically as shown in Fig. 3, where we have also included the back-reactions. The energy profiles for the two paths $\mathcal{F}$ and $\mathcal{B}$ are shown in Fig. 4, $a$ and $b$, respectively.

What features of the energy profiles allow the conversion $S \rightarrow P$ to more likely result in a step toward the B-terminus (to the right) by path $\mathcal{F}$ than to the A-terminus (to the left) by path $\mathcal{B}$?

Perhaps it is important to have the energy of state $L$ greater than that of state $R$ so that the large-amplitude conformational transition in path $\mathcal{F}$ is energetically downhill while the large amplitude conformational transition in path $\mathcal{B}$ is energetically uphill. Perhaps the position of the transition between states $L$ and $R$ nearer to $L$ is important so that once thermal activation from $L$ to the transition state (denoted $E_{LR}$) occurs, the molecule has a long, constant-force, slide downhill to state $R$ in path $\mathcal{F}$, where $d$, the distance covered in the power-stroke, is a large fraction on $l$, the total step length. Using microscopic reversibility we recognize that, while these factors are important for determining the magnitude and force dependence of a kinetic prefactor that is common to both the expression for the velocity of the motor and the rate at which substrate is converted to product, these factors are irrelevant for determining the direction, ratio of forward to backward steps, stopping force, or optimal efficiency of the motor. Instead, the thermodynamic properties of a molecular machine are governed solely by the external mechanical work, $FL$, done by the motor when it takes a step to the right; the chemical work, $D_m$, done when the motor converts a substrate to product; and on the differences $\Delta E_{LR} = E_{gL} - E_{gL} + E_{LR} - E_{LR}$ and $\Delta E_{RL} = (E_{fL} - E_{fL}) + (E_{fR} - E_{fR})$ of the transition state energies that determine the gating for the $f$ and $g$ processes at the right and left heads, respectively. The effects of the applied force on the differences $\Delta E_{LR}$ and $\Delta E_{RL}$ are parameterized by $Fa_R$ and $Fa_L$, respectively. The terms $Fa_R$ and $Fa_L$, unlike the thermodynamic dependence $FL$, may depend on all components of the applied force, and not just on the component along the axis of the track.
The ratio of the probability for any two of the four paths in Fig. 3 is given by the ratio of the products of the rate constants in the two paths:

\[
\frac{P_{FR}}{P_F} = \frac{g_{on}^R k_{LR} f_{on}^L}{g_{off} k_{RL} f_{off}^R} = \frac{g_{on}^L k_{RL} f_{on}^R}{g_{off} k_{LR} f_{off}^L} = e^{\frac{\Delta \mu}{k_B T}},
\]

\[
\frac{P_{BR}}{P_B} = \frac{g_{on}^R k_{RL} f_{on}^L}{g_{off} k_{LR} f_{off}^R} = \frac{g_{on}^L k_{RL} f_{on}^R}{g_{off} k_{LR} f_{off}^L} = e^{\frac{\Delta \mu}{k_B T}},
\]

\[
\frac{P_B}{P_F} = \frac{g_{on}^R k_{RL} f_{on}^L}{g_{off} k_{LR} f_{off}^R} = \frac{g_{on}^L f_{on}^R}{g_{off} f_{off}^L} = q e^{\frac{\Delta \mu}{k_B T}}.
\]

The second equality in each of the expressions in Eq. 2 is obtained using the expressions in Eq. 1 and show that none of these ratios depend on \( U_L, U_R, \alpha, D \) or any other parameter associated with the power-stroke, where

\[
U_L - U_R + Fd = k_B T \ln \frac{k_{LR}}{k_{RL}}.
\]

The two ratios \( P_{FR}/P_F \) and \( P_{BR}/P_B \) depend only on the chemical work, \( \Delta \mu \), and on the mechanical work, \( Fl \), and are independent of the structure of the molecular machine. The ratio \( P_B/P_F \) does depend on the structure of the machine through the factors

\[
\frac{f_{on}^L}{g_{on}^L} = \frac{[S] \ e^{\frac{\Delta \mu}{k_B T}}}{[P] \ e^{\frac{\Delta \mu}{k_B T}}},
\]

\[
\frac{f_{on}^R}{g_{on}^R} = \frac{[S] \ e^{\frac{\Delta \mu}{k_B T}}}{[P] \ e^{\frac{\Delta \mu}{k_B T}}},
\]

that describe the chemical gating at the left (trailing) and right (leading) heads. Here, we have parameterized the effect of the applied force on the gating by standard Boltzmann factors, \( e^{\Delta \mu/k_B T} \), \( i = R, L \). The terms \( \Delta \mathcal{E}_i(0) = \mathcal{E}_i - \mathcal{E}_i(0) \) and \( \Delta \mathcal{E}_i(I) = \mathcal{E}_i - \mathcal{E}_i \) are the zero load differences in activation energies for the \( g \) and \( f \) processes at the right and left heads, respectively, and we identify

\[
q = e^{\frac{\Delta \mu}{k_B T}}.
\]

as the no-load ratio of the number of motor molecules that complete a backward step while converting \( S \rightarrow P \) to the number that complete a forward step while converting \( S \rightarrow P \).

### Fully coupled motor

Irrespective of how many states are involved in the mechanism of a particular molecular machine, there is a one-to-one correspondence among forward \( (F) \), backward \( (B) \), forward-reverse \( (F_R) \), and backward-reverse \( (B_R) \) paths, although the barriers for some paths may be very large, and hence the path probabilities may be very small and experimentally undetected. As noted, the ratios between probabilities for paths that are the microscopic reverses of one another are thermodynamic identities. On the other hand, the ratio \( P_{FR}/P_B \) (or \( P_{FR}/P_{BR} \)) depends on the structure of the machine through the transition state energies. The selection between paths \( F \) and \( B \) is the sole determinant of the intrinsic (zero load) directionality of the machine.

There are also slip \( (S/S_R) \) paths in which the motor takes a step without conversion between substrate and product, and futile cycle \( (C/C_R) \) paths in which the motor catalyzes conversion between substrate and product without stepping. These slip and futile cycle paths can only reduce the intrinsic stepping ratio, efficiency, stopping force, and stoichiometry (number of steps per fuel molecule) of the motor without impacting the preferred direction of stepping. Thus, a fully coupled motor, in which every chemical conversion between \( S \) and \( P \) is accompanied by a step to the right or left, and vice versa, can be considered as the molecular machine equivalent of a Carnot engine, the function of which represents an ideal limit that is never attained in practice but nonetheless serves as a very important thermodynamic benchmark.

The velocity of a fully coupled motor can be written very simply as

\[
v_{fc} = \frac{t}{\tau} \left[ (P_F - P_{FR}) - (P_B - P_{BR}) \right],
\]

and the rate of conversion from \( S \) to \( P \) can be written as

\[
r_{fc} = \frac{t}{\tau} \left[ (P_F - P_{FR}) + (P_B - P_{BR}) \right],
\]

where \( \tau \) is a time constant that is common to both \( v_{fc} \) and \( r_{fc} \) (22) and where the subscript \( fc \) stands for fully coupled. The time constant \( \tau \), and hence kinetic quantities such as velocity, rate, and output power, in general depend on all state and transition state energies, \( U_L \) and \( \mathcal{E}_i \), on the concentrations of substrate and product, on the applied force, and on the position of the transition state relative to the pre- and post-power-stroke states, as well as on \( q_{LR} \) and \( q_{RL} \). See the Supporting Material for a derivation of \( \tau \) for the three-state model in Fig. 2. It is worth noting that if \( k_{LR} \) depends strongly on force, \( \tau^{-1} \) may asymptotically approach zero. In this case, it may be impossible to experimentally determine the stopping force, which may be mistakenly thought to be that force at which the velocity is within the experimental error of zero.

Thermodynamic quantities such as the step ratio,

\[
(P_F + P_{BR})/(P_B + P_{FR});
\]

stoichiometry, \( \nu_{fc}(lr_{fc}) \); efficiency, \( Fv_{fc}(\Delta \mu r_{fc}) \); and stopping force depend only on the chemical work, \( \Delta \mu \), the mechanical work \( Fl \), and on the difference in transition state energies for the chemical transitions at the front \( (\Delta \mathcal{E}_0) \) and rear \( (\Delta \mathcal{E}_r) \) head. Using Eqs. 1 and 2, the stepping ratio for a fully coupled motor works out to be
Irrelevance of the Power-Stroke

The approximation holds for \( e^{-\Delta \mu / k_B T} \ll q e^{-F(a_R-a_L)/k_B T} \) and \( q e^{-F(a_R-a_L)/k_B T} \ll 1 \). Using this approximation, the stopping force (the force at which the stepping ratio is unity) is

\[
F_{\text{stop,fc}} = \frac{k_B T}{[1 - (a_R - a_L)]} \ln q^{-1}.
\]

Note that in the limit \( q \to 0 \) in Eq. 5, the stepping ratio is simply \( e^{F_L - \Delta \mu / k_B T} \) and the stopping force is \( F_{\text{stop}} = \Delta \mu / l \), as expected for the tightly coupled limit where each conversion of substrate to product (S → P) causes a forward step. These two limits illustrate the difference between kinetic control, where the stepping ratio is proportional to \( q^{-1} \), and thermodynamic control, where the stepping ratio is proportional to \( e^{-\Delta \mu / k_B T} \).

Málnási-Csizmadia and Kovács (23) have argued that the myosin stepping cycle is under kinetic control, where the thermodynamically favorable futile lever-swing (power-stroke) of the myosin head—while not attached to actin, which would lead to futile cycling where ATP is hydrolyzed without stepping—is kinetically blocked.

We see from Eq. 5 that, given a sufficiently large applied force, even an intrinsically forward-directed molecular machine can move backward by a process in which a fuel molecule is consumed ([B] (24), as predicted by Astumian and Bier (25). This prediction was experimentally supported by Nishiyama et al. (26), and by Carter and Cross (27), who showed that back-stepping of kinesin is stimulated by increasing concentration of ATP. These authors both obtained data for the ratio of forward to backward stepping that was very well fit by the simple expression

\[
\frac{\langle N_{\text{right}} \rangle}{\langle N_{\text{left}} \rangle} = q^{-1} e^{-\frac{F_L - (a_R - a_L)}{k_B T}},
\]

with fit parameters of \( q^{-1} = 802 \) and \( (l - (a_R - a_L)) = 3.7 \) nm 27 or \( q^{-1} = 221 \) and \( (l - (a_R - a_L)) = 2.9 \) nm (26). Just as in this model, Nishiyama et al. (26) described the parameter \( q^{-1} \) in terms of the exponential of the difference in activation energies. Their interpretation, however, is different than in this article. In the model of Nishiyama et al. (26), the energy barriers governing \( q^{-1} \) are for transitions in which the motor moves along the track. Hence the relevant component of the force determining the external force-dependent work term in their model involves only the component of the force directed along the axis of the track.

In the model described here, the heights of the barriers for the chemical reactions (the \( f \) and \( g \) values), not for the transition \( L \) to \( R \) along the track, govern the stepping ratio and hence the work term involves all components of the force, not just the component along the track. And thus, the two models can be distinguished based on how the ratio of forward to backward stepping is influenced by off-axis components of an applied force.

In recent experiments, Clancy et al. (28) also observed ATP stimulated back-stepping of a truncated construct of kinesin (Kin6AA). In their experiments, the stepping ratio and stopping force were observed to depend on the ATP concentration, which is not consistent with the predictions of a fully coupled motor model. To explain the concentration dependence of the stopping force, it is necessary to include slip into the description of the motor stepping, which we can do by calculating the exact expression for the stepping ratio for Fig. 2.

**Slip and futile cycling**

In Fig. 2, each cycle through the states in the clockwise direction (E → L → R → E) describes a step to the right, and each cycle through the states in the counterclockwise direction (E → R → L → E) describes a step to the left, irrespective of whether there is interconversion between S and P. The ratio of the probability for a step to the right versus a step to the left, and hence the ratio of the average number of steps to the right (\( \langle N_{\text{right}} \rangle \)) to the average number of steps to the left (\( \langle N_{\text{left}} \rangle \)), is (29)

\[
\frac{\langle N_{\text{right}} \rangle}{\langle N_{\text{left}} \rangle} = \frac{k_L R \left[ f_{\text{off}}^R + g_{\text{off}}^R \right] \left( f_{\text{on}}^L + g_{\text{on}}^L \right)}{k_R L \left[ f_{\text{off}}^L + g_{\text{off}}^L \right] \left( f_{\text{on}}^R + g_{\text{on}}^R \right)}
\]

\[
= \frac{\left( 1 + q e^{-\frac{(a_R-a_L)}{k_B T}} \right) \left( f_{\text{on}}^L + g_{\text{on}}^L \right)}{q e^{-\frac{(a_R-a_L)}{k_B T}} + \left( g_{\text{on}}^L + g_{\text{on}}^R \right)}
\]

The second equality in Eq. 6 is obtained using microscopic reversibility (Eq. 1). It is self-evident that the ratio in brackets ([1]) on the right-hand side of Eq. 6 is unity if \( \Delta \mu = 0 \), but if \( q = 1 \) the ratio is unity irrespective of the value of \( \Delta \mu \). Further, we see that \( \langle N_{\text{right}} \rangle / \langle N_{\text{left}} \rangle \) depends only on the \( f \) and \( g \) rates and of course on \( \Delta \mu \) and \( F_L \) and not on \( U_L \), \( U_R \), \( \mathcal{E}_{LR} \), \( \alpha \), or \( d \). Thus we conclude that the ratio of forward to backward steps (and hence the directionality) is independent of the power-stroke. This conclusion is reiterated by the exact solution of the kinetic equations (see the Supporting Material) to obtain expressions for the velocity and rate of chemical catalysis by the motor. Note that if some of the \( f \) and \( g \) rates are simply assigned to be zero in Eq. 6, one can easily arrive at the opposite (and wrong) conclusion that
the stepping ratio is proportional to $k_{L,0}/k_{R,0}$. It is never ther-
modynamically correct to simply set a rate to be zero, and do-
ing so can lead to qualitatively incorrect conclusions, even if
the quantitative magnitude of the error introduced into nu-
merical calculations of kinetic behavior under particular
experimental conditions is vanishingly small.

Taking the large $\Delta \mu$ limit and dropping terms multiplied
by $e^{-\Delta \mu/k_BT}$ in Eq. 6, and then using Eq. 3, we obtain

$$\frac{\langle N_{\text{right}} \rangle}{\langle N_{\text{left}} \rangle} \approx \left( \frac{1 + \frac{A}{\langle S \rangle e^{|a_S|}}}{q \left(-\frac{a_S}{a_T} \right)^{|a_S|} + \frac{A}{\langle S \rangle e^{|a_S|}}} \right) e^{\frac{q_{10}}{k_BT}}, \quad (7)$$

where $A = [P]e^{A_E(0)/k_BT}$ is treated as a single fit parameter
because the concentration [ADP] is described as negligible and
not specified by Clancy et al. (28). The stepping ratio and stopping force are seen to be concentration-depen-
dent, and a reasonable fit to the data of Clancy et al. (28) (not
shown) is obtained with parameters $q = 0.005$, $A = 0.1$
$\mu$M$^{-1}$, and characteristic distances $a_R = 1.25$ nm, and
$a_L = 2.75$ nm. The values $q^{-1} = 200$ and $l - (a_R - a_L) =
4$ nm are quite consistent with the values measured for wild-type motor, suggesting that the major effect of the trun-
cation studied by Clancy et al. (28) is on the slip. The in-
creased importance of slip for the Kin6AA construct is con-
sistent with the experimental observation (28) of a large
mean-square displacement near-stall as compared to the wild-type motor.

The position-dependent gating by which $q \neq 1$ is the hall-
mark of a molecular information ratchet (30). When $q < 1$, the
predominant mechanism involves process $g$ between
states E and L and process $f$ between states E and R (i.e.,
path $P_F/P_{FR}$ is preferred over path $P_B/P_{BR}$) both at and
far from thermodynamic equilibrium. Free energy is
required to drive directed motion along the preferred path.
Only if $\Delta \mu > 0$, is the forward direction $\mathcal{F}$ preferred over
the reverse direction $\mathcal{R}$ so that the molecular walker
moves toward the B-terminus (i.e., to the right). The terms
$Fq_R$ and $Fq_L$ reflect the kinetic phenomenon known as strain
gating (31,32), by which increasing force enhances the ki-
etic selection between the forward and backward paths to
allow the motor to continue to function without being over-
whelmed by the applied load.

**Kinetic lattice description of a molecular machine**

We can appreciate how an approach based on pairs of trajec-
tories that are microscopic reverses of one another can be
used to obtain general expressions for the rate and velocity
of a molecular machine in terms of a two-dimensional ki-
etic lattice model for the motor (25). The two-dimensional
lattice describing the model in Figs. 1 and 2 is shown in
Fig. 5 (a). Starting at the center state, the system will

![Figure 5](image-url) (a) Kinetic lattice representation for the triangle reaction in Fig. 2 illustrating the possible paths for a molecular machine. (b) Energy landscape constructed from the model in Fig. 1 (for the area in the dashed rectangle in panel a), by assigning $U_E = -10 k_BT$, $E_{LR} = 10 k_BT$, $U_L = -8.5 k_BT$, $U_R = -11.5$
$k_BT$, $E_{SR} = E_{EL} = 8.5 k_BT$, and $E_{d} = E_{f} = 11.5 k_BT$ with a maximum energy of $+15 k_BT$ assigned to the point directly between states labeled $E_0$ and $E_0$, between $E_1$ and $E_1$, and between states $E_0$ and $E_1$ on the kinetic lattice, and then interpolating between these assigned values by spline curves using the software MATHEMATICA (Wolfram, www.wolfram.com/mathematica). Panels c–f show the landscape (two periods in each direction) with different values of
the state energy difference ($U_L - U_R = \pm 3 k_BT$) and transition state energies $\Delta E_R$, $\Delta E_L = \pm 3 k_BT$. The main point to note is that switching the
state energies $U_R \leftrightarrow U_L$ does not change the preferred path, but exchange of the transition state energies $\Delta E_R \leftrightarrow \Delta E_L$ does change the most probable path, with the path $\mathcal{F}_R/\mathcal{R}_R$ the preferred path in panels c and e, and $\mathcal{F}_B/\mathcal{F}_B$ the preferred path in panels d and f. Note that the transition state energies in general depend on an applied force giving rise to the kinetic phenomenon known as “strain gating” (31,32) as in Eq. 3. To see this figure in color, go online.
eventually reach one of the eight states on the edges by a biased random walk, having effected some change in the environment by stepping left and/or right and/or by having catalyzed interconversion between substrate (S) and product (P). The paths $F/F_R$ and $B/B_R$ are the coupled processes, and the paths $C/C_R$ and $S/S_R$ describe the uncoupled futile cycling in which the machine wastes energy without stepping ($P_R^C/C_R = e^{\Delta\mu/k_BT}$), or slip, in which the machine steps energetically downhill without being coupled to the chemical input ($P_S/S_R = e^{-F/k_BT}$). The average velocity is $v = l\tau^{-1}[(P_F - P_{F_R}) + (P_{B_R} - P_B) + (P_S - P_{S_R})]$. When the force is zero $P_S = P_{S_R}$, the essential question for determining the intrinsic direction of motion of the motor is whether $P_B/P_F < 1$, in which case the motor moves to the right when $F = 0$ and $\Delta\mu > 0$, or whether $P_B/P_F > 1$, in which case the motor moves to the left when $F = 0$ and $\Delta\mu > 0$. For Fig. 2, the ratio, $P_B/P_F$, is independent of the relative energies of the states and is governed solely by the transition state energies and by the applied force. Because of the periodicity on both mechanical and chemical coordinates, it is clear that this independence on the relative energies of the states must hold irrespective of how many additional states are added to the model.

Fisher and Kolomeisky (33) proposed a simplifying approximation to the two-dimensional kinetic lattice model in which the kinetic path that dominates in the absence of load is projected onto a linear one-dimensional mechanism

$$E_j \xrightarrow{w_1} E_{j+1} \quad \xrightarrow{w_2} \quad \ldots \quad \xrightarrow{w_N} E_{j+1}$$

with possibly many substeps. This simplification, which amounts to a tight coupling approximation where the backward (B) process is ignored, has the advantage that working out the velocity in terms of the rate coefficients is very easy. For a review of Fisher and Kolomeisky’s work on the kinetics of tightly coupled discrete stochastic models, see Kolomeisky and Fisher (34). The predictions of Fisher and Kolomeisky’s approximation are essentially identical to those of the earlier and more general kinetic lattice model of Astumian and Bier (25) at small load. However, at large load, the Astumian-Bier model (25) predicts that ATP can stimulate backward stepping through the B pathway in which ATP is hydrolyzed while the motor takes a step to the left. In contrast, the approximation by Fisher and Kolomeisky (33) predicts that any backward steps must be accompanied by ATP synthesis, and hence that ATP must inhibit backward (left) stepping. Experiments showing ATP stimulated back-stepping under super stall force (26–28) provide experimental evidence in support of the Astumian-Bier model (25) and falsify the approximation of Fisher and Kolomeisky (33) for large load.

Attention has been focused on the possibility of breaking the transitions of the motor into substeps with different force dependencies in order to fit experimental data. While this may well be important for fitting kinetic properties of the motor, and especially for understanding cooperative effects that arise when several motors work in tandem (35), the relationships dictated by microscopic reversibility (the expressions in Eq. 1 for the uncoupled processes and the expressions in Eq. 2 for the coupled processes) are independent of the number and force dependencies of the substeps. Thus the thermodynamic properties such as stall force and directionality, as well as the optimal efficiency and stoichiometry calculated for a fully coupled motor, do not depend on the details of the mechanism but on the difference in the activation energies ($\Delta\mathcal{E}_R$ and $\Delta\mathcal{E}_I$) for the chemical processes at the two heads.

The generality of this independence of the optimal steady-state thermodynamics of a molecular machine on the relative energies of the states of the machine can be appreciated by mapping the kinetic lattice shown in Fig. 5 onto a two-dimensional potential energy surface (25,36–38). Because the rate constants for the chemical conversion $k_{on}, k_{off}, f_{on},$ and $g_{off}$ obey microscopic reversibility, the combined mechanical and chemical processes can be described in terms of motion on a single time-independent potential energy landscape. Comparing the energy landscapes for four different cases, we see that the visually evident path of least thermodynamic action (39), indicated by the white line running from either the upper-left and lower-right corner ($F/F_R$) or from the upper-right and lower-left corner ($B/B_R$), is determined by the relative energies of the saddle points ($E_i$) and not by the relative energies of the states ($U_{EF}, U_{IL},$ and $U_{ER}$). For a more complete discussion of how the most probable paths are calculated, see the Supporting Material.

The specific features that govern the directionality for any chemically driven molecular machine are the relative energies of the kinetic barriers (saddle points on a multidimensional potential energy surface) as predicted by a Brownian motor model, and not the relative energies of the states themselves (minima on a multidimensional potential energy surface) as predicted by a power-stroke model. The two-dimensional energy surface for the $F_0F_1$ ATPase has recently been presented by Mukherjee and Warshel (40) where it is immediately seen that exchanging the energies of the wells makes no difference to the direction of rotation driven by ATP hydrolysis whereas exchanging the energies of the saddle points switches the preferred direction of rotation driven by ATP hydrolysis. This conclusion arises from the fact that the energy increases in every direction away from a minimum while at the saddle points there is one direction along which the energy decreases, and perpendicular (orthogonal) to that direction in which the energy increases. Thus there is an inherent selection of a preferred path at each saddle point. The overall preferred pathway for motion is determined by the relative energies of the different saddle points. The direction of motion on this overall preferred
path when the external force is zero is determined solely by mass action—i.e., by whether $\Delta \mu$ for the chemical reaction is greater than or less than zero.

The perspective based on motion on a potential energy landscape corrects two common misperceptions in the literature that arise from an overly mechanical and local perspective of the action of molecular machines.

First, Howard (1) asserted that "the power stroke can do work against an external loading force $F$ provided that $F l \leq \Delta U$, where $\Delta U$ is the decrease in free energy between the two chemical states." In fact, work can be done on the environment in the overall cycle so long as $0 < F l < \Delta \mu$, irrespective of whether the specific conformation change denoted the "power-stroke" is energetically downhill, uphill, or neutral, as seen in Fig. 5. Interestingly, the step labeled "power-stroke" in the forward functional path given by Mukherjee and Warshel (41) for myosin V is energetically uphill, while that in the backward nonfunctional path is energetically downhill. Also, the recent experimental work of Muretta et al. (42) showed that the equilibrium conformation of the eight rate constants for each path. Using the kinetic diagram arising from combining the mechanical and chemical states is shown in Fig. 6 (12,41).

The kinetic diagram for ATP hydrolysis under physiological conditions) as claimed by Fisher and Kolomeisky (33). Fisher and Kolomeisky argue that "in understanding the operation of a molecular motor, one should be concerned with the microscopically local release of free energy by ATP adsorbed on the motor-protein-track complex." Their claim articulates the common misunderstanding that there is a local and direct conversion of chemical free energy from the $\gamma$-phosphate of ATP to mechanical energy of the molecular machine. The local perspective of the action of ATP gives rise to such descriptions as "one microscopic picture to keep in mind is that of a nanoscale machine experiencing sudden violent 80 pN nm ($\sim 20 k_B T$) kicks originating from ATP hydrolysis" (43), and to depictions of the ATP hydrolysis event as a flash of light in cartoon movies of molecular machines. Such descriptions are totally wrong. Instead, the high chemical potential of ATP relative to ADP and Pi simply makes it much more likely that ATP will bind than that ADP and Pi will bind when the active site of the machine is unoccupied. The relatively high likelihood to bind ATP makes the path $P_F / P_{B_F}$ directional in favor of $P_F$ and the path $P_B / P_{B_B}$ directional in favor of $P_B$ by mass action. The directionality along each of these two paths is quantified by $e^{\Delta \mu/k_B T}$. If $q \neq 1$, there is then net directional motion as a result of breaking the symmetry between $P_F$ and $P_B$, a symmetry breaking that is independent of whether the system is at or away from thermodynamic equilibrium.

Analysis of a more realistic model for a molecular motor

A similar analysis to that given above for the three-state model in Fig. 1 can be applied to much more complicated, and arguably more realistic mechanisms. Consider the mechanical and chemical cycles of a model for a single head of myosin shown in Fig. 6 (12,41).

The kinetic diagram for ATP hydrolysis (14) where mechanical state 2 is specific for release of products $P_i$ and ADP and for binding ATP (i.e., the activation energies for binding/release of ADP, $P_i$ and ATP is small in state 2) and mechanical state 0 is specific for hydrolysis of ATP to ADP (the activation energy for hydrolysis of ATP at the active site is small in state 0). Fig. 6 d illustrates the consequence of switching these specificities—the intrinsic directionality reverses. The ratio of the probabilities for the paths shown in green in Fig. 6 c ($F$) and in Fig. 6 d ($B$) is given by the ratio of the products of the eight rate constants for each path. Using the thermodynamic identities $K_{12}^{T} K_{30}^{T} = K_{12}^{R} K_{30}^{R}$ and $K_{i0}^{DP} K_{j2}^{DP} K_{10}^{T} = K_{i0}^{DP} K_{j2}^{DP} K_{10}^{T}$ with $K_{ij}^{DP} = k_{j \rightarrow i}^{DP}/k_{i \rightarrow j}^{DP}$, we can write this ratio in the form of Eqs. 2, where

$$q = \frac{e^{\Delta U_{02}^{DP} + \Delta U_{02}^{DP} - \Delta U_{02}^{DP} - \Delta U_{02}^{DP}}}{e^{\Delta U_{02}^{DP} + \Delta U_{02}^{DP} - \Delta U_{02}^{DP} - \Delta U_{02}^{DP}}}$$

and $\Delta U_{02}^{DP} - \Delta U_{02}^{DP}$ are the no-load difference in activation energies for the chemical transition $i \rightarrow j$ in mechanical states 0 and 2. Because every forward path is associated with one backward path where the specificities are switched, the intrinsic directionality of the motor can be expressed solely in terms of the activation barriers for the chemical processes ($\varnothing \rightarrow T, T \rightarrow DP, DP \rightarrow D, D \rightarrow \varnothing$) and is independent of the equilibrium and rate constants.
for the mechanical transitions. This result suggests, e.g., that the structural differences evident in the crystal structures of myosin V (a + directed motor) and myosin VI (a − directed motor) (44) must control the chemical specificities rather than the equilibrium constant for the conformational change in which the myosin light chain rotates.

Let us now examine a model based on Fig. 6 that is similar to the original model of Huxley where the chemistry is described in terms of overall rate constants $f_{on}$ and $f_{off}$ for processes in which the connections between the motor and track are made and undone, respectively, and where the potential energy profile depends on whether the motor is strongly bound ($U_3$) or weakly bound ($U_w$). Huxley (21), and subsequently Hill et al. (45), used such models to describe the kinetic behavior of muscle—specifically the force-velocity curve—and obtained very good agreement between theory and experiment.

The picture in Fig. 7a gives rise to a plausible and easy to understand story about how the rightward slopes of the potentials allow a motor, starting in state $M_0$, to use chemical energy to drive directed motion to the right. (b) An intuitively implausible, but nonetheless thermodynamically possible sequence in which chemical energy is harnessed to drive directed motion to the left. There is a relationship between the relative probabilities for sequence a and for sequence b dictated by microscopic reversibility. With $\Delta \mu > 0$ and $q < 1$, the motor functions predominantly according to sequence a along the lines suggested by macroscopic intuition. When $q > 1$, however, the motor functions predominately according to sequence b, contrary to what might be intuitively expected.

Based on experience with marble ramps or gum-ball machines. The blockades placed at the tops of the barriers emphasize that we are considering the limit in which slip—i.e., stepping without hydrolysis—is taken to be negligible. In panel a, the mechanical processes are energetically downhill, and do not require thermal energy (although the chemical processes are thermally activated), whereas in panel b, the mechanical processes are energetically uphill and require thermal energy to occur.
energy to move one step forward (to the right). In state M₀ on the weakly bound potential, \(U_W\), hydrolysis or release of bound ATP and concomitant attachment of the motor to the track occurs with total rate \(k_0^0 + \gamma_{on}^0\), placing the motor in a “force-generating” state. The motor then spontaneously slides downhill through state M₁ on the potential for the strongly bound state, \(U_S\), coming to rest in state M₂. The motor remains until products ADP and Pᵢ dissociate and ATP binds or until ADP and Pᵢ are converted to ATP at the active site with total rate \(f_{off}^2 + s_{off}^2\) causing transition to the weakly bound state with potential \(U_W\). The motor then spontaneously slides down the potential (reprimers) through state M₃ back to state M₀, completing a step to the right and, in the thermodynamically most likely scenario, having hydrolyzed one ATP. This straightforward scenario depicted in Fig. 7a is similar to a marble ramp or gum-ball machine in which all of the mechanical motion occurs by deterministic processes.

Based on a figure similar to Fig. 7a, with the potential for the weakly bound state taken to be flat (slope of zero), Hill et al. (45) asserted that the maximal efficiency of the motor is \(\Delta U/\Delta \mu_{\text{ATP}}\) where \(\Delta U_S\) is the difference between the maximum and minimum on the strongly bound potential \(U_S\) and hence the maximum possible energy released by the power-stroke. Unfortunately, the illustration in Fig. 7a conveys the illusion, but not the reality, of understanding, and the assertion of Hill et al. (45) is wrong for a chemically driven motor.

To develop a complete picture it is necessary to consider the seemingly far less plausible mechanism shown in Fig. 7b in which a motor, starting in state M₀, moves uphill by thermal energy through state M₃ to state M₂ while weakly attached. Hydrolysis of ATP or release of bound ATP occurs with total rate \(f_{off}^2 + s_{off}^2\), switching the motor to the strongly bound stable state M₂ on potential \(U_S\). From the bottom of this well, the particle once again moves uphill because of thermal energy to move to unstable state M₀ (passing state M₁) on potential \(U_S\), at which point either the products ADP and Pᵢ dissociate and ATP binds, or ADP and Pᵢ are converted to ATP at the active site, with total rate \(f_{off}^0 + s_{off}^0\), switching the particle back to potential \(U_W\) to complete one step to the left. The ratio of the probabilities for the two processes shown in Fig. 7a and b, is

\[
\frac{\langle N_{\text{right}} \rangle}{\langle N_{\text{left}} \rangle} = \frac{(f_{off}^0 + s_{off}^0)(f_{off}^2 + s_{off}^2)P_W(M_0 \rightarrow M_2)P_S(M_2 \rightarrow M_0)}{(f_{off}^2 + s_{off}^2)(f_{off}^0 + s_{off}^0)P_W(M_2 \rightarrow M_0)P_S(M_0 \rightarrow M_2)}
\]

\[
= \frac{(f_{off}^0 + s_{off}^0)(f_{off}^2 + s_{off}^2)\Delta U_S - \Delta U_W - \delta f}{(f_{off}^2 + s_{off}^2)(f_{off}^0 + s_{off}^0)} e^{\frac{U_W - U_S}{k_B T}}
\]

\[
(9)
\]

where \(\Delta U_i = U_i(0) - U_i(2)\) for \(i = S,W\). The second equality follows from the relation between the conditional probabilities

\[
P(r' \rightarrow r'', \Delta t) = e^{\frac{i(r' -q(r'' - r))}{\eta t}}
\]

derived using microscopic reversibility and that holds for any time interval \(\Delta t\) and for any points \(r'\) and \(r''\) on an energy surface (15). The rate constants must obey microscopic reversibility (Eq. 1) and Eq. 9 reduces identically to Eq. 6 with 0 substituted for \(R\) and 2 substituted for \(L\) in Eq. 6 and in the expression for \(q\) in Eq. 4. See the Supporting Material for details of the evaluation of the ratio. Contrary to macroscopic experience with marble ramps, gum-ball machines, turbines, or even with light-driven molecular machines, the intrinsic direction of motion and optimal efficiency of a molecular machine driven by catalysis of a free-energy releasing chemical reaction is determined by the chemical specificities (the activation energies for the chemical processes of binding substrate, catalytic conversion to product, and release of product), and is independent of the energy released during the recovery- and power-strokes.

The independence of the net direction of the motor on the symmetry of the potentials can be verified directly, by checking the symmetry properties of the solution to the reaction-diffusion equations describing combined motion on several potentials with transitions between the potentials coupled to the catalysis of a chemical reaction (25). In the absence of load (\(F = 0\)), the velocity switches sign under the transformation \(\Delta \mu_{\text{ATP}} \rightarrow -\Delta \mu_{\text{ATP}}\). This fact should immediately raise a red flag concerning the macroscopically intuitive, but naive, interpretation of Fig. 7a as suggesting that the slope of the potential determines the direction of motion when driven by a catalyzed reaction. We then find that the sign of the velocity is invariant to the transformations \(U_W(x) \rightarrow U_W(-x), U_S(x) \rightarrow U_S(-x)\), or both. Clearly, the signs of the slopes of the potentials and the energy differences \(\Delta U_W\) and \(\Delta U_S\) do not govern the direction of motion. In contrast, the velocity does change sign under the transformation \(q \rightarrow 1/q\), and is identically zero if \(q = 1\) (25), irrespective of the value of \(\Delta \mu_{\text{ATP}}\) or of the shapes
and amplitudes of the potentials \( U_p(x) \) and \( U_q(x) \). Thus we conclude that the intrinsic direction of motion for a chemically driven motor is governed by the specificities through \( q \), and not by the slopes of the potentials or by the energy released during the power- and recovery-strokes.

It is perhaps tempting to designate those mechanisms in which the mechanical motion is predominantly energetically downhill (when \( q < 1 \)) as power-stroke motors, and those in which the motion is predominantly uphill (when \( q > 1 \)) as Brownian motors (46,47), but this distinction is misleading. In both cases the machine functions as an information ratchet (30,48) where allosteric feedback allows the motor to react rapidly with substrate (ATP) and slowly with product (ADP and P) at some positions along the mechanical coordinate, and to react rapidly with product and slowly with substrate at other positions. The importance of gated specificities has been discussed for ion pumps by Jencks (49), and chemically based information ratchets have been designed and synthesized by Alvarez-Perez et al. (50) and Carlone et al. (51). A very significant insight offered in this article is that molecular recognition (52)—the ability of a molecular machine to discriminate between substrate and product depending on the state of the machine—is far more important for determining the intrinsic directionality and thermodynamics of chemo-mechanical coupling than are the details of the internal mechanical conformational motions of the machine.

**DISCUSSION**

Microscopic reversibility guarantees that the dynamics of a molecular machine in which directed mechanical movement is coupled to a catalyzed chemical reaction such as ATP hydrolysis can be described in terms of motion on a single potential energy landscape—a scalar field. This gives rise to an unreasonable effectiveness of equilibrium theory for describing these nonequilibrium systems (53). For overdamped systems, including all molecular machines in solution, the velocity on the energy surface is proportional to \( q \), and not by the slopes of the potentials or by the energy released during the power- and recovery-strokes.

The major result of this article is the understanding that the directionality and optimal thermodynamic behavior of an autonomous chemically driven molecular machine is specified entirely by the relative heights of the barriers between the states—the saddle points on the potential. The relative energies of the states of a chemically driven molecular machine—energy minima on the potential—have no role whatsoever in determining the intrinsic directionality of the machine, the stopping force, or the optimal thermodynamic performance (i.e., the power-stroke is irrelevant). This result is obtained irrespective of whether the system’s dynamical behavior is modeled as transitions on a Markov chain, a random walk on a lattice of states, motion on a multidimensional potential energy surface described by coupled Langevin equations, or by reaction diffusion equations with chemically driven transitions between different potential energy surfaces. The glue that maintains consistency between these different approaches is the principle of microscopic reversibility.

**CONCLUSIONS**

The mechanism for a chemically driven molecular motor is much gentler than that for an optically or externally driven molecular machine. No violent kicks (43), judo throws (55), windup toys (56), or toy steam engines (57) are necessary. The effect of the disequilibrium between substrate and product in the bulk is based solely on mass action—there is no, nor can there be, a mechanical (58) smoking gun such as a power-stroke to which we can point as the essential step in free-energy transduction (59). As discussed by Moore (60), when viewed uncritically, the animated movies of molecular machines such as the ribosome, kinesin, myosin, or F0F1-ATP synthase can be very misleading. Certainly such a movie could never reveal the fundamental difference between, e.g., a light-driven and a chemically driven molecular machine, but the confusion is exacerbated by the common (mis)representations of ATP hydrolysis as a flash of light. Qualitatively different conclusions concerning the importance of a power-stroke are drawn for superficially similar mechanisms depending on whether microscopic reversibility does or does not apply (16) (see the Supporting Material).

In designing chemically driven molecular machines (61) that can perform work against a significant load, it will be very important to accomplish two goals.

1. It will be necessary to incorporate allosteric interactions to drive switching of bilabile specificities (62) so that the rates of binding/release of the fuel and product molecules depend strongly on the position or mechanical state of the machine. Accomplishing this goal will be sufficient to guarantee directed motion in the absence of load.

2. For the machine to do work against a significant mechanical load, it will be necessary to design strain-dependent gating of the specificities. Accomplishing this goal will assure that the kinetic effects of the specificity differences are not overwhelmed by the load (31,32).

Achieving goals 1 and 2 will provide a large step forward toward mechanized molecular catalysts that use chemical energy to perform mechanical work (61,63).

**SUPPORTING MATERIAL**

Supporting Materials and Methods, two schemes, one figure, and twenty equations are available at http://www.biophysj.org/biophysj/supplemental/S0006-3495(14)04672-4.
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