Thermodynamics and Kinetics of Molecular Motors

R. Dean Astumian*
Department of Physics and Astronomy, University of Maine, Orono, Maine; and Department of Physics, University of Munich, Munich, Germany

ABSTRACT Molecular motors are first and foremost molecules, governed by the laws of chemistry rather than of mechanics. The dynamical behavior of motors based on chemical principles can be described as a random walk on a network of states. A key insight is that any molecular motor in solution explores all possible motions and configurations at thermodynamic equilibrium. By using input energy and chemical design to prevent motion that is not wanted, what is left behind is the motion that is desired. This review is focused on two-headed motors such as kinesin and Myosin V that move on a polymeric track. By use of microscopic reversibility, it is shown that the ratio between the number of forward steps and the number of backward steps in any sufficiently long time period does not directly depend on the mechanical properties of the linker between the two heads. Instead, this ratio is governed by the relative chemical specificity of the heads in the front-versus-rear position for the fuel, adenosine triphosphate and its products, adenosine diphosphate and inorganic phosphate. These insights have been key factors in the design of biologically inspired synthetic molecular walkers constructed out of DNA or out of small organic molecules.

INTRODUCTION

Much work on molecular motors has been inspired by a single, central question—what is the mechanism by which a molecular motor uses energy from adenosine triphosphate (ATP) hydrolysis or some other chemical reaction to cause forward motion and generate a forward-directed force? In this review, I will focus on a different question—what is the mechanism by which a molecular motor uses energy from ATP hydrolysis or some other chemical reaction to prevent backward motion even in the presence of a backward-directed force (1)? To understand the subtle but very important differences between these two questions, and the descriptions that naturally arise from attempts to answer them, let us compare a very small macroscopic motor with a molecular motor, both at equilibrium.

In his seminal talk in 1959, ''Plenty of Room at the Bottom,'' Richard Feynman offered a $1000 reward to the first person to construct a motor that would fit in a cube 1/64th of an inch on a side, not counting wires and power source. This reward was claimed within a year by an engineer, William McClellan. When turned off, the motor does what we expect at static equilibrium—nothing. The motor must be connected to a power supply to get any motion at all.

Now, consider a molecular motor in aqueous solution, e.g., kinesin, at chemical equilibrium where the chemical potential of ATP is equal to the sum of the chemical potentials of adenosine diphosphate (ADP) and inorganic phosphate (Pi), $\mu_{ATP} = \mu_{ADP} + \mu_{Pi}$. The kinesin molecule moves to and fro, sometimes stepping left, sometimes stepping right, sometimes binding ATP and hydrolyzing it to ADP and Pi, and sometimes binding ADP and Pi and synthesizing ATP. The dynamic chemical equilibrium is maintained because each forward process is exactly as likely as the microscopic reverse of that process—on average, for every ATP hydrolyzed there is an ATP synthesized, and for every step to the right taken by the motor, there is a step to the left. Importantly, every motion that we associate with the normal function of the molecule under physiological conditions—hydrolyzing one ATP while taking one step to the +end of the microtubule track—is present also at chemical equilibrium. At equilibrium, however, each reverse motion is as likely as the forward motion.

What happens when we remove ADP (or add ATP) so that $\mu_{ATP} >> \mu_{ADP} + \mu_{Pi}$? Do the states accessible to the molecule change? Certainly not—there is no way for an individual kinesin molecule to sense the bulk chemical potentials of ATP and ADP. Similarly, the character of motion by which the protein undergoes a transition from one state to another does not change when the system is removed from chemical equilibrium by taking away ADP (or by adding of ATP). The only thing that changes is the relative likelihood that a kinesin molecule in which the active site for ATP hydrolysis is unoccupied will next bind ATP rather than ADP. Remarkably, this single change in the boundary conditions for the stochastic process results in a situation where the motor, in the absence of load, takes one step to the +end of microtubule for each ATP hydrolyzed, with almost deterministic precision.

In this review, I will discuss the implications of the ineluctable stochasticity of molecules due to interactions with their thermal environment for understanding the mechanisms of molecular motors and pumps. The focus will be on development of a framework for description of molecular motors rather than on interpretation of experimental results for any particular motor in terms of its structure or kinetics. Much of the discussion will be inspired by recent work on synthetic molecular motors (2,3) such as DNA (4,5) and small molecule (6) walkers, catenane-based molecular motors (7–9), and synthetic molecular rotors (10,11).

Submitted September 29, 2009, and accepted for publication February 16, 2010.

*Correspondence: astumian@maine.edu

Editor: Yale E. Goldman.

0006-3495/10/06/2401/9 $2.00

doi: 10.1016/j.bpj.2010.02.040

Stochastic cycles of molecular motors

Many molecular motors (12,13) convert chemical energy into directional motion along a polar macromolecular track (with ends designated "+" and "−"), possibly against an applied force. This transduction between chemical and mechanical free-energy is accomplished by coupling the chemical transformations involved in the catalytic conversion of a fuel molecule (e.g., ATP) to waste product (ADP and Pi) to the conformational transitions of the motor molecule that allow motion along the track. The overall process forms a conformational cycle in which the protein fluctuates away from and then regresses back to some initial conformational state (14). Consider, for example, a two-headed motor such as kinesin or Myosin V. These motors appear to move in a hand-over-hand fashion in which the rear head detaches from the track and reattaches to the front. When a head is detached from the track, it is prevented from diffusing away by being bound to the other head through a linker region of the protein. Sometimes it is also argued that this linker region acts like a spring, releasing stored elastic energy as it snaps forward in a power-stroke. Here we will examine the relative importance of the linker's passive role of preventing unwanted motion versus its active role of storing and releasing elastic energy to drive a power-stroke, first in the context of a general model and then for the specific cases of Myosin V and of a recent synthetic DNA walker (5).

Focusing on the internal conformation of the motor molecule irrespective of its location along the track, we arbitrarily choose as an initial internal conformational state a configuration in which both heads are attached to the track and in which ADP is bound at both active sites for ATP hydrolysis. Whether at, or far from, thermodynamic equilibrium, the motor will certainly fluctuate away from this initial state by releasing ADP from the front or from the rear head which then detaches from the track. It is equally certain (unless the other head dissociates from the track also) that eventually the motor will return to the initial state where both heads are attached and ADP is again bound at both active sites. In each conformational cycle the motor will have taken some number i of steps and will have hydrolyzed some number j of ATPs. The quantities i and j are stochastic, i.e., they vary from one conformational cycle to the next, with probability $P_{i,\,j}$. For many motors operating under normal physiological conditions in the absence of a strong opposing force, one step is taken for each ATP hydrolyzed (i.e., $P_{1,\,1} \approx 1$, and for i or $j \neq 1$, $P_{i,\,j} \approx 0$), giving the appearance of a completely (tightly) coupled mechanism. However, this is at best an approximation and when the motor is challenged, e.g., by a strong opposing force, or by concentrations of ATP or ADP and Pi that are very different than the normal physiological values, other pathways, for which the number of ATPs hydrolyzed (j) and the number of steps taken (i) in a conformational cycle are not +1, come into play. An example would be any process in which the motor takes a step backward—$i = -1$, or where an ATP is hydrolyzed but the molecule does not step (i.e., slip), with $i = 0, j = 1$.

For any cycle in which i steps are taken in the +-direction and j ATPs are hydrolyzed, $-i$ steps are taken in the +direction (i.e., the molecule takes i steps in the −direction) and $-j$ ATPs are hydrolyzed (i.e., j ATPs are synthesized) in the microscopic reverse of that cycle. By microscopic reversibility, the ratio of the probabilities for any cycle and its microscopic reverse is the exponential of the energy change due to the forward process, so we find (2,15,16)

$$\frac{P_{i,j}}{P_{-i,-j}} = e^{\frac{j\Delta\mu + iLF}{k_B T}}, \tag{1}$$

where

$$\Delta\mu = \mu_{\mathrm{ATP}} - \mu_{\mathrm{ADP}} - \mu_{P_i}$$

is the difference in the chemical potentials of the substrate and product, F is the component of the load force along the track (we take positive force to be in the +-direction), L is the step size along the track, and $k_B T (\approx 4 \times 10^{-21}$ J at room temperature) is the product of the Boltzmann constant and the Kelvin temperature. Note that the ratio $P_{i,\,j}/P_{-i,\,j}$ is not thermodynamically constrained since a trajectory in which the motor takes i steps in the +-direction while hydrolyzing j ATPs is not the microscopic reverse of any trajectory in which the motor takes i steps in the −direction and hydrolyzes j ATPs. Indeed, it is this ratio that is the key quantitative measure of the strength of coupling between the chemical reaction and stepping along the track. The average number of steps taken, and ATPs hydrolyzed in a conformational cycle of the protein motor, can be written as

$$\begin{aligned} \langle N_{\mathrm{step}} \rangle &= \sum_{i,j=1}^{\infty} i\big(P_{i,0}\phi_{i,0} + P_{i,j}\phi_{i,j} - P_{-i,j}\phi_{-i,j}\big), \\ \langle N_{\mathrm{ATP}} \rangle &= \sum_{i,j=1}^{\infty} j\big(P_{0,j}\phi_{0,j} + P_{i,j}\phi_{i,j} + P_{-i,j}\phi_{-i,j}\big), \end{aligned} \tag{2}$$

where

$$\phi_{i,j} = \left[1 - e^{(-i\Delta\mu - jLF)/(KBT)}\right]$$

and we use Eq. 1 to allow us to write the sums over only positive integers i and j (16). The velocity of the motor, v, and rate of ATP hydrolysis, r, can be obtained by dividing these averages by the average time for a conformational cycle, τ_{cyc}, $v = \langle N_{\mathrm{step}} \rangle L/\tau_{\mathrm{cyc}}$ and $r = \langle N_{\mathrm{ATP}} \rangle/\tau_{\mathrm{cyc}}$. The $\phi_{i,\,j}$ contain all of the thermodynamic dependence of the velocity and rate on the external force and chemical potential gradient while the terms $P_{i,\,j}$ and τ_{cyc} contain all of the dependence of the velocity and rate on the external force and chemical potential gradient due to kinetic and structural factors.

The expressions in Eq. 2 are quite general, but not directly useful to model molecular motors, as there are too many (an infinite number!) of the unspecified coefficient functions.

Relations between the coefficient functions have been derived, however, that generalize the linear Onsager reciprocal relations and fluctuation-dissipation relation to the case of large chemical potential difference and external force, $\Delta\mu, LF >> k_BT$ (16). A natural and often very good approximation for chemically driven molecular motors is to restrict the number of steps taken and ATPs hydrolyzed in a conformational cycle to $i = -1, 0, +1$ and $j = -1, 0, +1$, respectively. Aside from the nonproductive cycle $(i, j) = (0, 0)$, there are then eight possibilities—$(i, j) = (1,1)$, $(1,0)$ $(1,-1)$, $(0,1)$, $(0,-1)$, $(-1,1)$, $(-1,0)$, and $(-1,-1)$.

We define a forward coupled process ($\mathcal{F}$) as a conformational cycle that involves hydrolysis of one molecule of ATP while taking one step toward the +end of the polymeric track, $(i, j) = (1,1)$. There are in principle many possible paths—sequences of positions and chemical states—by which a forward process can occur. One such path is shown schematically as the green solid curve in Fig. 1 *a*, where the horizontal coordinate represents the mechanical processes, and the vertical coordinate the progress of the chemical process $ADP + Pi \rightarrow ATP$ (17–21). For each possible forward path there is a microscopic reverse path ($\mathcal{F}_\mathcal{R}$)—the ''movie'' of the forward path played backward—in which the sequence of positions and states in the forward trajectory is exactly reversed, and hence in which one molecule of ATP is synthesized from ADP and Pi while taking one step in the −direction with $(i, j) = (-1,-1)$. This exact microscopic reverse curve would start at the point labeled $\mathcal{F}$ and end at the center, following along the solid green curve backward. We take advantage of periodicity to recognize that the probability of such a path is exactly the same as that of a path starting at the center and ending at the point labeled $\mathcal{F}_\mathcal{R}$, as shown as the dotted green curve in Fig. 1 *a*. There is also a coupled backward process (labeled $\mathcal{B}$) in which one molecule of ATP is hydrolyzed while the motor takes one step in the −direction, with $(i, j) = (-1,1)$, shown as the solid red curve in Fig. 1 *a*. For each backward path there is also a microscopic reverse path ($\mathcal{B}_\mathcal{R}$) in which one molecule of ATP is synthesized while the motor takes one step in the +direction $(i, j) = (1,-1)$ shown as the dotted red curve in Fig. 1 *a* . In addition to the coupled transitions, there are also paths (denoted $\mathcal{C}$) in which ATP is hydrolyzed without stepping in either direction, $(i, j) = (0,1)$, and the reverse ($\mathcal{C}_\mathcal{R}$) in which ATP is synthesized without taking a step, $(i, j) = (0,-1)$, and there are paths in which the motor takes a step to the +end of the track without hydrolysis or synthesis of ATP, with $(i, j) = (1,0)$, denoted $\mathcal{S}$) and reverse paths ($\mathcal{S}_\mathcal{R}$) in which the motor takes a step to the −end of the track without hydrolysis or synthesis of ATP with $(i, j) = (-1,0)$. From Eq. 1, we have relationships between each path and its reverse,

$$\begin{aligned} \text{Coupled } \frac{P_\mathcal{F}}{P_{\mathcal{F}_\mathcal{R}}} &= e^{\frac{\Delta\mu + LF}{k_BT}}; \frac{P_\mathcal{B}}{P_{\mathcal{B}_\mathcal{R}}} = e^{\frac{\Delta\mu - LF}{k_BT}}, \\ \text{Uncoupled } \frac{P_\mathcal{C}}{P_{\mathcal{C}_\mathcal{R}}} &= e^{\frac{\Delta\mu}{k_BT}}; \frac{P_\mathcal{S}}{P_{\mathcal{S}_\mathcal{R}}} = e^{\frac{LF}{k_BT}}. \end{aligned} \tag{3}$$

Note that the ratio $P_\mathcal{B}/P_\mathcal{F}$ is not thermodynamically constrained and can, in principle, take on any positive value.

By using the expressions in Eq. 3, we can write the motor velocity in terms of the ratios of probabilities $P_\mathcal{B}/P_\mathcal{F}, P_\mathcal{S}/P_\mathcal{F}$, and $P_\mathcal{C}/P_\mathcal{F}$,

$$\begin{aligned} v &= \left[\left(1 - e^{\frac{-\Delta\mu - LF}{k_BT}}\right) - \frac{P_\mathcal{B}}{P_\mathcal{F}}\left(1 - e^{\frac{-\Delta\mu + LF}{k_BT}}\right) + \frac{P_\mathcal{S}}{P_\mathcal{F}}\left(1 - e^{\frac{-LF}{k_BT}}\right)\right]\frac{LP_\mathcal{F}}{\tau_{cyc}} \\ r &= \left[\left(1 - e^{\frac{-\Delta\mu - LF}{k_BT}}\right) + \frac{P_\mathcal{B}}{P_\mathcal{F}}\left(1 - e^{\frac{-\Delta\mu + LF}{k_BT}}\right) + \frac{P_\mathcal{C}}{P_\mathcal{F}}\left(1 - e^{\frac{-\Delta\mu}{k_BT}}\right)\right]\frac{P_\mathcal{F}}{\tau_{cyc}}, \end{aligned} \tag{4}$$

where τ_{cyc} is the average time for completion of a conformational cycle. In the absence of a load force ($F = 0$) if $\Delta\mu > 0$ then $P_\mathcal{F} > P_{\mathcal{F}_\mathcal{R}}$ and $P_\mathcal{B} > P_{\mathcal{B}_\mathcal{R}}$. Thus, in order to have a motor that steps to the +direction in the absence of load we require only that $P_\mathcal{F} > P_\mathcal{B}$. To assure that the motor is effective in moving against a load force, the uncoupled stepping probability $P_\mathcal{S}$ must also be small, and in order to assure that the motor does not waste chemical fuel, the uncoupled chemical conversion probabilities $P_\mathcal{C}$ should also be small. Our challenge then is to understand how to use chemical design principles to arrange such a situation.

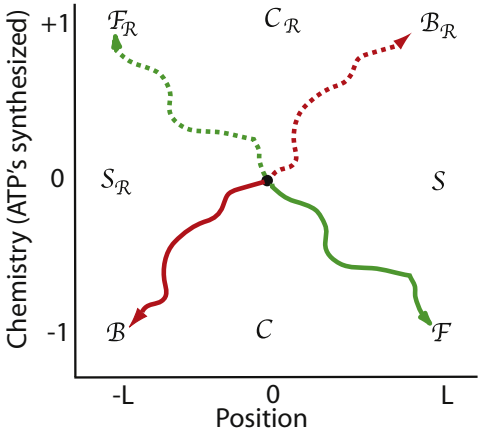

FIGURE 1 Illustration of possible conformational cycles for a molecular motor in which the motor fluctuates away from and then returns to some arbitrary initial conformational state, having performed work on or received work from the environment by moving a distance $\pm L$ on a periodic track (where L is the step length) and/or catalyzing a chemical reaction, $ATP \rightleftharpoons ADP + Pi$. For each trajectory, there is a microscopic reverse trajectory related by simple thermodynamic relations (see Eq. 3).

A theoretical prediction supported by experiment

In the general formulation given here, there is naturally a backward process, $\mathcal{B}$, in which ATP is hydrolyzed, as a mechanism for stepping to the −end of the track in addition to the reverse of the forward process, $\mathcal{F}_\mathcal{R}$, in which ATP is synthesized. Thus, when a molecular motor steps

backward in response to an external force, it does not always do so by the microscopic reverse of the forward stepping mechanism. Further, in contrast to most macroscopic motors, there is not a single mechanism by which the system functions, but, instead, several preferred pathways selected that depend on the prevailing environmental conditions, external force, chemical potentials of reactants and products, temperature, etc.

Based on a minimal kinetic model that incorporates the symmetry relations of Eq. 1, Martin Bier and I made the prediction in 1996 (19) that back-stepping of a molecular motor such as kinesin does not necessarily involve synthesis of ATP. We observed that "as an external force is applied, the system responds by changing the stoichiometry. Interestingly, a large applied force actually stimulates ATP hydrolysis." This suggestion is in striking opposition to predictions based on completely coupled single-cycle models (22,23) in which back-stepping can only arise by reversal of the single-cycle and which should, hence, be inexorably accompanied by ATP synthesis and inhibited by adding ATP. The behavior predicted by Astumian and Bier has been observed experimentally for kinesin by Nishiyama et al. (24) and by Carter and Cross (25), who showed that back-stepping in the presence of a large −end-directed external force is stimulated rather than inhibited by ATP, and suggested that the back-stepping may be accompanied by ATP hydrolysis. The observed behavior is a predicted signature of a gently coupled Brownian motor, in which the preferred mechanism (pathway through the states) shifts under the influence of changing environmental conditions such as the applied force (26). A number of recent authors (27,28) have proposed, subsequent to the experiments of Carter and Cross, models very similar to that of Astumian and Bier (19), but with many more states (up to seven or eight) in efforts to fit experimental curves.

The expressions in Eq. 4 are in the best traditions of theoretical physics—rather general (they can be used to describe many very different coupled transport processes (16)), and in and of themselves not very useful for calculations, as the time constant and the ratios of probabilities involved are functions of the forces and can only be derived in the context of a specific model or obtained from experiment. Let us consider how the general theory can be applied to understand the mechanism of motion of a specific biological motor, Myosin V.

Application of the theory to Myosin V stepping

The kinetic mechanism for Myosin V stepping is typically written as a completely coupled cycle (Fig. 2 *a*) in which each ATP hydrolyzed results in one step in the +direction (29). This picture is certainly only an approximation, and is not consistent with experiments showing back-stepping that is not chemically the reverse of the forward stepping (30). When we look at the mechanism of Reif et al. (29) closely, we see that there are two branching points, indicated by the gray boxes in Fig. 2 *a*, at which stochastic decisions affecting the outcome of a cycle must be made. These decisions are independent of the chemical potential difference between ATP, ADP, and Pi, $\Delta\mu$, that drives the reaction.

One branching point is the state in which both heads are attached to actin, and the active site of each head is occupied by ADP (D_iD_{i+1}). The transition out of this state is usually release of ADP, setting the stage for binding ATP and

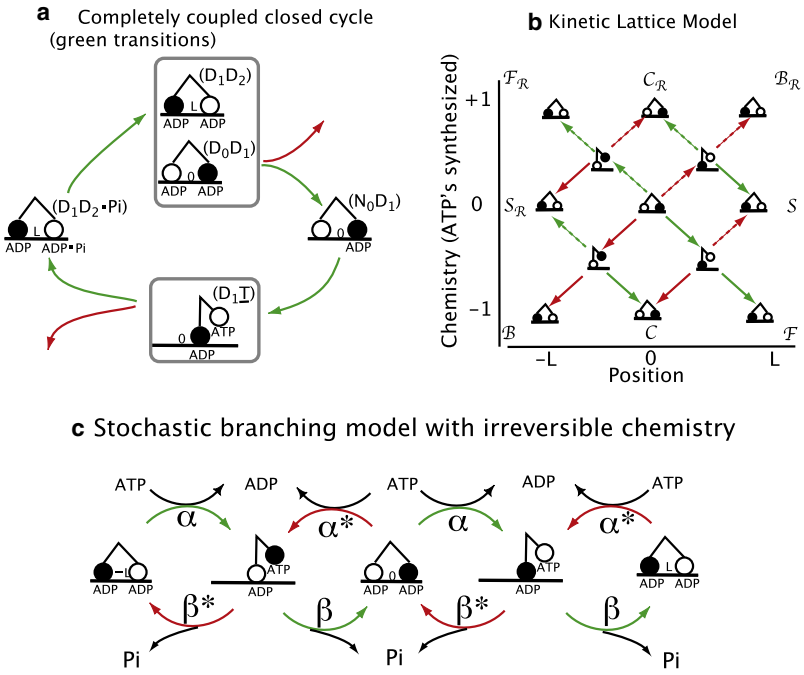

FIGURE 2 (*a*) Completely coupled cycle for Myosin V stepping denoted by the green arrows. This cycle is driven clockwise by hydrolysis of ATP. The gray boxes indicate branching states, and the red arrows indicate branching transitions by which the mechanism can deviate from this completely coupled picture. Note that although the two heads are colored differently (*black* and *white*), the two states in the upper-shaded box are chemically indistinguishable. (*b*) A kinetic lattice model picture in which all possible paths discussed in the text are shown in the context of transitions between the two branching states. Dashed arrows are used to denote backward chemistry transitions. Note that only transitions leading away from the center state are shown, consistent with the schematic diagram in Fig. 1. (*c*) A kinetic branching mechanism with irreversible chemistry and where the backward chemical transitions are ignored. The stochastic branching provides for the possibility of a backward step combined with hydrolysis of ATP. For simplicity, the intermediate states are not shown.

detachment of that head from actin, but from which head is ADP released? Normally it is taken to be the rear head (shown by the *green arrow*) from which ADP is released, but certainly release of ADP from the front head (shown as the *red arrow* out of this state) is a physical possibility.

Another branch point occurs at the state in which one head is attached with ADP at the active site and the other head is detached with ATP at the active site ($D_{i+1}\underline{T}$). The next transition is typically hydrolysis of ATP to ADP·Pi at the active site and reattachment of the head to actin, followed by release of inorganic phosphate to return to the original configuration in which ADP occupies both active sites and both heads are attached to actin, but does the head reattach to the front or to the rear? Normally, it is accepted that reattachment occurs to the front (following the *green arrow* in Fig. 2) with overwhelming probability due to the molecule having completed a power-stroke (31). However, it is certainly physically possible for the head to reattach to the rear (if the *red arrow* in Fig. 2 is followed), and clearly the probability for this to happen is strongly dependent on the magnitude and direction of any external load force.

The outcome of any given cycle starting and ending at the state in which both heads are attached with ADP at each active site depends on the stochastic decision at each of the two branch points. This is illustrated in Fig. 2 *b*, where the mechanism is drawn as a kinetic lattice (19) with only the two branching states shown explicitly, and with only transitions leading away from the center shown, in consistency with the schematic continuum picture in Fig. 1.

In the forward path, $\mathcal{F}$, ADP is released from the rear head, and reattachment after ATP hydrolysis occurs to the front, with probability

$$P_{\mathcal{F}} \equiv P\left[D_{i-1}D_i \rightarrow N_{i-1}D_i \rightarrow D_i\underline{T} \rightarrow D_i(D\cdot Pi)_{i+1} \rightarrow D_iD_{i+1}\right].$$

In the reverse of the forward path ($\mathcal{F}_{\mathcal{R}}$), the chemical and the mechanical steps occur in the reverse sequence, obtained by inverting the arrows describing the transitions of the forward process ($\mathcal{F}$), i.e.,

$$P_{\mathcal{F}_{\mathcal{R}}} \equiv P\left[D_iD_{i+1} \rightarrow D_i(D\cdot Pi)_{i+1} \rightarrow D_i\underline{T} \rightarrow N_{i-1}D_i \rightarrow D_{i-1}D_i\right].$$

Because of the large amount of free energy released to the environment by hydrolysis of ATP (and hence the large amount of free energy required from the environment to synthesize ATP), this reverse process is very unlikely under physiological conditions. In contrast, the backward process involves forward chemistry (ATP hydrolysis) but the opposite decision at each branch point—release of ADP from the front head in state D_0D_1, and reattachment to the rear from state $D_0\underline{T}$. The probability for this process is

$$P_{\mathcal{B}} \equiv P\left[D_iD_{i+1} \rightarrow D_iN_{i+1} \rightarrow D_i\underline{T} \rightarrow (D\cdot Pi)_{i-1}D_i \rightarrow D_{i-1}D_i\right].$$

In the reverse of the backward process ($\mathcal{B}_{\mathcal{R}}$), the chemical and the mechanical steps occur in the reverse sequence obtained by inverting the arrows of the backward process ($\mathcal{B}$), i.e.,

$$P_{\mathcal{B}_{\mathcal{R}}} \equiv P\left[D_{i-1}D_i \rightarrow (D\cdot Pi)_{i-1}D_i \rightarrow D_i\underline{T} \rightarrow D_iN_{i+1} \rightarrow D_iD_{i+1}\right].$$

Once again, because of the large amount of free energy released to the environment by hydrolysis of ATP (and hence large amount of free energy required from the environment to synthesize ATP), this reverse process is very unlikely.

In addition to the forward and backward processes, there are also two forward/reverse futile cycles ($2\times P_{\mathcal{C}}/P_{\mathcal{C}_{\mathcal{R}}}$) in which ATP is hydrolyzed/synthesized but there is no stepping, and two possible forward/reverse cycles in which the motor takes a step to the front/back in which no ATP is hydrolyzed/synthesized ($2\times P_{\mathcal{S}}/P_{\mathcal{S}_{\mathcal{R}}}$). These uncoupled cycles influence the quantitative but not qualitative aspects of the ATP-driven stepping of Myosin V.

A power-stroke (31) is invoked in many explanations of why forward stepping dominates backward stepping. The story line goes something like this. When ADP dissociates from the rear head, ATP binds, causing the head to detach from myosin. Because of stored elastic energy, the neck linker attached to the bound head undergoes a large energy-releasing conformational change, bringing the free head to a position in front of the bound head. Because the free head is now near the forward binding site, when ATP is hydrolyzed at the active site, reattachment occurs most probably to the front. This picture is represented by drawing the state $D_i\underline{T}$ with the detached head in front of the bound head.

When we analyze this plausible sounding description in the light of microscopic reversibility, however, we are forced to recognize that this picture is not correct and that the drawing of the state $D_i\underline{T}$ is a trompe l'oeil—it fools the eye into unquestioning acceptance that the proximity of the unbound head to the front of the bound head is important for determining whether the head will reattach to the front, state D_iD_{i+1}, or to the rear, state D_iD_{i-1}. In fact, it is not important at all. The determining factor is instead the chemical specificity. In the limit that the rear head is absolutely chemically specific for ADP release/binding and the front head is absolutely chemically specific for Pi release/binding (i.e., Pi release/binding at the rear head and ADP release/binding at the front head are totally prevented), the motor will take one step to the front for each ATP hydrolyzed irrespective of the mechanical properties of the neck linker. To see this, let us compare the probabilities for the processes $D_i\underline{T} \rightarrow D_iD_{i+1}$ and $D_i\underline{T} \rightarrow D_{i-1}D_i$.

We can break the overall mechanism into two subprocesses connecting the branching states as shown in Fig. 2 *c*, where, for simplicity, neither the intermediate states nor the reverse-chemistry transitions are shown. From each branching state, transition to the right (*green arrow*) or to the left (*red arrow*) is possible, with different zero-load probabilities α and β for rightward transitions and α^* and β^* for leftward

transitions. In the absence of a load force ($F = 0$), microscopic reversibility requires the following relations between the conditional probabilities for these subprocesses to hold irrespective of the magnitude of $\Delta\mu$:

$$\frac{P[D_iD_{i+1} \rightarrow N_iD_{i+1} \rightarrow D_{i+1}\underline{T}]}{P[D_iD_{i+1} \rightarrow D_iN_{i+1} \rightarrow D_i\underline{T}]} = \frac{\alpha}{\alpha^*}$$
$$\therefore \quad \frac{P[D_i\underline{T} \rightarrow N_{i-1}D_i \rightarrow D_{i-1}D_i]}{P[D_i\underline{T} \rightarrow D_iN_{i+1} \rightarrow D_iD_{i+1}]} = \frac{\alpha}{\alpha^*} \tag{5}$$

and

$$\frac{P\left[D_i\underline{T} \rightarrow D_i(D\cdot Pi)_{i+1} \rightarrow D_iD_{i+1}\right]}{P\left[D_i\underline{T} \rightarrow (D\cdot Pi)_{i-1}D_i \rightarrow D_{i-1}D_i\right]} = \frac{\beta}{\beta^*}$$
$$\therefore \quad \frac{P\left[D_iD_{i+1} \rightarrow D_i(D\cdot Pi)_{i+1} \rightarrow D_i\underline{T}\right]}{P\left[D_{i-1}D_i \rightarrow (D\cdot Pi)_{i-1}D_i \rightarrow D_i\underline{T}\right]} = \frac{\beta}{\beta^*}. \tag{6}$$

Note that the second of each of the relations in Eqs. 5 and 6 pertain to reverse chemistry (*dashed arrows* in Fig. 2 *b*)—dissociation of ATP from the active site, and synthesis of ATP from ADP and Pi at the active site, respectively. By comparing the second relation of Eq. 5 with the first relation of Eq. 6, we see that despite appearances, the proximity of a detached head to the forward-versus-rear binding site is not a reliable indicator of the relative probability to reattach to the forward-versus-rear binding sites. The fate of a molecule in the state $D_i\underline{T}$ depends on the chemical path by which reattachment occurs, and not on the position of the detached head relative to the front or rear binding site. For α/α^*, $\beta/\beta^* > 1$, if ATP directly dissociates from state $D_i\underline{T}$, followed by association of ADP, then the head is most likely to bind to the rear, but if ATP is hydrolyzed in state $D_i\underline{T}$ the head is most likely to bind to the front, followed by dissociation of inorganic phosphate.

It is not the mechanics of the motor molecule that directly governs the likelihood of attachment/detachment to/from the front or rear, but, instead, the relative rates of the chemical steps at the front versus rear heads—i.e., the chemical specificities. If dissociation/association of ADP is much faster at the rear head than at the front head, and dissociation/association of Pi is much faster at the front head than at the rear head, then in the absence of a load force, the forward process will dominate the backward process, $P_{\mathcal{F}} > P_{\mathcal{B}}$, at and away from thermodynamic equilibrium. Then, when ATP is in excess and ADP and Pi are in deficit relative to the equilibrium ratio, ATP binding will be more likely than ATP dissociation, and Pi dissociation will be more likely than Pi association so that $P_{\mathcal{F}} > P_{\mathcal{F}_{\mathcal{R}}}$. When both of these conditions hold, the motor will step toward the +end of the track.

The only communication between the heads necessary to assure this coordinated hydrolysis of ATP such that the stepping is almost perfectly synchronized is that by which the front head is distinguished chemically from the rear head, allowing the rear head to be specific for release/binding of ADP and the front head to be specific for release/binding of Pi. The relative specificities of the heads can, of course, depend on the angle of the neck linker with each head by a mechanism similar to any other allosteric interaction. Bond angles in the vicinity of the neck-linker attachment can influence the bond angles, and hence specificities and affinities, at the ATP hydrolysis active site even if that site is remote from the linker attachment region. The linker-head angles can be influenced by properties such as the persistence length of the linker, the intramolecular strain, and even mechanical load, as seems to be the case for both Myosin V (32) and kinesin (33–35).

The independence of the ratio of forward to backward steps on any special nonequilibrium conformational change or power-stroke has been shown here in the context of an effective two-state model. This result, however, is general. Already from Eq. 3, we see that in the absence of a load force, $F = 0$, we have the relation $P_{\mathcal{F}}/P_{\mathcal{B}} = P_{\mathcal{F}_{\mathcal{R}}}/P_{\mathcal{B}_{\mathcal{R}}}$. This equality means that the ratio of the probability to take a forward-versus-backward step with forward chemistry is equal to the ratio between the probability to take a backward-versus-forward step with reverse chemistry irrespective of any power-stroke or special conformational change whatsoever. The direction of motion of a molecular motor depends on the concentrations of substrate and product in the bulk solution and on the relative affinities of heads in the front and rear positions for substrate and product, not on any local consideration of the mechanical properties or power-stroke of the motor molecule itself. This conclusion is based on the fundamental principle of microscopic reversibility and is valid for all molecular motors and pumps, i.e., for molecular machines that mediate the transduction between two types of free energy.

The mechanism of vectorial free-energy transduction by switching specificities and affinities seems to be universal for molecular motors and pumps, ranging from the sarcoplasmic reticulum Ca^{+2} ATPase and other ion-motive ATPases (36–38) to Myosin V and kinesin, and even to DNA and small molecule synthetic walkers (5). On the other hand, the details of how the affinities and specificities are influenced by structural changes for each motor or pump, and the kinetic regulation of the motor or pump, depends on the individual molecule in question.

Although the relative probability for forward-versus-backward steps does not directly depend on the mechanical properties of the linker by which the two heads are attached to one another, the rate of the stepping does depend on the mechanical properties of the linker through the term

$$\tau_{\text{cyc}}^{-1} = \mathcal{A}\exp(cFL/k_{\text{B}}T)$$

in Eq. 4. From Kramer's theory (15), both forward and backward rates are faster (i.e., A is greater) if the effective spring constant for the linker is large (stiff) than if it is small (soft). The factor c in the exponential ranges from zero, in the case that only the chemically backward (ATP synthesis) steps

depend on the force, to one in the case that only the chemically forward (ATP hydrolysis) steps depend on force.

Two-headed biological motors such as kinesin and Myosin V have inspired much recent work on both theoretical (2,21) and experimental aspects of the design of artificial molecular motors using DNA (4,5) and small synthetic organic molecules (6). Here I examine a specific theoretical model (21) to obtain an operation curve that illustrates coupled transport in the near-to-thermodynamic equilibrium ($\Delta\mu/(k_BT)$, $FL/(k_BT) < 1$) regime in which the velocity and rate depend linearly on the thermodynamic forces $\Delta\mu$ and FL, and also in the far from thermodynamic equilibrium regime ($\Delta\mu/(k_BT)$, $FL/(k_BT) > 1$) where strong deviations from linearity occur. The mechanism designed experimentally by Green et al. (5) is very similar to the theoretical proposal (21), and thus shows explicitly how these ideas can be implemented experimentally.

A simple symmetry-based, hand-over-hand molecular motor

Consider a flexible dimeric motor molecule, *a*-*b*—*b*-*a*, that interacts with and walks along a rigid polymeric track, (−)–*A*-*B*—*A*-*B*—*A*-*B*—*A*-*B*–(+), where *a* binds tightly to *A* and *b* binds tightly to *B*. The dimer has mirror symmetry when extended while the track has translational (periodic), but not mirror, symmetry. Because the linker is attached to *b* and not to *a*, when the motor is attached to the track the front *a*-*b* monomer will not be equivalent to the rear *a*-*b* monomer (see Fig. 3).

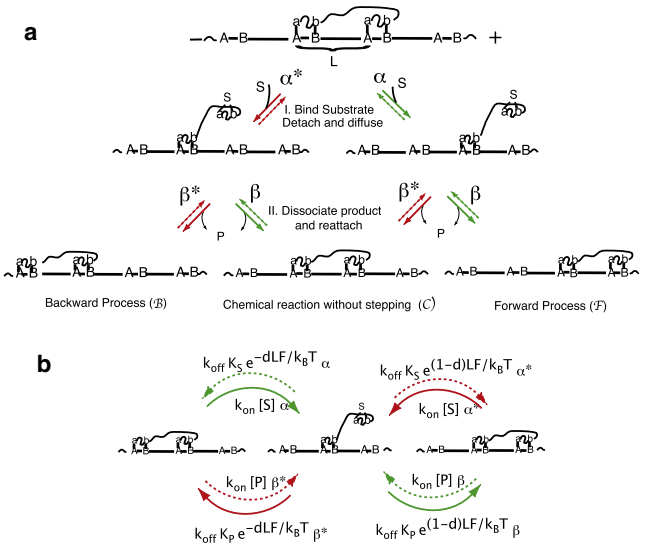

FIGURE 3 (*a*) Simple mechanism for unidirectional motion of a dimeric polymer on a rigid track, adapted from Astumian (21). (*b*) Mechanism shown as coupled kinetic cycles with possible values for rate constants (i.e., expressions consistent with microscopic reversibility) given for all transitions. The intrinsic affinity constants K_S and K_P parameterize how tightly substrate and product bind to the motor, respectively, and the kinetic factors k_{off} and k_{on} are introduced to allow the specificities α, β, α^*, and β^* to remain as dimensionless numbers, as in Fig. 2.

Let *a*-*b* held together at the fixed distance *A*-*B* form a catalytically active complex that catalyzes the reaction $S \rightleftharpoons P$, such that substrate binding leads to dissociation of *a*-*b* from *A*-*B* (see Fig. 3).

In Step 1, the substrate binds to the stably bound dimer causing that monomer to which *S* associates to detach from the track. Although the two dimers are a priori equivalent in solution, the probability for *S* to bind to the front *a*-*b* may well be significantly different than the probability for *S* to bind to the rear monomer, as the configuration of linker in the region of the two bound monomers will be quite different. We parameterize the kinetic splitting in the absence of load for binding/dissociation of *S* and subsequent detachment/attachment of the head from the track at the rear versus front by the ratio α/α^*. Interaction between the *a*-end of the front monomer and the *b*-end of the rear monomer may also favor binding of *S* to the rear monomer as in the DNA walker of Green et al. (5). In the case of the DNA walker, the catalyzed reaction is hybridization of two complementary DNA hairpin loops $H1 + H2 \rightleftharpoons H1 \times H2$, where spontaneous (noncatalyzed) hybridization is inhibited by closure of their necks, the opening of which is facilitated by interaction with the DNA walker (5). By designing a motor in which the interaction of the *a*-part of the forward head favored binding of *S* to the *b*-part of the trailing head, these authors obtained a splitting ratio $\alpha^*/\alpha = 0.01$.

In Step 2, substrate is converted to product at the active site, the product dissociates, and the free monomer reattaches to the track. For many different reasons, the dissociation/binding of *P* at the front head may be more or less probable than at the rear. We parameterize this kinetic splitting by the ratio β/β^*. In the DNA walker (5), the release of product was equally likely from both leading and trailing positions so there was no bias in this part of the mechanism, i.e., the ratio $\beta/\beta^* = 1$.

In Fig. 3 *b*, the mechanism is shown as a kinetic branching process with thermodynamically consistent expressions for all rate constants given explicitly. It is important to note that there is no thermodynamically mandated relationship between the ratios α^*/α and β^*/β (2,21,39), and the values of these two ratios are not thermodynamically constrained at or away from equilibrium.

The ratios between the probabilities for a backward and forward path, between an uncoupled +directed step and a forward step, and between an uncoupled conversion of $S \rightarrow P$ and a forward step, can be calculated from the rate constants in Fig. 3 *b* to be

$$\begin{aligned}\frac{P_B}{P_F} &= \frac{\alpha^*\beta^*}{\alpha\beta}e^{\frac{-LF}{k_BT}},\\ \frac{P_C}{P_F} &= \frac{\alpha^*}{\alpha} + \frac{\beta^*}{\beta}e^{\frac{-LF}{k_BT}},\\ \frac{P_S}{P_F} &= \frac{\alpha K_S}{\beta K_P}\frac{\alpha^*}{\alpha} + \frac{\beta K_P}{\alpha K_S}\frac{\beta^*}{\beta}e^{\frac{-\Delta\mu}{k_BT}},\end{aligned} \tag{7}$$

where we used the identity

$$[S]K_P/([P]K_S) = e^{\frac{\Delta\mu}{k_BT}}.$$

Note the kinetic constants $[S]k_{on}$, $[P]k_{on}$, and k_{off} do not appear in these probability ratios.

Inserting the expressions in Eq. 7 into Eq. 4, we obtain a diagram (Fig. 4) for the operational regimes of the motor (40) demarcated by the axes of the graph and the curves for which $v = 0$ (*dotted curve*) and for which $r = 0$ (*dashed curve*). The shaded areas are those in which free-energy transduction occurs. In the green region, chemical energy is used to do mechanical work against an applied force, while in the blue regions energetically downhill mechanical motion does chemical work against a chemical potential gradient. The solid straight line ($\Delta\mu = LF$) denotes the ideal operating curves with $\alpha^*/\alpha = \beta^*/\beta = 0$. A possible synthetic approach to achieve this ideal case has recently been suggested by Wang (41). This limit is approached when the trailing head is absolutely specific for binding/release of S, and the leading head is absolutely specific for binding/release of P.

The mechanical properties of the linker (e.g., the persistence length and stiffness when bent) are irrelevant for determining the operation diagram. Further, quantities such as the ratio of forward to backward steps, stopping force, and efficiency that depend only on the ratios $P_B/P_F, P_C/P_F$, and P_S/P_F are also independent of the mechanical properties of the linker. For the case in which the $\Delta\mu$ is large and the intrinsic affinity for substrate is much greater than the intrinsic affinity for product ($K_S << K_P$), the stopping force—that force beyond which the motor reverses direction—is simply

$$F_{stop} = \frac{k_BT}{L}\ln\frac{\alpha\beta}{\alpha^*\beta^*},$$

in agreement with the result of Green et al. (5).

On the other hand, the velocity of the motor, and hence its output power, do depend on the linker properties through the average cycle time τ_{cyc}, which can be easily worked out in terms of the rate constants (21). Thus, the conditions under which the power output is maximized can be adjusted to coincide with the conditions under which the efficiency is optimized (21). This ability to simultaneously optimize output power and efficiency for molecular free-energy transducing machines is in striking contrast to heat engines for which the conditions of maximum efficiency and maximum power output are intrinsically very different (42).

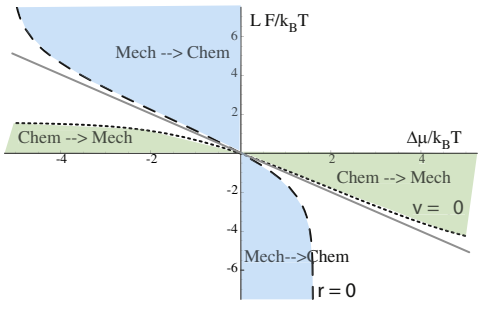

FIGURE 4 Operational regimes of molecular motor delineated by curves of $v = 0$ (*dotted curve*) and $r = 0$ (*dashed curve*) for the parameters $\alpha^*/\alpha = 0.005$, $\beta^*/\beta = 0.25$, and $\alpha K_S = \beta K_P$ (adapted from (21)). (*Solid straight line*) Ideal operating curves with $\alpha^*/\alpha = \beta^*/\beta = 0$. In this ideal case, the curves describing $v = 0$ and $r = 0$ are identical, and hence, are on top of one another.

CONCLUSIONS

In an amusingly titled article in the journal *Cell*, ''Fifty ways to love your lever: myosin motors'' (43), Steve Block made the quixotic, but prescient, observation that ''the kinesin lever may be more like a leash than a pry bar.'' The function of a pry bar is, of course, to transmit force and hence to cause desired motion. In contrast, a leash functions to prevent unwanted motion.

Here we have shown, by using microscopic reversibility, that the mechanical properties of the linker region can be irrelevant for determining the relative probability for a motor to take a forward versus a backward step. A thermodynamically efficient motor that takes one step in the +direction for every fuel molecule consumed can be designed by assuring that a head in the trailing position associates/dissociates substrate much more rapidly than a head in the leading position, and that a head in the leading position associates/dissociates product much more rapidly than a head in the trailing position. The linker between the two heads could just as well be made of cooked spaghetti. The velocity of a motor with cooked spaghetti for a linker, however, would of course be limited by diffusion, and the velocity would fall off precipitously in the presence of a significant load force, although the stopping force—that force beyond which the motor begins to move backward—is independent of the mechanical property of the linker. Put another way, the so-called power-stroke has nothing whatsoever to do with determining the direction of motion of a molecular motor, the ratio of the number of forward steps to the number of backward steps, the thermodynamic efficiency, or the stopping force of the motor—all thermodynamic quantities derived as the ratio of probabilities, or as zeros of the equations for the velocity and rate. The power-stroke does influence the kinetic properties such as velocity of the motor, and how the velocity changes with applied force.

The take-home message of this review is that, in order to understand how molecular motors couple chemical energy with directed motion and mechanical work, we need to pay much more attention to how structure influences chemical specificities, and in particular, to how changing physical position can lead to structural changes by which chemical specificity can be controlled. In this regard, the mechanism for molecular motors such as kinesin and myosin is identical to that by which ion pumps such as the Ca-ATPase of sarcoplasmic reticulum (36–38,44,45) couple ATP hydrolysis to transport ions across a membrane from low to high chemical potential reservoirs.

The stochastic chemical approach for modeling molecular motors reviewed here, and the insights provided by this approach have been very successfully used in the recent design of synthetic molecular motors. Although protein motors are far more complicated than synthetic molecular motors (6) or even DNA-based walkers (4,5) it seems likely that evolution has adopted similar strategies in the design of biomolecular motors, thereby working with the unavoidable thermal noise as a mechanism for transition from one state to another, rather than working against noise and friction by attempting to design miniaturized engines and the like that function according to macroscopic mechanical concepts (46).

I am very grateful to Andrew Turberfield, Imre Derenyi, Jan Neumann, and Changbong Hyeon for very useful discussions, and for critical reading of the manuscript.

I am also very grateful to the German Humboldt Foundation for facilitating this work through conferment of a Humboldt Research Award.

REFERENCES

1. Astumian, R. D. 2001. Making molecules into motors. *Sci. Am.* 285: 45–51.
2. Astumian, R. D. 2007. Design principles for Brownian molecular machines: how to swim in molasses and walk in a hurricane. *Phys. Chem. Chem. Phys.* 9:5067–5083.
3. Browne, W. R., and B. L. Feringa. 2006. Making molecular machines work. *Nat. Nanotechnol.* 1:25–35.
4. Omabegho, T., R. Sha, and N. C. Seeman. 2009. A bipedal DNA Brownian motor with coordinated legs. *Science.* 324:67–71.
5. Green, S. J., J. Bath, and A. J. Turberfield. 2008. Coordinated chemomechanical cycles: a mechanism for autonomous molecular motion. *Phys. Rev. Lett.* 101:238104.
6. Delius, M., E. M. Geertsema, and D. A. Leigh. 2010. A synthetic small molecule that can walk down a track. *Nat. Chem.* 10.1038/nchem.481.
7. Kay, E. R., D. A. Leigh, and F. Zerbetto. 2007. Synthetic molecular motors and mechanical machines. *Angew. Chem. Int. Ed.* 46:72–191.
8. Hernández, J. V., E. R. Kay, and D. A. Leigh. 2004. A reversible synthetic rotary molecular motor. *Science.* 306:1532–1537.
9. Astumian, R. D. 2007. Adiabatic operation of a molecular machine. *Proc. Natl. Acad. Sci. USA.* 104:19715–19718.
10. Kottas, G. S., L. I. Clarke, …, J. Michl. 2005. Artificial molecular rotors. *Chem. Rev.* 105:1281–1376.
11. Michl, J., and E. C. H. Sykes. 2009. Molecular rotors and motors: recent advances and future challenges. *ACS Nano.* 3:1042–1048.
12. Schliwa, M., and G. Woehlke. 2003. Molecular motors. *Nature.* 422:759–765.
13. Howard, J. 2001. Mechanics of Motor Proteins and the Cytoskeleton. Sinauer, Sunderland, MA.
14. Hill, T. L. 1977. Free Energy Transduction in Biology: The Steady State Kinetic and Thermodynamic Formalism. Academic Press, New York, NY.
15. Bier, M., I. Derényi, …, R. D. Astumian. 1999. Intrawell relaxation of overdamped Brownian particles. *Phys. Rev. E Stat. Phys. Plasmas Fluids Relat. Interdiscip. Topics.* 59:6422–6432.
16. Astumian, R. D. 2009. Generalized fluctuation-dissipation and reciprocal relations for Brownian sieves and molecular machines. *Phys. Rev. E Stat. Nonlin. Soft Matter Phys.* 79:021119.
17. Astumian, R. D., and M. Bier. 1994. Fluctuation driven ratchets: molecular motors. *Phys. Rev. Lett.* 72:1766–1769.
18. Magnasco, M. O. 1994. Molecular combustion motors. *Phys. Rev. Lett.* 72:2656–2659.
19. Astumian, R. D., and M. Bier. 1996. Mechanochemical coupling of the motion of molecular motors to ATP hydrolysis. *Biophys. J.* 70:637–653.
20. Keller, D., and C. Bustamante. 2000. The mechanochemistry of molecular motors. *Biophys. J.* 78:541–556.
21. Astumian, R. D. 2008. Symmetry based mechanism for hand-over-hand molecular motors. *Biosystems.* 93:8–15.
22. Cross, R. A. 2004. The kinetic mechanism of kinesin. *Trends Biochem. Sci.* 29:301–309.
23. Kolomeisky, A., and M. E. Fisher. 2007. Molecular motors: a theorist's perspective. *Annu. Rev. Phys. Chem.* 98:675–695.
24. Nishiyama, M., H. Higuchi, and T. Yanagida. 2002. Chemomechanical coupling of the forward and backward steps of single kinesin molecules. *Nat. Cell Biol.* 4:790–797.
25. Carter, N. J., and R. A. Cross. 2005. Mechanics of the kinesin step. *Nature.* 435:308–312.
26. Astumian, R. D. 2005. Biasing the random walk of a molecular motor. *J. Phys. Condens. Matter.* 17:S3753–S3766.
27. Liepelt, S., and R. Lipowsky. 2007. Kinesin's network of chemomechanical motor cycles. *Phys. Rev. Lett.* 98:258102.
28. Hyeon, C., S. Klumpp, and J. N. Onuchic. 2009. Kinesin's backsteps under mechanical load. *Phys. Chem. Chem. Phys.* 11:4899–4910.
29. Rief, M., R. S. Rock, …, J. A. Spudich. 2000. Myosin-V stepping kinetics: a molecular model for processivity. *Proc. Natl. Acad. Sci. USA.* 97:9482–9486.
30. Kad, N. M., K. M. Trybus, and D. M. Warshaw. 2008. Load and Pi control flux through the branched kinetic cycle of Myosin V. *J. Biol. Chem.* 283:17477–17484.
31. Howard, J. 2006. Protein power strokes. *Curr. Biol.* 16:R517–R519.
32. Purcell, T. J., H. A. Sweeny, and J. A. Spudich. 2005. A force-dependent state controls the coordination of processive Myosin V. *Proc. Natl. Acad. Sci. USA.* 102:13873–13878.
33. Uemura, S., and S. Ishiwata. 2004. Loading direction regulates the affinity of ADP for kinesin. *Nat. Struct. Biol.* 10:308–311.
34. Guydosh, N. R., and S. M. Block. 2006. Backsteps induced by nucleotide analogs suggest the front head of kinesin is gated by strain. *Proc. Natl. Acad. Sci. USA.* 103:8054–8059.
35. Hyeon, C. B., and J. N. Onuchic. 2007. Internal strain regulates the nucleotide binding site of the kinesin leading head. *Proc. Natl. Acad. Sci. USA.* 104:2175–2180.
36. Eisenberg, E., and T. L. Hill. 1985. Muscle contraction and free energy transduction in biological systems. *Science.* 227:999–1006.
37. Jencks, W. P. 1995. The mechanism of coupling chemical and physical reactions by the calcium ATPase of sarcoplasmic reticulum and other coupled vectorial systems. *Biosci. Rep.* 15:283–287.
38. Astumian, R. D., and I. Derényi. 1998. Fluctuation driven transport and models of molecular motors and pumps. *Eur. Biophys. J.* 27:474–489.
39. Green, S. J., J. Bath, and A. J. Turberfield. 2009. Erratum: Coordinated chemomechanical cycles: a mechanism for autonomous molecular motion. *Phys. Rev. Lett.* 102:139901.
40. Julicher, F., A. Ajdari, and J. Prost. 1997. Modeling molecular motors. *Rev. Mod. Phys.* 69:1269–1281.
41. Wang, Z. 2007. Synergic mechanism and fabrication target for bipedal nanomotors. *Proc. Natl. Acad. Sci. USA.* 104:17921–17926.
42. Chen, J. 1994. The maximum power output and maximum efficiency of an irreversible Carnot heat engine. *J. Phys. D.* 27:1144–1149.
43. Block, S. M. 1996. Fifty ways to love your lever: myosin motors. *Cell.* 87:151–157.
44. Astumian, R. D., and I. Derényi. 2001. Towards a chemically driven molecular electron pump. *Phys. Rev. Lett.* 86:3859–3862.
45. Astumian, R. D. 2003. Adiabatic pumping mechanism for ion motive ATPases. *Phys. Rev. Lett.* 91:118102.
46. Astumian, R. D. 1997. Kinetics and thermodynamics of a Brownian motor. *Science.* 276:917–922.