Biasing the random walk of a molecular motor

2005 J. Phys.: Condens. Matter 17 S3753

(http://iopscience.iop.org/0953-8984/17/47/008)

INSTITUTE OF PHYSICS PUBLISHING JOURNAL OF PHYSICS: CONDENSED MATTER

J. Phys.: Condens. Matter **17** (2005) S3753–S3766 doi:10.1088/0953-8984/17/47/008

Biasing the random walk of a molecular motor

R Dean Astumian

Department of Physics, University of Maine, Orono, ME 04469-5709, USA

E-mail: astumian@maine.edu

Received 19 October 2005
Published 4 November 2005
Online at stacks.iop.org/JPhysCM/17/S3753

Abstract

Biomolecular motors are often described in mechanical terms, with analogy to cars, turbines, judo throws, levers, etc. It is important to remember however that because of their small size, and because of the aqueous environment in which molecular motors move, viscous drag and thermal noise dominate the inertial forces that drive macroscopic machines. The sequence of motions—conformational changes—by which a motor protein moves can best be described as a random walk, with transitions from one state to another occurring by thermal activation over energy barriers. In this paper I will address the question of how this random walk is biased by a non-equilibrium chemical reaction (ATP hydrolysis) so that the motor molecule moves preferentially (with almost unit certainty) in one direction, even when an external force is applied to drive it in the opposite direction. I will also discuss how these 'soft matter' motors can achieve thermodynamic efficiencies of nearly 100%.

1. Introduction

Biomolecular motors are molecules (nanometre scale) that convert chemical free energy (often provided by hydrolysis of adenosine triphosphate (ATP)) into directed motion and into the performance of work on the environment. Pictures of these molecules obtained through x-ray diffraction and other structural studies are highly reminiscent of macroscopic machines [1, 2]. Animations widely available on the web further tend to depict them as carrying out a deterministic machine-like motion along their biopolymeric tracks (supplementary material to [3]). However, this notion of a molecular motor as a miniaturized version of a macroscopic machine is inconsistent with the physics of very small things in solution, where viscous drag and thermal noise totally dominate the inertial forces with which we are familiar from our macroscopic world. Rather than talking about levers, springs and dashpots, judo throws, rowing cross-bridges, or power strokes, let us start our discussion of how molecular motors work on the firm ground of equilibrium processes.

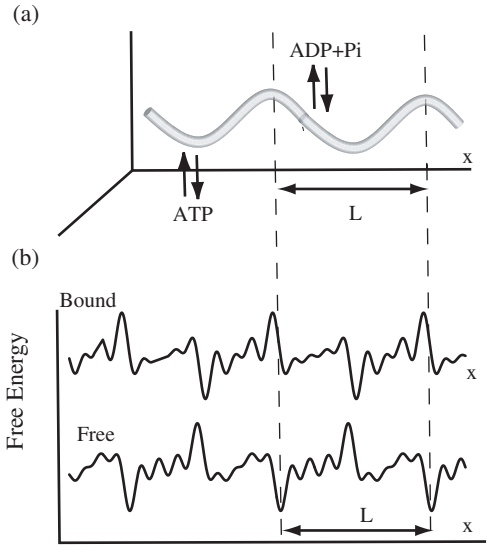

Figure 1. Schematic depiction of the motion of a molecular motor inside a tube describing the possible configurations of the protein. Progress through the tube corresponds to performance of the function—in the case of a motor, displacement by a distance L corresponds to a single 'step'. Motion perpendicular to the axis represents conformational fluctuation. Projection of the free energy onto the single position coordinate reveals a rough free energy landscape, the shape of which depends on whether the protein is bound to one of its ligands (ATP or ADP) or not.

2. Statement of the problem

Proteins and other macromolecules can assume many different configurations—arrangements of the monomeric subunits relative to one another. Whether we refer to an enzyme, to an ion channel, to a pump protein, or to a biomolecular motor, thermal interaction with the environment causes the molecule to randomly explore its various configurations.

Consider the case of a molecular motor moving on a biopolymeric track. At chemical and thermal equilibrium a motor molecule undergoes a random walk on its track, moving sometimes left, sometimes right, sometimes binding to its fuel, adenosine triphosphate (ATP), and products, adenosine diphosphate (ADP) and inorganic phosphate (Pi), and sometimes dissociating from them. Sometimes ATP is hydrolysed, and just as often ADP and Pi combine to make ATP. Equilibrium is maintained not by a static opposition of equal magnitude forces, but by dynamic processes in which every forward motion is exactly as likely as the microscopic reverse of that motion.

A schematic picture is shown in figure 1(a). We imagine the allowed (energetically accessible) protein configurations to describe a 'tube'. Directional progress along the axis of the tube corresponds to performance of the function of the motor and motion perpendicular to the axis represents conformational fluctuation. The thicker the tube the 'softer' (i.e., more conformationally flexible) the protein.

The motor interacts with the environment by associating and dissociating ATP and ADP and Pi as it moves, and of course by thermal exchange of energy with the aqueous solution. If the fluctuation of the protein perpendicular to the axis of the tube is fast compared to binding and release of reactants and products, we can project the contribution of the conformational flexibility onto the single coordinate along the axis of the polymeric track on which the motor

moves. The static contribution of the flexibility enters the free energy as entropy, and the dynamic contribution enters as friction and concomitant thermal noise forces in the description of the motion [4]. Schematic illustrations of rough potential energy landscapes on which the protein moves are shown in figure 1(b), one for the 'bound' state of the protein (i.e., the state where the active site for ATP hydrolysis is occupied) and the other for the 'free' state of the protein. These profiles reflect the underlying periodicity of the stepping process as well as the anisotropy of the polymeric track. At equilibrium there are transitions between the two landscapes due to binding and release of reactants and products, as well as diffusion along the axis of motion, but of course no directed motion.

What changes when we add more ATP so that the chemical reaction (ATP hydrolysis) is no longer at equilibrium? Are the accessible states of the protein different? Certainly not: there is no way for a protein to know what the chemical potential of ATP in the bulk solution is; the protein senses only whether it is bound or it is not bound to ATP. Do the transitions between the states of the protein have a different character when the chemical potential of ATP is higher than that of ADP and Pi in the bulk solution? For the same reason—the protein cannot be directly influenced by the chemical potentials of reactants in the bulk—the answer is also certainly no: the character of a transition between two states of the protein when the chemical reaction is away from equilibrium is exactly the same as the character of that transition when ATP is at equilibrium with ADP and Pi. Logically, the *only* difference in the presence of excess ATP is that a motor molecule that is not bound to ATP has a greater chance of binding to ATP. How does this increase in the rate of binding ATP translate into biasing the random walk of the motor protein so strongly that the motor can do work on the environment with almost unit efficiency and where the motor molecule takes almost one step for each ATP that is hydrolysed? That is the question I will address in this paper.

3. A specific example—kinesin moving on microtubule

The rough free energy surface can often be dramatically simplified by grouping families of rapidly equilibrating configurations into only a few conformational states. The transitions over the energy barriers between the states require thermal activation—reversible borrowing of energy from the environment—with rate coefficients of the form

$$k_{ij} = A \exp\left(\frac{g_i^0 - g_{ij}^{\ddagger}}{k_B T}\right) \tag{1}$$

where g_i^0 is the standard free energy of state 'i' and $g_{ij}^{\ddagger}$ is the free energy of the barrier separating state 'i' and 'j', k_B is Boltzmann's constant, T is the temperature (in Kelvin), and A is a frequency factor modelled either based on Eyring or Kramers rate theory [5]. The ratio of the equilibrium state probabilities $\overline{W_i}$ is

$$\frac{\overline{W_j}}{\overline{W_i}} = \frac{k_{ij}}{k_{ji}} = K_{ij} = \exp\left(\frac{g_i^0 - g_j^0}{k_B T}\right). \tag{2}$$

With this in mind let us consider a specific example.

Kinesin is a two-headed biomolecular motor that moves on a polymeric 'track' called a microtubule, that is polar in the sense that the two ends, '+' (front) and '−' (back), are distinguishable [6]. There is good evidence that kinesin moves on its track in a walking motion where the two heads move hand-over-hand. (Admittedly, this is an anatomically challenged nomenclature.) In a very simple picture we can talk about four states, {right head front, left head back} = {1}; {right head attached, left head free} = {2}; {right head back, left head front} = {3}; {right head free, left head attached} = {4} as shown in figure 2. It is clear that

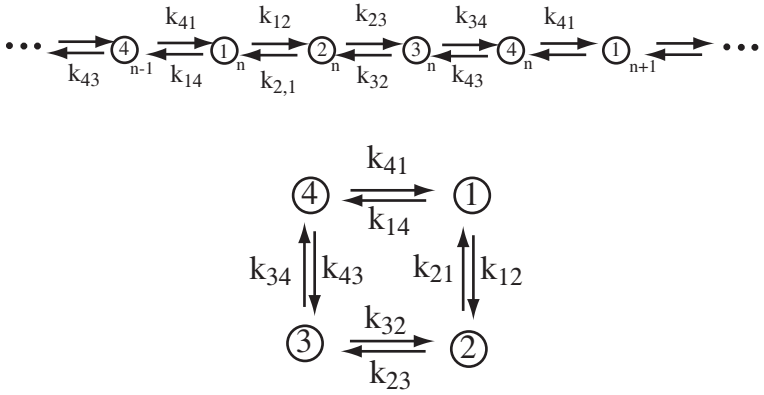

Figure 2. A four-state random walk. An interpretation of the states for a kinesin molecule is given in the text. The random walk can be represented either linearly, or as a cycle. In the latter case completion of a clockwise cycle corresponds to executing one full step to the right and completion of an anticlockwise cycle corresponds to making one step to the left.

cycling through these states in the order $1 \to 2 \to 3 \to 4 \to 1$ leads to a step to the front ('+' end) and cycling in the order $1 \to 4 \to 3 \to 2 \to 1$ leads to a step to the back ('−' end) of the microtubule [7]. For simplicity we ignore the possibility that the molecule can dissociate entirely from microtubule.

In the figure I do not draw the traditional pictures with feet, heads, springs and various other mechanically inspired accoutrement. Doing so would hide the generality of the theory, and would also call to mind mechanical principles that really have nothing to do with how molecular motors work.

4. Detailed balance

Even in the absence of an energy source the kinesin molecule undergoes a random walk along the microtubule by thermally activated transitions between the states. At equilibrium, however, on average each forward transition occurs just as often as the reverse of that transition. The principle describing this equilibrium balancing between forward and reverse transitions is known as detailed balance, and can be written mathematically as

$$\begin{aligned} k_{12}\overline{W_1} &= k_{21}\overline{W_2} \\ k_{23}\overline{W_2} &= k_{32}\overline{W_3} \\ k_{34}\overline{W_3} &= k_{43}\overline{W_4} \\ k_{41}\overline{W_4} &= k_{14}\overline{W_1}. \end{aligned} \tag{3}$$

The probabilities W_i are normalized so that $\sum_{i=1}^{4} W_\mathrm{i} = 1$ whether at or away from equilibrium.

4.1. A necessary but not sufficient condition for detailed balance

A corollary relation between the rate coefficients,

$$\frac{k_{12}k_{23}k_{34}k_{41}}{k_{14}k_{43}k_{32}k_{21}} = 1, \tag{4}$$

can easily be derived from equation (3). Note that equation (4) can also be derived from the more fundamental thermodynamic relation equation (2). The relation equation (4) is often

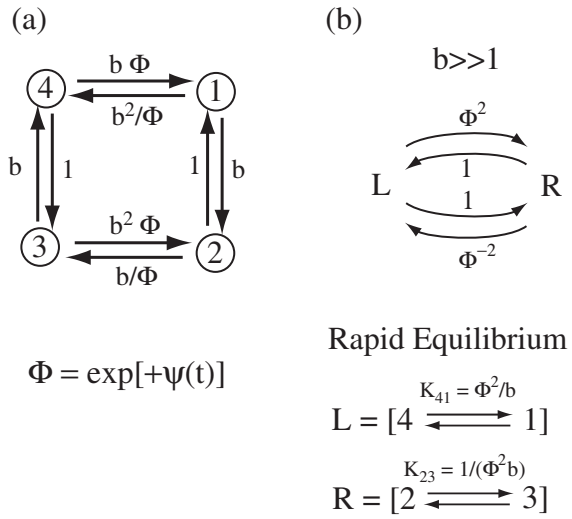

Figure 3. (a) A four state cycle with rate coefficients that allow oscillation of an external control parameter $\psi(t)$ to drive net cycling even while equation (4), a corollary derivable from the condition of detailed balance at equilibrium, holds at every instant. The system undergoes directional cycling even if ψ varies randomly [10]. Particularly interesting is the case where $\psi(t)$ fluctuates between two values, say $+\Psi$ and $-\Psi$, with a Poisson distributed lifetime, thus mimicking the kinetics of a simple two-state reaction. (b) Two-state 'reduction' of the four-state cycle. With $b \gg 1$ states 1 and 4 are in rapid equilibrium, as are states 2 and 3. We denote the overall state {1} + {4} as 'L' (for left) and the overall state {2} + {3} as 'R' (for right).

mistakenly identified as the condition of detailed balance itself. Indeed, Joe Howard in his well known text on molecular motors [8] explicitly claims that equation (4) is a necessary and sufficient condition for detailed balance, equation (3), to hold. If this were the case it would be impossible for a non-equilibrium chemical reaction such as ATP hydrolysis to drive directed motion—equation (4) must hold irrespective of what chemical species is bound to the protein. Cycling through a series of chemical states where detailed balance for the protein stepping transitions holds in each chemical state could not possibly lead to directed motion. However, while equation (4) is a necessary condition, it is *not* a sufficient condition for detailed balance if the rate coefficients for stepping depend on time, as they must, due to binding and release of ATP, ADP, and Pi [9, 10].

5. Time dependent rate coefficients

As the protein motor undergoes transitions between different chemical states—free, bound to ATP, bound to ADP, etc—the transition coefficients fluctuate depending on the chemical state of the protein. For each chemical state individually of course the rate coefficients conform to equation (4). How can fluctuations that preserve the relation expressed by equation (4) at every instant in time still drive directed motion? This was addressed in a series of papers in *PNAS* in the mid-1980s [9–11]. Consider the cycle with rate coefficients shown in figure 3, where $\psi(t)$ is an external control parameter (an energy normalized by $k_B T$) and b is a bias that governs the relative stability of the states. For $b > 1$ states $\{4\}$ and $\{2\}$ are more stable than states $\{1\}$ and $\{3\}$ when $\psi = 0$. Equation (4) is satisfied irrespective of the value of b or of ψ. Nevertheless, oscillation or fluctuation of ψ causes directed motion to the right (clockwise cycling) if $b > 1$ and to the left (anticlockwise cycling) if $b < 1$.

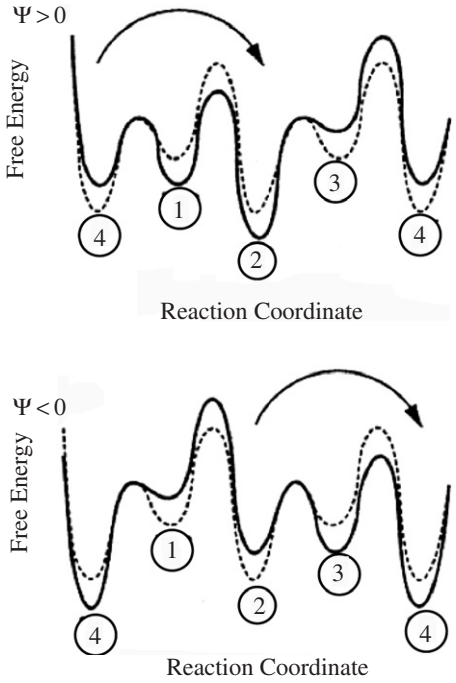

Figure 4. Free energy diagrams for the four-state cycle in figure 3 with positive and negative ψ. The net free energy change through a cycle is zero (the free energy of state four on the left and right of both profiles is the same) so equation (4) holds at every instant. However, changing ψ between two values (here positive and negative) induces net clockwise flow as indicated by the arrows showing the kinetically preferred path for relaxation between states 4 and 2 each time ψ changes sign. This figure was adapted from that given by Robertson and Astumian [12].

Consider the case where $b > 1$. When $\psi > 0$ state $\{2\}$ is the globally most stable state, and when $\psi < 0$ state $\{4\}$ is the most stable state. Therefore, as ψ changes back and forth between positive and negative values the molecule shifts back and forth between preferentially being in state $\{2\}$ and in state $\{4\}$. Figure 4 (adapted from Robertson and Astumian [12]) illustrates heuristically how a fluctuation of the control parameter 'ψ' leads to directed cycling where the *clockwise* path to the instantaneously most stable state is always favoured.

The dashed curves show the potential energy landscape consistent with rate coefficients in figure 3 with $\psi = 0$, and hence with states $\{2\}$ and $\{4\}$ equally stable. When ψ is positive ($\psi > 0$) state $\{2\}$ is energetically more stable than state $\{4\}$. Those motor molecules initially in state $\{4\}$ relax to state $\{2\}$ predominantly through state $\{3\}$ since the path through state $\{1\}$ is kinetically blocked. Then, when ψ becomes less than zero molecules in state $\{2\}$ relax to the now energetically favoured state $\{4\}$, this time predominantly through state $\{1\}$ since the path through state $\{3\}$ is kinetically blocked. Thus oscillation or fluctuation of the control parameter ψ leads preferentially to the sequence $\{4\} \to \{1\} \to \{2\} \to \{3\} \to \{4\}$, clockwise cycling which corresponds to stepping to the '+' end of the microtubule. With the relatively symmetric rate coefficients used in figure 3, there are two relaxation frequencies for the four-state cycle. For large b, the smaller relaxation frequency (in relative units) is approximately unity, while the larger frequency is approximately $b/2$. The optimal frequency for external modulation of ψ lies between these two values. The molecule takes one step to the right for each cycle $+\psi \to -\psi \to +\psi$ (and hence the rate of cycling is proportional to frequency)

up to frequencies approaching $b/2$, beyond which the number of steps per cycle of ψ drops quadratically (and hence the rate is proportional to the inverse of the frequency).

External oscillation or fluctuation of ψ requires energy to be pumped into the system. Some of this energy can be used to drive directed cycling of a molecular machine and can be used to do work on the environment—pumping molecules from low to high electrochemical potential, synthesizing a high free energy chemical, or moving a molecule against an external force. The thermodynamic efficiency of this energy conversion has no fundamental limit less than unity [13].

6. Driving motion with a non-equilibrium chemical reaction

It is quite natural to identify the control parameter with the effect of a chemical reaction, where, e.g., ψ takes on two different values, say $+\Psi$ and $-\Psi$, depending on whether the nucleotide binding site of the motor is occupied (denoted by subscript 'o') or unoccupied (denoted by subscript 'u'). In this case, we can write a kinetic lattice model [14] where the progress along the microtubule is plotted on one coordinate, and the chemical reaction on another coordinate (figure 5). If the association/dissociation of ATP is slow compared to relaxation $4_u \rightarrow 1_u \rightarrow 2_u$ but fast compared to $4_u \rightarrow 3_u \rightarrow 2_u$, and the association/dissociation of ADP+Pi is slow compared to relaxation $2_o \rightarrow 3_o \rightarrow 4_o$ but fast compared to $2_o \rightarrow 1_o \rightarrow 4_o$, the preferred pathway—the mechanism through which most of the kinetic 'traffic' passes—is that outlined by the dashed lines on figure 5. At chemical equilibrium, this traffic is bidirectional, equally heavy in both directions, but when ATP in excess of the equilibrium concentration is added the mechanism is biased toward motion from top to bottom (and hence from left to right) along the zigzag pathway hewn out by the bias (and the interaction parameter ψ) which have their ultimate origin in the structure of the molecule. For simplicity we have taken the case where the chemical and mechanical coordinates are orthogonal so that in a single step the protein either undergoes a conformational change in which it moves along the microtubule *or* it changes chemical state by association/dissociation of ATP, or ADP and Pi. It is of course possible that a motor could simultaneously move and associate/dissociate a ligand in a single step. For a treatment of this and other possibilities see the elegant extension of our model [14] by Keller and Bustamante [15].

In the limit where b is very large, the four-state cycle can be reduced to a two-state cycle [16] shown in figure 3(b), where states 4 and 1 are in rapid equilibrium and designated as state L (left head attached), and states 3 and 2 are in rapid equibrium and designated as state R (right head attached). The resulting two-state lattice model [14] (figure 6) predicts an interesting result recently observed experimentally.

6.1. Experimental verification of a prediction of the Brownian motor model

In a recent paper, Carter and Cross [17] discuss the mechanics of kinesin stepping, focusing on the effect of an applied load force. They show that a sufficiently large force can induce processive backward stepping, i.e., the motor takes many steps toward the '−' end of the microtubule without dissociating from the microtubule. Surprisingly, this backward stepping is stimulated at large ATP concentration, and may in fact be coupled to ATP hydrolysis. This is surprising because simple mechanisms based on completely coupled kinetic cycles predict that backward stepping should be accompanied by ATP synthesis [18], and hence inhibited by large ATP concentrations.

The backward stepping of kinesin and its stimulation by ATP reported by Carter and Cross are explicitly predicted by a Brownian motor model (figure 7(d) in [14]) in which the

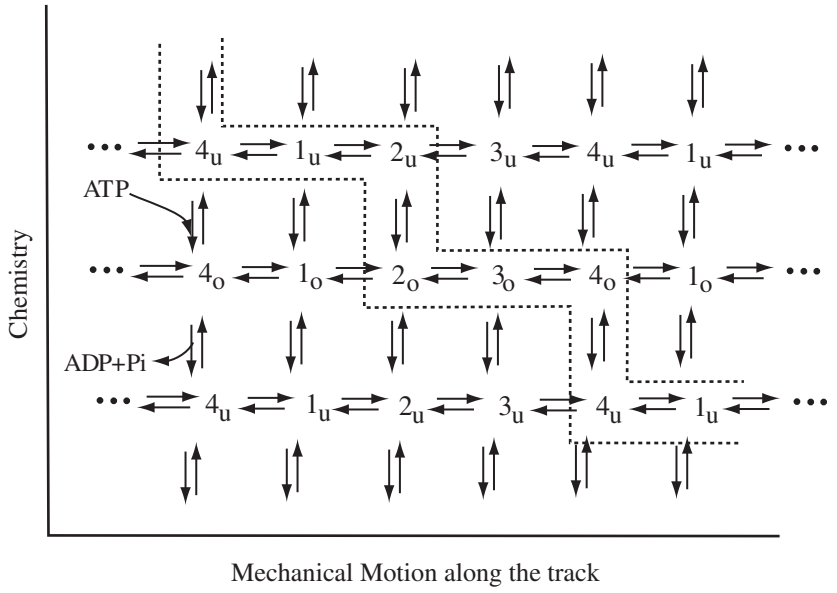

Figure 5. Kinetic lattice model described in text. The dashed line indicates the preferred pathway under no-load conditions.

conformational changes in the molecule leading to translation along the microtubule are treated as thermally activated diffusion over an energy barrier. The binding of ATP and release of ADP and Pi change the free energy landscape along the microtubule depending on whether the active site of the molecule is or is not occupied, but transitions from one state to the next arise by Brownian motion. The switching between the landscape for the states of the protein where the ATP active site is 'occupied' and 'unoccupied' produces a flashing ratchet effect [20, 21] that moves the kinesin unidirectionally along the microtubule.

The mechanism is 'loosely' coupled in that slip—ATP hydrolysis uncoupled from translation along the microtubule, and vice versa—is explicitly possible, but if the interaction is sufficiently strong the slip can be very small under a wide range of circumstances, reproducing the experimental observation that there is a one-to-one stoichiometry between ATP hydrolysis and motor stepping.

The motor can exist in two distinct chemical states, where the active site for ATP hydrolysis is either occupied (subscript o) or unoccupied (subscript u), and two distinct conformational state, one with the left head bound (denoted L) and the other with the right head bound (denoted R). Thus there are a total of four states, L_o, L_u, R_o, and R_u. A molecule in state L (R) can undergo transition to state R (L) either forward or backward (to the '+' end or '−' end, respectively), and an unoccupied (occupied) state can undergo transition to an occupied (unoccupied) state by associating (dissociating) ATP or ADP and Pi.

The simplest way to take the effect of the ATP reaction into account is to multiply the rate coefficients for the clockwise curved arrows by a factor $\Gamma = \exp(\Delta G_{ATP}/4)$ and those for the anticlockwise curved arrows by $\Gamma = \exp(-\Delta G_{ATP}/4)$. Similarly, in the simplest case, an applied force F (we take a force directed to the left to be positive) causes all left–right transition coefficients to be multiplied by $\Lambda^{-1} = \exp(-FL/4)$, and all right–left transition coefficients are multiplied by $\Lambda = \exp(+FL/4)$. The different energy landscape for the motor when the nucleotide binding site is occupied versus when the binding site is unoccupied is described

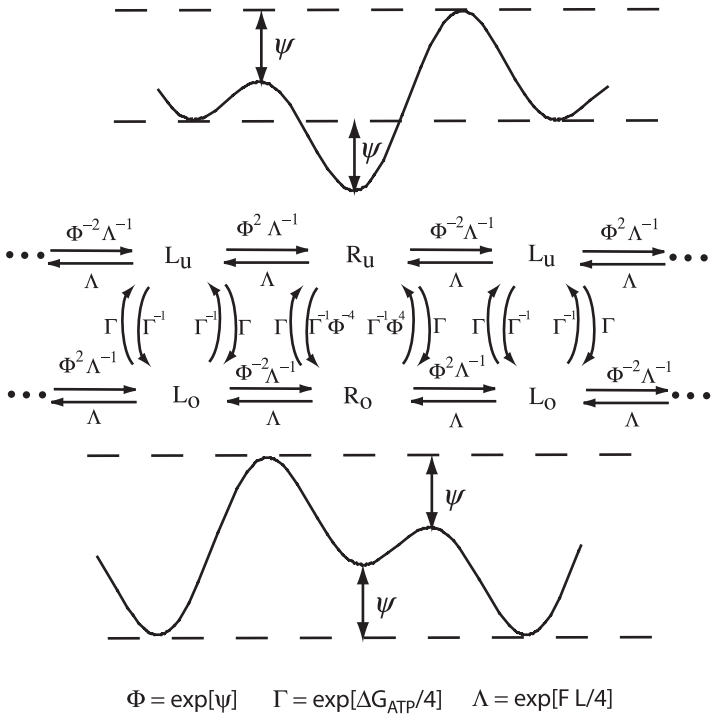

Figure 6. Basic kinetic model of a Brownian motor mechanism for kinesin. When the ATP hydrolysis reaction is away from equilibrium the random walk is biased to the right even against an applied force FL $< \Delta G_{\rm ATP}$ that tends to bias the walk to the left. The coupling requires that the free energy surface for the stepping be different when the nucleotide binding site is occupied than when it is unoccupied. Model free-energy landscapes for the occupied and unoccupied chemical states are shown, respectively, above the diagrams for the kinetic random walk for the two chemical states. The x-axis can be thought of as the centre-of-mass position of the motor along the microtubule.

with the interaction parameter $\Phi = \exp(\Psi)$. All energies are written in units of $k_{\rm B}T$, the Boltzmann constant multiplied by the Kelvin temperature. With these rate coefficients the motor velocity and the rate of ATP hydrolysis can be written in terms of the state probabilities as

$$\begin{aligned} &\text{motor velocity}/L = \Phi^2\Lambda^{-1}L_{\rm u} - \Lambda R_{\rm u} + \Phi^{-2}\Lambda^{-1}L_{\rm o} - \Lambda R_{\rm o} \\ &\text{ATP hydrolysis rate} = \Gamma L_{\rm u} + \Gamma R_{\rm u} - \Gamma^{-1}L_{\rm o} - \Gamma^{-1}\Phi^4 R_{\rm o}. \end{aligned} \tag{5}$$

When the interaction term $\Psi = 0$ the state probabilities are all one-quarter irrespective of the value of F or of $\Delta G_{\rm ATP}$ since the rate coefficients into and out of each state are identical. In this case, the motor velocity depends only on the applied force, F, and the rate of ATP hydrolysis depends only on $\Delta G_{\rm ATP}$. However, with $\Psi \neq 0$ the state probabilities do depend on both F and $\Delta G_{\rm ATP}$, and hence the motor velocity is coupled to $\Delta G_{\rm ATP}$, and the rate of ATP hydrolysis is coupled to the applied force. This is despite the fact that none of the rate coefficients for the stepping depend on $\Delta G_{\rm ATP}$, nor do the rate coefficients for ATP hydrolysis depend on the applied force. In consistency with the second law of thermodynamics, when $\Delta G_{\rm ATP} = 0$ and $F = 0$ there is diffusion, but no directed motion of the motor irrespective of the value of Ψ. In this model, the role of ATP in excess of its equilibrium concentration is to overcome by mass action the energetically unfavourable transition $R_{\rm u} \rightarrow R_{\rm o}$, but the motion out of state $R_{\rm o}$ is due to thermal noise, hence the description of this mechanism as a Brownian motor [19].

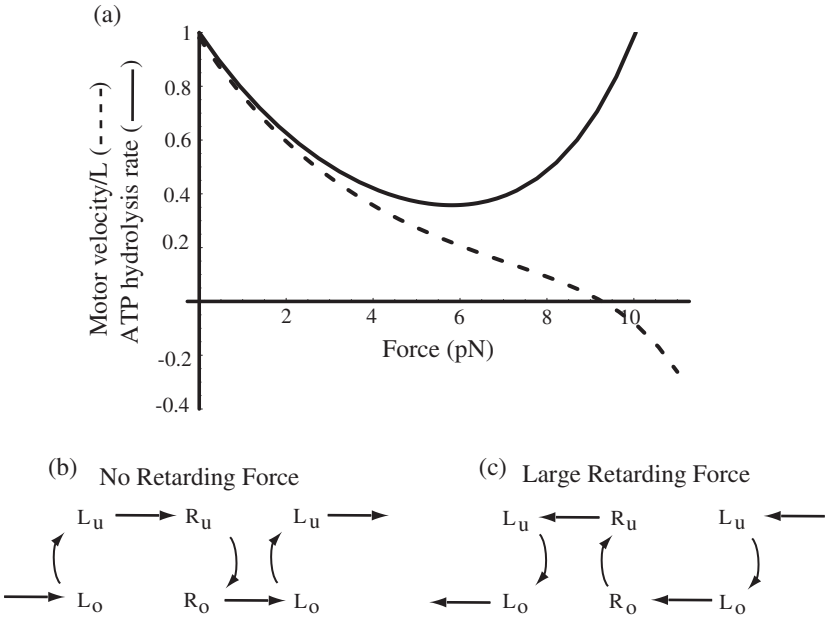

Figure 7. (a) Graph of the ATP hydrolysis rate (in ATP molecules hydrolysed per unit time) and motor velocity (in steps of distance L per ATP hydrolysed) as a function of the applied load force calculated from equation (5), where the state probabilities were calculated as described in [14]. (b) A skeleton mechanism obtained by drawing an arrow for only the thermodynamically spontaneous transition with the largest rate constant out of each state (see figure 6) for no load force. (c) The same as for (b) but now with a large load force.

This model directly predicts that a sufficiently strong load force should induce backstepping of the motor, and that this backstepping is facilitated by *hydrolysis* of ATP [14] (figure 7(a)). This result can be understood by considering a simplifying limiting case. In the absence of an applied force we take the thermodynamically favoured path out of each state without retracing a step to obtain the graph labelled 'No Retarding Force', figure 7(b). For a large force, carrying out the same procedure leads to the graph labelled 'Large Retarding Force', figure 7(c). These graphs represent the most probable (though certainly not the only) pathways. As predicted by the quantitative calculation (figure 7(a)), both forward and backward motion along the microtubule is stimulated by ATP hydrolysis, although it is certainly *not* the case where ATP hydrolysis 'fuels' the backward motion. At intermediate force, the factor Γ is comparable to $\Phi\Lambda^{-1}$. In this case, on average several ATPs are hydrolysed for each forward step.

7. Efficiency

While there is no reason to think that the conversion of chemical free energy to directed motion of a motor against an applied force should be constrained by any Carnot-like relation (the process is after all conversion of one form of free energy to another, not conversion of heat to free energy), it is surprising that molecules made out of soft materials, with many degrees of freedom, can be used to construct almost perfectly efficient machines. This is quite a contrast with the approach taken in constructing macroscopic machines where great care is take to eliminate extra vibrations, jitter, and any motion other than that directly linking the input with

the output—no one would make a macroscopic motor out of modelling clay! How is a high efficiency for a molecular motor possible? We can understand the answer to this question by comparing a macroscopic mechanical approach with a chemical approach for accomplishing a simple task (figure 8)—the transfer of a particle from a low energy ($E_0 \equiv 0$) well on the left to a higher energy (μ) well on the right, thereby storing an amount of energy μ.

In the mechanical approach, an external force is used to *push* the particle from the lower to the higher well. The external source must supply *at least* the activation energy ($E^{\ddagger}$, the energy gap between the bottom of the low well and the saddle point connecting the two wells) and generally will need to provide more energy than this since the particle will not necessarily take the shortest path but will wander from side to side due to the softness of the potential and the presence of thermal noise. The maximum thermodynamic efficiency (energy stored/energy consumed) is thus $\eta_{\max} = \mu/(E^{\ddagger})$, and will generally be much less than this for a soft, conformationally flexible (squishy) system.

In the chemical approach, external energy (e.g., binding of a high free-energy substrate) is used to destabilize the lower energy well relative to the higher well by $\mu + \epsilon$. If this is accomplished slowly relative to the very rapid intra-well relaxation [22], the system remains in local equilibrium and the energy required is just $\mu + \epsilon$. Following this, thermal activation provides the mechanism for relaxation to the right-hand well. After resetting the left-hand well (e.g., by dissociation of a low free-energy product) the system is restored to the original state, but now with the particle in the higher free-energy well. When this mechanism is set up to carry out continuous energy conversion (as in a molecular pump [23, 24] or molecular motor [25]), the maximum efficiency can be written

$$\eta_{\max} = \frac{\mu}{\epsilon + \mu} \tanh(\epsilon/2). \tag{6}$$

The hyperbolic tangent function incorporates the unavoidable possibility for the mechanism to slip backward, thereby losing energy. With $\mu + \epsilon = 22\ k_{\mathrm{B}}T = 90$ pN nm, consistent with the energy available from ATP hydrolysis, this efficiency is maximized at about 80% when the external load $FL = 20\ k_{\mathrm{B}}T$, corresponding to a force of about 8 pN for a motor with $L = 10^{-8}$ m.

It has been argued that the mechanism for a molecular motor must be very different than that for, say, an ion pump, since a molecular motor must often drive a load (typically an organelle or vesicle) through a viscous medium [26]. This argument is wrong for several reasons. First, viscosity does enter a chemical kinetic model through the frequency factor in equation (1), which must be less than D/L^2, where the diffusion coefficient $D = k_{\mathrm{B}}T/\gamma$ is the thermal energy divided by the coefficient of viscous friction according to the Stokes–Einstein relation. What this means is that the coupled mechanism of ATP-driven forward motion is self-regulating. When the viscosity increases, both ATP hydrolysis and motor transport slow down concomitantly, preventing the motor from spinning its wheels.

Further, while viscous drag clearly can be a major energy constraint for an artificial construct such as an F0F1 ATPase forced to twirl a long actin filament through water [27], it is far less important for kinesin dragging a vesicle. The power $(6\pi\eta r)v^2$ required to pull a 1 μm sphere ($r = 10^{-6}$) m through water [$\eta = 10^{-3}$ kg m^{-1} s^{-1}] at 1 μm s^{-1} ($v = 10^{-6}$ m s^{-1}) is $\approx 2 \times 10^{-20}$ W, which is very small compared to the energy released by the typical hydrolysis rate of 100 ATPs per second (90×10^{-21} J $\times$ 100 s^{-1} $\approx 10^{-17}$ W). Certainly under some circumstances the generalized efficiency [28] (see also [29] in this volume), which includes work done pulling or pushing a load through a viscous solution, may play an important role in interpreting how well molecular motors perform their jobs [30]. It is however unlikely to be a key factor under most conditions for single biomolecular motors.

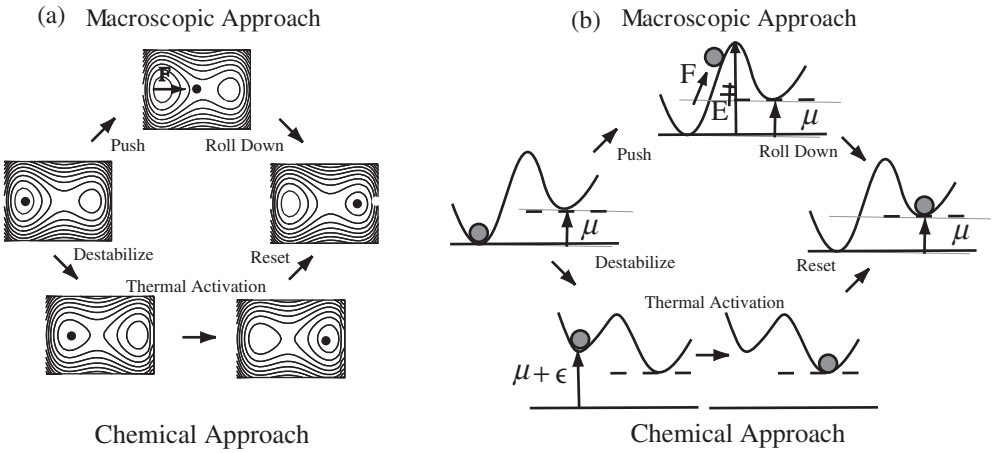

Figure 8. Cartoon illustration of the difference between a mechanical and a chemical approach for storing energy by transferring a particle from a low energy well to a well at a higher energy. (a) A two-dimensional representation. Because of the 'softness' of the potential, in the 'macroscopic approach' an external source provides a force to push the molecule over the barrier. Side to side motion due to thermal noise would result in a longer path to the top, and thus require more energy from the external source than the minimum, $E^{\ddagger}$, resulting in an inefficient motor. In the chemical approach however the input energy is used to destabilize the system in the left-hand well, but the motion itself is driven by thermal activation over the barrier. In this case, the side to side jitter does not decrease the efficiency. (b) A one-dimensional representation of the same process.

8. Conclusion

The random walk (Brownian motor) model for a molecular motor is doubtless unsatisfying to many. There is no specific reference to the structure of the molecule, only to an interaction energy (which does of course have a structural origin), and there is no special, crucial conformational step (power stroke?) that can be identified as that point at which energy transduction occurs. Mechano-chemical coupling is a property of the system as a whole in the Brownian motor picture. Further, the description of the mechanism involves only probabilities, in contrast to the comfortingly familiar mechanical analogies often used to describe the motion of biomolecular motors.

Nevertheless, there is good reason to believe that the motions of molecular motors are not qualitatively different than the motions of other proteins, and are best described as thermally activated stochastic processes with Poisson distributed lifetimes. There may well be a large global conformational change in the molecule as it goes through its coupled chemical and conformational cycle, but if so the same conformational change also occurs at chemical equilibrium, and hence it is hardly reasonable to call it a 'power stroke'. The Brownian motor mechanism is based on thermally activated transitions between states that are very close to 'local' equilibrium with respect to their internal degrees of freedom despite the fact that the chemical reaction that drives motion, ATP hydrolysis, is very far from equilibrium. In this way it is analogous to the way in which ion pumps function [31]. By operating where all conformational states are near to local equilibrium and by using a chemical (stabilization/de-stabilization) approach rather than a mechanical approach, a biomolecular motor made of 'soft', conformationally flexible, protein can operate at nearly perfect efficiency. The Brownian motor model has been implemented in a synthetic molecular motor, where the raising and lowering

of the barriers and wells is accomplished by external manipulation of the chemistry (pH, reduction/oxidation potential, etc) [32].

Soon, the ultimate arbiter—experiment—will be in a position to speak as to the mechanism of biomolecular motors. By observing in the same experiment at a single-molecule level an individual ATP hydrolysis event followed by the completion of a step of the motor along its track, the distribution of time lapse between these two events can be determined. The random walk (Brownian motor) model presented here predicts that the time between ATP hydrolysis and completion of a step will be randomly (Poisson) distributed rather than deterministic as expected from a mechanical model. Resolution of this question will be important not only for understanding the mechanisms of biomolecular motors, but also in the design of synthetic molecular motors [32].

References

[1] Kull F J, Sablin E P, Lau R, Fletterick R J and Vale R D 1996 Crystal structure of the kinesin motor domain reveals a structural similarity to myosin *Nature* **380** 550–4

[2] Rayment I 1996 Kinesin and myosin: molecular motors with similar engines *Structure* **4** 501–4

[3] Vale R D and Milligan R M 2000 The way things move: looking under the hood of molecular motor proteins *Science* **288** 88–95

[4] Frauenfelder H, Sligar S G and Wolynes P G 1991 The energy landscapes and motions of proteins *Science* **254** 1598–603

[5] Hanggi P, Talkner P and Borkovec M 1990 Reaction rate theory: fifty years after Kramers *Rev. Mod. Phys.* **62** 251–341

[6] Howard J 1996 The movement of kinesin along microtubules *Annu. Rev. Physiol.* **58** 703–29

[7] Hill T L 1977 *Free Energy Transduction in Biology* (New York: Academic)

[8] Howard J 2001 *Mechanics of Motor Proteins and the Cytoskeleton* (Sunderland, MA: Sinauer)

[9] Westerhoff H V, Tsong T Y, Chock P B, Chen Y D and Astumian R D 1986 How enzymes can capture and transmit free energy contained in an oscillating electric field *Proc. Natl Acad. Sci. USA* **83** 4734–8

[10] Astumian R D, Chock P B, Tsong T Y, Chen Y D and Westerhoff H V 1987 Can free energy be transduced from electrical noise? *Proc. Natl Acad. Sci. USA* **84** 434–8

[11] Kamp F, Astumian R D and Westerhoff H V 1988 Coupling of vectorial proton-flow to a biochemical reaction by localized electric interactions *Proc. Natl Acad. Sci. USA* **85** 3792–6

[12] Robertson B and Astumian R D 1990 Michaelis–Menten equation for an enzyme in an oscillating electric field *Biophys. J.* **58** 969–74

[13] Markin V S, Tsong T Y, Astumian R D and Robertson B 1990 Energy transduction between a concentration gradient and an alternating electric field *J. Chem. Phys.* **93** 5062–6

[14] Astumian R D and Bier M 1996 Mechanochemical coupling of the motion of molecular motors to ATP hydrolysis *Biophys. J.* **70** 637–53

[15] Keller D and Bustamante C 2000 The mechanochemistry of molecular motors *Biophys. J.* **78** 541–56

[16] Astumian R D and Robertson B 1989 Nonlinear effect of an oscillating electric field on membrane proteins *J. Chem. Phys.* **91** 4891–901

[17] Carter N J and Cross R A 2005 Mechanics of the kinesin step *Nature* **435** 03528

[18] Fisher M E and Kolomeisky A B 2001 Simple mechano-chemistry describes the dynamics of kinesin molecules *Proc. Natl Acad. Sci. USA* **98** 7748–53

[19] Astumian R D and Hönggi P 2002 Brownian motors *Phys. Today* **55** (11) 33–9

[20] Astumian R D and Bier M 1994 Fluctuation driven ratchets—molecular motors *Phys. Rev. Lett.* **72** 1766–9

[21] Prost J, Chauwin J-F, Peliti L and Ajdari A 1994 Asymmetric pumping of particles *Phys. Rev. Lett.* **72** 2652–5

[22] Derenyi I and Astumian R D 1999 Intrawell relaxation time: the limit of the adiabatic approximation *Phys. Rev. Lett.* **82** 2623–7

[23] Astumian R D and Derenyi I 2001 Towards a chemically driven electron pump *Phys. Rev. Lett.* **86** 3859–62

[24] Astumian R D 2003 Adiabatic pumping mechanism for ion motive ATPases *Phys. Rev. Lett.* **91** 118102

[25] Astumian R D 2005 Chemical peristalsis *Proc. Natl Acad. Sci. USA* **102** 1843–7

[26] Bier M 2004 Processive motor protein as an overdamped Brownian stepper *Phys. Rev. Lett.* **91** 148104

[27] Yasuda Y, Noji H, Kinoshita K and Yoshida M 1998 F_1-ATPase is a highly efficient molecular motor that rotates with discrete 120° steps *Cell* **93** 1117–24

[28] Derenyi I, Bier M and Astumian R D 1999 Generalized efficiency and its application to microscopic engines *Phys. Rev. Lett.* **83** 903–6
[29] Wang H 2005 Chemical and mechanical efficiencies of molecular motors and implications for motor mechanisms *J. Phys.: Condens. Matter* **17** S3997–4014
[30] Linke H, Downton M and Zuckermann M 2005 Performance charateristics of brownian motors *Chaos* **15** 026111
[31] Lauger P 1990 *Electrogenic Ion Pumps* (Sunderland, MA: Sinauer)
[32] Hernandez J V, Kay E R and Leigh D A 2004 A reversible synthetic rotary molecular motor *Science* **306** 1532–7