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The role of thermal activation in motion and force generation by molecular motors

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The currently accepted mechanism for ATP-driven motion of kinesin is called the hand-over-hand model, where some chemical transition during the ATP hydrolysis cycle stretches a spring, and motion and force production result from the subsequent relaxation. It is essential in this mechanism for the moving head of kinesin to dissociate, while the other head remains firmly attached to the microtubule. Here we propose an alternative Brownian motor model where the action of ATP modulates the interaction potential between kinesin and the microtubule rather than a spring internal to the kinesin molecule alone. In this model neither head need dissociate (which predicts that under some circumstances a single-headed kinesin can display processive motion) and the transitions by which the motor moves are best described as thermally activated steps. This model is consistent with a wide range of experimental data on the force–velocity curves, the one ATP to one-step stoichiometry observed at small load, and the stochastic properties of the stepping.

Keywords: molecular motors; ion pumps; Brownian ratchets; thermal activation

1. INTRODUCTION

An important question for understanding biomolecular motors is whether these proteins are best modelled as miniature versions of macroscopic deterministic devices, or whether they are intrinsically Brownian motors that work on the very different principle of biasing thermal noise. A common misconception is that this question boils down to whether a conformational change of the motor molecule is required for motion, or whether motion takes place by simple diffusion of the protein motor as a whole. This is a red herring—it seems almost certain that some conformational change is involved. The critical issue is whether the conformational change requires thermal activation, with a Poisson-distributed stochastic completion time, or whether the process is more like a viscoelastic relaxation with a deterministic completion time—a 'power stroke'.

To highlight this point, we first consider the mechanism by which an ion pump is able to use chemical or electrical energy to drive transport of ions against an electrochemical gradient. It is very well established that this involves a conformational change of the pump protein between states each of which is close to thermal equilibrium. Transitions between the states are activated by thermal noise, and are well modelled by chemical kinetic theory. This picture is able to explain recent work in which externally applied oscillating or fluctuating electric fields substitute for the energy normally provided by ATP hydrolysis to drive ion transport.

Using the concepts developed for ion pumps, we describe a simple model for the molecular motors kinesin and Ncd. These motors have a similar structure but use chemical energy from ATP hydrolysis to drive motion in opposite directions along microtubules. The model is based on a 'Brownian ratchet' in which the direction of motion of the motor is controlled by the chemical mechanism of ATP hydrolysis and is an inherent property of a single head. In contrast to conventional 'power stroke' models, dissociation of the individual heads is not obligatory in the chemomechanical cycle, and the steps during which motion and force generation occur are best described as one-dimensional thermally activated transitions that take place while both heads are attached to the microtubule. The predictions of this model are consistent with all major experimentally observed characteristics of kinesin: one-to-one stoichiometry, maximum velocity of about $1\,\mu m\,s^{-1}$, and a stopping force of about 5 pN. Furthermore, the thermodynamic efficiency for this Brownian motor can approach unity, even at finite velocity. We also discuss how in single-molecule experiments the variance of the distance moved in a given time is expected to depend on concentration of fuel, ATP, and compare this model with the observed behaviour of kinesin.

2. THERMAL NOISE AND ACTIVATION OVER ENERGY BARRIERS

A particle in solution is subject to random collisions with solvent molecules giving rise to the erratic 'Brownian' motion first observed and reported by Robert Brown in 1826. This dynamic behaviour was described theoretically by Langevin, who hypothesized that the forces on the particle due to the solvent can be split into two components: (i) a fluctuating force that changes magnitude and direction very frequently compared to any other time-scale of the system; and (ii) a viscous drag

orce that always acts to slow the motion induced by the uctuation term. Einstein derived a (fluctuation–dissipa-ion) relationship between the magnitude of the fluctua-ion term and the viscous drag coefficient that dampens s effect. Because the strength of the fluctuation increases ith temperature, the fluctuating force is often called hermal noise. If the particle is a molecule, bombardment y the solvent also allows exploration of the different olecular configurations, i.e. the arrangements of the toms of the molecule relative to each other. Biological and many other) macromolecules often have only a few table configurations, called conformations, with large nergy barriers separating them. Thermal noise 'activates' ransitions over these barriers, allowing passage from one onformation to another. Almost all chemical reaction athways are described in terms of rate constants that pecify the probability that thermal noise will provide ufficient energy to surmount barriers separating hemical states.

Despite sharing the similar function of using chemical nergy to drive vectorial transport, the effect of thermal oise on molecular motors and pumps is typically epicted from entirely different standpoints. Molecular umps are most often modelled in terms of chemical inetics, where ATP energy is used to change the relative ffinities of and barrier heights between different binding tes by sequentially favouring different conformational tates of the protein as ATP is bound, hydrolysed, and the roducts released. The conformational relaxation and olecular transport across the membrane are treated as hermally activated steps.

Models for molecular motors, on the other hand, have ocused on an ATP-driven 'power stroke', a viscoelastic elaxation process where the protein starts from a non-quilibrium, 'strained' conformation following product elease. The subsequent relaxation does not require hermal activation and can be visualized much as the ontraction of a stretched rubber band. In many ways rotein motors have been modelled as miniature versions f macroscopic devices, employing springs, cogs, levers, nd the like, to effect motion and force generation, where he inescapable molecular fluctuations arising from inter-ction with the medium are viewed as a nuisance to be vercome rather than as an essential feature that can be arnessed to allow for regulation of the timing between hemical and mechanical steps.

At first it may seem that the mechanism for using hemical energy to allow molecular motors to move ver great distances and exert large forces must indeed e fundamentally different from the way that mole-ular pumps sit in place in a membrane and use nergy from ATP hydrolysis to bias the diffusion of mall molecules and ions, and do work against an elec-rochemical gradient. However, the physics of motion f small things in viscous solution (low Reynolds umber motion) shows that these processes may not be s different as our macroscopically based intuition ould suggest and that perhaps the functions of mole-ular motors and pumps share a common mechanism. ecent work on 'Brownian ratchets' (Astumian & Bier 994; Astumian 1997; Hänggi & Bartussek 1996; ülicher *et al*. 1997; Prost *et al*. 1994) may provide the nifying link.

3. BROWNIAN RATCHETS AND ION PUMPS

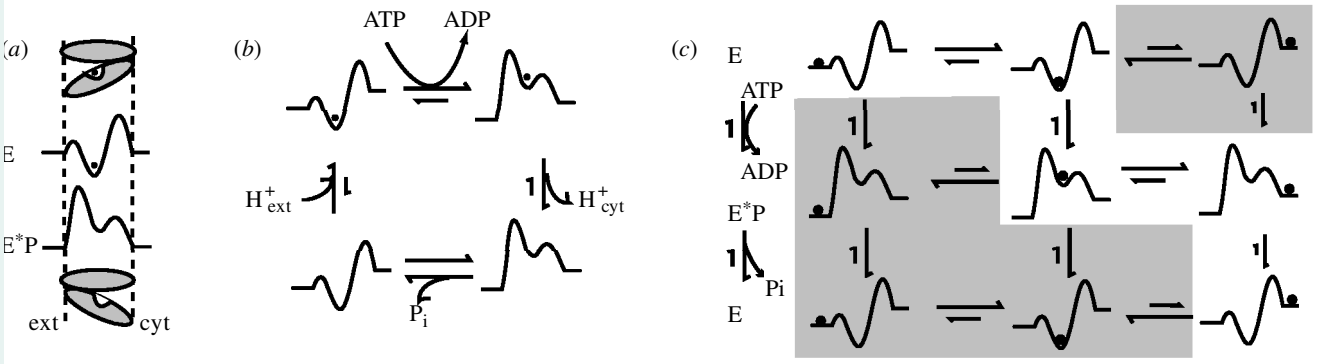
Perhaps the strongest direct evidence for a ratchet mechanism for free energy transduction by a biomolecule comes from recent experiments showing that the Na,K-ATPase, a biomolecular ion pump can use an external oscillating (Liu *et al*. 1990) or randomly fluctuating (Xie *et al*. 1994, 1997) electric field to drive unidirectional transport.

Much work has been done on characterization of the Na,K-ATPase pump (Skou 1957; Läuger 1990). This enzyme is found in almost all mammalian cells, and is important in the maintenance of the osmotic balance of cells, and for using the metabolic energy of ATP hydrolysis to form the Na and K ion gradients rapidly depleted during the action potential in excitable cells. Much of the modelling of the data has revolved around refinement of a kinetic mechanism first proposed by Albers (1967) and Post (1989). The essential feature of this mechanism is the idea that the pump can assume two principal conformations, E_1 (with inward facing ion binding sites) and E_2 (with outward facing ion binding sites). E_1 has a high affinity for Na^+ and/or ATP and is stabilized by these ligands, while E_2 has a high affinity for K^+ and/or inorganic phosphate (Pi) and is stabilized by these ligands.

Läuger (1990) has proposed a simple four-state minimal mechanism for the similar (but simpler) p-type proton ATPase shown in figure 1*b* illustrating this principle. A key feature is that phosphorylation–dephosphorylation of the enzyme serves to switch the protein between the two conformational states shown in figure 1*a*. In the phosphorylated state, the enzyme binds proton tightly, with easy access to the binding site from the outside (left). In the dephosphorylated state proton binds much more weakly, and access is easiest from the inside (right). This picture also explains how an external perturbation can drive directed transport, even without energy from ATP hydrolysis.

If the enzyme is caused by an external field to alternate between the E and E^* states sufficiently slowly, the system seeks its lowest free energy in each state—proton bound in the E state and proton not bound in the E^* state. The most likely path is that which presents the lowest energy barrier—binding from the exterior in the E state and release to the cytosol in the E^* state. The net result is that on average, one proton is pumped across the membrane for each cycle of the field if the proton electrochemical gradient is not too big. As the frequency increases, the number of protons pumped per unit time increases. At very large frequencies, however, the conformational transition $E \rightleftharpoons E^*$ cannot keep up and the pumping rate decreases with further increase in the frequency.

The way that we have drawn the mechanism in figure 1*b* implies that proton transport is completely coupled to ATP hydrolysis. This is, of course, only an approximation, and in principle it is always possible (though perhaps not likely) for proton to leak across the membrane through the protein without hydrolysis of ATP, or for ATP to be hydrolysed without pumping a proton. We can see the connection between this type of alternating access model for membrane transport and a



igure 1. Ratchet model for ion transport by a molecular pump. (*a*) Cartoon illustration of a protein with two conformational tates—one with a high affinity and easy access from the left (exterior), and one with low affinity and easy access from the right cytoplasm side). Switching between the two conformations is induced by phosphorylation–dephosphorylation of the enzyme. *b*) How this can be incorporated into a four-state mechanism for active proton transport driven by ATP hydrolysis. *c*) Illustration of a more general model for the proton transporter that includes slip transitions. As explained in the text, the referred pathway is controlled either by switching the for binding ATP and releasing Pi depending on whether the proton inding site is occupied, or by using differences in the affinities for proton binding in the two states such that the chemical steps re slow compared to thermal activation of proton over the low barriers, but fast compared to thermal activation of proton over ne high barriers.

rownian ratchet by rewriting the mechanism in figure *b* to explicitly incorporate the possibility of a leak, as nown in figure 1*c*. Here, we have written all the hemical transitions along the vertical axis, and the ransitions in which proton moves across the membrane n the horizontal axis. This emphasizes the fact that the wo processes are *a priori* independent, and that coupling s mediated by the conformational switching of the rotein between two states with different affinities and ccess. The mechanism in figure 1*c* is a Brownian atchet. The protein conformational changes are driven y ATP hydrolysis, but the transition of the proton from ulk solution to the binding site requires thermal activa- on over an energy barrier.

The preferred (coupled) pathway is shown as the white igzag, and follows the same sequence of states as the four- tate cycle in figure 1*b*. In order to achieve tight coupling it s necessary for two 'rules' to be followed (Jencks 1989*a*). irst, the binding of proton from the external solution in ne E state must be fast compared to the phosphorylation f the enzyme by ATP, and second, the dissociation of ound proton to the inside must be faster than release of norganic phosphate in the E^*P state.

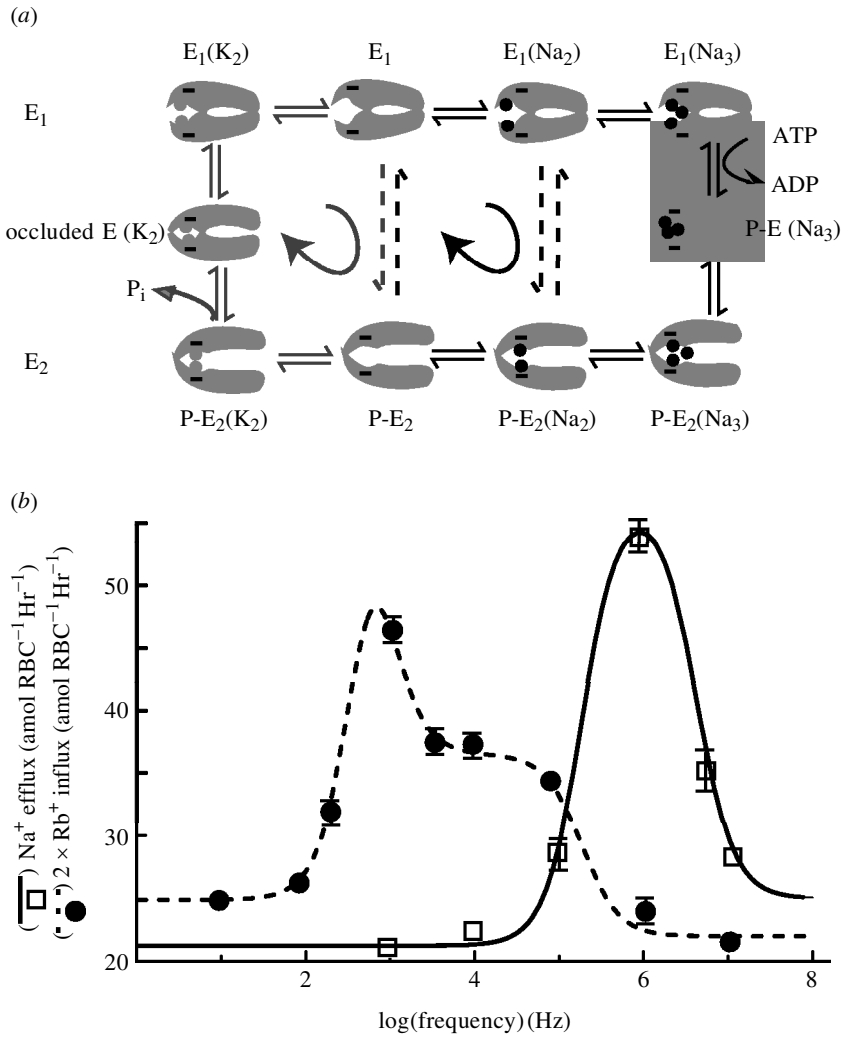
One way this can be achieved is for the transition etween the E and E^* states to be slow compared to the opping of the proton over the low energy barrier, but ast compared to hopping of the proton over the higher arrier. This situation can be achieved only if there is a arge difference in proton binding energy (affinity) etween the E and E^* states.

A second possibility is the control of the chemical pecificity of the reactions by allosteric interactions etween the protein and its ligands. If the protein can be hosphorylated by ATP (or transfer Pi to ADP) only hen the proton binding site is occupied, and can be ephosphorylated by transfer of Pi to water (or phos- horylated by Pi from water) only when the proton inding site is unoccupied, essentially complete coupling f proton transfer to ATP hydrolysis occurs.

Both of these mechanisms for enforcing a sequential kinetic pathway can be achieved by purely structural features of the enzyme—no continual energy input is required. But these considerations only provide a preferred pathway, and not directionality. If the proton electrochemical gradient would be zero, and the ATP hydrolysis reaction at equilibrium, most of the kinetic traffic would indeed be along the zigzag white path in figure 1*c*, but the number of transitions from the upper left corner to the lower right corner would exactly equal the number of transitions from the lower right corner to the upper left corner. The directionality is specified by the signs of the chemical and osmotic free energies—if the ΔG for ATP hydrolysis is greater than the electrochemical potential of proton, there will be more transitions from upper left to lower right, and ATP-driven pumping of proton.

Because the individual steps of ATP hydrolysis are stochastic, it has long been held that strictly regulated coupling between the chemical events of ATP hydrolysis and mechanical events of ion transport is essential for the function of an ion pump (Jencks 1989*b*). Allosteric interactions between the protein and ligands could ensure that neither ATP hydrolysis nor transport can be completed without the other process occurring, resulting in a strictly ordered sequential kinetic mechanism.

The rigid requirements for such clock-like coupling have recently been challenged by experiments of Tsong and colleagues (Liu *et al*. 1990; Xie *et al*. 1994) on Na,K-ATPase. In these experiments ATP hydrolysis is suppressed (either by low temperature or by depletion of ATP concentration) and energy for uphill transport provided by externally applied oscillating or fluctuating electric fields. Because the fields are external, there is no mechanism whatsoever for control of the timing of an electric pulse by the occupancy of the ion binding site of the protein. Nevertheless, these external fields are able to drive significant uphill transport. This has been described in terms of a mechanism known as electroconformational coupling



igure 2. Ion pumping by the Na,K-ATPase. (*a*) Electrostatic model for Na,K-ATPase (Wuddel & Apell 1995). In the ATP-riven coupled cycle, the step $P\text{-}E_2Na_3 \rightleftharpoons P\text{-}E_2Na_2$ is the most electrogenic, while $P\text{-}E_2Na_2 \rightleftharpoons P\text{-}E_2$ and $P\text{-}E_2 \rightleftharpoons P\text{-}E_2K_2$ are less lectrogenic, and $E_1 \rightleftharpoons E_1K_2$ and $E_1 \rightleftharpoons E_1Na_2$ are not electrogenic, indicating that the access channel for E_2 is more resistive than hat for E_1. The transition $E_1Na_2 \rightleftharpoons E_1Na_3$ is moderately electrogenic, showing that the binding sites for Na are not equivalent. he net transition $P\text{-}E_2 \rightarrow P\text{-}E_2K_2 \rightarrow E(K_2) \rightarrow E_1K_2 \rightarrow E_1$, in which two K are transported across the membrane is also not lectrogenic, suggesting that the binding site itself bears a charge of −2. The direct transition $E_1 \rightleftharpoons P\text{-}E_2$, while not directly ccessible to measurement using the technique of Wuddel & Apell, is predicted to be very strongly electrogenic. (*b*) Data howing the effect of an AC electric field on the ion transport modes of the Na,K-ATPase (Liu *et al.* 1990) where Rb^+ and Na^+ ransport are induced at different frequencies. The dashed line for Rb^+ and solid line for Na^+ are fits of Lorentzian curves to the ata as predicted by a nonlinear extension of relaxation kinetic theory (Robertson & Astumian 1991).

Tsong & Astumian 1986). The key feature of this ypothesis is that the field alters the relative energy evels of the different conformational states of the rotein, thus enforcing an external switching between he two states in figure 1 even without phosphorylation.

The rate of movement of ions across the membrane nduced by the AC electric field is independent of ATP oncentration, but does depend on the frequency ω of the eld as shown in figure 2*b*, where the solid lines are the t curves calculated from an extension of relaxation inetic theory (Robertson & Astumian 1991). The net ransport was in the direction stimulated by ATP ydrolysis *in vivo* in both cases, and from low to high oncentration under the experimental conditions. This ehaviour can be understood in terms of the recently roposed electrostatic model of the Na,K-ATPase shown n figure 2*a* (Wuddel & Apell 1995; Rakowski *et al.* 1997). Mechanistically the effect of the field can be interpreted as stimulation of non-canonical flux modes of the enzyme, slip cycles that operate when either Na^+ or K^+ are omitted from the medium. The energy from the field drives the 'slip' cycles in a direction opposite to that predicted based on the chemical driving force of the cycle. The 'slip' transitions are shown as dashed arrows. The conformational transition $E_1 \rightleftharpoons P\text{-}E_2$ confers the electrical sensitivity on these processes. Although the charge movement is minimal, the electric work is $2e\Delta\psi$ (where e is the elementary charge), because the access of the negatively charge binding site is changed from the outside to the cytosol and so the charge effectively moves through the entire membrane potential difference $\Delta\psi$.

For large fields, the thermodynamic efficiency of the external pumping can approach 100%, and the maximum gradient that can be supported is given by the ratio of the affinity in the high and low affinity states (Markin *et al.* 1990).

4. BROWNIAN RATCHETS AND MOLECULAR MOTORS

Now, let us apply the principles discussed for ion umps to consideration of the mechanism of mechano-hemical energy conversion by the molecular motors inesin and Ncd.

Kinesin and Ncd are two members of the kinesin super-amily of microtubule (MT)-based molecular motors. owered by ATP hydrolysis, these two molecules move in pposite directions along a MT. They are, however, struc-urally very similar (Kull *et al*. 1996; Sablin *et al*. 1996), nd bind with similar orientations on MTs, eliminating ne possibility that the origin of the oppositely directed notion comes about because the motors bind facing oppo-te directions (Hirose *et al*. 1996). The mystery is deepened y a recent elegant experiment in which a chimera was ormed by attaching the motor domain of Ncd to the neck egion of *Neurospora* kinesin (Henningsen & Schliwa 1997; ase *et al*. 1997). Surprisingly, the resulting motor cata-ysed the '+' end-directed motion characteristic of kinesin rom which the neck (and not the motor) region was aken. In addition to structural studies, there has been an xplosion of work on the mechanical behaviour of kinesin, eading to a consensus in the field that with saturating TP the velocity of a single kinesin dimer moving proces-ively on MTs is between 0.5 and 1 $\mu m\,s^{-1}$, and that the orce (either elastic (Svoboda & Block 1994; Coppin *et al*. 997; Meyerhofer & Howard 1995) or viscous (Hunt *et al*. 994)) needed to stop the forward progress is around pN. Furthermore, single-molecule studies of kinesin notion have shown that the motor moves in single steps of bout 8 nm (Svoboda *et al*. 1993), corresponding well with ne lattice spacing $d \approx 8$ nm of tubulin monomers along ne axis of the MT. Recently, it has been established that n the absence of a load the stoichiometry is one ATP per nm step of the motor (Schnitzer & Block 1997; Hua *et al*. 997).

Here we discuss a model, based on a 'Brownian ratchet' Huxley 1957; Hänggi & Bartussek 1996; Astumian 1997; ülicher *et al*. 1997) where the direction of motion is ontrolled by the chemical mechanism of ATP hydrolysis Astumian & Derényi 1999). A key assumption is that the TP bound state has a large one-dimensional diffusion oefficient for lateral motion along the MT backbone, lthough this state has a very small dissociation constant llowing the motor to retain energetic contact with its olymeric track while undergoing motion. In contrast to ne standard 'hand-over-hand' mechanism, the model oes not require either head of the motor to dissociate at ny time during a mechanochemical cycle. The steps in hich motion and force production occur are pictured as ermally activated transitions over an energy barrier on one-dimensional potential between molecular states, ach of which is close to thermal equilibrium even in the resence of large (5–10 pN) external forces. The system is us appropriately modelled by chemical kinetics, and no ower stroke (i.e. a viscoelastic relaxation from a non-quilibrium conformation) is involved. This mechanism is ndamentally similar to that used to describe the oupling of ATP hydrolysis to drive uphill transport of ns by ion pumps (Läuger 1990; Astumian & Derényi 998) discussed above.

To compare our model with experimental results for the effect of external force on the velocity of dimeric kinesin (few data are available for Ncd), we provide an extension to a two-headed model, and incorporate alternating site kinetics for the ATP hydrolysis because this seems to be well established experimentally. In this extended picture the mechanical motion is still described in terms of thermal activation on a one-dimensional potential. The presence of the second head significantly stabilizes the overall interaction between the kinesin and MT, so that highly processive motion is possible. In addition to reproducing quantitative aspects of the effect of an external force on the velocity of the motor, and the stoichiometry of one ATP per step at zero load, our picture is consistent with four key observations: (i) a force applied in the direction of motion increases the velocity of the motor but the effect saturates (Coppin *et al*. 1997); (ii) although the motor seems to be completely coupled at zero load, experiments show that at low ATP concentration the motion is more random even than predicted based on a single rate-limiting step (Schnitzer & Block 1997); (iii) increasing significantly the strength of the coiled-coil interaction between the two necks of a kinesin dimer does not abolish processive motion (Romberg *et al*. 1998); and (iv) the motion driven by single-headed kinesin seems to be consistent with a small duty cycle motor, while that driven by dimeric kinesin is consistent with a large duty cycle motor (Young *et al*. 1998; Hancock & Howard 1998).

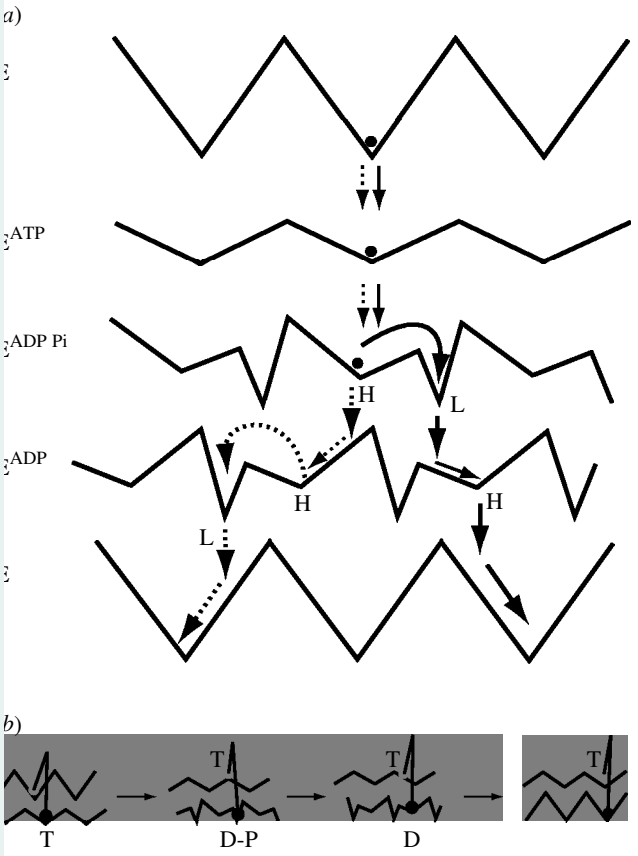
5. A CHEMICALLY REVERSIBLE BROWNIAN MOTOR

Consider the model shown in figure 3*a*, which describes the energy profile for movement of a single motor head along a MT in each of four different chemical states. Transitions between chemical states of the motor are shown on the *y*-axis.

In the E state where nucleotide phosphate is not bound, the motor is tightly pinned to one binding site on the MT. When ATP binds, the activation energy for lateral movement is decreased and transitions to the monomer on the left or right are fairly fast, but the motor is still tightly associated to MT. This makes the prediction that the one-dimensional diffusion coefficient will increase upon binding ATP to the motor even though the motor remains tightly bound to the MT.

Hydrolysis of ATP at the active site changes the interaction between the motor and track such that there are two ways the motor can bind in the $E^{ADP\times Pi}$ state—a relatively high-energy (H) position and a lower-energy (L) position. The barriers between the H and L positions are asymmetrical—transition from the H to the L position on the right is much faster than transition to the L position on the left. Dissociation of Pi again changes the interaction between the motor and the MT such that the binding positions on the one-dimensional coordinate are shifted in the E^{ADP} state, and the barriers are interchanged such that a transition from the H to the L position on the left is much more rapid than a transition to the L position on the right. Release of ADP completes a chemical cycle of ATP hydrolysis, returning the motor to the tightly pinned E state.

One simple possibility for controlling the direction of motion in this model is by the relative rates for release of



igure 3. Ratchet mechanism for chemically reversible notion. (*a*) The chemically reversible Brownian motor. The rotein concomitantly cycles through its chemical states while atalysing ATP hydrolysis (on the *y*-coordinate) and trans- ocates through space along a MT (possibly varying its onformation in the process) as plotted on the *x*-coordinate. *b*) Coupled transport. Illustration of how this mechanism ould work with a two-headed motor. We show only the case or coupled motion directed to the right. Initially, either head an bind ATP (T) and the interaction of that head with the IT is weakened. This is followed by hydrolysis of ATP at the ctive site changing the interaction with MT, and inducing inding of ATP to the other head. As the catalytic and echanical cycle of the first head proceeds, the second head ollows along. Finally, ADP dissociates from the first head and new cycle begins by hydrolysing the ATP in the second ead.

i and ADP (Astumian & Derényi 1998). This is similar o recent models for physical ratchets where a position- ndependent modulation of the potential coupled with patial anisotropy allows directed motion (Astumian & ier 1994; Prost *et al*. 1994; Bier & Astumian 1996). If elease of Pi is slow and release of ADP fast compared to he H → L transition, the motor will probably make a ransition to the L position while Pi is bound, but will elease ADP while in the transient H position, following he trajectory outlined by the solid arrows. In contrast, if elease of Pi is fast and release of ADP slow compared to he H → L transition, the motor will most probably elease Pi in the transient H position, but will make a tran- ition to the L position before release of ADP, following the rajectory outlined by the dashed arrows. Sadly, this legant mechanism alone is not sufficient to explain the echanical data—it predicts that application of a modest external force opposing the ATP-driven motion should cause the motor to begin stepping backward, and this is not seen. Experimentally, a force of 5 pN is sufficient to halt kinesin, but the motor remains fixed and does not undergo significant backwards motion even when challenged by forces as large as 12 pN (Coppin *et al*. 1997).

A second possibility, on which we focus here, is that the direction is controlled by the specificities for release of ADP and Pi from the H and L positions. This is closely related to Huxley's model for muscle contraction, where the rate constants for the chemical transitions are anisotropic along the reaction coordinate but the potential itself can be symmetrical (Huxley 1957). Once again this closely parallels ideas taken from the coupling mechanisms of ion pumps (Jencks 1989*a*).

Consider that the L position of the $E^{ADP\times Pi}$ state is specific for release of Pi and that the H position of the E^{ADP} state is specific for release of ADP (solid arrows). First, ATP binds to the motor, decreasing the interaction energy holding the motor to a fixed site. Most probably, ATP is hydrolysed before a transition to the left or right occurs. Because the H position is not specific for release of Pi, a transition to the L position on the right most probably occurs, triggering release of Pi. The motor then rapidly equilibrates in the H position in which it finds itself. Now, ADP release most probably occurs from the ADP-specific H position, completing a chemical cycle. Rapid equilibration in the tight binding site completes a mechanical cycle of movement one period to the right of the starting point.

If the H position of the $E^{ADP\times Pi}$ state is specific for release of Pi, and the L position of the E^{ADP} state is specific for release of ADP, the direction is reversed (dotted arrows). ATP hydrolysis is followed by release of inorganic phosphate from the Pi-specific H position. Then, because the H position is not specific for release of ADP, a transition over the low barrier to the L position on the left is quite likely. The L position is specific for ADP release, thus completing one chemical cycle of ATP hydrolysis, and the motor equilibrates in the tight binding site one period to the left of where it started, completing a mechanical cycle.

6. KINETIC MECHANISM FOR A SINGLE-HEADED MOTOR

If the local equilibration within a state is fast compared to any chemical transitions and to relaxation between the H and L positions, we can rewrite the model in terms of chemical kinetics (Astumian & Bier 1996) (figure 4*a*). For simplicity we assume that ATP hydrolysis is irreversible. With this assumption, the steady-state rate of ATP hydrolysis is $\mathcal{J}_{ATP} = k_{hyd}P(E^{ATP})$, where $P(E^{ATP})$ is the steady-state probability for the motor to be in the weakly constrained ATP-bound state E^{ATP}. We assume that the transition over the high barrier in the E, $E^{ADP\times Pi}$, and E^{ADP} states is essentially precluded. The constant s parameterizes the specificity difference for Pi and ADP release for the H and L positions. When $s \gg 1$, the L position is highly specific for release of Pi, and the H position is highly specific for release of ADP, and vice versa when $s \ll 1$. The parameter K is the equilibrium constant for transition from the H to the L position, and α and β are

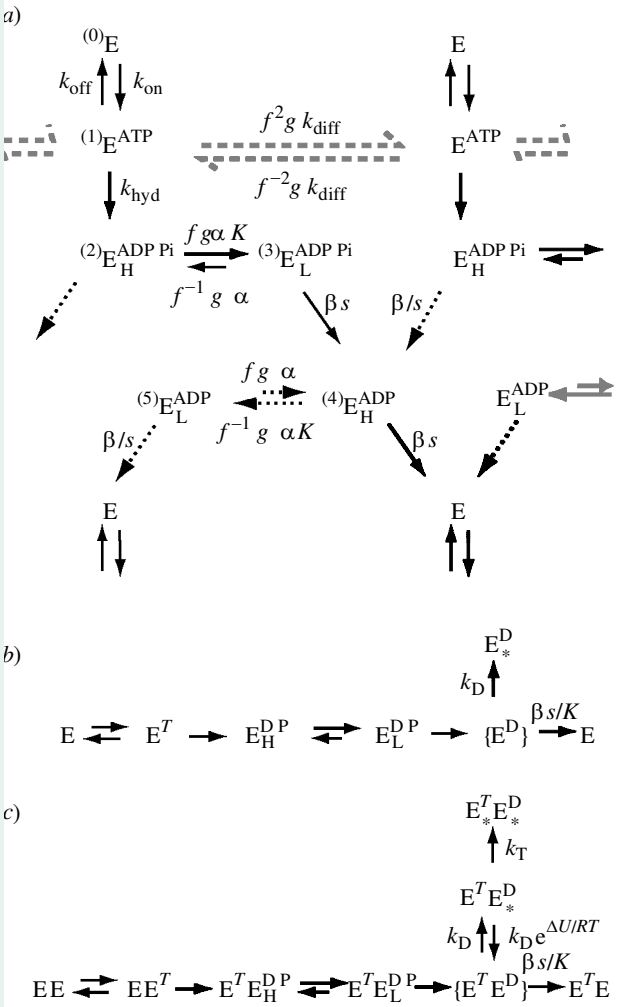


Figure 4. Kinetic mechanisms for kinesin. (*a*) Kinetic mechanism for a chemically reversible ratchet. k_{on} is a unimolecular rate constant which when multiplied by the concentration of ATP ([ATP]) gives the on rate for ATP, k_{off} and k_{hyd} are unimolecular rate constants and represent the 'off' rate and hydrolysis rate for ATP, respectively, and k_{diff} is the rate for a transition to the binding site on the monomer to the left or right while in the weakly attached ATP bound state. K is the equilibrium constant for the H to the L transition. α and β are rate constants that set the relative time-scales for the mechanical and chemical transitions, respectively, and f parametrizes the effect of external elastic force. (*b*) Reaction along the predominant pathway for a monomer showing the side reaction of dissociation in the ADP-bound state. (*c*) Reaction along the predominant pathway for a dimer showing the side reaction of dissociation in the ADP bound state. Here, two sequential steps are required—dissociation of one head followed by dissociation of the second head—before the dimer can be considered dissociated.

the rate constants for the translocation and chemical transitions, respectively.

An externally applied homogeneous force F can be visualized as superimposing a net tilt on each of the energy profiles in figure 1 ($U(x) \rightarrow U(x) + Fx$, where the origin is arbitrary). The energy difference between neighbouring binding sites in both the E and E^{ATP} states is then Fd. If we assume that the physical distance between the H and L positions is $d/2$, and that the barrier is halfway between them, the energies of the H and L positions change relative to each other by $Fd/2$ due to the force, and the effect of the external force on the transition rates can be parameterized by $f = \exp(-Fd/(4k_BT))$. In our model the effect of an external force appears only in the lateral transitions between the H and L positions, and the diffusive step (dashed arrows) in the weakly pinned ATP-bound state. The force dependencies of the chemical steps required by thermodynamics are subsumed in the rate constants for binding ADP and Pi. Far from equilibrium we can assume that Pi and ADP release are irreversible, and that these binding steps do not occur. This reflects a minimal mechanochemical coupling (Duke & Leibler 1996). This apportionment of the external force, while by no means unique, seems to be the simplest possibility.

The kinetic equations for the model can be easily worked out in terms of the time-scales of the individual steps to obtain the net rate of ATP hydrolysis, and the velocity of the motor along the MT. For sufficiently large values of s the stoichiometry approaches unity and ATP hydrolysis is described by the closed Markov chain $(0) \rightarrow (1) \rightarrow (2) \rightarrow (3) \rightarrow (4) \rightarrow (1)$. The rate of ATP hydrolysis can then be written in Michaelis–Menten form:

$$\mathcal{J}_{ATP} = \frac{k_{cat} \times [ATP]}{K_M + [ATP]}, \tag{1}$$

with

$$k_{cat} = \frac{1}{\frac{1}{k_{hyd}} + \frac{1}{f\alpha K} + \frac{2 + Kf^{-2}}{s\beta}},$$

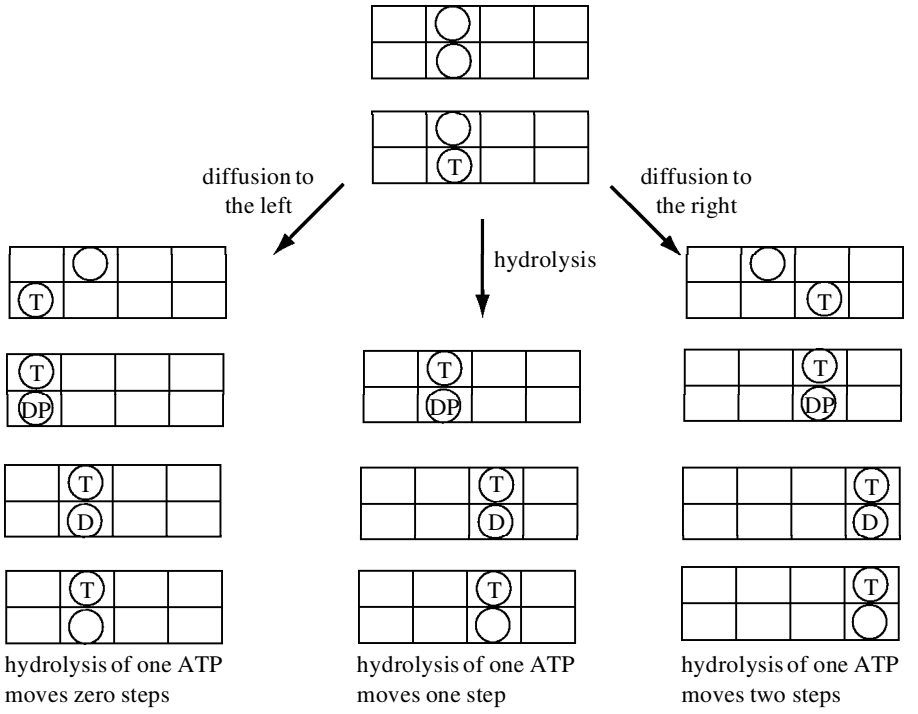
and

$$K_M = \frac{(k_{hyd} + k_{off})}{k_{hyd}} \times \frac{k_{cat}}{k_{on}}. \tag{2}$$

For $s \ll 1$, the equations are the same except with the transformation $f \rightarrow f^{-1}$. For large values of s, the stoichiometry is +1 step for each ATP hydrolysed, so the ATP-driven mechanical velocity is $v_{ATP} = d\mathcal{J}_{ATP}$, where d is the step size (8 nm for kinesin). However, in the weakly pinned ATP-bound state, an applied force can cause slip via the transition indicated by the dashed line in the kinetic mechanism (figure 4*a*). For a single head, or two independent heads, the term $dk_{diff}g(f^2 - f^{-2})P(E^{ATP})$ would have to be added to v_{ATP} to obtain the net velocity, predicting that a force applied in the direction of ATP-catalysed motion would increase the observed velocity without bound. Coppin *et al.* (1997) carried out such an experiment and found that while a force applied in the direction of motion does in fact increase the velocity of the motor, the effect saturates. This can be explained by a cooperative two-headed model (Hackney 1994; Peskin & Oster 1995) where only one head can bind ATP at a time, as schematically shown in figures 3*b* and 5.

7. COOPERATIVE TWO-HEADED MOTOR

In our two-headed model (see figure 5), we consider that the heads can either be together (the minimum energy configuration, where the heads occupy neighbouring subunits) or apart (where the heads occupy subunits that are displaced relative to each other). We assume



igure 5. Pattern of kinesin stepping during normal coupled cycle (middle column), when a diffusive step to the left occurs efore hydrolysis at the active site (left column), and when a diffusive step to the right occurs before hydrolysis at the active site ight column).

hat ATP hydrolysis at the active site of one head co-peratively induces binding of ATP to the other, but that TP hydrolysis at the second head cannot proceed until DP dissociates on the first head. This ensures alternating te kinetics for the ATP hydrolysis which is well estab-shed experimentally (Gilbert *et al*. 1998). In this case, here are three possibilities following ATP binding to the rst head. (i) ATP hydrolysis occurs while the heads are ogether (figure 5, middle column), inducing binding of TP to the second head. The first head completes its echanical and chemical cycle, hydrolysing one ATP and oving the motor one period to the right. (ii) The first ead might diffuse a period to the right before ATP ydrolysis at the active site occurs and induces ATP to ind to the second head which then rapidly moves to a osition adjacent to the first head (figure 5, right column). t this point, the motor is one period to the right of its tarting position. Completion of the mechanical and hemical cycle of the first head results in movement an dditional period to the right. Thus, the motor will have oved two steps while hydrolysing only one ATP. iii) The first head might diffuse a period to the left efore ATP hydrolysis at the active site occurs (figure 5, eft column). Hydrolysis induces ATP to bind to the econd head and rapidly move to a position adjacent to he first head. At this point, the motor is one period to he left of its starting position. Completion of the mechan-cal and chemical cycle of the first head results in move-ent one period to the right, back to the starting osition. Thus, the motor will have moved zero steps hile hydrolysing one ATP. In the absence of an applied orce, possibilities (ii) and (iii) are equally likely and do ot contribute to the net rate. These possibilities are consistent with the observations that occasionally a motor may step back and then forward, but almost never takes two steps backwards in a row (Schnitzer & Block 1997; Coppin *et al*. 1997).

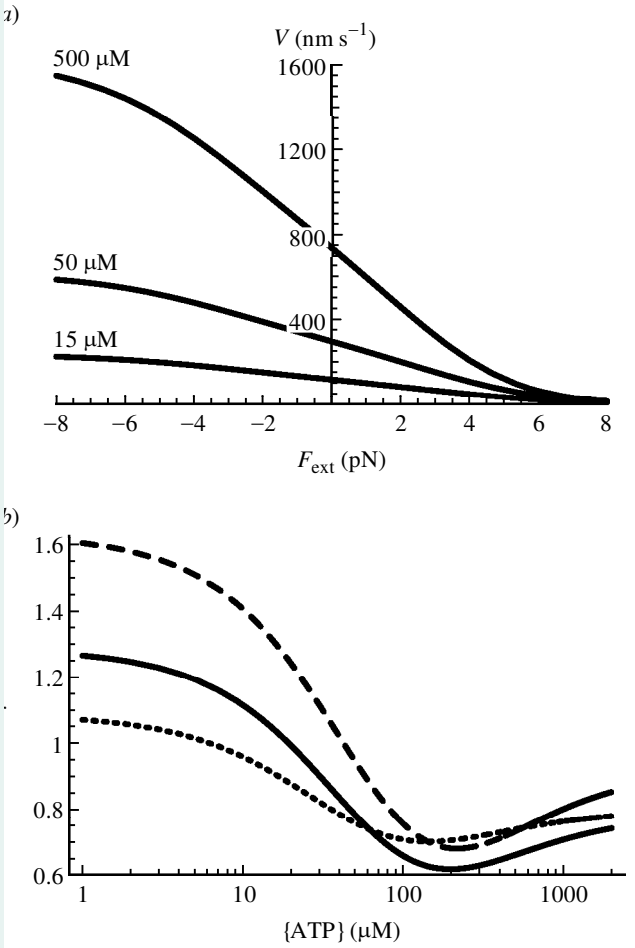
An external force biases the diffusive steps, making one more likely than the other. The effect on the net velocity can easily be calculated in terms of the splitting probabilities at the branch point E^{ATP}:

$$P_{\mathrm{right}} = \frac{k_{\mathrm{diff}} f^2}{k_{\mathrm{diff}}(f^2 + f^{-2}) + k_{\mathrm{hyd}}}, \qquad P_{\mathrm{left}} = \frac{k_{\mathrm{diff}} f^{-2}}{k_{\mathrm{diff}}(f^2 + f^{-2}) + k_{\mathrm{hyd}}}. \tag{3}$$

These probabilities are the fraction of molecules that, having bound ATP, diffuse to the right or left before hydrolysing ATP and are thus the fraction of events in which the motor moves two steps for one ATP and zero steps for one ATP, respectively. The net velocity can be written as

$$v_{\mathrm{net}} = L\mathcal{J}_{\mathrm{ATP}}(1 + P_{\mathrm{right}} - P_{\mathrm{left}}), \tag{4}$$

where $(1 + P_{\mathrm{right}} - P_{\mathrm{left}})$ is the average number of steps per ATP. Figure 6*a* shows a plot of the velocity versus external force at various ATP concentrations calculated using equations (1), (3) and (4). With the parameters used, the Michaelis–Menten constants at zero force are $K_{\mathrm{M}} = 60\,\mu\mathrm{M}$ and $k_{\mathrm{cat}} = 100\,\mathrm{s}^{-1}$, in good agreement with experimental evidence (Schnitzer & Block 1997; Hua *et al*. 1997). The velocity is a nearly linear function of the applied elastic force, and the extrapolated intercept ('stopping force'), above which no further forward



ig. 6. (*a*) Plot of velocity versus external elastic force at three TP concentrations, with $s = 10^5$, $K = 1000$, $\alpha = 10\,s^{-1}$, $= 1\,s^{-1}$, $k_{diff} = 25\,s^{-1}$, $k_{hyd} = 125\,s^{-1}$, $k_{on} = 2\,\mu M^{-1}\,s^{-1}$, and $_{off} = 100\,s^{-1}$. (*b*) Plot of the randomness as a function of ATP oncentration for zero load (black curve), a force of 3 pN pposing ATP catalysed motion (dashed curve), and a force f 3 pN in the direction of ATP catalysed motion (dotted urve). We used the same parameters as in (*a*), with $r_\infty = 0.5$. his reflects two approximately equal rate-controlling steps in he chemical cycle at large [ATP]. In our model with the arameters used these are ATP hydrolysis $k_{hyd} = 125\,s^{-1}$ and DP release, with an effective off rate $\beta s/K \approx 100\,s^{-1}$.

rogress can be observed, is around 5 pN and indepen- ent of ATP concentration, consistent with experimental esults (Svoboda & Block 1994). This stopping force is mited by the free energy available from ATP hydrolysis. he actual intercept, where the velocity crosses zero and ecomes negative, can be arbitrarily large, limited only y the largest kinetic barrier to motion found in any hemical state. This is consistent with the results of oppin *et al.* (1997) who found that even at forces as high s 12 pN the molecule does not step backwards.

8. STOCHASTIC BEHAVIOUR OF SINGLE-MOTOR STEPPING

Recently, several groups have studied the stepping notion of single motors (Svoboda *et al.* 1993; Vale *et al.* 996; Higuchi *et al.* 1997). Because the individual transi- ons are stochastic, the displacement of a motor in a given time is characterized by an average value and a variance. If the stepping is controlled by a single rate-limiting process, the variance is large, but if a step is made up of many discrete sub-transitions each of which on average takes about the same time, the variance is much smaller. Svoboda *et al.* (1994) defined a randomness parameter r in terms of the variance in the displacement of the motor due to ATP hydrolysis, the average displacement, and the step size d evaluated in the limit of very long observation time.

For a completely coupled kinetic cycle where hydrolysis of one ATP always produces one mechanical step of fixed length, the randomness varies between zero if many transitions of similar lifetime make up a single step (a clock-like mechanism), and unity if there is a single rate-limiting process (a 'Poisson' stepper). Thus for any model, r depends on ATP concentration (Schnitzer & Block 1997). At very low ATP concentration, ATP binding must be the single rate-limiting step in the reaction and r is unity. At intermediate ATP concentration, the number of rate-controlling transitions is maximum because ATP binding and other relatively slow steps will have similar characteristic times, thus minimizing the randomness. Finally, at very high ATP concentration, ATP binding no longer plays any rate-controlling role and the randomness approaches a value r_∞ characteristic of the number of rate-limiting transitions in the mechanism.

If the pathway is not completely coupled, hydrolysis of ATP can sometimes produce more or less than one mechanical step as described above. This situation is somewhat more complicated, and the randomness can be larger than unity. For the kinetic model in figure 6, r can be derived to be

$$r = \frac{1 - P_{left} + 3P_{right}}{1 - P_{left} + P_{right}} + (r_0 - 1)(1 - P_{left} + P_{right}), \qquad (5)$$

where

$$r_0 = \frac{r_\infty + \dfrac{K_M^2}{[ATP]^2}}{\left(1 + \dfrac{K_M}{[ATP]}\right)^2}, \qquad (6)$$

is the randomness for the completely coupled cycle. A plot of r versus [ATP] is shown in figure 6*b* for several values of applied force. The black line is that for zero force and is consistent with the experiments of Schnitzer & Block (1997). The dashed and dotted lines are for -3 pN and $+3$ pN applied force, respectively.

An important point to note is that in the limit of very small k_{diff} the model is very tightly coupled and slowing of the motor is accompanied by a commensurate decrease in the rate of ATP hydrolysis, analogous to the Fenn effect in myosin (Fenn 1924). In this limit the randomness cannot be greater than unity. Schnitzer & Block (1997), however, found a randomness of about 1.25 for kinesin at low ATP concentration. With larger k_{diff} the motor is not completely coupled, and at low ATP the randomness can be greater than unity. Also, at large force, significant slip occurs and ATP hydrolysis continues even when the motor comes to a halt. As seen in figure 6*b*, the randomness depends strongly on the applied force for $k_{diff} = 25\,s^{-1}$. However, for $k_{diff} < 1\,s^{-1}$ (not shown), the

andomness is far less sensitive to the applied force. Thus neasuring the randomness at several forces will allow irect determination of k_{diff} and discrimination between ightly and loosely coupled models.

9. PROCESSIVITY

Dimeric kinesin is highly processive, and can move for ver a hundred steps before dissociating from MTs. Monomeric kinesin (and apparently also Ncd) is much ess processive, moving at most two to four steps before issociating. In experiments where the motors are dsorbed onto a surface, MT motion driven by mono- neric kinesin is also qualitatively different than that of he dimeric wild-type. The MT velocity increases almost nearly with increasing surface density of monomers, nd is effectively zero in the limit that only one nonomer interacts with the MT. This is similar to the ehaviour of myosin, and is consistent with a motor that neither pulling nor offering appreciable resistance to notion a large fraction of the time, i.e. a small duty atio (Howard 1997). Dimeric kinesin, however, catalyses rocessive motion in the limit of very small surface ensity, and the velocity quickly saturates with increasing urface density of motors. This is consistent with a high uty ratio motor that spends most of the time either ulling or immobile on the surface.

This behaviour is most often interpreted in terms of a nand-over-hand' mechanism for motion of dimers, where ne head dissociates and swings forward while the other ead remains attached. This swinging head then binds, llowing the other head to release and swing forward. he process continues, with the heads strictly alternating oles as swinging arm and anchor. Because one head is lways firmly attached, the duty cycle is very high, and he velocity saturates at low motor surface density. Motion catalysed by single-headed kinesin is pictured as ccurring in a much more haphazard fashion, where an ndividual motor must release the MT altogether before noving forward (Young *et al.* 1998). In the detached state n individual motor offers no resistance to motion caused y other motor molecules, so the velocity increases with ncreasing surface density.

Our mechanism is entirely different. Neither head need issociate at all during a chemomechanical cycle. However, in the ATP-bound state (in which an individual ead spends about 50% of the time) a monomer offers ttle resistance to lateral motion even though it is attached, ut in the case of dimers at least one of the heads is tightly inned, reproducing the observed dependence of velocity n surface density of the motor. Dissociation is a side reac- ion and not an essential element of the chemomechanical ycle (see figure 4*b*,*c*). This picture is analogous to the reatment of Young *et al.* (1994) for processivity of ATP- riven translocases such as DNA helicase. If dissociation is llowed mainly from the ADP-bound state, the probability hat a monomeric motor (figure 4*b*) dissociates in a given ATP hydrolysis cycle is $P_{mon} = k_d/[k_d + \beta s/(1 + Kf^{-2})]$, where k_d is the rate constant for dissociation in the ADP- ound state. The average number of steps per encounter ith the MT is $\mathcal{N}_{mon} = P_{mon}^{-1} - 1 = \beta s/[(1 + Kf^{-2})k_d]$. With $k_d = 100\,s^{-1}$ and the parameters used to obtain the t shown in figure 6, $\mathcal{N}_{mon} \approx 2$.

Dissociation of a dimer, in contrast, requires two sequential dissociation events. Following dissociation of the ADP-bound head, the other head remains tightly bound. The effective rate constant for dissociation of this tightly bound head is probably much smaller than k_d and we label it k_d^*. While the one head is bound, the dissociated ADP-bound head has a high local concentration (of order 1 M), and the recombination rate constant is $k_d \exp(\Delta U/k_B T)$, where ΔU is the binding energy. For this mechanism the probability per cycle that the dimer dissociates can be calculated from

$$P_{dim} = P_{mon}\left[\frac{k_d^*}{k_d^* + k_d \exp[\Delta U/(k_B T)]} + \left|1 - \frac{k_d^*}{k_d^* + k_d \exp[\Delta U/(k_B T)]}\right| P_{dim}\right], \quad (7)$$

and thus the number of steps before dissociation is

$$\mathcal{N}_{dim} = P_{dim}^{-1} - 1 = \mathcal{N}_{mon}\frac{k_d^* + k_d \exp[\Delta U/(k_B T)]}{k_d^*}. \quad (8)$$

We see that with very reasonable values for the binding energy of only 10–20 kJ mol^{-1} a dimer can take a hundred steps per encounter even if the monomer takes only two with $k_d \geqslant k_d^*$.

10. DISCUSSION AND CONCLUSIONS

We have discussed a 'Brownian ratchet' mechanism for motion of motor proteins in the kinesin family where the direction of motion is governed by the rates and specificities of different binding states for ADP and Pi release. This mechanism is very similar to that for how ion pumps couple ATP hydrolysis to ion transport across membranes. Motion and force generation involve transitions between states that are close to thermal equilibrium even at a very large driving force. ATP energy is used to change the relative affinities and barrier heights between neighbouring binding sites. The timing and regulation is controlled by thermally activated steps from the H to L sites, and the H sites act as switching stations where the chemical rates are compared to the mechanical H→L transition rate. The H and L sites may represent either different physical locations along the MT or different conformations of the kinesin head. This simple model shows that Brownian ratchet mechanisms can have a stoichiometry very close to unity and offers a new way of thinking about the how molecular motors work.

Our picture of how ATP hydrolysis causes directed motion is entirely different from the mechanical hand-over-hand model often used to interpret the observation that kinesin dimers can move many steps along a MT without dissociating. The hand-over-hand model requires each head to successively detach from the MT, swing forward, and reattach to it. In contrast, our mechanism does not require dissociation as an obligatory step in the mechanochemical cycle, but does require relatively free lateral diffusion of a head while in the tightly associated ATP-bound state. The dissociation due to ADP binding observed experimentally is viewed as a side reaction.

To directly compare our model with mechanical experiments on kinesin in which the effect of external

orce on the velocity of motion was studied, we ntroduced a cooperative two-headed model. In this nodel one head of kinesin at random binds ATP. Hydro-ysis of ATP induces binding of ATP to the other head, educing the activation barrier for transition to a neigh-ouring binding site. As the first head continues through s catalytic cycle, moving a period to the right in igure 3*b*, the second head is more or less 'dragged' along or the ride. This model is able to explain how a random-ess greater than unity is obtained, and predicts that a orce opposing the ATP-driven motion will decrease the andomness at low ATP concentration and increase it at igh ATP concentration, and that a force acting in the irection of ATP-driven motion will increase the random-ess at all ATP concentrations.

An interesting prediction of the model is that if the nteraction between the heads were stiffened by substi-uting a different neck region, the motor could still work ell, but the probability for diffusion to the left or right n the E^{ATP} state would be significantly reduced. This ould cause a more complete coupling, resulting in a yperbolic flow–force curve, and the randomness would e decreased. This should be testable using the construct f Romberg *et al*. (1998).

We made several simplifying assumptions to allow us to xpress the chemical and mechanical rates in terms of only few parameters not taken directly from experiment: K, $_{diff}$, α, β and s. Nevertheless, the model fits experimental ata on kinesin for velocity as a function of external force, nd the observed stoichiometry and statistical behaviour f single-molecule stepping extremely well. We anticipate hat transient experiments on the biochemical mechanisms f ATP hydrolysis by kinesin and Ncd (Gilbert *et al*. 1995; Ma & Taylor 1997; Pechatnikova & Taylor 1997) can be sed to further constrain the rate constants.

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Discussion

L. Cruzeiro-Hansson (*Department of Mathematics, Heriot-Watt University, Edinburgh, UK*). Ratchets are a clever way of explaining directional motion. However, I have a bit of difficulty in mapping them to the realities of kinesin and Ncd. It would be simple if we could think of MT as providing a ratchet potential in which kinesin and Ncd were the moving particles. But even if we did not know any better, just the fact that kinesin and Ncd move in different directions means that the shape of the potential, which determines these directions, is defined by the interaction between kinesin and Ncd with MT. But, as we heard yesterday, the direction of motion is determined by a part of kinesin and Ncd that does not interact with MT. So, the shape of the potential is determined by parts of the molecules that are relatively far from MT.

There is the idea that if you have a kinetic or a thermodynamic model for a conformational change, and measure or calculate rate constants or dissociation constants that necessarily means that the conformational change takes place by thermal activation, i.e. random fluctuations. But kinetic or thermodynamic models apply equally if the conformational change takes place in a more deterministic fashion. The only way we can distinguish is by measuring how fast a conformational change takes place after the action of the trigger. If it is say nanoseconds, then it is not by thermal activation.