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Modelling processive motor proteins: moving on two legs in the microscopic realm

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Biological motion is for a large part powered by motor proteins. These are tiny engines (about a millionth of an inch) that convert chemical energy into mechanical force and motion. Processive motor proteins are among the most sophisticated and well studied of the motor proteins. They consist of two identical 'feet' that literally step forward on a long polymer as the fuel is consumed. When a human or other large animal steps, the physics involves mass, gravity and inertia. But for the walking protein the physics is different. Inertial forces are negligible compared to the frictional forces and Brownian motion, i.e. the random movements of molecules at a microscopic level, becomes an issue. Much of the research of the last decades has been directed towards figuring out the amino acid sequence and three-dimensional structure of proteins, but less effort and progress has been made towards understanding the operation of proteins in action. With a simple but rigorous model it is shown how Brownian motion and the generation of real force actually team up to make the motor protein step. The model, moreover, accounts accurately for recently obtained data on moving motor proteins.

1. Macroscopic versus microscopic stepping

When a human is 'stepping', most power is consumed by the repeated acceleration as the foot that was in touch with the ground is brought forward to a position in front of the torso. About a hundred times per minute the entire mass of a foot is accelerated from zero speed to a speed higher than that of the upper body so it can be brought to a position in front of the centre of mass. To overcome the inertia of the foot a force $F = ma$ is necessary, where m is the mass of the foot and a is the acceleration. For a quantitative treatment a good starting point is the energy $E = \frac{1}{2}mv^2$, where v is a speed slightly larger than the average speed of the walk. This energy E is generated each time a foot is moved forward. A small amount of energy also goes into the repeated lifting of the foot against gravity. In the end the stepping mechanism completely eliminates drag or friction as a factor in the energetics. How good a solution stepping is becomes obvious when we see how a 'non-stepper', like a seal or walrus, scoots on land. The aerodynamic resistance is generally negligible when walking or jogging, it does not become an issue until we go from a stepping mechanism to a much faster rolling mechanism, with e.g. a bicycle, and reach a speed of about 30 km h^{-1}.

Biomechanics does not scale. When, for instance, a swan is made twice as large in every length L, he will be eight times as heavy. This is because mass is proportional to volume and volume is proportional to L^3. His strength, however, will increase with the cross-sectional area of his muscles. Area is proportional to L^2, so strength will thus only quadruple and he will probably no longer be strong enough to ever get his weight off the ground.

When going to the microscopic realm the significance of inertia becomes smaller relative to the significance of friction. For a human swimming in water, the inertia of his motions is still a significant part of the mechanics. But for a

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bacteria swimming in water 'feels' like swimming in molasses would feel to us [1]. This is not hard to understand. For a cube-shaped particle with length, width and height L, the mass is again proportional to the volume. The volume, in turn, is proportional to L^3. The frictional force between the particle and the medium is roughly proportional to the surface area, i.e. L^2. Scientists and engineers often use the notion of the Reynolds number; this is an 'inertia-over-friction' ratio and it is obviously proportional to the scale L.

A piece of chalk at different scales can illustrate the concept. When dropped, a piece of about an inch long will fall to the ground with almost the gravitational acceleration of $g = 9.8\ \mathrm{m\ s^{-2}}$. Air resistance will be practically negligible. When, however, chalk powder is blown out of an eraser no such rapid falling takes place. The explanation is that for the micrometre size particles, the force of gravity, i.e. $F_{\mathrm{gr}} = mg$ (where m is proportional to L^3), is negligible compared to the frictional forces that go with L^2.

For microscopically sized particles in a fluid (i.e. a gas or a liquid) the Reynolds number is effectively zero and we call this the overdamped realm. Motion here follows a law that was actually first formulated by Aristotle: $F = \beta v$. So the velocity v of an object is directly proportional to the force F acting on that object. The proportionality factor β is called the coefficient of friction.

The chalk example illustrates another concept. When an eraser is knocked on the table a spherical cloud of chalk powder develops and spreads. Collisions with air molecules add a random component to the trajectory of the small particles. This is called Brownian motion or Brownian noise. For a Brownian particle moving in one dimension and located at $x = 0$ at $t = 0$, the average square displacement is directly proportional to the time t, i.e. $\langle x^2(t)\rangle = 2Dt$. Here D is the diffusion coefficient. In a fluid friction and Brownian motion are related to one another since both come about as a result of random collisions of the particle with molecules of the medium. Mathematically this relationship is expressed in a formula, due to Einstein, that is as beautiful as it is concise: $D = kT/\beta$. On the molecular scale there is Brownian motion in every degree of freedom. In each degree of freedom the Brownian noise-band carries an energy of kT, where k is the Boltzmann constant ($1.4 \times 10^{-23}\ \mathrm{J\ K^{-1}}$) and T is the absolute temperature expressed in degrees Kelvin.

The upshot of the above is that the world of biomolecules is not ruled by the same set of principles as the world of walking humans. Enzymes often couple an energetically downhill chemical conversion to a process or conversion that requires energy. But it is wrong to think of such an enzyme as a kind of miniaturized car engine. When the involved energies are comparable to kT, Brownian motion becomes part of the picture. The resulting picture is one of an enzyme that is operating in the middle of a hurricane. Moreover, it is a hurricane in which every motion is damped out as soon as it occurs. In this environment the mass of a particle is entirely irrelevant.

2. A quantitative model for a microscopic stepper

Motor proteins are agents that convert chemical energy into motion. There are many kinds of motor proteins. Muscle motion, for instance, comes about when *myosin* attaches itself to the biopolymer *actin* and makes a 'stroke' of about 5 nm [2–4]. Subsequently the myosin lets loose and gets ready for another stroke. The necessary energy is made available when an inorganic phosphate group is 'broken off' from ATP (adenosine triphosphate). ATP is the currency of energy in a living cell. When a phosphate group is broken off from ATP, i.e. ATP $\rightarrow$ ADP + $\mathrm{P_i}$ (where ADP stands for adenosine diphosphate), about 22 kT of energy is released under physiological conditions. This reaction is called ATP hydrolysis. It does not easily occur on 'naked' ATP, because of a high activation barrier. Enzymes, however, can bind ATP, bring this barrier down and effectively catalyse the reaction. Myosin catalyses ATP hydrolysis, but it picks up some of the the released 22 kT and uses it for the stroke.

The motor protein *kinesin* is mostly employed for intracellular transport. In any cell that is bigger than a bacteria kinesin pulls organelles (like mitochondria) to a part of the cell where they are needed. It also pulls vesicles that are filled with chemicals (see figure 1). Kinesin is a processive motor protein, i.e. it can make up to a hundred strokes and stay attached to its biopolymer before it comes loose again. As a biopolymer track kinesin uses the *microtubule*. Each unit of the microtubule is an actual protein of 862 amino acids. The polymer winds up in a tight spiral with a diameter of 25 nm and 13 units per winding. The microtubule also helps constitute the cytoskeletal network that gives the living cell structural reinforcement [2].

The remarkable thing about kinesin is that it literally 'steps' over the microtubule (see http://valelab.ucsf.edu for an animation). Kinesin is a dimer and the two identical units, of 340 amino acids each, essentially function as feet (see figure 2 (*a*)). Each unit measures about 7 nm. Ironically though, these feet are generally called 'heads'. Of course, it is not gravity that keeps the attached head attached to the microtubule. Instead, it is chemical bonds. In the catalytic cycle that couples ATP hydrolysis to a forward step such a bond is broken and then re-established. Via the state of the linker at the neck the two heads 'communicate' to each other what state they are in. Detachment of the entire protein from the polymer is thus prevented [5, 6].

Without other energy inputs there will be equilibrium fluctuations between state A, in which a particular kinesin head is attached to the microtubule, and the detached state

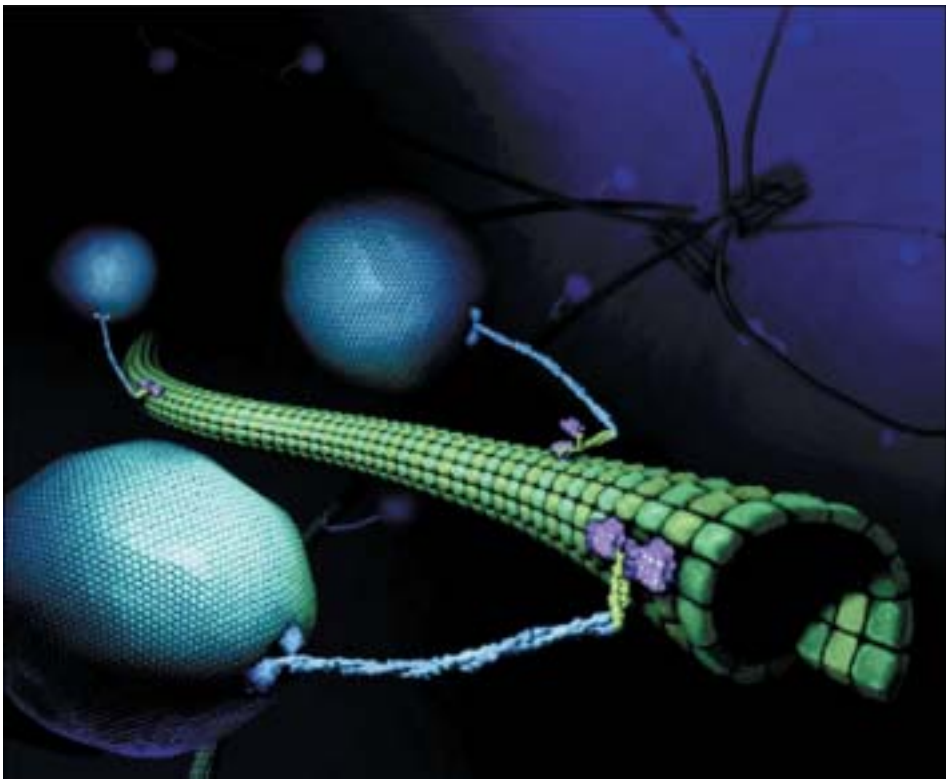

Figure 1. An on-scale artist's impression of kinesin motor proteins transporting vesicles along the microtubule. The two heads (purple) move in a hand-over-hand manner along a protofilament row of tubulin subunits. Reproduced from *The Journal of Cell Biology* **151** (2000), cover image, by copyright permission of The Rockefeller University Press.

D. If the attaching and detaching were not correlated with the ATP hydrolysis, the transition rates $k_{D\to A}$ and $k_{A\to D}$ would satisfy the Boltzmann equilibrium $k_{D\to A}/k_{A\to D} = \exp\{(E_D - E_A)/kT\}$, where E_D is the energy of the detached state and E_A is the energy of the attached state. The Boltzmann equilibrium gives $P_D/P_A = \exp\{-(E_D - E_A)/kT\}$ for the ratio of the probabilities to be in the detached versus the attached state. For the stepping motor protein it is important to break the Boltzmann equilibrium and the randomness of the fluctuations between states A and D. Binding and unbinding are to be well timed events within the catalytic sequence and it is therefore that they are coupled to the energetically downhill steps of ATP hydrolysis. So, like for a stepping human, energy dissipation is involved in the repeated docking and undocking of the motor protein heads.

Kinesin can only walk along the microtubule in one direction. Transport in the opposite direction on the microtubule is actually taken care of by another motor protein called NCD [2, 3]. The walking of motor proteins is different from human walking in that this directionality originates from the 'road' and not from the walker. The microtubule track is not isotropic, i.e. when walking from left to right you 'see' another pattern than you see when you walk from right to left. This is, of course, to be expected since each unit is a complicated, folded protein with no symmetries. When kinesin attaches to the microtubule it immediately 'knows' which way to go (see figure 2 (*a*)).

Directed walking of kinesin over the microtubule requires a fixed course of events. After the anterior head detaches it should be brought into the vicinity of the posterior binding site before it again goes into a conformational state in which it can attach. An effective way to proceed would then be for the attached head to reorient and bring the neck linker into a position from where the 'dangling' detached head can reach the posterior docking site. Such a reorientation would be very similar to the well-studied 'power stroke' of myosin. Rice *et al*. have described these changes as the 'zippering' and 'unzippering' of the neck linker and, based on their structural evidence, they have suggested that force is generated as the attached head turns forward upon ATP binding [5]. When the detached head is 'dangling' in the cytosol it is subject to Brownian motion (see figure 2 (*a*)). Eventually and inevitably this Brownian motion will make the detached head hit the posterior docking site. Attachment will then occur and next it is the turn of the other head to make a forward step. The Brownian trajectory that the detached head follows

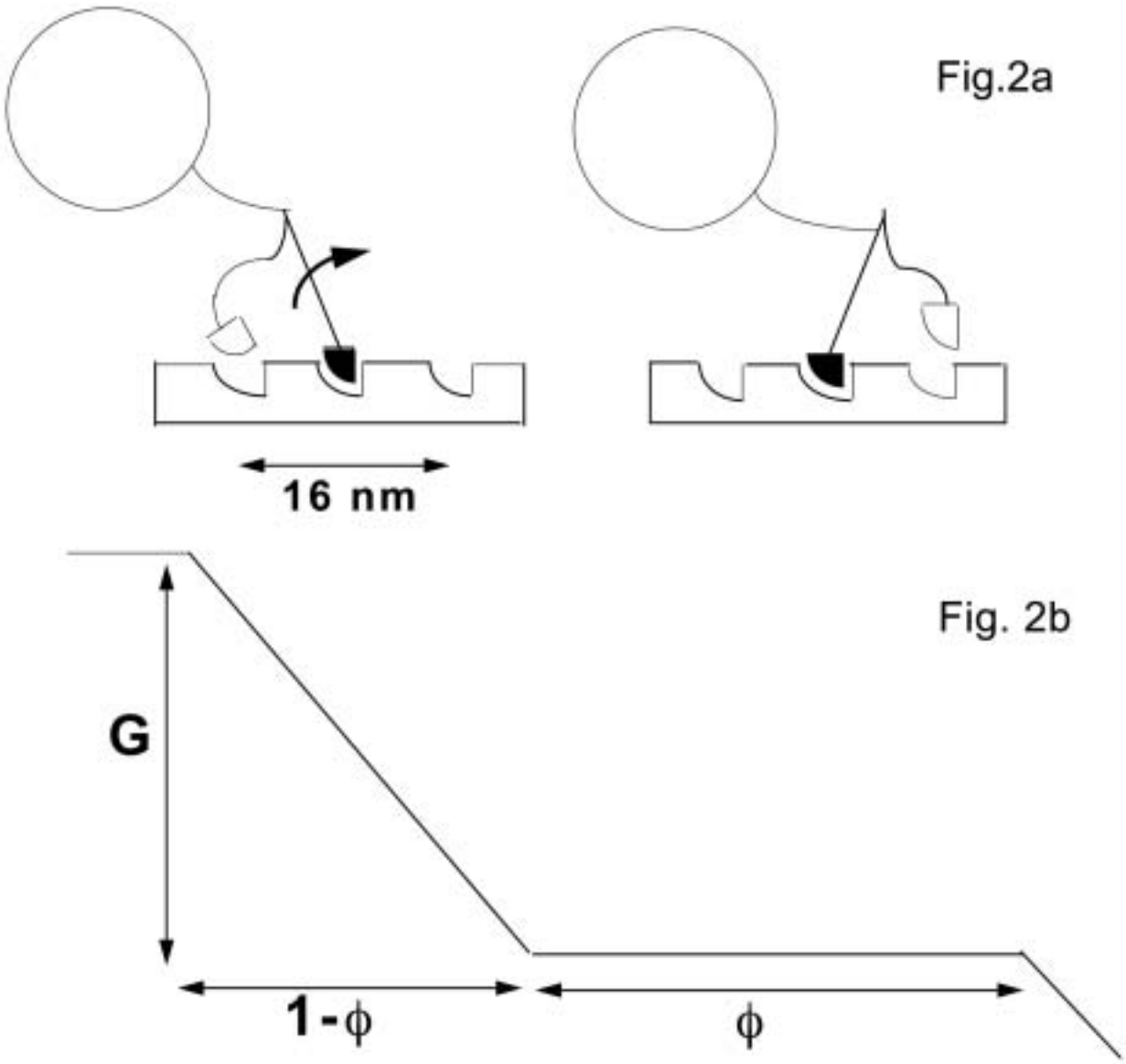

Figure 2. The set-up for our model. One step of the two-headed motor protein (*a*) corresponds to traversing one unit in a 1D reaction space (*b*). The reorientation of the attached head is the power stroke with energy G that covers a fraction $(1-\phi)$ of the cycle. The subsequent diffusion and docking of the detached head does not dissipate any energy and covers the remaining fraction ϕ.

immediately prior to attachment has been described as 'fluctuational interactions', 'conformational fluctuations' [7] or as a 'random diffusional search' [6]. A statistical physicist would immediately recognize such a process as a diffusive trajectory from a reflecting barrier to an absorbing barrier. It is not unlike a mosquito flying through a closed room until it hits a flytrap. For a one-dimensional path from a reflecting to an absorbing barrier we have $\langle T\rangle_{\mathrm{esc}} = L^2/(2D)$, where $\langle T\rangle_{\mathrm{esc}}$ is the average escape time, D is the diffusion coefficient and L is the distance between the reflecting and the absorbing barrier.

In a living cell kinesin can tow an organelle or a vesicle filled with a substance that is assembled on one end of the cell and needed on another end of the cell. Over the past decade increasingly accurate experiments have been performed following the action of one individual kinesin. In these experiments the role of the organelle or vesicle is played by a silica bead of micrometre (μm) order size that has been hooked up to the tail of the motor protein [8]. The position of the silica bead can be accurately followed with a microscope. It appears that the speed of the motor protein is unaffected by the size of the bead up until a bead size of

several micrometres. This simply means that, for a small bead, the internal friction of the motor protein exceeds the hydrodynamic friction of the bead. The way to intuit the notion of 'internal friction' is by comparing the motor protein and its cargo to a bicyclist carrying a large balloon. The hydrodynamic friction of the bead can be compared to the aerodynamic friction of the balloon. The internal friction of the motor protein would be analogous to the rolling resistance of the bicycle that is due to ball bearings, chain etc. For the microscopically small motor protein a high internal friction makes sense in order for the process not to be too much affected and disrupted by random diffusion (remember $D = kT/\beta$!).

When a particle in an overdamped, homogeneous medium is to be transported over a distance L along a straight line in a time T, the most energy efficient way is doing this with a constant speed $v = L/T$. This leads to an amount of energy being dissipated of $E = \beta L^2/T$. Any variation of speed around this average will lead to more energy dissipation. As an example consider a bullet falling to the bottom of a bottle of honey. $F_{\mathrm{gr}} = mg$ provides a constant force and this leads to a constant speed of $v = F_{\mathrm{gr}}/\beta$. During the fall the energy is converted from potential energy to heat that goes into the bullet and the medium, i.e. entropy. It is because of the Second Law of Thermodynamics that nature 'wants' this conversion to take place. Any bullet will always fall to the bottom when dropped in a bottle of honey. The power stroke of the attached head can be compared to such a trajectory through an overdamped medium. Energy is not converted from one stored form into another stored form. Instead it is irreversibly converted into heat.

The action of the motor protein is therefore fundamentally different from that of, for instance, an ion pump like Na,K-ATPase. Na,K-ATPase is abundantly present in the cell membrane and it uses the energy from the hydrolysis of one ATP to pump 3 Na^+ ions and 2 K^+ ions against the electro-osmotic gradient. It thus maintains the membrane potential of about 100 mV between inside and outside of the cell and the about tenfold difference between extracellular and intracellular concentration for both ions. Na,K-ATPase converts energy from one storable form to another, i.e. from the chemical energy of ATP to an electro-osmotic potential across the cell membrane. Such conversion can never take place with 100% efficiency if it is to take place within finite time. Part of the input energy has to be converted into entropy, i.e. heat, for the process to occur. Another feature of the Na,K-ATPase is its reversible mode of operation. In the case of a high electro-osmotic potential and low ATP concentration the Na,K-ATPase will start to let Na^+ ions and K^+ ions flow down the potential and use part of the released energy to produce ATP. No such conversion can occur for a motor protein. Utilizing the energy of thermal fluctuations, i.e. the Brownian motion, of the medium and turning this energy into ATP production and backward motor protein motion would be in obvious and flagrant violation of the Second Law of Thermodynamics. That would be like the bullet extracting thermal energy from the medium to propel itself from the bottom to the top of the bottle of honey. We thus assume that all of the energy G of ATP hydrolysis (22 kT units at physiological conditions) goes into the power stroke. As was explained in the previous paragraph, a smooth power stroke is an efficient power stroke, and it is likely that 3.5 billion years of evolution has led to a power stroke in which the energy of ATP hydrolysis transforms smoothly into the generation of a constant force driving the reorientation.

If the power stroke were not a completely smooth linear downslide, but, instead, had some variation in the slope (which is likely as it represents a sequence of many different conformational changes), the time to slide down would be longer. The effect of such a longer downslide time would be equivalent to having a smooth, linear power stroke with a G that is a few units smaller than 22.

It is possible to translate the stepping of the two heads into movement of a point along an abstract reaction coordinate (figure 2 (*b*)). One step of the motor protein corresponds to one cycle in reaction space. In terms of energy the forward reorientation of the attached head, i.e. the power stroke, can be interpreted as a downhill slide. It is here that force is generated and energy is used. No energy, however, is dissipated when the detached head is following its diffusive path. This diffusive segment is the ratchet part of the cycle and it can be thought of as a flat stretch from a reflecting barrier to an absorbing barrier. It is important to realize that the friction in the power stroke is constituted mainly by internal friction of the motor protein. The probably somewhat lower friction of the subsequent diffusive path of the detached head is due to the viscosity of the cytosol medium. The horizontal coordinate in figure 2 (*b*) represents a reaction coordinate and not the position of the centre of mass of the kinesin relative to the biopolymer. In principle it is possible to take the biopolymer as a reaction coordinate and the position of the centre of mass on the polymer as a measure for the progress towards the completion of one step. However, in that case one has to work with the position-dependent friction and thus with a position-dependent diffusion coefficient $D(x)$ in order to be realistic. Such a $D(x)$ would complicate the analysis. The way we have it now, the position of the point along the reaction coordinate indicates how far the motor protein has progressed in its path through the cycle. The essence of the reaction coordinate is that the diffusion coefficient D is the same everywhere. This means that there is an isomorphism between the position along the reaction coordinate and the position of the centre of mass of the protein and the position on the reaction coordinate. More detailed struc-

tural knowledge of the internal operation of the motor protein could lead to an explicit formulation of this isomorphism. All we know at this point is that one cycle in reaction space corresponds to an 8 nm step of the motor. By setting the period along the reaction coordinate equal to the 8 nm of the actual movement, one can obtain a diffusion coefficient D that represents the average $\langle D(x)\rangle$ for the kinesin on the microtubule.

We simplify our analysis by a few scaling operations. We let the 8 nm period be our unit of length. We, furthermore, take $D = \beta = 1$, which implies that energy is expressed in units of kT. This scaling will lead to nice and concise formulae. Upon completion of the analysis we will transform back to metres and seconds again so we can relate predictions of the model to actual experimental data. In figure 2 (*b*) the force driving the motor protein down the power stroke section equals $F_{\mathrm{ps}} = G/(1-\phi)$. With a scaled $\beta = 1$ we have a speed in reaction space that is equal to F_{ps}. The time to complete the power stroke thus equals $T_{\mathrm{ps}} = (1-\phi)/F_{\mathrm{ps}} = (1/G)(1-\phi)^2$. This formula is based on the assumption that the power stroke is like a deterministic downslide in reaction space. Diffusive effects have been neglected. This assumption is only valid when G is significantly larger than 2. This is because the time to diffuse over a distance $(1-\phi)$ equals $\frac{1}{2}(1-\phi)^2$. Using methods that are described in the textbook by Gardiner [9] it can be rigorously shown that for $G \approx 20$, the identity $T_{\mathrm{ps}} = (1/G)(1-\phi)^2$ is about 95% accurate.

The average time to diffuse from a reflecting barrier at $x = 0$ to an absorbing barrier at $x = \phi$ equals $T_{\mathrm{diff}} = \frac{1}{2}\phi^2$. For the edges of the flat segment to act like a reflecting barrier on the left and an absorbing barrier on the right we again need a steep slope for the power stroke. Once more, $G \approx 20$ is sufficient to warrant such approximation.

For the entire catalytic cycle we thus derive a duration of

$$T = T_{\mathrm{ps}} + T_{\mathrm{diff}} = \frac{1}{G}(1-\phi)^2 + \frac{1}{2}\phi^2. \tag{1}$$

For the motion of kinesin over the microtubule the completion of one catalytic cycle amounts to moving one $\varepsilon = 8$ nm period. Since length is in units of ε, we have for the average speed of the motor protein

$$v = \left\{\frac{1}{G}(1-\phi)^2 + \frac{1}{2}\phi^2\right\}^{-1}. \tag{2}$$

The motor protein is subject to diffusion and its stepping is therefore a stochastic process. The average speed is the first moment. But there is also information about the underlying dynamics in the second moment, i.e. the variations in speed from one period to another. What researchers have been doing boils down to the following. You take the motor protein and let it run over multiple periods from $x = 0$ at $t = 0$ to $x = L$. The different arrival times are recorded. If you think of the motor proteins all starting together at $x = 0$ at $t = 0$, then it is obvious that they will undergo a spreading in the course of drifting toward $x = L$. This spreading will be described by a widening Gaussian distribution. The centre of this Gaussian moves with a speed v according to (2). An effective diffusion coefficient for this spreading can be expressed as follows [10]:

$$D_{\mathrm{eff}} = \frac{1}{2}\frac{L^2(\Delta t)^2}{\langle t\rangle^3}. \tag{3}$$

Here $(\Delta t)^2$ represents the variance in the arrival times at L and $\langle t\rangle$ represents the average arrival time. For a sequence of subsequent stochastic processes the time variance of the total is the sum of the individual time variances. So with a distance that is α times as long, L, $(\Delta t)^2$ and $\langle t\rangle$ all increase with that same factor α, leaving D_{eff} in (3) eventually unaffected as it should be. It is important to realize that D_{eff} is different from the diffusion coefficient D that indicates the strength of the Brownian jolts. D_{eff} describes the spread of the drifting particles and, as such, it also takes account of the shape of the energy profile. Formula (3) is commonly used in the study of enhanced diffusion [10], i.e. the study of situations in which D_{eff} is orders of magnitude larger than D. It turns out that energy profiles like the one depicted in figure 2 (*b*) can actually lead to such enhanced diffusion. Experimentalists have often preferred to express the 'diffusive spreading' during transport in terms of a dimensionless quantity that expresses a diffusion-drift ratio and is called the randomness r [11]:

$$r = \frac{2D_{\mathrm{eff}}}{v\varepsilon}. \tag{4}$$

Here v is again the average speed and ε is the length of a period. Different mechanisms lead to different values of r. If every step of length ε were like one chemical transition with the ordinary, exponentially distributed waiting time of a Markov process, the randomness would come out to be $r = 1$. More chemical transitions per period lead to smaller values of r. Already in 1994, Svoboda *et al.* measured the randomness for moving motor proteins [8] and they used their data to rule out certain models and mechanisms. Engineers have faced situations with drift and diffusion for decades. Think, for instance, of the spread of pollutants in a flowing river. In such set-ups the Peclet number has been employed. Roughly speaking, the Peclet number is the inverse of the randomness. In other contexts, the Peclet number can also be interpreted as a kind of signal-to-noise ratio.

Because we consider the power stroke to be a deterministic downslide, the only source of stochasticity in our model is the flat segment. In order to obtain D_{eff} and r we

need to evaluate the time variance, $(\Delta t)^2 = \langle t^2\rangle - \langle t\rangle^2$, for a diffusive trajectory on a flat stretch from a reflecting barrier at $x = 0$ to an absorbing barrier at $x = \phi$. This requires the second moment $\langle t^2\rangle$. The second moment is $\langle t^2\rangle = \int_0^\infty t^2\, P(t)\mathrm{d}t$ where $P(t)$ is the distribution of arrival times at $x = \phi$. There are standard and straightforward methods to compute the second moment [9]. In this case we obtain $\langle t^2\rangle = \frac{5}{12}\phi^4$. With $\langle t\rangle = \frac{1}{2}\phi^2$ we then derive for the time variance $(\Delta t)^2 = \langle t^2\rangle - \langle t\rangle^2 = \frac{1}{6}\phi^4$. Taking $L = 1$ in formula (3) we find:

$$D_{\mathrm{eff}} = \frac{\frac{1}{12}\phi^4}{\left\{(1/G)(1-\phi)^2 + \frac{1}{2}\phi^2\right\}^3}. \tag{5}$$

For the randomness this leads to

$$r = \frac{\frac{1}{6}\phi^4}{\left\{(1/G)(1-\phi)^2 + \frac{1}{2}\phi^2\right\}^2}. \tag{6}$$

Taking the diffusion on the downslide into account leads to extra terms in the numerator of (6). But these terms are again negligible for physiological values of G ($\approx$20).

Before we check our model against experimental results there is a complication we have to take care of. It appears that in practice 5% to 10% of kinesin's steps are backward [12]. In the framework of our model the most likely explanation for this would be that the forward power stroke is followed by an accidental anterior docking of the detached head. This would then lead to a subsequent backward power stroke and an observed backward step. In order to relate our model to the observed speed, we have to multiply v in (2) with $p-q$, where q equals the backward stepping probability, and $p = 1-q$ is the forward stepping probability. Obviously, the randomness is going to increases with the percentage of backward steps. It is straightforward to derive

$$r_{\mathrm{obs}} = (p-q)r + \frac{4pq}{p-q}. \tag{7}$$

3. The comparison against experiment

In 1997 Yong-Ze Ma and Ed Taylor published two back to back papers [13, 14] in which they showed how they had used a variety of biochemical methods to determine conformational states and transition rates in the stepping cycle of kinesin. At the end they present a picture that looks very similar to our figure 2 (*a*). They found $T_{\mathrm{ps}}/T_{\mathrm{diff}} \approx 0.75$. We will call this ratio ξ. From equation (1) it is easily derived that the model of figure 2 leads to $\xi = T_{\mathrm{ps}}/T_{\mathrm{diff}} = (2/G)[1-(1/\phi)]^2$. We can thus get a quantitative estimate for the variable ϕ:

$$\phi = \left\{1 + \left(\frac{1}{2}G\xi\right)^{1/2}\right\}^{-1}. \tag{8}$$

For $G = 22$ we obtain $\phi = 0.26$. The form of expression (8) is such that a change in G leads to a much smaller relative change in ϕ. As was explained before, the effect of a possible bumpiness on the downslide in figure 2 (*b*) could be modelled by a smaller value for G. But taking $G = 16$, for instance, we get $\phi = 0.29$.

When we take the above formula for ϕ and substitute it in equation (2) for the speed, we find for the speed in terms of G and ξ:

$$v = 2\frac{\left\{1 + \left(\frac{1}{2}\xi G\right)^{1/2}\right\}^2}{1+\xi}. \tag{9}$$

Substituting (8) in equation (6) for the randomness r we find, remarkably, that G cancels out of the expression and a very simple and exclusive dependence of r on ξ remains:

$$r = \frac{2}{3(1+\xi)^2}. \tag{10}$$

There is no obvious intuitive explanation as to why, given the model of figure 2, the randomness r should solely depend on ξ. However, since the only stochasticity occurs during T_{diff}, it does make sense that r increases with the diffusion time T_{diff} and decreases with the power stroke time T_{ps}. Substituting $\xi = 0.75$ and $G = 22$, we get for the speed without backstepping and in scaled dimensionless units: $v = 17$. The randomness is already a dimensionless number and, with formula (6), we get a backstep free randomness of $r = 0.22$.

Several groups have made accurate recordings of the motion of an individual kinesin over the microtubule. Some of the most precise simultaneous measurements of both the speed and the randomness were made by the group around Steve Block. They have actually been able to resolve individual steps of moving kinesin and they thus came up with an experimentally observed value for the backstep probability q. In the course of more than ten identical experiments they found, at saturating ATP concentration, an average speed of $v_{\mathrm{obs}} = 810$ nm s^{-1}. The standard deviation was less than 4%. They found a randomness of $r_{\mathrm{obs}} = 0.44$ with again about 4% standard deviation [12]. Using equation (7) and $p + q = 1$ we obtain a simple quadratic equation for the backstep probability q that yields $q = 5.8\%$ for $\xi = 0.75$. Since q is generally smaller than 0.1 it makes sense to neglect terms of order q^2 in equation (7). We then obtain an approximate, but concise, expression for the backstep probability:

$$q = \frac{r_{\mathrm{obs}} - r}{4 + 2r_{\mathrm{obs}} - 4r}. \tag{11}$$

Equations (10) and (11) make it possible to predict the backstep probability given only the experimental values of $\xi = T_{ps}/T_{diff}$ and r_{obs}. Equation (11) predicts $q = 5.5\%$ for $\xi = 0.75$ and $r_{obs} = 0.44$. As was mentioned before, in the experiments described in [12] the individual 8 nm backward steps could actually be resolved. The observed backstep probability was between 5% and 10%. The prediction of our model is within this range.

In the previous section we already conjectured that a backward step occurs when the detached head accidentally docks on the anterior binding site instead of on the posterior site. It is important to realize that this picture does not correspond to an accidental sequence of stochastic Brownian kicks that drives the particle in figure 2 (*b*) up the slope. With a noise strength of 1 kT, the likelihood of an accidental mounting of the barrier of about 20 kT to the left is many orders of magnitude smaller than the likelihood of sliding down the 20 kT well to the right. Incorporating the above described backstepping scenario would require the addition of a second dimension to the 1D reaction space of figure 2 (*b*). Motion of an overdamped, Brownian point particle in a 2D energy landscape would then describe the progress of the chemical process.

If v_{obs} is to be expressed in metres per second, $v_{obs} = (p-q)v$ needs to include a redimensionalization factor on the right-hand side. The reader can check for himself that D/ε has the required dimension of metres per second. We thus get

$$v_{obs} = (p - q)\frac{D}{\varepsilon}v. \tag{12}$$

Given the observed values for v_{obs} and ε, and the derived values for v and q, this formula allows us to estimate D, i.e. the average strength of the Brownian jolts that the motor protein is subjected to. Through $\beta = kT/D$ we then also obtain the average internal friction of the motor protein. For $G = 22$, $\xi = 0.75$ and $v = 813$ nm s^{-1} we find $D = 4.3 \times 10^{-16}$ m^2 s^{-1} and an associated friction β of about 10^{-5} Ns m^{-1}.

The estimate of 4.3×10^{-16} m^2 s^{-1} that we find for the value of the diffusion coefficient D of the stepping kinesin turns out to be reasonable. Diffusion coefficients of proteins inside a cell are actually important for transport and for signalling. These diffusion coefficients have therefore been the subject of a lot of measurement and research. A kinesin head has a diameter of about 8 nm. A protein of that size has a diffusion coefficient in water of about 5×10^{-11} m^2 s^{-1}. In the more viscous cytosol such a protein has a diffusion coefficient of approximately 5×10^{-12} m^2 s^{-1} (see [3, 4], and references in [4]). In the model of figure 2 the motor protein is firmly connected to the biopolymer and it should, on average, be less subject to random jolts than in free solution. The experiments that led to the speed of 810 nm s^{-1} and the randomness of 0.44 were performed, not in the cell, but in an aqueous solution. Water has a viscosity of $\eta = 10^{-3}$ kg m^{-1}s^{-1}. With $\beta = 6\pi\eta r$ for the hydrodynamic friction of a spherical bead with radius r in water, one easily checks that the internal friction of the motor protein of 10^{-5} Ns m^{-1} is equivalent to that of a 400 μm bead in water. So the hydrodynamic friction of the submicrometre bead in the aforementioned experiments can be legitimately and safely neglected as a factor in the motion.

Substituting $G = 22$ and $\phi = 0.26$ into equation (5) we find $D_{eff} = 1.9$, i.e. the effect of the 'stepping potential' in figure 2 is to blow up diffusion by a factor of 2. Incorporating the backward steps further enhances the diffusion to $D_{eff} = 3.6$. So our stochastic stepping model for motor protein movement does exhibit an almost fourfold enhancement of the diffusion [10].

Finally, it is worth noting that the model we have studied is not equivalent to a model where the flat stretch is distributed over several segments across the period (cf. figure 2 (*b*)) with these segments adding up to a fraction ϕ. A split-up, for instance, of the diffusive stretch into two disjoint segments of equal length would result in a T_{diff} that is half of what it is in our current model.

4. Discussion

A common approach to modelling the action of proteins has been to take the minima along the reaction coordinate and interpret these as representing distinct chemical states. Noise activated transitions from one such state to another can then next be modelled as Markov processes. Chemical kinetics assumes that such transitions are instantaneous. This assumption may be adequate when evaluating how, for instance, the aforementioned Na,K-ATPase, converts energy. In the case of Na,K-ATPase the actual movement and the energy invested into overcoming friction is of minor significance for the energetics. But for a motor protein fast and efficient transport against friction is the entire point.

Suppose that a certain transition in the catalytic cycle of kinesin requires a time Δt and involves a displacement Δx of the centre of mass. The friction force that is overcome in that transition is $F_{fr} = \beta\Delta x/\Delta t$, where β represents the coefficient of friction. The energy dissipated in the displacement equals $E = F_{fr}\Delta x = \beta(\Delta x)^2/\Delta t$. It is obvious that the assumption of an instantaneous transition (i.e. $\Delta t \to 0$) with finite displacement Δx leads to the absurd implication of this step requiring an infinite amount of energy. The Brownian noise that is jolting the protein around may obscure the issue. But it has been shown rigorously that this added noise does not alter the energy transduction from ATP hydrolysis to motion against friction [15, 16]; the Brownian kicks fluctuate as much

energy in as that they dissipate out. $F = \beta v$ for the friction and $P = \beta v^2$ for the dissipated power still hold. It is therefore important, even in a Brownian environment, to convert the available energy into motion in as smooth a fashion as possible. Stepwise transitions are inefficient.

Even when energy is not being dissipated into friction, but, instead, being transduced from one stored form to another, a smooth adiabatic conversion that keeps the system close to equilibrium is the most efficient one. The optimal Carnot efficiency for the heat-to-work conversion is achieved only when the heating, cooling, expansion and compression are imposed sufficiently slowly for the system to be at equilibrium all the time [17]. Also in the context of microscopic molecular systems energy conversion is most efficient when changes are slow and adiabatic, and when the system is kept close to equilibrium [18, 19]. This is also the reason that, for instance, the breakdown of glucose in a living cell is a process that is distributed over a great number of chemical reactions, none of which stands out as particularly rate limiting.

All in all, for kinesin to be optimally efficient it is important that the energy is dissipated in as smooth and continuous a fashion as possible.

Furthermore, the 'random diffusional search' on the diffusive segment that was discussed in section 2 cannot adequately be described as a Markov state. Chemical kinetics assumes that the transition rate k from a state S_1 to another state S_2 is time independent. This leads to an exponentially distributed waiting time $P(t) = k \exp(-kt)$ for the transition out of state S_1. For the first moment, i.e. the average waiting time in S_1, we find $T_1 = \langle t \rangle = \int_0^\infty tP(t)\, \mathrm{d}t = 1/k$. For the second moment we find $T_2 = \langle t^2 \rangle = \int_0^\infty t^2\, P(t)\, \mathrm{d}t = 2/k^2$. So in this case we find that the standard deviation in the waiting times, i.e. $(T_2 - T_1^2)^{1/2} = (\langle t^2 \rangle - \langle t \rangle^2)^{1/2}$, equals the actual average waiting time itself. For the diffusive trajectory of section 2 the standard deviation in the waiting times is found to be $\frac{1}{3}6^{1/2}\, T_1$, i.e. only 82% of the actual average waiting time. It is possible to approximate the 'random diffusional search' as a sequence of Markov transitions leading from the reflecting barrier to the absorbing barrier. However, going to the underlying diffusive description is more accurate as well as mathematically simpler.

We have modelled the processive motor protein as a molecular stepper. The noise and the overdamped mechanics of the molecular realm that make a microscopic stepper different from a macroscopic one have been included in the set-up. The resulting model is simple. It contains only a very few assumptions and very few free parameters. It is remarkable how well everything eventually fits together. The model predicts a value for the backstep probability that is in agreement with experimental observation. Furthermore, the average value for the diffusion coefficient D that is derived is within an acceptable range. The above methods and reasoning should, in principle, be applicable also to other processive motor proteins like Myosin V or RNA polymerase. However, the amount of data available for these motor proteins is not as abundant as for kinesin.

As was mentioned before, little is known about how the motor protein exactly operates and moves. The stepping model that is the basis of our calculations is far from being a certainty. Recently a paper appeared in which evidence was presented for a so-called 'inchworm' model [20]. In this model the head that is up front always stays up front and after a forward step of the front head, the head that is behind makes an equally long step to 'catch up'. But this 'inchworm'-motion could be equally well described by the above presented model of a power stroke and a subsequent diffusive segment.

When under water and in a beam of laser light, a silica bead will 'pull' towards the centre of the beam where the light is brightest. This is the operating principle of the so-called *optical tweezer*. With an optical tweezer it is possible to apply forces of piconewton magnitude in a very accurate and pinpoint manner. This possibility to 'pull back' has enabled researchers to put together force–velocity diagrams for motor proteins at different ATP concentrations [12, 21]. These force–velocity diagrams have become increasingly accurate and they can be checked against the predictions of different models. In [22] it is described how the model presented in this article can account for the observed force–velocity and force–randomness graphs.

The amino acid sequence and the 3D structure of kinesin are known at this point (see figure 3). Fast progress is made in understanding how the different parts of the motor are organized, i.e. how they move and how they coordinate their movement [5, 6]. Each head appears to 'know' what the other head is doing and it is thus that the rear head only detaches when the front head is attached. A sequence of 15 amino acids in the neck linker (where the two heads 'meet') plays a crucial role in this coordination. The site where ATP is bound and hydrolysed appears to be far removed from the site where the kinesin is bound to its biopolymer. The mechanical transduction literally occurs via an α-helix that acts as a relay coil. A more complete description of the motor protein should take all these structural features into account and translate them into an energy landscape that is more sophisticated than the one presented in figure 2 (*b*). Eventually motor protein action is noisy, overdamped motion on such an energy landscape. When modelling motor proteins the researcher should no longer operate with a Newtonian intuition based on mass, inertia, momentum and conservative force fields. Even chemical kinetics is not an adequate paradigm. Instead a new intuition should be developed for a world of overdamped, Brownian motion on an energy landscape. It is helpful to keep in mind that, in the course of $3\frac{1}{2}$ billion years of

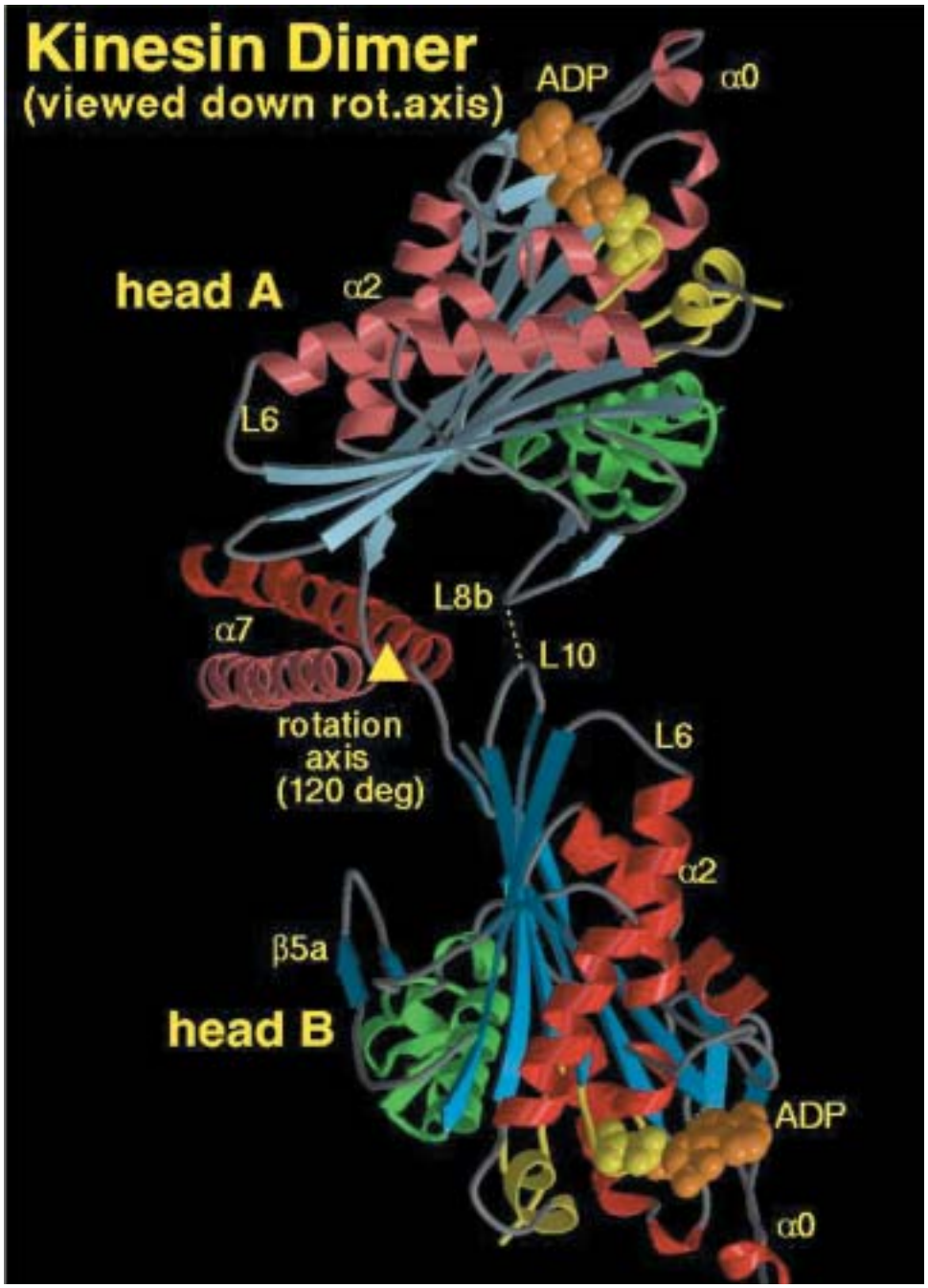

Figure 3. The structure of kinesin as determined through X-ray crystallography with a resolution of 0.3 nm. The ATP binding sites are in yellow. The microtubule binding sites are in green. From: Kozielski *et al*., *Cell* **91** 985 (1997), see also http://www.proweb.org/kinesin/CrystalStruc/CrysStruc_Rn-Dim.html

evolution, i.e. natural selection, the structure of the motor protein should have converged to an optimally efficient one. A catalytic cycle with high activation barriers and rapid downhill leaps in energy is likely to have been superseded by one with many small transitions of equal duration.

At present there is somewhat of a dichotomy in the world of motor protein research. Theoreticians are seeking to understand how chemomechanical energy transduction and directed motion can in principle occur in the Brownian, overdamped environment. Their research has concentrated around simple ratchet mechanisms [23]. Experimental biophysicists have largely focused on acquiring detailed empirical descriptions on the structure and motion of motor proteins [6]. Their theories have been phenomen-

ological more than explanatory. Real progress and innovation can be expected when researchers will start exploring the middle ground between these two approaches. The operation of the motor protein is not just an academic issue. Many drugs work by interfering with a protein's catalytic cycle. A good comprehension of how the processive motor protein works can directly result in the fabrication of new and effective pharmacological agents.

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