Processive Motor Protein as an Overdamped Brownian Stepper

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The two headed motor protein kinesin appears to "walk" along the biopolymer microtubule in 8 nm steps. There is ample justification for a model where the motion of the detached head to the next docking site on the biopolymer is described as ratcheted diffusion. The forward reorientation of an attached head can be conceived of as a power stroke. A model that is based on these premises can accurately predict parameters of motor protein motion.

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When a human is walking, the physics involves mass, gravity, and inertia. 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. Friction plays only a minor role in the energetics.

Biomechanics does not scale. Going into the microscopic realm, the significance of inertia and mass (which are proportional to volume, i.e., L^3) becomes smaller relative to the significance of friction (which is proportional to surface area, i.e., L^2). For a bacteria, swimming in water feels like swimming in molasses would feel to us [1].

With submicrometer size particles in a liquid, such as proteins in an aqueous solution like the cytosol, we are in the overdamped realm, and motion follows $F = \beta v$. So the velocity v of an object is directly proportional to the force F acting on that object at that moment. The proportionality factor β is the coefficient of friction. It is collisions with molecules of the liquid medium that cause the friction. These same collisions also cause Brownian motion, i.e., a diffusion D. The connection is expressed by Einstein's fluctuation-dissipation theorem: $D = kT/\beta$, where kT represents the average energy in the noiseband.

The remarkable thing about processive motor proteins is that they literally walk from one unit to the next along a biopolymer [2,3]. Kinesin is the motor protein that has been most extensively studied. The motor domain of kinesin consists of two identical heads, of 340 amino acids each, that essentially function as feet [see Fig. 1(a)]. They measure about 7 nm each. Kinesin takes 8 nm steps on the biopolymer microtubule and can take up to a hundred steps before it detaches (see [4] for an animation). Kinesin is mostly employed for intracellular transport. In any cell that is bigger than a bacteria, kinesin pulls organelles (such as mitochondria) and vesicles that are filled with chemicals.

Over the past decade increasingly accurate data and insights on motor proteins have become available (see [5] and references therein). Concurrently, many researchers have theorized about the design of underlying Brownian motor mechanisms (see [6] and references therein).

It is chemical bonds that keep the motor attached to the biopolymer and, in the course of stepping, these bonds are repeatedly broken and reestablished. This attachment and detachment cannot just take place in an equilibrium fashion. If that were the case, no forward motion would occur. Coordinated forward stepping is achieved by coupling the stepping cycle to the hydrolysis of adenosine triphosphate (ATP) [5]. Under physiological conditions, such hydrolysis releases about $22kT$ units of energy. Generally, the conformational changes that the motor protein goes through as it catalyzes the hydrolysis of one ATP also constitute one forward step.

The way the binding sites have been drawn in Fig. 1(a) indicates how a biopolymer is an anisotropic track. Kinesin can step in only one direction and this direction is determined by the orientation of the microtubule.

In the stepping cycle of kinesin, two phases can be distinguished: (i) A power stroke phase [Fig. 1(a), left-hand

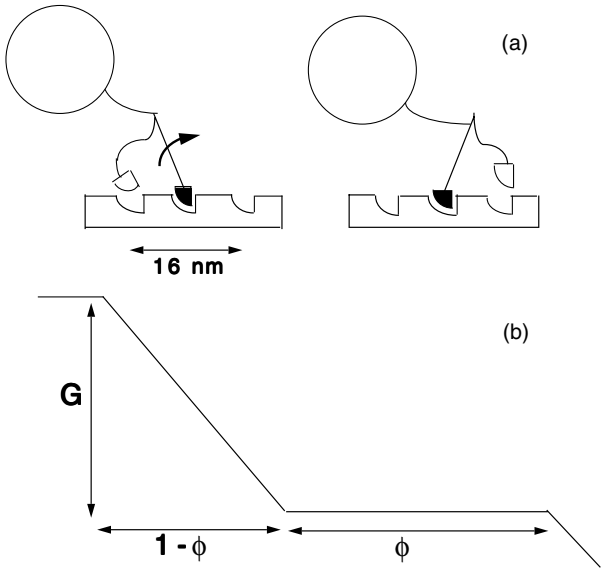

FIG. 1. The setup for our model. One step of the two headed motor protein (a) corresponds to traversing one unit in a 1D reaction space (b). The reorientation of the attached head is the power stroke with energy G that covers a fraction $(1 - \phi)$ of the cycle. The subsequent diffusion and docking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

side], i.e., the reorientation of the attached head. This is when force is generated, when power is dissipated, and when a load is being pulled. (ii) A ratcheted diffusion phase [Fig. 1(a), right-hand side], i.e., the detached head is randomly diffusing around the neck linker until it hits the posterior docking site. After attachment, a next step can commence.

The ratcheted diffusion phase has been characterized as a "random diffusional search" [3] and has been described as "fluctuational interactions" or "conformational fluctuations" [7]. But, from a physics perspective, it looks very much like a random walk between a reflecting barrier and an absorbing barrier (where docking occurs). A 1D random walk that starts at the reflecting barrier at $t = 0$ will reach the absorbing barrier at a distance L after an average escape time of $\langle T_{\rm esc} \rangle = L^2/2D$.

Figure 1(b) shows how the stepping process translates into a profile along a reaction coordinate. The power stroke occupies a fraction $1 - \phi$ and the diffusive stretch occupies the remaining fraction ϕ. The trajectory of an overdamped, Brownian point particle on this profile describes the progress of the motor protein's catalytic cycle. Many authors have identified the position along the reaction coordinate with the position of the center of mass of the motor protein on the biopolymer [6]. But this may be inaccurate. The essence of the reaction coordinate is that the point particle faces a constant, position independent diffusion coefficient D. For an actual motor protein, the different segments on the biopolymer may involve very different D's. The analysis of the motion on the reaction coordinate can lead us to durations for the power stroke and for the diffusive stretch, but not to their distances.

We simplify our analysis of the motion on the reaction coordinate by a few scaling operations. We take the step size of kinesin, which equals $\varepsilon = 8$ nm, to be our unit of length. We, furthermore, take $D = \beta = 1$, which implies that energy is expressed in units of kT. In Fig. 1(b), the force driving the motor protein down the power stroke section is $F_{\rm ps} = G/(1 - \phi)$. With a scaled $\beta = 1$, we have a speed in reaction space that is equal to $F_{\rm ps}$. The time to complete the power stroke thus equals $T_{\rm ps} = (1 - \phi)/F_{\rm ps} = (1 - \phi)^2/G$. We neglect diffusion here and take the power stroke as a deterministic downslide. It can be rigorously shown that for $G \approx 20$ the identity $T_{\rm ps} = (1 - \phi)^2/G$ is about 95% accurate [8].

The average time to diffuse from a reflecting barrier at $x = 0$ to an absorbing barrier at $x = \phi$ equals $T_{\rm diff} = \frac{1}{2}\phi^2$. 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 an approximation.

For the entire catalytic cycle, we obtain a duration of

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

With length in units of ε, we have, for the average speed of the motor protein,

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

It is important to realize that a motor protein is fundamentally different from an energy converting protein such as Na,K-ATPase. Na,K-ATPase and many other proteins transduce energy from one storable form to another. Na,K-ATPase takes the chemical energy in ATP and turns it into an electrochemical gradient across the cell membrane. Such conversion from one storable form to another can never be accomplished with 100% efficiency if it is to take place within a finite time. Some entropy production, i.e., heat loss, must occur. The operation of Na,K-ATPase is also reversible: at low ATP concentration, Na,K-ATPase can actually let Na ions and K ions flow down the potential and use part of the released energy to produce ATP [9]. A motor protein is fundamentally different. A motor protein employs the energy of ATP hydrolysis to work against friction. The power stroke can actually be compared best to a bullet falling down in a bottle of maple syrup. This process converts potential energy, via friction, into heat and it is obviously irreversible. It is also a process that can achieve 100% efficiency. It is therefore reasonable to take the full $G = 22$ of ATP hydrolysis as the energy for the power stroke.

When a particle in an overdamped, homogeneous medium without other external forces 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 L/T will lead to more energy dissipation. In this sense, motion in an overdamped medium is fundamentally different from motion in a conservative force field [10,11]. In the context of Fig. 1(b), this also means that any variation in slope will lead to a larger $T_{\rm ps}$ and a decreased efficiency. It is likely that 3.5×10^9 years of evolution has led to a smooth power stroke with a constant force.

A common approach to modeling 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 be modeled 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 [12]. In the case of Na,K-ATPase, the actual movement and the energy invested into overcoming friction is of negligible 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 center of mass. The friction force that is overcome in that transition is $F_{\rm fr} = \beta \Delta x/\Delta t$, where β represents the coefficient of friction. The energy dissipated in

the displacement equals $E = F_{\rm fr}\Delta x = \beta(\Delta x)^2/\Delta t$. 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. However, it has been shown rigorously that this added noise does not alter the energy transduction from ATP hydrolysis to motion against friction [10,11]; the Brownian kicks fluctuate as much energy in as 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.

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. 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 center of this Gaussian moves with a speed v according to (2). An effective diffusion coefficient for the spreading can be expressed as follows [13]:

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

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 $D_{\rm eff}$ in (3) eventually unaffected as it should be. It is important to realize that $D_{\rm eff}$ is different from the diffusion coefficient D that indicates the strength of the Brownian jolts. $D_{\rm eff}$ describes the spread of the drifting particles and, as such, it also takes account of the shape of the energy profile. Experimentalists have commonly expressed the "diffusive spreading" during transport in terms of a dimensionless quantity that expresses a diffusion-drift ratio and is called the randomness r [14]:

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

Here v is again the average speed and ε is the length of a period. Different mechanisms lead to different values of r. In 1994, Svoboda *et al.* measured the randomness for moving motor proteins [14] and they used their data to rule out certain models and mechanisms.

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 $D_{\rm eff}$ 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 $x = \phi$. There are standard and straightforward methods to compute the second moment [8]. In this case, we obtain $\langle t^2\rangle = \frac{5}{12}\phi^4$. So for the variance we get $(\Delta t)^2 = \frac{1}{6}\phi^4$. Taking $L = 1$ in formula (3), we find

$$D_{\rm eff} = \frac{\frac{1}{12}\phi^4}{[\frac{1}{G}(1-\phi)^2 + \frac{1}{2}\phi^2]^3}. \tag{5}$$

For the randomness, this leads to

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

Taking the diffusion on the downslide into account leads to extra terms in the numerator of (6). But with G in the physiological range (≈ 20), these terms are again negligible. In [13] it is shown how v and $D_{\rm eff}$ can be evaluated exactly on any tilted periodic potential in the presence of a constant nonzero D everywhere.

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 [15]. 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 $v_{\rm obs}$, 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. For the randomness one derives [16]

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

Ma and Taylor used a variety of biochemical methods to determine conformational states and transition rates in the stepping cycle of kinesin. In [17] they present a picture that looks similar to our Fig. 1(a). They found $T_{\rm ps}/T_{\rm diff} \approx 0.75$. We will call this ratio ξ. From Eq. (1) it is easily derived that the model of Fig. 1 leads to $\xi = T_{\rm ps}/T_{\rm diff} = 2(1 - \frac{1}{\phi})^2/G$. We thus get for the variable ϕ

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

For $G = 22$, we obtain $\phi = 0.26$.

When we take the above formula for ϕ and substitute it in Eqs. (2) and (6), we obtain for speed and randomness in terms of G and ξ

$$v = 2\frac{(1 + \sqrt{\frac{1}{2}\xi G})^2}{1+\xi}, \qquad r = \frac{2}{3(1+\xi)^2}. \tag{9}$$

Remarkably, G cancels out of the expression for the randomness r. With $\xi = 0.75$ and $G = 22$, we get $v = 17$ and $r = 0.22$.

In a large number of identical experiments at saturating ATP concentration, it was found that $v_{\mathrm{obs}} = 810\ \mathrm{nm/s}$ and $r_{\mathrm{obs}} = 0.44$ [15]. Both results carried a standard error of about 4%. Using $r = 0.22$, Eq. (7), and $p + q = 1$, we obtain a simple quadratic equation for the backstep probability q that yields $q = 5.8\%$ for $\xi = 0.75$. As was mentioned before, in the experiments described in [15], the individual 8 nm steps could actually be resolved. The experimentally 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. This picture does not correspond to an accidental sequence of Brownian kicks that drives the particle in Fig. 1(b) up the slope. With a noise strength of $1kT$, the likelihood of such an accidental mounting of the barrier of about $20kT$ to the left is many orders of magnitude smaller than the likelihood of sliding down the $20kT$ well to the right. Incorporating the above described backstepping scenario would require the addition of a second dimension to the 1D reaction space of Fig. 1(b).

Much experimental work has focused on the duty ratio [5], i.e., the fraction of time that a head is attached to the biopolymer. It should be pointed out that, in our model, it is entirely possible for the trailing head to remain attached for any part of the power stroke. For $T_{\mathrm{ps}}/T_{\mathrm{diff}} \approx 0.75$ this means that the model is consistent with any duty ratio between 50% and 70%.

If v_{obs} is to be expressed in meters per second, $v_{\mathrm{obs}} = (p - q)v$ needs to include a redimensionalization factor D/ε on the right-hand side, i.e., $v_{\mathrm{obs}} = (p - q)Dv/\varepsilon$. Given the observed values for v_{obs} and ε, and the derived values for v and q, this formula allows us to estimate D. Through $\beta = kT/D$ we then also obtain the average internal friction of the motor protein. For $G = 22$, $\xi = 0.75$, and $v_{\mathrm{obs}} = 810\ \mathrm{nm/s}$, we find $D = 4.3 \times 10^{-16}\ \mathrm{m^2/s}$ and an associated friction β of about $10^{-5}\ \mathrm{Ns/m}$.

The estimate $D = 4.3 \times 10^{-16}\ \mathrm{m^2/s}$ is reasonable. It is about 4 orders of magnitude smaller than the diffusion coefficient for a freely dissolved kinesin size protein in the cytosol [2]. With $\beta = 6\pi\eta r$ for the friction of a spherical bead and $\eta_{\mathrm{H_2O}} = 10^{-3}\ \mathrm{kg/ms}$ for the viscosity of water, one easily verifies that $\beta = 10^{-5}\ \mathrm{Ns/m}$ is equivalent to the friction of a 400 μm bead in water. So the hydrodynamic friction of the submicrometer bead in the aforementioned experiments can be legitimately and safely neglected as a factor in the motion.

Motor protein action has most commonly been modeled as a succession of discrete chemical, i.e., Markov, steps. Many free parameters are involved in fitting theory to experiment. This makes these models to some extent immune to experimental falsification. However, for reasons pointed out in this Letter, such Markov descriptions may be fundamentally unrealistic for the modeling of motor protein action. Diffusive motion along a reaction coordinate underlies chemical kinetics. Such diffusive motion is the basis for the model presented in this Letter. The construction of the piecewise linear profile in Fig. 1(b) was based on the observed stepping mechanism and guided by the premise that natural selection must have led to an optimal shape. The resulting formulas are simple and concise, there are no free parameters, and the inputs are observed data. The model correctly predicts other observed data. On processive motor proteins other than kinesin, such as myosin V, dynein, and RNA polymerase, the available experimental data are less abundant. The above model should, in principle, also apply to these motor proteins.

The animation of Ref. [4] was created by R. D. Vale, R. A. Milligan, and G. Johnson, and was included as supplementary material to Ref. [3] of this Letter.

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