The Noisy Steps of a Motor Protein

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Abstract. There is experimental evidence to show that the motor protein *kinesin* literally "walks" along the biopolymer *microtubule*. It is possible to depict this "stepping" as the movement of a point along a reaction coordinate. The reorientation of the attached head is an energetically downhill "power stroke." No energy is dissipated (i.e. a flat stretch along the reaction coordinate) during the subsequent diffusive route of the detached head to a new forward docking site. A model that is based on these premises can quantitatively account for the experimentally measured load-velocity diagrams and for the diffusion-drift ratios at different loads. Presently there is a divide in the research on molecular motors and directed transport. Theoreticians study simple, ratchet based mechanisms to come to an understanding of the physics of motion in the overdamped, Brownian realm. Many biophysicists seek to clarify the operation of the motor protein through a detailed study of the 3D molecular structure. This paper attempts to bridge the gap by rigorously modeling the stepping process in an overdamped, Brownian environment.

Over the past decade the amino acid sequence and the 3D structure of an overwhelming amount of proteins has been figured out and catalogued (see http://www.pdb.org). Less progress, however, has been made in understanding how protein structure translates into protein function. The motor protein kinesin is a case in point. The entire 3D structure has been known for a few years now, but it is still largely unclear how the protein operates. Kinesin is a dimer, i.e., it consists of two identical units called "heads." Most textbooks present a model in which these heads function as feet that literally step from one unit of the microtubule to the next unit 8 nanometers further [1] (for an animation see http://valelab.ucsf.edu). But the last word on this issue has not been spoken. The "hand-over-hand" stepping model is far from a certainty. Recently a paper appeared in which evidence for a so-called "inchworm" model was presented [2]. In this model the front head always stays up front and the forward step of the front head is followed by an equally long forward "catch up" step of the hind leg.

In this paper I will use the "hand-over-hand" model as a working hypothesis. But the results, or at least the ideas, are equally well applicable to an inchworm model.

Macroscopic human stepping may look similar to the microscopic stepping of the motor protein, but the underlying physics is entirely different. For a walking human the physics consists of mass, gravity and inertia. A walking human uses energy for the repeated lifting of the anterior leg against gravity and for the subsequent forward acceleration of the same leg to a speed larger than the speed of the torso, so he can eventually put the foot down again in front of him. On the nanometer scale in a liquid the only significant force is friction [3]. Of course, it is not gravity that keeps the motor protein connected to the biopolymer. It is chemical bonds. In the course of stepping these bonds are repeatedly broken and then reestablished. This attachment and detachment cannot just take place in a random and equilibrium fashion. If that were the case no

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forward motion would occur and there would be a significant likelihood for both heads to be simultaneously detached after which the entire dimer would diffuse away from the biopolymer. Directionality and coordination are achieved by coupling the stepping cycle to the hydrolysis of ATP. The conformational changes of the protein as it catalyzes the hydrolysis of one ATP molecule also constitute one forward step. The hydrolysis of ATP is thus energetically coupled to the stepping cycle and, effectively, driving the movement.

When the involved energies are of the order of kT, Brownian noise, i.e., random collisions with molecules from the medium, becomes part of the physics. An important formula is Einstein's Fluctuation-Dissipation Theorem, $D = kT/\beta$, which relates the friction to the strength of the diffusion D.

Microtubule, the biopolymer that kinesin steps on, is anisotropic. Each unit of this polymer is an actual protein of almost 900 amino acids. Going in one direction on the microtubule one sees a different profile than when traveling in the opposite direction. Fig. 1a indicates this with the way the binding sites have been drawn. Kinesin can only step in one direction and this direction is determined by the orientation of the microtubule. Transport over microtubule in the other direction is taken care of by motor proteins other than kinesin [1, 4].

In the stepping cycle of kinesin we can distinguish two phases:

1. A power stroke phase (Fig. 1a left side), i.e. the reorientation of the attached head after the anterior head detaches. This is when force is generated, when power is dissipated and when a load (like a vesicle with chemicals or an organelle) is being pulled.
2. A ratcheted diffusion phase (Fig. 1a right 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. This phase has been called a "random diffusional search" [5] and has been described as "fluctuational interactions" or "conformational fluctuations" [6]. But from a physics perspective it looks very much like a random walk between a reflecting barrier (the neck linker attachment) and an absorbing barrier (where docking occurs). Starting at the reflecting barrier at $t = 0$ the average escape time to reach the absorbing barrier is $< T_{esc} >= L^2/2D$ if the distance between the reflecting barrier and the absorbing barrier equals L.

Fig. 1b 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. But this may be seriously 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 parts of the cycle and the corresponding 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.

In Fig. 1b a power stroke driven by a constant force is represented by a downward

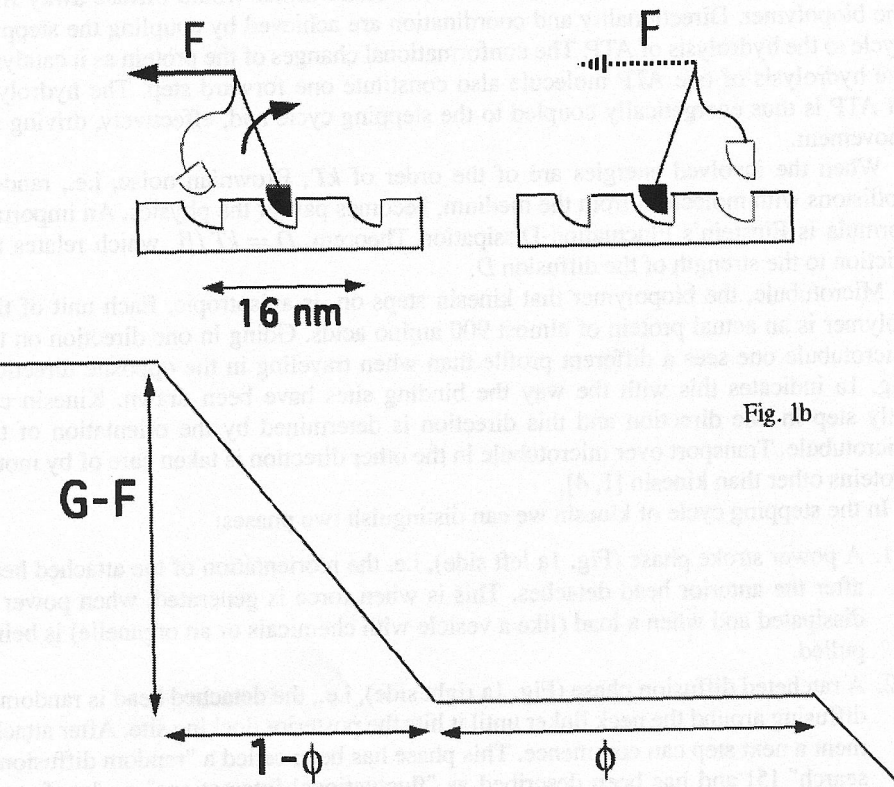

Fig. 1b

IGURE 1. The setup for our model. One step of the two headed motor protein (1a) corresponds aversing one unit in a 1D reaction space (1b). The reorientation of the attached head is the power stro ith energy G that covers a fraction $(1-\phi)$ of the cycle. The subsequent diffusion and docking of etached head does not dissipate any energy and covers the remaining fraction ϕ. When the motor prot pulling against a load force F, it is the power stroke that provides the energy. The diffusive segmer naffected.

lope. We take the stepsize of kinesin, which equals $\lambda = 8$ nm, to be our unit of leng his implies that if the kinesin is pulling against a load F it releases an energy G - t every power stroke, where G is the energy of ATP hydrolysis. With energy measu n units of kT, G equals about 22 at physiological conditions. When we also scale iffusion coefficient D to unity, $\beta = kT/D$ implies a friction coefficient β that equ nity. For $(G-F) > 2$ the diffusive noise on the power stroke constitutes a neglig

effect and we take the power stroke to be a deterministic downslide in Fig. 1a. The force driving the power stroke thus equals the power stroke velocity: $F_{ps} = (G-F)/(1-\phi)$. By inverting the total time to traverse one period we obtain a nice and concise formula for the speed of the motor protein:

$$v = \{T_{ps} + T_{diff}\}^{-1} = \{\frac{1}{G-F}(1-\phi)^2 + \frac{1}{2}\phi^2\}^{-1}. \quad (1)$$

It is important to realize that a motor protein is fundamentally different from another energy converter like Na,K-ATPase. Na,K-ATPase and many other proteins convert energy from one storable form to another. Na,K-ATPase takes the chemical energy in ATP and turns it into an electro-osmotic gradient across the cell membrane. Such conversion from one storable form to another can never be accomplished with 100% accuracy if it is to take place within finite time. Some entropy production, i.e. heat loss, must occur. The operation of Na,K-ATPase is, furthermore, 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. A motor protein is fundamentally different. A motor protein employs the energy of ATP hydrolysis to work against friction. The power stroke can actually best be compared 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 a 100% efficiency. It is therefore reasonable to take the full $G = 22$ of ATP hydrolysis as the energy for the power stroke.

When in the overdamped realm without other external forces, the most energy efficient way to move over a distance L in time T is with a constant velocity $v = L/T$ brought about by the work of a constant force. Any variations in speed, i.e. force, will lead to more energy necessary to cover the distance L in time T. In the context of our Fig. 1b this also means that any variation in slope will lead to a larger T_{ps}. It is likely that the motor protein exhibits some variations in the force of the power stroke. After all, the power stroke is constituted by a sequence of discrete conformational changes. This longer time can be construed to derive from a constant slope at a G smaller than 22. Eventually we will see how the best fit is obtained with a constant slope of $G = 16$.

A motor protein is a stochastic stepper. This means that if we do identical experiments starting the motor protein at $x = 0$ at $t = 0$, that we will then measure different arrival times at some $x = L$ in the forward direction down the biopolymer. Formula (1) for the average speed can give only the average arrival time. In 1994 Svoboda et al. were the first to measure standard deviations in arrival times and use the observations to rule out certain models [7]. The standard deviation can be used to formulate an effective diffusion coefficient: $D_{eff} = \frac{1}{2}L^2(\Delta t)^2/ < t >^3$, where $< t >$ is the average time to cover distance L and Δt is the standard deviation. D_{eff} describes how a drifting ensemble of motor proteins spreads and it can be significantly different from D [8]. D_{eff} is commonly absorbed into a dimensionless quantity, $r = 2D_{eff}/(v\lambda)$, which is called the randomness. Here λ represents the spatial period (i.e. the 8 nm steplength) and v represents the average speed. The randomness can be interpreted as a diffusion/drift ratio per period.

It is straightforward to calculate the randomness when drifting down the profile in Fig. 1b. Since we go down the power stroke deterministically, the only source of stochasticity is constituted by the flat segment. When diffusing from a reflecting barrier

to an absorbing barrier at a distance ϕ the second moment can be evaluated to be $\frac{5}{12}\phi^4$ [9]. Eventually we derive for the randomness:

$$r = \frac{\frac{1}{6}\phi^4}{\{\frac{1}{G-F}(1-\phi)^2 + \frac{1}{2}\phi^2\}^2}. \quad (2)$$

There is one more complication we have to take care of before we can compare the predictions of our model against actually measured velocity-load and randomness-load graphs. In experimental practice the motion of the motor protein is measured through a silica bead that is connected to the neck linker and visible through a microscope. It appears that between 5% and 10% of kinesin's steps are backward [10]. In the context of the above model the soundest way to explain these backward steps would be as follows. After a full power stroke (i.e. a forward motion of the bead) the detached head accidentally hits and binds at the anterior docking site instead of the posterior one. The anterior binding is then followed by a backward stroke and a detachment of the front head. In the context of Fig. 1b it would be wrong to think of the backstep as an accidental, Brownian upslide up the $G-F$ barrier. With a barrier of the order of 10 kT an upslide in Fig. 1b is many orders of magnitude less likely than the downslide. Incorporating anterior binding would involve adding a dimension to the reaction coordinate. The progress of the motor protein would then be represented by the motion of an overdamped, Brownian point particle in a 2D landscape.

When backward stepping occurs with a probability q (and for the forward stepping probability we thus have $p = 1-q$), the v and r of formulae (1) and (2) need to undergo the following corrections to represent the observed speed and randomness:

$$v_{obs} = (p-q)v, \quad (3)$$

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

Fig. 2 depicts the power stroke. The power stroke doesn't just drive the orientation of the attached head from θ_2 to θ_1. At the end of the power stroke a Boltzmann equilibrium is established and we have an average orientation $\theta_{eq} = \theta_1 + \Delta\theta$. The power stroke is driven by an energy release $G-F$. Assuming that this energy difference translates into a constant and homogeneous force, we find after integration of θ over the Boltzmann distribution for sufficiently large $G-F$ (larger than a few kT's) that $\Delta\theta = \frac{kT}{(G-F)}(\theta_2 - \theta_1)$. Picturing the detached head as "dangling" from the neck it is realistic to assume that the backstep probability goes up linearly with $\Delta\theta$, i.e. $q(F) = q_0(1-\frac{F}{G})^{-1}$. Obviously q_0 is the backstep probability when $F = 0$.

Next we take the backward stepping probability at $F = 0$ to be equal to $q = 0.05$, which is the lower limit of the 5% to 10% reported in [6, 10]. Schnitzer et al. report no "substantial" increase in the backstep probability for increasing load [6]. Coppin et al. do claim to see an increase in backstepping when the load reaches the stall force [11]. Because a motor protein easily detaches when the load is high, the involved measurements are hard and the margin of error is high. To redimensionalize the F in our model it must be multiplied with $kT/\lambda = 0.53$ pN (where λ represents the 8 nm period

Fig. 2

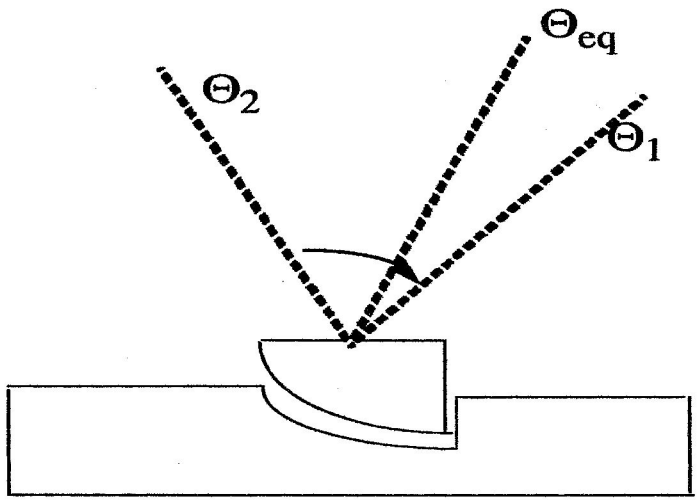

FIGURE 2. A more detailed consideration of the power stroke depicted in Fig. 1a. We assume that going from orientation θ_2 to orientation θ_1 the release of the energy $G-F$ leads to a uniform and homogeneous force. In a Brownian environment a Boltzmann distribution is established at the end of the power stroke and the average position is $\theta_{eq} = \frac{kT}{G-F}(\theta_2 - \theta_1) + \theta_1$. Assuming that the backward stepping probability q increases linearly with θ_{eq} we are led to $q(F) = q_0(1 - \frac{F}{G})^{-1}$, where q_0 is the backward stepping probability at zero load.

of the motion). At $G = 16kT$ we thus get a stall force of about 8 pN. 5 pN corresponds to $F = 9$ and leads to a backstep probability of 11%. These estimates are not inconsistent with experimental observations and constitute a legitimate working assumption.

Fig. 3 shows the experimental data together with the predictions of our model for velocity vs. load and randomness vs. load. The fit was for $G = 16kT$, $\phi = 0.28$ and $D = 5.4\ 10^{-16}\ \mathrm{m}^2/\mathrm{s}$. Our result for the average D is of the same order of magnitude as what other authors have estimated. The average internal friction of the motor protein, i.e. $\beta = kT/D$, does then indeed come out to be an order of magnitude larger than that of the micrometer magnitude bead that is dragged along in the experiments. With $G = 16kT$, $\phi = 0.28$ and $D = 5.4\ 10^{-16}\ \mathrm{m}^2/\mathrm{s}$ we also find that D_{eff} is about 10 times as large as D. This means that the stepping motor protein may provide an example of the recently discussed "enhanced diffusion" [8], i.e., cases where drift can significantly blow up diffusion.

It is remarkable that a simple model, with only very few assumptions and free parameters, can quantitatively account for the experimental data. An independent corroboration of the above results is provided by the work of Yong-Ze Ma and Edwin Taylor. These

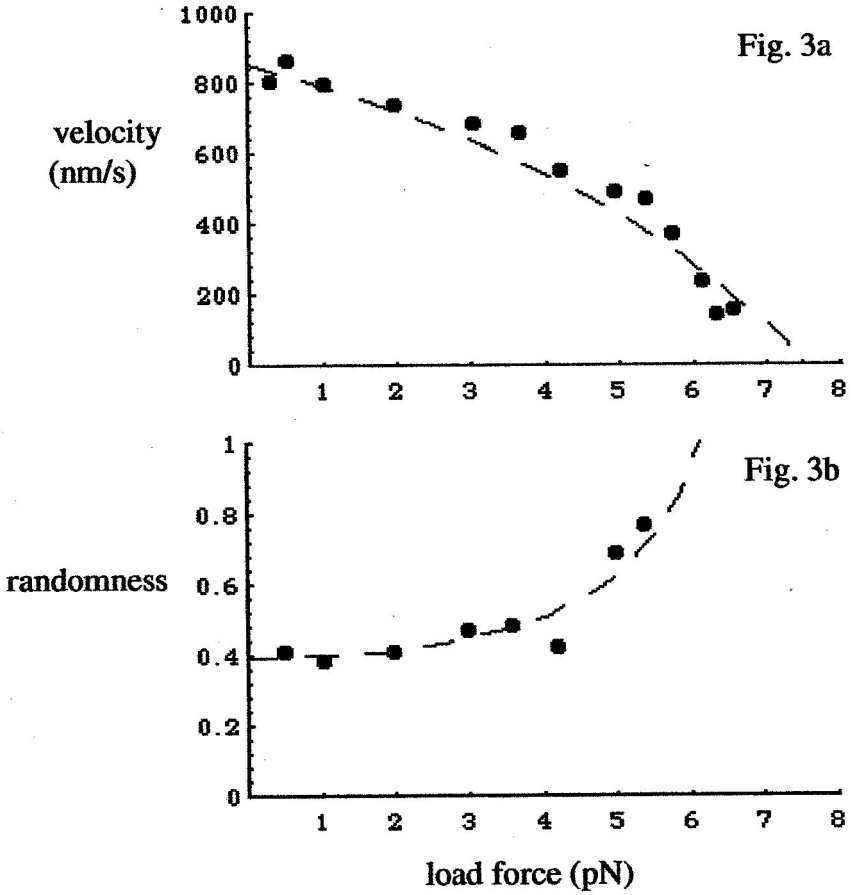

FIGURE 3. A force-velocity characteristic (3a) and a force-randomness characteristic (3b) for kinesin "walking" over microtubule. The dashed curves are the results of the simple stepping model described in the text. The dots represent the experimental data, cf. [10]. In the final analysis these graphs compare theory and experiment for the first and second moment of the stochastic motion.

researchers used a variety of biochemical methods to determine the different conformational states in the stepping cycle of kinesin. At the end of two back-to-back articles in 1997 [12, 13] they present a picture that looks very similar to Fig. 1a. They determined the rates from one state to a next and they found $T_{ps}/T_{diff} \approx 0.75$. At zero load the basic setup of our model (cf. Fig. 1b) leads to $T_{ps}/T_{diff} = \frac{2}{G}(1-\frac{1}{\phi})^2$. Numerically this works out to $T_{ps}/T_{diff} = 0.82$ for the G and ϕ that led to the fit in Fig. 3. All in all, there is excellent agreement between the simple model presented in Fig. 1 and the available data.

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