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The stepping motor protein as a feedback control ratchet

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Abstract

It is explained how going from a one headed motor protein to a stepping two headed motor protein is equivalent to going from a stochastically flashing ratchet to a feedback control ratchet. Both these ratchets have been well studied in the literature and their speeds and efficiencies are briefly reviewed. Next it is shown how a feedback control ratchet mechanism model can account for very accurate recent data obtained on kinesin. Finally, the role of internal friction in the operation of stepping motor proteins is discussed.

Keywords: Processive motor protein; Feedback control Brownian ratchet; Polypeptide internal friction

1. The Brownian ratchet

A little over 10 years ago interest in "fluctuating potentials" or "flashing ratchets" emerged (Ajdari and Prost, 1992; Astumian and Bier, 1994; Prost et al., 1994, for reviews, see: Reimann, 2002; Reimann and Hänggi, 2002; Astumian and Hänggi, 2002). The basic concept is clarified in Fig. 1. The figure shows a periodic, anisotropic, piecewise linear potential $V(x)$. The period is normalized to unity. The maximum is at $x = \phi$ and it is obvious that $V(x)$ is anisotropic for $\phi \neq 1/2$. When at equilibrium and with the maxima sufficiently high, Brownian particles in $V(x)$ can assumed to be concentrated around the minima at $x = 0$, $x = 1, \ldots$. Upon a switch from $V(x)$ to a flat potential, the Brownian particles will start diffusing and, given enough time, they will randomize their position within a period, i.e, a fraction ϕ will end up on the short segment and a fraction $(1 - \phi)$ will end up on the long segment. When the potential cycles back to $V(x)$, the Brownian particles on the long segment will be pushed towards the right by an average distance of $(1/2)(1 - \phi)$. The particles on the short slope will, on average, undergo a $(1/2)\phi$ displacement to the left. So the net result of one cycle is an average displacement of $((1/2) - \phi)$ to the right. Undoing the normalization and letting the period be L, we find a speed of:

$$v \approx \left(\frac{1}{2} - \phi\right) \frac{L}{\tau}, \tag{1}$$

where τ is the average time to complete one cycle.

In order to maximize the speed the Brownian particle should have sufficient time τ_0 on the flat potential to at least diffuse more than the length ϕ of the short segment. If τ_0 is too short, then the distribution does not have opportunity to spread from the initial delta function. If τ_0 is too long, then τ will become too long and, following Eq. (1), this will take away from the achieved speed. The time τ_V in state $V(x)$ should be long enough for particles to "slide down" the long slope from the maxima all the way to the minima and form a delta function-like distributions there. The τ_0 and τ_V that maximize the speed can be found as follows. Let the maximum in Fig. 1 be at $G\,(k_B T)$-units of energy. On the molecular level in an overdamped

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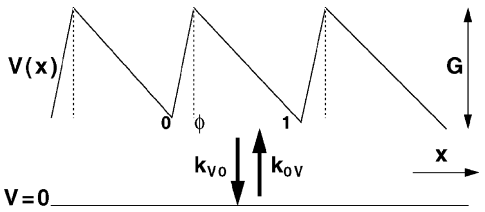

Fig. 1. The setup for the flashing ratchet. A Brownian particle is moving in a potential that fluctuates or oscillates between $V(x)$ and $V = 0$. $V(x)$ is an anisotropic potential the period of which is normalized to unity. G is the height of the barriers. If the diffusing particle is given sufficient time in $V = 0$ to randomize its position within a period, then it will be more likely be pushed to the right than to the left after the return to $V(x)$. k_{V0} and k_{0V} denote the transition rates between the potentials. The maximal magnitude of the ensuing flux is estimated in the text.

medium we have $F = \beta v$ for the relation between force and velocity. Here β represents the coefficient of friction. With $\tau_V = \beta L(1-\phi)/F$ and $F = Gk_BT/[L(1-\phi)]$, we then find for the optimal time in $V(x)$: $\tau_V = [L(1-\phi)]^2/(GD)$. In this derivation we made use of Einstein's Fluctuation–Dissipation Theorem, i.e. $D = k_BT/\beta$. The time to diffuse across the length ϕL of the short segment in Fig. 1 equals $\tau_0 = (1/2)(\phi L)^2/D$. We thus find that maximal speed is achieved with $\tau = \tau_0 + \tau_V = [(1/G)(1-\phi)^2 + (1/2)\phi^2]L^2/D$. For the speed itself we then have:

$$v_{\max} \approx \left(\frac{1}{2} - \phi\right) \frac{1}{\{(1/G)(1-\phi)^2 + (1/2)\phi^2\}} \frac{D}{L}. \quad (2)$$

Eq. (2) is a back-of-the-envelope estimate. However, this estimate turns out to correspond well with an exact solution that can be derived for the case of constant transition rates between $V(x)$ and $V = 0$ (Astumian and Bier, 1994). Constant transition rates are a good description when the "flips" are chemically driven. In that case the times τ_0 and τ_V that we used in the derivation are actually averages with $\tau_0 = k_{0V}^{-1}$ and $\tau_V = k_{V0}^{-1}$.

The remarkable thing about the mechanism in Fig. 1 is that there is no net macroscopic force, i.e. tilt, in either of the two potentials. The necessary energy input occurs when, during a k_{0V} flip from $V = 0$ to $V(x)$, the energy level of the particle is lifted. The transport is due to the anisotropy that breaks the right-left symmetry. Without diffusion, i.e. thermal noise, everything would obviously come to a standstill. It is therefore that the terms "thermal ratchet" and "Brownian ratchet" have been employed. Part of the excitement about thermal ratchets came about because they may constitute the operating mechanism of processive motor proteins (Alberts et al., 1994; Boal, 2002; Bray, 2001; Howard, 2001). In the case of these motor proteins the anisotropy would be on a molecular scale.

2. Processive motor protein as feedback control ratchet

In 1993 Karel Svoboda and his coworkers succeeded in resolving the movement of an individual motor protein on the nanometer scale (Svoboda et al., 1993). They followed kinesin as it moved in 8 nm steps on the biopolymer microtubule (Alberts et al., 1994; Boal, 2002; Bray, 2001; Howard, 2001). They measured speeds and stopping forces. The mechanism depicted in Fig. 1 could well be the way that such a molecular size engine works. The motor protein cycles through a number of conformational states as adenosine triphosphate (ATP; the fuel in a living cell) is bound to the motor protein, hydrolyzed, and released as ADP (adenosine diphosphate) and an inorganic phosphate group. As the motor protein is forced through its cycle, the potential between the kinesin and the microtubule to which it is bound changes. Of course, the potential is 8 nm-periodic and without any net tilt as the microtubule repeats itself every 8 nm. Each period of the microtubule track on which kinesin moves actually consists of a polypepetide of many hundreds of amino acids. So, more likely than not, the potential will be anisotropic. Finally, the presence of diffusion is obvious on the molecular scale. Models are taken to the test as more variables can be measured and/or controlled in an experiment. Even though the model of Fig. 1 is rough, it can account for the data of Svoboda et al. within an order of magnitude (Astumian and Bier, 1994). Substituting some of the experimental data, the model correctly "predicts" other data that were obtained in the same experiment.

It is possible to enhance the setup of Fig. 1 to make for higher speeds and efficiencies. One can, for instance, construct models with three or more states. Another possible venue is changing the $V = 0$ state in Fig. 1 into another periodic profile and/or turning to more complicated profiles than the two-segment piecewise linear one. The enhancement that will be our focus in the remainder of this article consists in making the flipping rates depend on the position x, i.e. $k_{0V} = k_{0V}(x)$ and $k_{V0} = k_{V0}(x)$. The resulting systems have been characterized as "information ratchets," "intelligence ratchets," or "control ratchets" as the system actually reads where it is and adjusts its flipping behavior accordingly (Bier, 1997).

In a 2004 paper Cao, Dinis, and Parrondo turned the basic system of Fig. 1 into a control ratchet through the following modification (Cao et al., 2004). Whenever the Brownian particle is on the interval $\langle 0, \phi\rangle$, the potential is kept on $V = 0$. Whenever the particle is on $\langle \phi, 1\rangle$, the potential $V = V(x)$ is kept on. Basically, an observing controller is sitting at a switch and flips the potential

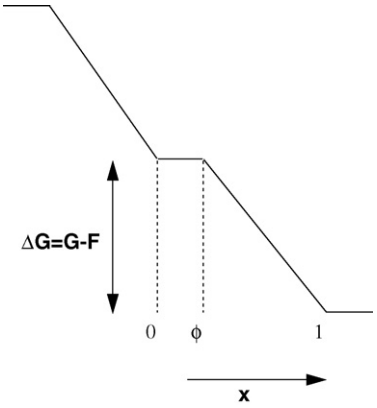

Fig. 2. The effective potential if the transitions between $V(x)$ and $V = 0$ in Fig. 1 are coordinated with the motion of the Brownian particle such that $V = 0$ is turned on if the particle is on $\langle 0, \phi \rangle$ and that $V(x)$ is turned on if the particle is on $\langle \phi, 1 \rangle$. The flux in this feedback control ratchet is about double the maximal flux that can be achieved with the setup of Fig. 1. This effective potential is also shown to be the shape of the reaction coordinate for the operation of a two headed motor protein. G represents the free energy made available from ATP hydrolysis. F is the scaled load force pulling the motor protein back in optical tweezer experiments.

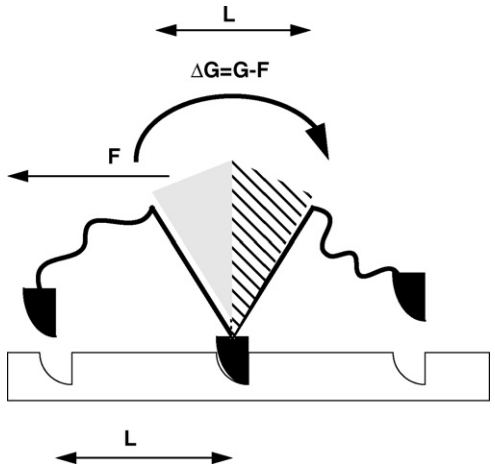

Fig. 3. For a stepping motor protein the power stroke occurs when the head that is attached to the biopolymer reorients and brings the detached head to the vicinity of the next forward binding site. The diffusive route of the detached head to the binding site corresponds to the flat stretch in Fig. 2. The power stroke corresponds to the downward slope in Fig. 2. The power stroke dissipates an energy $\Delta G = G - F$. Here G is the energy from ATP hydrolysis and F is the scaled force with which the protein is pulled back. We take the probability of the detached head accidentally binding to the binding site in the back to be equal to the probability of the Boltzmann equilibrated head to be in the grey area. This assumption leads to a remarkably good fit with recently obtained experimental backstep data.

whenever a crossing takes place at $x = 0$ or $x = \phi$. In this case we effectively get the particle to slide down the potential shown in Fig. 2. In the reference by Cao et al. the average speed on the profile in Fig. 2 is analytically evaluated. Unscaled, i.e. with a spatial period L and a diffusion coefficient D, we have:

$$v = \frac{1}{\{(1/G)(1-\phi^2) + (1/2)\phi^2\}} \frac{D}{L}, \tag{3}$$

where G is again in units of $k_B T$. For a sufficiently large value of G, we can take $x = 0$ to be a reflecting barrier and $x = \phi$ to be an absorbing barrier, i.e. backward movement of the Brownian particle is not allowed at $x = 0$ and $x = \phi$. For large G, we can, furthermore, take the trajectory from $x = \phi$ to $x = 1$ to be a deterministic downslide. In that case evaluation of the average speed is simpler and one derives (Bier, 2003a,b, 2005):

$$v \approx \frac{1}{\{(1/G)(1-\phi)^2 + (1/2)\phi^2\}} \frac{D}{L}. \tag{4}$$

This is exactly like Eq. (2) but without the $((1/2) - \phi)$ prefactor! So going from the stochastic ratchet of Fig. 1 to Parrondo's feedback control ratchet increases the speed by at least a factor 2.

When it comes to the anisotropy parameter, the resulting flux in the setup of Fig. 1 is obviously maximal for $\phi = 0$ and $\phi = 1$. However, because of the finite size of binding sites and charged groups, the distance between the maximum and the minimum in Fig. 1 should be no smaller than about 1 nm. For a period of $L = 8$ nm we then have $v \approx L/(3\tau)$, i.e. one traversed period for every three cycles. In the context of the motor protein every cycle involves the hydrolysis of one ATP. Three futile ATP conversions for every successful one would obviously be wasteful.

At first sight, Parrondo's feedback control ratchet looks like a construct that may be significant for nanotechnological applications more than that it may be relevant for understanding the operation of motor proteins. Surprisingly however, feedback control holds the key to comprehending some key features of motor proteins. Processive motor proteins are generally dimers. When kinesin moves along microtubule it literally "steps." When one head is tightly bound, the other moves randomly until it hits the next forward binding site and attaches (see Fig. 3). At that point the first head detaches and a next step can commence. KIF1A is a kinesin motor that will dimerize when given the opportunity (Tomishige et al., 2002). However, also as a monomer it can move forward on microtubule and hydrolyze ATP in the process. But in its monomeric form KIF1A moves with a slower net speed and with many more backsteps and random wanderings (i.e. a higher effective diffusion coefficient) than in its dimeric form. In 1999 Okada and

Hirokawa reported their observations on the motion of monomeric KIF1A (Okada and Hirokawa, 1999). They explained the observed movement with the stochastically flashing ratchet mechanism of Fig. 1. For the dimer's operation it would be sensible to assume that the head in front makes a forward reorientation just after the head in the back has detached (see Fig. 3). The detached head is then brought into the vicinity of the forward binding site. It attaches there after a brief random search. With two heads acting in a coordinated manner, backstepping can be reduced and almost perfect chemo-mechanical coupling, i.e. one step per hydrolyzed ATP, can be achieved. Such 1:1 coupling has also been observed experimentally (Hackney, 1995). So, in terms of speed and efficiency, going from a stochastically flashing ratchet (cf. Fig. 1) to a feedback control ratchet (cf. Figs. 2 and 3) represents an about threefold improvement. In a thorough experimental study of of monomeric and dimeric kinesin motion, Inoue et al. (2001) indeed found such threefold speed increases after dimerization.

The reaction coordinate for the stepping process is exactly the profile in Fig. 2. During the random diffusional search of the detached head, no energy is dissipated and this translates into the flat segment $\langle 0, \phi \rangle$. The reorientation of the attached head constitutes the power stroke (cf. Fig. 3). G represents the free energy that the hydrolysis of ATP makes available. If the motor protein has to also work against a load, i.e. a conservative force F, then less energy will be available to work against friction. With the power stroke distance L (cf. Fig. 3) as the unit of distance, we have $\Delta G = G - F$ as the available energy for the power stroke.

It is in the power stroke that the energy $\Delta G = G - F$ is dissipated. As the motor protein is moving along the biopolymer, none of that energy is stored. It all goes into overcoming friction. Friction forces do not derive from a conservative field. The amount of friction that a moving particle "feels" is roughly proportional to the speed of the particle. So $F_{\mathrm{fr}} = \beta v$, where β represents the coefficient of friction. If friction is the force to overcome when moving a distance L in time T, then moving with a *constant* speed $v = L/T$ leads to the minimum amount of dissipated energy (Derényi et al., 1999; Bier, 2001). So, through natural selection, there is pressure towards a power stroke with a constant force, i.e. a smooth "twist" in Fig. 3 and a straight line from $x = \phi$ to $x = 1$ in Fig. 2. If there is an amount of energy ΔG available for movement from $x = \phi$ to $x = 1$ in an overdamped medium, then a constant force $\Delta G/(1 - \phi)$ is the fastest way to traverse that distance. Locally varying the slope, for instance by putting little activation barriers on the power stroke profile, takes away from the efficiency.

Suppose $F = 0$. If the power stroke were a perfect straight segment, then G would be the about 22 $k_{\mathrm{B}}T$ units of energy released in ATP hydrolysis that would be available in physiological conditions. The slope variations of the actual power stroke make the power stroke correspond to a smooth stroke of an energy G that is actually smaller than 22 $k_{\mathrm{B}}T$ units. A few $k_{\mathrm{B}}T$s may furthermore be utilized to drive the binding of ATP to the motor protein and the release of ADP and inorganic phosphate from the motor protein. Matching the experimental data with our model, we will derive a value for such an "adjusted" G.

3. Comparison with experiment

The model of Figs. 2 and 3 can account (Bier, 2003a,b, 2005) for measurements that were done by the group of Steve Block (Visscher et al., 1999). Ever more accurate measurements, however, are done on the motion of motor proteins. Block's group reported the number of accidental backsteps of kinesin as being between 5% and 10% of the total number of steps. These researchers, furthermore, could not resolve any change of this percentage as they varied the load F. Very recently, Carter and Cross (2005) of the Marie Curie Research Institute were actually able to resolve the backstepping with great accuracy. Empirically they found the following exponential relation between the load and the forward to backward step ratio:

$$\frac{P_{\mathrm{f}}}{P_{\mathrm{b}}} = 802\, \mathrm{e}^{-0.95 F_{\mathrm{pN}}}. \tag{5}$$

Here F_{pN} represents the load in piconewtons. At the stall force there are just as many forward steps as that there are backward steps, so $P_{\mathrm{f}}/P_{\mathrm{b}} = 1$. The 802 prefactor is thus implicit in the 0.95 and the measured stall force of about 7 pN. Datapoints appear to follow Eq. (5) very tightly. To explain Eq. (5) we assume that it is after the completion of the power stroke, i.e. after the relaxation on the downward slope in Fig. 2, that the detached head reaches a conformational state in which it is ready to bind. The time $(1 - \phi)^2/(G - F)$ that it takes to deterministically slide down the power stroke part of Fig. 2 can legitimately be identified with the relaxation time to come to a Boltzmann equilibrium (Bier and Astumian, 1998; Bier et al., 1999). In the context of Figs. 2 and 3 it is reasonable to assume that, after the aforementioned relaxation, the probability of attaching to the forward binding site equals the probability to be in the striped segment in Fig. 3. The probability of binding to the backward binding site is equal to the probability to be in the grey segment. We put the borderline exactly

halfway down the power stroke. With a Boltzmann distribution we have $P_f = (1 - e^{-\Delta G/2})/(1 - e^{-\Delta G})$ and $P_b = (e^{-\Delta G/2} - e^{-\Delta G})/(1 - e^{-\Delta G})$. We thus find for the ratio of Eq. (5):

$$\frac{P_f}{P_b} = e^{(1/2)\Delta G} = e^{(1/2)G}\, e^{-(1/2)F}. \tag{6}$$

Eq. (6) employs the dimensionless force F in the second exponent. If we want to use the force in piconewtons F_{pN}, then we must insert a redimensionalization factor, i.e. $F/F_{pN} = L/(k_B T) \times 10^{-12}$. With $L = 8\,\text{nm}$, $k_B = 1.4 \times 10^{-23}\,\text{J/K}$, and $T = 300\,\text{K}$, the factor $L/(2k_B T) \times 10^{-12}$ comes out to be exactly the 0.95 that Carter and Cross observed!

Carter and Cross observed that kinesin still binds ATP when it is pulled back with a force larger than the stopping force. In the *News and Views* column that accompanied the article of Carter and Cross (Molloy and Schmitz, 2005) it was pointed out how remarkable this is, as it points to a fundamental difference between kinesin and customary ion pumps like, for instance, F_0F_1-ATPase. F_0F_1-ATPase is a rotary engine that couples a proton flow down a transmembrane proton gradient to the ADP → ATP reaction. If the proton gradient is reversed, then the axis starts spinning the other way and ATP is then consumed instead of produced (Nelson, 2003; Läuger, 1991). Kinesin's continuation of ATP hydrolysis under backward pulling is implicit in our model. It is the ATP hydrolysis that drives the coordinated attachment and detachment. Fig. 3 and the reasoning in the previous paragraph make clear how backward binding becomes more likely than forward binding for $F > G$.

Adjusting Eq. (4) for the presence of a load and for the presence of a nonzero backstep probability, we have:

$$v \approx (P_f - P_b)\frac{1}{\{(1/(G-F))(1-\phi)^2 + (1/2)\phi^2\}}\frac{D}{L}. \tag{7}$$

Fig. 4 shows the load–velocity data that Carter and Cross recorded. Fig. 4 also shows the theoretical curve according to Eq. (7). We use a G, i.e. the free energy made available in ATP hydrolysis, that we derive from Eqs. (5) and (6): $G = 2\ln 802 = 13.4$. We, furthermore, use $L = 8\,\text{nm}$ and the aforementioned $P_f = (1 - e^{-\Delta G/2})/(1 - e^{-\Delta G})$ and $P_b = (e^{-\Delta G/2} - e^{-\Delta G})/(1 - e^{-\Delta G})$. The theoretical curve in Fig. 4 was fitted to the data points with $\phi = 0.3$ and $D = 3.5 \times 10^{-16}\,\text{m}^2/\text{s}$. From fits to other experimental work (Visscher et al., 1999) similar values for ϕ and D were obtained (Bier, 2003a,b, 2005).

A striking feature in the experimental data points of Fig. 4 is that the motor protein appears to move fastest at $F = 0$. So even when the load is actually pulling in the direction of the kinesin movement, i.e. $F < 0$, the resulting speed is slower than for $F = 0$. What is probably happening is that the load force distorts the shape of the motor protein; it puts the neck linker of the two heads in a position from where it takes longer for the detached head to reach the next binding site. In vivo, kinesin is not commonly working against a conservative load. It is working against friction, i.e. a zero load. So evolution can be expected to have optimized the processive motor protein for operation in a high friction environment without distorting static forces. Without more structural knowledge it is impossible to quantitatively incorporate such distortion effects in Eq. (7). It is therefore meaningless to excessively belabor the fit of Eq. (7) to the experimental datapoints.

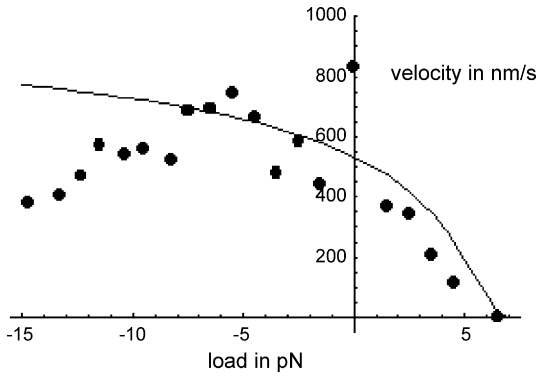

Fig. 4. A load–velocity diagram for the motion of kinesin on microtubule. The datapoints are from the reference by Carter and Cross (2005). The fit is according to the model that was set up in Figs. 2 and 3 and the ensuing Eq. (7). $D = 3.5 \times 10^{-16}\,\text{m}^2/\text{s}$ and $\phi = 0.3$ leads to a good fit that is consistent with previous estimates.

4. Concluding remarks on the role of polypeptide internal friction

To conclude, I will make a few remarks on the diffusion coefficient D. D represents the strength of the Brownian kicks. The flat part in the cycle of conformational states in Fig. 2 could never be crossed without diffusion. Going from $x = 0$ to $x = 1$ along the reaction coordinate in Fig. 2 corresponds to one mechanical step of the motor protein. However, it would be wrong to straightforwardly take the position between $x = 0$ and $x = 1$ of the Brownian particle on the reaction coordinate (cf. Fig. 2) and interpret it as the fraction of the period traversed by the center of mass of the moving motor protein on the biopolymer. As the processive motor protein is moving along the biopolymer, it may face a diffusion coefficient that varies with position. The reaction coordinate in Fig. 2 is set up such that D is constant along it.

There should be an isomorphism connecting the position on the reaction coordinate and the position of the center of mass along the biopolymer. But without more detailed knowledge this isomorphism is hard to construct. The $D = 3.5 \times 10^{-16}\,\mathrm{m}^2/\mathrm{s}$ that we derived in the previous section is therefore to be taken as a spatial average.

In vivo, a motor protein generally pulls an organelle or a vesicle filled with chemicals from one side of a cell to another. In vitro, researchers have commonly attached a silica bead to the motor protein. For a bead or a vesicle in a liquid, diffusion comes about as a consequence of the random collisions with molecules from the medium. When the particle is moving (for instance, when it is pulled by an electric field) there will be more collisions that hinder that motion than collisons that further propel that motion. It is thus that diffusion is connected to friction. It is in this way that we get to Einstein's fluctuation–dissipation theorem, i.e. $\beta = k_\mathrm{B}T/D$. The theorem, derived independently a century ago by Sutherland (1905) and Einstein (1905), is as simple as it is profound. k_B is the Boltzmann constant and T is the absolute temperature. $k_\mathrm{B}T$ thus represents the average energy in the thermal noiseband at temperature T.

Already Svoboda et al. (1993) let kinesin pull a little bead of about a micrometer in diameter. They observed that the speed of the stepping motor protein is not affected when the radius of the bead is varied. If the friction of the bead (i.e. $\beta = 6\pi\eta r$, where η is the viscosity of the liquid and r is the radius of the bead) had determined the speed of the entire motor protein-bead complex, then there should have been an inverse proportionality between speed and bead diameter. So apparently it is the internal friction of the motor protein that dominates. The internal friction of a protein that is going through a sequence of conformational changes is harder to intuit than the hydrodynamic friction of a bead. However, in his classic textbook "*Scaling Concepts in Polymer Physics*" (de Gennes, 1979), de Gennes already pointed out that intrachain collisions within a polymer can lead to a diffusion in conformational space as well as to a resistance when the protein is forced to move through that space. Intrachain collisions would thus lead to a fluctuation–dissipation relation of their own.

The $D = 3.5 \times 10^{-16}\,\mathrm{m}^2/\mathrm{s}$ that we found in our fit leads, through the fluctuation–dissipation theorem, to a friction of $\beta = 1.2 \times 10^{-5}\,\mathrm{s}^{-1}$. Such friction is indeed much larger than the hydrodynamic friction of a micrometer size bead and it has to be explained through internal friction. Recently, there have been measurements and studies on the internal friction of polypeptides other than motor proteins and it will be interesting to see whether the β-estimates derived therein are compatible with ours. I will briefly summarize a line of research that is more comprehensively described in the reference by Hagen et al. (2005).

When a protein is forced through a conformational cycle it is found that the conformational cycle takes more time to complete in a solution of higher viscosity. This can be understood by realizing that the protein has to move the surrounding liquid when a conformational change is accompanied by a change of shape. The relation is generally found to be linear. However, extrapolating to zero viscosity turns out to still lead to nonzero turnover time for the protein. This remainder is due to intrachain friction.

For a protein consisting of N peptides going through a conformational change, the internal friction would be expected to be proportional to N if every amino acid in the chain only interacted with its two direct neighbors in the chain and it would be expected to be proportional to N^2 if every amino acid in the chain interacted with all other amino acids in the chain. So as an *Ansatz* we take $\beta_\mathrm{int} \propto N^\alpha$, where $1 < \alpha < 2$, for the coefficient of internal friction. In the course of a conformational change a peptide will, most likely, interact with only a limited fraction of the peptides that are not its direct neighbors. So α is expected to be close to 1.

TrpCage is a peptide of only 20 amino acids long that folds within a microsecond. In its folded form the peptide fluoresces differently from the way it does in its unfolded form. The folding and unfolding can thus be probed with fluorescence measurements. The zero-viscosity folding speed of TrpCage has been observed to be independent of temperature. The zero-viscosity unfolding speed, on the other hand, follows an Arrhenius dependence (Hagen et al., 2005).

The interpretation of this is clear. Folding is an energetically downhill process. There is no activation barrier. The energy profile in conformational space is like the power stroke in Fig. 2. In the course of the folding a free energy $E = F_\mathrm{fr}L = \beta L^2/\tau$ is dissipated. Here L is the distance covered in conformational space, τ is the time that the folding takes, and β is the friction coefficient that we are after. As the unfolding is apparently energetically uphill, it requires thermal activation. By measuring the average unfolding time at different temperatures and making an Arrhenius fit, the energy E can be obtained (Pauling, 1988). For the unfolding and folding of TrpCage it was found that $E \approx 20k_\mathrm{B}T$ at $T = 300\,\mathrm{K}$ and $\tau = 0.7\,\mu$ sec. Substituting this in $E = \beta L^2/\tau$, we see that the quantity βL^2 equals about $6 \times 10^{-26}\,\mathrm{m}^2/\mathrm{s}$. As was mentioned before, L represents the distance traveled in conformational space. We do not have an exact relationship between L and the physical

displacement. What we will do here is employ the physical displacement nevertheless in order to set up some benchmarks and make an order of magnitude assessment. An α-helix of 20 peptides measures about 4 nm in length. So the average displacement of a an atom in TrpCage when folding is about 1 nm. Taking this as an estimate for L, we find $\beta_{\mathrm{TrpC}} \approx 6 \times 10^{-8}\,\mathrm{s}^{-1}$. For the stepping kinesin we had $\beta_{\mathrm{kin}} \approx 10^{-5}\,\mathrm{s}^{-1}$. The two stepping kinesin heads together consist of about 800 amino acids. From $\beta_{\mathrm{kin}}/\beta_{\mathrm{TrpC}} \approx (N_{\mathrm{kin}}/N_{\mathrm{TrpC}})^{\alpha}$, we derive $\alpha \approx 1.4$. This number is consistent with our earlier *Ansatz*.

For a tightly folded protein the volume will be proportional to the number of peptides, i.e. $V \propto N$. The surface area of such a protein is proportional to $V^{2/3} \propto N^{2/3}$. When a protein moves against the viscosity of a surrounding liquid, its friction can be taken to be proportional to the surface area, so $\beta \propto N^{2/3}$. Comparing this to $\beta_{\mathrm{int}} \propto N^{\alpha}$, with $1 < \alpha < 2$, it is obvious that the internal friction takes on more relative significance for larger proteins. Kinesin is a large protein that has to do a lot of internal rearrangement in the course of its step. It is thus not entirely implausible that the internal friction of the stepping kinesin exceeds the combined hydrodynamic friction of the protein and the pulled bead.

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