SZILARD-MACHINE-LIKE FEATURES IN A PROCESSIVE MOTOR PROTEIN*

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The motor protein kinesin literally walks on two legs along the biopolymer microtubule as it hydrolyzes ATP for its fuel supply. The fraction of accidental backsteps that kinesin takes appears to be about seven orders of magnitude larger than what one would expect given the amount of free energy that ATP hydrolysis makes available. This is puzzling, as more than a billion years of natural selection should have optimized the motor protein for its speed and efficiency. With an imagined device, Szilard has shown that the dissipation of information can drive motion. A higher backstepping probability creates more randomness in the walk and, consequently, leads to production of more entropy. If the product state of a transition has a higher entropy, then the free energy of that product state is lower. With the free energy that is made available by the production of "backstepping entropy", the catalytic cycle of the kinesin can be speeded up. We show quantitatively how the actually measured backstepping rate represents an optimum at which maximal net forward speed is achieved. We, furthermore, show how this thermodynamic mechanism can realistically operate on a biomolecular level. The results suggest that kinesin uses backstepping as a source of energy and that natural selection has manipulated the backstepping rate to optimize kinesin's speed.

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1. Introduction: how to model a processive motor protein?

In the early 1990s experimentalists developed the ability to follow and manipulate an individual kinesin as it is stepping along a microtubule polymer. The speed could be measured and separate steps could be resolved [1]. It became possible to vary the ATP concentration and apply a piconewton magnitude load at the same time. The ensuing speeds and backstep frequencies could be measured. The plethora of new data opened up a true Valhalla for theoreticians. A new world of molecular size engines had become accessible for experimental probing. Ideas could be worked out and next falsified or verified [2].

The first models that were developed were bottom-up. Kinesin "walks" with 8 nm steps on microtubule, a polymer with an 8 nm period. Kinesin and microtubule, like proteins in general, are very polar molecules. So it is envisioned that the kinesin faces an 8 nm periodic potential as it moves along the microtubule. As the kinesin is hydrolyzing ATP, its 3D architecture is changing in the course of the catalytic cycle. Such changes mean that the aforementioned periodic potential will fluctuate. We also need to be aware that kinesin, because of its size and because of the fact that it is moving in a liquid, is doing overdamped motion and is subject to Brownian fluctuations. With these ingredients it is possible to set up a crude "Brownian Ratchet" model without freely adjustable parameters. Next, one can take measured input parameters and use the model to "predict" other measured parameters. The ensuing Brownian Ratchet "predictions" generally turn out correct within an order of magnitude [3,4]. The relative success of a crude model in describing the operation of a complicated protein that consists of about 700 amino acids is a rare feat and very encouraging. But what the Brownian Ratchet models fail to predict is the tight coupling that kinesin and other motors exhibit: one step for every ATP hydrolyzed and one ATP for every step [5,6,7]. Also backstepping appears to be much rarer than simple Brownian Ratchet models predict. Real motors are ultimately more efficient than Brownian Ratchets.

It is possible to get to the higher efficiency by putting embellishments on the Brownian Ratchet models. One can put more states in the cycle, employ chemical kinetics, and add states and rates until a good fit is achieved [8]. The shape of the potentials is also something that can be tinkered with. Working out such larger and more complicated models generally requires extensive numerics. The usefulness of this approach is questionable. The rates are not known experimentally and can be freely fitted to achieve good fit.

Another approach has consisted in raw molecular dynamics simulation. A successful numerical simulation of a processive motor protein may be a worthy goal and leads to an ultimate validation of the bottom-up approach. However, it will do little to help increase understanding and build intuition.

What we will describe in this article is an approach to molecular motors from the opposite direction, *i.e.* a top–down approach very much like the one that Statistical Mechanics takes. In Statistical Mechanics the details of many molecular interactions are left for what they are. Instead, it is realized, that in the course of the motion in a many dimensional phase space, one does not need to specify the microstate of the system at each point in time. The state of the system is appropriately described by just a few observables that describe the macrostate of the system. With the idea of microstates and macrostates the values of some macroscopic observables can be derived as optima.

Awareness is building in the motor protein community that modeling in terms of jumps between discrete states along a one-dimensional coordinate does not yield an accurate picture. In Ref. [9] it is described how the catalytic cycle of a motor protein should be viewed as diffusive motion through a corridor in a many-dimensional conformational space. As this motion is taking place, the motor protein is functioning as a conduit for a chemical-to-mechanical energy conversion. In the course of about one billion years of evolution, natural selection should have optimized kinesin for its task. As we will see below, this optimization allows us to derive some features. Features that can be checked against experimental data.

2. Why speed is an issue for a processive motor protein

One criterion in the aforementioned natural selection of kinesin should be speed. As it runs along microtubule, kinesin pulls vesicles with chemicals. Generally, chemicals are made near the centrosome of the cell and needed where the action is taking place, which is often at the edges near the membrane. The speed with which a cell or organism can respond to a stimulus or environmental challenge can hinge on the speed with which kinesin runs along microtubule.

This is particularly salient for the case of fast neuronal transport [10]. For a nerve cell, the distance between the cell body and a synapse can be up to about a meter. It is kinesin walking on microtubule that pulls cargo (*e.g.* neurotransmitter) from the cell body along the axon to the synapses. At about 40 centimeter per day, such transport can take days. Alzheimer's disease is an example of an ailment that is associated with obstruction of fast neuronal transport [11]. It is obvious that faster fast-neuronal-transport can give an organism an advantage.

In the next few sections, we will show how kinesin can employ a "trick" to move a little faster. The trick works because the motor operates in an overdamped, Brownian environment. No similar mechanism would work for a macroscopic engine.

The "trick" mentioned in the last paragraph explains why the actual backstep fraction for a stepping kinesin is about 7 orders of magnitude higher than expected if backsteps were reversed forward steps. Under physiological conditions the hydrolysis of ATP makes about $22\,k_BT$ units of free energy available. The simplest possible kinetic scheme for a two-legged kinesin would be one cycle in which the different necessary chemical transitions (binding of ATP, hydrolysis of ATP, release of ADP and P, detachment of the rear leg, forward move of detached leg, and forward attachment of detached leg) follow each other up. In a scheme with tight coupling between the chemistry and the stepping mechanics, a backstep would originate from the reverse process and correspond to ATP synthesis. Such a reverse process would require a very unlikely Brownian fluctuation. With $22\,k_BT$ for the ATP hydrolysis, we would have one backstep for every $\exp[22] \approx 3 \times 10^9$ forward steps. What is, instead, observed in the most recent and most accurate experiments is a backstep fraction of between $1/100$ and $1/1000$ [12, 13].

The "trick" we will show increases the speed of kinesin by less than 1%. Among those not familiar with the more quantitative approach to evolution, it may be surprising that such a small selective advantage can drive evolution. Nevertheless, that appears to be the case. In the 1930s the so-called "New Evolutionary Synthesis" took place. Evolutionary biologists, geneticists, population dynamicists, and mathematicians came together to combine Darwin's basic idea with Mendelian genetics and with the study of partial differential equations that describe the spread of a gene through a population. The textbook by D. Futuyma [14] summarizes some of the findings: "... a character state with even a miniscule advantage will be fixed by natural selection. Hence even very slight differences among species, in seemingly trivial characters such as the distribution of hairs on a fly or veins on a leaf, could conceivably have evolved as adaptations."

The California kangaroo rat provides a nice illustrative example from the field [15]. This burrowing rodent lives in a California desert that is, about once in every 50 years, hit by an earthquake that is strong enough to collapse the burrows. The kangaroo rat, however, has evolved a seismic-escape response. It picks up on precursors of the earthquake and utilizes the less than one minute time that it has to make a run for the surface. The earthquake happens about once every 200 generations. So the seismic escape cannot be a trained response. If, without seismic response, 10% of the colony dies in the collapse (this is a low estimate), then the genetic fixation of the response implies a selective advantage of 0.05%. With computer

simulations Kirschvink shows that it takes about 12 seismic events, *i.e.* 600 years, to eliminate the fraction of the population that does not have the seismic escape response. "Simulations in which the trait (no seismic-escape response) is present in small amounts indicate that it is quickly eliminated from the population even after only a few dozen seismic events, even for only a 10% advantage of the competing A genes expressed only once every 50 years (a net selective advantage of only 0.05%)." Futuyma's aforementioned textbook actually gives analytic formulae for the speed of genetic fixation.

Eukaryotic cells use processive motor proteins for active transport needs. Faster active transport leads to a selective advantage that is independent of the fitness landscape around the organism. Even the smallest selective advantage is likely to have been genetically fixed in the more than one billion years that eukaryotic cells have existed.

3. The Szilard machine

In 1929 Leo Szilard connected the concepts of information and energy. The unit of information, the "bit", is owed to Szilard. He derived how one bit is associated with $k_{\mathrm{B}}T \ln 2$ of energy [16].

Figure 1 shows what we will call a "Szilard machine". A unit consists of two vacuum compartments with a movable partition in between. The unit also contains one molecule and the molecule can be either in the left compartment (corresponding to a 0) or in the right compartment (corresponding to a 1). Knowledge of the molecule's location thus corresponds to one bit of information. If we have that bit of information, then the information can be put to work in the following way. Insert a piston into the vacant compartment, *i.e.* the compartment that does not contain the molecule. Next, take away the partition and allow for isothermal expansion. With one molecule involved, Boyle's Law for an ideal gas is $PV = k_{\mathrm{B}}T$. The work that is delivered by the isothermal expansion is $W = \int P \, dV = k_{\mathrm{B}}T \int (1/V) \, dV = k_{\mathrm{B}}T \ln(V_2/V_1)$. For $V_2 = 2V_1$, this leads to $W = k_{\mathrm{B}}T \ln 2$. In this way, information can be used to fuel a motor (see [17] for a general theory). If it has to be guessed in which compartment the molecule is located and if the probability of the guess being right is p, then the average work made available will be $(1 - 2p)k_{\mathrm{B}}T \ln 2$. Obviously, the machine can be reversed. Work can be turned into information. Forcing the molecule into one of the compartments involves compression to half the original volume and requires $k_{\mathrm{B}}T \ln 2$ of work. After completion of the compression, the partition can be brought into the cell at $x = 1/2$. Doing that, one creates one bit of information. Good explanations of Szilard's machine are found in Refs. [18, 19, 20].
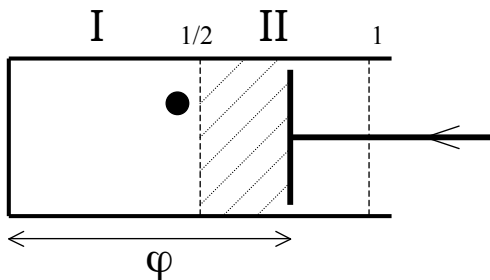
Fig. 1. In a Szilard engine the operator knows on which side of the partition at $x = 1/2$ the particle is located. He brings in the piston on the other side and removes the partition. The isothermal doubling of the volume then releases $k_{\mathrm{B}}T \ln 2$ of energy. In the "molecular motor"-context, compartment I corresponds to a forward step and compartment II corresponds to a backstep. Eliminating the backstep requires the $k_{\mathrm{B}}T \ln 2$ of energy that it takes to push the piston from $x = 1$ to $x = 1/2$. By not pushing the piston to $x = 1/2$, but to $x = \varphi$, we save some energy, but at the expense of a possible mistake, *i.e.* backstep.

The Szilard machine was intended as an abstraction to show that mere information can actually be turned into work. But with today's experiments on the molecular scale, it has become a quantitative reality [21]

It does not have to be a real space that is halved. If a system has two equally likely options (*e.g.* spin up and spin down), then forcing that system into one of the two requires an energy of at least $k_{\mathrm{B}}T \ln 2$. The best way to understand this is through recognizing that such forcing implies a compression of the available phase space to half of the original volume. The number of available microstates for the system reduces from Ω to $\Omega/2$ and the concurrent entropy decrease is $k_{\mathrm{B}} [\ln \Omega - \ln(\Omega/2)] = k_{\mathrm{B}} \ln 2$. The associated free energy, $k_{\mathrm{B}}T \ln 2$, is too small to be of any consequence in most engineering applications. Even in the smallest computers, the energies involved in storing and processing a bit of information are orders of magnitude larger than $k_{\mathrm{B}}T$. They actually have to be in order to not be affected by thermal noise and stay deterministic.

Now consider the possibility of not going all the way to $x = 1/2$ in Fig. 1 when creating the bit of information. One saves energy in that case, but at the expense of making a possible "mistake" (corresponding to the striped area in Fig. 1). There is then, in the end, a small probability that the molecule will end up in the wrong compartment when the partition is brought in.

The energies involved in biomolecular conversions and transitions are commonly between 1 and 10 $k_{\mathrm{B}}T$ units. In the optimization of such biomolecular operations the $k_{\mathrm{B}}T \ln 2$ can be an issue. If the processive motor

protein can make a forward step or backward step with equal probability, the step will involve a doubling of the available phase space, implying $k_{\mathrm{B}}T \ln 2$ of free energy becoming available to do work (Fig. 2). No such expansion of the available phase space occurs when the motor moves forward like clockwork and has the negligible backstep probability of $\exp[-22]$ that corresponds to running the entire ATP-driven cycle in reverse. Allowing for a small backstep probability, $0 < p_{\mathrm{b}} < 1/2$, will produce entropy and make an amount of free energy available that is between 0 and $k_{\mathrm{B}}T \ln 2$. The backstep probability corresponds to the striped area in Fig. 1. The backstep is the possible "mistake" and it is the price paid for a small amount of free energy. That free energy can be put towards speeding up the catalytic cycle. In Ref. [22] variational calculus is employed to quantitatively show that, for kinesin, the startlingly high backstep fraction is a "programmed erring" that actually optimizes the stepper for speed. Below we will add rigor to the argument and work out the correspondence between the Brownian stepper and the Szilard unit.

In the remainder of the paper we will keep the formulae concise by taking $k_{\mathrm{B}} = 1$ and $T = 1$ (which implies $k_{\mathrm{B}}T$ as the unit of energy).
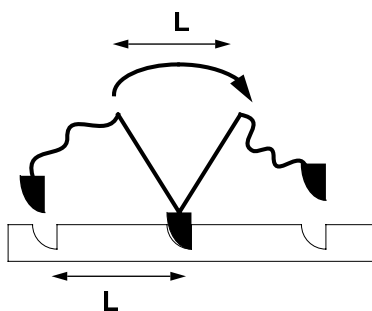

Fig. 2. Reprinted from [22]. The processive motor protein kinesin literally makes steps of a length $L = 8\,\mathrm{nm}$ along the biopolymer microtubule. As kinesin is hydrolyzing ATP, it also goes through the mechanical cycle. After the rear leg detaches, the attached head reorients so that the detached leg is brought to the vicinity of the next forward binding site. After attachment there, a new step can commence. There is, however, a probability for the detached leg to rebind at the rear binding site. Such rebinding is generally assumed to lead to a backstep. With the shape of the indents on the track, it is indicated that the directionality of kinesin's motion is due to the anisotropy of the microtubule.

4. The Szilard machine and its thermodynamic consistency

We take a "Szilard piston" as depicted in Fig. 1. Compartment I corresponds to a forward step of our molecular motor. Compartment II corresponds to a backstep. To completely eliminate the probability for a backstep requires compression of the volume to half of the original volume, *i.e.* an energy of $\ln 2$. Pushing only up till $x = \varphi$ requires $W = -\ln \varphi$. We next put in the partition. The probability p_b now corresponds to the probability of the molecule being in the striped volume. We have $p_f = 1/(2\varphi)$ and $p_b = 1 - 1/(2\varphi)$. This leads to $\varphi = 1/(2p_f) = 1/(2(1-p_b))$. In terms of p_f and p_b the "saved" work equals $\Delta W = \ln 2 - \ln[2(1-p_b)] = -\ln(1-p_b) = -\ln p_f$. For small p_b this boils down to simply $\Delta W \approx p_b$.

After compression to $x = \varphi$ and putting in the partition, we need to reset the piston to $x = 1$ to get it ready for the next step. Furthermore, the bit of information is only established if we have two compartments of equal volume (*i.e.* the piston at $x = 1$) and the molecule in one known compartment while the other is empty. No work would have been involved in the reset if the piston had gone all the way to $x = 1/2$. But if the molecule happens to be in the striped p_b-area, then energy will be released in the expansion. If the p_b-volume is expanded to the same volume as p_f, then the released energy is $\ln(p_f/p_b)$. The probability of this happening is p_b. So per step the released energy is $p_b \ln(p_f/p_b)$.

So, all in all, we have saved $\Delta G = -\ln p_f + p_b \ln(p_f/p_b)$ in the course of the cycle by going to $x = \varphi$ instead of going all the way to $x = 1/2$. After some algebra this expression can be put in a very well-known form [20]

$$\Delta G = -(p_f \ln p_f + p_b \ln p_b) . \tag{1}$$

This is the entropy production per step when a finite p_b is allowed. The indeterminacy in the stepping direction leads to an expansion of the available phase space at every step.

Suppose that, in the absence of backsteps, we have a speed $v_0 = f_0 L$, where f_0 is the stepping frequency and L is the steplength. Upon allowing for a nonzero backstep probability p_b we have

$$v = fL(p_f - p_b) . \tag{2}$$

As was mentioned before, the idea is that the energy ΔG that we save by allowing for backsteps can be put towards increasing the stepping frequency, *i.e.* towards getting $f > f_0$. So we do not put in the entire $k_B T \ln 2$ that is required to get into the p_f compartment for the full 100%. We save some energy by allowing for a small p_b. There is an explicit dependence on p_b

in Eq. (2) that reduces the net velocity, but the "$f > f_0$"-effect can more than compensate for this. In the next section we will derive the p_b that leads to the optimum speed.

On a more intuitive level, one can also think as follows of how backstepping increases the number of microstates. Imagine a large number, N, of motor proteins that are all at the same position. There is only one possible microstate for this macrostate. Next, let all of these N motors take a step. If all of these motors step forward, then we will continue to have only one microstate for the macrostate of the system. But if, on the other hand, we allow for one single backstep in these N steps, then N possible microstates ensue. This is because the backstep can occur with any one of the N motors. The increase in the number of microstates implies an increase in entropy that follows Boltzmann's well-known formula: $\Delta S = \ln N$.

5. Speeding up the catalytic cycle

The catalytic cycle of kinesin consists of about 10 identifiable chemical transitions [23]. The cycle includes transitions like the binding of ATP, the release of ADP, the release of an inorganic phosphate, the attachment of a detached head, and the detachment of an attached head. Every catalytic cycle also includes the actual 8 nanometer mechanical steps. It appears that there is no single rate limiting transition in kinesin's cycle [23]. From an evolutionary point of view this is understandable. If there is selectional pressure for a process to be fast and if there is a single bottleneck in the chemical cycle, then the course of evolution will be to speed up the bottleneck transition. However, once the bottleneck transition is as fast as the second slowest transition there will be equal selectional pressure on both transitions to be speeded up. It is thus that in most metabolic networks and enzymatically driven cycles there is no single rate limiting transition. Instead, control is distributed over several transitions. This is also the case for kinesin [23].

Below we will first perform a simple calculation in which we assume that all of the ΔG that is gained by allowing for a finite p_b goes towards speeding up a rate limiting step. This will give us an upper bound for the p_b that maximizes the net walking speed of the processive motor protein. Subsequently, we will explain how a situation with distributed control is to be dealt with. An explanation to that effect is also found in [22].

If there is a single rate limiting transition in the catalytic cycle of the processive motor protein, then the mechanical stepping rate equals the rate of this rate-limiting transition. Figure 3 depicts the reaction coordinate of this presumed rate-limiting transition. If this transition involves the thermally activated crossing of a barrier of height G_0, then we have $f_0 \propto \exp[-G_0]$. Now suppose that the ΔG of Eq. (1) goes towards lowering the energy of

both the product state and the activation barrier. In Section 7 we will explain how this can occur on the biomolecular level. After the lowering of the barrier we have $f \propto \exp\left[-G_0 + \Delta G\right]$. With this proportionality, with Eqs. (2) and (1), and with $p_f = 1 - p_b$, we can express the full dependence of the stepping speed v on p_b

$$v \propto e^{\Delta G}\left(1 - 2p_b\right) = e^{-(1-p_b)\ln(1-p_b) - p_b \ln p_b}\left(1 - 2p_b\right). \qquad (3)$$

Figure 3 (b) depicts v as a function of p_b according to Eq. (3). There is a maximum for v, where $v/v_0 = 1.11$, at $p_b^* = 0.08$.
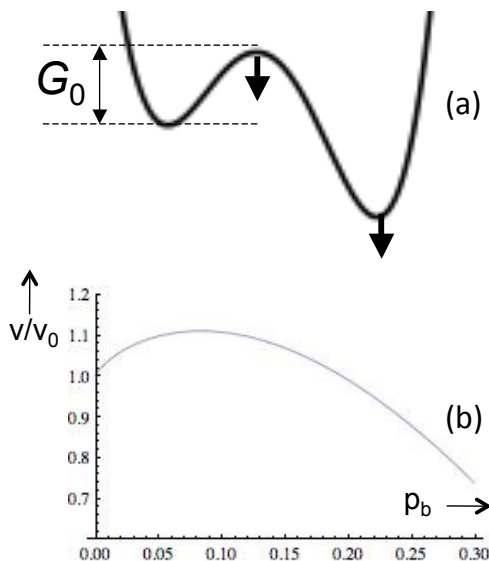

Fig. 3. (a) The reaction coordinate for a presumed rate limiting transition in the catalytic cycle of a processive motor protein. The activation barrier has a height G_0. The arrows show how the energy ΔG (*cf.* Eq. (1)) brings down the activation barrier and the product state. (b) The speed v as a function of the backstep probability p_b according to Eq. (3). v_0 is the speed if no backstepping occurs ($p_b = 0$). The speed v is seen to have a maximum for $p_b = 0.08$.

The value $p_b^* = 0.08$ constitutes an upper bound. It results when the energy that is made available following Eq. (1) goes for the full 100% towards lowering the activation barrier of the rate-limiting transition. A more common situation in chemical kinetics is that when the energy of the product state is being varied by ΔG, the activation barrier varies along by only $\alpha \Delta G$, where α is the so-called apportionment factor. This apportionment factor also expresses how the effect of an energy change of one of the two involved states is distributed over the forward and backward transition rates

between these states. The apportionment factor is generally found to be between 0.2 and 0.8 [24]. Implementing the effect of the apportionment brings in the factor α as a prefactor for the exponent in Eq. (3). The exponential term in Eq. (3) expresses how the walking speed v increases with p_{b}. The $(1 - 2p_{\mathrm{b}})$-term describes a decrease with p_{b}. It is obvious that introducing the smaller than unity prefactor α in the exponent reduces the ability of the backstepping to speed up the motor protein. The effect of this is that the optimum backstep probability, p_{b}^*, is pushed towards values smaller than 0.08.

Another issue arises when the transition that takes on the ΔG of Eq. (1) is no longer the obvious rate-limiting transition. In that case we have to utilize the formalism of control coefficients [22,25,26]. The control coefficient C_i^v can be thought of as the percentage by which the walking speed v of the processive motor protein increases if the transition i in the chemical cycle is speeded up by 1%. Obviously, C_i^v is a number between 0 and 1; we have $C_i^v \to 1$ if the transition i is rate-limiting and we have $C_i^v \to 0$ if the transition i is already so fast that a small variation is not of any consequence for the time to go through the catalytic cycle as a whole. Implementing the effect of distributed control over the speed v means that control coefficients enter our formalism.

For small ΔG we can take $\exp[\Delta G] \approx 1 + \Delta G$. So for a small p_{b} the exponent in Eq. (3) simply describes the relative increase of the speed due to the activation barrier going down by ΔG. However, if the transition of which the activation barrier is affected comes with a control coefficient of C_i^v, then the relative change in the speed will be $C_i^v \Delta G$. Next, we enter the apportionment factor α and the control coefficient C_i^v into Eq. (3) and obtain a concise formula

$$v \propto e^{\alpha C_i^v \Delta G}(1 - 2p_{\mathrm{b}}). \tag{4}$$

Upon using a first order approximation in p_{b} in the exponent, *i.e.* $\Delta G \approx (1 - \ln p_{\mathrm{b}})p_{\mathrm{b}}$ (*cf.* Eq. (1)), we obtain

$$v \propto \exp\left[\alpha C_i^v \left(1 - \ln p_{\mathrm{b}}\right) p_{\mathrm{b}}\right] \left(1 - 2p_{\mathrm{b}}\right). \tag{5}$$

Using again the approximation $\exp[\delta] \approx 1 + \delta$ for small δ, we establish the following simple first order approximation for the speed v in p_{b}

$$v \propto 1 + \left[\alpha C_i^v \left(1 - \ln p_{\mathrm{b}}\right) - 2\right] p_{\mathrm{b}} \ldots . \tag{6}$$

For very small p_{b}, the term in square brackets is positive and v increases as a function of p_{b}. Clearly, for larger p_{b} ($p_{\mathrm{b}} \to 1$) the -2 will dominate and the term in square brackets will become negative. The walking speed v will

then decrease as a function of p_b. Taking the derivative of Eq. (6) w.r.t. p_b and setting it equal to zero leads to a simple approximate formula for the optimal backstep probability p_b^*

$$-\ln p_b^* \approx \frac{2}{\alpha C_i^v} \,. \tag{7}$$

With methods of variational calculus, a similar formula was derived in [22].

For the aforementioned upper limit case of $p_b^* = 0.08$, the maximal v/v_0 in Eq. (3) becomes $v^*/v_0 = 1.11$. So the free-energy-out-of-backstepping scenario that we describe speeds up the processive motor protein by at most about 11%. Dynein is a processive motor protein that walks on microtubule, but in the direction opposite to kinesin's walking. Recent measurements have revealed that about 13% of dynein's steps are backward [27]. To our knowledge, that is the highest load-free backstep fraction that has been measured for a processive motor protein. However, we do not have sufficient additional data to link dynein's backstepping to our mechanism. In Eq. (7) we see that it is the logarithm of p_b^* that is related to the kinetic parameters α and C_i^v. Parameters of chemical kinetics are generally not easy to accurately determine. So, ultimately, an order-of-magnitude estimate of p_b^* is the best we can get out of Eq. (7). But within these confines, Eq. (7) accounts for experimentally obtained data on kinesin. References [12] and [13] report backstep fractions of between 10^{-2} and 10^{-3}. This means that $-\ln p_b^*$ is between about 4.5 and 7. For kinesin, the steps with the highest control come with coefficients of 0.3 and 0.4 [23, 22]. As was mentioned before, α should be between 0.2 and 0.8. Operating within these allowable ranges for α and C_i^v, the right-hand side of Eq. (7) can be brought to between 6 and 7. Of course, for such small p_b^*'s, the speeding up is much smaller than the maximal 10%. Take, for instance, as p_b^* the $p_b = 1/220$ that was measured and reported for kinesin in [12]. We then have $\Delta G = 0.03$ (*cf.* Eq. (1)) and we find $v/v_0 = 1.0016$ upon substitution in Eq. (4) (using $\alpha C_i^v = -2/\ln p_b^*$), *i.e.* a speeding up of about 0.16%. If we take the $p_b = 1/802$ that is found for kinesin in [13], then we derive $\Delta G = 0.01$ (*cf.* Eq. (1)) and a speeding up of about 0.04%. Although the improvement may seem small, it is worthwhile to note that this is a selective advantage comparable to the one for the aforementioned seismic escape response of the California kangaroo rat.

That Eq. (7) accounts for measured data indicates that the backstepping-to-increase-speed mechanism may be real, that a Szilard machine is actually at work in a processive motor protein, and that the machine has evolved to make the motor perform optimally.

6. Free energy from entropy production and the situation near equilibrium

Entropy production can make free energy available. Decreasing entropy requires an input of free energy. Biology is full of examples of this. Bringing an electroneutral molecule from where it has a low concentration to where it has a high concentration requires energy. This is a purely entropic effect. Usable free energy can, in turn, be acquired if the molecule is allowed to go the opposite way. This is the way in which the operation of membrane pumps and transporters can be understood.

The Na,K,2Cl-cotransporter [28] picks up one sodium ion, one potassium ion, and two chloride ions on the outside of the cell. It next brings them to the inside of the cell. The net charge transport is zero, so it is only concentration differences that matter. Sodium and chloride have high concentrations ($\sim 100\,\mathrm{mM}$) outside the cell and low concentrations ($\sim 10\,\mathrm{mM}$) inside the cell. For potassium it is the other way round. The transporter thus effectively uses the chemical potentials of sodium and chloride as an energy source to pump a potassium ion against its chemical potential.

Another good example is entropic coiling. Take a polymer consisting of N "rigid rods" of length l. When it is stretched out, it has an end-to-end distance of $L = Nl$. There is only one microstate for an end-to-end distance of L. It is obvious that for smaller end-to-end distances, the polymer can have many different configurations (*i.e.* microstates). For an unconstrained polymer we have an average end-to-end distance of about $L \approx l\sqrt{N}$ [29]. When a stretched-out polymer coils to a smaller end-to-end distance, it generates an actual force. Such a so-called "entropic force" is encountered in many biological systems [30, 31]. Entropic forces of coiling polymers are also thought to play a key role in the force-generation of the stepping kinesin [32, 33, 34].

A cute case of diffusion being put to work is the so-called "drinking bird" or "dippy bird" [35, 36]. It is a popular gadget in the instruction of undergraduates and there is also a good Wikipedia page about it. Evaporation of the water is what eventually drives the up and down motion of the bird. The felt covered beak of the bird gets submerged in the water and absorbs some of that water. Because of the large surface area of the beak and because surface tension is no longer an issue, the water evaporates relatively fast from the felt-covered beak once the bird is upright. What ultimately drives the cycle is the water going from the confinement in a liquid to the much larger space that is has in vapor form, *i.e.* phase space expansion and the ensuing entropy production. In essence, the bird with its felt beak catalyzes the evaporation of water and harnesses some of the released free energy of that evaporation to drive the up-and-down motion. So it catalyzes an ener-

getically downhill process (the evaporation) and couples the downhill energy flow to push the reciprocal motion. In its traditional form, the dippy bird "uses" the evaporation to cool part of the bird. Next, the up and down motion of the bird is effectively the work of a heat engine [35, 36]. But a dippy bird variation with no temperature gradients has been constructed and analyzed [37]. In this construction the coupling of evaporation to motion, *i.e.* of entropy production to mechanical work, is direct.

To check on the thermodynamic consistency of the mechanism that we propose, we will look at what happens when the system is near equilibrium. This will occur when the ATP-ADP chemical potential is close to zero, *i.e.* $G_{T\to D} \to 0$. Life is a far-from-equilibrium phenomenon, so such a situation is nonphysiological. *In vitro* the equilibrium situation can be achieved with the right (but unphysiologically high) ADP-to-ATP concentration ratio in the bath. The motor's speed should vanish linearly as the driving potential is made to vanish. As we saw earlier in this section, it is possible for diffusion to be a source of energy. However, it is not possible for mere diffusion to bias its own direction to the left or right on a periodic track (*cf.* Fig. 2). Such biasing requires a macroscopic force, an explicit conversion mechanism (as with the aforementioned transporter), or some input of other energy [38]. The fact that the track is obviously anisotropic (*i.e.*, the left-to-right and the right-to-left direction are distinguishable) does not change that.

Figure 4 helps us understand, in terms of the basic ingredients of the system, how there is indeed no directionality to the diffusion at chemical equilibrium. The circles in the horizontal plane represent the chemical cycle. The ordinary physiological ATP-to-ADP conversion corresponds to the clockwise direction. The mechanical direction is perpendicular to the horizontal plane. It is at the mechanical junction that a "decision" is made whether to take a forward (solid arrow) or backward (dashed arrow) step. Near equilibrium we have $(f_+ - f_-) \propto G_{T\to D}$, where f_+ is the number of clockwise cycles that is circled per unit of time and f_- is the number of counterclockwise cycles that is circled per unit of time. When circling clockwise, the route of the solid arrow (p_f) is taken more frequently because it has a lower activation barrier. That difference in activation barrier height is the same when moving counterclockwise. Therefore, the solid arrows depict the dominant route also for counterclockwise motion. Backstepping will thus dominate for ATP synthesis. For the stepping velocity due to the clockwise cycles we have $v_+ \propto (p_f - p_b)f_+$ and for stepping due to counterclockwise cycles we have $v_- \propto (p_b - p_f)f_-$, which is negative. Inspection of the arrows at the chemical–mechanical junction in Fig. 4 makes clear why the difference $(p_f - p_b)$ gets a minus sign for a cycle in the counterclockwise direction. For the net velocity we obtain

$$v_{\text{net}} = v_+ + v_- \propto (p_f - p_b)(f_+ - f_-) \propto (p_f - p_b)G_{T\to D}\,. \qquad (8)$$

So near equilibrium the motor's speed is proportional to the chemical potential and to the difference $(p_f - p_b)$. Close-to-equilibrium our mechanism reduces to a simple linear force-flow relationship as it should [39].
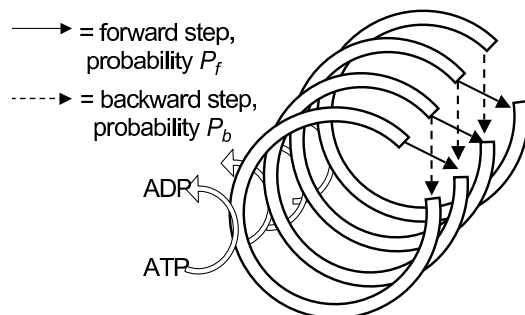

Fig. 4. Reprinted from [22]. Kinesin catalyzes the ATP-to-ADP conversion. Under physiological conditions such conversion makes $22\,k_BT$-units of energy available. Kinesin couples the chemical conversion to the mechanical stepping and some of the released energy is thus used to drive the stepping. The chemical cycle is in the horizontal plane. The mechanical direction is perpendicular to the chemical plane. On the right side of the circle, the solid and dashed arrows indicate the chemo-mechanical junction. This is where the actual coupling occurs and where the forward *vs.* backward "decision" is made. This picture also helps us understand how near chemical equilibrium, the net speed of the molecular motor vanishes even if $p_f \neq p_b$. At chemical equilibrium there are as many clockwise (ATP-to-ADP) chemical conversions as that there are counterclockwise (ADP-to-ATP) chemical conversions.

7. The biomolecular realization of Szilard's mechanism

The model presented in the previous sections is thermodynamically sound and consistent. It is, however, still legitimate to ask how a biomolecule can operate like the piston in Fig. 1. Next, we will point our how features of the Szilard machine can be realized in a working protein.

Before we start this explanation, it is important to realize the following again. A cycle that is driven by $22\,k_BT$-units of energy has a probability to be run in the reverse direction just through a "Brownian conspiracy". For such a cycle, on average one out of every exp[22] cycles will be in the reverse direction. The observed backstep fraction is much higher and that means that a mechanism has been built into the molecule to "deliberately" increase the backstep fraction. Below we will ignore the extremely rare backsteps that originate in a reversal of the cycle.

Kinesin is a dimer each unit of which consists of about 350 amino acids. Within this large protein there are many clusters of amino acids with considerable flexibility. Imagine that, in a certain state S_1 in kinesin's catalytic cycle, the position of such a cluster within the kinesin determines whether a step will be forward or backward. We can depict this situation with the double well as shown in figures 5 (a) and 5 (b). When the cluster is in the left well a forward step is made. When the cluster is in the right well a backstep occurs. The symmetric double well of Fig. 5 (a) corresponds to $p_f = p_b = 1/2$. For a smaller backstep probability, the right well has a correspondingly higher level than the left well (see Fig. 5 (b)). Complete removal of the backstep possibility corresponds to eliminating the right well from the reaction coordinate. Such removal means that, in the state S_1, the number of possible positions for the cluster is reduced. Removal of part of the available phase space implies an effective increase of the free energy of state S_1. For the case of the symmetric double well of Fig. 5 (a), it is obvious that taking away the right well (i.e. going from the dashed to the dotted situation) halves the available phase space and thus increases the free energy level of S_1 by $\ln 2 \ k_B T$-units.
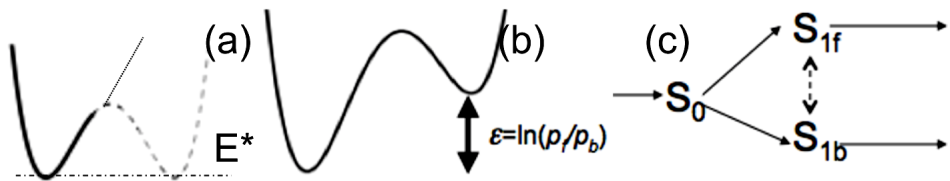

Fig. 5. A model for the mechanism behind the backstepping and the acquiring of the associated entropic energy. Whether a step goes forward or backward depends on the position of a cluster within the processive motor protein. The corresponding reaction coordinate for the cluster is depicted. The left and right well stand for forward and backward step, respectively. A $p_f = p_b = 1/2$ situation is depicted by a symmetric double well as in (a). Eliminating the backstepping would mean eliminating the right well, i.e. replacing the dashed part by the dotted part. Such a change halves the number of states at level E^* and effectively increases the free energy of that level by $k_B T \ln 2$. The situation with a finite small p_b corresponds to an energy level difference ε between the wells (b). Panel (c) depicts the mechanism in terms of chemical kinetics. The states S_{1f} and S_{1b} correspond to the left and right well of (b), respectively. The $S_0 \to S_1$ transition is speeded up by adding the state S_{1b}.

Implementing a small nonzero backstep probability means that we insert the small higher well as in Fig. 5 (b). Within state S_1 of the catalytic cycle we then get a situation where the cluster faces a reaction coordinate as in Fig. 5 (b) and, effectively, makes a "choice". The activation barrier

between the states in Fig. 5 (b) needs to be sufficiently low for quick equilibration to take place. A Boltzmann distribution between the two wells then determines the forward-*versus*-backward step probability. For the energy difference between the wells we have, following Boltzmann, $\varepsilon = \ln(p_{\mathrm{f}}/p_{\mathrm{b}})$. For kinesin, with a backstep fraction of $1/802$ [13], the resulting energy difference is $\varepsilon \approx 7$ $k_{\mathrm{B}}T$-units. The energy ε is related to, but not identical to the "saved" energy ΔW that was introduced in Section 2. The energy ΔW that is saved on the $\ln 2$ $k_{\mathrm{B}}T$-units of energy that guarantee a forward step is much smaller than ε. Putting that rightmost well at a height $\varepsilon = \ln [p_{\mathrm{f}}/p_{\mathrm{b}}]$ above the left well leads to $\Delta W = -\ln p_{\mathrm{f}} = -\ln [1 - p_{\mathrm{b}}]$. The derivation of that quantity is found in Section 2 in the context of Fig. 1. For small p_{b} we have $-\ln [1 - p_{\mathrm{b}}] \approx p_{\mathrm{b}} \approx p_{\mathrm{b}}/p_{\mathrm{f}}$. This leads to $\Delta W \approx \exp[-\varepsilon]$ for the connection between ε and ΔW.

In Section 2 we saw that there was also an energy release of $p_{\mathrm{b}} \ln [p_{\mathrm{f}}/p_{\mathrm{b}}] = p_{\mathrm{b}}\varepsilon$ involved in the reset of the piston. In the case of the biomolecule, this energy can be acquired after a next conformational change in which the position of our cluster becomes energetically integrated into the progress of the catalytic cycle. In that case an energy $\varepsilon = \ln [p_{\mathrm{f}}/p_{\mathrm{b}}]$ can help drive the catalytic cycle forward if the cluster is in the backstep well on the right. With a Boltzmann distribution, the probability to be in that right well is p_{b}. So, with our biomolecule, we indeed collect $\Delta G = \Delta W + p_{\mathrm{b}} \ln [p_{\mathrm{f}}/p_{\mathrm{b}}] = -\ln p_{\mathrm{f}} + p_{\mathrm{b}} \ln [p_{\mathrm{f}}/p_{\mathrm{b}}]$ of energy per stepping cycle from the backstepping. This is the same expression for ΔG that we found in Section 2 with the system of Fig. 1, and that ultimately reduced to Eq. (1).

Figure 5 (c) shows what this boils down to in the language of chemical kinetics. Let S_0, S_1, ..., S_k be the subsequent states in the course of the full catalytic cycle. We took S_1 for the state where the forward–backward "split" occurs. This means that we effectively get a "fork" at the $S_0 \rightarrow S_1$ transition. The dotted double arrow corresponds to the reaction coordinate depicted in Fig. 5 (b). Quick equilibration between the wells is assumed to occur there. The lower and the higher well in Fig. 5 (b) correspond to $S_{1\mathrm{f}}$ and $S_{1\mathrm{b}}$, respectively. The addition of the state $S_{1\mathrm{b}}$ speeds up the transition from S_0 to S_1. Proceeding from $S_{1\mathrm{f}}$ means that a forward step has occurred. Proceeding from $S_{1\mathrm{b}}$ means a backstep has occurred.

8. Discussion

Boltzmann's entropy formula $\Delta S = k_{\mathrm{B}} \ln(\Omega_f/\Omega_i)$ and the ensuing $\Delta W = k_{\mathrm{B}}T \ln(\Omega_f/\Omega_i)$ establish a connection between work and statistics. The connection is counterintuitive. On a macroscopic level no energy release is involved when an apple is put in a larger basket. But when a thermally agitated molecule is involved, the shrinking or expanding of the available phase space implies changes in free energy.

On the biomolecular scale, where the energy k_BT is significant, information can be traded for energy. The Szilard machine was originally devised as a thought experiment to illustrate this. But, as that scale has become experimentally accessible, the machine has become part of the experimentalist's reality [21]. At the end of Ref. [21] it is written "We demonstrated that free energy is obtained by a feedback control using the information about the system; information is converted to free energy, as the first realization of Szilard-type Maxwell's demon". It would be misguided to think that, in the course of evolution, such an information-for-energy trading has not occurred where it can occur and where it can help a processive motor protein to go faster. The formula $\ln p_b^* = -2/(\alpha C_i^v)$ that we derived for the optimum, appears to be followed by actual processive motor proteins. Indications are strong that a processive motor protein has indeed exploited backstepping statistics to maximize its speed.

That, in a Brownian environment, statistical "wiggle room" is equivalent to energy is well-known in the study of protein folding. In the textbook by Jackson [40] it is pointed out how entropic energy can play an important role in protein folding. For a polypeptide to go from a random coil to a correctly folded protein generally requires many k_BT's. After all, energy has to be invested into driving the protein from a macrostate with a large number of microstates to the unique folded state. In order to reduce this energy difference, some proteins apparently retain flexible clusters in their folded form.

Myosin V and myosin VI are processive motor proteins that walk on actin. These motor proteins have been more recently discovered. It appears that for these motor proteins the stepsize does not consistently cover one period of the biopolymer. Instead, stepsizes are large and there appears to be a fairly wide distribution of forward as well as backward stepsizes [41,42]. Such variable steplength is also a way to produce entropy and make free energy available. After all, the variable stepsize means that there are different ways to cover a fixed distance along the biopolymer. So for the same initial and final state, we can have multiple pathways, involving combinations of different microstates. An analysis analogous to that in this paper could, in principle, apply.

Many ailments involve a malfunctioning of processive motor protein transport. Getting a good understanding of the operation of processive motor proteins, therefore, has a medical significance and is essential for effective drug development. As biology is going down to the molecular level, it is becoming an ever more quantitative science. The study of motor proteins is now the realm of physicists as much as it is of biologists. A theoretical physicist may look at a helium atom and ask *how* he can manipulate the Schrödinger equation most effectively to compute the atom's energy levels.

But the hard core bottom-up methodology that yielded success in atomic physics may not be so fruitful when facing the catalytic cycle of a complex protein. Ultimately, the mere complexity of motor proteins may be well beyond what simulations and fits can capture anyway. At this point it makes sense to step back and ask the *why* question again. In his famous 1973 essay *Nothing in Biology Makes Sense Except in the Light of Evolution*, the evolutionary biologist T. Dobzhansky wrote: "Seen in the light of evolution, biology is, perhaps, intellectually the most satisfying and inspiring science. Without that light it becomes a pile of sundry facts, some of them interesting or curious, but making no meaningful picture as a whole" [43]. Unlike helium atoms, motor proteins, and proteins in general, have evolved to be robust and efficient. Sense can be made of the operation of a motor protein upon the realization that the dynamics represent a stable optimum. Physicists have many methods at their disposal to compute optima. It is with the application of these methods that they may put some quantitative rigor behind Dobzhansky's insight and behind the understanding of motor protein operation.

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