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Ergodic pumping: A mechanism to drive biomolecular conformation changes

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Abstract

We propose that a significant contribution to the power stroke of myosin and similar conformation changes in other biomolecules is the pressure of a single molecule (e.g. a phosphate ion) expanding a trap, a mechanism we call "ergodic pumping". We demonstrate the principle with a toy computer model and discuss the mathematics governing the evolution of slow degrees of freedom in large Hamiltonian systems. We indicate in detail how the mechanism could fit with known features of the myosin cycle. Many other biomolecular conformation changes could be driven in part by ergodic pumping. We suggest the use of ergodic pumping as a design principle in nanobiotechnology.
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1. Introduction

Conformation changes are an important feature of much biochemistry, e.g. G-proteins, ion channels, photoreceptors, chaperonins, haemoglobin, and of particular interest to us, biomotors, especially myosin. For surveys of biomotors see [30, 32,27]. What are the design principles that evolution has discovered for making efficient molecular motors?

Many mechanisms have been proposed for driving conformation changes. Some have received strong experimental support and wide acclaim (e.g. ATP-synthase [8]). Most proposals focus on binding of ligands as the driving force. The basic requirement, however, is just a decrease in free energy [22]. Thus, in principle, release of ligands could also drive conformation changes if the departing ligands gain enough entropy to compensate for their loss of binding energy. Indeed, release of ligands must occur at some stage in any cycle of conformation change involving binding of ligands, so its effects should be considered.

Release of ligands can decrease free energy by increasing entropy. For example, opening a trap of small volume v_0 containing one molecule of an ideal gas into a large container with density $\rho = 1/v_1$ molecules per unit volume (Fig. 1(a)) increases the entropy by approximately $k \ln \frac{v_1}{v_0}$ (k being Boltzmann's constant). This can be quite large [21,49,67]. Taking rough values for the volume $v_0 = 1$ Å^3 available to the centre of mass of a phosphate ion in the ATP-binding pocket of myosin after hydrolysis, and concentration $\rho = 2$ mM in the cellular solution outside (while fully conscious that phosphate ions in solution do not form an ideal gas), we find $\Delta S = 13k$ for the release of the phosphate ion, and so $\Delta G = 13kT$ at temperature T. If such a phosphate ion is simply 'released' into the cellular solution, then $13kT$ of free energy is wasted. But $13kT$ is a similar energy to the work done in the power stroke of myosin under optimal conditions, $15kT$ according to [30]. It is far too much to allow to go to waste! Nature must use it somehow.

We heed warnings that it does not make sense to evaluate partial molar entropy changes without specifying whether one is operating at constant volume or pressure [51,67], and that it does not make sense to attribute free energy changes to components of a system except after a full cycle [23]; but the above arguments still provide strong motivation for considering

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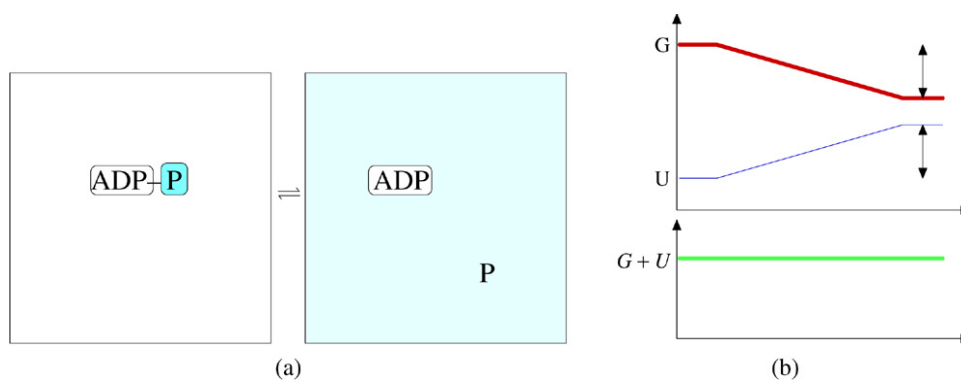*E-mail address:* mackay@maths.warwick.ac.uk (R.S. MacKay).

Fig. 1. (a) When one molecule is released from a confined space into a larger space, there is a substantial reduction in chemical free energy. If an idealized phosphate ion, for example, is initially confined to a tiny accessible volume $v_0 \simeq 1$ Å^3 adjacent to an ADP molecule and later it is free to move around a larger volume, say $v_1 \simeq 800\,000$ Å^3, then its entropy has increased by $k \ln \frac{v_1}{v_0} \simeq 13k$, and the associated free energy decrease is $13kT$. (b) For such a drop in the free energy G to be efficiently converted into mechanical work, U, we believe the falling free energy must be directly coupled to rising work in such a way that the total $G + U$ remains roughly constant. Our paper presents an explicit model for this coupling.

whether release of ligands could drive conformation changes. As well as by entropy increases, release can also decrease free energy by descent of an electrostatic gradient, by hydration or by other processes. We focus on entropy increases now, and discuss electrostatic effects later, as we believe both are essential to understand the power stroke of actin–myosin.

How can entropy increase drive conformation changes? Thermodynamics says that to do so efficiently, it must be done slowly enough to allow the system to remain close to a state of constrained equilibrium at all stages (Fig. 1(b)); but what could the mechanism be?

There is a natural way to turn the entropy increase of release of a particle from a trap into work. The particle exerts a pressure P on the walls of the trap, for example $P = kT/v_0$ for an ideal gas molecule, which tries to expand the trap. In the case of a trap of size $v_0 = 1$ Å^3 at temperature $T = 300$ K, this pressure is enormous: 4×10^9 Pa $\approx 40\,000$ atmospheres! If the trap has a mode of expansion (which we will represent by a piston, but this is not to be interpreted literally for biomolecules: instead it should be a cavity within the biomolecule) coupled to a conformation change then the pressure can push the piston to provide a force to accomplish the conformation change (Fig. 2). If the opposing force is matched to the pressure in such a way that the expansion is done slowly then the pressure does net work $\int_{v_0}^{v_1} P(v)\,\mathrm{d}v$, e.g. $kT \ln(v_1/v_0)$ for an ideal gas molecule. If the final volume v_1 is equal to $1/\rho$, the work done is $-kT \ln \rho v_0$, which is T times the entropy change for the release.

This cartoon neglects the external pressure acting on the system and assumes the particle is an ideal gas. The mechanism is not restricted to ideal gases, however. In liquids, the same entropic effect goes by the name "osmotic pressure". Osmotic pressure is exerted on a semi-permeable membrane (permeable to the solvent but not the solute particles) when there is a difference in density of solute on the two sides; the pressure reflects the entropy increase that would be achieved by moving the membrane to equalize the densities. For dilute macroscopic solutions the pressure difference is given by van t'Hoff's formula $\Delta P = \Delta \rho kT$ where ρ on each side is the number of solute particles per unit volume [35]. Osmotic pressure is usually regarded as an effect of large numbers of solute
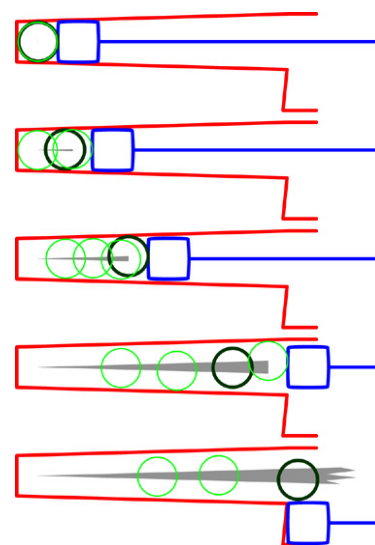

Fig. 2. Cartoon of the power stroke of a model engine that converts chemical free energy to work. The circle represents a phosphate ion, trapped in a cleft by a piston (the square) that is coupled to an external load (not shown). The shaded triangular region shows the accessible volume for the centre of mass of the phosphate ion. The phosphate bounces against the piston; if it succeeds in pushing the piston back, the accessible volume for the phosphate increases; if the phosphate loses kinetic energy when it bounces against the piston, its kinetic energy will be rapidly restored to its thermal value by subsequent interaction with the surrounding thermal reservoir. If the piston is coupled to a load given by a potential $U(x)$ (as a function of piston displacement x), such that $\frac{\partial U}{\partial x} \simeq kT \frac{\partial}{\partial x} \ln v(x)$, where $v(x)$ is the volume accessible to the centre of mass of the phosphate, then the work done when the cleft's size has increased from $v(x_0)$ to $v(x_1)$ will be $kT \ln \frac{v(x_1)}{v(x_0)}$. As viewed by the piston, the load should be large (big $\frac{\partial U}{\partial x}$) for small x and smaller for large x, so appropriate nonlinear gearing would be required to match to a constant load.

particles, but osmotic pressure can be defined equally well, in a time-averaged sense, when one or both sides have a small number of solute particles. The pressure due to a single solute ion in volume v_0 is approximately $P = kT/v_0$, though for small v_0 one should expect excluded-volume and surface effects to produce deviations.

When the number of relevant molecules is small on at least one side of a membrane, we call the effect "ergodic pumping".

The terminology reflects the fact that the molecules exert a *time-averaged* force ("ergodic theory" is the study of time averages for dynamical systems). It seems rare for biochemists to think about forces on conformation changes other than attraction and repulsion, though the idea of pressure forces is used in cell mechanics, e.g. [24,20]. In this paper, the 'membrane' is embodied by a single protein molecule, and the two sides of the membrane are the interior and exterior of that molecule.

The idea that entropy increases can drive biochemical processes is not new [22]. For example, the entropy increase of released water is responsible for the binding of hydrophobic residues to each other [36]. Our point is to suggest an apparently overlooked mechanism, ergodic pumping, by which entropy increases can drive conformation changes. In general ergodic pumping can be expected to be responsible for only certain parts of a cycle of conformation change, but we believe that it is likely to be an important ingredient in many biomolecular systems.

First we describe some computer demonstrations of ergodic pumping. Then we review the relevant mathematical theory of kinetics and show how it can lead to ergodic pumping. Next we discuss the idea in some detail in the context of actin–myosin. We hope that this three-pass approach, presenting the same idea from different viewpoints and in different styles, will permit a broad range of readers to appreciate at least some parts of the paper. Finally we speculate on a wide range of other biomolecular systems in which we suspect ergodic pumping plays a significant role, ending by proposing it as a design principle for nanobiotechnology.

The idea was presented at Equadiff in July 2003 [38], a Royal Society of Chemistry Forum in September 2003 [39], a Newton Institute discussion meeting in March 2004, and at the May 2005 Nonlinear Physics conference in honour of Serge Aubry.

2. Computer demonstrations

Consider a cycling ATP-hydrolysing system that quickly and nearly reversibly converts chemical energy to work. We focus on the key step in this system, before which the chemical free energy is large and the work done is small, and after which the chemical energy has dropped by a large amount (say $13kT$) and the work done has risen by a similar amount. Our idealized system starts this step with a single phosphate particle confined in a tiny volume v_0. In order to capture the free energy, the phosphate release is directly associated with the doing of work, as depicted in Fig. 2.

2.1. The system

As a minimal demonstration of the principle of ergodic pumping, we simulated a one-dimensional system containing just two particles — the driver (phosphate) and the piston. The state space of the system is shown in Fig. 3(b). The driver's coordinate q is free to vary between 0 and the piston coordinate Q. We couple the piston to an appropriate load $U(Q)$ such that

$$\frac{\partial U}{\partial Q} \simeq kT \frac{\partial}{\partial Q} \ln v(Q), \tag{1}$$

where $v(Q)$ is the volume accessible to the centre of mass of the driver, taken here to be simply $v(Q) = Q$.

The potential energy of the system is chosen to be (Fig. 3(a)):

$$U(Q) = \ln \frac{Q}{Q_0} + 0.1Q, \tag{2}$$

where $Q_0 = 0.01$ is the leftmost permitted coordinate for the piston, so as to approximately satisfy (1) but also include a long slope representing a small additional external force. We fix the mass of the driver at $m = 1$. We choose the piston to have a mass $M(Q)$ depending on its position, specifically

$$M(Q) = 10 \left(\frac{\partial U}{\partial Q} \right)^2. \tag{3}$$

We chose a position-dependent mass for two reasons. Firstly, in a real biomolecule there is no actual piston — the boundary of the phosphate trap is formed from pieces of protein which move relative to each other in complicated ways; the effective mass $M(Q)$ (defined by writing the kinetic energy of the full linkage in terms of the coordinate Q of the trap as $\frac{1}{2}M(Q)\dot{Q}^2$) is unlikely to be independent of the trap volume. Secondly, if we put a constant-mass piston in a potential like (2), which has very little configuration space with energy within kT of the minimum, thermal fluctuations would take the energy several kT above the minimum even when no driver is present; such spontaneous fluctuations would obscure the effect of ergodic pumping by the driver. For a piston whose effective mass increases sharply near the minimum of the potential, the small configuration-space volume available near the minimum is compensated for by a large momentum-space volume. The specific choice (3) makes the probability distribution for the energy of the piston proportional to

$$\exp\left[-(U - U_0)/kT\right], \tag{4}$$

where U_0 is the minimum possible energy, $U(Q_0)$, so the average potential energy relative to that minimum is $1kT$. Thus when we introduce the driver, we can attribute increases of potential energy significantly bigger than kT to the presence of the driver.

The system is maintained at temperature T with $kT = 1$ by thermalizations of the driver's velocity whenever it hits the left-hand wall. Whenever the driver hits the wall $q = 0$, the driver's velocity v is drawn from the appropriate thermal distribution $\propto |v| \exp(-mv^2/2)$ (the factor of $|v|$ must be included to give the thermal distribution for particles passing a particular point, in contrast to the thermal distribution for particles at a particular time, which is $\propto \exp(-mv^2/2)$). When the piston hits the wall $Q = Q_0$, the piston's velocity V is thermalized similarly. Hamiltonian dynamics apply everywhere else. 199 out of every 200 collisions between the piston and driver ($q = Q$) are elastic (energy and momentum are conserved). On every 200th collision between piston and driver, momentum is conserved, and the relative velocity is thermalized (this rule was added to avoid infinitely long simulations when the piston and driver chatter against each other). Between collisions, the Hamiltonian dynamics are simulated using the leapfrog
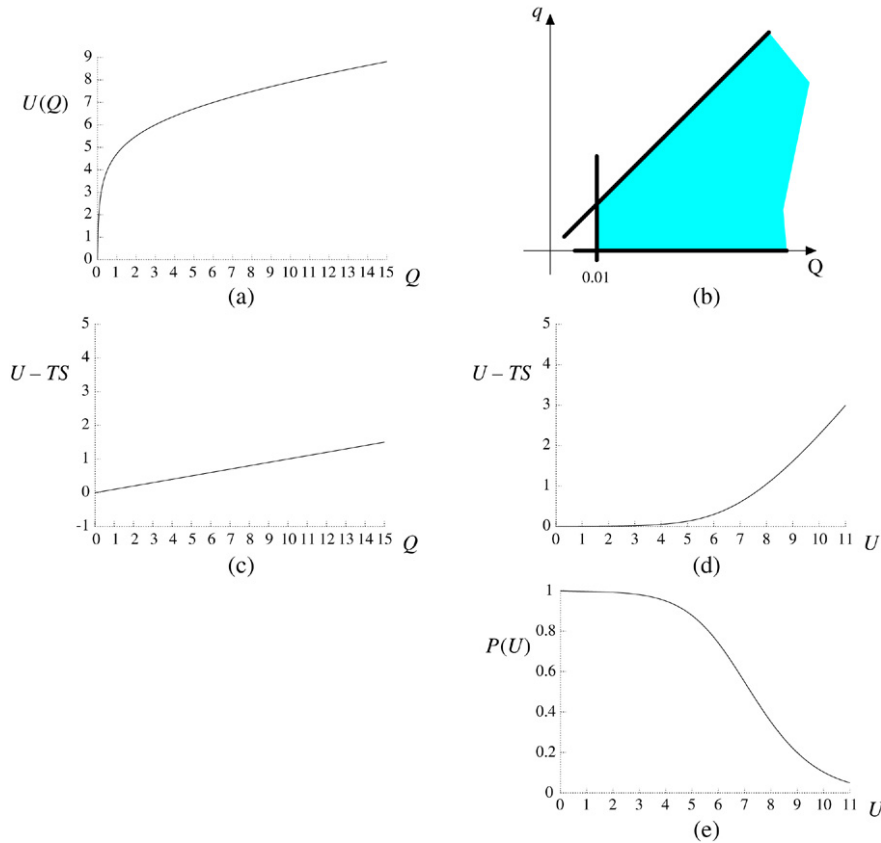
Fig. 3. Parameters of the computer demonstration. (a) Work done versus piston coordinate Q, in units of kT: $U(Q) = \ln \frac{Q}{Q_0} + 0.1Q$. (b) The accessible state space for the driver coordinate q and piston coordinate Q is bounded by the constraints $q > 0$, $q < Q$, and $Q > Q_0 = 0.01$. (c) The free energy $U - TS$ varies little with Q, by design. (d) The free energy $U - TS$ as a function of the work done, U. (f) The equilibrium probability distribution of the work done, $P(U) \propto \exp(U - TS)$. A range of values of U from 0 to 7 have substantial probability, so the system is expected to pass reversibly between states whose potential energy differs by $6kT$.

method. Friction forces are not included explicitly: friction effects arise automatically in accordance with the fluctuation-dissipation theorem. Source code may be viewed at http://www.inference.phy.cam.ac.uk/mackay/dynamics/myosin/c/.

2.2. Predictions

We can predict the behaviour of the piston coordinate Q by marginalizing over q, whose accessible volume, conditional on Q, is $v(Q) = Q$. In energy terms, the driver adds an entropic contribution

$$TS(Q) = kT \ln \frac{Q}{Q_0} \tag{5}$$

to the energy landscape $U(Q)$ (Fig. 3(c)). The predictions for the probability distribution of the work done, U, are plotted in Fig. 3(e).

2.3. Results

A single simulation of the system is shown in Fig. 4. At time zero, the driver is at $q = 0.005$ and the pocket is almost at its smallest permitted volume: $Q = 0.0101$. We set the driver velocity and piston velocity to typical thermal values. Between

collisions, the driver coordinate q varies linearly with time, because the potential is independent of q.

The characteristic pattern while work is being done (e.g., Fig. 4(a), where the piston is moving away from zero) is this: after each collision of piston and driver, the speed of the driver is smaller than it was before; the driver then collides with the wall $q = 0$ and its velocity is re-thermalized, leading, typically, to an increase in speed; so the zigzag trajectory of the driver in that period consists of steep upward segments and slightly less steep downward segments. Thus heat is sucked from the surroundings into the driver and turned into work, just as in the isothermal expansion of a heat engine.

This simulation used a simple one-dimensional pocket, rather than the triangular region depicted in Fig. 2. If we simulate a pocket whose volume grows as Q^2, as shown in that figure, or, most realistically, as Q^3, the entropy increases as $2k \ln Q$ or $3k \ln Q$, so the force exerted by the driver is two or three times greater.

2.4. The charged exit path

Our model so far has a defect: to extract *all* the free energy associated with the release of phosphate into a solution where the concentration is one phosphate per 800 000 Å^3, a tube of
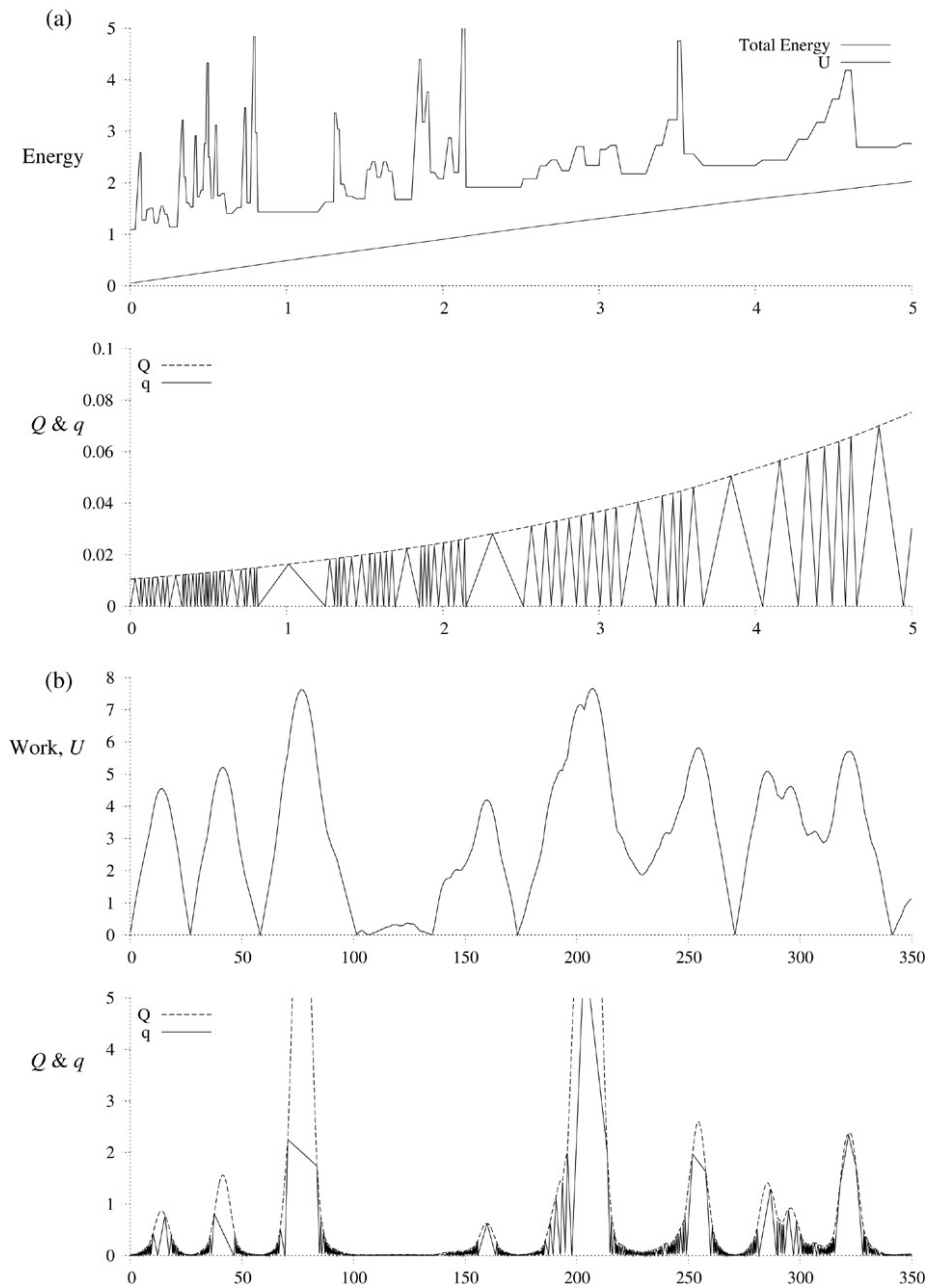
Fig. 4. System energy and state as a function of simulation time. (a) The first 5 time units, starting from initial condition $q \simeq 0$, $Q \simeq 0.01$. After each collision between the driver and the retreating piston, a little energy is transferred from driver to piston. The driver's energy is typically boosted again by the thermalizations when $q = 0$. (b) The first 350 time units. As predicted, the work done wanders reversibly between 1 and $7kT$.

size 800 000 Å^3 is required. This is implausibly large. We now describe a fix for this defect that allows the full free energy to be reversibly extracted with a tube whose volume is a thousand times smaller.

We add positive charges to the mouth of the tube, as shown in Fig. 5. As we explain below, the energy associated with bringing a negatively charged phosphate ion close to some positive charges could easily be about $7kT$, which makes the equilibrium density at the mouth of the tube higher by a factor of $e^7 \approx 1000$: one phosphate per 800 Å^3 instead of one phosphate per 800 000 Å^3. If the volume of the tube is

about 800 Å^3 then the release of phosphate can be a reversible process.

How does this relate to getting the second half of the free energy turned into work? The attraction of phosphate to the positive charge will increase the pressure exerted by the phosphate on the piston as the piston approaches the end of the tube. The potential $U(x)$ should be designed such that this extra force is exploited to obtain extra work.

This model embodies several concrete predictions. We predict that all ATP-hydrolysing motors, if they are efficient, will have positive charges in the vicinity of the phosphate exit-port. We predict that the exit-port will have a fairly strong
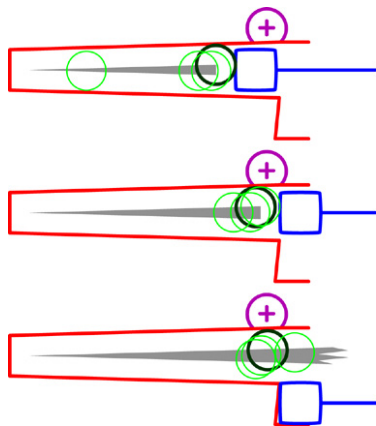
Fig. 5. Enhanced model engine with a positive charge near the exit-port for the negatively charged phosphate ion. This charge increases the local equilibrium concentration of phosphate so that phosphate release becomes a reversible step that does not waste free energy. Extra work can be done during the expansion if the external load is increased appropriately to match the increased pressure exerted by the phosphate; the phosphate exerts a higher pressure during the expansion because it is attracted to the positive charge.

Fig. 6. New potentials. From top to bottom: the new potential for the driver, as a function of Q; the old potential for the driver, as a function of Q; the extra resisting potential for the driver $U_{\text{increment}}^{(2)}(Q)$; and the attractive potential for the driver, $U_{\text{increment}}^{(1)}(q)$, as a function of q. All potentials are shown in units of kT.

binding affinity for phosphate. We also predict that mutations that reduce the affinity of the exit-port for binding phosphate will lead to a reduction in efficiency of the motor: the motor may still run as fast, but the maximum work it can do will be reduced (this last prediction may not be obvious if one thinks of phosphate as a reaction product that should simply be expelled so that the motor can continue its cycle; in such a view, surely it would be best for the phosphate *not* to feel any attraction back to the exit-port, the better to be expelled?)

2.5. Simulation of the charged exit-path model

We modify the one-dimensional system by adding an attractive potential for the driver

$$U_{\text{increment}}^{(1)}(q) = \begin{cases} -q & q < 3.5 \\ -3.5 & q \geq 3.5 \end{cases} \qquad (6)$$

and an additional resisting potential for the piston

$$U_{\text{increment}}^{(2)}(Q) = \begin{cases} Q & Q < 3.5 \\ 3.5 & Q \geq 3.5 \end{cases}. \qquad (7)$$

The new potential for the piston is the top curve in Fig. 6. If the driver gets past coordinate $q = 3.5$, then an extra $3.5kT$ of work will have been done. In exchange, the released driver will now be in a potential of $-3.5kT$; escaping from this potential well is difficult, so the released driver will be at equilibrium with an external solution where the driver concentration is smaller by a factor of $e^{3.5}$.

For efficient simulation, we increase the rate of thermalizations of the driver. We introduce additional thermalization events, one per time unit, at which the velocity of the driver is drawn from the Boltzmann distribution. These can be thought of as the effect of collisions of the driver with water molecules. To clarify the effect of these two modifications to the model,
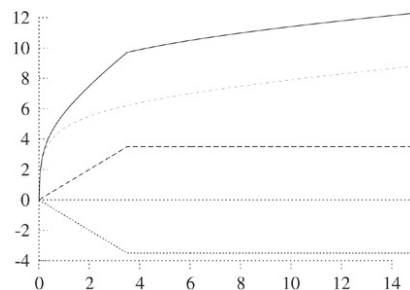
Fig. 7 shows two simulations, one (7(a)) with the extra randomization events only, and one (7(b)) with the charged exit-path modification.

In the detail inset in Fig. 7(a), we see that the driver, which in the first simulation moved in straight lines until it encountered wall or piston, now pursues an undirected random walk. The piston, as before, moves along slightly curved trajectories because of the force $\partial U/\partial Q$.

In the detail of Fig. 7(b), we see that (for $q \in (0, 3.5)$) the driver follows parabolas directed towards the piston; so the driver tends to be closer to the piston and hit it more frequently than before. The piston moves along more curved trajectories because the force $\partial U/\partial Q$ is now greater by 1, for $Q \in (0, 3.5)$. During the period 270–330, the driver drives the piston up this additional $3.5kT$ barrier, and the amount of work that can be reversibly done and undone is increased.

As a check on these simulations, predicted and actual histograms of the work done are shown in Fig. 8. In the final simulation, the system frequently reaches a state in which the work done is $11kT$.

3. Isothermal kinetics

The possibility of driving conformation changes by ergodic pumping follows from thermodynamics, but thermodynamics is usually formulated for macroscopic quantities so that fluctuations from averages are negligible, whereas we wish to study the behaviour of a single biomolecule or small group of biomolecules subject to their environment. Furthermore, thermodynamics does not predict the rates at which changes will happen, nor quantify what counts as a slow enough change for free energy not to be wasted. So one has to consider microscopic models.

We follow the standard belief [10] that slow degrees of freedom of a large system in thermal equilibrium can be regarded as evolving via a Langevin equation with thermal forcing noise and associated damping, a line of thought initiated by Einstein 100 years ago [12] and brought into chemistry by Kramers [19]. Its foundations will be examined elsewhere, but here we spell out what we believe to be a correct general formulation.
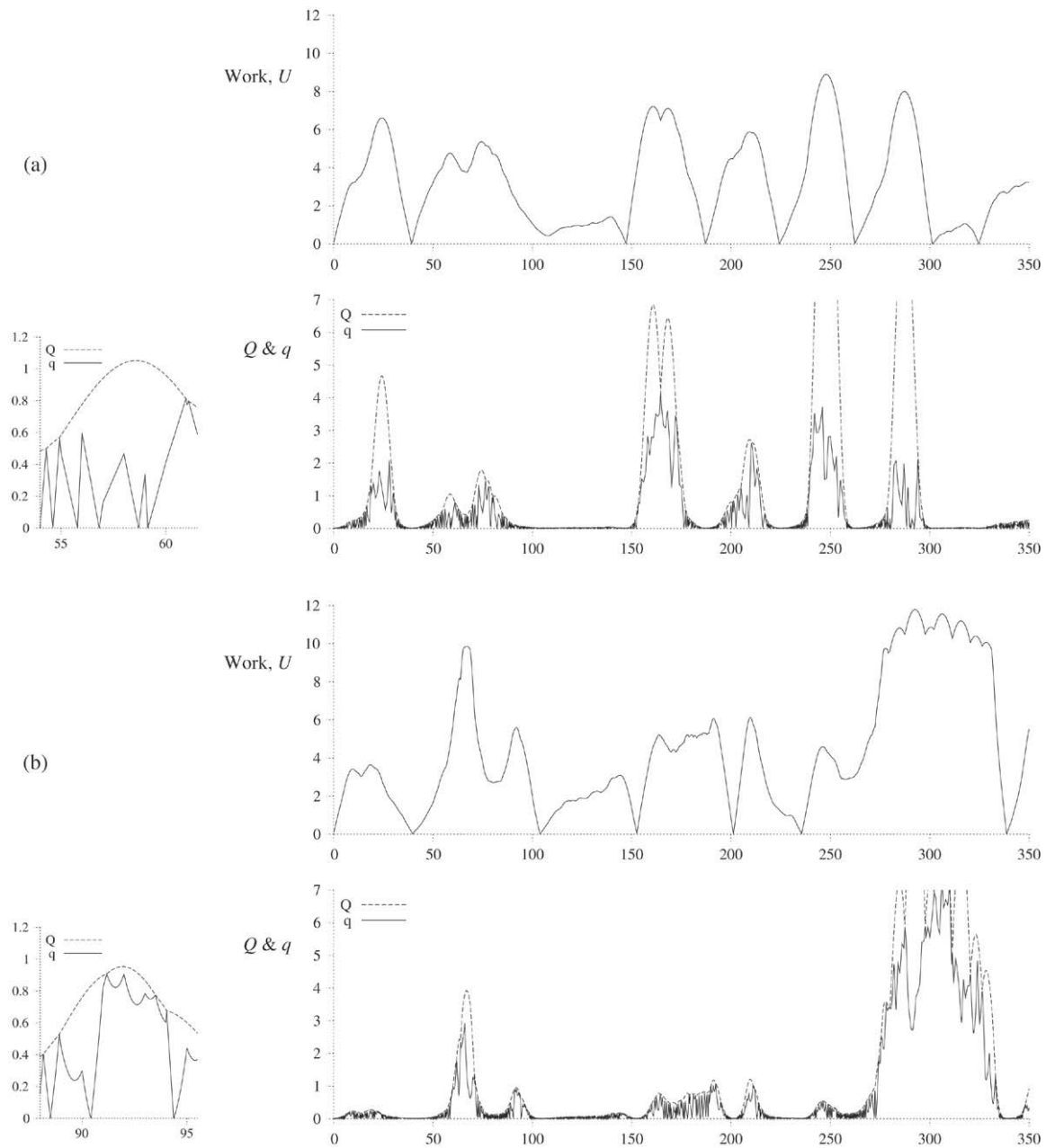
Fig. 7. (a) Simulation of a system identical to that of Fig. 4, except that the velocity of the driver is thermalized every 1 time unit. (b) Simulation of the system with the charged exit path. The piston and driver potentials are modified as shown in Fig. 6. In all figures, the horizontal axis is simulation time.
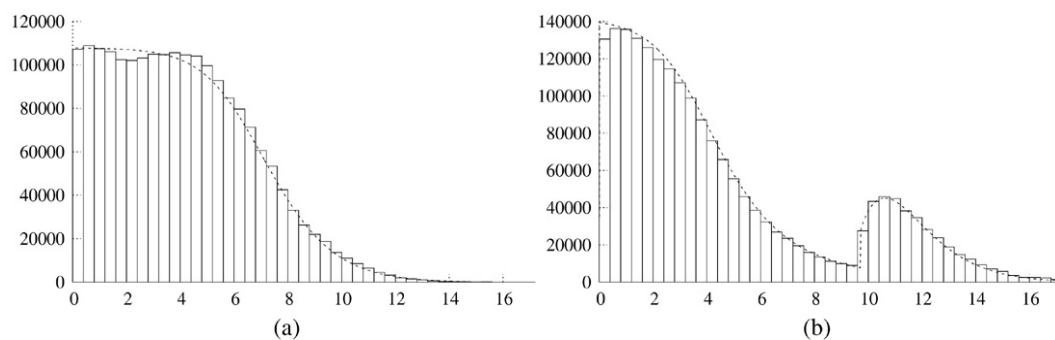
Fig. 8. Predicted (dashed) and actual (solid) histograms of work done, for 2000 000 samples from a simulation of duration 200 000 time units. In both graphs, the horizontal axis is the work done, U, in units of kT. (a) Predictions and results for the simulation with frequent thermalizations but without the charged exit-path modification (these predictions were first plotted in Fig. 3(e)). (b) Predictions and results for the charged exit-path simulation.

We consider the slow variables in canonically conjugate pairs $Z_i = (Q_i, P_i)$ (configuration and momentum) for $i = 1, \ldots, N$, and denote the whole set of slow variables by Z. Reduction to only configuration coordinates Q will be discussed later. We let $\beta = 1/kT$, and denote the free energy given Z by $F(Z)$ (F also depends on T). In most treatments F is regarded as a function of only Q, not P, and it is then called the "potential of mean force" or the "configurational energy landscape", but it can be defined equally well for $Z = (Q, P)$. We denote by an overbar averages with respect to the conditional distribution of the canonical ensemble on a prescribed value of Z. For the present exposition we work at constant volume, but the discussion could be adapted to constant pressure. For subsystems that catalyse a chemical reaction that is maintained out of equilibrium, it is appropriate to consider Z to include information about the numbers of molecules on each side of the reaction (mathematically we would say we are working in a covering space over the slow variables). So, during a cycle in Z, $F(Z)$ changes by the free energy change for the reaction.

Then $\dot{Z} = J\nabla F(Z)$, where $J = \begin{bmatrix} 0 & I \\ -I & 0 \end{bmatrix}$ and ∇ is the gradient with respect to Z. We denote the deviations of $\dot{Z}$ from this average by ζ, and let $D_{ij}(Z) = \frac{1}{2}\int_0^\infty \overline{\zeta_i(t)\zeta_j(0)}\mathrm{d}t$ (assuming the integral converges), where the time evolution is that of the system constrained to constant Z. Then $Z(t)$ is believed to evolve via a Langevin equation

$$\dot{Z}_i = (J_{ij} - \beta D_{ij}(Z))\nabla_j F(Z) + \gamma_{ij}(Z)\dot{W}_j(t), \quad (8)$$

where W_j are independent Wiener processes, $\gamma(Z)$ is a matrix with $\gamma\gamma^{\mathrm{T}} = D + D^{\mathrm{T}}$ (superscript T denotes transpose), summation over repeated indices is assumed, and the equation is to be interpreted in the *backwards Ito* sense, i.e. as the limit as $\tau \to 0$ of the discrete-time process

$$Z_i((n+1)\tau) - Z_i(n\tau)$$
$$= \tau(J_{ij} - \beta D_{ij})\nabla_j F + \gamma_{ij}(Z((n+1)\tau))w_j(n), \quad (9)$$

with $w_j(n)$ independent random steps of zero mean and variance τ ("backwards" means that the coefficient of $\dot{W}$ is evaluated at the end of each step, rather than the beginning). Although we are aware neither of a rigorous derivation (for a review, see [17]), nor anyone pointing out before that one should use the backward Ito convention, this (or special cases or extensions to time-dependent damping and time-correlated noise) is what most biochemists assume, possibly in different notation. In particular, it follows that the probability density ρ for Z, with respect to Liouville measure $\mathrm{d}Z$ for the slow system, evolves according to a Fokker–Planck equation

$$\frac{\partial\rho}{\partial t} = -\nabla_i([(J_{ij} - \beta D_{ij})\nabla_j F]\rho - D_{ij}\nabla_j\rho), \quad \text{for } t > 0. (10)$$

In the special case where the Hamiltonian has the simple form

$$H(Q, P, z) = \frac{1}{2}P^{\mathrm{T}}M^{-1}P + h(Q, z) \quad (11)$$

for some (possibly Q-dependent) positive-definite symmetric matrix M, where z denotes all the remaining variables,

$F(Q, P)$ takes the form $\frac{1}{2}P^{\mathrm{T}}M^{-1}P + G(Q)$, with G the usual free energy function of configuration coordinates Q (though still at constant volume rather than constant pressure, so this is Helmholtz rather than Gibbs free energy). Then $\dot{Q} = M^{-1}P$ and ζ has only P-components, so D and γ reduce to matrices relating only components of P and depending only on Q. The stochastic equation reduces to

$$M_{ij}\ddot{Q}_j = -\nabla_i G(Q) - \frac{1}{2}\dot{Q}_k\nabla_k M_{ij}\dot{Q}_j - \beta D_{ij}\dot{Q}_j$$
$$+ \gamma_{ij}\dot{W}_j(t). \quad (12)$$

If the slowest damping rate $\beta\|MD^{-1}\|^{-1}$ is fast compared to the maximal rate of change of M, D and G along solutions (due to their Q-dependence), then the second derivative and ∇M terms can be eliminated, to a good approximation, resulting in a stochastic equation that is more commonly used than (8):

$$\beta D_{ij}\dot{Q}_j = -\nabla_i G(Q) + \gamma_{ij}\dot{W}_j, \quad (13)$$

which can alternatively be written as

$$\dot{Q} = -kTD^{-1}\nabla G + 2kT\gamma^{-\mathrm{T}}\dot{W}, \quad (14)$$

where $\gamma^{-\mathrm{T}}$ denotes the inverse of γ^{T}. The associated equation for the evolution of the probability density ρ for Q with respect to volume $\mathrm{d}Q$ in slow configuration space is the Smoluchowski equation:

$$\frac{\partial\rho}{\partial t} = \mathrm{div}((kTD^{-1}\nabla G)\rho + k^2T^2D^{-1}\nabla\rho), \quad (15)$$

which may be more familiar (e.g. [41]) than (10).

We prefer to stick with the general form (8), however, because it allows the kinetic energy to contain cross-terms involving both slow and fast momenta, which is generically the case; for example, if one component of z represents the state of a side chain relative to the conformation change variables Z, then its contribution to the kinetic energy will include terms of the form $(P + p)^2$.

Computing F and D ab initio is fairly hopeless, but in small enough systems they can be estimated from molecular dynamics simulations where constraint forces are added to keep the slow variables fixed [4,41].

Now consider what (8) tells us about the dynamics of conformation changes. Using the backwards Ito version of stochastic calculus (which we have not found written anywhere, but can be developed analogously to the forward case [48]), the rate of change of $F(Z)$ along a solution is

$$\frac{\mathrm{d}}{\mathrm{d}t}F(Z) = \nabla F^{\mathrm{T}}(J - \beta D)\nabla F + \nabla F^{\mathrm{T}}\gamma\dot{W} - D_{ij}\nabla_i\nabla_j F. \quad (16)$$

Since J is antisymmetric, the contribution $\nabla F^{\mathrm{T}}J\nabla F$ is zero, and since D is non-negative, the contribution $-\beta\nabla F^{\mathrm{T}}D\nabla F$ is non-positive. The final term $-D_{ij}\nabla_i\nabla_j F$ is non-positive near a local minimum of F, but the noise term has non-zero mean $\gamma_{ij}\nabla_i(\gamma_{kj}\nabla_k F)$, which more than compensates. Thus $F(Z(t))$ typically decreases as t increases, until close enough to a local minimum of F that the remaining terms may cause it to settle to thermal equilibrium. So $Z(t)$ drifts generally downhill in the free energy landscape (albeit with a dominant component along

level sets of F because of the $J\nabla F$ term in (8), though this is eliminated if the system is reduced to first order in Q, as in (14)), until it reaches a neighbourhood of a local minimum (transitions over barriers to other local minima may take place on a longer timescale). Under "hypoellipticity" assumptions on D, (8) has a globally attracting equilibrium density for Z, namely $e^{-\beta F(Z)}$, up to normalization.

It is convenient to think of many conformation changes (e.g. actin–myosin) as transferring free energy from an internal system to an external one, albeit with some inevitable loss. For example, actin–myosin can transfer free energy from muscle cells into lifting weights. Even for conformation changes that do not do such obvious external work, this point of view is useful, e.g. kinesins turn free energy of hydrolysis of ATP into motion of cargo, which does work against viscous forces; even though the viscous forces are in principle just the result of the same Langevin equation applied to the cargo, it is convenient to regard them as external.

It is simplest to think about the case where the external forces are conservative, so the system can be represented by a Hamiltonian of the form $H_{\text{ext}}(Z) + H_{\text{int}}(Z, z)$, where H_{ext} is considered as "external energy". Then

$$F(Z) = H_{\text{ext}}(Z) + F_{\text{int}}(Z), \tag{17}$$

with F_{int} being the internal free energy. Suppose Z is started in a state that is not a minimum of F_{int}. To obtain a significant increase in H_{ext}, we had better have at least a corresponding and nearly monotone decrease in F_{int}. To convert a large fraction of the decrease in F_{int} to an increase in H_{ext}, we had better have the dissipation rate $\beta\nabla_i F D_{ij}\nabla_j F$ small along the path. An internal free energy decrease can be converted to work with high efficiency if and only if the function H_{ext} matches F_{int} well, in the sense that there is a probable path for (8) with only small downhill slope for $F = F_{\text{int}} + H_{\text{ext}}$.

This point of view is fundamentally different from that of [22], who considers a partition of the state space into a discrete set of "states", which are assumed to be in internal equilibrium even if they possess parameters like conformation state variables, which would imply that free energy is not dissipated at all within a state, ignoring the well known phenomenon of viscous dissipation. Hill's view also makes it impossible to account for the fact that entry into a state like the power stroke state for actin–myosin has to occur in some window of the state variables and exit from a different window, or to account for the time spent in achieving change of the state variables in between. Also Hill's view makes it complicated to include continuously progressive binding — one has to introduce a sequence of states which may not really have clear boundaries between them.

Our point of view is also different from that of many "thermal ratchet" models, which are popular among physicists [53]. They often invoke an artificial external switching between chemical states and study the resulting motion of a biomotor along a (linear or circular) track. Our criticisms of such models are firstly that in reality the switching between chemical states is a result of the chemical process, not an externally imposed cycle. Secondly, thermal ratchet models
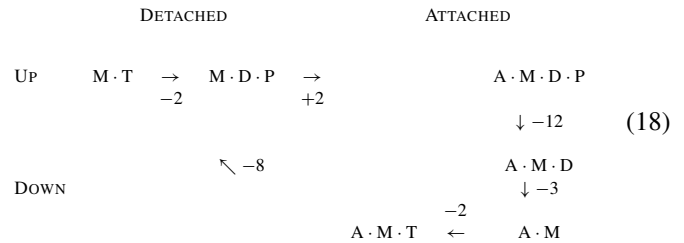
tend to employ significantly irreversible steps which make it difficult to explain the high free-energy-to-work conversion efficiencies achieved by many biological motors. A synthesis of the ideas of ratchet models and Langevin equations, however, is presented in [10].

4. Actin–myosin

4.1. Background

Now we make our main application. Actin and myosin are the principal players in the generation of force and movement by muscle. Actin polymers form long filaments (the "thin" fibres). The myosin tails mat together in long filaments parallel to these (the "thick" fibres). After a myosin head attaches to a binding site on actin, it undergoes a conformation change pulling the thick fibre relative to the thin fibre. After this "power stroke" goes to completion, the head detaches and changes back to its original conformation, ready to go round the cycle again. The cycle is driven by hydrolysis of ATP (adenosine triphosphate) to ADP + P (ADP = adenosine diphosphate, P = phosphate ion), a reaction that is maintained far from equilibrium in most cells. Following [16] we refer to the conformation of myosin before the power stroke as "up" and after as "down".

At a biochemical level, the basic cycle is believed to be

DETACHED   ATTACHED

UP   M·T   →   M·D·P   →   A·M·D·P
                −2   +2
                                                     ↓ −12   (18)

                      ↖ −8   A·M·D
DOWN   ↓ −3
                                       −2
                              A·M·T   ←   A·M

where M = myosin, A = actin, T = MgATP, D = MgADP (ATP and ADP carry a magnesium ion with them most of the time), and the numbers indicate approximate free energy changes in units of kT per molecule (for rabbit skeletal muscle under no tension at physiological conditions, adapted from [30]). On the left side the myosin is detached from the actin, whereas on the right it is attached (though with varying degrees of binding which are not shown). The free energy drops by $25kT$ per cycle. For comparison, the work done by muscle under optimal conditions is estimated at a maximum of $15kT$ per cycle. This can be presumed to come mainly from the right-hand part of the cycle where the myosin is attached to the actin and changes conformation from up to down (though the $8kT$ decrease from AMT to MT also looks too good to waste and merits future consideration).

It should be noted that the above cycle is not universally accepted, and the details may depend on the type of myosin (of which there are 18 main classes [7]). For example, [59] indicates Mg^{2+} release as a separate step between release of P and ADP for myosin V (and shows a slightly different cycle in [29]). The most serious issue under dispute is whether the AMDP to AMD step has an intermediate UP AMD* state. Some authors believe P is pushed out, creating a "highly

strained AMD* state" [30] which then undergoes the major conformation change (see also [1,59,54]), but others are sure that P release occurs after the main working stroke (e.g. [16,58, 60]). We will work with the second hypothesis, though we will indicate later how we could incorporate the first if it turns out to be the case for some myosins. Another detail, which will not be relevant for us, is that it appears the MT to MDP transition proceeds via an intermediate MT* state [40].

4.2. Proposed mechanism

How is the free energy decrease in the power stroke turned into work? We propose that it is principally by ergodic pumping. Here is our proposed story, based on the above principles, the experimental literature, and structures determined by crystallography, studied with the aid of Protein Explorer. Details might be wrong, but we believe that something like this story must be true.

Let us first dispose of a common misconception. Contrary to the cartoon of [62], hydrolysis of ATP in the ATP-binding pocket of myosin does not liberate a flash of energy which is then stored in some unidentified place such as vibrations or springs and released later as work in the power stroke. In fact, the hydrolysis of ATP in the myosin pocket is endothermic ($\Delta H = +27kT$) and reversible with an equilibrium constant of about 10 [2,43] (so $\Delta G = -2.3kT$). Thus, an amount $T\Delta S = +29kT$ of heat is sucked *in* from the environment on hydrolysis. Part of the entropy increase this represents could be due to tumbling of the P in the pocket (inferred from ^{18}O exchange [64]), but there are myosin conformation changes too [40] which could account for the rest.

Thus, to begin our story, before attachment to actin, the ATP in the ATP-binding pocket of the myosin has already hydrolysed. The resulting P is confined, however, to a small volume at the inner end of the binding pocket. We claim it exerts a pressure on this part of the pocket, attempting to expand it. It is not until actin begins to bind to the myosin, however, that the expansion is able to start (initial actin binding breaks a salt bridge and hydrogen bonds between switches 1 and 2 that stabilized the pre-power stroke conformation [14]). We presume the actin binding weakens the tightness of the pocket (compatible with suggestions of [16]), perhaps initiates the expansion, and in particular allows the P to detach from the magnesium ion which has still been holding it to some extent. The P expands the inner end of the pocket (the "cleft" of [14]), driving the beginning of the believed rotation of one domain relative to another (for summaries of the believed system of gears, levers and clutch, see [16,25]) and permitting strengthening of the actin binding (which contributes further free energy). The trap is permeable to water but not to P, so water enters as it expands. At the same time that the P expands its trap, the resulting expanding "bubble" made by the P moves away from its original location, initially along the tunnel, since it is almost universally agreed that P cannot leave past the ADP. There is no consensus, however, about where the P finally leaves myosin. As we explained in Section 2.4, we believe it is essential that it leave via a surface region of significant positive charge. Thus, as one candidate for the final location

of the bubble, we propose the region between the switch-2 helix, switch-2 loop (known together as the "relay") and the SH1-helix (shown in Figure 6(b) of [16]). This region is also plausible because it is known to change shape significantly during the power stroke. The end of the main phase of the power stroke comes with the P expanding its available volume to about 800 Å^3, and we propose that this expansion drives the conformation from up to down. The changes produced by the combination of P pressure and actin binding also drive detachment of the ADP from its binding site (possibly via release of the Mg^{2+} which probably also does work by ergodic pumping) and it begins to exert a pressure in its pocket. In some myosins it might be that it is only this ADP pressure which drives the main conformation change, allowing for P release before the power stroke, as some authors claim.

This would not be the end of the available free energy from the P, however, since the volume available in the cellular fluid is about 800 000 Å^3 per P, one thousand times larger than the final volume proposed above. It is unrealistic to expect an expansion chamber of this size in a biomolecule. So without further ingredients, ergodic pumping could convert to work at most about half of the free energy corresponding to entropy increase associated with phosphate release. We propose that the system extracts the remaining free energy of release of P by electrostatics, as sketched in Section 2.4.

We now fill in the quantitative details of the 'charged exit-path' model that we introduced there. The P carries a charge of between –1 and –2. The believed in-line hydrolysis reaction produces HPO$_4^{2-}$ + H$^+$ [30], but pK_a for the H$_2$PO$_4^-$ to HPO$_4^{2-}$ transition is about 6.8 and the hydrolysis is observed to release 0.4 H$^+$ [43], so perhaps 60% of the 2− form is converted to 1−. Outside the myosin, the pH is about 7.2, so we would expect 75% of the P to end up as 2− (though [47] suggest P is released as 1−). As the proposed pocket expands to 800 Å^3, we propose that a mouth opens up, carrying positively charged residues. Possibly these are the highly conserved arginines along the SH1-helix at locations[1] SH2 + 8, +11, +17; see Table 1 of [16] and view Protein Data bank structure 1VOM for Dictyostelium cytoplasmic myosin II, which is believed to correspond to UP-DETACHED in the cycle. Now the equilibrium density of an ion of charge $-c$ with external concentration C is enhanced in a positive potential ϕ by a Boltzmann factor to $Ce^{\beta c\phi}$. Thus ϕ of the order of 0.11 V averaged over the pocket would suffice to match the external concentration (since the thermal energy at 300 K is $kT \approx 0.026$ eV). One might worry that the charges would be screened and so have less effect, but the screening length associated with water's dipoles is about 5 nm, so one can expect charged residues to influence the potential over a

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[1] To allow cross-species comparison we adopt a relative-addressing notation as opposed to the standard absolute addressing: we choose a conserved residue like the cysteine SH2 (697 in chicken and rabbit, 678 in dictyostelium, 693 in scallop) and write nearby locations as SH2 + n with the integer n (positive or negative) indicating how many amino acids to step along the chain. Thus the SH1-helix extends from SH2 + 2 to SH2 + 13 and SH1 is SH2 + 10. The composition of the SH1-helix and residues near each end is well conserved: SH2 − 2 to SH2 + 15 in chicken and rabbit is LRCNGVLEGIRICRKGFP; in dictyostelium the only change is that SH1 is a threonine instead of cysteine; in scallop SH2-1 is a glutamine instead of arginine.

volume of up to about 500 nm³. So in addition to the work done by expansion, the P would also "descend" an electrostatic gradient, turning the resulting energy into further work on the expansion. The P starts in an environment that looks neutral or negatively charged, because apart from the Mg^{2+}, the two phosphate groups of ADP carry a total charge of about 3−, and there are several negative residues around the P.

Supporting evidence for our suggestion of the region behind the SH1-helix as the end location for the phosphate pocket also comes from p. 466 of [28], who say the SH1-helix "fits into a kind of cavity between this subdomain on one side and the relay on the other . . . . The size and shape of the cavity depends strongly on the relative positions of the lower 50 kDa and N-terminal subdomains. In states I and II, the cavity is filled by the helix, which makes van der Waals contacts – but no specific interactions – along its length. . . a change in location [of the relay] that separates [the] two joints [at either end of the SH1-helix] can produce a major destabilization and unwinding of the helix, as seen in the scallop structure". We would suggest it is the phosphate ion trapped between the SH1-helix and the rest that pushes the relay and the SH1-helix. [28] make similar comments on p.467 about motion of switch-2 triggering unwinding of the SH1-helix. Although they argue that a "structure in which the SH1-helix is unwound cannot correspond to the strong actin-binding "ADP" state of the contractile cycle", they propose a picture for the strong actin-binding state which seems to us might have a considerable space behind the SH1-helix. One point to bear in mind is that there is considerable negative charge along the switch-2 loop and switch-2 helix.

Another possible location for the expanded phosphate pocket is the region of the "converter" domain adjacent to the ends of the SH1-helix in the conformation of [28]: most of the SH1-helix (SH2 + 3 to +10) is not resolved, presumably because floppy, but it reveals a large hole right through the converter, so a double mouth, surrounded by 11 positively charged residues (locations 57, 71, 108, 116, 144, 502, 704, 710, 719, 759 and 774 in Scallop myosin; see PDB structure 1B7T).

Additional evidence that the SH1-helix is on the route of phosphate release comes from experiments on genetic modifications. For example, [50] found that modification of Gly691 in Dictyostelium myosin (SH2 + 13) to Cys led to "promiscuous release of P$_i$". This seems pretty clear evidence that the P goes to this region of the SH1-helix; presumably it leaks out because the modification makes the backbone less flexible, but it might also be because the cysteine bonds somewhere it should not. They also found that modification of Gly680 (SH2 + 2) to Val makes it bind strongly to actin (following an analogous finding for Gly699 in chicken to Ala [33]). Similarly, the (natural) mutation Arg702 (SH2 + 8) to Cys in human nonmuscle myosin IIA reduces actin activation and speed [31].

4.3. What happens next?

Once the P is in equilibrium with the outside, it is free to leave, and can do no more work. At this stage some sort of energetic obstacle is required to prevent the conformation change from reversing; one possible mechanism would exploit

the charge of the phosphate again: the pocket might have some positive charges inside, placed such that in the absence of phosphate there is a substantial energy barrier against the pocket's collapse; the phosphate, if present, neutralizes these charges and lowers the barrier. A more likely alternative is that the thiols SH1 and SH2 link, preventing immediate recollapse. There is evidence that they move from 18 Å apart to 3 Å during the power stroke [9,45,46,55].

When the phosphate is near release, we propose that the ADP takes over as the main force exerter and the P expansion pocket decouples from the lever arm movement. Detachment of the ADP having been initiated by the P expansion, ADP exerts force by ergodic pumping, with rotational and flexural as well as translational entropy increases contributing to the force (the Mg^{2+} may also exert pressure if it separates from the ADP, but would require a negatively charged exit route). Expansion of the ADP-binding pocket provides a little more movement (observed in some slow myosins; see references on p. 717 of [16]), before the ADP in turn reaches a positively charged pocket whose size is such that the ADP is close to equilibrium with its concentration outside; the ADP can then leave nearly reversibly. The positive charges for the ADP exit are clear, namely the lysines and arginines around the mouth of the ATP-binding pocket (e.g. residues 130, 131, 190, 191, 265, 435, 445 and 587 in Dictyostelium cytoplasmic myosin II). The large number of positive charges is required to equilibrate with 20 μM ADP outside (80 000 nm³ per ADP). In the process of the ADP pocket expansion, the myosin becomes strongly bound to actin [26], thus preventing recollapse of the ADP-binding pocket. This is now the rigor state.

To complete the cycle, myosin has to wait for an ATP to turn up and get attracted into the binding pocket; as the ATP binds, it weakens the binding of the myosin to actin, and the myosin floats off the actin. Estimates suggest a significant free energy decrease during this step, which is too large to waste, so we suspect that during the unbinding from actin some further work is done. On the other hand, an irreversible step here might be useful to ensure the cycle can operate at a high speed when necessary. It would be interesting to understand the mechanics of the release of myosin. A different type of ergodic pumping might occur in which partial release of the myosin allows parts to move more vigorously and hence exert a net repulsive force. Completion of the binding of ATP closes the ATP-binding pocket, reverting the myosin to its UP state. Once the pocket is closed, the appropriate residues are in place to catalyse hydrolysis of the ATP.

4.4. Further evidence

We have incorporated much experimental evidence into the above proposal. We defer a detailed comparison with experiments to a paper aimed directly at biologists, but list here some further experimental results we must take into account. Increase of [P] decreases isometric tension and has no effect on the speed of shortening [11]; it also reduces stiffness and increases the rate of attainment of final tension [60]. Increase of the ratio [ADP]/[ATP] increases isometric force and

decreases speed [11,15,47]. Addition of osmotic equivalents of P, e.g. phenyl phosphate, arsenate or vanadate, reduces maximum tension [47]. 10 MPa external pressure reduces tension by 10% [15]. pH also has effects [43,3] (the power stroke releases about 0.4 of a proton). There are effects of chemical modification of SH1 and SH2 [55,42] and effects of replacing Mg^{2+} by Ca^{2+} or K^+ [56]. There are further results on P release and force generation in [29,58]. There are various attempts to ascertain the route by which P exits myosin, e.g. [66,54] give two conflicting proposals (neither of which corresponds to our tentative one). There are several studies of natural or artificially induced mutations of myosins [16, 68]. Temperature is also highly relevant to the operation of muscle; cold-blooded animals have to warm up in the sun before attempting significant movement. This is consistent with the emphasis we place on the entropic drive, whose effect is proportional to temperature. The effect of temperature on the SH1-helix has been studied by [50,45].

ATP, GTP, CTP, UTP and their 2-deoxy forms all drive myosin [52], but with differing speed and force. Some non-nucleoside triphosphates also drive myosin but others are only hydrolysed by it [18]. These experiments tell one something about the function of the adenosine part of ATP: in our story it must be retained as a plug on the phosphate trap until the phosphate trap has moved far enough away.

Added to the positive evidence for our proposal is the failure of anyone else to propose a mechanism (in the sense of how free energy is turned into work). It is generally believed that binding of the ATP to myosin winds up a "spring" in myosin whose energy is released on binding to actin [29]. But where is the spring? We would say the energy source is the pressure of the confined P. Similarly, it is sometimes claimed that the phosphate offers no net free energy of release because the entropy gained pays to unbind it. The evidence, however, is that it is not bound in the pocket immediately after hydrolysis [64]. Nor does it appear to be in an electrostatic well; as remarked at the end of Section 4.2, it appears to be surrounded by mainly negative charge.

Finally, we propose some tests of the idea.

Genetic modifications

One could modify residues that are crucial for our explanation. For example, if one made extra space at the γ-phosphate end of the ATP-binding pocket, in a way that still leads to hydrolysis, we would predict that this change would waste a significant amount of entropy of expansion of available volume for the P and hence reduce the work that can be done.

One could remove some of the positive charges at the proposed exit route for the phosphate. We predict that the maximum work extractable would decrease (the E683K mutation studied in [68] is relevant to this region, though it adds a positive charge rather than removing one).

Solute effects

We predict that the addition of small negatively charged solutes, e.g. F^- ions, to the solution might shield the positive charges at the exit route, reducing the work that can be done.

Adding large osmotically active molecules to the surrounding solution is predicted to reduce the force exerted, even if those molecules have no relationship to the molecules involved in the ATP reaction: if the external concentration of osmotically active molecules is increased, the expansion of the phosphate's pocket will be opposed by a larger osmotic pressure.

Molecular dynamics simulations

Molecular dynamics simulations could be done in which some external assistance is given to the phosphate ion to expand a pocket, in order to speed up the otherwise prohibitively (for simulations) slow power stroke (taking about 1 ms). For example, the phosphate ion could be artificially maintained at a higher temperature. On the basis of our proposal, this would be much more fruitful than simulations like those of [65,34] in which the phosphate ion is pulled along a promising path, for which the energy barriers are found to be enormous. The important thing in our view is to allow the P to rattle around, exerting pressure and expanding a trap.

5. Other speculative applications

5.1. Tubulin motors

A similar story might be expected for ATP-driven biomotors on tubulin tracks, like kinesins and dyneins, but the phasing of their mechanical and chemical cycles is believed to be somewhat different from actin–myosin [30]. So instead of ergodic pumping occurring in the power stroke, we believe it occurs in a recovery phase. Nonetheless, it does work in just the same way (though perhaps of a more internal nature), and requires the same positive charge exit.

5.2. F_1 ATP-ase

F_1 ATP-ase is the part of ATP-synthase that in "normal" operation synthesizes ATP from ADP and P [8]. But if it is detached from the F_0 proton motor, or the proton-motive force for the F_0 motor is insufficient, then F_1 ATP-ase hydrolyses ATP and rotates in the opposite direction (pumping protons against the proton-motive force in the second case). The standard model for F_1 ATP-ase is Boyer's binding change mechanism. But we believe this explanation is incomplete, since it does not describe how the large entropy increase of the escaping phosphate is used. ATP-synthase is freely reversible and makes negligible losses, so we believe ergodic pumping must be involved.

5.3. Some ion pumps

There are several ATP-driven ion pumps, for example 3Na/2K ATP-ase, sarcoplasmic Ca ATP-ase, and the H/K exchanger in the stomach wall [6]. Phosphate release must play a role in their function.

5.4. DNA and RNA motors

There are several types of ATP-driven motors which use DNA or RNA as their "track" rather than actin or tubulin. In

particular, DNA helicase moves along double-stranded DNA to separate the strands: release of ADP + P opens a cleft between two subdomains of the helicase, thus advancing the separation by one base pair. As another example, to terminate RNA transcription, when ρ-protein detects a region of RNA rich in C and poor in G it attaches to the RNA and pulls itself along towards the RNA polymerase by ATP hydrolysis and causes the RNA polymerase to release its product. As a third example, the bacteriophage $\Phi 29$ has an ATP-powered motor for packing its DNA into its capsid; it is immensely powerful [57]. Ergodic pumping by P + ADP must be a major driving force in all three of these motors.

5.5. Protein translocators

There are ATP-driven motors which translocate proteins across membranes, e.g. SecA [63] and Hsp-70 [13]. Again, ergodic pumping is likely to be used to turn the entropy of release of phosphate into work.

5.6. Proton and sodium motors

Ergodic pumping could also happen with drivers other than phosphate ions. For example, consider torque-producing proton motors like the F_0 of ATP-synthase and many bacterial flagellar motors [5]. These motors are driven by transfer of protons across a membrane from a low pH (high proton concentration) to a high one. Thus the final step is release of a proton into a low concentration environment. To make use of the resulting entropy increase, we believe the motor must use ergodic pumping (combined with the charged exit-path trick, with negative charges in this case).

Other bacterial flagellar motors are driven by a flow of Na^+ ions through a membrane across which a difference in Na^+ concentration is maintained by pumps. Release of the Na^+ implies ergodic pumping if the entropy increase is not to be wasted.

5.7. Others

Some other biomolecular systems where ergodic pumping could contribute to driving conformation changes are chaperonins [37], G-proteins [61,44] and cytoskeletal polymerization (actin and tubulin) [30,65].

5.8. Nanobiotechnology

In addition to contributing to understanding conformation changes in natural biomolecules, ergodic pumping has potential as a design principle for nanobiotechnology. It might be possible to design artificial motors, switches and other devices performing conformation changes, driven at least in part by ergodic pumping. Perhaps simplest is to hijack the ATP production in natural cells and design a new device to use it, perhaps also making use of a natural ATP-binding pocket, but modifying the use to which the expansion by the phosphate ion is put. There is also the potential to design and use entirely different fuels, binding sites, and expansion chambers.

It is worth noting a quantum-mechanical limitation, which Nature seems to have recognized. At temperature T, a particle of mass m has a thermal length $a = \frac{\hbar}{\sqrt{mkT}}$, where $\hbar$ is Planck's constant divided by 2π; the particle's position cannot be pinned down more precisely than this (Heisenberg's uncertainty principle says that, for each coordinate x and its conjugate momentum p, their standard deviations satisfy $\Delta x \Delta p \geq \hbar/2$; $\Delta p = \sqrt{mkT}$ in thermal equilibrium). So there is a smallest feasible volume from which ergodic pumping can start, of order $(\sqrt{3}a)^3$ (the factor of $\sqrt{3}$ is for the case of a cubic trap; an alternative used commonly in thermodynamics is to take the thermal volume per particle to be $(\sqrt{2\pi}a)^3$). For example, for a phosphate ion at $T = 300$ K, taking $m = 150$ atomic units (rather than just the bare 97 or 98, to allow for the fact that it carries along some water molecules), $\sqrt{3}a \approx 0.07$ Å.

One interesting question is whether the mass of the driver plays a crucial role. The phosphate ion is intermediate in mass between a water molecule and a short segment of protein, and perhaps it is near optimal for some aspect of ergodic pumping.

6. Conclusion

We have proposed a mechanism for converting the entropy increase on release of a ligand into useful work under isothermal conditions, by letting the pressure it exerts expand a pocket until it equilibrates with conditions outside. We proposed three important refinements of the idea, in order to make it biologically plausible. Firstly, not only the ligand ('driver') but also the mode of expansion ('piston') have entropic effects, and the total of these entropic contributions must be balanced by the work done; one way of eliminating the piston's contribution is for the effective mass of the piston to decrease from a large to a small value during the process. Secondly, the expansion must be accompanied by descent of the expansion bubble into an electrostatic well relative to the external potential. Thirdly, after release of the driver, some other process (possibly ergodic pumping of another species) must take over to prevent recollapse. We have described a computer demonstration of the phenomenon. We have proposed applications to a range of conformation changes, in particular in myosin. We propose ergodic pumping as a design principle in nanobiotechnology.

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