

ARTICLES

The Physics of Molecular Motors[†]CARLOS BUSTAMANTE,^{*,‡} DAVID KELLER,[§] AND GEORGE OSTER[⊥]

Howard Hughes Medical Institute, Departments of Physics and Molecular and Cell Biology, and ESPM, University of California, Berkeley, California 94720, and Department of Chemistry, University of New Mexico, Albuquerque, New Mexico 87131

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ABSTRACT

Molecular motors convert chemical energy into mechanical force and movement. Operating at energies just above those of the thermal bath, these motors experience large fluctuations, and their physical description must be necessarily stochastic. Here, motor operation is described as a biased diffusion on a potential energy surface defined by the interactions of the motor with its track and its fuel. These ideas are illustrated with a model of the rotary movement of the F_0 motor.

1. Introduction

A quarter of a century ago, the cell and its many internal compartments were still thought of as microscopic reaction vessels containing complex chemical mixtures held at constant temperature and pressure. As the intricate pathways of the intermediate metabolism became available, scientists hoped to describe cellular processes as the result of a complex series of parallel and sequential second-order chemical reactions brought about by the diffusion and random collisions of chemical species in these confined spaces. During the intervening time, this view has changed dramatically. Cells are polar structures, and their interior is neither homogeneous nor isotropic. Moreover, most of the essential cellular functions such as chromosomal segregation during cell division, trans-

location of organelles from one part of the cell to another, or the maintenance of a voltage across the membrane all involve *directional* movement and transport of chemical species. Processes such as replication, transcription, and translation require the information encoded in the sequence of linear polymers to be read and copied in a directional manner, and cells must often move and orient in response to external chemical gradients and other signals.

To overcome the randomizing effect of Brownian motion and carry out these directional processes, cells possess molecular structures that behave as tiny machine-like devices. These devices operate as *molecular motors*, converting chemical energy into mechanical work. However, they are unlike macroscopic engines in that, because of their dimensions, the many small parts that make up these molecular motors must operate at energies only marginally higher than that of the thermal bath and hence are subjected to large fluctuations. Sitting astride the line that separates stochastic from deterministic phenomena, the function of these molecular motors can be thought to be that of “taming” the randomness of molecular events and generating directional processes in the cell.

Cells have hundreds of different types of molecular motors, each specialized for a particular function. Many biological motor-like proteins have been discovered and characterized in recent years (see, for example, ref 1). Although there is much variation in design and performance among them, several lines of evidence suggest that many such “mechanochemical” proteins share fundamental underlying features that can be understood with the same basic concepts and theories. Such theories seek to describe the physical principles that govern the behavior of molecular motors, to explain the role of fluctuations in their operation, to describe the nature of the coupling between chemical reaction and physical coordinates, and to understand specific aspects of this conversion, such as its efficiency and reversibility.

In this paper we describe the basic physical ideas behind the stochastic description of molecular motors, and some of the general formalism of stochastic processes. To illustrate how these ideas work in practice, a simple model for the ion-powered F_0 rotary motor is then presented.

Carlos Bustamante is a Howard Hughes Medical Investigator and a professor in the Departments of Physics and Molecular and Cell Biology at the University of California, Berkeley. His research interests include the theory of molecular motors, the development of methods of single-molecule manipulation and detection, and the use of these methods to investigate nucleic acid-binding motors and protein and RNA folding.

David Keller is a professor of biophysical chemistry in the Chemistry Department at the University of New Mexico, Albuquerque. His research interests are in single-molecule measurements, molecular machines, and the theory of molecular motors.

George Oster is a professor in the Department of Molecular and Cell Biology at the University of California, Berkeley. He is also a professor at the College of Natural Resources. His main research interests are in the area of biophysics of cell locomotion, molecular motors, and membrane biophysics.

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^{*} To whom correspondence should be addressed.

[‡] Howard Hughes Medical Institute and Departments of Physics and Molecular and Cell Biology, University of California, Berkeley, and Physical Biosciences Division, Lawrence Berkeley National Laboratory.

[§] University of New Mexico, Albuquerque.

[⊥] Department of Molecular and Cell Biology and ESPM, University of California, Berkeley.

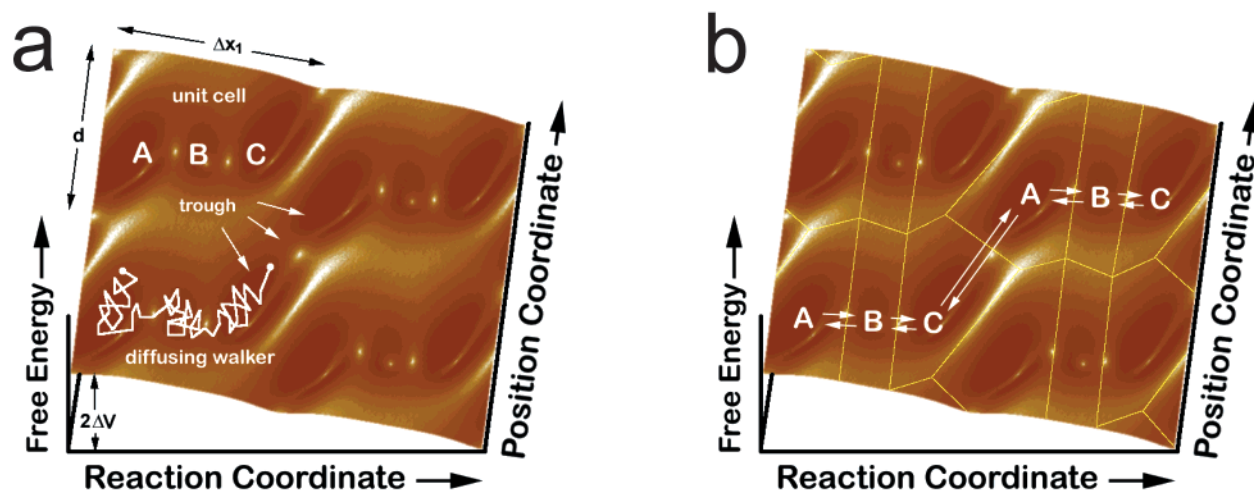


FIGURE 1. (a) Minimal potential energy surface for a molecular motor. The surface is periodic in both the reaction coordinate and position coordinate, reflecting the cyclic nature of both enzymatic turnovers and motor cycles. The surface has three local minima (labeled A, B, C) connected by low-energy passes and is tilted along the chemical axis, representing the driving force for the motor, i.e., the free energy of reaction. An externally applied load force would appear as a tilt of the surface along the position coordinate. The long trough in the center couples chemical energy to mechanical motion. The system point moves by random walk over this surface. (b) Correspondence between the potential energy surface and the kinetic mechanism of the motor. The regions around the local minima represent intermediate species. Diffusion between minima is equivalent to chemical transitions, which can be described by kinetic rate constants.

2. Basic Theory

Molecular motors come in a wide variety of designs. Some motors operate in a cyclic fashion, undergoing a number of steps that correspond to changes in conformation and/or in chemical state and eventually resetting themselves to their initial configuration. The steps of the mechanical cycle are coupled to the states of a chemical cycle that generates the energy that fuels the movement. The chemical steps in cyclic motors typically involve catalytic turnover of a high-energy molecule, generally a nucleotide (e.g., myosin, kinesin, helicases), or translocation of an ion across an electrochemical gradient (e.g., F_0 ATPase, the bacterial flagellar motor).² Other motors are “one-shot” motors that release previously stored elastic energy and then are disassembled (e.g., spasmoneme, actin polymerization).³

The central difference between macroscopic and molecular motors is that the latter are so small that Brownian motion dominates their operation. This means that thermal fluctuations are an essential component of the molecular mechanism of the motor/enzyme. Enzymatic catalysis depends on thermally induced crossing of potential energy barriers. Similarly, the ability of molecular motors to generate forces and motion may depend on thermally driven diffusion from one site on a filament (such as actin, DNA, or a microtubule) to the next.

A motor molecule interacting with a track and with molecules in solution possesses many degrees of freedom, but most of these fluctuate very rapidly and are approximately at equilibrium on the experimental time scale. These extra degrees of freedom in the protein and in the solvent that surrounds it will be called *bath variables*.⁴ Bath variables do not appear explicitly in the description of the motor, but they affect its motion implicitly as fluctuating stochastic forces, as sources of

friction, and as entropic contributions to the potential energy surface that governs the motor. The rest of the variables, called the *system variables*, define an n -dimensional state space in which the motor moves. Each point on the state space represents a unique configuration of the motor and has an associated free energy, the *potential of mean force*. The potential of mean force depends on the system variables and defines the potential energy surface on which the motor moves. It arises primarily from three sources: (1) interactions within the motor molecule itself, and between the motor and its track (if it has one), (2) interactions between the motor and fuel molecules, and (3) interactions of all of the above with the solvent environment. All three include entropic contributions associated with the bath degrees of freedom. Since a molecular motor must have a source of chemical energy, at least one of the system variables must measure the progress of the chemical reaction and will be called the *chemical variable* or *reaction coordinate*. All others will be called *mechanical variables*. At least one of the mechanical variables must describe the progress of the motor along its track or, for rotary motors, the angle of rotation about its axis.

In the simplest case, when the motor can be described by only one chemical and one mechanical variable, the potential energy surface can be easily visualized (see Figure 1a). A cut through the surface along the chemical coordinate gives a reaction free energy diagram. A change in the concentration of reactants or products changes the net free energy of the reaction and tilts the diagram forward or backward along the chemical coordinate axis. A cut through the surface along the mechanical (position) variable gives the potential for movement of the motor along its track in the absence of any chemistry. Application of an external force against or in favor of the direction of

motion tilts the surface along the mechanical coordinate axis. The trough in the center of Figure 1a (arrows) is the region where chemistry is coupled to mechanical motion. In this region, the slope of the low-energy pathway in the chemical direction drives movement along the mechanical direction. Thus, chemical energy is converted into motion in this region of the potential energy surface.

For a macroscopic system, motion on this potential would be a smooth trajectory determined by the shape of the surface, but in the presence of random thermal forces, the motion is stochastic and only statistically biased by the potential. If inertial forces are negligible compared to friction (as is almost always the case at the nanometer scale), and if the random forces are rapid on the time scale of interest, so that they lose correlation between steps of the stochastic walk, the motion is Markovian and amounts to a biased diffusion of the system point on the potential energy surface.

The movement over the potential energy surface in the mechanochemical space is then well described by Langevin equations, which, in simple cases, are Newtonian equations of motion for over-damped particles subject to a fluctuating force:

$$\begin{aligned} \gamma_1 \frac{dx_1}{dt} &= -\frac{\partial V(x_1, x_2)}{\partial x_1} + f_1(t) + F_{B1}(t) \\ \gamma_2 \frac{dx_2}{dt} &= -\frac{\partial V(x_1, x_2)}{\partial x_2} + f_2(t) + F_{B2}(t) \end{aligned} \quad (1)$$

where subscripts 1 and 2 refer to the chemical and spatial position coordinates, respectively, of the motor. Here, V is the motor potential, the γ 's are friction coefficients, the F_{B_i} 's are random (Brownian) bath forces, and the f 's are external mechanical forces acting on the motor. Note that molecular motors always operate in the low Reynolds' number regime where inertial forces can be neglected.

Because of the random force, individual trajectories obtained by solving these equations are meaningless; only statistical distributions over many trajectories are useful. The appropriate solution of the Langevin equations is thus a probability distribution, $w(x_1, x_2, t)$, for the location of the system point at each time t . Since total probability is conserved, this probability distribution must satisfy a continuity equation:

$$\frac{\partial w}{\partial t} + \nabla \cdot \mathbf{J} = \frac{\partial w}{\partial t} + \sum_{i=1}^2 \frac{\partial J_i}{\partial x_i} = 0 \quad (2)$$

where \mathbf{J} is the two-component probability current. For a biased diffusion process \mathbf{J} is the sum of two contributions, a diffusion current and a drift current:

$$\mathbf{J}_i = \underbrace{\left(-\frac{kT}{\gamma_i} \frac{\partial w}{\partial x_i} \right)}_{\text{Diffusion}} + \underbrace{\left(\frac{f_i}{\gamma_i} w \right)}_{\text{Advection}} \quad (3)$$

where f_i are the forces acting on the i th component of the state space due to potential and external forces, but

excluding the stochastic force, which is accounted for by the first term. The equilibrium state corresponds to $J_i = 0$, in which case eq 3 yields a Boltzmann distribution for w ensuring detailed balance. Substituting eq 3 into eq 2 yields the Smoluchowski equation:⁵

$$\frac{\partial w}{\partial t} + \underbrace{\left[\sum_{i=1}^2 \left(-\frac{k_B T}{\gamma_i} \frac{\partial^2}{\partial x_i^2} + \frac{1}{\gamma_i} \frac{\partial}{\partial x_i} f_i \right) \right]}_{\mathbf{K}} w = 0 \quad (4)$$

which has the form of a biased diffusion equation, as expected. The Smoluchowski equation gives the probability density, $w(x_1, x_2, t)$; eq 3 gives the probability currents and hence the rates at which the system moves over the potential energy surface through the stages of its mechanochemical cycle. Formally, the Smoluchowski equation can be written

$$\frac{\partial w}{\partial t} = \mathbf{K} w \quad (5)$$

where \mathbf{K} is the operator in square brackets in eq 4. This form will be useful for comparison with the results of the next section.

2.1. Connecting Mechanics to Kinetics. The picture of a molecular motor outlined so far is essentially mechanical and stochastic: only a few degrees of freedom are important, movement is stochastic and diffusive, friction dominates inertia, and the combination of external forces and the potential energy surface leads to diffusive currents that couple chemistry to force and movement. In many circumstances, this mechanical picture can be mapped directly onto the standard kinetic view that is more familiar to chemists. Figure 1b shows how this is done. A typical free energy surface will have minima (labeled A, B, and C in Figure 1b) around which the system point tends to fluctuate for long periods of time. These correspond to the recognizable intermediate species in a chemical kinetic mechanism. The "concentration" or population corresponding to each intermediate is proportional to the integral of the probability density in the region surrounding the minimum. The minima are connected to each other by low-energy "passes", and occasionally fluctuations will cause the system point to wander through one of these to a new intermediate state. This amounts to a chemical transition or reaction, and the statistical rate at which such transitions occur is governed by kinetic rate constants. Like the populations, the rate constants can be calculated from the diffusion currents and probability densities of the Smoluchowski equation.⁴ Since the diffusion of the system is affected by external mechanical forces, these calculated rate constants will also depend on external forces. Note that in this approximation all mechanical motions are thermally excited transitions, and so the rate constants contain Boltzmann factors of the form $\exp(-lL/k_B T)$, where L is the mechanical transition distance. However, many mechanical motions cannot always be accurately described by thermally excited processes.⁶⁻⁸ Nonetheless, for most

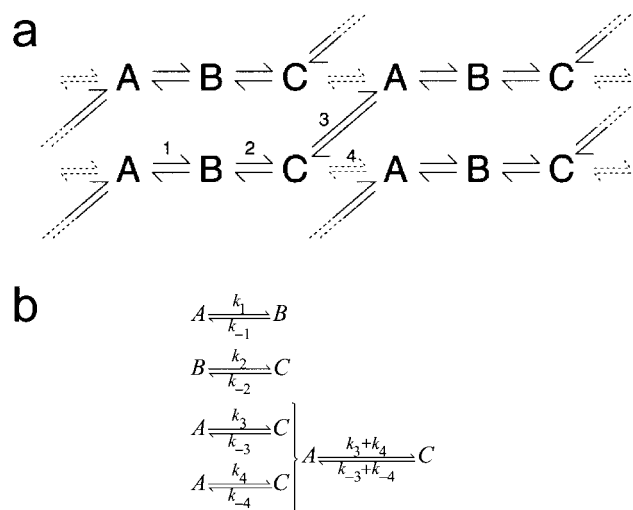


FIGURE 2. (a) Kinetic mechanism equivalent to the potential energy surface in Figure 1. This “mechanism” is a periodic network of chemical states (A, B, C) and transition pathways. The four unique pathways are labeled 1, 2, 3, and 4. Notice that states A and C are connected by two distinct pathways with different kinetic rates and different dependence on external force. (b) Set of chemical equations equivalent to the network in a). Two kinetic equations appear for $A \rightleftharpoons C$, corresponding to the two distinct transition pathways in (a).

applications, it is possible to go in a systematic way from the relatively detailed, mechanical view represented by eqs 1–4 to a more phenomenological, course-grained, chemical kinetic view. The potential energy surface determines the kinetic mechanism, and, conversely, the kinetic mechanism can reveal the main features of the potential energy surface. This equivalence is true for any small molecular system, not just a molecular motor, but two things separate a molecular motor from an ordinary enzyme or chemical system.

(1) Some of the rate constants in the kinetic mechanism depend on the spatial position of the motor. Others may depend on externally applied forces. These rate constants identify the steps where motion and work are produced. The way in which the rate constants depend on external force, and the way they contribute to the overall kinetic mechanism, determine all the crucial properties of the motor: how it responds to a load, its maximum generated force, its maximum velocity, its chemical turnover rate, the number of fuel molecules burned per motor step taken, its efficiency and reversibility, etc.

(2) It is possible for two intermediate species to be connected by several different kinetic pathways. Figure 2a shows the kinetic mechanism corresponding to the potential energy surface in Figure 1. On this diagram, a horizontal transition is a purely chemical process (with no net movement by the motor), and a vertical transition is a purely mechanical movement (with no progress in the chemical reaction). Because the potential energy surface is periodic, the “kinetic mechanism” is also a periodic array of states connected by transition paths. The important point is that pathways 3 and 4 are different. Comparing Figure 2b to Figure 1b, path 3 corresponds to movement between states A and C through the long

trough, and path 4 corresponds to movement between the same two states, but over a steep, narrow barrier. Figure 2b shows the equivalent kinetic mechanism as it is usually written. Because states A and C are connected by two distinct paths, two chemical equations of the form $A \rightleftharpoons C$ appear. These can always be combined into a single equation with total effective rate constants (as shown at right in Figure 2b), but it must then be borne in mind that the combined equation no longer corresponds to a single pathway on the free energy surface.

2.1.1. Tight and Loose Coupling. Finally, notice that path 4 corresponds to a nonproductive chemical turnover, i.e., to a “leak” in the coupling between chemistry and movement. If the rate constants (diffusion currents) along this pathway are significant (compared to the productive path 3), a fraction of the fuel molecules burned will produce no movement. On the other hand, if path 4 is negligibly slow, every chemical turnover must result, sooner or later, in a forward physical step by the motor. The former case is a *loose coupled* motor and the latter a *tight coupled* motor. One practical measure of mechanochemical coupling in a given motor is

$$\chi \equiv \frac{\langle v \rangle}{L \langle r \rangle} \quad (6)$$

where $\langle v \rangle$ is the mean velocity, $\langle r \rangle$ the mean reaction rate, and L the step size (i.e., the periodicity of the track or rotation angle or, in general, of the potential along the position coordinate). If the motor is tight coupled, $L \langle r \rangle$ will equal the mean motor velocity, $\langle v \rangle$, and χ will be close to unity, but if a significant number of chemical steps do not lead to motion (or somehow lead to partial steps), χ will be less than 1. Other definitions are possible, but χ has the advantage that it can be computed from quantities that can be measured experimentally.

2.1.2. Discrete Kinetic Equations. The equivalent of the Smoluchowski equation in the discrete case is a set of first-order or pseudo-first-order rate equations governing the populations of the discrete species. These can be written in the form

$$\frac{\partial \rho}{\partial t} = \mathbf{K} \rho \quad (7)$$

where $(\rho_1, \rho_2, \dots, \rho_n)$ is the vector of populations, one for each discrete species, and

$$\mathbf{K} = \begin{pmatrix} k_{11} & k_{12} & \cdots & k_{1n} \\ k_{21} & k_{22} & & \\ \vdots & \vdots & \ddots & \\ k_{n1} & k_{n2} & \cdots & k_{nn} \end{pmatrix}$$

is a matrix of rate constants or step transition probabilities. For a molecular motor, some of the elements of \mathbf{K} will be functions of the motor position and/or applied force. The condition that all the populations add to 1 (the system must always be in one state or another) means that the sum of all rate equations must be zero, which requires

that the diagonal elements of the rate matrix equal the negative of the sum of all other elements in the same column. Comparing eq 7 with eq 5 above shows that eq 7 is simply a discrete version of the Smoluchowski equation. In fact, both are forms of the master equation, which governs all Markov processes.^{5,9}

2.2. Discrete Chemistry, Continuous Mechanics. The Smoluchowski equation gives a fully continuous description of a molecular motor, and the kinetic equations give a fully discrete description. A “mixed” description is also possible, in which the chemistry is discrete but the mechanics are continuous.

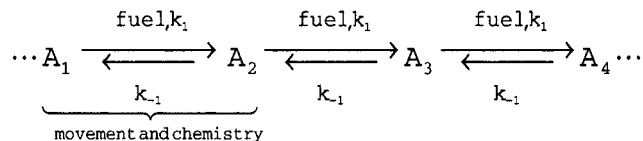
In most cases, physical movements associated with the chemical reaction are much smaller than those associated with motor movements. Moreover, the chemical reaction often proceeds by long pauses in which nothing happens, followed by very rapid transitions to a new chemical state. This is the case, for example, when the motor is waiting for a new fuel molecule to bind or for reaction products to release, or whenever a high, narrow kinetic barrier must be crossed. In these circumstances, little is lost by replacing the continuous reaction coordinate with a discrete “reaction index” that merely labels the intermediate chemical states. A two-dimensional potential energy surface then becomes a series of one-dimensional potentials (suitably averaged over the regions corresponding to each chemical state) connected to each other by phenomenological kinetic transition rates. The system diffuses smoothly along the continuous position variables but jumps discontinuously between the various potential energy surfaces when chemical transitions occur. The model for the F_0 rotary motor presented in section 3, below, is a theory of this type.

All three of the approaches outlined above are simple but well-founded basic methods for describing molecular motors. Many other variants are possible. The simplicity of the mathematics in the discrete cases may lead some to question whether they are appropriate for systems as complex as molecular motors. But as indicated by the development above, each approximation in the process of going from a detailed atomic description to a simple discrete description is understood. It is therefore possible to build simple models that can be justified quite rigorously. The main difficulty is usually in defining the correct model—choosing the important degrees of freedom and their physics—rather than in justifying the final relatively simple mathematical description.

2.2.1. Modes of Mechanochemical Coupling. Apart from the details of particular systems, two general mechanisms have been identified by which chemical energy can be converted into mechanical motion: the power stroke and the Brownian ratchet. The model for the F_0 rotary motor described in section 3, below, is particularly appropriate in this regard, since it includes both a power stroke and a Brownian ratchet within the same overall motor mechanism.

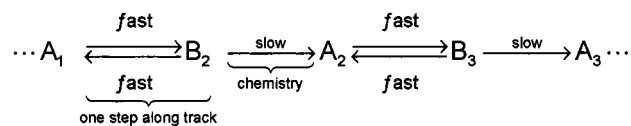
The motor designs most closely resembling a macroscopic engine involve “power strokes”, in which some step of the chemical reaction is mechanically coupled to move-

ment and generation of force by some part of the motor. For example, the chemical reaction can be binding of a substrate molecule, and the movement, the corresponding conformational change in the protein. The surface in Figure 1, with its trough that diverts a chemical step into a movement step, is an example of a power stroke model. The simplest power stroke model is just a series of these troughs, with no purely chemical steps at all:



This example shows that in many ways the power stroke mechanism is the molecular analogue of an inclined plane: a sloping groove in the potential energy surface that converts forces in one direction (along the chemical axis) to forces in another direction (along the position axis). Like an inclined plane, the slope of the trough with respect to the position axis determines the mechanical advantage of the machine. A large slope means that a small chemical force (gradient of the potential with respect to the chemical coordinate) will produce a large (mechanical) output force, that is, a *mechanochemical* advantage greater than 1. But a large slope also implies a small step size (per chemical turnover), so large mechanochemical advantage comes at the expense of small maximum velocity. Conversely, a small slope means a small mechanochemical advantage, but high velocity.

Much attention has been given in recent years to “Brownian ratchet” models for molecular motors.^{7,10–15} The original ratchet model was devised by Feynman,¹⁶ but his example was driven by a temperature gradient and does not apply directly to molecular motors, which always operate in a constant-temperature bath. In a Brownian ratchet, the role of the chemistry is to select forward fluctuations (or prevent backward fluctuations) of the *load*, rather than to apply a mechanical force directly to the load. That is, the load force is driven by its own Brownian fluctuations, and the chemistry provides the energy to rectify the diffusive motion of the load. Though this is a slightly more subtle mechanism than the power stroke, even very simple mechanisms can act as Brownian ratchets. For example, consider the simple model for a DNA polymerase shown in Figure 3. The polymerase molecule is bound at the junction between a region of double-stranded DNA and a region of single-stranded DNA. It can slide rapidly between a “closed” state and an “open” state one step forward. While in the open state, a new nucleotide can bind and be incorporated into the growing strand, thus locking the polymerase one step ahead. This physical model can be described by a two-step kinetic scheme,



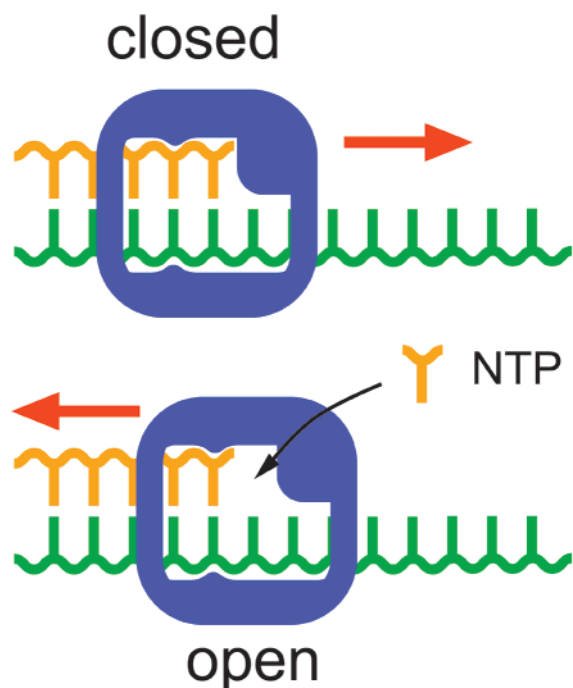


FIGURE 3. Simple Brownian ratchet model. A DNA polymerase enzyme is bound at the junction between double-stranded DNA and single-stranded DNA. It can slide one step forward, opening a space for a new nucleotide, but no farther. Both open and closed states have equal free energy, so, though it can step back and forth under the influence of thermal fluctuations, neither is preferred statistically, and no net mechanical forces push the molecule either forward or backward. Net forward motion occurs because binding of a new nucleotide in the open state prevents the backward step.

where the single chemical step includes both binding and incorporation of the nucleotide into the chain. The potential energy surface for this motor might look much like the surface in Figure 1, except there would be only two potential energy minima per unit cell (A_i and B_i), and the trough would be a purely mechanical transition, i.e., it would be almost vertical, along the position coordinate. From the viewpoint of the basic free energy surface, the difference between a Brownian ratchet and a power stroke may be rather subtle because the motor potential has been combined with the load potential. A power stroke surface always has an “inclined plane” like the trough in Figure 1. A ratchet surface always has a zigzag of pairs of transitions at right angles, one nearly parallel to the chemical axis, one nearly parallel to the position axis. A plot of the potential energy following the low-energy path then resembles a staircase with step height much larger than $k_B T$. The flat regions allow mechanical diffusion along the position axis, and the vertical drops are (nearly) irreversible chemical steps that prevent the load from diffusing backward.

In this example, the motor moves forward because the chemical step is irreversible, i.e., its free energy of reaction is large and negative. This example clearly illustrates that the chemical energy is expended to preferentially select forward steps (or prevent backward steps) and hence to favor forward motion, rather than doing mechanical work on the motor directly. It is also worth noting that a

Brownian ratchet can be tightly coupled and efficient in the sense that each fuel molecule burned results in exactly one step forward.

2.2.2. The Efficiency of Molecular Motors. The *thermodynamic efficiency* is defined only when the motor works against a *conservative* external force, $F = -\partial\psi/\partial x$ (e.g., a laser trap or atomic force microscope). The thermodynamic efficiency can be defined, $\eta_{TD} \equiv FL/(-\Delta G)$, where L is a characteristic spatial distance, usually the “step size” of the motor, and ΔG is the free energy drop per reaction cycle. For a tightly coupled motor, the average velocity is the step size times its average reaction rate, $\langle v \rangle = L\langle r \rangle$, where $\langle r \rangle$ is the average reaction rate (eq 6). In this case, $\eta_{TD} = F\langle v \rangle/(-\Delta G\langle r \rangle)$, where $\langle v \rangle$ is the average motor velocity, and $\langle r \rangle$ is the average reaction rate. Many motors appear to be tightly coupled when they are loaded to near their stall force. This means that, near stall, they have a very high thermodynamic efficiency. A Brownian ratchet can be very efficient near its stall force, although its power output ($F\langle v \rangle$) is small because it moves very slowly; that is, one must wait a long time between steps.

The situation is more subtle when the motor is working only against the viscous load of the fluid medium. It is usually easy to measure the average velocity, $\langle v \rangle$, and from this one can define the *Stokes efficiency* as $\eta_{ST} \equiv \gamma\langle v \rangle^2/(-\Delta G\langle r \rangle)$, where γ is the drag coefficient. It can be shown that $\eta_{ST} \leq 1$, and $\eta_{ST} \approx 1$ means that the motor driving force is nearly constant (i.e., the motor potential has a nearly constant slope).¹⁷ Note that, although the numerator, $\gamma\langle v \rangle^2$ has the units of energy/time, it is *not* the rate of work done on the fluid by the frictional drag due to the motor motion; this is given by $\gamma\langle v \rangle^2$.¹⁷

3. The F_0 Motor of ATP Synthase: A Brownian Ratchet with a Power Stroke

Cells store chemical energy in several ways. The two most common energy repositories are in the phosphate bonds of nucleotides, generally ATP or GTP, and in transmembrane electrochemical gradients. Molecular motors have evolved to use one or the other of these energy sources. Rotary motors, such as the bacterial flagellar motor, use ion gradients, while track motors, such as myosin and kinesin, use nucleotide hydrolysis. However, there is one protein that uses both energy sources. ATP synthase is the ubiquitous enzyme that manufactures ATP. This amazing protein consists of two rotary molecular motors attached to a common shaft, each attempting to rotate in the opposite direction. The F_1 motor uses the free energy of ATP hydrolysis to rotate in one direction, while the F_0 motor uses the energy stored in a transmembrane electrochemical gradient to turn in the opposite direction. Which motor “wins”—that is, develops more torque—depends on cellular conditions. When F_0 wins—the normal situation—it drives the F_1 motor in reverse whereupon it synthesizes ATP from its constituents, ADP and phosphate. When F_1 wins, it hydrolyzes ATP and drives the F_0 motor in reverse, turning it into an ion pump that drives ions across the membrane against the electrochemical

gradient. The mechanochemistry of ATP synthase has been studied in great detail; in-depth treatments, reviews, and references can be found in refs 7, 8, 18–23. Here we will give a simplified account of the mechanochemistry of the sodium-driven F_o motor of *Propionigenium modestum*. This system provides a minimal realistic example of a motor that combines a ratchet mechanism and a power stroke. We refer the reader to the references for a more comprehensive treatment.

A transmembrane electrochemical gradient provides the energy reservoir that the motor will convert into a rotary torque. The thermodynamic measure of this energy gradient is the chemical potential difference (measured in millivolts) between the periplasm (high ion concentration) and the cytoplasm (low ion concentration):

$$\Delta\mu_{\text{Na}^+} = 2.3 \left(\frac{k_B T}{e} \right) \Delta\rho_{\text{Na}^+} + \Delta\psi \quad (\text{mV}) \quad (8)$$

where e is the electronic charge, $\text{pNa}^+ = -\log [\text{Na}^+]$ (the sodium analogue of pH), and $\Delta\psi$ is the transmembrane electrical potential.²⁴

Figure 4A shows the overall geometry of the F_o motor. It consists of two counter-rotating subunits:

- The “rotor” carries 10–12 negatively charged ion-binding sites equally spaced around the periphery, and lying below the level of the membrane. The sites are in equilibrium with the low ion concentration in the cytoplasm.

- The “stator” provides a hydrophobic seal, preventing ions from leaking across the membrane. An aqueous input channel provides access for the sodium ions to bind to the rotor charges. The bottom of the input channel is connected to the cytoplasm by a polar strip that permits the rotor charges to rotate into the input channel. A single positive charge on the stator located close to the strip repels the sodium ions; this prevents them from leaking from the input channel into the cytoplasm.

Figure 4B shows a face-on view of the stator. The only way for an ion to pass through the stator is for it to bind a rotor site at the bottom of the input channel. This neutralizes the site sufficiently for it to rotate to the left through the hydrophobic interface. Once exposed to the low concentration in the cytoplasm, the bound ion quickly dissociates. However, a bound site is not completely neutral but forms a dipole. If the bound site moves to the right, the positive stator charge presents a high electrostatic barrier that forces the ion to dissociate back into the input channel if the site comes too close.

Given this structure, how does the rotor–stator assembly convert the electrochemical gradient into a rotary torque? First, the chemistry. For simplicity, we assume that the stator dimensions are such that only one rotor site is in the rotor–stator interface at a time. During the transit through the interface, only one reaction takes place: the binding and dissociation of sodium ions to the rotor sites. Let us follow one rotor site as it passes through the stator. We can characterize the state of the rotor site by giving its probability of being unoccupied (0) or occupied (1), i.e., binding a sodium ion. Denote the probability densities

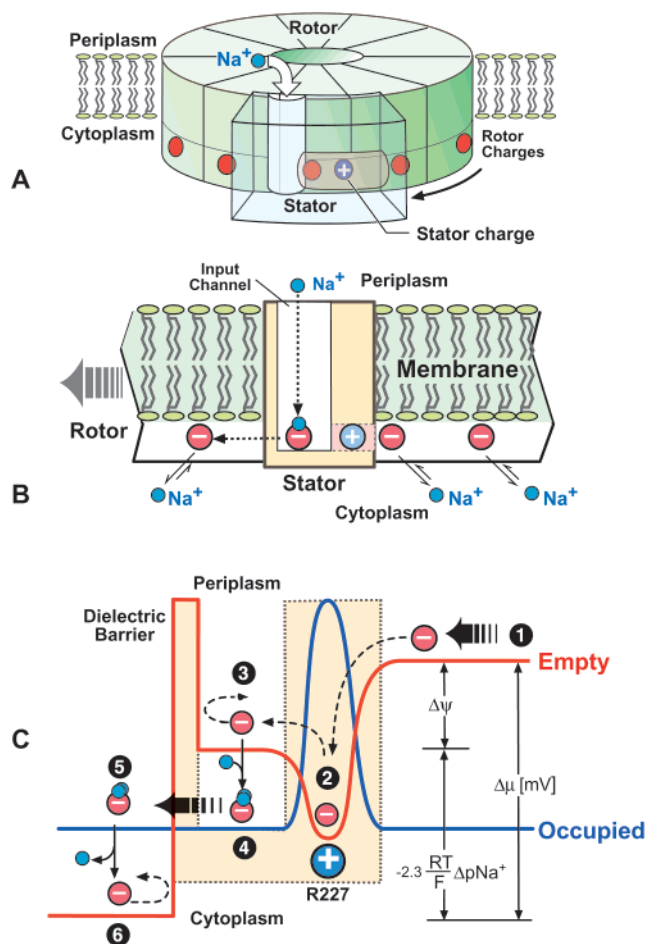


FIGURE 4. (A) Simplified geometry of the sodium-driven F_o motor.¹⁸ (B) Face-on view of the rotor–stator assembly showing the path of ions through the stator. (C) Free energy diagram of one rotor site as it passes through the rotor–stator interface. An empty site sees the red potential, while an occupied site sees the blue potential. Binding of an ion switches between the two potentials. Step 1 → 2: the rotor diffuses to the left, bringing the empty (negatively charged) site into the attractive field of the positive stator charge. Step 2 → 3: once the site is captured, the membrane potential biases the thermal escape of the site to the left (by tilting the potential and lowering the left edge). Step 3 → 4: the site quickly picks up an ion from the input channel; this switches the site to the blue potential. Step 4 → 5: an occupied site, being nearly electrically neutral, can pass through the dielectric barrier. If the occupied site diffuses to the right, the ion quickly dissociates back into the input channel as it approaches the stator charge. Step 5 → 6: upon exiting the stator, the site quickly loses its sodium ion. The empty (charged) site once again sees the stator dielectric barrier, which prevents the rotor from diffusing backward. The cycle decreases the free energy of the system by an amount equal to the electromotive force (eq 3).

Ⓜ An animated image in QuickTime format is available.

of each state by $\rho(\theta, \tau) = (\rho_0, \rho_1)$; then the reaction can be described by a Markov model analogous to eq 7:

$$\frac{d}{dt} \begin{pmatrix} \rho_0 \\ \rho_1 \end{pmatrix} = \mathbf{K}(\theta) \begin{pmatrix} \rho_0 \\ \rho_1 \end{pmatrix} \quad (9)$$

where $\mathbf{K}(\theta)$ is the matrix of binding and dissociation rate constants in the input channel. Note that the probabilities

and transition rates depend on the angular position of the site, since binding can occur only when the site is exposed to the input channel.

Next, we formulate the mechanical equations of motion. The rotary motion of the rotor is driven by the torque developed at the rotor–stator interface. This torque originates from three interactions:

- The Coulomb interaction between the rotor site and the fixed stator charge, which depends on the rotor occupancy: $\phi_Q(\theta, \mathbf{s})$, where $\mathbf{s} = (0, 1)$ denotes the occupancy state of the site.

- The dielectric barrier, $\phi_{\Delta\epsilon}(\theta, \mathbf{s})$, that prevents unoccupied rotor sites from passing through the rotor. The height of the hydrophobic barrier is given approximately by $200[(1/\epsilon_1) - (1/\epsilon_2)] \approx 45k_B T = 27 \text{ kcal/mol}$, where ϵ_1 and ϵ_2 are the dielectric constants of the aqueous channel and the rotor–stator interface, respectively.²⁵

- The membrane potential, $\phi_M(\theta, \mathbf{s})$, that tilts the Coulomb interaction potential.

Therefore, the motor torque, τ_M , can be written in terms of a potential function, Φ , as

$$\tau_M(\theta, \mathbf{s}) = - \frac{\partial \Phi(\theta, \mathbf{s})}{\partial \theta} = - \frac{\partial}{\partial \theta} (\underbrace{\phi_Q(\theta, \mathbf{s})}_{\text{charge interaction}} + \underbrace{\phi_{\Delta\epsilon}(\theta, \mathbf{s})}_{\text{dielectric barrier}} + \underbrace{\phi_M(\theta, \mathbf{s})}_{\text{membrane potential}}) \quad (10)$$

Thus, the Langevin equation governing the rotor motion is

$$\underbrace{\zeta \frac{d\theta}{dt}}_{\text{frictional drag}} = \underbrace{\tau_M(\theta, \mathbf{s})}_{\text{motor torque}} - \underbrace{\tau_L(\theta)}_{\text{load torque}} + \underbrace{\tau_B(\theta)}_{\text{Brownian torque}} \quad (11)$$

$\mathbf{s} = (0, 1)$
chemical states

Or, in the Smoluchowski formulation:

$$\frac{\partial \rho}{\partial t} = \underbrace{\frac{1}{\zeta} \frac{\partial}{\partial \theta} (\Phi'(\theta, \mathbf{s}) \rho)}_{\text{force between rotor and stator}} + \underbrace{\frac{1}{\zeta} \frac{\partial}{\partial \theta} (\tau_L(\theta) \rho)}_{\text{load torque}} + \underbrace{D \frac{\partial^2 \rho}{\partial \theta^2}}_{\text{Brownian motion}} + \underbrace{\mathbf{K}(\theta) \rho}_{\text{transitions between rotor states}} \quad (12)$$

$$\rho(\theta, t) = \begin{pmatrix} \rho_0(\theta, t) \\ \rho_1(\theta, t) \end{pmatrix}$$

where Φ is the rotor–stator interaction potential and \mathbf{K} is the matrix of transition rates between chemical states.¹⁹ Single-molecule experiments are generally designed to measure the load–velocity relationship, which characterizes the mechanical behavior of the motor. Equations 9 and 12 can be solved numerically to yield the load–velocity curve shown in Figure 5. The motor develops its torque by using electrostatic forces and the transmembrane concentration difference to rectify the rotor’s Brownian fluctuations so that its diffusion is biased to the left. Ions bind quickly to the rotor sites in the input

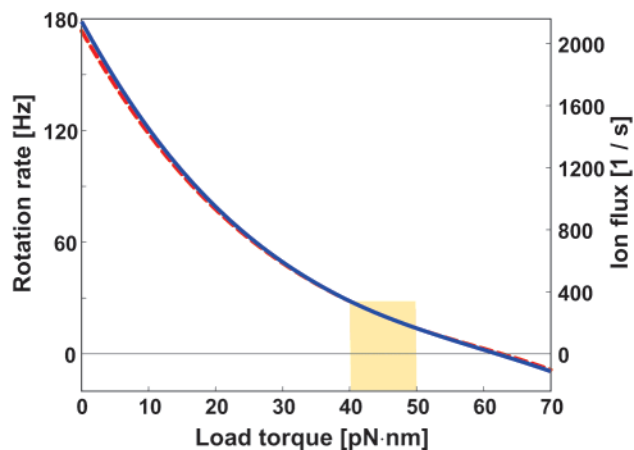


FIGURE 5. Load–velocity curve computed for the F_0 motor from eqs 3 and 2 for the case when the stator can accommodate two rotor sites. The ion flux is shown on the right-hand axis. It is nearly superimposed on the velocity curve, indicating that the motor is almost tightly coupled: $\langle d\theta/dt \rangle \propto \langle \dot{r} \rangle$.

channel and dissociate quickly once the rotor has passed the dielectric barrier and entered the cytoplasmic reservoir. The binding–dissociation reaction switches the electrostatic potential seen by the rotor sites so that its diffusion to the left is rectified. Note that without the membrane potential, the motor would be a pure Brownian ratchet: the motion of the rotor would be driven only by its own diffusion. By tilting the electrostatic potential, the membrane potential provides a unidirectional force to the left on the rotor, biasing the escape of the rotor site from the grip of the stator charge. The caption to Figure 4C gives a heuristic description of the motor cycle.

If the stator is wide enough to accommodate two rotor sites, then the stator charge can supply an additional power stroke to assist the ratchet mechanism. (In this case, there are $2^4 = 16$ states.¹⁹) A typical sequence of events that advance the rotor by one step of $2\pi/12$ is as follows. An empty rotor site fluctuates into the rotor–stator interface, where it is captured by the electrostatic attraction of the stator charge. The rotor diffuses in the well until it eventually escapes. This escape is biased to the left by the transmembrane potential and is helped by the dielectric barrier, preventing the empty rotor site to the left of the stator from re-entering the low-dielectric medium of the stator. Once out of the potential well of the stator charge, the rotor site quickly binds a sodium ion from the input reservoir. Now neutral, it can pass through the dielectric barrier when the stator charge pulls the next rotor site into its potential. When the rotor site passes out of the stator, its sodium ion quickly dissociates into the cytoplasmic reservoir. Once empty, it cannot go back into the low-dielectric rotor–stator interface. This is the ratchet step. Note that the ion flux in Figure 5 can be scaled to be congruent to the velocity. This means that the motor is tightly coupled: $\langle d\theta/dt \rangle \propto \text{ion flux}$, which in turn is proportional to the reaction rate. That is, on average, one ion passes through the rotor for every step of $L = 2\pi/12$.

This example demonstrates how a protein can extract energy from an electrochemical gradient and convert it into a mechanical force. The other large class of protein motors that employ nucleotide hydrolysis operate on a completely different principle. These motors use the binding energy of the fuel molecule to the enzyme to produce mechanical force, and they use the hydrolysis reaction to weaken the binding between the enzyme and products so that they can be released and the cycle can repeat. A detailed analysis of this process is reviewed in ref 8.

4. Summary

The fundamental distinction between chemistry and motor mechanics is one of scale: chemical reactions proceed by means of small, rapid movements on the atomic scale, while motors proceed by means of larger, slower movements on the molecular scale. Both are strongly influenced by the presence of thermal fluctuations and in most cases make use of the fluctuations in their mechanisms.

In building a detailed physical model, the question is not so much how to wed chemistry and mechanics as to find a mechanism by which the small, rapid, stochastic motions and the energy derived from chemical reactions can produce the larger, slower forces and movements of the motor. Because of the great variety in protein geometry, there are many ways this can be done.

Molecular motors' operation can be conveniently visualized as stochastic motion on a potential energy surface. Most motor models proposed recently make use of one variant or another of the stochastic formalism outlined above. The key elements are the identity of the important degrees of freedom—that is, molecular geometry—and some knowledge of the important intermediate states and the rates of transition between them. In principle, these could be determined by detailed atomic-scale simulations, but this is usually not practical.

Finally, using a theoretical understanding of motor mechanisms combined with numerous examples of biological molecular motors, it is possible, in principle, to design new motors of our own. The main problem with such “nano-motor engineering” is not the basic principles but the more pedestrian difficulties of protein engineering. A motor requires specific interactions between its parts and a catalytic interaction with its fuel molecule. These necessitate precise control over the final structure of the motor, and this cannot yet be designed de novo with present technology. Eons of evolution remains the best protein engineer. One way to avoid this difficulty might be to assemble new motors from pre-existing “unit machines”—protein domains with known structure and behavior that can be combined without much change in their properties. Then all that we have learned, both theoretically and experimentally, about microscopic motors can be brought to bear. It seems likely that many new motors, and even basic motor mechanisms, could be built by this approach.

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References

- (1) Vale, R. D.; Milligan, R. A. The way things move: looking under the hood of molecular motor proteins. *Science* **2000**, *288*, 88–95.
- (2) In reality, ATP is not a particularly “high-energy” compound, but its phosphate transfer potential is *intermediate*, so it is a fairly good donor and acceptor. This makes it a good fuel molecule. In reactions involving ATP, the “high energy” is often supplied by ATP *binding*. Hydrolysis allows the products, phosphate and ADP, to be released so the cycle can repeat. In the case of spasmoneme and actin polymerization, it is also the binding reaction that provides the energy to ratchet up the stored elastic energy.
- (3) Mahadevan, L.; Matsudaira, P. Motility powered by supramolecular springs and ratchets. *Science* **2000**, *88*, 95–100.
- (4) Keller, D.; Bustamante, C. The mechanochemistry of molecular motors. *Biophys. J.* **2000**, *v78*, 541–556.
- (5) Risken, H. *The Fokker–Planck Equation*, 2nd ed.; Springer-Verlag: New York, 1989.
- (6) Elston, T. C.; Oster, G. Protein turbines. I: The bacterial flagellar motor. *Biophys. J.* **1997**, *73*, 703–721.
- (7) Elston, T.; Wang, H.; Oster, G. Energy transduction in ATP synthase. *Nature* **1998**, *391*, 510–513.
- (8) Oster, G.; Wang, H. Reverse engineering a protein: the mechanochemistry of ATP synthase. *Biochim. Biophys. Acta* **2000**, *1458*, 482–510.
- (9) Oster, G.; Wang, H. *Stochastic Processes in Physics and Chemistry*; North Holland: Amsterdam, 1992.
- (10) Oster, G.; Peskin, C. The speed of a Brownian Ratchet. In *Theoretical and Experimental Advances in Pattern Formation*; Maini, P. K., Murray, J. D., Othmer, H. G., Eds.; Plenum Press: New York, 1993; pp 267–276.
- (11) Simon, S.; Peskin, C.; Oster, G. What drives the translocation of proteins? *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 3770–3774.
- (12) Peskin, C. S.; Odell, G. M.; Oster, G. Cellular motions and thermal fluctuations: the Brownian ratchet. *Biophys. J.* **1993**, *65*, 316–324.
- (13) Wang, H. Y.; Elston, T.; Mogilner, A.; Oster, G. Force generation in RNA polymerase. *Biophys. J.* **1998**, *74*, 1186–1202.
- (14) Jülicher, F.; Bruinsma, R. Motion of RNA polymerase along DNA: a stochastic model. *Biophys. J.* **1998**, *74*, 1169–1185.
- (15) Derényi, I.; Vicsek, T. The kinesin walk: a dynamic model with elastically coupled heads. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 6775–6779.
- (16) Feynman, R.; Leighton, R.; Sands, M. *The Feynman Lectures on Physics*; Addison-Wesley: Reading, MA, 1963.
- (17) Wang, H.; Oster, G. The efficiency of molecular motors. *Eur. J. Phys.* **2001**, in press.
- (18) Wang, H.; Oster, G. Energy transduction in the F1 motor of ATP synthase. *Nature* **1998**, *396*, 279–282.
- (19) Dimroth, P.; Wang, H.; Grabe, M.; Oster, G. Energy transduction in the sodium F-ATPase of *Propionigenium modestum*. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 4924–4929.
- (20) Oster, G.; Wang, H. ATP Synthase: The rotary molecular motors working together. In *Encyclopedia of Molecular Biology*; Creighton, T., Ed.; Wiley: New York, 1999.
- (21) Oster, G.; Wang, H. ATP synthase: two motors, two fuels. *Structure* **1999**, *7* (4), R67–R72.
- (22) Oster, G.; Wang, H.; Grabe, M. How Fo-ATPase generates rotary torque. *Philos. Trans. R. Soc. London, Ser. B: Biol. Sci.* **2000**, *355*, 523–528.
- (23) Dimroth, P.; Matthey, U.; Kaim, G. The motor of the ATP synthase. *Biochim. Biophys. Acta* **2000**, *1459*, 506–513.
- (24) Equation 7 is an equilibrium relation that implies that the concentration and electrical potential differences are energetically equivalent. This is not true in the nonequilibrium regime where the motor operates.
- (25) Israelachvili, J. *Intermolecular and Surface Forces*, 2nd ed.; Academic Press: New York, 1992.

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