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Enzyme as catalytic wheel powered by a Markovian engine: conformational coupling and barrier surfing models

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Abstract

We examine a typical Michaelis–Menten Enzyme (MME) and redress it to form a transducer of free energy, and electric, acoustic, or other types of energy. This amendment and extension is necessary in lieu of recent experiments in which enzymes are shown to perform pump, motor, and locomotion functions resembling their macroscopic counterparts. Classical textbook depicts enzyme, or an MME, as biocatalyst which can enhance the rate of a chemical reaction by lowering the activation barrier but cannot shift the thermodynamic equilibrium of the biochemical reaction. An energy transducer, on the other hand, must also be able to harvest, store, or divert energy and in doing so alter the chemical equilibrium, change the energy form, fuel an energy consuming process, or perform all these functions stepwise in one catalytic turnover. The catalytic wheel presented in this communication is both a catalyst and an energy transducer and can perform all these tasks with ease. A Conformational Coupling Model for the rotary motors and a Barrier Surfing Model for the track-guided stepping motors and transporters, are presented and compared. It is shown that the core engine of the catalytic wheel, or a Brownian motor, is a Markovian engine. It remains to be seen if this core engine is

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the basic mechanism for a wide variety of bio-molecular energy transducers, as well as certain other dynamic systems, for example, the Parrondo's Games.
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1. Introduction

Energy dissipates in a spontaneous process. Any order vanishes with time. Life must evolve against this odd. For a biological cell to survive under such conditions and yet sustain its life activities must be able to extract energy from its surrounding and couple it to complex biochemical reactions to attain off-equilibrium steady-state, in which energy flow is controlled and dissipation is checked to its own best advantage. A class of molecules, enzymes, performs such tasks. They are mostly made of proteins, but some RNAs or DNAs have limited activity. In 1913 Michaelis and Menten proposed a mechanism of enzyme action which has since become the standard model for explaining simple, as well as complex kinetics of the biocatalytic reactions [1,2].

2. Michaelis–Menten Enzyme (MME)

Let us review some essential features of a MME which are relevant to our discussion. Here an enzyme is treated as a mere catalyst. The simplest biochemical reaction involving the conversion of a substrate (S) to product (P), e.g. urea to ammonia and carboxylic acid, respectively, is

$$S \xrightarrow{k} P\,. \tag{1}$$

The velocity or rate of the product formation is given by

$$v = \frac{\delta C_P}{\delta t} = -\frac{\delta C_S}{\delta t} = kC_S\,. \tag{2}$$

Here k is the rate constant, and the concentration of S and P are given in C_S and C_P, respectively. This relationship assumes either that the product is rapidly defused, sequestered, or converted to other chemical form, or that the reverse reaction, P to S, is much slower than the forward reaction, S to P.

Most un-catalyzed biochemical reactions are slow and inadequate for supporting the activity of life. When an enzyme is present, the rate of a reaction is greatly enhanced, often by several orders of magnitude or higher. By what mechanism can an enzyme achieve such efficiency? The MME model assumes that an enzyme (E) has the affinity for and can bind the substrate to become an enzyme–substrate complex (ES), and the bound substrate is then converted to the product by certain surface

reaction, and subsequently released. The rate of the catalytic reaction is limited by the rate of the product release. Because of special Key–Lock relationship and surface activation mechanism, an enzyme can substantially reduce the activation barrier that prevented a rapid conversion of S to P [1,2]. Once S is converted to P it is released and the enzyme turnovers and recycles. The second step in the reaction is the rate-limiting step

$$S + E \underset{k_d}{\overset{k_a}{\rightleftharpoons}} ES \xrightarrow{k_{cat}} P + E. \tag{3}$$

Here k_a is the association rate of E and S, k_d, the dissociation rate of ES, and k_{cat}, the catalytic rate of the enzyme. The catalytic power is defined as the ratio k_{cat}/k, which, for the decarboxylation of orotidine monophosphate by its decarboxylase, has a value of 3×10^{17} [1]. Other refined MME models, for example, the induced-fit model of the substrate to product considers the dynamics of the protein molecule but the basic construct of the MME remains unchanged [2]. Where more complex systems or cascades of enzymatic reactions are concerned catalytic network, each consisting of several simple MME mechanism, is often constructed to solve the overall kinetics.

3. Enzyme as energy transducer, not mere rate enhancer

An enzyme catalyzed reaction can be very fast and difficult to handle except with advanced instrumentation. In 1925 Briggs and Haldane proposed that by choosing the condition, $C_E \ll C_S$ to study the speed of the product formation

$$\frac{\delta C_P}{\delta t} = k_{cat} C_{ES} \tag{4}$$

can be greatly reduced and the catalytic reaction would also reach a steady state. Classical kinetic study of enzyme action all adopted such a strategy. Note that under such conditions C_E is negligibly low and so is C_{ES} compared to C_S or to C_P and the free energy of the catalyzed reaction

$$\Delta G_{cat} = \Delta G_o - RT \ln\left(\frac{C_P}{C_S - C_{ES}}\right) \tag{5}$$

is indistinguishable from the free energy of the un-catalyzed reaction

$$\Delta G = \Delta G_o - RT \ln\left(\frac{C_P}{C_S}\right). \tag{6}$$

Ever since, standard biochemistry textbook depicts an enzyme as a biological catalyst, which does not alter the chemical equilibrium of a biochemical reaction. The effect of an enzyme on a reaction is purely kinetic, i.e., an enzyme simply enhances the forward S to P conversion rate [1,2].

How does an enzyme enhance the rate? According to the transition state theory the rate of a chemical reaction in the absence of a kinetic barrier is simply $k_B T/h$, in

which k_B, T, and h are, respectively, the Boltzmann constant, Kelvin temperature, and the Planck constant. At 25 °C this term has a value of $6.25 \times 10^{12}\,\mathrm{s}^{-1}$, which is the frequency of a critical vibrational mode. The rate of a chemical reaction slows down if the reactants must cross over an activation barrier

$$k = (k_B T/h)\exp\left(\frac{-G^*}{RT}\right). \tag{7}$$

Most biochemical reactions have a non-zero G^* and k is thus smaller than $k_B T/h$. Fig. 1 illustrates this enzymatic effect. For example, the decarboxylation of orotidine monophosphate, we have just mentioned, the half-time of the reaction is 78 million years. The G^* of the reaction is thus, $65\,k_B T$. The decarboxylase enhances the rate by 10^{17} fold or reduces the activation barrier by $39\,k_B T$.

We now know that in the cytoplasm of a cell, the concentrations of substrate, product and enzyme vary widely and in most cases, they are similar and in the mM range. Under such conditions, $\Delta G_{cat} \neq \Delta G$. Furthermore, an enzyme in a cell does not act alone. Holistically, it is in constant on-and-off interactions with other molecules. Therefore, its action cannot be described by a classical enzyme model that accounts only for the simple catalytic activity. The ATPase activity in a myosin head group, S1, for example, cannot be treated as a simple sequence of substrate–enzyme–product reaction. An S1 interacts with an actin filament in cyclic yet stochastic fashion and during each catalytic cycle the ATPase in the S1 inevitably performs a variety of roles that comprise the power stroke or the locomotive function of the muscle contraction cycle [3,4]. In the following pages we will present two prototypes of the catalytic wheel that by the formulation of Brownian motor can perform the task of an energy transducer with amazing effectiveness and efficacy. A Markovian

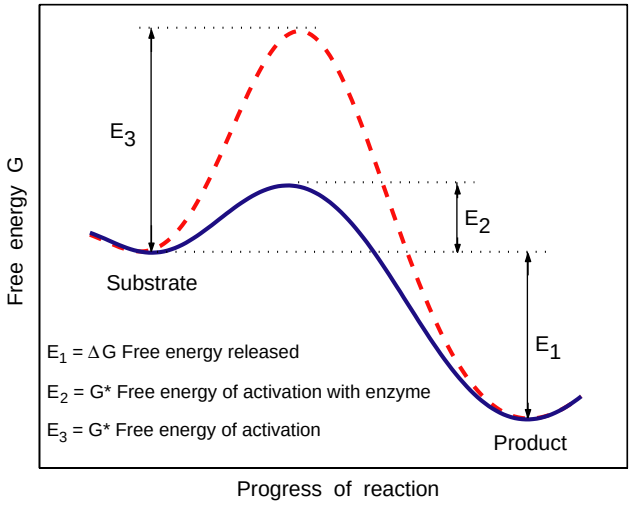

Fig. 1. Reaction profile showing large activation energy G^* (broken curve) for the $S \rightarrow P$ reaction; the enzyme catalyst lowers the G^* (dark curve), thereby accelerating the rate.

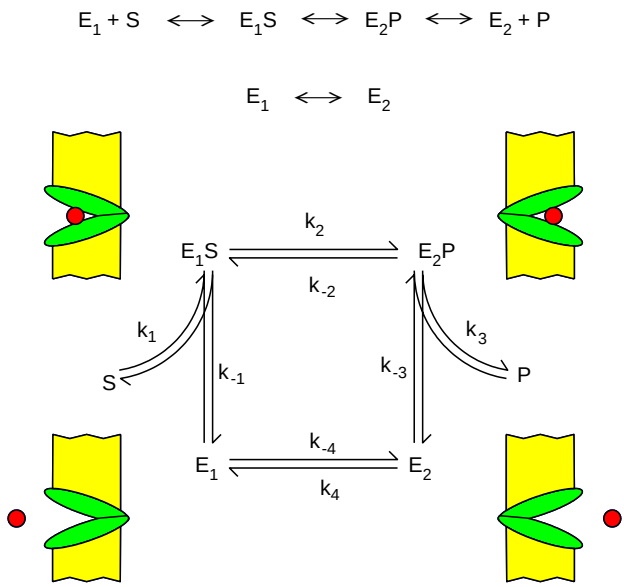

Fig. 2. MME as catalytic wheel. The two chemical equations at the top of the figure represent the complete catalytic process of the MME mechanism. The enzyme binds a substrate and the substrate is converted to the product and released. The enzyme then recycles, or turns over. The enzyme has two conformational states. E_1 favors binding of the substrate while E_2 is the form that associates with the product. In general, the rate limiting step is the product dissociation. In this figure a membrane associated MME is shown. Because the enzyme turns over in each catalytic cycle, it is better treated as a catalytic wheel. The wheel turns clockwise if the ΔG_{cat} is negative. Conversely, it turns counterclockwise if ΔG_{cat} is positive.

engine constitutes its core mechanism. Figs. 2 and 3 illustrate essential features of the catalytic wheel.

4. Catalytic wheel type I: conformational coupling model, or the rotary motor

An ion or molecular channel requires the ability to select ion or molecule for diffusive passage. The direction of flux is downhill, i.e., the flux is driven by the chemical potential of the ion or molecule. In contrast, in an ion or molecular pump not only the specificity is preserved, the transport flux is also uphill. Thus, the pump requires, in addition, the ability to convert and transfer energy from one form to another. For example, in the plasma membrane of biological cells, there exists a Na,K-Pump which can transport K^+ from the external medium to the cytoplasm and simultaneously Na^+ from the cytoplasm to the external medium [5,6]. Under the physiological condition of the human red blood cells, each ion must overcome a concentration gradient of 10–15 folds. Thus, an input of approximately $2.3\,k_BT$ per

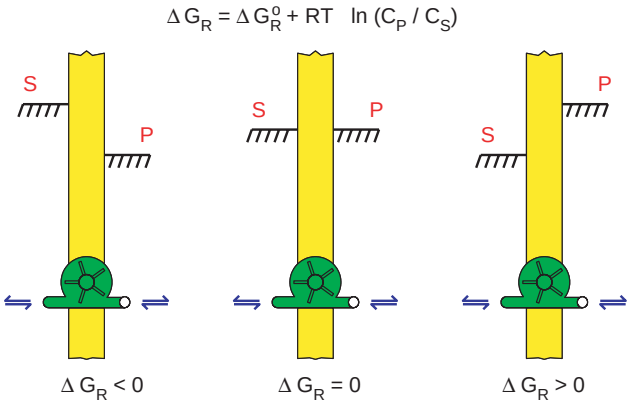

Fig. 3. Enzyme as analog of motor. A MME exhibits many characteristics of a motor. In the first case, the enzyme is fueled by the free energy of the substrate to product conversion. The wheel of Fig. 2 spins clockwise. In the second case, the free energy levels on both sides of the partition are equal and there will be no net spin of the wheel. However, the conversion still takes place dynamically in both ways. Net changes in the level of product or substrate will not occur. In the third case, the free energy level on the product side is higher, *P* is converted to *S*, reversely. In a cell case 3 is a common occurrence. To convert *S* into *P* the enzyme must be fueled by an input of energy. F_oF_1ATPase may hydrolyze ATP to pump proton uphill, or conversely, use the proton electrochemical gradient energy to synthesize ATP. The enzyme is a reversible molecular motor, which uses an external energy source to spin the wheel counterclockwise.

ion is required. The enzyme pumps 3 Na^+ out of and 2 K^+ into the cytoplasm on each ATP hydrolyzed. The hydrolysis of an ATP to ADP and P_i (inorganic phosphate) provides $15\,k_BT$ of energy. The efficiency of energy conversion is roughly 75%. By its pump activity Na,K-ATPase maintains the cytoplasmic ionic environment for the cellular reactions, a steady-state negative transmembrane electric potential, and an osmotic pressure that regulates the cell volume. The ATP hydrolysis site and the pump sites involve different functional groups and energy coupling must take place within the protein molecule. The biochemical process of this energy coupling has been worked out to certain details [5,6]. An amazing feature among others is the electrogenic nature of the pump. The asymmetry of the charge transport produces the deficit of cations in the cytoplasm and the establishment of a negative electric potential inside the plasma membrane. There are many consequences of this electrogenic pumping one of which serves as the basis of the electroconformational coupling (ECC) model [7–10].

In the catalytic wheel of Fig. 2, Na,K-ATPase is shown to have two functionally relevant conformational states E_1 and E_2 and their conversion into or out of a specific state is controlled by ATP-dependent phosphorylation–dephosphorylation reactions. However, the chemical equilibrium between E_1 and E_2 is regulated by the transmembrane electric potential. This latter property affords us to perform electric activation experiment with the pump [11–15]. Work with periodic electric field and analysis of data has been published elsewhere [7,8,13–17]. The discussion here limits

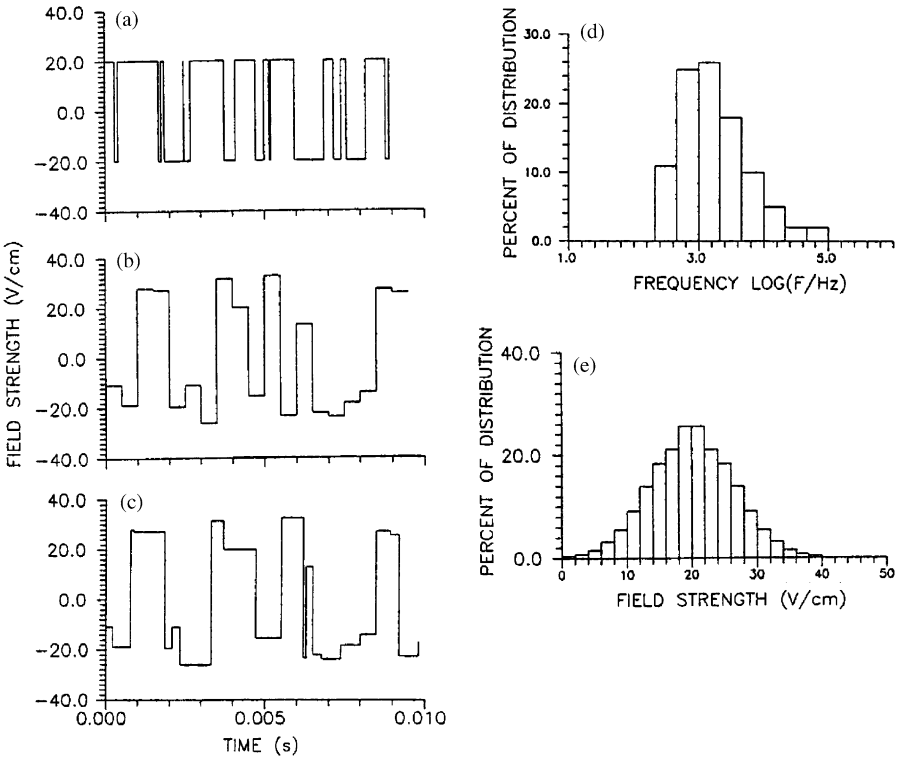

Fig. 4. Typical waveforms of electric fields used in the experiment. (a) A random telegraph function (RTF) with a constant amplitude (±20 V/cm) and a fluctuating lifetime. (b) A waveform with a constant lifetime (1 kHz) and a fluctuating Gaussian-distributed amplitude. (c) A RTF with fluctuating amplitude and lifetime. (d) A histogram of frequency distribution for waveforms shown in (a) and (c). (e) Histogram of Gaussian amplitude distribution, with $\sigma = 10$ V/cm, for the waveforms shown in (b) and (c). See Ref. [14] for details.

the scope to those experiments that are done with stochastic electric field (Fig. 4). Specifically, random telegraph electric pulses have been applied to the suspension of the human red blood cell which activates Na,K-ATPase and produces the influx of Rb^+ (a K^+ replacement) and the efflux of Na^+. A systematic study shows that the voltage stimulated pump activity can be completely suppressed with ouabain, a highly specific inhibitor of the Na,K-ATPase. Furthermore, ATP hydrolysis is not required for this transport activity. In other word, when the conformational state of the enzyme is forced to fluctuate between E_1 and E_2 it can extract energy from the applied electric field to pump cations uphill. Experiment has found that the voltage induced Rb^+ or K^+ pumping is dependent on amplitude, frequency, and waveform of the pulsed electric field. So is the Na^+ pumping. The optimal amplitude of the bipolar electric field for the two pumps is identical at $20\,\mathrm{V\,cm^{-1}}$.

The thermodynamic relationship governing the electric field distribution of a sphere of radius R_{cell}, made of non-conducting shell of thickness d, suspended in a conducting medium is

$$\phi_m(t) = 1.5 R_{cell}\mathbf{e}(t)/d\ , \tag{8}$$

where $\phi_m(t)$ is the effective electric field across the cell membrane and $\mathbf{e}(t)$ is the applied electric field [8,9]. This field strength $20\,\mathrm{V\,cm^{-1}}$ can cause a membrane potential fluctuation of $\pm 25\,\mathrm{mV}$, which corresponds to a transmembrane fluctuation of $\pm 5 \times 10^6\,\mathrm{V\,m^{-1}}$ of effective electric field when d is taken to be 5 nm. The optimal mean frequency is 1.0 kHz for the Rb^+ pump and 1.0 MHz for the Na^+ pump. The waveforms shown in Fig. 4 are all effective for the activation of the Na^+- and the K^+-pumps.

The basic premise of the ECC model is that E_1 and E_2 exhibit different values of electric moments ($\mathbf{m}$) which is the sum of charge separations ($z_i d_i$), dipole moments (μ_i), and induced dipoles ($\alpha\phi_m(t)$, α being the electric susceptibility of the structure) in the molecule. For the enzyme in E_1 state it is expressed as

$$\mathbf{m}_1 = \sum (z_{1,i} d_{1,i} + \mu_{1,i}) + \alpha_1 \phi_m(t)\ . \tag{9}$$

Similar situation applies to enzyme in the E_2 state. The fact that an electric field ($\mathbf{e}$) can shift the chemical equilibrium between the two states implies that the molar electric moments of the two states are different and their net difference is

$$\Delta\mathbf{m} = \mathbf{m}_2 - \mathbf{m}_1\ . \tag{10}$$

The thermodynamic relationship that governs the interaction of an electric field with the system is

$$K^e_{ij}(t) = \frac{C^e_{ij}(t)}{C^e_{ji}(t)} = K^0_{ij}[N_{av}\Delta\mathbf{m}\cdot\phi(t)/RT]\ . \tag{11}$$

Here K^0_{ij} is the equilibrium constant for $i \to j$ step in the absence of the applied field and $K^e_{ij}(t)$ is the concentration ratio in the presence of the applied field and N_{av} is the Avogadro number. Note that $\mathbf{m}$ and $\phi(t)$ are vector quantities and their dot product is equivalent to the free energy of the electric interaction with the system, $-\Delta G_e$. Because $\phi(t)$ fluctuates with respect to time ΔG_e also fluctuates with respect to time. As shown in Fig. 2, the enzyme is embedded in a cell membrane and the degree of freedom is severely restricted. In the absence of rapid tumbling of the protein molecule, its molar electric moment $\mathbf{m}_i$ is anisotropic, pointing normal to the membrane. Previously, this catalytic wheel has been shown, in great detail, to harvest electric energy to pump the ligand uphill by the ECC mechanism [7–10,13–17]. The essence of the ECC is reiterated in Fig. 5. The potential energy profile shown for the E_1 state allows a rapid loading of a substrate or ligand approaching from the left reservoir of the membrane and conversely the potential energy profile shown for the E_2 state allows unloading of the product or ligand into the right reservoir. From this figure one might expect any conformational fluctuation between E_1 and E_2 would lead to directional flow of energy or pumping of the ligand rightward. The leftward transport would be prevented because of the asymmetry of the potential profiles built

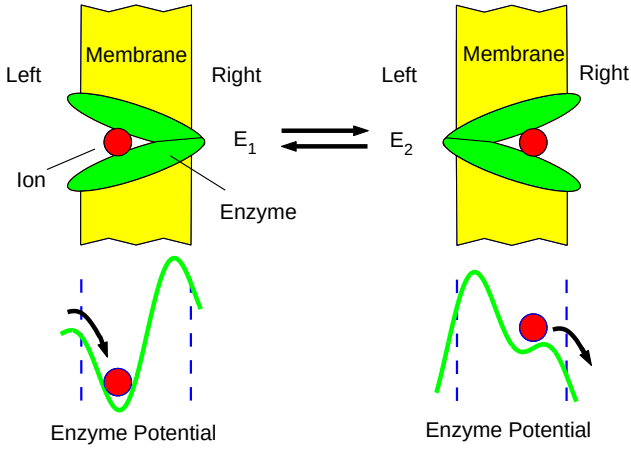

Fig. 5. Brownian motor and Brownian ratchet. The MME enzyme of Fig. 2 will behave like a Brownian motor. The E_1 to E_2 transition and vice versa can be effectuated by the binding of S and P and the binding of S and P is stochastic. In the experiment discussed in the main text, the E_1 to E_2 transition is induced by an applied electric field. The electric field-enforced conformational oscillation or fluctuation induces the pumping of a ligand from the left compartment to the right when the activation barriers of the ligand and protein interactions are as shown. An enzyme or molecular pump is constructed to pump a ligand leftward or rightward by the design of the chemical activation barriers on the two compartments. Ratchet analysis of a Brownian motor may simplify mathematics as shown in Refs. [19,20].

into the system. If this concept were carried to an extreme any ambient thermal noise or thermal electric noise would propel the ligand rightward. The system so constructed would have all the property of the perpetuum mobile of the second kind. This is not to be the case when detailed balance and reversibility are strictly observed [8,9]. Rigorous analysis indicates that only conformational fluctuation induced by off equilibrium thermal or electric noise can perform directional pumping of the ligand.

A pump that directs the ligand leftward by the same mechanism can also be easily constructed by reversing the potential profiles shown in this figure. Our analysis indicates that the directional flow of energy is an inherent property of the symmetry breaking, which is built into the two interaction potentials, one for S and E_1 and the other for P and E_2.

5. Catalytic wheel type II: barrier surfing model, or the track-guided stepping motor

Note that the basic construction of Fig. 5 is based on Eq. (7) and the simple cyclic kinetic scheme of Fig. 2. Here the rate is determined by a potential energy profile and net flux of a chemical species by the ratio of the forward and the backward rates. The wheel is propelled by a coupled driving force either originating internally through a chemical reaction or externally through an applied potential, chemical, electric,

acoustic, or thermal. When a track-guided stepping motor or locomotion is concerned similar chemical interaction profiles may be applied. If the step size has a length of L the velocity of motor on the track is described by Astumian and Derenyi [18]

$$v = \frac{k_B T}{\gamma L} \exp\left(\frac{-F^* L}{k_B T}\right). \tag{12}$$

Here γ is the frictional coefficient or the viscous drag. The pre-exponential term is simply D/L, D being the diffusion coefficient. F^* is a quantity characteristic of the motor-track interaction, and F^*L is equivalent to the activation barrier G^* shown in Eq. (7). The model assumes that there are two different states of motor–track interaction although the number of states need not be limited to two. With such construction stochastic switching of the two states becomes a generally known model of the Brownian motor, the flashing ratchet. Fig. 6 illustrates a track-guided stepping motor and Fig. 7 parameterizes the potential barrier of a state and the switching between the two states. In this particular system the potential profiles of the two states are identical but spatially shifted by $L/2$ [19,20]. The directed transport of the particle is easily visualized because of the presence of spatial symmetry breaking, as in the case of the Conformational Coupling Model.

6. Markovian engine (ME)

The two types of catalytic wheel or Brownian motor shown above have identical core engine. This engine is Markovian meaning that the probability of the enzyme in current state does not depend on its previous state, i.e., there is no effect of memory in transition. Furthermore, the transition from one state to the other is much faster than other dynamics of the system. The core engine (ME) rectifies the flow of energy by virtue of the symmetry breakdown in the interaction energy profiles of the two thermodynamic states of the system (See Figs. 5 and 7). The applied potential, e.g. electrical, acoustic, chemical, etc. can actuate the switching of state. However, the switching dynamics is stochastic and may be considered a stationary Markov process which is symbolically expressed by the rate equation [7,8,15,19,20]

$$+\Delta \underset{\gamma^-}{\overset{\gamma^+}{\rightleftharpoons}} -\Delta. \tag{13}$$

According to this scheme, the probability of finding the system in the $+\Delta$ state is simply $P^+ = (\gamma^+)^{-1}$ and the probability in the $-\Delta$ state is $P^- = (\gamma^-)^{-1}$. In Fig. 8(a) the random telegraph function $t = t^* \ln R$ is employed to actuate the switching of states, where R is a random number between 0.01 and 0.99 and t^* is the mean lifetime (see Fig. 4 for the symbols and the properties of the function). The pulses have two polarities $+$ and $-$, with equal amplitude and in each reversal of polarity, the switching of states is complete. Each switching in this case moves the potential profile of Fig. 5 and of Fig. 7 by one-half a cycle, i.e., 180° resembling the turn of

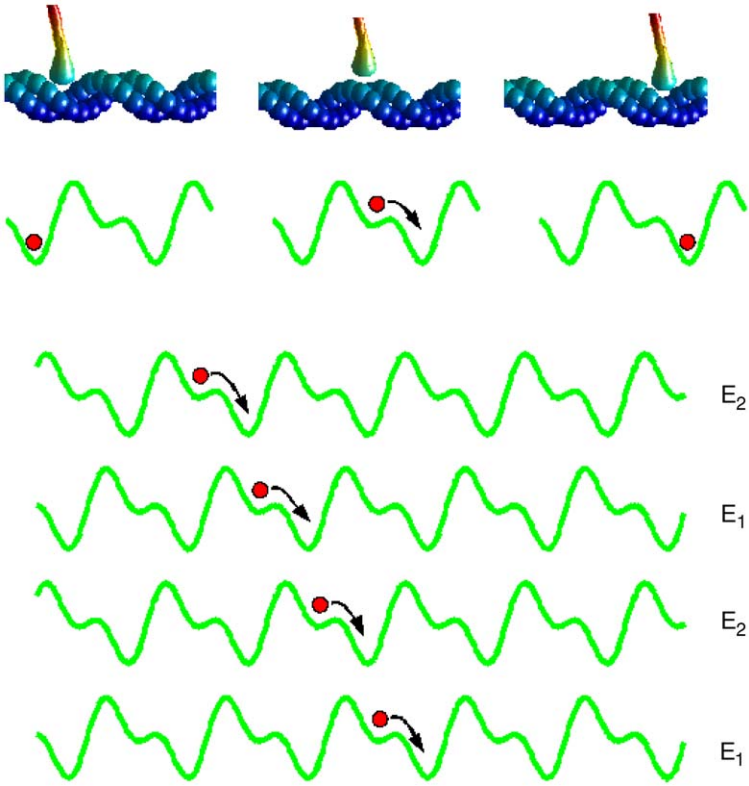

Fig. 6. Biased transport of Brownian particle by flashing ratchet mechanism. The Brownian ratchet model of Fig. 5 is a genetic model known as the flashing ratchet. It can easily be adapted to other biological motors and engines. Here the two activation barriers shown are repeated to form a one-dimensional track, with a periodicity. An external force is applied to effectuate an oscillation or a fluctuation between the E_1 and E_2 states. Each flip or flash of states moves the track rightward by one-half of the period. The particle rolls rightward in each flashing. The model may be used to mimic the directional transport of S-2 head of myosin on the F-actin track. The direction of the particle motion is inherent in the built-in asymmetry of the barriers. This asymmetry may be determined by experiment or by design. By a proper design of the shape and height of the activation barriers, the system may reserve its direction of transport. It may also achieve any arbitrary efficiency in energy transduction. An optimal design will achieve nearly 100% of efficiency. See Refs. [19,20] for details.

motor by half a turn. The energy transferred from the external power source to the engine for chemical work can be precisely evaluated by considering the shape of the interaction profiles of Fig. 7, which is explicitly expressed in Eq. (7). As shown elsewhere [15,19,20] the maximum efficiency of the engine is 100%.

Fig. 8(b) presents yet another scenario; here each switching of states does not give an uniform complete conversion to $+\Delta$ or $-\Delta$. Instead the extent of state changes follows a Gaussian function $G(\Delta_i)$ for the $+\Delta$ state and $G(\Delta_j)$ for the $-\Delta$ state. The behavior of such a system is discussed in detail elsewhere [15,19,20].

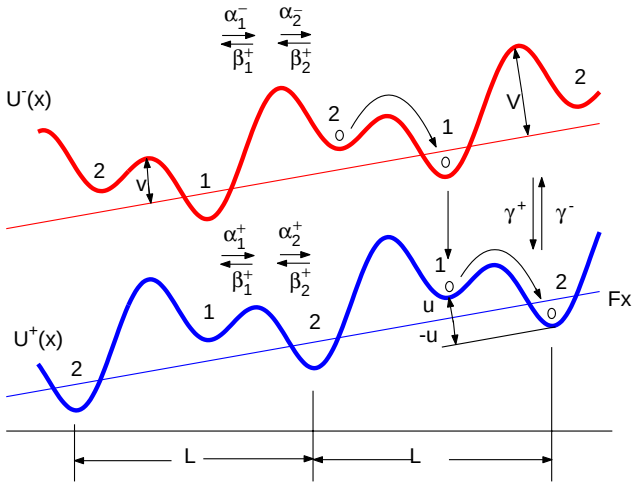

Fig. 7. Schematic representation of the model for a Brownian motor. For the definitions of symbols and its working orders please refer to Ref. [19].

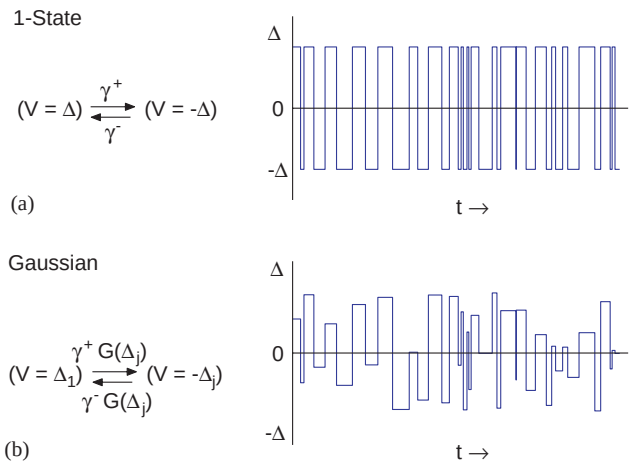

Fig. 8. Kinetic schemes and histograms of some RTF signals. The dichotomous signal V fluctuates between positive and negative values with a mean frequency of $\gamma/2$. $G(\Delta_j)$ denotes amplitude as a Gaussian distribution, with a standard deviation. See Ref. [15] for details.

As mentioned above this core engine may use fuels of different forms. Thermal fluctuation [21] and membrane potential fluctuation come to mind [22]. In this respect as emphasized earlier, one must caution the difference between an equilibrium fluctuation and an off-equilibrium fluctuation [21,23]. Huxley has proposed that the energy derived from the hydrolysis of ATP may fuel muscle

contraction by a Brownian ratchet mechanism via the off-equilibrium thermal fluctuation of states [17]. Such is the first known case of a qualitative model of the biological relevance. We have proposed experimental test with Na,K-ATPase using the telegraph function to drive an off-equilibrium fluctuation of the E_1 and the E_2 states [24] and performed experiment to verify the prediction of the model and analyze the data quantitatively [14,15]. A catalytic wheel is a reversible device. An example in case is the F_oF_1 ATPase. This catalytic wheel can synthesize ATP by a proton gradient energy [2], an electric potential energy [25] or a mechanical energy [4]. But it can also use the γ-phosphorous bond energy of ATP to pump proton uphill or build up an electric potential. In either forward or reverse direction its dynamics is Markovian. We as well as other investigators [26–29] have shown that this core engine is also applicable to other dynamic systems, for example, to game theory [28,29] or prediction of market performance [30]. Enzyme catalysis is cyclic. Coupling of two or more catalytic cycles into one complex mechanism is known to generate directional flow of energy [31,32]. Often more than one enzyme works in cascade or in network. Our work specifically considers the off-equilibrium fluctuation of enzyme conformation as a core mechanism for performing its many complex functions.

In summary, we have redressed the classical MME to become a catalytic wheel, and by the analysis of the Brownian motor shown that in most catalytic wheels there is a core engine, Markovian engine, at work. In the Markovian engine two types of dynamics come to play: the conformational dynamics of the wheel and its catalyzed reaction, and the dynamics of the applied force. For the engine to function properly the reaction dynamics, or the activation barriers, must have an asymmetric element and the force input must match its dynamics according to a set of rules [7,8,14,15,19,20]. However, an asymmetry is not a prerequisite for the applied force. This article reviews and summarizes these results. The catalytic wheel is made of soft matter that is rich in modes of motion. The barrier height determines the rate and when a barrier is high the rate is greatly slowed down. A reaction with slow rate is like a reaction which never occurs and it is like a high wall and it shows a property that may be conceived as hard. In other words, rapid dynamics are equivalent to soft events and slow dynamics are equivalent to hard events. We wish to point out also that although our catalytic wheel model is Markovian, whether the Na,K-ATPase is purely Markovian or it also contains some element of non-Markovian dynamics remains unknown. Ionic current fluctuations in cell membrane are known to show non-Markovian behavior [33,34].

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