Catalytic Wheel, Brownian Motor, and Biological Energy Transduction

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An enzyme turns over, or recycles during each catalytic process. It is a catalytic wheel, analogous to a motor, or an engine. The fuel, or the driving force for the wheel, is the free energy of the substrate (S) to product (P) conversion (ΔG_R), or a free energy output of a coupled chemical reaction. When ΔG_R of the $S \rightarrow P$ reaction is negative the reaction is spontaneous and the downhill wheel motion requires no external input of energy. When ΔG_R is positive the wheel must turn uphill against a workload, a reaction that produces energy must be coupled to the wheel. The synthesis of ATP from ADP and P_i in a cell is an uphill reaction and the energy required for the synthesis is derived from the dissipation of a proton gradient, or an electric potential. This article focuses on mechanisms of action of the uphill catalytic wheels. It is shown that the coupling of an external energy source to an uphill catalytic wheel can be done, with nearly 100% efficiency, by mechanisms of the Brownian Motor. Theory of electroconformational coupling (TEC) is used to construct a Brownian motor, and electric activation of an ion pump, Na, K-ATPase is used to demonstrate its basic principles. A TEC Motor has three essential elements: 1) the ability of the protein to interact with an electric field, 2) existence of at least two conformations of protein that oscillate or fluctuate on interaction with the applied field, and 3) a built-in asymmetry in the molecular interactions with substrate and product. It is shown that the TEC Motor is a generic model and applicable to other types of biological energy transducers.

1. SOFT-MATTER CHARACTERISTICS AND CONFORMATIONAL FLEXIBILITY OF PROTEINS

The nanometer-scale motors and engines that tend our life are mostly made of "*soft-matter*" proteins. The soft characteristic, that is the limited stability of its structure and highly flexible conformation in solution, is essential for a protein to work as a Brownian Motor.

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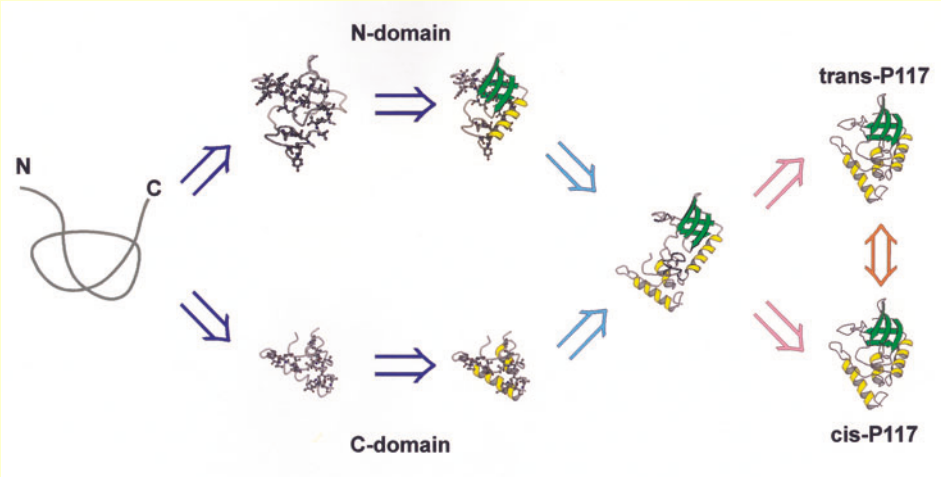
Except water, proteins are the most abundant molecules in cells. They perform nearly all of the structural, mechanical, pump, motor, transport and locomotion functions of cells [1]. Proteins range in sizes and, by the current buzzwords, are the nanometer-scale devices of the living cell. What makes a protein unique compared to other classes of molecule? Why a

Modular Assembly Model (MAM) of protein folding

Folding of newly synthesized peptide(s) into an active, native form of a protein encompasses broad ranges of structural dynamics. The model is proposed to explain the folding of staphylococcal nuclease (SNase) by T. Y. Tsong and colleagues (to be published). The protein in its active form assumes the conformation shown in the lower rightmost structure (cis-P117). The structure is composed of two discernible domains, the N-domain and the C-domain. Folding starts from presumably an ensemble of conformations with no intra-molecular interaction energy. These reactions, taking place between ns and μs, represent events of small activation processes. They give rise to less than 10% of the circular dichoism signal. They are not represented in this figure. The events that follow are the formation of the hydrophobic clusters, semi-independently, in the N-domain and the C-domain. These events take place in 10-50 ms. The accumulation of helices and sheets inside the newly formed hydrophobic clusters is slow, in 50 ms to 2 s, presumably because of the crowded environment. The interlocking of the N-domain and C-domain then takes place in 6 s. The formation of the two active forms (trans-P117 and cis-P117) requires further structural fine-tuning, and it takes place in 35 s. The trans to cis isomerization of proline 117 is slow. However, both forms of the enzyme exhibit full nuclease activity. The stability of enzyme in cis-P117 and trans-P117 is similar, approximately 8 k_BT. Almost the entire 8 k_BT of the conformational stability of the native state is acquired at the last step in folding. However, these kinetic steps have activation barriers ranging from 25 k_BT to 30 k_BT [12]. Experiments show that the Least Activation Path (LAP) dictates the pathway of protein folding [12]. In other words, the population trots the deepest valleys of the activation energy landscape to arrive at the global free energy minimum of the native state. Multiple pathways will result if the activation barriers of different pathways have similar heights.



Box 1

protein catalyst, enzyme can speed up a chemical reaction by as much as 10^{14} times, and why proteins are so versatile compared to other types of molecules or manmade devices? Protein chemists would point out several facts but none as prominent as its three dimensional structure. By virtue of its 3-D structure a protein or an enzyme can properly align several chemically active functional groups in space to carry out chemical reactions efficiently. The structure also provides high specificity in substrate recognition and binding and product release.

Another aspect of a protein concerns its stability. A protein denatures or unfolds and loses its functionality by exposure to certain chemical agents or to high or low temperatures. The range of temperature in which a protein can function is very limited indeed, typically between 20-50℃. Temperatures above or below this range cause it to denature or unfold. There are exceptions, for examples, proteins of archaebacteria such as halophiles and thermoacidophiles, may tolerate higher concentration of acids, salts, and a bigger range of temperature, than usual but the range of tolerance is still very limited.

Interestingly, excess stability of a protein may hamper its ability to perform. A typical protein of molecular weight 20,000 D (Dalton) consists of over thousand of atoms, and the intra-molecular interaction energy for hydrogen bonding, electrostatic interaction, hydrophobic effects, van der Waals' interaction, etc. may amount to hundreds of k_BT for each category. A direct calorimetry measurement indicates that the

enthalpy of unfolding a typical small globular protein, is approximately 150 k_BT [2]. However, due to the highly unusual properties of solvent water, the enthalpy-entropy compensation comes into play, and the resulting net conformational energy of SNase in an aqueous solution amounts to a mere 8 k_BT. This value is in the same order of magnitude to that of the ambient thermal noise 1.5 k_BT. In other word, the conformational energy of a typical globular protein may fluctuate between 6.5 k_BT and 9.5 k_BT. When the temperature deviates from its optimal value, the stability of a protein is further reduced. Today we know that some components of muscle proteins may unfold or undergo helix-coil transition even at the physiological temperature 310°K. Conformational changes relevant to protein functions involve less free energy changes than that of the folding/unfolding reaction. These relatively minor conformational changes are even more susceptible to influence by the ambient thermal noise. Folding/unfolding of proteins involves many modes of motion and conformational changes, and is the most instructive. A proposed mechanism for the folding/unfolding of a typical globular protein is given in Box 1, which summarizes various molecular dynamics and energetics of the protein structure.

2. INTERNAL MOTIONS AND NATURAL FREQUENCY OF A PROTEIN

There are many internal degrees of freedom in a protein molecule and the relaxation time of these modes of motion falls in broad time ranges [1, 3]. Vibration and rotation of chemical bond takes place in 10^{-14} to 10^{-13}s. Electron transport between two prosthetic groups, for example, hemes can take place in 10^{-15} to 10^{-9}s. Flip-flop of organic rings of amino acids may occur in 10^{-9} to 10^{-3}s. Segmental motions and local folding/unfolding events are slow, in the 10^{-6}s range. Folding/unfolding of a protein have many modes of motion ranging from ns to s. Rotational relaxation time of rotary motors occur in ms and locomotion of myosin and kinesin head groups is also in the ms time ranges. Thus, the internal motions of protein span nearly 20 decades in time [1, 3].

Are these motions essential for protein function? If so, what modes of motion are important and what are not? Apparently, the modes of motion important to function are those relevant to the chemical reactions of the catalytic wheel. For Na, K-ATPase, a channel-enzyme, the frequency of motion for K^+ transport is 1.0 kHz and that for Na^+ transport is 1.0 MHz. For F_oF_1ATPase, synthesis of ATP involves motion of around 10 Hz. Any activity required internal motion has a characteristic frequency, which for all practical purposes is termed the Natural Frequency of the catalytic process [4]. Every enzyme is expected to manifest its natural frequency under appropriate conditions as discussed below.

3. ENZYME AS CATALYTIC WHEEL AND SOFT-MATTER MOTOR

Enzyme as biological catalyst has certain properties that make it a catalytic wheel. Consider the most widely used enzyme model, the Michaelis-Menten Enzyme (MME). A MME satisfies these three basic premises [1]: 1) it has the ability to bind substrate(s) and while the substrate(s) are still bound to the enzyme convert them to product(s), and 2) these product(s) then dissociate from the enzyme, and 3) the enzyme returns to its initial conformational state for reuse. The second step is considered the rate-determining step. The complete catalytic process is represented by the two chemical equations shown in the upper part of Fig. 1.

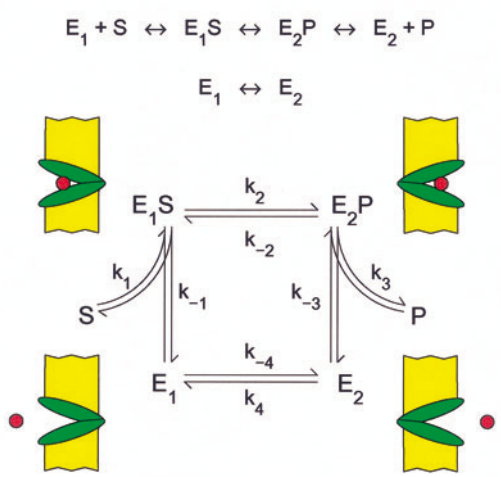


Fig. 1: ***Michaelis-Menten Enzyme (MME) as catalytic wheel*** *The two chemical equations on top of the figure represent the complete catalytic process of the Mechaelis-Menten Enzyme mechanism. The enzyme binds a substrate and the substrate is converted to the product and released. The enzyme then recycles, 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.*

In the two chemical equations the enzyme in E_1S and in E_2P has different conformations. This is evident because S and P are different chemicals and enzyme conformation, that favors binding of S, E_1 is necessarily different from the conformation, E_2 that favors binding of P. E_1 and E_2 coexist in the solution mixture. Because the enzyme recycles the two chemical equations are combined to become a catalytic wheel that embodies all reaction steps.

Figure 2 illustrates that this catalytic wheel also displays property of a motor. The wheel spins, or the motor turns, clock-

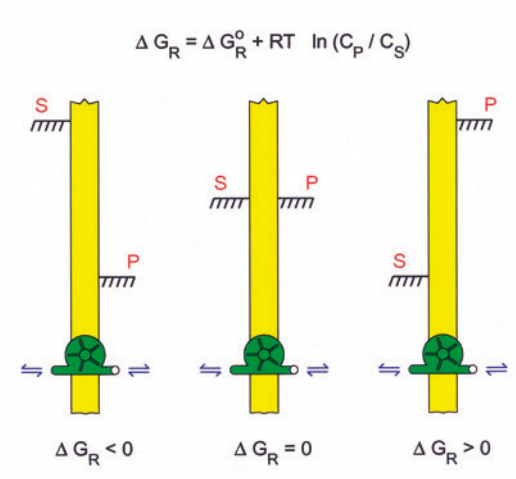


Fig. 2: ***Enzyme as analog of motor***

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. 1 spins clockwise. In the second case, the free energy levels on both side 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, reversibly. In a cell case 3 is a common occurrence. To convert S into P the enzyme must be fueled by an input of energy. $F_o F_1$ATPase 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 clockwise or counterclockwise.

wise or counterclockwise in each enzyme turnover. The fuel that feeds the motor is the free energy of the S to P conversion. If the free energy is negative, i.e. P is more stable than S in solution, the wheel turns clockwise. Conversely if the free energy is positive, i.e. P is less stable than S, the wheel turns counterclockwise. The catalytic wheel is reversible and obeys the detailed balancing in every step of the way.

In a cell or an organism, the need of S to P conversion under an uphill condition is common. Input of energy into the mechanism will be required. The process is known as the energy coupling [1]. Synthesis of ATP from ADP and P_i under the physiological condition requires an input of approximately 15 k_BT and this energy is supplied by the proton electrochemical potential energy. Fig. 1 is a catalytic wheel specifically adapted for an uphill membrane transporter in which the conformational change of the enzyme is to flip its ligand-binding site from one side of the membrane to the other side. The energetic relationship of this catalytic wheel is explained in Fig. 2. Here the enzyme is treated as the motor that drives the whole mechanism into action. The fuel is ATP or in the case discussed below, the applied electric potential.

4. THEORY OF ELECTROCONFORMATIONAL COUPLING (TEC)

As emphasized above, conformational change or fluctuation of the protein catalyst is an essential part of the MME mechanism. The change may be triggered by interaction with a ligand, by a change in temperature, or by other chemical reactions. An effective mean to alter the conformational equilibrium of a membrane protein is to change the magnitude of the transmembrane electric potential $\Delta\Psi_m$. $\Delta\Psi_m$ of cell membranes ranges in value between -200 mV and +200 mV. For example, in an actively respiring mitochondrion, in which ATP is synthesized, the $\Delta\Psi_m$ is approximately -200 mV. Because the membrane is a bilayer of lipids 5 nm thick, the effective transmembrane electric field, E_{eff} (= $\Delta\Psi_m$ / d ; d being membrane thickness) is 4×10^7 V m^{-1} [5]. This is a tremendous electric field. Most cell membranes can sustain a steady-state $\Delta\Psi_m$ of about 250 mV. For a short pulsed-electric field (PEF), the tolerance of the bilayer is higher. It can reach 500 mV or 1000 mV. In a cell membrane the magnitude of membrane potential oscillation is small, a few mV. However, this value is the spatial and temporal average. Local potential change in a neuron impulse is of the order of 100 mV which gives an E_{eff} of 2×10^7 V m^{-1}.

The TEC postulates that a membrane protein may interact with a strong, physiologically relevant electric field and undergo a conformational change [4, 6]. Note that a $\Delta\Psi_m$ is a vector quantity. Its field direction points, or oscillates normal to the membrane surface. A membrane protein is also restricted in motion. It may be allowed to rotate in the lipid bilayer but flip-flop or tumbling motion may be severely restricted. The molar electric moment, M_e of a protein is also a vector quantity and its interaction with E_{eff} is a vector-to-vector interaction. The interaction energy is simply the dot product of the two quantities $M_e \cdot E_{eff}$ and we call reaction of this type Anisotropic chemical reaction. Furthermore, the cell membrane is an "anisotropic medium", meaning that the orientation and reactivity of reactants is not uniform spatially or temporally. The interaction energy $M_e \cdot E_{eff}$, which is the free energy of interaction with the applied electric field, G_{Elc}, is a scaler quantity.

Now let us turn our attention back to the transporter shown in Fig. 1. If E_1 and E_2 have a difference in molar electric moment, $\delta M_e = M_{e,2} - M_{e,1}$, an applied electric field will shift the conformational equilibrium of the transporter, and if the applied field E_{eff} is oscillatory or fluctuating, it will induce conformational oscillation of the enzyme species. The speed of conformational oscillation or fluctuation is dictated by the rate constants of the chemical events. The wheel is fed with electric energy ΔG_{Elc} in the amount of $\delta M_e \cdot E_{eff}$ in each

turnover, and the wheel becomes an energy transducer, or a motor, to convert it into other forms of energy.

5. TEC MOTOR, ION PUMP AND BROWNIAN MOTOR

Until now we have made no use of the concept of the Brownian motion or of the Brownian ratchet. The catalytic wheel of Fig. 1 can be made to pump a substrate, or ion, across the cell membrane when a sinusoidal electric field is applied. The TEC motor will spin at an appropriate frequency defined already by the chemical rate constants of the kinetic steps [4, 6]. This frequency is the natural frequency of the channel enzyme as we have just discussed. Our analysis indicates that the efficiency of energy coupling, i.e. the conversion of the electric energy into the chemical gradient energy, depends on many factors. One of these factors is the frequency of the oscillating field. When this frequency coincides with the natural frequency of the pump, the efficiency reaches a peak value [4 - 8].The maximum efficiency approaches unity when the system is optimized. Much information on the pump design and its optimization has been worked out and published.

The TEC motor discussed so far follows the formulation of the classic chemical kinetics. No extraordinary assumptions or stochastic elements have been built into the mechanism. However, in a cell a molecular motor must pitch its minute wattage against the much more violent dissipation power of the water environment. Astumian and Hanggi in their recent article on Brownian Motor have given a shocking fact about an ATP-powered molecular motor [9]. From the ATP hydrolysis activity of the enzyme one may estimate the wattage of the motor to be in the range 10^{-16} to 10^{-17} W. Water molecules have an average speed of 350 m s^{-1} and the energy fluctuation of 1.5 k_BT [3]. These molecules bump into the molecular motor and exchange with it heat at a rate equivalent to 10^{-8} W. How can the enzyme work as pump, engine or locomobile under such a violently dissipating environment?

To approach this question we simplify the four-state transporter of Fig. 1 into a two-state molecular ratchet mechanism by assuming that the $E_1 \longleftrightarrow E_2$ and the $E_1S \longleftrightarrow E_2P$ steps are fast compared to the frequency of the PEF and the rates of the substrate binding and product dissociation [4, 6, 7].

$$k_{conf} >> f_{ele} >> k_{chem}$$

Here the three constants are respectively, the rate of the protein conformational changes, the frequency of the applied field, and the rate of the substrate binding and product dissociation. With this condition Fig. 1 becomes a two-state ratchet of Fig. 3. It is a membrane transporter if S is replaced with L_{left} and P with L_{right}. The transporter oscillates or ratchets between E and EL depending on the barrier oscillation or fluctuation that is triggered by the applied electric field. If the PEF is stochastic rather than deterministic or periodic, the ratchet becomes a Brownian ratchet.

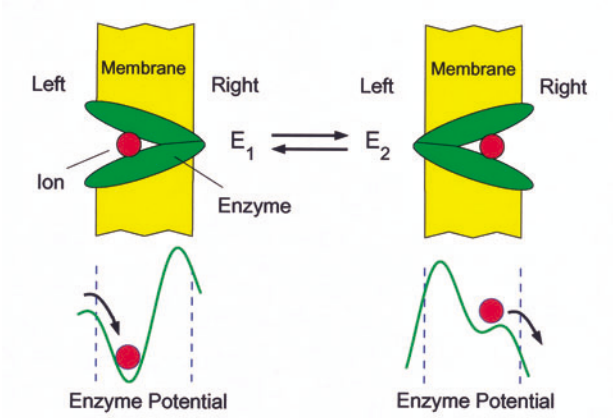


Fig. 3: ***Brownian motor and Brownian ratchet***

The MME enzyme of Fig. 1 will behave like a Brownian motor. The E_1 to E_2 transition and vice versa can be effectuated by the binding of S or 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 the literature.

In fact, Fig. 3 is considered a generic model and it can easily be modified to other biological motors and locomobiles. An essential attribute of Fig. 3 is the asymmetry of the barriers for the enzyme to bind ligand on the left compartment L_{left} and the right compartment L_{right}. In other words, E_1 has a higher association constant, or lower barrier, for L_{left} than that of E_2 for L_{right}, and vice versa. When a pulsed electric field (PEF) is applied to induce $E_1 \longleftrightarrow E_2$ transition, accompanied with the transition an L is pumped from the left compartment to the right compartment. This mechanism is adapted to the transport of a myosin S-2 moiety on an actin cable in Fig. 4. In this model S-2 may interact with a G-actin either in a strong-interaction mode or in a weak interaction mode, with corresponding activation barriers shown in Fig. 3. The modes of interaction are determined by the chemical event of the ATP hydrolysis. In Fig. 4 the oscillation or fluctuation between the two modes of interaction will drive directional transport of the red ball, i.e. a Brownian particle on the one-dimensional activation barriers. The mechanism shown in Fig. 4 for the locomotion of S-2 on actin cable, can achieve a high efficiency

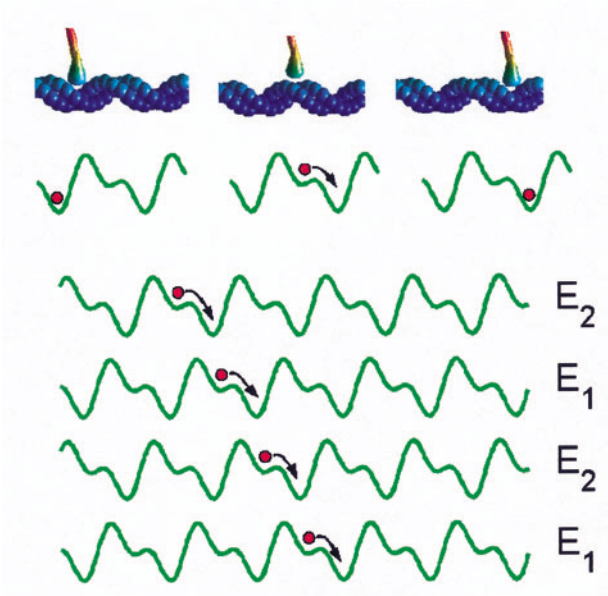


Fig. 4: ***Biased transport of Brownian particle by flashing ratchet mechanism***

The Brownian ratchet model of Fig. 3 is a generic 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 leftward by one-half of the period. The particle rolls leftward in each flashing. The model may be used to mimic the directional transport of S-2 head of myosin on the F-acting 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 reverse its direction of transport. It may also achieve any arbitrary efficiency in energy transduction. An optimal design will achieve nearly 100 % of efficiency.

of energy transduction on a proper design of the barrier shape, as opposed to certain model in which the efficiency of energy transduction is demonstratively low [10].

Pulsed electric field (PEF) with the waveforms sinusoidal, squared with periodicity, and random telegraph function (RTF), have been applied to the electric activation experiment and also in the simulation based on the TEC concept [11]. The PEF enforced-conformational oscillation or fluctuation is in accord with the shape, frequency and amplitude of the PEF. There is a range of electric parameters that the pump will respond and outside of this range the pump will become erratic and no directional flow of energy will commence. The range of electric parameters is determined by the set of kinetic constants and their relationship with the applied field, E_{eff}. It's interesting to note that barrier shape of the enzyme-ligand interactions provides the asymmetry needed for the biased Brownian transport. A ratchet is a bare version of a motor and our discussion below will focus on the Brownian Motor of Fig. 1.

6. SIGNAL, NOISE AND STOCHASTIC RESONANCE IN A BROWNIAN MOTOR

The PEF induced pumping of K^+, Rb^+ and Na^+ exhibits windows of frequency and amplitude. Waveform of a PEF also plays an important role. Waveform, amplitude and frequency are three elements of a signal. Each element may have effects on the other two. A systematic study is needed to fully understand their mutual influence. This being the case, with a sinusoidal electric field the optimal frequency for the Rb^+ and K^+ pumping is found at 1.0 KHz and for the Na^+ pumping is found at 1.0 MHz. These values are, respectively, the natural frequencies of the two semi-independent ion pumps within the enzyme molecule. Experiment with the RTF electric pulse gives consistent result. For example the best RTF field to activate the Rb^+ pump has a mean frequency of 1.0 kHz. The best amplitude of PEF for the two pumps happens to be identical, at 2×10^3 V m^{-1}, which is equivalent to an E_{eff} of 5×10^6 V m^{-1} across the cell membrane.

Having defined an electric signal with these parameters any PEF that has much different electric parameters may be considered a noise. This concept presents us an opportunity to investigate effects of noise, or stochastic resonance on the signal transduction of a Brownian Motor. Sinusoidal electric signals are superimposed with different level of "white noise" and effects of added noise on Rb^+ pumping activity of Na, K-ATPase is monitored [11]. The salient features of the experiment are: 1) when the signal is at its optimum, any added noise reduces the pump activity. 2) When the signal is suboptimal low levels of noise increase pump activity but there is a maximal tolerance for the noise. Beyond this level increased noise reduces the pump activity. 3) A noise can carry a subthreshold signal over the threshold. In other words, the added noise elevates the energy level of the pump, and the activation barrier for the pumping is subdued in comparison. Other biological signal processing systems have shown similar properties. Analysis by TEC, currently in progress, reproduces these findings feature by feature [13]. This success of the TEC analysis is considered a strong evidence for the ion pump as the Brownian Motor. Some experimental results are shown in Fig. 5.

7. ANISOTROPIC CHEMICAL REACTION AND BIOLOGICAL ENERGY TRANSDUCTION

As discussed above enzyme catalysis is cyclic and the enzyme is the driver of the catalytic wheel. An enzyme is a nanometer scale motor, engine or locomobile. However, to generate a

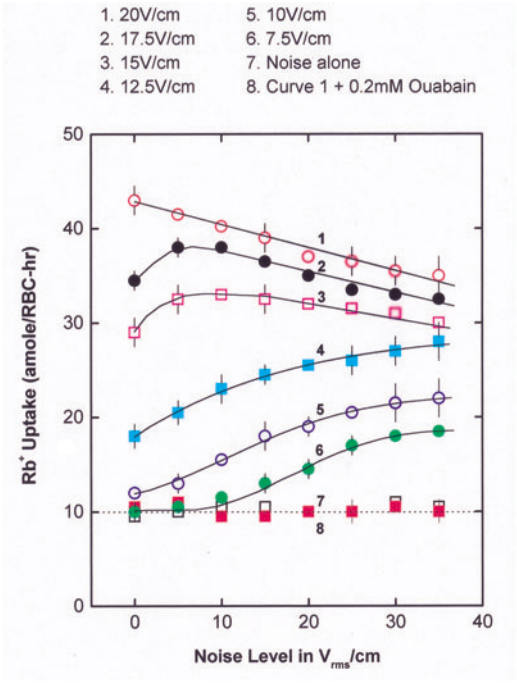


Fig. 5: ***Singal and noise, and stochastic resonance***

Based on the TEC motor a signal is characterized by the waveform, amplitude, and frequency of the electric field. These quantities in turn are determined by the chemical rate constants of the system, and their dependence on the field strength. Any electric field far removed from the natural frequency of the motor is considered an electric noise. For the electric activation of the Rb^+ pump of Na, K-ATPase, the optimal signal is 20 V cm^{-1} at 1.0 kHz and the threshold voltage is 10 V cm^{-1} [11]. Here electric white noise of varied power levels (root-mean-square) is superimposed on an electric signal for the stimulation of the Rb^+ pumping. Data show that when the pump is at its optimum (Curve 1), any level of added noise reduces the efficiency. When the signal is sub-optimal (Curves 2 - 5) an appropriate level of noise increases the efficiency of the pump. Furthermore, noise added to a sub-threshold signal may elevate the signal above the threshold (Curve 6). See [11, 13] for details.

directional flow of energy or a biased Brownian motion, its interaction with reactants and a driving force must be anisotropic. Indeed, chemical reactions of cells are rarely reactions among free-floating, fast tumbling molecules. Instead, they are reactions of molecules with highly restricted motions, for examples, in a cell membrane, supramolecular structure, or viscous and over-crowded medium. For an anisotropic reaction of nanometer scale molecules made of soft-matter, mechanisms of Brownian Motor are remarkably effective. TEC is an electric ratchet and motor. Nevertheless, most of the equations derived for TEC are also applicable to other types of ratchet or engine [4, 7]. For a chemical ratchet, oscillation of concentration or chemical potential will produce ratchet effects. In other words, oscillation or fluctuation of either one of a pair of *conjugate-variables* will produce ratchet effects and a Brownian motor may thus, be constructed [4, 7]. Most commonly encountered conjugate-variables in cells are pressure/molar volume, area/tension, length/tension, etc. Energy in the form of mechanical force, electrical potential, chemical bond, concentration gradient, etc. may inter-convert by mechanisms similar to TEC.

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