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...
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°C. Temperatures above or below this range cause it to denature or unfold. There are exceptions, for examples, proteins of archaeabacteria 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 $k_B T$ for each category. A direct calorimetry measurement indicates that the
enthalpy of unfolding a typical small globular protein, is approximately 150 kₜ [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 kₜ. This value is in the same order of magnitude to that of the ambient thermal noise 1.5 kₜ. In other word, the conformational energy of a typical globular protein may fluctuate between 6.5 kₜ and 9.5 kₜ. 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. 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 F₁/F₅ ATPase, 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](image)

The two chemical equations on top of the figure represent the complete catalytic process of the Michaelis-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. E₁ favors binding of the substrate while E₂ 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 E₁S and in E₂P have different conformations. This is evident because S and P are different chemicals and enzyme conformation, that favors binding of S, E₁ is necessarily different from the conformation, E₂ that favors binding of P. E₁ and E₂ 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-
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. A molecular motor, which uses an external energy source or conversely, use the proton electrochemical gradient energy to synthesize ATP. The enzyme is a reversible 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, \( E_{eff} = \frac{\Delta \Psi_m}{d} \) (d being membrane thickness) is \( 4 \times 10^7 \) 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 \( 2 \times 10^7 \) 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 scalar 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 activity of reactants is not uniform spatially or temporally. The speed of conformational oscillation or fluctuation is dictated by the rate constants of the chemical events. The wheel is fed with electric energy \( \Delta G_{elc} \) in the amount of \( \hat{\sigma} M_e \cdot E_{eff} \) 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 $350 \text{ m s}^{-1}$ and the energy fluctuation of $1.5 \text{kBT}$ [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 $E_1 \leftrightarrow E_2$ and the $E_1S \leftrightarrow E_2P$ steps are fast compared to the frequency of the PEF and the rates of the substrate binding and product dissociation [4, 6, 7].

$$k_{\text{conf}} >> f_{\text{pe}} >> k_{\text{chem}}$$

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 $L_{\text{left}}$ and P with $L_{\text{right}}$. 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.

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 $L_{\text{left}}$ and the right compartment $L_{\text{right}}$. In other words, $E_1$ has a higher association constant, or lower barrier, for $L_{\text{left}}$ than that of $E_2$ for $L_{\text{right}}$, and vice versa. When a pulsed electric field (PEF) is applied to induce $E_1 \leftrightarrow E_2$ transition, accompanied with the transition an L is pumped from the left compartment to the right. 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.
of energy transduction on a proper design of the barrier shape, as opposed to certain models 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, $E_{\text{eff}}$. 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 $2 \times 10^3$ V m⁻¹, which is equivalent to an $E_{\text{eff}}$ of $5 \times 10^6$ 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 sub-optimal 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 sub-threshold 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
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 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.

8. REFERENCES