ML-15
The Cell as a Machine

Molecular Mechanics

The consideration of how forces affect individual molecules is critical for our understanding of force transduction and motor activities which underlie many of the important functions that we have discussed. We will consider force effects on the kinetics of protein unfolding and on the energetics of motor function.

In the case of protein unfolding, the problem can be described as the transition of a folded protein to an unfolded state. We will consider here a long protein such as spectrin, which has many similar domains that can unfold to produce about a 3-fold increase in molecular length. There is normally an energy barrier that must be overcome to unfold a protein. A plot of the free energy of spectrin versus the overall length of spectrin will have a peak corresponding to the energy barrier to unfolding the weakest domain. If we consider the fact that Brownian forces are working at unfolding the molecule and domain motions are on the order of 10^9 - 10^{10} /s even a high energy barrier can be crossed occasionally. Force on the molecule lowers the energy barrier because there is a lengthening of the molecule as it crosses the barrier (the force times the distance to the peak of the energy barrier gives the degree to which the free energy was decreased).

$$\Delta G_{force} = \Delta G_{norm} - F \cdot \Delta X$$

The rate constant for the unfolding will be increase by the Boltzmann relationship from the original rate constant

$$k_1 = k_1^0 \exp [F \cdot \Delta X / kT]$$

Motor Proteins

In the case of motor proteins the hydrolysis of ATP (or other high energy compound) is coupled to the movement of the protein along a filament. Kinesin movement on microtubules is a good example in which the details are known. For kinesin, there appears to be one ATP hydrolyzed per 8 nm of movement. The equilibrium energetics of the process bring out some important general aspects of reversible force-dependent processes.

If we assume that the Substrate (S) is hydrolyzed to the Product (P) by the motor enzyme (E), then a simple description of the process is

$$S + E = ES = EP = E + P$$

We have described this previously in terms of the free energy of the reaction; however, it is obvious that the relative concentrations of substrate, [S], and product, [P], affect the free energy of the hydrolysis reaction. The standard free energy of hydrolysis of ATP is defined as the free energy of the conversion of ATP to ADP where both are at

1M concentration. The normal cellular values are 2-4 mM and 10-30 μM for ATP and ADP, respectively. Because ATP is much greater than ADP in concentration, there is more energy available (mass action favors the hydrolysis of ATP to ADP). Thus the free energy of conversion of S to P can be described as noted below.

$$\Delta G_{S\text{-}P} = \Delta G^0 + kT \ln [P]/[S] = kT \ln K/Keq = kT \ln [P][Seq]/[Peq][S]$$

As a motor, the protein generates force through a cycle of ATP hydrolysis and ADP release. When the force on the motor stalls the forward movement of the motor, the energy of hydrolysis is theoretically equal to zero. The additional term of the force times the distance traveled per ATP can theoretically give the stall force from the ATP and ADP concentrations.

$$\Delta G_{S\text{-}P} = 0 = \Delta G^0 + kT \ln [P]/[S] + F \cdot \Delta X \quad \text{or by rearranging}$$

$$F \cdot \Delta X = -\Delta G^0 - kT \ln [P]/[S]$$

The energy from a single molecule of ATP is estimated to be 80 pN·nm which would correspond to a force of 10 pN moving on a kinesin molecule that moves 8 nm. In fact, the measured stall force for kinesin is 5-7 pN and the estimated distance of movement is 8 nm. The motor can then be said to be 50-70% efficient.

Filament Mechanics

Another important aspect of the mechanics of a cell is the consideration of polymer or filament packing, which necessarily involves bending of the polymer or filament. A microtubule will have considerable rigidity and resists bending deformation. The mathematical description of the bending resistance is given by the beam equation,

$$M = E^*I / R$$

Where M is the bending moment (M = F X), and E*I is the flexural rigidity that is the product of the Young's modulus (E) and the second moment (I). The second moment is a geometrical factor that is related to the geometry of the beam. For a cylinder, $I = \pi r^4/4$. For a rectangular beam of width, b, and height, a, $I = ab^3/12$. To estimate the bending of a beam, we need to adopt a different formulation and a common formulation is to describe the tangent angle at each point along the beam, θ(s). The angle of the bend is related to the parallel length displacement, dx, and perpendicular displacement, dy, for a given displacement along the beam, ds, by the relationships, $dx/ds = \cos\theta$, $dy/ds = \sin\theta$, and it follows that $d\theta/ds = 1/R$. The beam equation can then be rewritten as

$$d\theta/ds\ (s) = M(s)/EI$$

or $$d^2y/dx^2 = M(x)/EI$$

and this formula can be used to derive the displacement of the end of a beam of length L, by a spring constant, K.

$$K = F/y(L) = 3EI/L^3$$

For a small glass rod of radius 0.25 μm and length of 100 μm. E = 70 Gpa, $I = (\pi/4)\, r^4 = 3x10^{-27}$ which gives a spring constant K = 0.64 pN/nm. Note that increasing the length to 400 μm will decrease K to 0.01 pN/nm whereas increasing the radius to 0.5 mm will increase K to 2.56 pN/nm

Persistence Length

For a polymer it is useful to describe the rigidity in terms of the length over which the rod loses any correlation between the angles at the ends. The principle of Equipartition of Energy can be used to derive the relationship that the persistence length, Lp, is proportional to the flexural rigidity

$$Lp = EI/kT$$

Freely Jointed Chain

Another way to treat the matter of the polymer length is to consider the polymer as a freely jointed chain with n links of a length, b. In terms of the persistence length, the length of the links in the freely jointed chain can be described as

$$2Lp = b$$

Thus, some polymers in cells can be considered as a freely jointed chain (for example, DNA (persistence length of 53 ± 2 nm), RNA and long fatty acid chains. However, many of the filaments have a very long persistence length that exceeds the average dimensions of a cell (persistence length of actin filaments is 10-20 μm and of microtubules is 1-6 mm).

Problems

1. Consider a piece of spaghetti 1mm in diameter. Yung's modulus is ~10^8 J/m^3.
    a. What is its persistence length at room temperature? Is the result consistent with your everyday observations?
    b. Please calculate spring constant for 1 cm spaghetti piece. If you consider spaghetti as linear spring, how many molecules of ATP have to be hydrolyzed in order to displace the end of a spaghetti piece in 3mm?
2. (extra credit) Suppose that the ratio of substrate to product in a mixture is ten times greater then the ratio at equilibrium. How much mechanical work could be obtained by converting one molecule of substrate to one molecule of product? Suppose that you have a total of N substrate plus product molecules and that the equilibrium ratio is 1. What is the total amount of mechanical work that could be done with mixture before it becomes completely spent?