

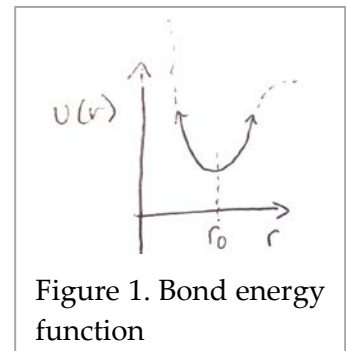
## BIOEN 326 2014 LECTURE 21: INTRODUCTION TO MOLECULAR BIOMECHANICS

When an element of materials is exposed to stress, the individual molecules within the materials are exposed to mechanical forces. The mechanical and biological response of the material depends on how these molecules respond to these mechanical forces. **Molecular Biomechanics** is the study of how biological molecules respond to forces.

Specifically, we ask how mechanical forces change the length (structure) of the molecule? The answer to this question determines the elastic and viscoelastic properties of the material composed of these molecules. We will consider two kinds of structural responses to force in molecules, and will learn how both depend on the energy landscape of the molecule or bond. First, a molecule may undergo a **continuous deformation**, or a gradual change in length due to the elasticity of the molecule. Second, a molecule may undergo a **discrete state change**, which is a sudden change in the conformation of the molecule accompanied by a change in length, such as unfolding and refolding of a protein. When a molecule undergoes a conformational change, this may affect the elastic properties of the material, but in living materials like cells and tissues, it can also change the functional properties of the molecule. In particular, the different states may initiate biochemical signaling pathways differently, so mechanical forces affect biochemistry, which is the molecular basis of **mechanotransduction**.

### Principles of Continuous Deformation

Materials are composed of atoms or molecules held together by molecular bonds, such as van der Waals bonds, ionic bonds, or covalent bonds. Many gels and biological materials are composed of molecular fibers that are cross-linked together with covalent or noncovalent energies. Both of these have in common the presence of **nodes** (atoms, molecules or cross-links) held together by **bonds** (bonds between atoms and molecules, or molecular fibers connecting cross-links). When the individual bonds stretch, the material stretches. Thus, we are interested in the effect of force on the length of the bonds. We can calculate this from the energy landscape of the bond as follows. We define the **bond energy function**,  $U(r)$  to be the energy of the bond at a length  $r$ . If  $r_0$  is the length where  $U(r)$  is a minimum, then we define  $r_0$  as the **equilibrium bond length**. By the definition of a local minimum,  $U(r) > U(r_0)$  for  $r$  near  $r_0$ . Figure 1 illustrates these definitions.



The bond force is the negative derivative of the energy function:  $f_b = -\frac{dU(r)}{dr}$ . If  $U$  is continuous and smooth (as in Figure 1), and  $r$  is sufficiently near  $r_0$ , then by definition of a local minimum  $\frac{dU(r)}{dr} < 0$  for  $r < r_0$ ,  $\frac{dU(r)}{dr} = 0$  for  $r = r_0$ , and  $\frac{dU(r)}{dr} > 0$  for  $r > r_0$ . Since  $f_b$  is the negative of this, then  $f_b > 0$  for  $r < r_0$ ,  $f_b = 0$  for  $r = r_0$ , and  $f_b < 0$  for  $r > r_0$ , so the bond force acts to restore the bond to its equilibrium length.

For  $r$  sufficiently near  $r_0$ , the second order Taylor expansion approximates the energy function:

$$U(r) \sim U(r_0) + (r - r_0) \frac{dU}{dr}(r_0) + \frac{1}{2}(r - r_0)^2 \frac{d^2U}{dr^2}(r_0)$$

However, we know that  $\frac{dU}{dr}(r_0) = 0$ , since  $r_0$  is a minimum, and we can define the bond stiffness as  $k = \frac{d^2U}{dr^2}(r_0)$ , so

$$U(r) \sim U(r_0) + \frac{1}{2}k(r - r_0)^2$$

That is, we can approximate the energy function as a linear spring within a sufficiently small range. This is illustrated in Figure 1 by the solid lines, while outside this range, linearity will be lost and we need to consider the full form of the function  $U(r)$  instead of the second order approximation.

Now we ask what happens when we add an externally applied force,  $f$ , to the bond. We will use the deformation sign convention, so  $f > 0$ , when the bond is under tension. We expect this to lengthen the bond. However, we defined  $f_b$  as a restoring force created by the bond, not acting on the bond, and specifically noted that  $f_b < 0$  when the bond is longer than its equilibrium length. The bond will stop lengthening when the restoring force balances the external force, or when  $f = -f_b$ . Thus, the external force needed to compress or stretch a bond to a length  $r$  depends on the energy landscape, with:

$$f(r) = \frac{dU}{dr}$$

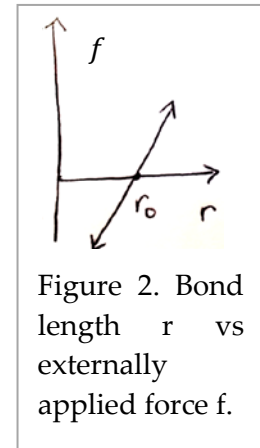
And for sufficiently small deformations,  $f(r) = k(r - r_0) = \frac{d^2U}{dr^2}(r_0) * (r - r_0)$ .

In the next lecture, we will learn how to estimate the Young's modulus of materials from the equilibrium length and stiffness of the bonds. This can help us understand the molecular basis of the Young's modulus. After that, we will see how the nonlinear properties of these bond energies can lead to nonlinear elastic behaviors. In short, the structure and energy of the molecular bonds that make up a material determine its elastic properties in a somewhat predictable way.

When we apply this theory, we can define 'bonds' very loosely. We will consider bonds between atoms, such as covalent, ionic, metallic, and van der Waals bonds, when we consider non-biological materials. We will also consider bonds between macromolecules or complexes when we consider biological materials. For example, many biomaterials, tissues and the cytoskeleton of cells are made up of cross-linked polymers, so each polymer region spanning two cross-links can be considered a bond. Adhesive proteins form bonds between cells and the extracellular matrix.

### Principles of Discrete Deformation

Some molecules or macromolecular complexes have not one, but two or more low-energy states. For example, proteins may be folded or unfolded, ion channels can be open or closed, and many proteins are allosteric, so that they have two distinct folded conformations, with different functions. As long as the energy of the two states is reasonably similar, the state of the molecule can be regulated by outside perturbations such as binding of other molecules, covalent modifications such as phosphorylation or truncation, or exposure to physical stimuli



such as voltage differences or mechanical force. Thus, the existence of multiple states allows for regulation of biological function. Here we learn quantitatively how mechanical forces affect macromolecular structure and function.

For our discussion of multi-state molecules or bonds, we will use  $G$  for the energy function to remind us that entropy is an important component of the energy of these macromolecular states, so we will use Gibb's free energy to describe the energy of states ( $G = H - ST$ ). We also use the superscript 0 to indicate standard conditions, which for the purposes of this discussion means in the absence of externally applied force. We are thus considering a molecule with two states with energy  $G_1^0$  and  $G_2^0$ . We will always use state 1 as the state that is lowest energy in standard conditions, which we will also call the **native state**, so by definition,  $G_1^0 < G_2^0$ .

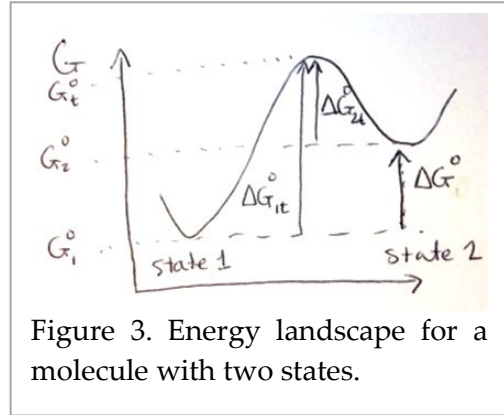


Figure 3. Energy landscape for a molecule with two states.

You may recall that the probability  $P_i$  of a state  $i$  is proportional to the **Boltzman factor**,  $\exp\left(-\frac{E_i}{RT}\right)$ , where  $R$  is the gas constant and  $E_i$  is the energy of a mole of the item in question when it is in state  $i$ . Here we are focusing on single molecules, so we will use  $P_i \propto \exp\left(-\frac{G_i}{k_B T}\right)$ , where  $k_B = \frac{R}{N_A} = 1.38 \times 10^{-23} \text{ J/K}$  is the **Boltzman constant**, which is the single molecule version of  $R$ , and  $G_i = E_i/N_A$ , is the energy of a single molecule in state  $i$ , and  $N_A$  is Avogadro's number.

We refer to  $k_B T$  as **thermal energy**. Most biomechanics calculations and measurements are done at either room temperature or body temperature, so it is convenient to memorize or look up thermal energy in these two conditions rather than calculating them from the Boltzman constant. Recall that  $K = C + 273$ , so body temperature is  $37C = 310K$ , and room temperature is about  $22C = 295K$ , so Thus, thermal energy is  $k_B T = 4.1 \times 10^{-21} \text{ J}$  or  $k_B T = 4.28 \times 10^{-21} \text{ J}$  for room and body temperature respectively. For this class, unless you are told otherwise, assume a measurement is performed at room temperature.

Since the probabilities must always sum to one,  $\sum_i P_i = 1$ , the probability of being in state  $i$  is determined by the **Boltzman distribution**, which is the Boltzman factor divided by the **partition function**  $Z$ , which is the sum of all Boltzman factors:  $Z = \sum_i \exp\left(-\frac{G_i}{k_B T}\right)$ . That is,  $P_i = \exp\left(-\frac{G_i}{k_B T}\right)/Z$ . When we want to compare the probability of two states, the  $Z$  falls out:  $\frac{P_2^0}{P_1^0} = \exp\left(-\frac{G_2^0}{k_B T}\right)/\exp\left(-\frac{G_1^0}{k_B T}\right) = \exp\left(\frac{G_1^0 - G_2^0}{k_B T}\right)$ . We then define  $\Delta G^0 = G_2^0 - G_1^0$  as the difference in energy between the native state 1 and the higher energy state 2 in standard conditions. Note that again by definition,  $\Delta G^0 > 0$ , as illustrated in Figure 3. From this, we can calculate the **equilibrium constant**,  $K_{eq}^0 = \frac{P_2^0}{P_1^0} = \exp\left(\frac{-\Delta G^0}{k_B T}\right)$ . This describes the thermodynamic equilibrium between the two states.

We also use the energy diagram to calculate the **transition rate constants** between the two states. If  $G_t^0$  is the energy of the transition state, then the transition rate constant from state 1 to 2,  $k_{12}^0$ , depends on the height of the **energy barrier**,  $\Delta G_{1t}^0 = G_t^0 - G_1^0$  and an **Arrhenius cofactor**,  $A$ , described as the attempt frequency:  $k_{12}^0 = A \exp\left(-\frac{\Delta G_{1t}^0}{k_B T}\right)$ .  $A$  is on the order of  $10^{10} \text{ s}^{-1}$ , but is different for each reaction, so this equation cannot be used to calculate the exact activation energy or the rate constant from the other, but can be used to estimate either. More importantly, this equation is used to see how a change like temperature or mechanical force affects a rate constant. Similarly,  $k_{21}^0 = A \exp\left(-\frac{\Delta G_{2t}^0}{k_B T}\right)$ .

The overall rate of transition from 1 to 2 is the rate constant times the probability of being in the state 1:  $P_1^0 k_{12}^0$ . At equilibrium, the forward rate must equal the reverse rate,  $P_2^0 k_{21}^0$ . Thus  $P_2^0 k_{21}^0 = P_1^0 k_{12}^0$ . This can be rearranged to show that  $\frac{P_2^0}{P_1^0} = \frac{k_{12}^0}{k_{21}^0}$ , which means that  $K_{eq}^0 = \frac{k_{12}^0}{k_{21}^0}$ . That is, the equilibrium constant is the ratio of the rate constants.

### Mechanics of Discrete State Changes

Now consider what happens when we add a force,  $f$ , across the molecule. The molecule may have some elastic properties that are independent of the state transition, but we addressed those in the first part of this lecture and now we only concern ourselves with changes in length that are associated with the state transition. That is, we are interested in changes in length or elastic properties between the two states. Specifically, we consider the length of each state as a function of force:  $x_1(f)$  is the length of state 1,  $x_2(f)$  is the length of state 2, and  $\Delta x(f) = x_2(f) - x_1(f)$  is the difference in length at the force  $f$ . Note that  $\Delta x(f) > 0$  if state 2 is longer in the presence of force. Figure 4 illustrates the meaning of  $x_1$ ,  $x_2$ , and  $\Delta x$ , by graphing the energy against the length of the bond.

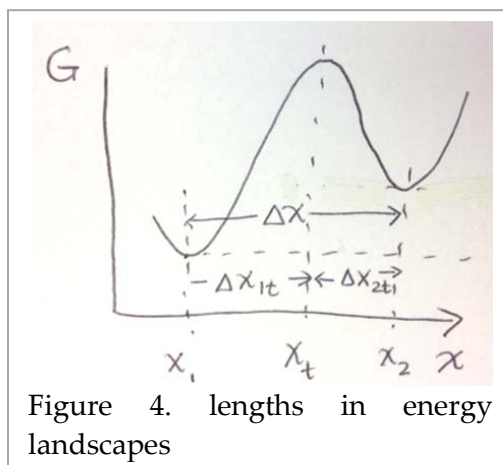


Figure 4. lengths in energy landscapes

The difference in energy between the two states in the presence of force includes the energy required to shorten or extend the molecule by that distance  $\Delta x(f)$ , which is  $f \cdot \Delta x(f)$ . Thus,  $\Delta G(f) = \Delta G^0 - f \cdot \Delta x(f)$ , as illustrated in Figure 5. Recall that energy is in units force times distance, so the units are consistent. Also check the signs intuitively: tensile force ( $f > 0$ ) favors the longer state, and the free energy of the longer state relative to the shorter should drop with force if  $\Delta x(f) > 0$ .

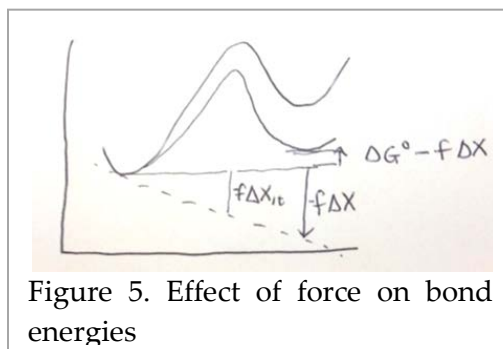


Figure 5. Effect of force on bond energies

First we ask how force affects the equilibrium between the two states. The probability of being in state  $i$  in the presence of force,  $P_i(f) = \exp\left(\frac{\Delta G^0 - f \cdot \Delta x(f)}{k_B T}\right) / Z(f)$ . From this, we can calculate the equilibrium constant in the presence of force  $K_{eq}(f) = \frac{P_2(f)}{P_1(f)} = \exp\left(\frac{-\Delta G(f)}{k_B T}\right) = \exp\left(\frac{-\Delta G^0 + f \cdot \Delta x(f)}{k_B T}\right)$ :

$$K_{eq}(f) = K_{eq}^0 \exp\left(\frac{f \Delta x(f)}{k_B T}\right)$$

From this, we can ask when the two states become equally likely. We call this the **equilibrium force**,  $f_{eq}$ . The two states are equally likely when  $\frac{P_2(f)}{P_1(f)} = 1$ , so when  $\exp\left(\frac{-\Delta G(f)}{k_B T}\right) = 1$ , which is when  $\Delta G(f_{eq}) = 0$ , which is when  $\Delta G^0 - f \cdot \Delta x(f) = 0$ . Thus, to find the equilibrium force, we solve for

$$f_{eq} \cdot \Delta x(f_{eq}) = \Delta G^0$$

Next we apply a similar logic to understand the effect of force on rate constants. Let  $\Delta x_{1t}(f) = x_t(f) - x_1(f)$  be the length change between state 1 and the transition state at the force  $f$ , as shown in Figure 4. Then the energy barrier in the presence of force is  $\Delta G_{1t}(f) = \Delta G_{1t}^0 - f \Delta x(f)$  and the rate constant with force is

$$k_{12}(f) = k_{12}^0 \exp\left(\frac{f \Delta x_{1t}(f)}{k_B T}\right)$$

Thus, the rate is increased if the transition state is longer than the original state ( $\Delta x_{1t} > 0$ ) and decreased if the transition state is shorter. This makes sense, as force will pull the bond into the transition state or away from the transition depending on whether the transition or ground state is longer.

Linear elasticity. If each state exhibits linear elasticity at the force range in question, then each state has a spring constant, which we will call  $k_1$  and  $k_2$ . In this case,  $x_1(f) = x_1^0 + f/\kappa_1$  and  $x_2(f) = x_2^0 + f/\kappa_2$ , where  $x_1^0$  and  $x_2^0$  are the lengths of the two states without external force. We can then calculate that  $\Delta x(f) = x_2^0 - x_1^0 + f\left(\frac{1}{\kappa_2} - \frac{1}{\kappa_1}\right)$ , or  $\Delta x(f) = \Delta x^0 + f\left(\frac{1}{\kappa_2} - \frac{1}{\kappa_1}\right)$ , where  $\Delta x^0 = x_2^0 - x_1^0$  is the difference in lengths without external force. This means that our equation for the equilibrium force is  $f_{eq} = \frac{\Delta G^0}{\Delta x^0 + f_{eq}\left(\frac{1}{\kappa_2} - \frac{1}{\kappa_1}\right)}$ , or  $f_{eq} \Delta x^0 + f_{eq}^2 \left(\frac{1}{\kappa_2} - \frac{1}{\kappa_1}\right) - \Delta G^0 = 0$ , which can be solved with the quadratic formula.

From the equation above, we can see that we can neglect the  $f\left(\frac{1}{\kappa_2} - \frac{1}{\kappa_1}\right)$  term if the two states have the same spring constant. In this case,  $\Delta x(f) = \Delta x^0$ , and  $f_{eq} = \Delta G^0 / \Delta x^0$ . This equation also applies if both spring constants are stiff enough that we can neglect the stretch in either state when compared to the difference in length between the states: if  $\frac{f}{\kappa_1} \ll \Delta x^0$  and  $\frac{f}{\kappa_2} \ll \Delta x^0$ , then  $f\left(\frac{1}{\kappa_2} - \frac{1}{\kappa_1}\right) \ll \Delta x^0$  and we can neglect the  $f\left(\frac{1}{\kappa_2} - \frac{1}{\kappa_1}\right)$  term.

### Summary:

If  $r$  is the length of a bond or molecule,  $r_0$  is the equilibrium length (the length at which energy is lowest), and  $U(r)$  is the energy function, then...

1. For sufficiently small deformations, the linear spring approximation can be used for a bond or molecular length:  $U(r) = U(r_0) + \frac{1}{2}k(r - r_0)^2$ , where  $k = \frac{d^2U}{dr^2}(r_0)$ .
2. The force needed to hold a bond or molecule at a length  $r$  is  $f(r) = \frac{dU}{dr} \sim k(r - r_0)$

If a bond or molecule has two low energy states (two minima in the energy function), and  $G_1^0$ ,  $G_t^0$ , and  $G_2^0$  are the energy of state 1, the transition state, and state 2 respectively, then...

1. The energy difference between the two states is  $\Delta G^0 = G_2^0 - G_1^0$
2. The equilibrium constant is  $K_{eq}^0 = \frac{p_2^0}{p_1^0} = \exp\left(\frac{-\Delta G^0}{k_B T}\right)$ , and determines the relative probabilities of being in each state.
3. The energy barrier from state 1 to 2 is  $\Delta G_{1t}^0 = G_t^0 - G_1^0$
4. The transition rate is  $k_{12}^0 = A \exp\left(-\frac{\Delta G_{1t}^0}{k_B T}\right)$ , where  $A$  is approximately  $10^{10} \text{ s}^{-1}$ , and similar for  $k_{21}^0$ .

If  $x_1(f)$ ,  $x_t(f)$ , and  $x_2(f)$  are the equilibrium lengths of each of these states at a force  $f$ , respectively, and  $\Delta x(f) = x_2(f) - x_1(f)$ , and  $\Delta x_{1t}(f) = x_t(f) - x_1(f)$ , then

1. The probability of being in each state with force is  $\frac{p_2(f)}{p_1(f)} = K_{eq}(f) = K_{eq}^0 \exp\left(\frac{f \Delta x(f)}{k_B T}\right)$
2. The equilibrium force at which the two states are equally likely is  $f_{eq} \cdot \Delta x(f_{eq}) = \Delta G^0$
3. The rate constant with force is  $k_{12}(f) = k_{12}^0 \exp\left(\frac{f \Delta x_{1t}(f)}{k_B T}\right)$ , and similar for  $k_{21}(f)$ .

We can usually use the linear approximations for changes in lengths:  $\Delta x(f) = \Delta x^0 + f \left(\frac{1}{\kappa_2} - \frac{1}{\kappa_1}\right)$  and can sometimes use the constant approximation, when the stiffnesses are the same or are very high:  $\Delta x(f) = \Delta x^0$ .

**Example:**

FimH is a bacterial adhesive protein that undergoes an allosteric conformational change that increases its affinity for mannose, which is found on host cells. Calculations here refer to the structure of the bond between FimH and mannose. This bond has two low energy states. State 1 is the low-affinity state, and state 2 is the high affinity state. Force affects the transition between the states because they are different in length. We can estimate lengths of the low-affinity, high-affinity and transition state from the structures:  $x_1^0 = 10 \text{ nm}$ ,  $x_2^0 = 12.4 \text{ nm}$ , and  $x_t^0 = 10.9 \text{ nm}$ . We can also estimate the transition rates in the absence of force:  $k_{12}^0 = 0.00125 \text{ s}^{-1}$  and  $k_{21}^0 = 0.025 \text{ s}^{-1}$  for the most common variant of FimH. We can assume that all three states have about the same spring constant,  $\kappa_2 = \kappa_1 = \kappa_t$ .

- What fraction of bonds are in the high-affinity state without force?
- At what force are the two states equally likely?
- At this force, what are the transition rates between the states?

First, I reword the questions using the notations we learned in lecture:

- find  $P_2^0$
- find  $f_{eq}$
- find  $k_{12}(f_{eq}), k_{21}(f_{eq})$

Then I calculate:

- I know that  $K_{eq}^0 = \frac{P_2}{P_1} = \exp\left(\frac{-\Delta G^0}{k_B T}\right)$ . However, I was given rate constants, not energies, so that's not helpful. However, I also know that  $K_{eq}^0 = \frac{P_2^0}{P_1^0} = \frac{k_{12}^0}{k_{21}^0}$ . That's better; I know  $\frac{k_{12}^0}{k_{21}^0} = \frac{0.00125}{0.025} = 0.05$ . Thus,  $\frac{P_2^0}{P_1^0} = 0.05$ . To find  $P_2^0$ , I need to use the fact that  $P_1^0 + P_2^0 = 1$ , since we are told the bond just has these two states. Thus,  $\frac{P_2^0}{1-P_2^0} = K_{eq}^0$ , which rearranges to  $P_2^0 = \frac{K_{eq}^0}{1+K_{eq}^0} = \frac{0.05}{1.05} = 0.048$ . (In general, when  $K_{eq} \ll 1$ , then  $p_2 \sim K_{eq}$ .)
- To find I might try to use  $f_{eq} \cdot \Delta x(f_{eq}) = \Delta G^0$ , but I was not told  $\Delta G^0$ . I could calculate  $\Delta G^0$  from  $K_{eq}^0$ , but it will be quicker to use  $K_{eq}(f) = K_{eq}^0 \exp\left(\frac{f \Delta x(f)}{k_B T}\right)$  and the fact that  $K_{eq}(f) = 1$  when  $f = f_{eq}$ . That is,  $K_{eq}^0 \exp\left(\frac{f_{eq} \Delta x(f_{eq})}{k_B T}\right) = 1$ . To solve this, I need an equation for  $\Delta x(f)$ . I know  $\Delta x^0 = x_2^0 - x_1^0 = 2.4 \text{ nm}$ ,  $\Delta x(f) = \Delta x^0 + f \left(\frac{1}{\kappa_2} - \frac{1}{\kappa_1}\right)$ , and  $\kappa_2 = \kappa_1$ , so  $\Delta x(f) = \Delta x^0$ . Thus,  $\exp\left(\frac{f_{eq} \Delta x^0}{k_B T}\right) = 1/K_{eq}^0$  so  $\frac{f_{eq} \Delta x^0}{k_B T} = \ln\left(\frac{1}{K_{eq}^0}\right)$ , or  $f_{eq} = \frac{k_B T}{\Delta x^0} \ln\left(\frac{1}{K_{eq}^0}\right) = \frac{4.1E-21J}{2.4E-9m} \ln(20) = 5.12E - 12N$ . That is,  $f_{eq} = 5.1 \text{ pN}$ .
- This time I need the force dependence of the rates, so I define  $\Delta x_{1t}^0 = x_t^0 - x_1^0 = 0.9 \text{ nm}$ , and  $\Delta x_{2t}^0 = x_t^0 - x_2^0 = -1.5 \text{ nm}$ . Again, the spring constants are all equal, so these length changes are not affected by force. Note that the two are the same:

$$k_{12}(f_{eq}) = f_{12}^0 \exp\left(\frac{f_{eq} \Delta x_{1t}^0}{k_B T}\right) = 0.00125 * \exp\left(\frac{(5.1E-12N * 0.9E-9m)}{4.1E-21J}\right) = 0.003844s^{-1}$$

$$k_{21}(f_{eq}) = f_{21}^0 \exp\left(\frac{f_{eq} \Delta x_{2t}^0}{k_B T}\right) = 0.025 * \exp\left(\frac{(5.1E - 12N * -1.5E - 9m)}{4.1E - 21J}\right) = 0.003844s^{-1}$$

## Appendix:

As indicated by our figures, the state of a molecule is described not by discrete states, but rather along a continuum. Why, then, do we only consider the lowest energy states? Briefly, because this is here the molecule spends most time. Moreover, we can show that while a molecule is in one state, meaning fluctuating around the local minima, that the most frequent length of the bond is the length at the local minima, which we refer to as  $r_0$ .

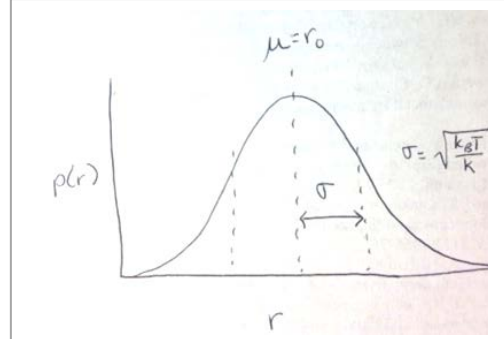
Here we define  $G(r)$  as the continuous energy function for the bond. For a continuous function, the

probabilities integrate to 1, so  $\int_r p(r)dr = 1$ . In this case, the integral version of the Boltzman distribution gives the probability density of being at length  $r$ :

$$p(r) = \frac{\exp\left(-\frac{G(r)}{k_B T}\right)}{\int_r \exp\left(-\frac{G(r)}{k_B T}\right) dr}$$

Regardless of the form of  $G(r)$ , we can use the second order Taylor expansion for  $r$  close to  $r_0$ :  $G(r) \sim G(r_0) + \frac{1}{2}k(r - r_0)^2$ , with  $k = \frac{d^2G}{dr^2}(r_0)$ . Thus the probability of having length  $r$  can be approximated as  $p(r) = \frac{1}{Z} \exp\left(-\frac{GU(r_0) + \frac{1}{2}k(r-r_0)^2}{k_B T}\right)$ . This can be rearranged to  $p(r) = \frac{\exp\left(-\frac{G(r_0)}{k_B T}\right)}{Z} \exp\left(-\frac{(r-r_0)^2}{2k_B T/k}\right)$ . This resembles the normal (Gaussian) distribution, or Bell curve, with mean  $\mu$  and a standard deviation  $\sigma$ :  $p(x) = \frac{1}{\sigma\sqrt{2\pi}} \exp\left(-\frac{(x-\mu)^2}{2\sigma^2}\right)$ , with  $\mu = r_0$  and  $\sigma = \sqrt{\frac{k_B T}{k}}$ , and  $\frac{\exp\left(-\frac{G(r_0)}{k_B T}\right)}{Z} = \frac{1}{\sigma\sqrt{2\pi}} = \sqrt{\frac{k}{2\pi k_B T}}$ . Thus  $p(r) = \sqrt{\frac{k}{2\pi k_B T}} \exp\left(-\frac{(r-r_0)^2}{2k_B T/k}\right)$ , which is the normal distribution as shown in the figure.

Thus, in any low-energy state, a bond vibrates with a mean length  $r_0$  and a standard deviation  $\sigma = \sqrt{\frac{k_B T}{k}}$ , where  $r_0$  is the length of the local minimum in energy and  $k$  is the second derivative at this position  $k = \frac{d^2G}{dr^2}(r_0)$ .



Probability distribution for a linear spring is the normal distribution.