1. Thermodynamics/Statistical Mechanics

1.A. Probability and entropy

Thermodynamics/statistical mechanics deal with the average properties of a large group of molecules. Many biochemical experiments are done on large groups (often called ensembles) of molecules so thermodynamics plays a large role in understanding the experimental results. We are going to spend some time doing derivation of thermodynamics/statistical mechanics expressions to show that they can be understood in large part from probability arguments.

The guiding principal in thermodynamics/statistical mechanics is that the properties reflect the most probable distributions of these molecules subject to whatever constraints the system is under (e.g. constant temperature and pressure). "Most probable" refers to having a large number of different ways of achieving the distribution.

For example, in a solution, the solute molecules are typically equally distributed throughout the solution volume. This is because there are many more ways to distribute the solute molecules throughout the container than to place them all on one side of the container.

So, we begin by thinking about probability – see page 15 of DB. Let us consider a residue in a surface region of a protein which has equal probability of being in a helix (A) or strand (B) conformation. For a single molecule, the probability of being in helix is 0.5 and in strand is 0.5.

$$P(\mathrm{A}) = 0.5 \tag{1.1}$$
$$P(\mathrm{B}) = 0.5 \tag{1.2}$$

Now consider an experiment done on two molecules. The probability of observing two A molecules is:

$$P(\mathrm{AA}) = P(\mathrm{A_2B_0}) = (0.5)^2 = 0.25 \tag{1.3}$$

Similarly, the probabilities of finding A and then B, $P(\mathrm{AB})$, or B and then A, $P(\mathrm{BA})$ are

$$P(\mathrm{AB}) = P(\mathrm{BA}) = 0.25 \tag{1.4}$$

And of the probability of finding two strand molecules is

$$P(\mathrm{BB}) = P(\mathrm{A_0B_2}) = 0.25 \tag{1.5}$$

Consider now that our experiment (say NMR chemical shift) only measures the total number of helix or strand residues. We see that our experimental probabilities are:

$$\begin{aligned} P(\mathrm{A_2B_0}) &= 0.25 \\ P(\mathrm{A_1B_1}) &= 0.5 \\ P(\mathrm{A_0B_2}) &= 0.25 \end{aligned} \tag{1.6}$$

Now consider three molecules. Using Pascal's triangle, we can show:

$$P(A_3) = 0.125$$
$$P(A_2B_1) = P(A_1B_2) = 0.375 \quad (1.7)$$
$$P(B_3) = 0.125$$

For N molecules, the general probability formula for observing n A and N-n B residues is:

$$P(A_nB_{N-n}) = (0.5)^N\{(N!/n!(N - n)!\} \quad (1.8)$$

If the probabilities are not equal with $P(A) = p$ and $P(B) = 1-p$, then (see DB 1.28)

$$P(A_nB_{N-n}) = (p)^n(1-p)^{N-n}\{(N!/n!(N - n)!\} \quad (1.9)$$

This is the binomial distribution.

For large N, $P(A_nB_{N-n})$ of Eq. 1.8 will be peaked at n = N/2. For example, for N = 10,

$$P(A_5B_5) = 0.246$$
$$P(A_3B_7) = P(A_7B_3) = 0.118$$
$$P(A_1B_9) = P(A_9B_1) = 0.001$$

For even larger N (say 10^{17} molecules), we can also convert Eq. 1.8 into an analytical form. First, consider ln $P(A_nB_{N-n})$

$$\ln\{P(A_nB_{N-n})\} = -N\ln2 + \ln(N!) - \ln(n!) - \ln\{(N-n)!\} \quad (1.10)$$

If we define the excess number of A residues as "s" then

$$n = N/2 + s = (N + 2s)/2 \quad (1.11)$$
$$N - n = N/2 - s = (N - 2s)/2$$

Eq. 1.10 is then

$$\ln\{P(A_{(N+2s)/2}B_{(N-2s)/2})\} = -N\ln2 + \ln(N!) - \ln\{[(N + 2s)/2]!\} - \ln\{[(N - 2s)/2]!\} \quad (1.12)$$

Stirling's approximation for large N is:

$$\ln(N!) \cong \ln(2\pi)/2 + (N + ½)\ln(N) - N \quad (1.13)$$

and for small x:

$$\ln(1+x) \cong x \quad (1.14)$$

These equations can be used to simplify Eq. 1.12 as:

$$\ln\{P(A_{(N+2s)/2}B_{(N-2s)/2})\} = \ln\{P(s)\} \cong \ln(2/\pi N)/2 - 2s^2/N \quad (1.15)$$

or

$$P(s) = (2/\pi N)^{1/2} \exp(-2s^2/N) \quad (1.16)$$

which is a Gaussian probability distribution. If we define $\sigma = (N)^{1/2}/2$, then (see DB, p. 22):

$$P(s) = (2\pi\sigma^2)^{-1/2} \exp(-s^2/2\sigma^2) \quad (1.17)$$

The average value of s or $\langle s \rangle$ can be calculated as an integral from $-\infty$ to $+\infty$:

$$\langle s \rangle = \int_{-\infty}^{+\infty} s\, P(s)\, ds = 0 \quad (1.18)$$

A measure of the width of a probability distribution is the variance (DB Eq. 1.41):

$$\langle (s - \langle s \rangle)^2 \rangle = \langle s^2 \rangle - \langle s \rangle^2 \quad (1.19)$$

In our example,

$$\langle s^2 \rangle = \int_{-\infty}^{+\infty} s^2\, P(s)\, ds = \sigma^2 \quad (1.20)$$

The most useful measure of the width of the probability distribution is the square root of the variance or the root-mean-squared deviation (RMSD) and is σ for the Gaussian distribution.

If for N molecules, the residue were helical in N/2 molecules and strand in N/2 molecules, then $s = 0$ and $P(0) = 0.40/\sigma$. If for N molecules, the residue were helical in N molecules ($N_A = N$) and strand in 0 molecules ($N_B = 0$), then $s = N/2$ and $P(N/2) = (0.40/\sigma) \times e^{-N/2}$. The ratio of the probabilities of the two results are:

$$P(N/2)/P(0) = e^{-N/2} \quad (1.21a)$$

For $N = 10^{17}$ molecules (~0.15 μmol), this ratio is $\sim 10^{-10^{16}}$, i.e. it is very, very, very unlikely that all of the molecules will have a helical residue. We can get some idea of the most probable results by considering $s = \langle s \rangle + \sigma = \sigma$.

$$P(\sigma)/P(0) = e^{-1/2} = 0.61 \quad (1.21b)$$

So, it is likely that $-\sigma < s < \sigma$. For $s = \sigma = (N)^{1/2}/2$ and $N = 10^{17}$ molecules, $N_A = 5.000000016 \times 10^{16}$ and $N_B = 4.999999984 \times 10^{16}$, i.e. there are nearly equal numbers of molecule with helical and strand residues. Another way of looking at this is that the most probable distributions of molecules have fractional excess helical residues:

$$|s/N| \langle \sigma/N = (N)^{-1/2}/2 \quad (1.22)$$

and for $N = 10^{17}$ molecules, $(N)^{-1/2}/2 \approx 10^{-9}$.

The bottom line of this example is that for typical numbers of molecules, our experimental results will likely reflect the most probable distributions of molecules and the range of these probable distributions is very narrow compared to the total possible range of distributions.

Also, remember that probability refers to the number of ways of arranging the molecules to achieve the distribution. For example, there is only one way that 10^{17} molecules can be arranged so that each molecule has a helical residue. There are 10^{17} ways of arranging the molecules so that one of the molecules has a strand residues. There are $\sim 10^{10^{16}}$ ways of arranging the molecules in which $N_A = N_B$. We associate the number of ways of achieving a particular distribution as being associated with its degree of disorder. So, having all of the molecules with a helical residue is a highly ordered distribution (and highly improbable) while having $N_A = N_B$ is highly disordered (and highly probable). You can understand that the second law of thermodynamics is based on a probability argument – i.e. that the universe is tending towards the most probable arrangement of atoms and molecules.

In DB, the number of ways of achieving a particular distribution is denoted *W* and for the numbers of molecules which we typically measure in the laboratory, it is clear that *W*s are usually very large. It is more convenient to work with the natural logarithm of *W*. The term entropy is defined as

$$S = k \times \ln W \tag{1.23}$$

where k is Boltzmann's constant and is the ideal gas constant divided by Avogadro's number.

Using $\ln W$ is also convenient because it allows for the following additive property described using an example.

Suppose we have two solutions of protein molecules with N_1 protein molecules in the first solution and N_2 molecules in the second solution. We wish to calculate W_{tot} for the two solutions and we do not allow the two solutions to mix.

We"ll do the simplest example where $N_1 = N_2 = 1$. The possible arrangements are:

Solution 1	Solution 2
Molecule 1	Molecule 2
helical	helical
helical	strand
strand	helical
strand	strand

So $W_{tot} = 4$. Note that if we only consider the first solution, $W_1 = 2$ and if we only consider the second solution, $W_2 = 2$. Clearly, $W_{tot} = W_1 \times W_2$. This is a specific example showing that W_{tot} for isolated systems is the product of *W*s of the systems. The convenient thing about the entropy is

that $\ln W_{tot} = \ln W_1 + \ln W_2$ and the total entropy is the sum of the entropies of the individual systems.

There is another common formula for entropy using probability rather than multiplicity (W). Consider a protein solution with two molecules and let us calculate the number of ways that we can achieve both molecules with helical residues $W(A_2B_0)$, one molecule with a helical residue and one molecule with a strand residue $W(A_1B_1)$, and both molecules with strand residues $W(A_0B_2)$. Going back to Eqs. 1.3–1.5, the results will be:

$$\begin{aligned} W(A_2) &= 1 \\ W(A_1B_1) &= 2 \\ W(B_2) &= 1 \end{aligned} \tag{1.24}$$

These multiplicities fit the general formula:

$$W(A_nB_{N-n}) = N!/n!(N - n)! \tag{1.25}$$

where N is the total number of molecules. Eq. 1.25 is the same as Eq. 1.8 with removal of the $(0.5)^{-N}$ factor which relates multiplicity to probability.

For a given $W(A_nB_{N-n})$, the probability that a particular molecule has a helical residue is:

$$p_A = n/N \tag{1.26}$$

and the probability that a particular molecule has a strand residue is:

$$p_B = (N\text{-}n)/N \tag{1.27}$$

so that Eq. 1.25 can be written as:

$$W(A_nB_{N-n}) = N!/[(N \times p_A]![(N \times p_B]! \tag{1.28}$$

For large N, Stirling's approximation (Eq. 1.13) can be further approximated to:

$$\ln(N!) \cong N\ln N - N \tag{1.29}$$

and

$$\ln W(A_NB_{N-n}) \cong N\ln N - (N \times p_A)\ln(N \times p_A) - (N \times p_B)\ln(N \times p_B) \tag{1.30}$$

which can be simplified to:

$$\ln W(A_nB_{N-n}) \cong N \times (-p_A \ln p_A - p_B \ln p_B) \tag{1.31}$$

More general expressions are given in DB, Eq. 6.4 and VJH, Eq. 2.18. The DB Eq. 6.4 is:

$$\ln W = \mathrm{N} \times (-\sum_{\mathrm{j}=1}^{\mathrm{t}} p_{\mathrm{j}} \ln p_{\mathrm{j}}) \qquad (1.32)$$

where p_j represents the probability that a single molecule is in a given state "j". For example, with t = 3, p_1 could be the probability that the residue is in α helix conformation, p_2 is the probability that the residue is in β strand conformation, and p_3 is the probability that the residue is in turn conformation.

Eq. 1.32 provides a general route to calculating the entropy of a large number of molecules provided that we know the p_j for the most probable distribution. In my example so far, the p_j have been given as ad hoc parameters with $p_A = p_B = 0.5$. This is a reasonable assumption if the energies of the two conformations ε_A and ε_B are equal but is not reasonable if the energies are not equal.

1.B. Probability under the constraint of constant total energy - temperature

We now consider how to calculate the p_A and p_B when $\varepsilon_A \neq \varepsilon_B$. We"ll assume that a molecule with the residue in the strand conformation has higher energy than a molecule with the residue in the helical conformation, i.e. $\varepsilon_A < \varepsilon_B$. We treat p_A and p_B as parameters which are varied to satisfy the conditions and constraints of our system. DB provides a description of the mathematical approach of constrained maximization on pp. 69-73 and pp. 86-89 and we will apply this approach to our system. The best values p_A* and p_B* will maximize $S(p_A,p_B)$ under the constraint that the total probability is fixed:

$$p_A + p_B = 1 \qquad (1.33)$$

We also have the experimentally reasonable constraint that the average energy per molecule $\langle\varepsilon\rangle$ is a fixed quantity in our solution of N protein molecules:

$$\langle\varepsilon\rangle = \sum_{\mathrm{m}=1}^{\mathrm{N}} \varepsilon_{\mathrm{m}}/\mathrm{N} \; = \; p_A\varepsilon_A + p_B\varepsilon_B \qquad (1.34)$$

Using Eq. 1.33, we define a function:

$$g(p_A,p_B) = p_A + p_B = 1 \qquad (1.35)$$

so that:

$$(\partial g/\partial p_A)_{pB} = (\partial g/\partial p_B)_{pA} = 1 \qquad (1.36)$$

and

$$dg = (\partial g/\partial p_A)_{pB}\, dp_A + (\partial g/\partial p_B)_{pA}\, dp_B \; = dp_A + dp_B = 0 \qquad (1.37)$$

The last equality comes from Eq. 1.33. The subscript in the partial derivative means that this parameter is held constant. For example, g is a function of p_A and p_B and $(\partial g/\partial p_A)_{pB}$ is the derivative of g with respect to p_A with p_B held constant. We can rewrite Eq. 1.37 as:

$$dp_B/dp_A = -(\partial g/\partial p_A)_{pB}/(\partial g/\partial p_B)_{pA} \tag{1.38}$$

Similarly, using Eq. 1.34, we define a function:

$$h(p_A,p_B) = p_A\varepsilon_A + p_B\varepsilon_B \tag{1.39}$$

so that:

$$\begin{aligned}(\partial h/\partial p_A)_{pB} &= \varepsilon_A \\ (\partial h/\partial p_B)_{pA} &= \varepsilon_B\end{aligned} \tag{1.40}$$

and:

$$dh = (\partial h/\partial p_A)_{pB}\, dp_A + (\partial h/\partial p_B)_{pA}\, dp_B = \varepsilon_A dp_A + \varepsilon_B dp_B = 0 \tag{1.41}$$

with:

$$dp_B/dp_A = -(\partial h/\partial p_A)_{pB}/(\partial h/\partial p_B)_{pA} \tag{1.42}$$

At $p_A = p_A^*$ and $p_B = p_B^*$, S is at a maximum so that:

$$dS = (\partial S/\partial p_A)_{pB}\, dp_A + (\partial S/\partial p_B)_{pA}\, dp_B = 0 \tag{1.43}$$

and:

$$dp_B/dp_A = -(\partial S/\partial p_A)_{pB}/(\partial S/\partial p_B)_{pA} \tag{1.44}$$

If we combine Eqs. 1.38, 1.42, and 1.44:

$$\begin{aligned}(\partial S/\partial p_A)_{pB}/(\partial S/\partial p_B)_{pA} &= (\partial g/\partial p_A)_{pB}/(\partial g/\partial p_B)_{pA} \\ (\partial S/\partial p_A)_{pB}/(\partial S/\partial p_B)_{pA} &= (\partial h/\partial p_A)_{pB}/(\partial h/\partial p_B)_{pA}\end{aligned} \tag{1.45}$$

These equations would be consistent with:

$$\begin{aligned}(\partial S/\partial p_A)_{pB}/2 &= N\alpha \times (\partial g/\partial p_A)_{pB} \\ (\partial S/\partial p_A)_{pB}/2 &= N\beta \times (\partial h/\partial p_A)_{pB} \\ (\partial S/\partial p_B)_{pA}/2 &= N\alpha \times (\partial g/\partial p_B)_{pA} \\ (\partial S/\partial p_B)_{pA}/2 &= N\beta \times (\partial h/\partial p_B)_{pA}\end{aligned} \tag{1.46}$$

where α and β are constants. Again these equations are only true when $p_A = p_A^*$ and $p_B = p_B^*$, i.e. maximum S. We will now proceed to solve for p_A, p_B, α, and β. I will not put in the asterisks

but the calculated p_A and p_B are meant to refer to the most likely probabalities. We add together the top two equations and add together the bottom two equations:

$$-(\partial S/\partial p_A)_{pB} + [N\alpha \times (\partial g/\partial p_A)_{pB}] + [N\beta \times (\partial h/\partial p_A)_{pB}] = 0$$
$$-(\partial S/\partial p_B)_{pA} + [N\alpha \times (\partial g/\partial p_B)_{pA}] + [N\beta \times (\partial h/\partial p_B)_{pA}] = 0 \quad (1.47)$$

Using Eqs. 1.23, 1.31, 1.36, and 1.40:

$$k\ln p_A + k + \alpha + \beta\varepsilon_A = 0$$
$$k\ln p_B + k + \alpha + \beta\varepsilon_B = 0 \quad (1.48)$$

or:

$$p_A = \exp(-1 - \alpha/k) \times \exp(-\beta\varepsilon_A/k)$$
$$p_B = \exp(-1 - \alpha/k) \times \exp(-\beta\varepsilon_B/k) \quad (1.49)$$

We know that $p_B + p_A = 1$ so:

$$q = 1/[\exp(-1 - \alpha/k) = \exp(-\beta\varepsilon_A/k) + \exp(-\beta\varepsilon_B/k) \quad (1.50)$$

where q is known as the *partition function*. It probably gets its name because it plays an important role in portioning the molecules between the different states. The probability ratio p_B/p_A:

$$p_B/p_A = \exp[-\Delta\varepsilon \times (\beta/k)] \quad (1.51)$$

where $\Delta\varepsilon = \varepsilon_B - \varepsilon_A$.

In order to evaluate β, we will consider a somewhat different approach taken from Kittel and Kroemer, *Thermal Physics*. We consider a protein solution with total energy $U = U_0 = N\langle\varepsilon\rangle$ where $\langle\varepsilon\rangle$ is the average molecular energy and then consider the probability that a single molecule ("molecule 1") in the solution has energy ε_A.

As a "thought experiment", we consider that all of the energy U_0 is initially in the N-1 other "reservoir" molecules. In order for molecule 1 to have energy ε_A, the energy ε_A must be transferred from the reservoir so that the reservoir now has energy $U_0 - \varepsilon_A$. We consider the multiplicity of the protein solution:

$$W = W_{\text{molecule1}} \times W_{\text{reservoir}} = W_{m1} \times W_r = W_r(U_0 - \varepsilon_A) \quad (1.52)$$

because $W_{m1} = 1$ (it is in state ε_A). W_r is written as an explicit function of reservoir energy. The probability of this state p_A:

$$p_A \propto \exp[\ln W_r(U_0 - \varepsilon_A)] \approx \exp\{\ln W_r(U_0) - [(\partial \ln W_r/\partial U)_{U=U0} \times \varepsilon_A]\}$$

$$= \exp\{S(U_0)/k - [\varepsilon_A \times (\partial S/\partial U)/k]\} \quad (1.53)$$

The Taylor series approximation has been used. For a function f:

$$f(x - x_o) = f(x_o) + [(df/dx)_{x=xo} \times (x - x_o)] + \{½ \times [(d^2f/dx^2)_{x=xo} \times (x - x_o)^2]\} + \ldots$$

For $x - x_o << 1$, we can approximate $f(x - x_o)$ by the first two terms of the series. We do a similar calculation for molecule 1 with energy ε_B:

$$p_B \propto \exp\{S(U_0)/k - [\varepsilon_B \times (\partial S/\partial U)/k]\} \quad (1.54)$$

The ratio of probabilities:

$$p_B/p_A = \exp[-\Delta\varepsilon \times (\partial S/\partial U)/k] \quad (1.55)$$

Comparing Eqs. 1.51 and 1.55:

$$\beta = (\partial S/\partial U) \quad (1.56)$$

This is a very significant result and tells us that the probabilities are based on the parameter $(\partial S/\partial U)$, the ratio of the change in total entropy with respect to the change in total energy or equivalently the change in the logarithm of multiplicity with the change in energy. The Boltzmann constant k also enters into the expression but to my knowledge the numerical value of this constant cannot be derived from probability arguments.

Now consider another property of $(\partial S/\partial U)$. Consider two protein solutions which are in thermal contact; i.e. they can exchange energy. The two solutions cannot exchange molecules and are thermally isolated from their surroundings. The solutions will exchange energy to maximize multiplicity or equivalently total entropy. Consider that solution 1 has initial energy U_1, solution 2 has initial energy U_2, and the total entropy $S = S_1(U_1) + S_2(U_2)$. Consider a small quantity of energy dU transferred from solution 2 to solution 1 and the new total entropy:

$$S = S_1(U_1 + dU) + S_2(U_2 - dU) \quad (1.57)$$

Because there is now more energy in solution 1, we would expect that it will be possible to have more protein molecules with strand conformation and hence higher multiplicity and entropy in solution 1. Equivalently, there will be lower multiplicity and entropy in solution 2. Using Taylor series expansions:

$$S = S_1(U_1) + S_2(U_2) + (\partial S_1/\partial U)_{U=U1}dU - (\partial S_2/\partial U)_{U=U2}dU \quad (1.58)$$

Consider:

$$dS = (\partial S_1/\partial U)_{U=U1}dU - (\partial S_2/\partial U)_{U=U2}dU \quad (1.59)$$

Energy exchange will occur until S is maximized; i.e. dS = 0:

$$(\partial S_1/\partial U)_{U=U1} = (\partial S_1/\partial U)_{U=U2} \quad (1.60)$$

This equation is showing that "equilibrium" (i.e. no more exchange of energy) and maximum total entropy are equivalent to the notion that transfer of a small amount of energy from solution 1 to solution 2 results in an increase in entropy of solution 1 and decrease in entropy of solution 2 of equal magnitudes. Note that it is quite possible that at equilibrium, $U_1 \neq U_2$; i.e. equal energies of the two solutions is not a criterion for equilibrium.

We also know from experience that energy exchange will occur until the temperatures (T) are equal. The derivative $(\partial S/\partial U)$ has units of inverse Kelvin which minimally suggests $1/T \propto (\partial S/\partial U)$. In fact, the value of k is calculated from the ratio of the ideal gas constant to Avogadro's number and the value of the ideal gas constant was determined from R=pV/nT where T is expressed in Kelvin units. So, in Kelvin units:

$$1/T = (\partial S/\partial U)$$
$$T = (\partial U/\partial S) \quad (1.61)$$

In this example, it was assumed that there were only two states and energies for the residue; i.e helical or strand conformation. The more general case is that there are a total of t states. The more general expressions are given in DB Eqs. 6.16 – 6.18 and VJH Eqs. 2.9 – 2.11. For this more general case, the partition function:

$$q = \sum_{j=1}^{t} \exp(-\varepsilon_j/kT) \quad (1.62)$$

This is the sum of the relative probabilities of all of the states. Eq. 1.50 is the specific application of Eq. 1.62 to a two-state system. In VJH, the sums are over energy levels rather than states and each energy level has a degeneracy g associated with it with value equal or greater than 1. Using the DB approach of sum over states rather than energy energy levels, the probability p_j that an individual molecule is in state j:

$$p_j = \exp(-\varepsilon_j/kT)/q \quad (1.63)$$

Eqs. 1.49 are the specific application of Eq. 1.63 to a two-state system. The average energy per molecule:

$$\langle\varepsilon\rangle = \sum_{j=1}^{t} p_j \times \varepsilon_j = kT^2 \times (\partial \ln q/\partial T) \quad (1.64)$$

Any other average molecular property could be determined with an expression similar to Eq. 1.64. Eqs. 1.62 – 1.64 are consistent with our intuition that as the temperature increases, higher energy states are more likely to be populated and the average energy per molecule is larger. These higher populations correlate with higher reactivity at higher temperature.

It is instructive to think about Eq. 1.61. We generally associate total energy and temperature but Eq. 1.61 shows that there is a more profound interpretation of temperature. Temperature is associated with maximizing total entropy under the constraint of fixed total energy.

It is probably easiest to think about $1/T = (\partial S/\partial U)$, i.e. the ratio of the increase in S per unit increase in U. Consider our protein solution at very low T with helical protein molecules (A) and strand protein molecules (B). From Eqs. 1.62 – 1.64, $p_A \approx 1$, $p_B \approx 0$, and $\langle\varepsilon\rangle \approx \varepsilon_A$; i.e. nearly all molecules are helical. Because of this restriction, there are relatively few different ways of arranging the molecules and the multiplicity and entropy are small. However, Eq. 1.61 means that small inputs of energy give large increases in multiplicity and entropy.

By contrast, at high T, $p_A \approx 0.5$, $p_B \approx 0.5$, and $\langle\varepsilon\rangle \approx (\varepsilon_A + \varepsilon_B)/2$. This is equivalent to the case initially discussed in this section and maximum multiplicity and entropy are achieved because of equal numbers of helical and strand molecules (cf. Eq. 1.17). However, Eq. 1.61 means that input of energy yields a very small increase in entropy. This can be understood by considering that with nearly equal helical and strand populations, the solution is close to the maximum possible multiplicity and entropy.

In summary, as T↑, U↑, S↑, but $(\partial S/\partial U)\downarrow$.

1.C. Classical thermodynamics

The properties of our protein solution are specified by a few parameters: U, S, V (volume), and N (number of protein molecules). By properties, we mean the average or sum over the molecules in the solution rather than the property of any single molecule in the solution. Eq. 1.22 shows that for a large number of equivalent molecules, an average property can be very well-defined.

U, S, V, and N are dependent on one another such that there are general equations:

$$U = U(S,V,N)$$
$$S = S(U,V,N) \qquad (1.65)$$

The most important parameters are typically changes in U or S and a general equation can be written for dU:

$$dU = (\partial U/\partial S)_{V,N}\, dS + (\partial U/\partial V)_{S,N}\, dV + (\partial U/\partial N)_{S,V}\, dN \qquad (1.66)$$

where the subscripts mean that these parameters are held constant while the one other parameter is varied. In subsequent equations, these subscripts are not shown. In Eq. 1.61, we proposed that $(\partial U/\partial S) = T$. Now we propose:

$$-(\partial U/\partial V) = p \text{ or pressure}$$
$$(\partial U/\partial N) = \mu \text{ or the “chemical potential”} \qquad (1.67)$$

The pressure part of Eq. 1.67 makes sense because energy/volume ≡ energy/(distance × area), force ≡ –energy/distance, and pressure ≡ force/area. Eq. 1.66 can be rewritten: