1. (10 points) Circle ALL correct answers, or fill in the blank, as appropriate.
   a) A reversible process is one that proceeds by a succession of very small incremental steps, all of which are (at equilibrium, spontaneous, enthalpically and entropically driven).
      
   b) The heat needed to change the phase of a substance is typically (smaller / larger) than the heat needed to increase its temperature by 10 K.
      
   c) \( \Delta H, \Delta S, \Delta G, \Delta E \) implies constant pressure.
      
   d) An ideal gas expands adiabatically against an external pressure of 2 atm. \( \Delta E \) for the system is (greater than zero / equal to zero / less than zero).
      
   e) An ideal gas expands isothermally against an external pressure of 2 atm. \( \Delta E \) for the system is (greater than zero / equal to zero / less than zero).
      
   f) An ideal gas expands adiabatically into a vacuum. \( \Delta E \) for the system is (greater than zero / equal to zero / less than zero).
      
   g) When a sample of liquid is converted reversibly to its vapor at its normal boiling point, \( (q, w, \Delta P, \Delta V, \Delta T, \Delta E, \Delta H, \Delta S, \Delta G) \) is equal to zero for the system.
      
   h) For a process that can be carried out by either a reversible or an irreversible path, the change in \( (P, T, V, q, E, H, w) \) must be the same for both paths.
      
   i) If the frictional coefficient of a protein doubles, but its molecular weight and partial specific volume remain the same, \( s \) (doubles, is halved, quadruples, remains the same).
      
   j) All other things being equal, the unhydrated form of a protein will show a (higher / lower / the same) diffusion coefficient compared with the hydrated form of the same protein.
      
2. (10 points) At a scientific conference on gene regulation some time back, a colleague was describing a system which he studied. It seems that there is a signaling molecule in the cell which undergoes phosphorylation as a part of a particular signaling pathway. My colleague told me that the enzyme he studies catalyzes the phosphorylation of this signaling molecule,
and that when a special cofactor binds to his enzyme, the enzyme-cofactor complex now catalyzes the reverse reaction. A very cute signaling mechanism, but as stated, wrong. Why?

Enzymes do not influence the thermodynamic direction of a reaction (or the thermodynamics at all!). Reverse and forward reactions are accelerated to the same extent.

3. (10 points) Imagine a very simple model for a cell, a 1 µm spherical membrane enclosing a volume of 0.50 pL (1 pL = 10^{-12} L). The membrane is permeable to water and salts, but not to proteins. The “cell” contains 600,000 molecules of trypsin and 300,000 molecules of trypsin inhibitor (a small protein). NaCl is present at 10 mM throughout, the temperature is 37°C, and the ambient pressure is 1.0 atm.

The key to this question is remembering that it is the difference in concentration of solutes on two sides of a membrane that gives rise to osmotic pressure. The membrane is permeable to NaCl (salt) and so its concentration is the same on each side. It does not enter into the calculation. This is a key concept!

a) Assuming that the proteins do not interact, what is the osmotic pressure in the cell? Is the pressure higher inside or outside?

\[
\Pi = cRT = (3.0 \times 10^{-6} M)(0.08206 \text{ L atm K}^{-1} \text{ mole}^{-1})(310K) \frac{1 \text{ mole}}{M} \frac{1}{L} = 7.6 \times 10^{-5} \text{ atm}
\]

The pressure is higher inside (1.000076 atm).

b) Assuming that trypsin inhibitor binds trypsin with a binding constant of 1.0 \times 10^9 M^{-1}, what is the osmotic pressure inside the cell? Is it lower or higher than in part (c)?

Note that binding is very strong, such that it will be 99.99% stoichiometric (should you care to do the calculation). We must then re-work the concentrations of solutes as follows.

\[
[Tryp] = \frac{6 \times 10^5 \text{ molecules}}{0.50 \times 10^{-12} \text{L}} \frac{1 \text{ mole}}{6.02 \times 10^{23} \text{ molecules}} = 2.0 \times 10^{-6} \text{ M} = 2.0 \mu \text{M}
\]

\[
[Inhib] = \frac{3 \times 10^5 \text{ molecules}}{0.50 \times 10^{-12} \text{L}} \frac{1 \text{ mole}}{6.02 \times 10^{23} \text{ molecules}} = 1.0 \times 10^{-6} \text{ M} = 1.0 \mu \text{M}
\]

\[
[solute] = (2.0 \times 10^{-6} M) + (1.0 \times 10^{-6} M) = 3.0 \times 10^{-6} M
\]

\[
\Pi = cRT = (3.0 \times 10^{-6} M)(0.08206 \text{ L atm K}^{-1} \text{ mole}^{-1})(310K) \frac{1 \text{ mole}}{M} \frac{1}{L} = 5.1 \times 10^{-5} \text{ atm}
\]

The osmotic pressure is lower than in part (a).
4. (10 points) Consider the favorable reaction:

\[ E + S \rightleftharpoons E + P \]

On the energy level diagram at right, indicate the following.

\( E^\dagger \) (forward), \( E^\ddagger \) (reverse), \( \Delta G \)

5. (15 points) For an NMR transition using radio waves of 800 MHz, what is the energy spacing between ground and excited states in J mol\(^{-1}\)? Remember that MHz = \(10^6\) s\(^{-1}\) and that the energy levels are not degenerate.

Although an “NMR” question, this really deals with concepts from previous chapters.

The first is the relationship for electromagnetic radiation (radio waves) that says \( E = hv \). That’s all you needed here. The “NMR” equation relating magnetic field strength (Bo) to E is not relevant to this question.

\[
\Delta E = \hbar v = \left(6.6262 \times 10^{-34} \text{ J s}\right) \left(800 \times 10^6 \text{ s}^{-1}\right) \frac{6.02 \times 10^{23} \text{ mole}}{\text{mole}} = 0.32 \text{ J}
\]

Calculate the equilibrium population ratio between ground and excited states for an NMR transition of 500 MHz at 298K. Report the ratio to 5 significant figures.

The second concept is the relationship between equilibrium populations and energy gaps – Boltzmann!!.

\[
\frac{N_i}{N_j} = \frac{g_i e^{-\frac{E_i}{kT}}}{g_j e^{-\frac{E_j}{kT}}} = e^{\frac{E_i - E_j}{kT}} = e^{\frac{-(E_i - E_j)}{RT}} = e^{\frac{-0.32 \text{ J mol}^{-1}}{(8.314 \text{ J K}^{-1} \text{ mol}^{-1})(298 \text{ K})}} = 0.99987
\]
6. (15 points) Try to stay in space allotted.
   a) You have a protein which binds ligand “L” and you discover that when you add the ligand to a solution of your protein, the fluorescence of the protein increases as shown at right.

What thermodynamic parameter for binding of ligand to your protein can you readily discern from this plot? What is its value?

\[ K_d = 0.05 \, \text{µM} \]

Noting that the concentration of ligand at which the binding site is half-saturated is \( K_d \), we can visually inspect the above and see that the change is half over at 0.05 µM ligand (the change is 3 units, so half maximal is a fluorescence of 2+1.5=3.5 fluorescence units).

b) A friend of yours does a similar titration with a different protein and a different ligand and gets the results shown at right. Can she do the same kind of analysis that you just did? Explain.

The above analysis assumes that ligand is in large excess over protein. This is true above, but looking at the data in part (b), we see that most of the titration is occurring from (0.2-1.0)x10^-3 µM, in other words, from 0.2 to 1 nM ligand. This is in a titration of 1 nM protein. The requirement underlying the (“concentration at half max”) equation is not satisfied.

You can’t be expected to figure it out, but the above titration actually follows a system with a \( K_d = 0.05 \) nM, not 0.5 nM. Off by a factor of ten!
c) Returning to the system in part (a) above, you now add different concentrations of NaI to the solution before starting your titration. The titrations appear as at right. What does this tell you?

You can see at right that NaI is not weakening binding, else you would need higher [L] to saturate. Rather NaI is quenching fluorescence. This is evident by a decrease in fluorescence which depends on [NaI]. The result suggests that ligand binding makes the Trp residue(s) in the protein exposed to solvent, and therefore exposed to the quencher, since in the absence of L, NaI does not lead to any quenching (the Trp is/are buried inside the protein).

7. (15 points) Consider a one dimensional box of length 15.0 Å, containing four electrons.

a) Calculate the energy of the ground state of the system. Remember that there are four electrons.

\[ E_n = \frac{\hbar^2 n^2}{8ma^2} = \frac{(6.6262 \times 10^{-34} \text{ J s})^2}{8(9.11 \times 10^{-31} \text{ kg})(15 \times 10^{-10} \text{ m})^3 n^2} \]

\[ E_n = 2.68 \times 10^{-20} \text{ J} \]

So adding the energies for each of the four electrons, we have

\[ E_{\text{Total}} = E_1 + E_1 + E_2 + E_2 = (2.68 \times 10^{-20} \text{ J})(1^2 + 1^2 + 2^2 + 2^2) = 2.68 \times 10^{-19} \text{ J} \]

b) Calculate the energy of the transition from the ground state to the first excited state.

\[ \Delta E_{2\rightarrow3} = E_3 - E_2 = (2.68 \times 10^{-20} \text{ J})(3^2 - 2^2) = 1.34 \times 10^{-19} \text{ J} \]

c) Calculate the wavelength of the transition in nanometers.

\[ E = \frac{hc}{\lambda} \quad \lambda = \frac{hc}{E} = \frac{(6.6262 \times 10^{-34} \text{ J s})(3.0 \times 10^{-8} \text{ m s}^{-1})}{1.34 \times 10^{-19} \text{ J}} = 1.48 \times 10^{-6} \text{ m} = 1480 \text{ nm} \]
8. (15 points) Consider the simple Michaelis-Menten mechanism for an enzyme-catalyzed reaction:

\[
E + S \overset{k_1}{\underset{k_{-1}}{\rightleftharpoons}} ES \overset{k_2}{\rightarrow} E + P
\]

The following data were obtained:
k_1 and k_{-1} very fast.
k_2 = 100 s\(^{-1}\) \(K_m = 1.0 \times 10^{-4}\) M at 280 K
k_2 = 200 s\(^{-1}\) \(K_m = 1.5 \times 10^{-4}\) M at 300 K

a) For [S] = 0.20 M and [E]₀ = 1.0 \times 10^{-6}\) M, calculate the rate of formation of product at 280 K.

\[
u_o = \frac{k_2 [E]_o}{1 + \frac{K_m}{[S]}} = \frac{(100 \text{ s}^{-1})(1.0 \times 10^{-6}\) M)}{1 + \frac{1.0 \times 10^{-4}\) M}{0.2 \) M} = 9.995 \times 10^{-5}\) M s\(^{-1}\) = 1.0 \times 10^{4}\) M s\(^{-1}\)

b) Calculate the activation energy for k_2.

\[
k = Ae^{\frac{E_a}{RT}}\quad \text{leads to} \quad \frac{k_2}{k_1} = Ae^{\frac{E_a}{RT_2} - \frac{E_a}{RT_1}} = e^{\frac{E_a}{RT_2} - \frac{E_a}{RT_1}}\]

\[
\ln \frac{k_2}{k_1} = -\left(\frac{E_a}{RT_2} - \frac{E_a}{RT_1}\right) = -\frac{E_a}{R} \left(\frac{1}{T_2} - \frac{1}{T_1}\right)
\]

\[
E_a = -R \ln \frac{k_2}{k_1} = \left(\frac{8.314 \text{ J mol}^{-1}\text{K}^{-1}}{100 \text{ s}^{-1}}\right) \ln \frac{200 \text{ s}^{-1}}{100 \text{ s}^{-1}} = 2.4 \times 10^{4}\text{ J mol}^{-1} = 24 \text{ kJ mol}^{-1}
\]

c) What is the sign and magnitude of the standard thermodynamic enthalpy (ΔH°) for the formation of ES from E and S?

We need the equilibrium (NOT kinetic) K’s.
Note that this is a pre-equilibrium situation, so \(K_m = K_d = K^{-1}\)

\[
\ln \frac{K_m}{K_1} = -\frac{\Delta H^o}{R} \left(\frac{1}{T_2} - \frac{1}{T_1}\right)
\]

\[
\Delta H^o = -R \ln \frac{K_m}{K_1} \ln \frac{K_m}{K_2} = -\left(\frac{8.314 \text{ J K}^{-1}\text{mol}^{-1}}{15 \times 10^{-4}\) M} \ln \frac{1.0 \times 10^{-3}\) M}{1 \times 300K - 1 \times 280K}\right) = 14.1 \text{ kJ mol}^{-1}
\]
Extra credit questions:

Remember that concise, to the point answers are scored more highly than long, rambling answers, in which it appears that you are searching for an answer. Stay within the space provided.

(2 points) **Do proteins fold against entropy?** If an unfolded protein is a large collection of a large number of random states, why do proteins fold into a single unique structure? Do proteins have a special exemption from the second law?

This was discussed in class. No they do not. Unfolded proteins expose hydrophobic side chains to solution, which results in the ordering of water molecules around those side chains. Protein folding buries the hydrophobics, releasing all of the ordered water. The increase in the entropy of the water is larger than the decrease in entropy of the protein.

(2 points) **Fine tuning light absorption.** In the latter half of the course, we saw how it is that nature builds molecules which absorb in the visible spectrum (benzene and other small molecules absorb in the UV). Porphyrins are big, naturally occurring molecules with absorbances in the visible spectrum. Recently some chemists took a cue from nature and, using porphyrins as building blocks, built molecules which absorb in the infrared. 1) How do you think they achieved this? What would be your design criteria and why?

Build a bigger box! This can be achieved by linking together multiple porphyrins. But this must be done in a way which links the π systems (ie, via double bond linkages set up appropriately.

(1 point) **Electrical conductor.** In the above experiment, the real goal was not making an absorber, but creating a good conductor of electricity. 2) With this goal in mind, explain why they did what they did.
In General Chemistry texts you will find that conductors work by having very small separations between the highest occupied orbital and the lowest unoccupied orbital. Thus an electron can hop up out of the highest orbital and then move through the empty unoccupied orbital. A small energy separation makes a small barrier for this hopping. A bigger box has smaller spacing between energy levels.