BE 25 Winter 2024
Homework #7
Due at 9 AM PST, February 29, 2024

Problem 7.1 (Stretching a single molecule, 40 pts)**.**
This problem is based on problem 9.14 from Dill and Bromberg. In single molecule experiments, researchers can grab on to the ends of single polymer molecules and pull on them. They typically apply a constant force f on the ends of the polymer (that is *total* force, not force on each end), and then measure the distance L between the ends of the polymer. This is commonly done for biopolymers, including proteins and DNA.

a) Write an expression $\mathrm{d}E$ for the total differential of the energy $E(S, V, L)$.

b) Write down the total differential for a thermodynamic potential $\Phi(T, p, L)$.

c) Use this potential to derive a Maxwell relation involving $(\partial S/\partial L)_{T,p}$.

d) By doing an experiment at various temperatures, we can use the Maxwell relation you derived to learn about the entropic effects of the polymer. For small displacements, we can write the force as a function of displacements to linear order in L (like a Hookean spring); $f = k(T, p)\, L$, where the spring constant is in general dependent on temperature and pressure. Operating at constant pressure, an experimenter find a linear dependence on temperature, $k = a_0 + a_1 T$. With this empirically determined relation, derive an expression for the entropy $S(L)$ of the polymer at fixed temperature and pressure.

e) Derive an expression for the enthalpy $H(L)$ of the polymer.

f) Show that if $a_0 = 0$, the resistance to stretching is entirely entropic.

Problem 7.2 (Convex Gibbs free energy, 15 pts)**.**
Prove that the Gibbs free energy of a dilute solution is a convex function of all of the n_i's, where n_i is the number of particles of solute i in the solution. This means that there exists a unique equilibrium concentration of solute species and thermodynamic stability is always maintained. (This is true in for *dilute* solutions, but is not generally true.)

Problem 7.3 (Turgor pressure and hypoosmotic shock, 10 pts)**.**
When a cell is suddenly moved from an environment with a given concentration of solute to on with a lower concentration, it is said to experience hypoosmotic shock. The osmotic pressure in the cell jumps and the cell may rupture. Cells have mechanisms for dealing with such shocks, including mechanosensitive ion channels, which open to allow the outflow of solute to help bring the osmotic pressure down. In a study of cellular responses to osmotic shock, Chure and coworkers (Chure, et al., *J.*

Bacteriol., **200**, e00460-18, 2018) used a microfluidic device to transfer cells at 37°C from an LB solution with 500 mM NaCl to an LB solution without any NaCl. Unperturbed, *E. coli* cells have an NaCl concentration of a few hundred mM (Szatmári, et al., *Sci. Reports*, **10**, 12002, 2020). What is the approximate osmotic pressure (which is called turgor pressure in the context of bacterial cells) when the cells are suddenly put in the salt-free solution? How does this pressure compare to the typical turgor pressure of unperturbed cells of about 30 kPa as measured by Deng, et al., *Phys. Rev. Lett.*, **107**, 158101, 2011? (Note that the Deng, et al. measurement is smaller than other measurements, which put the turgor pressure around 1–3 atm.) *Suggestion:* Do not use a calculator or computer for this. Estimates are much more fun without them!

Problem 7.4 (Inhibition of binding with full solution, 35 pts)**.**
In Box 12.1, Kuriyan, Komforti, and Wemmer consider the case where a protein may bind a ligand or an inhibitor. That is, the following two chemical reactions are possible.

$$\mathrm{PA} \rightleftharpoons \mathrm{P} + \mathrm{A}, \tag{7.1}$$

$$\mathrm{PB} \rightleftharpoons \mathrm{P} + \mathrm{B}. \tag{7.2}$$

We will define $K_{\mathrm{d,A}}$ as the dissociation constant for the binding of the protein with ligand A and $K_{\mathrm{d,B}}$ as the dissociation constant for the binding of inhibitor B with the protein. (This is different notation than KKW use.) They derive that, at equilibrium,

$$\text{activity} = \zeta\, c_{\mathrm{PA}} = \zeta\, c_{\mathrm{P}}^0\, \frac{c_{\mathrm{A}}/K_{\mathrm{d,A}}}{1 + c_{\mathrm{B}}/K_{\mathrm{d,B}} + c_{\mathrm{A}}/K_{\mathrm{d,A}}}, \tag{7.3}$$

where ζ is some constant of proportionality between activity and concentration of ligand-bound protein.

a) Assume there is another inhibitor C such that a third reaction, $\mathrm{PC} \rightleftharpoons \mathrm{P} + \mathrm{C}$ with dissociation constant $K_{\mathrm{d,C}}$ takes place. Derive a similar expression as above in this case. That is, derive an expression for c_{PA} in terms of the total protein concentration c_{P}^0 and c_{A}, c_{B} and c_{C}, and the dissociation constants.

b) These expressions are all fine and good, but when we study protein-ligand-inhibitor binding, we often do so in a test tube, where we pipette in pure amounts of each component. That is, we know c_{P}^0, c_{A}^0, c_{B}^0, and c_{C}^0. Solving for c_{PA} in this case, that is in terms of the *total* concentrations of all ligands and inhibitors rather than their equilibrium concentrations, is much more difficult. Fortunately, we can do so numerically. EQTK is a software package to do just that. Imagine you are doing an experiment where you fix the total concentrations of P, A, and B to 1 µM, 0.5 µM, and 1 µM, respectively. You titrate in C, such

that c_C^0 ranges from zero to 100 µM. Use $K_{d,A} = 0.001$ µM, $K_{d,B} = 0.003$ µM, and $K_{d,C} = 0.005$ µM. Use EQTK to compute the fraction of protein P that is bound to A and plot the titration curve. *Hints:* You should be sure you have the most up-to-date version of EQTK, which you can do by running `pip install --upgrade eqtk` on the command line. You should also be able to do the calculations just by reading the "Quick start" in the EQTK docs, found at https://eqtk.github.io/.