Assignment #5 – Advanced topics in macromolecular linked equilibria

Readings:

Handout on Wyman linkage relations

Problems, due Apr 14

1. The MWC model of allosteric control postulates that the $T_o <--> R_o$ equilibrium is described by a single dimensionless number $L$, a conformational equilibrium constant that must be very large to give good cooperativity. Derive for yourself that for a 3-site MWC protein binding ligand $x$ (with microscopic constant $k$):

$$v(x) = 3kx(1+kx)^2/[L+(1+kx)^3]$$

Plot this function for $L=0$, $L=1$, $L=10$, $L=1000$. (Note: In order to get a sense of the shape of the curves, you'll have to figure out over what range of $x$ you need to make the plots.)

Now, suppose $L$ is itself controlled by an allosteric inhibitor $Y$ because single $Y$ can bind ONLY to the $T_o$ state (with binding constant $k'$). The protein follows the "extended" MWC equilibrium:

$$k' L_o$$

$Y:T_o -> T_o -> R_o -> RX_1 -> RX_2 -> RX_3$

a. Show that the equation in problem #1 still holds, but that now

$$L=L_o(1+k'y)$$. These equilibria are complicated, so use the Q-method!

b. Suppose that $L_o=1$. (What does this mean?) Show that for this protein to give strong cooperative behavior, $Y$ must be present at concentrations much higher than its dissociation constant.

c. Derive an expression for the variation in bound $X$ as the concentration of $Y$ is varied at fixed $X$ concentration. Show that this is the SAME as the variation in bound $Y$ and with $x$, at fixed $y$. Why must these be the same? Hint: consider the “variation of bound $X$ with $Y$ conc” to be:

$$\frac{\partial v_x}{\partial n_y}$$

d. Can you think of any biological situations in which a case like this might operate?

2. Use the Q-method to derive eq (5) in the assigned paper by Ames et al.
3. The tryptophan-repressor protein (TrpR) provides a well-studied example of a DNA-binding protein interacting with a specific sequence on double-stranded DNA. Here are some facts.

1. The recognition sequence is palindromic, so that the DNA target presents 2 identical sites for TrpR.
2. TrpR binds to these two identical sites in a highly cooperative fashion, with the second molecule binding much more tightly than the first.
3. TrpR is able to bind only to double-stranded DNA, and only when its tryptophan-binding site is occupied.

a. What do you think underlies the high co-operativity in the binding of TrpR to DNA?

b. Let the dissociation constant for the first TrpR to bind is $K_1$; then, assuming that the second binding is of 100-fold higher affinity, derive an expression for the fractional occupancy of the DNA as a function of TrpR concentration. Plot this function, and compare it to an expression derived by assuming that the second binding is infinitely higher affinity than the first.

4. Aequorin (Aq) is a light-emitting protein found in a Pacific Ocean luminescent jellyfish that works the night shift. This protein has a useful property: the intensity of emitted light is a function of Ca concentration. This is because the protein has three identical Ca-binding sites, and that somehow, binding of Ca is linked to conformational changes leading to an "activated" luminescent form of Aq.

This problem asks you to consider three different ways in which Ca binding might drive the conformational changes. For each of these ways, you must do 3 things: (1) derive an equation for the relative light intensity as a function of Ca concentration, by clearly showing the equilibria involved, (2) plot this function on a linear-linear scale, (3) construct a ‘Hill plot’ of this function. Don't be over-precise: no more than 2 significant figures.) It’s OK to use computer programs to make these graphs (this really is NOT a course in arithmetic...)

a. Suppose the 3 Ca ions bind to the protein independently, with binding constant $k$, but that only the triply-liganded protein emits light.

b. Suppose the 3 Ca ions bind independently as above, and that the light emitted by a given protein molecule is directly proportional to the number of Ca ions bound to that molecule.

c. Suppose that after the first Ca binds with a binding constant $k$, the protein flips into a light-emitting conformation which increases the affinity of the remaining two sites a zillion-fold.

After you've done all this, state clearly how the three situations are experimentally distinguishable.
5. An ion channel may be blocked from the inside by blocker X and from the outside by blocker Y. These blockers bind reversibly to separate sites on the respective sides of the pore and plug it up; thus, only "unblocked" channels conduct ions. Each of these blocking reactions may be studied separately by applying the blocker, at varying concentrations, on the proper side, and determining its own dissociation constant, $K_X$ or $K_Y$, from the resulting "falling langmuir" inhibition curve.

After failing even to crystallize MgCl$_2$, Chris Catastrophe, a grad student in the Genick lab, decides to do some electrophysiological work, wondering whether the two blockers can plug up the channel simultaneously when added on both sides of the membrane. First, he determines that $K_X = 1$ mM and $K_Y = 0.1$ mM when measured separately. Then, he varies the concentration of internally applied X in the presence of a fixed external Y concentration of 0.3 mM. The experiment gives a nice falling langmuir inhibition curve, with an "observed dissociation constant of X," $K_{X_{\text{obs}}}$.

a. Draw a thermodynamic box describing the channel in its various unblocked and blocked states, and derive an equation describing the inhibition of channel activity in Chris's experiment.

b. What can you conclude, using this equation, if CC finds that:

- $K_{X_{\text{obs}}} = 1$ mM
- $K_{X_{\text{obs}}} = 3.5$ mM
- $K_{X_{\text{obs}}} = 4$ mM
- $K_{X_{\text{obs}}} = 0.3$ mM

c. In each case above, calculate the apparent standard-state free energy of binding of X to the channel already blocked by Y. Compare these to the free energy of binding of X to the unblocked channel, to get an idea of the nature of the "interaction" between the two blockers on the same channel.

d. Do problem #c for $K_{X_{\text{obs}}} = 5$ mM, and show that this is an "impossible" situation. Explain why it could never happen.
6. Calbindin is a small globular protein that is a homodimer, and that can undergo a conformational change as pictured below. Each of the identical subunits has a binding site for Ca\(^{2+}\). In the "T" state, the Ca\(^{2+}\)-binding sites are disrupted so that effectively they don't exist, but the transition to the "R" state creates the two sites. The "intrinsic" binding constant to the individual, isolated site is \(k_o\).

However, the 2 Ca\(^{2+}\) ions do not bind independently of one another in the R-state. This is because Ca\(^{2+}\) is charged, and the two sites are actually quite close together -- only 7 Å apart -- in the dimer. Thus, there is an electrostatic repulsion between the Ca\(^{2+}\) ions when they are both on their sites. This energy of electrostatic repulsion has a value \(\epsilon\) (kcal/mol).

a. Draw out a scheme for the linked equilibria involved here.

b. Suppose that the only form of the protein that has "activity" is the form with 2 Ca\(^{2+}\) ions bound. Derive an expression for how the protein's activity will vary with Ca\(^{2+}\) concentration, in terms of \(L_o\), \(k_o\) and \(\epsilon\).

c. Assuming that \(L_o = 100\), give a rough sketch of the activity as a function of [Ca\(^{2+}\)], and compare this to what this would look like if there were no electrostatic repulsion. Use a value of \(\epsilon\) that you calculate explicitly from coulombic repulsion.

7. Human growth hormone receptor (R) is an integral membrane protein that acts as the initiation point in the elaborate, complicated cellular proliferation response to growth hormone. Its structure is known, as is its mode of action, which is a "dimerization" mechanism. Monomeric R is randomly distributed and mobile in the plasma membrane. A single growth hormone molecule -- let's call it "L" for ligand -- binds with high affinity to two R molecules, but with low affinity to only one. The ternary complex \(R_2L\) can be considered a "sandwich" structure in which the two R proteins are the slices of bread glued together by the growth hormone jam. When dimerized in this way, the ternary complex becomes "activated" by autophosphorylation, and then an elaborate cascade of events occurs leading to cell growth. The binary complex, RL, is totally inactive in leading to these downstream responses.

a. Set up the appropriate equilibria to solve for the concentration of ternary complex, \(C\), as a function of total receptor concentration in the membrane, \(R_T\), and free circulating growth hormone, in the blood L. You may assume that the concentration of binary complex, RL, is negligibly low.

b. It is found that the response of a given cell to growth hormone is very sensitive to the expression level of receptor in that cell. Why is this, both intuitively, and according to your answer in (a)?

*Hint: Do not try to use the Q-method here -- it won't work! Just set up the equilibria involved, take a deep breath, and plow ahead.*