A Fundamental Wyman Linkage Relation

It is nearly impossible to find a biological function that does not ultimately depend on the binding of a ligand to a macromolecule and a concomitant conformational change of that macromolecule. (Think of 20 examples off the top of your head.) The particular way ligand binding is linked to conformational change -- the linkage model -- will differ for different examples. But there is an extremely general, model independent formulation of binding-conformational linkage that everyone should be aware of: the Wyman linkage relation. Its basic message, in words, is this: binding of a ligand will drive a conformational change if and only if the ligand binds more to one conformation than to the other.

We define several variables describing the situation. Let the macromolecule exist in 2 conformations, A and B, and let there exist n binding sites for ligand X on the macromolecule; in general these sites are not identical. Furthermore, we accept the possibility that the values of the ligand binding constants are different in the two conformations. This is described by the scheme:

\[
\begin{array}{c}
L_0 \\
A_0 \longrightarrow B_0 \\
K_{A1} | \quad | \quad K_{B1} \\
AX_1 \longrightarrow BX_1 \\
| \quad | \\
AX_2 \longrightarrow BX_2 \\
\vdots \\
AX_i \longrightarrow BX_i \\
\vdots \\
AX_n \longrightarrow BX_n \\
L_n
\end{array}
\]

We will define two kinds of equilibrium constants (illustrated above)

- **Conformational equilib const** \( L_i = [BX_i] / [AX_i] \)
- **Ligand binding const** \( K_{Ai} = [AX_i] / (x [AX_{i+1}]) \),

where \( x = \) ligand concentration
We further define an “observed” conformational equilibrium constant, something you would determine from an experimental measurement on the system. For instance, if the B conformation has a certain measurable activity (regardless of how many X ligands are bound), but the A conformation is inactive, then the measured “activity” would reflect the balance between all the B-forms and all the A-forms:

\[ L_{\text{obs}} = \frac{\sum_i [B X_i]}{\sum_i [A X_i]} \]

We also define the average number of ligands bound per macromolecule in the A conformation:

\[ \bar{v}_A = \frac{\sum_i i [A X_i]}{\sum_i [A X_i]} \]

- and a similar definition for the B conformation

Given these definitions, and the general result (see Q-method handout) that

\[ \bar{v} = \frac{\partial n Q}{\partial n x} \]

it is easy to prove that:

\[ \frac{\partial n L_{\text{obs}}}{\partial n x} = \bar{v}_B - \bar{v}_A = \Delta \bar{v} \]

Think about it – this is fantastic! It says that, irregardless of the particulars of ligand binding, or of the values of the various equilibrium constants involved, raising the concentration of ligand X will “drive” the equilibrium towards B only if the number of ligands bound to the B conformation is greater than the number bound to the A conformation. (Conversely, if there are more ligands bound to A than to B, raising the ligand concentration drives the conformational equilibrium towards A.) You might wonder – if there are the same number, n, of X-binding sites on A and B, as in the scheme above, why would there be different numbers of ligands bound to the different conformations? Well, at very high x, when all the sites are saturated, there wouldn’t be – but at lower concentrations of x, below saturation, if the binding affinities of the sites differ in the different conformations, then there will be, on average, differential binding of X to the two conformations. In other words, this result shows that only if the ligand affinities change upon conformational change will ligand binding “drive” the conformational change. That’s a big, important conclusion.

This result says more. It says that if you would make a log-log plot of the observed equilibrium constant as a function of concentration, the slope of this plot at any point is the difference in the number of ligands bound in the two conformations. This is usually called a “Hill plot”, and the “Hill slope” is a measure of the upper limit of the number of ligand-binding sites. Do you now see why?