Statisical Themodyanmics Approach to ligand binding allostery

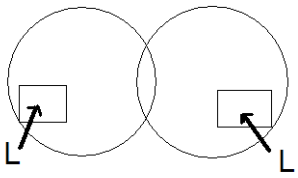

Figure 1: *Simple model for a dimeric protein binding a ligand "L"*

The partition function is the sum of the relative probabilities of all states. We can arbitrarily set the concentration of protein to be unity:

$$[P] = 1$$

$$\frac{[P_\alpha]}{[P][L]} = k$$

$$[P_\alpha] = k[L]; \quad [P_\beta] = k[L]$$

$$\frac{[P_{\alpha\beta}]}{[P_\alpha][L]} = k \;\Rightarrow\; [P_{\alpha\beta}] = k^2[L]^2$$

Note the the partition function is just the sum of the relative populations (concentrations) of all species:

$$Q = 1 + 2k[L] + k^2[L]^2 = q_0 + q_1\lambda + q_2\lambda^2 = \sum_{i=0}^{N} q_i\lambda^i$$

In general, Q will be a polynomial in the concentration of ligand; this is sometimes called the *binding polynomial*. Now, compute the fraction of binding sites that contain ligands:

$$f = \frac{\binom{0}{2}1 + \binom{1}{2}2k\lambda + \binom{2}{2}k^2\lambda^2}{1 + 2k\lambda + k^2\lambda^2} = \frac{1}{2}\frac{\sum i q_i\lambda^i}{\sum q_i\lambda^i}$$

$$= \frac{\lambda}{2}\frac{\sum i q_i\lambda^{i-1}}{\sum q_i\lambda^i} = \frac{\lambda}{2}\frac{\partial \ln Q}{\partial \lambda}$$

$$\boxed{f = \frac{\lambda}{N}\frac{\partial \ln Q}{\partial \lambda} = \frac{1}{N}\frac{\partial \ln Q}{\partial \ln \lambda}} \tag{1}$$

(See Appendix C in Dill and Bromberg.) Since this is *uncoupled* binding:

$$Q = 1 + 2k\lambda + k^2\lambda^2 = (1 + k\lambda)^2$$

Hence:

$$f = \frac{k\lambda}{1 + k\lambda} \qquad or \qquad \frac{f}{1 - f} = k\lambda \tag{2}$$

Now f is the fraction of sites that contain ligand; the average number of bound ligands per protein molecule (not per binding site) is then just $\nu = Nf$. [Very advanced: See Onufriev, Case, Ullmannn, *Biochemistry* **40**, 3413 (2001) for a generalization.]

Suppose we just have a simple acid-base equilibrium:

$$\begin{aligned} AH &\Leftrightarrow A^- + H^+ \\ Q &= 1 + k\lambda \end{aligned}$$

$$f = \lambda \frac{\partial \ln Q}{\partial \lambda} = \frac{\lambda k}{1+\lambda k}$$

Now, $k = 10^{pK_a}$ and $\lambda = 10^{-pH}$; hence:

$$f = \frac{10^{pK_a - pH}}{1 + 10^{pK_a - pH}} \tag{3}$$

This yields the usual sigmoidal binding curve.

Hemoglobin-like model

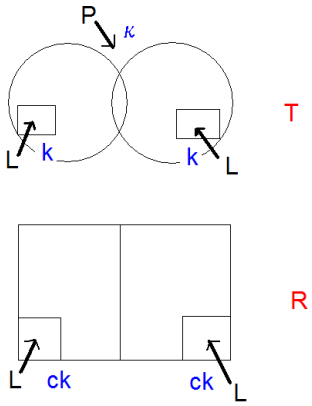

Figure 2: *A more complex model, binding two ligands, with a protein conformational change coupled to ligand binding.*

Now we consider the more complex model shown at the left. We can define some new constants, then make a table of relative probabilities:

$$\frac{[T^P]}{[T][P]} = \kappa; \quad \mu \equiv [P]$$

$$\frac{[R]}{[T]} = L$$

λ	T	T^P	R
0	1	$\mu\kappa$	L
1	$k\lambda$	$\mu\kappa k\lambda$	$Lck\lambda$
1	$k\lambda$	$\mu\kappa k\lambda$	$Lck\lambda$
2	$k^2\lambda^2$	$\mu\kappa k^2\lambda^2$	$Lc^2k^2\lambda^2$

Adding up all 12 elements of the Table gives the partition function:

$$Q = (1+k\lambda)^2(1+\mu\kappa) + L(1+ck\lambda)^2 \tag{4}$$

Now suppose we have no phosphate present, so that $\mu = 0$:

$$\begin{aligned} f &= \frac{1}{2}\frac{\partial \ln Q}{\partial \ln \lambda} = \frac{\lambda}{2Q}\left(\frac{\partial Q}{\partial \lambda}\right) \\ &= \frac{\lambda}{2Q}\frac{\partial}{\partial \lambda}\left[(1+k\lambda)^2 + L(1+ck\lambda)^2\right] \\ &= \frac{\lambda}{2Q}\left[2(1+k\lambda)k + 2L(1+ck\lambda)ck\right] \\ &= \frac{(1+k\lambda)k\lambda + L(1+ck\lambda)ck\lambda}{(1+k\lambda)^2 + L(1+ck\lambda)^2} \end{aligned} \tag{5}$$

If $L = 0$, get simple non-cooperative binding; for $L < 1$ and $c > 1$ (that is, T state is favored in the absence of ligand, but the R state has a higher affinity), get "hemoglobin-like" cooperative binding.

When $\mu > 0$, get a *linkage* between the binding of L and the binding of P. *See pp. 586-594 in Dill and Bromberg.*

Linkage relationships

The fraction f is often called $\bar{y}$. Suppose we have two different ligands, L and P, so that Q is a function of λ and μ:

$$N\overline{y_L} = \frac{\partial \ln Q}{\partial \ln \lambda}; \quad M\overline{y_P} = \frac{\partial \ln Q}{\partial \ln \mu}$$

$$\begin{aligned} d(\ln Q) &= \frac{\partial \ln Q}{\partial \ln \lambda} d\ln\lambda + \frac{\partial \ln Q}{\partial \ln \mu} d\ln\mu \\ &= N\overline{y_L} d\ln\lambda + M\overline{y_P} d\ln\mu \end{aligned}$$

or (*see pp. 593-594 in Dill and Bromberg*):

$$\boxed{\left(\frac{\partial \ln \lambda}{\partial \ln \mu}\right)_{y_P} = -\frac{M}{N}\left(\frac{\partial \overline{y_P}}{\partial \overline{y_L}}\right)}$$

Let $P = H^+$ and L=O_2 and $N = 4$:

$$\left(\frac{\partial \log[O_2]}{\partial pH}\right)_{\overline{y_{O2}}} = \frac{M}{4}\left(\frac{\partial \overline{y_{H+}}}{\partial \overline{y_{O_2}}}\right)_{pH} \simeq -(H^+_{deoxy} - H^+_{oxy}) \equiv -\Delta H^+$$

Here H^+_{deoxy} is the number of protons bound (per heme) in the deoxy form, similar for the oxy form. The figures below show one way this can work: In the top figure, values of ΔH^+ are measured as a function of pH. These results are then used in the bottom figure to integrate the affinity (setting $\bar{y_{O_2}} = 1/2$ to get a mean affinity. (Figures from Cantor & Schimmel, *Biophysical Chemistry*.)

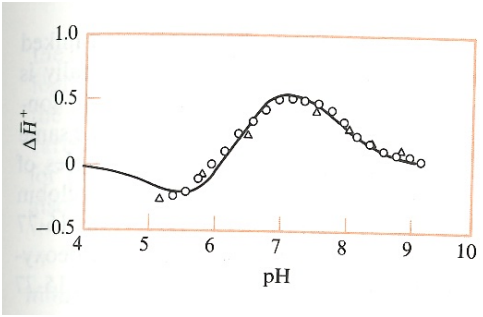

Figure 17-18

The effect of pH on $\Delta \bar{H}^+$ for human hemoglobin at 30°C. The curve is calculated from the constants in Table 17-2. The points correspond to two different types of experimental measurements. [After E. Antonini et al., *J. Biol. Chem.* 240:1096 (1965).]

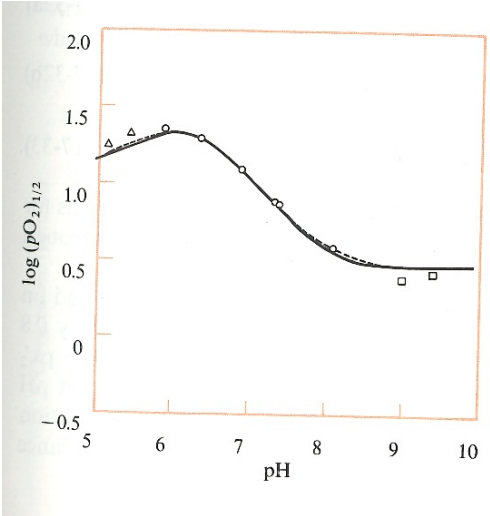

Figure 17-19

The Bohr effect in human hemoglobin at 30°C. The points are experimental values of log $(pO_2)_{1/2}$ obtained from oxygenation curves measured in three different buffer systems. The solid line is calculated from the constants in Table 17-2. The dashed line comes from a graphical integration of the data in Figure 17-18. [After E. Antonini et al., *J. Biol. Chem.* 240:1096 (1965).]