Statisical Themodyanmics Approach to ligand binding allostery

CQB 422/522, Statistical Mechanics, Spring 2021

1 A quick note about coarse-graining of models

Suppose we "coarse-grain" all states into "red" and "blue" subclasses. Then, since $A = -kT \ln Q$, we can write:

$$Q = \sum_i e^{-\beta E_i} = \sum_{i \subset red} e^{-\beta E_i} + \sum_{i \subset blue} e^{-\beta E_i} = e^{-\beta A_{red}} + e^{-\beta A_{blue}}$$

Further, remember that all the thermodynamic relations involve $\ln Q$, and not Q itself. So multiplying Q by a constant just adds a constant to A (and other thermodynamic parameters), which we can absorb into our choice of the zero of energy. So we are free to choose one coarse-grained state (say "red"), and set $A_{red} = 0$.

2 A supersimple model of ligand binding to a protein dimer

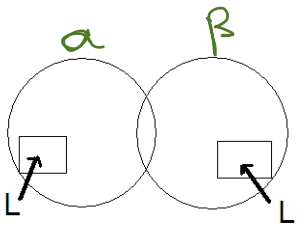


Figure 1: *Simple model for a dimeric protein binding a ligand "L"*

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

$$[P] = 1$$

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

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

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

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:

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

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

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

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

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

factorized Q characteristic of non-cooperative binding

Hence:

f is an average! $\langle f \rangle$ $\bar{f}$

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

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, Ullmann, *Biochemistry* **40**, 3413 (2001) for a generalization.]

Suppose we just have a simple acid-base equilibrium:

P_L P L

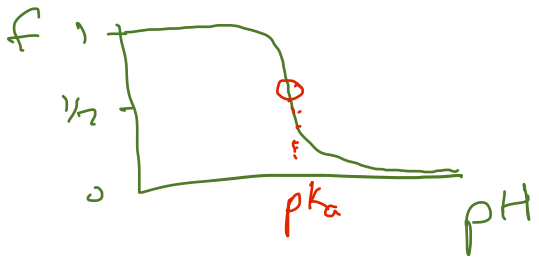
$$\begin{array}{rcl} AH & \Leftrightarrow & A^- + H^+ \\ Q & = & 1 + k\lambda \end{array}$$

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

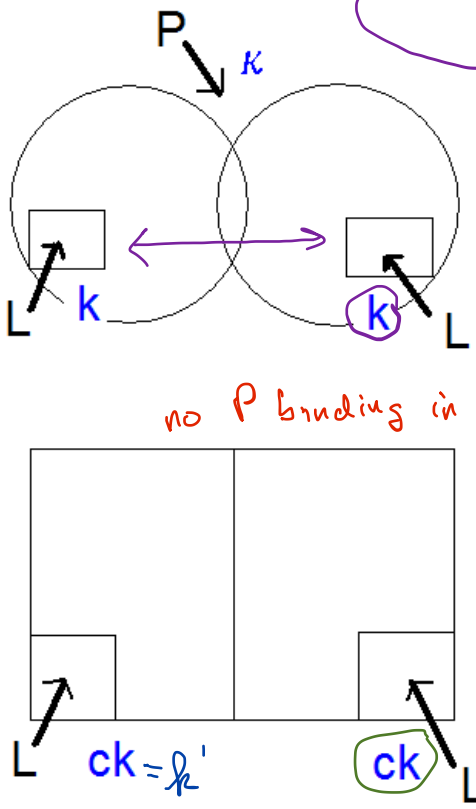
Now, $k = 10^{pK_a}$ and $\lambda = 10^{-pH}$; hence:

$$f = \frac{10^{pK_a - pH}}{1 + 10^{pK_a - pH}} = \frac{1}{1+1} = 1/2 \tag{3}$$

This yields the usual sigmoidal binding curve.



3 Hemoglobin-like model



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

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

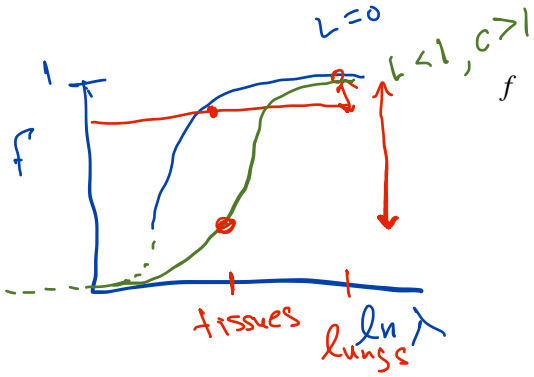
$$\frac{[R]}{[T]} = L$$

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 $\mu = 0$:



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

nothing to see for the form of this eq

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.*

bind many ligands of same type : homotropic
different heterotropic

4 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:

$f = \frac{1}{N}\left(\frac{\partial \ln Q}{\partial \ln \lambda}\right)$

$Q(\lambda, \mu)$

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

dependence of O_2 affinity on pH LHS

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

Maxwell relationship

Wyman-Gill linkage relationship

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

or in Slater

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

binding of O_2 → release of H^+ RHS

His

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

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

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*.)

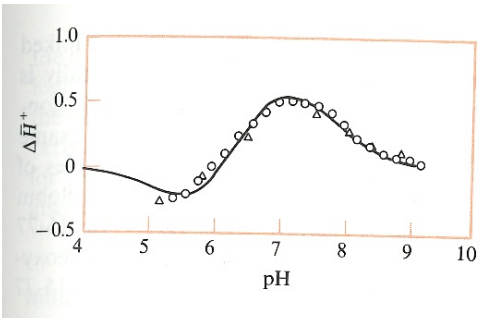


Figure 17-18
The effect of pH on $\Delta\bar{H}^+$ for human hemoglobin at 30°C. The curve is calculated from the constants in Table 17-2. The points correspond to two different types of experimental measurements. [After E. Antonini et al., *J. Biol. Chem.* 240:1096 (1965).]

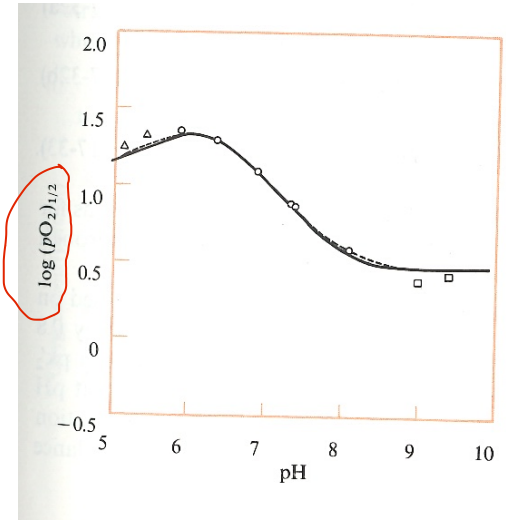


Figure 17-19
The Bohr effect in human hemoglobin at 30°C. The points are experimental values of $\log (pO_2)_{1/2}$ obtained from oxygenation curves measured in three different buffer systems. The solid line is calculated from the constants in Table 17-2. The dashed line comes from a graphical integration of the data in Figure 17-18. [After E. Antonini et al., *J. Biol. Chem.* 240:1096 (1965).]

5 Helix-coil transitions

poly A ↔ poly V

Please read pp. 527-535 in Dill & Bromberg. These same ideas can be used to study conformational changes in a single biomolecule, as well as to study ligand binding. Suppose, in a very simple model, each residue in a protein could be in either a helical configuration ("h") or a non-helical one ("c" for "coil"). The equilibrium constant for this change is traditionally called s, so that $s = [h]/[c] = \exp(-\beta\Delta\varepsilon)$, where $\Delta\varepsilon < 0$ represents the energy of forming a helical unit relative to a coil unit.

The simplest model says that this equilbrium constant is the same everywhere, and is not dependent on the conformation of the surrounding residues. Then, for a polypeptide containing N residues:

$$Q = (1+s)^N \quad \Rightarrow \quad f = \frac{s}{1+s}$$

$s = 1.2 \quad N = 15$

$f = 1.2/2.2 = 0.55$

where f is the fraction of residues in the helical conformation. (Derive this, using Eq. 1, where s now takes the place of $k\lambda$; note that this is Eq. 26.15 in MDF.) Note that s is a function of temperature: at low T, $s > 1$ and most of the residues will be in the helical configuration; and at high temperature, $s \simeq 1$ (why?), and half the residues will be helical and half will be coil.

Another simple model is one of complete cooperativity: either all the residues are helical, or they are all coil. Here:

$$Q = 1 + s^N \quad \Rightarrow \quad f = \frac{s^N}{1+s^N}$$

when $s = 1.2$ $N = 15$

$f \to 1$

In this model, f will be a sharper function of temperature than in the non-cooperative model.

The Zimm-Bragg model is a more complex model, with four rules for determining the required equilibrium constants (or relative probabilities, or statistical weights.) Although the math becomes somewhat more complex, the basic idea is the same:

1. • Compute Q as a function of the model parameters, like s and σ. (Note that these parameters may in turn depend on temperature or concentration or other aspects of the problem.
2. • Obtain (say) the average number of monomer units in the helical conformation per polymer molecule by evaluating $[\partial \ln Q / \partial \ln s]$.

s, adds in σ initiation equil. constant

$\frac{[h]}{[c]} = \sigma$ if preceding residue in coil

$\frac{[h]}{[c]} = s$ if preceding residue is already helical

2^N possible states

c c h h h c c h h

$\sigma\ \sigma\ \sigma\ s\ s\ s\ \sigma\ \sigma\ s$

if $s > \sigma$ tend to zip up the helix once it gets started

$$v(t) = v_0 e^{-\gamma t} + e^{-\gamma t} \int_0^t e^{\gamma t'} f(t')\,dt' \qquad (1)$$

$$\langle v^2 \rangle (t) \equiv \langle v(t)\, v(t) \rangle \qquad (2)$$

$$\langle f(t) \rangle = 0 \qquad \langle f(t)\, f(t') \rangle = 2B\,\delta(t-t')$$

white noise

squre of integral

$$\left\langle \int_0^t e^{\gamma t'} f(t')\,dt' \int_0^{t'} e^{\gamma t''} f(t'')\,dt'' \right\rangle$$