

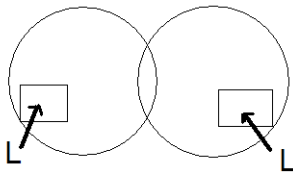
# Statistical Thermodynamics Approach to ligand binding and helix-coil transitions

CCB 341: Physical Chemistry of biochemical systems, Fall 2014

## 1 Partition function approach to allostery

Please read pp. 181-190 of your text.

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



$$[P] = 1$$

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

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

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

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

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

$$\boxed{f = \frac{\lambda}{N} \frac{\partial \ln Q}{\partial \lambda} = \frac{1}{N} \frac{\partial \ln Q}{\partial \ln \lambda}} \quad (1)$$

Since this is *uncoupled* binding:

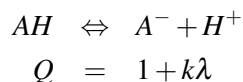
$$Q = 1 + 2k\lambda + k^2\lambda^2 = (1 + k\lambda)^2$$

Compare this to Eq. (5.78) in your text, where they use  $S$  for what we call  $k\lambda$ . Hence:

$$f = \frac{k\lambda}{1 + k\lambda} \quad \text{or} \quad \frac{f}{1-f} = k\lambda \quad (2)$$

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  $\nu = Nf$ ; see Eqs. 5.80 and 5.83 in your text. [Very advanced: See Onufriev, Case, Ullmann, *Biochemistry* **40**, 3413 (2001) for a generalization.]

Suppose we just have a simple acid-base equilibrium:



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

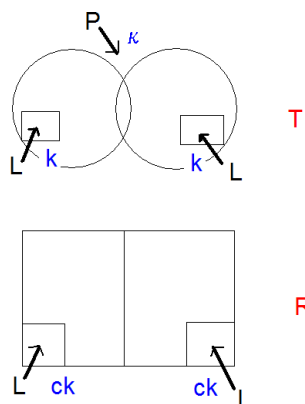
Now,  $k = 10^{pK_a}$  and  $\lambda = 10^{-pH}$ ; hence:

$$f = \frac{10^{pK_a - pH}}{1 + 10^{pK_a - pH}} \quad (3)$$

This yields the usual sigmoidal binding curve discussed in class. Look at the discussion of Fig. 5.13 in your text.

## 2 Hemoglobin-like model

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



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

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

$\lambda$	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:

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

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

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

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

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.

### 3 Helix-coil transitions

*Please read pp. 173-180 in your text.* 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  $s = [h]/[c] = \exp(-\beta\Delta\epsilon)$ , where  $\Delta\epsilon < 0$  represents the energy of forming a helical unit relative to a coil unit.

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

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

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  $s$  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:

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

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

On p. 174, your text discusses 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:

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