University of Washington
Department of Chemistry
Chemistry 453
Winter Quarter 2015

Lecture 11. 1/30/15

A. Bragg-Zimm Model: A Mathematical Epilog

- This section gives you some idea as to how the Bragg-Zimm model obtains the partition function for large N.
- In the last lecture we learned that the Bragg-Zimm partition function for a N monomer protein is

$$\frac{q}{q_0} = (1,0)M^N\begin{pmatrix}1\\1\end{pmatrix} = (1,0)\begin{pmatrix}1 & \sigma s\\ 1 & s\end{pmatrix}^N\begin{pmatrix}1\\1\end{pmatrix} \tag{11.1}$$

- This is a very difficult equation to use for large N because many matrix multiplications must be done to obtain q. Fortunately, matrix algebra has a solution to this difficulty.
- The solution is called diagonalization. Multiplying the matrix M by two other matrices T and T^{-1} transforms M into diagonal form:

$$T^{-1}\tilde{M}T = \begin{pmatrix}\lambda_1 & 0\\ 0 & \lambda_2\end{pmatrix} \tag{11.2}$$

- T^{-1} is the inverse of matrix T and these two matrices have the property

$$T^{-1}T = \begin{pmatrix}1 & 0\\ 0 & 1\end{pmatrix} \tag{11.3}$$

- Applying 11.3 and 11.2 to 11.1 where N=1 we obtain:

$$\frac{q}{q_0} = (1,0)TT^{-1}MTT^{-1}\begin{pmatrix}1\\1\end{pmatrix} = (1,0)T\begin{pmatrix}\lambda_1 & 0\\ 0 & \lambda_2\end{pmatrix}T^{-1}\begin{pmatrix}1\\1\end{pmatrix} \tag{11.4}$$

- Equation 11.4 can be generalized for N>1 and leads to:

$$\frac{q}{q_0} = (1,0)T\begin{pmatrix}\lambda_1 & 0\\ 0 & \lambda_2\end{pmatrix}^N T^{-1}\begin{pmatrix}1\\1\end{pmatrix} = (1,0)T\begin{pmatrix}\lambda_1^N & 0\\ 0 & \lambda_2^N\end{pmatrix}T^{-1}\begin{pmatrix}1\\1\end{pmatrix} \tag{11.5}$$

  where we have used the fact that $\begin{pmatrix}\lambda_1 & 0\\ 0 & \lambda_2\end{pmatrix}^N = \begin{pmatrix}\lambda_1^N & 0\\ 0 & \lambda_2^N\end{pmatrix}$.

- Equation 11.4 allows us to calculate the partition function with only three matrix multiplications, no matter how large N is. But we need to determine T, T^{-1}, λ_1, and λ_2.
- Calculating λ_1, and λ_2 is done by solving the determinant equation

$$0 = \begin{vmatrix}1-\lambda & \sigma s\\ 1 & s-\lambda\end{vmatrix} = (1-\lambda)(s-\lambda) - \sigma s = \lambda^2 - (s+1)\lambda + s(1-\sigma) \tag{11.6}$$

- Equation 11.6 is a quadratic equation and has the solution

$$\lambda_{1,2} = \frac{s+1 \pm \sqrt{(s+1)^2 - 4s(1-\sigma)}}{2} = \lambda_{1,2} = \frac{s+1 \pm \sqrt{(s-1)^2 + 4s\sigma}}{2} = \qquad (11.7)$$

- The matrices T and T-1 can also be determined. They are:

$$T = \begin{pmatrix} 1-\lambda_2 & 1-\lambda_1 \\ 1 & 1 \end{pmatrix} \text{ and } T^{-1} = \frac{1}{\lambda_1 - \lambda_2}\begin{pmatrix} 1 & \lambda_1 - 1 \\ -1 & 1-\lambda_2 \end{pmatrix} \qquad (11.8)$$

- You should confirm that these two matrices multiply together as shown in equation 11.3.
- Putting 11.7 and 11.8 into equation 11.5 we obtain:

$$\frac{q}{q_0} = \frac{\lambda_1^{N+1}(1-\lambda_2) - \lambda_2^{N+1}(1-\lambda_1)}{\lambda_1 - \lambda_2} \approx \frac{\lambda_1^{N+1}(1-\lambda_2)}{\lambda_1 - \lambda_2} \approx \frac{\lambda_1^{N}(1-\lambda_2)}{\lambda_1 - \lambda_2} \qquad (11.9)$$

where $\lambda_1 > \lambda_2$ so for large N $\lambda_1^{N+1} \gg \lambda_2^{N+1}$ and $N+1 \approx N$. Equation 11.9 was used in the last lecture and in the homework set.

B. Simple Ligand Binding Equilibria

- Let us consider the equilibrium between a free ligand L, free polymer P, and the 1:1 polymer: ligand complex PL $P + L \underset{}{\overset{K}{\rightleftharpoons}} PL$ where the equilibrium constant is given by

$$K = \frac{[PL]}{[P][L]} \qquad (11.10)$$

- In a solution containing this equilibrium we want to calculate the average number of ligands bound per polymer $\langle \nu \rangle$. For polymers with single binding sites $\langle \nu \rangle$ varies between zero and 1:

$$\langle \nu \rangle = \frac{[PL]}{[P]+[PL]} \qquad (11.11)$$

- We eliminate [PL] from equation 11.11 using equation 11.10:

$$\langle \nu \rangle = \frac{[PL]}{Q} = \frac{K[P][L]}{[P]+K[P][L]} = \frac{K[P][L]}{[P](1+K[L])} = \frac{K[L]}{1+K[L]} \qquad (11.12)$$

- The term in the denominator $Q = [P]+[PL] = [P](1+K[L])$ is called a binding polynomial. It plays the same role in binding equilibria as the partition function in helix-coil transitions...except Q is a sum over binding states instead of energy states. The role of Q is better appreciated for a more complicated system.
- Suppose a polymer has four ligand binding sites. The binding polynomial is:

$$\begin{aligned} Q &= [P]+[PL_1]+[PL_2]+[PL_3]+[PL_4] \\ &= [P]\left(1+\frac{[PL_1]}{[P]}+\frac{[PL_2]}{[P]}+\frac{[PL_3]}{[P]}+\frac{[PL_4]}{[P]}\right) \end{aligned} \qquad (11.13)$$

- Now as Figure 11.1 shows there are four equilibria of the form

$$P + L \underset{}{\overset{k_1}{\rightleftharpoons}} PL_1' \qquad (11.14)$$

where $k_1 = \dfrac{[PL_1']}{[P][L]}$ and the prime indicates that the complex is one of the four species in Figure 11.1, i.e. $[PL_1] = [PL_1'] + [PL_1''] + [PL_1'''] + [PL_1^{iv}]$.

Note we assume all four single site equilibria have the same constant k_1.

- As Figure 11.1 also shows there will be six equilibria of the form

$$PL_1' + L \underset{}{\overset{k_2}{\rightleftharpoons}} PL_2' \qquad (11.15)$$

where $k_2 = \dfrac{[PL_2']}{[PL_1'][L]}$ is assume the same for all six equilibria.

- Inserting the equilibrium expressions into equation 11.6 we obtain for Q:

$$Q = [P]\left(1 + 4k_1[L] + 6k_1k_2[L]^2 + 4k_1k_2k_3[L]^3 + k_1k_2k_3k_4[L]^4\right) \qquad (11.16)$$

- Now let us assume that the binding is independent such that sites already filled do not influence subsequent binding. If all binding sites are equivalent and independent then $k_1 = k_2 = k_3 = k_4 = k$ and Q becomes

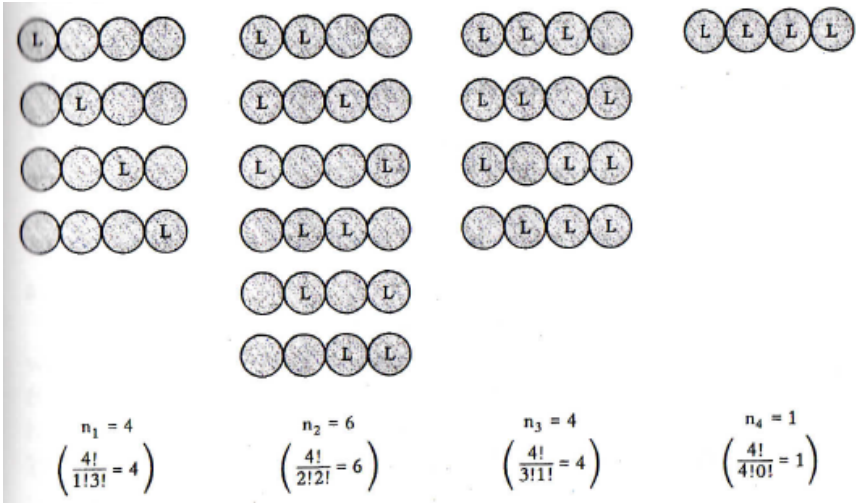
$$Q = [P]\left(1 + 4k[L] + 6k^2[L]^2 + 4k^3[L]^3 + k^4[L]^4\right) = [P]\left(1 + k[L]\right)^4 \qquad (11.17)$$

- Now we use the same expression used in the last lecture to obtain the average number of helical sites $\langle n \rangle$ to obtain the average number of sites bound:

$$\langle \nu \rangle = \frac{[L]}{Q}\frac{\partial Q}{\partial [L]} = \frac{[L]}{(1+k[L])^4}\frac{\partial}{\partial [L]}(1+k[L])^4 = \frac{4k[L]}{(1+k[L])^4}(1+k[L])^3 = \frac{4k[L]}{(1+k[L])}$$
$$\therefore f_B = \frac{\langle \nu \rangle}{4} = \frac{k[L]}{(1+k[L])} \qquad (11.18)$$

- In general for N binding sites $f_B = \dfrac{\langle \nu \rangle}{N} = \dfrac{k[L]}{(1+k[L])}$

Figure 11.1: The number of partly filled polymers is given by the binomial coefficient



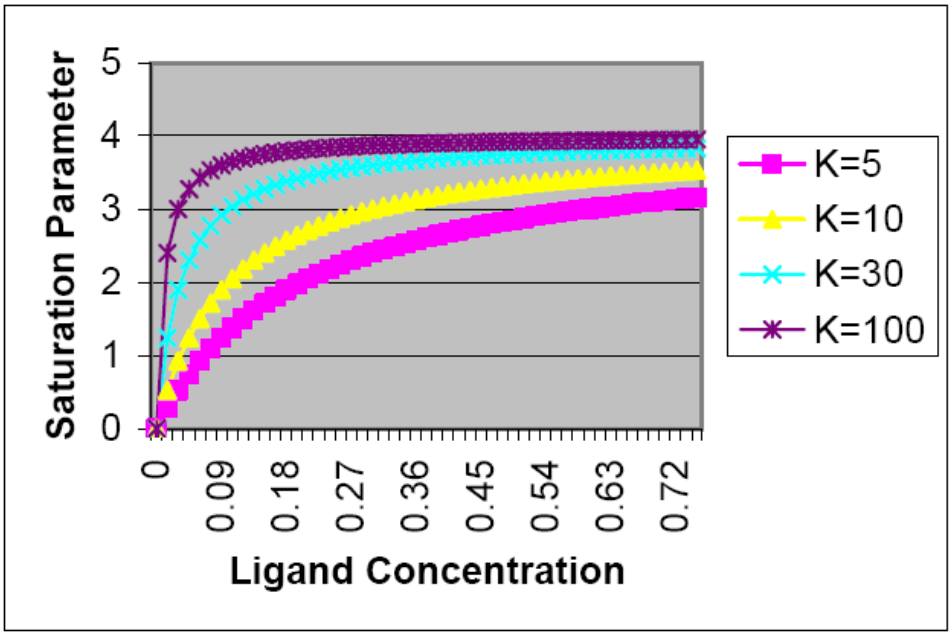


Figure 11.2: Equation 11.18 plotted for N=4 with varying values of K.

- Equation 11.18 shows plots sensitive to N and K: The curve rises with steeper slope as K increases and levels out at N.
- In substrate binding studies the objective is commonly to determine the number of sites bound and the binding affinity K. Therefore it is common to see the equation $\langle \nu \rangle = \frac{Nk[L]}{(1+k[L])}$ put into linear form:

$$\frac{\langle \nu \rangle}{[L]} = NK - K\langle \nu \rangle \qquad (11.19)$$

- Equation 11.19 is called the Scatchard equation. A Scratchard plot has the general appearance shown in Figure 11.3.

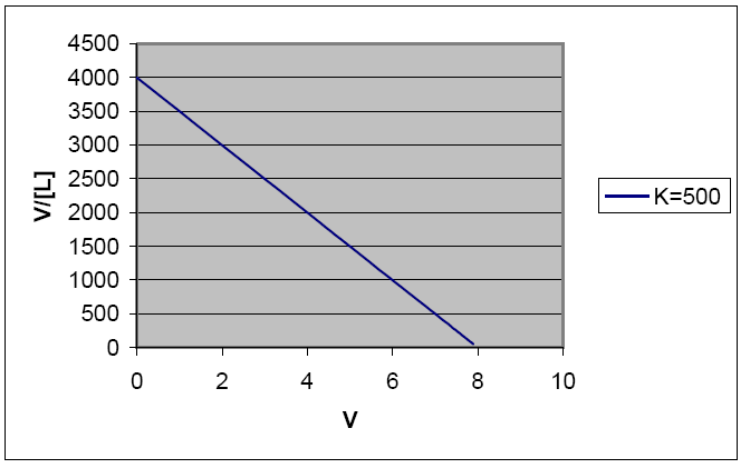


Figure 11.3: The Scatchard plot for N=8 and K=500 has a slope of –K=-500, a y-intercept of NK=4000, and a x intercept of N=8.

B. Fully Cooperative Binding

- Fully Cooperative binding is easy to describe. For fully cooperative ligand binding the equilibrium is $P + NL \overset{K}{\rightleftharpoons} PL_N$ and all partially filled polymers are excluded. Now the binding polynomial is:

$$Q = [P]\left(1 + k^4[L]^4\right) \tag{11.20}$$

- The average number of sites bound is as before

$$\langle \nu \rangle = \frac{[L]}{Q}\frac{\partial Q}{\partial [L]} = \frac{[L][P]}{[P]\left(1 + k^4[L]^4\right)}\frac{\partial}{\partial [L]}\left(1 + k^4[L]^4\right)$$

$$= \frac{[L]}{1 + k^4[L]^4}\left[4k^4[L]^3\right] = \frac{4k^4[L]^4}{1 + k^4[L]^4} \tag{11.21}$$

$$\therefore f_B = \frac{\langle \nu \rangle}{4} = \frac{k^4[L]^4}{1 + k^4[L]^4} = \frac{K[L]^4}{1 + K[L]^4}$$

- The fraction of sites bound can be generalized to N binding sites

$$f_B = \frac{\langle \nu \rangle}{N} = \frac{K[L]^N}{1 + K[L]^N} \tag{11.22}$$

where $K = k^4$.

- Equation 11.22 for fully cooperative ligand binding shows the same sigmoidal dependence that we observed for fully cooperative helix-coil transitions. In Figure 11.4 the blue line corresponds to non-cooperative binding and the red line corresponds to fully cooperative binding according to equation 11.22.

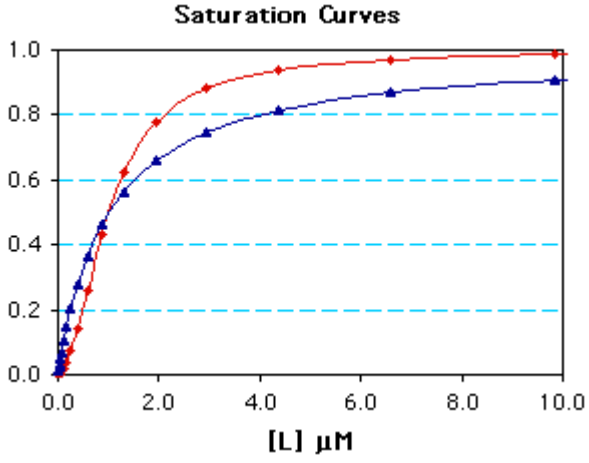


Figure 11.4: Non-cooperative (blue) versus fully cooperative(red) binding curves according to equations 11.18 and 11.22.

- If the binding is fully cooperative, the Scatchard plot is not linear. However, equation 11.22 can be rearranged to the form:

$$\frac{f_B}{1 - f_B} = K[L]^N \tag{11.23}$$

(11.24)

- Then we take the logarithm of both sides of equation 11.23:

$$\ln\left(\frac{f_B}{1-f_B}\right) = \ln K + N\ln[L] \qquad (11.25)$$

- Equation 11.25 is called the Hill equation. If $\ln\left(\frac{f_B}{1-f_B}\right)$ is plotted as a function of ln[L], the Hill plot is a straight line with a slope of N.

- Figure 11.5 is a Hill plot showing $\ln\left(\frac{f_B}{1-f_B}\right) = \ln\left(\frac{Y}{1-Y}\right)$ versus [L] for non-cooperative binding (blue) and a plot of binding data for oxygen to hemoglobin(Hb, red. Nohe the plot is slightly non-linear, with a maximum slope of 1.8. Hb has four oxygen binding sites, so while Hb does not bind oxygen non-cooperatively, neither does it bind oxygen with full cooperativity. We will consider more realistic binding models in the next lecture.

Figure 11.5: Hill plot for non-cooperative binding (blue) and for the binding of pxygen to Hemoglobin (red).