LIGAND EQUILIBRIA

Macroscopic and microscopic equilibrium constants

Titration of Glycine (dissociation of two proton ligands)

Macroscopic Equilibria:

\[
\begin{align*}
GH_2 & \rightleftharpoons K_1 GH + H^+ \\
GH & \rightleftharpoons K_2 G^- + H^+
\end{align*}
\]

where:

\[
K_1 = \frac{[GH][H^+]}{[GH_2]} \quad (pK_1 = 2.35)
\]

\[
K_2 = \frac{[G^-][H^+]}{[GH]} \quad (pK_2 = 9.78)
\]
Microscopic forms:

\[
\begin{align*}
\text{GH}_2 &= +H_3NCH_2COOH \\
\text{GH} &= +H_3NCH_2COO^- + H_2NCH_2COOH \\
\text{G}^- &= H_2NCH_2COO^- 
\end{align*}
\]

[2]

Microscopic equilibria:

Substituting Eq. 2 into Eq. 1:

\[
K_1 = \frac{\left\{ [+H_3NCH_2COO^-] + [H_2NCH_2COOH] \right\}[H^+]}{[+H_3NCH_2COOH]} = k_1 + k_2
\]

\[
K_2 = \frac{[H_2NCH_2COO^-][H^+]}{\left\{ [+H_3NCH_2COO^-] + [H_2NCH_2COOH] \right\}} = \frac{1}{k_3 + 1/k_4}
\]
Long chain diacid protonation (proton binding at two equivalent sites)

Macroscopic equilibria:

\[
A(H^+)_2 \rightleftharpoons K_1 A(H^+) + H^+ \\
A(H^+) \rightleftharpoons K_2 A + H^+
\]

Macroscopic dissociation constants:

\[
K_1 = \frac{[A(H^+)] [H^+]}{[A(H^+)_2]} \\
K_2 = \frac{[A] [H^+]}{[A(H^+) ]}
\]

Microscopic equilibria:

\[
H^+AH^+ \rightleftharpoons k AH^+ + H^+ \\
H^+AH^+ \rightleftharpoons k H^+A + H^+ \\
AH^+ \rightleftharpoons k A + H^+ \\
H^+A \rightleftharpoons k A + H^+
\]

Relationships between macroscopic and microscopic species:

\[
A = A \\
A(H^+) = AH^+ + H^+A \\
A(H^+)_2 = H^+AH^+
\]
Substituting Eq 5 into 4:

\[
K_1 = 2k \\
K_2 = k / 2 \\
K_1 = 4K_2
\]

Interesting! Even though the actual affinity of each site for H\(^+\) is identical, the apparent affinity of the second binding reaction is four times smaller than the first. This statistical effect looks like negative cooperativity, but it’s not!

**Ligand Binding at Multiple Identical Independent Sites**

Calculating the number of microscopic species (multiplicity of binding modes)

Macromolecule M with n binding sites for ligand L with a microscopic dissociation constant of \(k\)

\[
\begin{align*}
M + L &\rightleftharpoons M_1 \\
M_1 + L &\rightleftharpoons M_2 \\
\vdots & \quad \vdots \\
M_{n-1} + L &\rightleftharpoons M_n
\end{align*}
\]

\(M_i\) is the macroscopic state of macromolecule with \(i\) ligands bound. For example, if \(n=4\) then \(M_2\) is:

\[
M_2 = \begin{array}{c}
\text{LL} \\
\text{LL} \\
\text{LL} \\
\text{LL}
\end{array}
\]

Thus, there are six ways to put two ligands in four sites. In general:

\[
W(n,i) = \frac{n!}{(n-i)!i!}
\]
Calculation of ν (moles of L bound per mole of M)

\[ \nu = \frac{\sum_{i=0}^{n} i[M_i]}{\sum_{i=0}^{n} [M_i]} \]  \hspace{1cm} [7]

Macroscopic dissociation constants:

\[ K_1 = \frac{[M_0][L]}{[M_1]} \]
\[ K_i = \frac{[M_{i-1}][L]}{[M_i]} \] \hspace{1cm} [8]
\[ K_n = \frac{[M_{n-1}][L]}{[M_n]} \]

Solving for \([M_i]\):

\[ [M_i] = \frac{[M_{i-1}][L]}{K_i} = \frac{[M_0][L]^i}{i \prod_{j=1}^{i} K_j} \]  \hspace{1cm} [9]
Distinction between \( k \) (microscopic) and \( K \) (macroscopic) dissociation constants:

\[
k = \begin{bmatrix} 1 \\ 1 \\ 1 \end{bmatrix} [L] = \begin{bmatrix} 1 \\ 1 \\ 2 \end{bmatrix} [L] = \begin{bmatrix} 1 \\ 2 \\ 3 \end{bmatrix} [L] = \ldots
\]

whereas:

\[
K_1 = \begin{bmatrix} L \\ L \\ L \end{bmatrix} [L] = \begin{bmatrix} L \\ L \\ 2 \end{bmatrix} [L] + \begin{bmatrix} L \\ 2 \\ L \end{bmatrix} + \begin{bmatrix} 2 \\ L \\ L \end{bmatrix}
\]

Relationship between \( k \) and \( K_i \) determined by the multiplicity of state \( i \):

\[
K_i = \frac{W(n,i-1)}{W(n,i)} k = \frac{n!/(i-1)!(n-i+1)!}{n!/i!(n-i)!} k = \frac{i!(n-i)!}{(i-1)!(n-i+1)!} k = \frac{i}{n-i+1} k \quad [10]
\]

Substituting Eq. 10 into Eq. 9:

\[
[M_i] = [M_0] \left\{ \prod_{j=1}^{i} \left( \begin{bmatrix} (n-j+1) \\ j \end{bmatrix} \right) \right\} ([L] / k)^i \quad [11]
\]
Substituting Eq. 11 into Eq. 7:

\[
\nu = \sum_{i=1}^{n} i[M_0] \left\{ \prod_{j=1}^{i} \left\{ \frac{(n - j + 1)}{j} \right\} \right\} ([L]/k)^i \]

\[
[M_0] + \sum_{i=1}^{n} [M_0] \left\{ \prod_{j=1}^{i} \left\{ \frac{(n - j + 1)}{j} \right\} \right\} ([L]/k)^i \]

Notice that:

\[
\prod_{j=1}^{i} (n - j + 1) = \frac{n!}{(n - i)!} \]

so:

\[
\prod_{j=1}^{i} \left\{ \frac{(n - j + 1)}{j} \right\} = \frac{n!}{(n - i)!} = W(n,i) \quad [13]
\]

Substituting Eq. 13 into Eq. 12:

\[
\nu = \frac{\sum_{i=1}^{n} iW(n,i) ([L]/k)^i}{1 + \sum_{i=1}^{n} W(n,i) ([L]/k)^i} \quad [14]
\]

The denominator of Eq. 14 is the binomial expansion of \((1+[L]/k)^n\):

\[
1 + \sum_{i=1}^{n} W(n,i) ([L]/k)^i = (1 + [L]/k)^n \quad [15]
\]
Mathematical trick! Differentiate Eq. 15 with respect to \([L]/k\):

\[
\sum_{i=1}^{n} iW(n,i)\left(\frac{[L]}{k}\right)^{i-1} = n\left(1 + \frac{[L]}{k}\right)^{n-1}
\]  \[16\]

Multiply both sides by \([L]/k\):

\[
\sum_{i=1}^{n} iW(n,i)\left(\frac{[L]}{k}\right)^{i} = n\left(\frac{[L]}{k}\right)\left(1 + \frac{[L]}{k}\right)^{n-1}
\]  \[17\]

Substituting Eq. 17 for the numerator of Eq. 14 and Eq. 15 for the denominator of Eq. 14:

\[
\nu = \frac{n\left(\frac{[L]}{k}\right)\left(1 + \frac{[L]}{k}\right)^{n-1}}{(1 + \frac{[L]}{k})^{n}} = \frac{n\left(\frac{[L]}{k}\right)}{(1 + \frac{[L]}{k})}
\]  \[18\]

Linearize to get the **Scatchard Equation**:

\[
\frac{\nu \left(1 + \frac{[L]}{k}\right)}{[L]} = \frac{n}{k}
\]

\[
\frac{\nu}{[L]} = \frac{n}{k} - \frac{\nu}{k}
\]  \[19\]
Scatchard plot for a macromolecule with \( n \) independent and identical binding sites:

\[
\frac{n}{[L]} = \frac{n}{k} - \frac{\nu}{k}
\]

**Intercept:** \( n/k \)

**Slope:** \(-1/k\)

![Scatchard Plot Diagram](image_url)
Scatchard plot for a macromolecule with $n$ independent and identical binding sites:

\[
\frac{\nu}{[L]} = \frac{n/k}{1 + [L]/k} = \frac{n}{k} - \frac{\nu}{k}
\]

Scatchard plot for two classes of independent binding sites with different affinities

\[
\frac{\nu}{[L]} = \sum_i \frac{n_i/k_i}{1 + [L]/k_i}
\]

Intercept $= n/k$

Intercept $= n_1/n_2$

Slope $= -1/k$

Intercept $= n_1/n_2 + n_2/n_2$
The Hill Constant

For ligand binding that is infinitely cooperative over part of saturation range, i.e., an all-or-none reaction:

\[ M_0 + nL \xrightleftharpoons[K^n]{K^n} M_n \]

\[ K^n = \frac{[M_0][L]^n}{M^n} \]  \hspace{1cm} [20]

where K is the apparent dissociation constant for each of the interacting sites. For this situation:

\[ \nu = \frac{n[M_n]}{M_0 + M_n} = \frac{n[L]^n / K^n}{1 + [L]^n / K^n} \]

\[ \frac{\nu}{[L]} = \frac{n[L]^{n-1} / K^n}{1 + [L]^n / K^n} \]  \hspace{1cm} [21]

The fractional saturation (0→1) is:

\[ y = \frac{[L]^n / K^n}{1 + [L]^n / K^n} \]  \hspace{1cm} [22]
In reality, infinitely cooperative binding is not observed. However, in the saturation range of 25% to 75% semiempirical equations in the form of Eq.s 20 – 22 can be used:

\[
\nu = \frac{n[L]^{\alpha_H} / K^{\alpha_H}}{1 + [L]^{\alpha_H} / K^{\alpha_H}}
\]

\[
\frac{\nu}{[L]} = \frac{n[L]^{\alpha_H-1} / K^{\alpha_H}}{1 + [L]^{\alpha_H} / K^{\alpha_H}} \quad [23]
\]

\[
\overline{y} = \frac{[L]^{\alpha_H} / K^{\alpha_H}}{1 + [L]^{\alpha_H} / K^{\alpha_H}}
\]

where \(\alpha_H\) (Hill constant) is between 1 (no cooperativity between sites) and \(n\) (perfect cooperativity between sites)
Steepness at the midpoint sensitive to $\alpha_H$:

$$\frac{d\left\{\ln\left[\frac{\bar{y}}{1 - \bar{y}}\right]\right\}}{d(\ln[L])} = \alpha_H$$

at half-saturation ($[L]_{1/2}$):

$$\left(\frac{d[\bar{y} / (1 - \bar{y})]}{d[L]}\right)_{\bar{y}=1/2} = \frac{\alpha_{H,1/2}}{[L]_{1/2}}$$