

Lecture 14: Analysis of Cooperative Binding

Key Terms:

- Independent Binding Sites
- Positive and negative cooperativity
- Hill Plot
- Hill coefficient, n_H

Review of Ligand Binding:

The equilibrium constant for ligand binding, K_{eq} , is determined in large part by k_{off} , the *rate constant* with which a ligand dissociates from its binding site on a macromolecule. k_{on} , the *rate constant* with which a ligand associates with its binding site, is usually diffusion limited and does not vary widely.

$$K_{eq} = \frac{[ML]}{[M][L]} = \frac{k_{on}}{k_{off}} = K_A$$

Single Binding Site:

Saturation Binding Curve

Scatchard Plot

$$Y = \frac{[ML]}{[M] + [ML]} = \frac{[L]}{K_D + [L]}$$

Multiple Binding Sites: Non-cooperative (e.g. an antibody molecule with two sites, $n=2$): Non-cooperative binding to multiple sites (n sites) is analyzed in exactly the same fashion as to a single site. The only difference is that the fractional saturation, Y , is replaced by v , the **total** amount of ligand bound/macromolecule. v varies from 0 to n . For two binding sites:

Saturation Binding Curve

Scatchard Plot

$$v = \frac{[ML] + 2[ML_2]}{[M] + [ML] + [ML_2]} = \frac{2[L]}{K_D + [L]}$$

You can easily convert from v to Y by dividing by n , the number of sites: $Y = v/n$

Cooperative Binding – Multiple interacting sites:

- Positive cooperativity if $K_2 > K_1$ (or $K_{D2} < K_{D1}$) i.e., the second binding site has a higher affinity if the first site is already occupied by ligand.
- Negative cooperativity if $K_1 > K_2$ (or $K_{D2} > K_{D1}$) i.e., the second binding site has a lower affinity if the first site is already occupied by ligand.

Quantative Analysis of Cooperativity by the Hill Plot:

Consider a two step binding: $[M] + [L] \xrightleftharpoons{K_1} [ML]$ $[ML] + [L] \xrightleftharpoons{K_2} [ML_2]$

$$K_1 = \frac{[ML]}{[M][L]} \quad K_2 = \frac{[ML_2]}{[ML][L]}$$

Consider an **infinitely** cooperative system such that the second affinity constant (K_2) is much greater than the affinity constant for binding the first ligand (K_1). Then the only species present in solution are $[M]$ and $[ML_2]$. The fractional saturation under these conditions is:

$$Y = \frac{[ML_2]}{[M] + [ML_2]} = \frac{K_2[ML][L]}{[M] + K_2[ML][L]} = \frac{K_2K_1[M][L][L]}{[M] + K_2K_1[M][L][L]} = \frac{K_1K_2[L]^2}{1 + K_1K_2[L]^2} = \frac{K_D[L]^2}{1 + K_D[L]^2}$$

$$Y = \frac{K_D[L]^2}{1 + K_D[L]^2} = \frac{[L]^2}{K_{D\bar{}} + [L]^2} \quad (K_{D\bar{}} = 1/K_D = K_{D1} \cdot K_{D2}) \quad K_D^{Ave} = \sqrt{K_{D1}K_{D2}}$$

When $[L] = K_D^{Ave}$, $[L] = \sqrt{K_{D1}K_{D2}}$, $[L]^2 = K_{D1}K_{D2} = K_{D\bar{}}$
and $Y=0.5$ as before. However, this is now an **'average'** K_D .

An **infinitely** cooperative system with n-ligands: For a system that binds n ligands with *infinite* cooperativity:

$$Y = \frac{K_D[L]^n}{1 + K_D[L]^n} = \frac{[L]^n}{K_{D\bar{}} + [L]^n}$$

Again, when $[L] = K_D^{Ave}$, $[L] = \sqrt[n]{K_{D1}K_{D2} \dots K_{Dn}}$ $[L]^n = K_{D\bar{}}$ and once again $Y=0.5$.

However, most cooperative binding interactions are less than infinitely cooperative. For **less** cooperative systems, the fractional saturation can be approximated by:

$$Y = \frac{K_D[L]^{n_h}}{1 + K_D[L]^{n_h}} = \frac{[L]^{n_h}}{K_{D\bar{}} + [L]^{n_h}} \text{ where}$$

- n_h is the **Hill coefficient**. This is a measure of the *degree of cooperativity*.
- $\sqrt[n_h]{K_{D\bar{}}}$ is the 'average' K_D , giving the ligand concentration where $Y=0.5$.

The Hill coefficient, and the 'average' K_D can be obtained from a Hill Plot.

The Hill Plot is based on the following transformation of the above binding equation:

$$\log \frac{Y}{(1-Y)} = \log K_D + n_h \log [L]$$

Hill Plot:

1. Define: $\theta = Y/1 - Y$
2. Plot of $\log \theta$ versus $\log[L]$
3. Slope at $\log \theta = 0$ ($Y=0.5$) is the Hill coefficient, n_h .
4. Intercept at $\log \theta = 0$ ($Y=0.5$) gives $\log K_{D_{Ave}}$, or the average dissociation constant. This can be seen from the following:

At $\log \theta = 0$ ($Y=0.5$):

$$0 = \log K_D + n_h \log[L]$$

$$\frac{0}{n_h} \log K_D = \log[L]$$

$$\log K_D^{1/n} = \log[L]$$

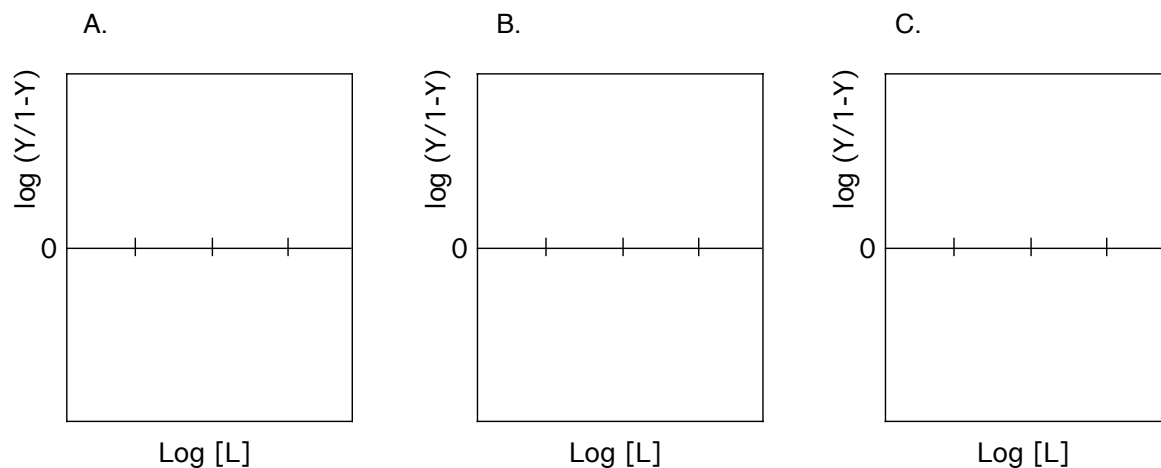
$$\sqrt[n]{1/K_D} = [L]$$

$$K_D^{Ave} = [L]$$

Qualitative interpretation of Hill Plot:

At *low* ligand concentrations, the binding measures essentially K_{D1} because most of the macromolecule is in the $[M]$ form. At *high* ligand concentrations, K_{D2} is measured because most of the macromolecule is in the $[ML]$ form.

	n_h
A. Hill Plot for a non-cooperative system: $K_{D1} = K_{D2}$	
B. Hill Plot for a positive cooperative system: $K_{D1} > K_{D2}$	
C. Hill Plot for a negative cooperative system: $K_{D1} < K_{D2}$	



Hill Plot for O₂ Binding to Hemoglobin

The Hill plot for O₂ binding to normal adult hemoglobin is shown below:

