A. Cooperative Binding

- Cooperativity refers to the influence that binding at one site has on binding to another site. If binding at one site enhances binding at another site, the effect is called positive cooperativity. If binding at one site diminishes the affinity for other sites, the effect is called negative cooperativity.

- In biochemistry the general phenomenon of long-range effects of ligand binding is called allostery.

- Homoallostery: influence on ligand-binding of ligands of the same kind

- Heteroallostery: influence on ligand-binding of ligands of a different kind

Hill Plots and Allosteric Effects

- If a straight line is observed over the entire range of ligand concentration \([A]\), the binding is non-cooperative and the sites are identical

- If the curve has a slope \(>1\) in some range of \([A]\), the binding must be positively cooperative.

- If the curve has a slope \(<1\) in some range of \([A]\), the biopolymer \(P\) either has more than one class of binding site or the binding is negatively cooperative.

- Example: Fully cooperative binding (all or nothing)

\[
\nu = \frac{NK[A]^N}{1 + K[A]^N} \Rightarrow \log \left( \frac{f}{1 - f} \right) = \log K + N \log [A] \quad \text{where} \quad f = \theta = \frac{\nu}{N}
\]

- In reality full cooperativity is not realized and the value of \(N\) derived from a Hill plot may not reflect the number of binding sites and may not even be an integer. For example, hemoglobin has four binding sites for oxygen gas, but in the Hill plot for oxygen binding to hemoglobin it is found that \(N=2.8\). This value for \(N\) indicates that some cooperativity exists between the binding sites, but binding to hemoglobin is not “all or nothing”. Also, the Hill plot may deviate from linearity, with regions of partial linearity or with slopes \(>1\) or \(<1\).

B. Models for Cooperative Binding:

a. There is no unique interpretation for a Hill plot with slope \(>1\) or \(<1\).

Models must be advanced to explain the data and more than one model may account for the data.

b. Monod-Wyman-Changeaux (MWC) Theory: In the absence of ligand, the biopolymer exists in two forms \(T\) and \(R\) that are in dynamic equilibrium

\(R \rightarrow T; \quad K = \begin{bmatrix} T \\ R \end{bmatrix} \)

- In the \(T\) state, all binding sites bind ligand \(A\) weakly
In the R state, all binding sites bind ligand A tightly.
In the absence of ligand, the T form is favored, i.e. $[T] \gg [R]$.
As ligand A is added, the R form is favored…

The equilibria between the various forms of bound and unbound T is characterized by the equilibrium constant $k_T$. The equilibria between the various forms of bound and unbound R is characterized by the equilibrium constant $k_R$. The ratio $C = \frac{k_T}{k_R}$. Assume two binding sites on T and R….

- $\bar{v} = \frac{[RA] + 2[RA_2] + [TA] + 2[TA_2]}{[R] + [RA] + [RA_2] + [T] + [TA] + [TA_2]}$
- Substitute $K = \frac{[T]}{[R]}$ and $C = \frac{k_T}{k_R} \Rightarrow f = \frac{1 + KC(1 + k_T[A])}{1 + k_T[A]}$
- Limiting behavior:

\[
\frac{f}{1-f} \approx k_T[A] \text{ as } [A] \to 0
\]

\[
\frac{f}{1-f} \approx k_R[A] \text{ as } [A] \to \infty
\]

\[
\ln \left( \frac{f}{1-f} \right) \approx \ln k_T + \ln [A] \ldots \text{as } A \to 0
\]

log-log limiting behavior…

\[
\ln \left( \frac{f}{1-f} \right) \approx \ln k_R + \ln [A] \ldots \text{as } A \to \infty
\]

This behavior produces a Hill plot with the following appearance…
C. Derivation of the Differential Heat of Adsorption from Isotherm Equations

- Consider the equilibrium $A(g) \rightleftharpoons A(\text{adsorbed})$. The equilibrium constant $K_A = \frac{1}{P_0}$, where $P_0$ is the equilibrium pressure of the gas.

- Then the simple Langmuir isotherm equation is

$$\frac{N}{V} = f = \theta = \frac{K_A P}{1 + K_A P} = \frac{P}{P_0 - P}, \text{or } P = P_0 \left( \frac{\theta}{1 - \theta} \right).$$

- From the equations above the general form for an isotherm is $P = f(\theta)$.

At equilibrium the chemical potential of the free gas is equal to the chemical potential of the adsorbed gas i.e. $\mu_{\text{free}} = \mu_{\text{adsorbed}}$.

- From the relationship $\mu_{\text{free}} = \mu_{\text{free}}^0 + RT \ln P$, the equilibrium condition $\mu_{\text{free}} = \mu_{\text{adsorbed}}$, and the general isotherm equation $P = f(\theta)$, we obtain...

$$\mu_{\text{adsorbed}} = \mu_{\text{free}}^0 + RT \ln(f(\theta)).$$

- We can now obtain the molar enthalpy of adsorption using the Gibbs-Helmholtz equation...

$$\frac{\partial (\mu_{\text{adsorbed}} / T)}{\partial (1/T)} = \Pi_{\text{adsorbed}} = \Pi_{\text{free gas}}^0 + R \left( \frac{\partial \ln(f(\theta))}{\partial (1/T)} \right)$$
• The differential heat of adsorption is then
\[
\Delta \tilde{H}_{\text{diff}} = \tilde{H}_{\text{free gas}}^0 - \tilde{H}_{\text{adsorbed}} = -R \left\{ \frac{\partial \ln f(\theta)}{\partial (1/T)} \right\}
\]

D. Isothermal Titration Calorimetry

• Enthalpies of binding between two molecules in solution or the enthalpy of binding between a molecule in solution and a surface may be measured by isothermal titration calorimetry (ITC). ITC instrumentation is schematized in Figure 21.2.

![Figure 21.2 Schematic of an isothermal titration calorimeter](image)

- Two vessels are thermally isolated from the surroundings. The control or reference vessel contains a buffered solution. The reaction vessel contains a buffered solution of a macromolecule or some other binding target. Initially the temperatures of the control and reaction vessels are identical (\(\Delta T = 0\)). The injection system delivers into the reaction vessel at a measured rate a ligand solution. Depending on whether the binding is exothermic or endothermic the temperature of the reaction vessel will increase (\(\Delta T > 0\)) or decrease (\(\Delta T < 0\)). The heat of binding \(q_b\) can be obtained given a knowledge of the solution heat capacity:
\[
q_b = C \Delta T
\]  
\[
(21.1)
\]

- The enthalpy of binding is related to the heat of binding by
\[
q_b = V c_L^b \Delta H_b
\]  
\[
(21.2)
\]

where \(V\) is the volume of the solution in the reaction vessel and \(c_L^b\) is the concentration of bound ligand.

- To calculate the enthalpy of binding from Equation (21.2) we require the concentration of bound ligand. The total ligand delivered into the reaction vessel
will partition into free ligand with concentration \( c_L \) and bound ligand with concentration \( c_L^b \):

\[
c_L^T = c_L^b + c_L = \nu c_p + c_L
\] (21.3)

Equation (21.3) can be solved for \( c_L \) if we assume an expression for \( \nu \). The simplest approach is to assume a model of independent ligand binding and use Equation (12.30) to substitute \( \nu = \frac{c_L NK_b}{(1 + K_b c_L)} \):

\[
c_L^T = \nu c_p + c_L = \left( \frac{c_L NK_b}{(1 + K_b c_L)} \right) c_p + c_L
\] (21.4)

where \( N \) is the number of ligand binding sites and \( K_b \) is the binding equilibrium constant. Equation (21.4) can be rearranged into standard quadratic form:

\[
K_b c_L^2 + \left( NK_b c_p - K_b c_L^T + 1 \right) c_L - c_L^T = 0
\] (21.5)

and solved using the quadratic formula

\[
c_L = \frac{-\left( NK_b c_p - K_b c_L^T + 1 \right) \pm \sqrt{\left( NK_b c_p - K_b c_L^T + 1 \right)^2 + 4 K_b c_L^T}}{2 K_b}
\] (21.6)

Combining Equation (21.6) with Equation (21.3) we obtain a final expression for the bound ligand concentration:

\[
c_L^b = c_L^T - c_L = c_L^T - \left\{ -\left( NK_b c_p - K_b c_L^T + 1 \right) + \sqrt{\left( NK_b c_p - K_b c_L^T + 1 \right)^2 + 4 K_b c_L^T} \right\} \frac{1}{2 K_b}
\] (21.7)

Near the saturation point where virtually all \( N \) sites on the target protein are filled, the bound ligand concentration approaches \( Nc_p \) in which case Equation (23.2) can be approximated by

\[
q_b \approx V Nc_p \Delta H_b
\] (21.8)

and the binding enthalpy is

\[
\Delta H_b \approx \frac{q_b}{V Nc_p}
\] (21.9)

Pierce, Raman, and Nall have used ITC to study the binding of two monoclonal antibodies 2B5 and 5F8 to horse heart cytochrome c. Because the volume of the solution in the reaction vessel changes upon addition of the titrant, calorimetric heat has to be corrected for the heat of dilution. Figure 12.25 shows an experimental calorimetric titration of a solution of MAb 5F8 with cytochrome c corrected for the heat of dilution. The quantities \( N \), \( \Delta H_b \), and \( K \) are obtained by fitting of the calorimetric data in Figure 21.3 using Equations (21.2) and (21.7).
Sample Problem 21.1

ITC study of binding between horse heart cytochrome c and MAb 5F8 yield $K_b = 1.4 \times 10^{10}$ and $\Delta H_b = -90.7 \text{ kJ mol}^{-1}$. From these data calculate the Gibbs energy of binding $\Delta G_b$ and the entropy of binding $\Delta S_b$. Assume $T=298 \text{ K}$.

Solution: The Gibbs energy is obtained from the relation

$$\Delta G_b = -RT \ln K_b = -\left(8.31 \text{ J mol}^{-1} \text{ K}^{-1}\right) \ln \left(1.40 \times 10^{10}\right) = -57.8 \text{ kJ mol}^{-1}$$

At constant temperature the entropy is obtained from

$$\Delta S_b = \frac{\Delta H_b - \Delta G_b}{T} = \frac{-90.7 \text{ kJ mol}^{-1} - (-57.8 \text{ kJ mol}^{-1})}{298 \text{ K}} = -110 \text{ JK}^{-1}$$

The change in heat capacity as a result of binding $\Delta C_p$ can also be obtained from ITC if the binding enthalpy is measured as a function of temperature. An example is shown in Figure 12.25C where $\Delta H_b$ for MAb 5F8 binding to cytochrome c is plotted as a function of temperature. In Figure 12.25C a straight line is fitted to the four experimental points to obtain
\[ \Delta C_p = \left( \frac{\partial \Delta H_p}{\partial T} \right)_p \approx \frac{\Delta \Delta H_p}{\Delta T} = -172 \text{cal mol}^{-1} \text{K}^{-1} = 719 \text{J mol}^{-1} \text{K}^{-1} \]