Lecture 15: Hill Plot & Introduction to Enzyme Kinetics

Assigned reading in Campbell: Chapter 6.1-6.5, 7.3, 7.4

Key Terms:

Hill Plot & Hill coefficient n_h
Active site, catalytic groups
Transition state theory
Transition state stabilization: Enthalpic
Transition state stabilization: Entropic

15.1 Hill Equation:

$$\log\left(\frac{Y}{(1-Y)}\right) = \log K_{\pi} + n_h \log[L]$$

Hill Plot:

1. Define: $\theta = Y/1 - Y$
2. Plot of log θ 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:

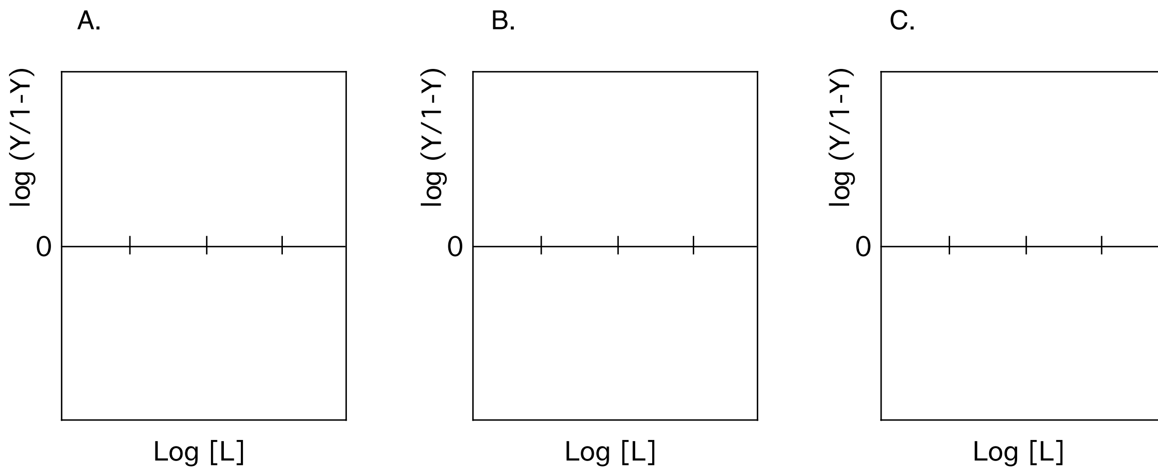
   At $\log\theta = 0$ (Y=0.5):

$$0 = \log K_{\pi} + n_h \log[L]$$
$$\frac{-1}{n_h}\log K_{\pi} = \log[L]$$
$$\log K_{\pi}^{-1/n} = \log[L]$$
$$\sqrt[n]{1/K\pi} = [L]$$
$$K_D^{Ave} = [L]$$

Qualitative interpretation of Hill Plot:

	nh
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: $K_{D1} < K_{D2}$	

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.



15.2 Enzyme Kinetics:

For the simple enzyme-catalyzed reaction: $S \xrightarrow{Enzyme} P$

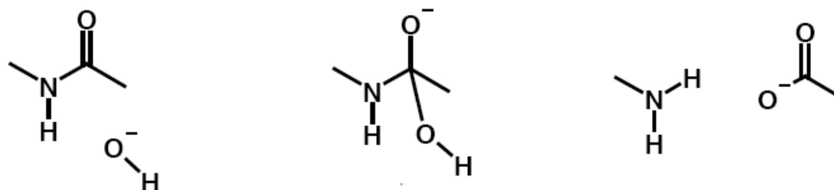
The enzyme forms a complex with the substrate (S) (similar to a protein-ligand complex) and performs some chemical reaction/transformation of the bound substrate. The resultant product (P) is released.

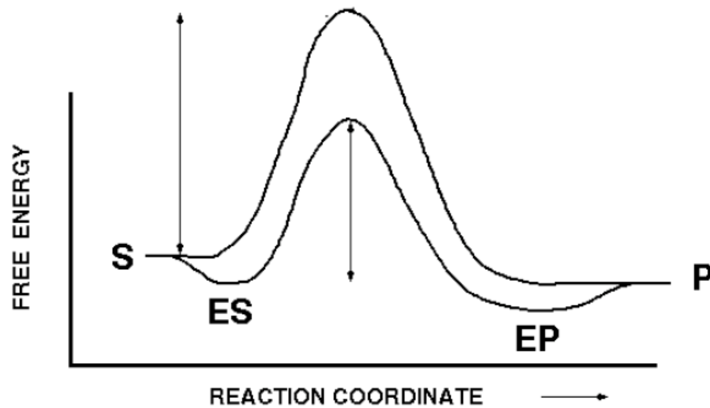
15.3 Important Features of Enzyme Catalysis:

1. Enzymes increase the rate of reactions.
2. Enzymes do not change the equilibrium point of reactions.
3. Enzymes are not changed by the overall reaction (although they may be reversibly modified *during* the reaction).
4. Catalysis occurs at the "active site", which is specific for certain substrates. The active site has the following with respect to its substrate:
   - Geometric complementarity
   - Energetic complementarity
5. Enzyme activity is regulated:
   - by abundance (the number of copies of the enzyme in the cell)
   - by concentration of substrate(s) and product(s)
   - by the presence of allosteric activators and inhibitors
   - by post-translational modification, e.g. phosphorylation
   - by proteolytic cleavage of zymogens

15.4 Transition State Theory:

The transition state is a high energy intermediate in the reaction, a distorted substrate on its way to becoming product. For example, in the hydrolysis of a peptide bond by hydroxide ion, the transition state is the oxyanion:





Transition state theory states that the rate or velocity (v) of the reaction is directly proportional to the concentration of molecules in the transition state:

$v \propto [X^{\ddagger}]$

The key assumption made in transition state theory is that the substrates and products are in equilibrium with the transition state. We can then write an equilibrium constant, e.g. for the forward reaction:

$K^{\ddagger}=[X^{\ddagger}]/[ES]$

Therefore the velocity of the reaction is: $v \propto K^{\ddagger}[ES]$.

Using the relationship between free energy and the equilibrium constant gives the following for the enzyme catalyzed reaction:

$$v_E \propto \exp(-\Delta G_E{}^{\ddagger}/RT)[ES]$$

For the uncatalyzed reaction:

$$v \propto \exp(-\Delta G^{\ddagger}/RT)[S]$$

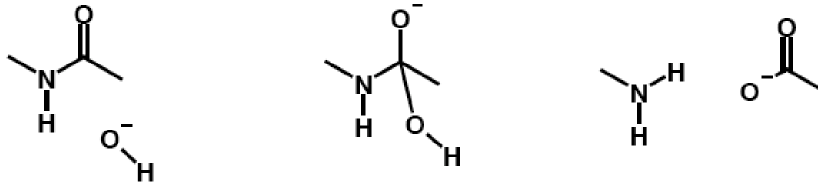
The rate *enhancement* by the enzyme (assuming all of the substrate is bound to the enzyme; i.e. [S] in the uncatalyzed reaction = [ES] in the catalyzed reaction):

$$\frac{v_E}{v} = \frac{e^{-\Delta G_E/RT}[ES]}{e^{-\Delta G/RT}[S]} = e^{(\Delta G - \Delta G_E)/RT}$$

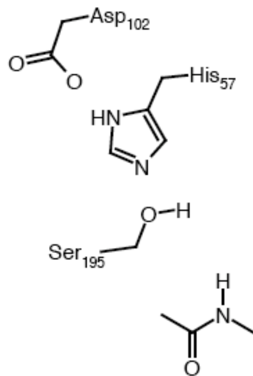
From the above equation it is clear how enzymes increase the rate of the reaction – they must do so by lowering the energy of the transition state in the enzyme substrate complex. Note the exponential dependence on the activation free energy: **small changes in $\Delta G^{\ddagger}$ lead to large changes in rates**.

The transition state of the enzyme substrate complex is stabilized in two ways:

1. **Enthalpic** - The enzyme transition-state complex is stabilized by direct interactions (e.g. H-bonds, electrostatic interactions) between the enzyme and the transition state. This reduces the free energy of the transition state due to ΔH (enthalpy).



2. **Entropic** – Formation of the transition state requires a precise geometric arrangement of sidechain groups. In the case of a reaction occurring in solution, this would require considerable ordering of these chemical groups, i.e., a reduction in the entropy of the system, which is unfavorable.



In an enzyme, these groups are already in the correct position because of the way the protein folded, therefore there is no loss of entropy in the complex. For example, the serine proteases utilize a serine, histidine, and aspartic acid to catalyze peptide bond hydrolysis. Consider the following changes in entropy between the initial state and the transition state: