BioChem 330 - Course Outline

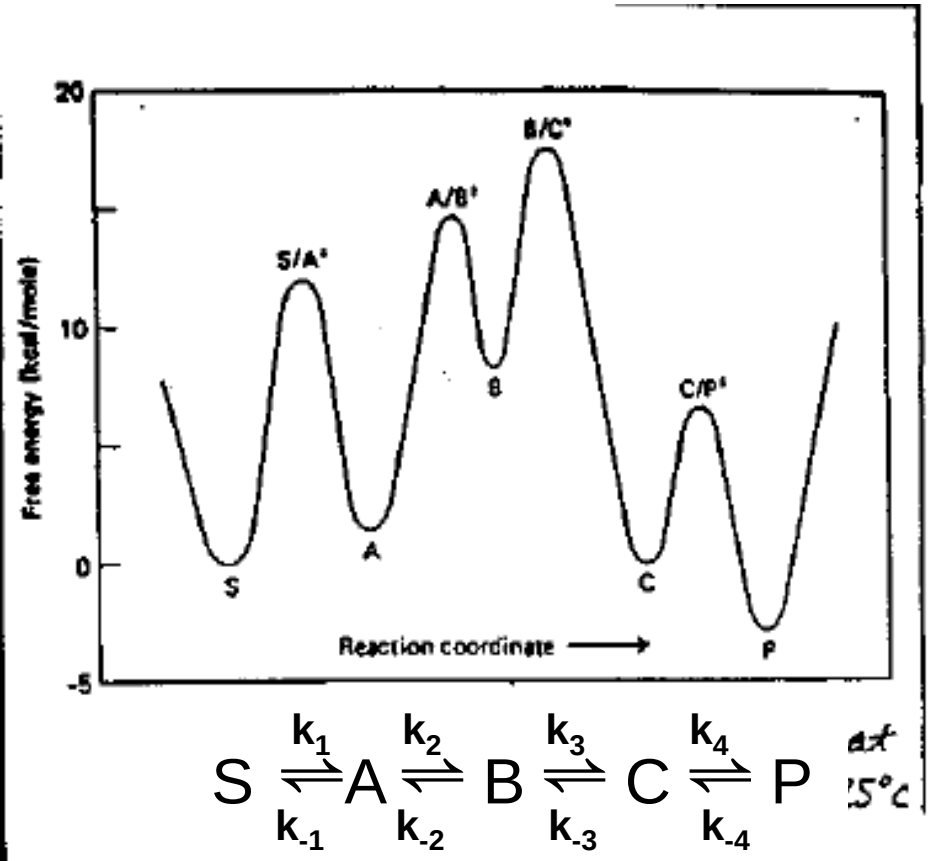
October 13,18, 2011

- **Bio-molecular Structure/Function (I cont'd)**
  - NUCLEIC ACID
    - DNA sequence and structure
    - Protein/nucleic acid interactions
  - CARBOHYDRATES
    - Sugars - mono and disaccharides
    - Polysaccharides
    - Glycerides and glycerol
  - FATS AND LIPIDS
    - Chemistry and nomenclature for fatty acids
    - Saturated and unsaturated fatty acids
    - Fluid mosaic model of membrane structure

BioChem 330 - Course Outline

- **Metabolism and Bioenergetics (II)**
  - ENZYME CATALYSIS:
    - kinetic constants k_{cat}, K_m
    - Catalytic strategies, the serine proteases
  - CATABOLISM (*breakdown*)
    - Carbohydrates
      - Glycolysis
      - Tricarboxylic Acid Cycle
      - Electron Transport
      - Chemiosmosis and ATPase
    - Fatty acids and amino acids

REACTION COORDINATE DIAGRAMS

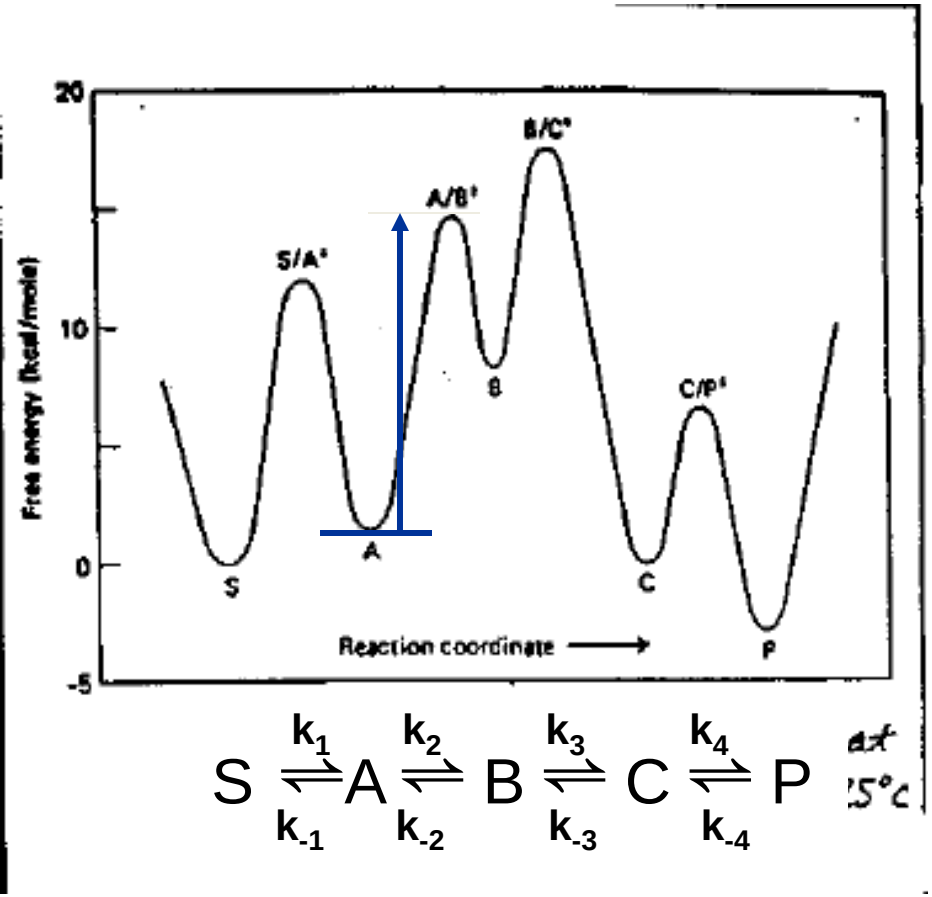


$$S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} A \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} B \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} C \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} P$$

Reaction Coordinate Diagram can be reconstructed once all the forward and reverse rate constants are measured at a particular T.

- Intermediates:
  - A, B and C are intermediates in the conversion of S to P. Intermediates can accumulate and reach steady states in concentration.
- TS Complexes:
  - $S/A^{‡}$, $A/B^{‡}$, $B/C^{‡}$, and $C/P^{‡}$ are transition state complexes which have a fleeting existence and can only be poorly defined.

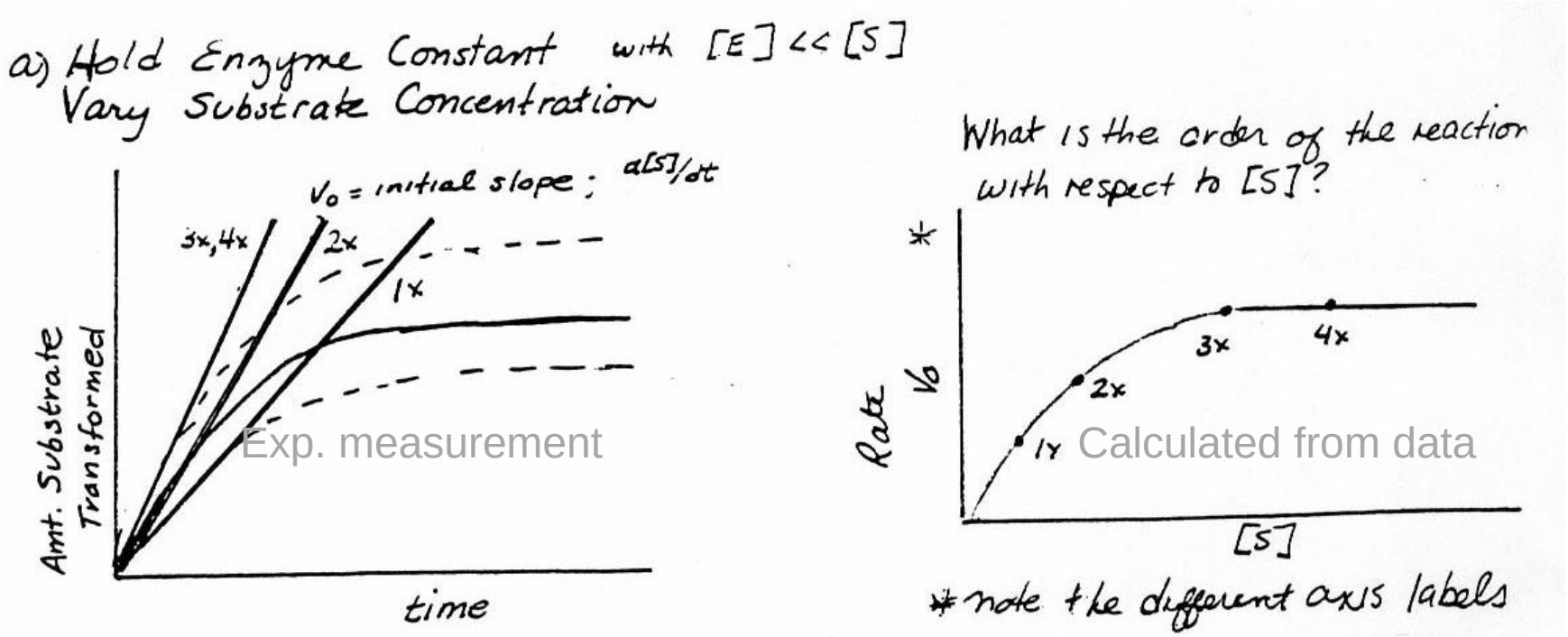
REACTION COORDINATE DIAGRAMS



$$S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} A \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} B \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} C \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} P$$

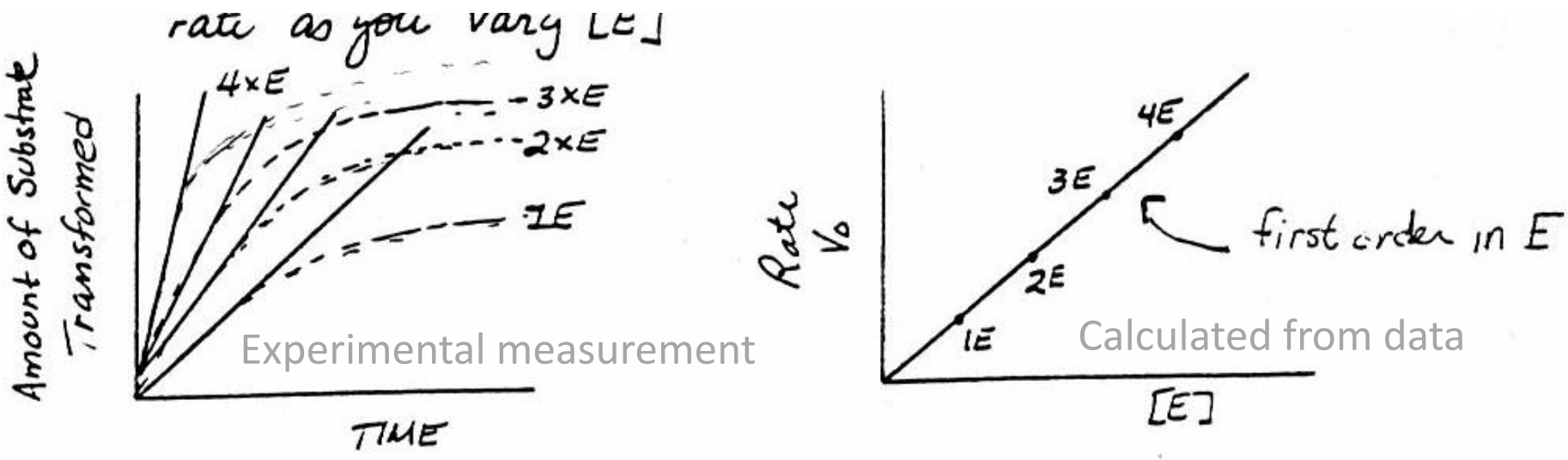
- The rate or velocity, V, is limited by the rate determining step; that step with the largest barrier, or smallest rate constant, here A→B, k_2
- V depends on the combined rates of all the elementary steps up to the slowest step, which determines [A].
- Conversion A →B $k_2 = 10^3 s^{-1}$
- **Activation Barrier:**
- $\Delta G^{\ddagger} = 17.6 - 1.36 \log k$
  - $= 17.6 - 1.36 \log 10^3$
  - **= 13.5 kcal/mole at 25.0°C**

What is the order of enzyme reaction w.r. to [S]?



- Biological conditions of small [E] compared to [S].
- Reaction is first order with respect to [S] at low concentrations of [S], then zero order at high [S]
- Can only be explained by presence of specific [ES] complex.

What is the order of the reaction with respect to [E]?



- Here, [S] is held constant and Rate is measured as a function of enzyme concentration (but still at concentrations of [E] much lower than [S]
- Rate is first order in [E].
- Whatever rate expression you propose, it must have the three characteristics, first order in [S] at low [S], zero order in [S] at high [S] and first order in E.

Proposed Michaelis Menten Mechanism

- $E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$

$[E] << [S]$, $[S] = [S_t]$

- Assume ES reaches a steady state with E and S
  - Rate of formation of ES equals the rate of breakdown
  - $k_1[E][S] = k_{-1}[ES] + k_2[ES] = (k_{-1} + k_2)[ES]$
  - $[ES] = k_1[E][S] / (k_{-1} + k_2) = [E][S] / K_m$

$K_m = (k_{-1} + k_2) / k_1$

- Assume k_2 is the catalytic step: product dissociates rapidly
  - **Velocity $= k_2 [ES]$**
  - $[ES] = [E][S] / K_m$
  - $[ES] = (([E_t] - [ES])[S_t]) / K_m$

$[E] = [E_t] - [ES]$

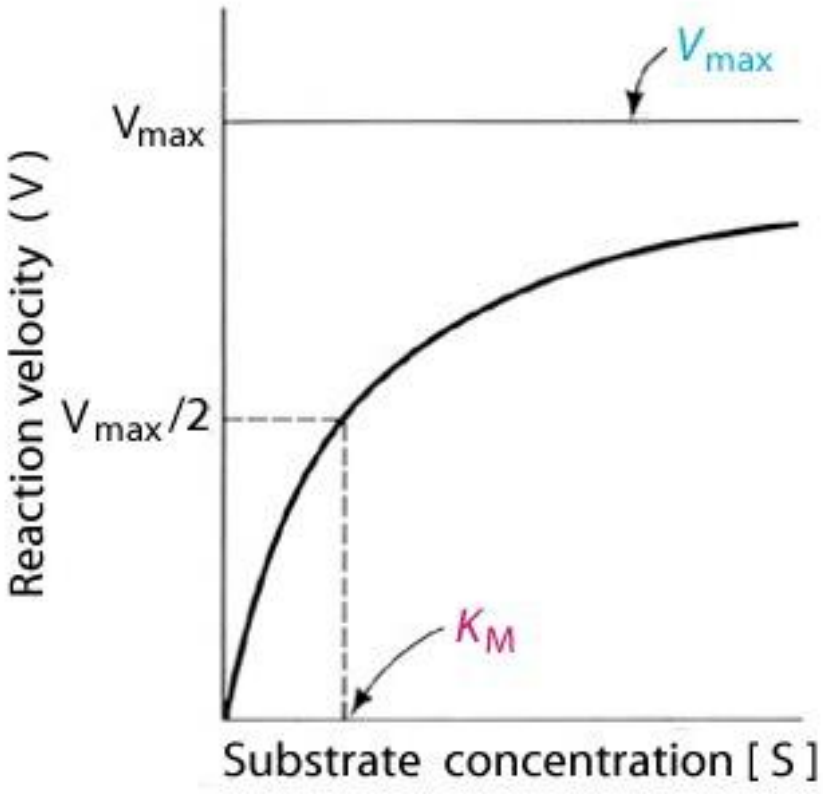
- *Solve this for ES* ⟶

$$[ES] = \frac{[E_t][S_t]}{K_m + [S_t]}$$

- *Plug into Velocity equation above*
- **Velocity** $= \frac{k_2 [E_t][S_t]}{K_m + [S_t]} = \frac{k_{cat} [E_t][S_t]}{K_m + [S_t]} = \frac{V_{max} [S_t]}{K_m + [S_t]}$

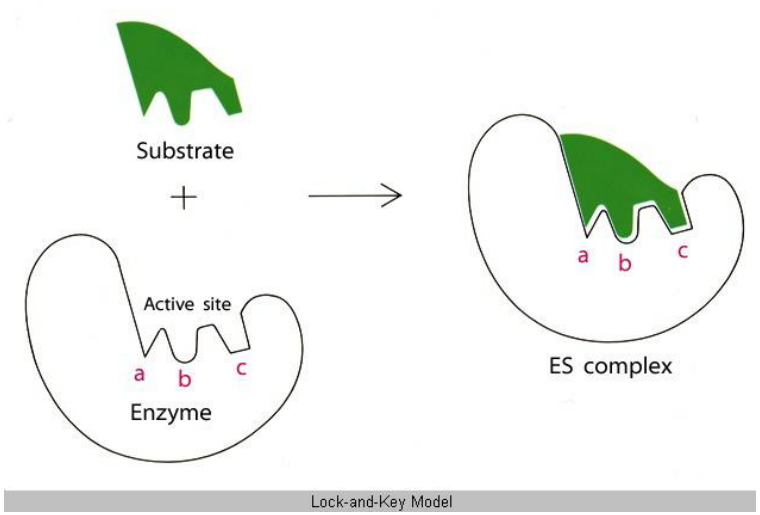
Define:
$V_{max} = k_{cat}[E_t]$

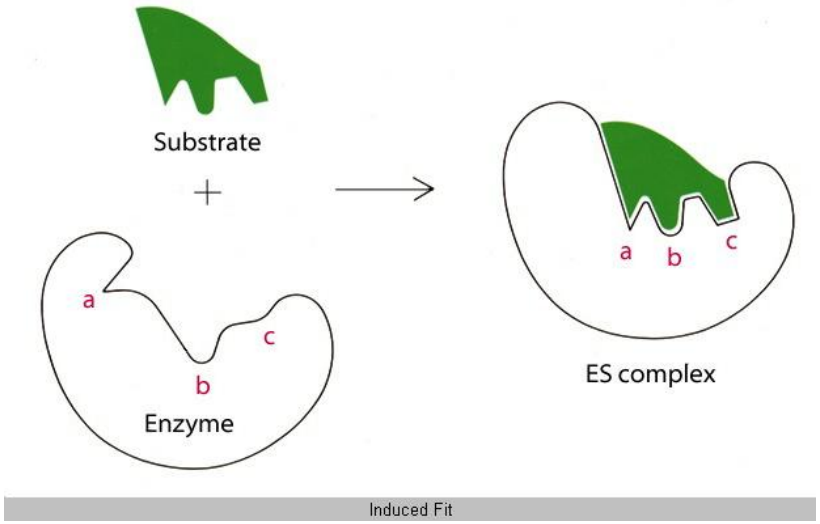
Michaelis Menten Kinetics



- Velocity = $\frac{V_{max}[S_t]}{K_m + [S_t]}$
- When [S] is small, the reaction is first order.
- When [S] is large, the reaction is zero order in [S], velocity is at V_{max}
- Reaction is always first order in [E]
- When [S] = K_m, V= ½V_{max}

ES Complex* Michaelis Complex

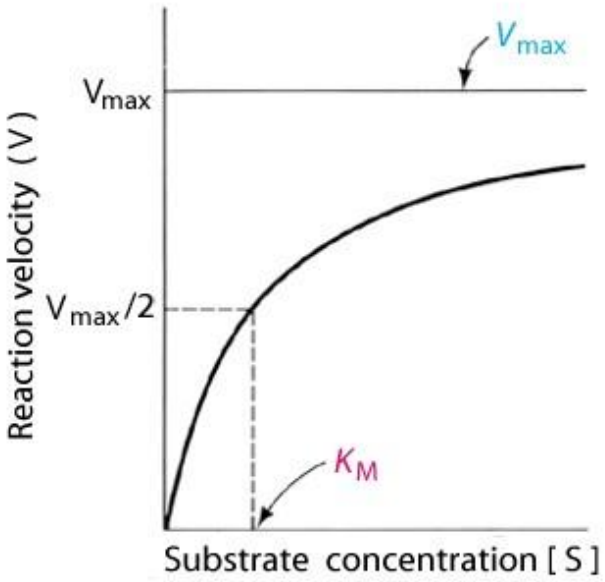


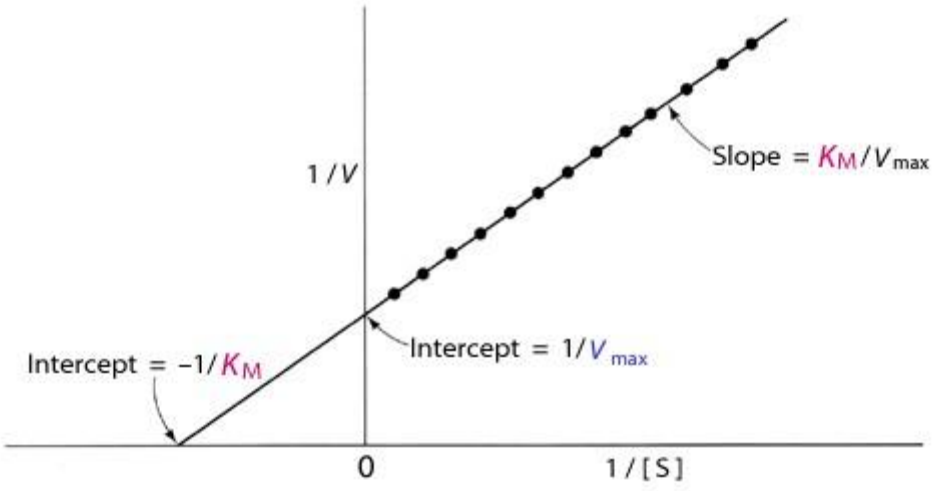


- **Lock and Key Model** **Induced Fit Model**
- K_m measures the affinity of an enzyme for a particular substrate (often an enzyme can bind and catalyze reaction on a family of closely related substrates)
- higher K_m, lower binding, K_m is like a dissociation constant

Graphical Means of Extracting M^2 Parameters

$$\text{Velocity} = \frac{k_{cat}[E_t][S_t]}{K_m + [S_t]} = \frac{V_{max}[S_t]}{K_m + [S_t]}$$





- Hyperbolic Curve
- V_{max} = asymptotic max.
- $k_{cat} = V_{max}/[E_t]$
- K_m = [S] at ½ V_{max}

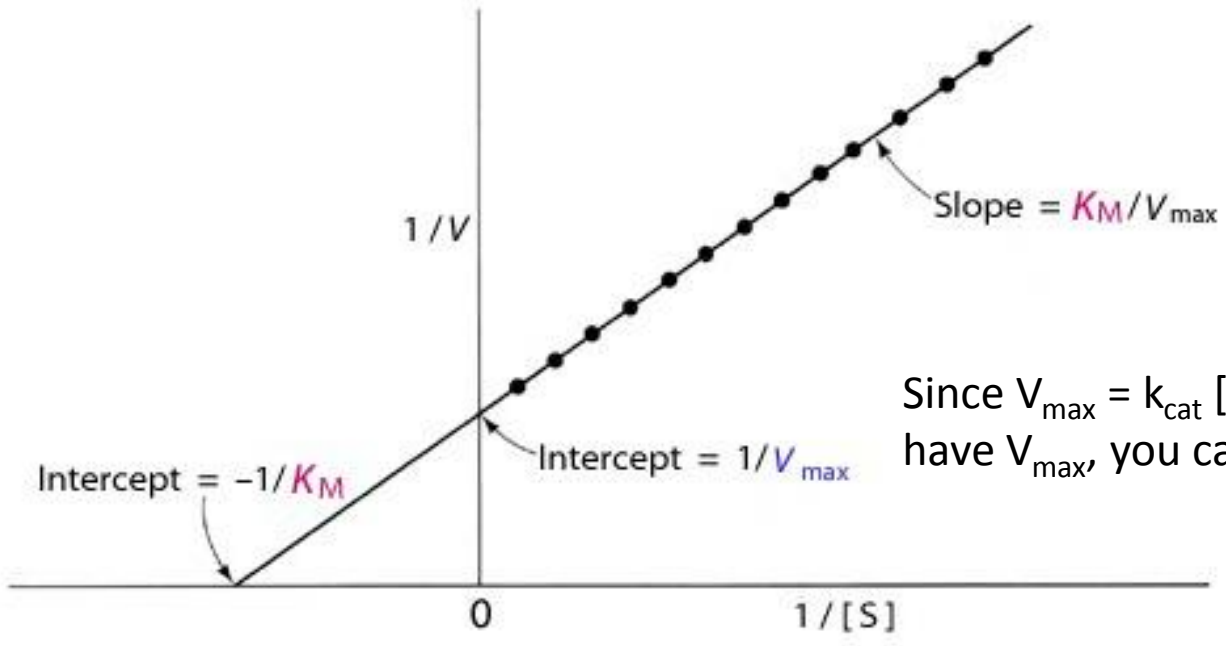
Lineweaver-Burk Plot

$V_{max} = 1/Y_{intercept}$

$k_{cat} = V_{max}/[E_t]$

$K_m = -1/X_{intercept}$

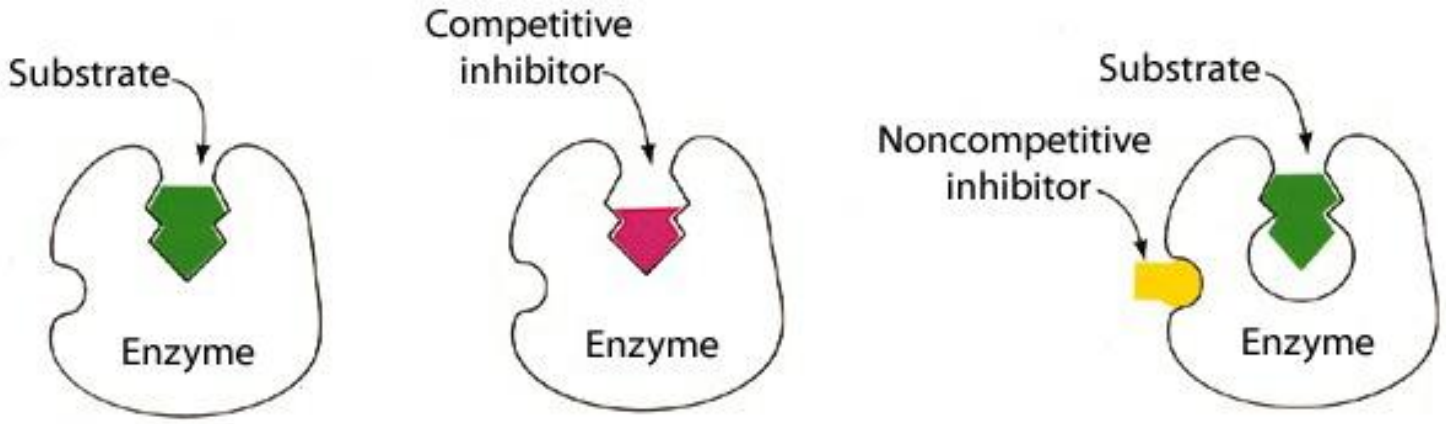
Lineweaver Burk Plots



Since $V_{max} = k_{cat}[E_T]$, once you have V_{max}, you can calculate k_{cat}

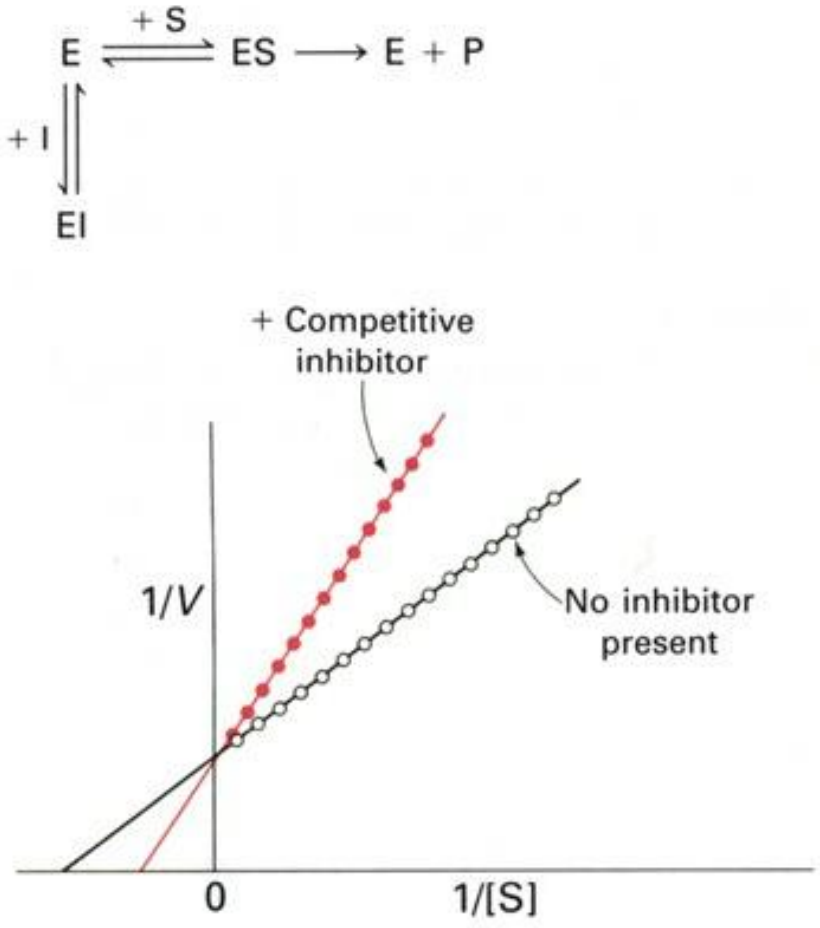
- These plots, known as double reciprocal plots, are a much preferred way of plotting data for an enzyme catalyzed reaction, though this idealizes error.
- Km is derived from the -1/X intercept
- V_{max} is derived from the 1/Y intercept

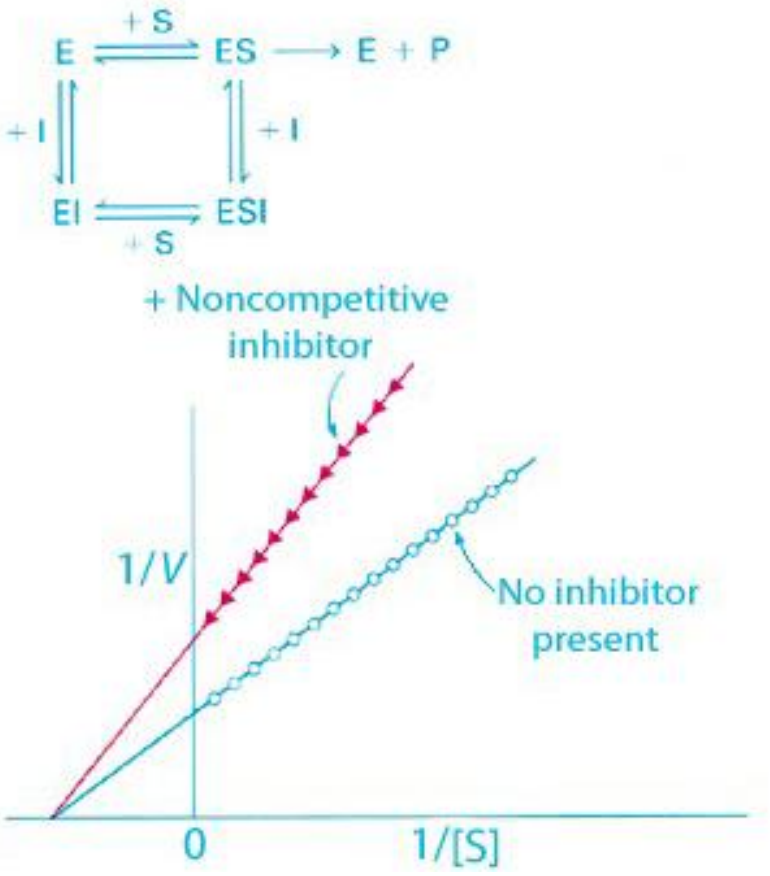
Reversible Enzyme Inhibition



- Competitive inhibitors slow down a reaction by competing with substrate for the active site, kcat not affected but Km appears to be higher (appears to be worse binding)
- Noncompetitive inhibitors slow down a reaction by binding at a remote site and slowing down kcat, Km not affected.

How to Distinguish Types of Inhibition





- Competitive and Noncompetitive Inhibition can be distinguished by how they affect the double reciprocal plots

Meaning of Michaelis Constants

- **I. k_{cat} (turnover number)....** the number of substrate molecules converted into product molecules by an enzyme molecule in a unit of time when the enzyme is fully saturated with substrate.
  - Turnover number is calculated from $k_{cat} = V_{max}/E_t$
  - Has units of s^{-1} (like a first order rate constant)
  - May or may not be a microscopic rate constant, in M^2 mechanism, $k_{cat} = k_2$
  - Range of about $1\text{-}10^7$ s^{-1}
  - The turnover number of most enzymes falls between 1 and 10,000 substrates converted to product per enzyme molecule per sec.

I. k_{cat} (turnover number) continued

Turnover numbers

Maximum turnover # of some enzymes

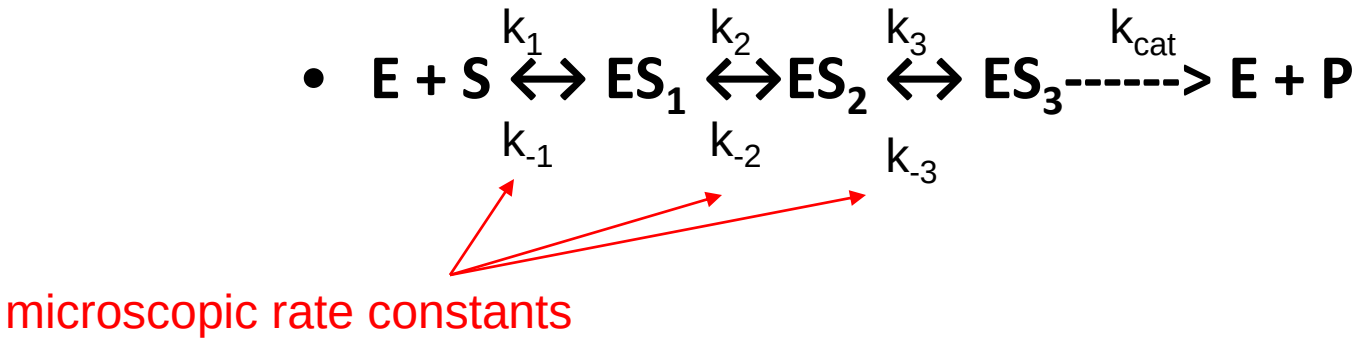
	k_{cat} (per s)	reaction time
carbonic anhydrase	600,000	1.7 us
3-ketosteroid isomerase	280,000	3.6 us
Acetylcholinesterase	25,000	40. us
Penicillinase	2,000	500. us
Chymotrypsin	100	10. ms
DNA polymerase I	15	67. ms
Tryptophan Synthase	2	500. ms
Lysozyme	0.5	2 s

NO MICROSCOPIC RATE CONSTANT CAN BE SLOWER THAN k_{cat}

k_{cat} cannot be greater than any first order rate constant along the forward reaction pathway, and thus helps to set a lower limit on all of the microscopic rate constants.

I. k_{cat} (turnover number) continued

- ***What is a microscopic rate constant?***
- **NO MICROSCOPIC RATE CONSTANT CAN BE SLOWER THAN k_{cat}**



- k_{cat} cannot be greater than any first order rate constant along the forward reaction pathway, and thus helps to set a lower limit on all of the microscopic rate constants.

II. K_m, the Michaelis constant

- experimentally measured from [S] at ½ V_{max}
- Units of M or mM or µM
- represents dissociation of Michaelis complex and is either a true or apparent dissociation equilibrium constant
- $K_m = [E]\,[S] / \sum[ES_n]$
- Range 10^{-7} to 10^{-3} M

Enzyme	**Substrate**	**K_m (mM)**
Chymotrypsin	acetyl-tryptophanamide	5000
Lysozyme	hexa-N-acetylglucosamine	6
b-Galactosidase	Lactose	4000
Thr deaminase	Threonine	5000
Carbonic Anhydrase	CO_2	8000
Pyruvate carboxylase	Pyruvate	400
–	HCO_3^-	1000
–	ATP	60

- **II. K_m, the Michaelis constant (cont'd)**
- It is informative to compare the natural substrate concentration and K_m, $[S]/K_m$
  - If $[S]/K_m < 1$; $[S] < K_m$; the enzyme is not very saturated and the system is not working at full capacity.
  - If $[S]/K_m = 1$; $[S] = K_m$; the enzyme is half saturated, and here, the system is both working at a decent velocity and quite sensitive to changing conditions.
  - If $[S]/K_{m;} > 1$; $[S] > K_m$; the enzyme is saturated with substrate and the system is working at full capacity, but NOT sensitive to changes in substrate concentration.
- **The $[S]/K_m$ ratio is typically between 0.01 and 1.0. This can be interpreted as most systems not working at full capacity, so that they function with a built in throttle to rapidly accelerate the rate should there be a sudden biological demand.**

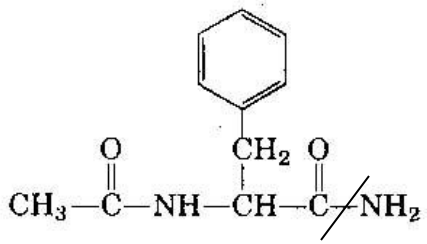
- **III. Kinetic Perfection in Enzymic Catalysis, The k_{cat}/K_m criterion**
  - Velocity = $\frac{k_{cat}[E_t][S_t]}{K_m + [S_t]}$
  -
  -
- When enzymes are not saturated with substrate,
  - $V = (k_{cat}/K_m)[E_t][S]$
- Graphically $(k_{cat}/K_m)[E_t]$ is the slope of V vs [S] at low [S]
- k_{cat}/K_m has units of $M^{-1}s^{-1}$
- k_{cat}/K_m is an apparent second order rate constant looks at free [E] with [S] and is really of interest when studying enzyme-substrate specificity

- **III. Kinetic Perfection in Enzymic Catalysis, The k_{cat}/K_m criterion**

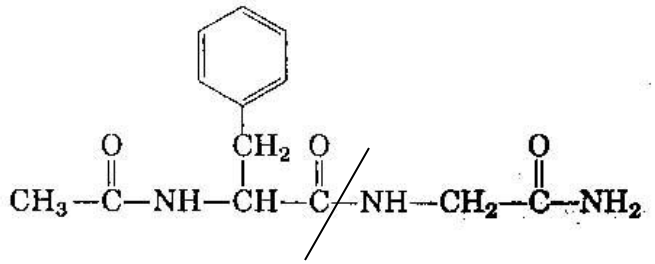
- k_{cat}/K_m is a critical parameter when assessing enzyme specificity for competing substrates, if k_{cat}/K_m increases, the specificity increases.
- Some enzymes have k_{cat}/K_m of 10^7 or 10^8 $M^{-1}s^{-1}$, which is amazing specificity! (diffusion controlled second order rate constants are about this value)
- Let's look at the effects of small structural changes in the substrate on the kinetic parameters for chymotrypsin catalyzed amide hydrolysis:

III. Kinetic Perfection in Enzymic Catalysis, The k_{cat}/K_m criterion

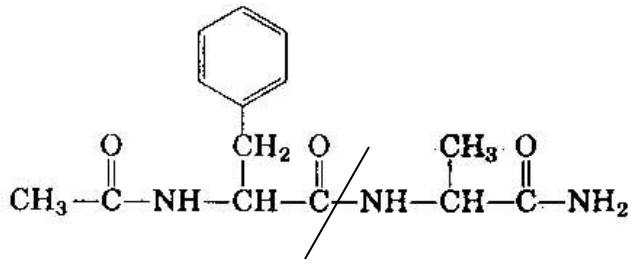
Effects of small structural changes in the substrate
on the kinetic parameters for chymotrypsin catalyzed amide hydrolysis



	k_{cat} (s^{-1})	K_m (mM)	k_{cat}/K_m ($M^{-1}\ s^{-1}$)
Substrate A	0.06	31	2

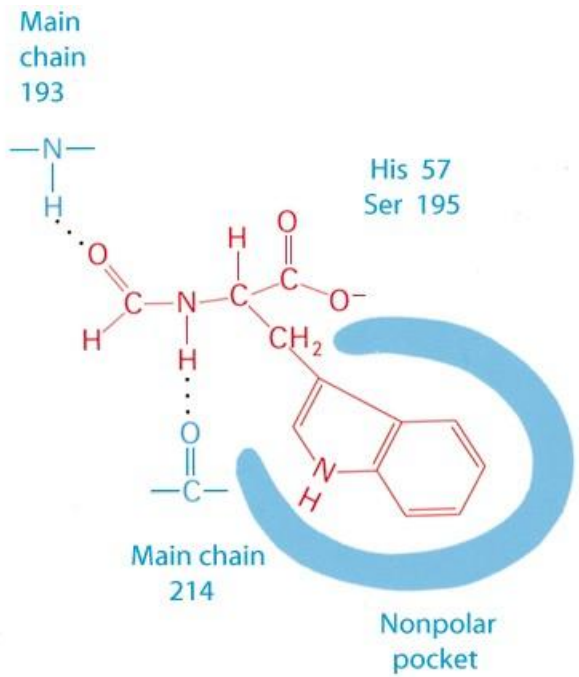


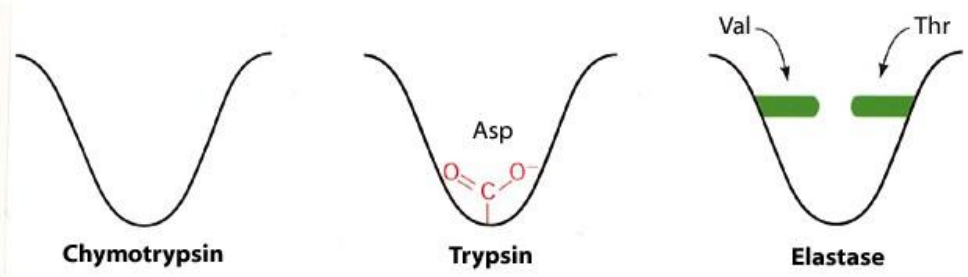
	k_{cat} (s^{-1})	K_m (mM)	k_{cat}/K_m ($M^{-1}\ s^{-1}$)
Substrate B	0.14	15	10



	k_{cat} (s^{-1})	K_m (mM)	k_{cat}/K_m ($M^{-1}\ s^{-1}$)
Substrate C	2.8	25	114

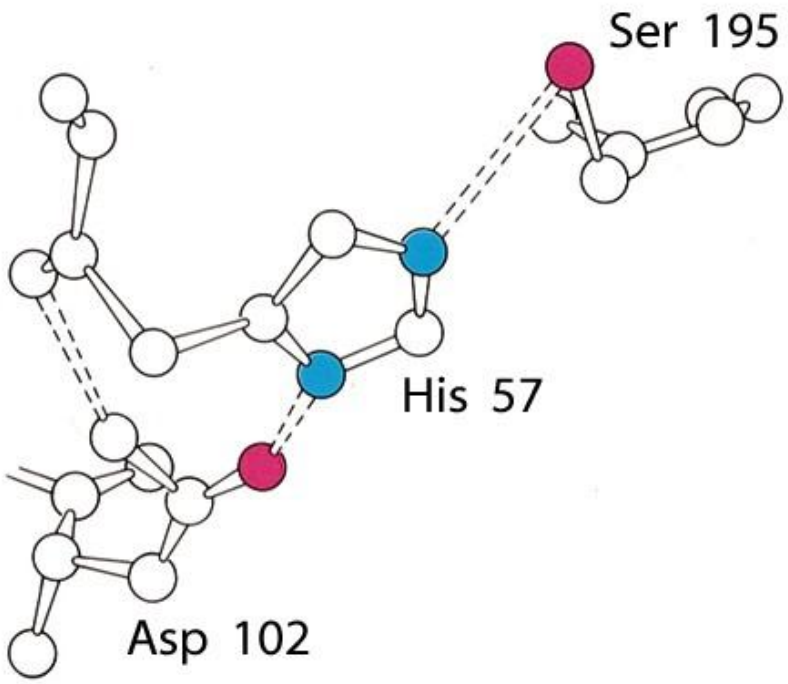
A Family of Serine Proteases





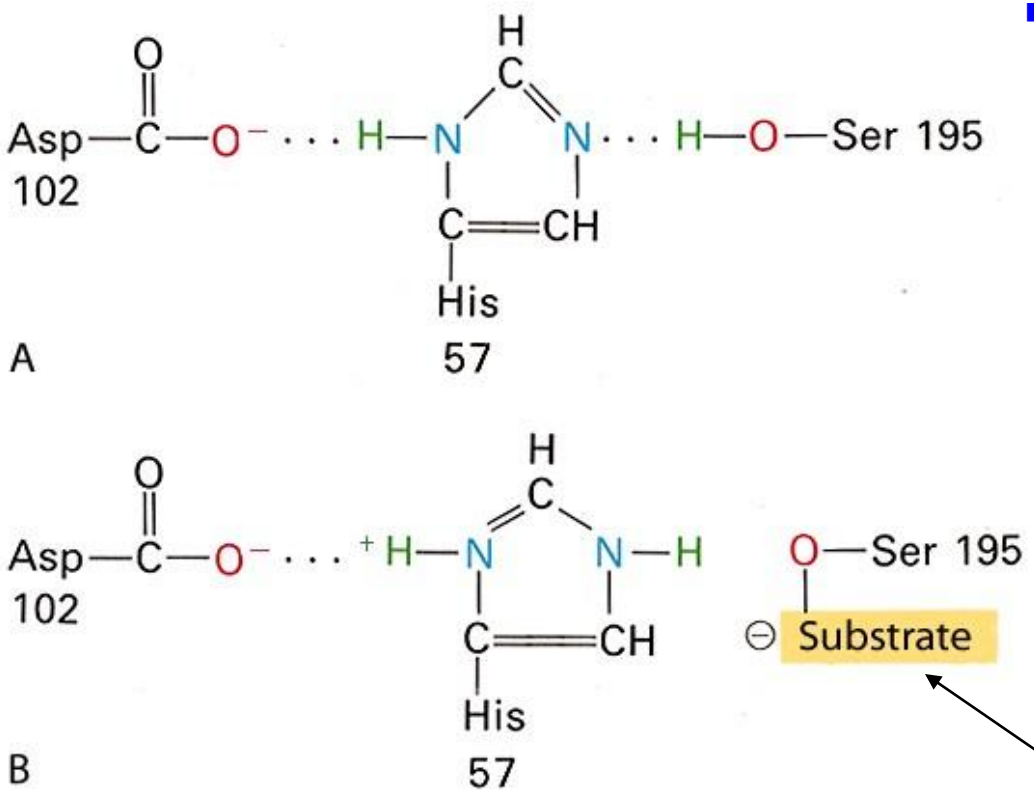
- Substrate specificity pocket helps chymotrypsin choose the right substrate
  - large nonpolar channel into which side group can be place adjacent to scissile bond (bond to be cut by enzyme)
- Other serine proteases have other specificity pockets
  - Lys, Arg for Trypsin (Asp in bottom of oxyanion hole
  - Ala, Val for Elastase

Proteases: Enzymes that Hydrolyze Peptide Bonds



- Catalytic Triad in Serine Proteases:
  - Serine 195 Nucleophile
  - His 57, neutral, acts as a proton shuttle
  - Asp 102, activates His through H bonding

Proteases: Enzymes that Hydrolyze Peptide Bonds

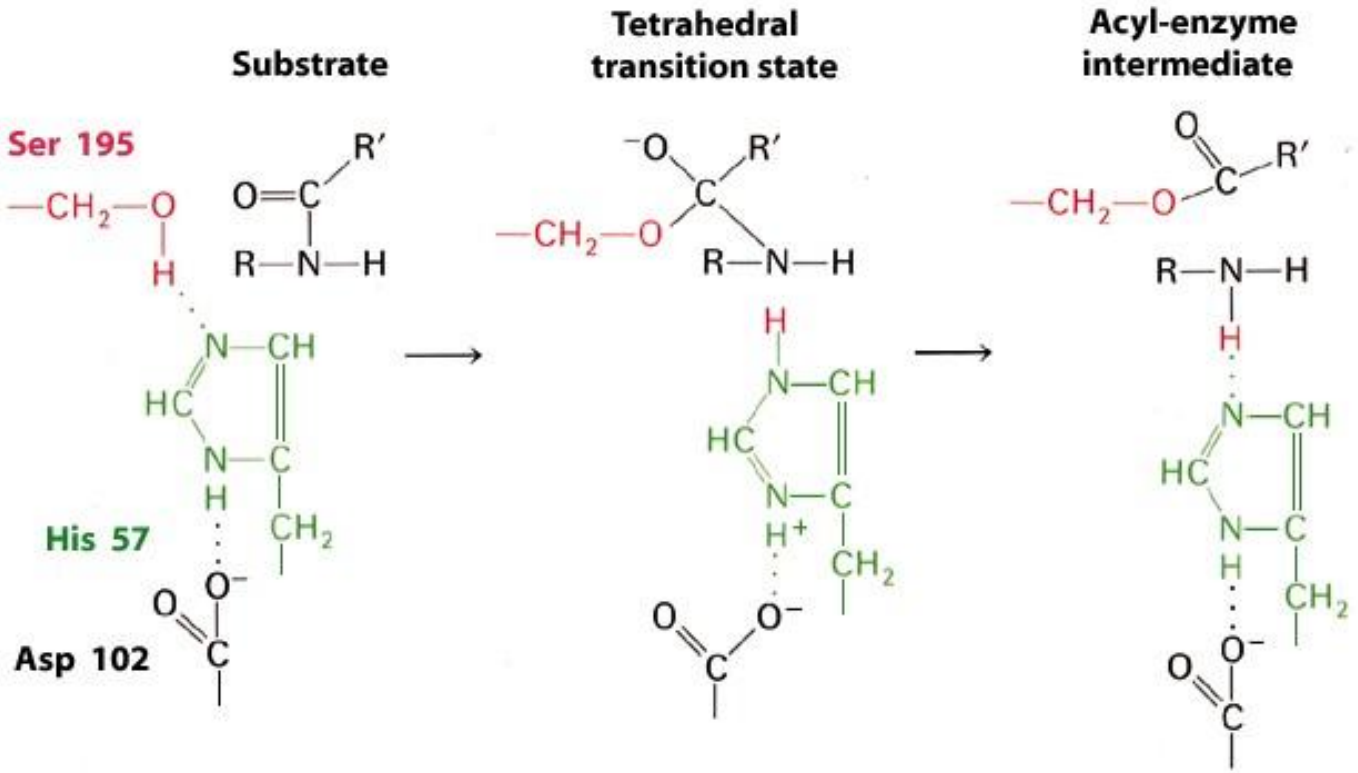


- How the catalytic triad works
  - Ser is activated by His, to which it transfers a H when it attacks the carbonyl of the substrate
  - His holds onto H from serine transiently, His+ stabilized by Asp- in acyl enzyme intermediate

http://www.amherst.edu/~pbohara/

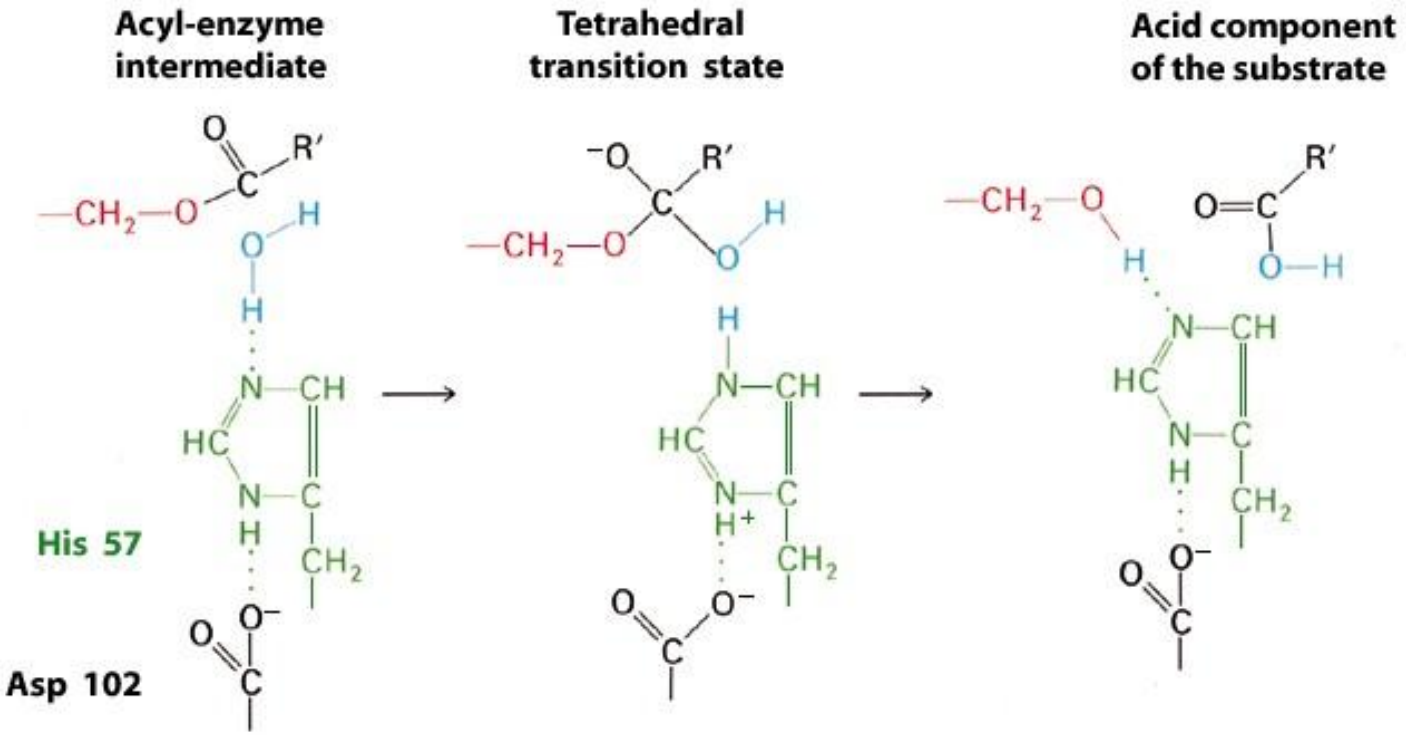
Catalytic Mechanism for Trypsin

- Phase I
- E + S = ES --> [ES]^{tt} --> EA + P_1 (amino)

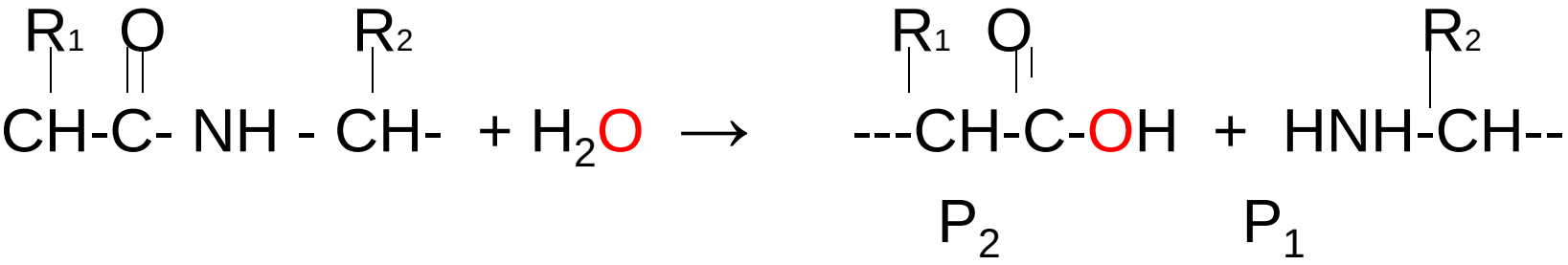


Catalytic Mechanism for Trypsin

- **Phase II**
- **E A --> [EA]^{tt} --> E + P2 (carboxylate)**



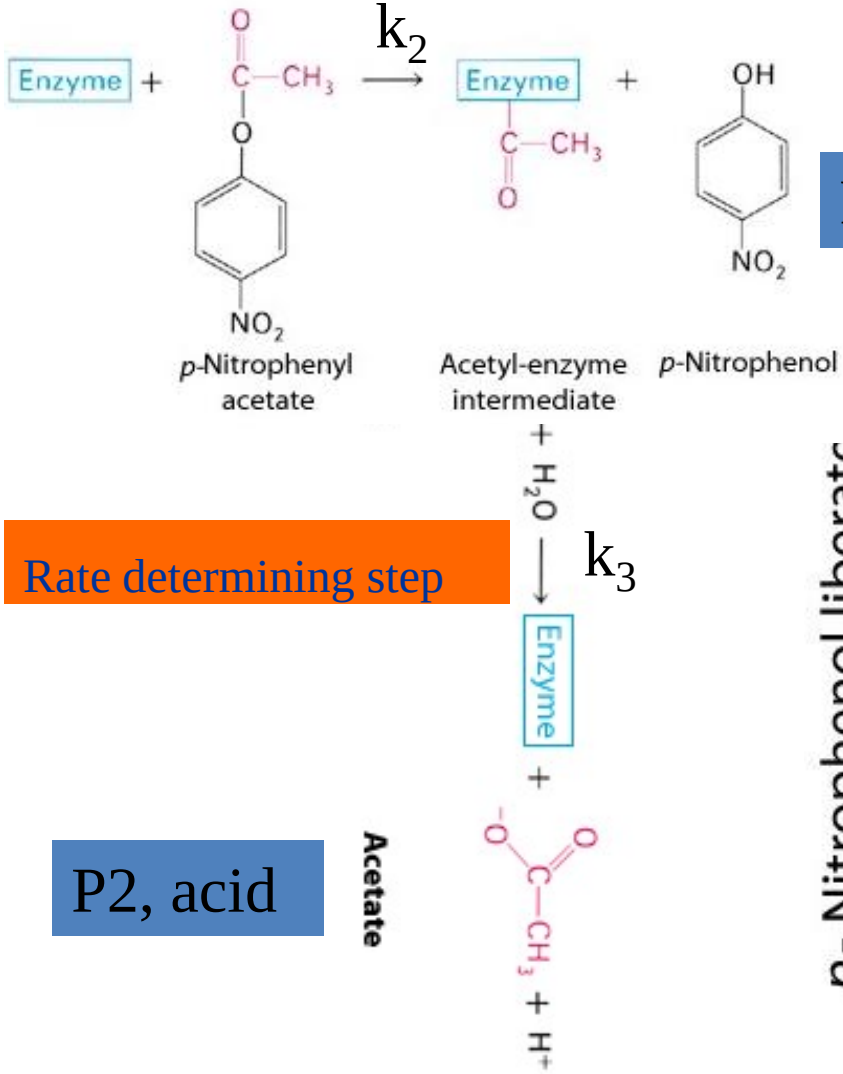
Proteases:Enzymes that Hydrolyze Peptide Bonds

- Amide Bond Hydrolysis
- 

- $E + S = ES \xrightarrow{k_2} EA + P_1 \xrightarrow{k_3} E + P_2$
- For amide bond hydrolysis, formation of EA is rate limiting, $k_2 < k_3$, , k_2 is rate limiting

Ping Pong Mechanism

Ester Bond Hydrolysis, k_2 is much faster, now k_3 is rate limiting



P_1, phenol

Esters, $k_2 >> k_3$

Rate determining step

P2, acid