ENZYMES: Binding & Catalysis

A. Binding
B. Catalysis
C. Nomenclature
D. Catalysis
   1. Transition State Theory
   2. **Catalytic** strategies (**What**)
   3. **Mechanistic** strategies (**How**)
E. Quantifying the Catalytic Power: Kinetics
   1. Review
   2. Enzyme Kinetics
   3. Rate vs. [S] for enzyme catalyzed reaction; initial rate (v_0)
   4. ES complex
      a. Reaction
         i. Binding reaction
         ii. Catalytic reaction
      b. Meaning of rate curve: hyperbolic curve
   5. Rate expression; Michaelis-Menten Kinetics (M-M)
      a. Assumptions
      b. M-M equation derivation
   6. Meaning of rate expression (M-M eqn)
      a. $[S] = K_m$
      b. $[S] >> K_m$
      c. $[S] << K_m$
   7. Collection and manipulation of data
      a. Lineweaver-Burk; double reciprocal; $1/v_0$ vs. $1/[S]$
      b. Eadie-Hofstee; v_0 vs. $v_0/[S]$
      c. Hanes-Woolf; $[S]/v_0$ vs. $1/[S]$
   8. Inhibition
      a. Irreversible: protein modification
      b. Reversible
         i. Competitive; like substrate; K_m affected by $(1 + [I]/K_I) = \alpha$
         ii. Uncompetitive; binds only ES; both K_m and V_{max} affected in opposite ways
         iii. Noncompetitive; binds both E & ES (mixed, non-equal binding); V_{max} affected
         iv. Mixed inhibition if I binds E differently than it binds ES

v. Using Steady-state kinetics: Active Site
vi. Energetics of Catalysis

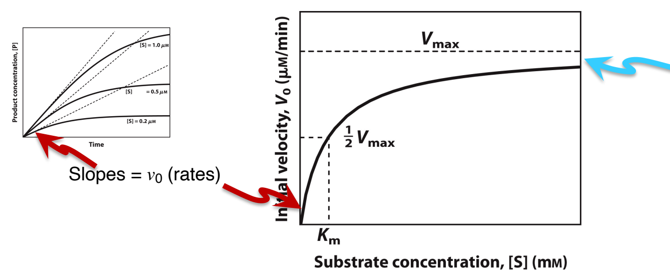
- Reading: Ch6; 191, 197-200
- Homework #15

NEXT
- Reading: Ch6; 181-186, 195-196
- Homework #16

Lecture 15 (10/18/23)

Enzyme Kinetics

Determination of Kinetic Parameters



A nonlinear Michaelis-Menten plot could be used to calculate parameters K_m and V_{max}.

Lineweaver-Burk derived a linear form of the M-M equation by taking the reciprocal of both sides. This is called the linearized double-reciprocal plot. Its good for analysis of enzyme kinetic data to get these kinetic parameters.

Enzyme Kinetics

Lineweaver-Burk Plot: Linearized, Double-Reciprocal

The Michaelis-Menten equation can be manipulated into one that yields a straight-line plot.

$$v_0 = \frac{V_{max}[S]}{K_m + [S]}$$

$$\frac{1}{v_0} = \frac{K_m + [S]}{V_{max}[S]}$$

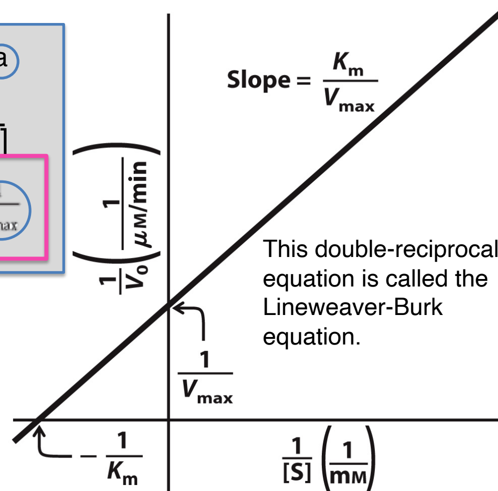
$$\frac{1}{v_0} = \frac{K_m}{V_{max}[S]} + \frac{[S]}{V_{max}[S]}$$

$$\frac{1}{v_0} = \frac{K_m}{V_{max}[S]} + \frac{1}{V_{max}}$$

y = bx + a

$y = \frac{1}{v_0}$ $x = \frac{1}{[S]}$

$$\frac{1}{V_0} = \frac{K_M}{V_{max}} \cdot \frac{1}{S} + \frac{1}{V_{max}}$$



This double-reciprocal equation is called the Lineweaver-Burk equation.

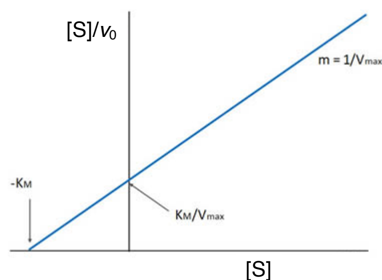
Enzyme Kinetics

Other Linearized Derivations of the M-M Equation

Hanes-Woolf

$$\frac{[S]}{v} = \frac{[S]}{V_{max}} + \frac{K_m}{V_{max}}$$

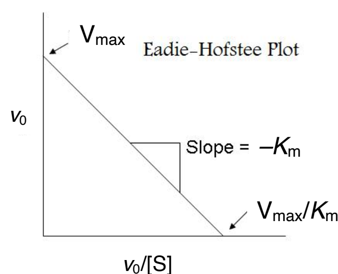
$\frac{[S]}{v_0}$ *vs.* [S]



Eadie-Hofstee

$$v_0 = V_{max} - K_m \frac{v_0}{[S]}$$

v_0 *vs.* $v_0/[S]$



Enzyme Kinetics

Linearized Derivations of the M-M Equation

Lineweaver-Burk

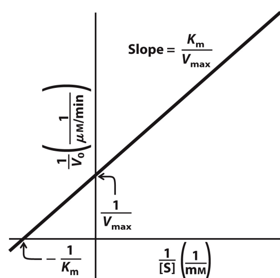
$$\frac{1}{V_0} = \frac{K_M}{V_{max}} \cdot \frac{1}{S} + \frac{1}{V_{max}}$$

Hanes-Woolf

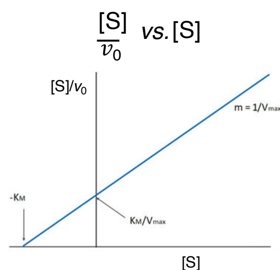
$$\frac{[S]}{v} = \frac{[S]}{V_{max}} + \frac{K_m}{V_{max}}$$

Eadie-Hofstee

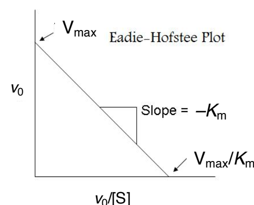
$$v_0 = V_{max} - K_m \frac{v_0}{[S]}$$

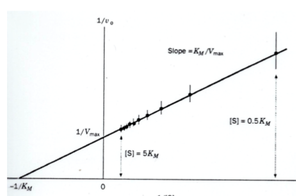


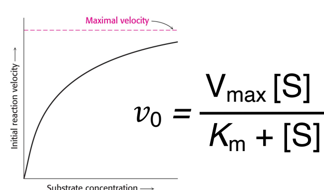
$\frac{[S]}{v_0}$ *vs.* [S]



v_0 *vs.* v_0/[S]







$$v_0 = \frac{V_{max}[S]}{K_m + [S]}$$

Enzyme Kinetics

ENZYME INHIBITION

Enzyme Kinetics

What is Enzyme Inhibition?

This is the action of a small molecule that results in loss of enzyme activity

This is not regulation by the action of another enzyme or protein
This is not loss of enzyme activity due to denaturation/unfolding of the enzyme.

Two major kinds of inhibition
1) Irreversible
2) Reversible

Enzyme Kinetics

Reversible Enzyme Inhibition:

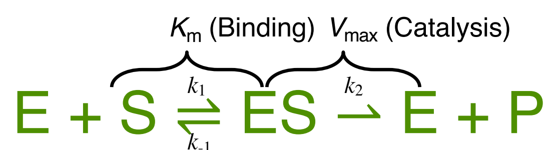
There are THREE-to-FOUR types of reversible inhibition:

1) Competitive
2) Un-Competitive
3) Non-Competitive
4) Mixed

(3 and 4: These are closely related)

Enzyme Kinetics

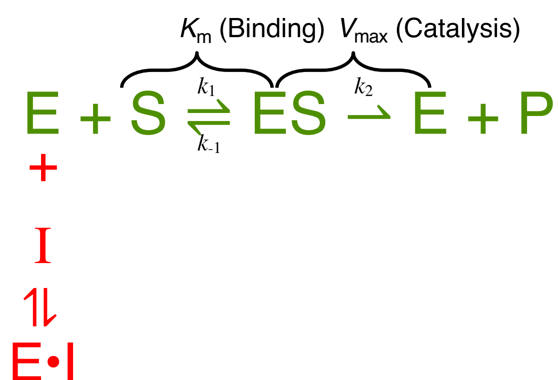
Reversible Enzyme Inhibition:



No Inhibition

Enzyme Kinetics

Reversible Enzyme Inhibition:



Competitive

Enzyme Kinetics

Reversible Enzyme Inhibition:

K_m (Binding) V_{max} (Catalysis)

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

$$ES + I \rightleftharpoons ES{\bullet}I$$

Un-Competitive

Enzyme Kinetics

Reversible Enzyme Inhibition:

K_m (Binding) V_{max} (Catalysis)

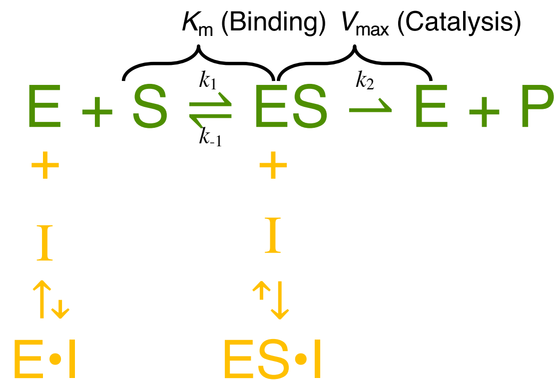
$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \overset{k_2}{\rightharpoonup} E + P$$

$$E + I \rightleftharpoons E{\bullet}I \qquad ES + I \rightleftharpoons ES{\bullet}I$$

Non-Competitive

Enzyme Kinetics

Reversible Enzyme Inhibition:



Mixed

Enzyme Kinetics

Reversible Enzyme Inhibition:

There are THREE-to-FOUR types of reversible inhibition:

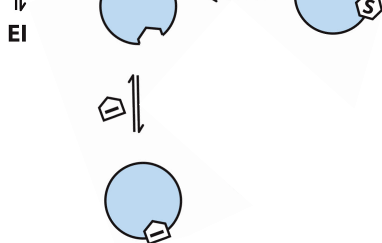
1) Competitive
2) Un-Competitive
3) Non-Competitive
4) Mixed

These are closely related

Competitive Inhibition

- Competes with substrate for binding
  - easiest to remember
  - binds active site
  - does not affect catalysis (e.g., once ES is formed, catalysis occurs)

Competitive inhibition



How does this inhibition affect the rate expression?

It pulls on the binding reaction (competing with S for free E)

Which kinetic constant will be affected?

K_m becomes an "Apparent" K_m (in the presence of inhibitor): ${}^{App}K_m$

$$v_0 = \frac{V_{max}[S]}{K_m + [S]}$$

$$\Downarrow$$

$$v_0 = \frac{V_{max}[S]}{K_m\left(1+\frac{[I]}{K_I}\right) + [S]}$$

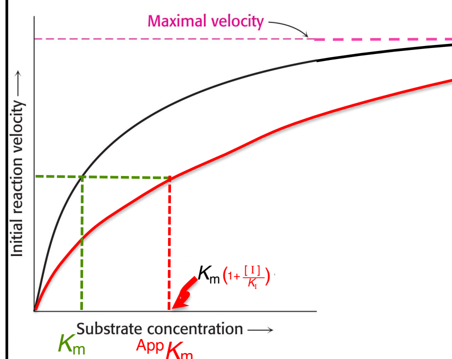
Derivation on line

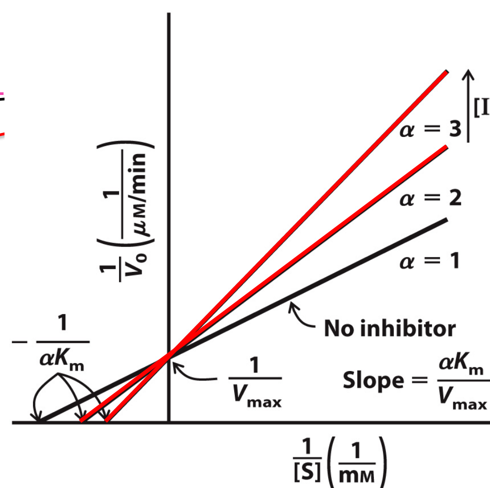
Competitive Inhibition

$$\left(1+\frac{[I]}{K_I}\right) = \alpha$$

$$K_I = \frac{[E][I]}{[EI]} \qquad v_0 = \frac{V_{max}[S]}{K_m\left(1+\frac{[I]}{K_I}\right) + [S]}$$

$$\frac{1}{V_0} = \left(\frac{\alpha K_m}{V_{max}}\right)\frac{1}{[S]} + \frac{1}{V_{max}}$$

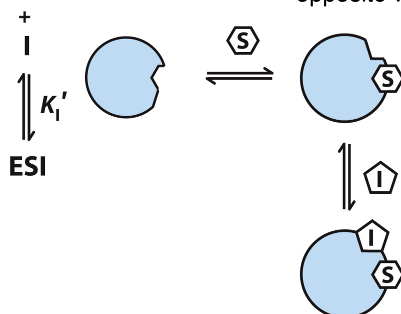




- No change in V_{max}; apparent increase in K_m
- Lineweaver-Burk: lines intersect at the *y*-axis.

Uncompetitive Inhibition

- Only binds to ES complex; AFTER S
  - The binding of S causes a conformational change and creates the site for inhibitor
  - affects substrate binding by pulling binding equilibrium (makes it look better!)
  - affects catalytic function by pulling catalysis equilibrium (depleting [ES])



How does this inhibition affect the rate expression?

Affects both Binding and Catalysis, but in opposite ways

Which kinetic constant will be affected?

Apparent K_m gets smaller

Apparent V_{max} gets smaller

$$v_0 = \frac{V_{max}[S]/(1+\frac{[I]}{K'_I})}{K_m/(1+\frac{[I]}{K'_I}) + [S]}$$

⇓

$$v_0 = \frac{V_{max}[S]}{K_m + [S](1+\frac{[I]}{K'_I})}$$

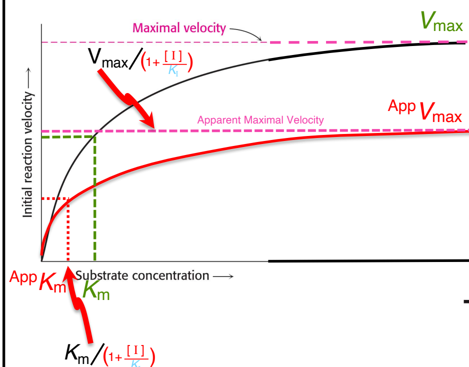
Uncompetitive Inhibition

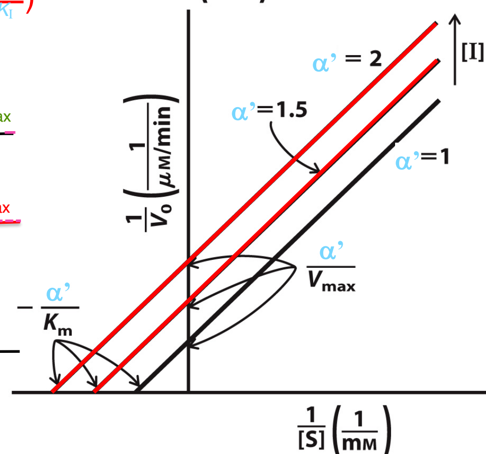
$(1+\frac{[I]}{K'_I}) = \alpha'$

$$K'_I = \frac{[ES][I]}{[ESI]}$$

$$v_0 = \frac{V_{max}[S]}{K_m + [S](1+\frac{[I]}{K'_I})}$$

$$\frac{1}{V_0} = \left(\frac{K_m}{V_{max}}\right)\frac{1}{[S]} + \frac{\alpha'}{V_{max}}$$





- Decrease in V_{max} & decrease in K_m (but to same extent!)
- No change in V_{max}/K_m
- Lineweaver-Burk: lines are parallel (recall slope is $1/V_{max}/K_m$)

Non-competitive Inhibition

- Binds BOTH free enzyme (E) and enzyme bound to substrate (ES)
  - binds to a entirely different site from the active site (regulatory/inhibitory site)
  - Essentially just titrates out the [enzyme]
  - inhibits both substrate binding and catalysis equally (rare)

Non-competitive inhibition

$$\begin{array}{ccccc} E + S & \rightleftharpoons & ES & \rightarrow & E + P \\ + & & + & & \\ I & & I & & \\ \Updownarrow K_I & & \Updownarrow K_I & & \\ EI + S & \rightleftharpoons & ESI & & \end{array}$$

How does this inhibition affect the rate expression?

Affects Catalysis; Binding is affected but to the same degree in opposite ways, so it cancels.

Which kinetic constant will be affected?

Apparent K_m stays the same as its pulled and pushed the same.

Apparent V_{max} gets smaller due to loss of $[E]_T$:

([E]+[ES]+[EI]+[ESI]).

Non-competitive Inhibition

Non-competitive inhibition

$$\begin{array}{ccccc} E + S & \rightleftharpoons & ES & \rightarrow & E + P \\ + & & + & & \\ I & & I & & \\ \Updownarrow K_I & & \Updownarrow K_I & & \\ EI + S & \rightleftharpoons & ESI & & \end{array}$$

$${}^{[E]}K_I = \frac{[E][I]}{[EI]}$$

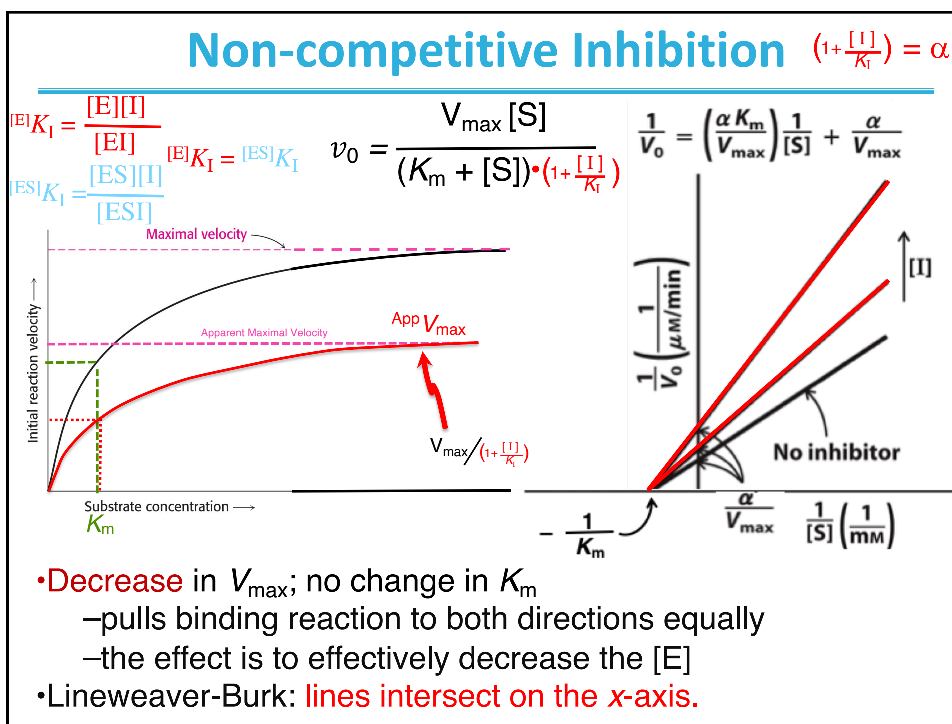
$${}^{[ES]}K_I = \frac{[ES][I]}{[ESI]}$$

$${}^{[E]}K_I = {}^{[ES]}K_I$$

$$v_0 = \frac{V_{max}[S]}{K_m + [S]}$$

$$v_0 = \frac{V_{max}[S] \Big/ \left(1 + \frac{[I]}{K_I}\right)}{K_m + [S]}$$

$$v_0 = \frac{V_{max}[S]}{(K_m + [S]) \cdot \left(1 + \frac{[I]}{K_I}\right)}$$



Enzyme Kinetics

Reversible Enzyme Inhibition:

There are THREE-to-FOUR types of reversible inhibition:

1) Competitive
2) Un-Competitive
3) Non-Competitive
4) Mixed

} These are closely related

Mixed Inhibition

- Variation of Non-competitive*
  - binds to regulatory/inhibitory site differently in free enzyme (E) versus enzyme-substrate complex (ES)

$$v_0 = \frac{V_{max}[S]}{K_m + [S]}$$

Mixed inhibition

E + S ⇌ ES ⟶ E + P
+ I ⇌ K_I ; + I ⇌ K_I'
EI + S ⇌ ESI

$$v_0 = \frac{V_{max}[S] / \left(1+\frac{[I]}{K_I'}\right)}{\left(1+\frac{[I]}{K_I}\right) K_m / \left(1+\frac{[I]}{K_I'}\right) + [S]}$$

$$v_0 = \frac{V_{max}[S]}{K_m\left(1+\frac{[I]}{K_I}\right) + [S]\left(1+\frac{[I]}{K_I'}\right)}$$

$^{[E]}K_I = \frac{[E][I]}{[EI]}$

$^{[ES]}K_I = \frac{[ES][I]}{[ESI]}$

$^{[E]}K_I \neq {}^{[ES]}K_I$

*Noncompetitive inhibitors are mixed inhibitors such that there is no change in K_m.

Mixed Inhibition

$\left(1+\frac{[I]}{K_I}\right) = \alpha$

$\left(1+\frac{[I]}{K_I'}\right) = \alpha'$

$^{[E]}K_I = \frac{[E][I]}{[EI]}$ $^{[ES]}K_I = \frac{[ES][I]}{[ESI]}$ $^{[E]}K_I \neq {}^{[ES]}K_I$

$$v_0 = \frac{V_{max}[S]}{K_m\left(1+\frac{[I]}{K_I}\right) + [S]\left(1+\frac{[I]}{K_I'}\right)}$$

$$\frac{1}{V_0} = \left(\frac{\alpha K_m}{V_{max}}\right)\frac{1}{[S]} + \frac{\alpha'}{V_{max}}$$

- Decrease in V_{max}; change in K_m
- Lineweaver-Burk: lines intersect left from the y-axis.

SUMMARY: Reversible Inhibition

= got worse = got better

K_m (Binding) V_{max} (Catalysis)

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

E + I ⇌ E•I

ES + I ⇌ ES•I

$\left(1+\frac{[I]}{K_I}\right) = \alpha$

$\left(1+\frac{[I]}{K'_I}\right) = \alpha'$

	App K_m	App V_{max}
Competitive	$K_m\left(1+\frac{[I]}{K_I}\right)$ (got worse)	V_{max}
Un-Competitive	$\frac{K_m}{\left(1+\frac{[I]}{K'_I}\right)}$ (got better)	$\frac{V_{max}}{\left(1+\frac{[I]}{K'_I}\right)}$ (got worse)
Non-Competitive	K_m	$\frac{V_{max}}{\left(1+\frac{[I]}{K'_I}\right)}$ (got worse)
Mixed	$\frac{K_m\left(1+\frac{[I]}{K_I}\right)}{\left(1+\frac{[I]}{K'_I}\right)}$ (got better / got worse)	$\frac{V_{max}}{\left(1+\frac{[I]}{K'_I}\right)}$ (got worse)

Enzymes

ACTIVE SITE

SUMMARY SO FAR:

We have described enzymes in general terms such as:

- catalytic cycle
- binding, even stereo-specific binding
- catalysis, turnover number & proficiency
- nomenclature
- transition state theory
- catalytic strategies (what to do)
- mechanistic strategies (how to do)
- enyzme kinetics and inhibition

ALL of this happens at the ACTIVE SITE

Now, we want to ask what all happens here & how do we determine what happens?

Enzymes

How do your determine what is going on at the active site?

We will discuss FOUR methods for study of the active site

1. Enzyme kinetics
2. pH studies
3. Protein modification
4. Structural studies