Lecture 15 (10/16/20)

- Reading: Ch6; 207-210
  Ch6; Box 6-1
- Problems: Ch6 (text); 15, 16, 18-21
  Ch6 (study guide-facts); 8,9,10, 11, 12
  Ch6 (study guide-applying); **2**

NEXT

- Reading: Ch6; 192-193, 195-196, 205-206
- Problems: Ch6 (text); 22,24
  Ch6 (study guide-facts); 15

Lecture 15 (10/16/20)

ENZYMES: Binding & Catalysis

A. Kinetics-review

B. Enzyme Kinetics

1. Rate vs. [S] for enzyme catalyzed reaction
   a. initial rate (v_0)
2. ES complex
   a. Reaction
      i. Binding reaction
      ii. Catalytic reaction
   b. Meaning of rate curve: hyperbolic curve
3. Rate expression; Michaelis-Menten Kinetics (M-M)
   a. Assumptions
   b. M-M equation derivation
4. Meaning of rate expression (M-M equation)
   a. [S] = Km
   b. [S] >> Km
   c. [S] << Km
5. Collection and manipulation of data
   a. Lineweaver-Burk; double reciprocal; 1/vo vs. 1/[S]
   b. Eadie-Hofstee; vo vs. vo/[S]; Similar to Scatchard Plot for binding; (Y vs. Y/[S])
   c. Hanes-Woolf; [S]/vo vs. 1/[S]
6. 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

Enzyme Kinetics

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

Binding Catalysis

What is the rate equation for enzyme kinetics?

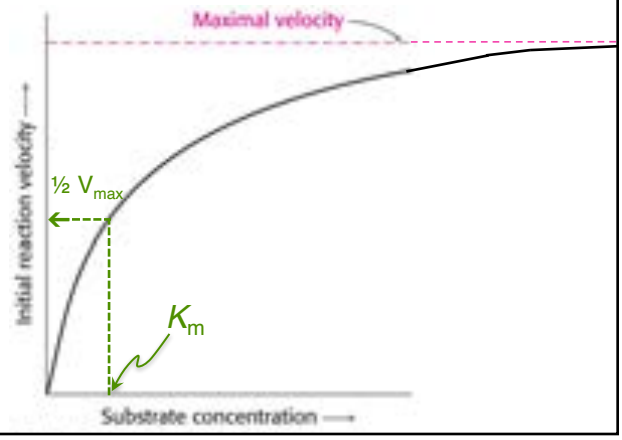
Michaelis-Menten Equation

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

① Special cases:

What is v_0 when [S] = K_m?

When K_M = [S], v_0 = ½ V_{max}. Thus, K_M is the substrate concentration that yields ½ V_{max}.



Enzyme Kinetics

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

Binding Catalysis

What is the rate equation for enzyme kinetics?

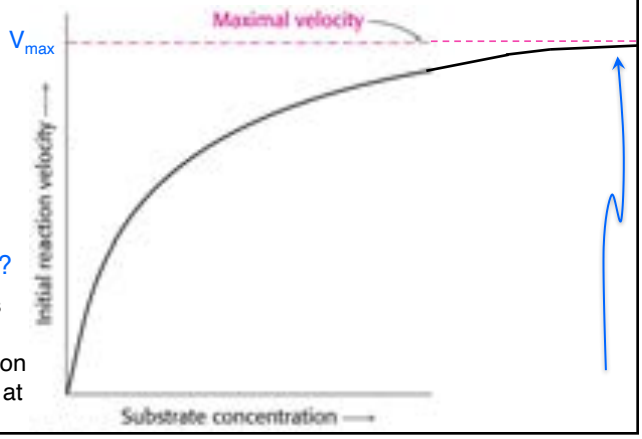
Michaelis-Menten Equation

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

② Special cases:

What is v_0 when [S] >> K_m?

When K_M << [S], its value is negligible and v_0 = V_{max}. Thus, at high [S], the equation tells us the obvious; we are at V_{max}.



Enzyme Kinetics

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

Binding Catalysis

What is the rate equation for enzyme kinetics?

Michaelis-Menten Equation

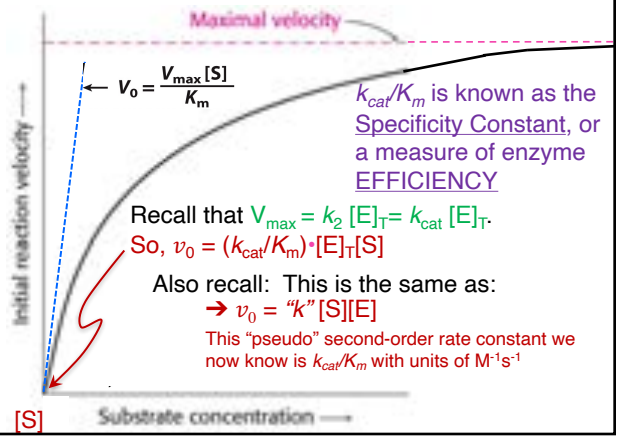
$$v_0 = \frac{V_{max}[S]}{K_m + [S]}$$

③ Special cases:

What is v_0 when [S] << K_m?

When K_M >> [S], v_0 = (V_{max}/K_m)•[S].

Thus, the rate is strictly dependent on the [S]. But, remember that it also depends on [E].



k_{cat}/K_m is known as the Specificity Constant, or a measure of enzyme EFFICIENCY

Recall that $V_{max} = k_2 [E]_T = k_{cat} [E]_T$.

So, $v_0 = (k_{cat}/K_m)•[E]_T[S]$

Also recall: This is the same as:

→ v_0 = "k" [S][E]

This "pseudo" second-order rate constant we now know is k_{cat}/K_m with units of $M^{-1}s^{-1}$

Enzyme Kinetics

Enzyme Efficiency is Limited by Specificity: k_{cat}/K_M

- Diffusion from the active site limits the maximum value for specificity/efficiency.
- Can gain efficiency by having high velocity or affinity for substrate
  - catalase vs. acetylcholinesterase

TABLE 6-8 Enzymes for Which k_{cat}/K_m is Close to the Diffusion-Controlled Limit (10^8 to 10^9 $M^{-1}s^{-1}$)

Enzyme	Substrate	k_{cat} (s^{-1})	K_m (M)	k_{cat}/K_m ($M^{-1}s^{-1}$)
Acetylcholinesterase	Acetylcholine	1.4×10^4	9×10^{-5}	1.6×10^8
Carbonic anhydrase	CO_2	1×10^6	1.2×10^{-2}	8.3×10^7
	HCO_3^-	4×10^5	2.6×10^{-2}	1.5×10^7
Catalase	H_2O_2	1×10^7	2.5×10^{-2}	4×10^8
Crotonase	Crotonyl-CoA	5.7×10^3	2×10^{-5}	2.8×10^8
Fumarase	Fumarate	8×10^2	5×10^{-6}	1.6×10^8
	Malate	9×10^2	2.5×10^{-5}	3.6×10^7
β-Lactamase	Benzylpenicillin	2.0×10^3	2×10^{-5}	1×10^8

Source: A. Fersht, *Structure and Mechanism in Protein Science*, p. 166, W. H. Freeman and Company, 1999.

Enzyme Kinetics

SUMMARY:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

- The final form in case of a single substrate is the Michaelis-Menten equation:

$$v_0 = \frac{k_{cat}[E_{tot}][S]}{K_m + [S]} = \frac{V_{max}[S]}{K_m + [S]}$$

- k_{cat} (turnover number): how many substrate molecules one enzyme molecule can convert per second
- K_m (Michaelis constant): an approximate measure of a substrate's affinity for an enzyme; actually ratio of rate constants for formation and loss intermediate involved in rate-limiting step.
- During steady state, the maximum velocity (V_{max}) occurs when all of the enzyme is in the ES complex and is dependent on the breakdown of that complex (k[ES]).
- The microscopic meaning of K_m and k_{cat} depends on the details of the mechanism.

Enzyme Kinetics

CLINICAL INSIGHT

Variations in K_M Can Have Physiological Consequences

Two enzymes play a key role in the metabolism of alcohol.

$$CH_3CH_2OH + NAD^+ \xrightleftharpoons{\text{Alcohol dehydrogenase}} CH_3CHO + NADH + H^+$$

Ethanol — Acetaldehyde

$$CH_3CHO + NAD^+ + H_2O \xrightleftharpoons{\text{Aldehyde dehydrogenase}} CH_3COO^- + NADH + 2H^+$$

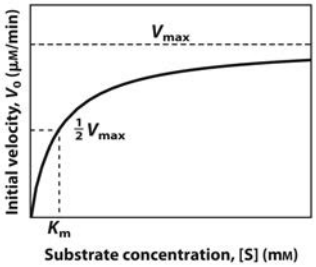
Some people respond to alcohol consumption with facial flushing and rapid heart beat, symptoms caused by excessive amounts of acetaldehyde in the blood. There are two different acetaldehyde dehydrogenases in most people, one with a low K_M and one with a high K_M.

The low K_M enzyme is genetically inactivated in some individuals. The enzyme with the high K_M cannot process all of the acetaldehyde, and so some acetaldehyde appears in the blood.

So, if knowing the values of the constants, K_m and V_{max}, for enzymes and their substrates is important, how are they determined?

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.

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

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

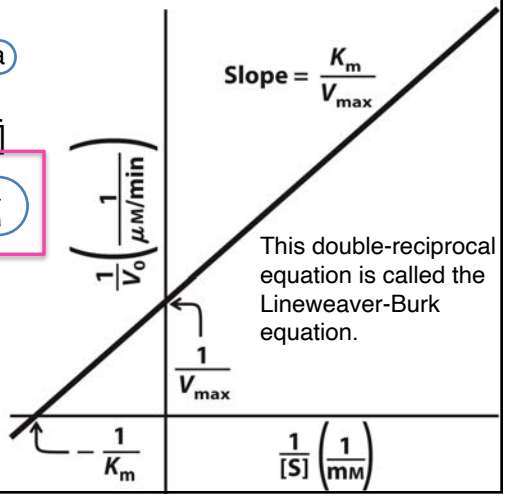
$$\frac{1}{v_0} = \frac{K_m}{V_{max}[S]} + \frac{[S]}{V_{max}[S]}$$

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

$y = bx + a$

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

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



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

Enzyme Kinetics

Lineweaver-Burk Plot: Linearized, Double-Reciprocal

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

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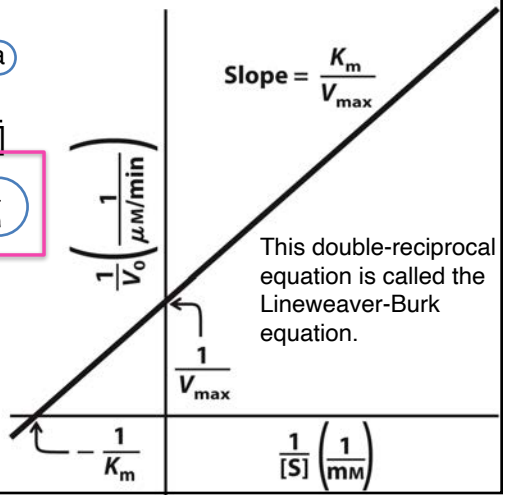
$$\frac{1}{v_0} = \frac{K_m}{V_{max}[S]} + \frac{[S]}{V_{max}[S]}$$

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

y = bx + a

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

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



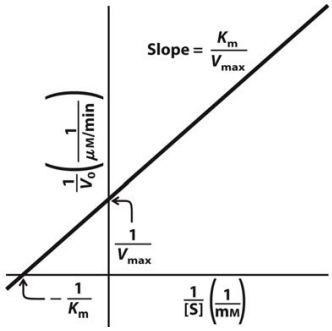
This double-reciprocal equation is called the Lineweaver-Burk equation.

Enzyme Kinetics

Linearized Derivations of the M-M Equation

Lineweaver-Burk

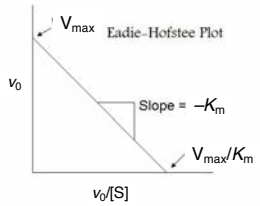
$$\frac{1}{V_0} = \frac{K_M}{V_{max}} \cdot \frac{1}{S} + \frac{1}{V_{max}}$$



Eadie-Hofstee

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

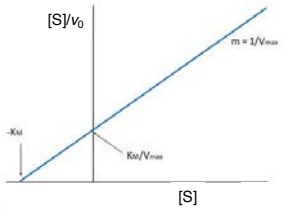
v_0 *vs.* $v_0/[S]$

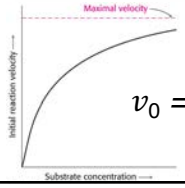


Hanes-Woolf

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

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

Enzyme Kinetics

Reversible Enzyme Inhibition:

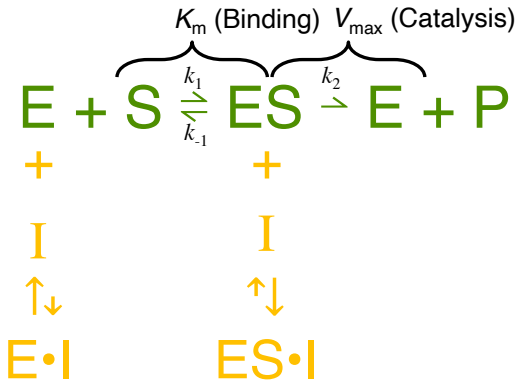
There are THREE-to-FOUR types of reversible inhibition:

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

} These are closely related

Enzyme Kinetics

Reversible Enzyme Inhibition:

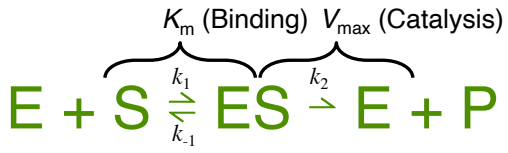


Competitive Un-Competitive

Non-Competitive Mixed

Enzyme Kinetics

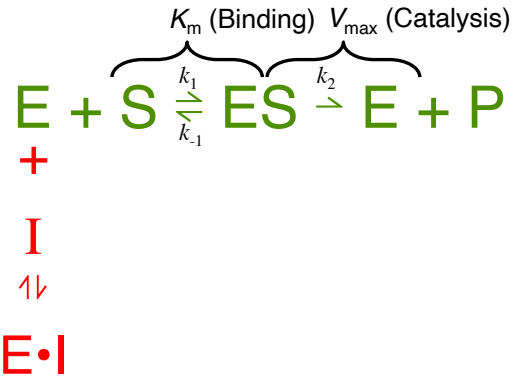
Reversible Enzyme Inhibition:



No Inhibition

Enzyme Kinetics

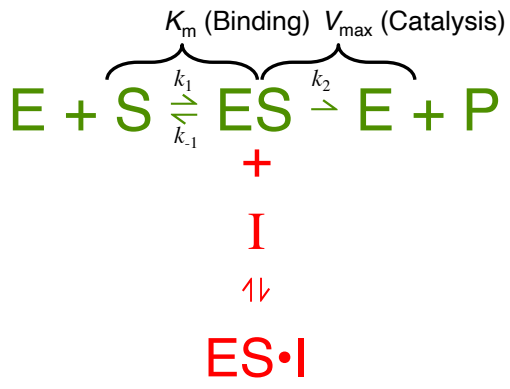
Reversible Enzyme Inhibition:



Competitive

Enzyme Kinetics

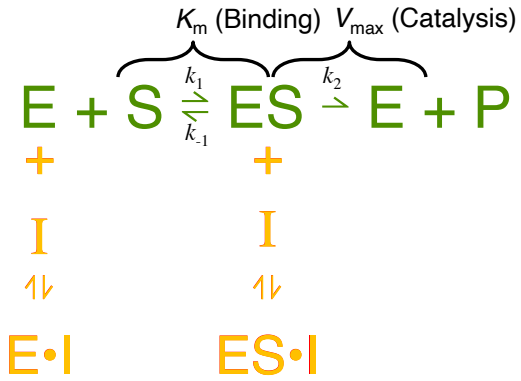
Reversible Enzyme Inhibition:



Un-Competitive

Enzyme Kinetics

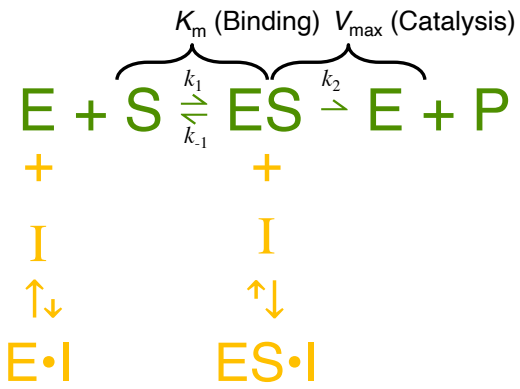
Reversible Enzyme Inhibition:



Non-Competitive

Enzyme Kinetics

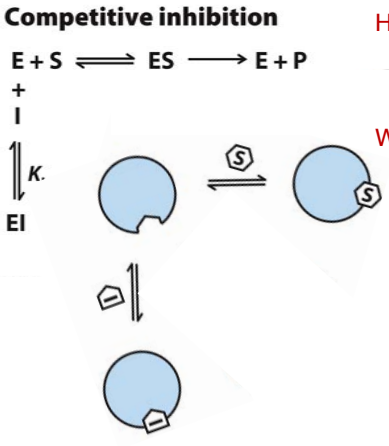
Reversible Enzyme Inhibition:



Mixed

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)



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): $^{App}K_m$

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

$$\Downarrow$$

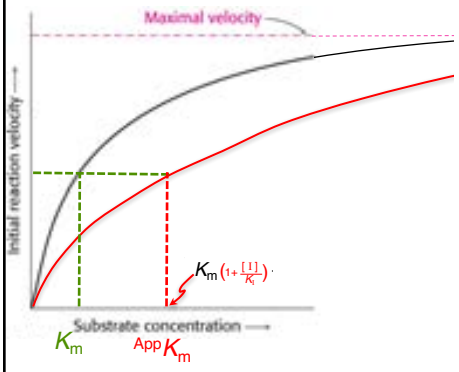
$$v_0 = \frac{V_{max}[S]}{K_m\left(1+\frac{[I]}{K_I}\right) + [S]}$$

Derivation on line

Competitive Inhibition

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

$K_I = \frac{[E][I]}{[EI]}$ $\quad v_0 = \frac{V_{max}[S]}{K_m\left(1+\frac{[I]}{K_I}\right)+[S]}$ $\quad \frac{1}{V_0} = \left(\frac{\alpha K_m}{V_{max}}\right)\frac{1}{[S]} + \frac{1}{V_{max}}$

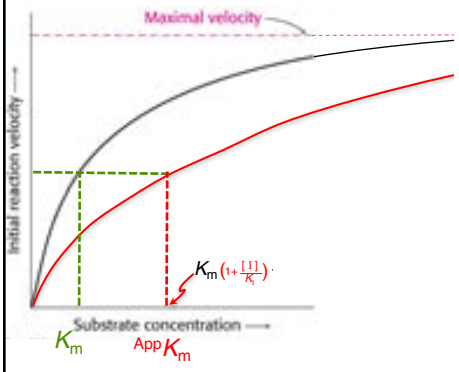


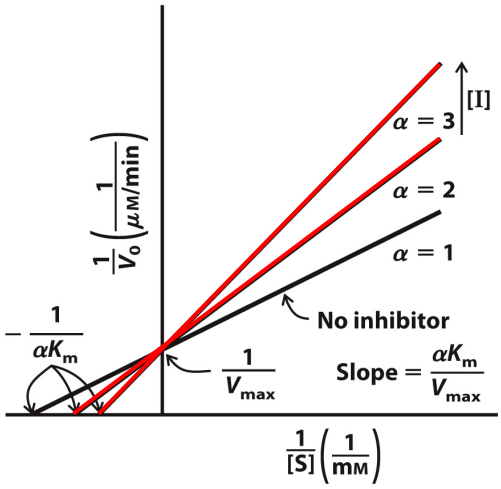
- No change in V_{max}; apparent increase in K_m
- Lineweaver-Burk: lines intersect at the *y*-axis.

Competitive Inhibition

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

$K_I = \frac{[E][I]}{[EI]}$ $\quad v_0 = \frac{V_{max}[S]}{K_m\left(1+\frac{[I]}{K_I}\right)+[S]}$ $\quad \frac{1}{V_0} = \left(\frac{\alpha K_m}{V_{max}}\right)\frac{1}{[S]} + \frac{1}{V_{max}}$





- 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])

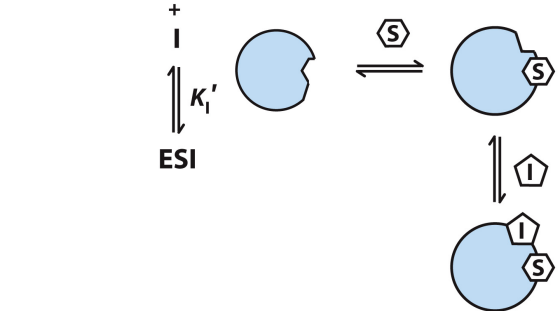
Uncompetitive inhibition

$E + S \rightleftharpoons ES \longrightarrow E + P$

$ES + I \rightleftharpoons ESI$ (K_I')

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

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

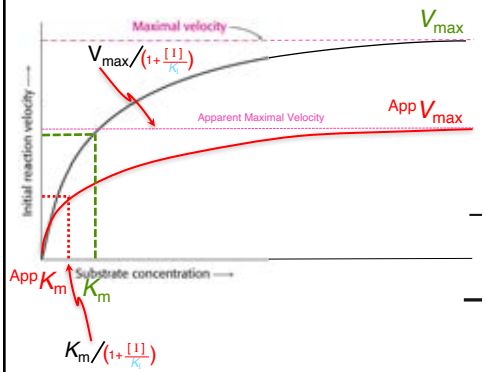
$$\Downarrow$$

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

Uncompetitive Inhibition

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

$K'_I = \frac{[ES][I]}{[ESI]}$ $\quad v_0 = \frac{V_{max}[S]}{K_m + [S](1+\frac{[I]}{K_I'})}$ $\quad \frac{1}{V_0} = \left(\frac{K_m}{V_{max}}\right)\frac{1}{[S]} + \frac{\alpha'}{V_{max}}$



- 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 $1/V_{max}/K_m$)

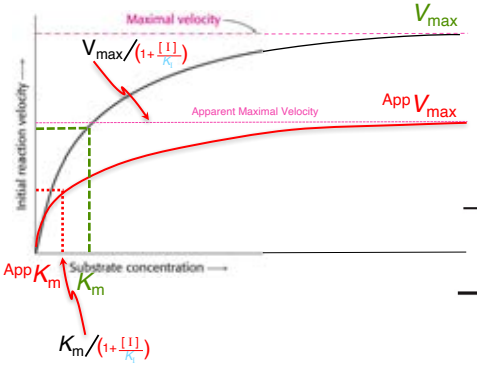
Uncompetitive Inhibition

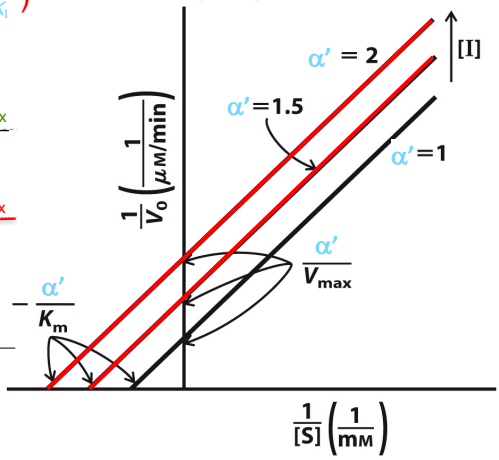
$(1+\frac{[I]}{K'_I}) = \alpha'$

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

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

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





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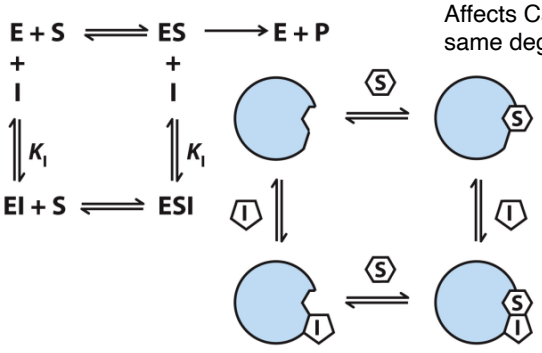
- No change in V_{max}/K_m
- Lineweaver-Burk: lines are parallel (recall slope is $1/V_{max}/K_m$)

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)
  - inhibits both substrate binding and catalysis equally
  - Essentially just titrates out the [enzyme]

Non-competitive inhibition



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]).