ENZYMES: Binding & Catalysis

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

Lecture 15 (10/17/25)

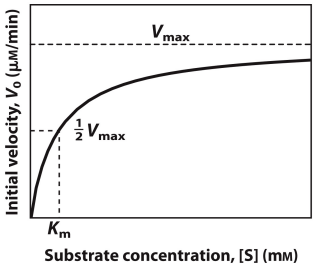
- Reading: Ch6; 191, 197-200
- Homework #15

NEXT

- Reading: Ch6; 181-186, 195-196
  Ch6; 203-208
- Homework #16, 17

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.

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

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

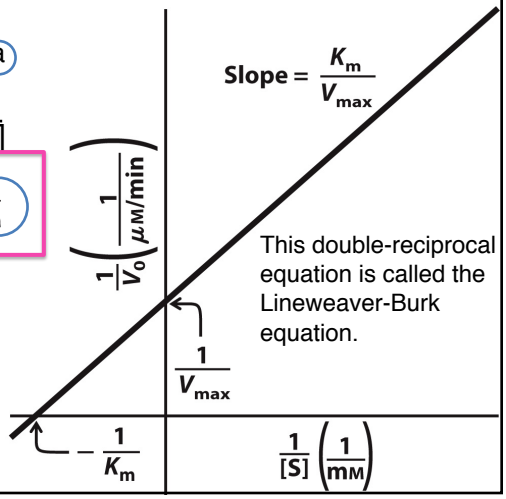
$$\frac{1}{v_0} = \frac{K_m}{V_{max}[S]} + \frac{[S]}{V_{max}[S]}$$

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

y = bx + a

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

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



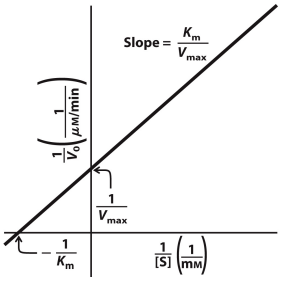
This double-reciprocal equation is called the Lineweaver-Burk equation.

Enzyme Kinetics

Linearized Derivations of the M-M Equation

Lineweaver-Burk

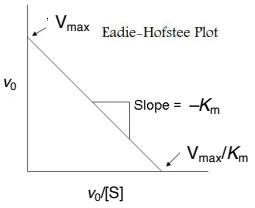
$$\frac{1}{V_0} = \frac{K_M}{V_{max}} \cdot \frac{1}{S} + \frac{1}{V_{max}}$$



Eadie-Hofstee

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

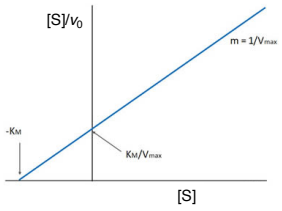
v_0 *vs.* $v_0/[S]$

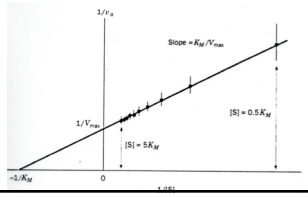


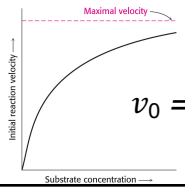
Hanes-Woolf

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

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







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

Enzyme Kinetics

ENZYME INHIBITION

Enzyme Kinetics

What is Enzyme Inhibition?

This is usually 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:

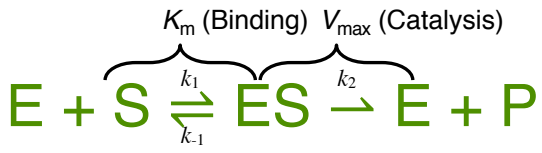
There are THREE-to-FOUR types of reversible inhibition:

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

} These are closely related

Enzyme Kinetics

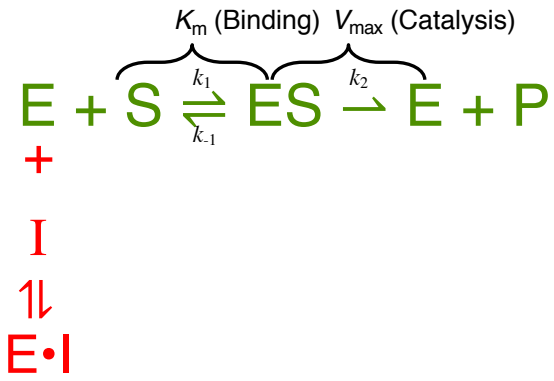
Reversible Enzyme Inhibition:



No Inhibition

Enzyme Kinetics

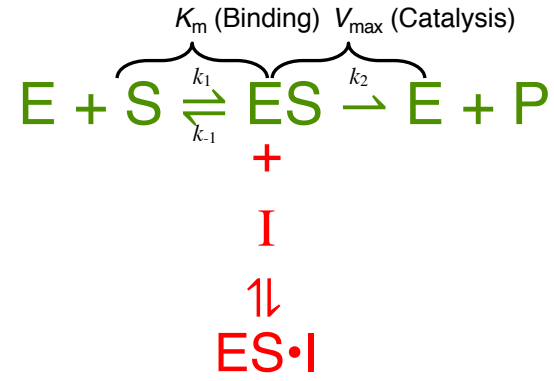
Reversible Enzyme Inhibition:



Competitive

Enzyme Kinetics

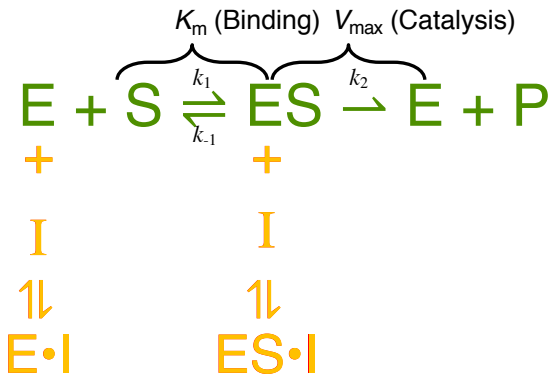
Reversible Enzyme Inhibition:



Un-Competitive

Enzyme Kinetics

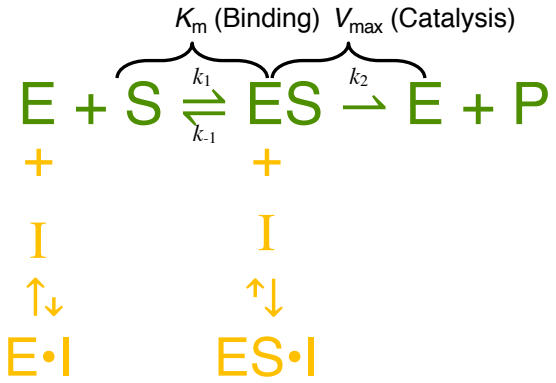
Reversible Enzyme Inhibition:



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)

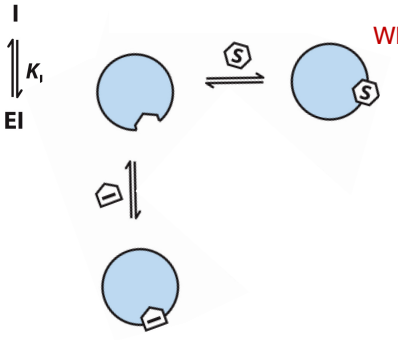
Competitive inhibition

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

$+ \; I$

$\Vert K_I$

EI



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): $^{App}K_m$

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

$$\Downarrow$$

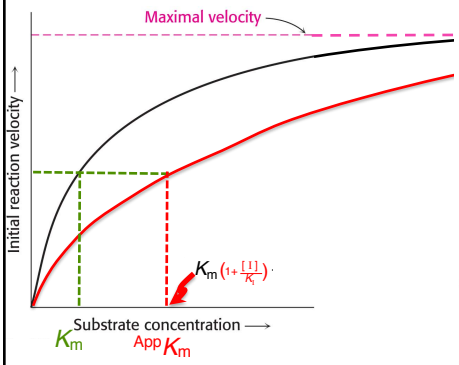
$$v_0 = \frac{V_{max}[S]}{K_m\left(1+\frac{[I]}{K_I}\right) + [S]}$$

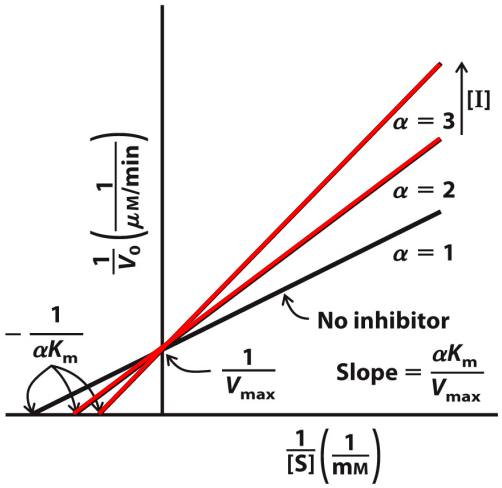
Derivation on line

Competitive Inhibition

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

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



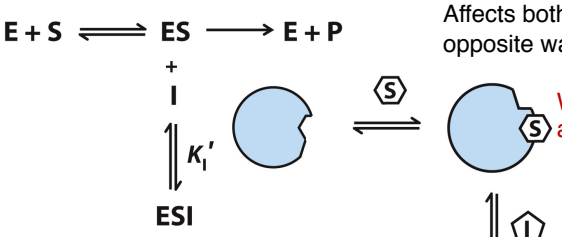


- No change in V_{max}; apparent increase in K_m
- Lineweaver-Burk: lines intersect at the *y*-axis.

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



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

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

$$\Downarrow$$

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

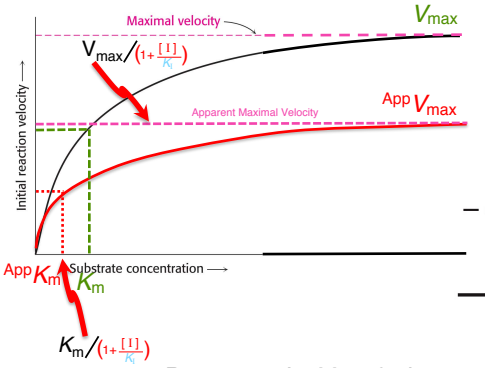
Uncompetitive Inhibition

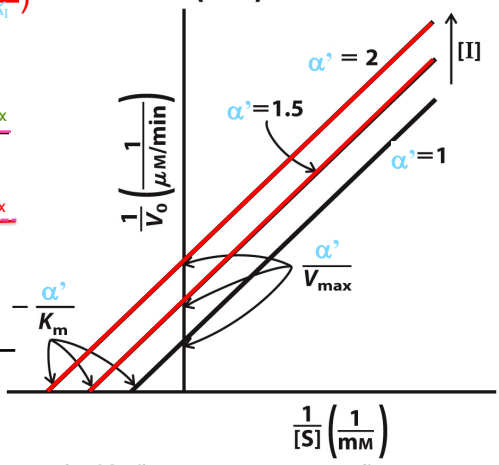
$(1+\frac{[I]}{K_I'}) = \alpha'$

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

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

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



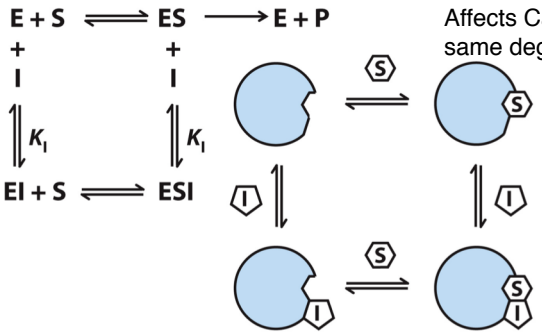


- Decrease in V_{max} & decrease in K_m (but to same extent!)
- No change in V_{max}/K_m
- Lineweaver-Burk: lines are parallel (recall slope is $1/V_{max}/K_m$)

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)
  - 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.

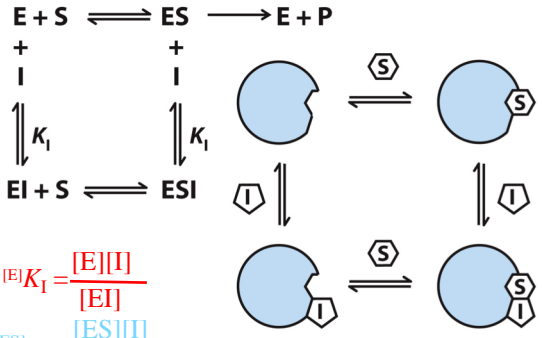
Which kinetic constant will be affected?

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]).

Non-competitive Inhibition

Non-competitive inhibition



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

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

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

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

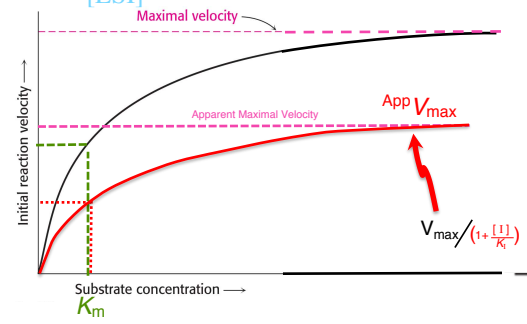
$$v_0 = \frac{V_{max}[S] \Big/ \left(1 + \frac{[I]}{K_I}\right)}{K_m + [S]}$$

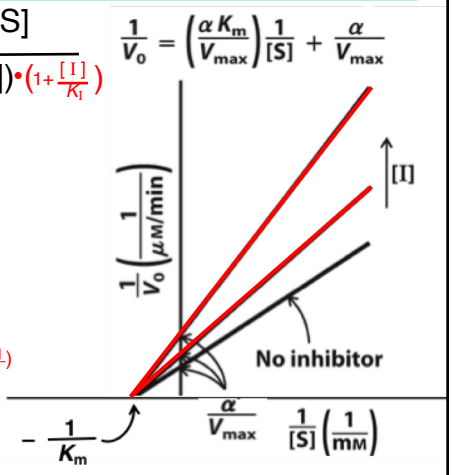
$$v_0 = \frac{V_{max}[S]}{(K_m + [S]) \cdot \left(1 + \frac{[I]}{K_I}\right)}$$

Non-competitive Inhibition $(1+\frac{[I]}{K_I}) = \alpha$

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

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





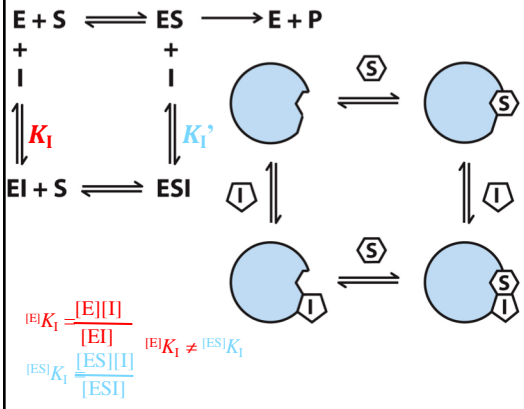
- Decrease in V_{max}; no change in K_m
  - –pulls binding reaction to both directions equally
  - –the effect is to effectively decrease the [E]
- Lineweaver-Burk: lines intersect on the *x*-axis.

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



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

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

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

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

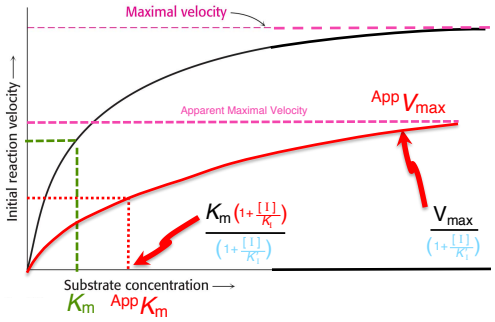
Mixed Inhibition

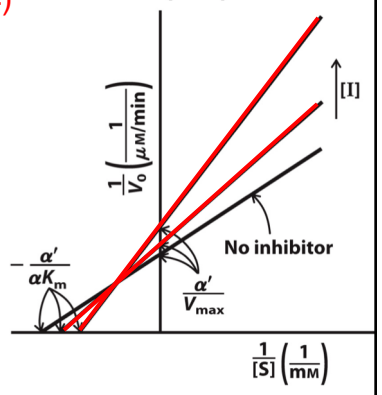
$(1+\frac{[I]}{K_I}) = \alpha$ $(1+\frac{[I]}{K_I}) = \alpha'$

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

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

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





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

Reversible Inhibition

= got worse = got better

	App K_m	App V_{max}
Competitive	$K_m(1+\frac{[I]}{K_I})$	V_{max}
Un-Competitive	$\frac{K_m}{(1+\frac{[I]}{K_I})}$	$\frac{V_{max}}{(1+\frac{[I]}{K_I})}$
Non-Competitive	K_m	$\frac{V_{max}}{(1+\frac{[I]}{K_I})}$
Mixed	$K_m\frac{(1+\frac{[I]}{K_I})}{(1+\frac{[I]}{K_I})}$	$\frac{V_{max}}{(1+\frac{[I]}{K_I})}$

K_m (Binding) V_{max} (Catalysis)

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

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

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

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