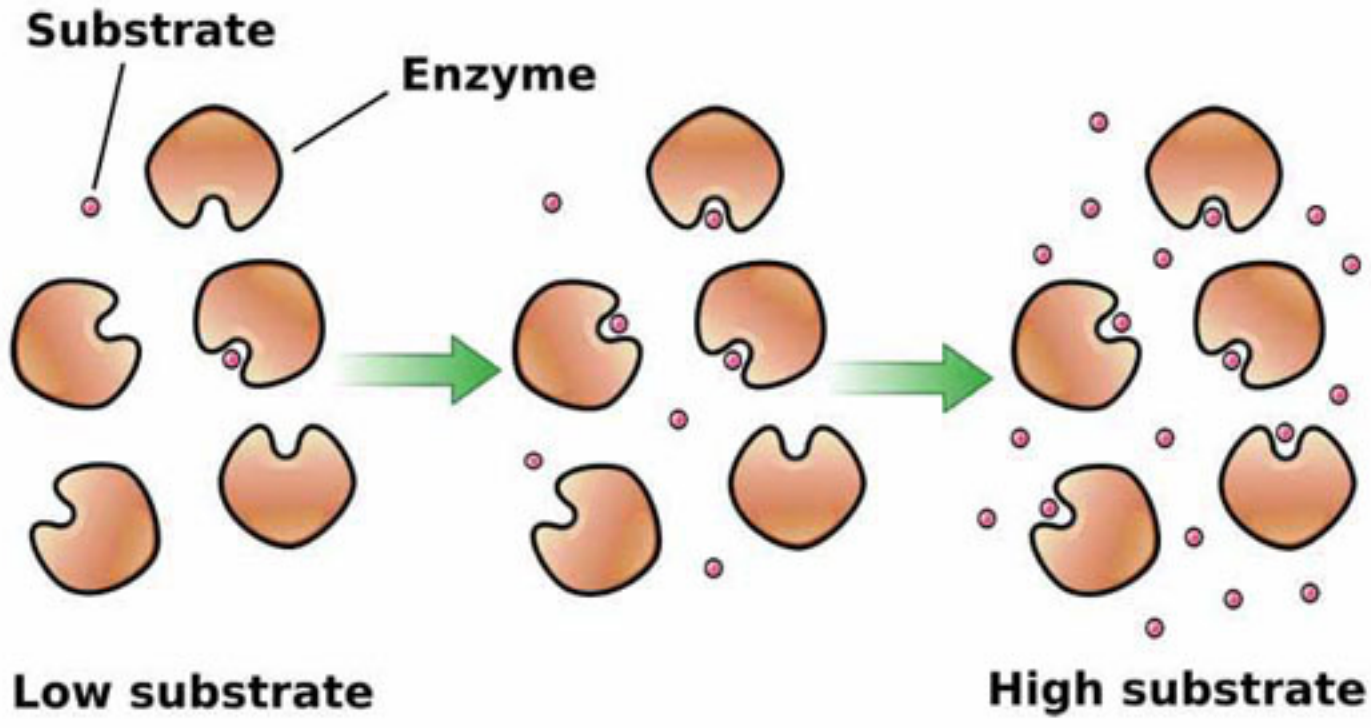
Module 8

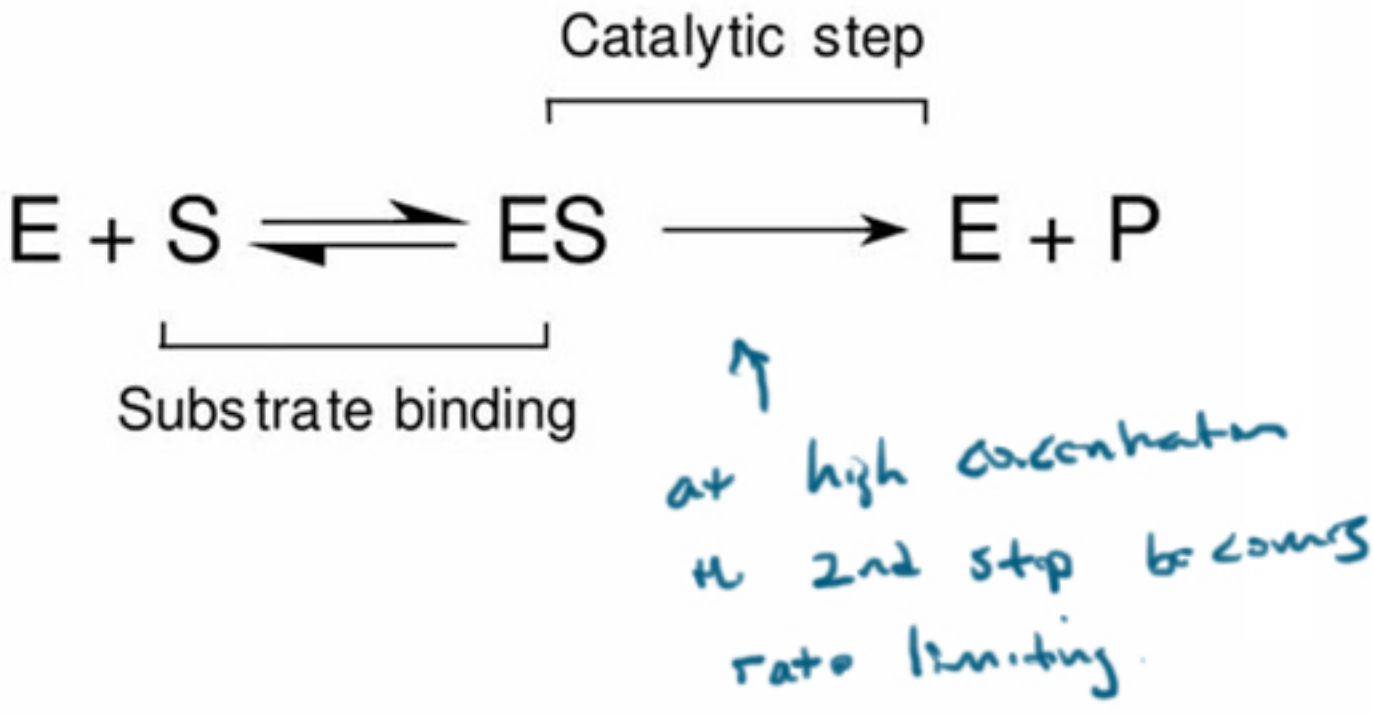
Enzyme Kinetics

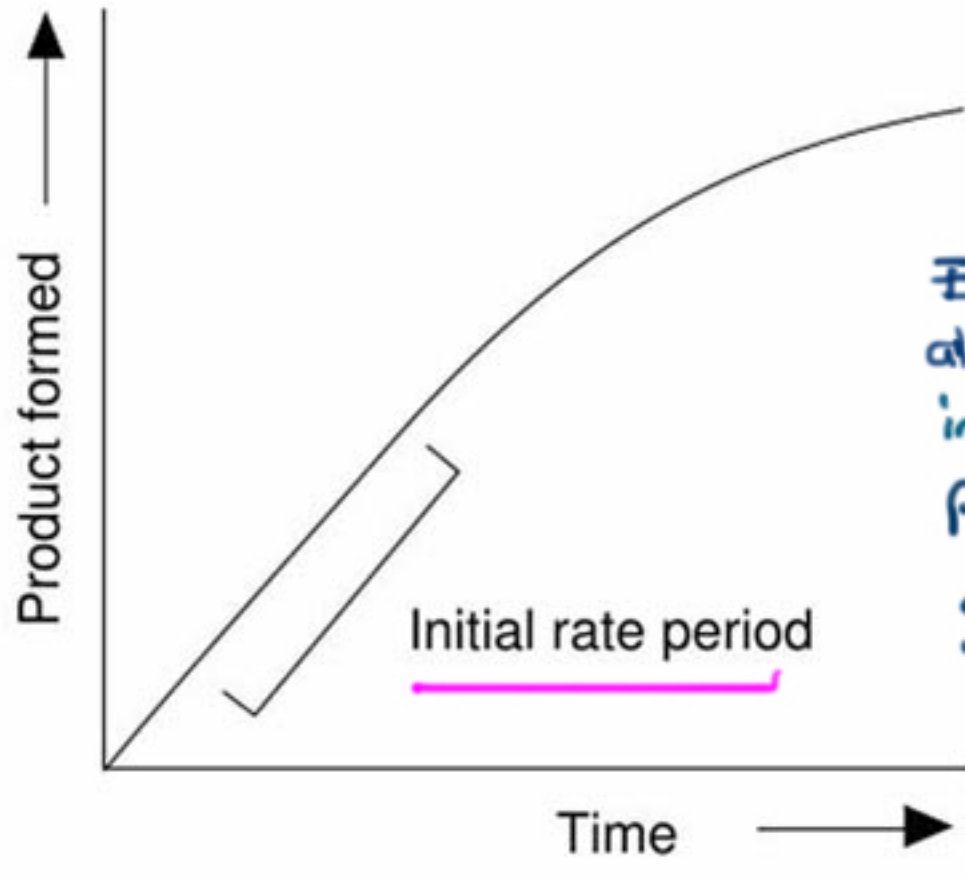
Session Slides with Notes

Enzyme kinetics



at high [S] the enzyme becomes saturated and the rate no longer increases with [S]


Catalytic step
E + S
ES
E + P
Substrate binding
at high concentration
the 2nd step becomes
rate limiting



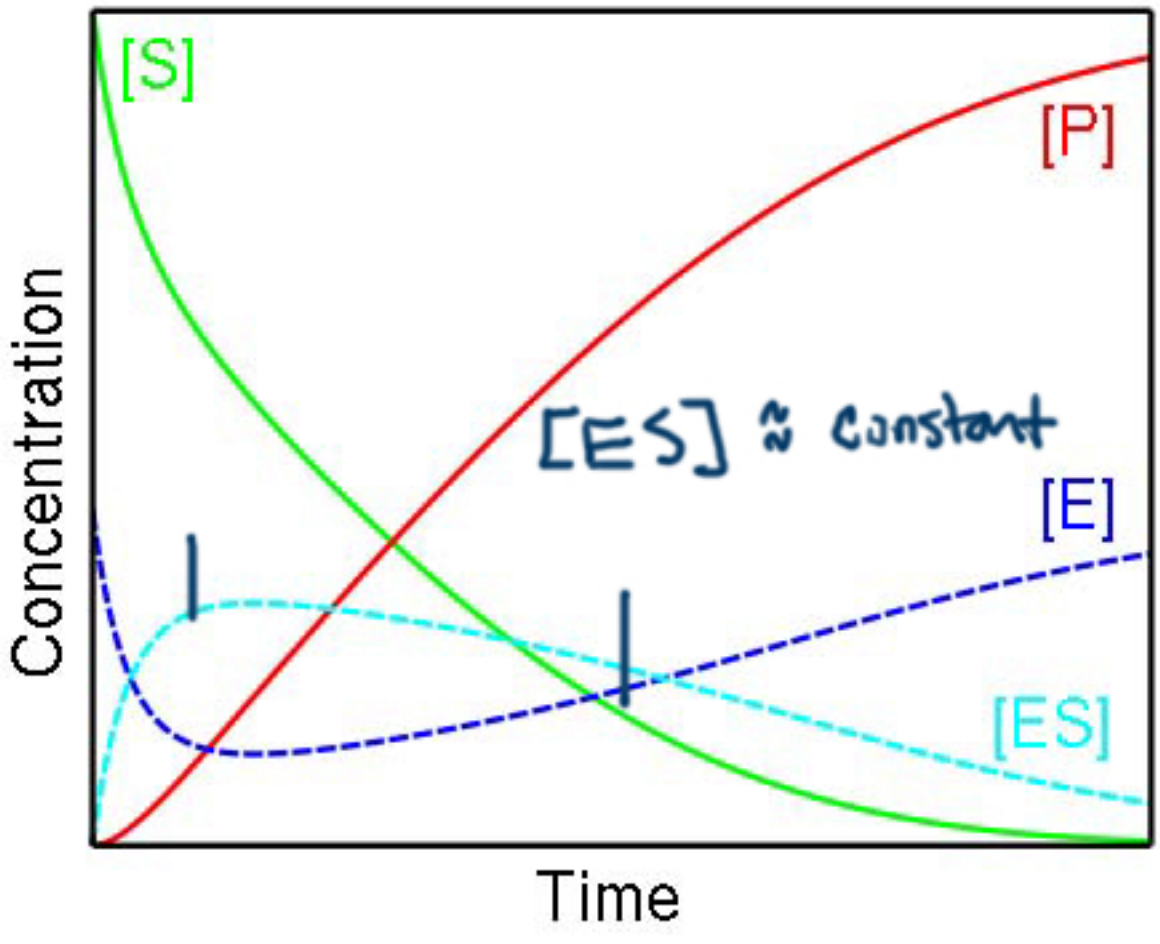
Enzyme measurements are almost always done in the initial rate period.

Sometimes you'll see

V_0

Sometimes, just V

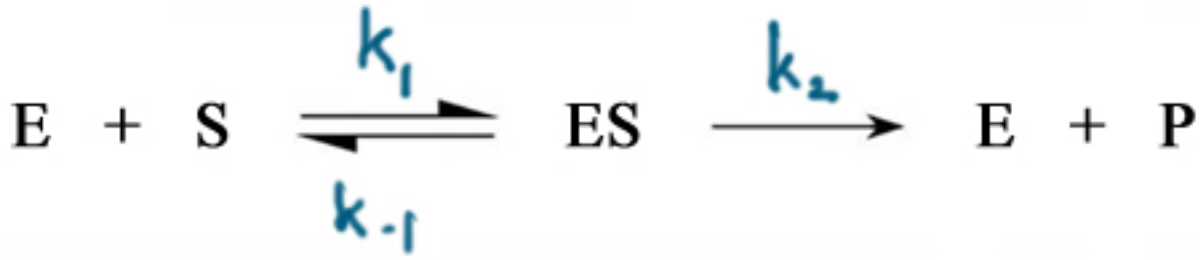
They're the same.



Underlies the steady state assumption

- foundation of Michaelis Menten kinetics.

k_2 - turnover number
(k_{cat})



If [ES] is constant

formation = breakdown

$$k_1[E][S] = k_{-1}[ES] + k_2[ES]$$

$$k_1[E][S] = (k_{-1} + k_2)[ES]$$

$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES} \xrightarrow{k_2} \mathrm{E} + \mathrm{P} \qquad v = k_2[\mathrm{ES}]$$

1) Assuming a steady state where [ES] is constant:

formation breakdown

$$k_1[\mathrm{E}][\mathrm{S}] = (k_{-1} + k_2)[\mathrm{ES}]$$ steady state

$$[\mathrm{ES}] = \frac{[\mathrm{E}][\mathrm{S}]}{(k_{-1} + k_2)/k_1}$$

$$K_M = \frac{k_{-1} + k_2}{k_1}$$ Michaelis constant

$$[\mathrm{ES}] = \frac{[\mathrm{E}][\mathrm{S}]}{K_M}$$

↑

The bigger K_M the harder to saturate the enzyme.

2) Assuming total enzyme doesn't change:

$$[\mathrm{E}] = [\mathrm{E_T}] - [\mathrm{ES}]$$

$$[\mathrm{ES}] = \frac{([\mathrm{E_T}] - [\mathrm{ES}])[\mathrm{S}]}{K_M}$$

$$[\mathrm{ES}] = [\mathrm{E_T}] \frac{[\mathrm{S}]}{[\mathrm{S}] + K_M}$$ $\frac{[S]}{[S]+K_M}$ ∝ % saturation

$$v = k_2[\mathrm{E_T}] \frac{[\mathrm{S}]}{[\mathrm{S}] + K_M}$$

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

$\frac{V}{V_{max}}$ = % saturation = $\frac{[S]}{[S]+K_M}$

$V_{max}[S]$

$V_0 = \frac{}{[S]+K_M}$

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

- If $[S] >> K_M$

  $V \approx V_{max}$

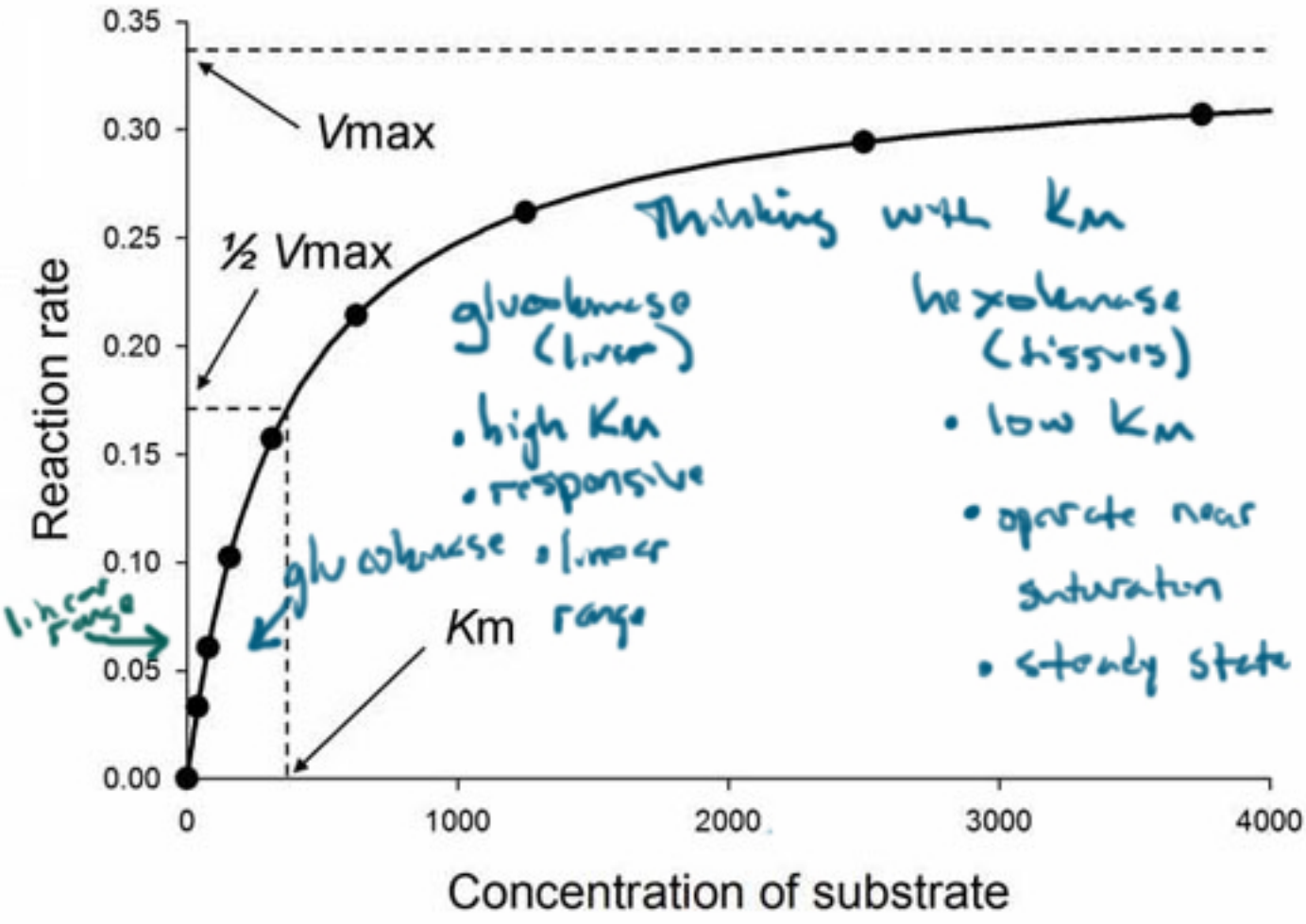
- If $[S] << K_M$

  $V \approx \frac{V_{max}}{K_M} [S]$

  (linear range)

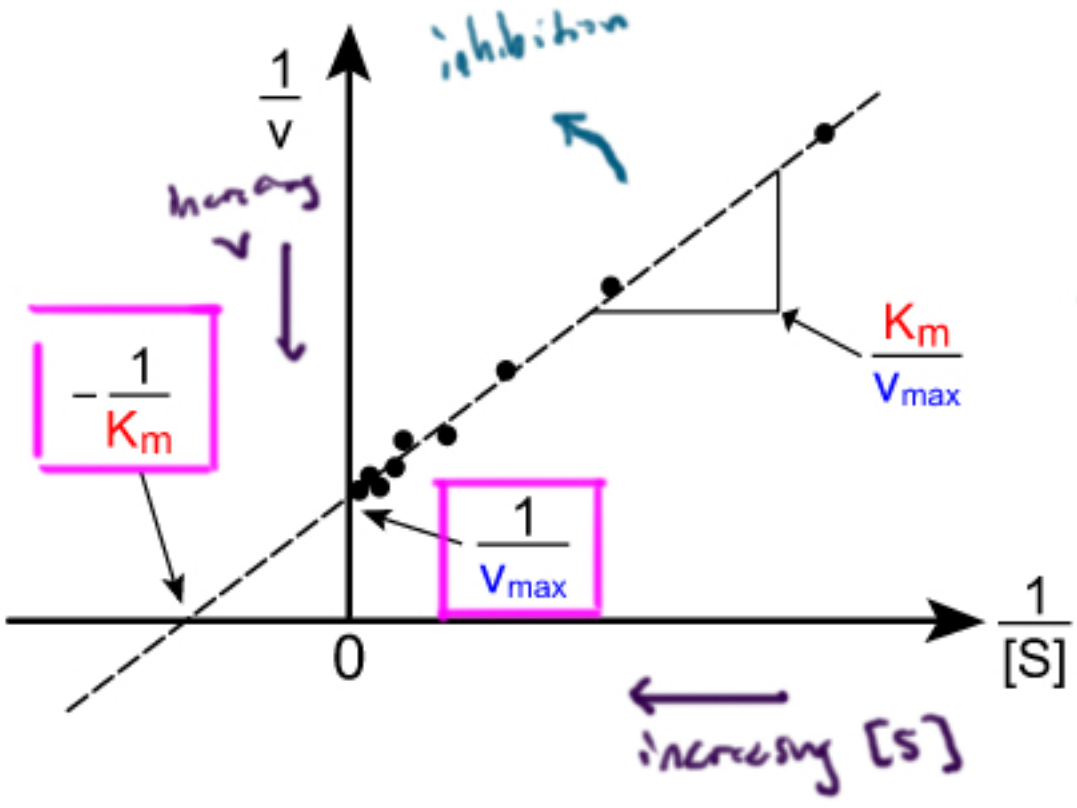
- If $[S] = K_M$

  $V = \frac{1}{2} V_{max}$



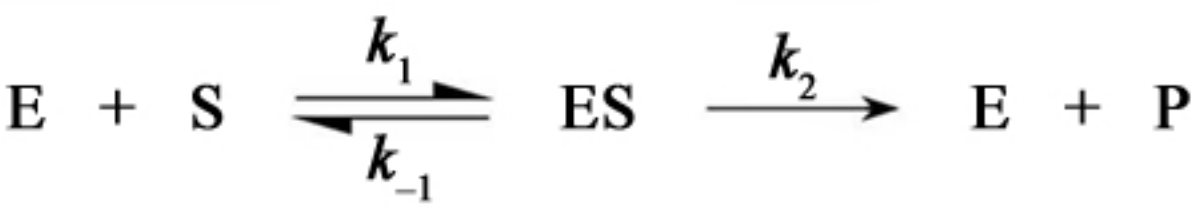
K_M is the [S] needed to half saturate the enzyme.

Lineweaver Burke Plot



$$\frac{1}{v} = \frac{1}{V_{max}} + \left(\frac{K_M}{V_{max}}\right)\left(\frac{1}{[S]}\right)$$

$y = b + m x$



Catalytic efficiency : $\frac{k_2}{K_M}$ or $\frac{k_{cat}}{K_M}$

Enzyme	K_m (M)	k_{cat} (1/s)	k_{cat}/K_m (1/M*s)
Chymotrypsin	1.5×10^{-2}	0.14	9.3
Pepsin	3.0×10^{-4}	0.50	1.7×10^{3}
Tyrosyl-tRNA synthetase	9.0×10^{-4}	7.6	8.4×10^{3}
Ribonuclease	7.9×10^{-3}	7.9×10^{2}	1.0×10^{5}
Carbonic anhydrase	2.6×10^{-2}	4.0×10^{5}	1.5×10^{7} ★
Fumarase	5.0×10^{-6}	8.0×10^{2}	1.6×10^{8} ★

when it's high the enzyme binds substrate, doesn't fall backwards, and turns over right away.

Is there a natural limit?

Yes. Diffusion limits k_1.

$$\frac{k_2}{K_M} = \frac{k_2 k_1}{k_{-1} + k_2}$$

Catalytic efficiency is limited to 10^8 to 10^9 $M^{-1} s^{-1}$

- to get close means enzymatic perfection ★

Hexokinase catalyzes the phosphorylation of both glucose and fructose. K_m for hexokinase with glucose is 0.15mM. K_m for fructose is 1.5mM. Assuming that V_{max} is the same for both enzymes, calculate the normalized initial velocity (v_0 / V_{max}) when the initial substrate concentration is 0.15mM.

glucose

$K_M = [S]$

$\frac{V_0}{V_{max}} = 50\%$

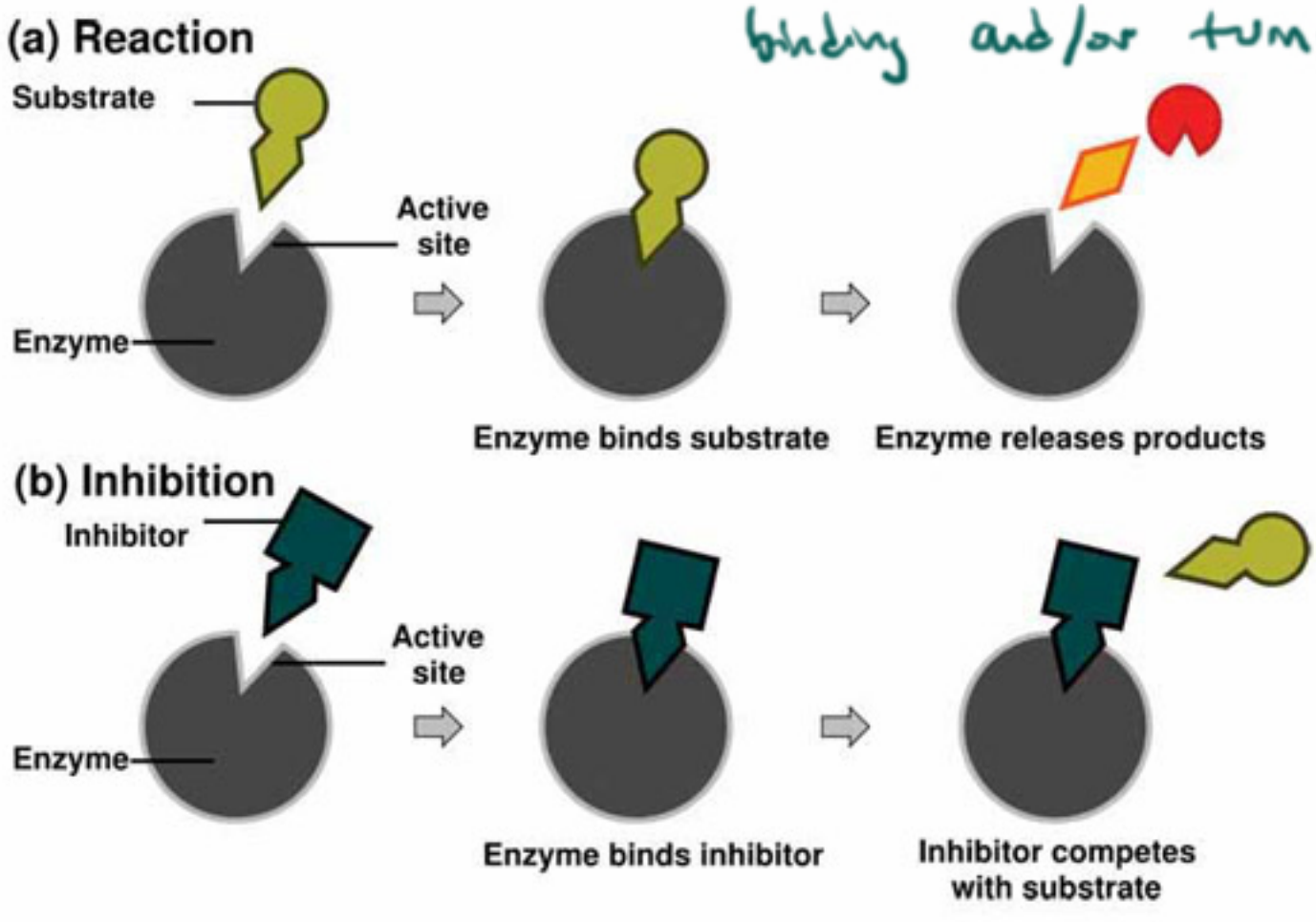
fructose

$\sim 9\%$

$V_0 = V_{max} \frac{[S]}{[S] + K_M}$

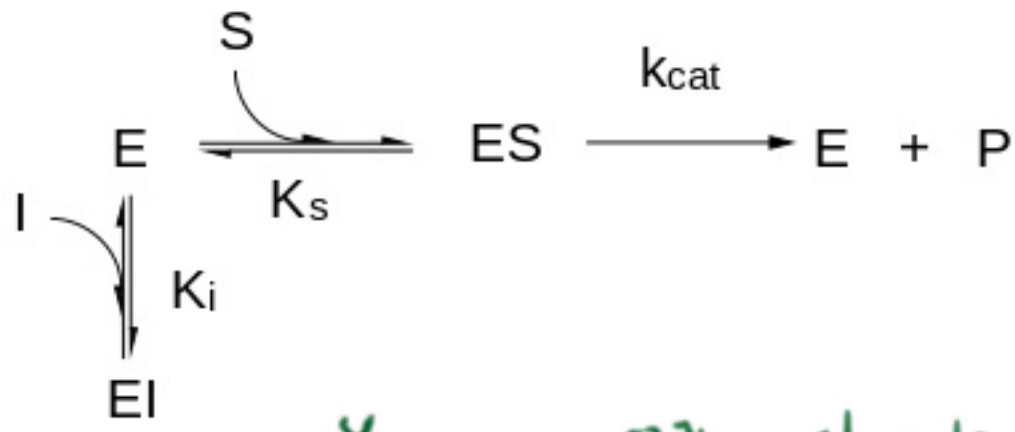
$\frac{V_0}{V_{max}} = \frac{[S]}{[S] + K_M}$

Enzyme inhibitors affect binding and/or turnover



Competitive inhibition

Competitive Inhibition



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

↑ apparent K_M

You can still saturate the enzyme

You can still get back to V_{max}

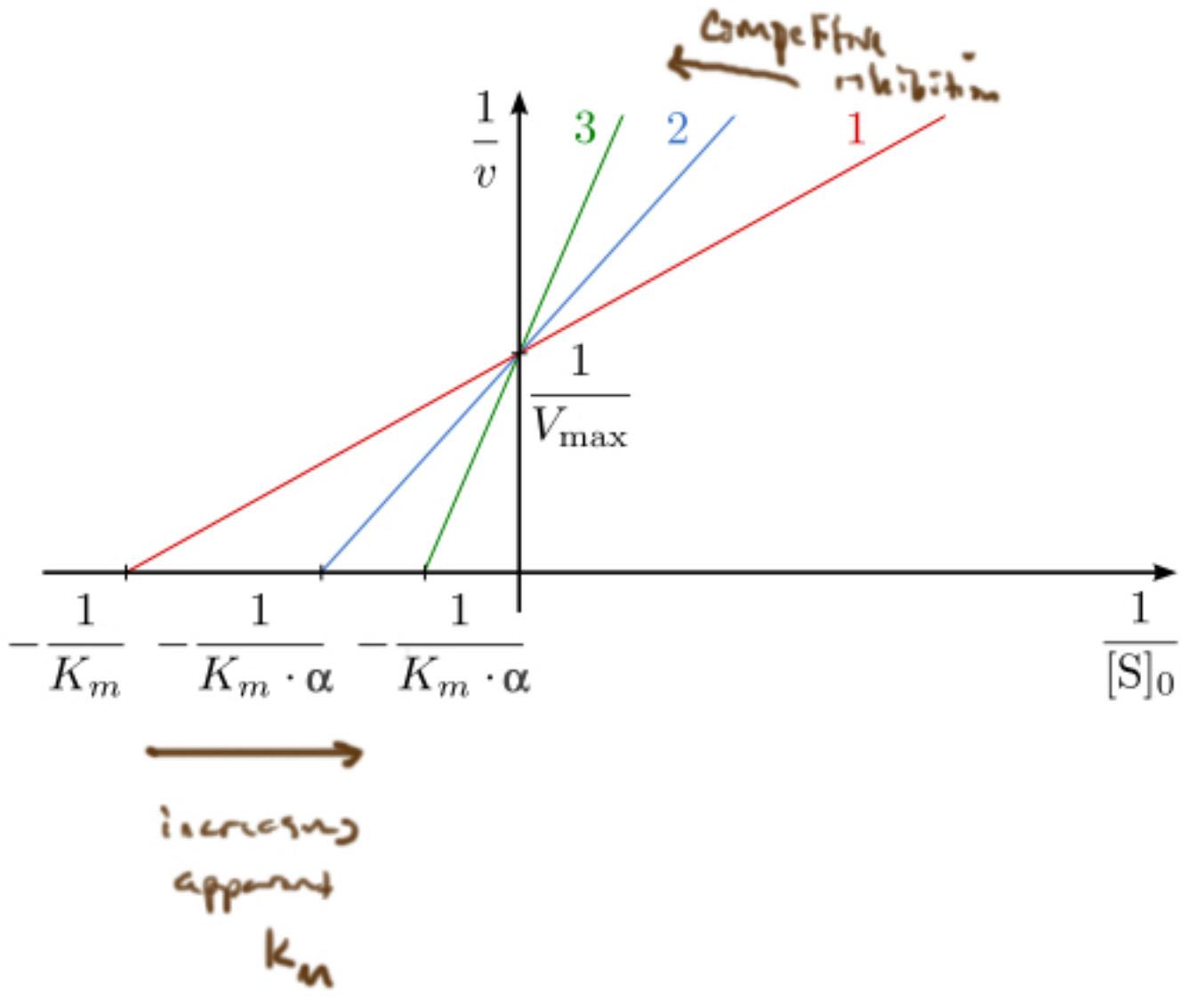
But it's harder to saturate the enzyme.

(Apparent) K_M has increased.

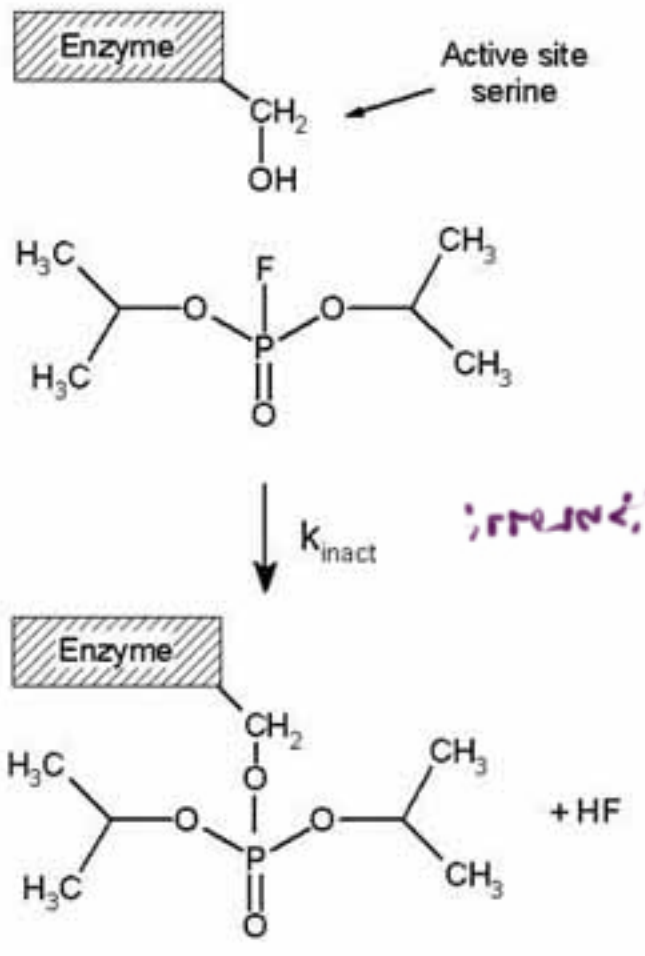
Competitive - V_{max} unchanged

K_M^* increased

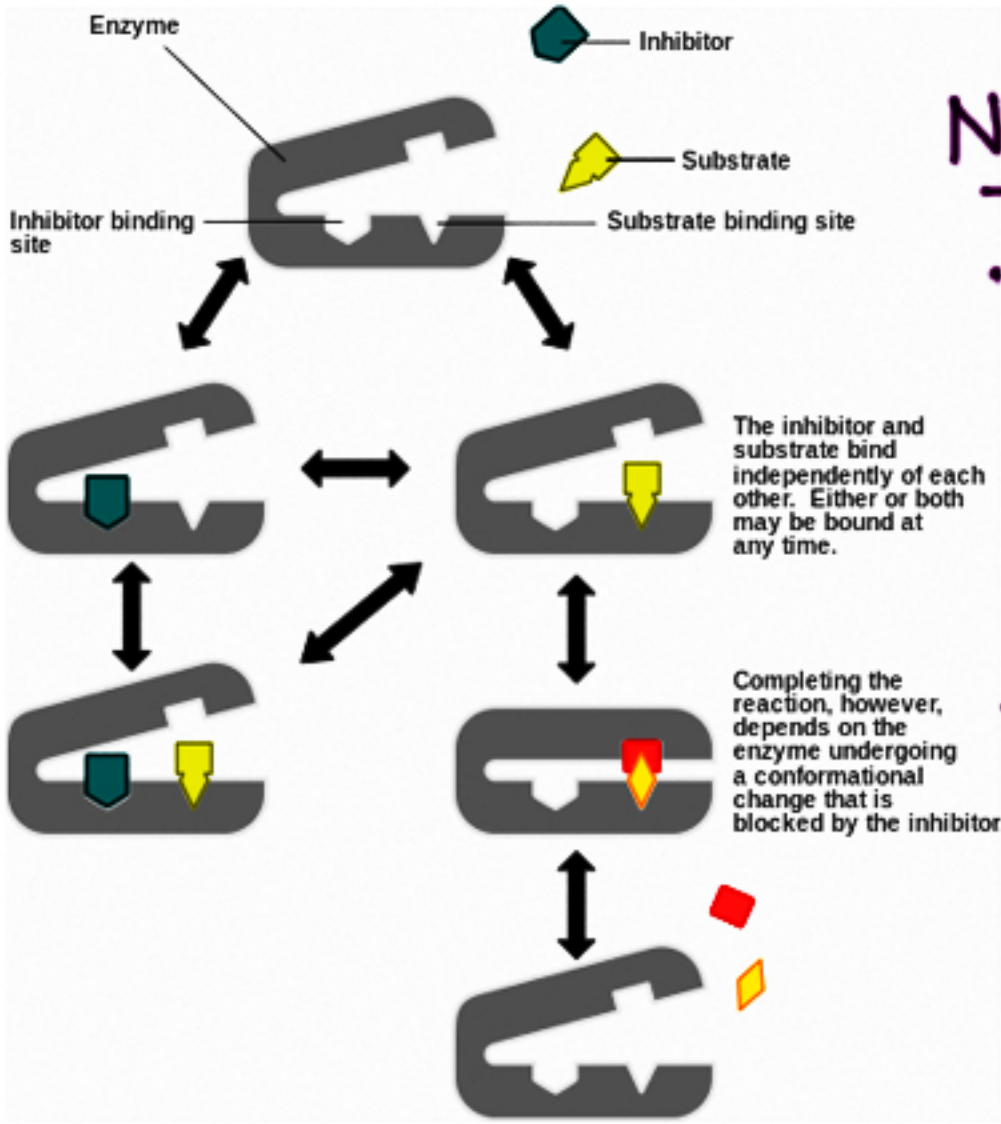
↑ apparent


Competitive inhibition
$\frac{1}{v}$
3
2
1
$\frac{1}{V_{\max}}$
$-\frac{1}{K_m}$
$-\frac{1}{K_m \cdot \alpha}$
$-\frac{1}{K_m \cdot \alpha}$
$\frac{1}{[S]_0}$
increasing apparent K_M

acetylcholine esterase →



suicide inhibitor
(not a form of competitive inhibition)



cannot turn over

Noncompetitive

- substrate binds just as easily
- enzyme is as easy to saturate as before

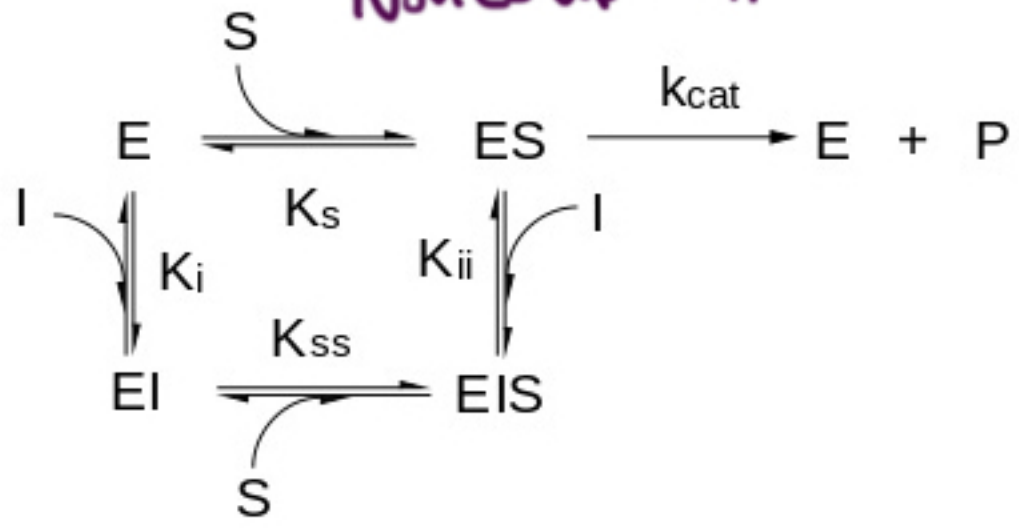
K_M is unchanged

- V_{max} decreased

Mixed

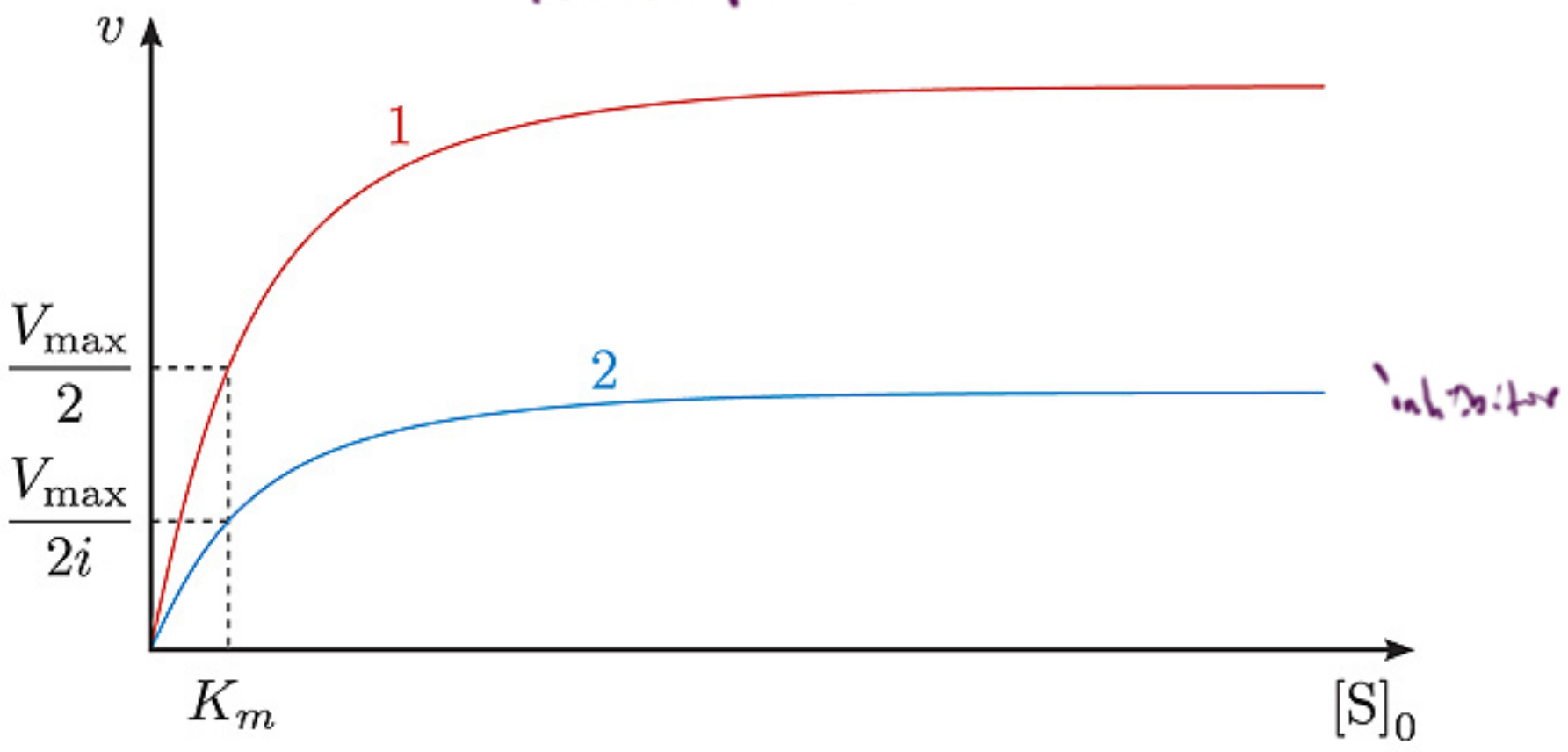
when inhibitor has some effect on binding both K_M and V_{max} change

Noncompetitive (or mixed)



$$v = \frac{V_{max}[S]}{\alpha K_M + \alpha'[S]}$$

Noncompetitive

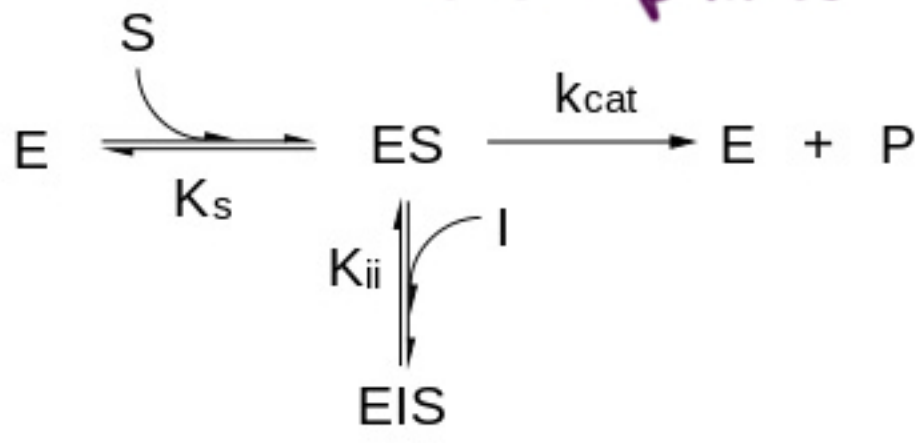


Inhibitor

V_{max} decreased

K_m unchanged.

uncompetitive

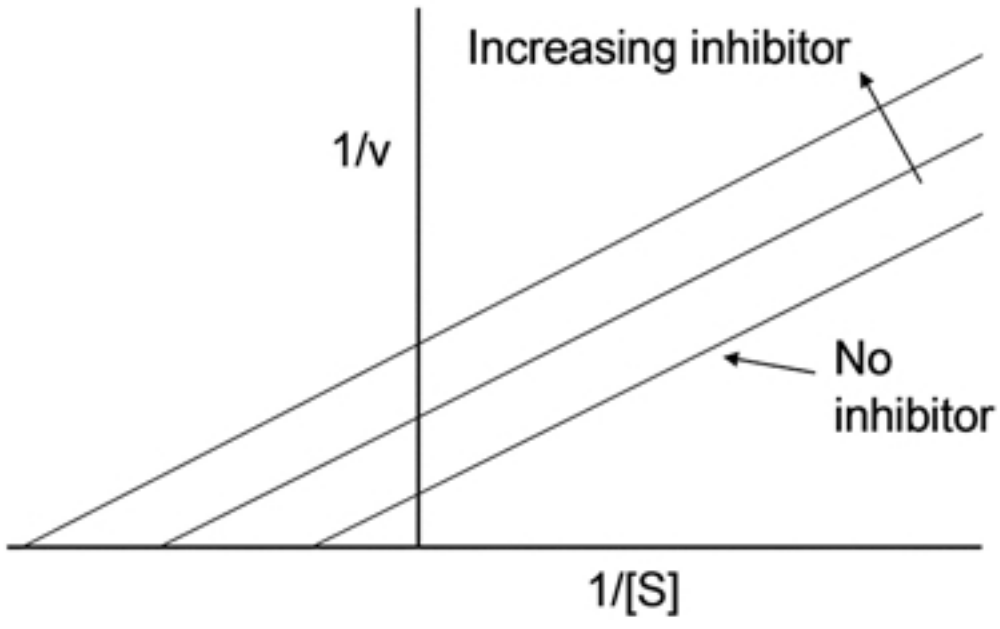


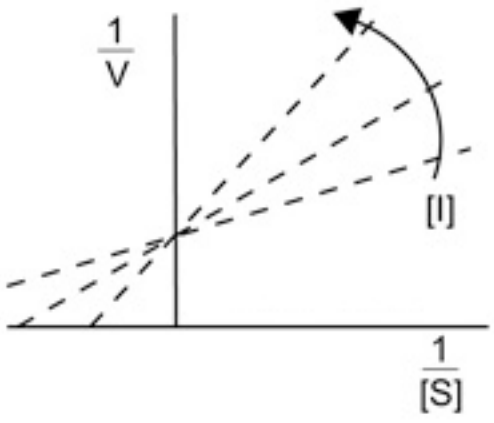
$$v = \frac{V_{max}[S]}{K_M + \alpha'[S]}$$

V_{max} decreased

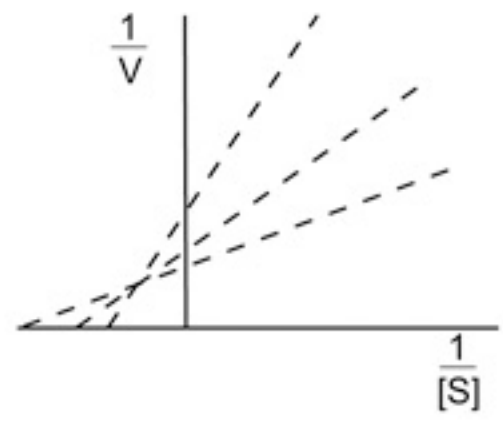
K_M decreases also (proportionally)

uncompetitive

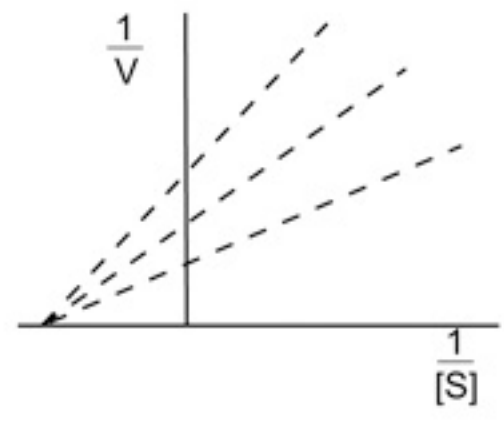




Competive



mixed



noncompetitive

Type of inhibition	K_m apparent	V_{max} apparent
competitive	$K_m\alpha$	$V_{\max}$
uncompetitive	$\frac{K_m}{\alpha'}$	$\frac{V_{\max}}{\alpha'}$
non-competitive	K_m	$\frac{V_{\max}}{\alpha'}$
mixed	$\frac{K_m\alpha}{\alpha'}$	$\frac{V_{\max}}{\alpha'}$

Bisubstrate Mechanisms

With sucrose phosphorylase, incubate with sucrose and isotopically labelled fructose* in the absence of phosphate - the label passes to sucrose.

Also, incubation with labelled glucose-1-phosphate* and phosphate, the label passes to phosphate.

The analagous process does not happen with maltose phosphorylase.

Sucrose Phosphorylase - Ping Pong Mechanism

Enzyme + sucrose ⟶ Enzyme-glucose + fructose

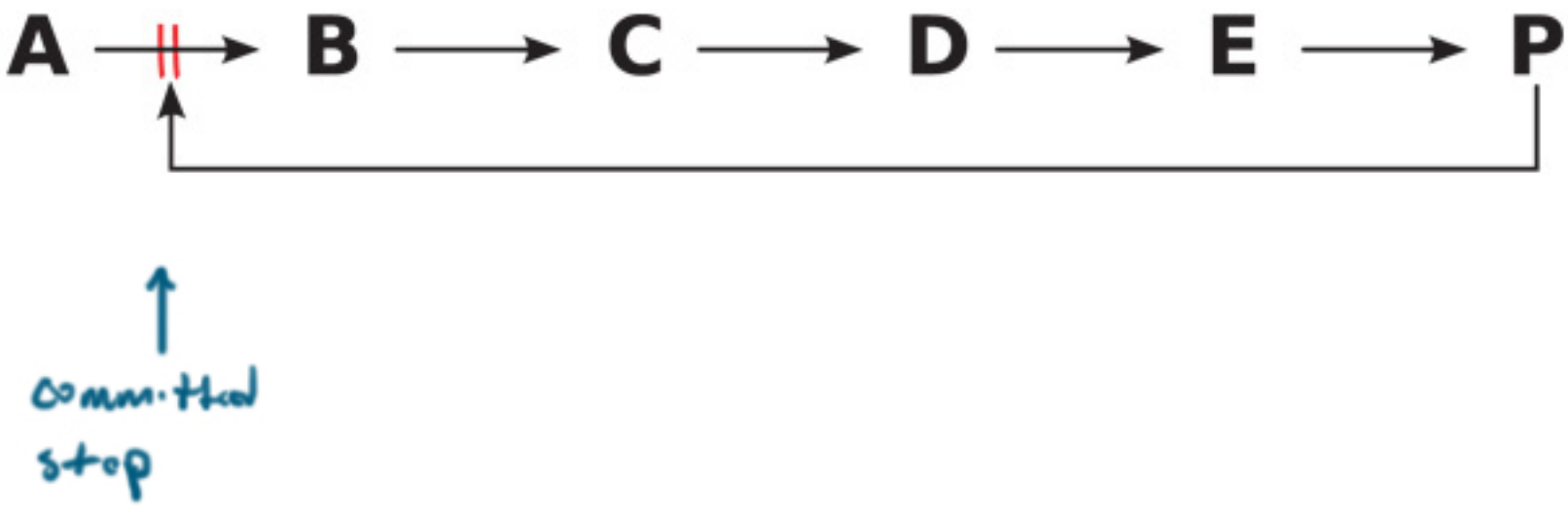
Enzyme-glucose + P_i ⟶ Enzyme + G1P

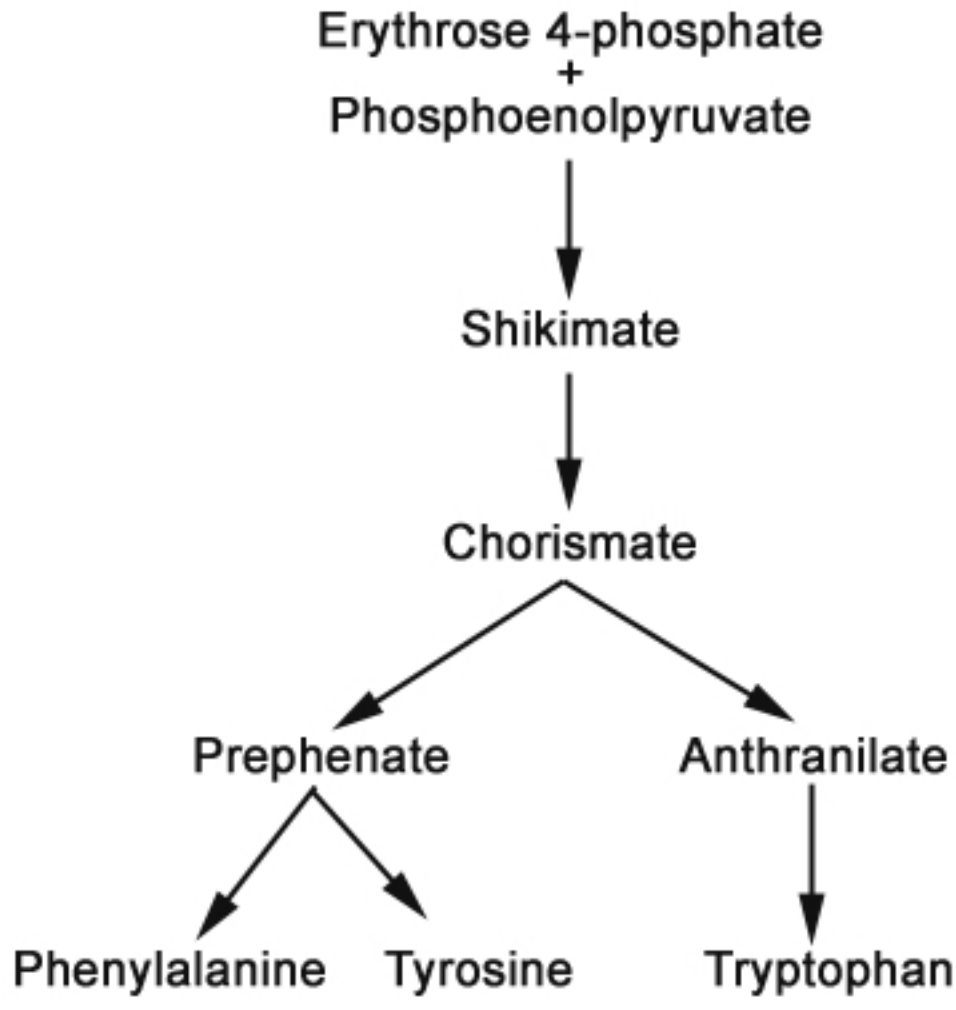
Maltose Phosphorylase - Sequential Mechanism

Enzyme + Maltose + P_i ⟶ Enzyme + G1P + Glucose

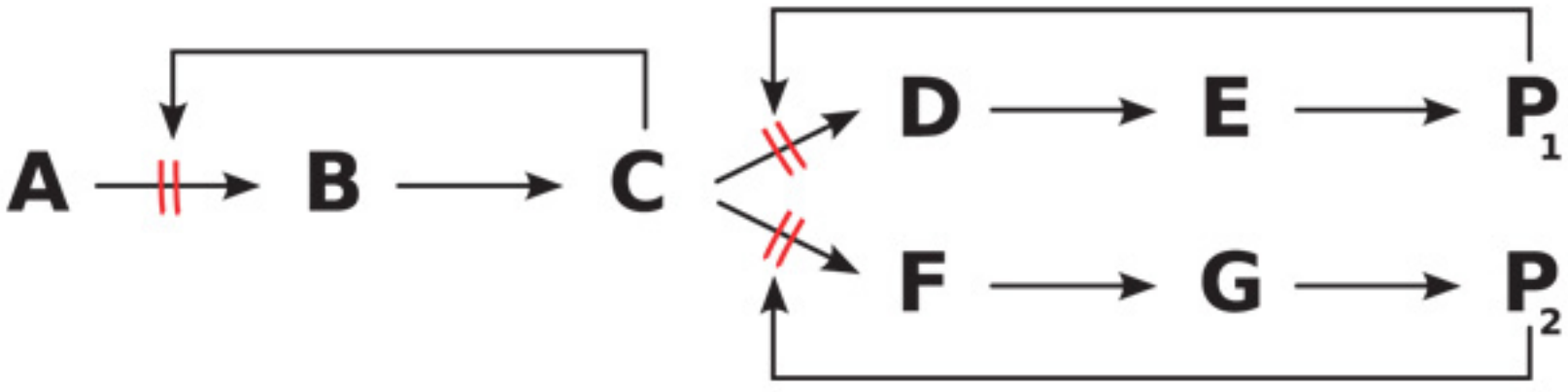
- Sequential mechanisms may be ordered or random.

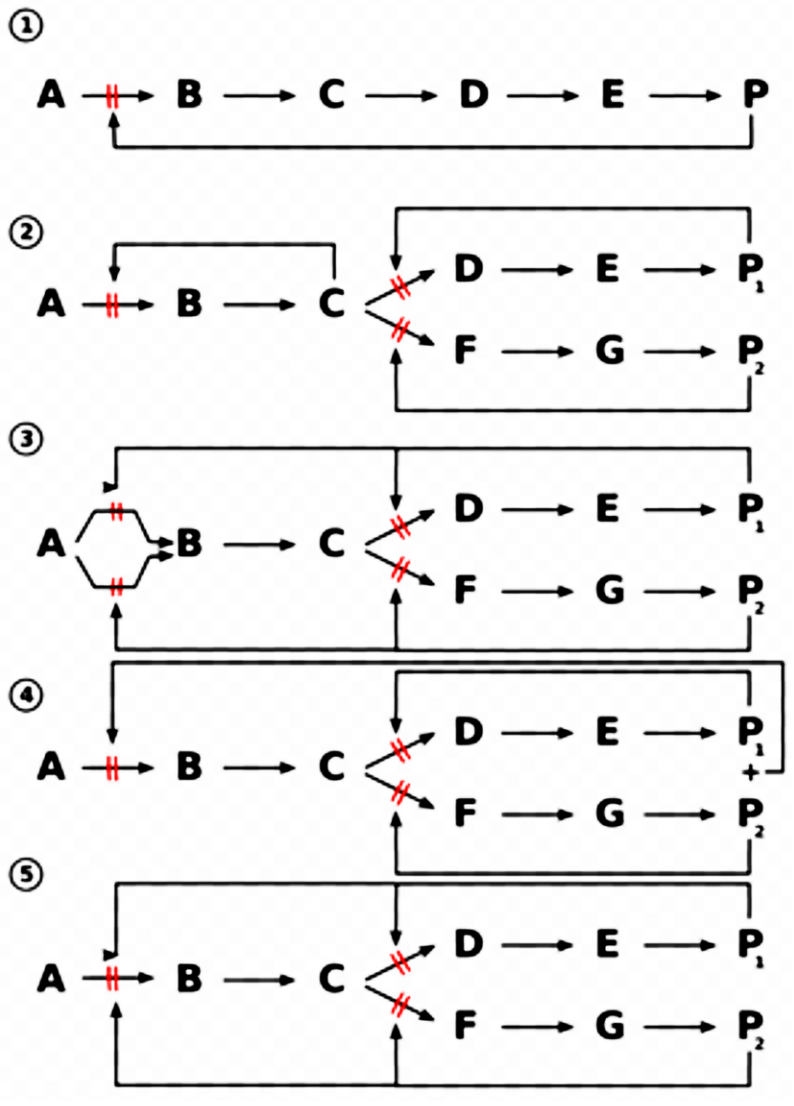
Basic Feedback




Erythrose 4-phosphate
+
Phosphoenolpyruvate
Shikimate
Chorismate
Prephenate
Anthranilate
Phenylalanine
Tyrosine
Tryptophan

Sequential Feedback





1. The basic feedback inhibition mechanism, where the product (P) inhibits the committed step (A→B).

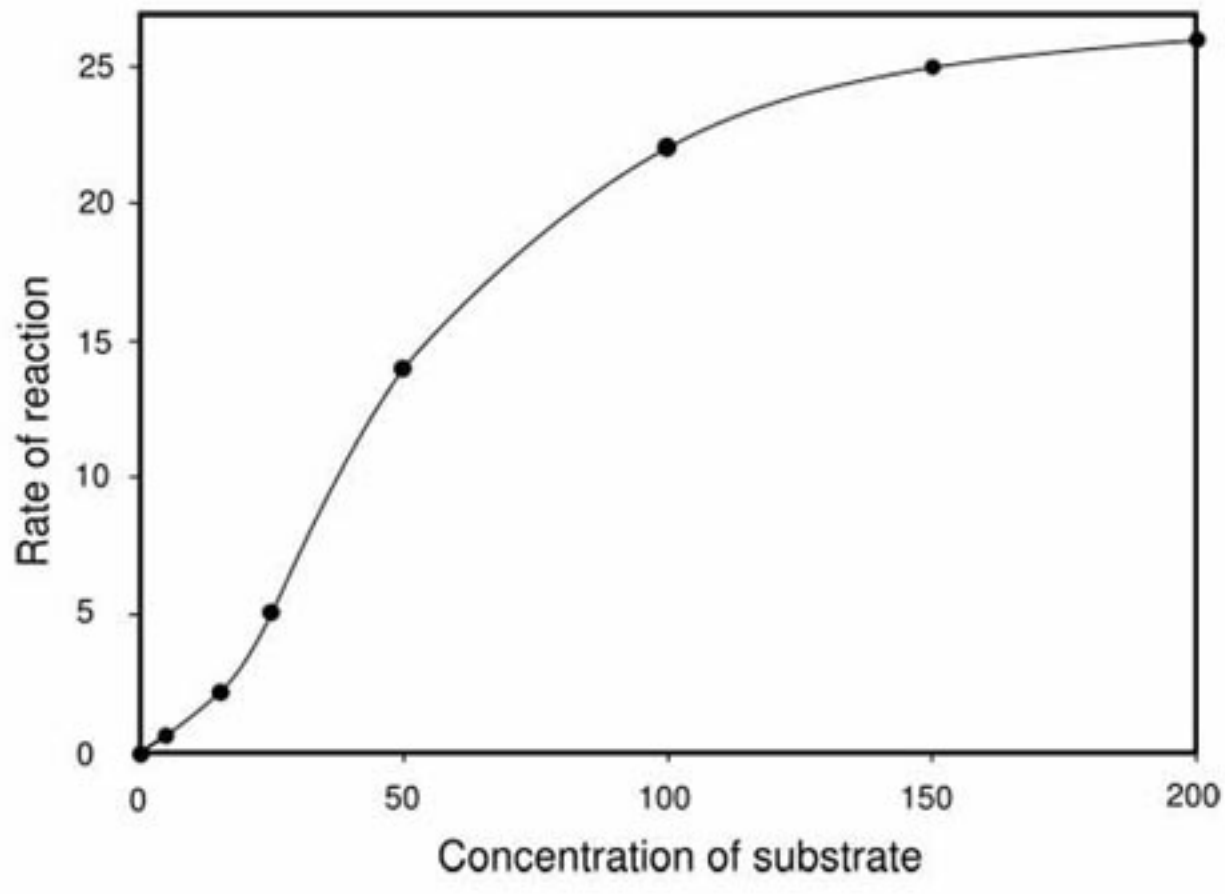
2. *Sequential feedback inhibition.* The end products P_1 and P_2 inhibit the first committed step of their individual pathway (C→D or C→F). If both products are present in abundance, all pathways from C are blocked. This leads to a buildup of C, which in turn inhibits the first common committed step A→B.

3. *Enzyme multiplicity.* Each end product inhibits both the first individual committed step and one of the enzymes performing the first common committed step.

4. *Concerted feedback inhibition.* Each end product inhibits the first individual committed step. *Together*, they inhibit the first common committed step.

5. *Cumulative feedback inhibition.* Each end product inhibits the first individual committed step. Also, each end product *partially* inhibits the first common committed step.

Supplemental



sigmoidal

- positive cooperativity
- multisubunit (almost certainly)
- Hill coefficient > 1


+S
+S


+S
+S

Monod Jacob Model