Enzymes Ch-6-

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Enzymes are mostly proteins , or catalytic RNAs.

- Some require nonprotein coenzymes (organic) / cofactors (metal ion).

-Some require both.

-Coenzyme / cofactor tightly and covalently bound= prosthetic group.

-Enzyme + cofactor and or coenzyme = holoenzyme

-Enzyme alone = apoprotein / apoenzyme

table 8–1

Some Inorganic Elements That Serve as Cofactors for Enzymes

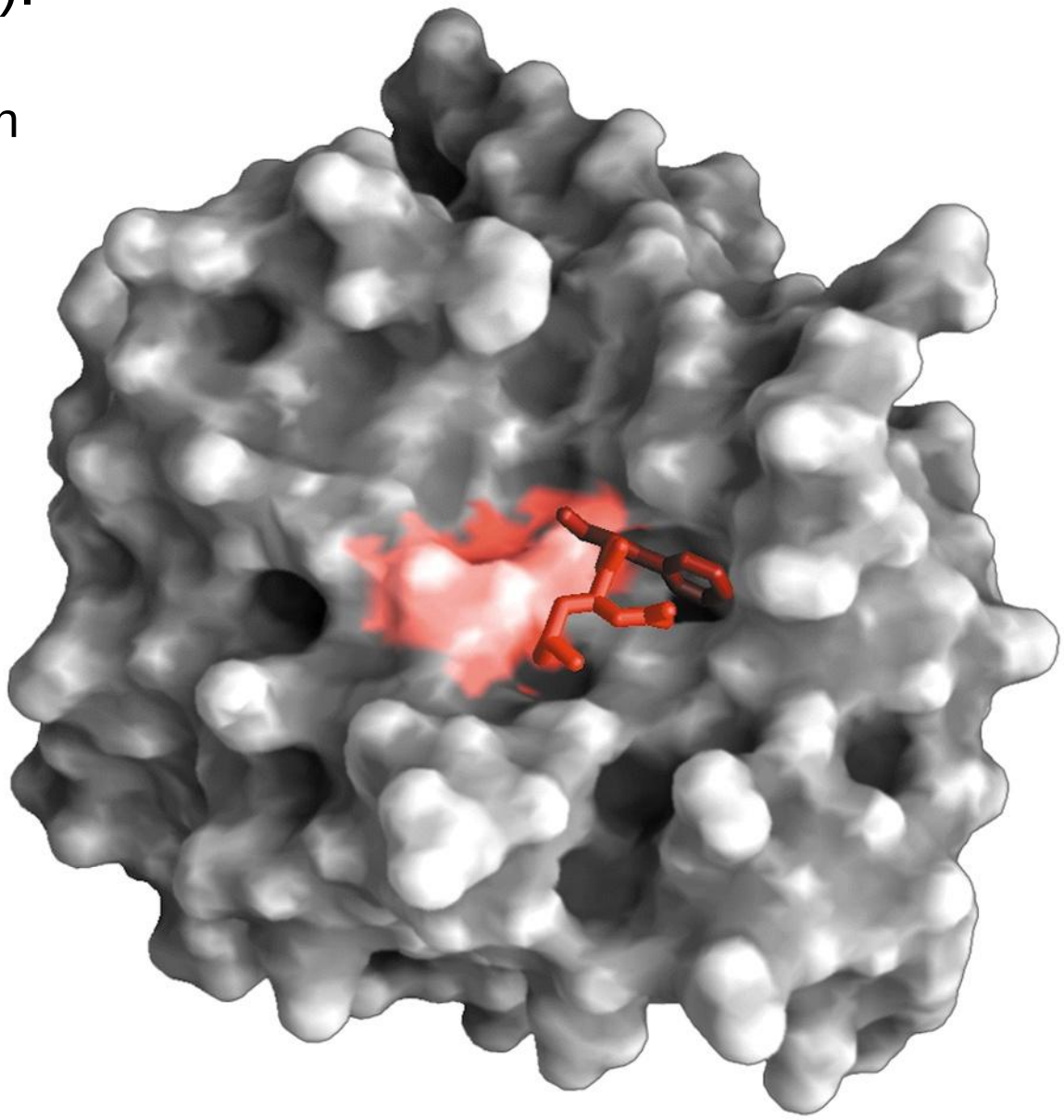
Cu^{2+}	Cytochrome oxidase
Fe^{2+} or Fe^{3+}	Cytochrome oxidase, catalase, peroxidase
K^{+}	Pyruvate kinase
Mg^{2+}	Hexokinase, glucose 6-phosphatase, pyruvate kinase
Mn^{2+}	Arginase, ribonucleotide reductase
Mo	Dinitrogenase
Ni^{2+}	Urease
Se	Glutathione peroxidase
Zn^{2+}	Carbonic anhydrase, alcohol dehydrogenase, carboxypeptidases A and B

Substrate binding to enzyme active site:

Chymotrypsin with S (red).

Active site: pocket of the E with a.a residues with groups that bind S

Substrate: molecule/ ligand bound to active site and acted on by the E.



Sucrose $C_{12}H_{22}O_{11} + O_2 \rightarrow 12CO_2 + 11\ H_2O$

In vitro (in container) : no rxn
In vivo : rxn + useful form of energy ATP
E overcomes the E barrier , important for control in metabolism.

Reaction intermediate: species on rxn pathway with finite chemical lifetime
S→ ES→EP→P

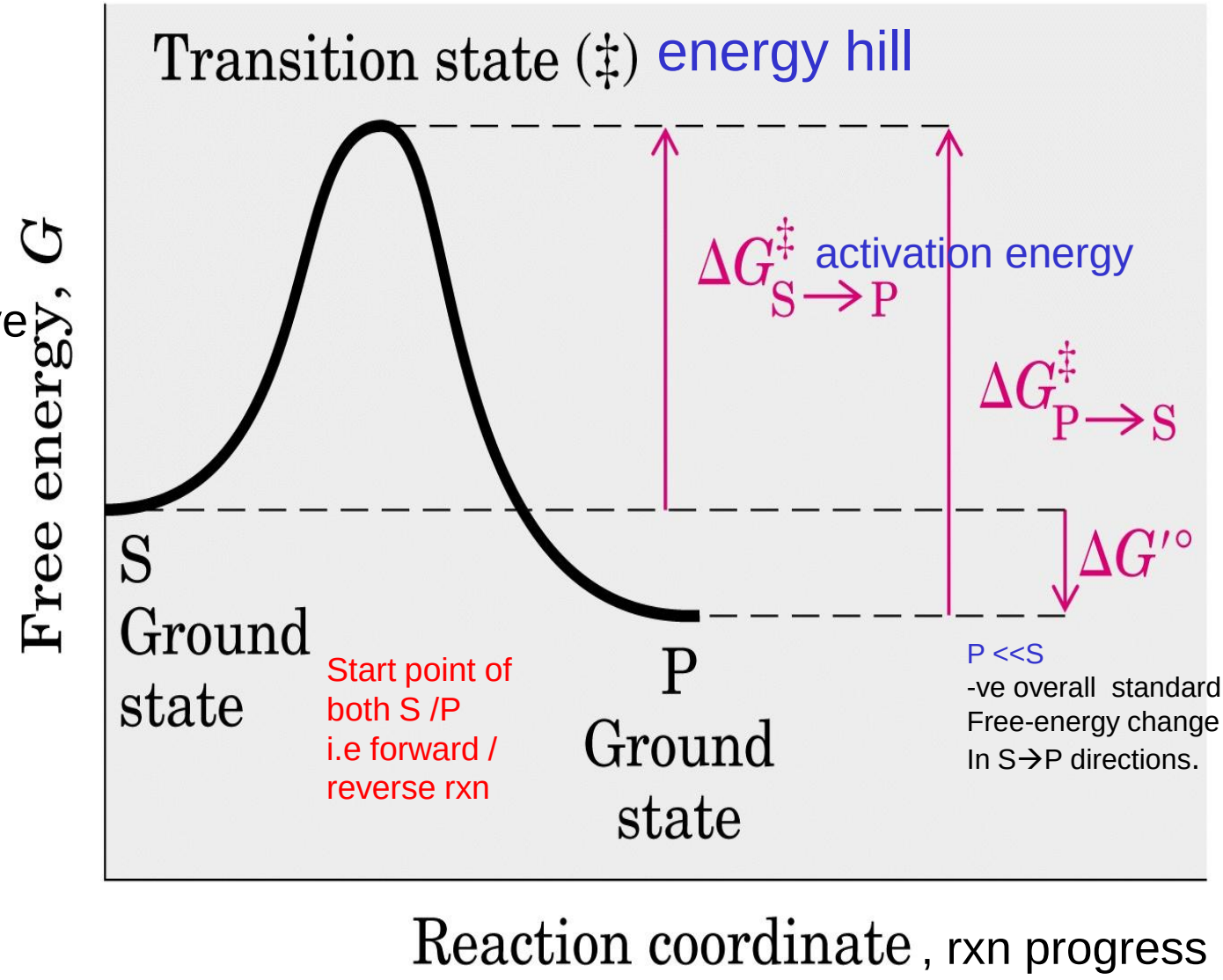
Rxn with several steps:
Step/s with highest activation energy
= highest energy point in S→P interconversion = rate limiting step.

Reaction coordinate diagram for a chemical rxn:

A description of energy changes during a rxn S→P

-Energy changes in biological systems expressed as free energy G

-rxn coordinate represent progressive chemical changes bond formation / cleavage as S→P



Enzyme specificity :

ability to discriminate bw a S and a competing molecule.

e.g. S with hydroxyl group form H-bond with Glu at active site, any other molecule excluded from active site.

Specificity → derived from many weak interactions.

1) Key and Lock model :

Emil Fischer →

E structurally complementary to S Fit in like key in lock

2) Induced fit :

Active site not 100% complementary to S but to transition states.

Enzyme kinetics

Rate of rxn and the effect of [S]

Initial rate = initial velocity = $V_{\circ}$

Catalytic power and specificity of enzymes:

Free energy released in forming bonds + interaction bw E and S = binding energy = ΔG_B

1) Transient covalent interactions bw S and catalytic site of E mainly functional groups of some a.a metal ion coenzyme. Rearrangement of bonds/ transfer of a functional group to E

2) Non covalent interactions bw E and S (hydrophobic / ionic / H-bonds)

Each bond formation associated with energy release→ stabilize transition state.

Effect of [S] on the initial velocity of an E – catalyzed rxn:

Michaelis constant = Km = [S] where Vo is half maximal.

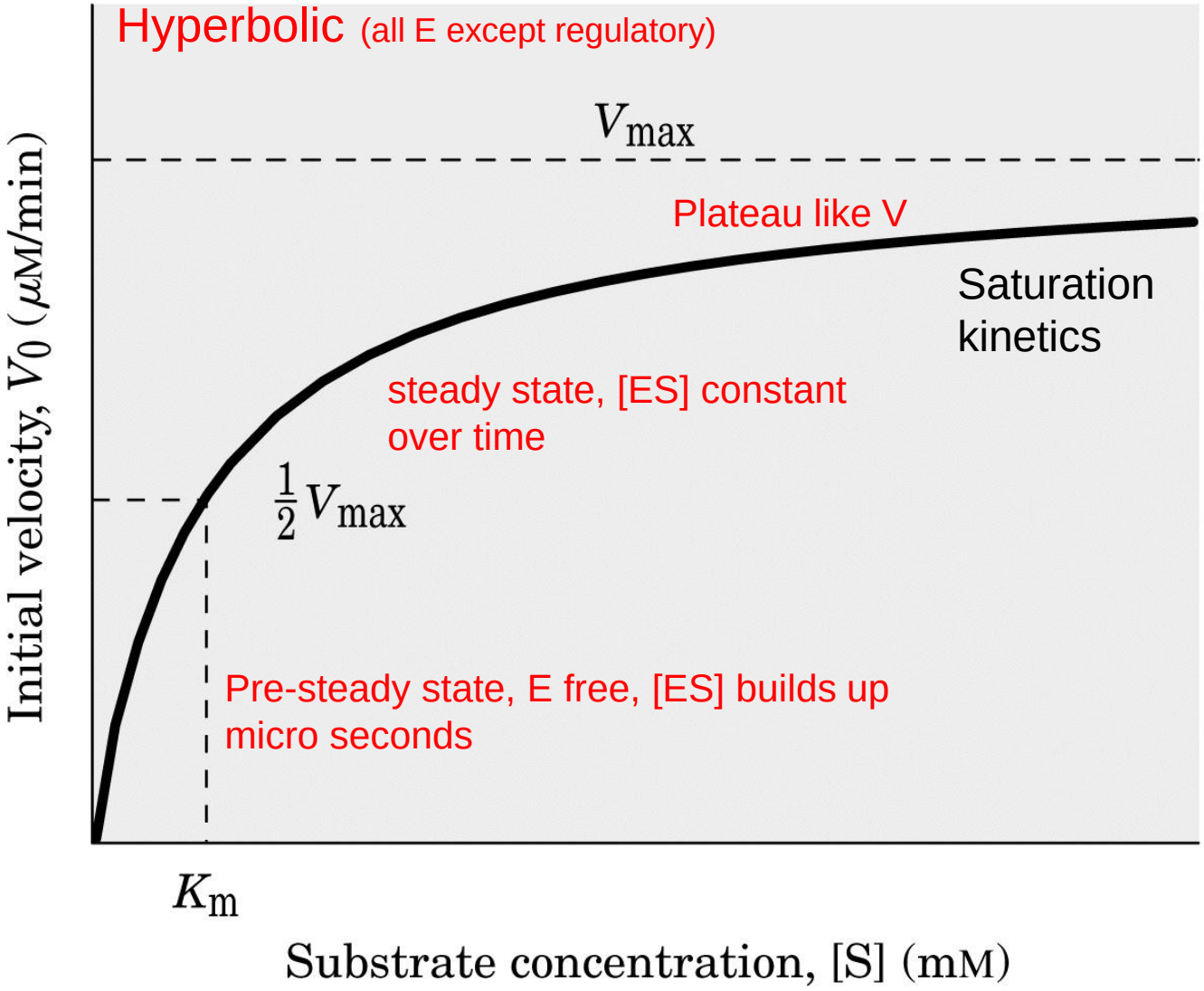
E + S ⇔ ES ⇔ P

At low [S] → Vo ↑ linearly with [S].

At higher [S]→ Vo ↑ by smaller amounts.

E saturated with S

[S] ⇑ but effect on V is smaller.



Michaelis - Menten equation / rate equation: Dependence of initial velocity Vo on [S]

The equation

$$Vo = \frac{Vmax\,[S]}{K_m + [S]}$$

$$Vo = 1/2\ Vmax$$

$$\frac{Vmax}{2} = \frac{Vmax\,[S]}{K_m + [S]}$$

$$K_m = [S]$$

Equation and Km provide little information about chemical nature/ steps of the rxn.

K_m indicator of the affinity of E to S.

Vary from E to another and from different S for the same E

E with low [S] in cell lower K_m than E with abundant S

table 8–6

K_m for Some Enzymes and Substrates

Enzyme	Substrate	K_m (mM)
Catalase	H_2O_2	25
Hexokinase (brain)	ATP	0.4
	D-Glucose	0.05
	D-Fructose	1.5
Carbonic anhydrase	HCO_3^-	26
Chymotrypsin	Glycyltyrosinylglycine	108
	N-Benzoyltyrosinamide	2.5
β-Galactosidase	D-Lactose	4.0
Threonine dehydratase	L-Threonine	5.0

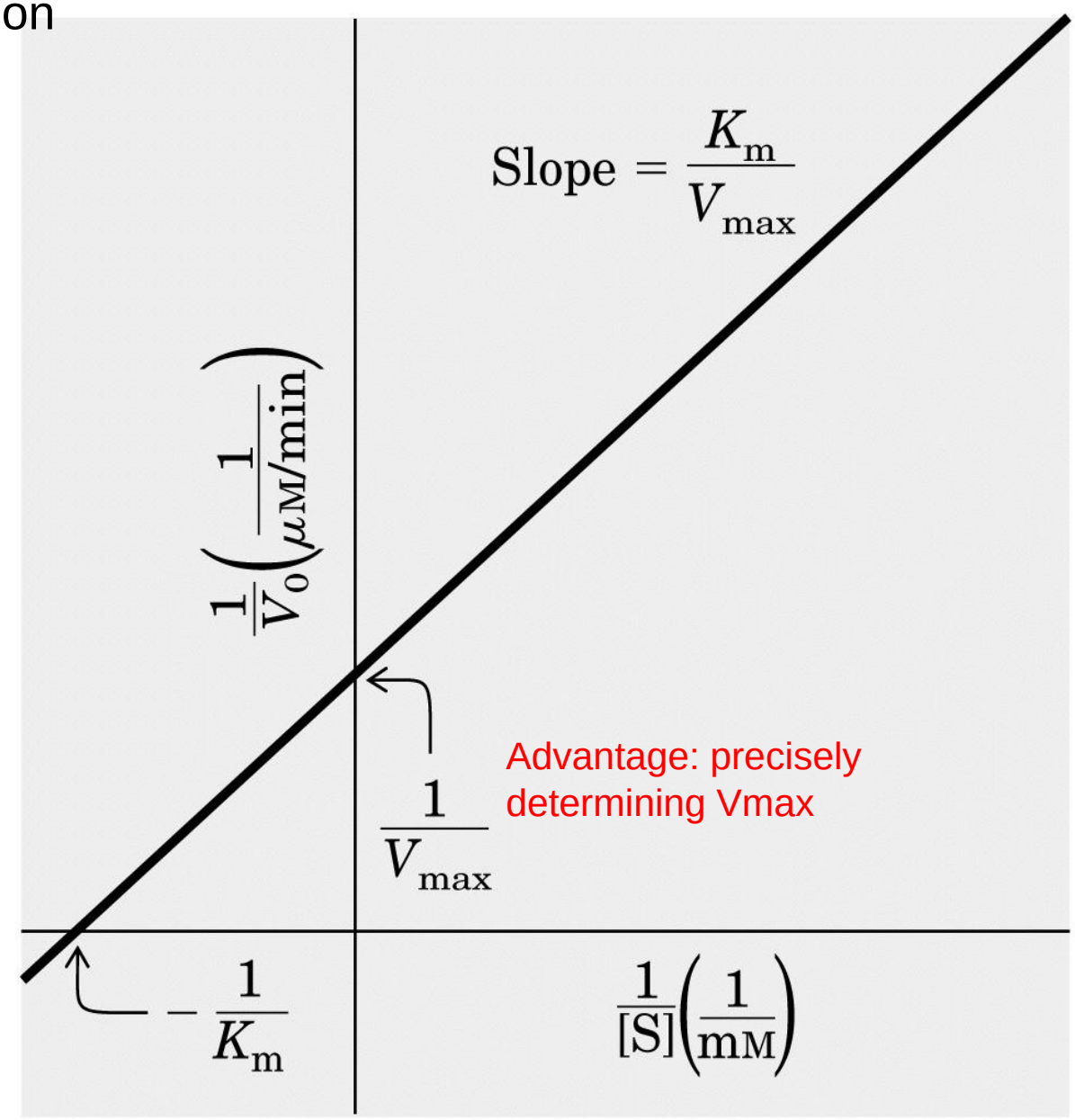
Double reciprocal plot or Lineweaver-Burk plot: (more convenient)

The Michaelis-Menten equation

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

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

Important in analyzing
E inhibition.



Enzymatic rxn with 2 or more S :

ATP + glucose → hexokinase → glucose-6-p + ADP

-Analyzed by M-M equation and M-M kinetics with Km for each S.

-Involve transfer of a functional group/ atom from one S to another.

Common mechanisms for rxn with more than one S:

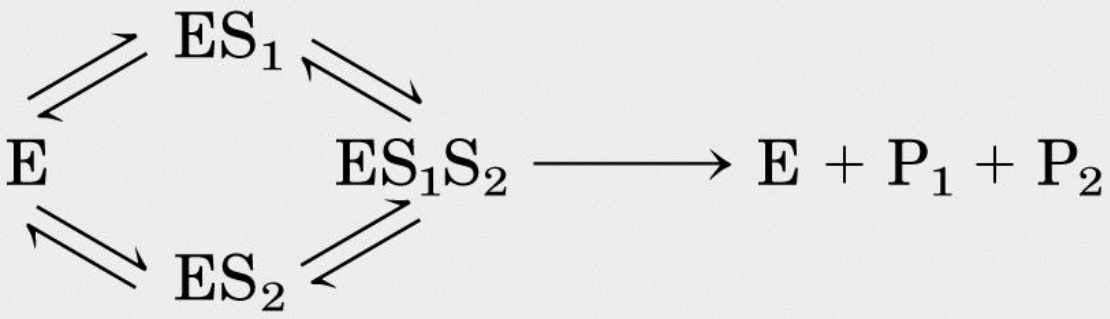
1) E and both S form a ternary complex.

In random binding substrates can bind in either order.

In ordered binding S_1 must bind before S_2 can bind productively.

(a) Enzyme reaction involving a ternary complex

Random order



Ordered

2) Ping-pong or double-displacement mechanism:

E-S complex forms a P_1 leaves the complex. Altered E forms a 2nd complex with another S, and P_2 leaves, regenerating E

S_1 transfer a functional group to E to form covalently modified E' which is transferred to S_2 .

(b) Enzyme reaction in which no ternary complex is formed

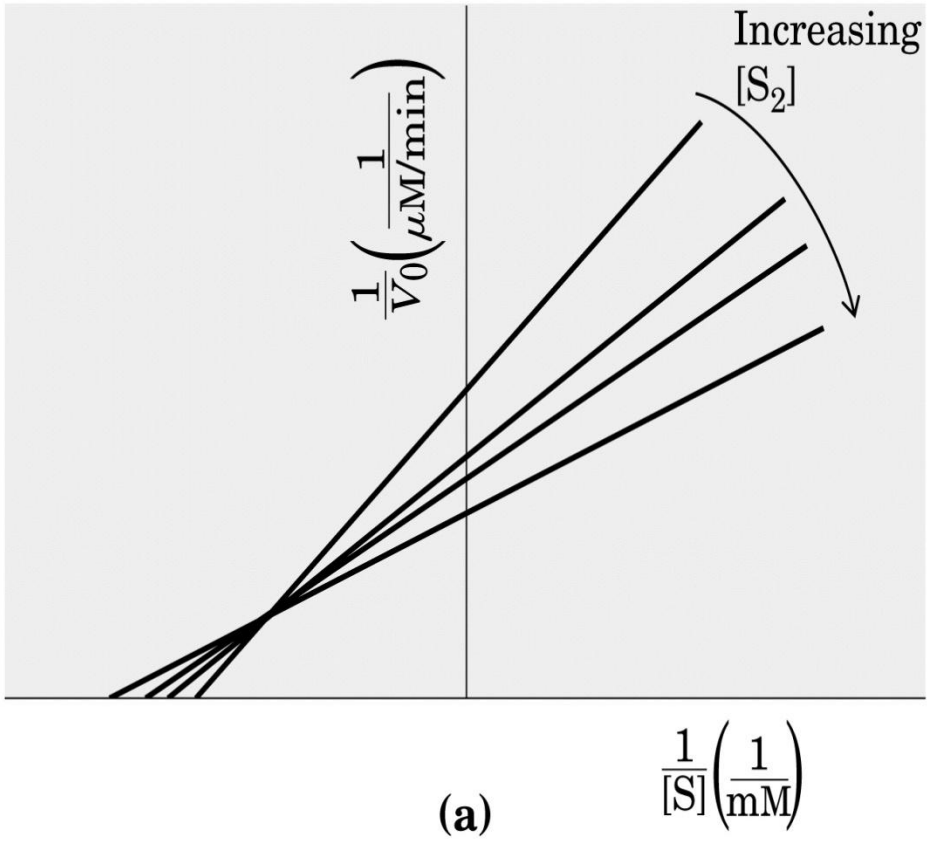
$$E + S_1 \rightleftharpoons ES_1 \rightleftharpoons E'P_1 \overset{P_1}{\rightleftharpoons} E' \overset{S_2}{\rightleftharpoons} E'S_2 \longrightarrow E + P_2$$

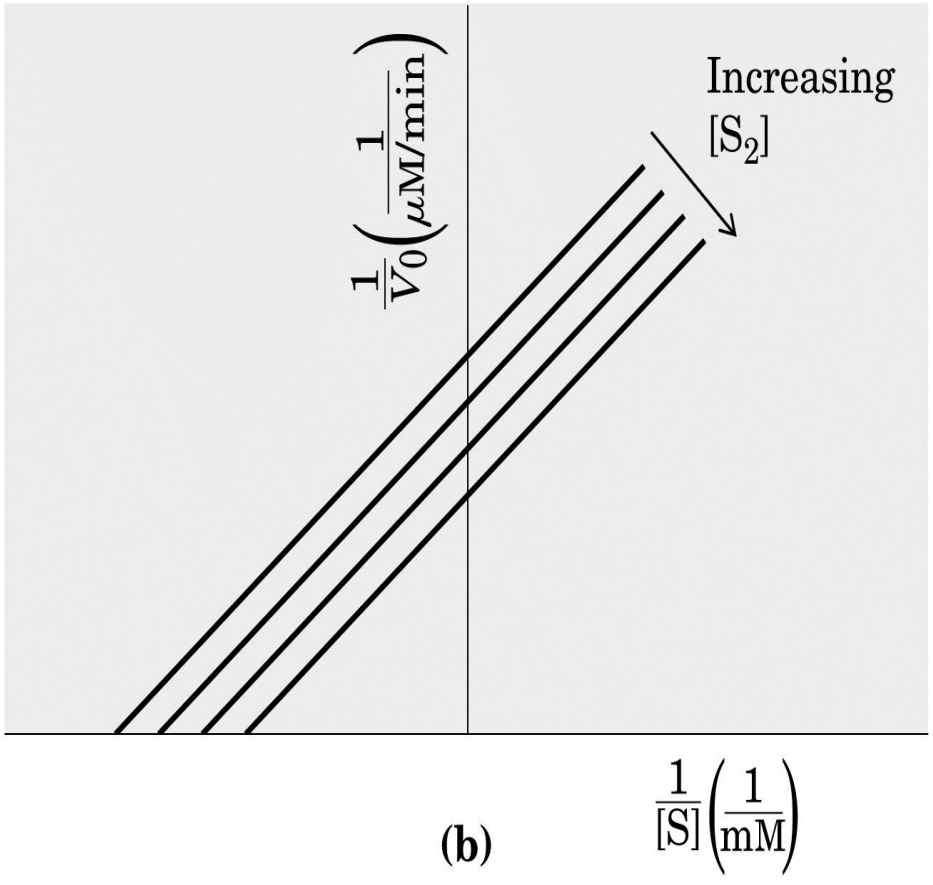
Steady-state kinetic analysis of bisubstrate rxns:

In double-reciprocal plots, S_1 conc is varied while the S_2 conc is held constant.

Intersecting lines indicate formation of ternary complex.

Parallel lines indicate a ping-pong /double displacement pathway.





Enzyme inhibition:

inhibitors: agents that interfere with catalysis slowing / halting enzymatic rxns.

1) Pharmaceutical agents:

   e.g aspirin (acetylsalicylate) inhibits prostaglandins synthesis (pain processes).

2) Discovery and define of metabolic pathway.

Inhibition is reversible / irreversible

Three types of reversible inhibition:

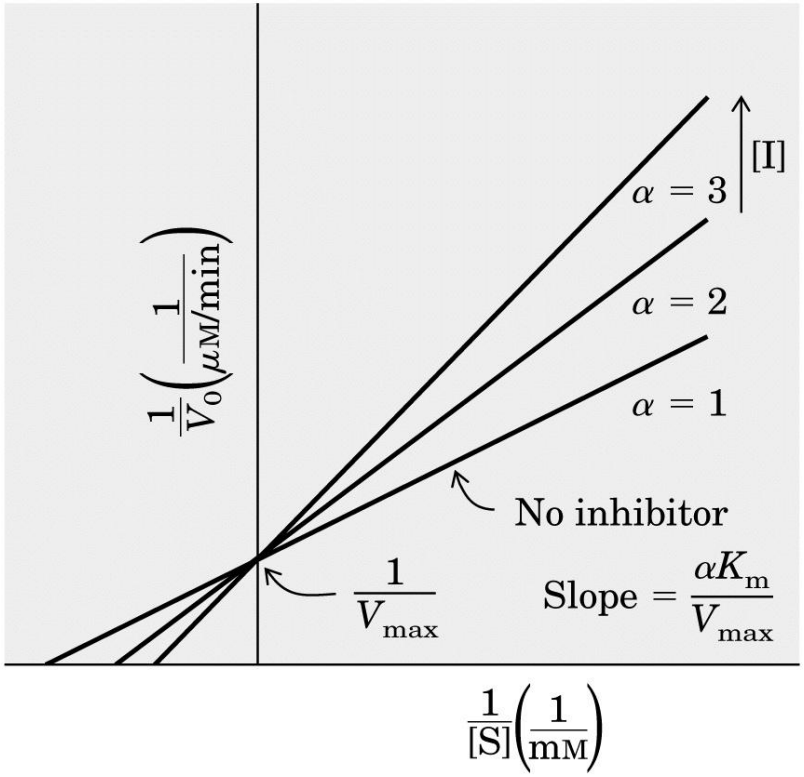
1) Competitive inhibitors:

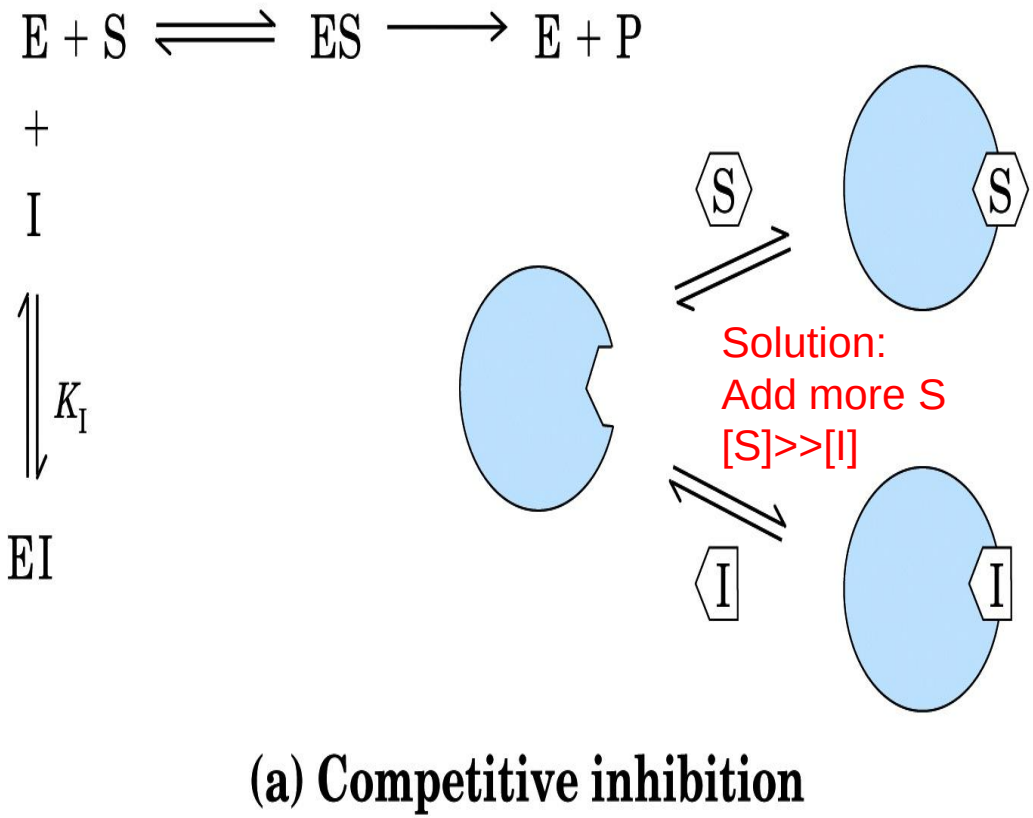
Compete with S by binding reversibly to the enzymes active site.

I resembles S and bind E → EI complex but no catalysis / P

αKm↑= “apparent”/ observed in I presence.

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





Use of competitive inhibitor in Medical therapy:

Case:

methanol poisoning usually from contaminated alcohol beverages

Methanol in liver → alcohol dehydrogenase→ formaldehyde → formic acid→ metabolic acidosis

Symptoms:

Vomiting, abdo pain, photophobia, tissue damage

Ingestion of 10ml →blindness , 30ml → fatal (2 tablespoons deadly to a child).

Treatment:

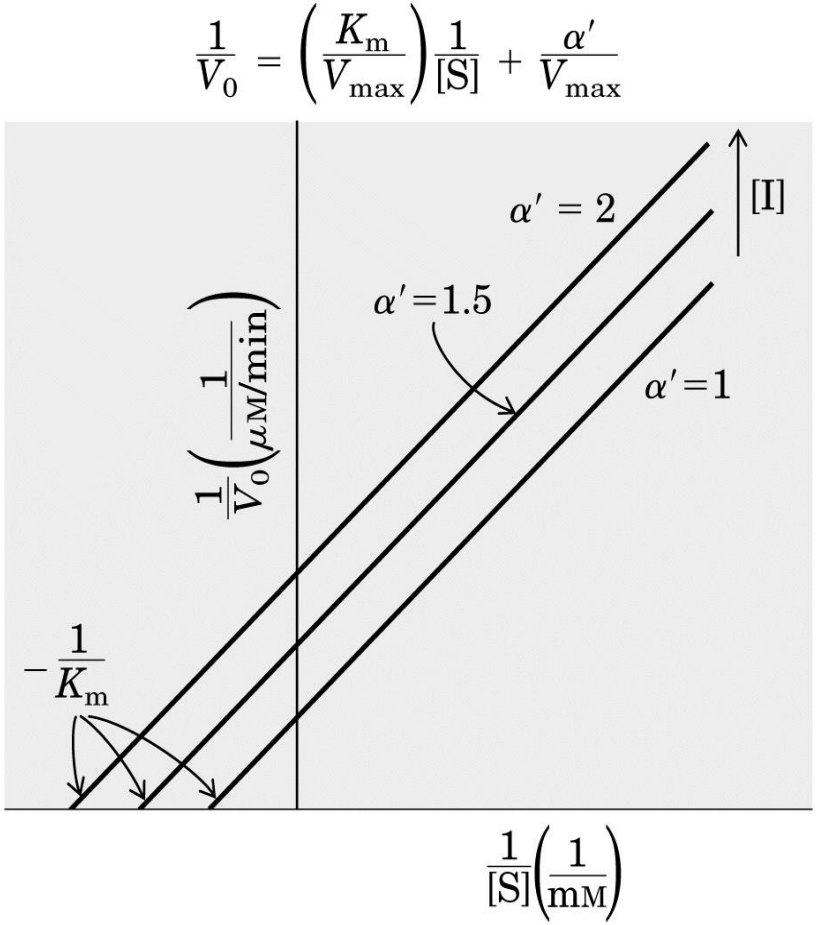
Antidote to reverse the effect of the poison intravenous infusion of ethanol = competitive inhibitor to alcohol dehydrogenase.

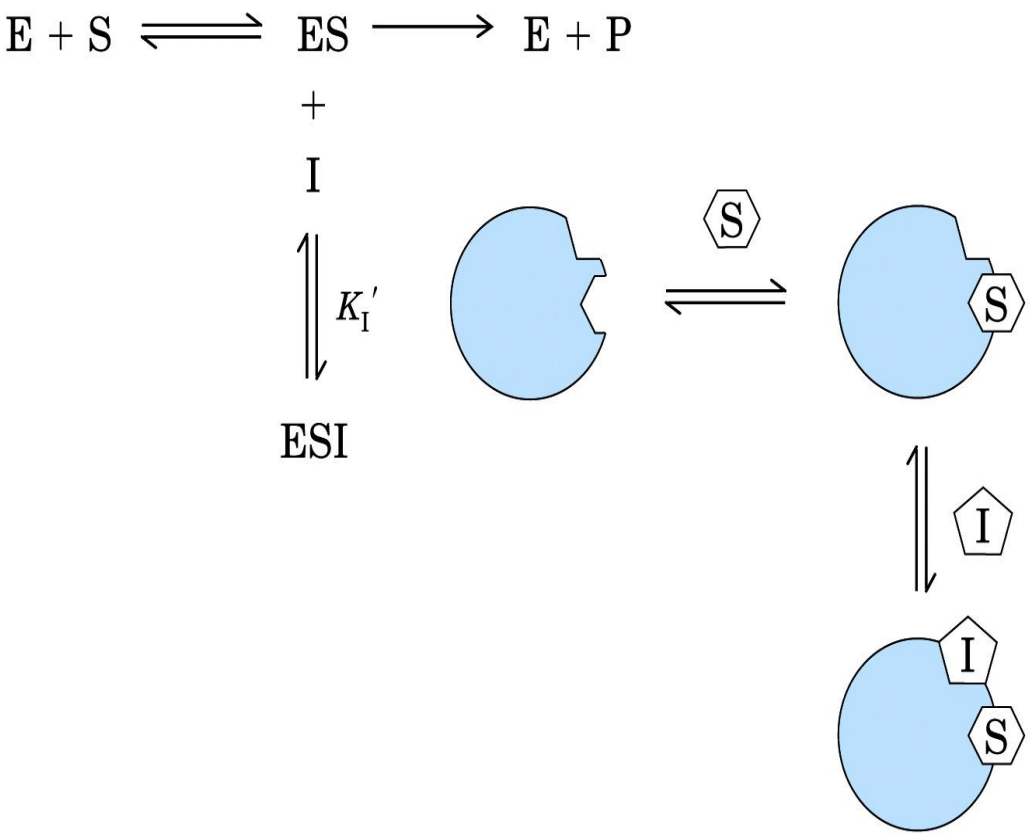
Methanol present in Copy machine fluids, De-icing fluid, Antifreeze, Fuel additives, Paint remover.

Uncompetitive inhibitors:

Bind at a separate site, but bind only to the ES complex (after S)

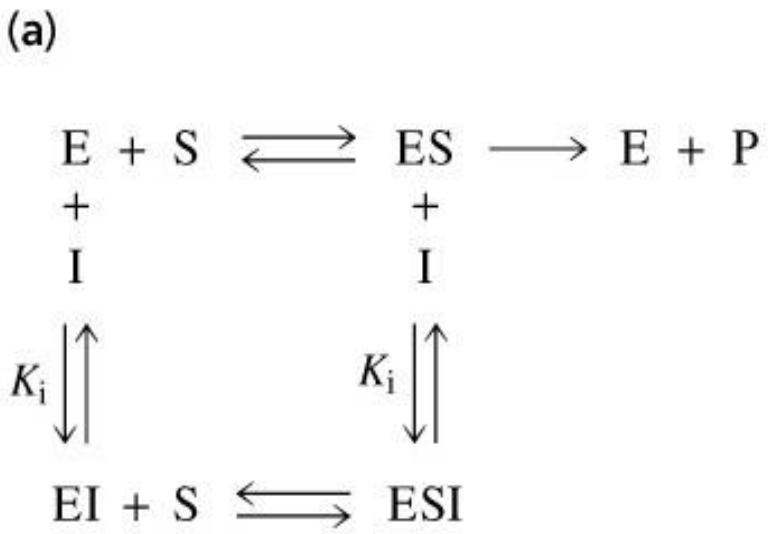
α Km ↓ and Vmax ↓

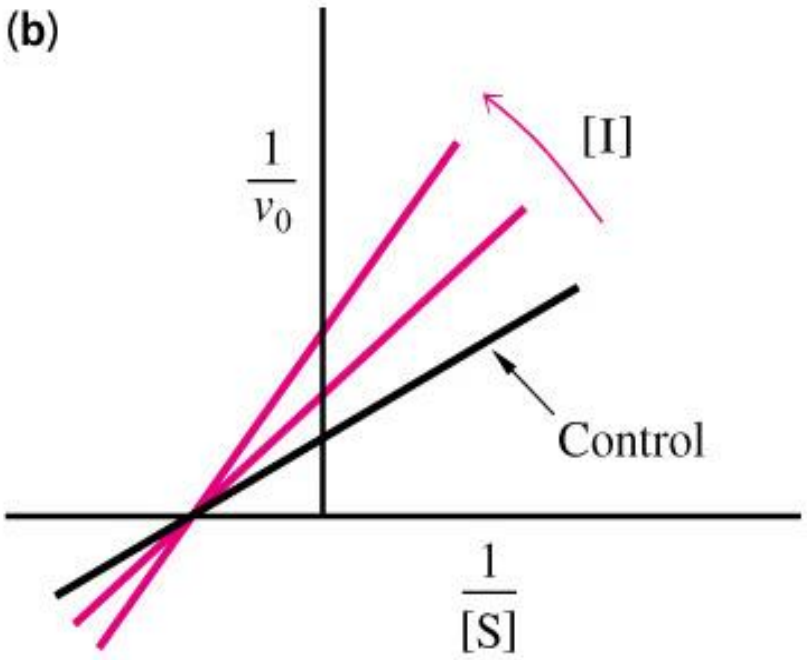




(b) Uncompetitive inhibition

Non-competitive Inhibitor



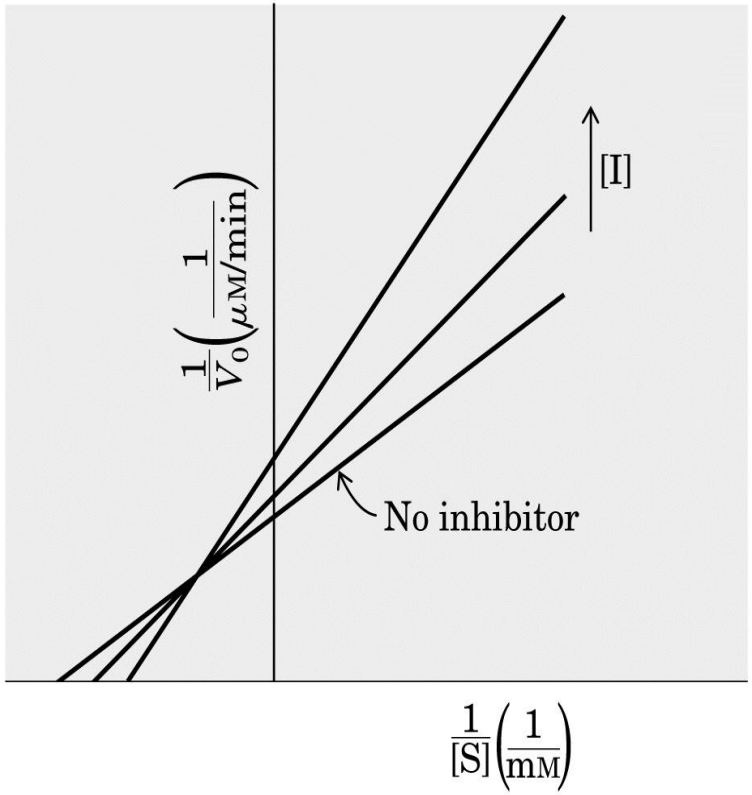


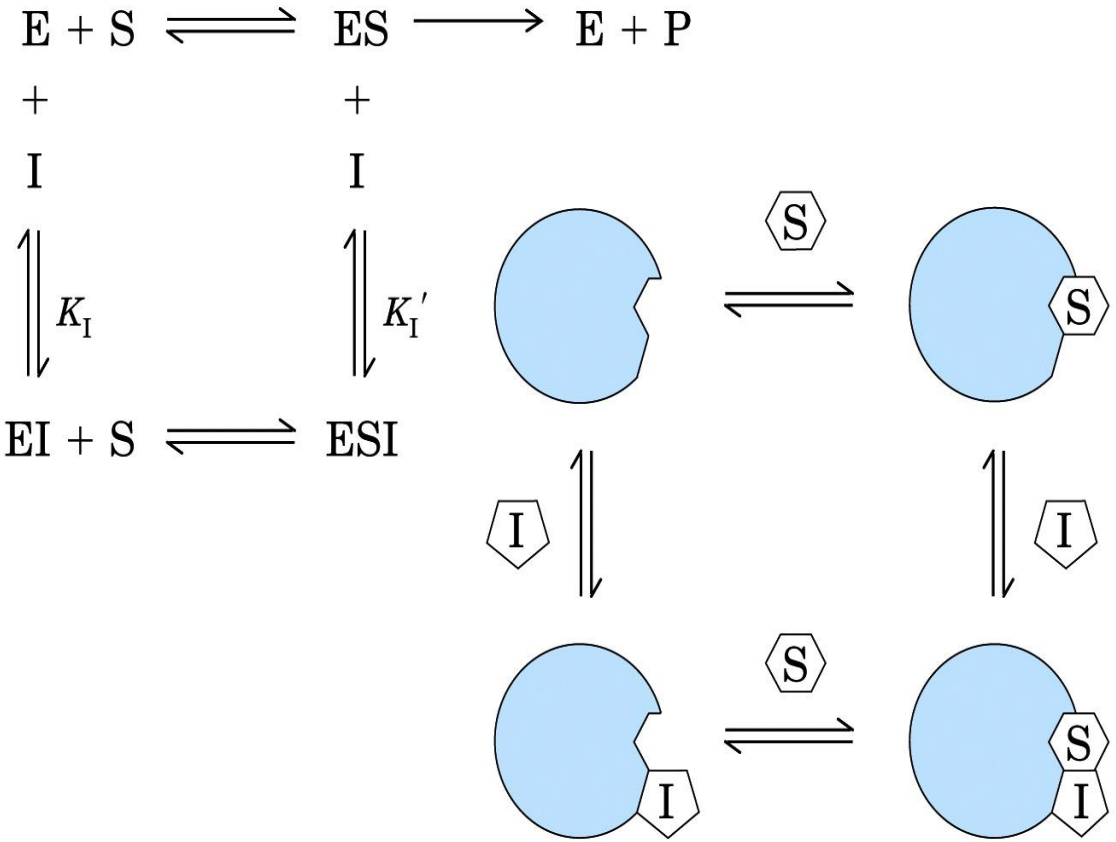
- can bind free E or ES complex
- Lowers Vmax, but Km remains the same
- Alters conformation of enzyme to affect catalysis but not substrate binding

Mixed inhibitors:

Also bind to a site separate than the active site, but may bind to either E or ES .

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





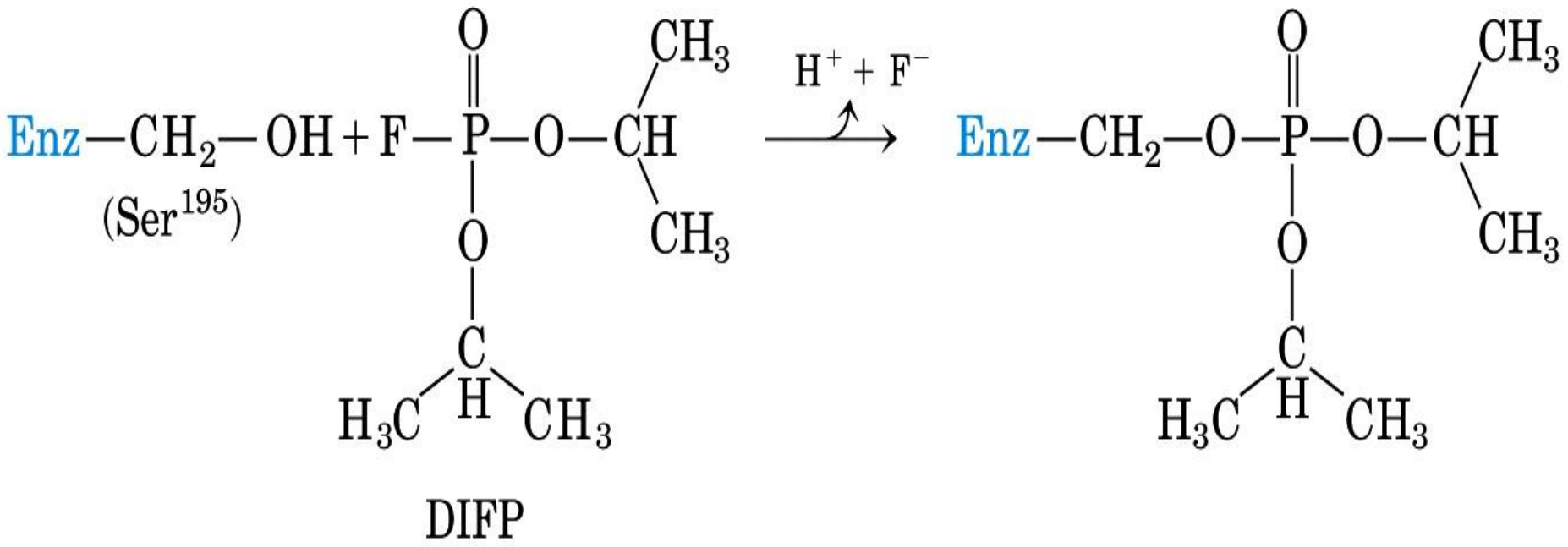
(c) Mixed inhibition

Irreversible inhibition:

Binds irreversibly to E active site by forming a covalent bond/ very stable non covalent interaction → destroy a functional group.

diisopropylflourophosphte (DIFP):

irreversibly inhibits chymotrypsin and serine proteases→ led to conclusion that Ser^{195} is the key active site residue in chymotrypsin.



DIFP

Suicide inactivators / mechanism-based inactivators:

Relatively unreactive until binding to E active site.

Perform first few chemical steps of rxn→ not transfomed to normal product.

AZT and other chain-terminating nucleoside analogues used to inhibit HIV-1 reverse transcriptase in the treatment of HIV/AIDS.

Penicillin which inhibits transpeptidase from building bacterial cell walls.

Important in DRUG DESIGN:

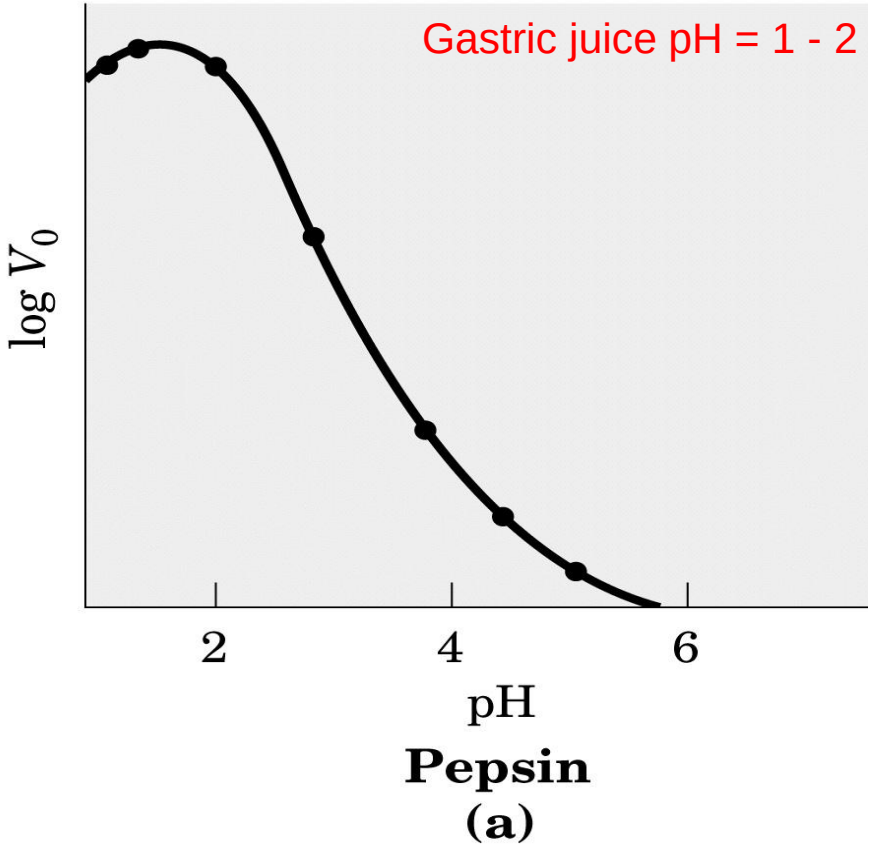
Specific for a single E unreactive until within E active site.

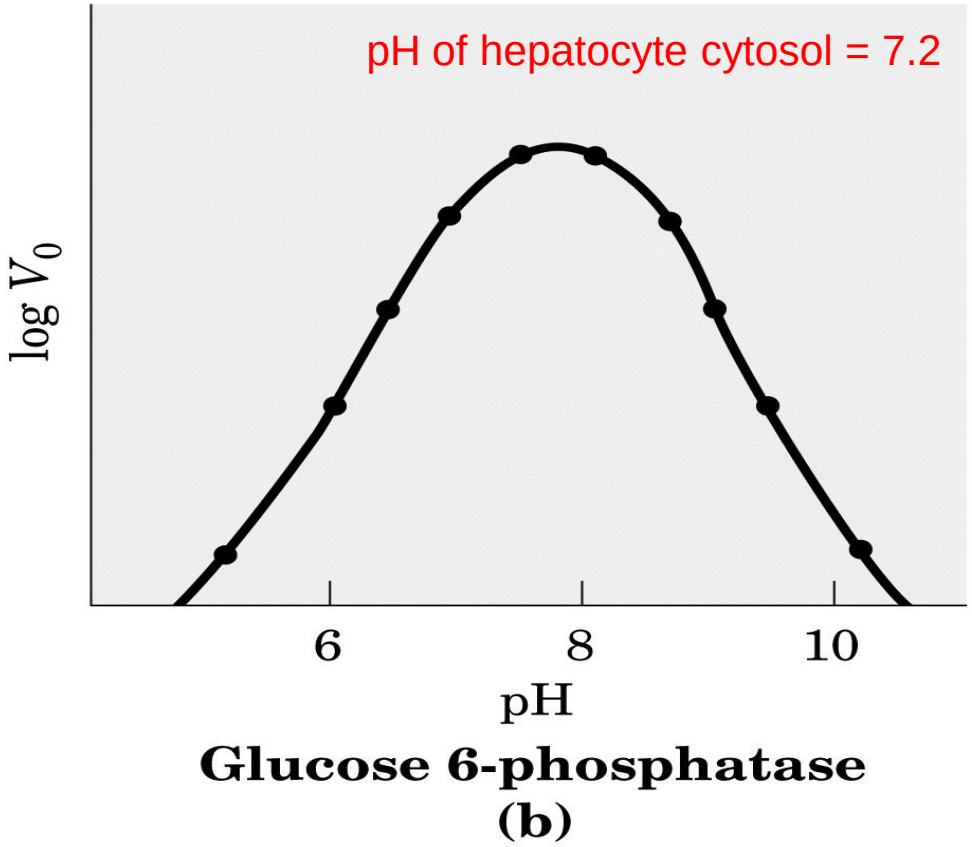
Advantage: few side effects

The pH activity profiles of two enzymes:

Optimum pH is close to the pH of the environment in which the enzyme is normally found.

Optimum pH→ maximum activity.





Regulatory Enzymes:

- One / more E in a metabolic pathway have great effect on the rate of the overall rxn.

- Activity of allosteric E (increase /decrease) regulated by reversible binding of a specific modulator to a regulatory site other than active site.

Effect of modulator = positive / negative.

Modulators maybe the S itself (Homotropic modulator) / other metabolites.

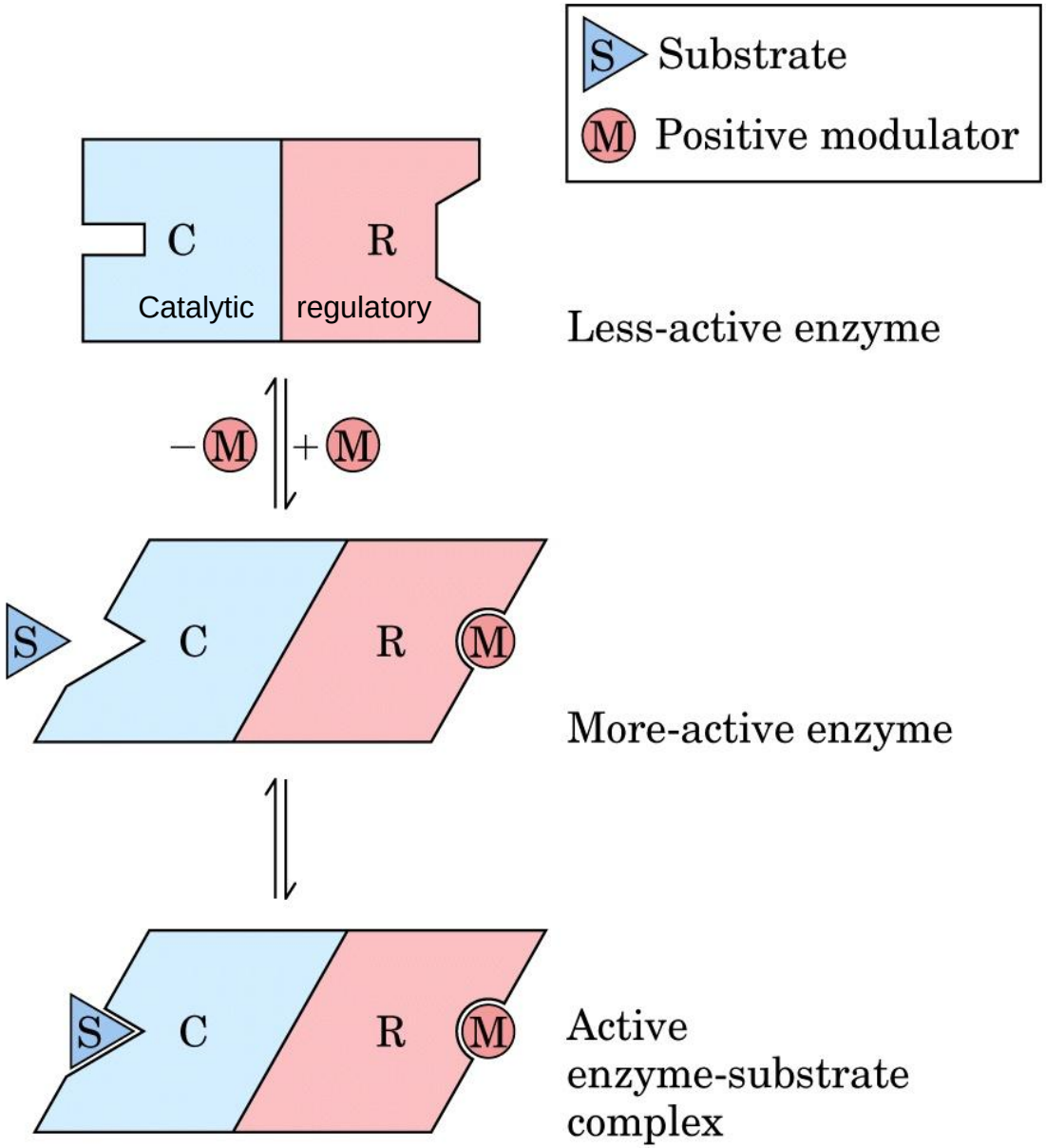
Kinetic behavior of allosteric E reflects cooperative interactions among E subunits (similar to O_2 – hemoglobin).

Subunit interactions in an allosteric E, interactions with inhibitors and activators:

S binding site and M sites on different subunits.

Binding of M to R →induce conformational change in C →active and capable of binding S with higher affinity.

When M dissociates. Enzyme→ inactive/ less active.



Feedback inhibition =

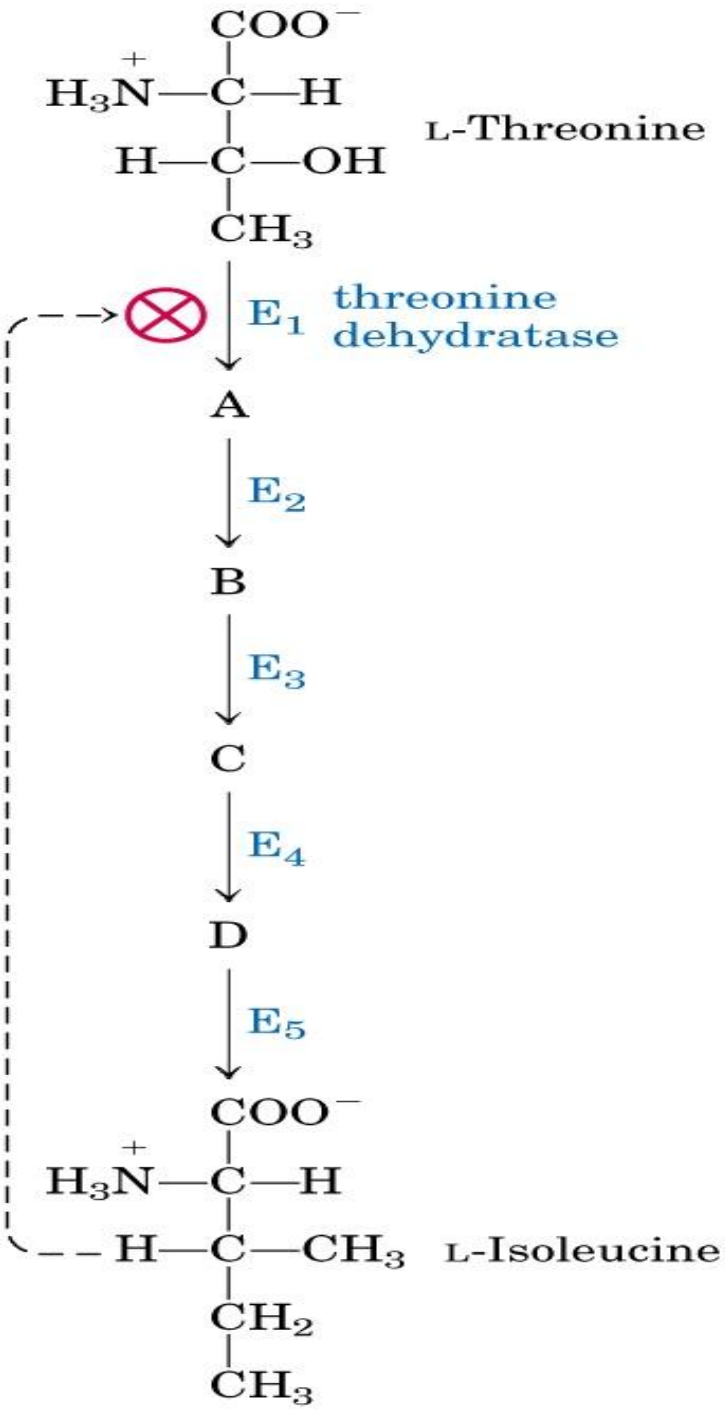
- The regulatory E in a metabolic pathway is inhibited by the end product of the pathway.
- Build up of end product slows entire pathway

Heterotropic allosteric inhibition =

Noncovalent + reversible.

Pathway inhibited allostericaly by L-isoleucine.

But not by any of the 4 intermediate.



Kinetics of allosteric enzymes:

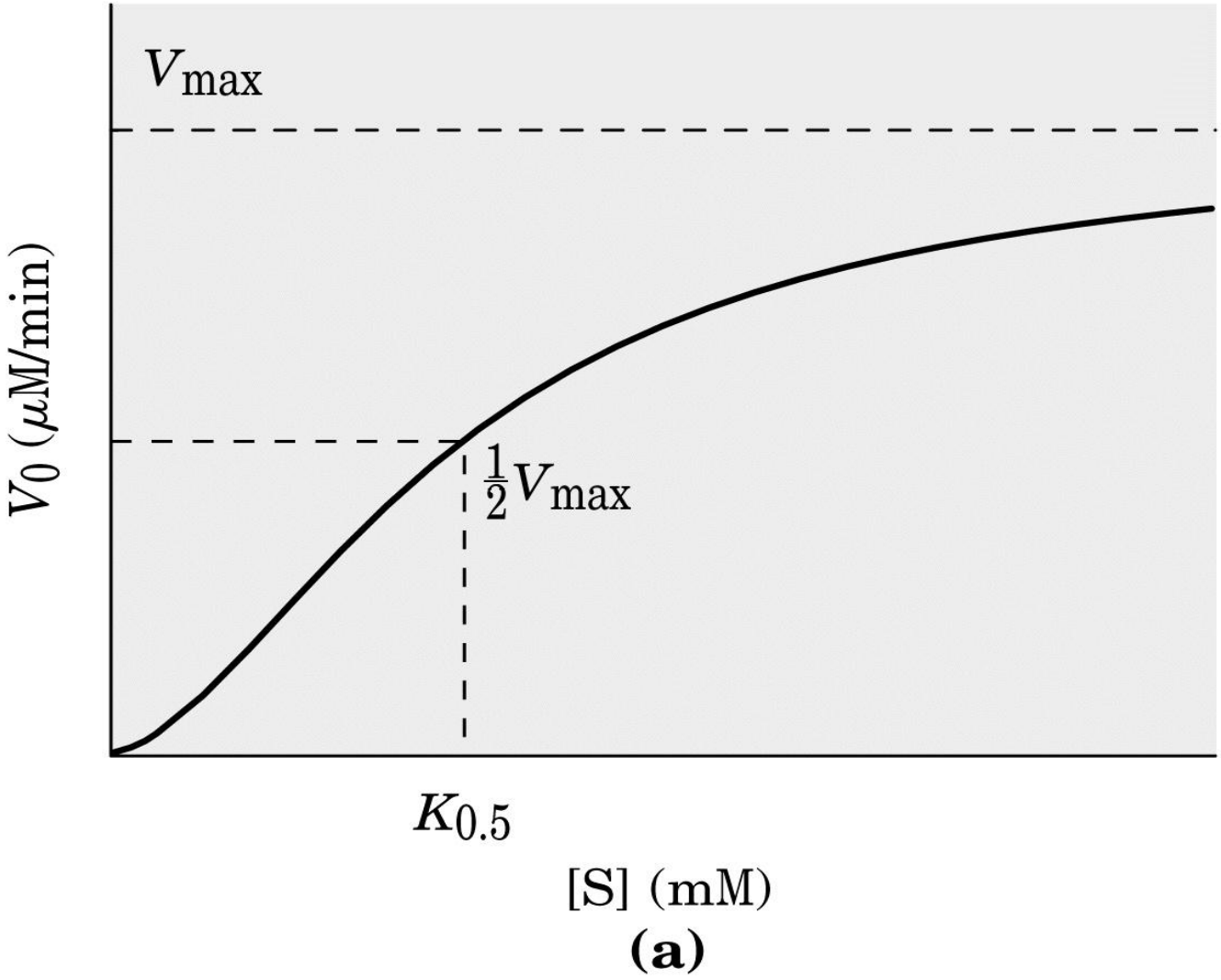
Differ than M-M kinetics. Sigmoid saturation curve not hyperbolic.

Reflects cooperative interaction bw subunits.

Small changes in [S]

Large effect on activity

$K_{0.5}$ not K_m



Sigmoid curve for allosteric enzymes:

An activator cause the curve to become → more hyperbolic.

$K_{0.5}$ decrease, no change in V_{max} and large change in activity.

An inhibitor cause the curve to become → more sigmoid