6| Enzymes

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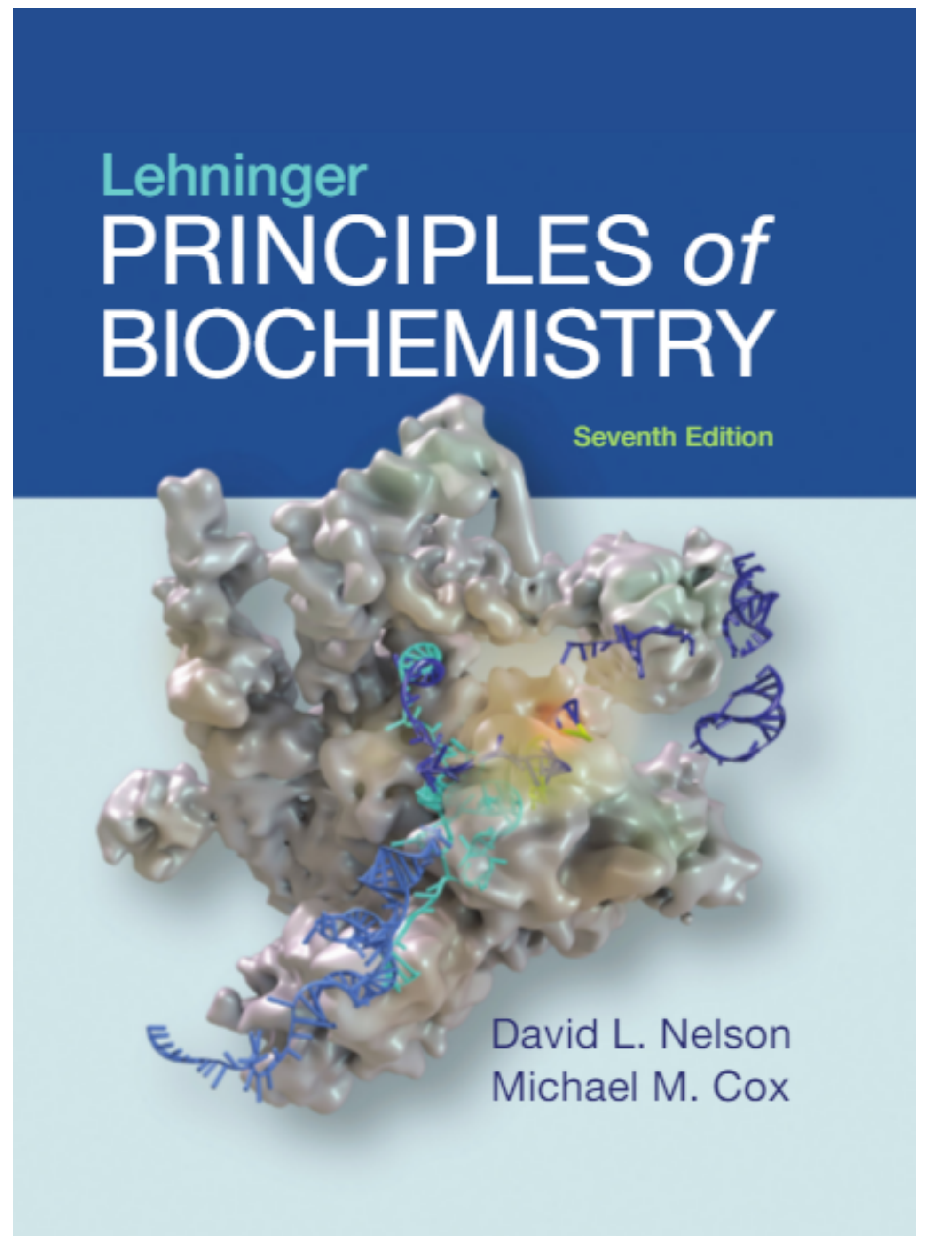


Catalysis

- Think of sugar:
  Our bodies use it to give energy within seconds (converting it to CO_2 + H_2O)

  But a bag of sugar can be on the shelve for years even though the process is very thermodynamically favorable

- The difference is catalysis

What Are Enzymes?

- The study of enzymatic processes is the oldest field of biochemistry, dating back to late 1700s.
- Enzymes are biological catalysts.
    - increase reaction rates without being used up
- Most enzymes are globular proteins.
    - However, some RNA (ribozymes and ribosomal RNA) also catalyze reactions.
- Extraordinary catalytic power
- High degree of specificity
- Accelerate chemical reactions tremendously
- Function in aqueous solutions under very mild conditions of temperature and pH
- *Very few non-biological molecules have all these properties*

What are Enzymes?

- Some enzymes need other chemical groups
- *Cofactors* – either one or more inorganic ions
- *Coenzymes* – complex organic or metalloorganic molecules
- Some enzymes require both
- *Prosthetic group* – a coenzyme or cofactor that is very tightly (or covalently) bound to an enzyme
- *Holoenzyme* – a complete active enzyme with its bound cofactor or coenzyme
- *Apoprotein* – the protein part of a holoenzyme

What are Enzymes?

- Enzyme classification
- Common names (DNA polymerase, urease; pepsin; lysozyme, etc.)
- 6 classes each with subclasses

What are Enzymes?

Group	**Reaction catalyzed**	**Typical reaction**	**Enzyme example(s) with trivial name**
EC 1 *Oxidoreductases*	To catalyze oxidation/reduction reactions; transfer of H and O atoms or electrons from one substance to another	AH + B → A + BH (reduced) A + O → AO (oxidized)	Dehydrogenase, oxidase
EC 2 *Transferases*	Transfer of a functional group from one substance to another. The group may be methyl-, acyl-, amino- or phosphate group	AB + C → A + BC	Transaminase, kinase
EC 3 *Hydrolases*	Formation of two products from a substrate by hydrolysis	AB + H_2O → AOH + BH	Lipase, amylase, peptidase
EC 4 *Lyases*	Non-hydrolytic addition or removal of groups from substrates. C-C, C-N, C-O or C-S bonds may be cleaved	RCOCOOH → RCOH + CO_2 or [X-A-B-Y] → [A=B + X-Y]	Decarboxylase
EC 5 *Isomerases*	Intramolecule rearrangement, i.e. isomerization changes within a single molecule	AB → BA	Isomerase, mutase
EC 6 *Ligases*	Join together two molecules by synthesis of new C-O, C-S, C-N or C-C bonds with simultaneous breakdown of ATP	X + Y+ ATP → XY + ADP + Pi	Synthetase

Example:

- ATP + $_D$-glucose → ADP + $_D$-glucose 6-phosphate

- **Formal name:** ATP:glucose phosphotransferase
- **Common name:** hexokinase
- **E.C. number** (enzyme commission): 2.7.1.1

2 → class name (transferases)

7 → subclass (phosphotransferases)

1 → phosphotransferase with –OH as acceptor

1 → $_D$-glucose is the phosphoryl group acceptor

Why Biocatalysis Over Inorganic Catalysts?

- Greater reaction specificity: avoids side products
- Milder reaction conditions: conducive to conditions in cells

  pH ~ 7, 37°C

- Higher reaction rates: in a biologically useful timeframe
- Capacity for regulation: control of biological pathways

Chorismate mutase

- Metabolites have many potential pathways of decomposition.
- Enzymes make the desired one most favorable.

Enzymatic Substrate Selectivity

→

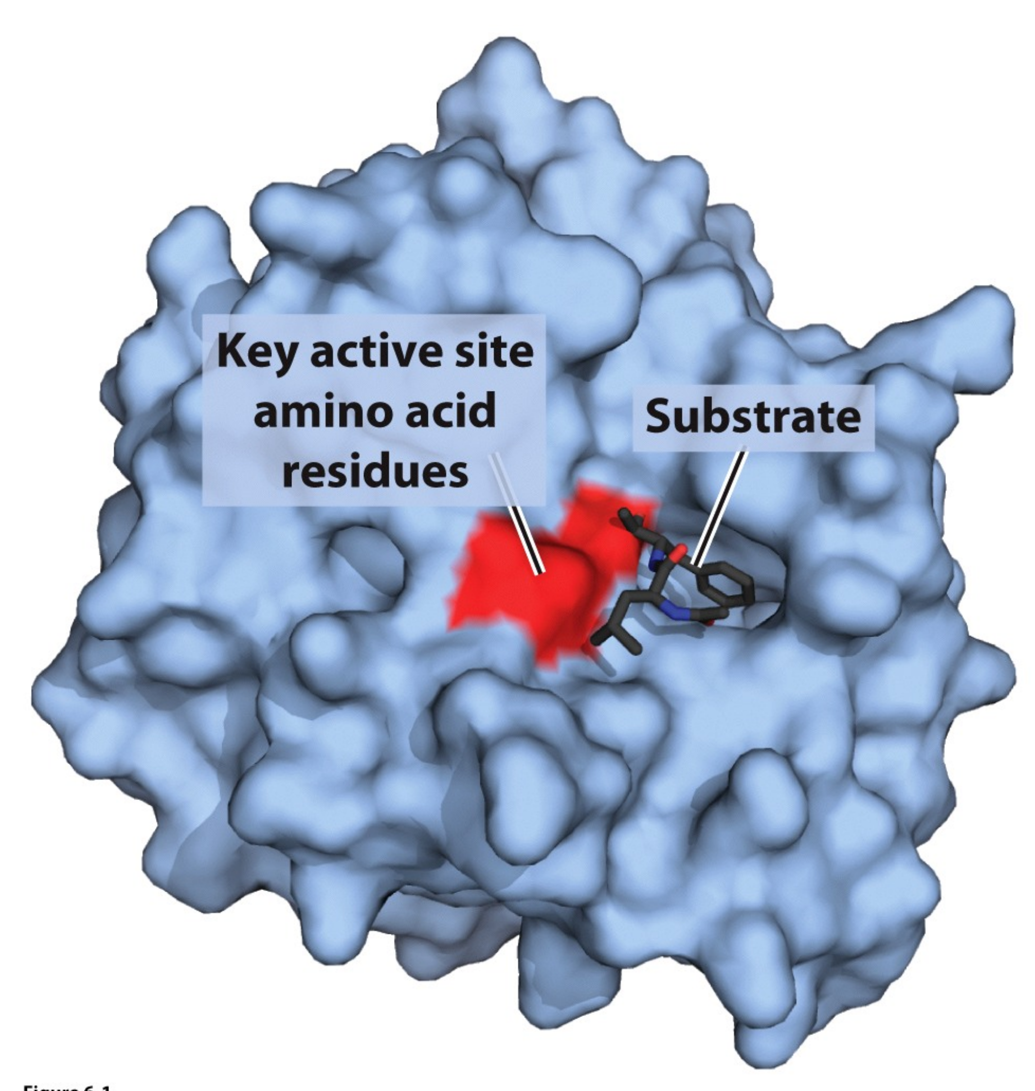
→ No binding

→ Binding but no reaction

Example: Phenylalanine hydroxylase

6.2 How Enzymes Work

- Recall:
- Active site
- Substrate



Binding of a substrate to an enzyme at the active site (chemotrypsin)

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Enzymatic Catalysis

- A simple enzyme reaction:

$$E + S \leftrightarrow ES \leftrightarrow EP \leftrightarrow E + P$$

  - The function of a catalyst is to increase the *rate* of the reaction.
  - Catalysts do not affect the *equilibrium*
  - Reaction coordinate diagram

Enzymatic Catalysis

- Slow reactions face **significant activation barriers** ($\Delta G^{\ddagger}$) that must be surmounted during the reaction
  - –transition state theory is applicable for catalysis
  - –rate constants and free energies can be related
  - –Enzymes increase reaction rates (k) by decreasing $\Delta G^{\ddagger}$

$$k = \left(\frac{k_B T}{h}\right) \exp\left(\frac{-\Delta G^{\neq}}{RT}\right)$$

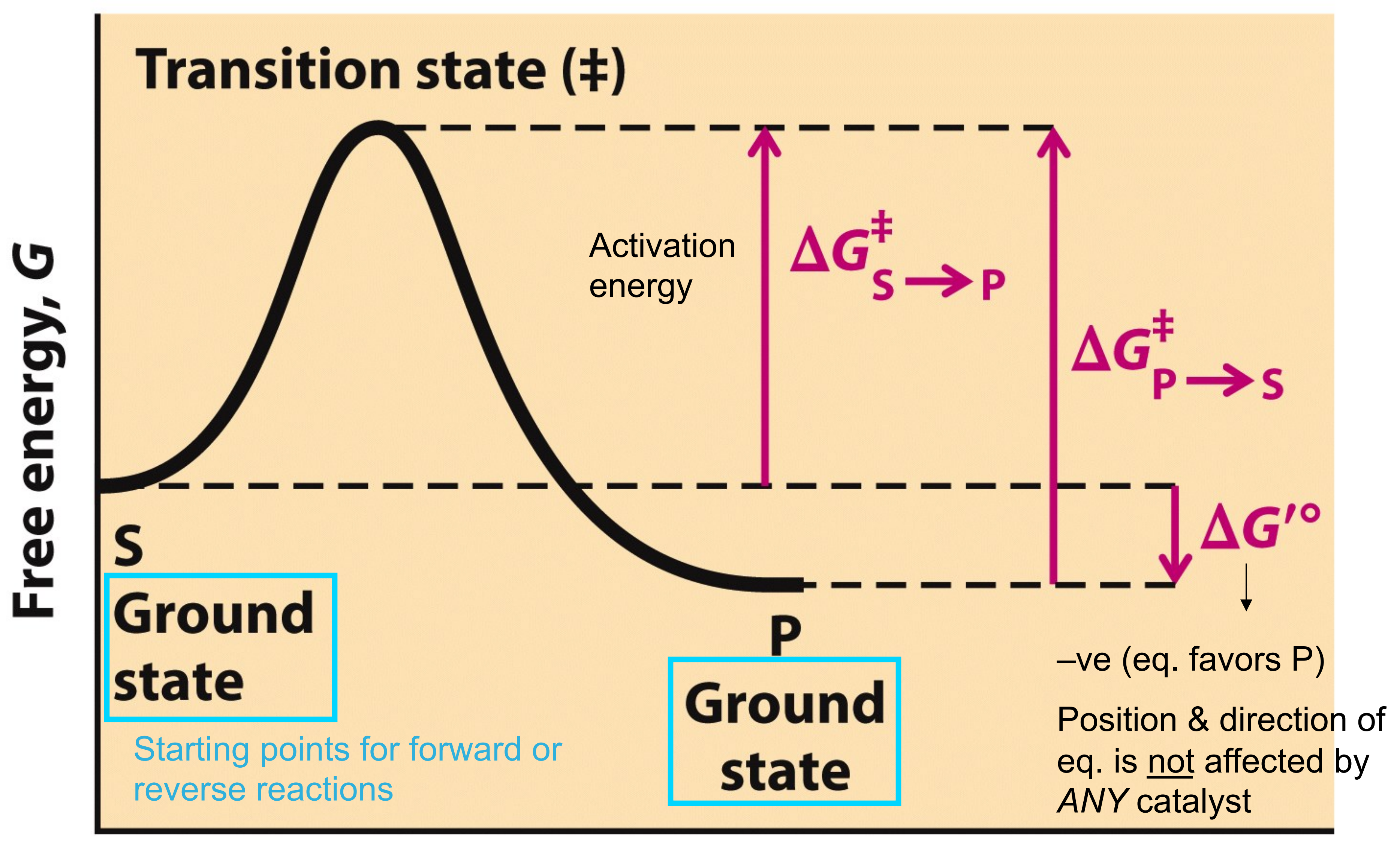
k_B: Boltzmann const.

h: Planck's const.

k is inversely proportional to $\Delta G^{\ddagger}$ and exponential

➔ a lower activation energy means a faster rate

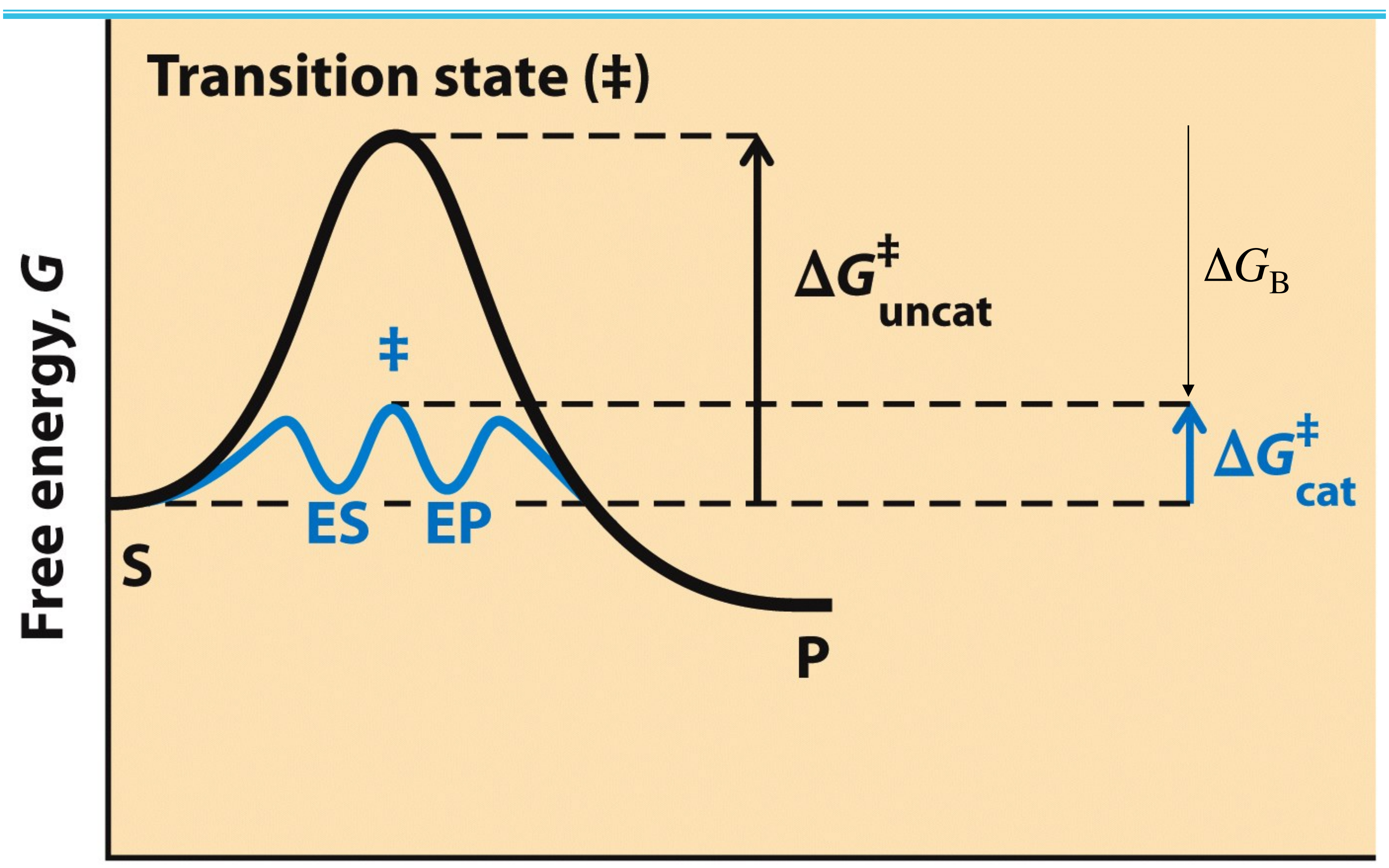
Reaction coordinate diagram. The free energy of the system is plotted against the progress of the reaction S → P.



Enzymes affect reaction rates not equilibria

- *A favorable equilibrium does not mean the S → P conversion would be detectable*
- The *rate* of the reaction depends on the energy barrier (the activation energy):
  - a higher activation energy → slower reaction
- Rates of reactions can be increased with:
  - increasing temperature
  - increasing pressure
  - the addition of catalysts

Enzymes Decrease $\Delta G^‡$



Reaction rates & equilibria have precise thermodynamic definitions

S ←→ P

- $K'_{eq} = [P] / [S]$ (equilibrium constant)
  and $\Delta G'^{o} = -RT \ln K'_{eq}$
- A large –ve free-energy change → a favorable reaction equilibrium (*but does not mean the reaction will proceed at a high rate!*)
- The rate of any reaction depends on [reactant] and k (**rate constant**)
- $V = k[S]$ (1st order reaction)
  units of k (s^{-1})
- $V = k[S_1][S_2]$ (2nd order reaction)
  units of k ($M^{-1}s^{-1}$)

Rate Enhancement by Enzymes

TABLE 6-5	Some Rate Enhancements Produced by Enzymes
Cyclophilin	10^5
Carbonic anhydrase	10^7
Triose phosphate isomerase	10^9
Carboxypeptidase A	10^{11}
Phosphoglucomutase	10^{12}
Succinyl-CoA transferase	10^{13}
Urease	10^{14}
Orotidine monophosphate decarboxylase	10^{17}

How do enzymes lower $\Delta G^{\neq}$?

Enzymes organize reactive groups into close proximity and proper orientation

- Uncatalyzed bimolecular reactions

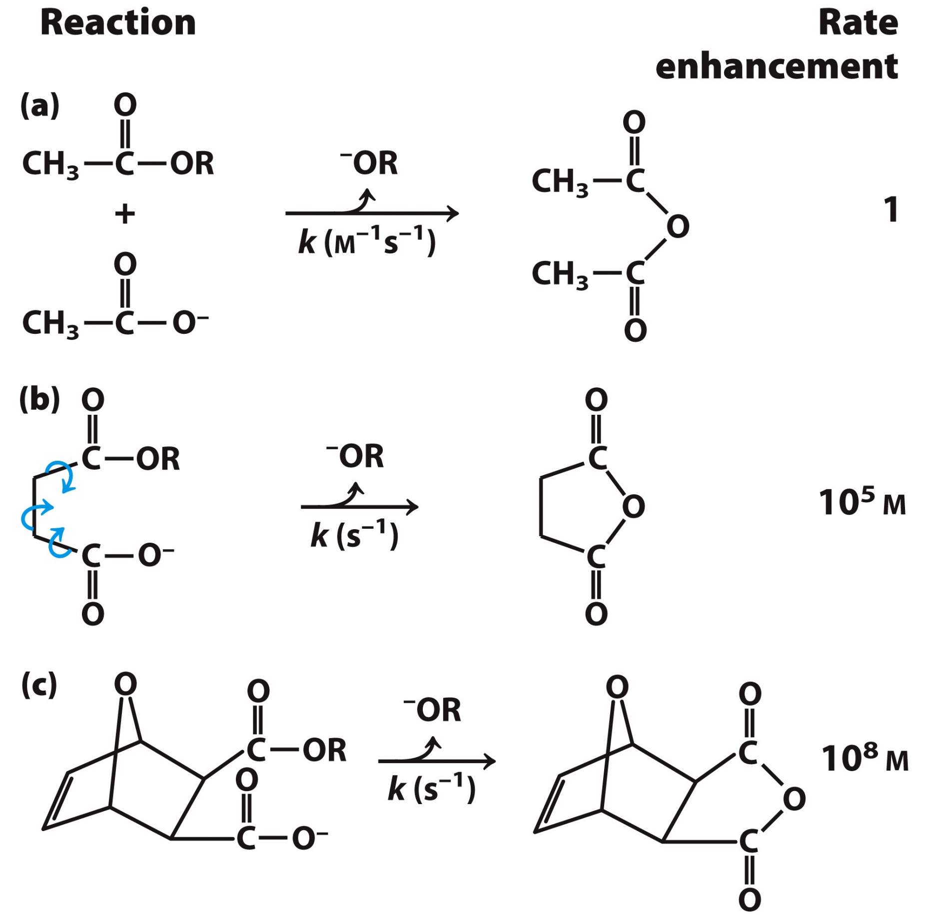
  two free reactants → single restricted transition state conversion is entropically unfavorable
- Uncatalyzed unimolecular reactions

  flexible reactant → rigid transition state conversion is entropically unfavorable for flexible reactants
- Catalyzed reactions

  Enzyme uses the binding energy of substrates to organize the reactants to a fairly rigid ES complex
  Entropy cost is paid during binding
  Rigid reactant complex → transition state conversion is entropically OK
  Weak interactions are optimized in the TS (active sites are complementary to TS)

Support for the Proximity Model

The rate of anhydride formation from esters and carboxylates shows a strong dependence on proximity of two reactive groups (work by Thomas C. Bruice’s group).



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How to Lower $\Delta G^{\neq}$

Enzymes bind transition states best

- The idea was proposed by Linus Pauling in 1946
  - Enzyme active sites are complimentary to the transition state of the reaction
  - Enzymes bind **transition states** better than **substrates**
  - Stronger/additional interactions with the transition state as compared to the ground state lower the activation barrier

Weak interactions are optimized in TS

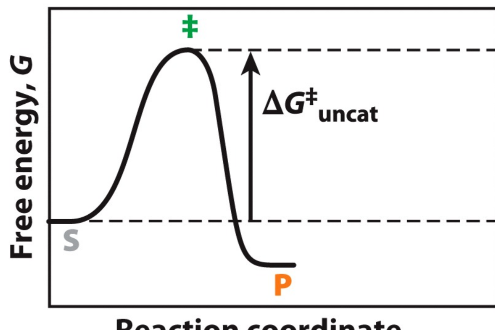
- "Lock and key" model – substrate fits the enzyme like a key in a lock
- This hypothesis can be misleading in terms of enzymatic reactions
- An enzyme completely complementary to its substrate is a very poor enzyme!

Illustration of TS Stabilization Idea

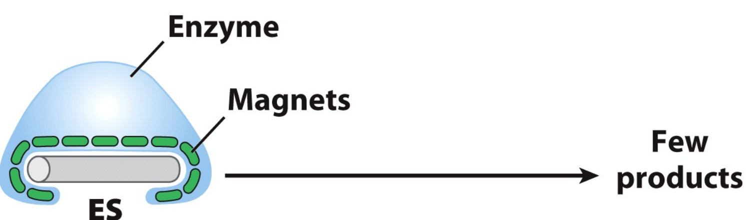
Imaginary Stickase

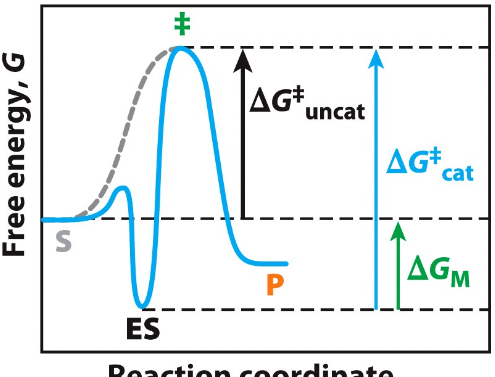
(a) No enzyme

Substrate (metal stick) | **Transition state (bent stick)** | **Products (broken stick)**

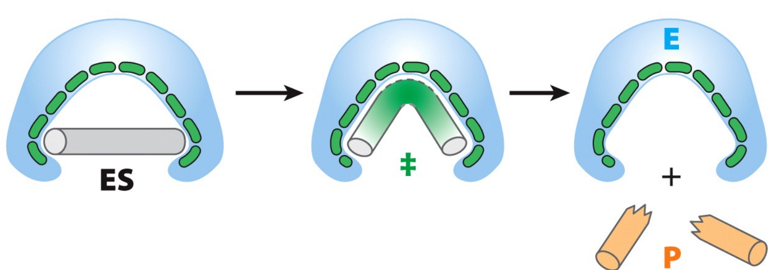


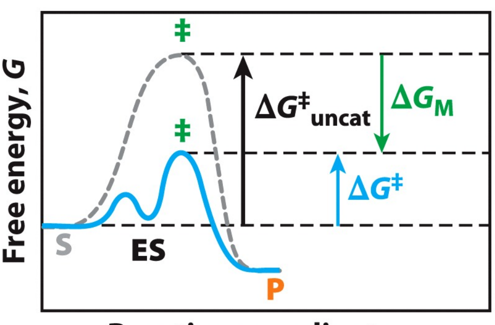
(b) Enzyme complementary to substrate





(c) Enzyme complementary to transition state





Specific catalytic groups contribute to catalysis

- When a substrate binds to an enzyme, catalytic functional groups which are place in proper positions help in the breaking and making bonds by many mechanisms:

Catalytic Mechanisms

– acid-base catalysis: give and take protons
– covalent catalysis: change reaction paths
– metal ion catalysis: use redox cofactors, pK_a shifters
– electrostatic catalysis: preferential interactions with TS

General Acid-Base Catalysis

Reactant species

Without catalysis, unstable (charged) intermediate breaks down rapidly to form reactants.

When proton transfer to or from H_2O is faster than the rate of break- down of intermediates, the presence of other proton donors or acceptors does not increase the rate of the reaction.

OH^- H_2OH^+ → HOH HOH

B: HA → $\overset{+}{B}H$ A^-

When proton transfer to or from H_2O is slower than the rate of breakdown of intermediates, only a fraction of the intermediates formed are stabilized. The presence of alternative proton donors (HA) or acceptors (B:) increases the rate of the reaction.

Products

In active sites, many aa side chains can act as H^+ donors or acceptors

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Amino Acids in General Acid-Base Catalysis

Amino acid residues	General acid form (proton donor)	General base form (proton acceptor)
Glu, Asp	R—COOH	$R—COO^-$
Lys, Arg	$R—\overset{+}{N}H_3$	$R—\ddot{N}H_2$
Cys	R—SH	$R—S^-$
His	R—C=CH, HN, C, H, $\overset{+}{N}H$ (imidazolium ring)	R—C=CH, HN, C, H, N: (imidazole ring)
Ser	R—OH	$R—O^-$
Tyr	R—(phenyl)—OH	$R—(phenyl)—O^-$

Figure 6-9
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Covalent Catalysis

- A transient covalent bond between the enzyme and the substrate
- Changes the reaction Pathway
  - Uncatalyzed: $A - B \xrightarrow{H_2O} A + B$
  - Catalyzed: $A - B + X: \rightarrow A - X + B \xrightarrow{H_2O} A + X: + B$
- Requires a nucleophile on the enzyme
  - Can be a reactive serine, thiolate, amine, or carboxylate

Metal-ion Catalysis

- Weak bonding between metal and substrate (similar to binding energy of substrate to enzyme)

- Redox reactions (metal ions can change their oxidation state reversibly)

- ~ $^{1}/_{3}$ of all known enzymes need one or more metal ions for catalysis

6.3 Enzyme Kinetics

- **Kinetics** is the study of the *rate* at which compounds react
- Rate of enzymatic reaction is affected by
  - Enzyme
  - Substrate
  - Effectors
  - Temperature
  - Etc.

Rockefeller Archive Center

Leonor Michaelis, 1875–1949

Unnumbered 6 p199a
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Maud Menten, 1879–1960

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Derivation of Enzyme Kinetics Equations

- Simplest Model Mechanism: $E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k_2} E + P$
  - One reactant, one product, no inhibitors
- Total enzyme concentration is constant
  - Mass balance equation for enzyme: $E_t = [E] + [ES]$
  - It is also implicitly assumed that: $S_t = [S] + [ES] \approx [S]$
- **Steady state assumption** (initially [ES] is constant!)

$$\frac{d[ES]}{dt} = \text{rate of formation of ES} - \text{rate of breakdown of ES} = 0$$

- What is the observed rate?
  - Rate of product formation

$$V_0 = \frac{dP}{dt} = k_2[ES]$$

Effect of [S] on reaction rate

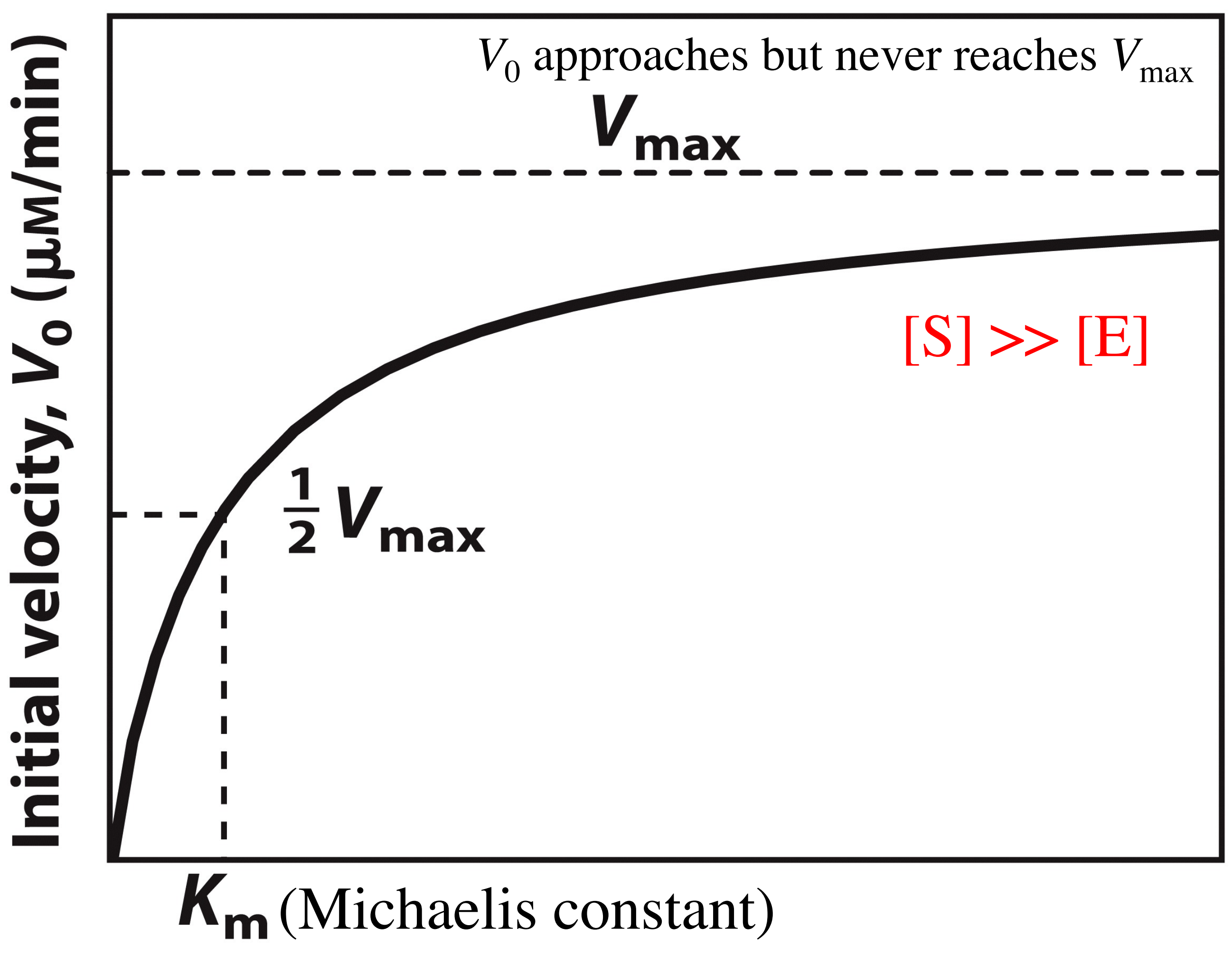
- The relationship between [S] and V_0 is similar for *most* enzymes (rectangular hyperbola)

- Mathematically expressed by **Michaelis-Menten** equation:

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

- Deviations due to:
  - – limitation of measurements
  - – substrate inhibition
  - – substrate prep contains inhibitors
  - – enzyme prep contains inhibitors

Effect of Substrate Concentration



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Some useful equations

- The term: $(k_{-1} + k_2)/k_1$ is **Michaelis constant** (K_m) for a two-step reaction

- The **maximum velocity** of an enzyme (V_{max}) for a two-step reaction occurs when the enzyme is fully saturated with substrate (i.e. when **E_t = [ES]**) ➔ $V_{max} = k_2[E_t]$

- K_m and V_{max} depend on the reaction mechanisms (2-step, 3-step, etc.) → more appropriately we use the general rate constant (***k_{cat}***, **turnover number**) which describes the **rate limiting step** of the reaction (for the previous example $k_{cat} = k_2$)

- k_{cat} is equivalent to the number of substrate molecules converted to product in a given unit of time on a single enzyme when enzyme is saturated with substrate

Carry out the algebra

- The final form in case of a single substrate is

$$V_0 = \frac{k_{cat}[E_t][S]}{K_m + [S]}$$

- The values of K_m and k_{cat} together are useful to evaluate the *kinetic efficiency* of enzymes

  * Two enzymes catalyzing different reactions may have the same k_{cat} but the uncatalyzed reaction rates can be different ➔ the rate enhancement rate will be different

  * **Specificity constant** is k_{cat}/K_m (used to compare the catalytic efficiencies of different enzymes or the turnover of different substrates by the same enzyme)

  * When [S] << K_m... What happens to the rate equation? *Second order reaction, how?*

Enzyme efficiency is limited by diffusion: k_{cat}/K_M

- 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	4×10^7	1.1×10^0	4×10^7
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: Fersht, A. (1999) ***Structure and Mechanism in Protein Science***, p. 166, W. H. Freeman and Company, New York.

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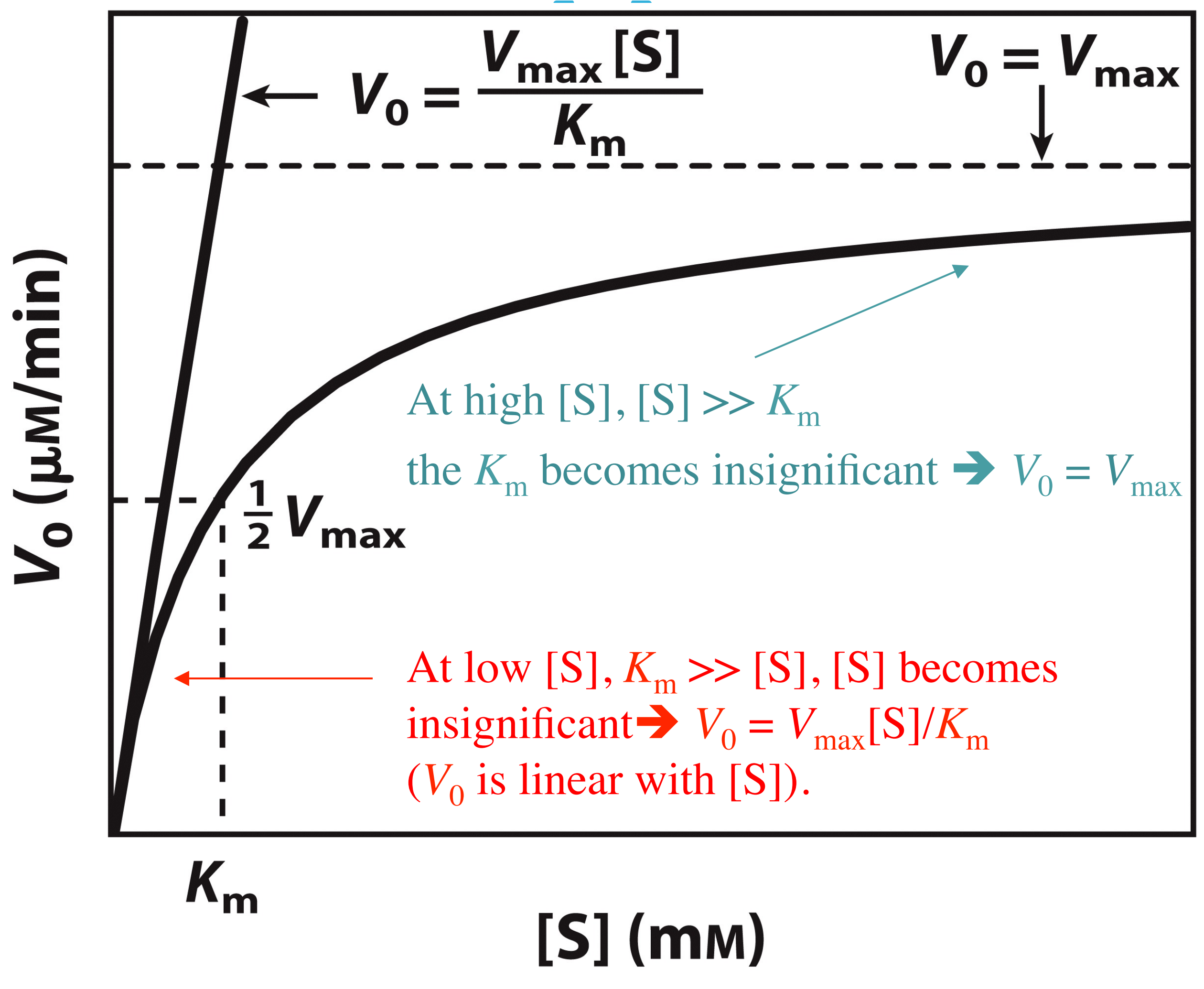
K_m for an enzyme tends to be similar to its substrate's cellular concentration

Saturation Kinetics: At high [S] velocity does not depend on [S]



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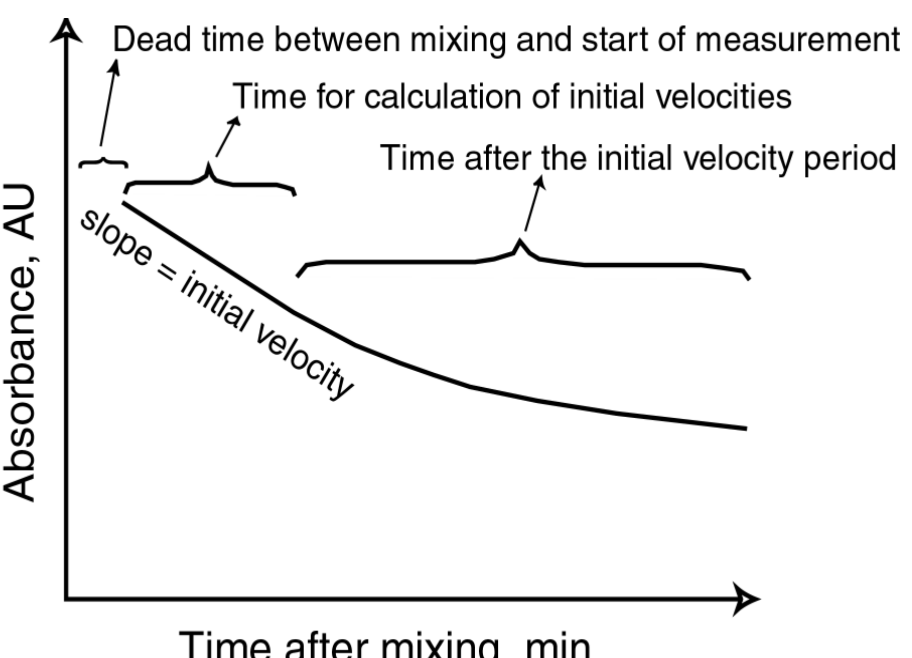
How to Do Kinetic Measurements

Experiment:

1) Mix enzyme + substrate
2) Record rate of substrate disappearance/product formation as a function of time (the velocity of reaction)
3) Plot initial velocity versus substrate concentration.
4) Change substrate concentration and repeat



A tangent to each curve taken at t = 0 defines the initial velocity, V_0, of each reaction.

[S] = 1.0 μM

[S] = K_m = 0.5 μM

[S] = 0.2 μM

Product concentration, [P]

Time

The rate of an enzyme-catalyzed reaction declines as substrate is converted to product.

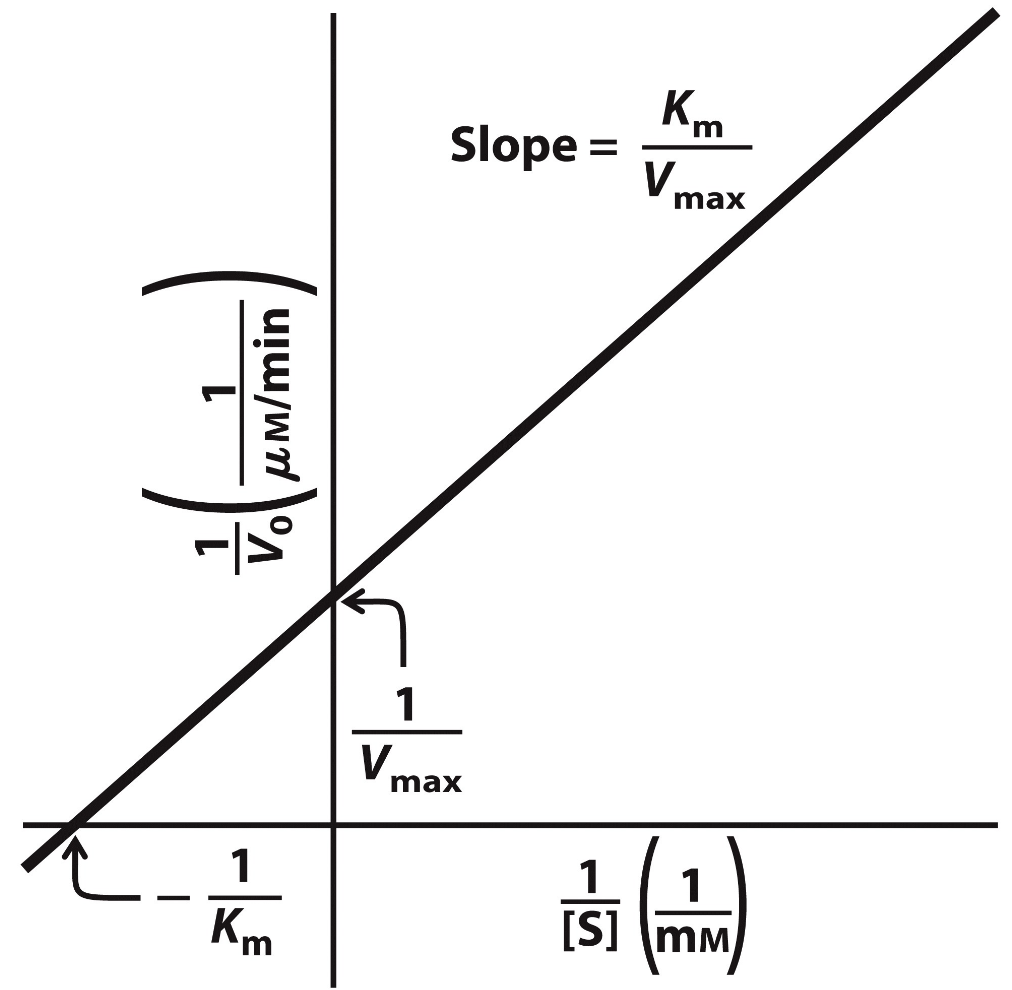
Determination of Kinetic Parameters

Nonlinear Michaelis-Menten plot should be used to calculate parameters K_m and V_{max}.

Linearized **double-reciprocal** (Lineweaver-Burk) plot is good for analysis of two-substrate data or inhibition.

Lineweaver-Burk Plot: Linearized, Double-Reciprocal

$$\frac{1}{V_0} = \frac{K_m + [S]}{V_{max}[S]}$$



Box 6-1 figure 1
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Two-Substrate Reactions

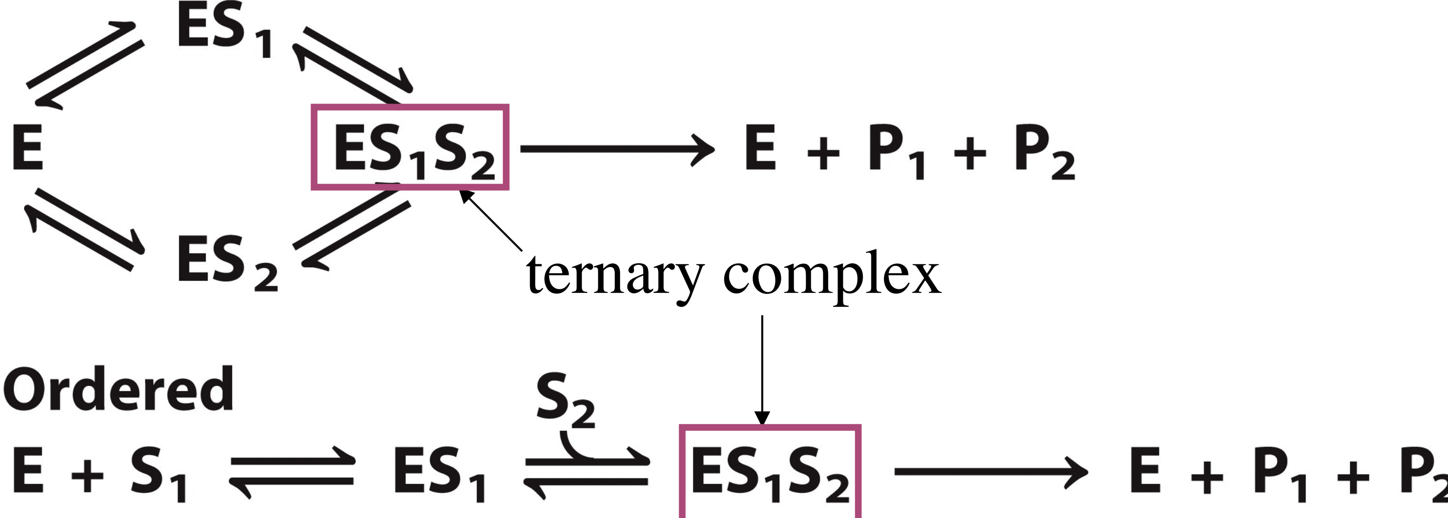
- The rate of a bisubstrate reaction can also be analyzed by Michaelis-Menten kinetics. Enzymes catalyzing polysubstrate reactions have K_m for each of their substrates

- Kinetic mechanism: the order of binding of substrates and release of products

- When two or more reactants are involved, enzyme kinetics allows to distinguish between different kinetic mechanisms
  - ***Sequential mechanism*** (involving a ternary complex)
  - ***Ping-Pong*** (double displacement) mechanism

(a) Enzyme reaction involving a ternary complex

Random order



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

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

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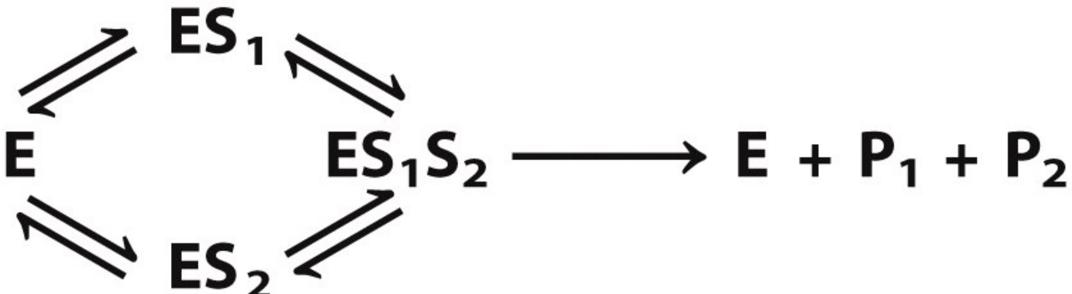
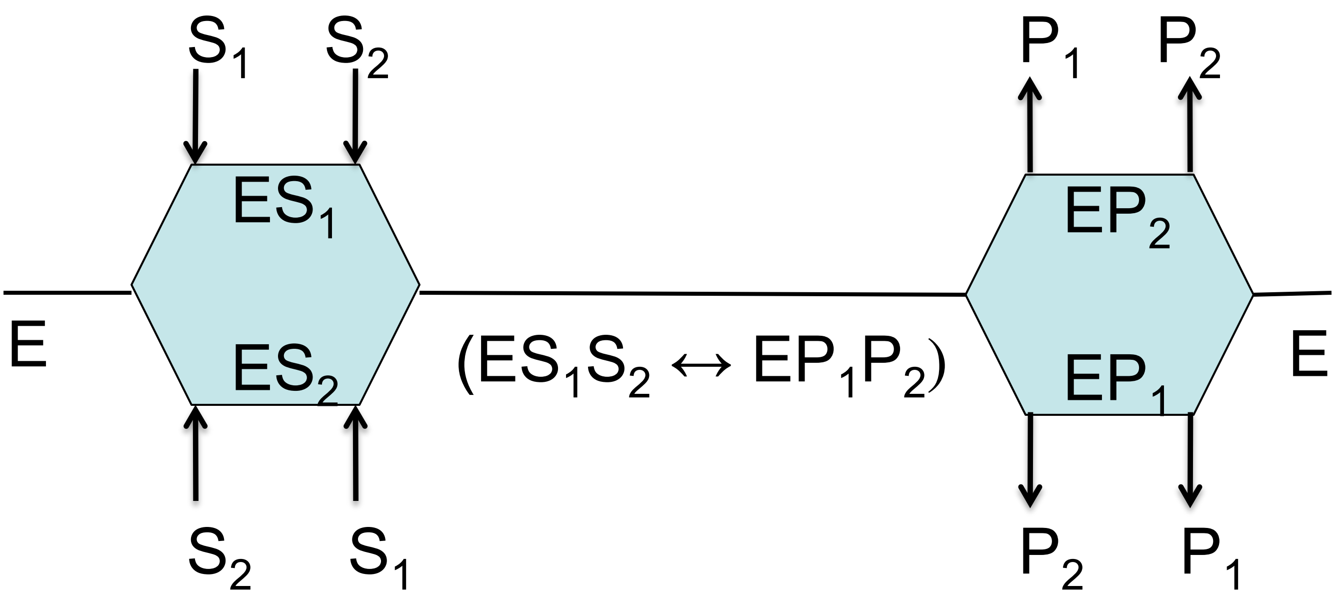


Cleland Diagram



$$E + S_1 \rightleftharpoons ES_1 \overset{S_2}{\rightleftharpoons} ES_1S_2 \longrightarrow E + P_1 + P_2$$

Cleland Diagram

S_1 ↓ S_2 ↓ P_1 ↑ P_2 ↑

E ES_1 $(ES_1S_2 \leftrightarrow EP_1P_2)$ EP_2 E

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

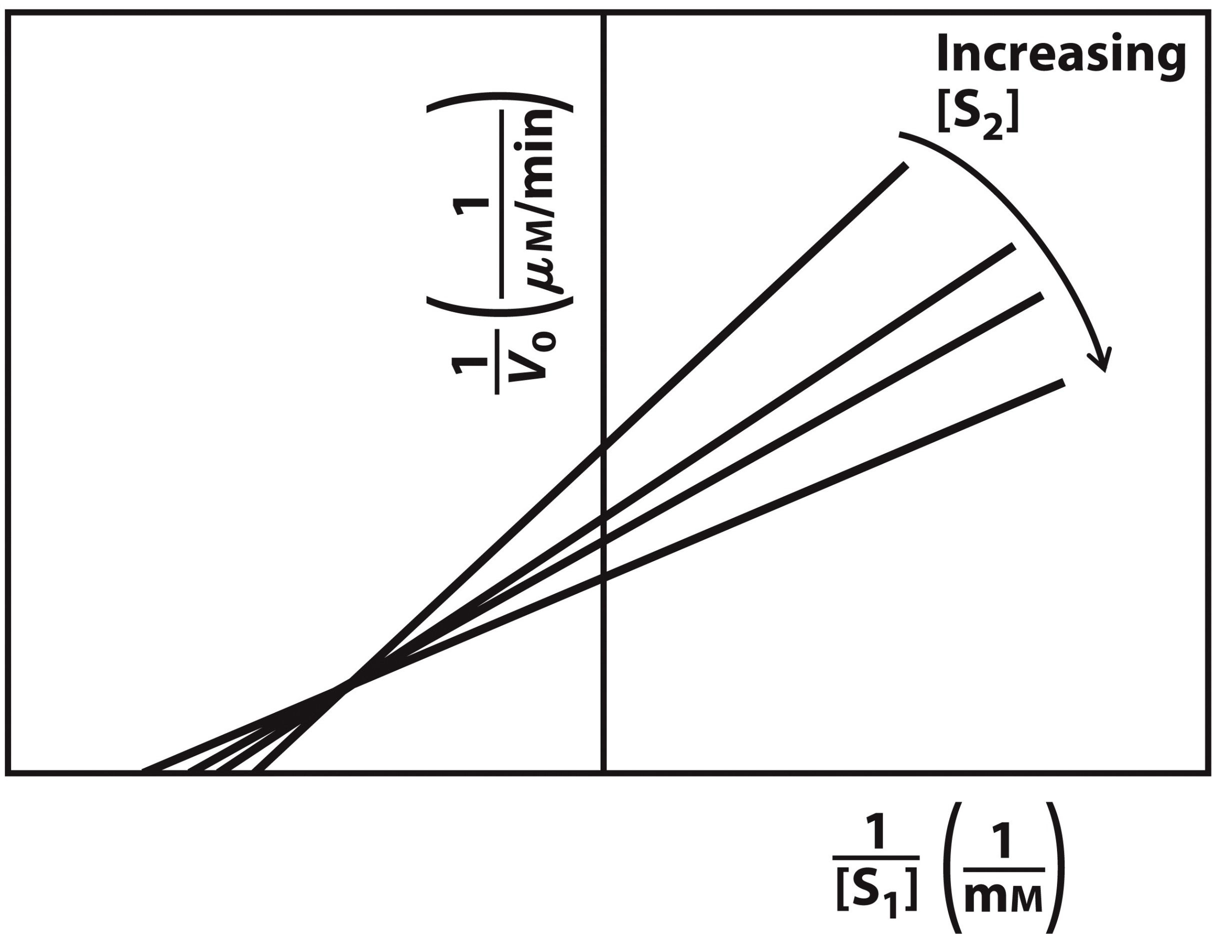
Cleland Diagram

S_1 ↓ P_1 ↑ S_2 ↓ P_2 ↑

E $(ES_1 \leftrightarrow E'P_1)$ E' $(E'S_2 \leftrightarrow EP_2)$ E

Sequential Kinetic Mechanism

- We cannot easily distinguish random from ordered
- Lineweaver-Burk: lines intersect



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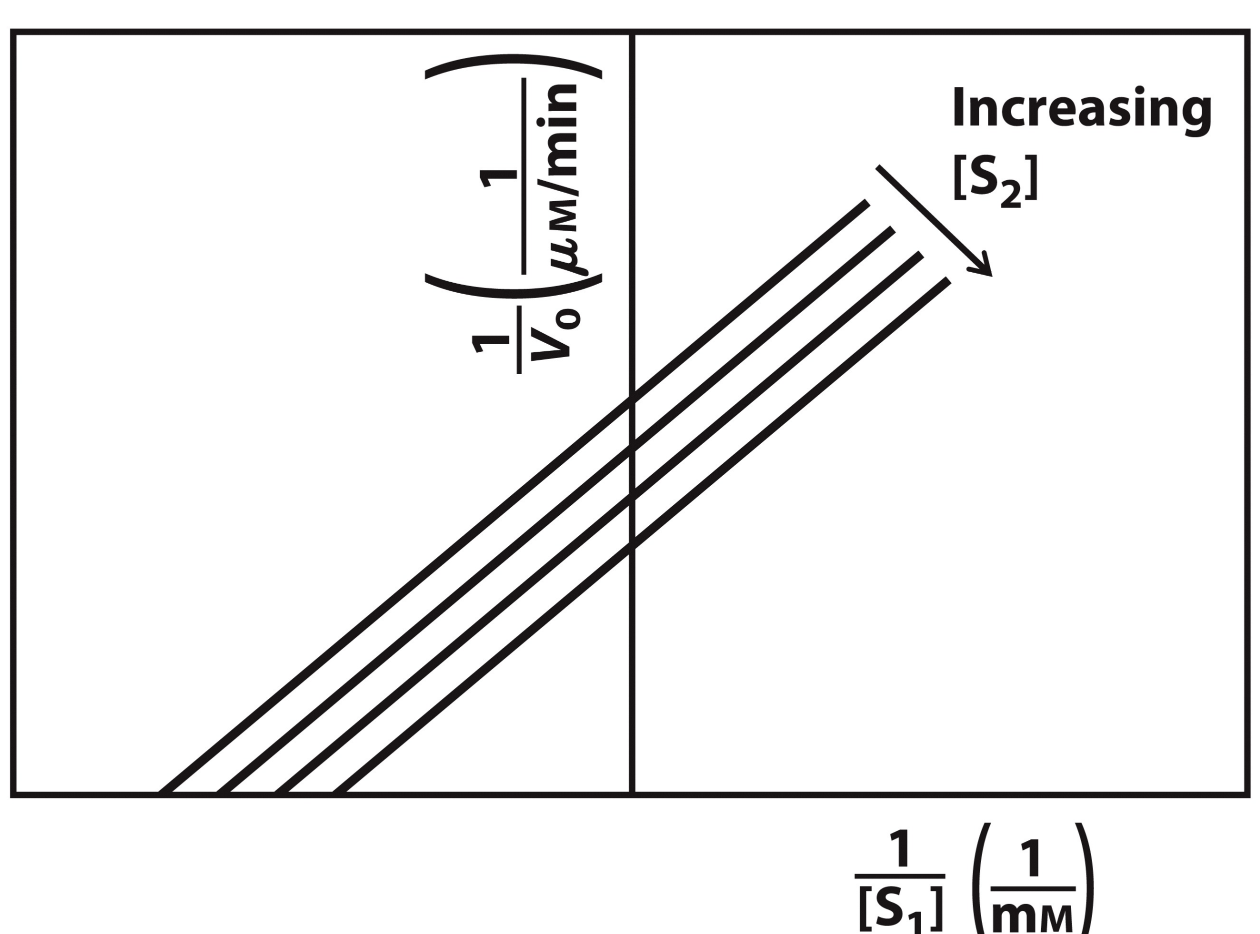
Ping-Pong Kinetic Mechanism

Lineweaver-Burk: lines are parallel



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Enzyme Inhibition

Inhibitors are compounds that decrease enzyme's activity

- Irreversible inhibitors (inactivators) react with the enzyme
  - One inhibitor molecule can permanently shut off one enzyme molecule
  - They are often powerful toxins but also may be used as drugs

- Reversible inhibitors bind to and can dissociate from the enzyme
  - They are often structural analogs of substrates or products
  - They are often used as drugs to slow down a specific enzyme

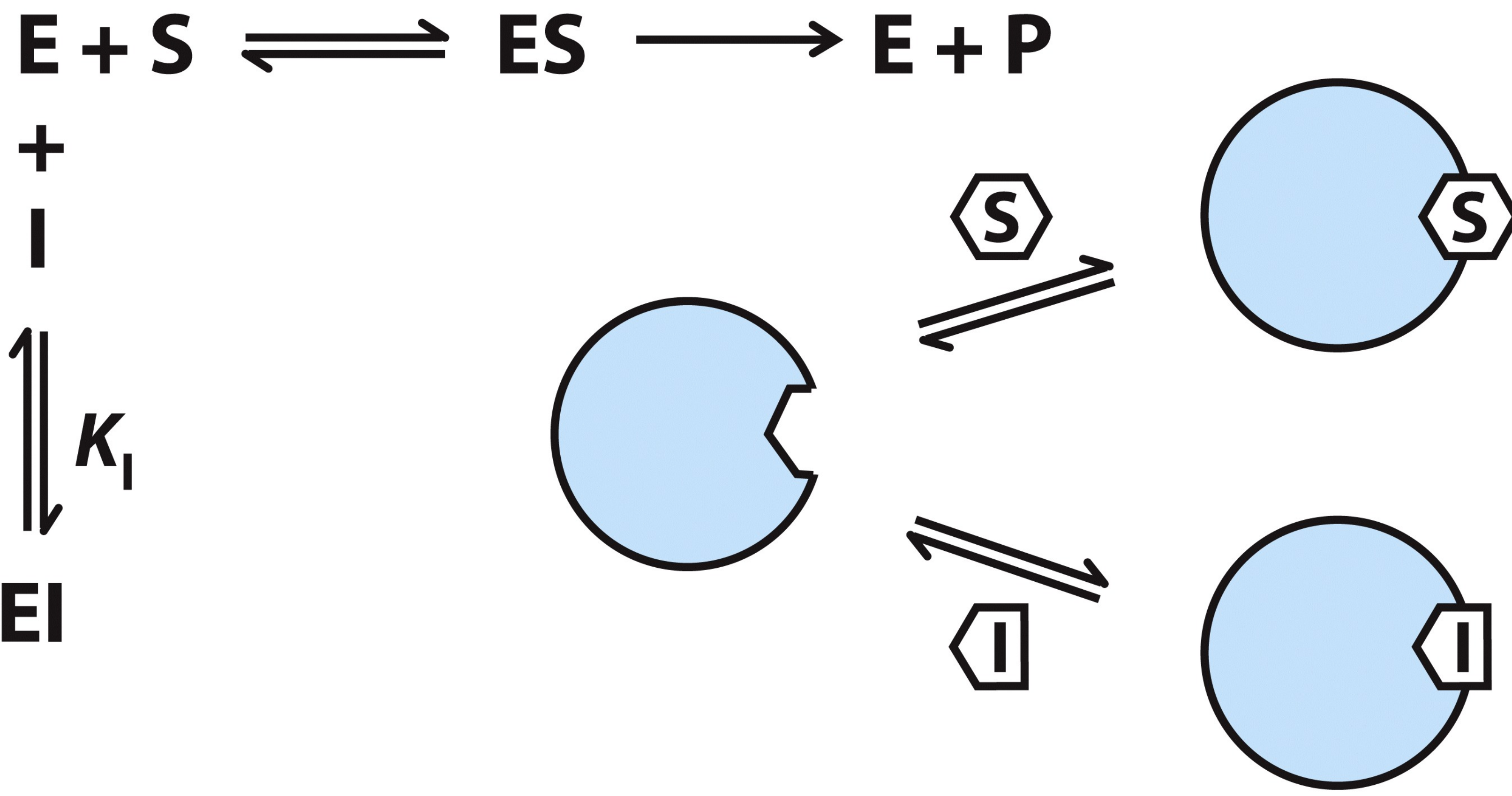
- Reversible inhibitor can bind:
  - to the free enzyme and prevent the binding of the substrate
  - to the enzyme-substrate complex and prevent the reaction

Competitive Inhibition

- **Competes** with substrate for binding
  - Binds active site
  - Does not affect catalysis
  - many competitive inhibitors are similar in structure to the substrate, and combine with the enzyme to form an EI complex

- No change in V_{max}; apparent increase in K_m
- Lineweaver-Burk: lines intersect at the y-axis at $-1/V_{max}$

Competitive Inhibition



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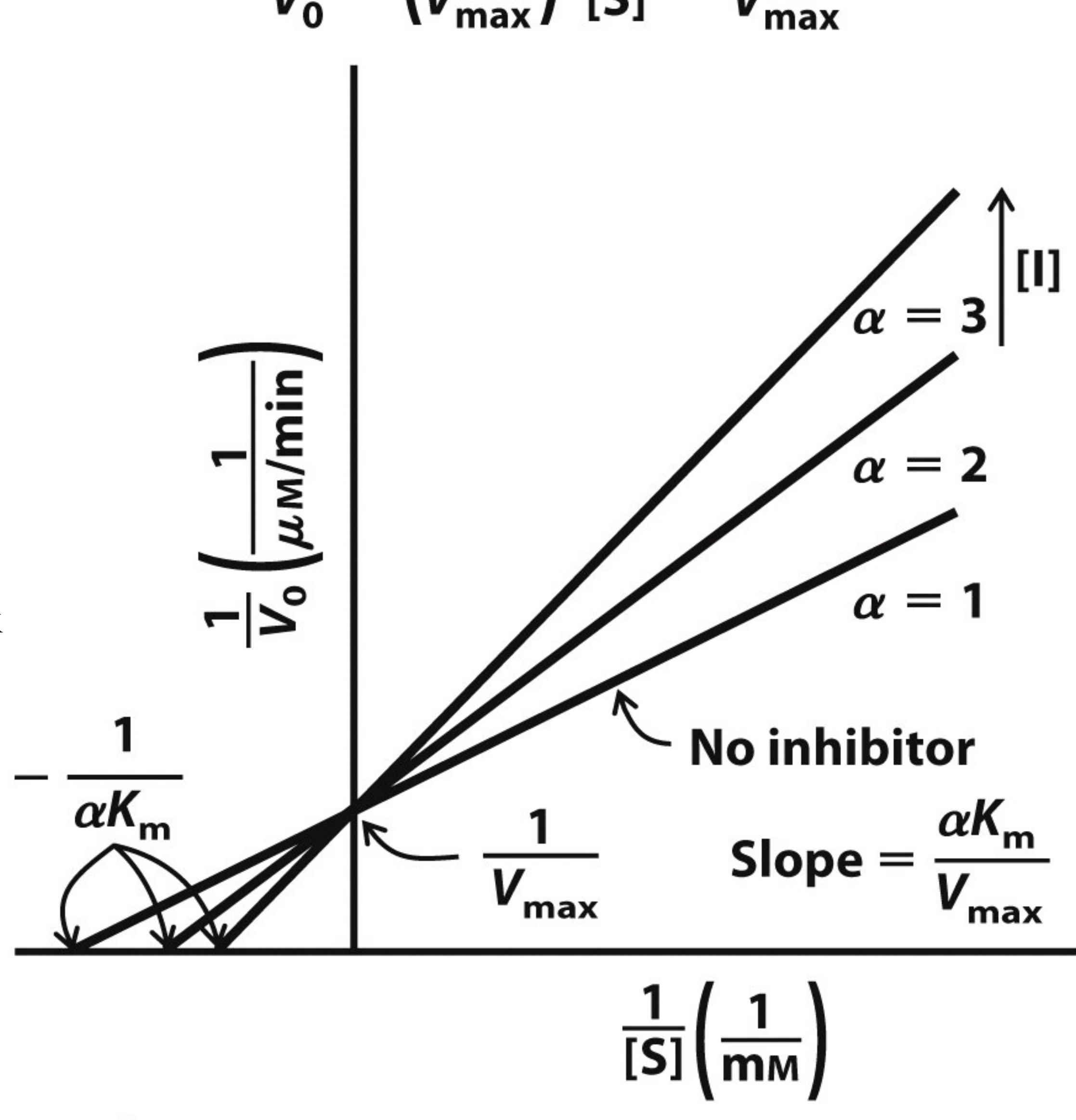
$\alpha = 1 + [I]/K_I$

$K_I = [E][I]/[EI]$

αK_m (the apparent K_m) is the K_m measured in the presence of an inhibitor

When [S] >> [I] → reaction shows normal V_{max} because the substrate competes out the inhibitor

No effect of the competitive inhibitor on V_{max}



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Uncompetitive Inhibition

- Only binds to ES complex
  - Does not affect substrate binding
  - Inhibits catalytic function

- Decrease in V_{max}; apparent decrease in K_m
- No change in K_m/V_{max}
- Lineweaver-Burk: lines are parallel

Uncompetitive Inhibition

Uncompetitive inhibition

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

$$+ \; I \; \underset{}{\overset{K_I'}{\rightleftharpoons}} \; ESI$$

S

S

I

I

S

Figure 6-15b
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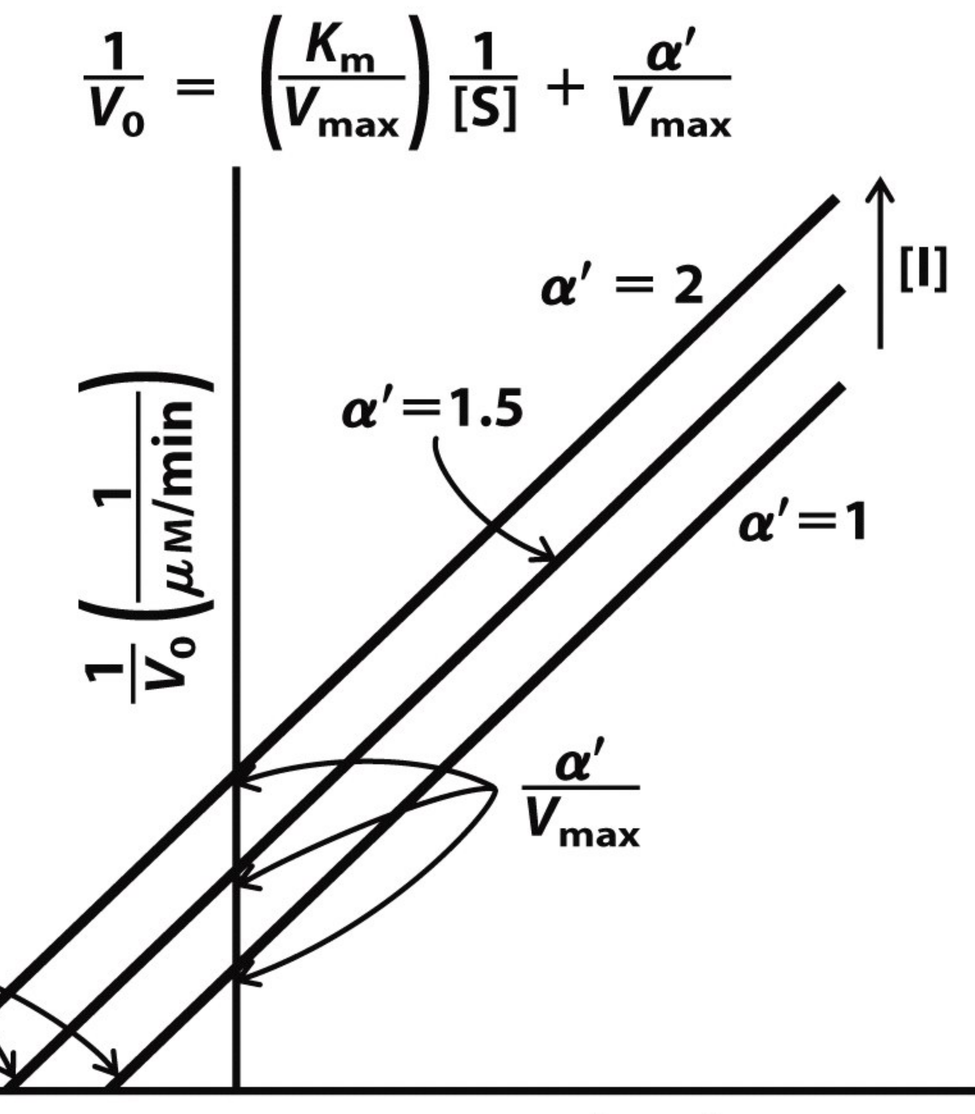
Uncompetitive Inhibition

$\alpha' = 1 + [I]/K_I$
$K'_I = [ES][I]/[ESI]$

At high [S], $V_0 \rightarrow V_{max}/\alpha'$

Therefore, an uncompetitive inhibitor lowers the measured V_{max}

K_m also decreases because [S] required to reach ½ V_{max} is reduced by the factor α'



Mixed Inhibition

- Binds enzyme with or without substrate
  - Binds to regulatory site
  - Inhibits both substrate binding and catalysis
- Decrease in V_{max}; apparent change in K_m
- Lineweaver-Burk: lines intersect left from the y-axis

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

Mixed Inhibition

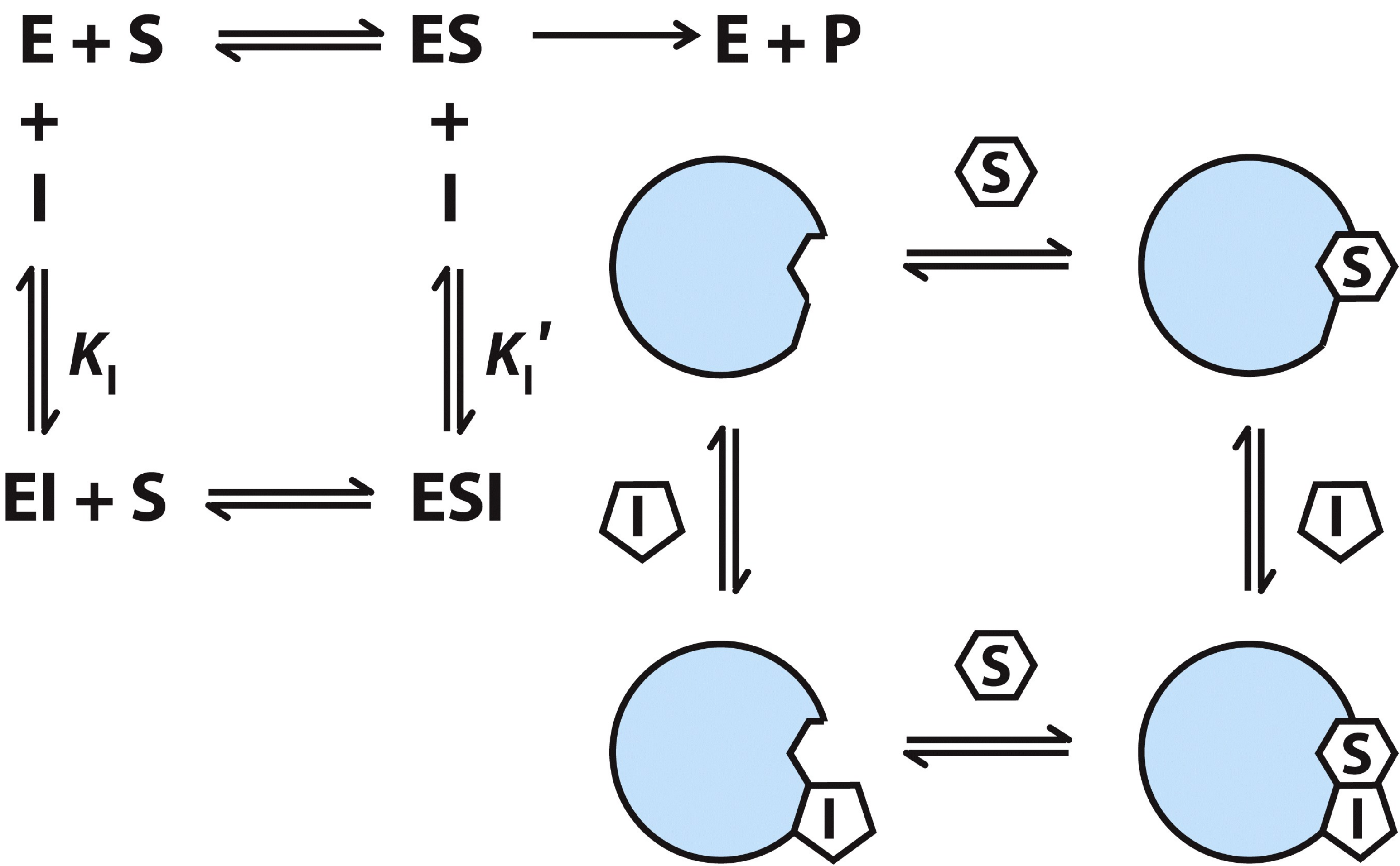


Figure 6-15c
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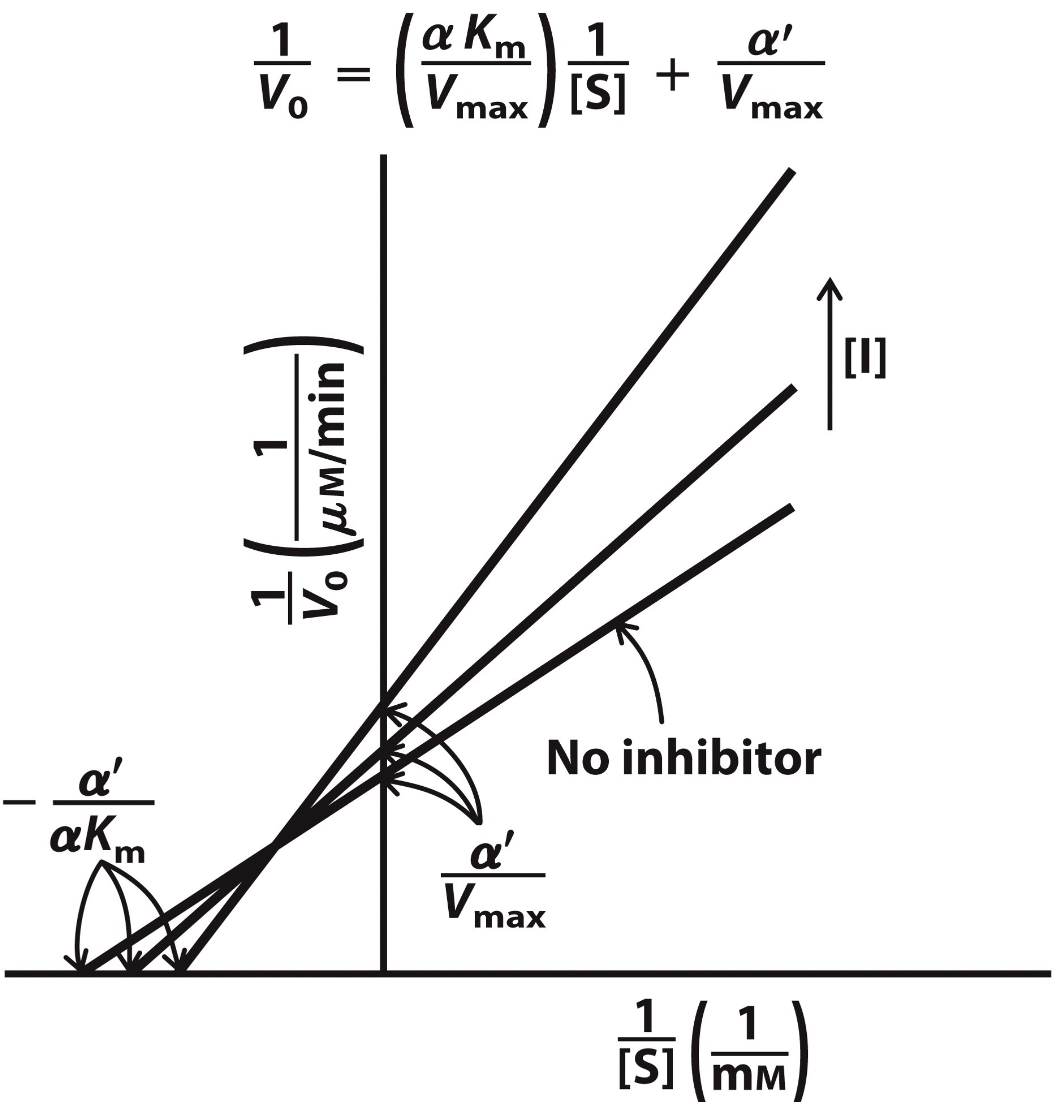
Mixed Inhibition



Box 6-2 figure 3
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A special case

- **Noncompetitve inhibitors**: a special (rare) case of mixed inhibitors when α and α' are equal
- x-intercept is $-1/K_m$ (no effect on K_m with increasing [I])
- V_{max} is lowered with increasing [I]

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

$\frac{1}{V_0}\left(\frac{1}{\mu M/\text{min}}\right)$

[I]

No inhibitor

$\frac{\alpha}{V_{max}}$

$\frac{1}{[S]}\left(\frac{1}{mM}\right)$

$-\frac{1}{K_m}$

$$V_0 = \frac{V_{max}[S]}{\alpha K_m + \alpha'[S]}$$

When $\alpha = 1 \rightarrow$ uncompetitive

When $\alpha' = 1 \rightarrow$ competitive

When $\alpha' = \alpha \neq 1 \rightarrow$ noncompetitive

TABLE 6–9 Effects of Reversible Inhibitors on Apparent V_{max} and Apparent K_m

Inhibitor type	Apparent V_{max}	Apparent K_m
None	V_{max}	K_m
Competitive	V_{max}	αK_m
Uncompetitive	V_{max}/α'	K_m/α'
Mixed	V_{max}/α'	$\alpha K_m/\alpha'$

Table 6-9
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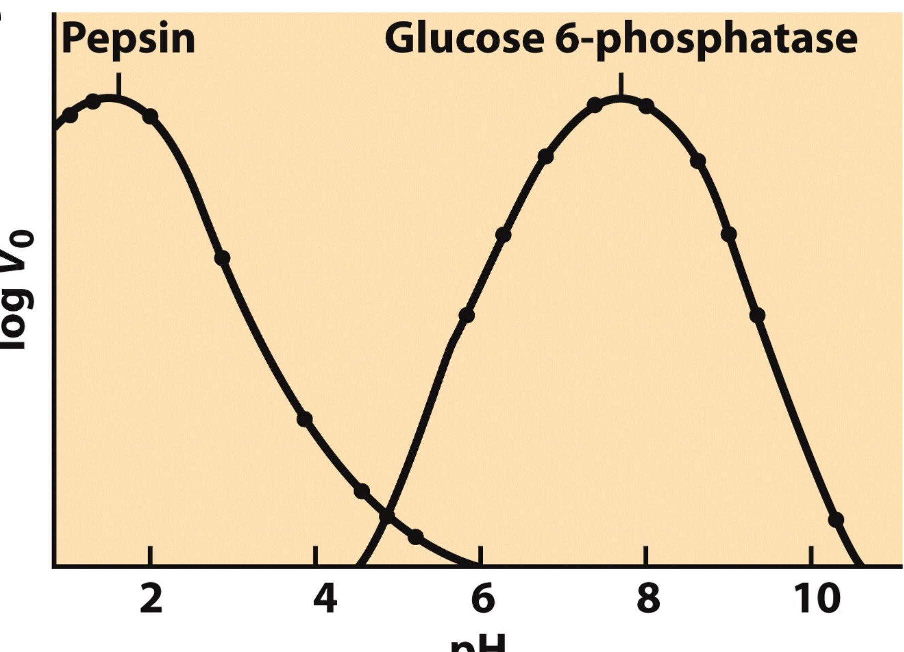
Enzyme activity depends on pH

- Enzymes have optimum pH ranges at which their activity is maximal:
  - Activity decreases at higher or lower pH values
  - Due to the physical and chemical properties of amino acids and their side chains



6.5 Regulatory Enzymes

- Each cellular metabolism pathway has one or more **regulatory enzymes** (enzymes that have a greater effect on the rate of the overall sequence)

- They show increased or decreased activities in response to certain signals (function as switches)

- Generally, the first enzyme in a pathway is a regulatory enzyme (not always true!)

Regulatory Enzymes

- Classes of regulatory enzymes:

❖ **allosteric enzymes** (affected by reversible noncovalent binding of allosteric modulators)
❖ **nonallosteric/covalent enzymes** (affected by reversible covalent modification)
❖ **regulatory protein binding enzymes** (stimulated or inhibited by the binding of separate regulatory proteins)
❖ **proteolytically activated enzymes** (activated by the removal of some segments of their polypeptide sequence by proteolytic cleavage)

Allosteric Enzymes

- **Allosteric enzymes** function through reversible, noncovalent binding of regulatory compounds (allosteric modulators, aka allosteric effectors)

- Allosteric enzymes are generally larger and more complex than nonallosteric enzymes with more subunits

- Modulators can be *stimulatory* or *inhibitory*

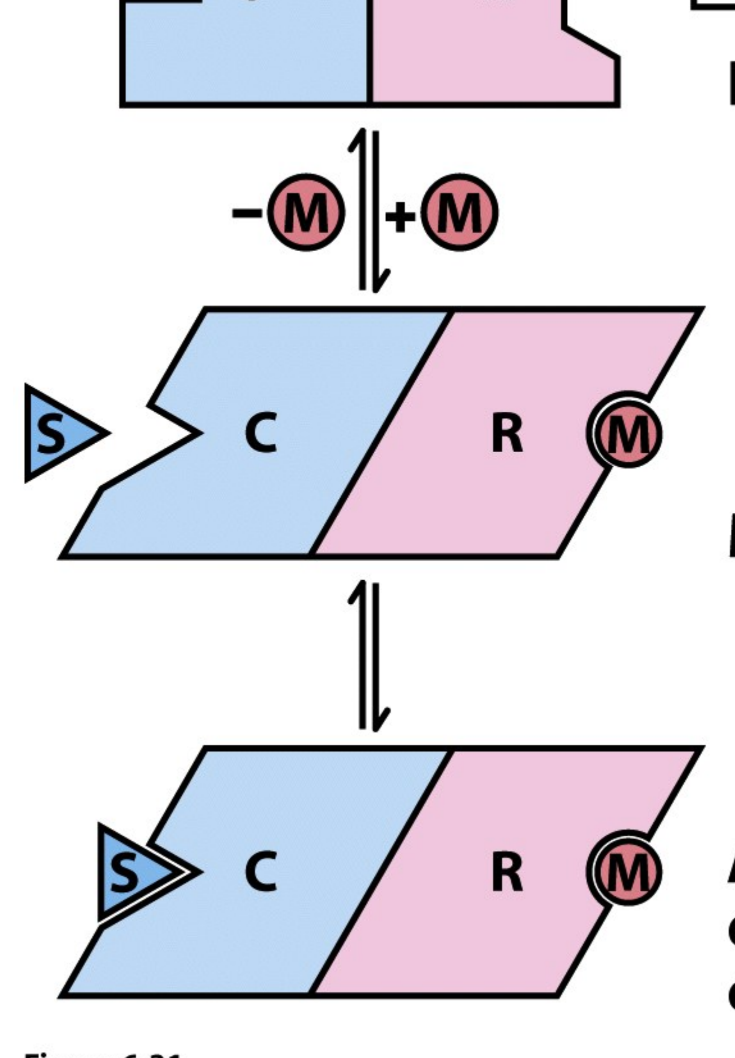
- Sometimes, the regulatory site and the catalytic site are in different subunits

- Recall: homotropic and heterotropic enzymes

- Conformational change from an inactive T state to an active R state and vice versa

(C) catalytic (R) regulatory subunits

- *Not to be confused with uncompetitive or mixed inhibitors…*
- *Those inhibitors are kinetically distinct and they do not necessarily induce conformational changes*



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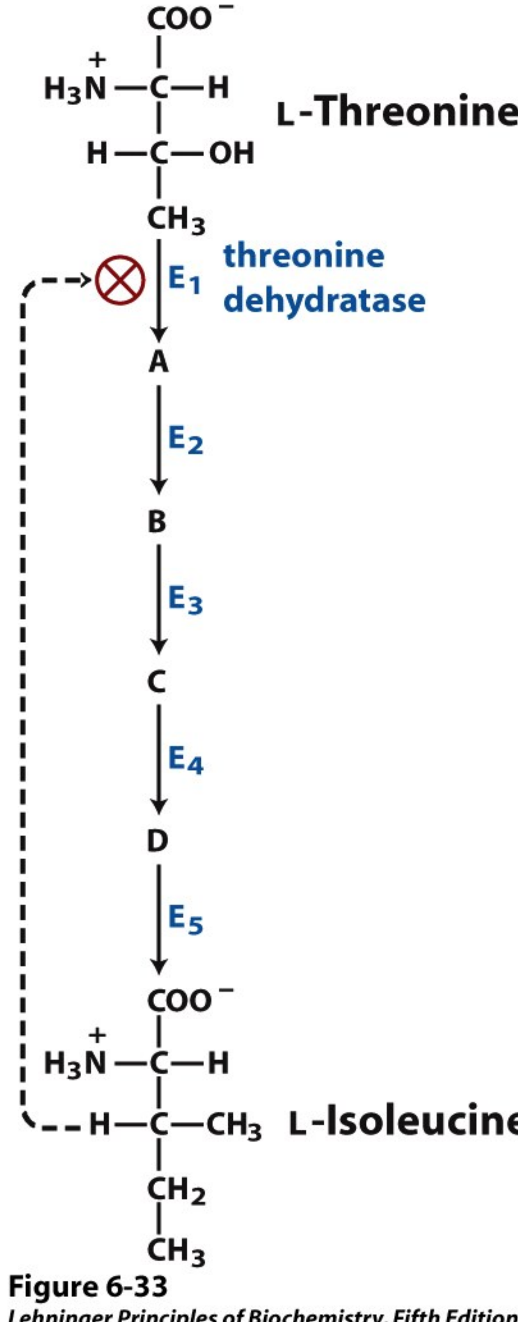
Regulated Steps Are Catalyzed by Allosteric Enzymes

- **Feedback inhibition** – regulatory enzymes are specifically inhibited by the end product of the pathway whenever the concentration of the end product exceeds the cell's requirements
- Heterotropic allosteric inhibition

Threonine dehydratase (E_1) is specifically inhibited allosterically by L-isoleucine, the end product of the sequence, but not by any of the four intermediates (A to D).

Ile does not binds to the active site but to a regulatory site on the enzyme.

The binding is reversible: if [Ile] ↓ → rate of Thr dehydration ↑



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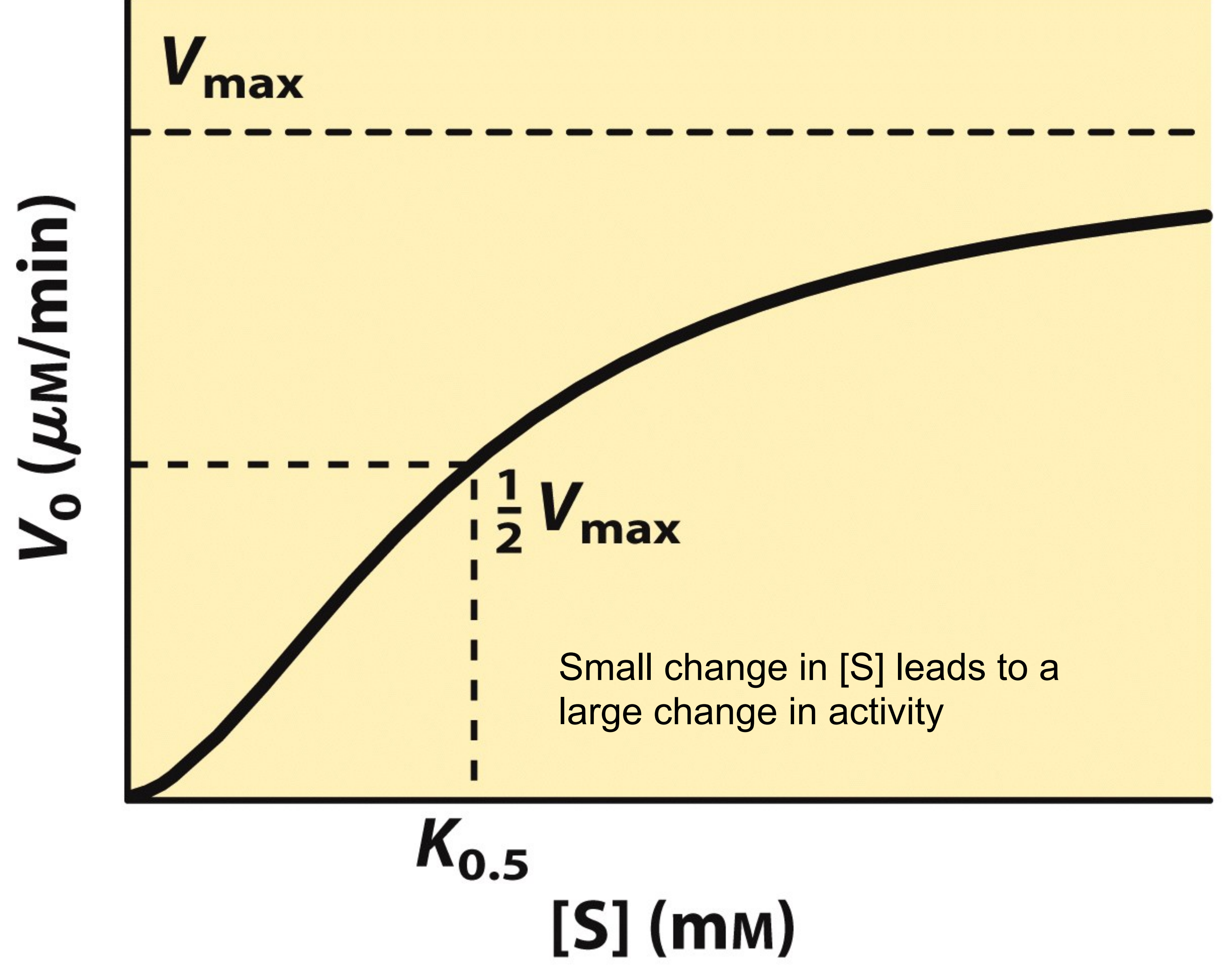
Kinetic Properties of Allosteric Enzymes Diverge from Michaelis-Menten Behavior

- For some allosteric enzymes, plots of V_0 vs [S] give sigmoid (not hyperbolic) saturation curves
- In a sigmoid curve, the [S] that gives ½ V_{max} is $K_{0.5}$
- Homotropic allosteric enzymes are multisubunit proteins. The same binding site on each subunit serves as both an active and regulatory site
- Heterotropic allosteric enzymes, an activator causes the curve to be more hyperbolic (also ↓in $K_{0.5}$; no change in V_{max}) and an inhibitor produces a more sigmoid curve (↑ in $K_{0.5}$; no change in V_{max})

The sigmoid curve of a homotropic enzyme, in which the substrate also serves as a positive (stimulatory) modulator, or activator



Noncovalent Modification: Allosteric Regulators

The kinetics of allosteric regulators differ from Michaelis-Menten kinetics.

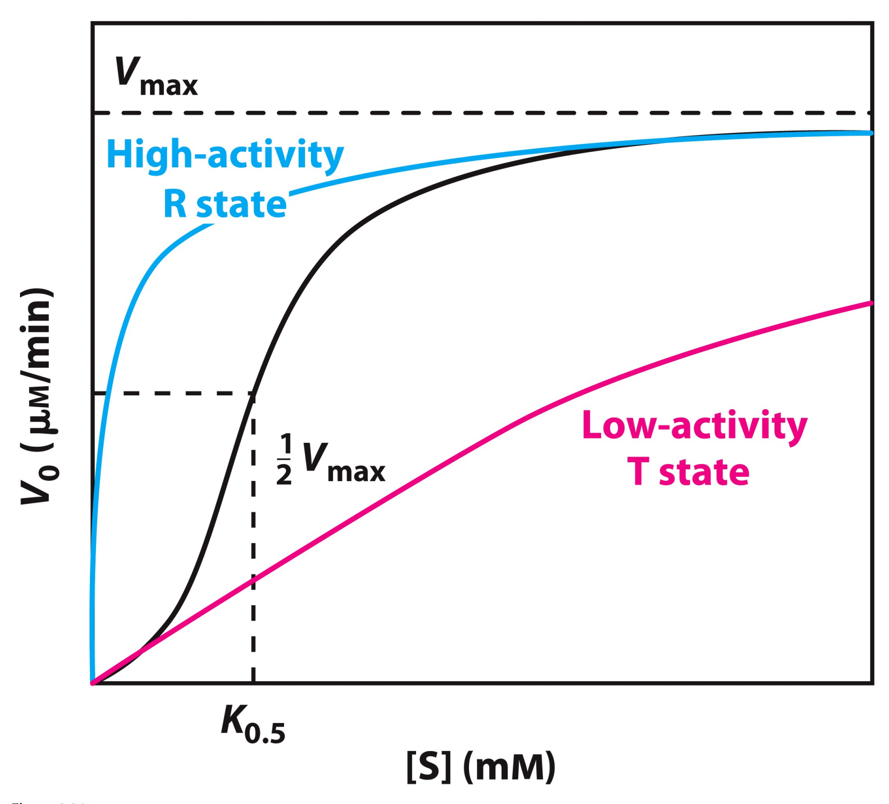


Figure 6-34a
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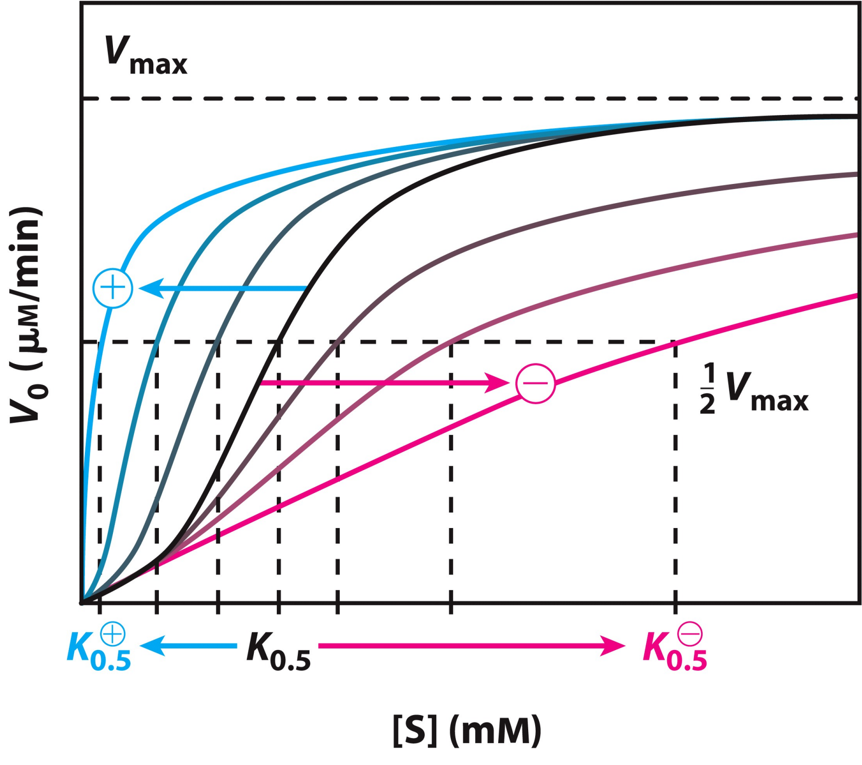

Figure 6-34b
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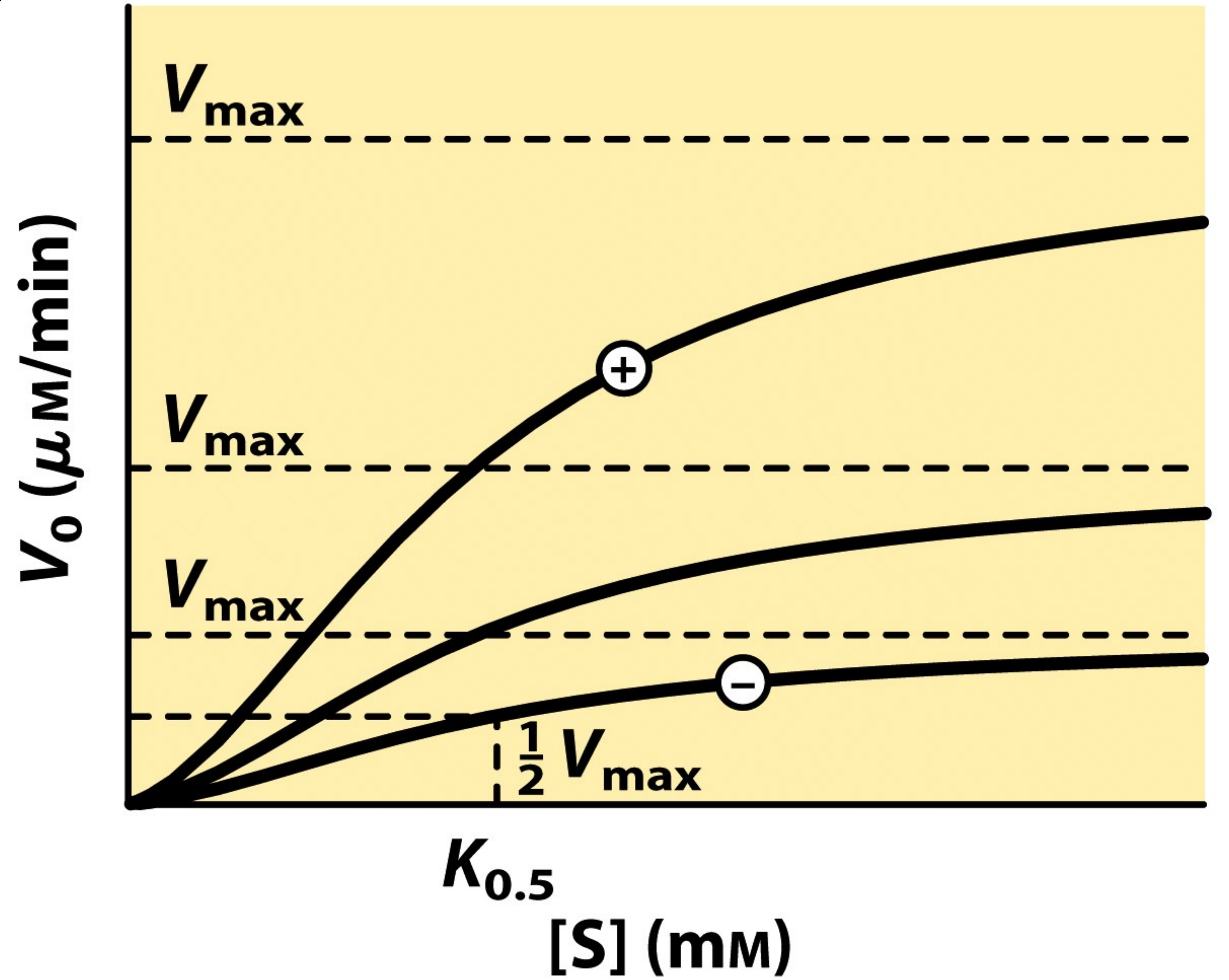
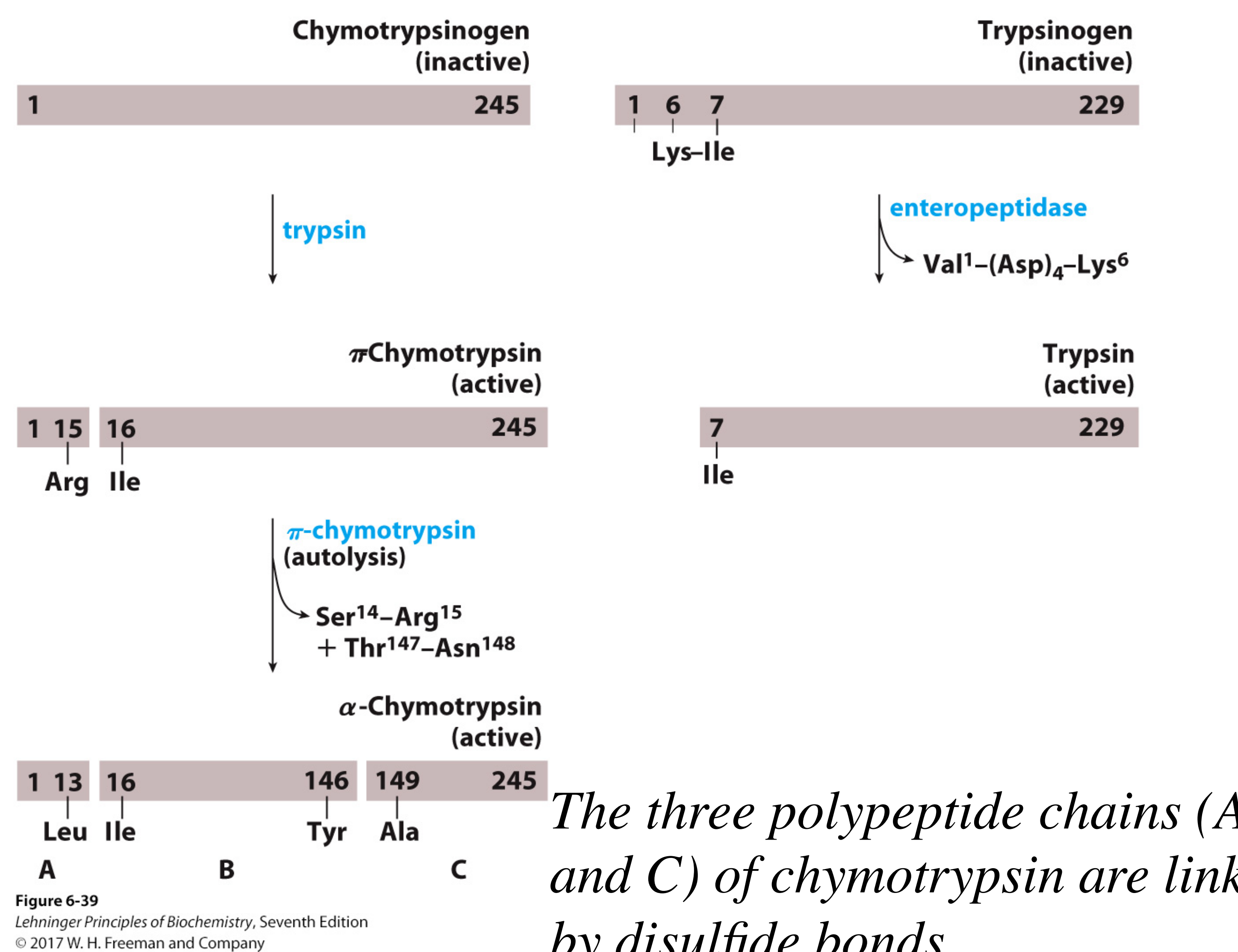
A less common type of modulation, in which V_{max} is changed and $K_{0.5}$ is nearly constant.



Zymogens Are Activated by Irreversible Proteolytic Cleavage



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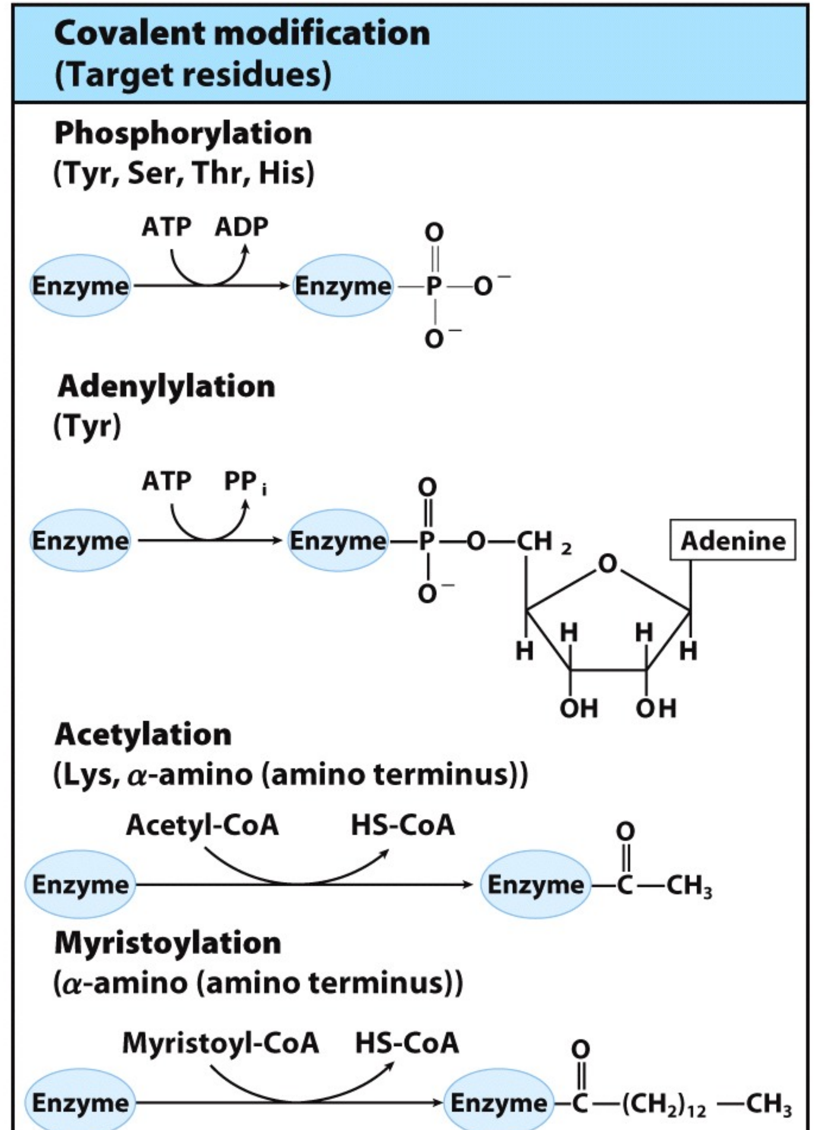
The three polypeptide chains (A, B, and C) of chymotrypsin are linked by disulfide bonds

Some Enzymes Are Regulated by Reversible Covalent Modification

- Over 500 different kinds of covalent modifications are found in proteins
- Covalent bonds form (reversibly) between regulatory molecules and aa residues in proteins
- When an aa residue is modified, a new aa with changed properties has effectively been introduced in the enzyme (for instance, Ser-OH can be phosphorylated to Ser-O-PO_3^- changing the properties → conformation → function, etc.)

Some Reversible Covalent Modifications



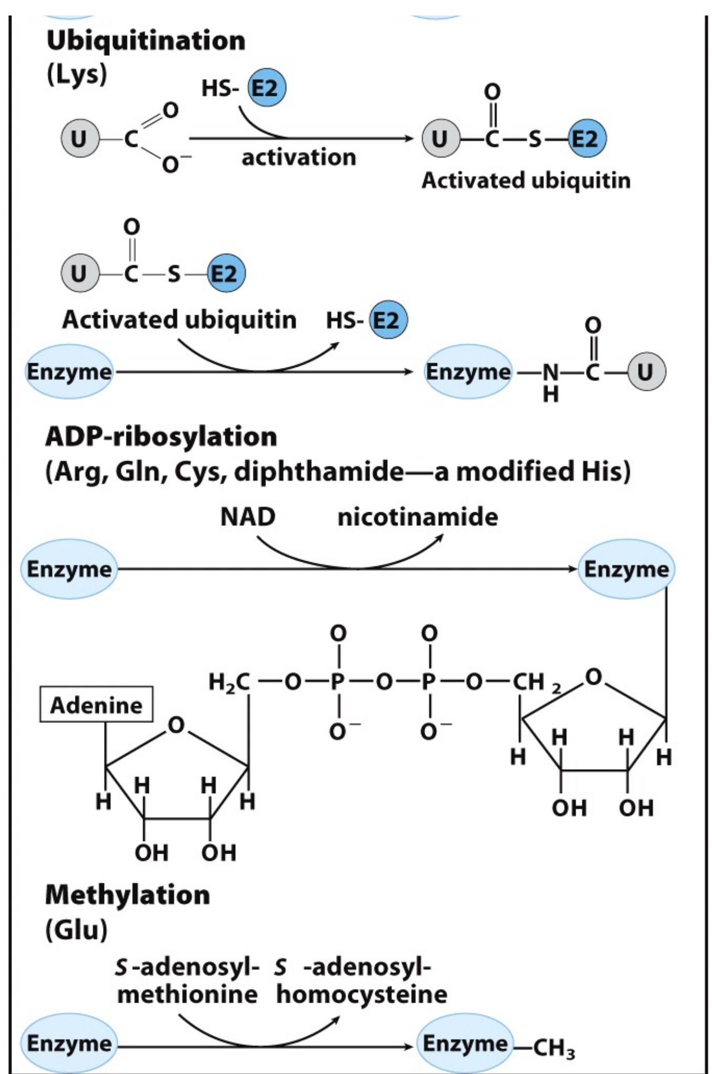


Figure 6-35

Example on Protein Phosphorylation

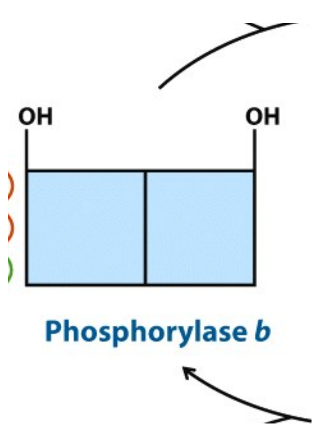
- **Protein kinases** – add phosphate groups on specific aa residues of other proteins
- **Protein phosphatases** – remove phosphate groups from phosphorylated proteins

glycogen phosphorylase

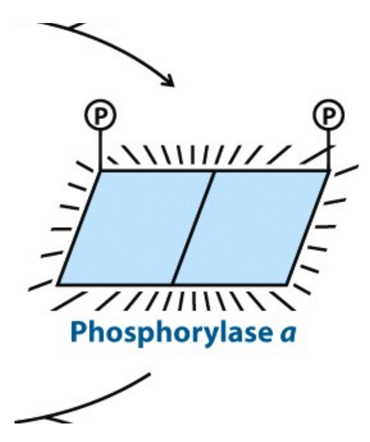
- $\text{Glucose}_n + P_i \rightarrow \text{glucose}_{n-1} + \text{glucose 1-phosphate}$

(Glycogen)

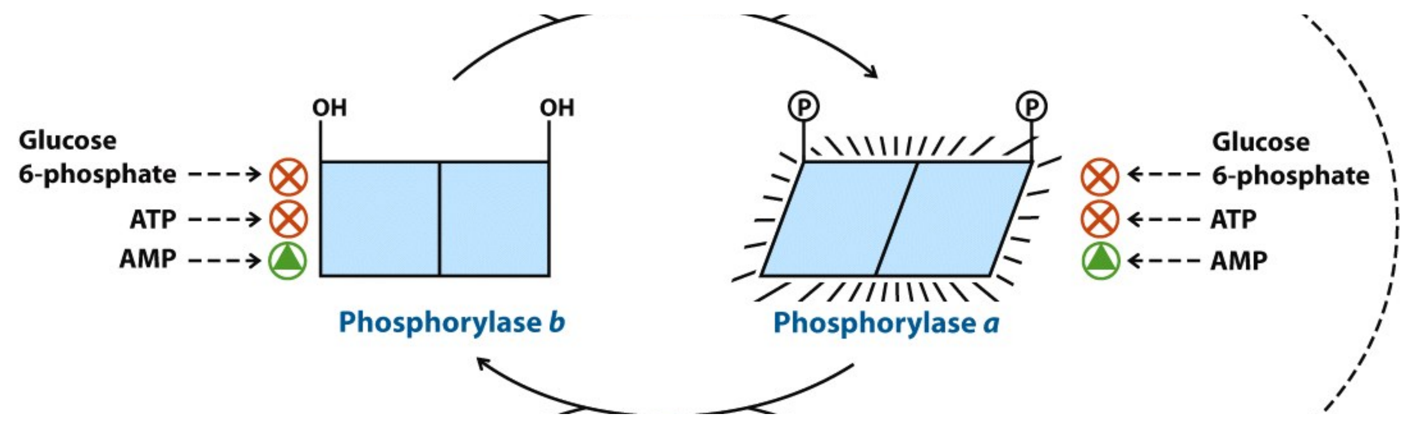
The less active form of the enzyme, *phosphorylase b*, specific Ser residues, one on each subunit, are <u>not phosphorylated</u>.



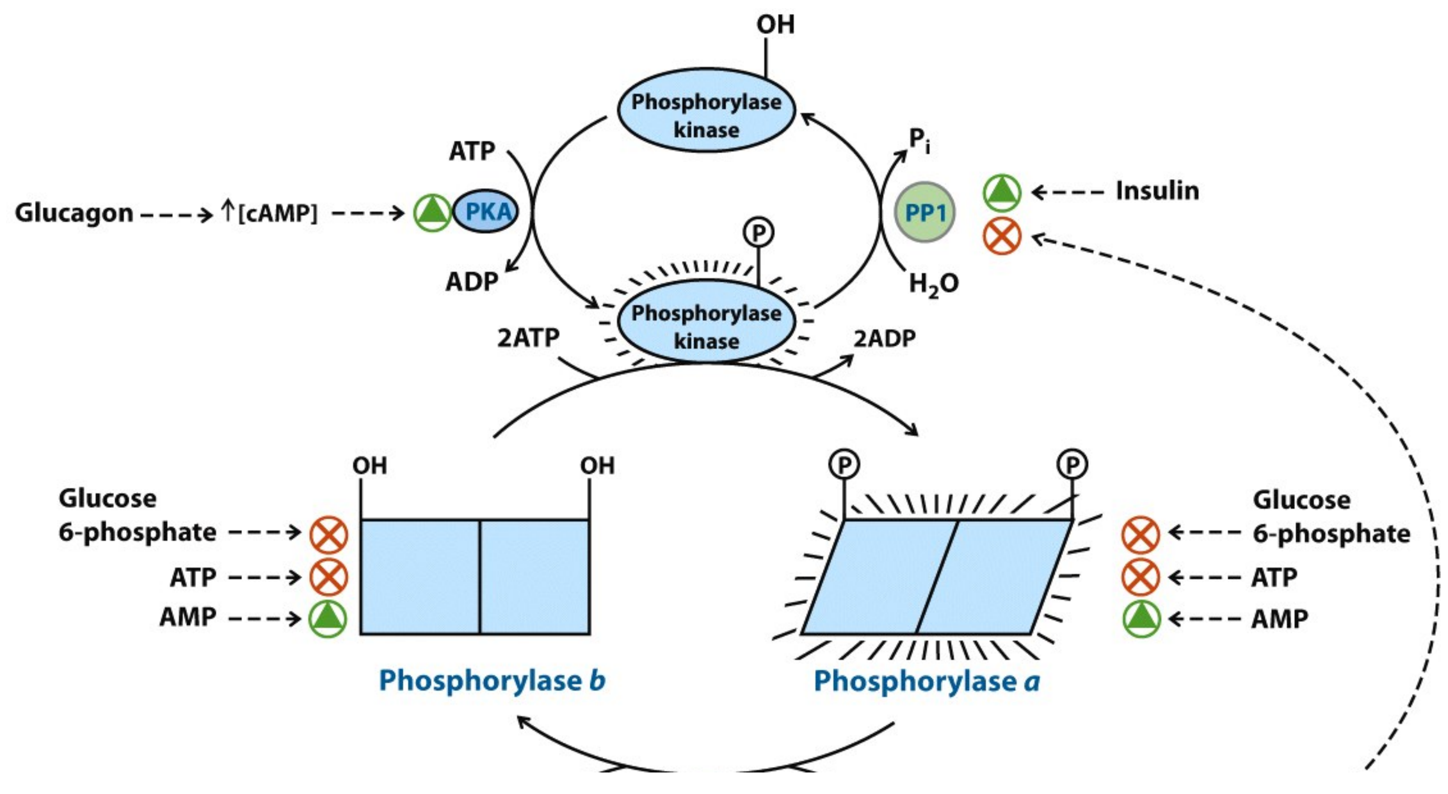
In the more active form of the enzyme, *phosphorylase a*, specific Ser residues, one on each subunit, are phosphorylated.



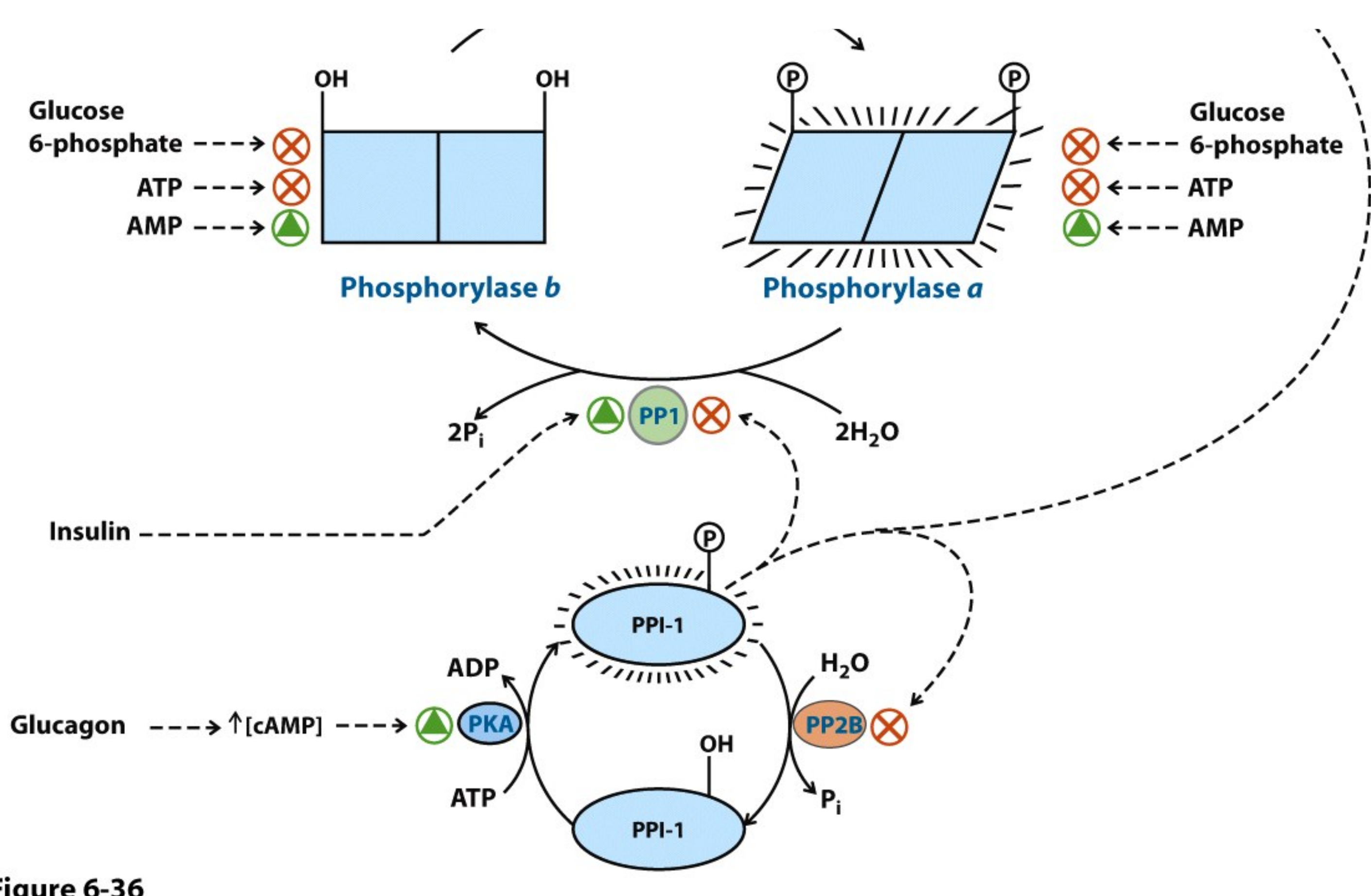
Phosphorylase *b* and phosphorylase *a* can be interconverted.



Phosphorylase *b* can be converted (activated) to phosphorylase *a* by the action of ***phosphorylase kinase***.



Phosphorylase *a* is converted to the less active phosphorylase *b* by enzymatic loss of these phosphoryl groups, promoted by *phosphoprotein phosphatase 1 (PP1)*.



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- The activity of both forms of the enzyme is allosterically regulated by an activator (AMP) and by inhibitors (glucose 6-phosphate and ATP) that bind to separate sites on the enzyme

- The activities of phosphorylase kinase and PP1 are also regulated via a short pathway that responds to the hormones glucagon and epinephrine

- When blood sugar levels are low, the pancreas and adrenal glands secrete glucagon and epinephrine.
- Epinephrine binds to its receptor in muscle and some other tissues, and activates the enzyme adenylyl cyclase.
- Glucagon plays a similar role, binding to receptors in the liver.
- This leads to the synthesis of high levels of cAMP, activating the enzyme cAMP-dependent protein kinase (PKA).
- PKA phosphorylates several target proteins, among them phosphorylase kinase and phosphoprotein phosphatase inhibitor 1 (PPI-1).
- The phosphorylated phosphorylase kinase is activated and in turn phosphorylates and activates glycogen phosphorylase.
- At the same time, the phosphorylated PPI-1 interacts with and inhibits PP1.
- PPI-1 also keeps itself active (phosphorylated) by inhibiting phosphoprotein phosphatase 2B (PP2B), the enzyme that dephosphorylates (inactivates) it.
- In this way, the equilibrium between the *a* and *b* forms of glycogen phosphorylase is shifted decisively toward the more active glycogen phosphorylase *a*