Lehninger

SIXTH EDITION

Principles of Biochemistry

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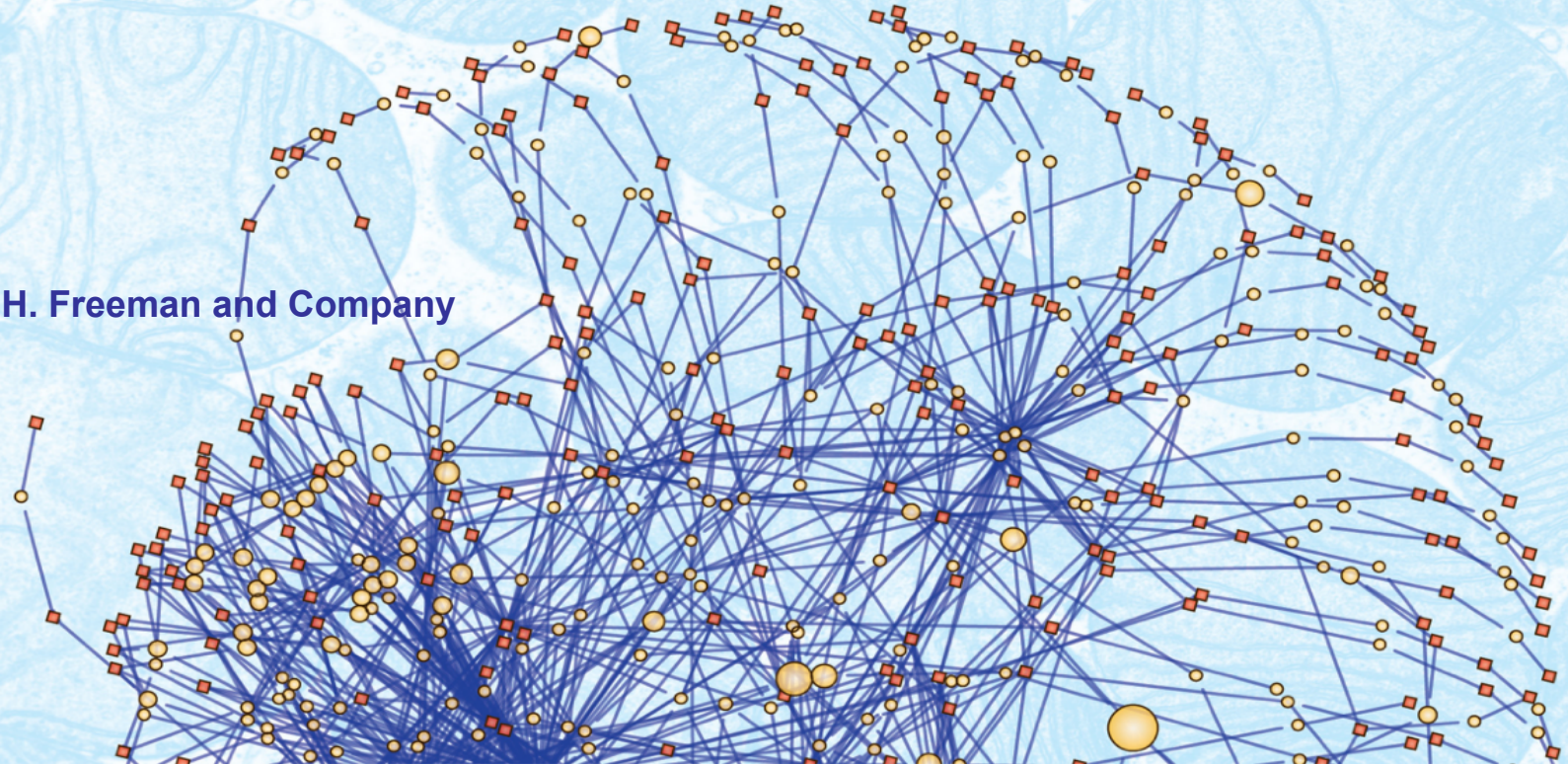
6| Enzymes



How to Speed Up a Chemical Reaction?

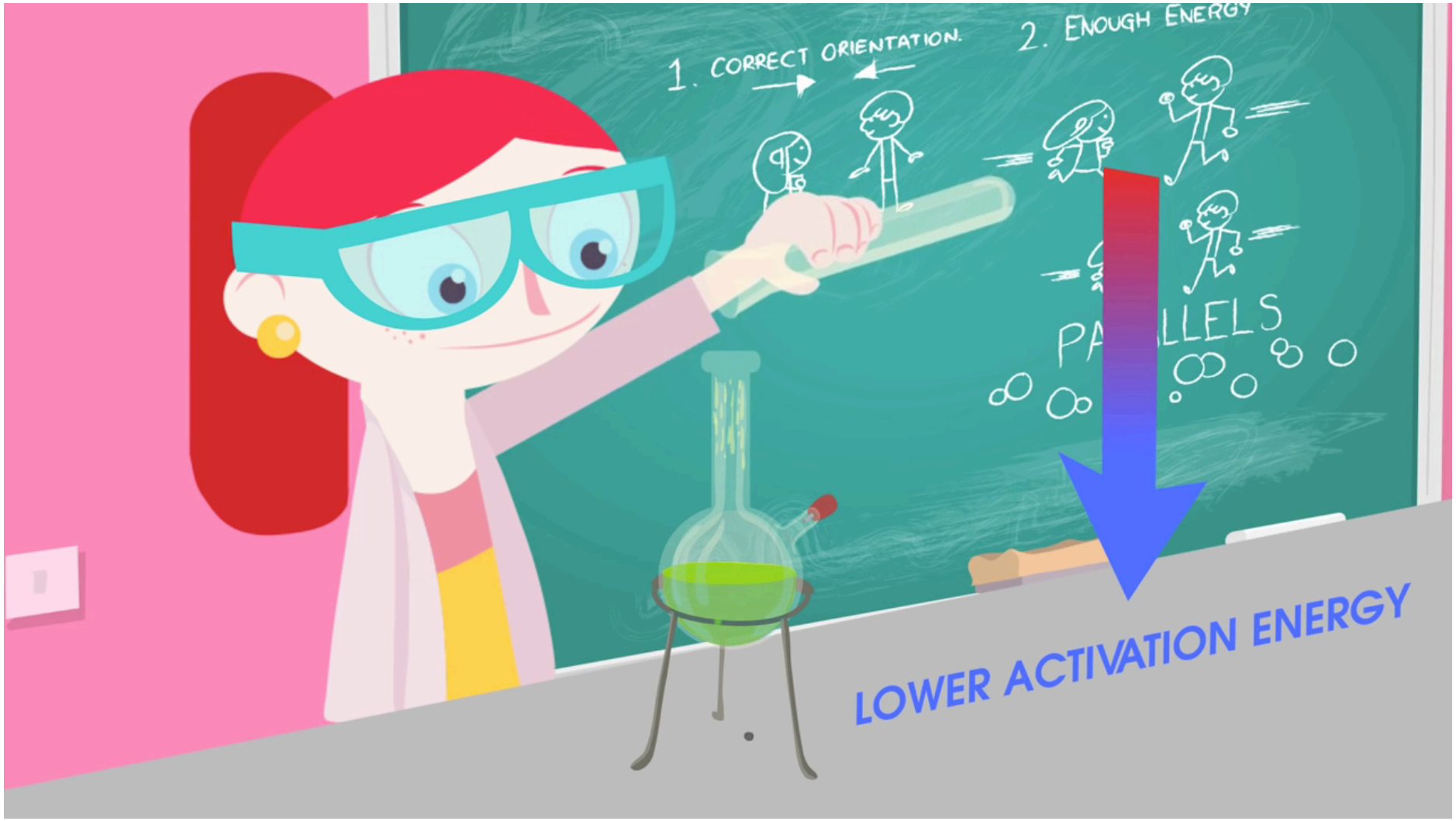
For a bimolecular reaction to occur, two reactant molecules must:

1. collide.
2. have correct orientation.
3. have enough energy.

To speed up a reaction, you can:

1. increase concentrations of reactants.
   a) lower reaction volume.
   b) add more reactants.
2. raise temperature to make molecules move faster.
3. disassemble aggregates into smaller particles.
4. use a catalyst.

How to Speed Up a Chemical Reaction?



Enzymes

6.1 An Introduction to Enzymes

6.2 How Enzymes Work

6.3 Enzyme Kinetics

6.4 Examples of Enzymatic Reactions

6.5 Regulatory Enzymes

What Are Enzymes?

- 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.
- Function depends on structure.
  - Denatured or dissociated -> activity lost
  - Holoenzyme = apoenzyme + cofactor(s)

Ions	Coenzyme	
Cu^{2+}	Biocytin	vitamin B_7
Fe^{2+} or Fe^{3+}	Coenzyme A	
K^+	5′-Deoxyadenosylcobalamin (coenzyme B_{12})	B_{12}
Mg^{2+}	Flavin adenine dinucleotide	B_2
	Lipoate	
Mn^{2+}	Nicotinamide adenine dinucleotide	B_3
Mo	Pyridoxal phosphate	B_6
Ni^{2+}	Tetrahydrofolate	B_9
Zn^{2+}	Thiamine pyrophosphate	B_1

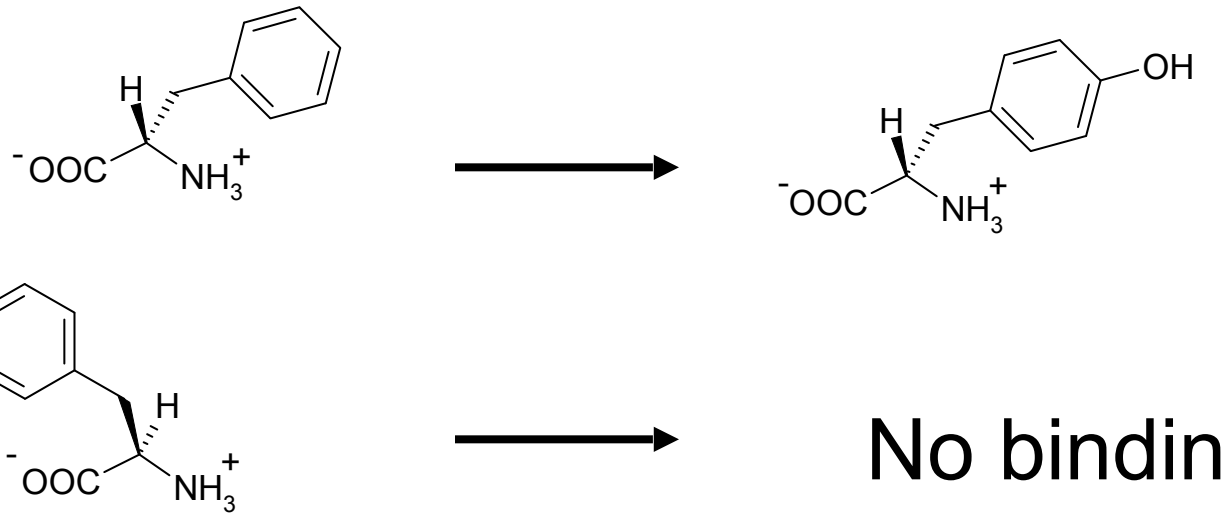
Biological vs. Inorganic Catalysts

- Greater reaction specificity
  - Avoids side products.
- Milder reaction conditions
  - Conducive to conditions in cells.
- Higher reaction rates
  - In a biologically useful timeframe.
- Capacity for regulation
  - Control of biological pathways.

Enzymatic Substrate Selectivity

Phenylalanine hydroxylase (PAH)

catalyzes hydroxylation of Phe to generate Tyr



Phenylketonuria (PKU)

- Caused by absent PAH activity. Affects brain development.
- Phe accumulates and converted into phenylketone (detected in urine).
- Protein-rich food and aspartame act as poisons to PKU patients.
- Inherited disease. Not curable. Treatable by a strict diet.

Conditions Compatible with Life

- 37˚C
- pH ≈ 7

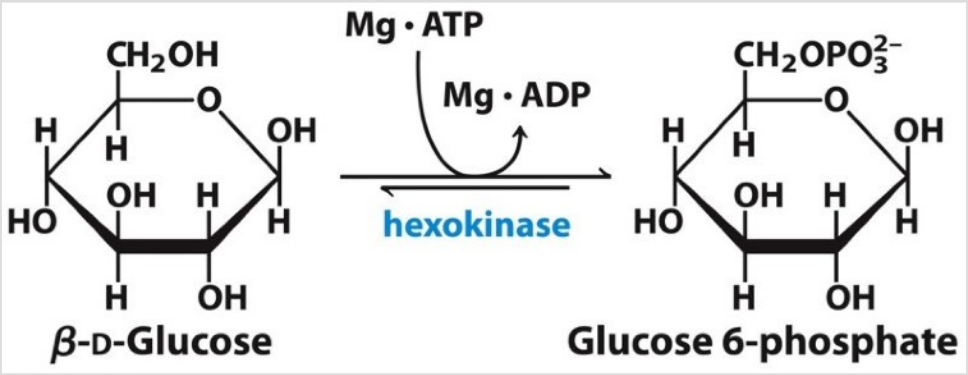
- Extremophile.
  - Organism that live in extreme conditions that most life on Earth cannot tolerate.
  - Thermophile (45–122°C).
  - Acidophile (pH ≤ 3).
  - Alkaliphile (pH ≥ 9).

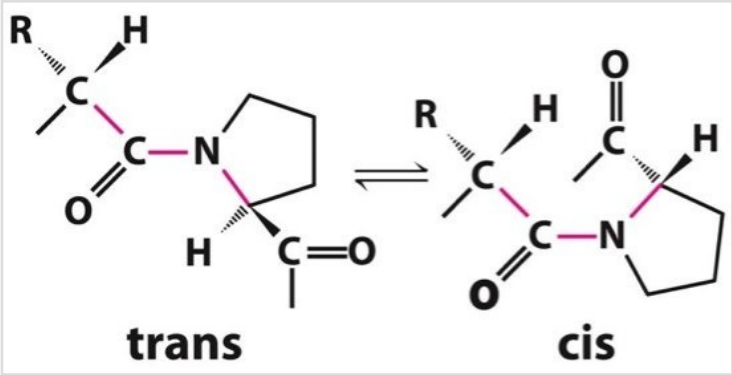
Six Classes of Enzymes

TABLE 6–3 International Classification of Enzymes

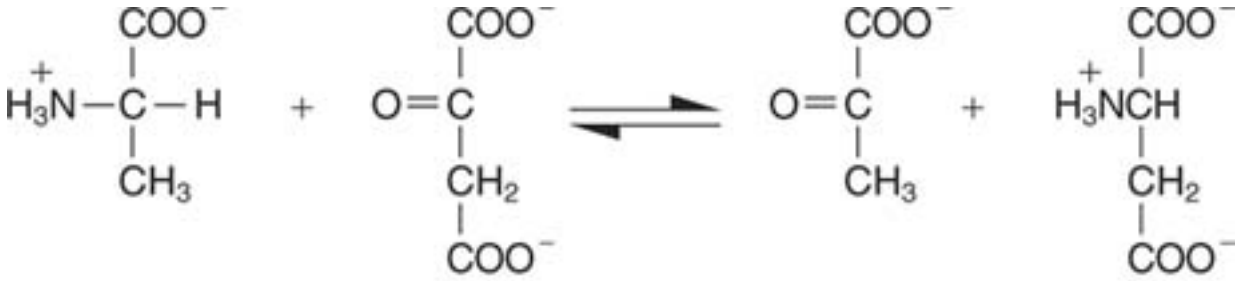
Class no.	Class name	Type of reaction catalyzed
1	Oxidoreductases	Transfer of electrons (hydride ions or H atoms)
2	Transferases	Group transfer reactions
3	Hydrolases	Hydrolysis reactions (transfer of functional groups to water)
4	Lyases	Cleavage of C—C, C—O, C—N, or other bonds by elimination, leaving double bonds or rings, or addition of groups to double bonds
5	Isomerases	Transfer of groups within molecules to yield isomeric forms
6	Ligases	Formation of C—C, C—S, C—O, and C—N bonds by condensation reactions coupled to cleavage of ATP or similar cofactor

ATP + glucose -> ADP + glucose 6-phosphate



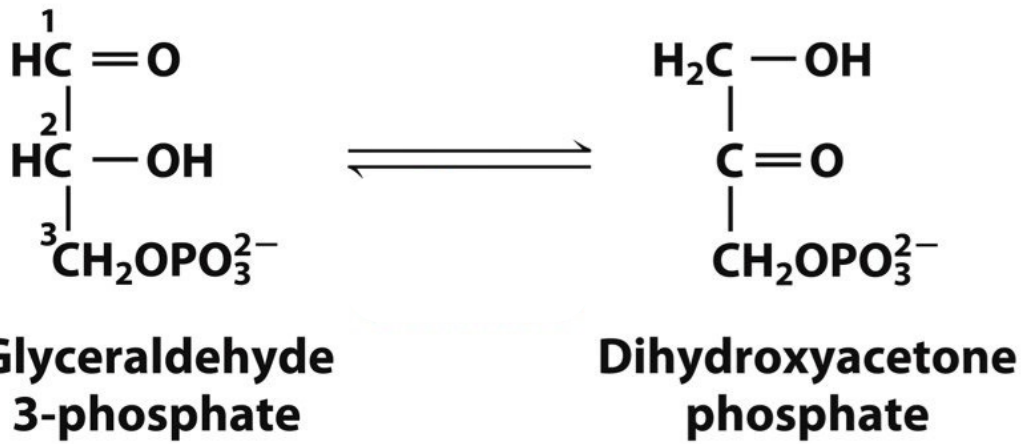


Six Classes of Enzymes



aminotransferase
OR transaminase

1	Oxidoreductases
2	Transferases
3	Hydrolases
4	Lyases
5	Isomerases
6	Ligases



triose phosphate isomerase

Summary 6.1 Introduction to Enzymes

- Life depends on catalysis. Enzymes are powerful and specific biological catalysts.
- All known biological enzymes are proteins, with the exception of a few catalytic RNAs.
- Enzymes are classified by the type of reaction they catalyze, into six classes.

Example Question

Which one of the following is not among the six internationally accepted classes of enzymes?

A) Hydrolases

B) Ligases

C) Isomerases

D) Polymerases

E) Transferases

Enzymes

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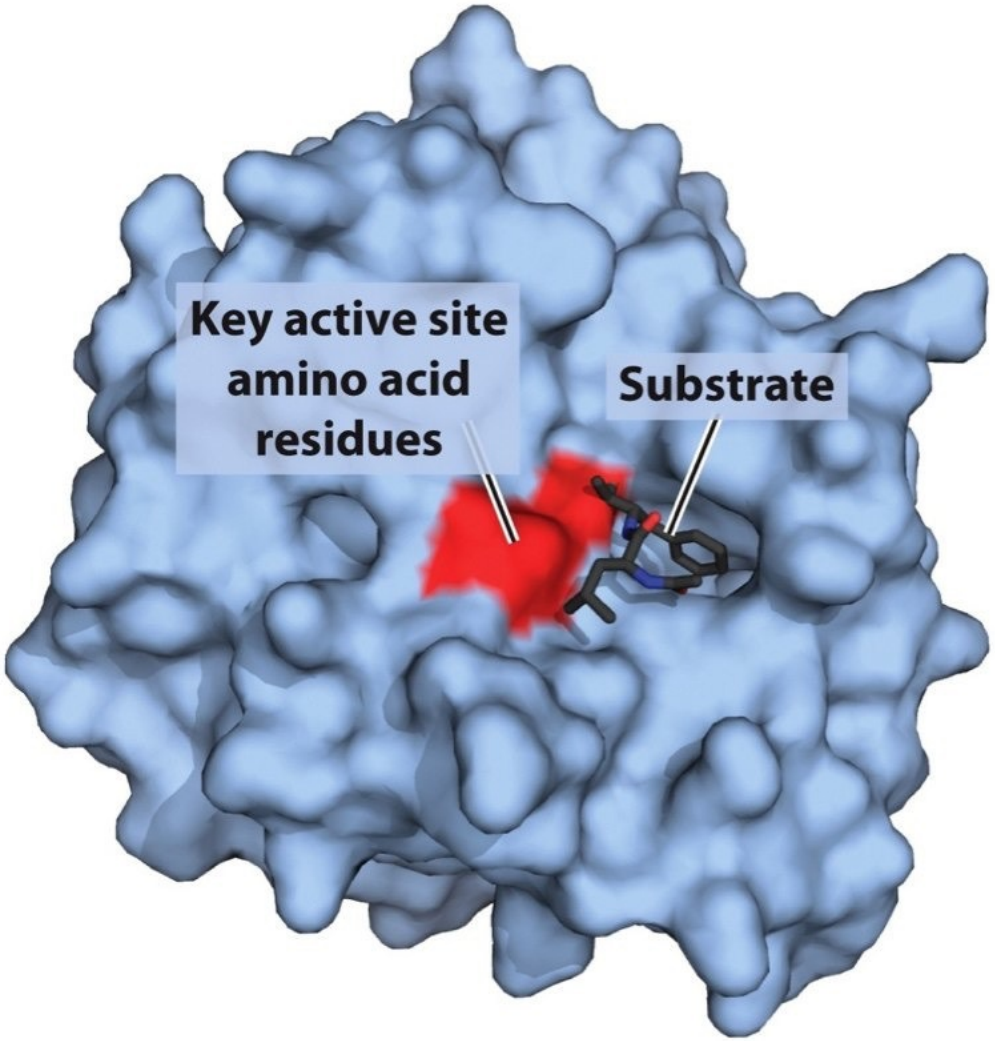
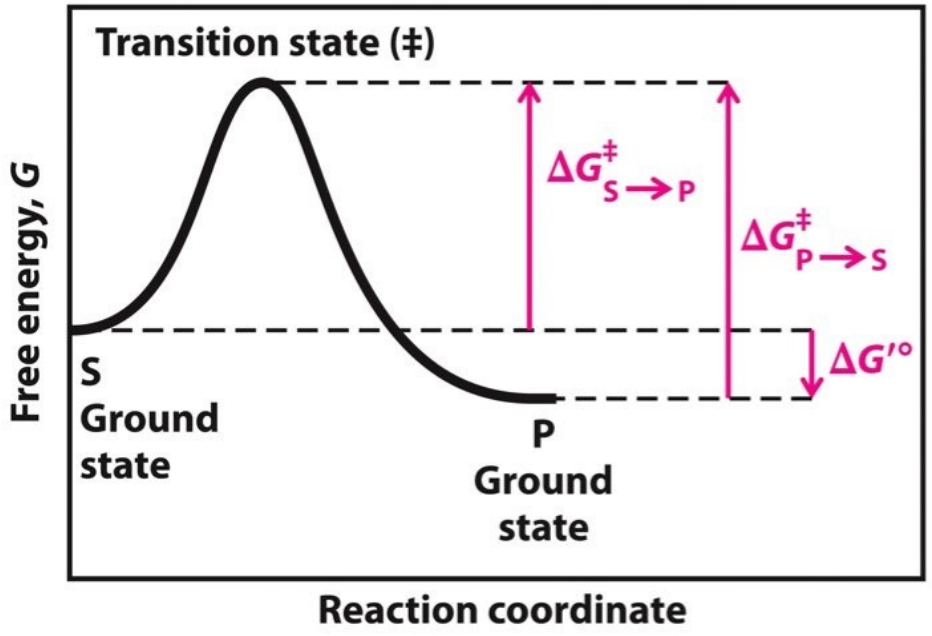
Enzyme-Substrate Complex



- **Active Site**
- **Substrate**

Enzymes Affect Rates, Not Equilibria

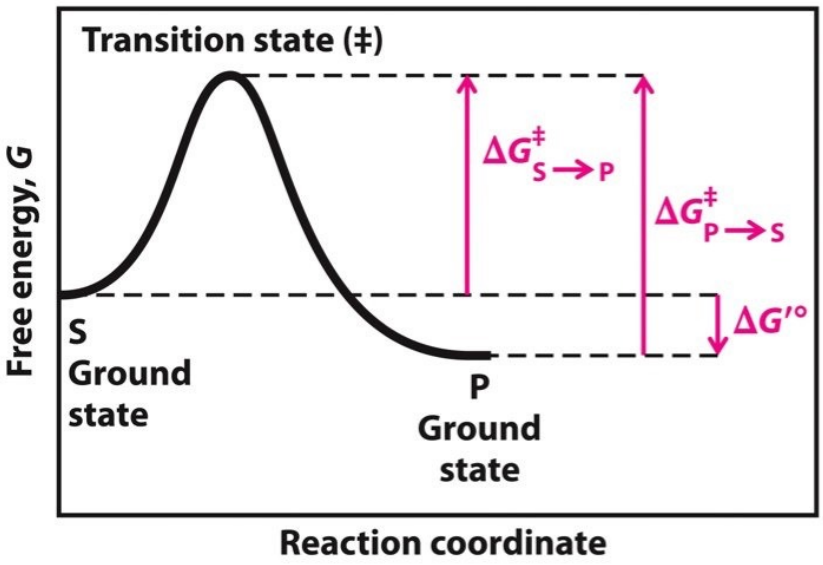
- Enzymes increase reaction rates.
- Enzyme do NOT affect reaction equilibrium.
- A reaction can be described by a reaction coordinate diagram.



free energy plotted against reaction process

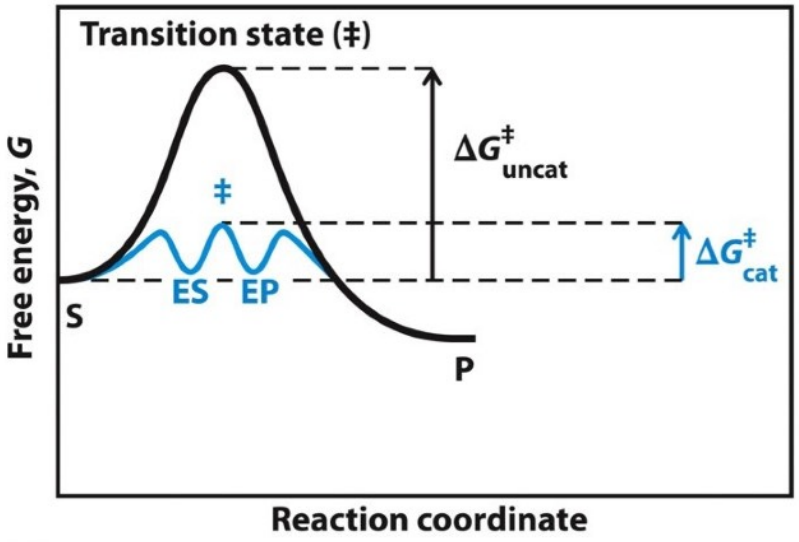
Reaction Coordinate Diagram

- Ground State.
  - Starting point for forward or reverse reaction
- Biochemical standard free-energy change.
  - Free-energy change under biochemically standard conditions.
- Transition state.
  - A point at top of energy hill where decay to S or P state is equally probable.
- Activation energy.
  - Energy difference between ground state and the transition state.



Enzymes Affect Rates, Not Equilibria

- Equilibrium (constant) related to free-energy change, NOT affected by any catalyst.
- Reaction rate reflects activation energy.
- Catalysts enhance reaction rates by lowering activation energies.

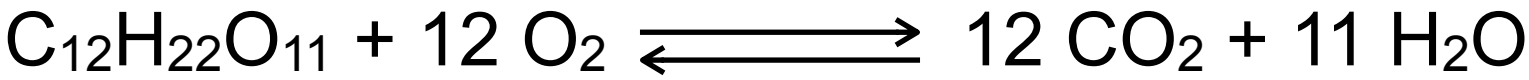


Enzymes Affect Rates, Not Equilibria

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

- Enzymes catalyze S -> P also catalyze P -> S.
- Enzymes NOT used up in the process.
- Enzymes accelerate interconversion of S and P.
  - Reaction reaches equilibrium faster.
  - Equilibrium point NOT affected.

Example: Sucrose Oxidation

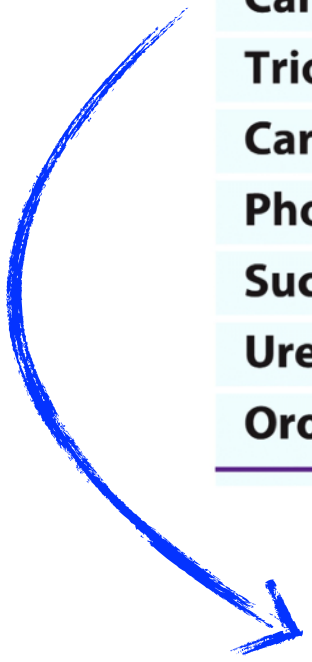


- A very large and negative free-energy change.
  - At equilibrium amount of sucrose is negligible.
- Sucrose is a stable compound.
  - Activation energy barrier quite high.
- Enzymes make reactions proceed on a biologically useful time scale.

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}



s found in red blood cells and catalyzes the reactio

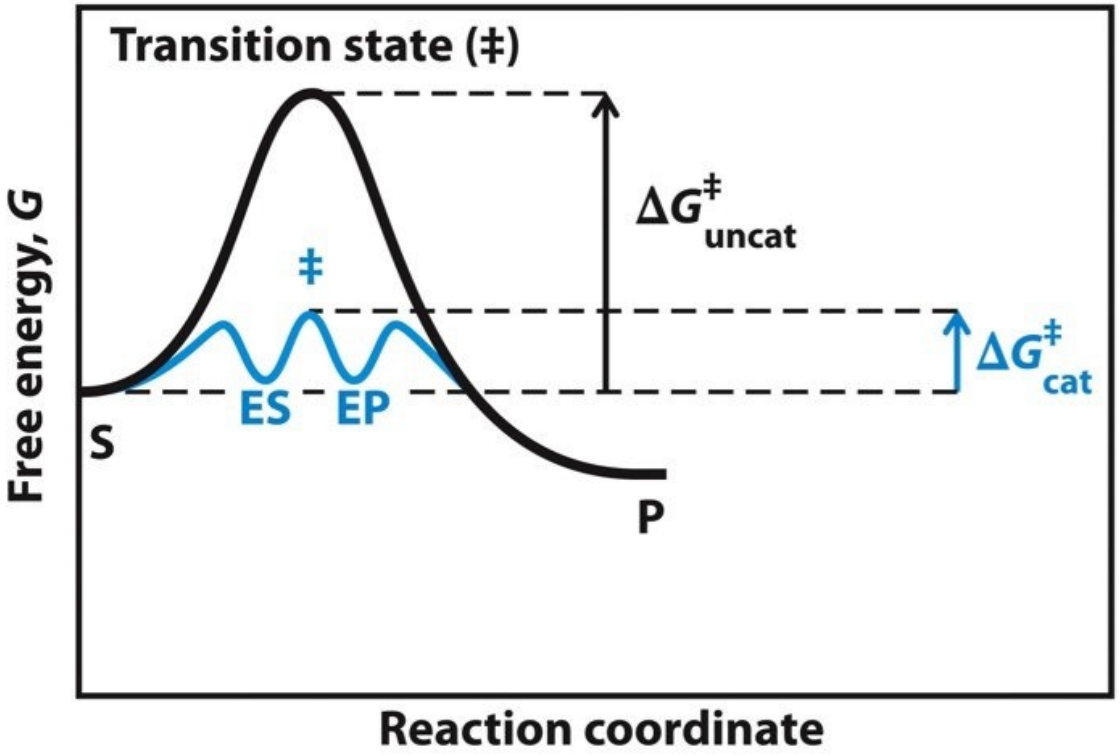
$$CO_2(g) + H_2O(l) \rightleftharpoons H^+(aq) + HCO_3^-(aq)$$

e absence of a catalyst, it is too slow to absorb a

What is source of energy for lowering activation energy?

Enzyme-Substrate Covalent Bond

- Transient covalent bond between enzyme and substrate.
- Provide alternative, low-energy reaction path.
- Enzymes restored and seem unchanged.



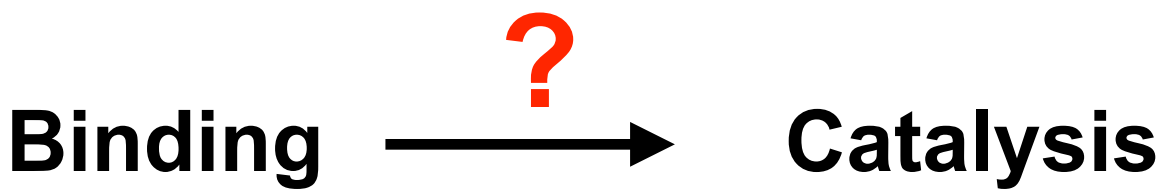
Enzyme-Substrate **Noncovalent** Bond

- Weak, noncovalent interactions stabilize protein structure and protein-ligand interactions.
- Much of energy required to lower activation energy is derived from weak, noncovalent interactions between substrate and enzyme.
- **Binding energy.**
  - Energy derived from enzyme-substrate interaction.
  - Stabilize enzyme-substrate complex.
  - **Major** source of energy to lower activation energy barrier.

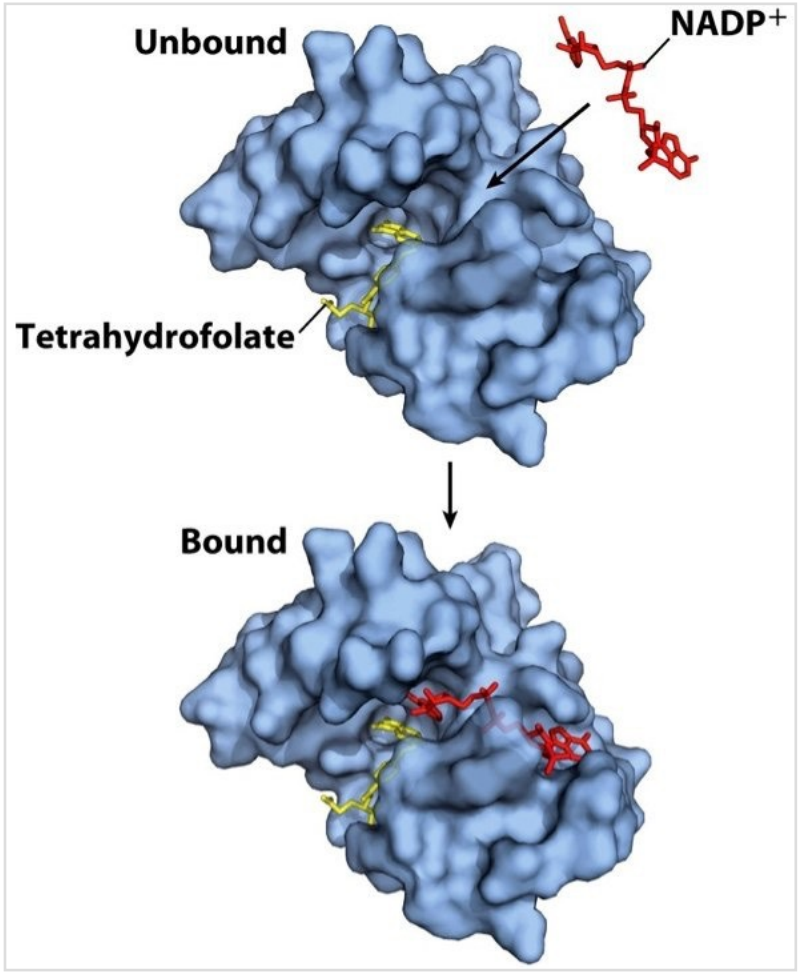
How Enzymes Use Binding Energy

1. Binding energy contributes to **specificity** and **catalysis**.
2. Weak interactions are optimized in transition state.

   Enzyme active site NOT complementary to substrate per se but complementary to **transition state**.



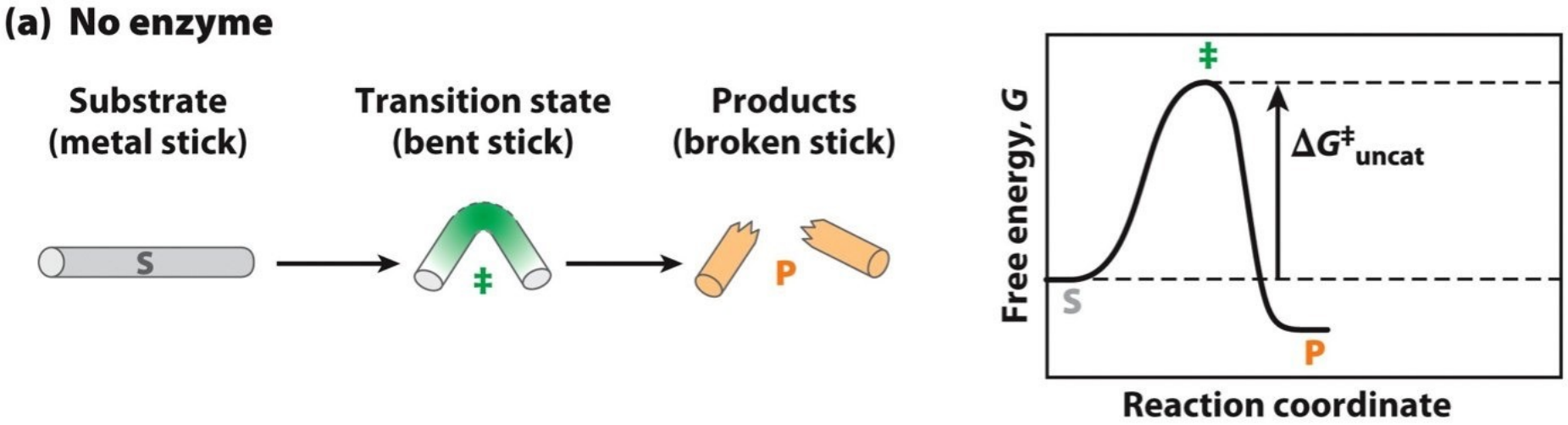
“Lock and Key” Hypothesis



dihydrofolate reductase

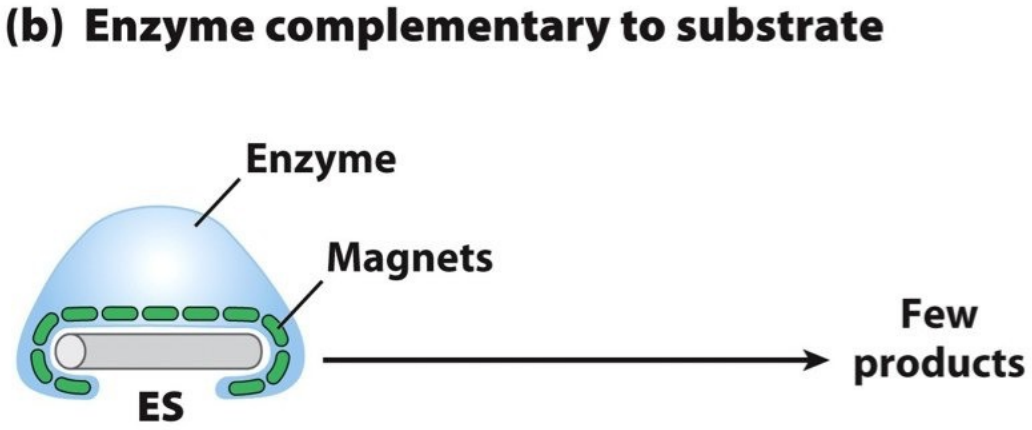
- Enzymes structurally complementary to substrates.
- In reality, complementarity between protein and ligand is rarely perfect.
- An enzyme completely complementary to substrate would be a poor enzyme.

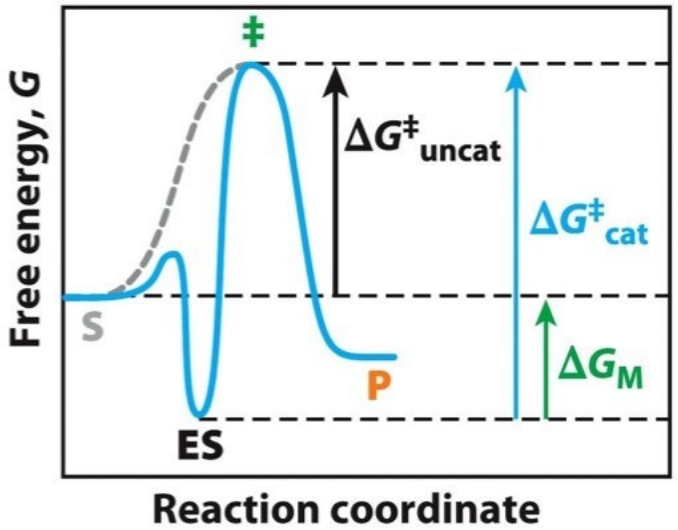
Transition State Stabilization Idea



An imaginary reaction: breaking of a metal stick

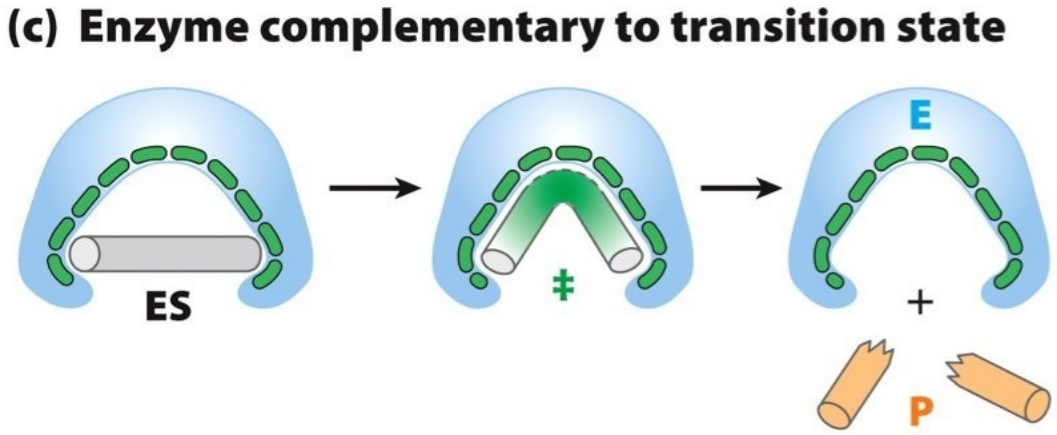
Transition State Stabilization Idea

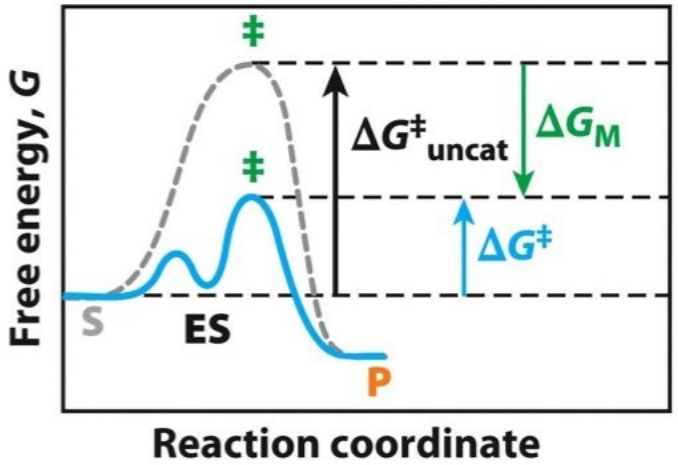




- A “stickase” enzyme perfectly complementary to substrate.
- Use magnetic forces as binding energy.
- Bending would eliminate some magnetic interactions.
- Enzyme does NOT catalyze reaction; instead, it stabilizes substrate.

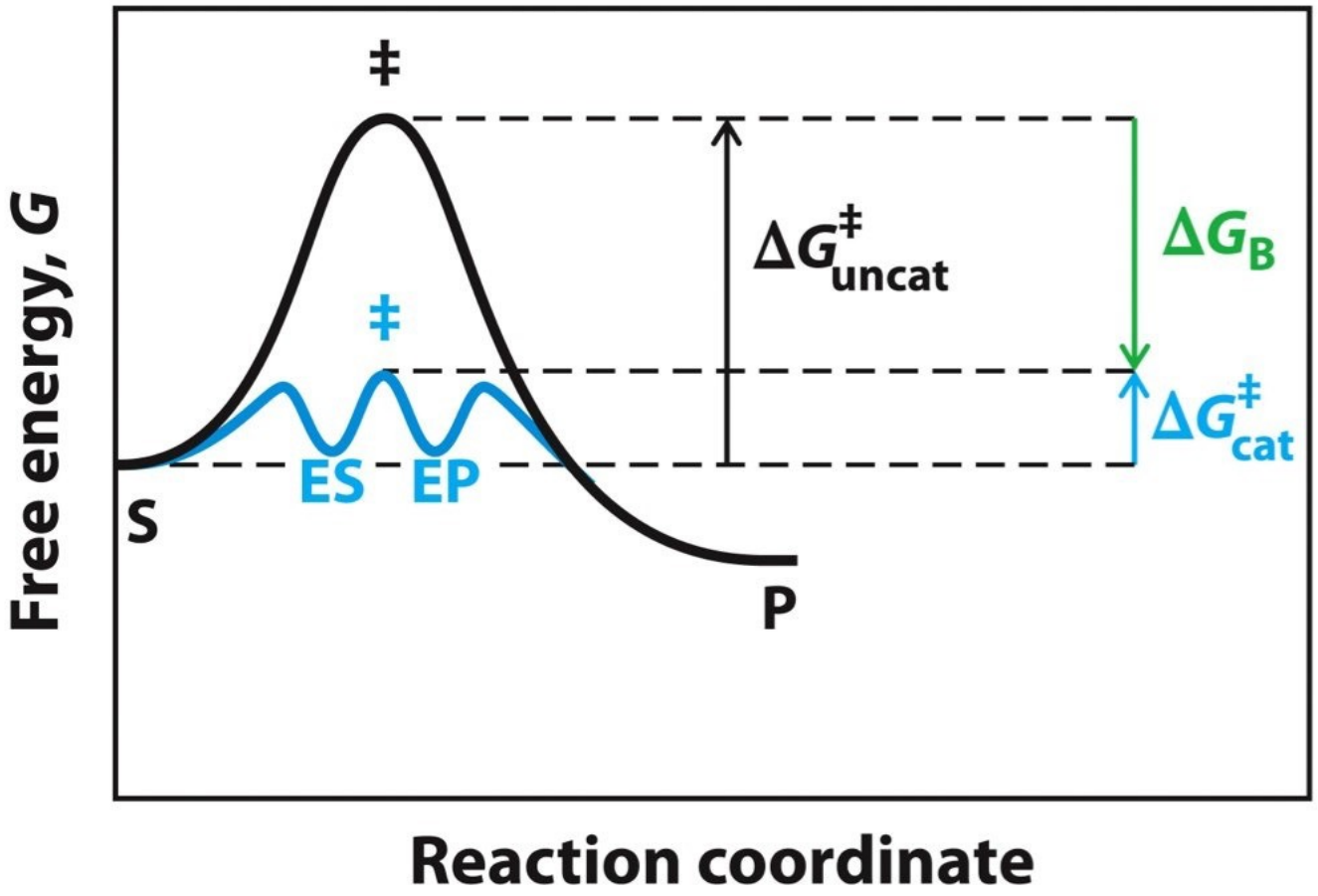
Transition State Stabilization Idea





- A “stickase” enzyme complementary to transition state.
- Use magnetic forces as binding energy.
- Optimal interaction occurs only in transition state.
- Free energy increase offset by additional magnetic interactions.

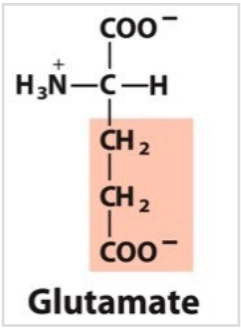
Binding Energy in Catalysis



Binding energy (ΔG_B) used to lower activation energy

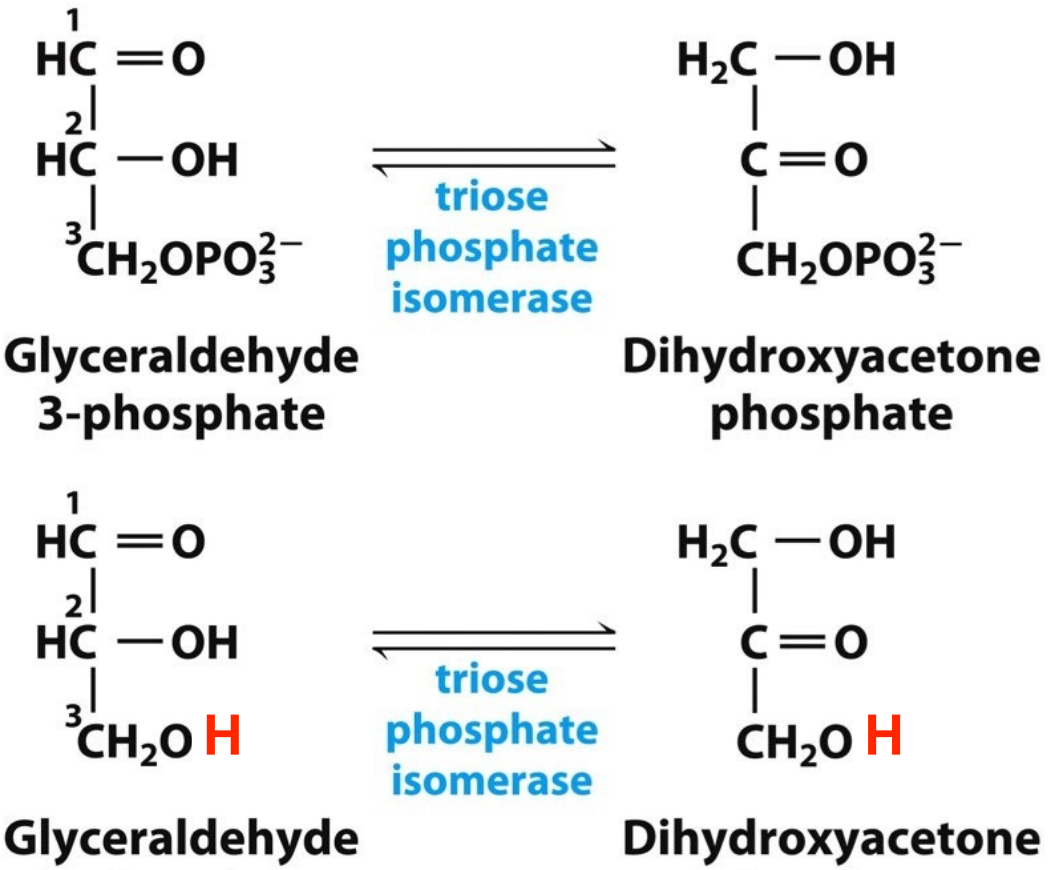
Binding Energy -> Specificity & Catalysis

- Specificity: ability to discriminate between a substrate and a competing molecule.
- If substrate has a -OH group that forms a H-bond with a Glutamate residue on enzyme:
  - any molecule lacking -OH group would be a poor substrate.
  - any molecule with extra groups, for which the enzyme has no binding site, is also likely to be excluded.
- Specificity is derived from many weak interactions between enzyme and its substrate molecule.



Glutamate

Binding Energy -> Specificity & Catalysis



Much of enzymatic rate acceleration comes from enzyme binding to phosphate group of substrate.

Binding Energy Overcome Reaction Barriers

1. Entropy reduction.
   - Restrict relative motions of two substrates

Entropy

2. Desolvation.
   - Replace H-bonds between substrate and water.

Solvation shell

3. Additional interactions formed only in transition state.
   - Compensate for high energy of transition state.

Distortion

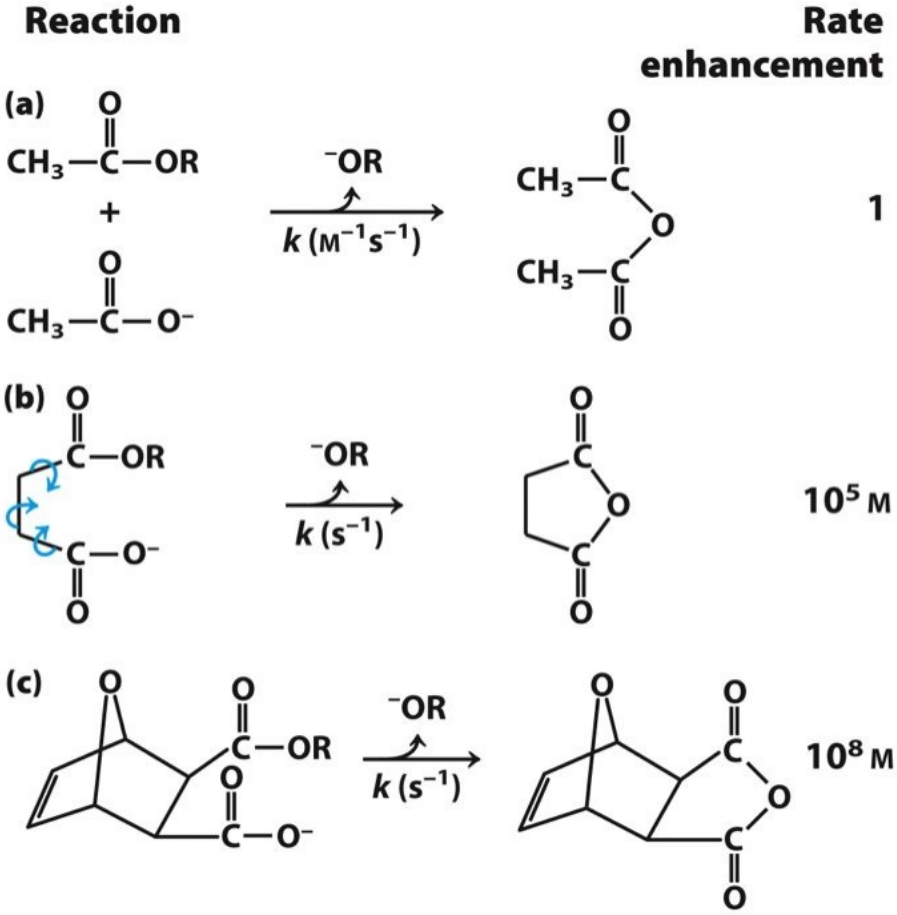
4. Induced fit.

Enzyme functional group alignment

   - Substrate binding induces conformational change in enzyme.
   - Position functional groups for catalysis.
   - Form additional weak bonding in transition state.

Rate Enhancement by Entropy Reduction

Ester reacts with carboxylate to form anhydride



bimolecular or inter-molecular reaction

intra-molecular reaction
less freedom of motion

bond rotation
constrained
entropy further reduced

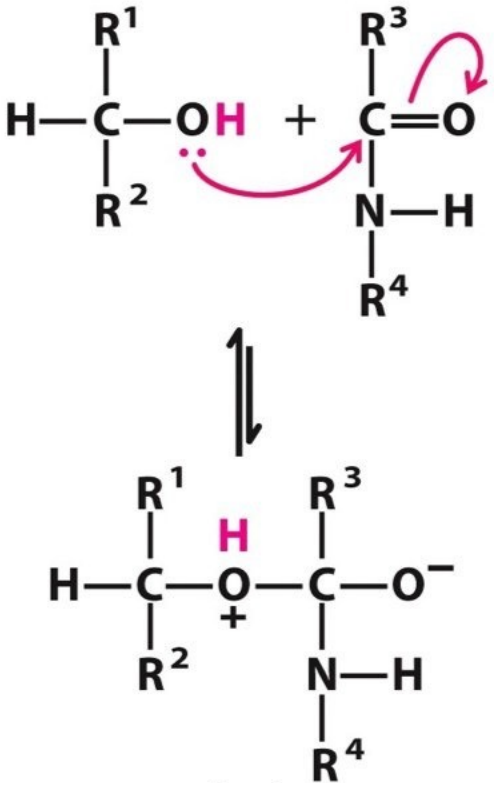
Strong dependence on proximity of two reactive groups

Catalytic Mechanisms

- Acid-base catalysis.
  - Give and take protons.
  - Water as proton donor or acceptor (specific).
  - Weak acids or bases other than water (general).
- Covalent catalysis.
  - Change reaction path.
- Metal ion catalysis.
  - Ionic interactions similar to binding interactions.
  - Reversible changes in oxidation state.

Acid-Base Catalysis

Reactant species

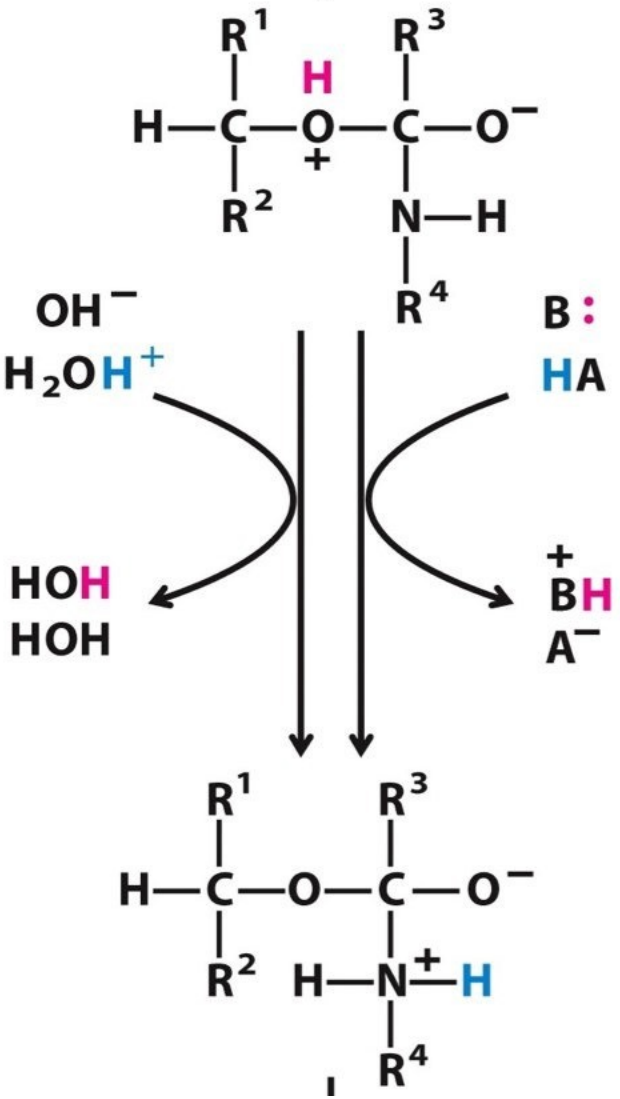


Without catalysis, unstable (charged) intermediate breaks down rapidly to form reactants.

Acid-Base Catalysis

Specific acid-base catalysis

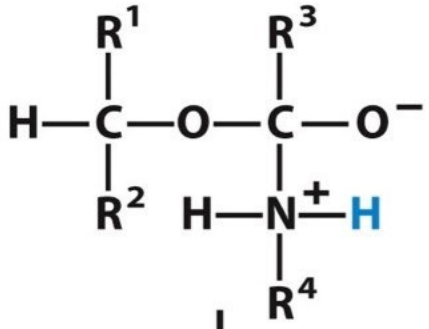
water as proton donor and/or acceptor

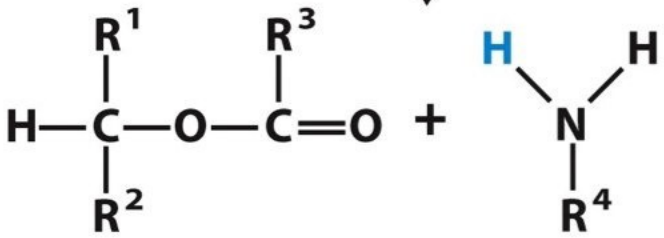


General acid-base catalysis

weak acid (HA) or base (B:) as proton donor and/or acceptor

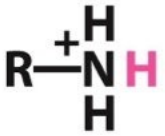
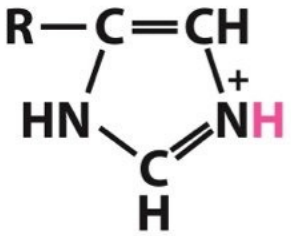
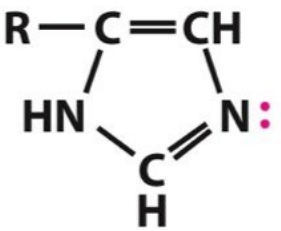
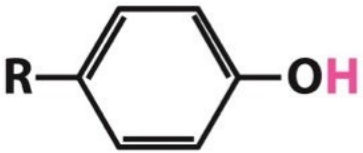
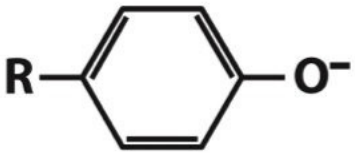
Acid-Base Catalysis





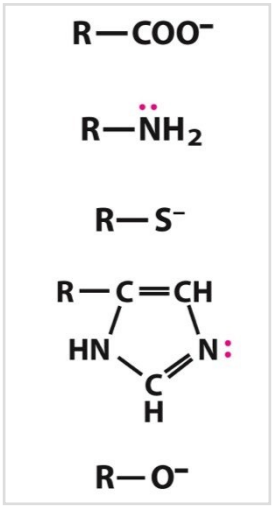
Products

Amino Acids in Acid-Base Catalysis

Amino acid residues	General acid form (proton donor)	General base form (proton acceptor)
Glu, Asp		
Lys, Arg		
Cys		
His		
Ser		
Tyr		

Covalent Catalysis

- A transient covalent bond between enzyme and substrate.
- Changes the hydrolysis 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:
    - Carboxylate.
    - Amine.
    - Thiolate.
    - Serine.

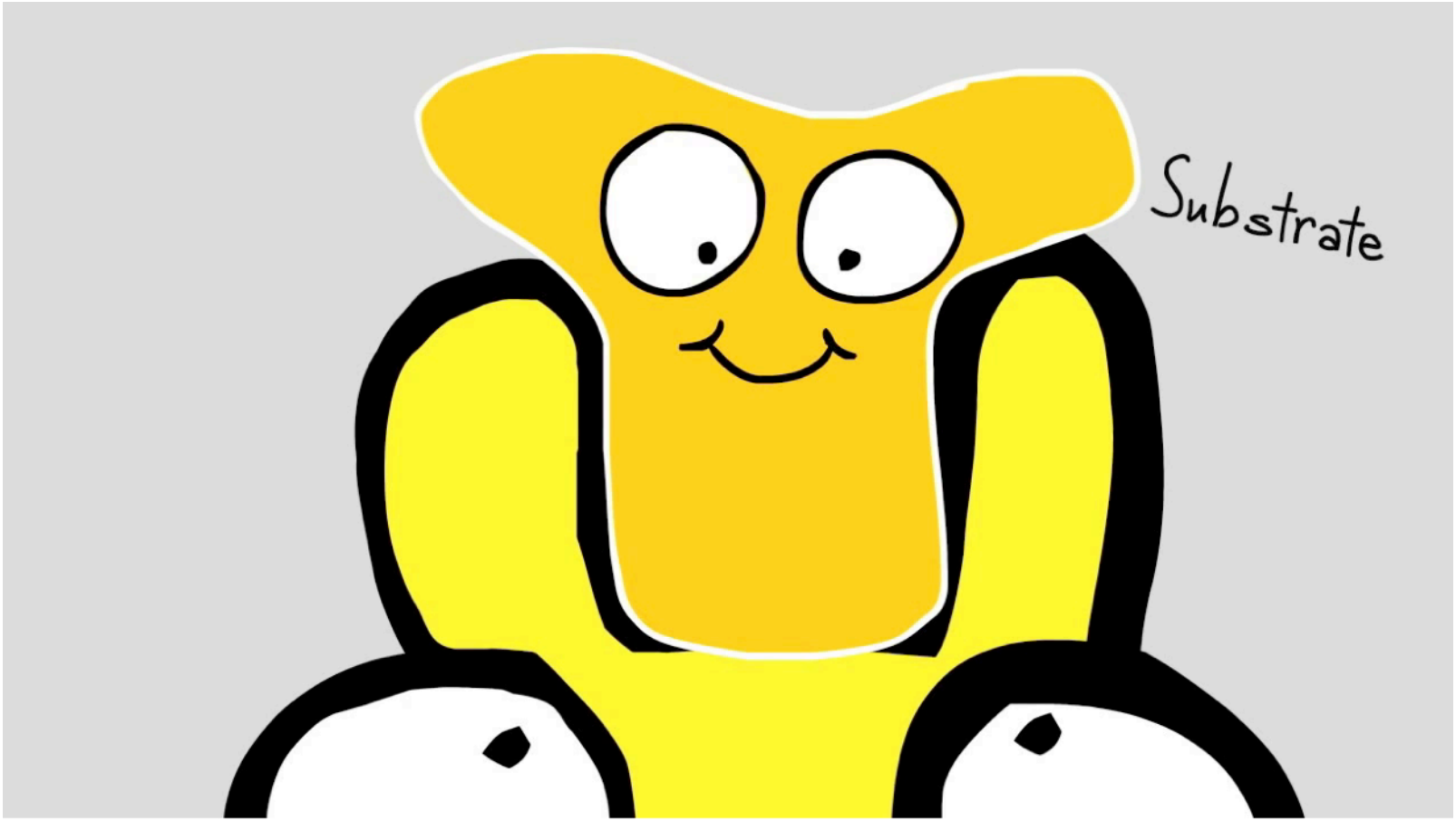


further reaction to regenerate enzyme

Summary 6.2 How Enzymes Work

- Substrate binding occurs in active site on enzyme. Enzyme enhances reaction rates (10^5 - 10^{17}) by lowering activation energy. Equilibrium is unaffected by enzyme.
- Active site allows additional weak interactions with transition state. Binding energy is used to offset activation energy.
- Additional catalytic mechanisms include acid-base catalysis, covalent catalysis, and metal ion catalysis.

What Are Enzymes?



What Are Enzymes?

- Digesting a steak (or any protein-rich food).
  - Acid in stomach and enzyme in acidic environment.
- Many reactions too slow to support life without enzymes.
  - Examples: Pepsin, Amylase, and Lysozyme.
- What are enzymes and their common characteristics?
  - Active site and substrate.
    - Example: starch -> glucose -> glycogen.
  - Enzymes are specific.
    - Example: lipase.
  - Enzymes are recycled.
    - NOT altered by reaction.

Pepsin

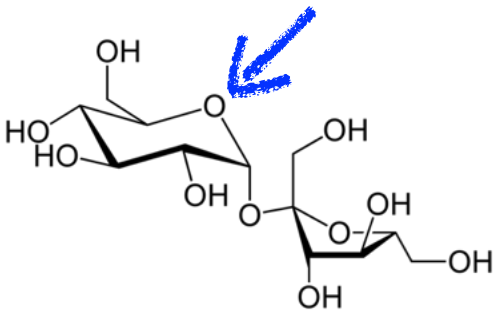
- Breaks down proteins to small peptides (**protease**).
  - Produced in stomach and help digest proteins in food.
  - Cleaves peptide bonds before Phe, Tyr and Trp.
- Synthesized as an inactive proenzyme pepsinogen.
  - Activated by hydrochloric acid (HCl).
  - Additional 44 amino acid residues cleaved to create pepsin.
- Most active in acidic environments (37 °C - 42 °C).
  - Maximal activity at pH 2.0
  - Inactive at pH 6.5
  - Irreversibly denatured at pH 8.0

Amylase

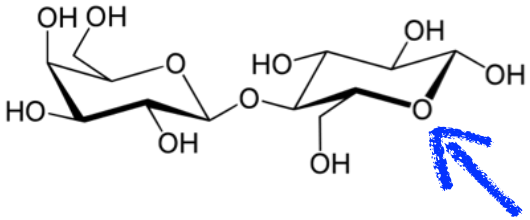
- Catalyze hydrolysis of starch to sugars.
  - Present in saliva and pancreas.
  - Hydrolyze starch to disaccharides and trisaccharides.
- α-amylase.
  - Major digestive enzyme in human and other animals.
  - Calcium metalloenzyme. Optimal pH around 7.
- β-amylase.
  - Break starch into maltose during ripening of fruit.
  - Result in sweet flavor of ripe fruit.
  - Optimal pH is 4.0 - 5.0.

Disaccharides and Enzymes

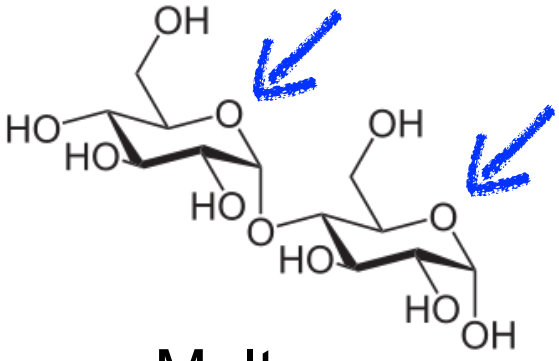
- Sucrose
  - Sucrase catalyzes hydrolysis of sucrose to glucose and fructose.
- Lactose.
  - Sugar that gives milk its sweetness.
  - Lactase catalyzes hydrolysis of lactose to glucose and galactose.
- Maltose.
  - Maltase catalyzes hydrolysis of maltose to glucose and glucose.



Sucrose



Lactose



Maltose

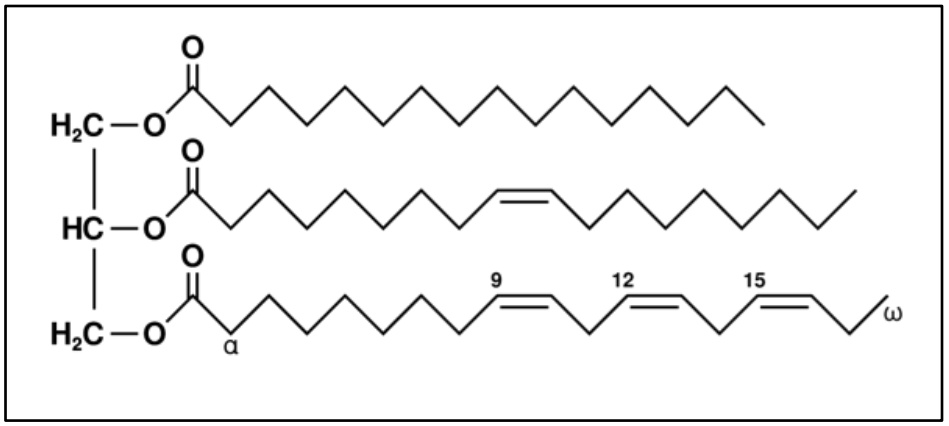
Lysozyme

- Attack peptidoglycan (bacterial cell wall).
  - Abundant in secretions such as tear, saliva, milk and mucus.
  - Also present inside a cell (macrophage).
  - Found in egg white.
- Antibacterial action (1922 Alexander Fleming).

In the first experiment nasal mucus from the patient, with coryza, was shaken up with five times its volume of normal salt solution, and the mixture was centrifuged. A drop of the clear supernatant fluid was placed on an agar plate, which had previously been thickly planted with *M. lysodeikticus*, and the plate was incubated at 37° C. for 24 hours, when it showed a copious growth of the coccus, except in the region where the nasal mucus had been placed. Here there was complete inhibition of growth, and this inhibition extended for a distance of about 1 cm. beyond the limits of the mucus.

Lipase

- Catalyze hydrolysis of fats.
  - Convert triglyceride to monoglyceride and two fatty acids.
  - Human pancreatic lipase secreted into extracellular space.
  - Also present inside a cell, within an organelle called lysosome.
- Catalysis.
  - Chymotrypsin-like mechanism using a catalytic triad.
    - Serine nucleophile (-OH).
    - Histidine base.
    - Acidic residue (Asp).



Example Question

Which one of the following statements is *true* of enzyme catalysts?

A) Their catalytic activity is independent of pH.
B) They are generally equally active on D and L isomers of a given substrate.
C) They can increase the equilibrium constant for a given reaction by a thousand fold or more.
D) They can increase the reaction rate for a given reaction by a thousand-fold or more.
E) To be effective, they must be present at the same concentration as their substrate.

Example Question

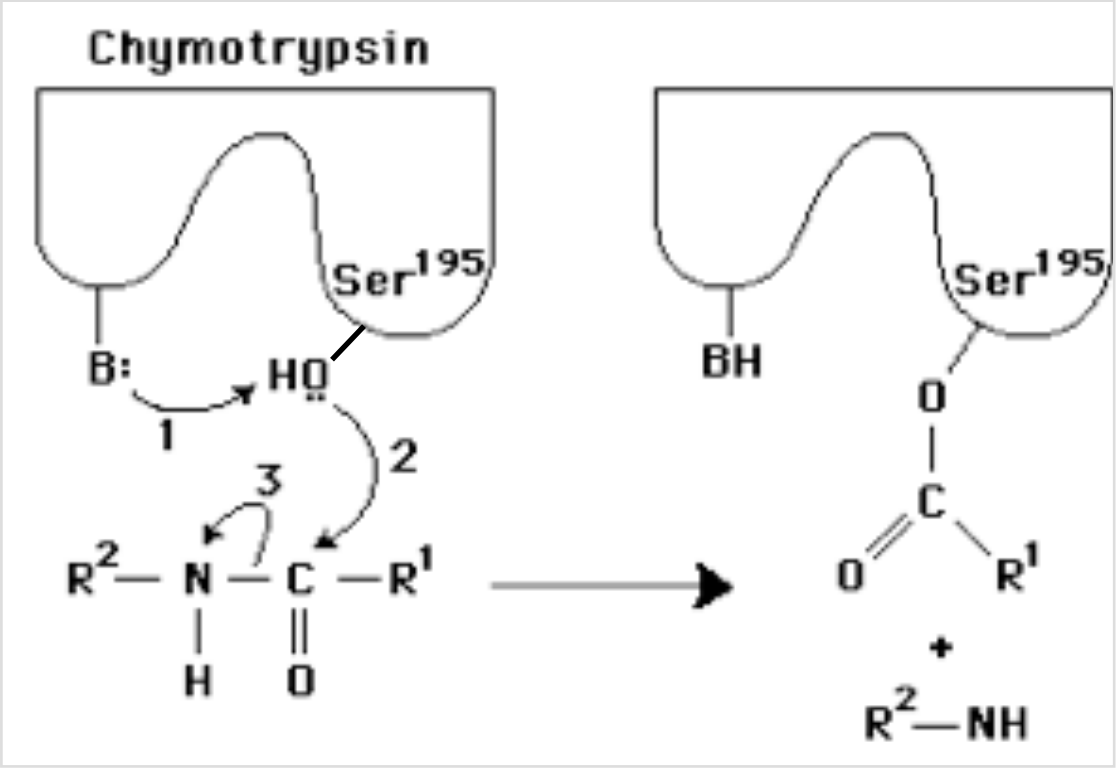
Enzymes are potent catalysts because they:

A) are consumed in the reactions they catalyze.
B) are very specific and can prevent the conversion of products back to substrates.
C) drive reactions to completion while other catalysts drive reactions to equilibrium.
D) increase the equilibrium constants for the reactions they catalyze.
E) lower the activation energy for the reactions they catalyze.

Example Question

In the following diagram of the first step in the reaction catalyzed by the protease chymotrypsin, the process of general base catalysis is illustrated by the number _____, and the process of covalent catalysis is illustrated by the number _____.

A) 1; 2
B) 1; 3
C) 2; 3
D) 2; 3
E) 3; 2



Enzymes

6.1 An Introduction to Enzymes

6.2 How Enzymes Work

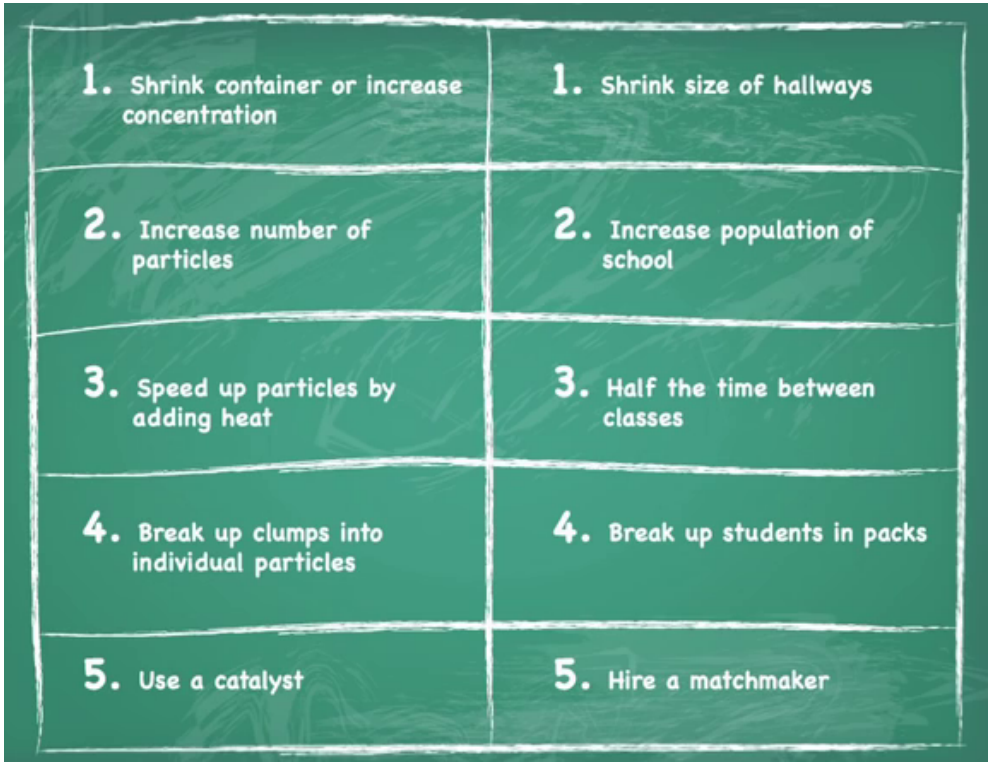
6.3 Enzyme Kinetics

6.4 Examples of Enzymatic Reactions

6.5 Regulatory Enzymes

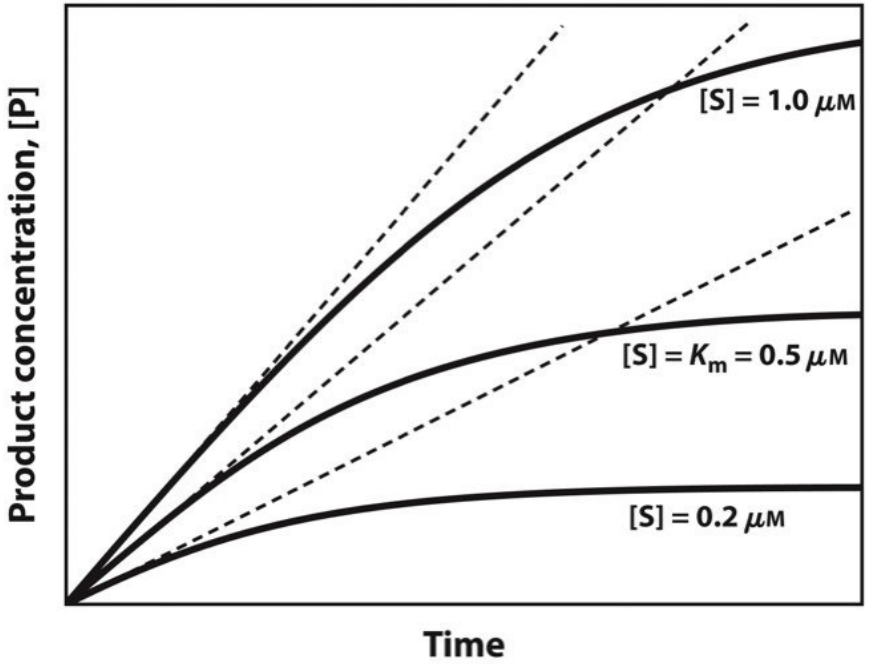
What is Enzyme Kinetics?

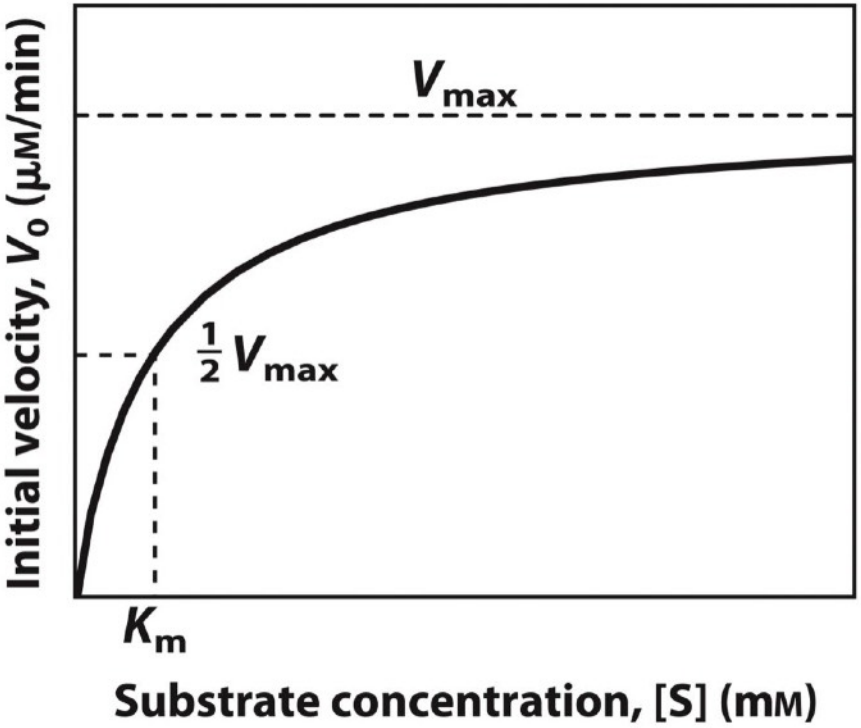
- Kinetics is the study of the **rate** at which compounds react
- Rate of enzymatic reaction is affected by:
  - enzyme
  - substrate
  - temperature



How to Do Kinetic Measurements

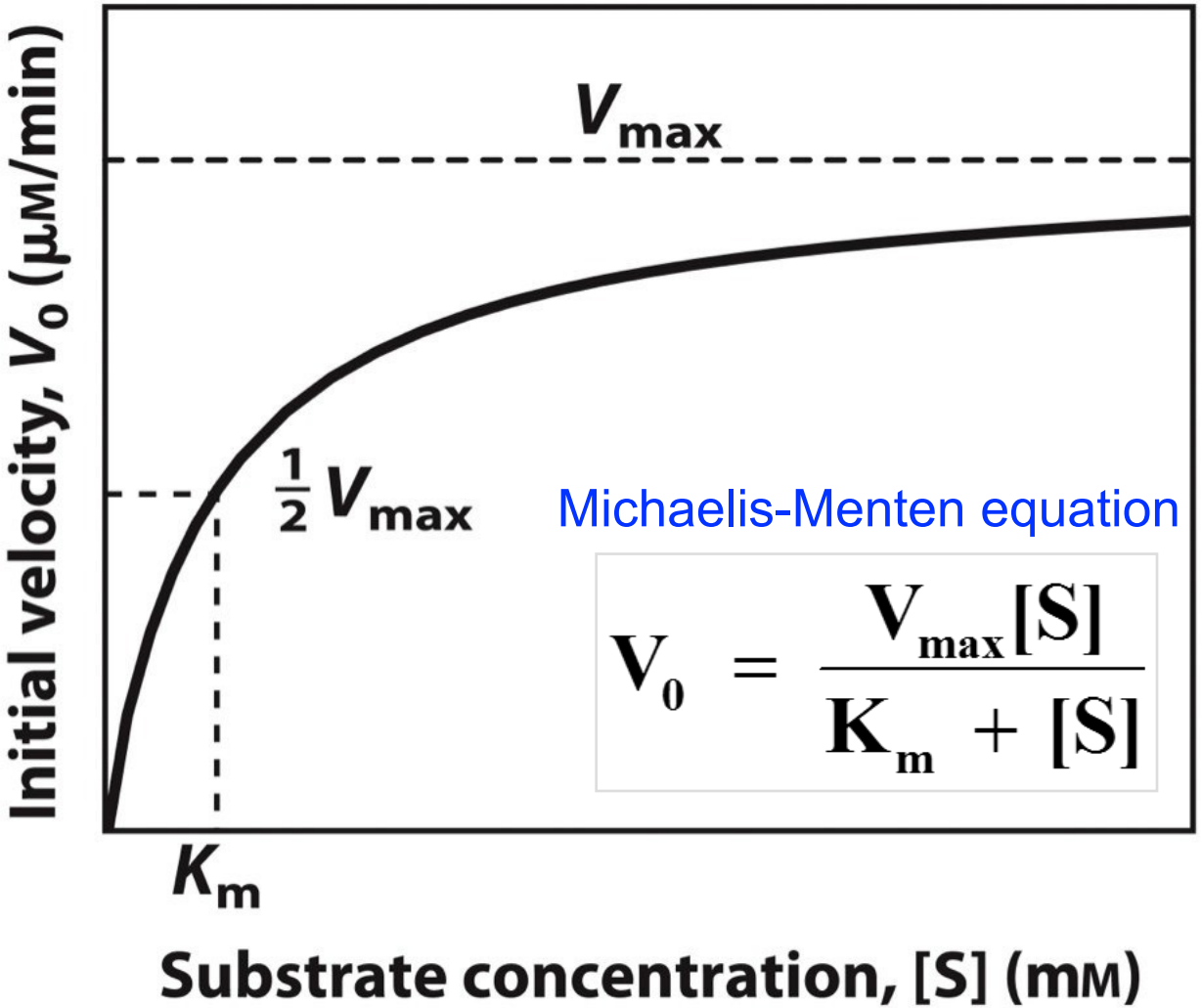
1. Mix enzyme and substrate ([S] is generally much greater than [E])
2. Record product concentration as a function of time (one curve in left figure)
3. Change substrate concentration and repeat (multiple curves in left figure)
4. Plot **initial velocity/rate (V_0)** versus substrate concentration (right figure)





$$V = k[E][S]$$

Effect of Substrate Concentration



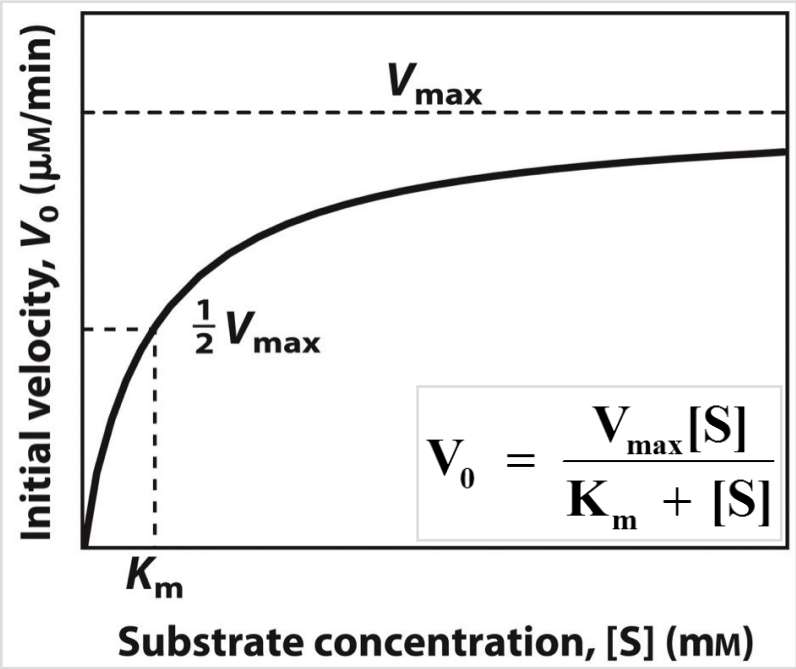
Michaelis-Menten equation

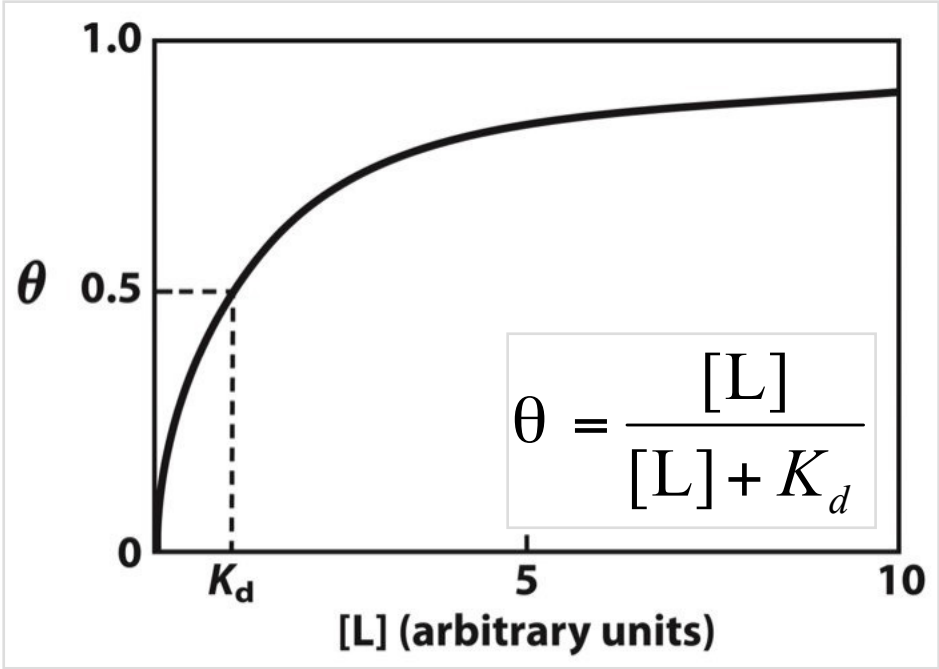
$$V_0 = \frac{V_{max}[S]}{K_m + [S]}$$

Rate Curve vs Binding Curve

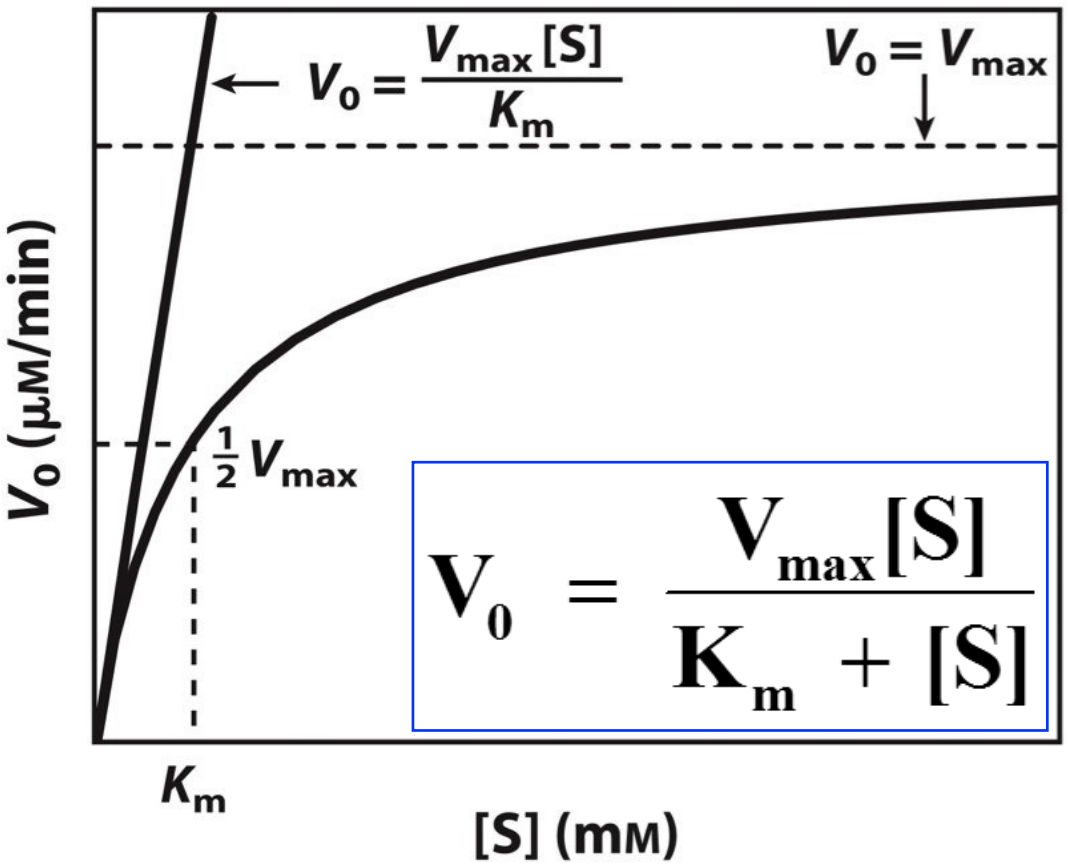
Hyperbolic curve.

- $[S] >> K_m$ → V_0 -> V_{max}.
- $[L] >> K_d$ → θ -> 100%.
- $[S] = K_m$ → V_0 -> 0.5 * V_{max}.
- $[L] = K_d$ → θ -> 50%.





Saturation Kinetics



- [S] << K_m
  - V_0 proportional to [S].
  - More substrate leads to faster reaction.
- [S] >> K_m
  - V_0 not affected by [S].
  - More substrate leads to little increase in reaction rate, because enzyme is saturated.
  - V_0 proportional to [E].

Interpret V_{max} and K_m

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

- V_{max} (maximum velocity): reflect initial reaction rate when virtually all enzyme is present as ES complex
- K_m (Michaelis constant): an approximate measure of substrate's affinity for enzyme

TABLE 6–6 K_m for Some Enzymes and Substrates

Enzyme	Substrate	K_m (mM)
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

Interpret k_{cat}

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

- $V_{max} = k_{cat}\,[E_{total}]$
- k_{cat} (turnover number): how many substrate molecules can one enzyme molecule convert per second

TABLE 6–7 Turnover Number, k_{cat}, of Some Enzymes

Enzyme	Substrate	k_{cat} (s^{-1})
Catalase	H_2O_2	40,000,000
Carbonic anhydrase	HCO_3^-	400,000
Acetylcholinesterase	Acetylcholine	14,000
β-Lactamase	Benzylpenicillin	2,000
Fumarase	Fumarate	800
RecA protein (an ATPase)	ATP	0.5

Compare enzyme efficiency: k_{cat} / K_m

When [S] << Km, left equation reduces to the right form

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

- Upper limit to k_{cat}/K_m imposed by diffusion rates of E and S
- Diffusion-controlled limit is 10^8 - 10^9 $M^{-1}s^{-1}$
- Such enzymes are said to have achieved catalytic perfection

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

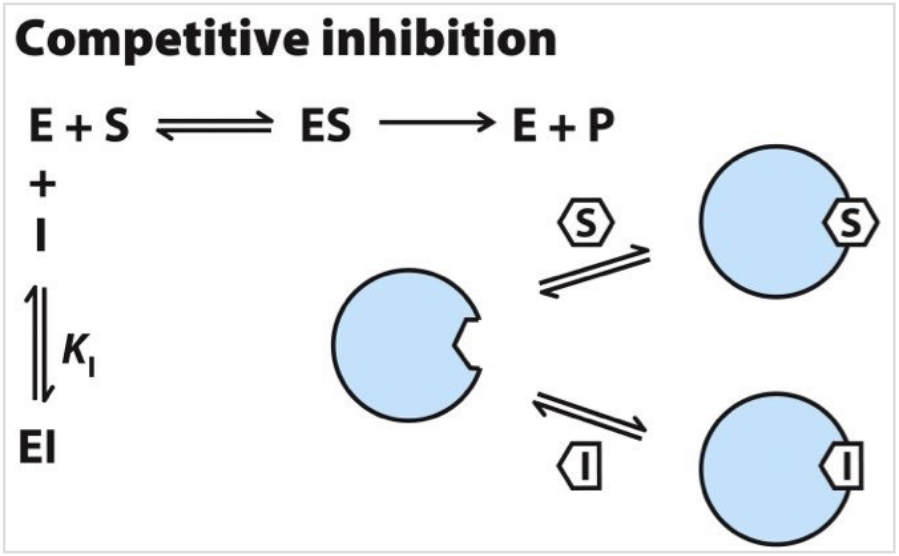
Enzyme Inhibition

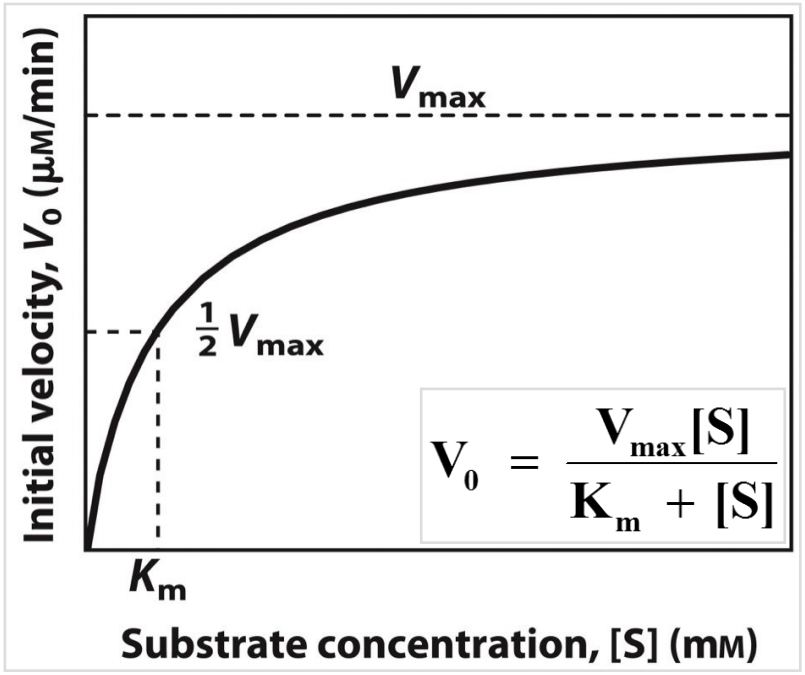
- **Inhibitors** are compounds that decrease enzyme's activity
- Reversible inhibitors bind to and can dissociate from the enzyme
  - Competitive Inhibition.
    - Bind free enzyme and prevent binding of substrate.
  - Uncompetitive Inhibition.
    - Bind enzyme-substrate complex and prevent catalysis.
- Irreversible inhibitors (inactivators) react covalently with enzyme, or form a particularly stable noncovalent association.
  - One inhibitor molecule can permanently shut off one enzyme molecule.

Competitive Inhibition

- Competes with substrate for binding
  - Binds active site reversibly
  - Does not affect catalysis
  - Apparent increase in K_m
  - No change in V_{max}

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



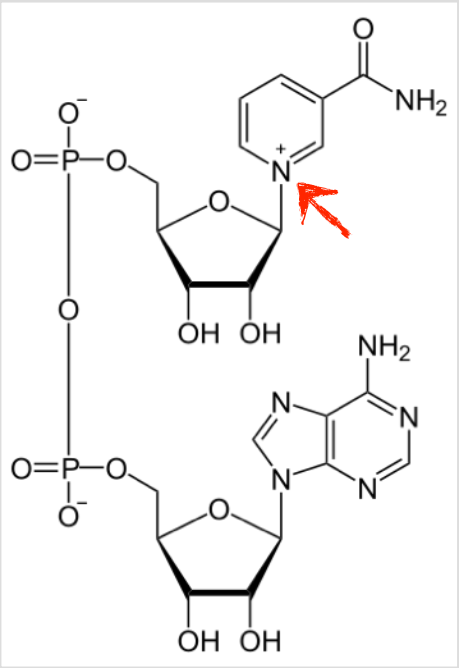


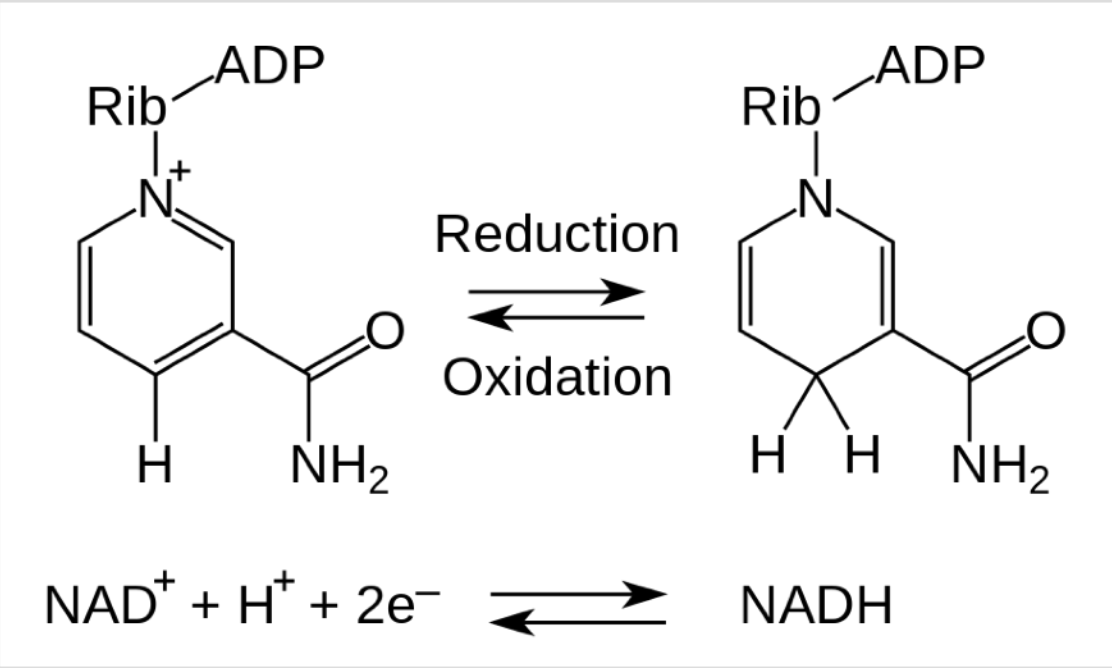
Example of Competitive Inhibition

- Why is methanol toxic?
  - Methanol (CH_3OH) converted to formaldehyde (CH_2O).
  - Formaldehyde causes damages, including blindness.
  - CH_3OH to CH_2O catalyzed by alcohol dehydrogenase (ADH).
- Ethanol competes with methanol.
  - CH_3CH_2OH also binds to active site of ADH.
  - Similar to competitive inhibitor, but also a substrate.
- Methanol detoxification.
  - Slow intravenous infusion of ethanol.
    - Slows formation of formaldehyde.
    - Methanol filtered out by kidney.

Alcohol Dehydrogenase (ADH)

- Interconversion of alcohol and aldehyde or ketone (CH_3CH_2OH -> CH_3CHO).
  - Coenzyme nicotinamide adenine dinucleotide (NAD^+ -> NADH).
  - Break down alcohol in human and other animals.
  - Catalyze opposite reaction in yeast (fermentation).





Alcohol Dehydrogenase (ADH)

- A dimer
  - Have two zinc atoms per subunit.
  - One zinc atom at active site, involved in catalysis.
  - The other zinc plays a structural role, crucial for stability.

Example Questions

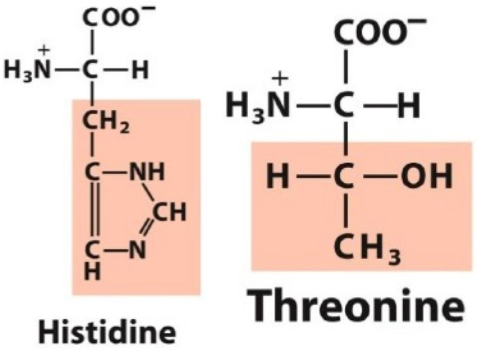
- (True or false) The tertiary structure of alcohol dehydrogenase refers to the spatial arrangement of its two polypeptide chains.
- (True or false) Without zinc atoms, ADH is not stable and not functional.

Example Questions

- Is alcohol dehydrogenase a simple protein or a conjugated protein?
- Is alcohol dehydrogenase a glycoprotein or metalloenzyme?
- Is alcohol dehydrogenase a transferase or oxidoreductase?
- (True or false) Alcohol dehydrogenase catalyzes only conversion of alcohol to aldehyde but not the reverse reaction.
- (True or false) Alcohol dehydrogenase in the absence of NAD^+ is called an apoenzyme.

Catalytic Mechanism of ADH in Human

1. Binding of the coenzyme NAD^+
2. Binding of the alcohol substrate by coordination to zinc.
3. Deprotonation of His-51.
4. Deprotonation of nicotinamide ribose.
5. Deprotonation of Thr-48.
6. Deprotonation of alcohol.
7. Hydride (H^-) transfer to NAD^+, leading to NADH and a zinc bound aldehyde or ketone.
8. Release of the product.



(True or false) ADH has an active site that is completely complementary to substrate alcohol.

Catalytic Mechanism of ADH in Human

1. Binding of the coenzyme NAD^+
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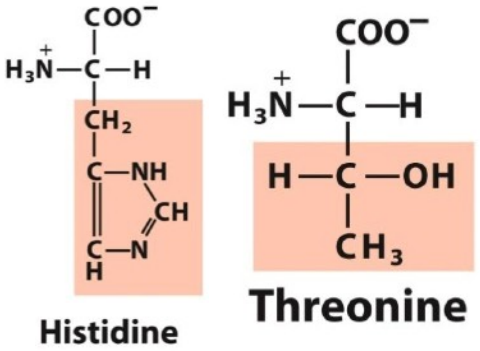

(True or false) ADH shifts the equilibrium of reaction (alcohol -> aldehyde) to the right.

Catalytic Mechanism of ADH in Human

1. Binding of the coenzyme NAD^+
2. Binding of the alcohol substrate by coordination to zinc.
3. Deprotonation of His-51.
4. Deprotonation of nicotinamide ribose.
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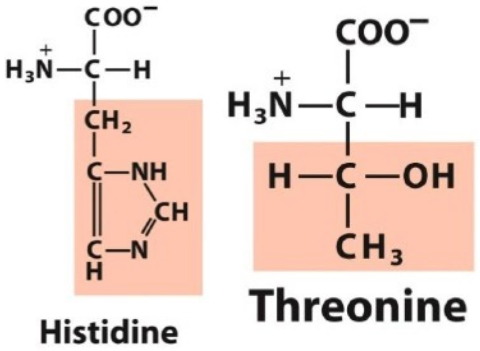

(True or false) Interaction of ADH binding to substrate alcohol contributes to a lower activation energy.

Catalytic Mechanism of ADH in Human

1. Binding of the coenzyme NAD^+
2. Binding of the alcohol substrate by coordination to zinc.
3. Deprotonation of His-51.
4. Deprotonation of nicotinamide ribose.
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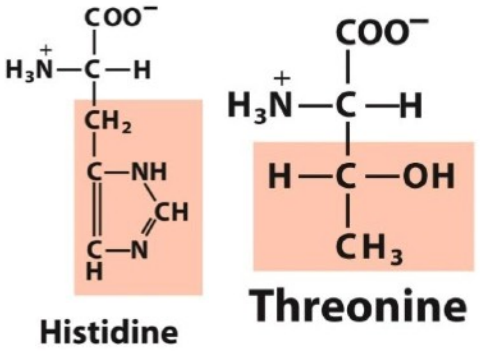
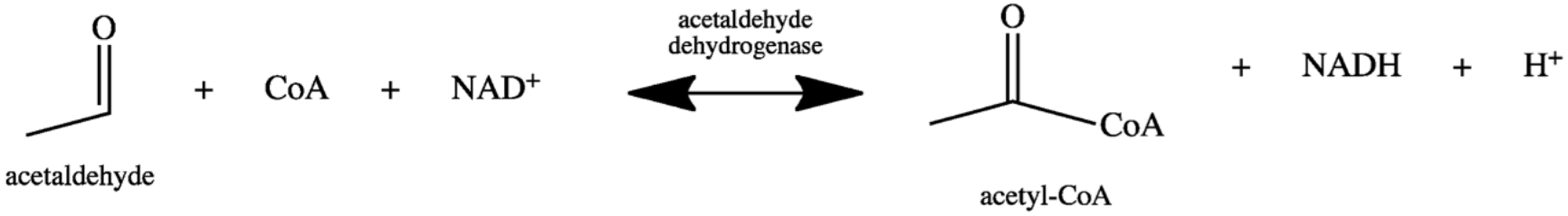


(True or false) ADH catalysis involves general acid-base catalysis.

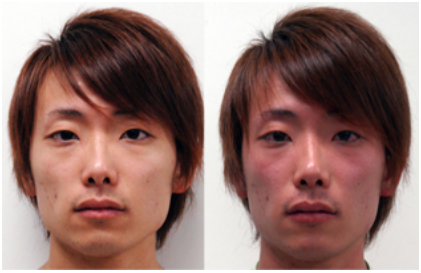
Acetaldehyde Dehydrogenase (ALDH)

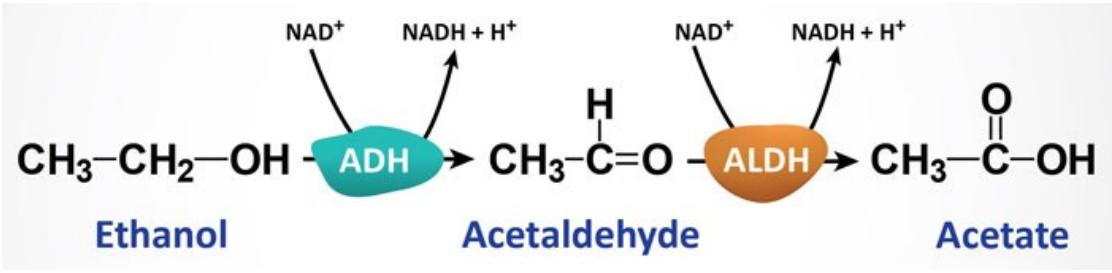
- Interconversion of acetaldehyde and acetic acid (CH_3CHO -> CH_3COOH)
  - When ALDH not active enough, acetaldehyde accumulates.
  - Acetaldehyde poisoning, including flushing of skin, increased heart rate, shortness of breath, nausea, vomiting, headache, visual disturbance, and mental confusion.
- Drug disulfiram (Antabuse)
  - ALDH inhibitor.
  - Covalent binding to a catalytically important Cys residue.
  - Used to treat alcoholism by producing unpleasant effects.



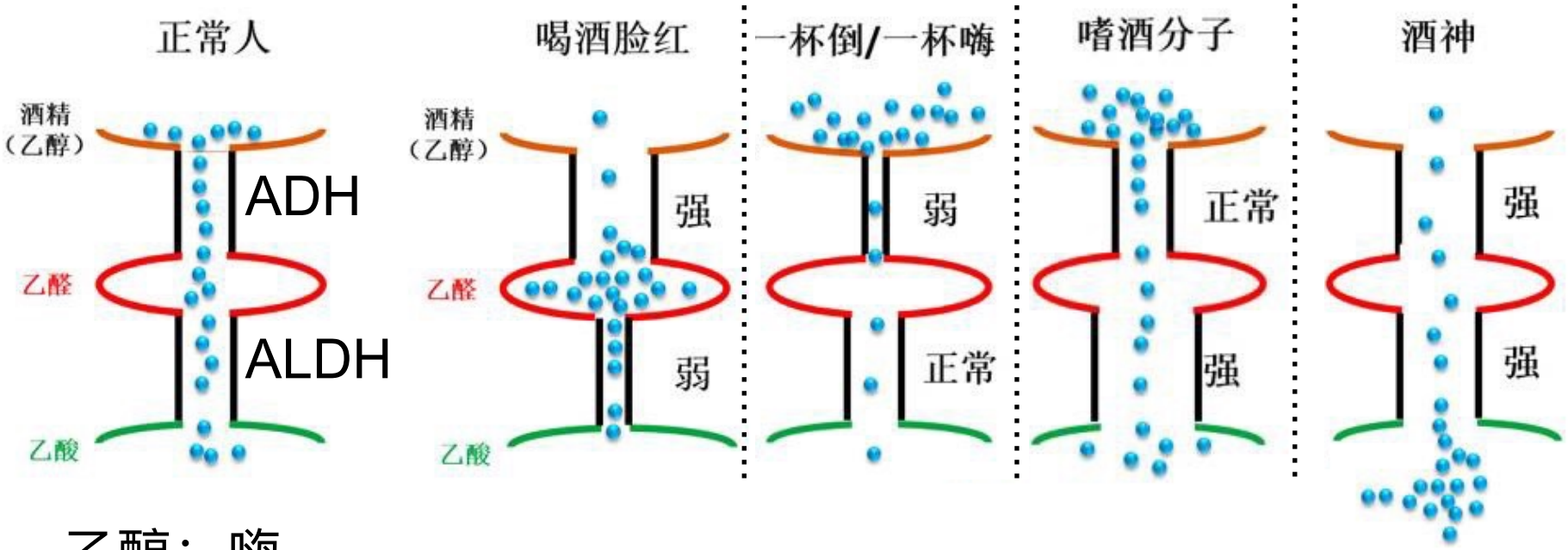
ALDH Deficiency (Alcohol Flush)

- Flush after consuming alcohol.
  - Face, neck, shoulders, and in some cases, entire body.
  - Result of accumulation of acetaldehyde.
  - Caused by ALDH deficiency.
- Also called Asian Flush.
  - 1/3 East Asians (Chinese, Japanese, and Koreans).
  - Less prone to alcoholism.
  - More active ADH and less functional ALDH.





ALDH Deficiency (Alcohol Flush)



乙醇：嗨

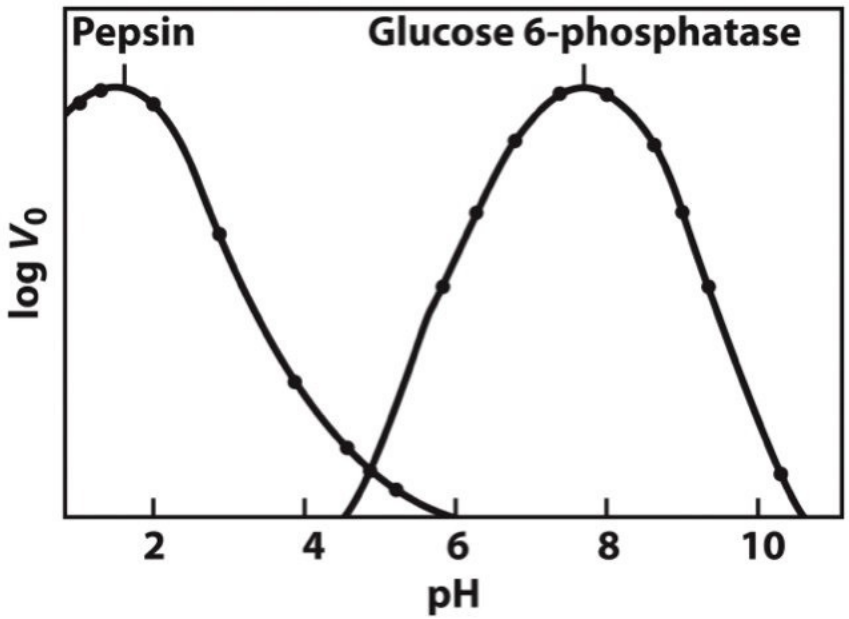
乙醛：毒

乙酸：醋

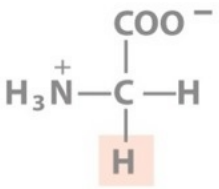
易红易吐　易醉易疯　易瘾易毒　易进易出

Enzyme Activity Depends on pH

- Optimal pH.
  - Activity is maximal.
  - At higher or lower pH, activity decreases.
- pH optimum generally close to pH where enzyme is found.
  - Pepsin optimum pH about 2.
  - Glucose 6-phosphatase optimum pH about 8.

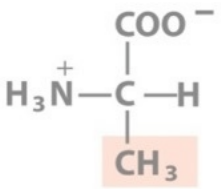


- pH in stomach is about 2.
- pH in liver cells is about 8.



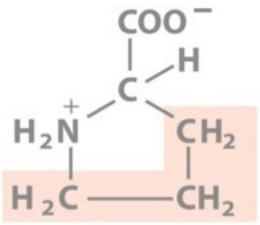
Glycine

Gly, G



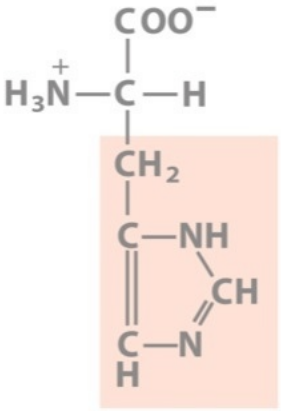
Alanine

Ala, A



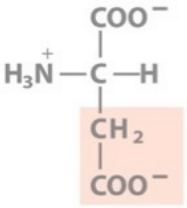
Proline

Pro, P

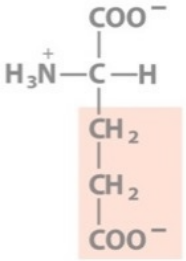


Histidine

His, H

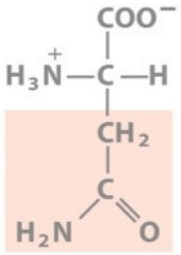


Aspartate

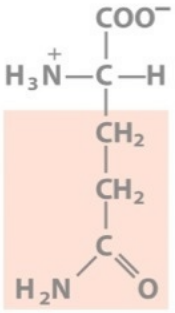


Glutamate

Asp, D Glu, E



Asparagine

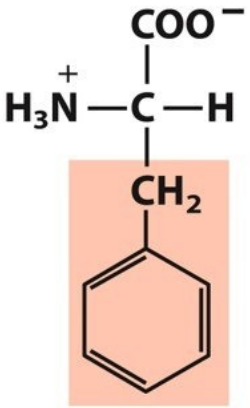


Glutamine

Asn, N Gln, Q

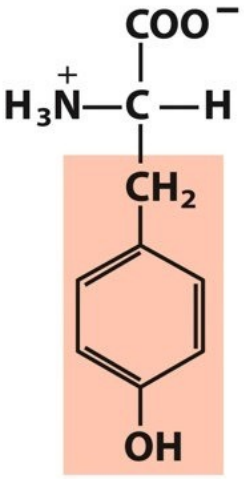
Amino acid

for 5th week



Phe, F

Phenylalanine



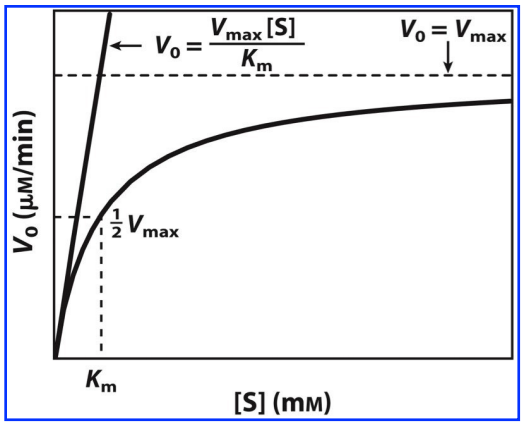
Tyrosine

Tyr, Y

Summary 6.3 Enzymes Kinetics

- As [S] increases, initial reaction rate increases in a hyperbolic fashion, approaching V_{max}
- Michaelis constant K_m is the substrate concentration at which reaction rate is equal to one-half V_{max}
- Competitive inhibitors compete with substrate by binding reversibly to active site. Every enzyme has an optimum pH (or pH range)

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



Example Question

The following data were obtained in a study of an enzyme known to follow Michaelis-Menten kinetics:

V_0 (μmol/min)	Substrate added (mmol/L)
217	0.8
325	2
433	4
488	6
647	1,000

The K_m for this enzyme is approximately:

A) 1 mM.
B) 1000 mM.
C) 2 mM.
D) 4 mM.
E) 6 mM.

Example Question

An enzyme-catalyzed reaction was carried out with the substrate concentration initially 1000 times greater than the K_m for that substrate. After 9 minutes, 1% of the substrate had been converted to product, and the amount of product formed in the reaction mixture was 12 μmol. If, in a separate experiment, one-third as much enzyme and twice as much substrate had been combined, how long would it take for the same amount (12 μmol) of product to be formed?

A) 1.5 min

B) 13.5 min

C) 27 min

D) 3 min

E) 6 min