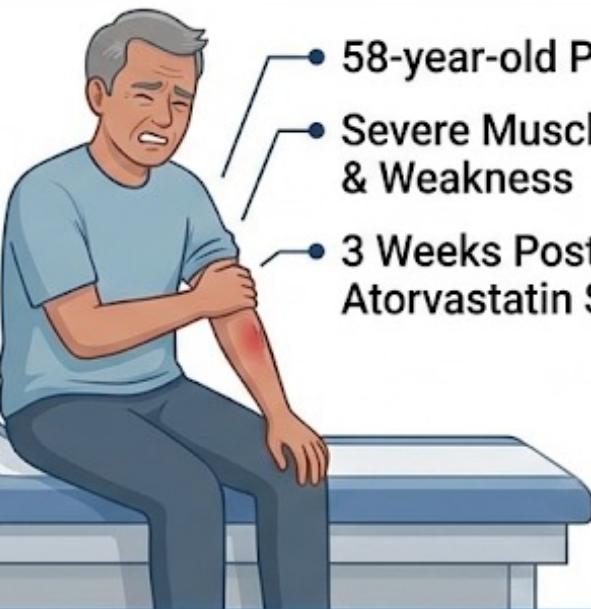


Announcements.

- Thoughts on how to study
- Final exam dates are out (May 1st or 4th)
- Recording the lecture for next Tuesday.
- I had slide decks from the textbook maker.
- Quick comment on procedure for taking the exam next Tuesday.
 - Bringing ID.
 - 4 function calculator.
 - Room assignments according to pod.
 - Literally cutting corners.

CLINICAL CASE STUDY: THE STATIN MYSTERY

PATIENT PRESENTATION



- 58-year-old Patient
- Severe Muscle Pain & Weakness
- 3 Weeks Post-Atorvastatin Start



KEY LAB FINDING

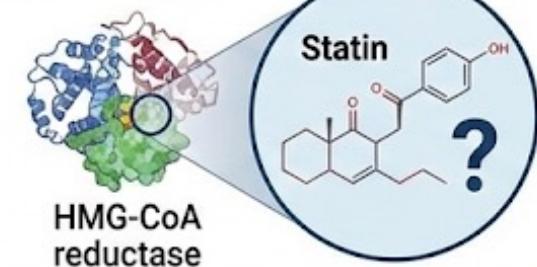


CK (Creatine Kinase) Levels:
ELEVATED 10-FOLD



WHAT'S HAPPENING?

The answer lies
at the molecular
level.



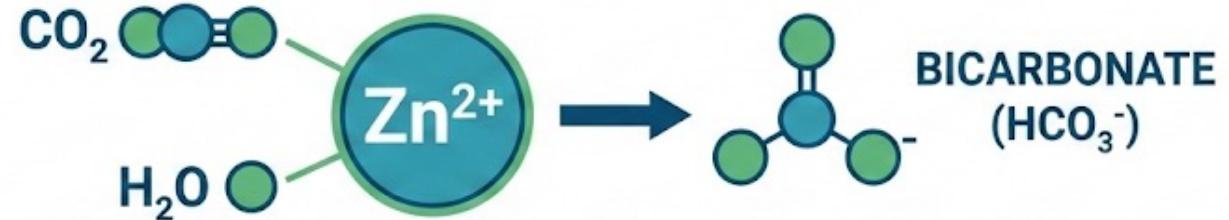
HMG-CoA
reductase

By the end of this lecture, you'll understand the **drug-enzyme interaction** causing **rhabdomyolysis** and why **enzyme kinetics** is essential for every prescription. 

CHAPTER 7

Basic Concepts of Enzyme Action

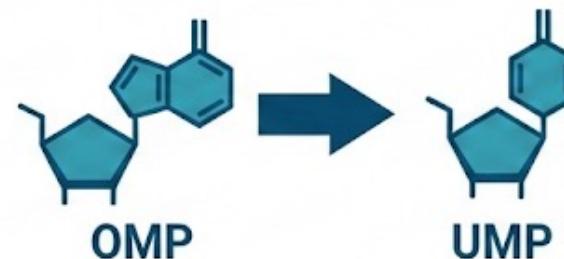
CARBONIC ANHYDRASE



PROTEASE



OMP DECARBOXYLASE



RATE ENHANCEMENT:
 10^{17}

Chapter 7: Outline

7.1 Enzymes Are Powerful and Highly Specific Catalysts

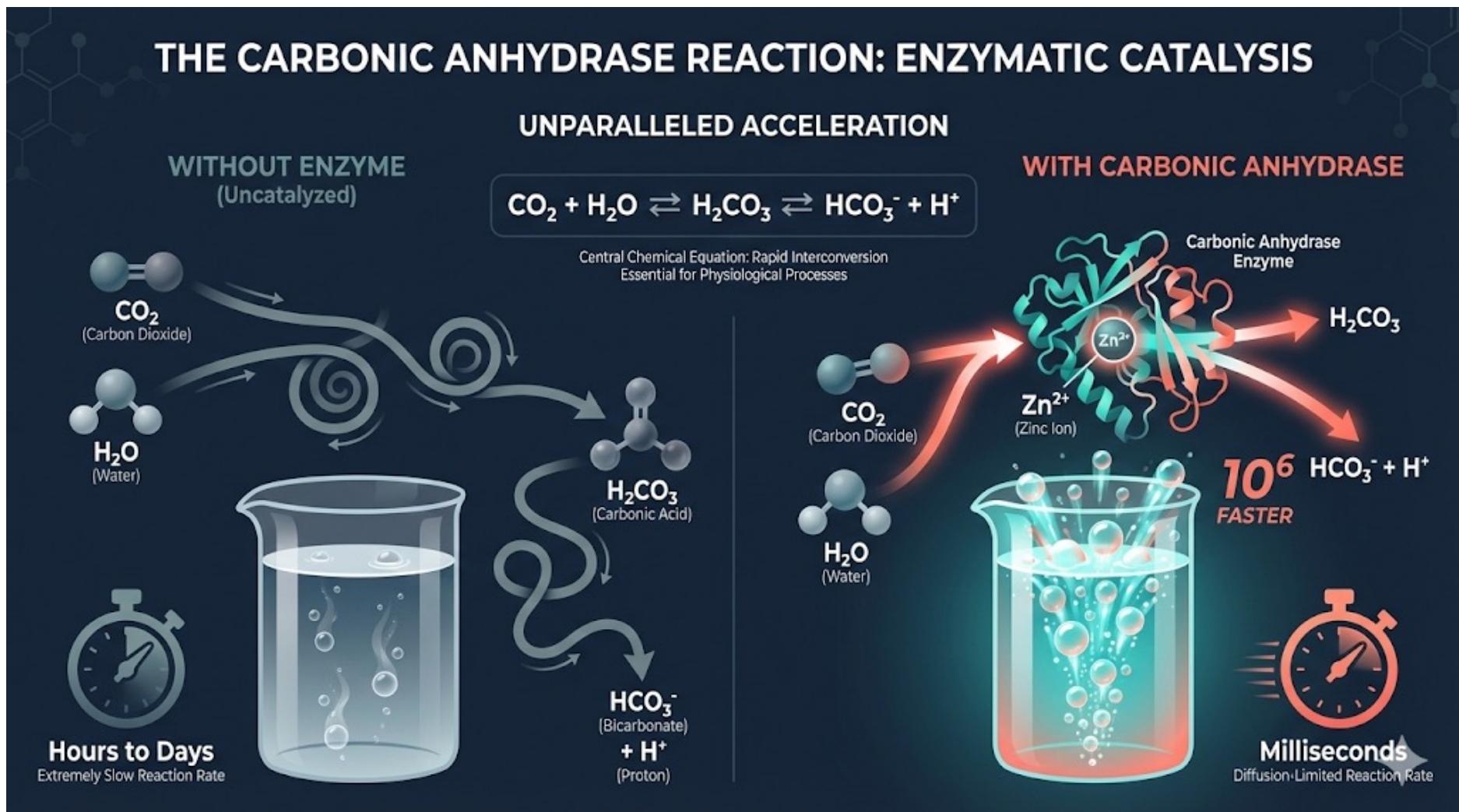
7.2 Many Enzymes Require Cofactors for Activity

7.3 Gibbs Free Energy Is a Useful Thermodynamic Function for Understanding Enzymes

7.4 Enzymes Facilitate the Formation of the Transition State

Section 7.1 Enzymes Are Powerful and Highly Specific Catalysts

- Enzymes are protein catalysts that can accelerate the rate of a reaction by factors of as much as a million or more.



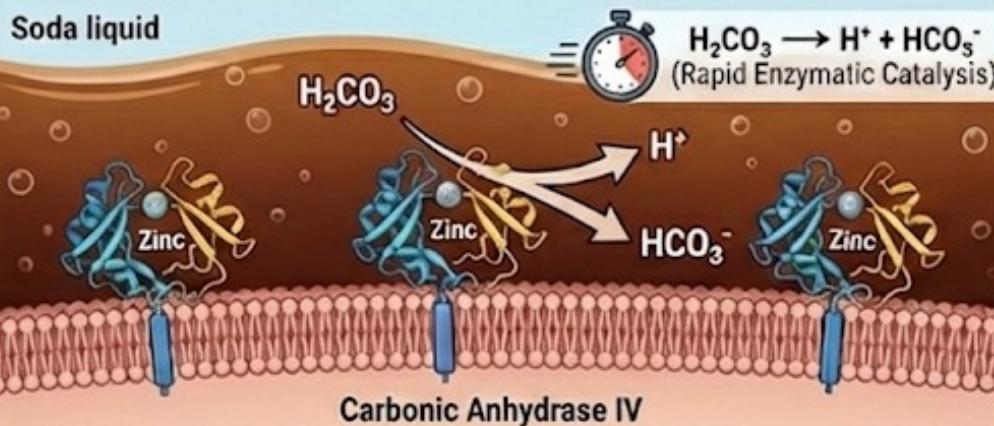
THE SODA FIZZ PHENOMENON: FROM PRESSURE TO ENZYMATIC SENSORY BURST

1. CARBONATION UNDER PRESSURE (Physics & Chemistry)

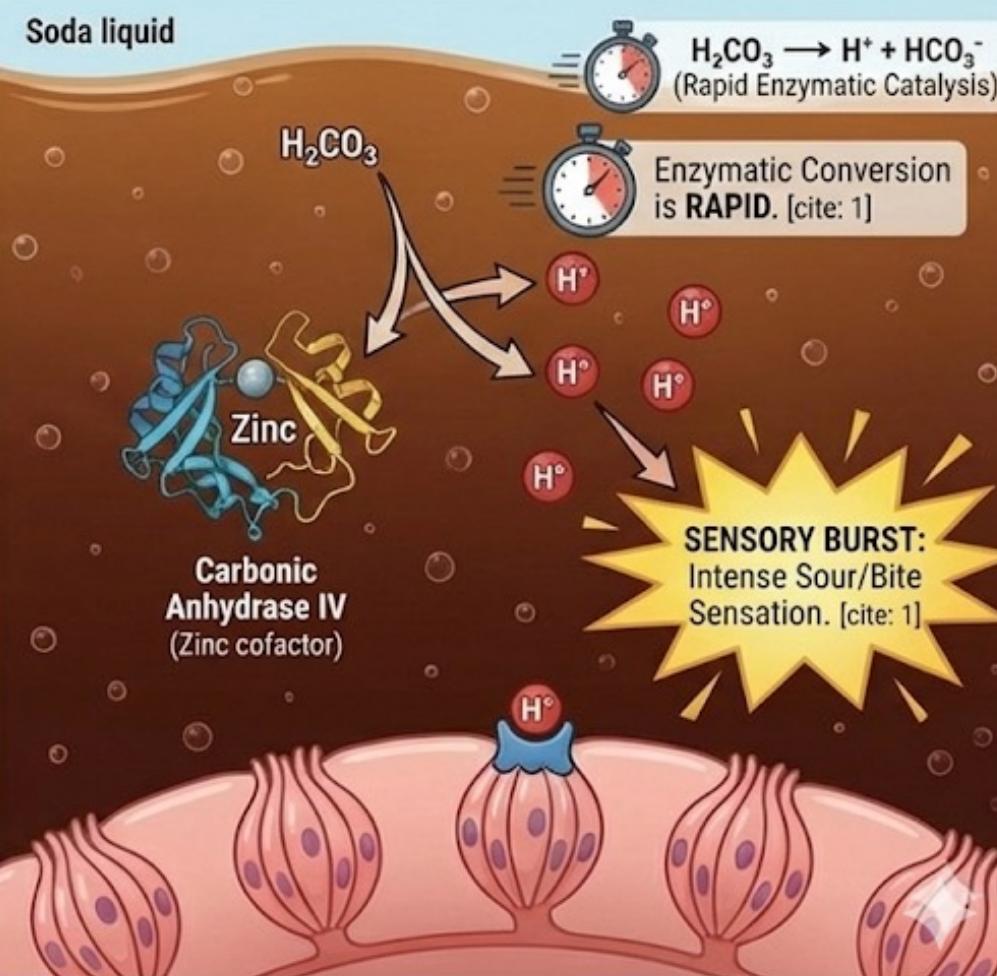
High pressure forces CO_2 into solution;
 H_2CO_3 is a minor component.



3. ON TONGUE: ENZYMATIC ACCELERATION (Biochemistry)



2. ON TONGUE: ENZYMATIC ACCELERATION



How much does an enzyme speed up a reaction? A lot

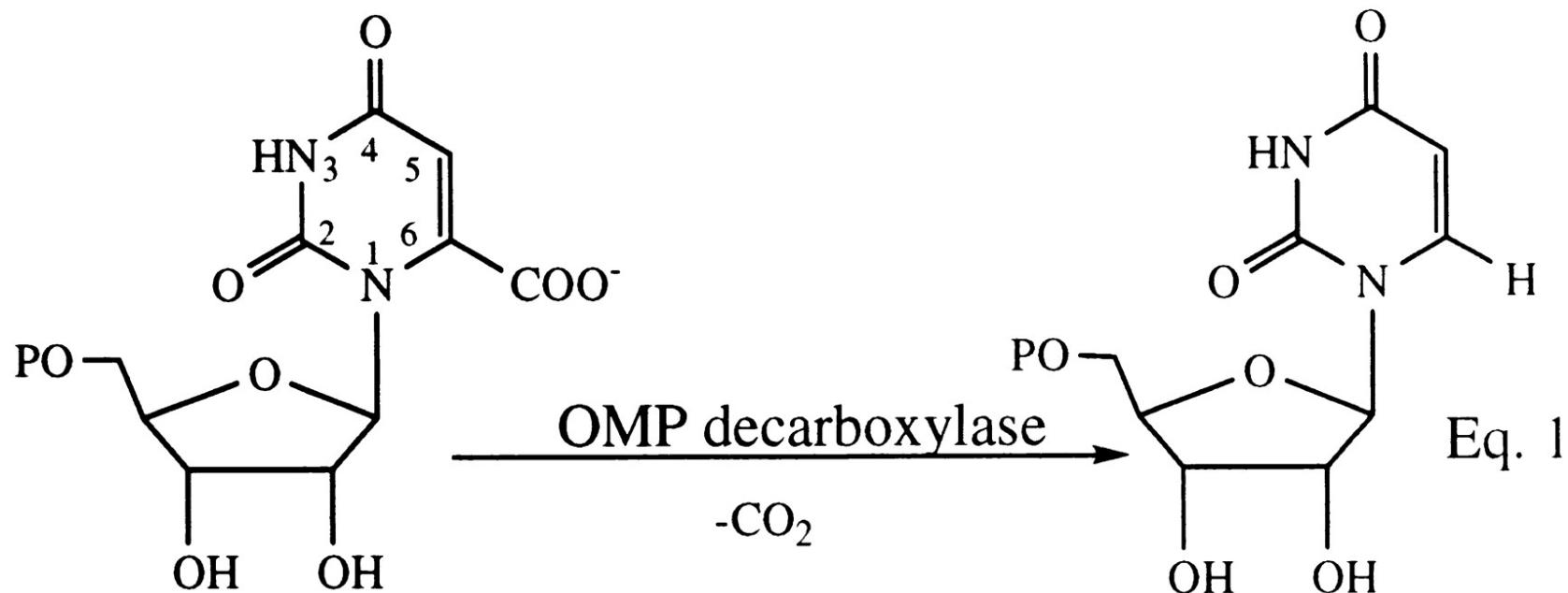


TABLE 6.1 Rate enhancement by selected enzymes

Enzyme	Nonenzymatic half-life years	Uncatalyzed rate ($k_{un} s^{-1}$)	Catalyzed rate ($k_{cat} s^{-1}$)	Rate enhancement ($k_{cat} s^{-1} / k_{un} s^{-1}$)
OMP decarboxylase	78,000,000 years	2.8×10^{-16}	39	1.4×10^{17}

What is the rate enhancement of an enzyme with an uncatalyzed rate of $2.9 \times 10^{-6} s^{-1}$ and a catalyzed rate of $540 s^{-1}$?

- A. 1.0×10^6
- B. 1.9×10^8
- C. 1.9×10^9
- D. 1.9×10^{11}



THE MOST IMPRESSIVE NUMBER IN BIOLOGY

78 MILLION YEARS



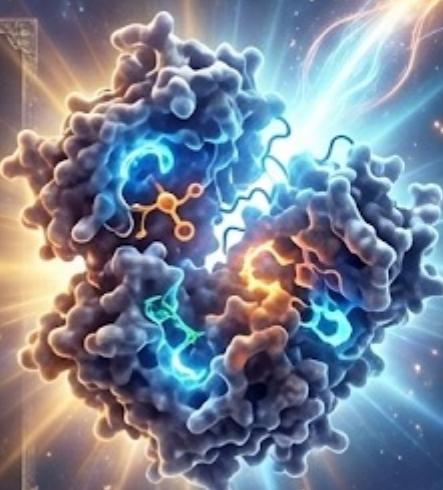
DINOSAURS
EXTINCTION
(~66 MYA)



EARLY HUMANS



PYRAMIDS BUILT
(~4,500 YA)



MILLISECONDS

00:00:00.020

10¹⁷ RATE
ENHANCEMENT

Uncatalyzed vs. Catalyzed
Reaction

OMP decarboxylase

Table of Rate Enhancement by Selected Enzymes

TABLE 6.1 Rate enhancement by selected enzymes

Enzyme	Nonenzymatic half-life	Uncatalyzed rate ($k_{\text{un}} \text{ s}^{-1}$)	Catalyzed rate ($k_{\text{cat}} \text{ s}^{-1}$)	Rate enhancement ($k_{\text{cat}} \text{ s}^{-1}/k_{\text{un}} \text{ s}^{-1}$)
OMP decarboxylase	78,000,000 years	2.8×10^{-16}	39	1.4×10^{17}
Staphylococcal nuclease	130,000 years	1.7×10^{-13}	95	5.6×10^{14}
AMP nucleosidase	69,000 years	1.0×10^{-11}	60	6.0×10^{12}
Carboxypeptidase A	7.3 years	3.0×10^{-9}	578	1.9×10^{11}
Ketosteroid isomerase	7 weeks	1.7×10^{-7}	66,000	3.9×10^{11}
Triose phosphate isomerase	1.9 days	4.3×10^{-6}	4300	1.0×10^9
Chorismate mutase	7.4 hours	2.6×10^{-5}	50	1.9×10^6
Carbonic anhydrase	5 seconds	1.3×10^{-1}	1×10^6	7.7×10^6

Abbreviations: OMP, orotidine monophosphate; AMP, adenosine monophosphate. Source: Data from A. Radzicka and R. Wolfenden, *Science* 267:90–93, 1995.

Tymoczko et al., *Biochemistry: A Short Course*, 4e, © 2019 W. H. Freeman and Company

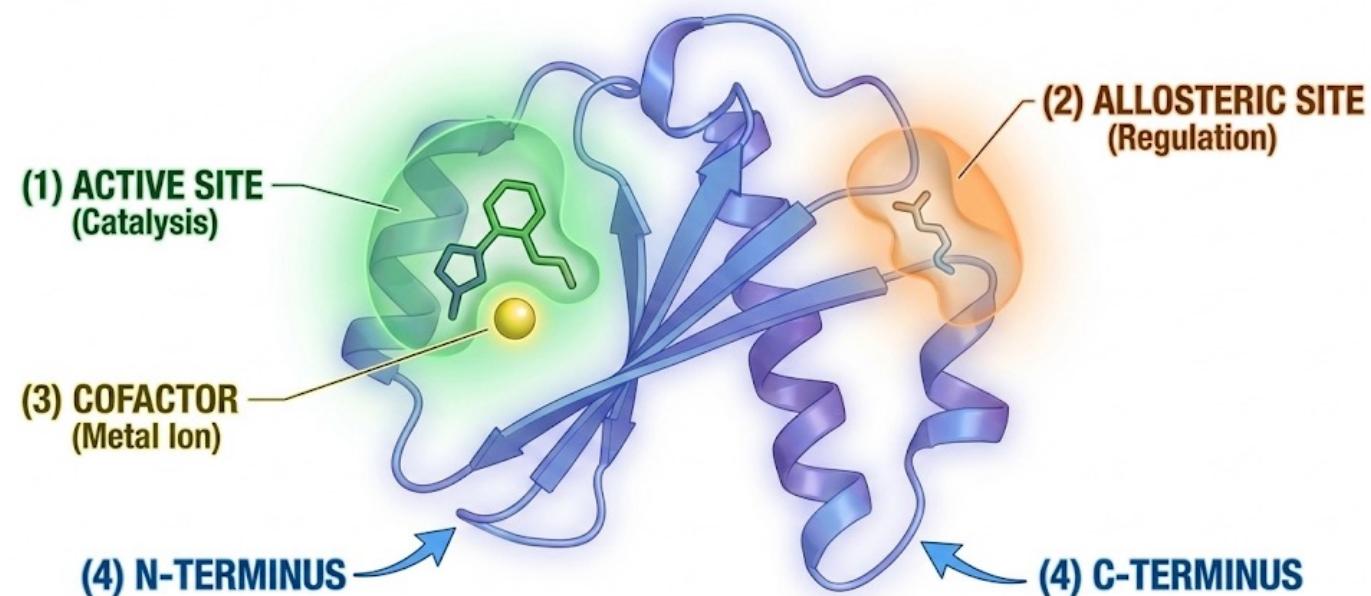
Catalysts speed up chemical reactions without being consumed by them.

Enzymes are powerful biological catalysts.

- They are made by all living organisms.
- They dramatically enhance and control the rates of chemical reactions.
- They are almost all proteins.

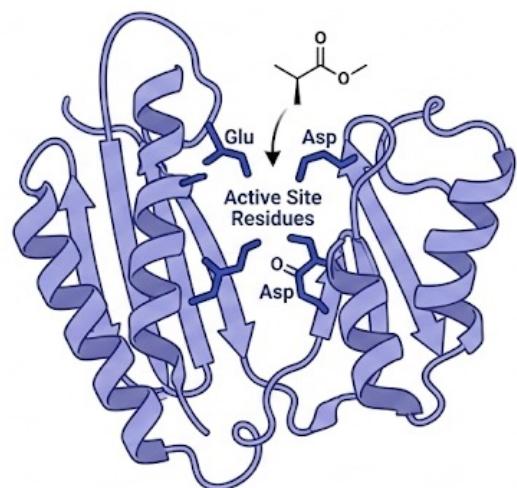
The discovery of catalytically active RNA molecules, called **ribozymes**, provides compelling evidence that RNA was a biocatalyst early in evolution.

ENZYME STRUCTURE AND FUNCTIONAL REGIONS

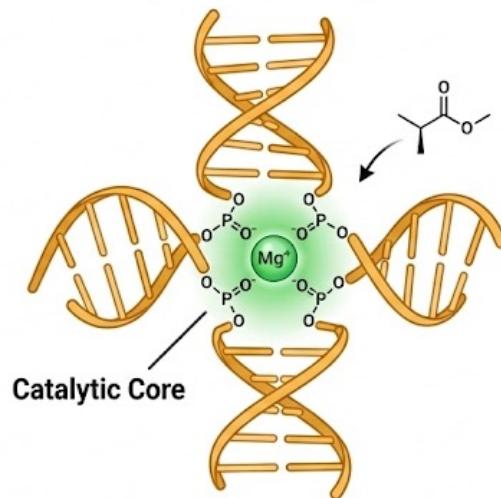


CATALYTIC COMPARISON: PROTEIN vs. RNA

PROTEIN ENZYME
(Lysozyme)



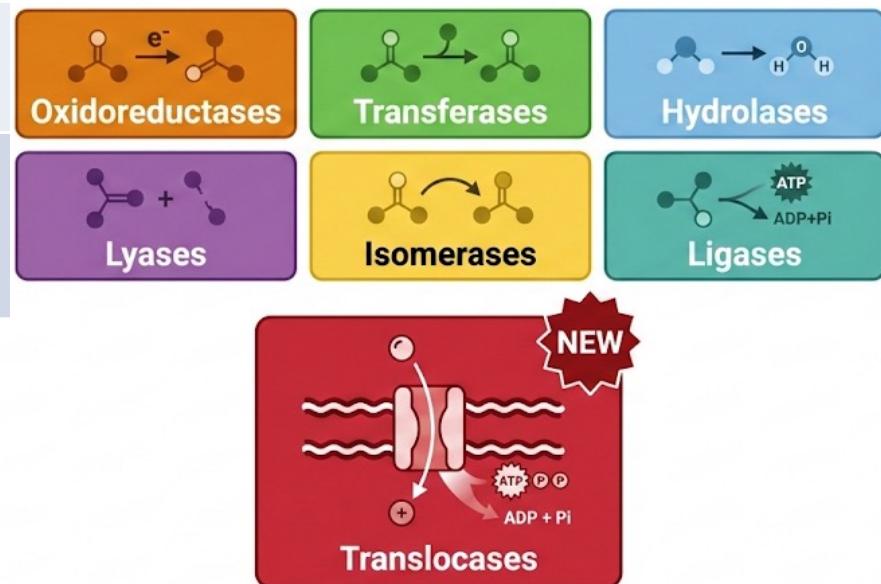
RIBOZYME
(Hammerhead)



'Catalysis requires precise 3D positioning—not necessarily protein.'

There Are Six 7 Major Classes of Enzymes

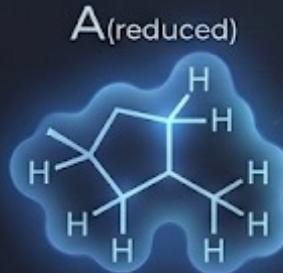
Class	Type of reaction
1. Oxidoreductases	Oxidation–reduction
2. Transferases	Group transfer
3. Hydrolases	Hydrolysis reactions (transfer of functional groups to water)
4. Lyases	Addition or removal of groups to form double bonds
5. Isomerases	Isomerization (intramolecular group transfer)
6. Ligases	Ligation of two substrates at the expense of ATP hydrolysis
7. Translocases	Movement of ions or molecules across membranes or within membranes



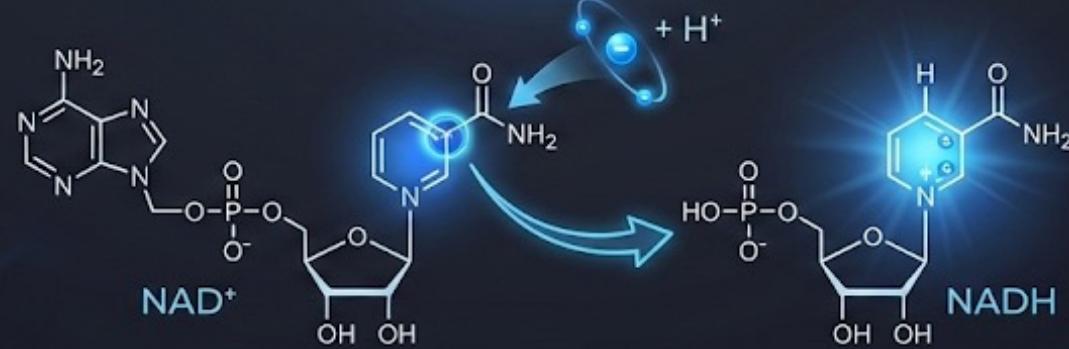
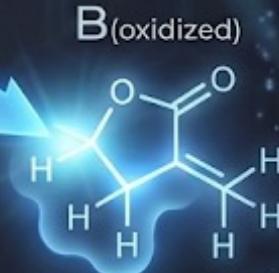
OXIDOREDUCTASES



EC 1.x.x.x



A(reduced)



Example: Lactate Dehydrogenase

These enzymes transfer electrons between molecules. In other words, these enzymes catalyze oxidation-reduction reactions.

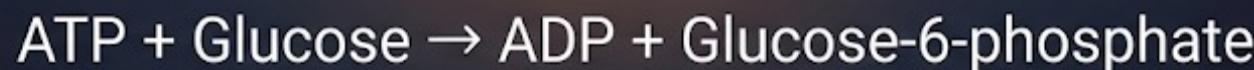
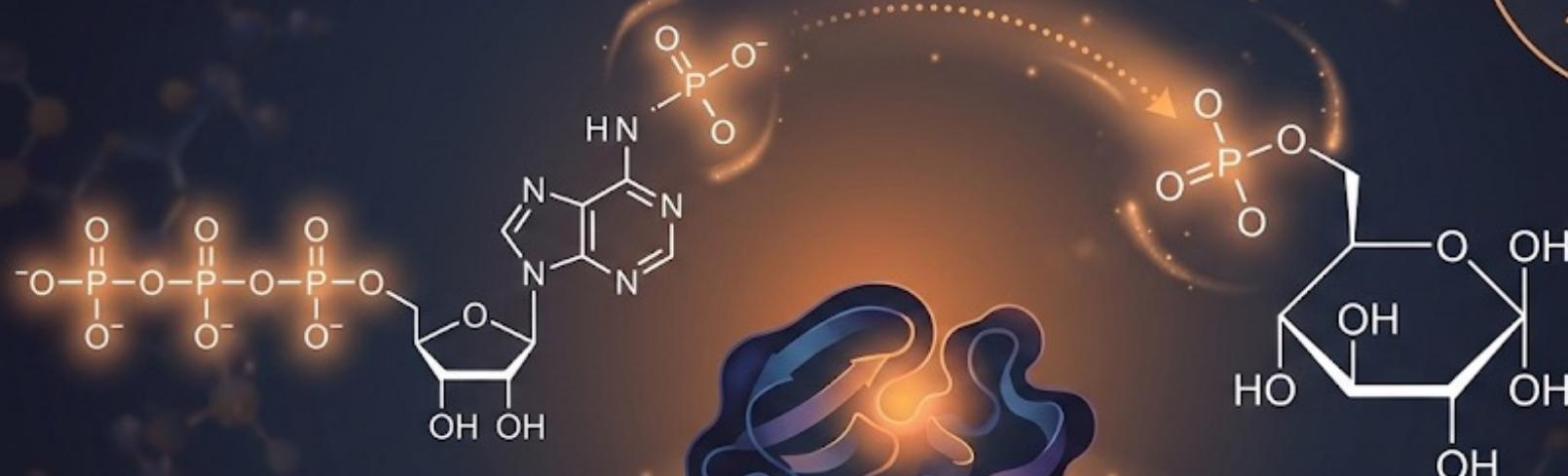
The Importance of Oxidoreductase: Elephant toothpaste

- **A Fun Example of an oxidoreductase: Catalase**
- Catalase is present in the peroxisomes of eukaryotic cells and serves to detoxify hydrogen peroxide (H_2O_2), a potentially harmful by-product of cellular metabolism. The enzyme catalyzes the decomposition of hydrogen peroxide into water and oxygen gas:
 $2H_2O_2 \rightarrow 2H_2O + O_2$
- **Fun Experiment:** One fun demonstration involving catalase is the "Elephant Toothpaste" experiment. When a sample rich in catalase (like liver puree or yeast solution) is mixed with hydrogen peroxide and a drop of dish soap, the rapid decomposition of hydrogen peroxide produces oxygen gas. The soap catches this oxygen, creating a large foam resembling toothpaste big enough for an "elephant."



TRANSFERASES

EC 2.x.x.x



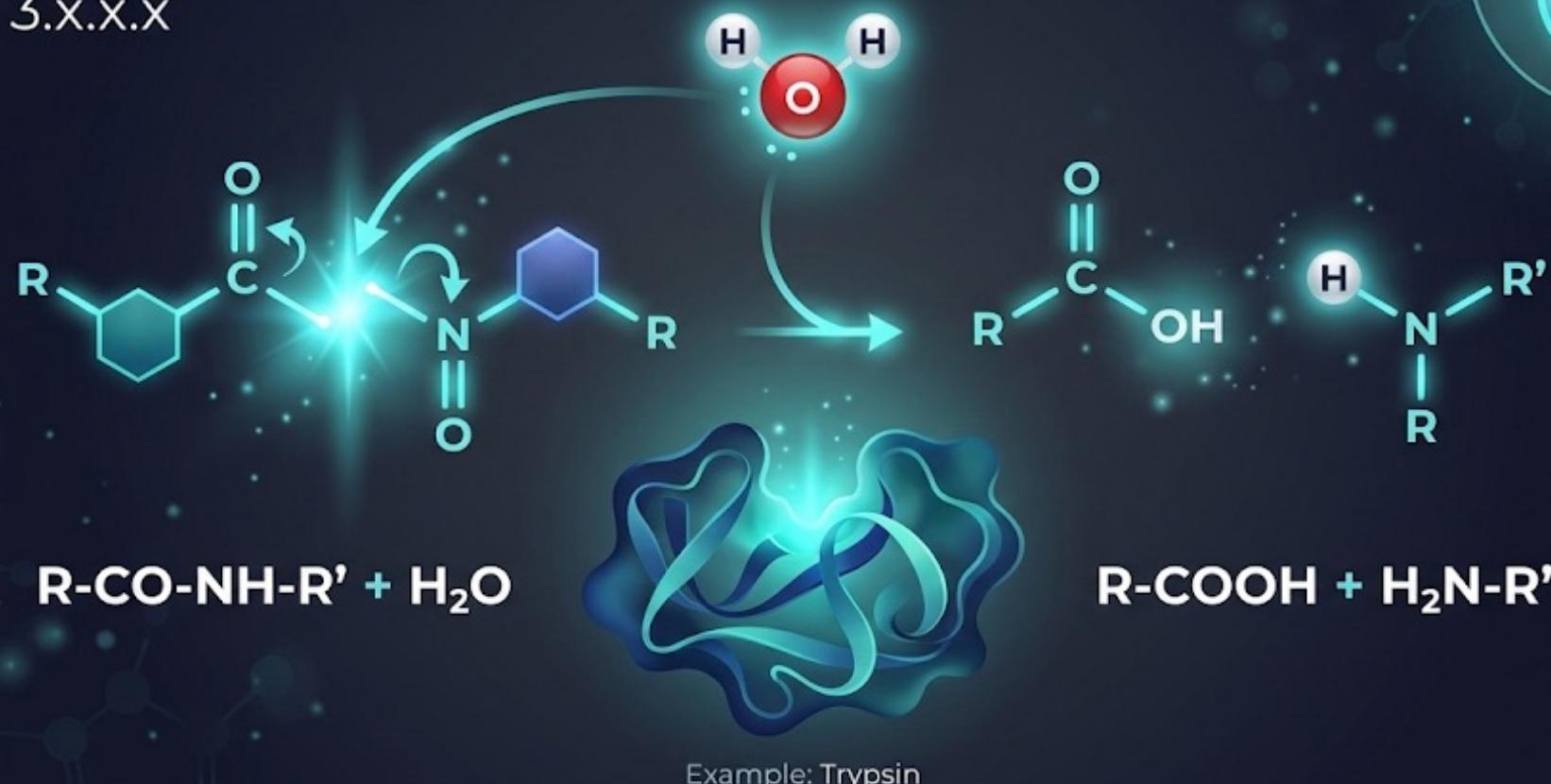
Example: Hexokinase

These enzymes transfer functional groups between molecules. Aminotransferases are prominent in amino acid synthesis and degradation, where they shuffle amine groups between donor and acceptor molecules.



HYDROLASES

EC 3.x.x.x



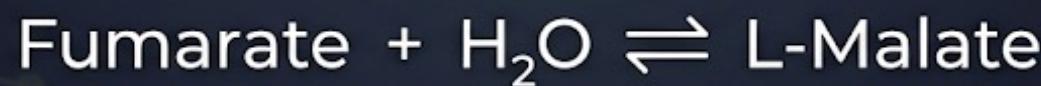
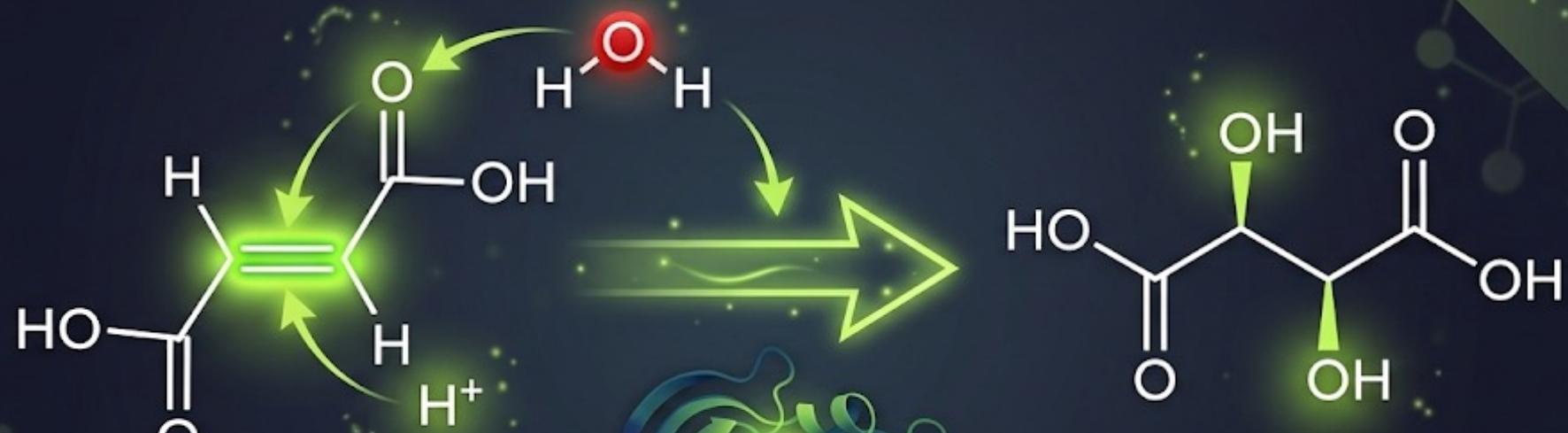
Example: Trypsin

A hydrolase cleaves molecules by the addition of water. Trypsin, the proteolytic enzyme already discussed, is a hydrolase.



LYASES

EC 4.x.x.x

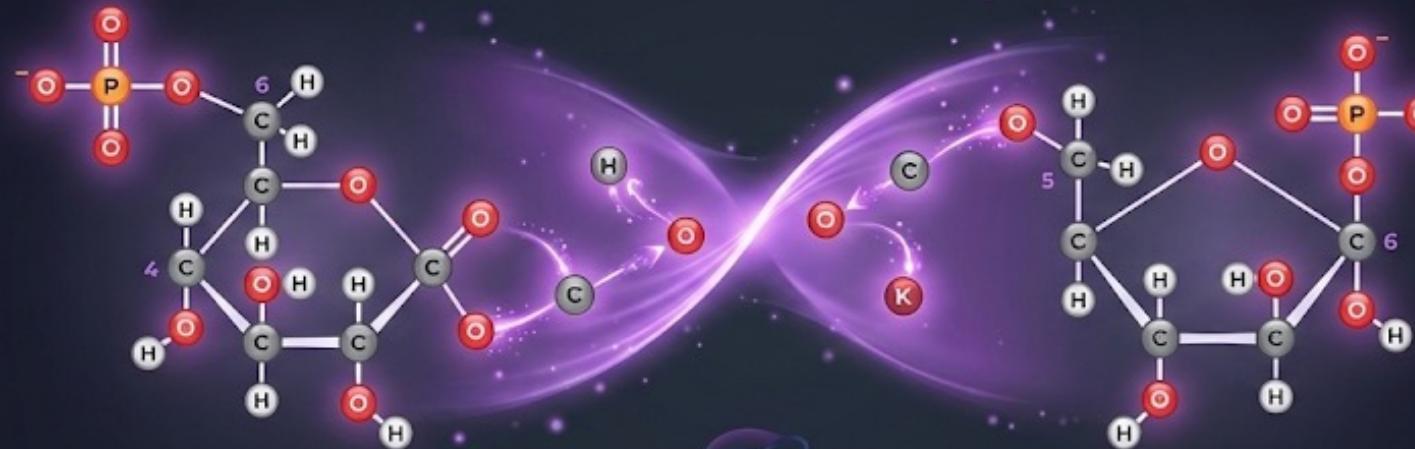
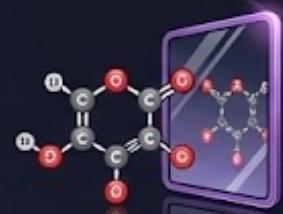


Example: Fumarase

A lyase adds atoms or functional groups to a double bond or removes them to form double bonds. Does not usually require extra energy.

ISOMERASES

EC 5.X.X.X



Glucose-6-phosphate

Fructose-6-phosphate

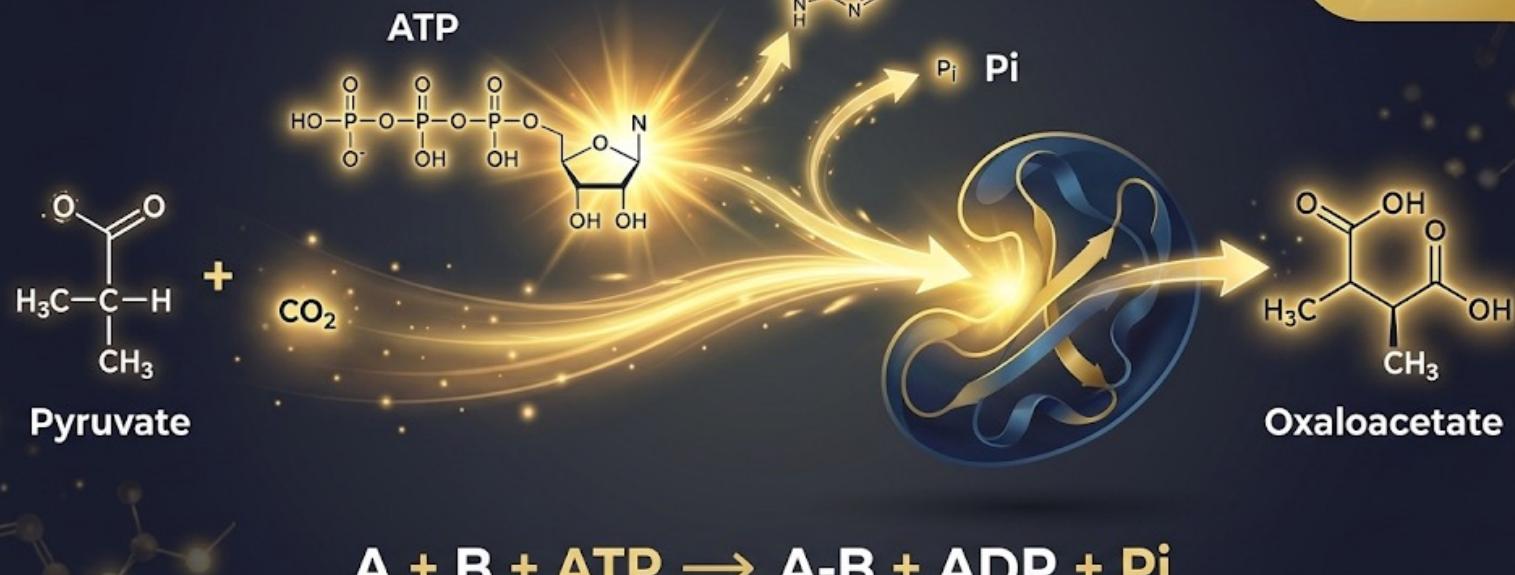


Example: Phosphoglucomutase

These enzymes move functional groups within a molecule. We will meet triose phosphate isomerase in glycolysis.

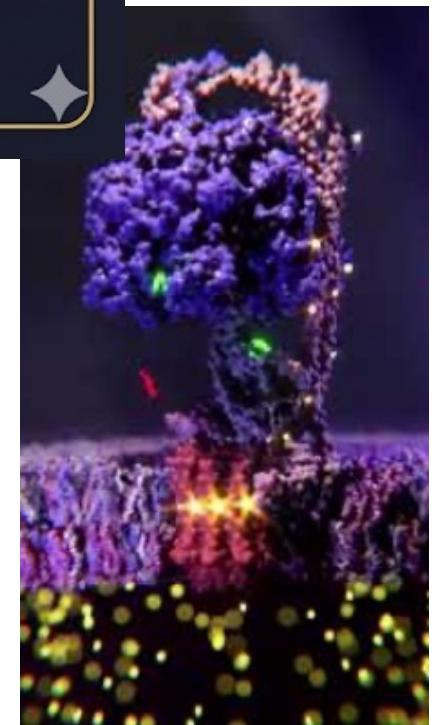
LIGASES

EC 6.x.x.x



Example: Pyruvate Carboxylase

Ligases join two molecules in a reaction powered by ATP hydrolysis. DNA ligase, an important enzyme in DNA replication, is representative of this class. ATP synthase shows that enzyme classification is based on the reaction mechanism, not physiological function.



ATP Synthase

EC naming convention

1. Oxidoreductase

- 1.1 Acting on the CH-OH group of donors
 - 1.1.1 With NAD⁺ or NADP⁺ as acceptor
 - 1.1.1.1 alcohol dehydrogenase (NAD)
 - 1.1.1.2 alcohol dehydrogenase (NADP⁺)
 - 1.1.2 With a cytochrome as acceptor
 - 1.1.3 With oxygen as acceptor
- 1.2 Acting on the aldehyde or oxo group of donors
 - 1.2.1 With NAD⁺ or NADP⁺ as acceptor
 - 1.2.2 With a cytochrome as acceptor
 - 1.2.3 With oxygen as acceptor

2. Transferase

- 2.1 Transferring one-carbon groups
 - 2.1.1 Methyltransferases
 - 2.1.2 Hydroxymethyl-, formyl- and related transferases
 - 2.1.3 Carboxy- and carbamoyltransferases
- 2.2 Transferring aldehyde or ketonic groups
- 2.3 Acyltransferases
 - 2.3.1 Transferring groups other than aminoacyl groups
 - 2.3.2 Aminoacyltransferases

3. Hydrolase

- 3.1 Acting on ester bonds
- 3.2 Glycosylases

4. Lyase

- 4.1 Carbon-carbon lyases
- 4.2 Carbon-oxygen lyases

5. Isomerase

- 5.1 Racemases and epimerases
- 5.2 cis-trans-Isomerases

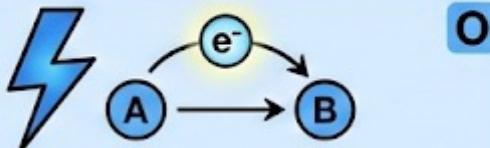
6. Ligase

- 6.1 Forming carbon-oxygen bonds
- 6.2 Forming carbon-sulfur bonds

THE SIX ENZYME CLASSES

Old Tiresome Harold Loves Indoor Lamps

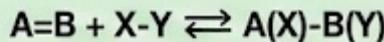
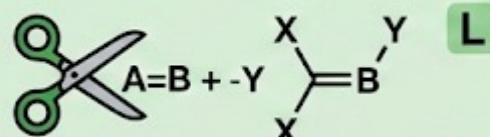
Class 1: OXIDOREDUCTASES



Example: Lactate dehydrogenase

Key phrase: Electron transfer.

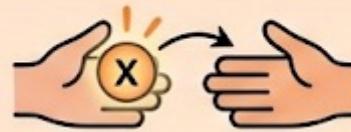
Class 4: LYASES



Example: Fumarase

Key phrase: Add/remove from double bonds.

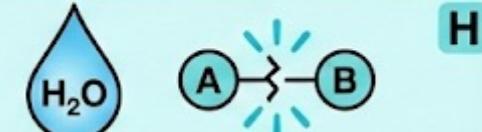
Class 2: TRANSFERASES



Example: Hexokinase

Key phrase: Group transfer.

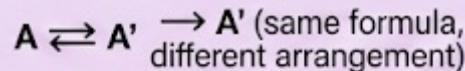
Class 3: HYDROLASES



Example: Trypsin

Key phrase: Water breaks bonds.

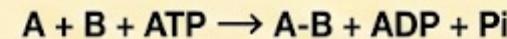
Class 5: ISOMERASES



Example: Phosphogluucose isomerase

Key phrase: Rearrangement

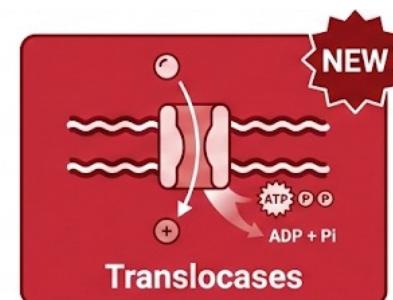
Class 6: LIGASES



Example: DNA ligase

Key phrase: Join with ATP energy.

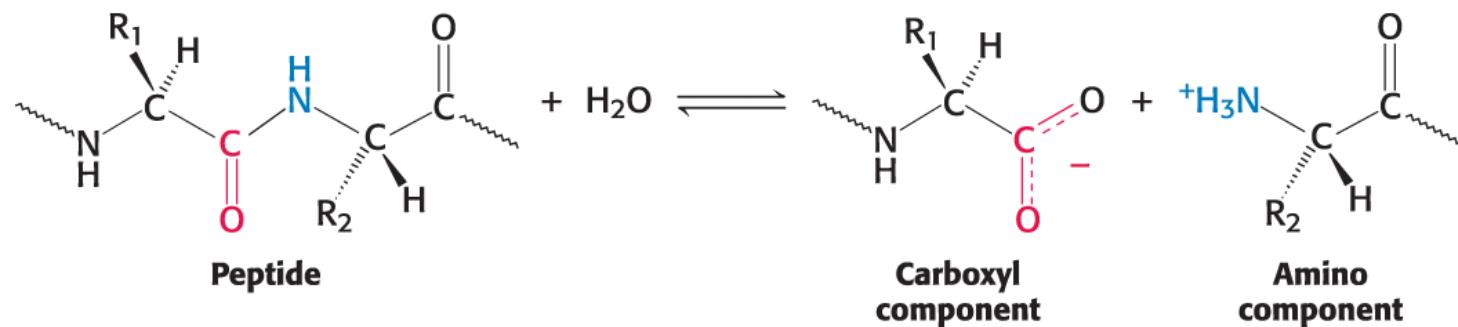
FOOTER | EC Number: 1.x.x.x = Oxidoreductase, 2.x.x.x = Transferase, 3.x.x.x = Hydrolase, 4.x.x.x = Lyase, 5.x.x.x = Isomerase, 6.x.x.x = Ligase



EC 7 in modern classifications

Proteases

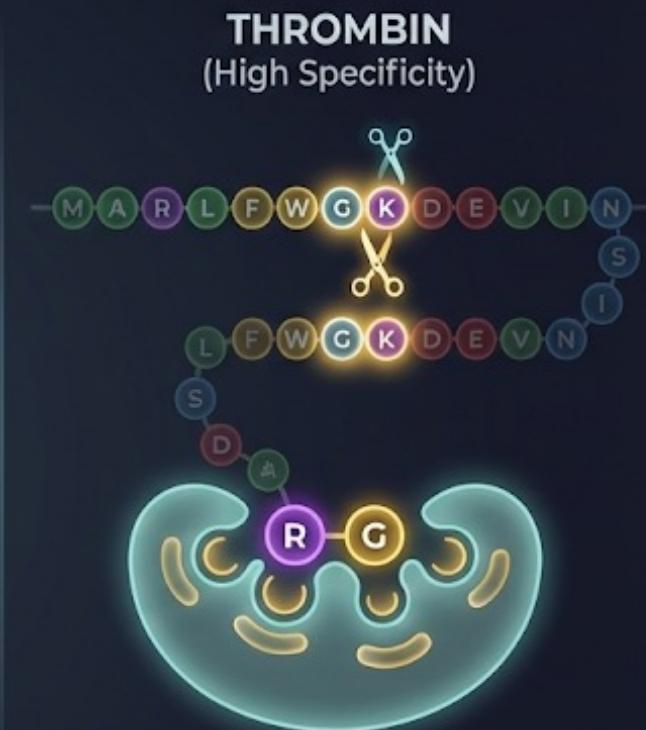
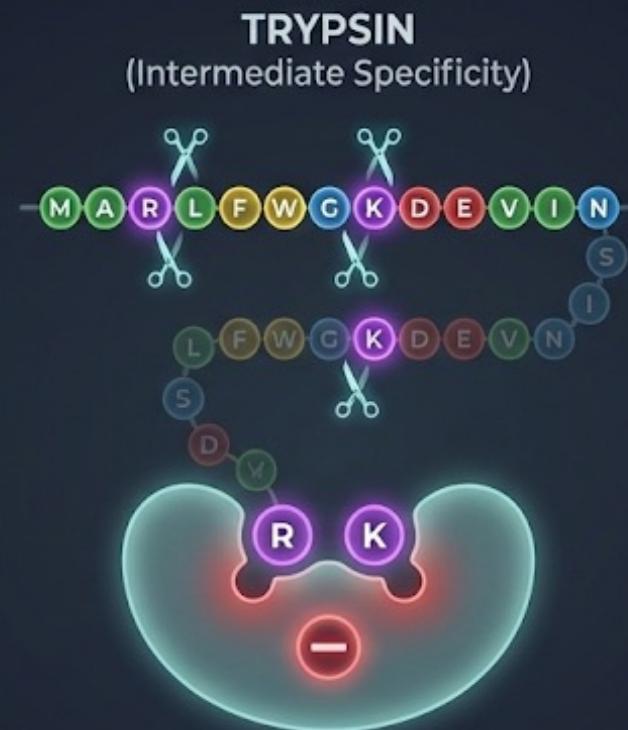
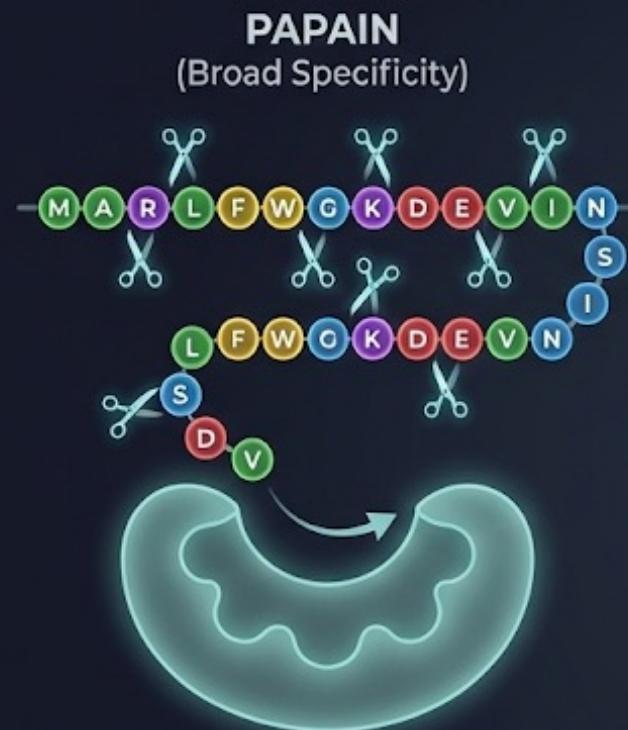
Proteases (proteolytic enzymes) catalyze proteolysis, the hydrolysis of a peptide bond.



All proteases break a chemical bond by the addition of a water molecule.

Diagram of Enzyme Specificity

PROTEASE SUBSTRATE SPECIFICITY COMPARISON



Broad Specificity
- cleaves at many residues

Cleaves after Lys or Arg
(positively charged)

Cleaves only Arg-Gly
sequence



Section 7.2 Many Enzymes Require Cofactors for Activity

- Cofactors are small molecules that some enzymes require for activity. **The two main classes of cofactors are coenzymes—organic molecules derived from vitamins—and metals.**
- Tightly bound coenzymes are called prosthetic groups.
- An enzyme with its cofactor is a holoenzyme. Without the cofactor, the enzyme is called an apoenzyme.

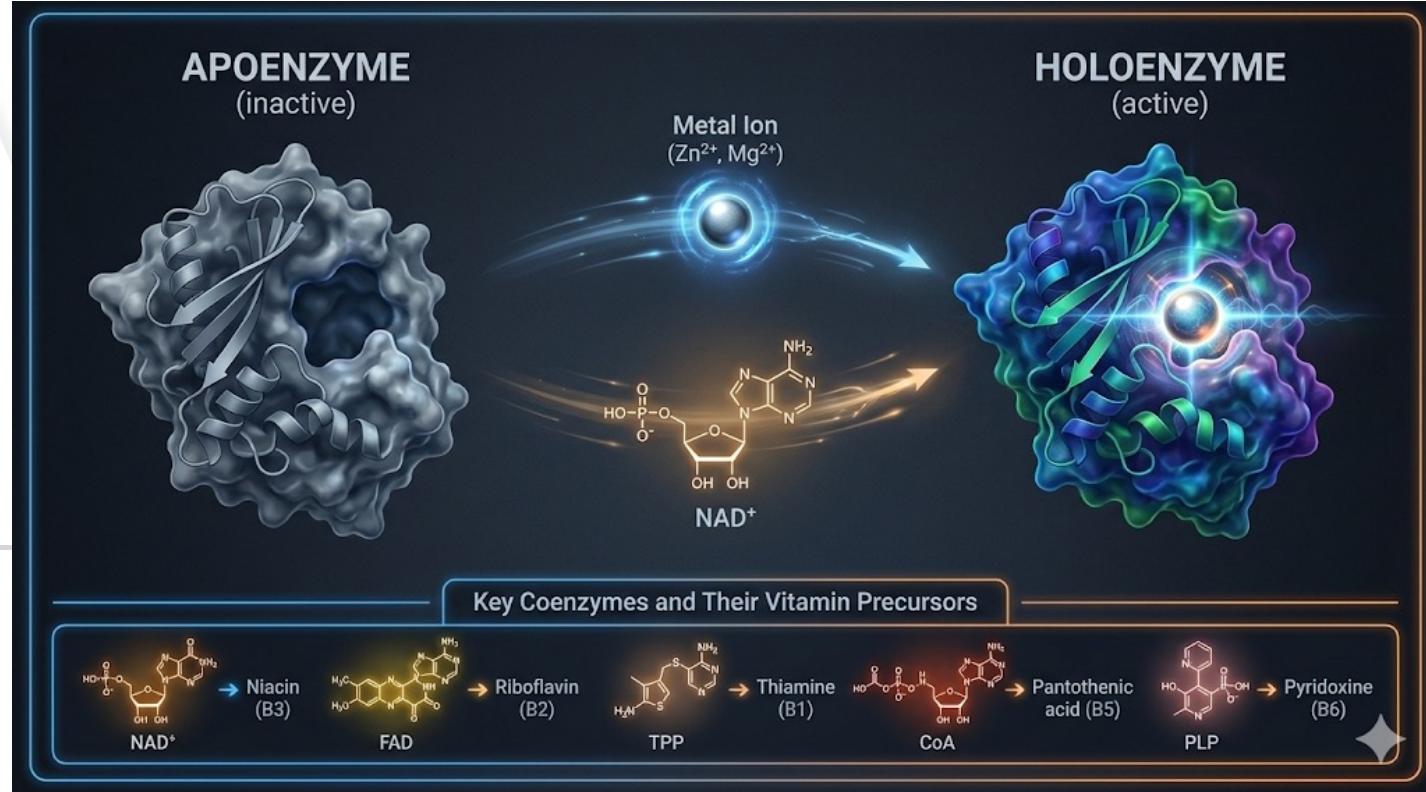


TABLE 6.2 Enzyme cofactors

Cofactor	Enzyme*
Coenzyme[†]	
Thiamine pyrophosphate (TPP)	Pyruvate dehydrogenase
Flavin adenine dinucleotide (FAD)	Monoamine oxidase
Nicotinamide adenine dinucleotide (NAD^+)	Lactate dehydrogenase
Pyridoxal phosphate (PLP)	Glycogen phosphorylase
Coenzyme A (CoA)	Acetyl CoA carboxylase
Biotin	Pyruvate carboxylase
5'-Deoxyadenosylcobalamin	Methylmalonyl mutase
Tetrahydrofolate	Thymidylate synthase
Metal	
Zn^{2+}	Carbonic anhydrase
Mg^{2+}	EcoRV
Ni^{2+}	Urease
Mo	Nitrogenase
Se	Glutathione peroxidase
$Mn^{2+} \leftrightarrow 3+$	Superoxide dismutase
K^+	Acetoacetyl CoA thiolase

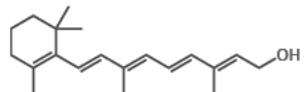
*The enzymes listed are examples of enzymes that employ the indicated cofactor.

[†] Often derived from vitamins, coenzymes can be either tightly or loosely bound to the enzyme.

THE CHEMICAL STRUCTURES OF VITAMINS

Vitamins are the essential nutrients that our body needs in small amounts. More specifically, an organic compound is defined as a vitamin when it is required by an organism, but not synthesised by that organism in the required amounts (or at all). There are thirteen recognised vitamins.

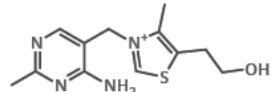
VITAMIN A



RETINOL
active form in mammalian tissues

Important for eyesight. Also strengthens immune system and keeps skin and linings of parts of the body healthy.

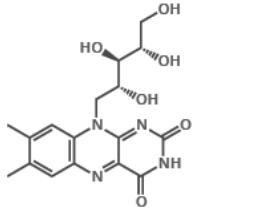
VITAMIN B1



THIAMIN
can also occur in pyrophosphate ester form

Used to keep nerves & muscle tissue healthy. Also important for processing of carbohydrates and some proteins.

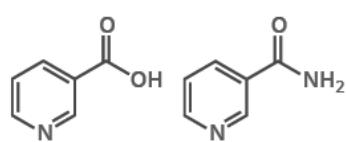
VITAMIN B2



RIBOFLAVIN
excess turns urine bright yellow

Important for body growth, red blood cell production, and keeping the eyes healthy. Also helps processing of carbohydrates.

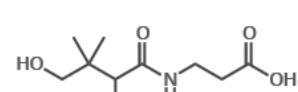
VITAMIN B3



NICOTINIC ACID
niacin is collective name for these compounds

Helps with digestion and digestive system health. Also helps with the processing of carbohydrates.

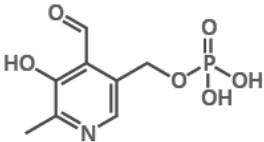
VITAMIN B5



PANTOTHENIC ACID
also occurs in pyrophosphate ester form

Important for manufacturing red blood cells and maintaining a healthy digestive system. Also helps process carbohydrates.

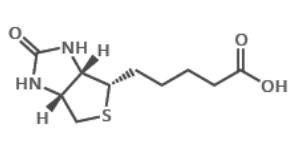
VITAMIN B6



PYRIDOXAL PHOSPHATE
active form in mammalian tissues

Helps make some brain chemicals; needed for normal brain function. Also helps make red blood cells and immune system cells.

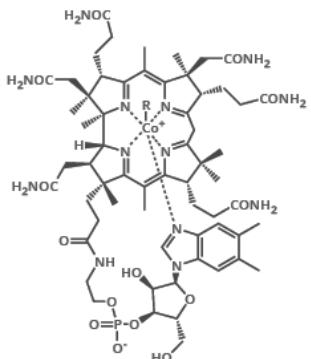
VITAMIN B7



BIOTIN
produced by intestinal bacteria

Needed for metabolism of various compounds. Often recommended for strengthening hair, but evidence is variable.

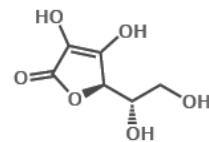
VITAMIN B12



COBALAMIN
usually contains CN as the R group

Important for the nervous system, for making red blood cells, and helps in the production of DNA and RNA.

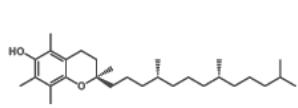
VITAMIN C



ASCORBIC ACID
deficiency can cause scurvy

Important for a healthy immune system; helps produce collagen, used to make skin and other tissues. Also helps wound healing.

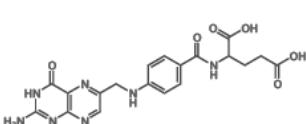
VITAMIN E



ALPHA-TOCOPHEROL
group includes tocopherols & tocotrienols

An antioxidant that helps prevent damage to cells and may have a preventative role in cancer. Also helps make red blood cells.

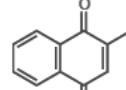
VITAMIN B9



FOLIC ACID
found as tetrahydrofolate in food

Important for brain function & mental health. Aids production of DNA & RNA. Important when tissues are growing quickly.

VITAMIN K



MENADIONE
all K vitamins are menadione or derivatives

Helps blood clot properly, & plays a key role in bone health. Newborns receive vitamin K injections to prevent bleeding.

Key

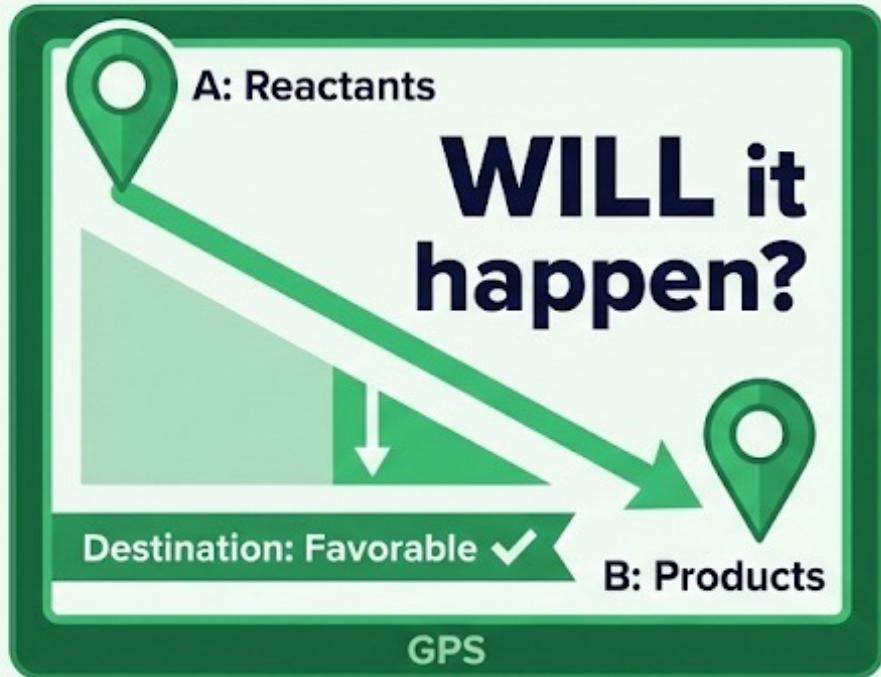
Vitamins can be divided broadly into two classes.

WATER-SOLUBLE VITAMINS
These vitamins are not stored in the body. As such, generally, they are required more frequently than the fat-soluble vitamins.

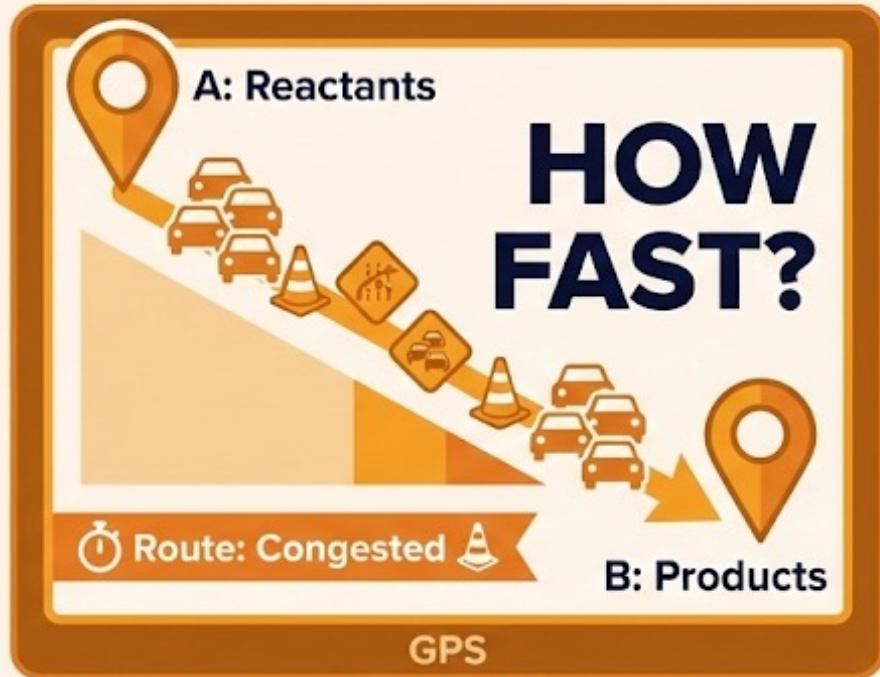
FAT-SOLUBLE VITAMINS
These vitamins are stored in the liver and fatty tissues until required. As such, they can be harmful if too much is taken in.



Before We Talk About HOW Enzymes Work, Let's Ask: What CAN Enzymes Do?



VS.



THERMODYNAMICS

ΔG (Thermodynamics)

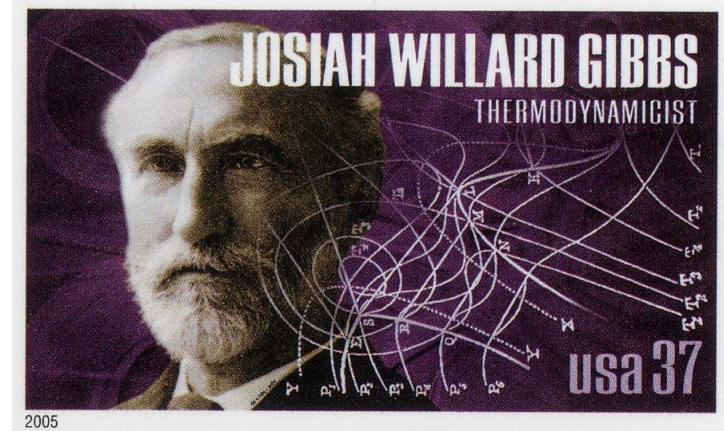
KINETICS

ΔG^\ddagger (Kinetics)

Enzymes answer the second question—not the first.

Let's forget about enzymes for a second and just think about reactions.

Section 7.3 Gibbs Free Energy is a Useful Thermodynamic Function for Understanding Enzymes



The properties of chemical reactions and whether they can take place at all depend on free-energy differences.

Gibbs free energy, or **free energy (G)**, is a thermodynamic property that is a measure of the energy that is capable of doing work.

To understand how enzymes operate, we need to consider two thermodynamic properties of reactions:

1. The free-energy difference (ΔG) between the products and the reactants
 - Determines whether the reaction will take place spontaneously.
2. The free energy required to initiate the conversion of reactants into products
 - Determines the rate of the reaction
 - Enzymes affect only this property



Free energy (G) is a measure of energy capable of doing work. The change in free energy when a reaction occurs is symbolized by ΔG .



Enzymes do not alter the ΔG of a reaction.



ZEROTH LAW:

If A is in equilibrium with C, and B is in equilibrium with C, then A is in equilibrium with B



FIRST LAW:

Energy cannot be created or destroyed, only transformed

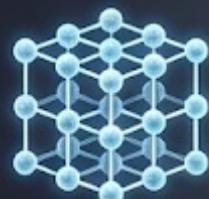
$$\Delta U = q - w$$



SECOND LAW:

The entropy of an isolated system always increases

$$\Delta S_{\text{universe}} \geq 0$$



THIRD LAW:

The entropy of a perfect crystal at absolute zero is exactly zero

H

U

S

S

T

$$\Delta G = \underline{\Delta H} - T \underline{\Delta S}$$

Gibbs Free Energy (ΔG) combines Enthalpy (from First Law), Entropy (from Second Law), and Temperature (related to Third Law) to determine spontaneity at constant temperature and pressure.

Gibb's free energy equation describes thermodynamic processes

Gibb's free energy equation

$$G = H - TS$$

free enthalpy temperature
energy x x
entropy

Enthalpy (H)
Total energy of the system
(energy in bonds)

$$G = H - TS$$



Randomness in the system

Free energy (G) (energy that can do work)

$$G = H - TS$$

The diagram illustrates the components of free energy (G) using the equation $G = H - TS$. Three arrows point from descriptive text below the equation to the terms in the equation: one arrow points to H (all energy), another to $-TS$ (portion of energy that can't do work), and a third to G (portion of energy that can do work).

portion of
energy
that can
do work

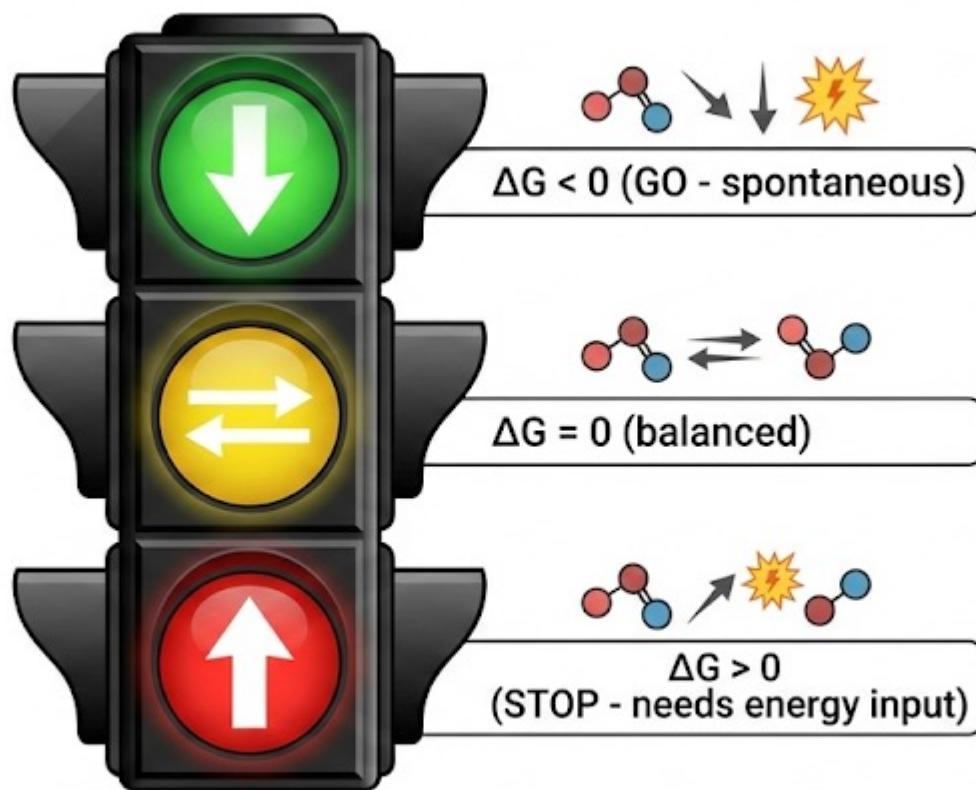
all
energy

portion of
energy
that can't
do work

How do we tell if reaction is:

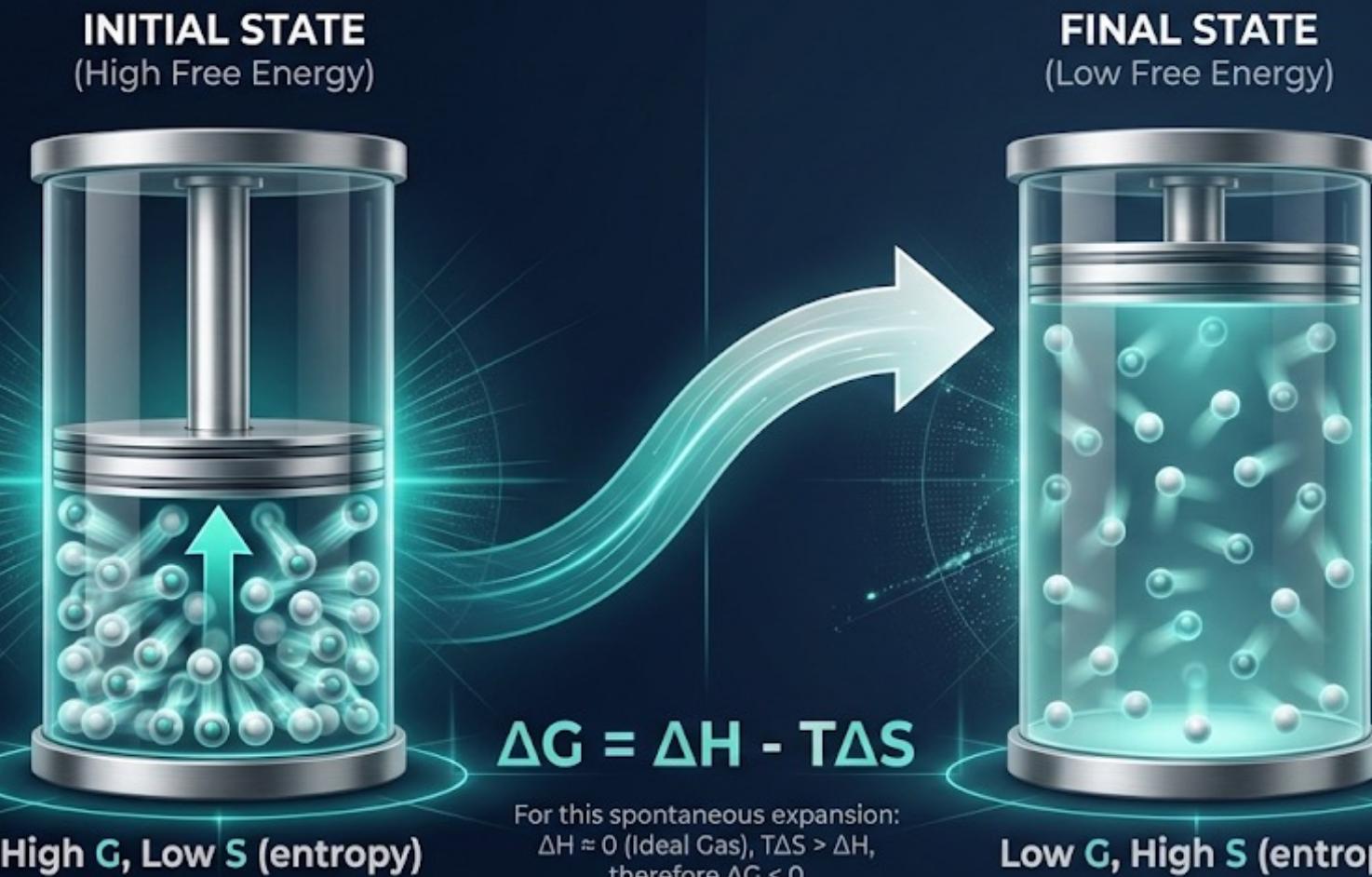
1. Spontaneous or not
2. Equilibrium constant
3. Directionality
4. Velocity

THE THERMODYNAMIC TRAFFIC LIGHT



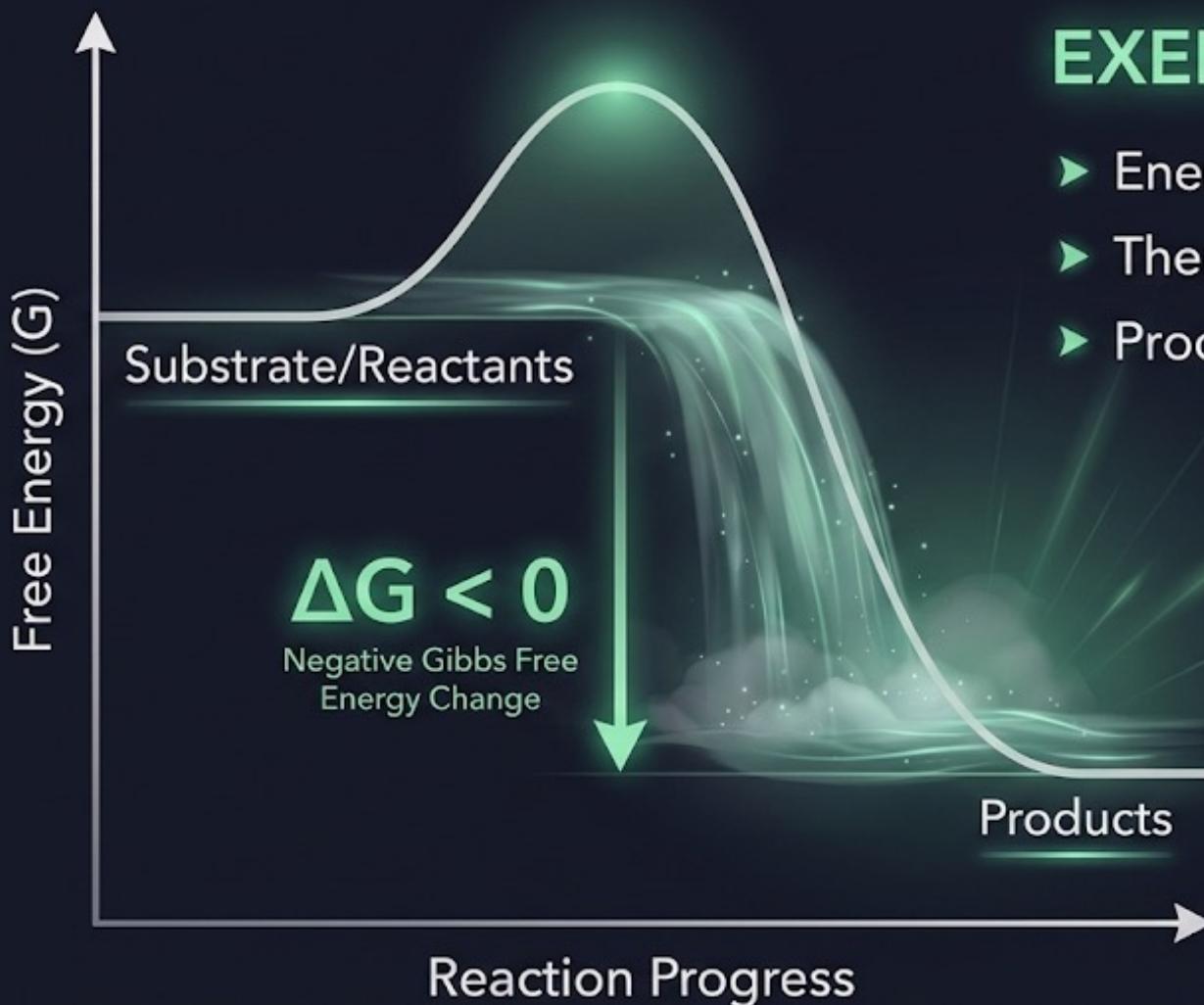
Enzymes cannot change the light color.
They only speed up the passage through a green light.

Reactions will spontaneously evolve toward something with lower energy
(2nd law of thermodynamics)



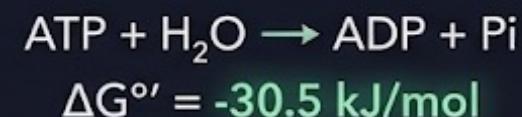
Spontaneous processes move toward lower free energy

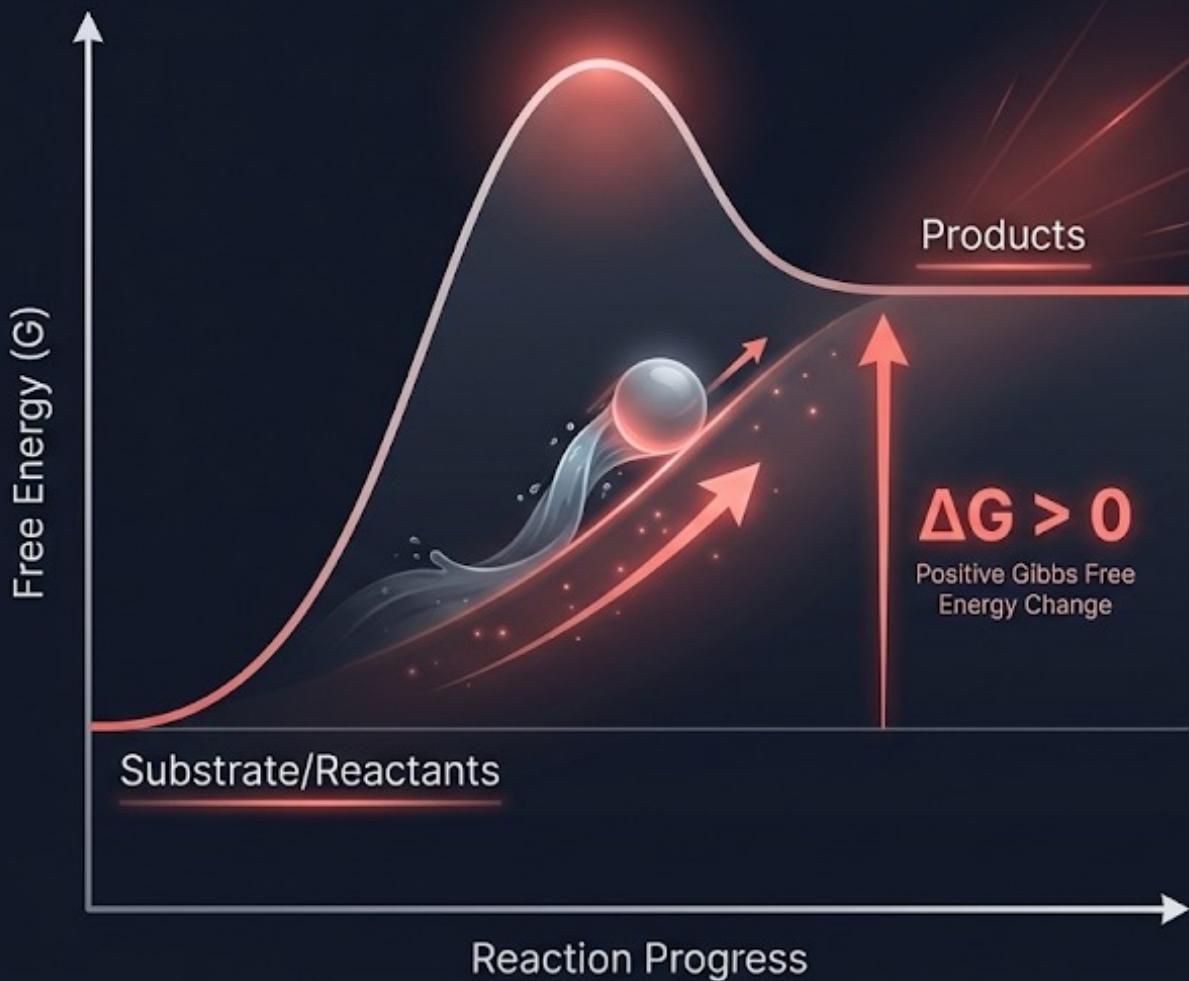
Spontaneous event involves a decrease in free energy, is exergonic
(thermodynamically favorable)



EXERGONIC REACTION

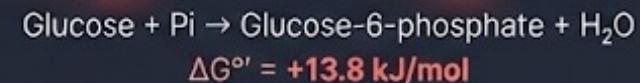
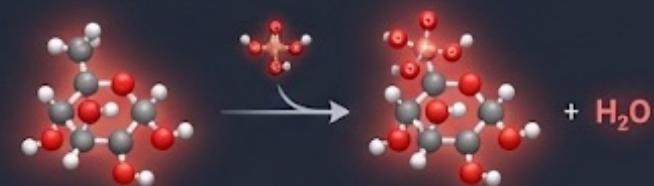
- Energy is **RELEASED**
- Thermodynamically **FAVORABLE**
- Proceeds **SPONTANEOUSLY**





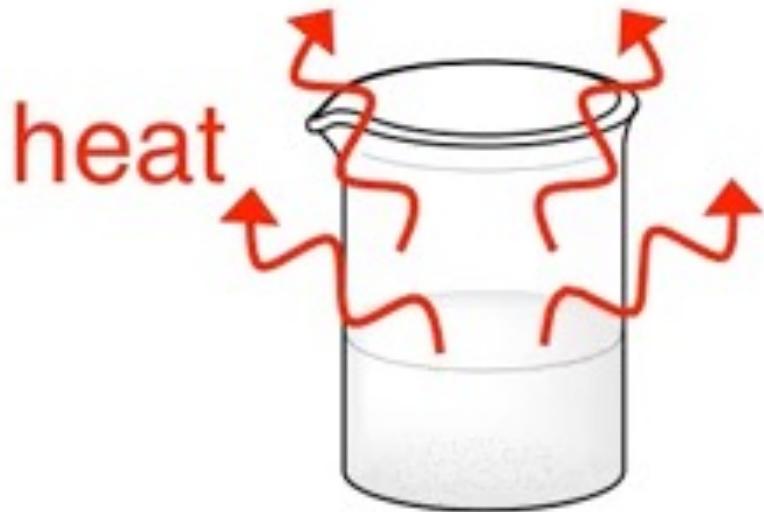
ENDERGONIC REACTION

- Energy is **REQUIRED**
- Thermodynamically **UNFAVORABLE** alone
- Does **NOT** proceed spontaneously
- Must be **COUPLED** to exergonic reaction

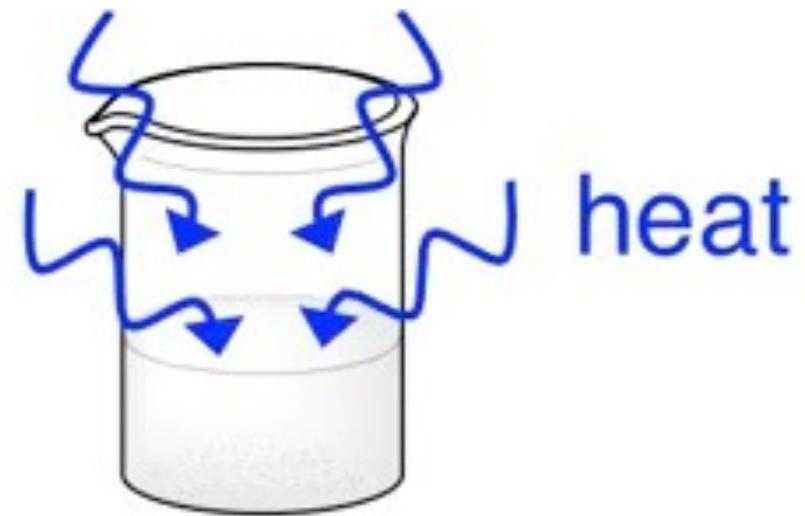


REACTION COUPLING





(-) ΔH
exothermic

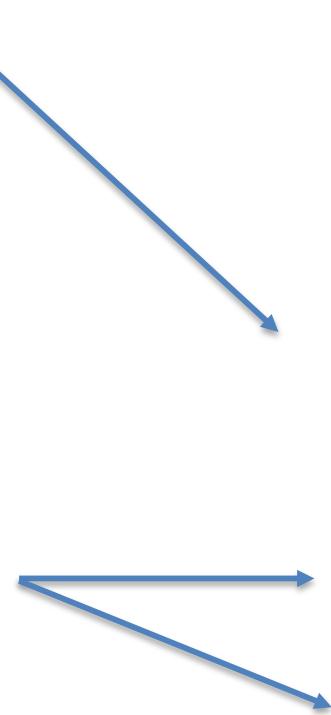


(+) ΔH
endothermic **H X**

Exo/endo THERMIC = heat. Exo/endo GONIC = free energy. Don't mix them up



The Free-Energy Change Provides Information About the Spontaneity but Not the Rate of a Reaction

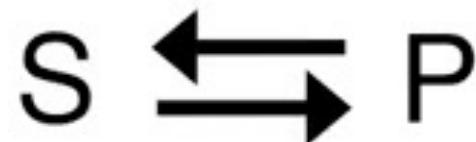


- A reaction will occur without the input of energy, or spontaneously, only if ΔG is negative. Such reactions are called exergonic reactions.
- A reaction will not occur if the ΔG is positive. These reactions are called endergonic reactions.
- If a reaction is at equilibrium, there is no net change in the amount of reactant or product. At equilibrium, $\Delta G = 0$.
- The ΔG of a reaction depends only on the free energy difference between reactants and products and is independent of how the reaction occurs.
- The ΔG of a reaction provides no information about the rate of the reaction.

How do we tell if reaction is:

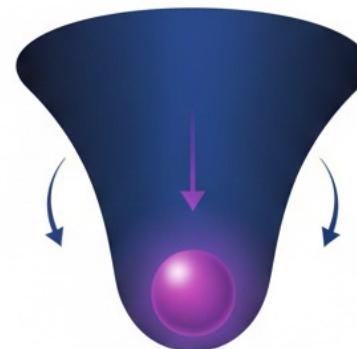
1. Spontaneous or not
2. Equilibrium constant
3. Directionality
4. Velocity

Equilibrium constant (K_{eq})

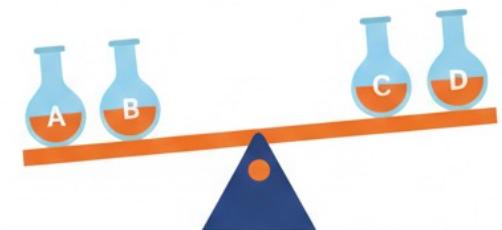


$$K_{\text{eq}} = \frac{[P]_{\text{eq}}}{[S]_{\text{eq}}}$$

Energy Perspective (ΔG)



Concentration Perspective (K_{eq})

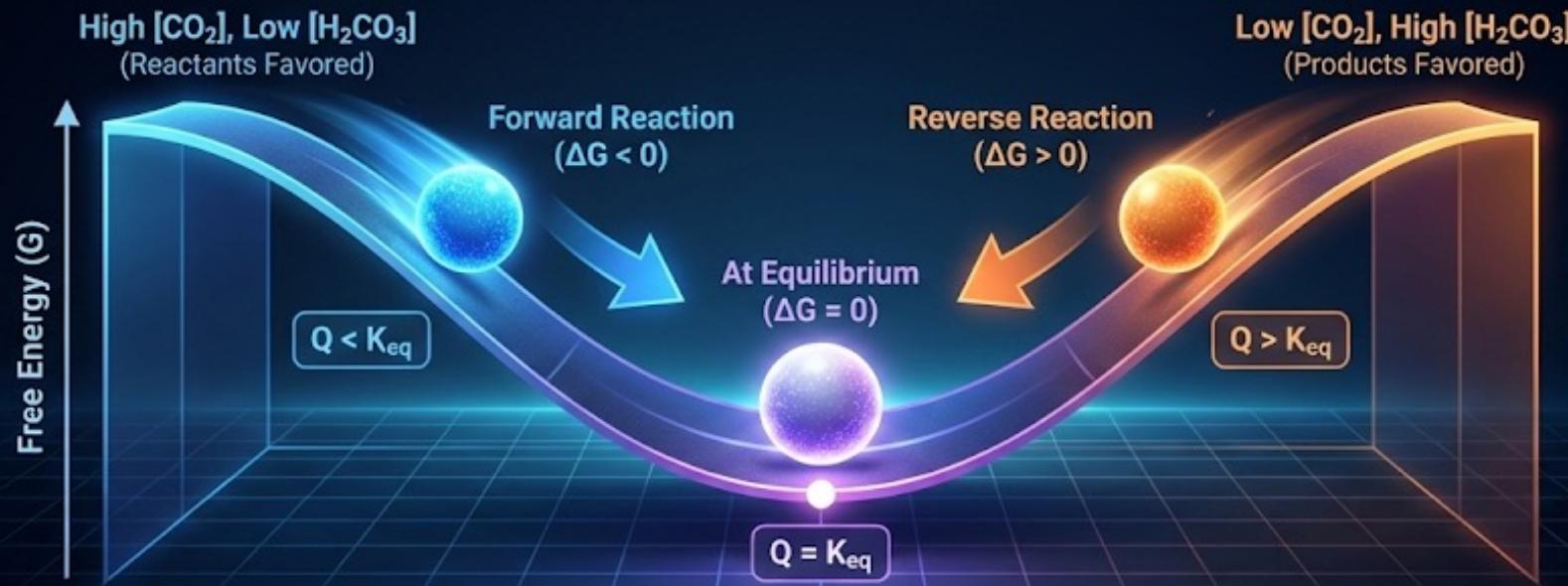


1. System “wants” to reach lowest energy state.
2. Lowest energy = Equilibrium.
3. Equilibrium is a specific concentration ratio (K_{eq}).

∴ Energy (ΔG) \rightleftharpoons Concentration Ratio (K_{eq})

Directionality depends on the concentration of the components

REACTION DIRECTION DEPENDS ON COMPONENT CONCENTRATIONS



KEY INSIGHT: The reaction direction depends on where you **START** relative to equilibrium, not on ΔG° alone. The system always spontaneously moves towards the lowest energy state (equilibrium).

$$\Delta G = \Delta G^\circ + RT \ln(Q) \text{ where } Q = [\text{Products}]/[\text{Reactants}]$$

FORWARD ($Q < K_{\text{eq}}$) $\rightarrow \Delta G$ is negative, reaction proceeds to products.

EQUILIBRIUM ($Q = K_{\text{eq}}$) $\rightarrow \Delta G = 0$, reaction is balanced.

REVERSE ($Q > K_{\text{eq}}$) $\rightarrow \Delta G$ is positive, reaction proceeds to reactants.



PROBLEM STATEMENT:

Hexokinase catalyzes glucose phosphorylation with

$$\Delta G^\circ' = -17 \text{ kJ/mol}$$
 at 298K. Calculate K_{eq} .

SOLUTION STEPS:

$$\Delta G^\circ' = -RT \ln K_{\text{eq}}$$

$$\hookrightarrow K_{\text{eq}} = e^{(-\Delta G^\circ'/RT)}$$

$$\rightarrow K_{\text{eq}} = e^{[-(-17 \text{ kJ/mol} \times 1000 \text{ J/kJ}) \div (8.314 \text{ J/(mol}\cdot\text{K}) \times 298 \text{ K})]}$$

$$K_{\text{eq}} = e^{(17,000 \text{ J/mol} \div 2,478 \text{ J/mol})} = e^{6.86}$$

$$\hookrightarrow K_{\text{eq}} \approx 950$$

INTERPRETATION:

$K_{\text{eq}} \approx 10^3$ means products favored 1000:1 at equilibrium.

The Standard Free-Energy Change of a Reaction Is Related to the Equilibrium Constant

- The more exergonic a reaction is, the larger the equilibrium constant will be. The more endergonic a reaction is, the smaller the equilibrium constant will be.
- Note that the ΔG of the reaction can be larger than, smaller than, or equal to $\Delta G^\circ'$, depending on the concentrations of the reactants and products.
- A *kilojoule* (kJ) is equal to 1000 J.
- A *joule* (J) is the amount of energy needed to apply a 1-newton force over a distance of 1 meter.
- A *kilocalorie* (kcal) is equal to 1000 cal.
- A *calorie* (cal) is equivalent to the amount of heat required to raise the temperature of 1 gram of water from 14.5°C to 15.5°C.
- 1 kJ = 0.239 kcal

TABLE 6.3 Relation between $\Delta G^\circ'$ and K'_{eq} (at 25°C)

K'_{eq}	$\Delta G^\circ'$	
	kJ mol^{-1}	kcal mol^{-1}
10^{-5}	28.53	6.82
10^{-4}	22.84	5.46
10^{-3}	17.11	4.09
10^{-2}	11.42	2.73
10^{-1}	5.69	1.36
1	0	0
10	-5.69	-1.36
10^2	-11.42	-2.73
10^3	-17.11	-4.09
10^4	-22.84	-5.46
10^5	-28.53	-6.82

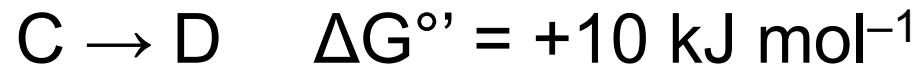
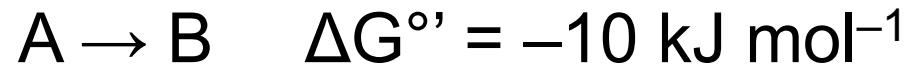
Tymoczko et al., *Biochemistry: A Short Course*, 4e, © 2019 W. H. Freeman and Company

Quick Quiz



QUICK QUIZ

Which of the following two reactions will take place spontaneously? What are the $\Delta G^\circ'$ values for the reverse reactions?



WHAT ENZYMES DO

✓ CAN DO



Accelerate reactions



Lower activation energy (ΔG^\ddagger)



Speed up equilibrium attainment

✗ CANNOT DO



Change equilibrium position



Change ΔG or ΔG°



Force unfavorable reactions

Same destination. Faster arrival.



1. Spontaneous or not
2. Equilibrium constant
3. Directionality
4. Velocity



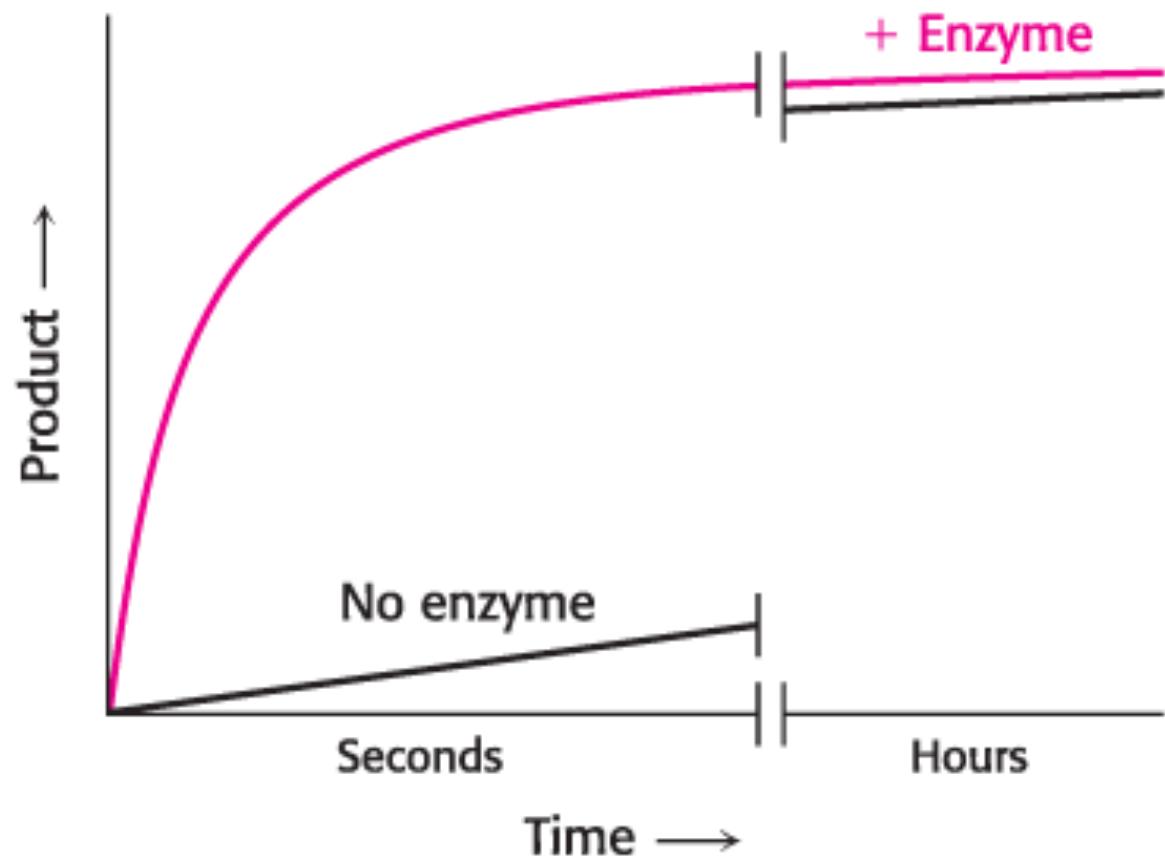
These qualities are about the rxn and are independent of the enzyme



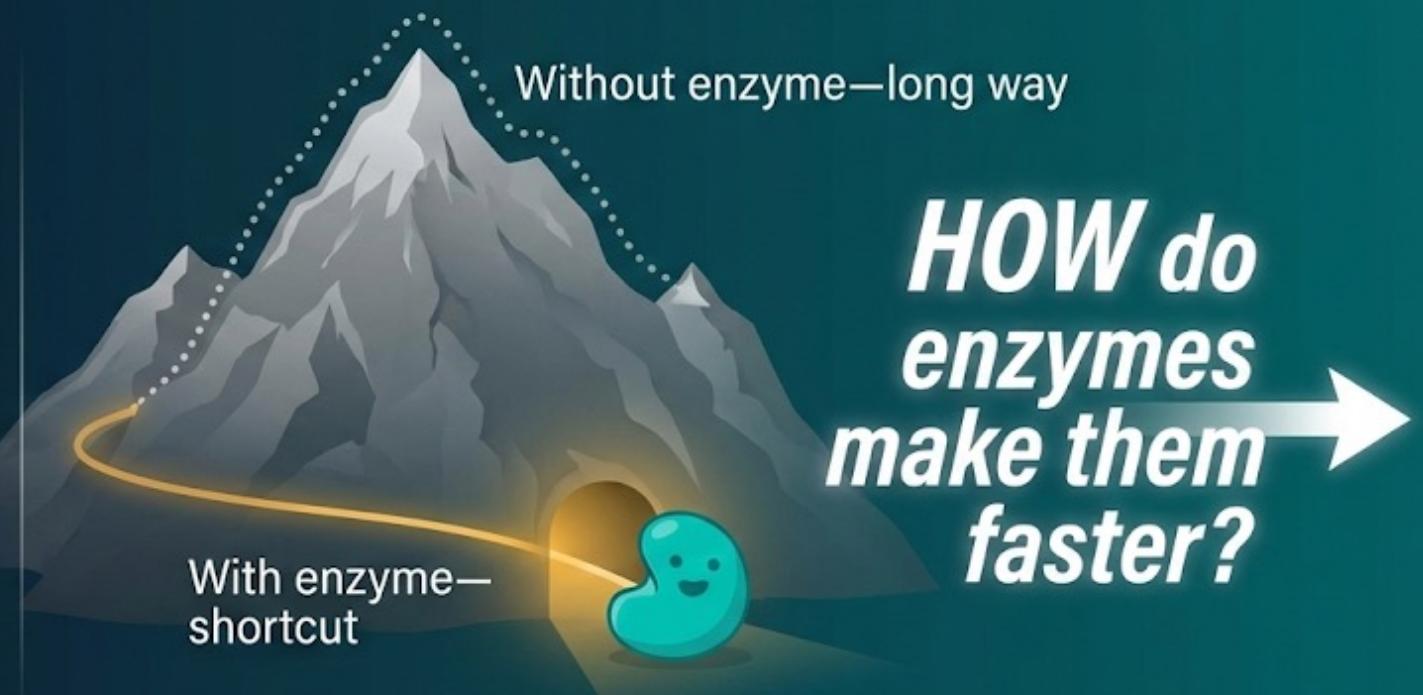
This is where enzymes work

Enzymes Alter the Reaction Rate but Not the Reaction Equilibrium

- The reaction equilibrium is determined only by the free energy difference between the products and reactants. Enzymes cannot alter this difference.



WHY do reactions happen? ✓



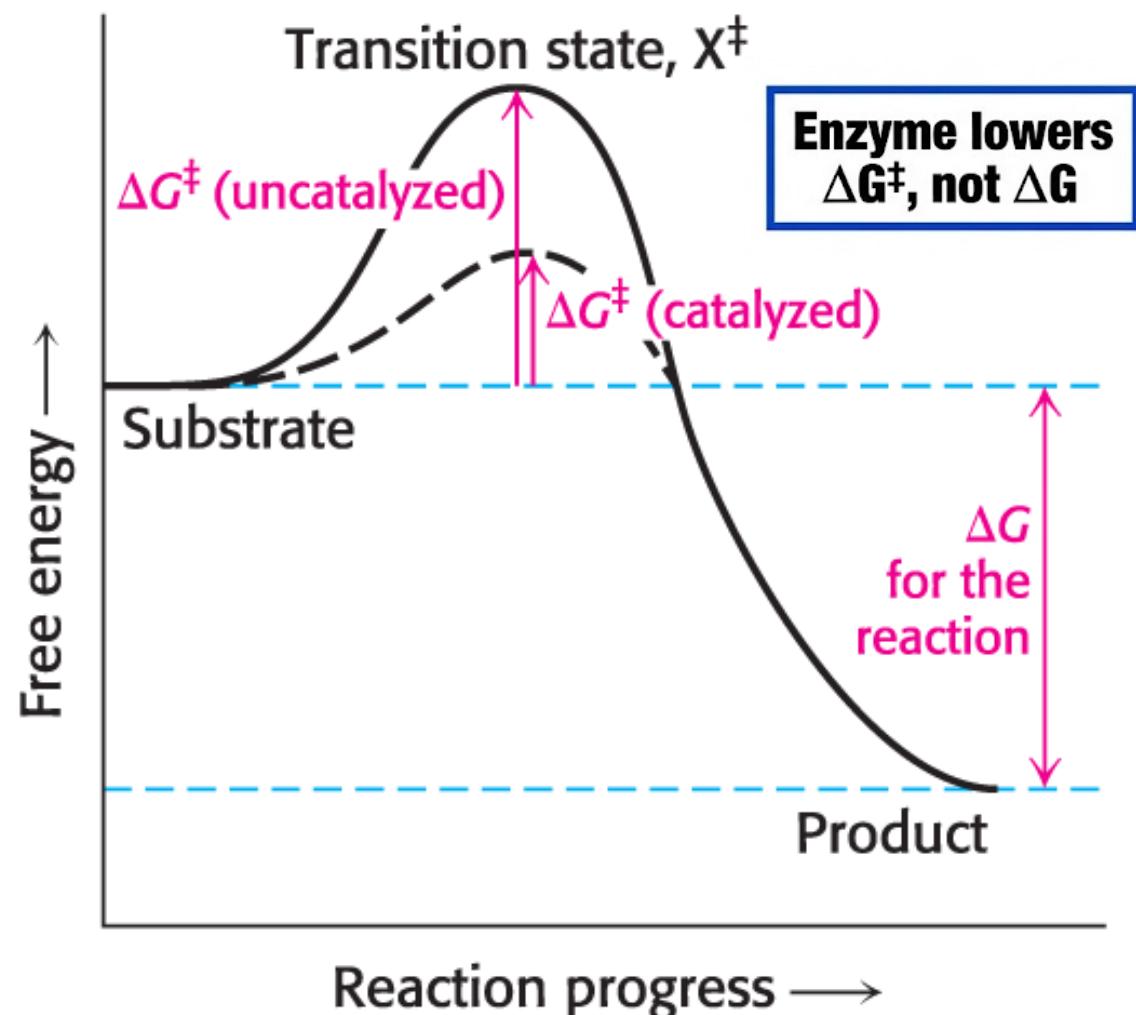
HOW do enzymes make them faster? ➔

Coming up: The Transition State Secret



Section 7.4 Enzymes Facilitate the Formation of the Transition State

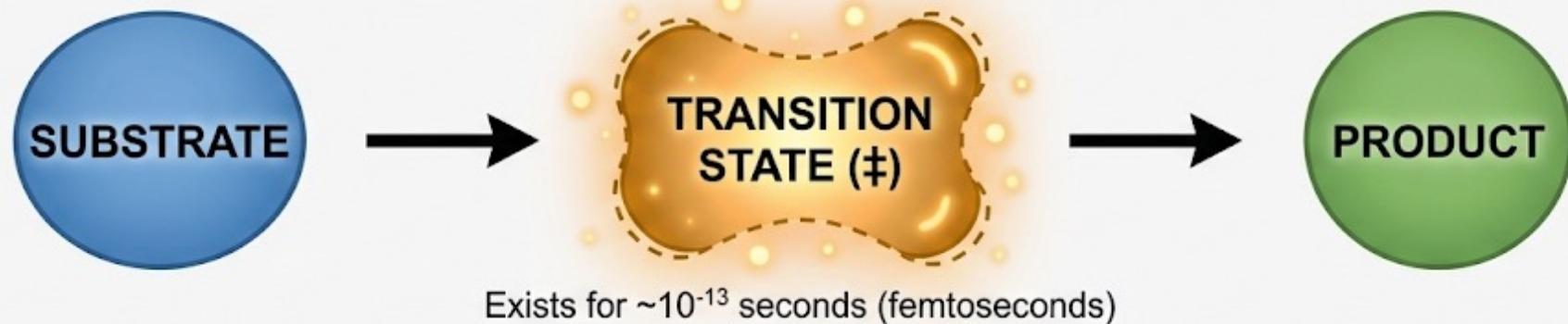
- Learning objective 2: Explain the relation between the transition state and the active site of an enzyme and list the characteristics of active sites.
- A chemical reaction proceeds through a transition state, a molecular form that is no longer substrate but not yet product.
- $S \rightleftharpoons X^\ddagger \rightarrow P$
- The transition state is designated by the double dagger.
- The energy required to form the transition state from the substrate is called the activation energy, symbolized by ΔG^\ddagger .
- $\Delta G^\ddagger = G_{X^\ddagger} - G_S$
- Enzymes facilitate the formation of the transition state.



What is the transition state?

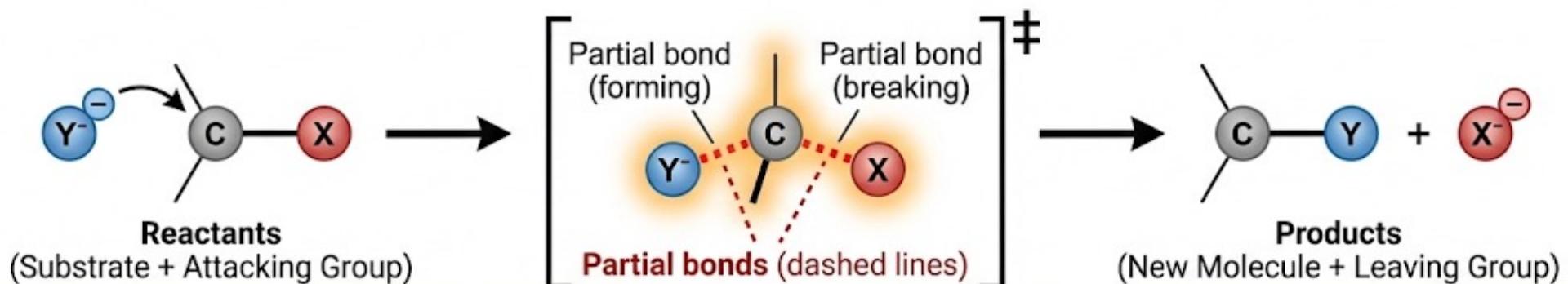
THE TRANSITION STATE (\ddagger)

ROW 1: CONCEPTUAL PROGRESSION



ROW 2:

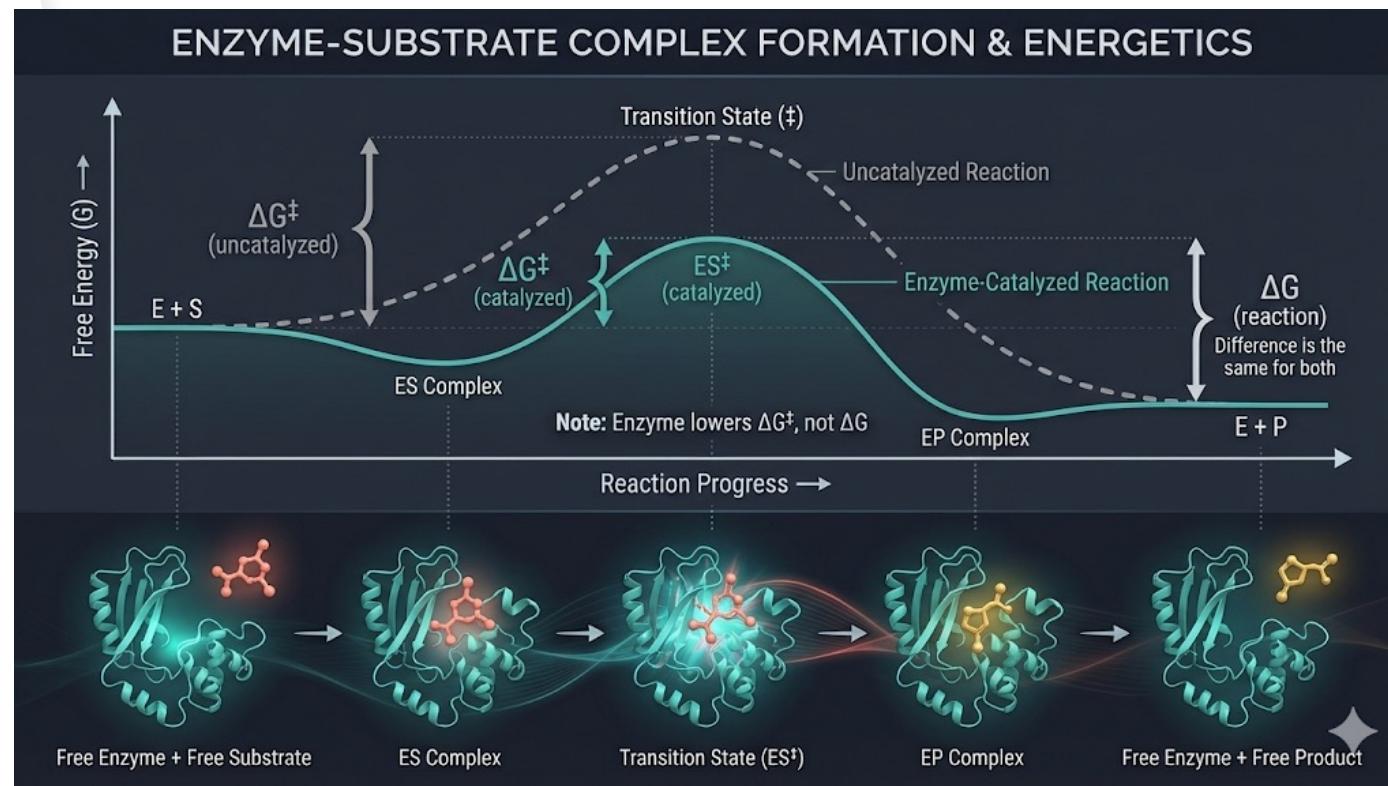
MOLECULAR VIEW: BOND BREAKING & FORMING



Key insight: The transition state has a specific **GEOMETRY** that enzymes evolved to recognize.

The Formation of an Enzyme–Substrate Complex Is the First Step in Enzymatic Catalysis

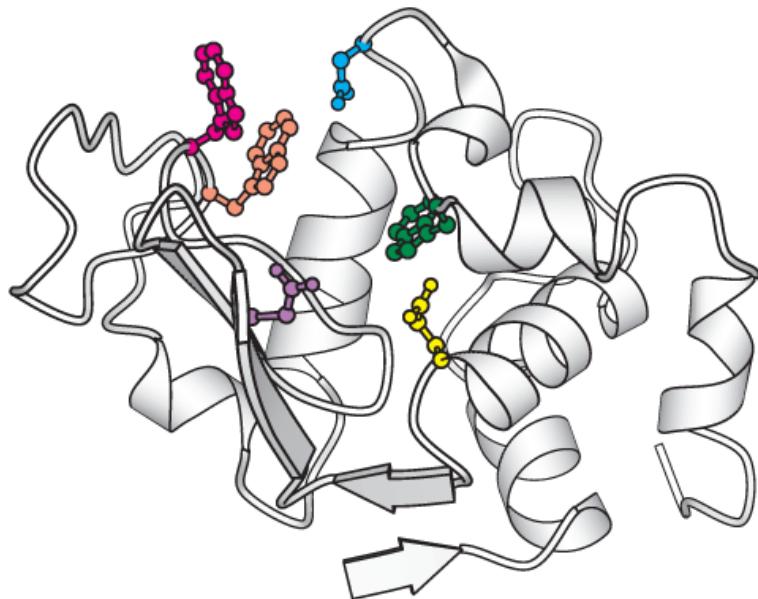
- Enzymes bring substrates together to form an enzyme–substrate complex on a particular region of the enzyme called the active site.
- The interaction of the enzyme and substrates at the active site promotes the formation of the transition state.



The Active Sites of Enzymes Have Some Common Features (1/2)

- The active site is a three-dimensional cleft or crevice created by amino acids from different parts of the primary structure.
- The active site constitutes a small portion of the enzyme volume.
- Active sites create unique microenvironments.
- The interaction of the enzyme and substrate at the active site involves multiple weak interactions.
- Enzyme specificity depends on the molecular architecture at the active site.

(A)



(B)



Tymoczko et al., *Biochemistry: A Short Course*, 4e,
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The Active Sites of Enzymes Have Some Common Features (2/2)

- Enzymes do not interact with their substrates like a lock and key.
- Rather, the enzyme changes shape upon substrate binding, a phenomenon called induced fit.

ENZYME-SUBSTRATE BINDING: LOCK-AND-KEY vs. INDUCED FIT MODELS

LOCK AND KEY MODEL (Outdated)

Rigid Enzyme (Fischer, 1894)
Lock and Key: Emil Fischer, 1894

OUTDATED

KEY PREDICTION (Incorrect)
Predicts enzyme binds substrate most tightly - **BUT THIS IS WRONG**. No conformational change upon binding.

INDUCED FIT MODEL (Current Understanding)

Flexible Enzyme (Koshland, 1958)
Induced Fit: Daniel Koshland, 1958

KEY INSIGHT (Correct)
Enzyme binds **TRANSITION STATE** most tightly. Conformational change optimizes active site for transition state stabilization.

SCIENTIFIC ACCURACY ELEMENT

ENERGY DIAGRAM: Binding Affinity & Catalysis

Free Energy (G)

Reaction Coordinate

ES[‡] (Transition State)
Strongest binding to Substrate (Incorrect)

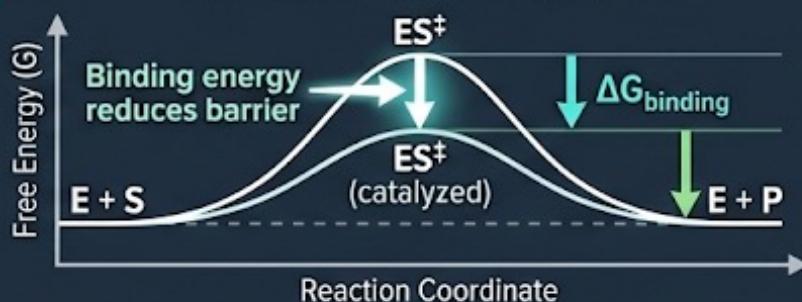
ES[‡] (Lock and Key Prediction)
Induced Fit Reality

ES (Substrate Bound)
Strongest binding to Transition State (Correct, Lower Activation Energy)

Binding energy facilitates catalysis

1

ENERGY LANDSCAPE



The binding energy between enzyme and transition state lowers the activation energy barrier.

2

SHAPE COMPLEMENTARITY



Substrate
(#3498db)



Transition State
(#f39c12)



Active site
(#00cec9)

Active site is complementary to TRANSITION STATE, not substrate.

3

BINDING ENERGY EQUATION

$$\Delta G_{\text{catalyzed}}^{\ddagger} = \Delta G_{\text{uncatalyzed}}^{\ddagger} - \Delta G_{\text{binding}}$$



Tighter binding to TS → More energy released → Lower barrier

4

CLINICAL APPLICATION: Transition-State Analogs as Drugs



Drugs like HIV protease inhibitors mimic TS geometry → Bind 10⁶× tighter than substrate → Potent inhibition

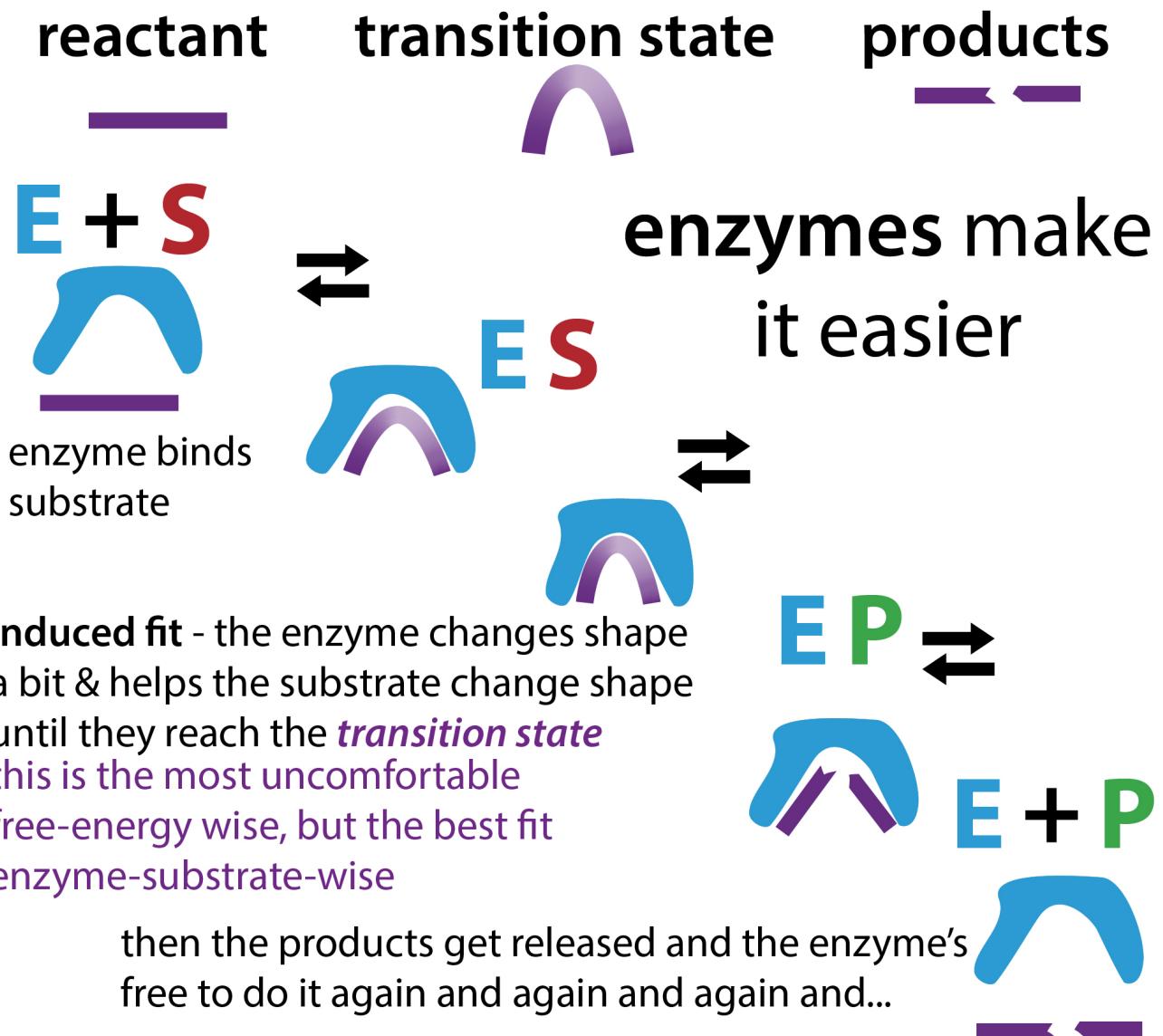
By mimicking the transition state, drugs can tightly bind and inhibit the enzyme.

The enzyme's job: Bind the transition state tighter than the substrate.

The Binding Energy Between Enzyme and Substrate Is Important for Catalysis

- Binding energy is the free energy released upon interaction of the enzyme and substrate.
- Binding energy is greatest when the enzyme interacts with the transition state, thus facilitating the formation of the transition state.
- Transition-State Analogs Are Potent Inhibitors of Enzymes

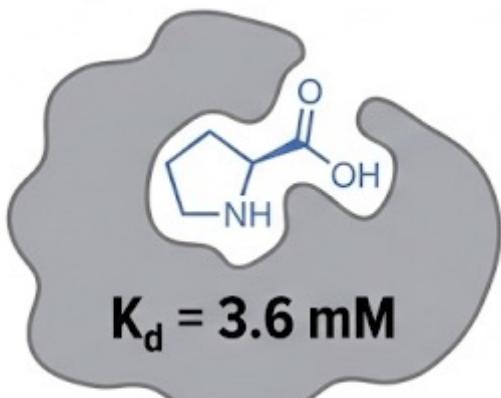
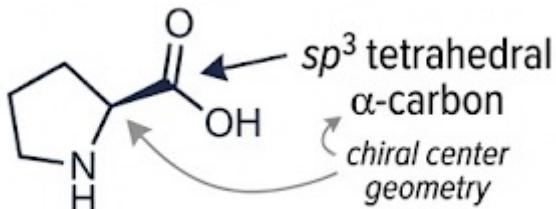
breaking up is hard



Transition state analog evidence.

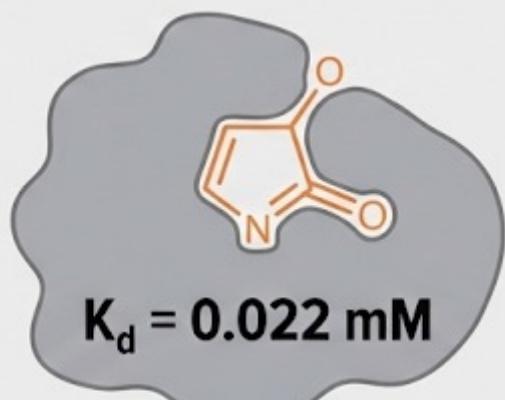
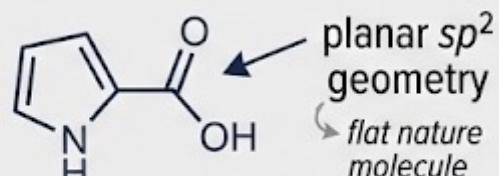
SUBSTRATE BINDING

Proline (Substrate)

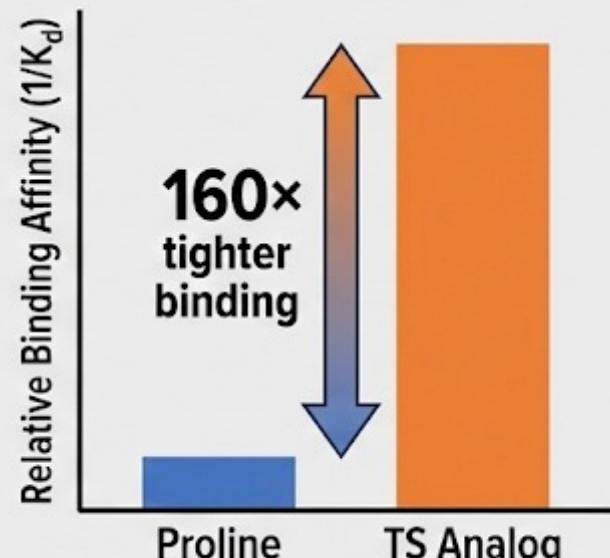


TRANSITION-STATE ANALOG BINDING

Pyrrole-2-carboxylate (TS Analog)



BINDING COMPARISON

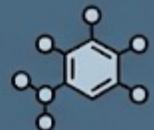


Enzymes bind transition state geometry preferentially—this is the source of catalytic power.

Evolution designed enzymes to grip the TRANSITION STATE shape

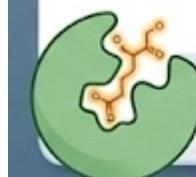
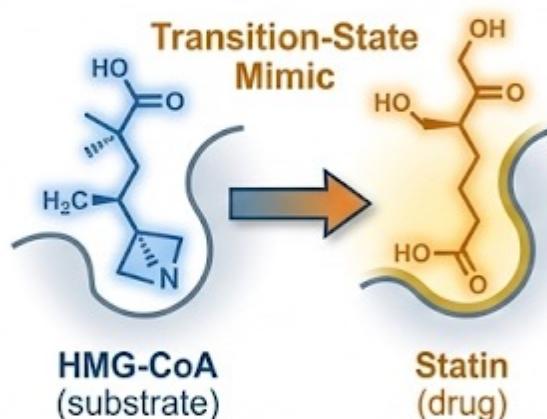


CLINICAL CASE RESOLUTION: The Statin Mystery Solved



1

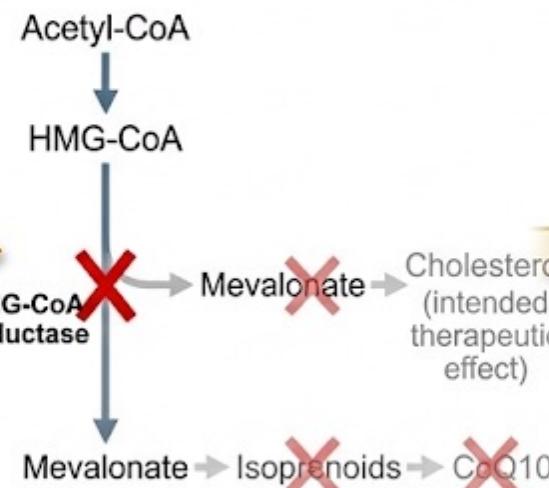
1. STATINS ARE TRANSITION-STATE ANALOGS



Statins bind enzyme active site.

2

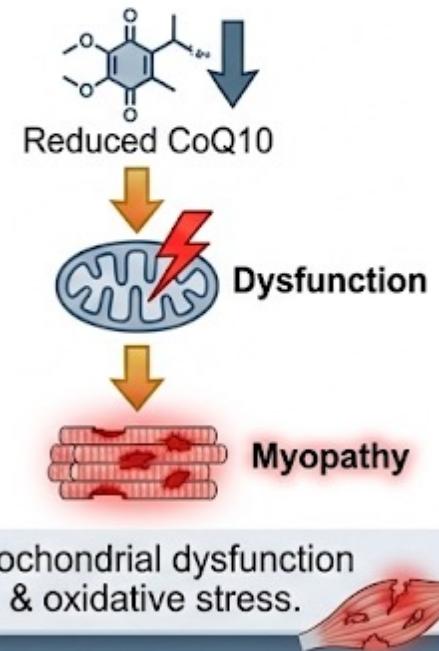
2. BLOCKS CHOLESTEROL AND ISOPRENOID SYNTHESIS



Inhibition of both pathways.

3

3. REDUCED CoQ10 → MITOCHONDRIAL DYSFUNCTION → MYOPATHY

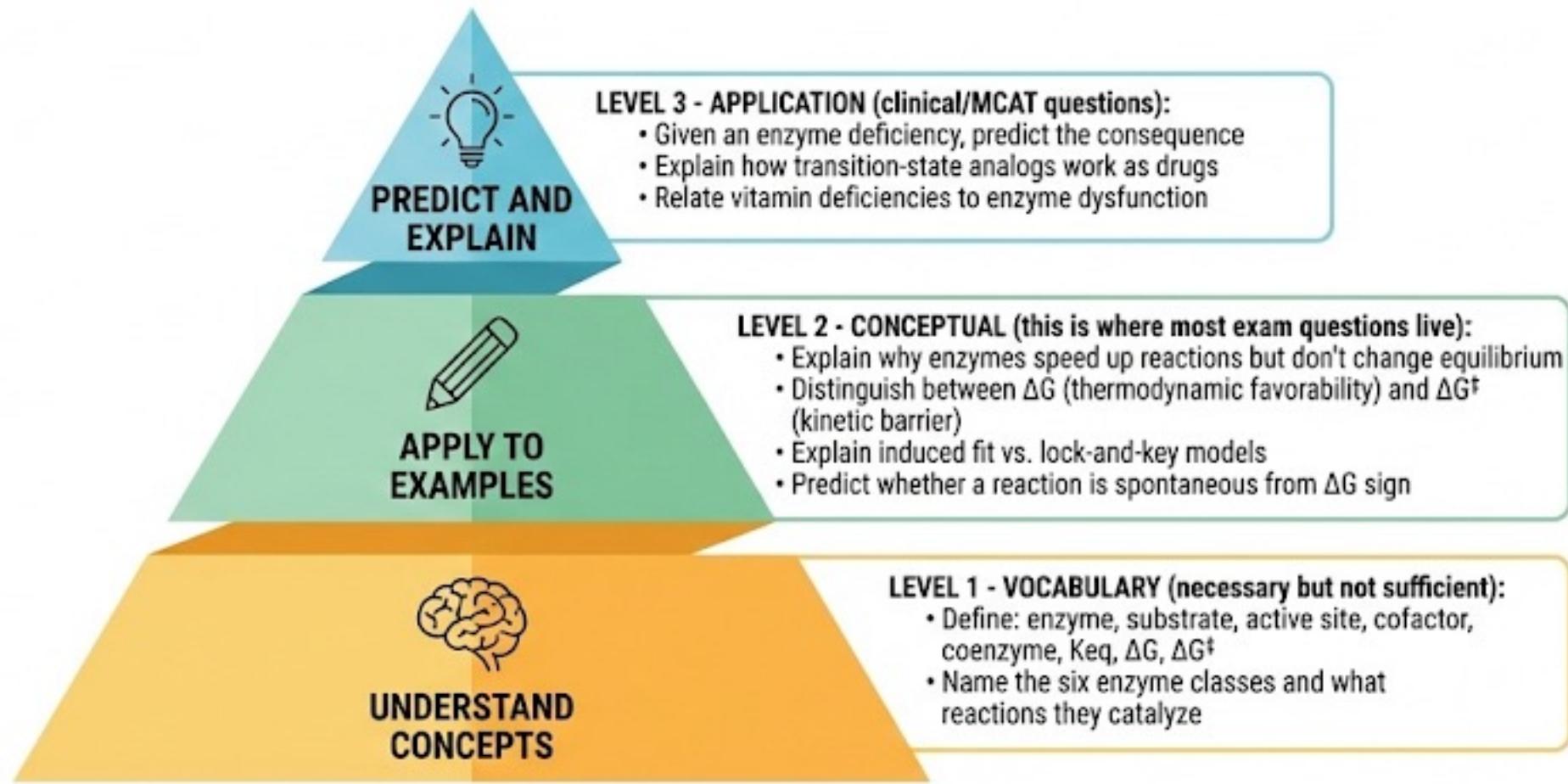


Mitochondrial dysfunction & oxidative stress.



Clinical Pearl: Statins bind HMG-CoA reductase $\sim 10,000\times$ tighter than substrate—same principle as proline racemase example.

HOW TO STUDY THIS CHAPTER: ENZYME FUNDAMENTALS STUDY STRATEGY



COMMON EXAM TRAPS

- Confusing rate with equilibrium
- Thinking negative ΔG means fast reaction (it doesn't—it means favorable)
- Believing enzymes "force" reactions in one direction
- Mixing up exergonic/endergonic with exothermic/endothermic