

CHEM1200: Discovery in Biology

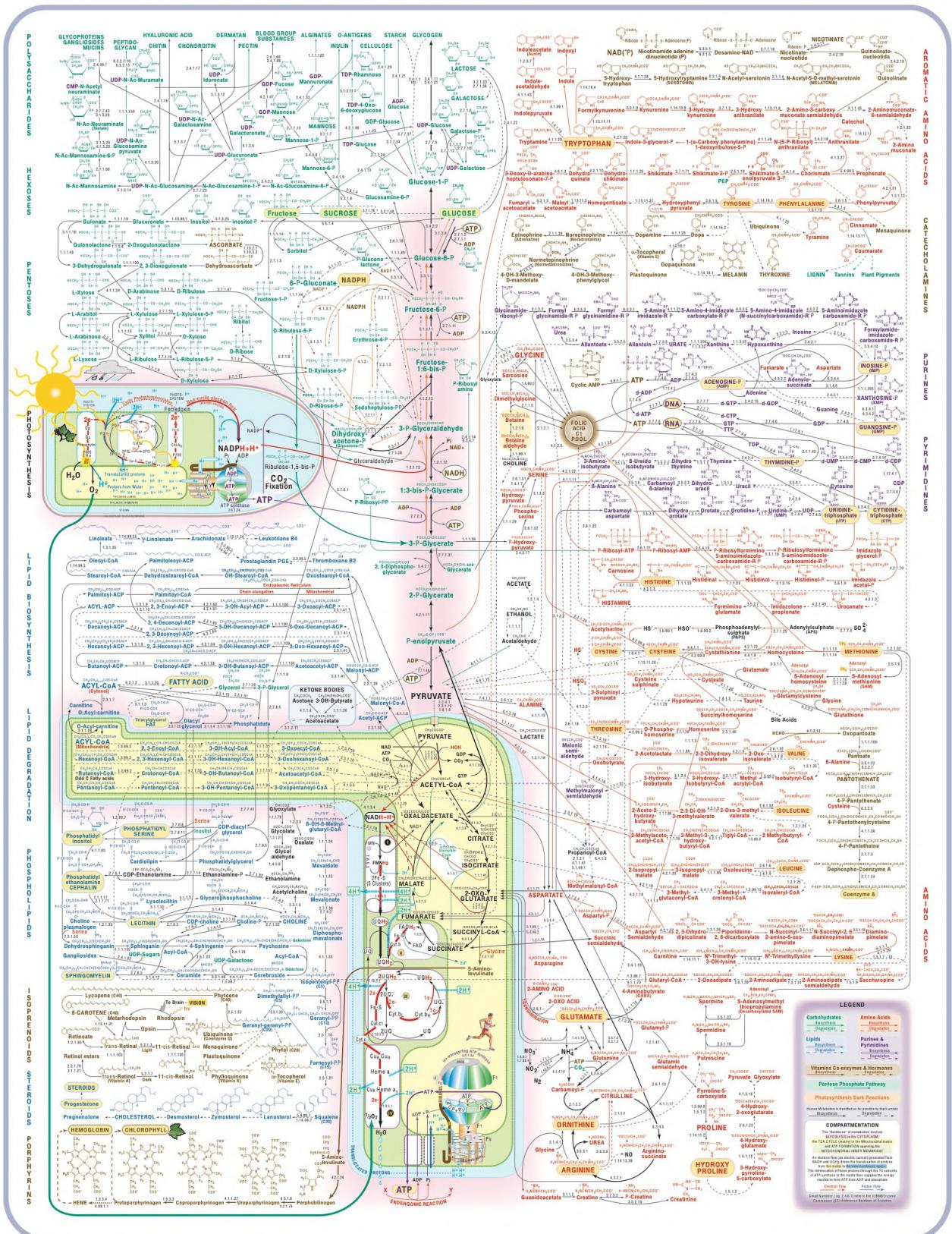
Week 6: Metabolism

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Metabolic Pathways Map

Metabolic Pathways



- We are performing a large number of chemical reactions in our body to maintain our lives; there exist many metabolic pathways.
- These reactions are catalyzed by enzymes with different specificities (substrate specificity & product specificity).
- What reactions are we performing? What enzymes are involved? How are the enzymatic activities controlled?

Metabolism

Metabolism

- Series of life-sustaining chemical reactions within the cells of organisms
- Basically performed by **enzymes**

Catabolism

- Metabolic process whereby cells break down complex substances into simpler, smaller ones to obtain energy

Anabolism

- Metabolic process whereby cells convert simple substances into more complex ones, including carbohydrates, proteins, lipids, and nucleic acids

How to Draw Chemical Structures

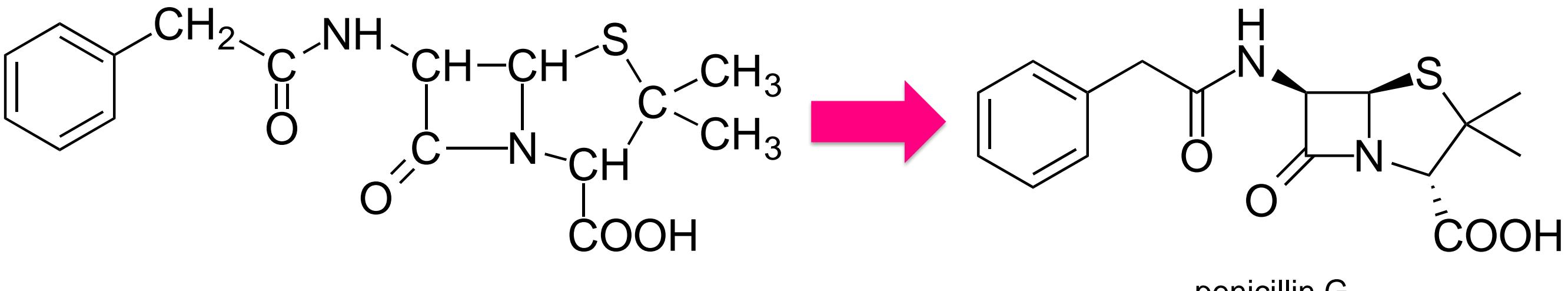
Drawing chemical structures...

1. Organic molecules normally have many carbon and hydrogen atoms, but it is tough to draw all carbon and hydrogen atoms.

- Carbon atoms and hydrogen atoms bound to a carbon are often not shown

2. Organic molecules often contain one or more chiral centers

- Wedged  or dashed  lines are used to draw bond coming forward or going backward out of the plane of the paper, respectively



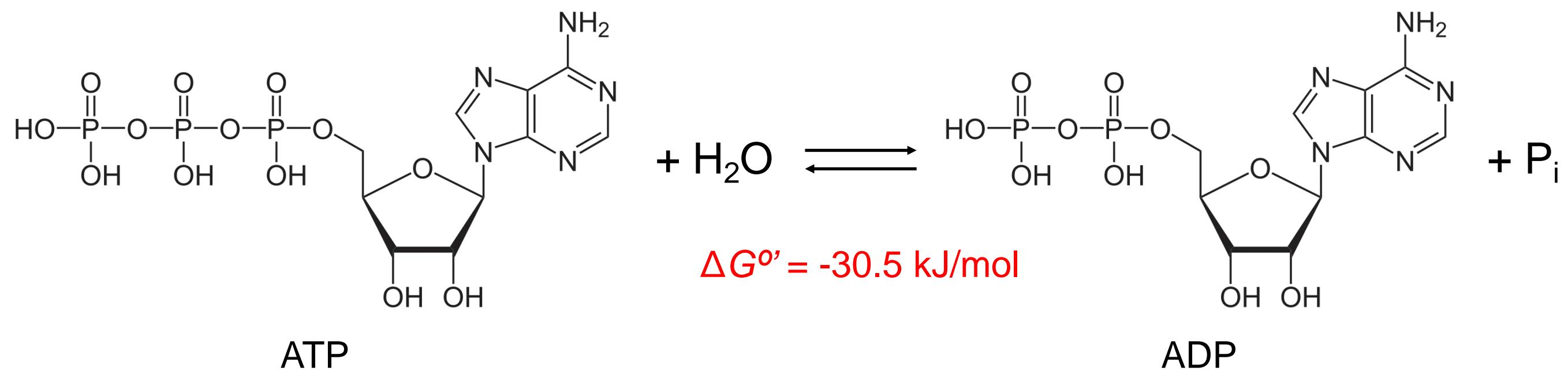
penicillin G

antibiotic from the fungus Penicillium rubens

ATP: The Energy Currency

Adenosine Triphosphate (ATP)

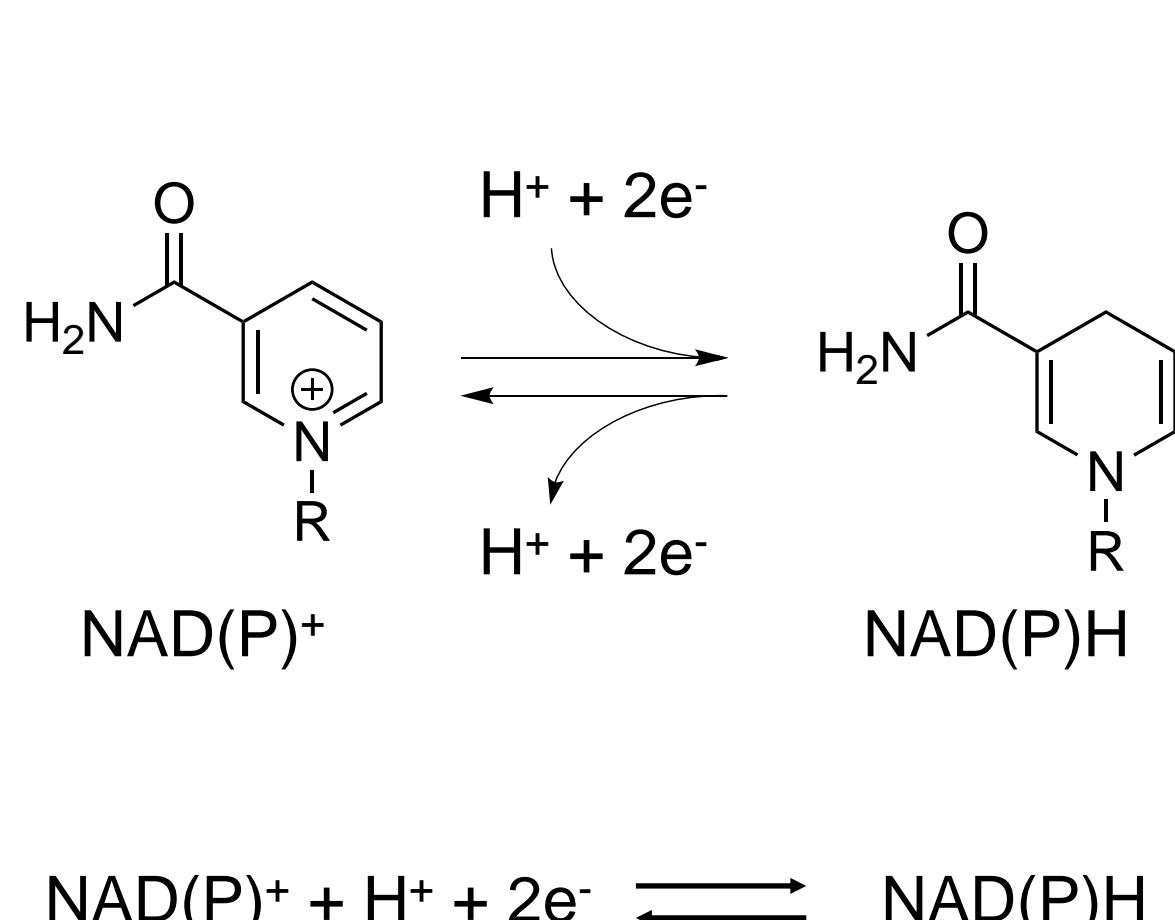
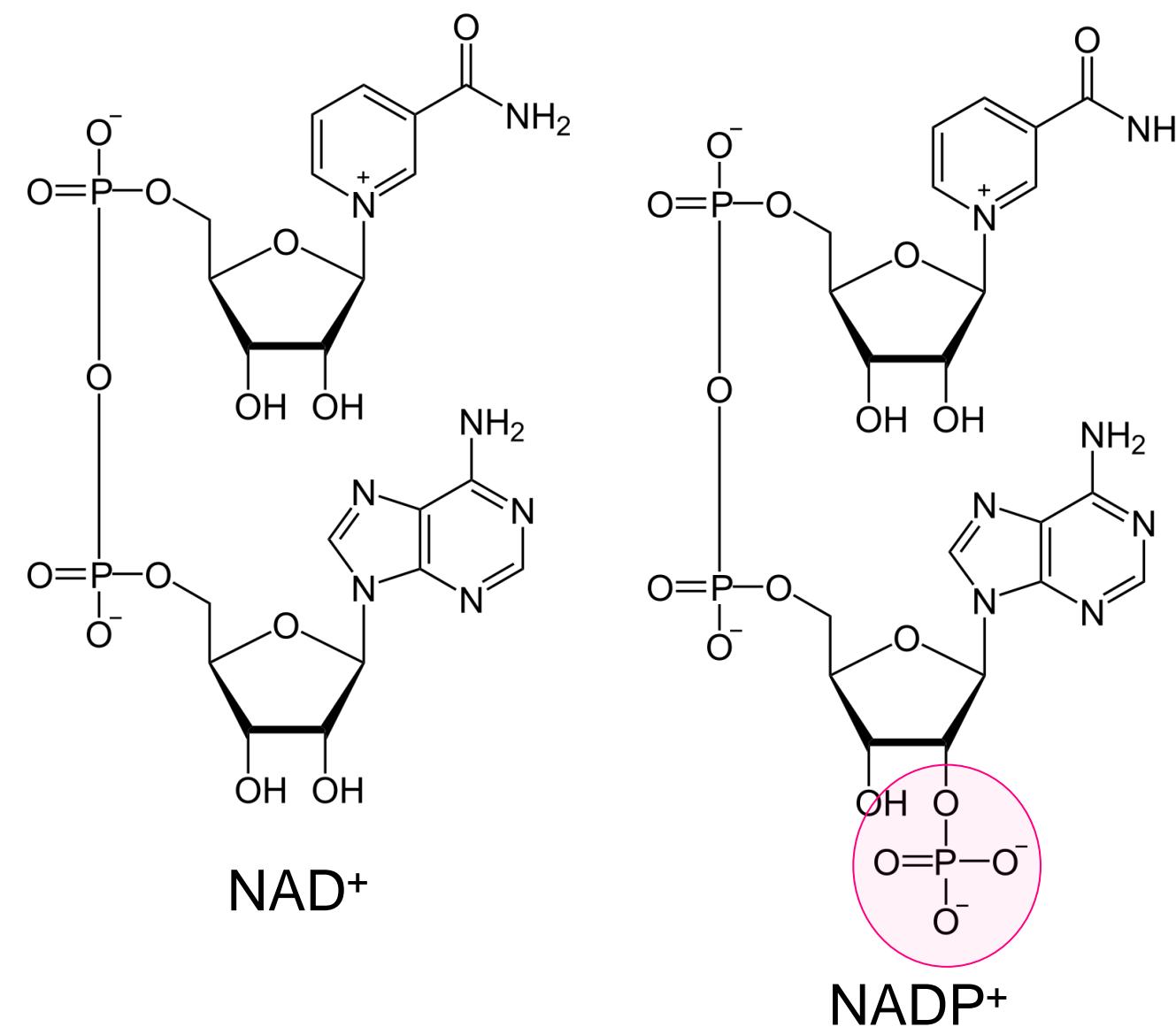
- Serves as the universal energy currency that kick-starts many energy-releasing processes and powers energy consuming processes
- High energy molecules, as its hydrolysis to adenosine diphosphate (ADP) and inorganic phosphate (P_i) releases a large amount of energy
- The reverse reaction (ADP to ATP) can also occur, which captures energy from the oxidation of energy nutrients



Electron Carriers

Nicotinamide Adenine Dinucleotide (NAD^+)

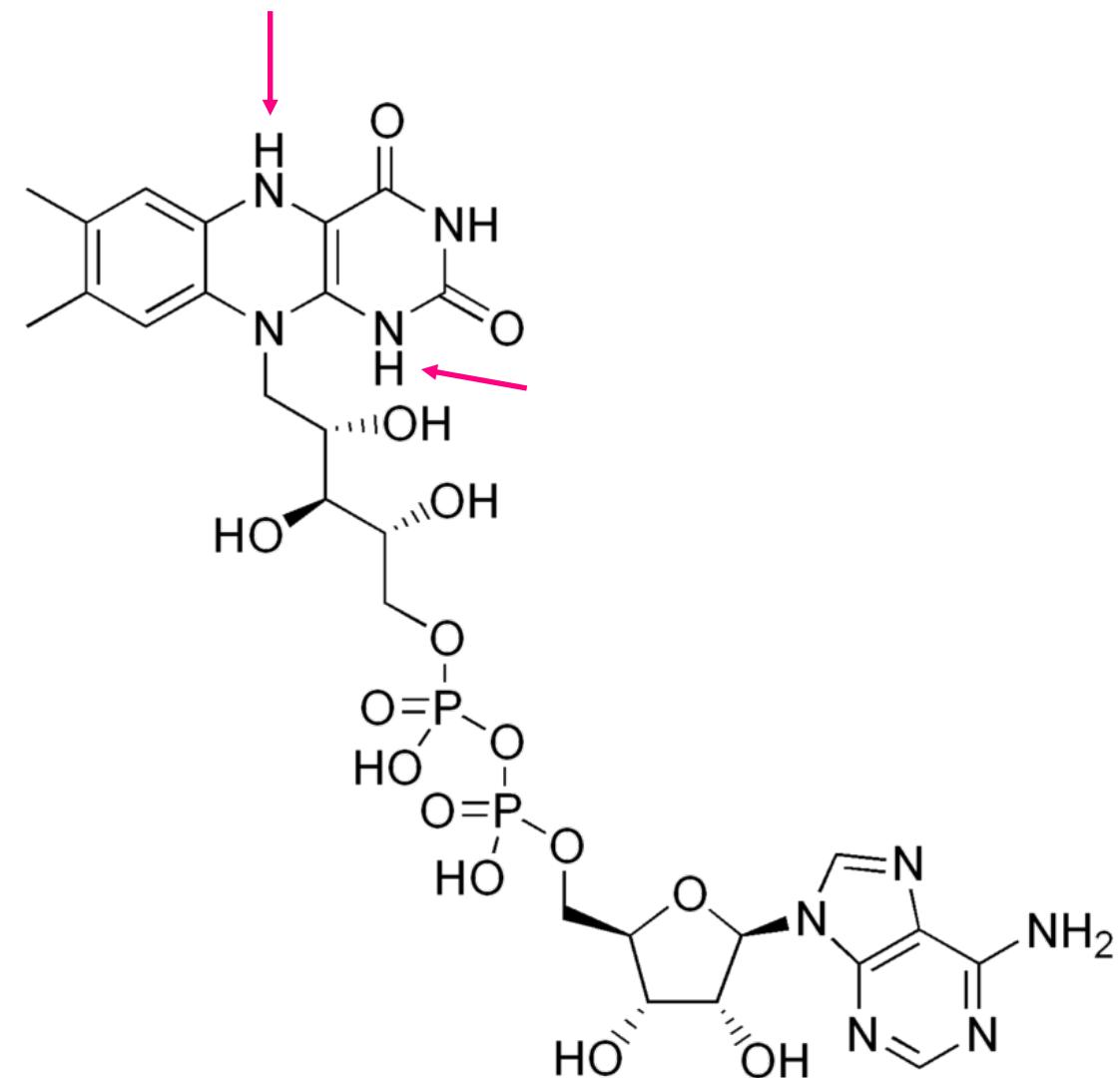
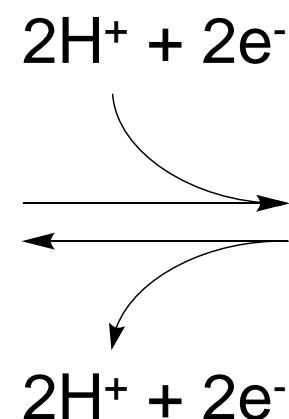
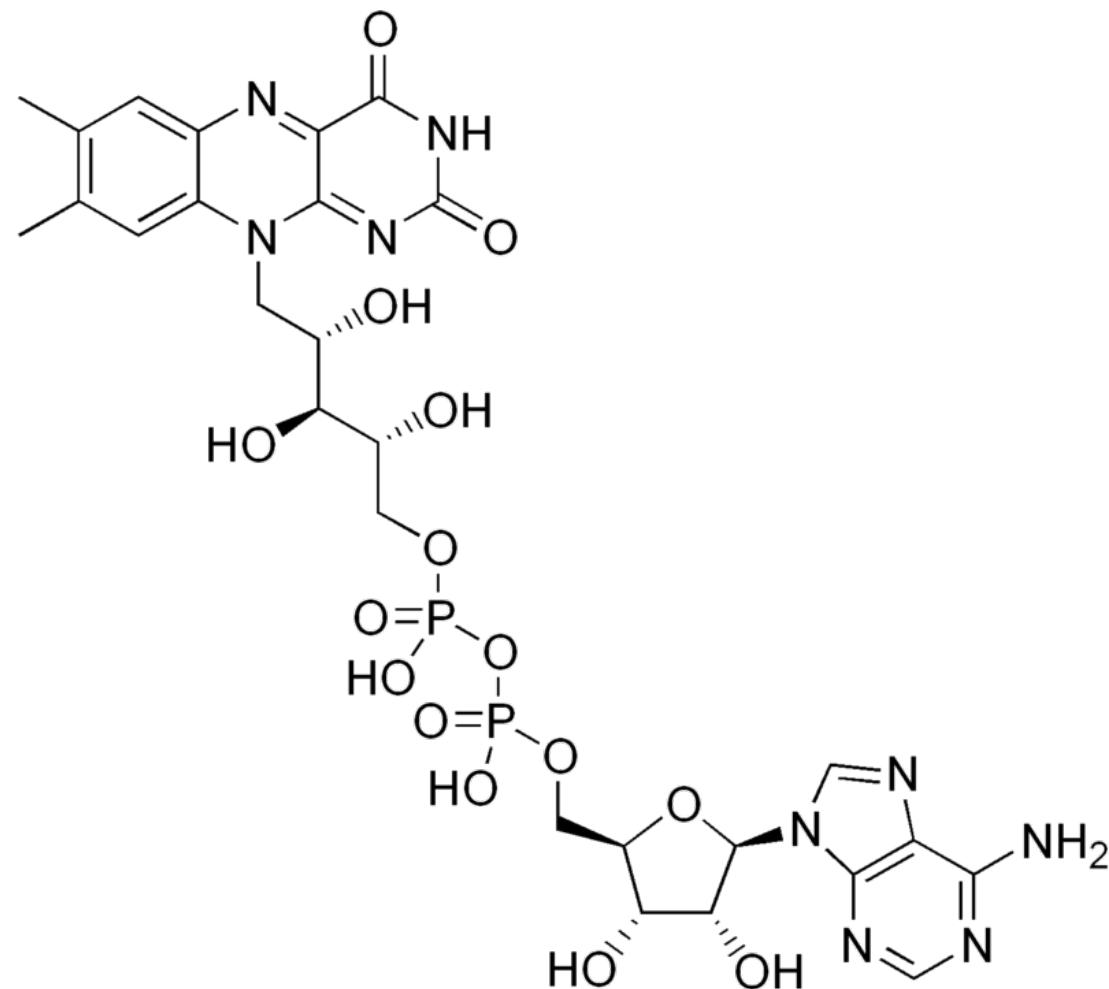
- One of the major electron acceptors, which can accept two electrons
- Its phosphorylated analogue NADP^+ is also widely utilized in metabolic reactions; both of them often involve redox reactions



Electron Carriers

Flavin Adenine Dinucleotide (FAD)

- Another major electron acceptor, which can accept two electrons
- Derivative of riboflavin (vitamin B₂)



FAD

FADH₂

Proteins / Enzymes

Proteins

- Biomacromolecules with one or more chains of amino acid residues
- 20 proteinogenic amino acids

Enzymes

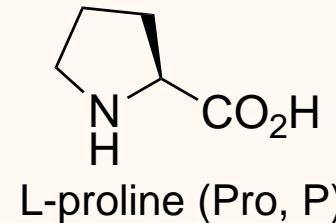
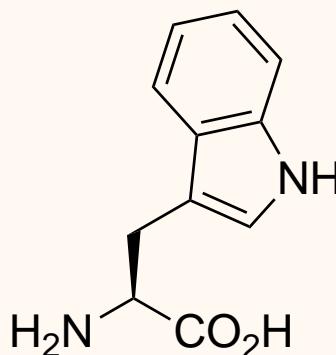
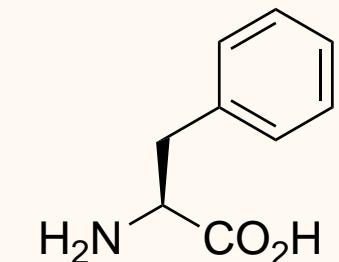
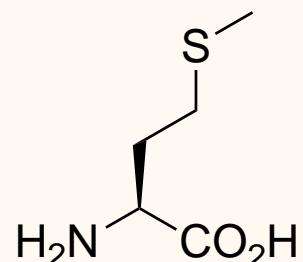
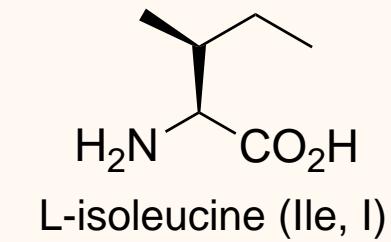
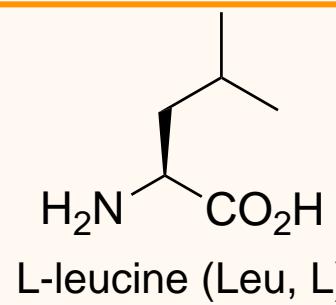
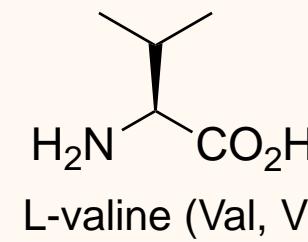
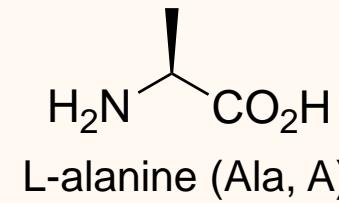
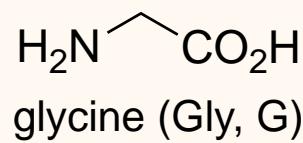
- Biological catalysts and mostly proteins: key players in metabolic pathways
- Generally exhibit high substrate and product selectivities
- Some require non-protein chemicals for activity (cofactors)

Cofactors

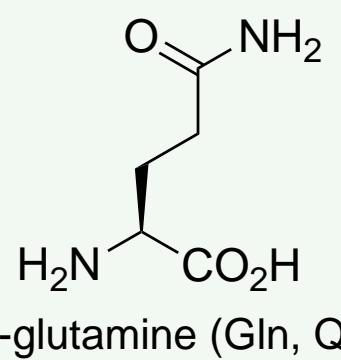
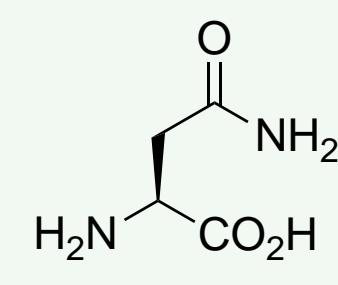
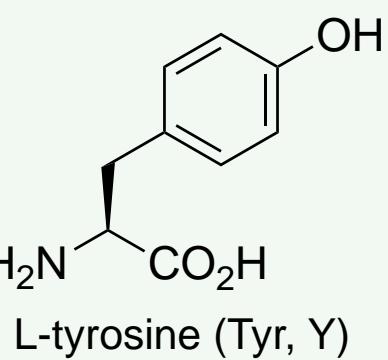
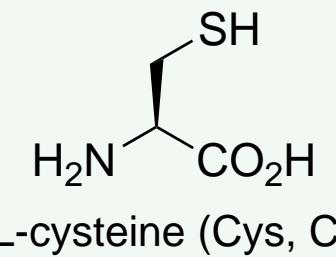
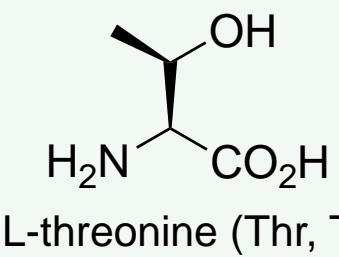
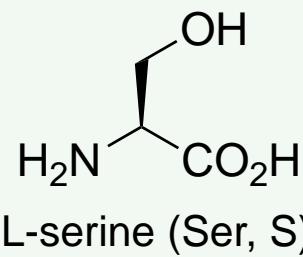
- Inorganic ions (e.g. Fe^{2+} , Fe^{3+} , Cu^{2+} , Mg^{2+} , etc.)
- Coenzymes: Prosthetic groups (covalently bound) or cosubstrates (non-covalently bound)

Proteinogenic Amino Acids

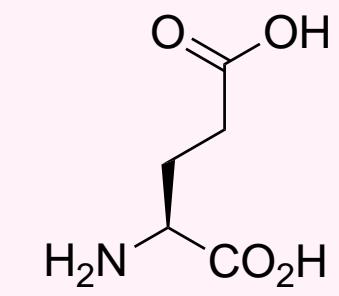
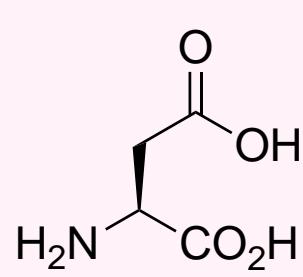
Nonpolar side chains



Uncharged polar side chains

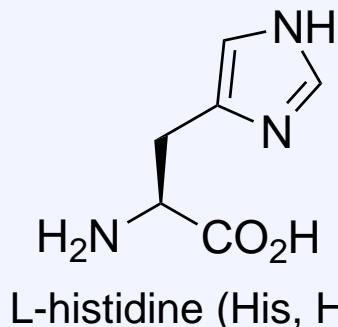
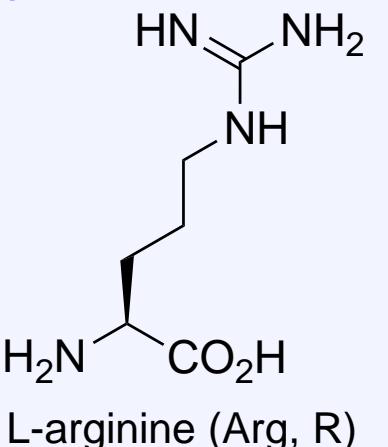
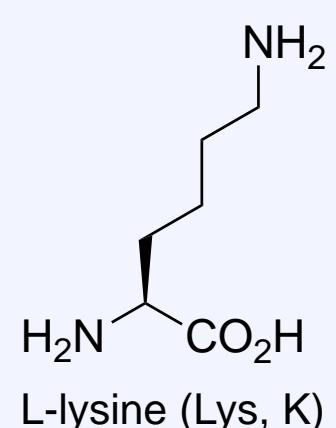


Negatively charged polar side chains



L-aspartic acid (Asp, D) L-glutamic acid (Glu, E)

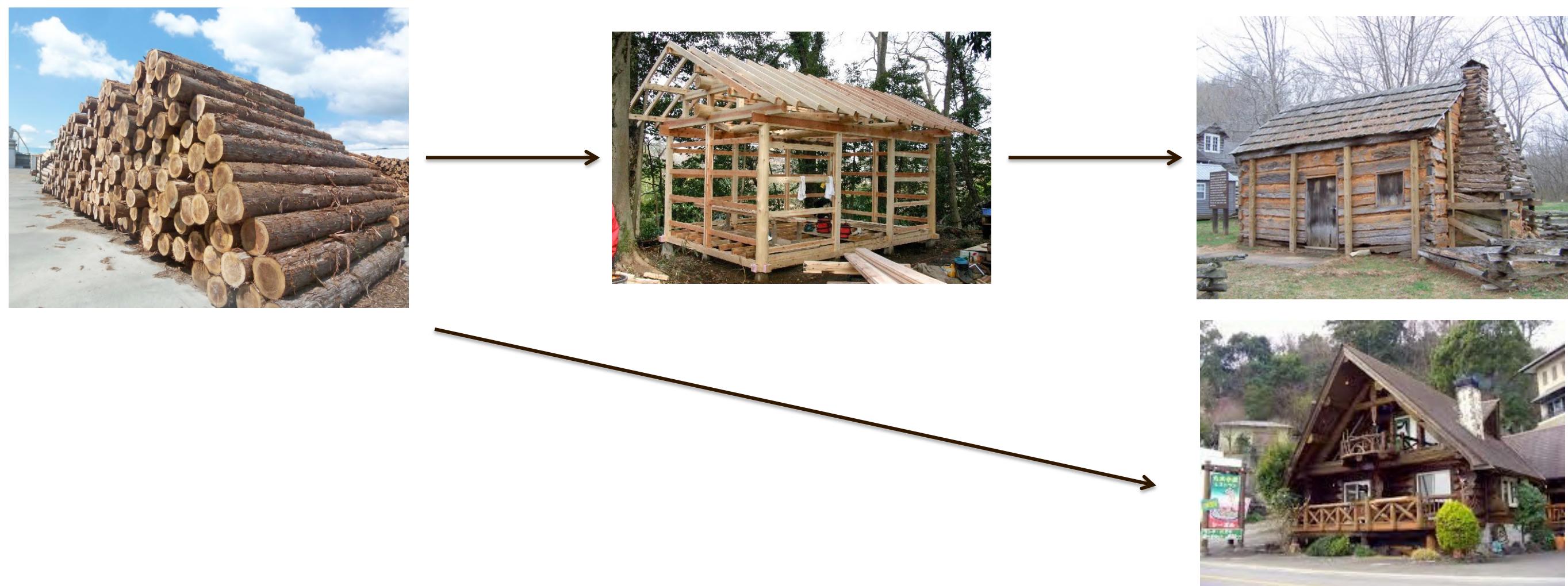
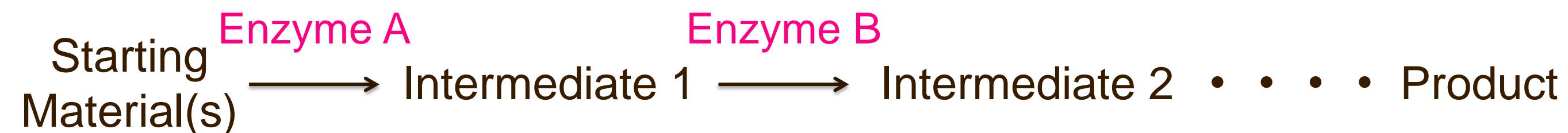
Positively charged polar side chains



L-lysine (Lys, K) L-arginine (Arg, R) L-histidine (His, H)

Enzymatic Reactions

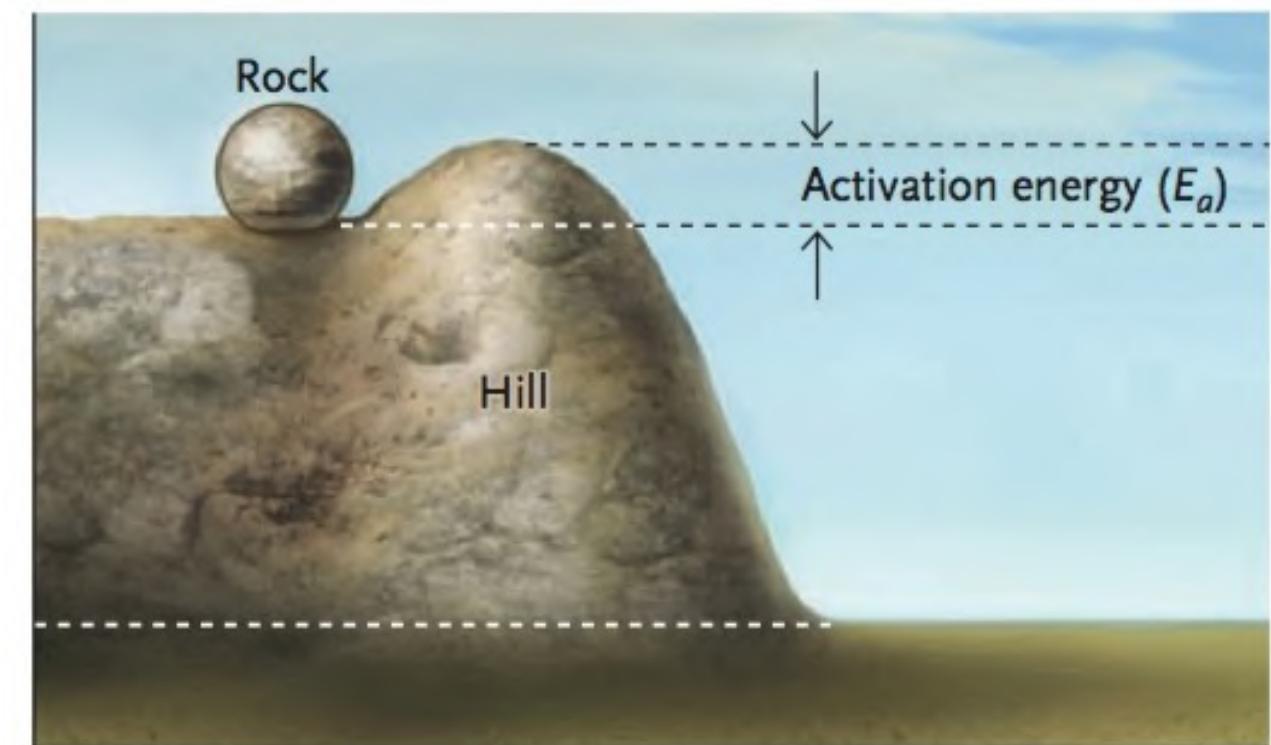
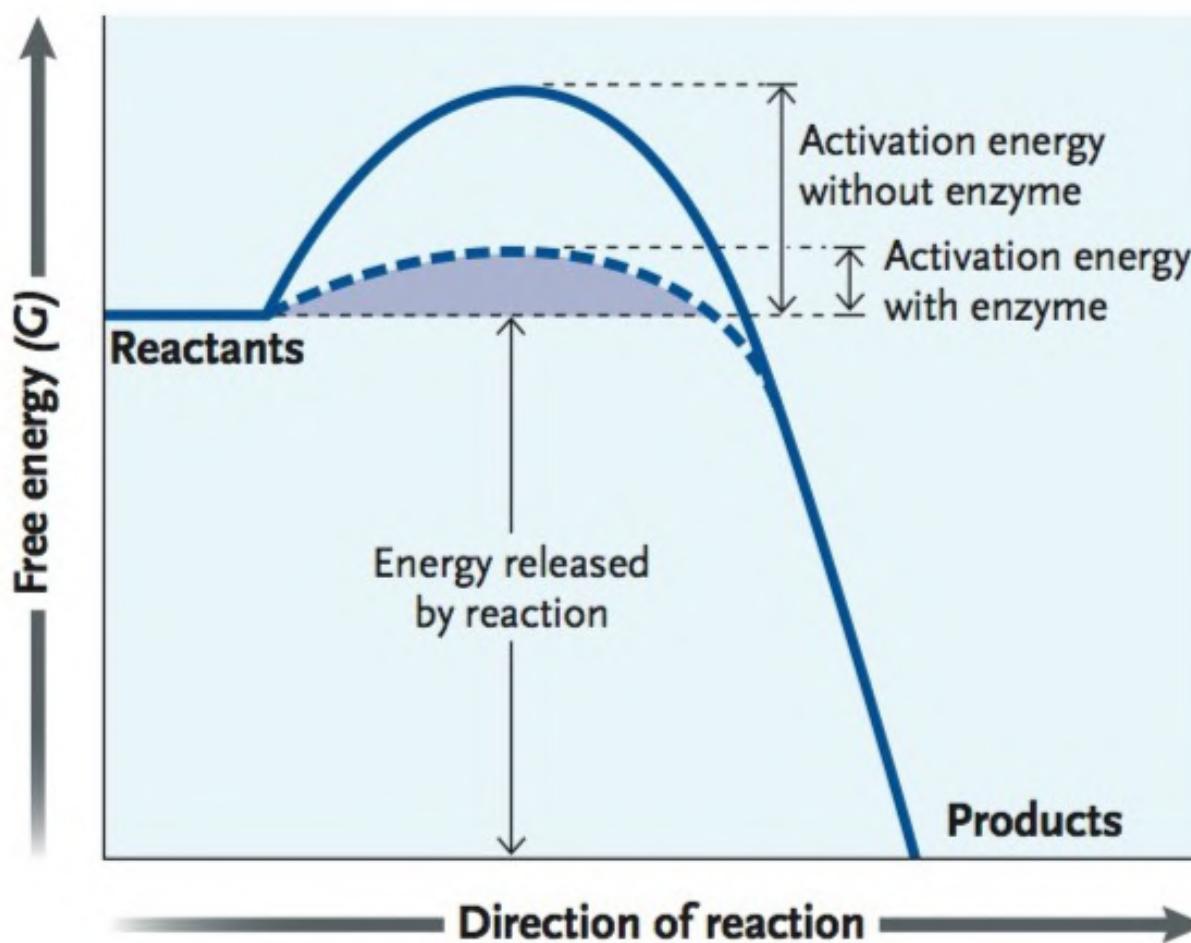
Biological compounds, including secondary metabolites, are synthesized by enzymes. Enzymes are catalysts.



Enzymes Are Catalysts

Enzyme

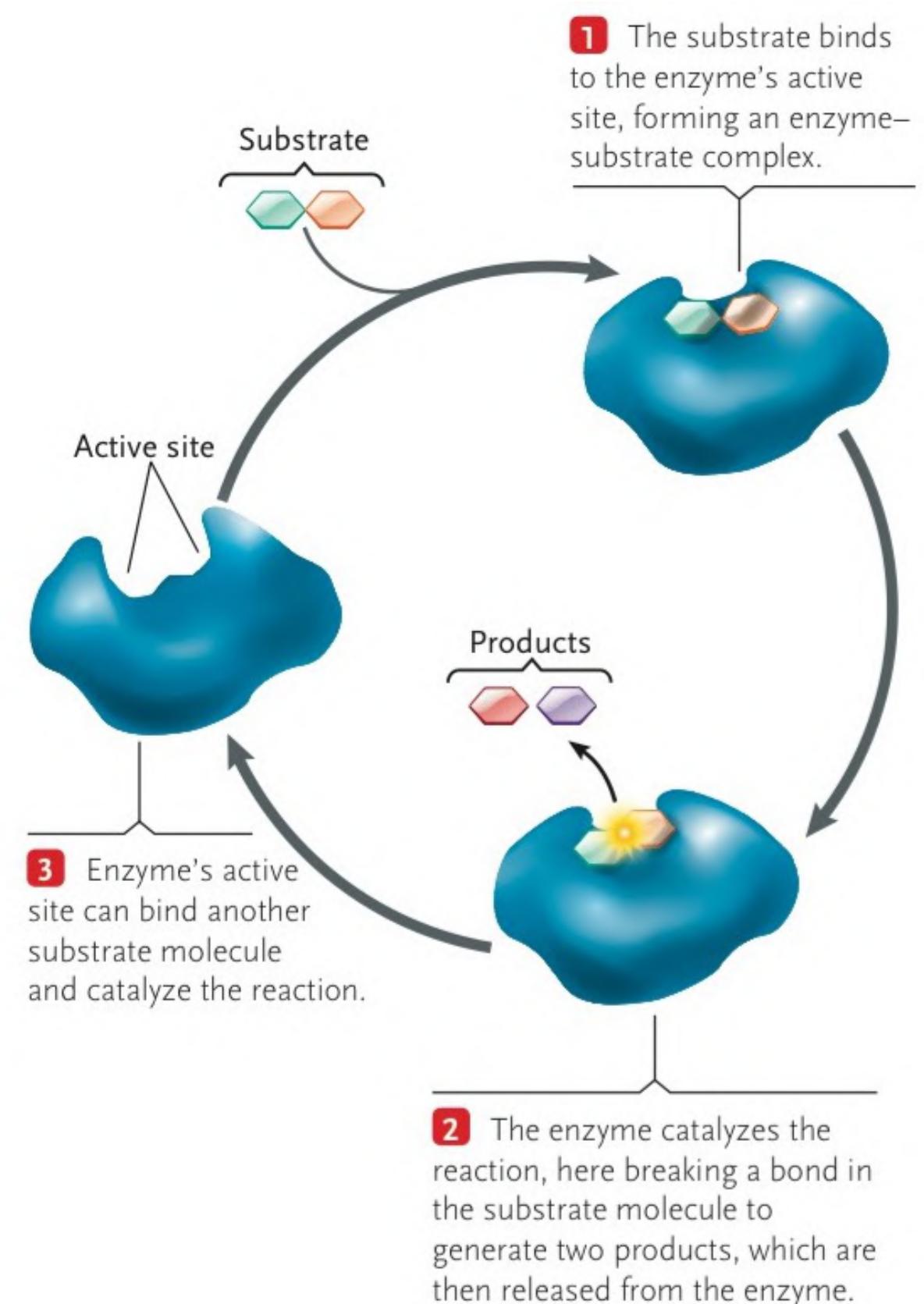
- A catalyst, which accelerates the reaction rate without being changed by the reaction
- Lowers the activation energy and speed up the rate of exergonic reactions
- Do NOT supply free energy to a reaction, so CANNOT make an endergonic reaction spontaneously; ATP hydrolysis can be coupled to an endergonic reaction to make it proceed spontaneously



Catalytic Cycle of Enzymes

Enzyme

- Generally shows a high specificity toward its substrate (**substrate specificity**), so only accepts one or closely related molecules
- Has an **active site**, to which the substrate binds
- Catalyzes a reaction once an enzyme-substrate complex is formed to yield one or more products
- Goes back to the original state after the reaction and accepts another substrate;
An enzyme is a catalyst!

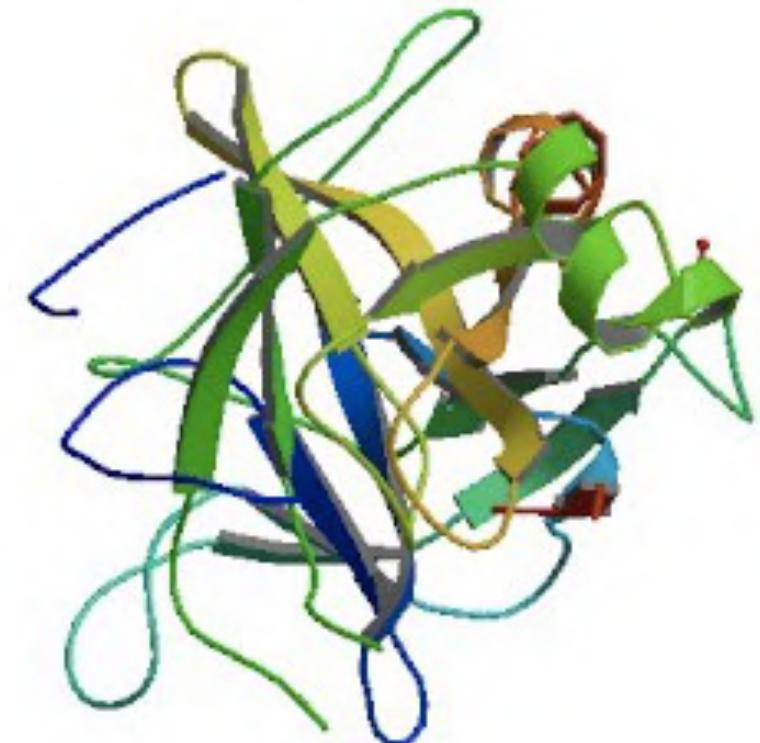


Example of an Enzymatic Reaction

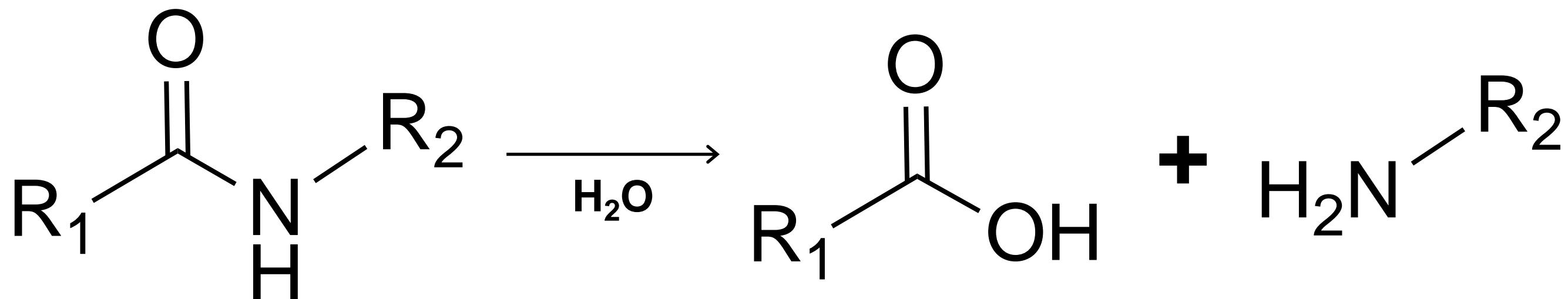
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Chymotrypsin

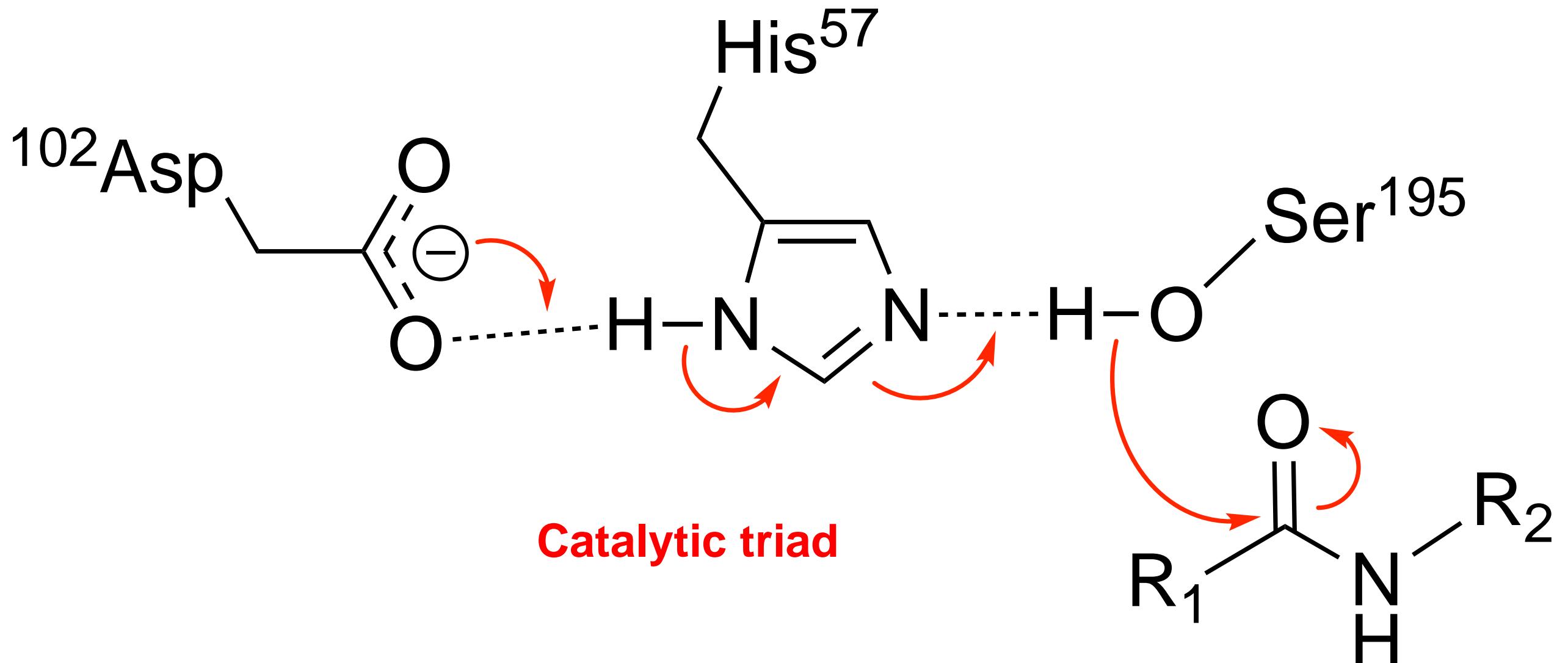
- Proteolytic enzyme (serine hydrolase)
- Performs amide hydrolysis



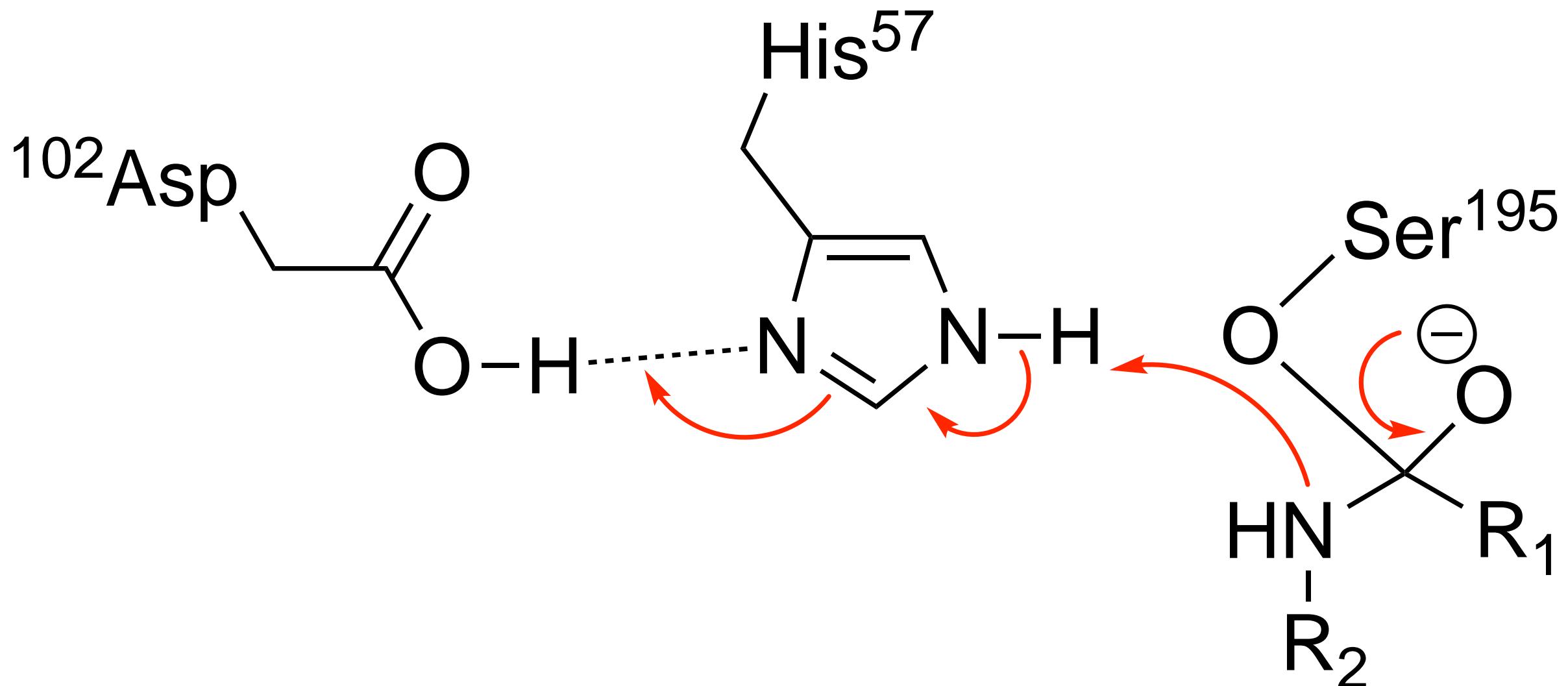
Reaction catalyzed by chymotrypsin



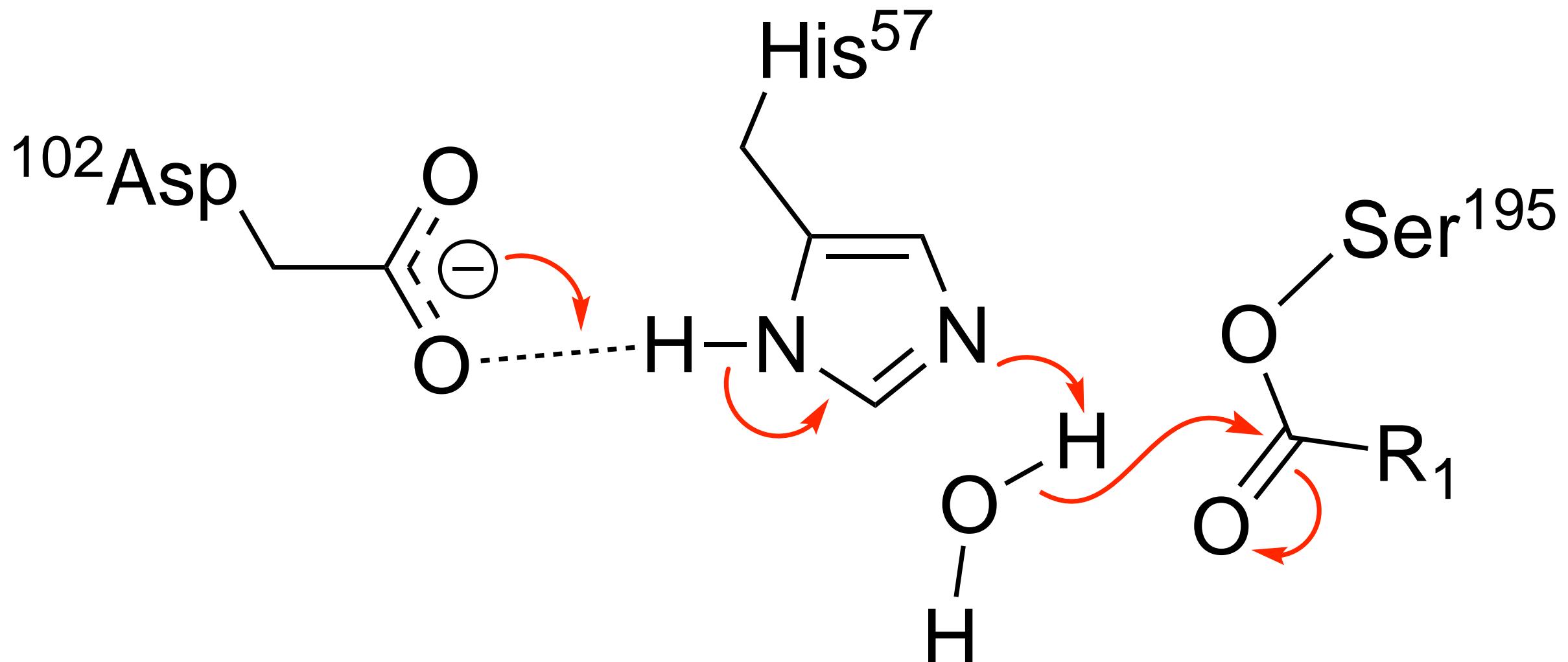
Example of an Enzymatic Reaction



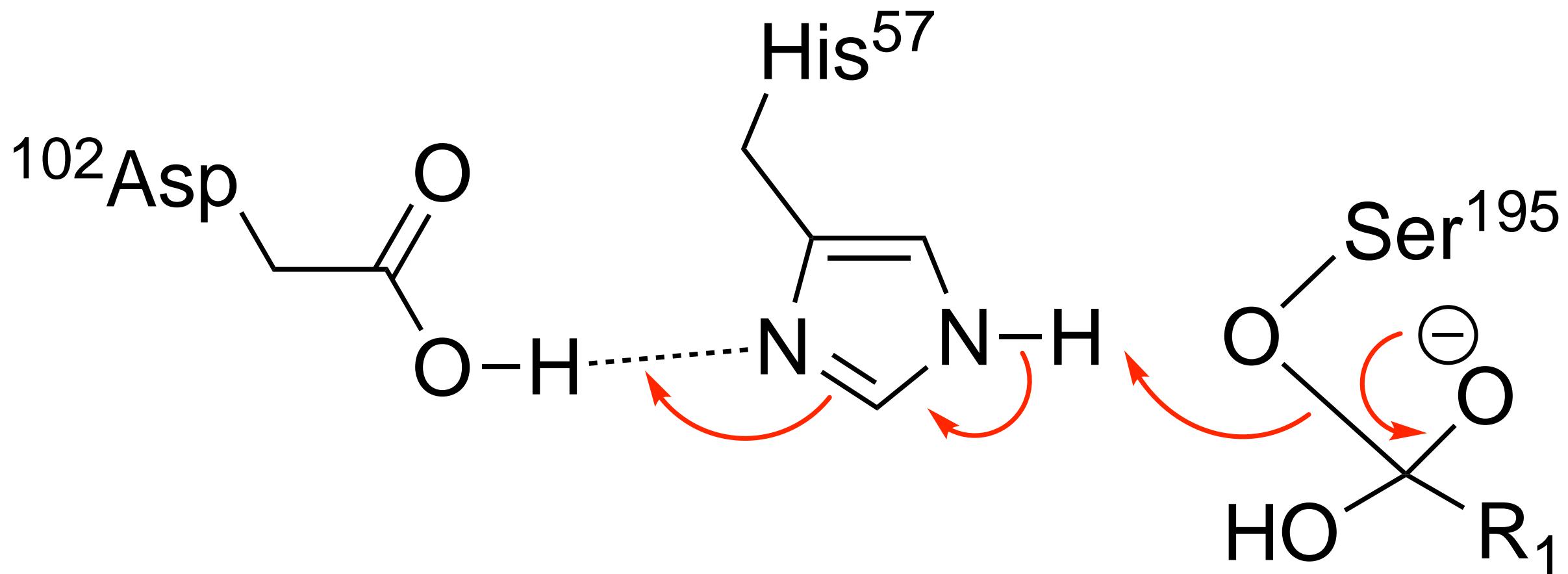
Example of an Enzymatic Reaction



Example of an Enzymatic Reaction



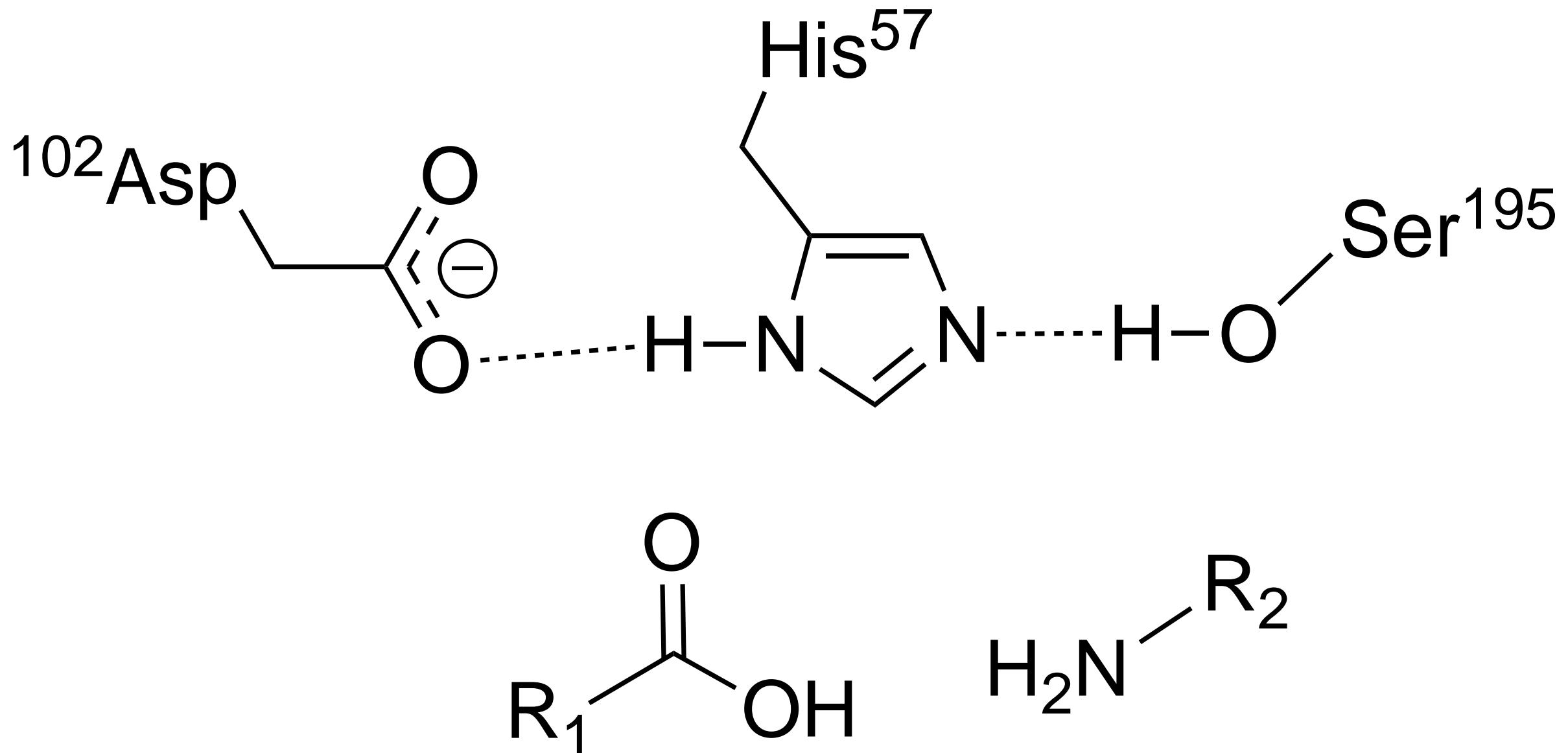
Example of an Enzymatic Reaction



Example of an Enzymatic Reaction

17

Returned to the original state

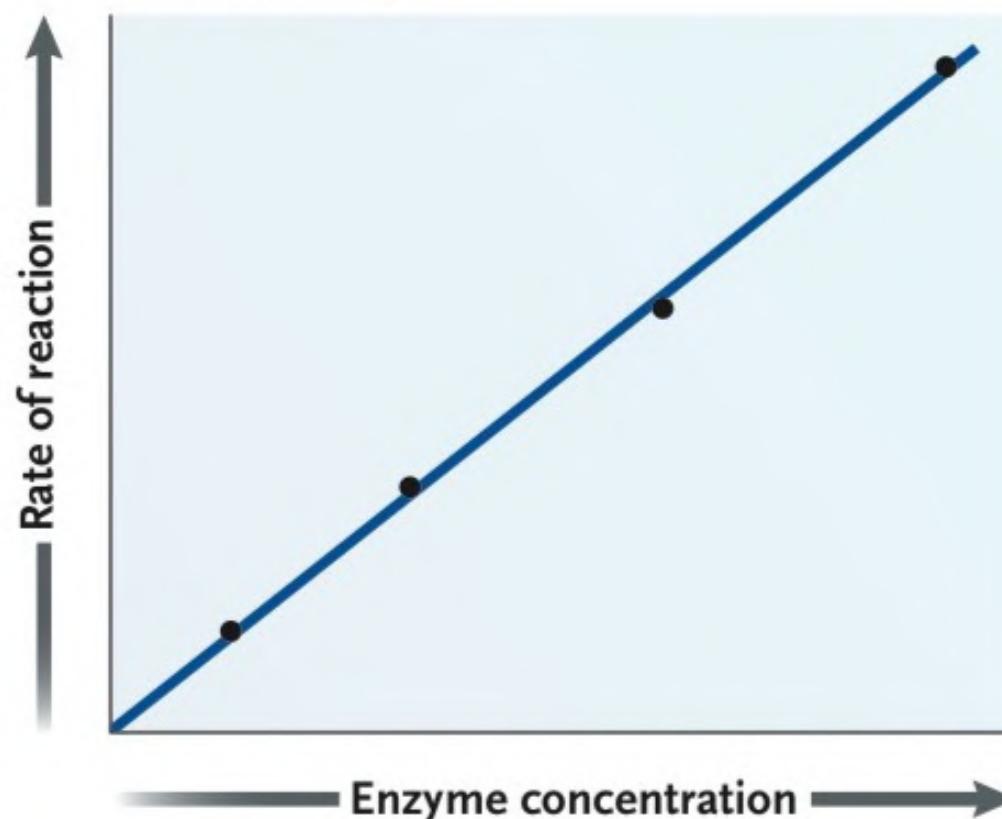


Enzyme Kinetics

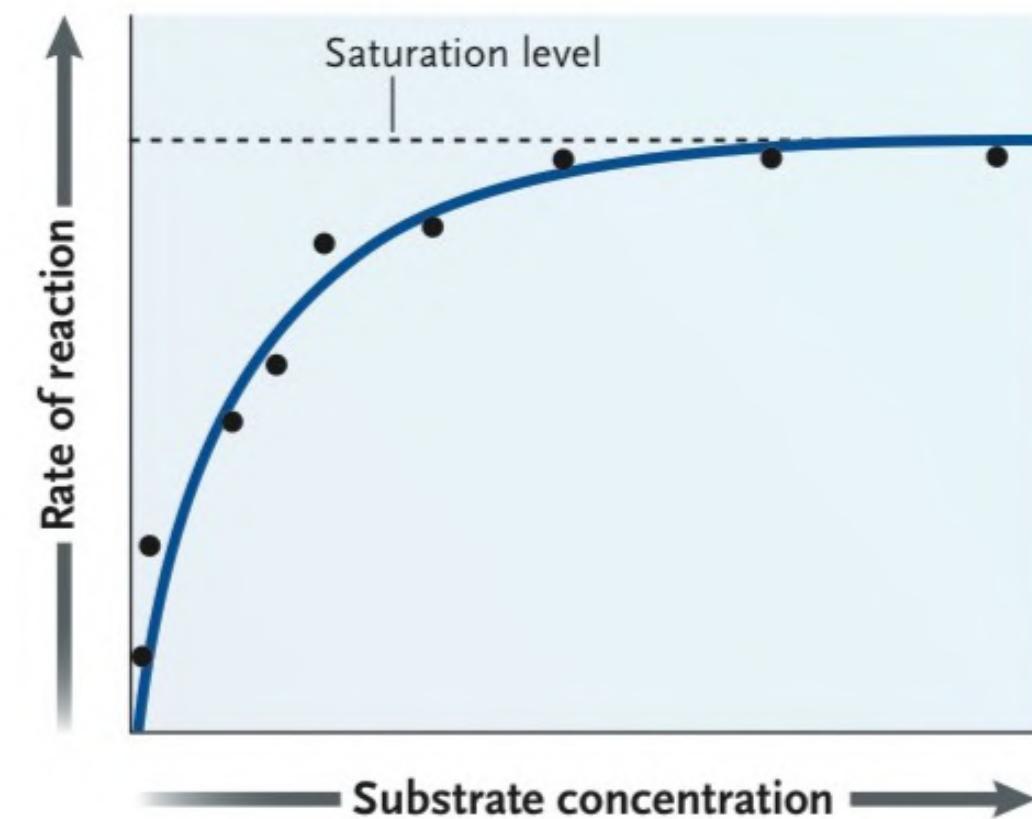
Reaction rate of an enzymatic reaction

- Proportional to the amount of enzyme in the presence of excess substrate
- Initially increases as the substrate concentration increases in case the enzyme concentration is constant, but reaches the maximum rate (saturation with the enzyme) at higher substrate concentration

A. Rate of reaction as function of enzyme concentration (substrate at high concentration)



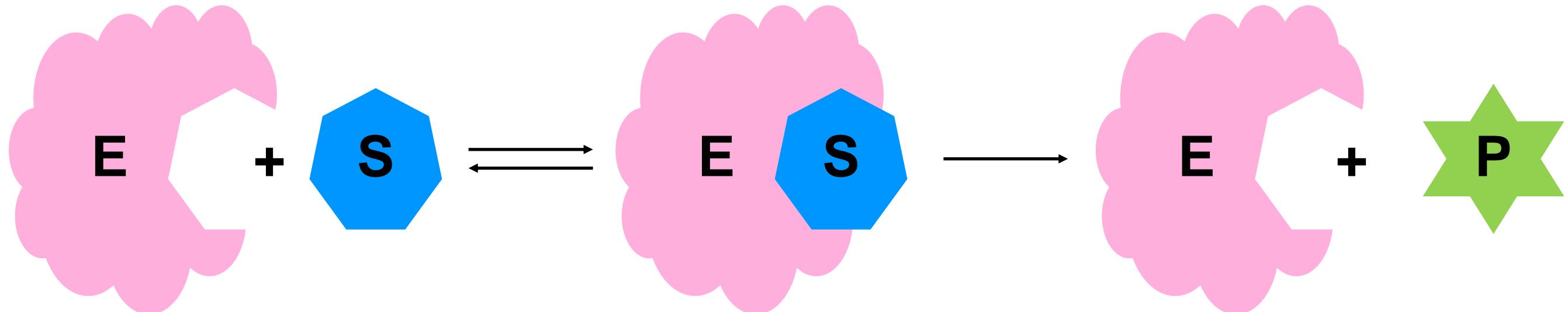
B. Rate of reaction as function of substrate concentration (enzyme amount constant)



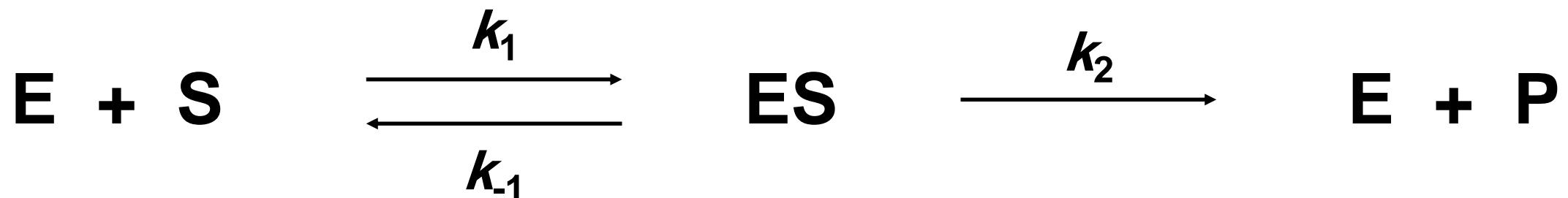
Enzyme Kinetics

Let's think about the following enzymatic reaction

enzyme-substrate complex

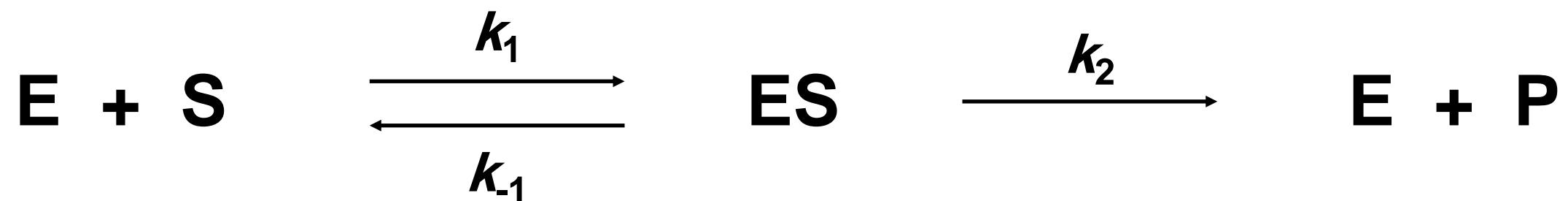


E: enzyme, S: substrate, P: product



k_1 , k_{-1} , k_2 : rate constants

Enzyme Kinetics

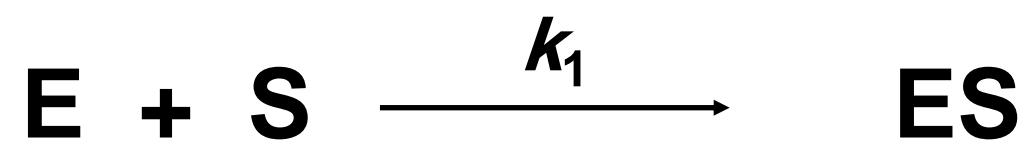


k_1, k_{-1}, k_2 : rate constants

Reaction rates

Reaction rate for ES formation

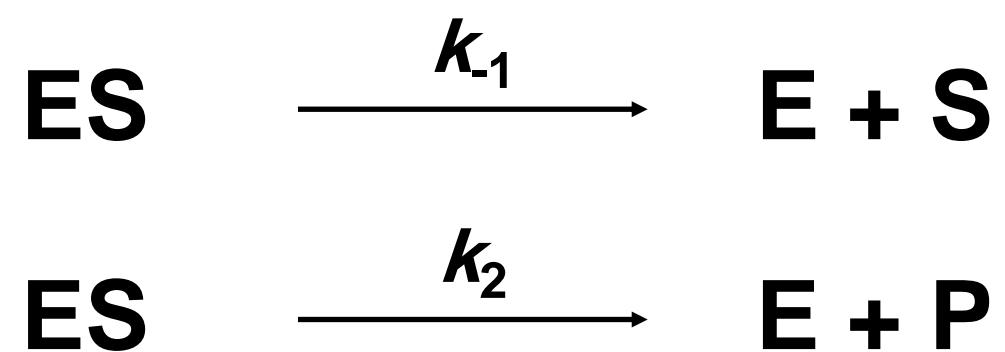
$$\text{rate} = k_1[E][S]$$



Reaction rate for ES break down

$$\text{rate} = k_{-1}[ES] + k_2[ES]$$

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



note: $[X]$ is the concentration of X

Enzyme Kinetics

Steady state assumption

- Assumption that [ES] (concentration of ES) is constant; the rate of ES formation equals the rate of ES break down

With the assumption, how can we express the product formation rate V ?

$$V = k_2[\text{ES}] \text{ (normally } k_2 \ll k_1, k_{-1} \text{)} \quad \textcolor{red}{\textit{It is difficult to measure [ES]}}$$

Based on the steady state assumption:

Here, consider the total enzyme concentration $[E]_0$:

$$[E]_0 = [E] + [ES] \dots \dots \dots \quad \textcircled{2}$$

Eliminate [E] from the equations ① and ②:

$$[ES] = \frac{[E]_0[S]}{\frac{k_{-1} + k_2}{k_1} + [S]}$$

$$\therefore V = k_2[ES] = \frac{k_2[E]_0[S]}{\frac{k_{-1} + k_2}{k_1} + [S]}$$

Michaelis-Menten Equation

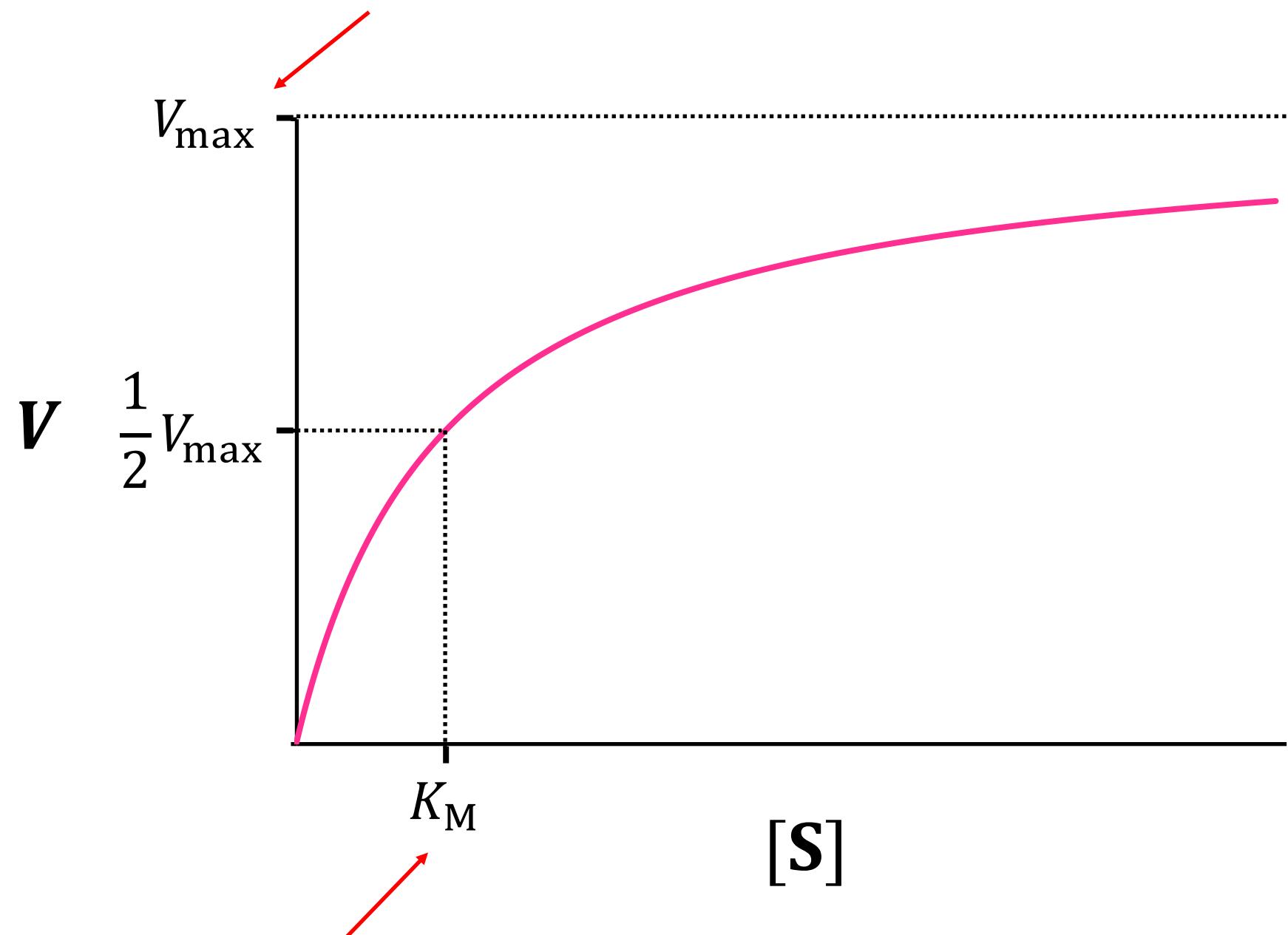
$$V = k_2[\text{ES}] = \frac{k_2[\text{E}]_0[\text{S}]}{\frac{k_{-1} + k_2}{k_1} + [\text{S}]} = \frac{V_{\max}[\text{S}]}{K_M + [\text{S}]}$$

$$\text{where } V_{\max} = k_2[\text{E}]_0 \text{ and } K_M = \frac{k_{-1} + k_2}{k_1}$$

This equation is called the **Michaelis–Menten equation**, which is a well-known model used in enzyme kinetics and can be applied to many (but not all) enzymatic reactions. The constant K_M is known as **Michaelis constant**.

Michaelis-Menten Equation

V_{\max} : Maximum rate of an enzyme-catalyzed reaction; the rate when the enzyme is saturated with the substrate but not achieved in reality

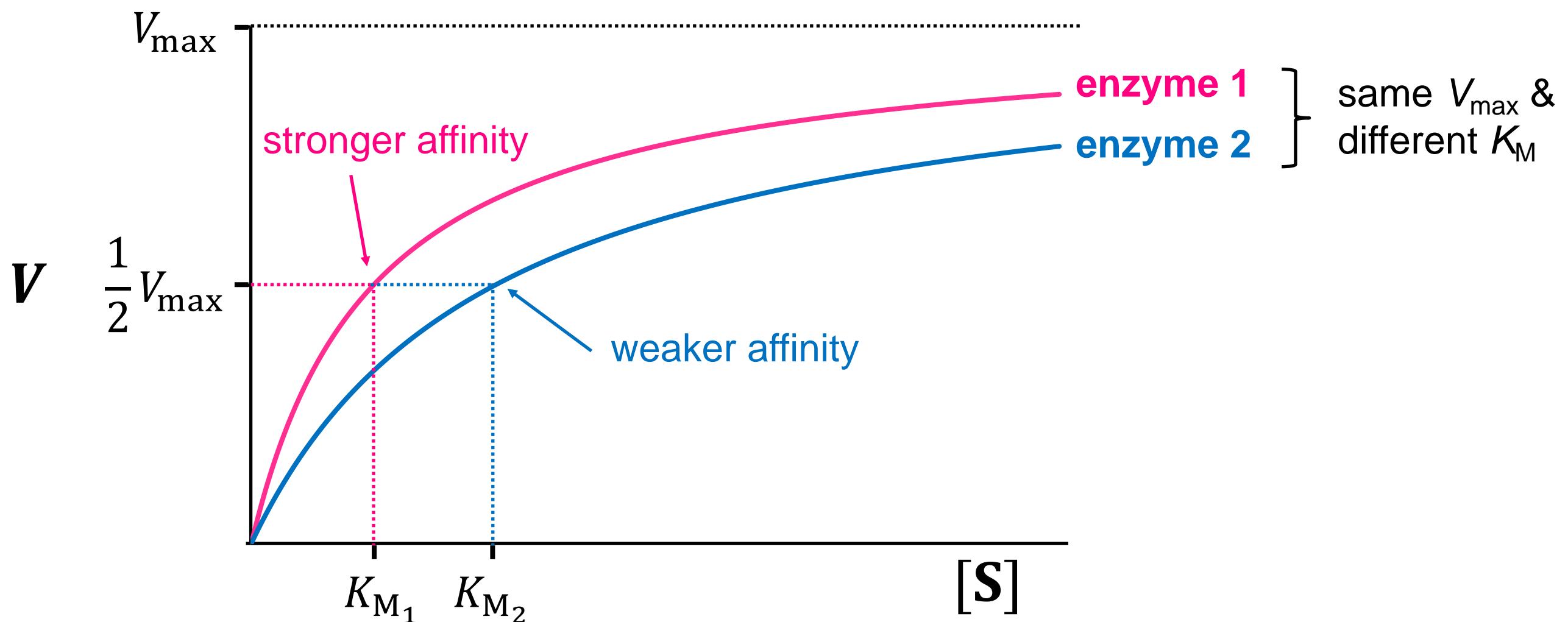


K_M : Substrate concentration at which the reaction rate is equal to $1/2 V_{\max}$

Michaelis-Menten Equation

More about K_M

- $K_M = [S]$ at $1/2 V_{max}$
- K_M represents the concentration of the substrate required to bind half of the available enzyme (**binding constant** of the enzyme for substrate)
- K_M can be used to evaluate the binding affinity of an enzyme for a substrate
- Under normal physiological condition: $0 < [S] < K_M$



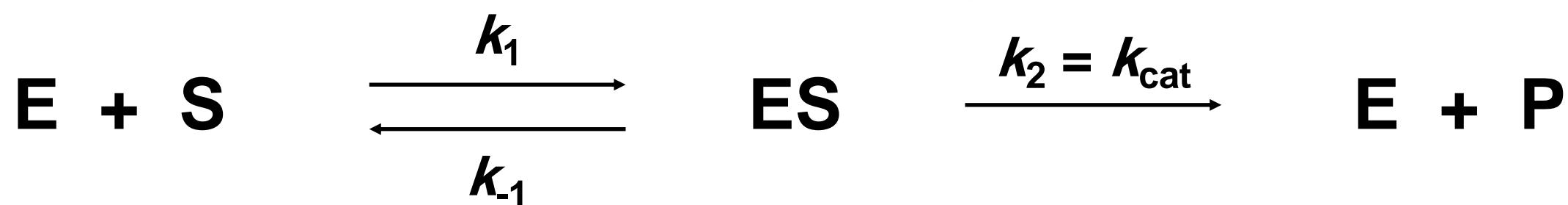
Turnover Number

Turnover number

- Termed k_{cat} ; maximum number of substrate molecules converted to product per enzyme molecule per unit of time (normally second)
- If the Michaelis-Menten model fits:

$$k_{\text{cat}} = k_2 = V_{\text{max}} / [E]_0$$

Enzyme	Turnover Number (per second)
Carbonic anhydrase	600,000
3-Ketoesteroid isomerase	280,000
Acetylcholinesterase	25,000
Penicillinase	2,000
Lactate dehydrogenase	1,000
Chymotrypsin	100
DNA Polymerase I	15
Tryptophan synthetase	2
Lysozyme	0.5



Catalytic Efficiency

Let's think about how we can evaluate the catalytic efficiency of an enzyme

Based on the steady state assumption:

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

$$\Leftrightarrow [\text{ES}] = \frac{[\text{E}][\text{S}]}{K_M} \left(\because K_M = \frac{k_{-1} + k_2}{k_1} \right) \dots \dots \quad \textcircled{1}$$

$$\therefore V = k_2[\text{ES}] = k_{\text{cat}}[\text{ES}] = \frac{k_{\text{cat}}}{K_M} [\text{E}][\text{S}]$$

Under typical physiological condition,

$$[S] \ll K_M \Rightarrow [E]_0 = [E] + [ES] \approx [E]$$

Therefore,

$$V = \frac{k_{\text{cat}}}{K_M} [E]_0 [S] \longleftarrow \textcolor{red}{\text{Bimolecular second order chemical reaction}}$$

Catalytic Efficiency

$$V = \frac{k_{\text{cat}}}{K_M} [E]_0 [S]$$

Based on the equation, the rates of enzyme-catalyzed reaction in our cells depend on:

$[S]$; concentration of substrate

$[E]_0$; total concentration of enzyme

$\frac{k_{\text{cat}}}{K_M}$, which is termed **specificity constant** or **kinetic efficiency**

Catalytic efficiency

➤ $\frac{k_{\text{cat}}}{K_M}$ can be used as a measure of the enzyme's efficiency

Catalytic Efficiency

k_{cat} / K_M values of some enzymes

Enzyme	$K_{Cat} / K_M (s^{-1}M^{-1})$
Acetylcholinesterase	1.6×10^8
Carbonic anhydrase	8.3×10^7
Catalase	4.0×10^7
Crotonase	2.8×10^8
Fumarase	1.6×10^8
Triose phosphate isomerase	2.4×10^8
β -Lactamase	1.0×10^8
Superoxide dismutase	7.0×10^9

Regulations of Enzymatic Activities

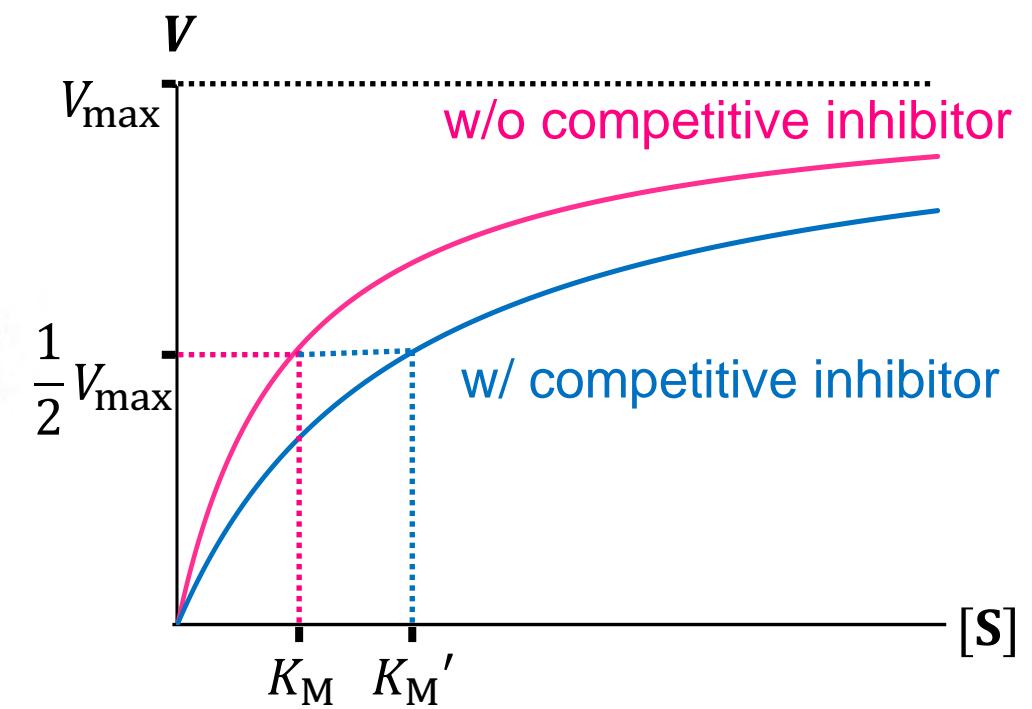
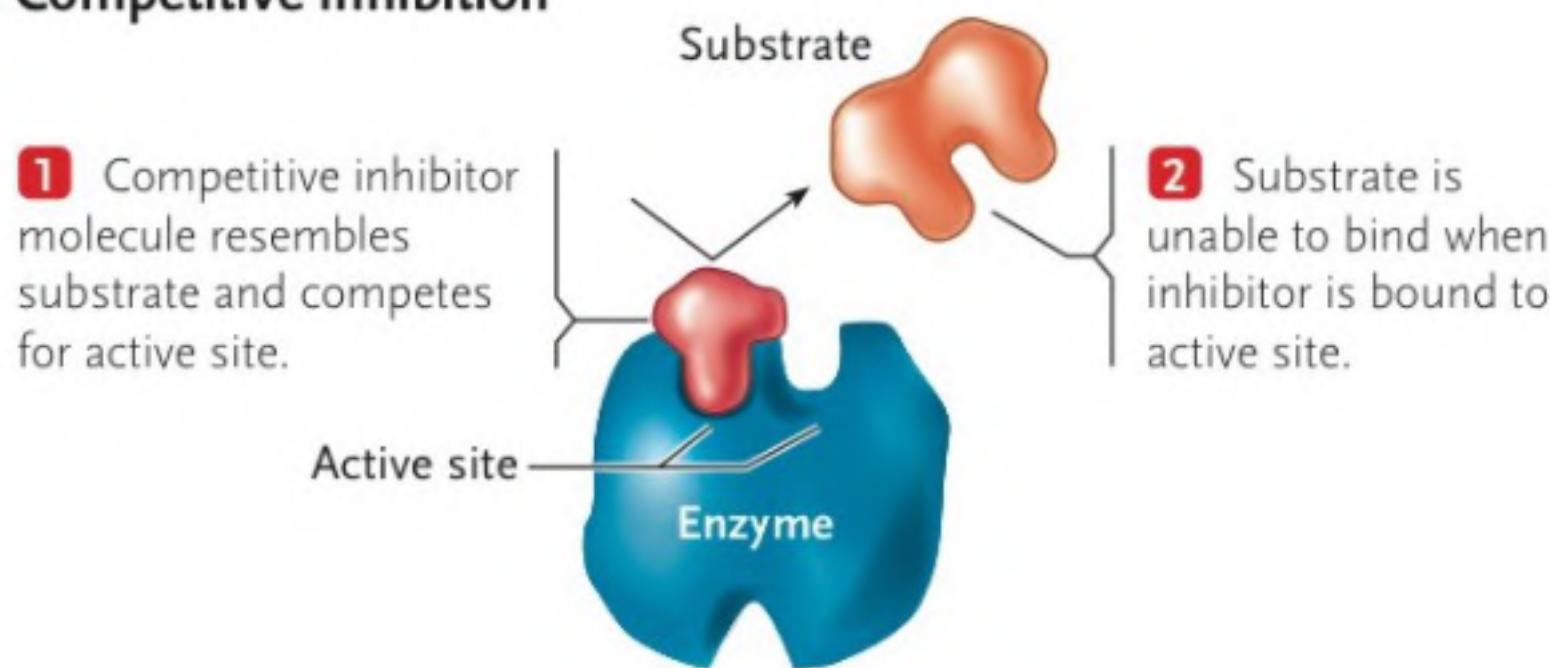
- Cells adjust the activity of many enzymes upward or downward to meet their needs for reaction products.
- Normally, cells regulate the most important enzyme (key enzyme) in a pathway rather than regulating all of the enzymes in the pathway.
- Mechanisms for the regulation include:
 - (1) **Competitive and noncompetitive inhibitions**
 - (2) **Allosteric regulation**, which is a form of noncompetitive inhibition
 - (3) **Covalent modification** by the addition or removal of chemical groups, such as phosphate groups

Competitive Inhibition

Competitive inhibition

- An inhibitor competes with the normal substrate for binding to an enzyme's active site, so it blocks access for the normal substrate and slows down the reaction rate
- Competitive inhibitors normally have an analogous structure to the substrate
- In the presence of a competitive inhibitor, apparent K_M (K_M') increases, whereas V_{max} remains unchanged

A. Competitive inhibition

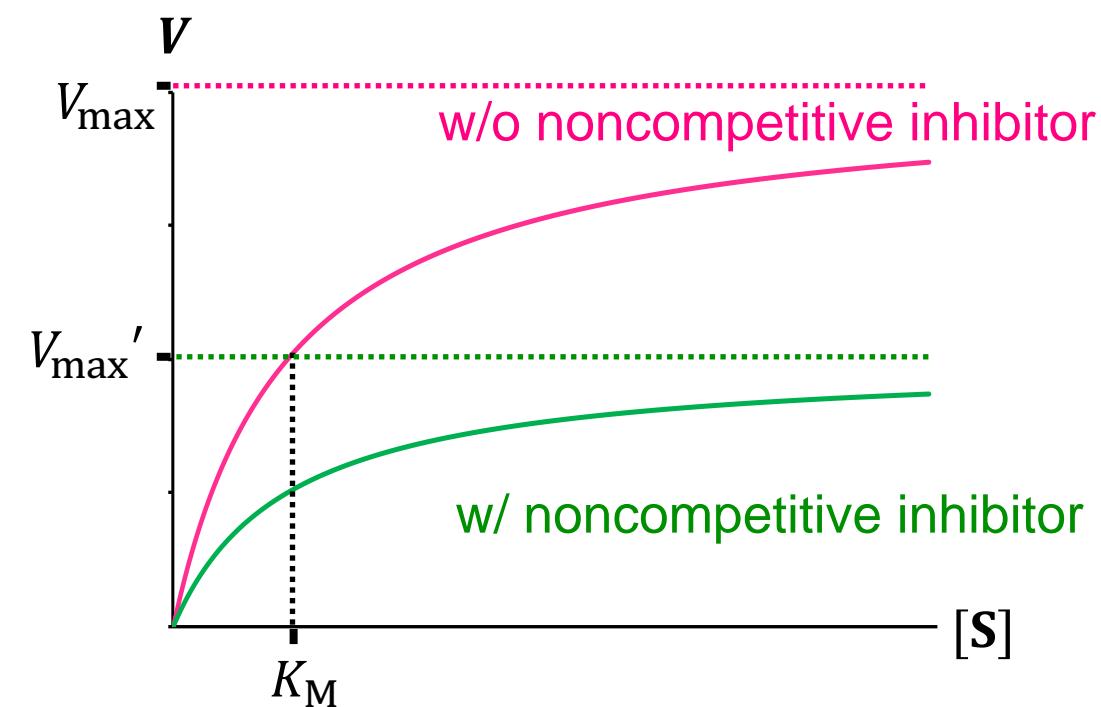
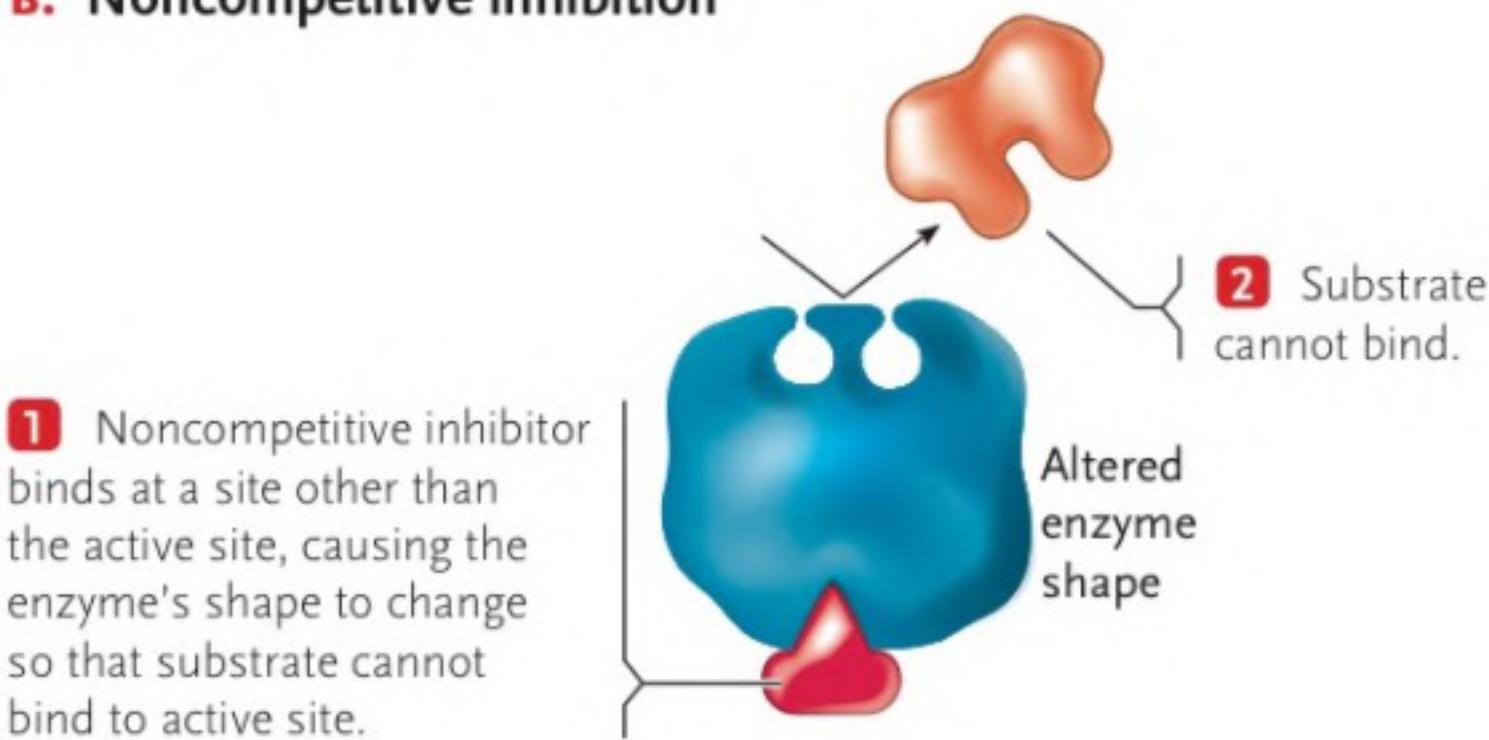


Noncompetitive Inhibition

Noncompetitive inhibition

- An inhibitor binds to an enzyme at a site other than its active site and changes the conformation of the enzyme so that the ability of the active site to bond substrate is reduced
- In the presence of a noncompetitive inhibitor, apparent V_{max} (V_{max}') is lowered, whereas K_M remains unchanged

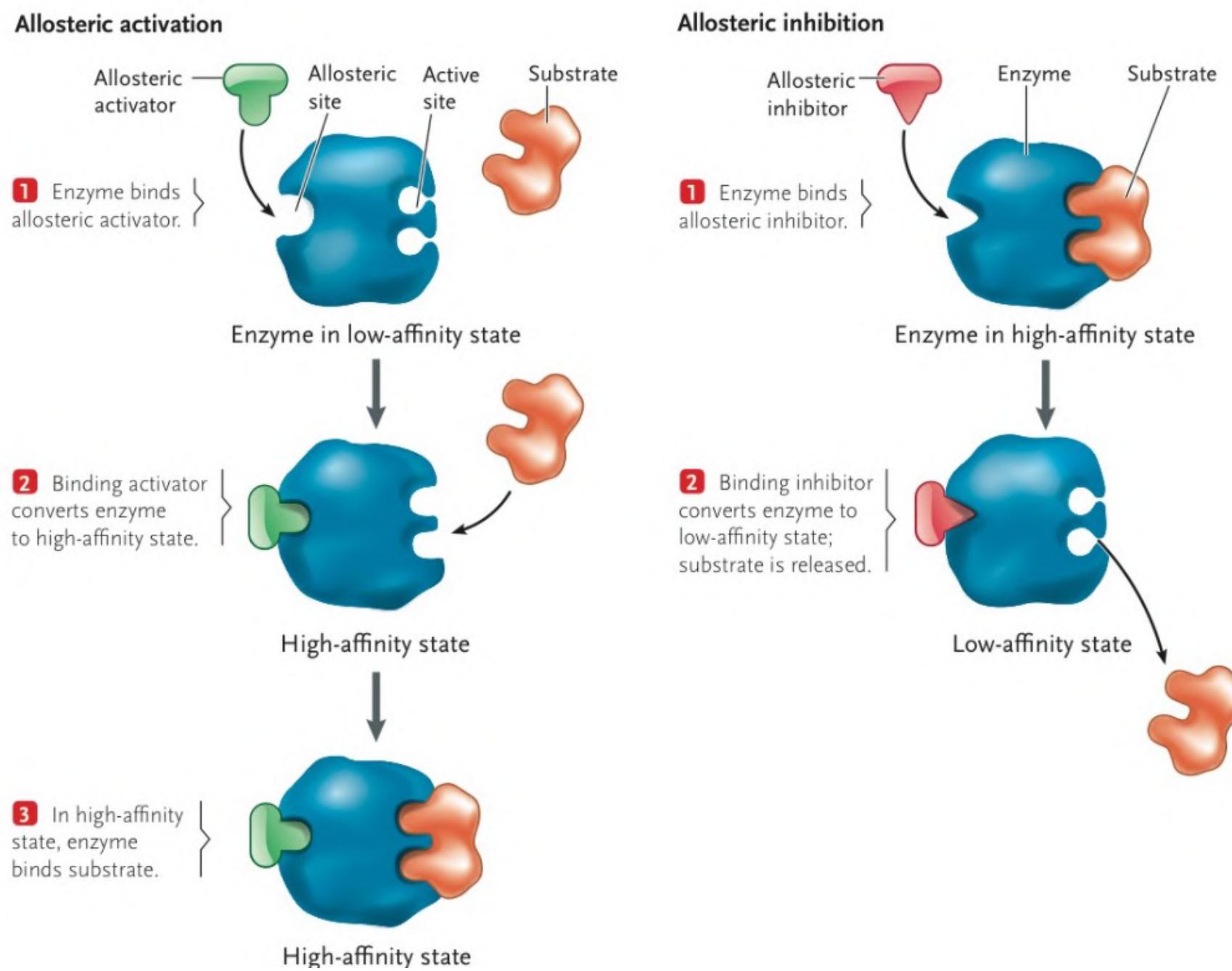
B. Noncompetitive inhibition



Allosteric Regulation

Allosteric regulation

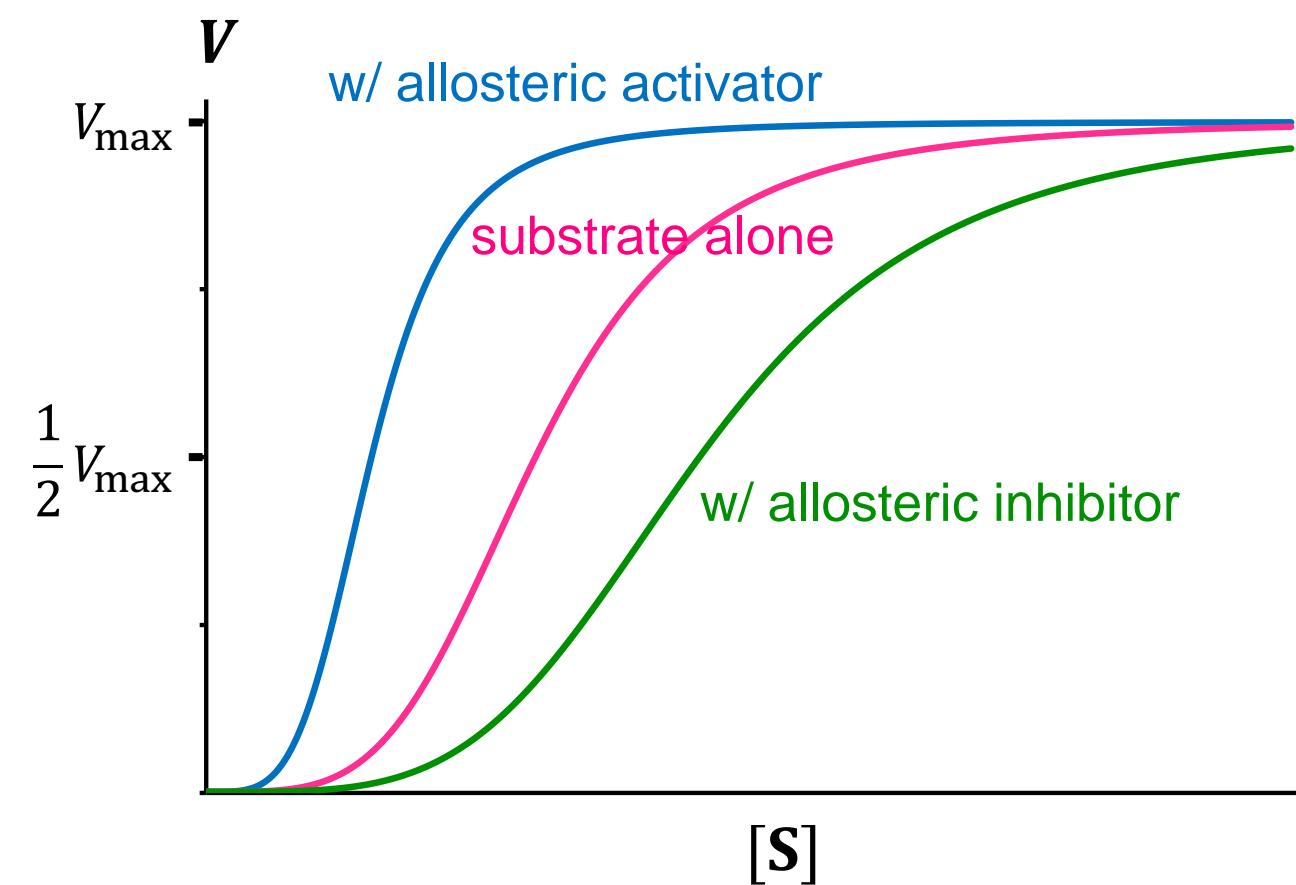
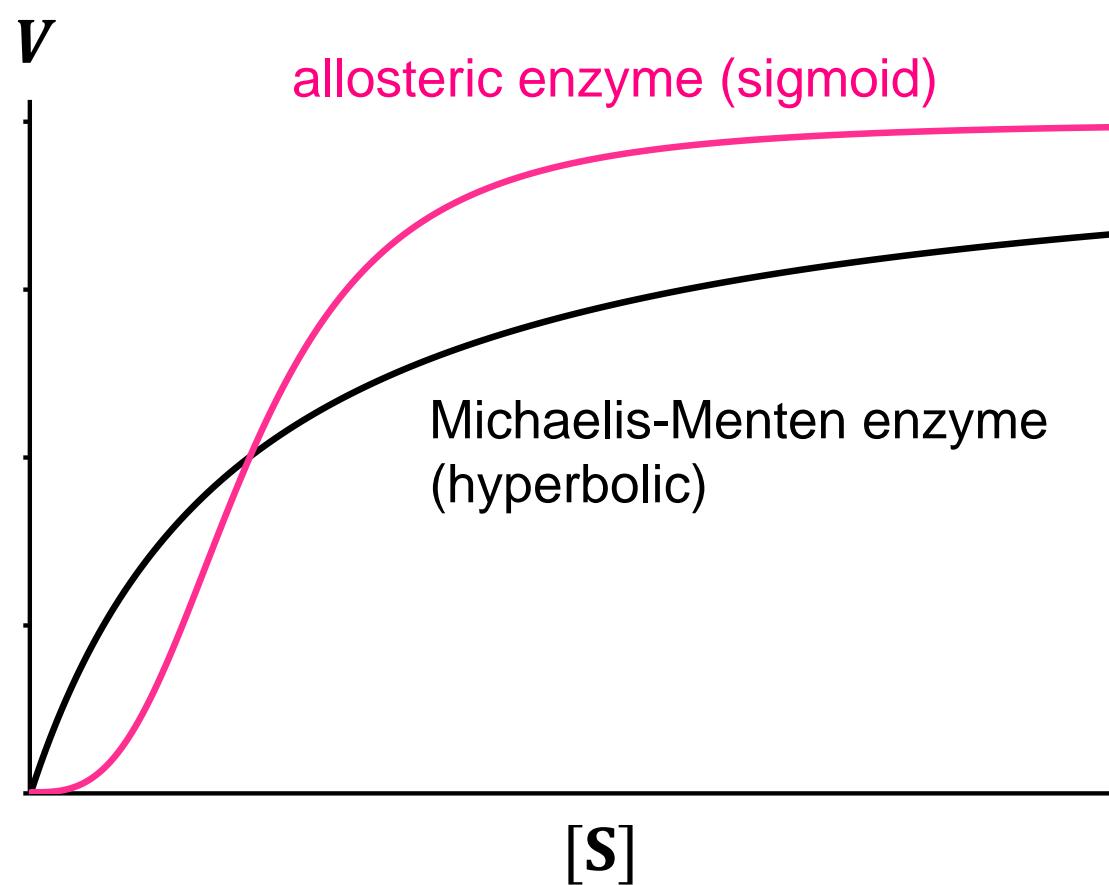
- Enzyme activity is controlled by the reversible binding of a regulatory molecule (allosteric effector) to the allosteric site, a location on the enzyme outside the active site.



Allosteric Enzymes

Allosteric enzymes

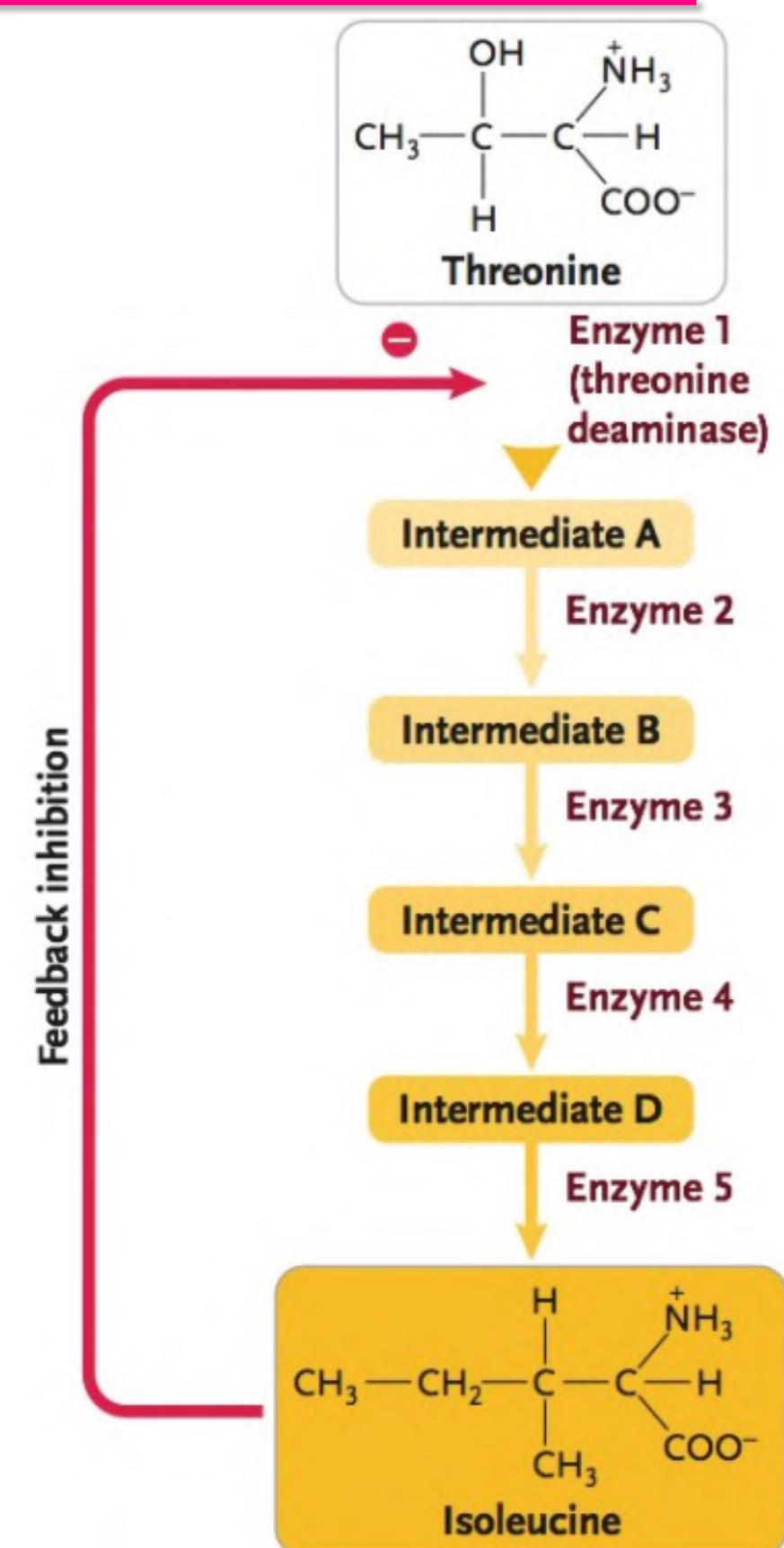
- Enzymes regulated by allosteric effector(s)
- Generally multimeric proteins in which the activity of one subunit affects that of the other subunits
- Display a sigmoidal curve on a kinetics plot; even a small amount of effector can significantly regulate the enzymatic activity



Feedback Inhibition

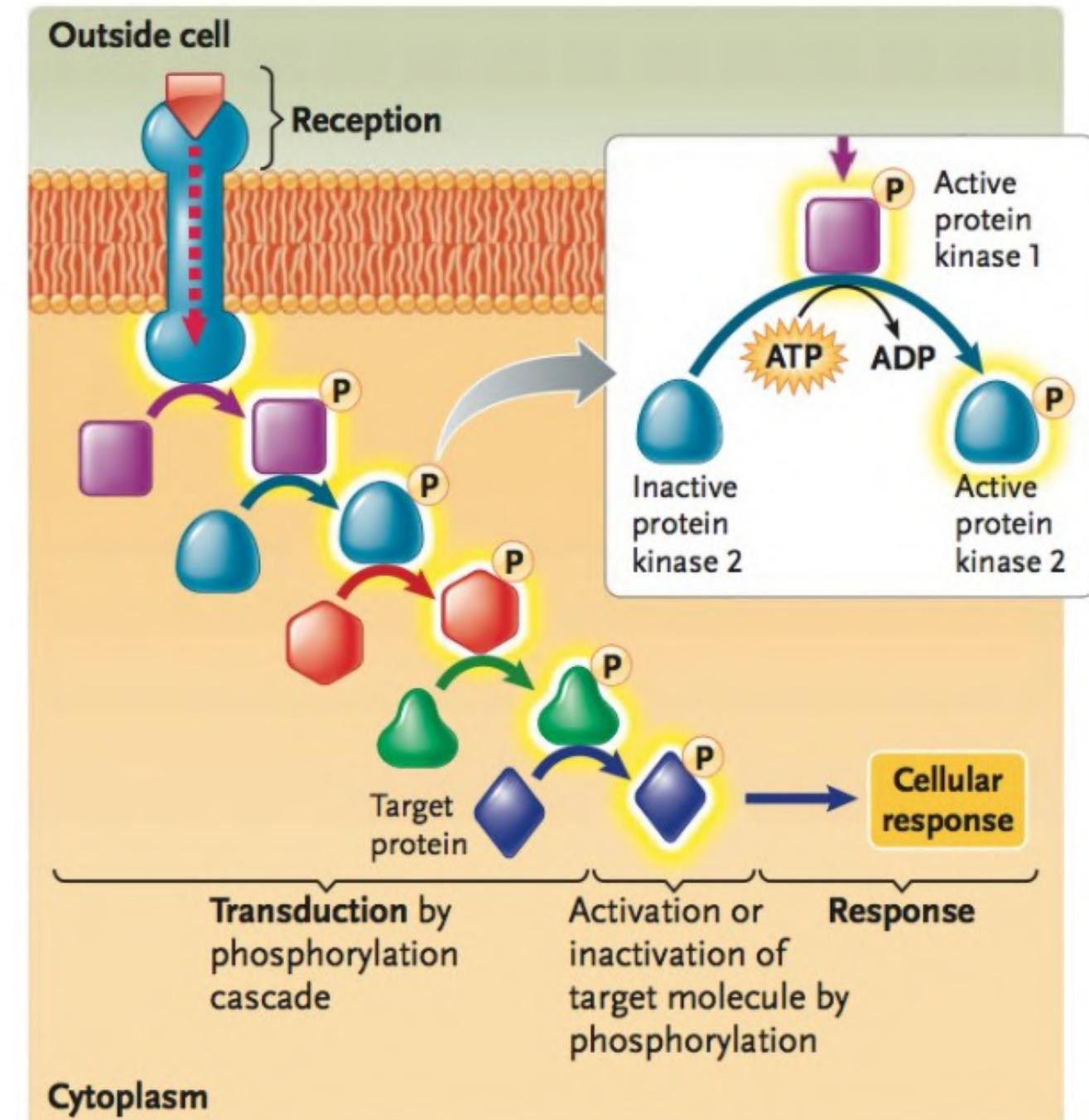
Feedback inhibition

- Allosteric inhibitors are often a product of the metabolic pathway they regulate; typically, they inhibit the enzyme that catalyzes the first reaction of the pathway: **Feedback inhibition**
- The isoleucine biosynthesis adopts feedback inhibition; isoleucine serves as the allosteric inhibitor of the first enzyme of the pathway (threonine deaminase)
- Feedback inhibition prevents cellular resources from being wasted in the synthesis of molecules made at intermediate steps of the pathway



Chemical modifications

- Many key enzymes are regulated by chemical linkage to other substances
- Addition or removal of phosphate group is highly significant mechanism in cellular regulation.
- **Protein kinases** perform phosphorylation reaction using ATP or other nucleotides as a source of phosphate group
- **Protein phosphatases** perform dephosphorylation reaction



Catabolism

Cellular Respiration

Cellular respiration

- Include both the reactions that transfer electrons from organic molecules to oxygen and reactions that synthesize ATP

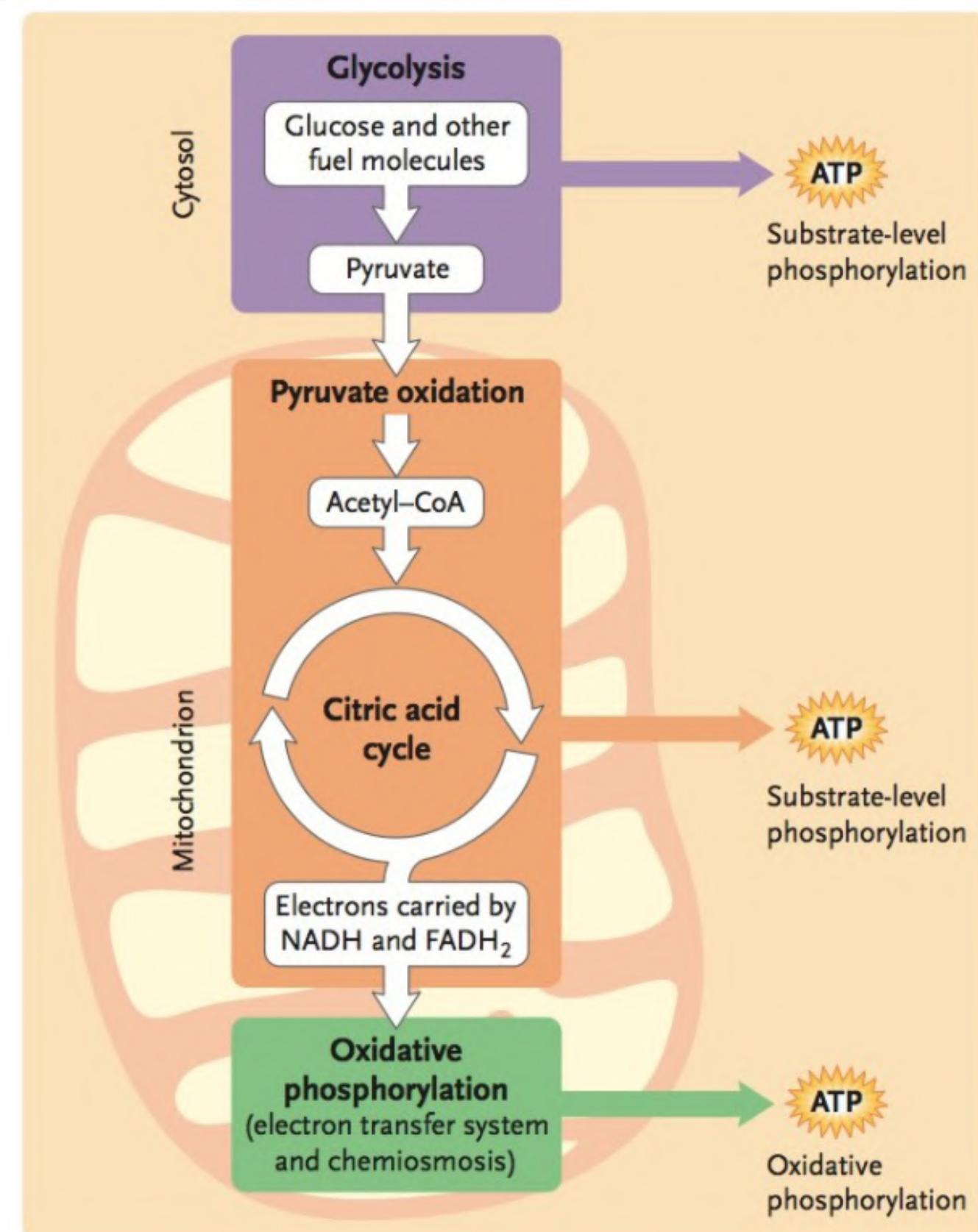
- The process can be divided into three stages:

- 1) Glycolysis

- 2) Pyruvate oxidation and citric acid cycle

- 3) Oxidative phosphorylation

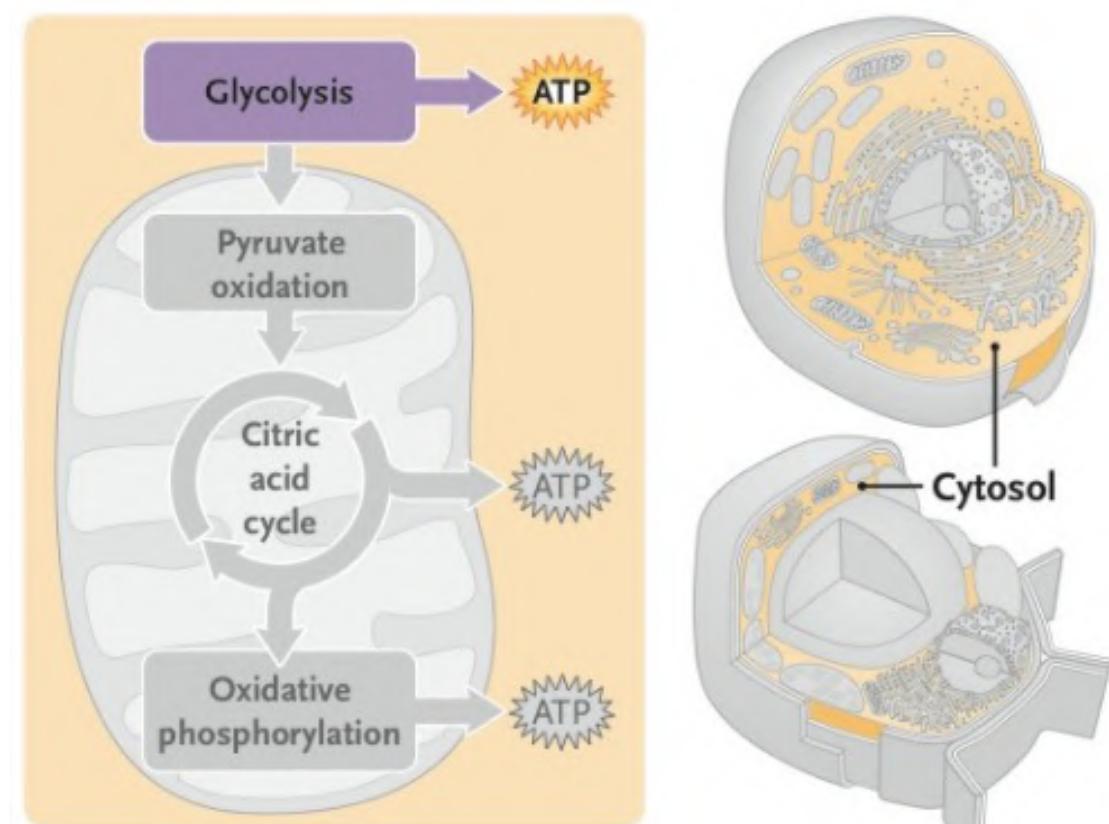
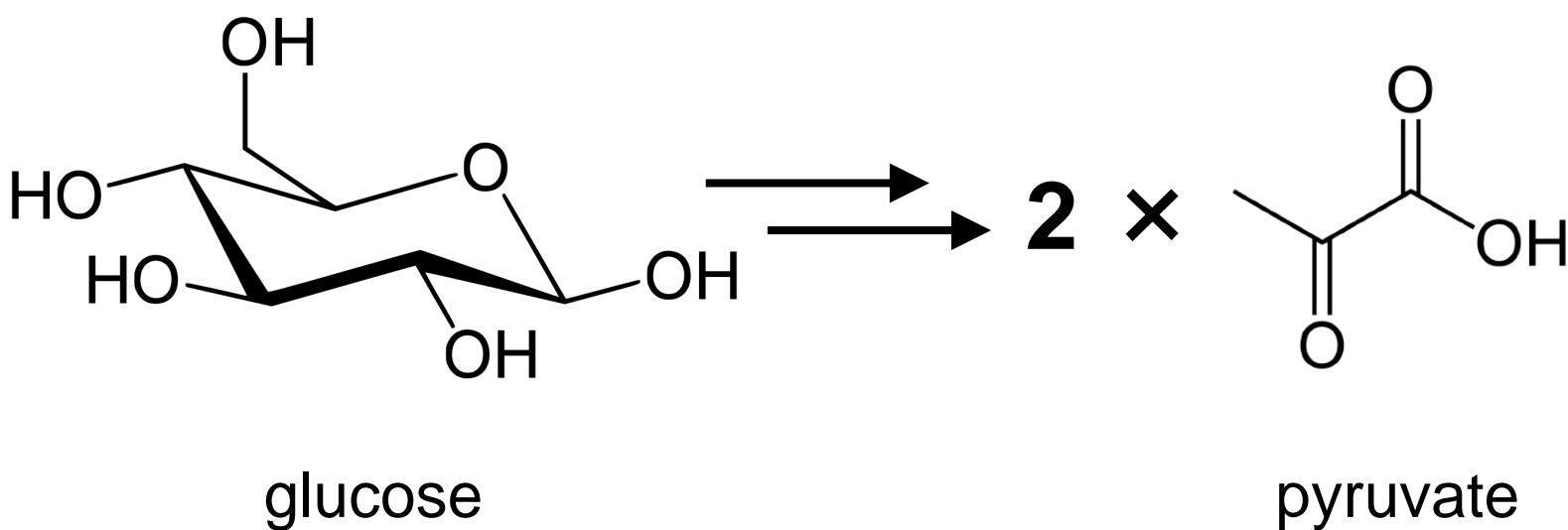
- Overall, 32 molecules of ATP are synthesized from one molecule of glucose



Glycolysis

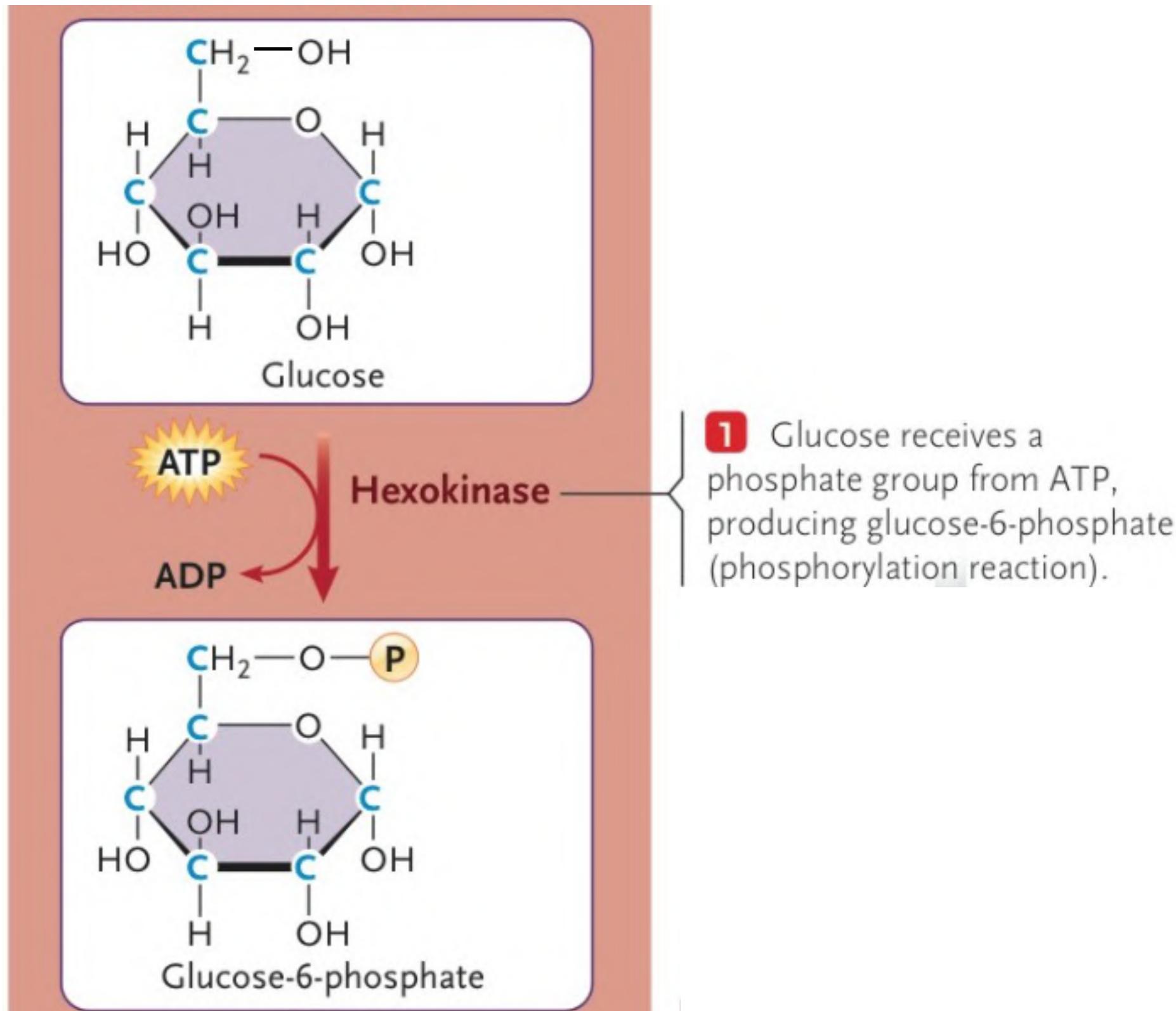
Glycolysis (“glucose splitting”)

- Anaerobic process (= no oxygen required) that splits each 6-carbon **glucose** molecule into two 3-carbon **pyruvate** molecules, producing a relatively small amount of energy
- The process occurs in cytosol
- Two ATP molecules are initially required; the process eventually produces four molecules of ATP and two molecules of NADH
- Some reactions are irreversible, preventing glycolysis from running backward



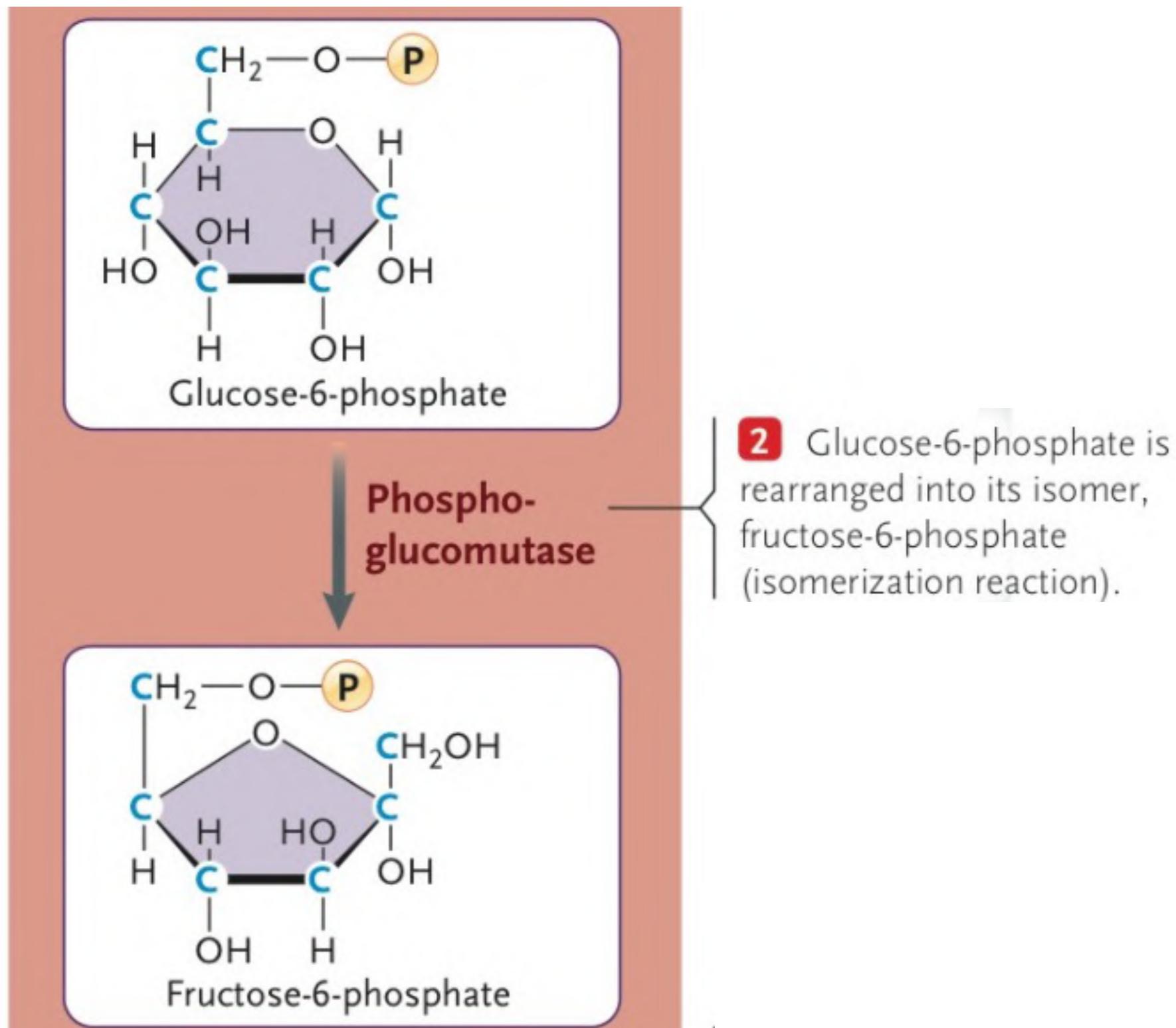
Glycolysis

Rxn 1: Glucose to glucose-6-phosphate



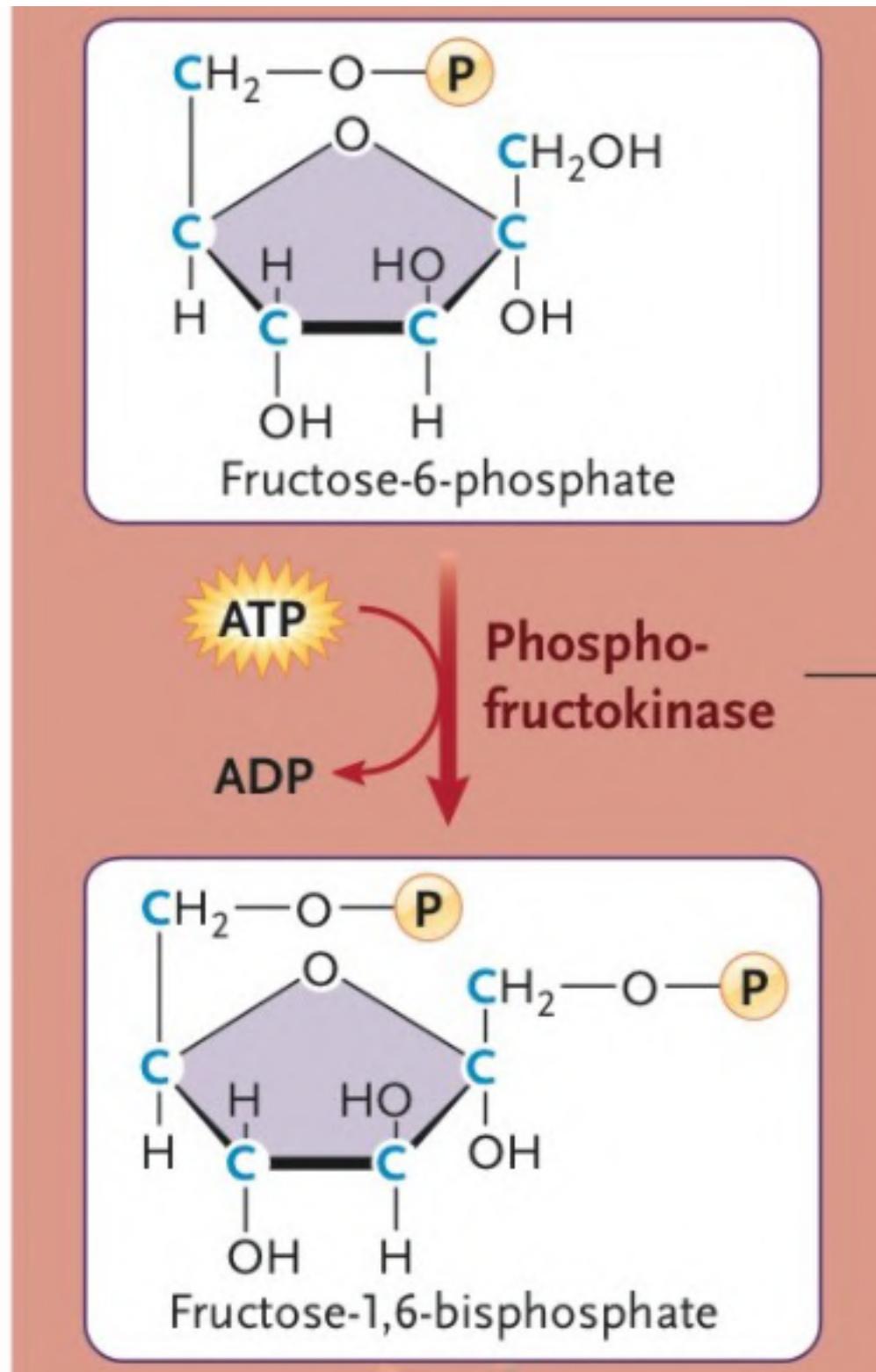
Glycolysis

Rxn 2: Glucose-6-phosphate to fructose-6-phosphate



Glycolysis

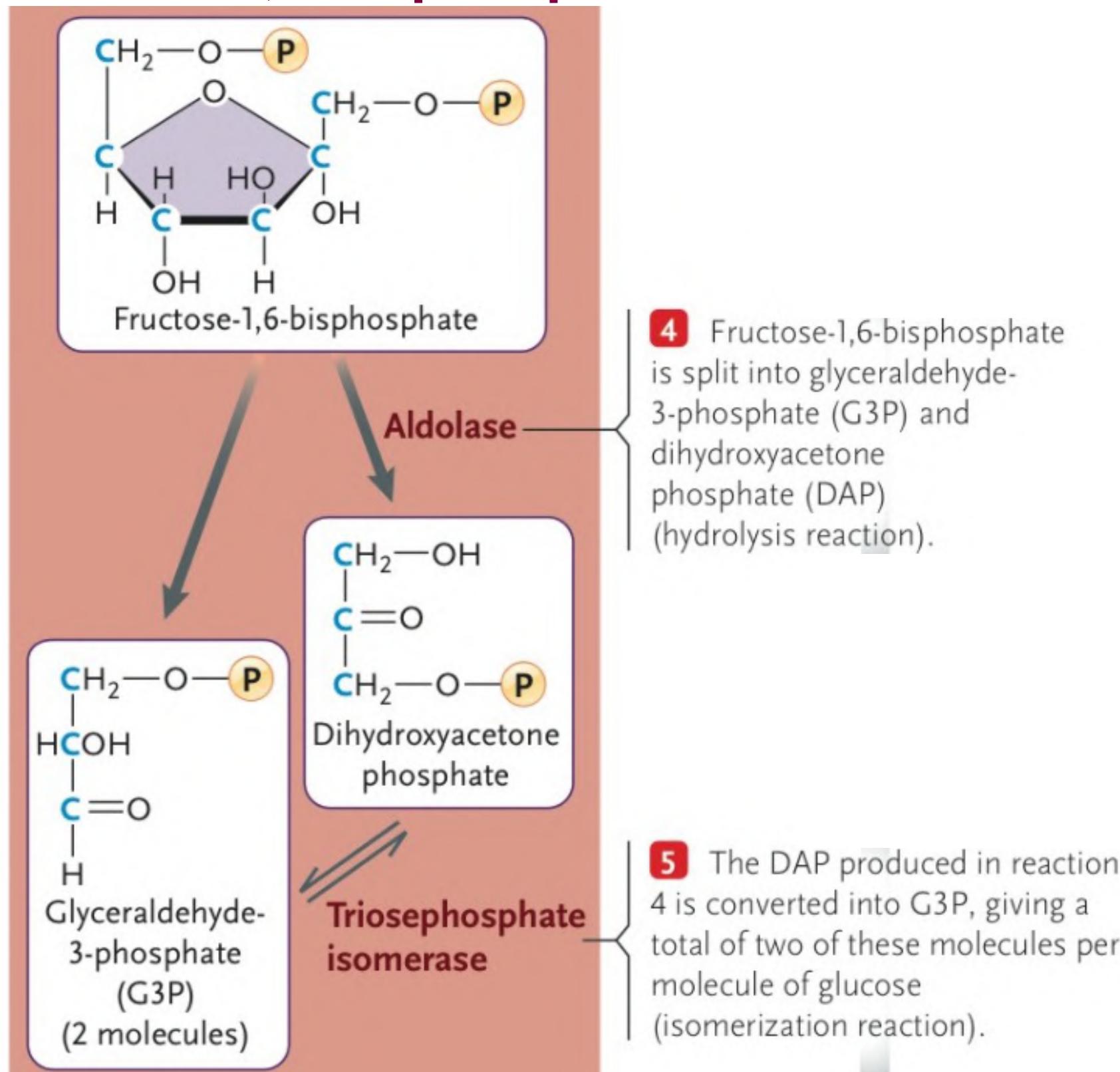
Rxn 3: Fructose-6-phosphate to fructose-1,6-bisphosphate



3 Another phosphate group derived from ATP is attached to fructose-6-phosphate, producing fructose-1,6-bisphosphate (phosphorylation reaction).

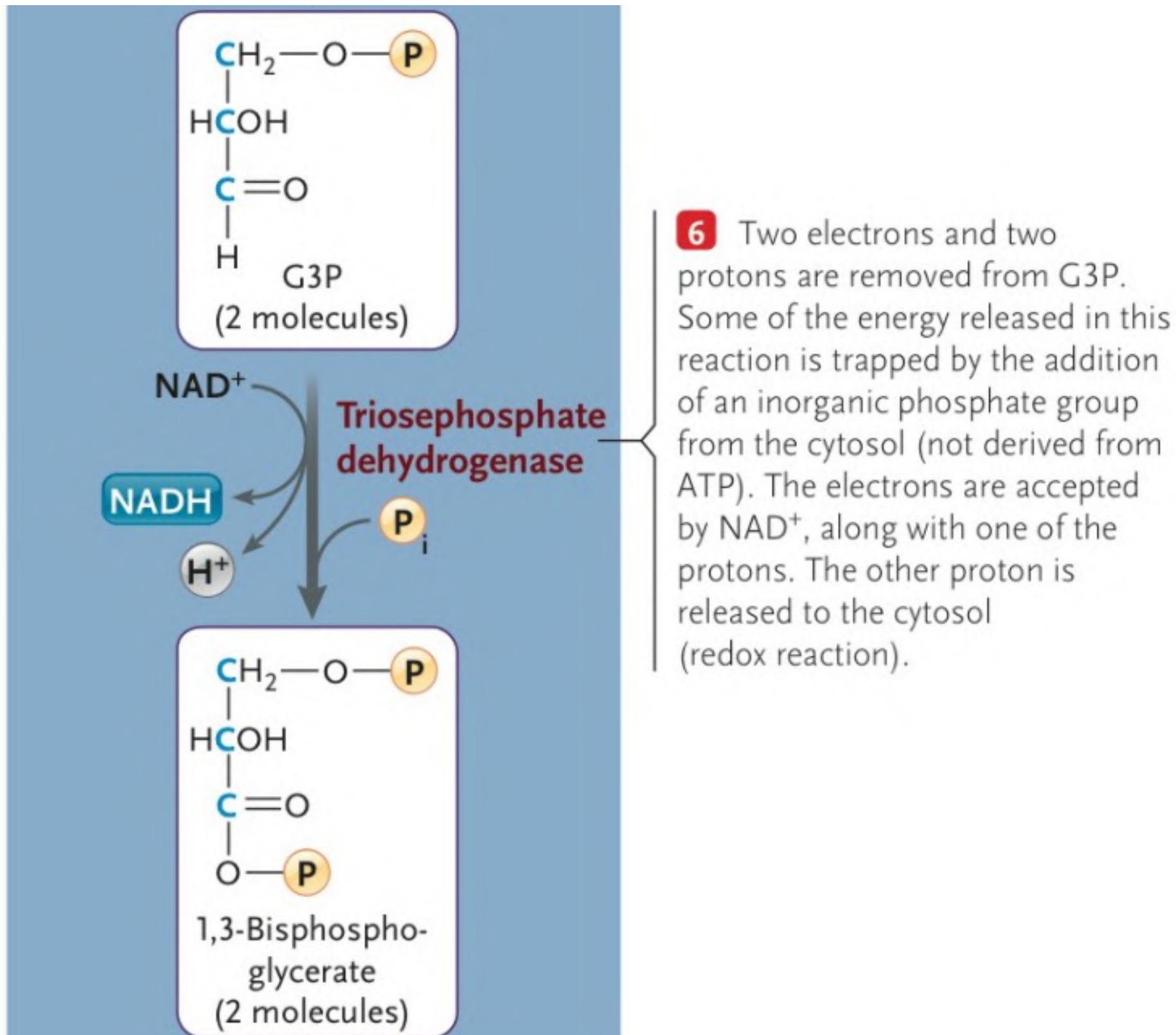
Glycolysis

Rxn 4 & 5: Fructose-1,6-bisphosphate to 2 × G3P



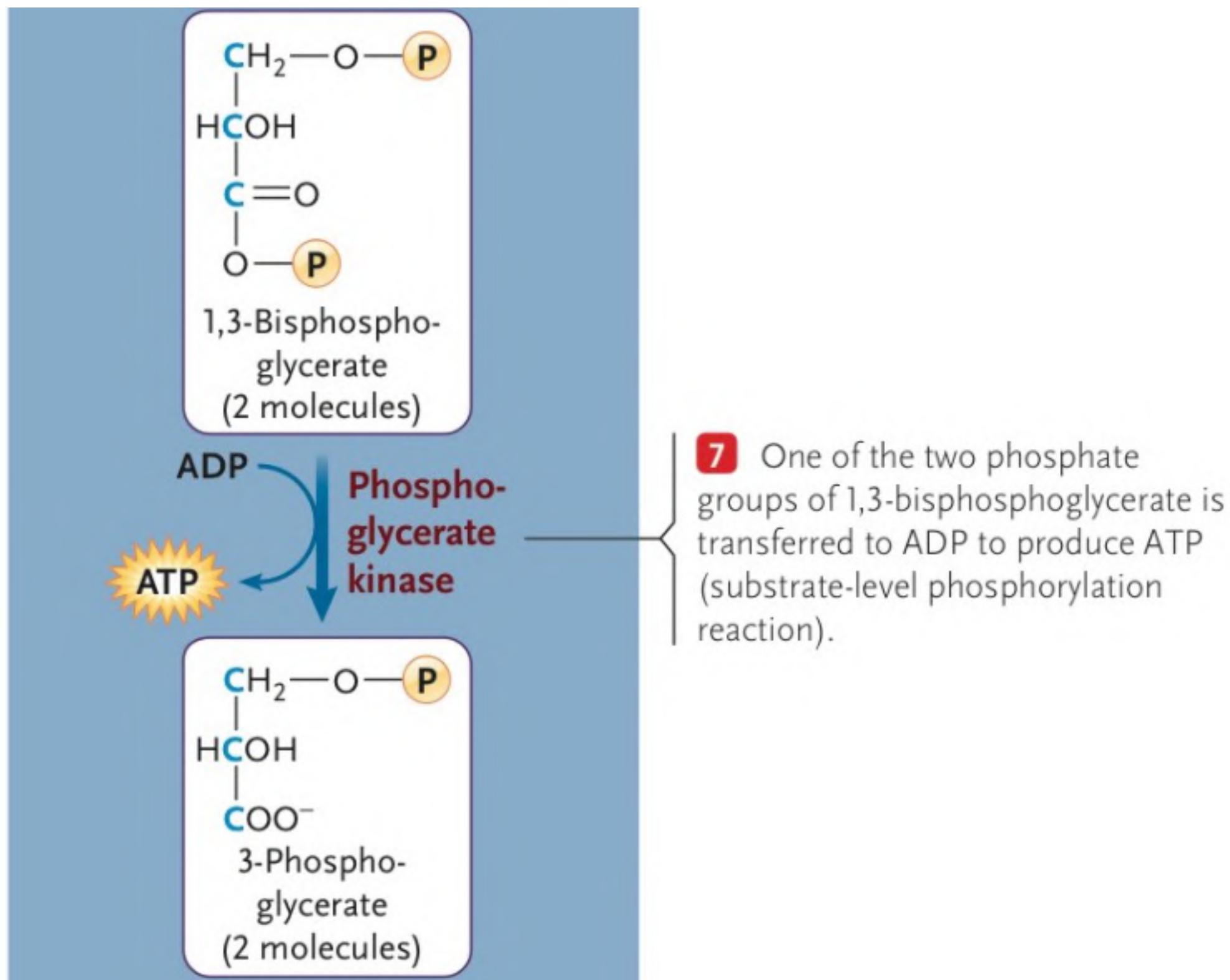
Glycolysis

Rxn 6: G3P to 1,3-bisphosphoglycerate



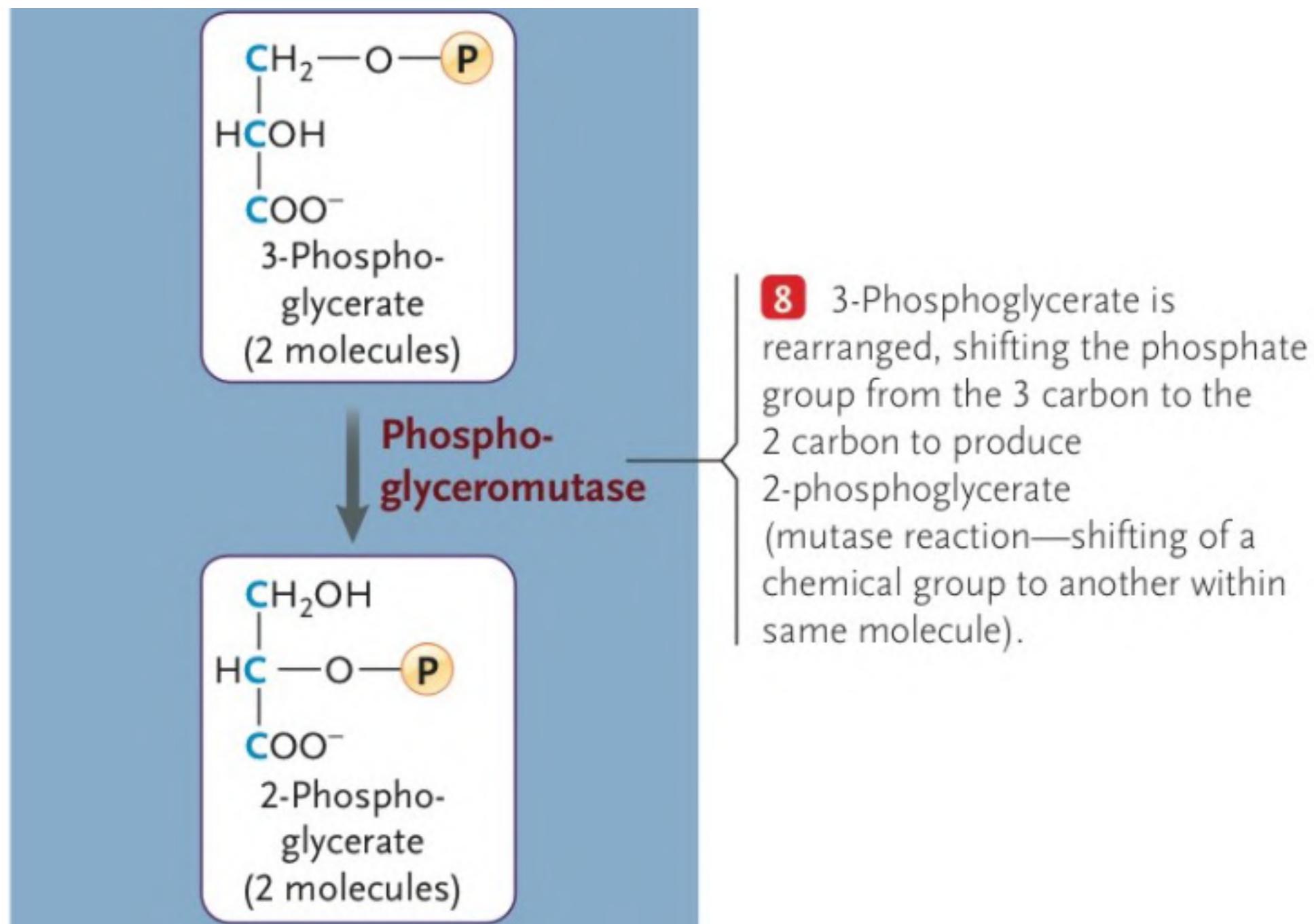
Glycolysis

Rxn 7: 1,3-Bisphosphoglycerate to 3-phosphoglycerate



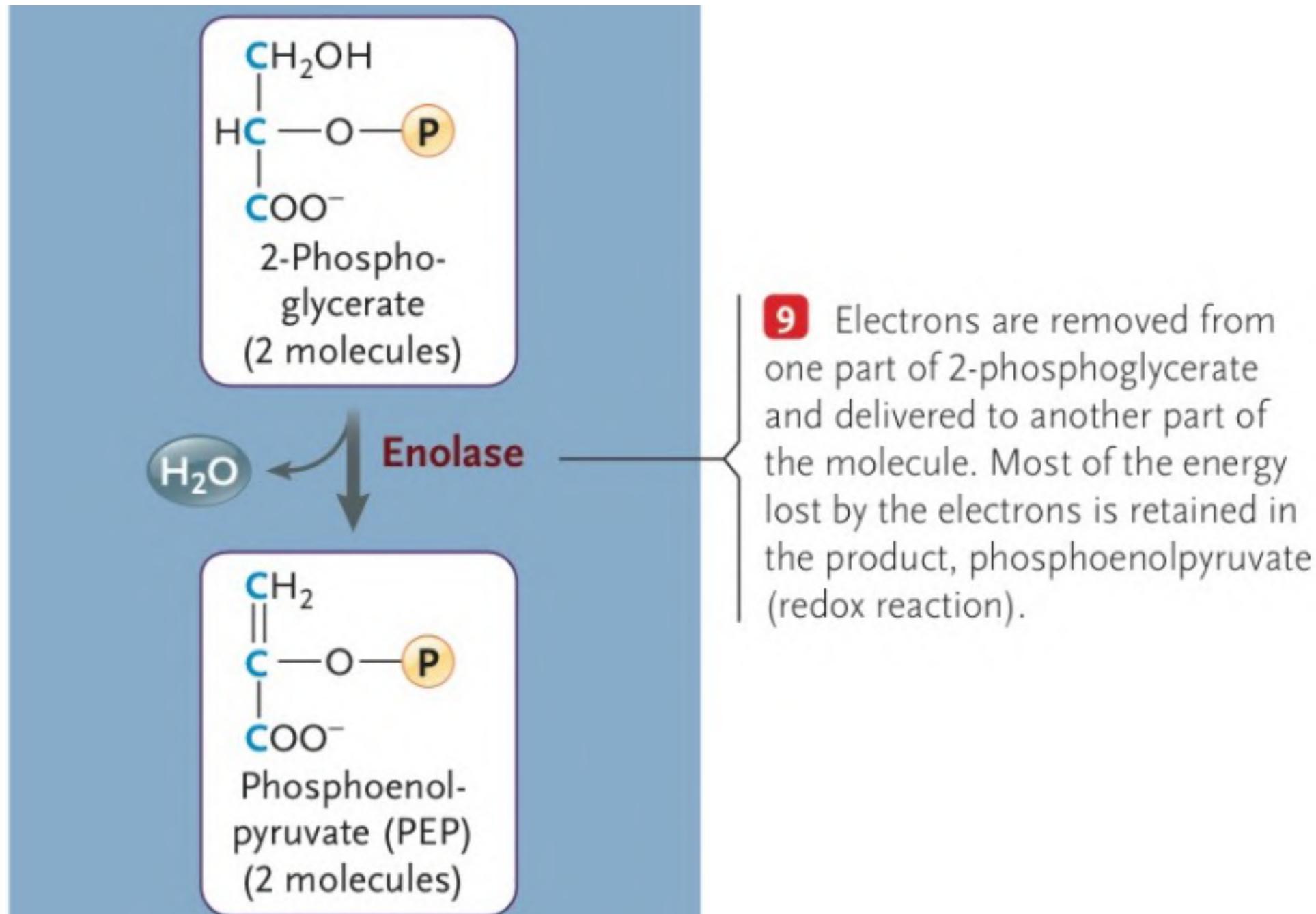
Glycolysis

Rxn 8: 3-Phosphoglycerate to 2-phosphoglycerate



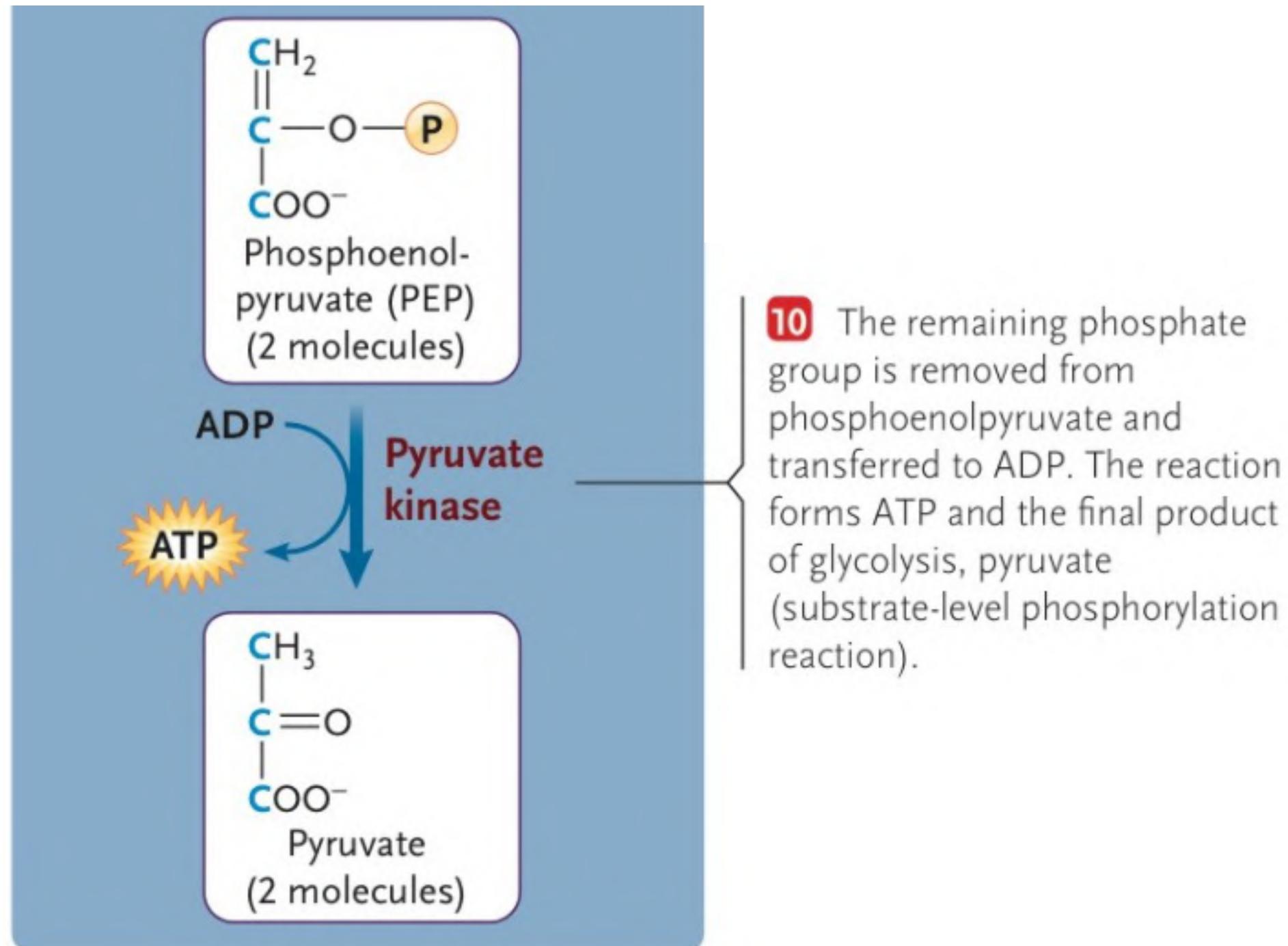
Glycolysis

Rxn 9: 2-Phosphoglycerate to phosphoenolpyruvate

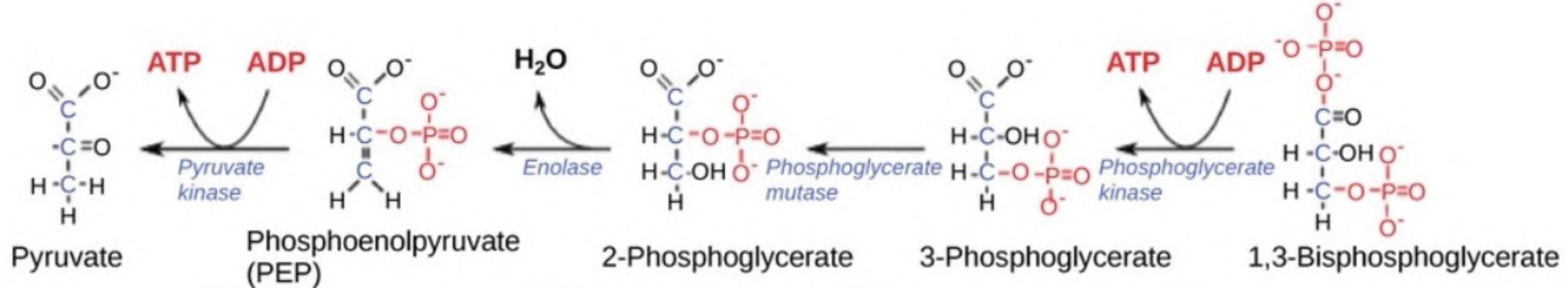
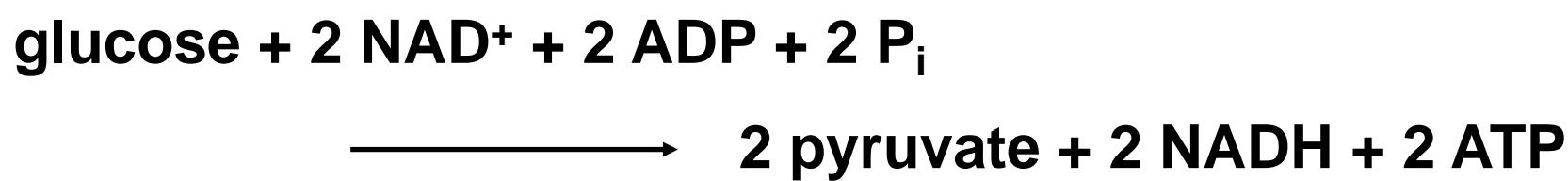
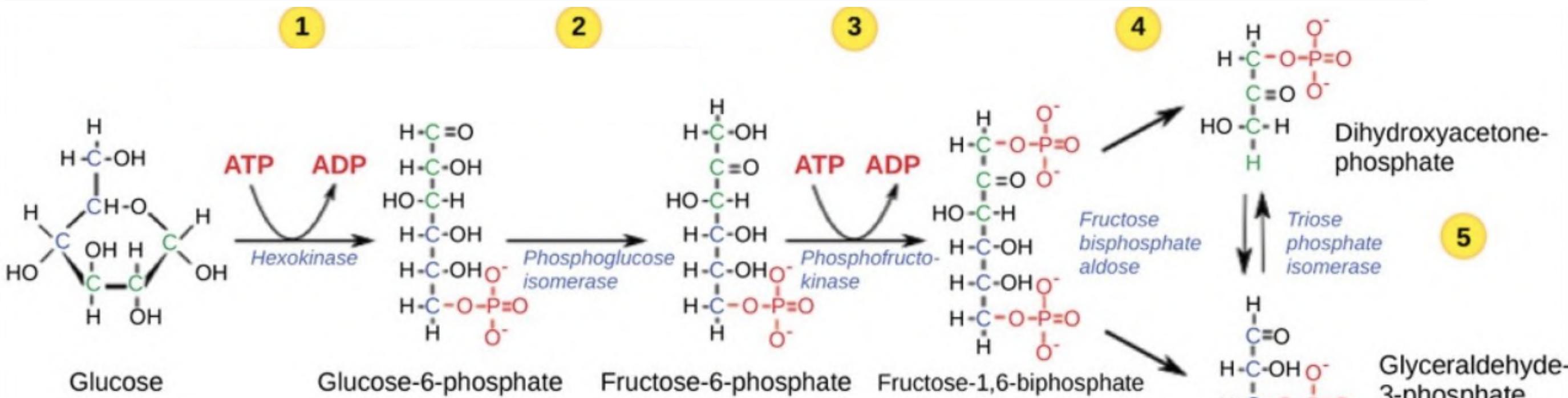


Glycolysis

Rxn 10: Phosphoenolpyruvate to pyruvate



Glycolysis



10

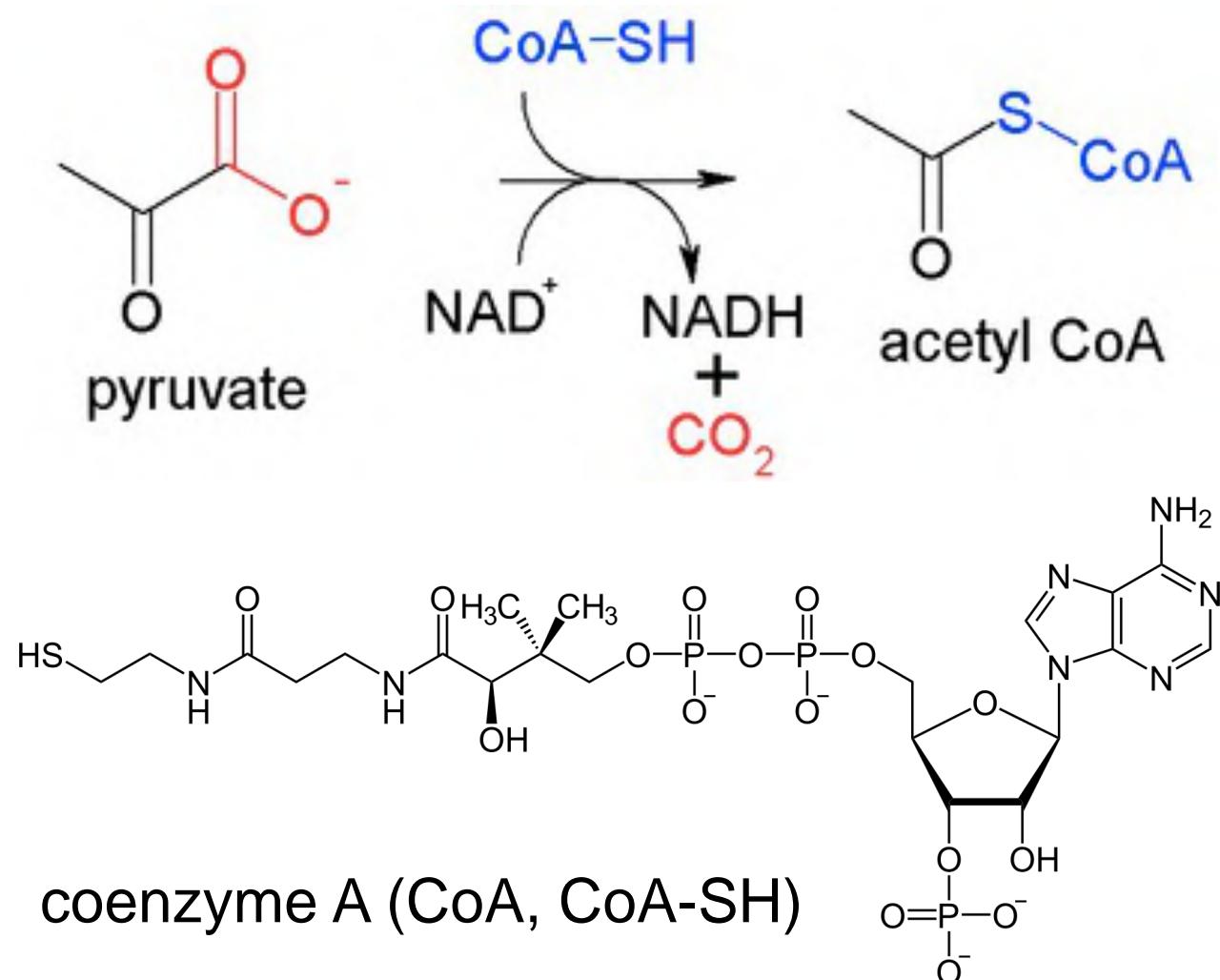
9

8

7

Conversion of Pyruvate to Acetyl-CoA

- Pyruvate is transformed into acetyl coenzyme A (acetyl-CoA) by pyruvate dehydrogenase complex (PDC)
- The reaction occurs inside the mitochondrial matrix in eukaryotic cells; acetyl-CoA synthesized herein is trapped inside the mitochondria, as the mitochondrial membrane is impervious to acetyl-CoA
- Acetyl-CoA synthesized from pyruvate enters the Krebs cycle



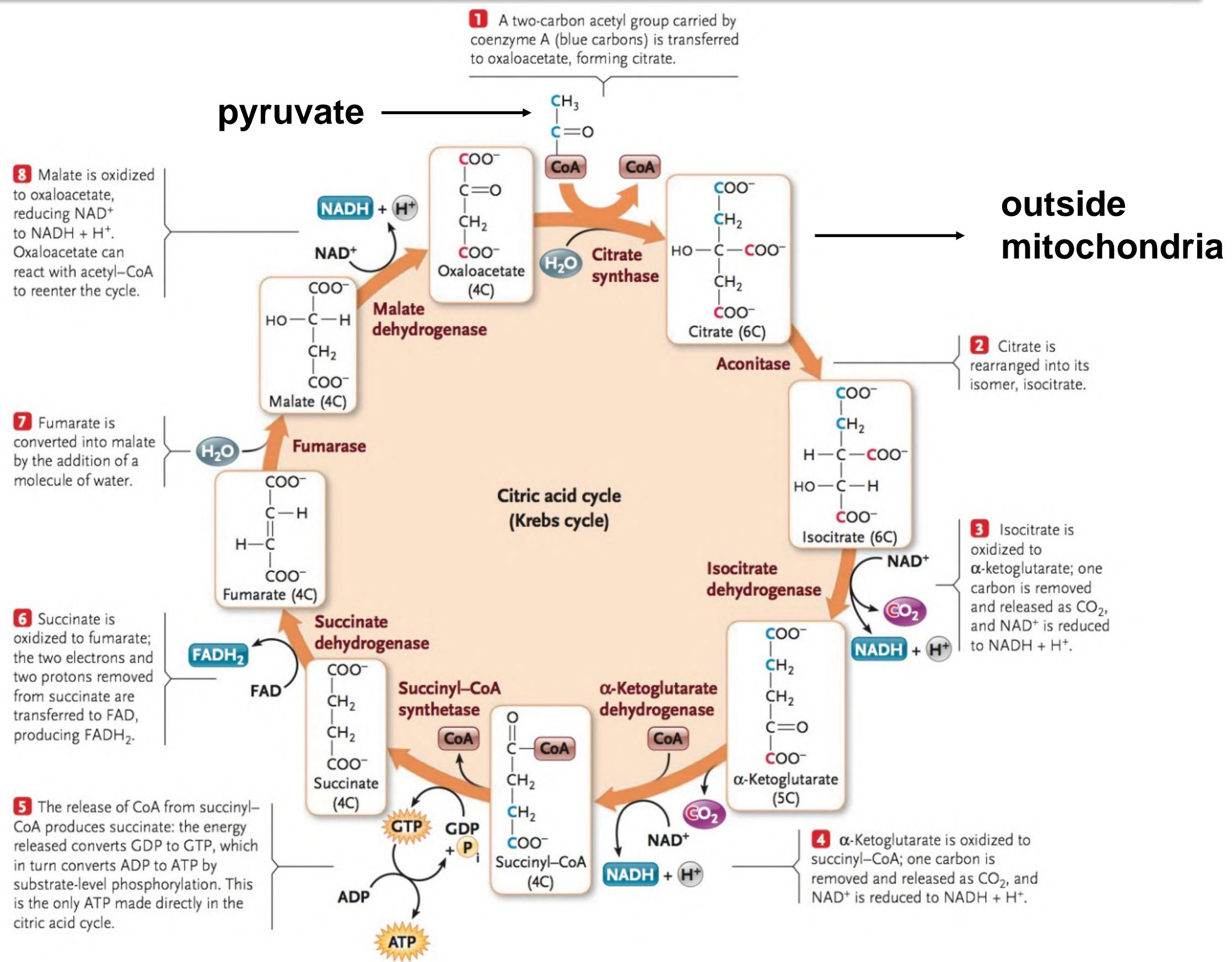
Krebs Cycle

Krebs cycle (TCA cycle, citric acid cycle)

- Aerobic process in which acetyl portion of acetyl-CoA is oxidized to yield two molecules of CO_2 , three molecules of NADH, and one molecule each of FADH_2 , and GTP (guanosine triphosphate)
- The process occurs in mitochondria in eukaryotic cells
- The process is initiated by the formation of six-carbon compound called **citrate** from acetyl-CoA and oxaloacetate
- Acetyl-CoA adds two carbons to the cycle, and the cycle releases two carbons as CO_2 ; there is no net gain or loss of carbon atoms
- The final step in the Krebs cycle regenerates oxaloacetate

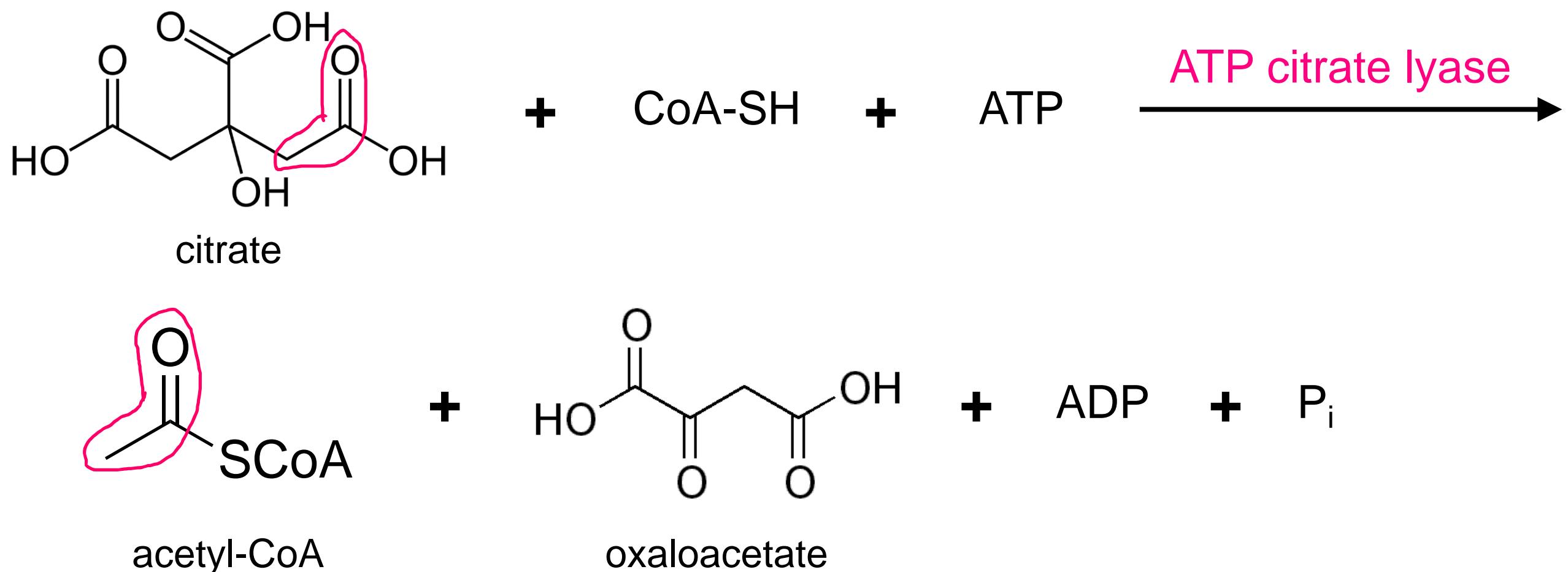


Krebs Cycle



Conversion of Citrate to Acetyl-CoA

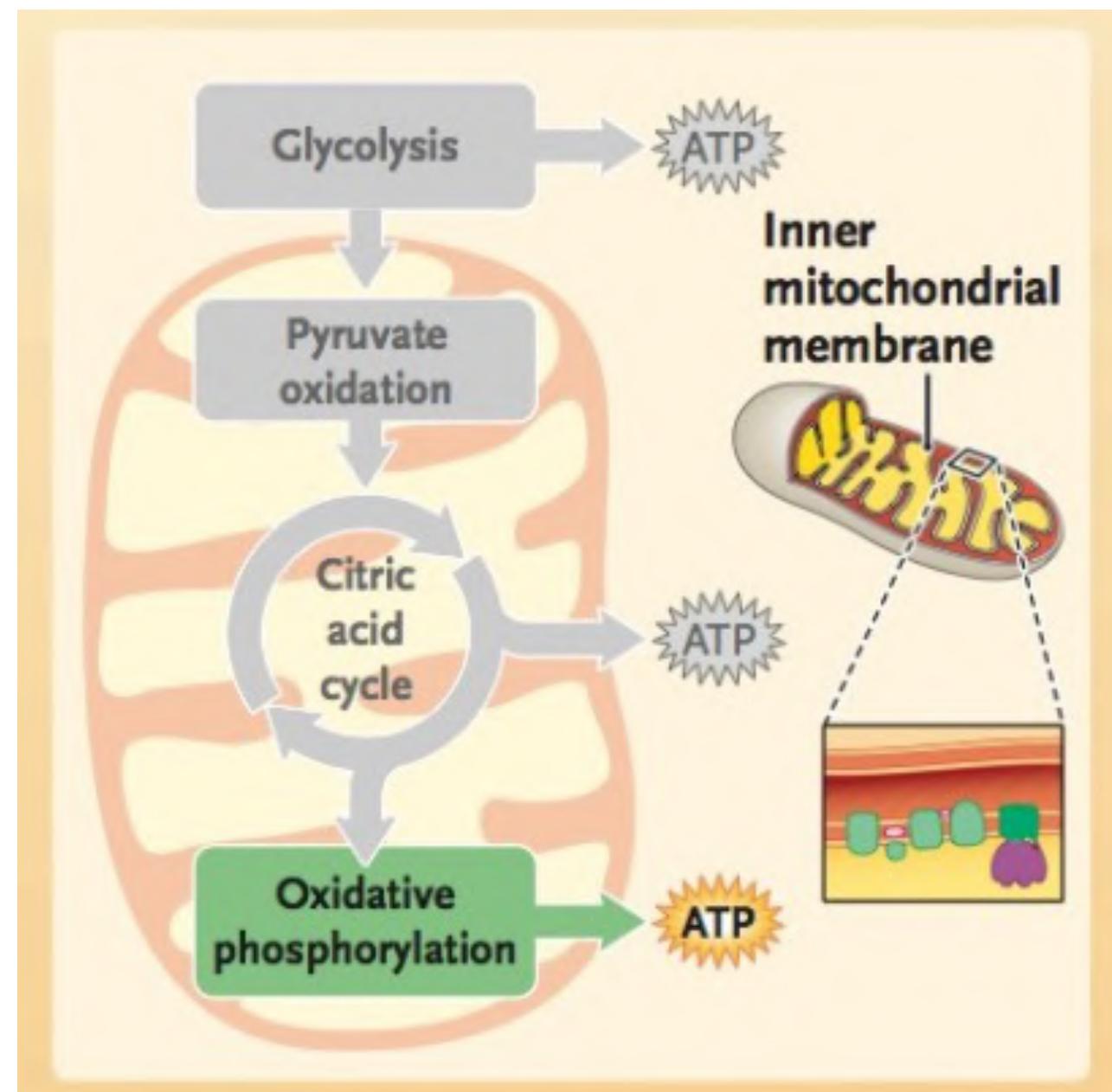
- Citrate can be transported to cytosol from mitochondria
- **ATP citrate lyase** (ACLY) converts citrate and CoA into acetyl-CoA and oxaloacetate; acetyl CoA synthesized herein can be utilized as a starting material for many biosynthetic processes, such as fatty acid and sterol biosynthesis



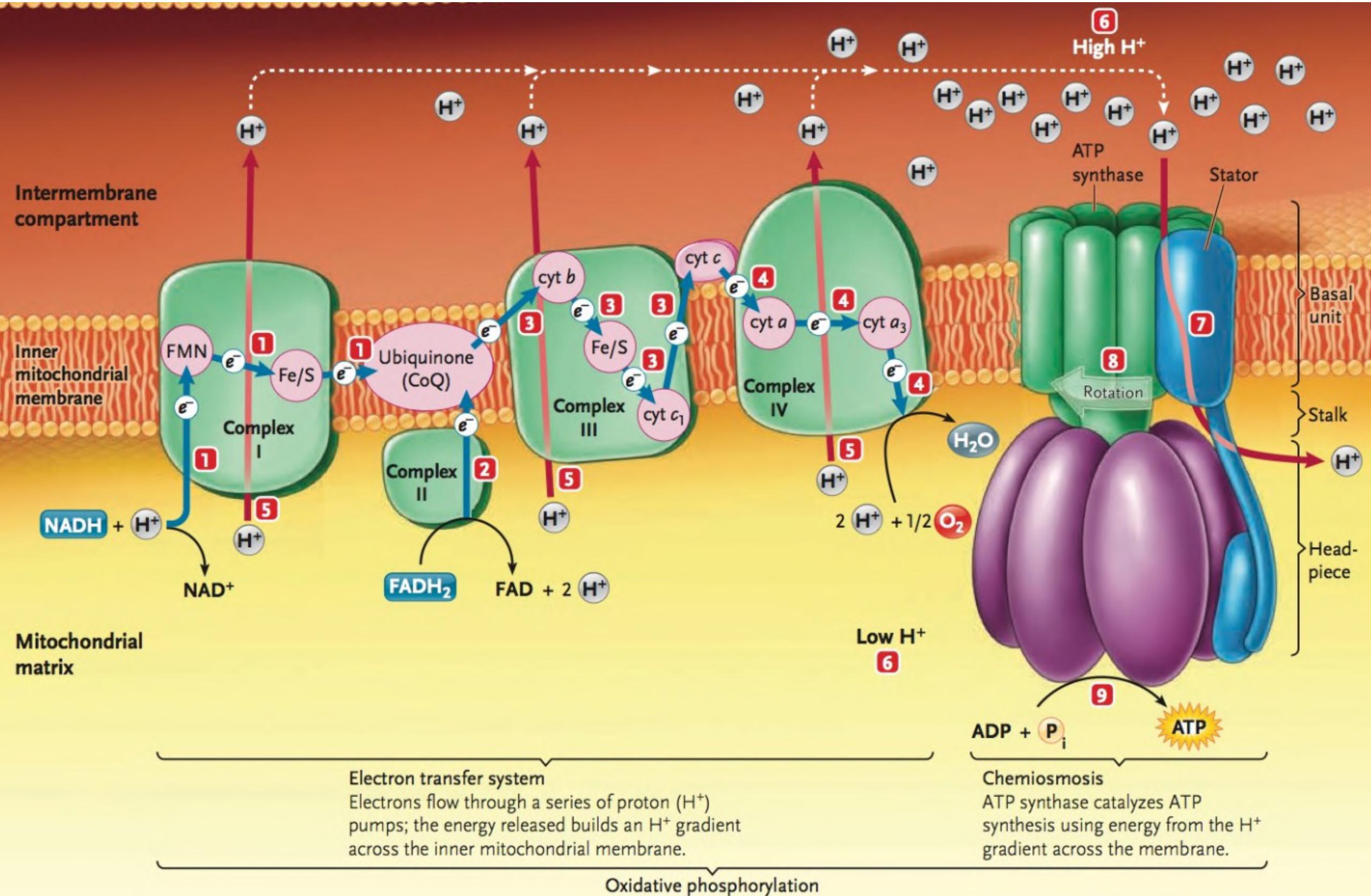
Oxidative Phosphorylation

Oxidative phosphorylation

- High energy electrons generated by glycolysis and Krebs cycle are released into the electron transfer system.
- The **mitochondrial electron transfer system** consists of a series of electron carriers that alternately pick up and release electrons
- The electron flow releases free energy, which is used to generate a proton (H^+) gradient across the inner mitochondrial membrane
- The proton gradient provides the energy that drives ATP synthesis by mitochondrial **ATP synthase**



Oxidative Phosphorylation



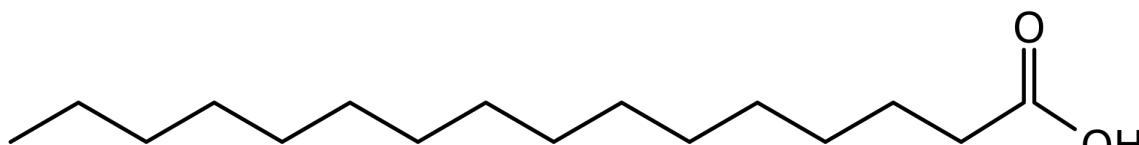
Anabolism

Fatty Acids

Fatty acids

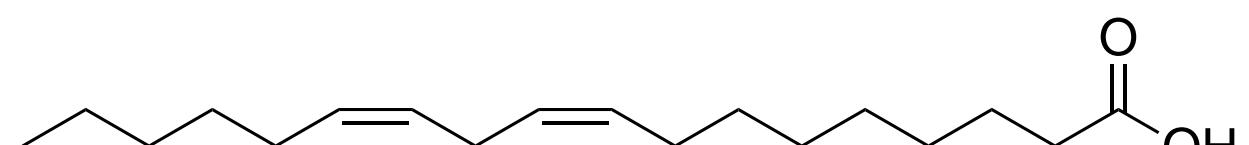
- Carboxylic acids with a long aliphatic chain
- Either saturated or unsaturated
- Biosynthesized from acetyl-CoA and malonyl-CoA

Saturated

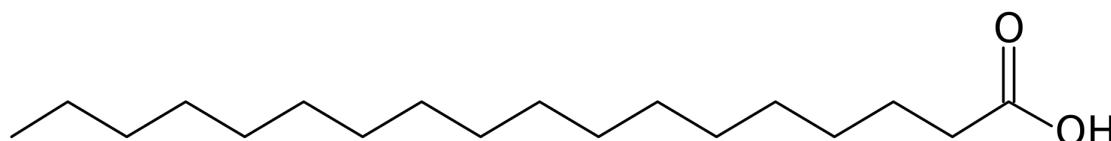


palmitic acid

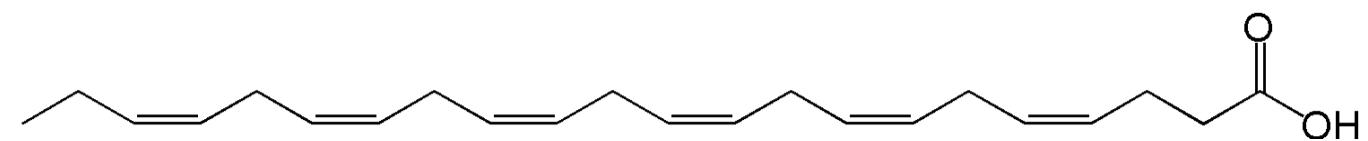
Unsaturated



linoleic acid



stearic acid



docosahexaenoic acid

Fatty Acid Biosynthesis

Fatty acid synthase (FAS)

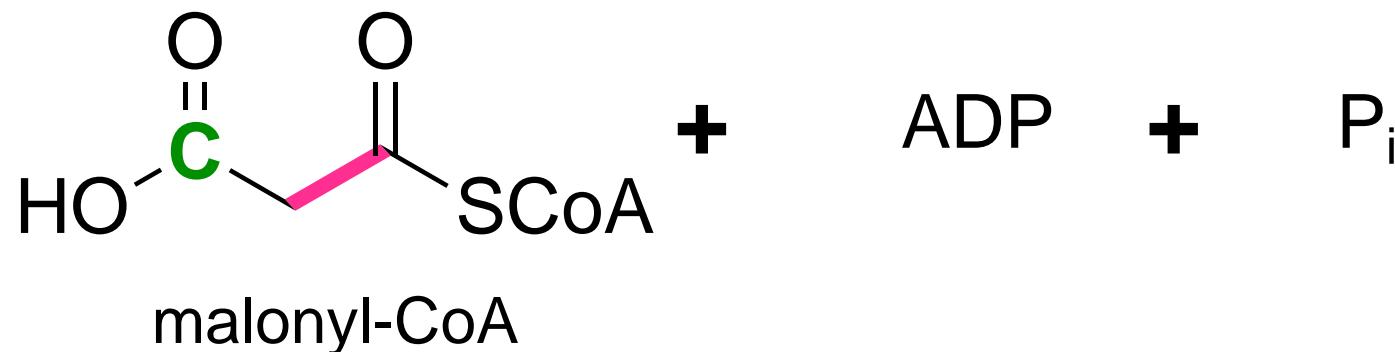
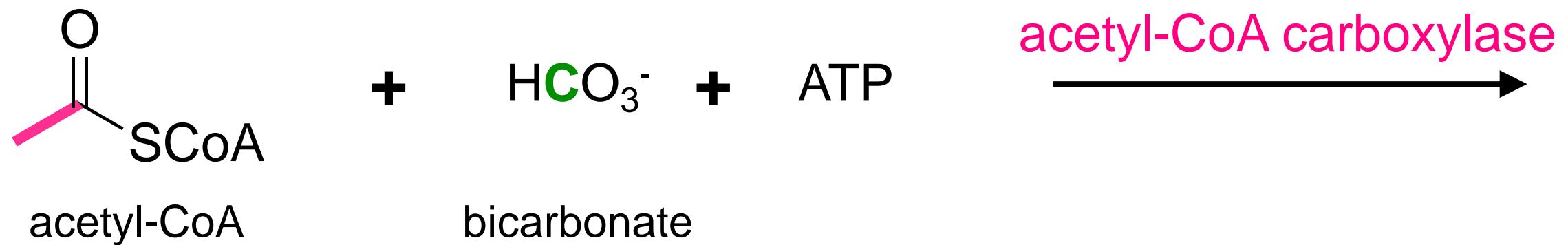
- Enzyme for fatty acid biosynthesis
- Catalyzes repeated condensation of C₂ (acetate) units to provide a long alkyl chain
- Shows great structural differences among different organisms

Type I FAS: Found in animals and fungi. Multifunctional large protein with different catalytic domains. Animal and fungal FASs have distinct structural features.

Type II FAS: Found in plants and bacteria. Consists of a series of monofunctional enzymes.

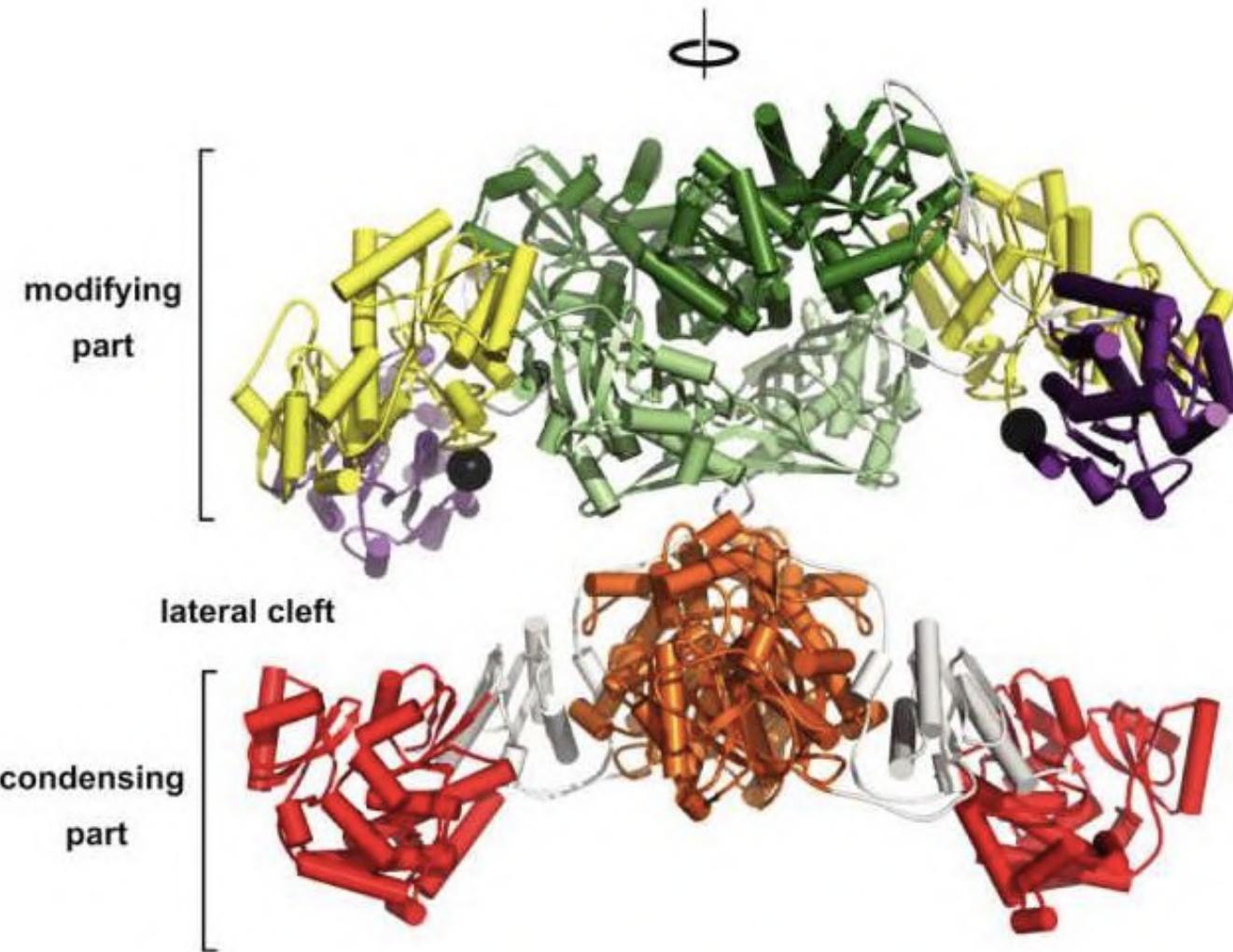
Synthesis of Malonyl-CoA from Acetyl-CoA

- Acetyl-CoA carboxylase converts acetyl-CoA and bicarbonate into malonyl-CoA
- Acetyl-CoA serves as the starter unit for the fatty acid synthesis, whereas malonyl-CoA is utilized as the extender unit for the fatty acid synthesis

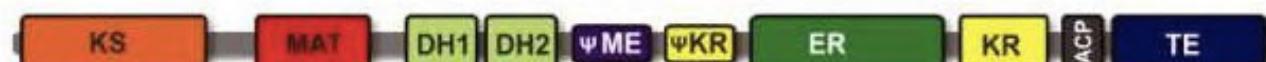
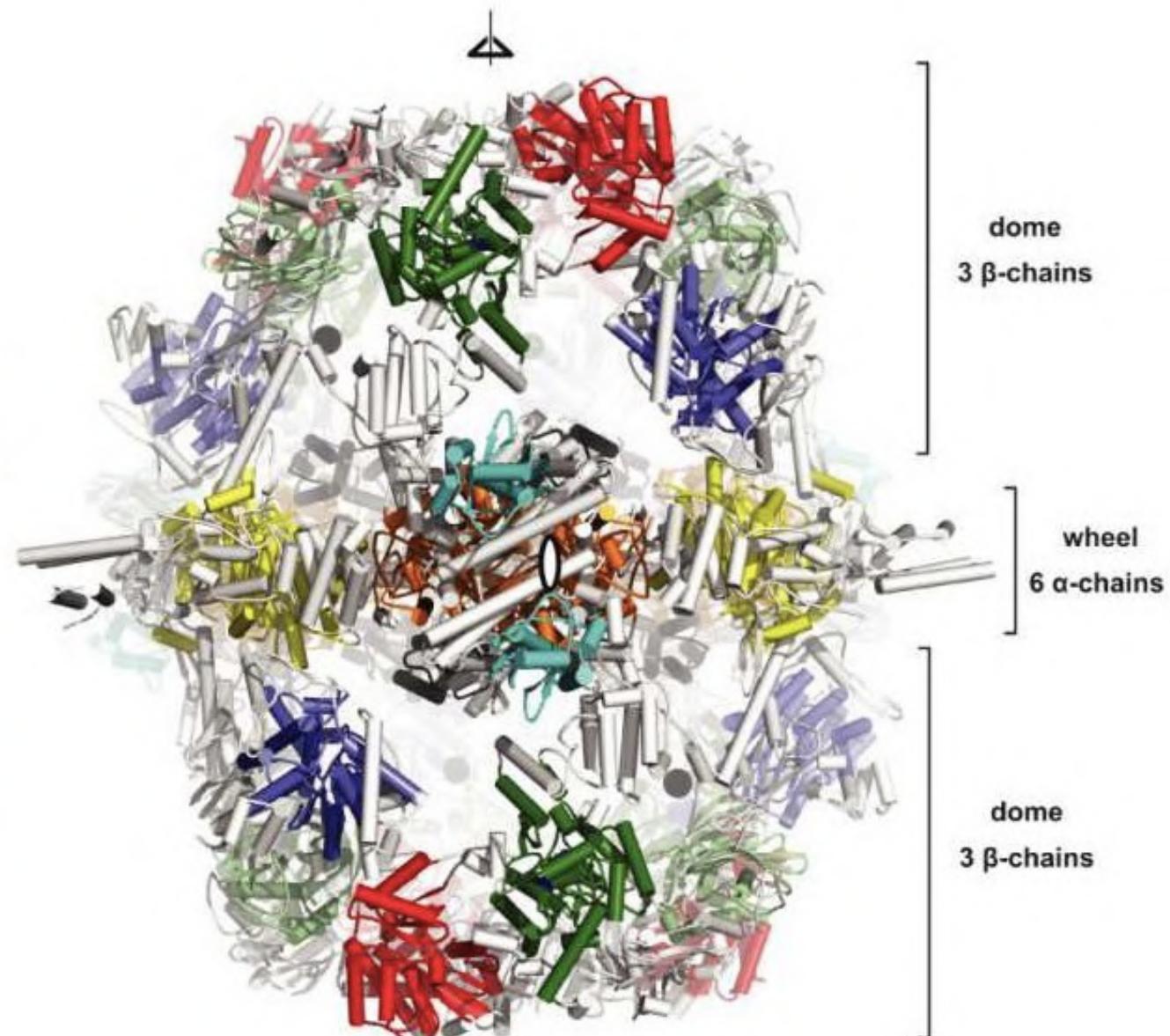


Fatty Acid Biosynthesis

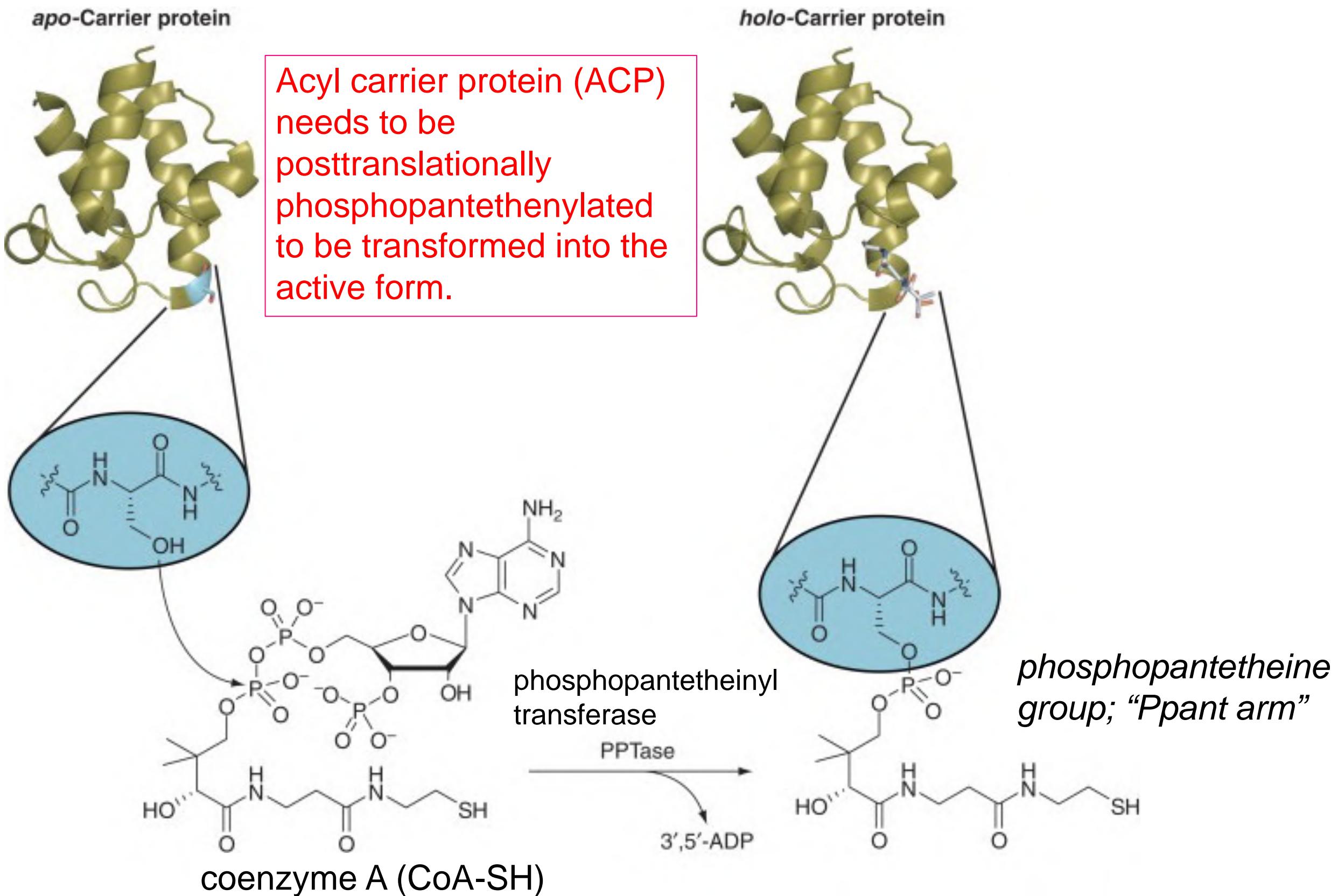
mammalian FAS



yeast FAS

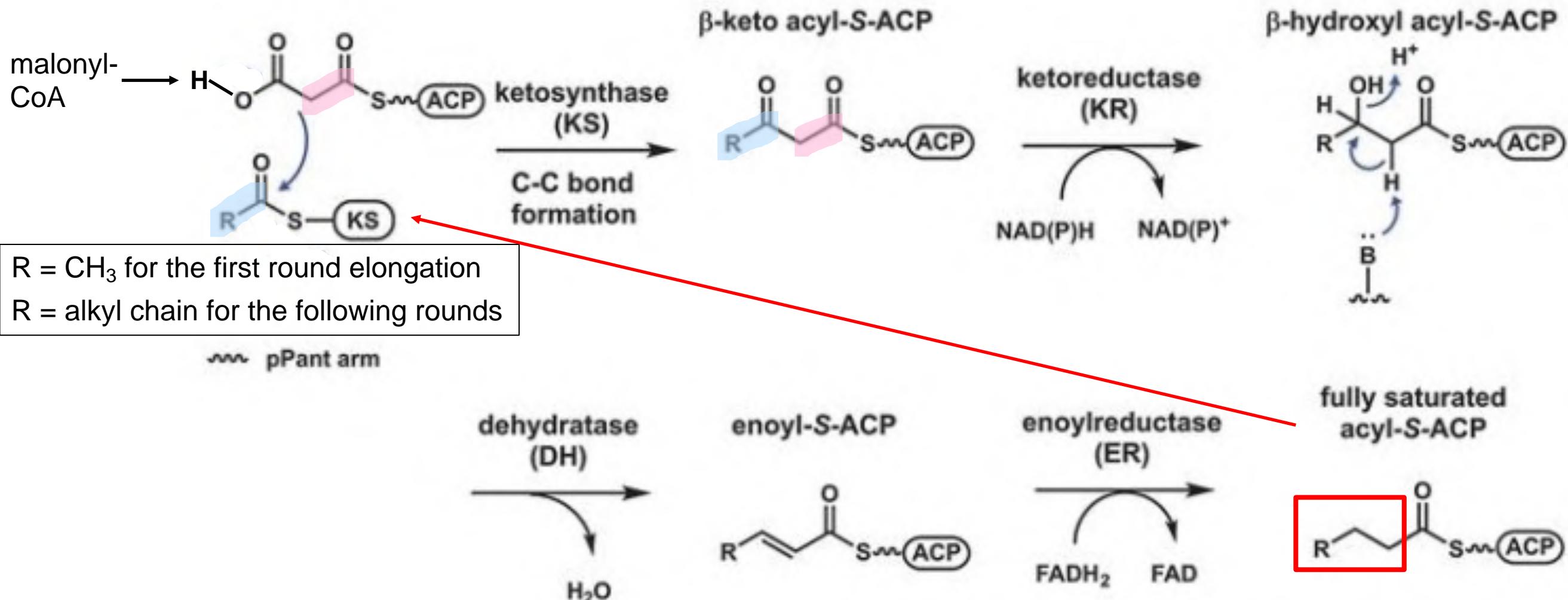


Fatty Acid Biosynthesis

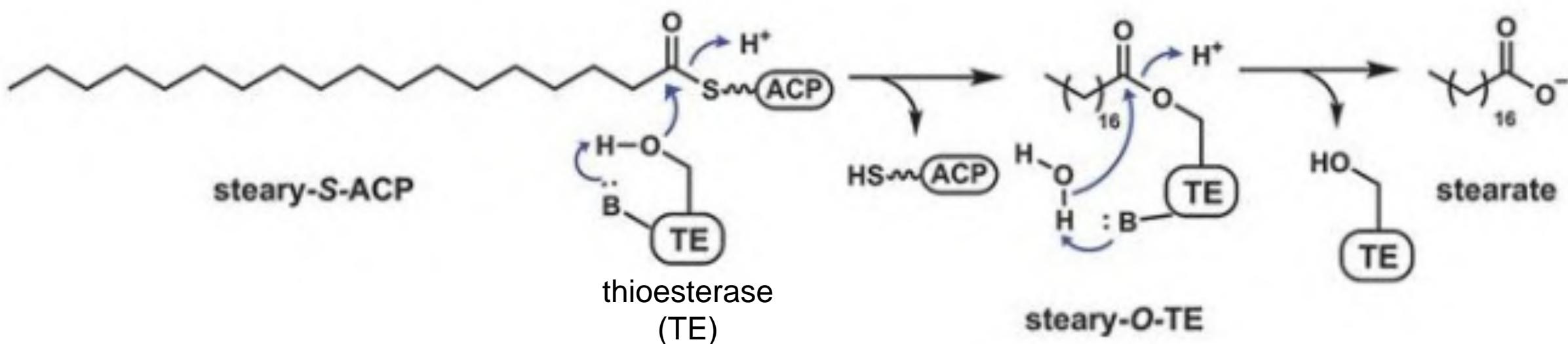


Biosynthetic Mechanism of Fatty Acids

Chain elongation



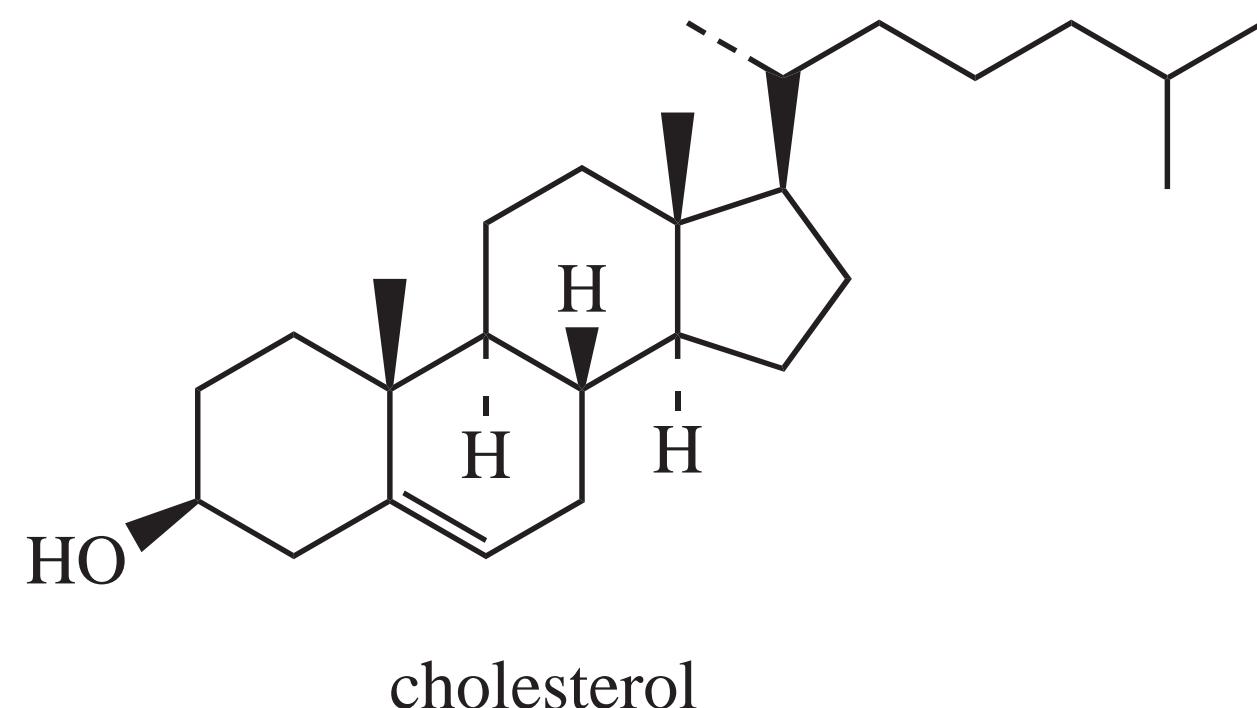
Chain release



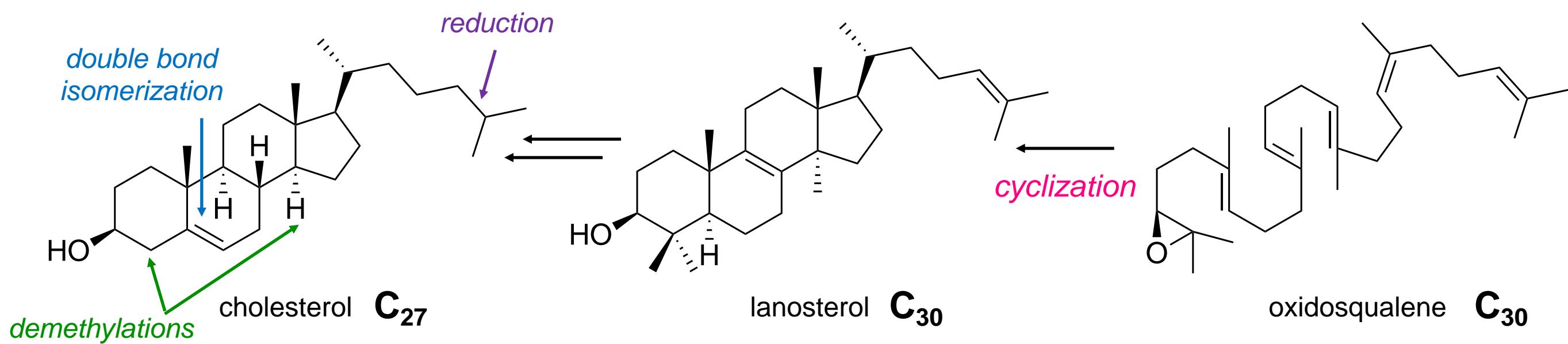
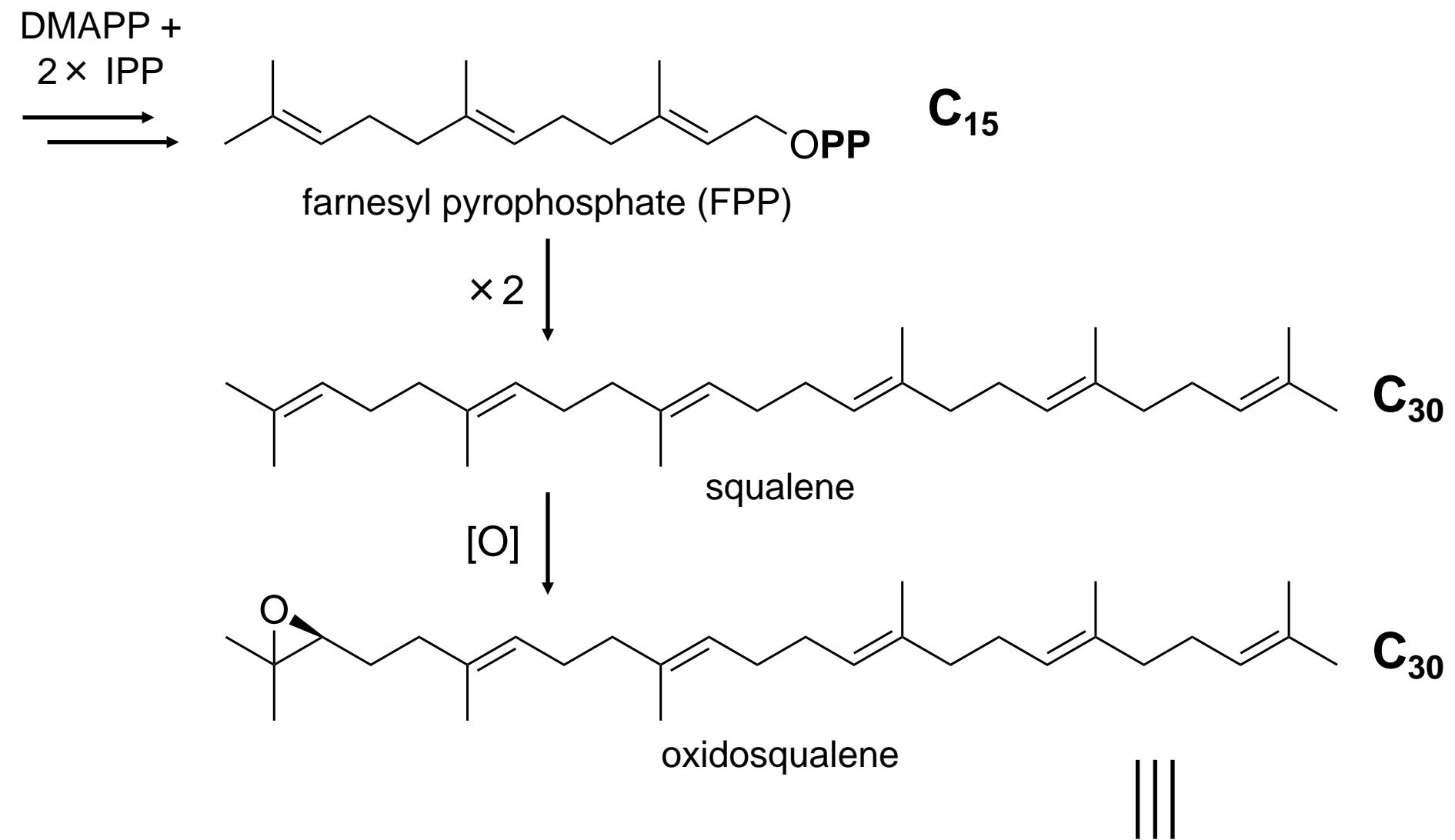
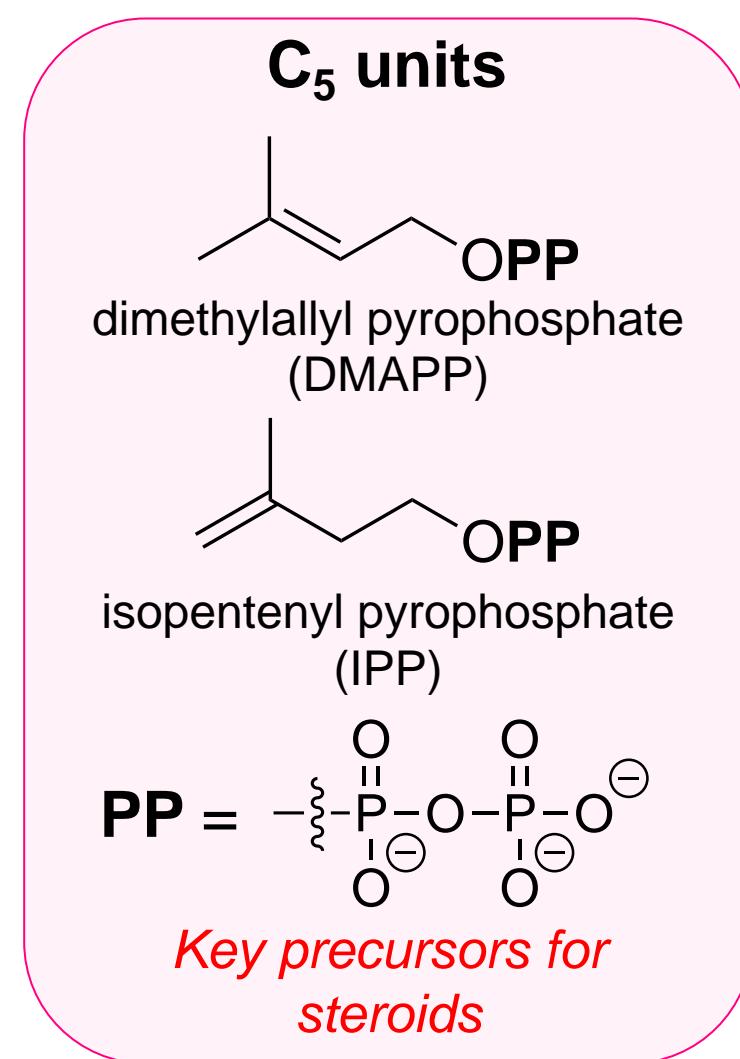
Cholesterol

Cholesterol

- Principal animal sterol and a constituent of cell membranes
- Maintains membrane fluidity, microdomain structure, and permeability
- Functions as a precursor for steroid hormones, bile acids, vitamin D, and lipoproteins
- Correlated with some diseases, such as cardiovascular disease; a molecule that can inhibit the cholesterol biosynthesis can serve as a cholesterol-lowering drug!



Overview of Cholesterol Biosynthesis

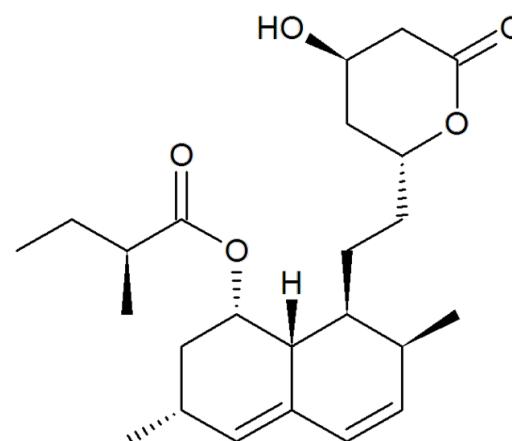


Statins

Statins

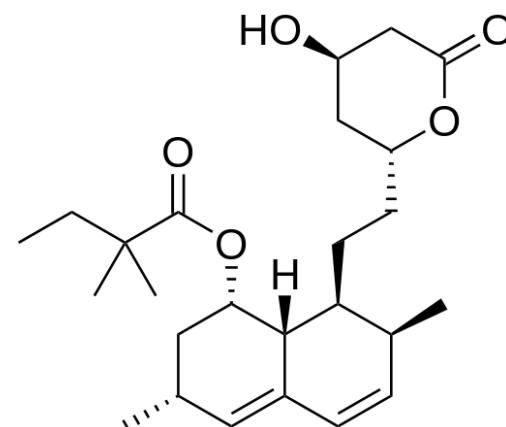
- Cholesterol-lowering medications
- Structurally analogous to HMG-CoA and thus inhibit HMG-CoA reductase

Natural product

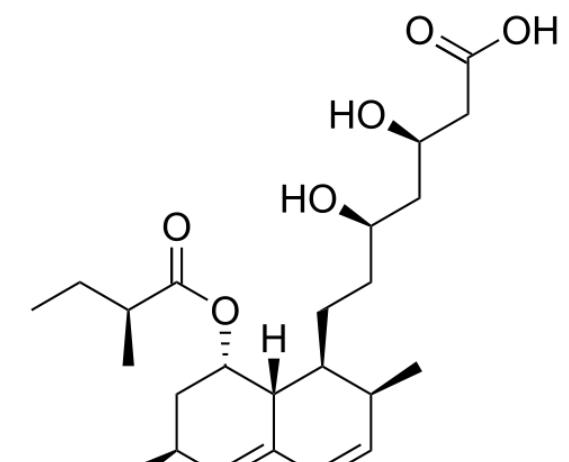


lovastatin

Natural product-derived

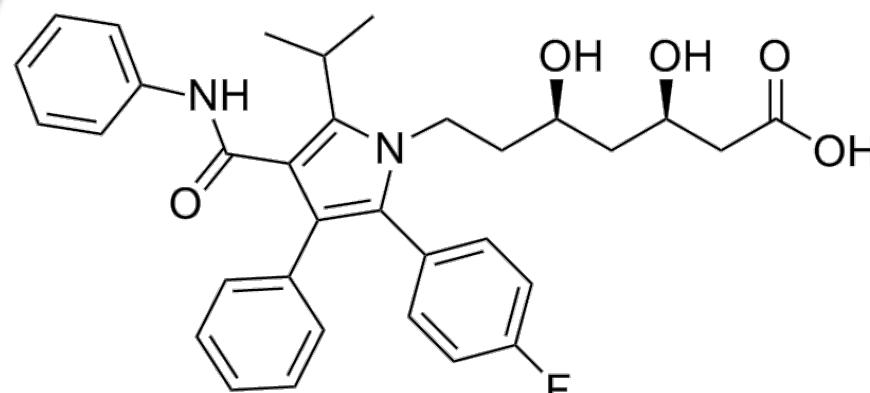


simvastatin

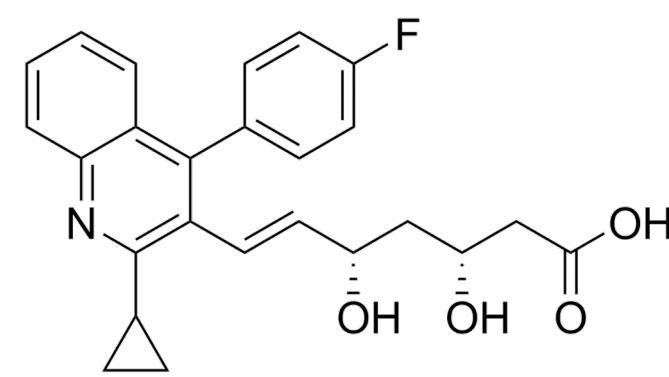


pravastatin

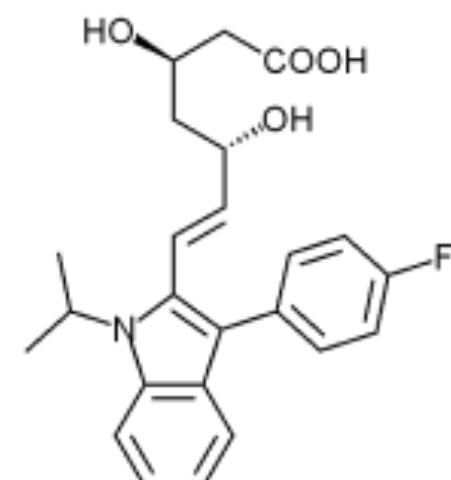
Synthetic



atorvastatin

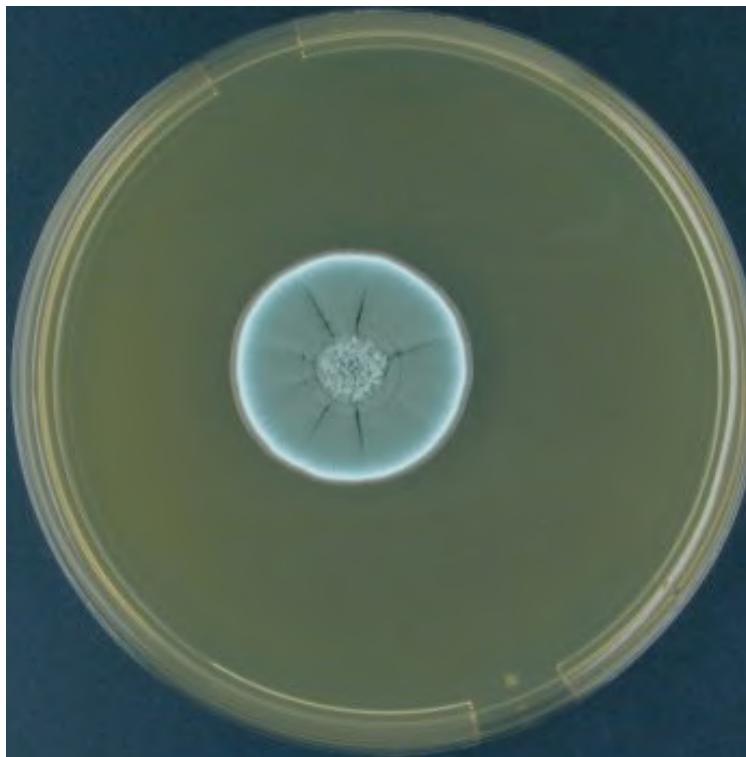
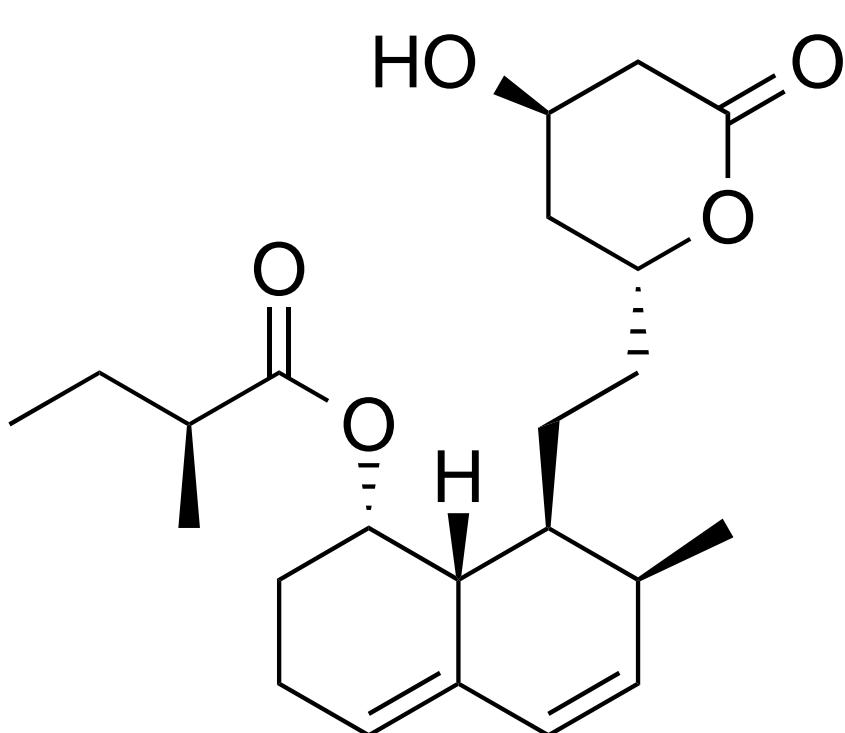


pitavastatin



fluvastatin

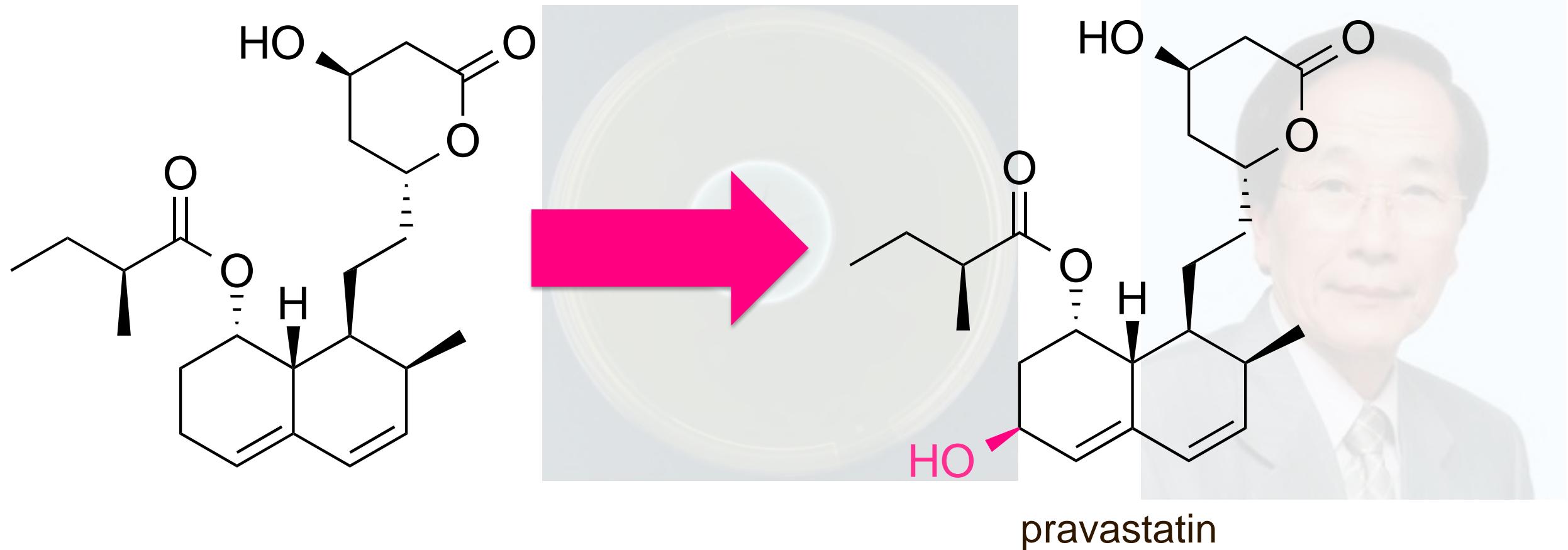
Discovery of Statins



Compactin

- Discovered from *Penicillium citrinum* in 1973 by Akira Endo and his colleagues; >6,000 fungal strains were investigated!

Discovery of Statins

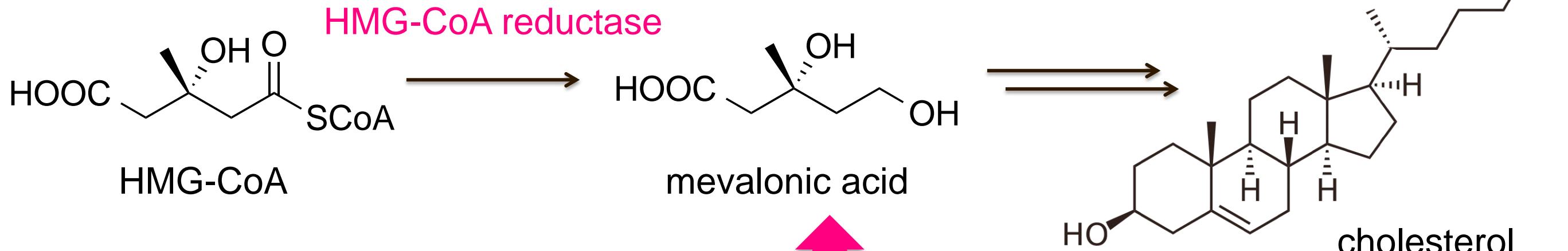


Compactin

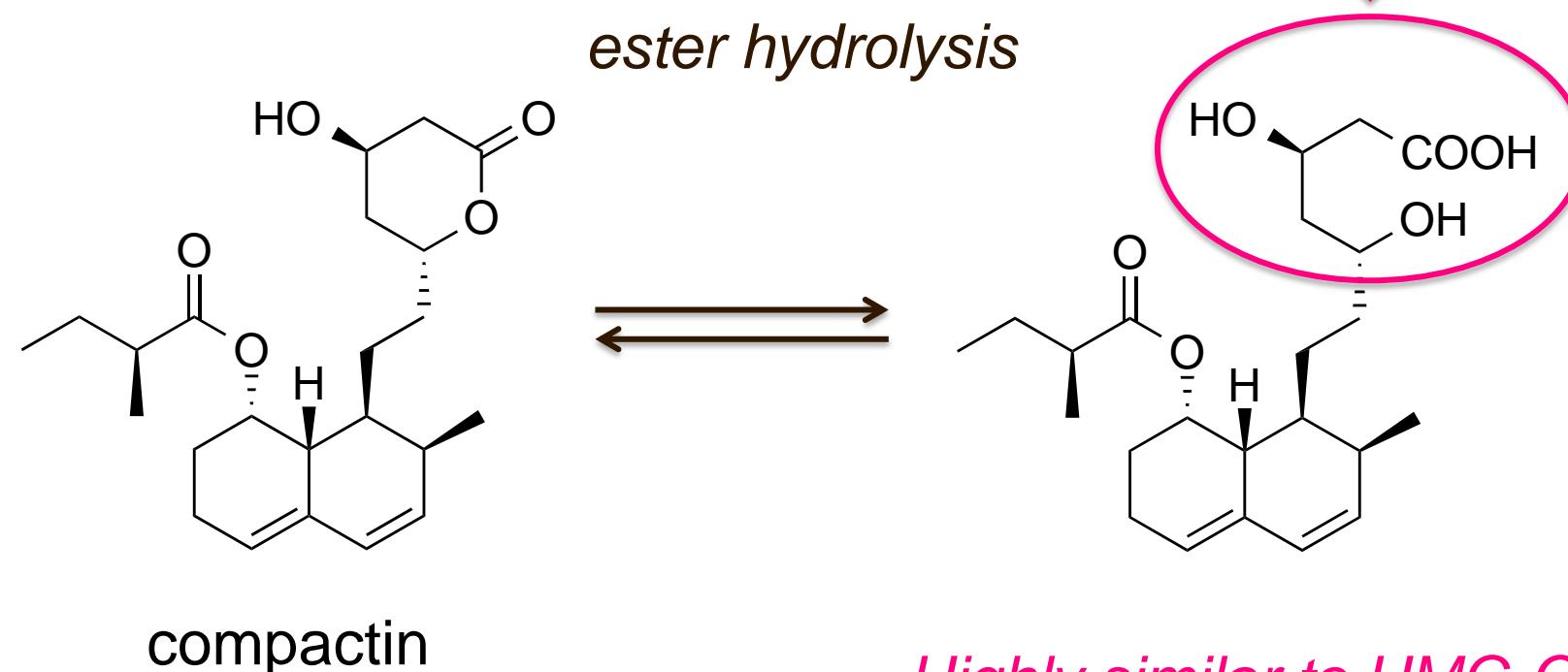
- Discovered from *Penicillium citrinum* in 1973 by Akira Endo and his colleagues; >6,000 fungal strains were investigated!
- Compactin itself was never marketed, but its hydroxylated analogue, pravastatin, is used as a cholesterol-lowering drug

How Statins Work?

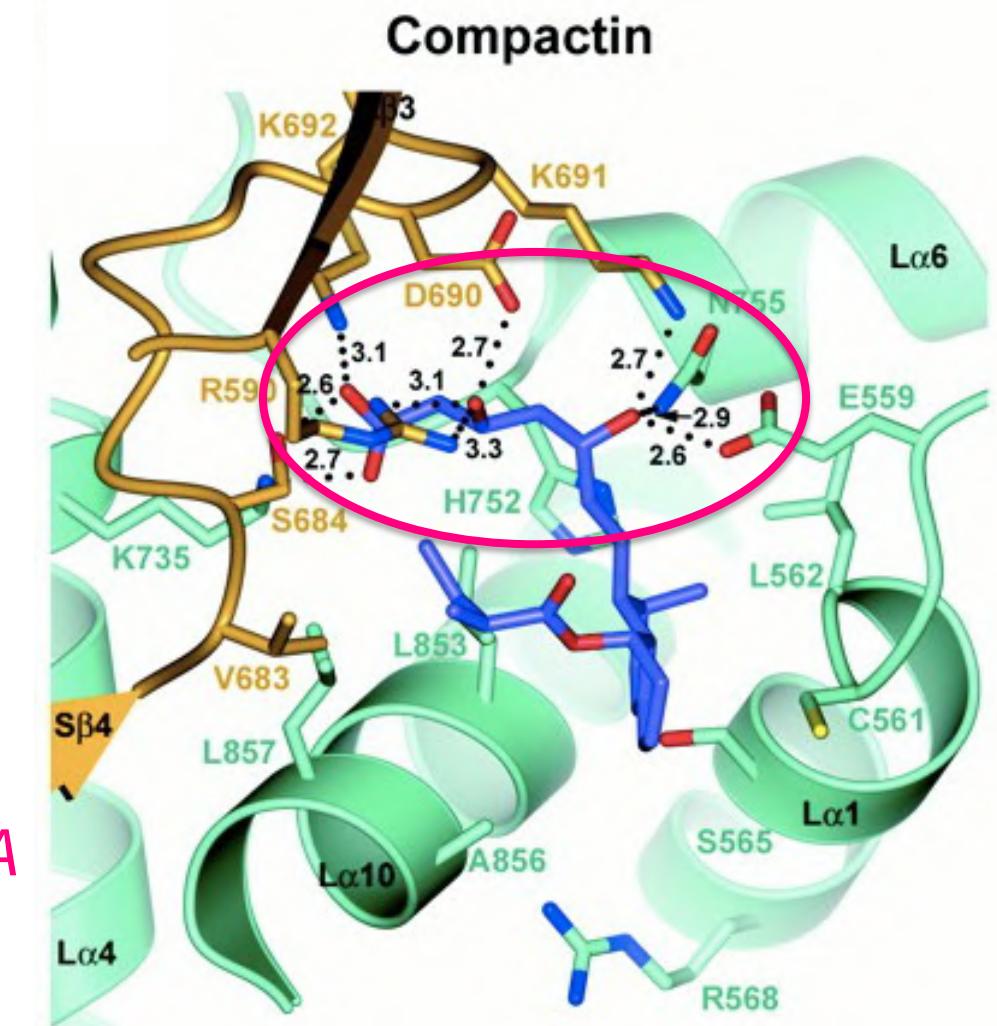
Biosynthesis of cholesterol



How do stains work??



Highly similar to HMG-CoA and mevalonate!



Summary

What you have learned today are:

- General function of enzymes
- How to characterize an enzyme
- Regulation mechanism of enzymes in cells
- Some important metabolic pathways: glycolysis, Krebs cycle, and oxidative phosphorylation
- Understanding metabolic pathways is important for drug discovery and development

References) Ch. 6 and 7 in
Biology: The Dynamic Science, 4th Ed.