Environmental Microbiology

Microbial Metabolism

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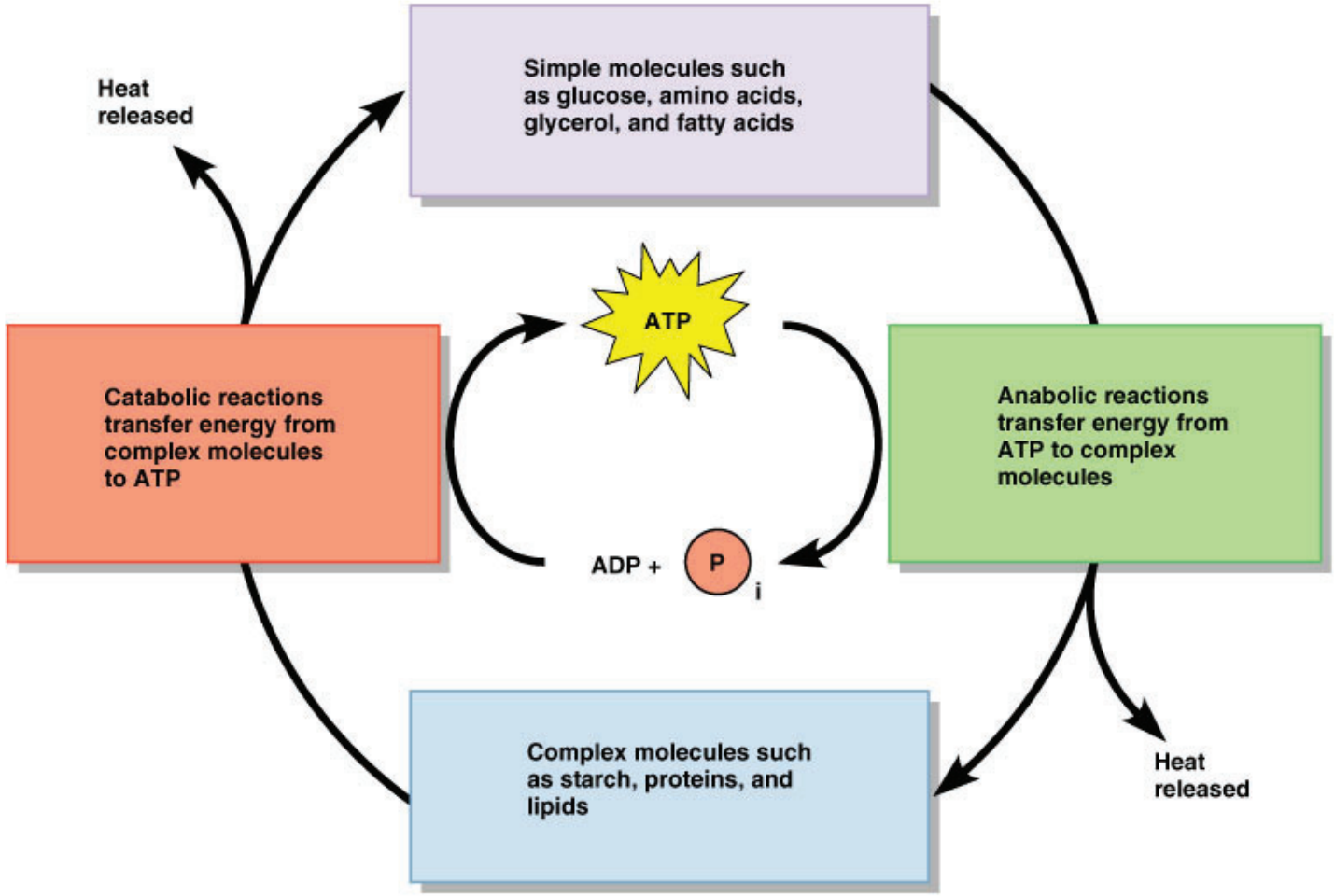
Microbial Metabolism

- Metabolism is the sum of the chemical reactions in an organism.
- Catabolism is the energy-releasing processes.
- Anabolism is the energy-using processes.

Microbial Metabolism

- Catabolism provides the building blocks and energy for anabolism.



Bioenergetics

- Energy is defined in units of kilojoules (kJ), a measure of heat energy
- In any chemical reaction, some energy is lost as heat
- Free energy (G): energy released that is available to do work
- The change in free energy during a reaction is referred to as $\Delta G^{0\prime}$
- $\Delta G^{0\prime}$: under standard conditions;1 M, pH 7, 25oC, 1 atm

Bioenergetics

- Reactions with a negative $\Delta G^{0\prime}$ release free energy (exergonic)
- Reactions with a positive $\Delta G^{0\prime}$ require energy (endergonic)
- To calculate free-energy yield of a reaction, we need to know the free energy of formation(G_f^0; the energy released or required during formation of a given molecule from the elements).

Bioenergetics

- For the reaction A + B C + D,
- $\Delta G_0' = G_f^0$ [C+D] - G_f^0 [A+B]
- $\Delta G_0'$ not always a good estimate of actual free-energy changes
- ΔG: free energy that occurs under actual conditions
- $\Delta G = \Delta G_0' + RT \ln k$
- where R (8.29 J/mol/kelvin) and T (K=^{o}C+273.15) are physical constants and k is the equilibrium constant for the reaction in question

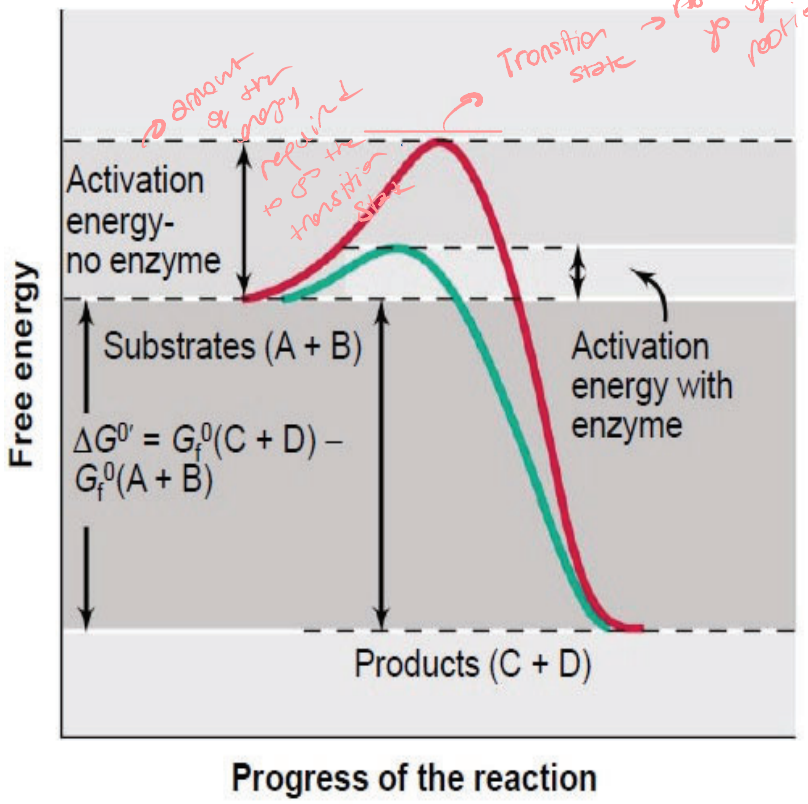
Catalysis and Enzymes

- Free-energy calculations do not provide information on reaction rates
- Activation energy: energy required to bring all molecules in a chemical reaction into the reactive state
- A catalysis is usually required to breach activation energy barrier
- A metabolic pathway is a sequence of enzymatically catalysed chemical reactions in a cell.
- Metabolic pathways are determined by enzymes.
- Enzymes are encoded by genes.

Activation Energy and Catalysis

Catalyst:
substance that

- Lowers the activation energy of a reaction
- Increases reaction rate
- Does not affect energetics or equilibrium of a reaction



- Even chemical reactions that release energy may not proceed spontaneously, because the reactants must first be activated.
- Once they are activated, the reaction proceeds spontaneously.
- Catalysts such as enzymes lower the required activation energy.

Catalysis and Enzymes

Enzymes

- Biological catalysts
- Typically proteins (some RNAs)
- Highly specific
- Generally larger than substrate
- Typically rely on weak bonds

•Examples: hydrogen bonds, van der Waals forces, hydrophobic interactions

Active site: region of enzyme that binds substrate

Increase the rate of chemical reactions by 10^8 to 10^{20} times the spontaneous rate

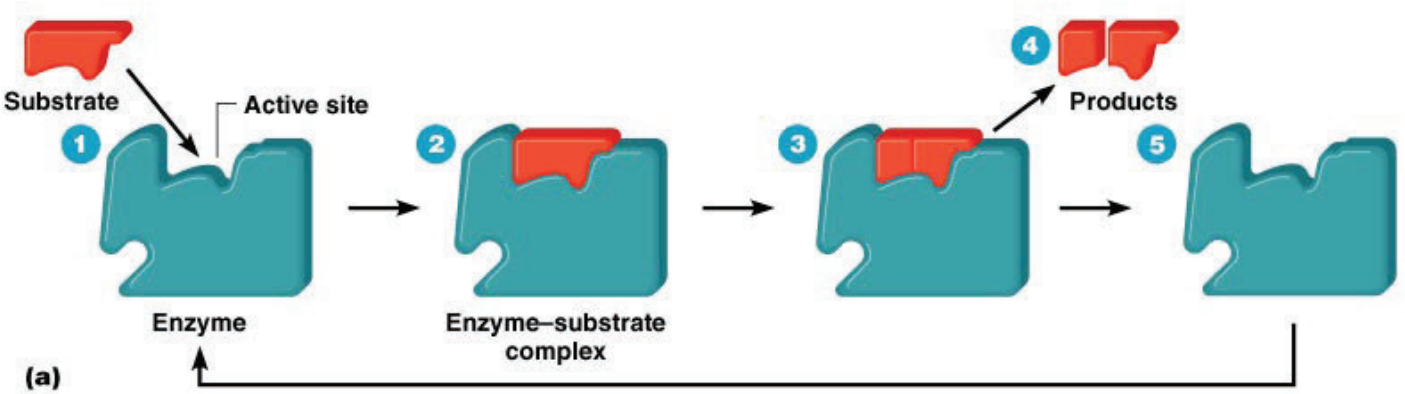
Enzyme catalysis: E + S $\Longleftrightarrow$ E-S $\Longleftrightarrow$ E + P

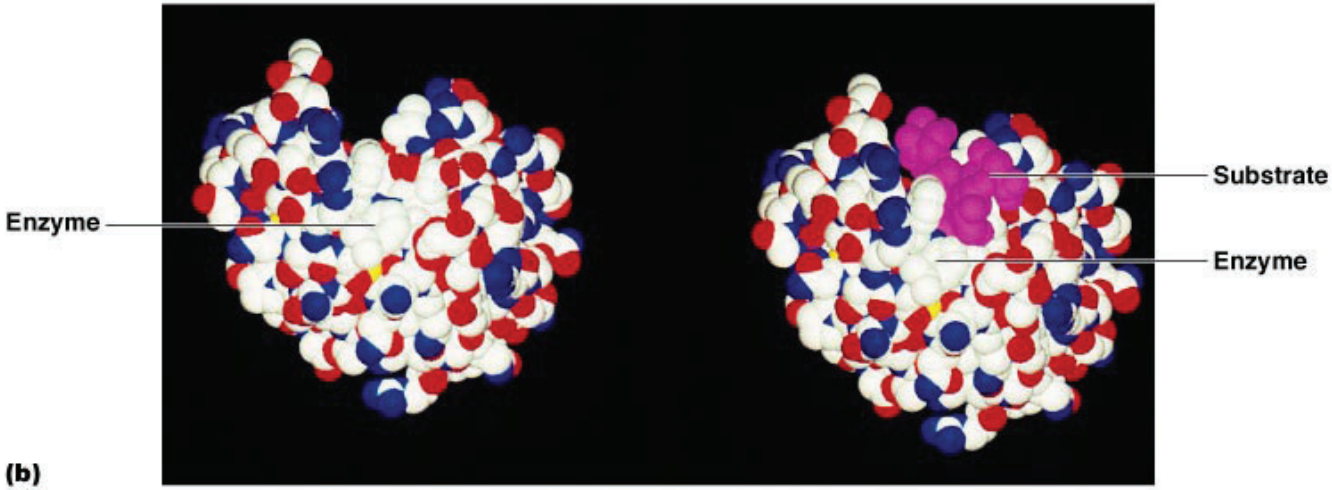
Catalysis dependent on

- Substrate binding
- Position of substrate relative to catalytically active amino acids in active site

Enzymes

- The turnover number is generally 1-10,000 molecules per second.





Enzyme Classification

• Oxidoreductase	Oxidation-reduction reactions
• Transferase	Transfer functional groups
• Hydrolase	Hydrolysis
• Lyase	Removal of atoms without hydrolysis
• Isomerase	Rearrangement of atoms
• Ligase	Joining of molecules, uses ATP

Enzymes

- Biological catalysts

Specific for a chemical reaction; not used up in that reaction

Many enzymes contain small nonprotein molecules that participate in catalysis but are not substrates

  - Prosthetic groups (Cofactor: Nonprotein component)

    Bind tightly to enzymes. Usually bind covalently and permanently (e.g., heme group in cytochromes)

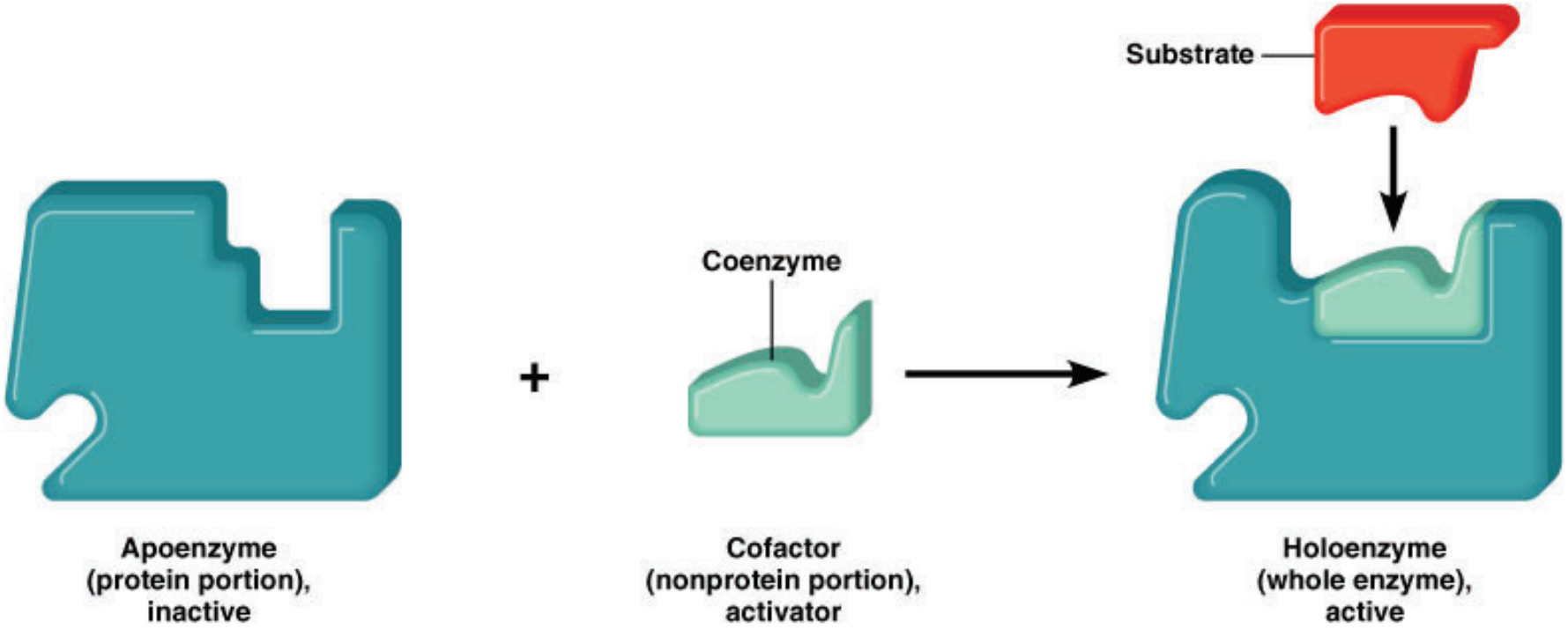
  - Coenzymes: Organic compounds

    Loosely bound to enzymes. Most are derivatives of vitamins (e.g., NAD+/NADH)

- Apoenzyme: protein
- Cofactor: Nonprotein component (If it is metal ion Ca^{+2}, Mg^{+2})
- Coenzyme: Organic cofactor (Again non-protein)
- Holoenzyme: Apoenzyme + cofactor

Active enzyme → functional enzyme

Enzymes

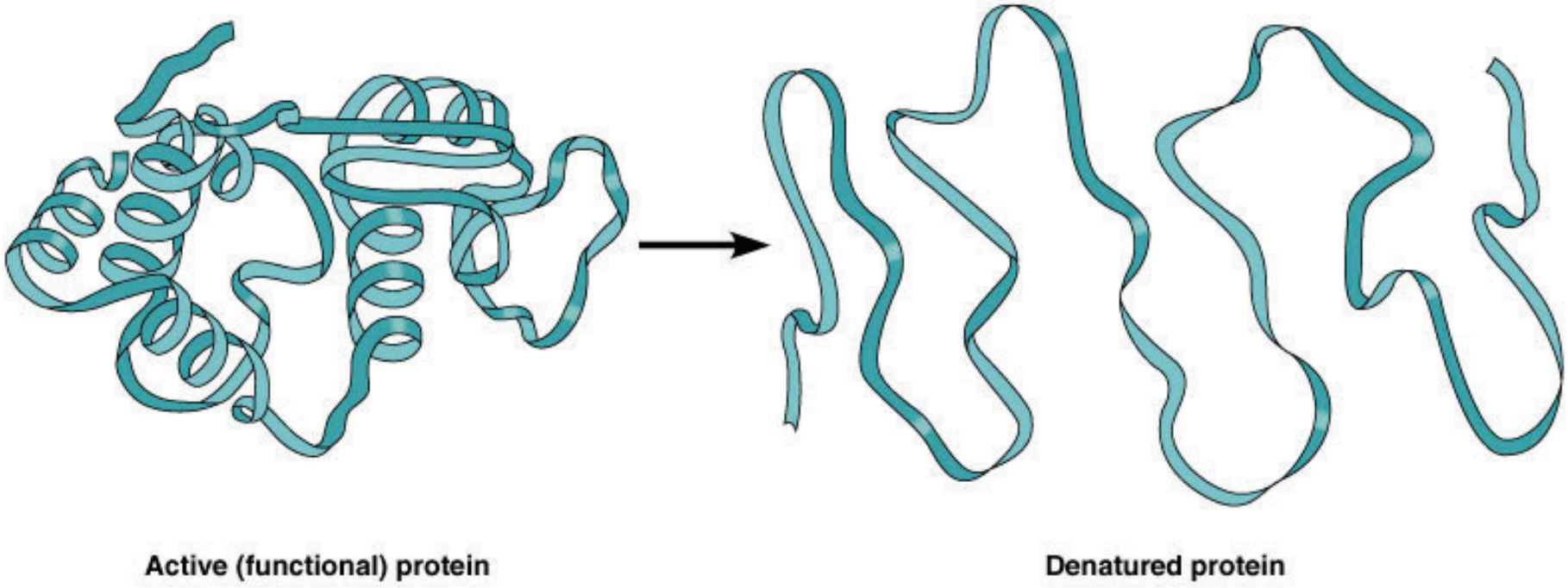

Substrate
Coenzyme
+
Apoenzyme
(protein portion),
inactive
Cofactor
(nonprotein portion),
activator
Holoenzyme
(whole enzyme),
active

Important Coenzymes

- NAD^+
- $NADP^+$
- FAD
- Coenzyme A

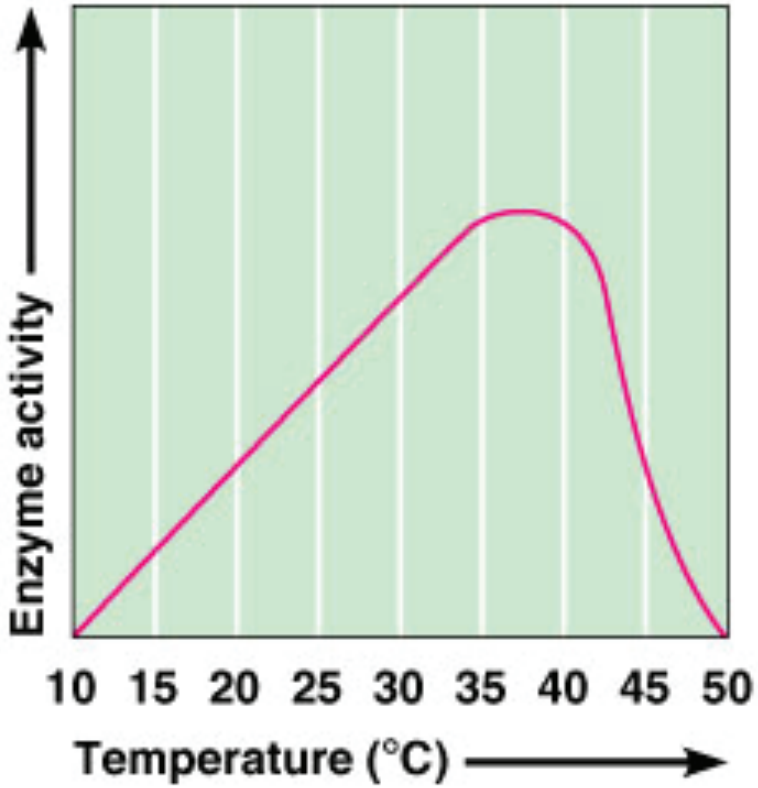
Factors Influencing Enzyme Activity

- Enzymes can be denatured by temperature and pH



Factors Influencing Enzyme Activity

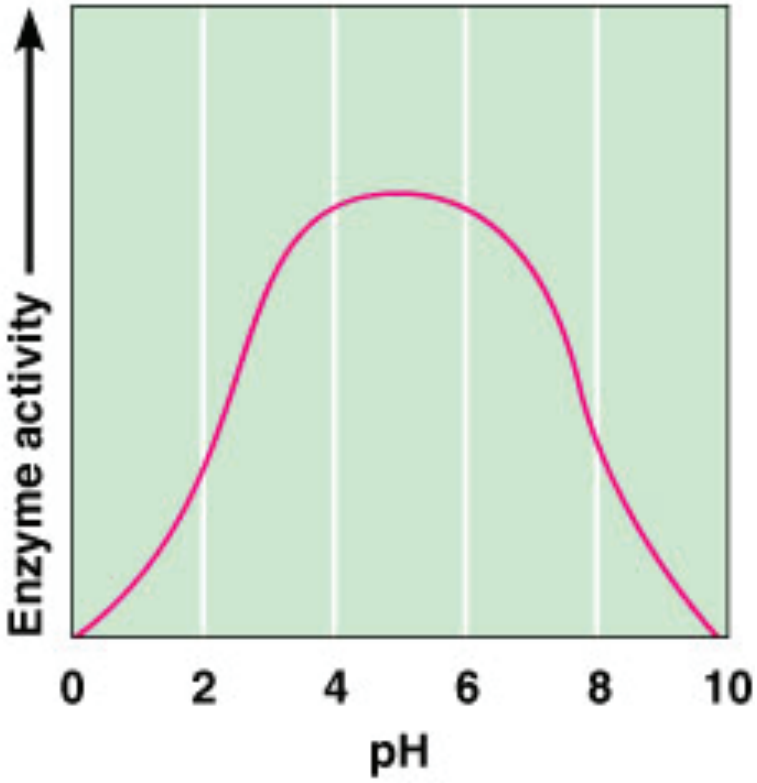
- Temperature



(a) Temperature

Factors Influencing Enzyme Activity

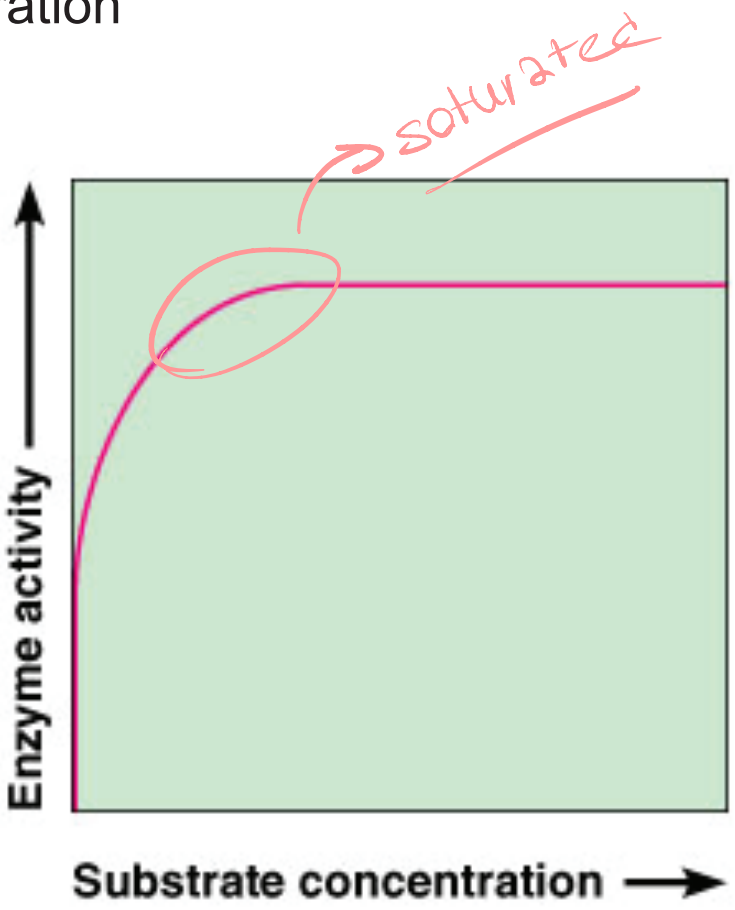
- pH



(b) pH

Factors Influencing Enzyme Activity

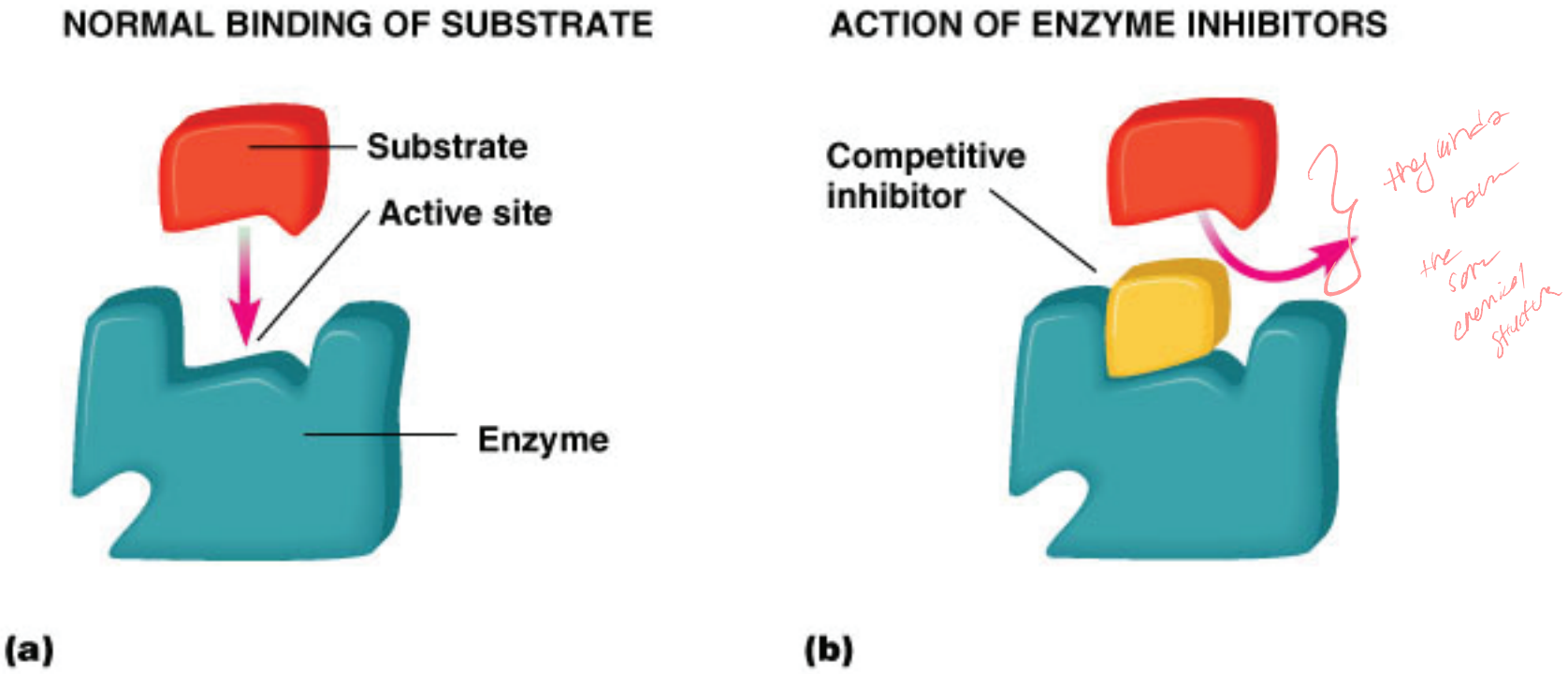
- Substrate concentration



(c) Substrate concentration

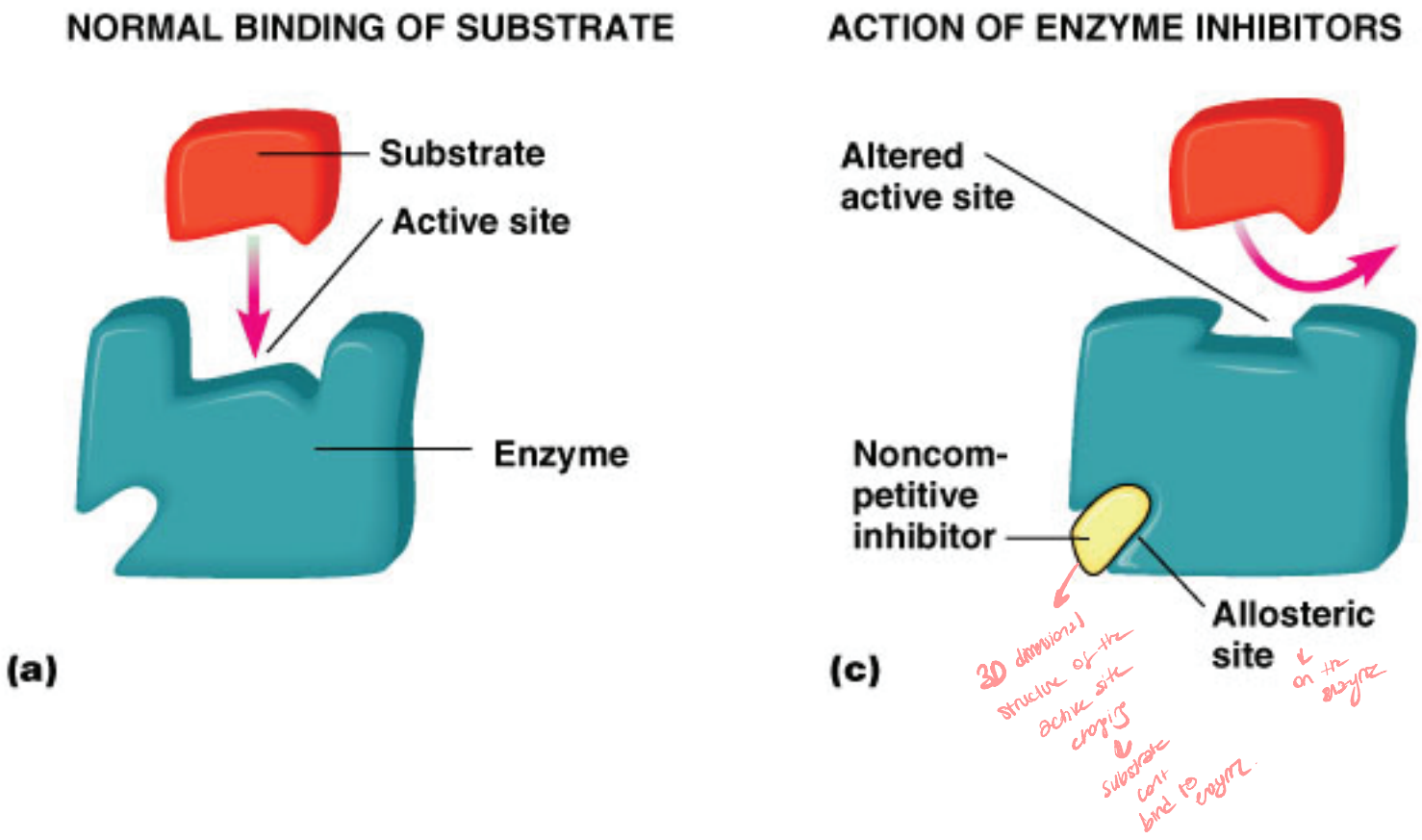
Factors Influencing Enzyme Activity

- Competitive inhibition



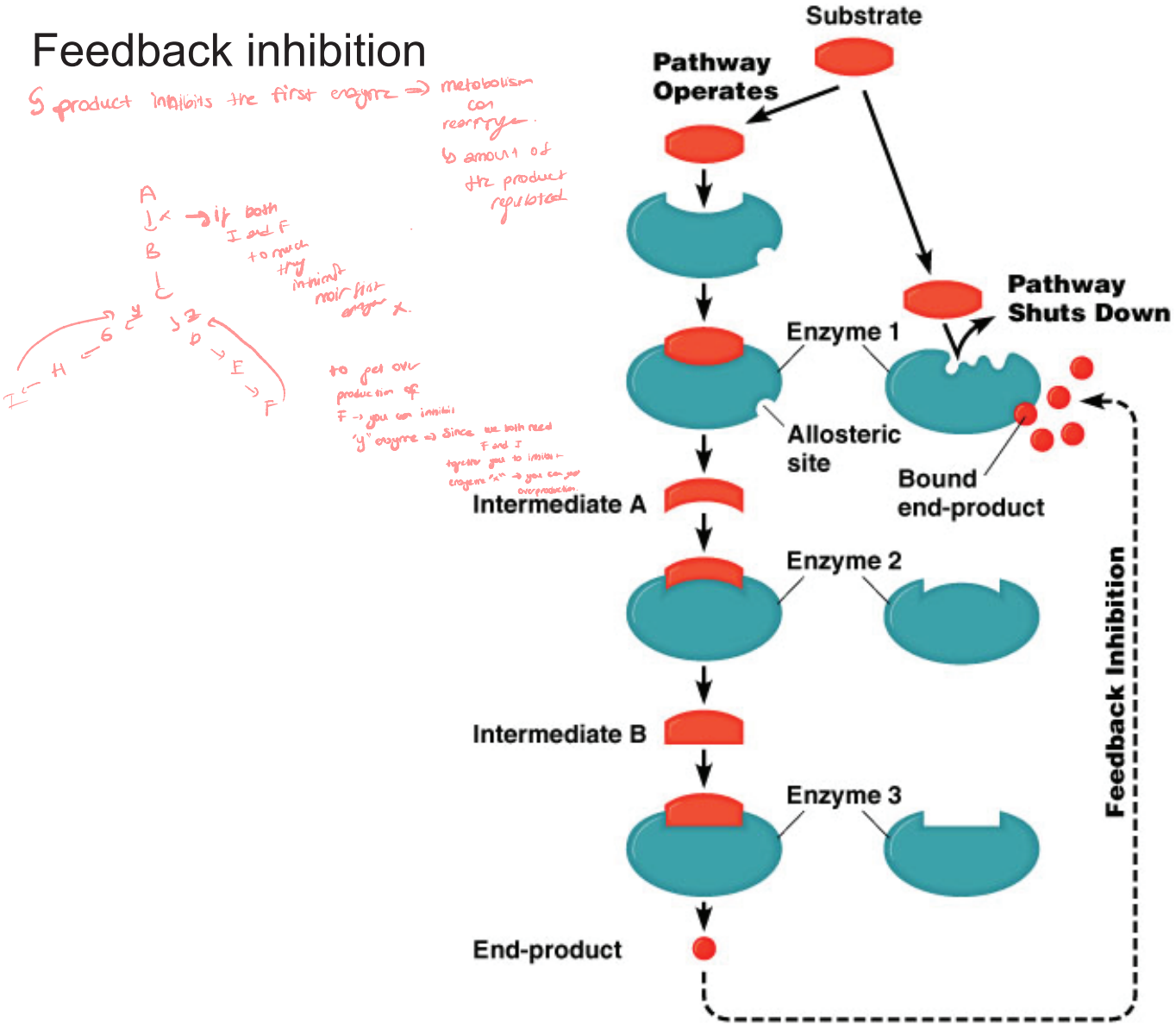
Factors Influencing Enzyme Activity

- Noncompetitive inhibition



Factors Influencing Enzyme Activity

- Feedback inhibition



Enzyme Kinetics

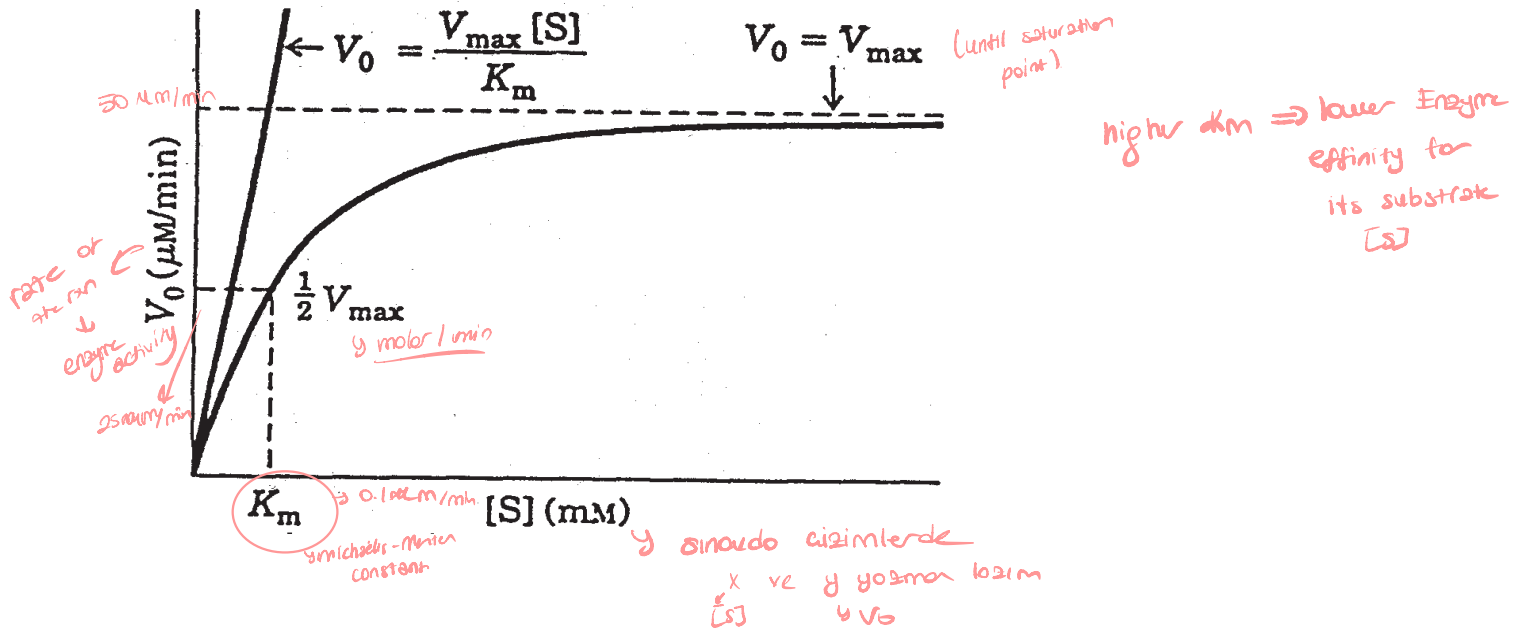
- Michaelis-Menten kinetics
- Interpretations and uses of the Michaelis-Menten equation
- Enzyme inhibitors: types and kinetics

Enzyme Kinetics Equation

$$E + S \underset{k_{-1}}{\overset{k_1}{\leftrightharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

E	enzyme
S	substrate
P	product
ES	enzyme-substrate complex
k_1, k_2, k_{-1}, k_{-2}	are rate constants

Michaelis-Menten Curve



- When initial velocity is plotted against [S], a hyperbolic curve results, where V_{max} represents the maximum reaction velocity. At this point in the reaction, if [S] >> E, all available enzyme is "saturated" with bound substrate, meaning only the ES complex is present.

Michaelis-Menten Equation

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

V_0	initial reaction velocity
V_{max}	maximal velocity
[S]	substrate concentration

$$K_m = (k_{-1} + k_2) / k_1$$

If k2 ≪
i.e., E +P back to ES is minimal

Initial Velocity (V_o) and [S]

The concentration of substrate [S] present will greatly influence the rate of product formation, termed the velocity (v) of a reaction. Studying the effects of [S] on the velocity of a reaction is complicated by the reversibility of enzyme reactions, e.g. conversion of product back to substrate. To overcome this problem, the use of **initial velocity** (v_o) measurements are used. At the start of a reaction, [S] is in large excess of [P], thus the initial velocity of the reaction will be dependent on substrate concentration

Meaning of K_m

An important relationship that can be derived from the Michaelis-Menten equation is the following: If **V_o is set equal to 1/2 V_{max}, then** the relation $V_{max}/2 = V_{max}[S]/K_m + [S]$ can be simplied to $K_m + [S] = 2[S]$, or **$K_m = [S]$. This means that at one half of the maximal velocity, the substrate concentration at this velocity will be equal to the K_m.** This relationship has been shown experimentally to be valid for many enzymes much more complex in regards to the number of substrates and catalytic steps than the simple single substrate model used to derive it.

Uses of K_m

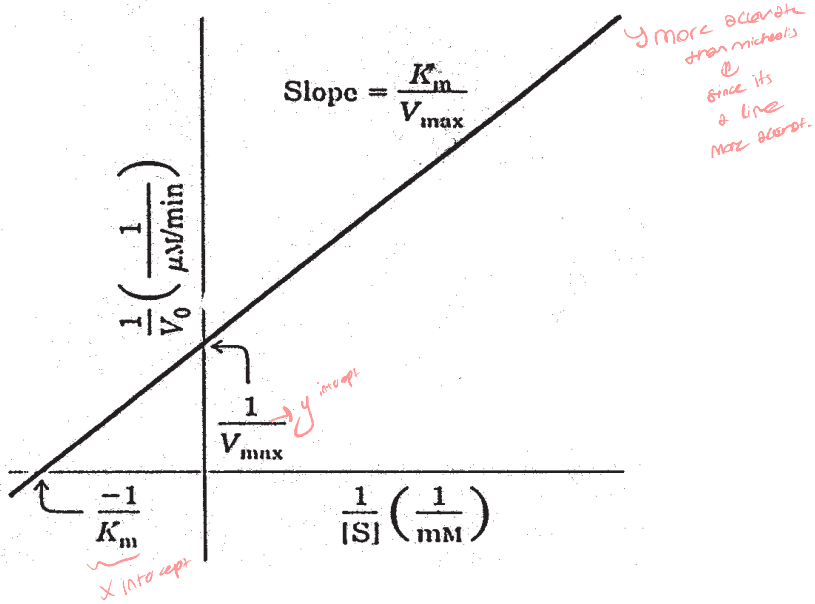
Experimentally, K_m is a useful parameter for characterizing the number and/or types of substrates that a particular enzyme will utilize. It is also useful for comparing similar enzymes from different tissues or different organisms. Also, it is the K_m of the rate-limiting enzyme in many of the biochemical metabolic pathways that determines the amount of product and overall regulation of a given pathway. Clinically, K_m comparisons are useful for evaluating the effects mutations have on protein function for some inherited genetic diseases.

Lineweaver-Burk (double reciprocal plot)

- If the reciprocal (1/X) of the Michaelis-Menten equation is done, after algebraic simplification the following equation results:

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

- This relation is written in the format of the equation for a straight line, $y = mx + b$, where $y = 1/v_o$, m (slope) = K_m/V_{max}, $x = 1/[S]$ and the y-intercept, $b = 1/V_{max}$. When this relation is plotted,the result is a straight line graph



Uses of Double Reciprocal Plot

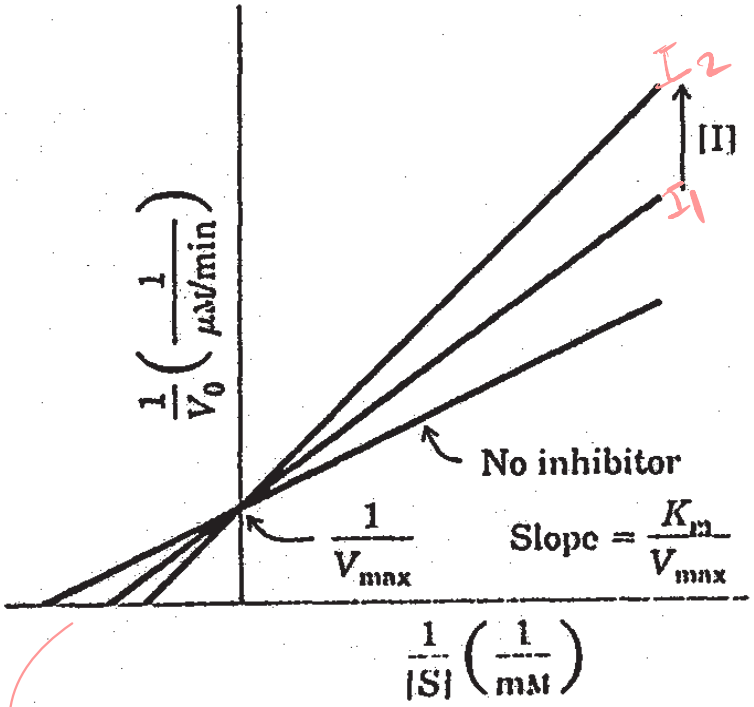
- The x intercept value is equal to $-1/K_m$. The biggest advantage to using the double reciprocal plot is a more accurate determination of V_{max}, and hence K_m. It is also useful in characterizing the effects of enzyme inhibitors and distinguishing between different enzyme mechanisms.

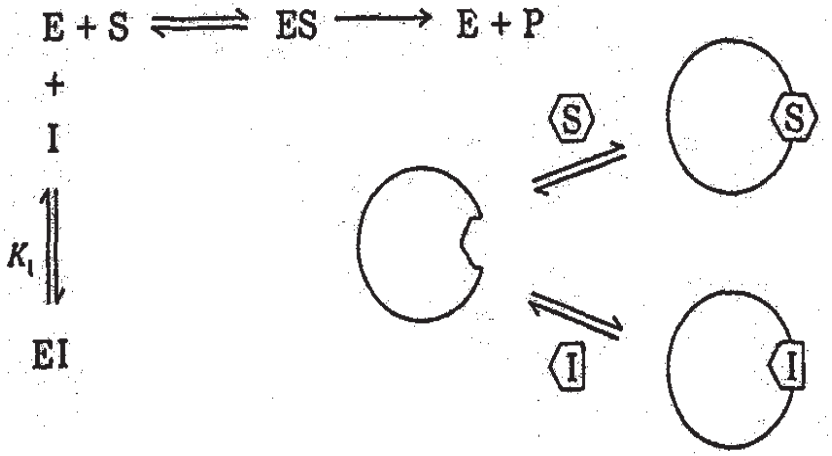
Enzyme Inhibitor Types

Inhibitors of enzymes are generally molecules which resemble or mimic a particular enzymes substrate(s). Therefore, it is not surprising that many therapeutic drugs are some type of enzyme inhibitor. The modes and types of inhibitors have been classified by their kinetic activities and sites of actions. These include:

- Competitive Inhibition
- Noncompetitive Inhibition
- Uncompetitive Inhibition
- Substrate Inhibition
- Irreversible Inhibitors

Competitive Inhibition





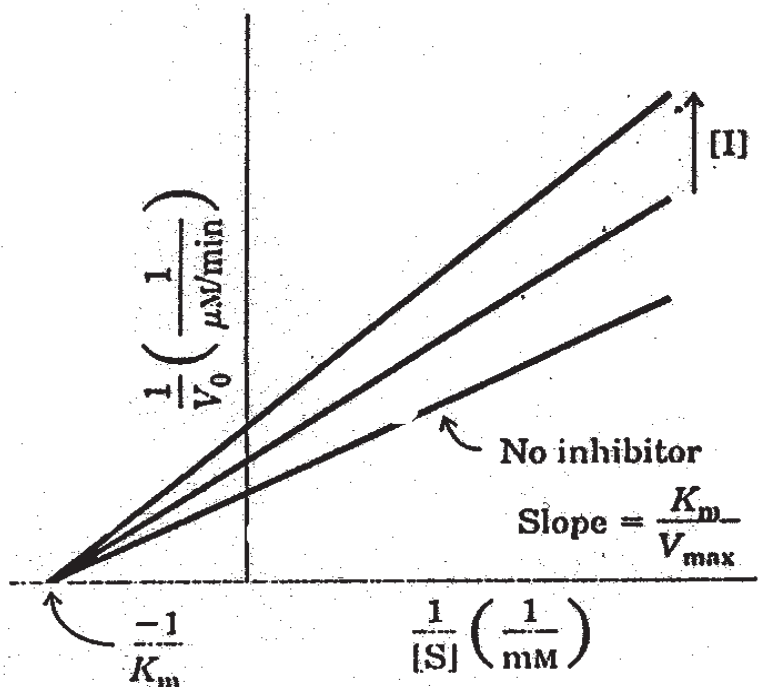
Inhibitor is an analog of the substrate, and binds to the active site of the enzyme.

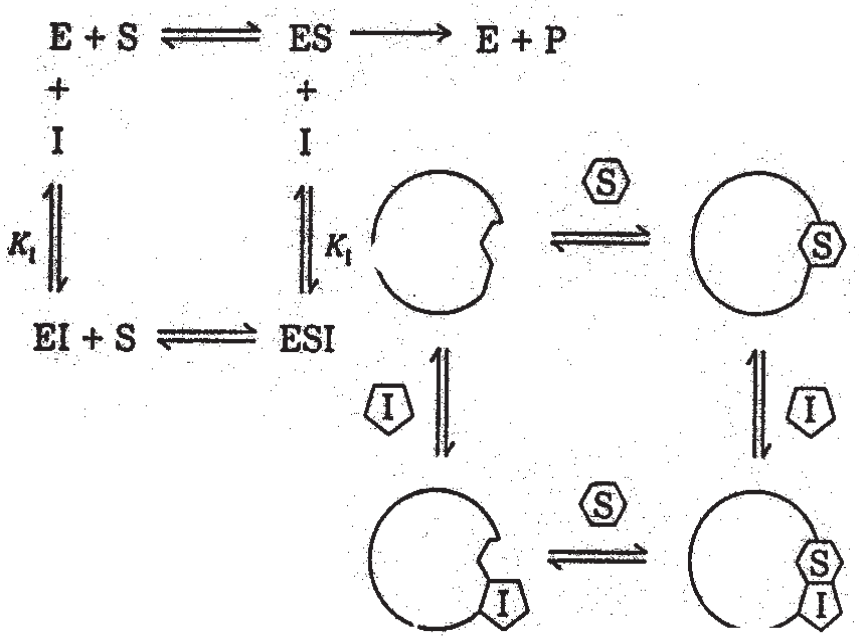
V_{max} No change

K_m INCREASES - indicates a direct interaction of the inhibitor in the active site

Using inhibitor concentration increases.

Non-Competitive Inhibition



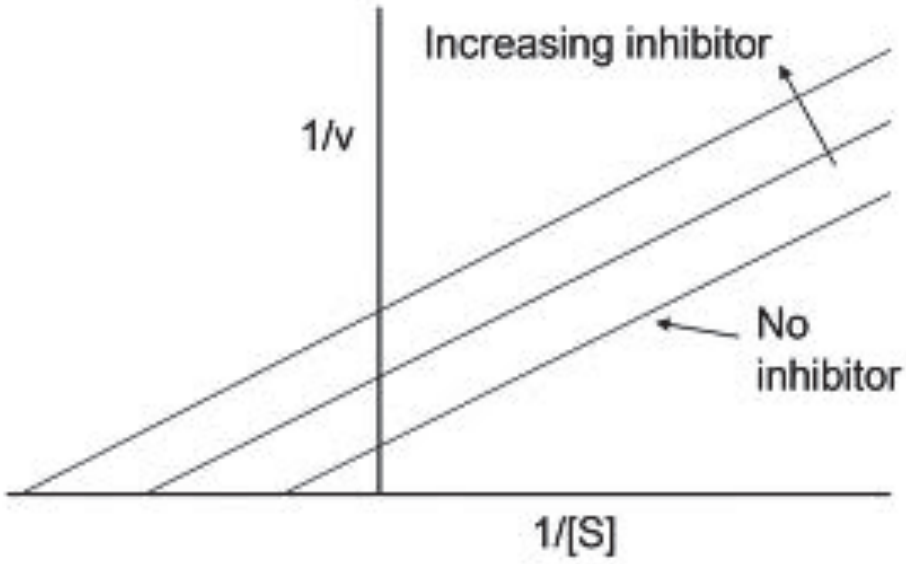


V_{max} DECREASES - inhibitor affects rate of reaction by binding to site other than substrate active-site

K_m No change

Inhibitor binds to the enzyme, but not at the active site. However, the enzyme affinity for substrate is reduced.

Uncompetitive Inhibition



V_{max} and K_m both changed

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_{cat}} E + P$$

$$+I \downarrow\uparrow -I$$

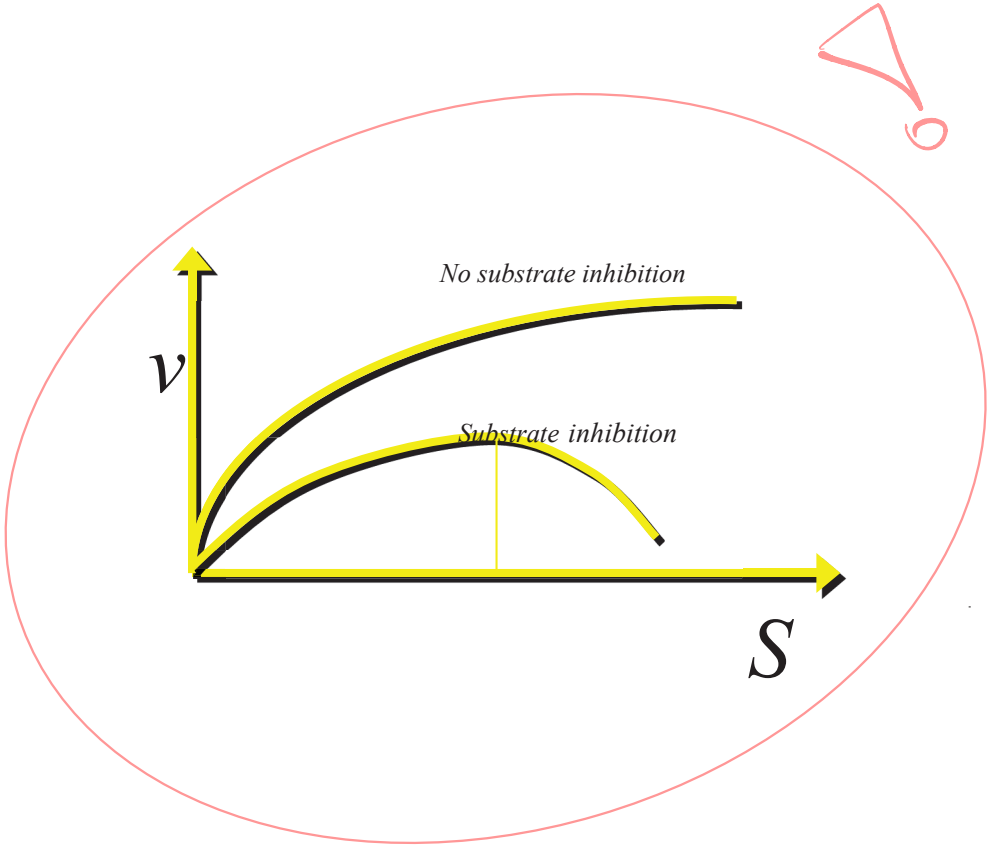
$$K_{Iu} = \frac{[ES][I]}{[ESI]} \qquad ESI \not\rightarrow \text{(N.R.)}$$

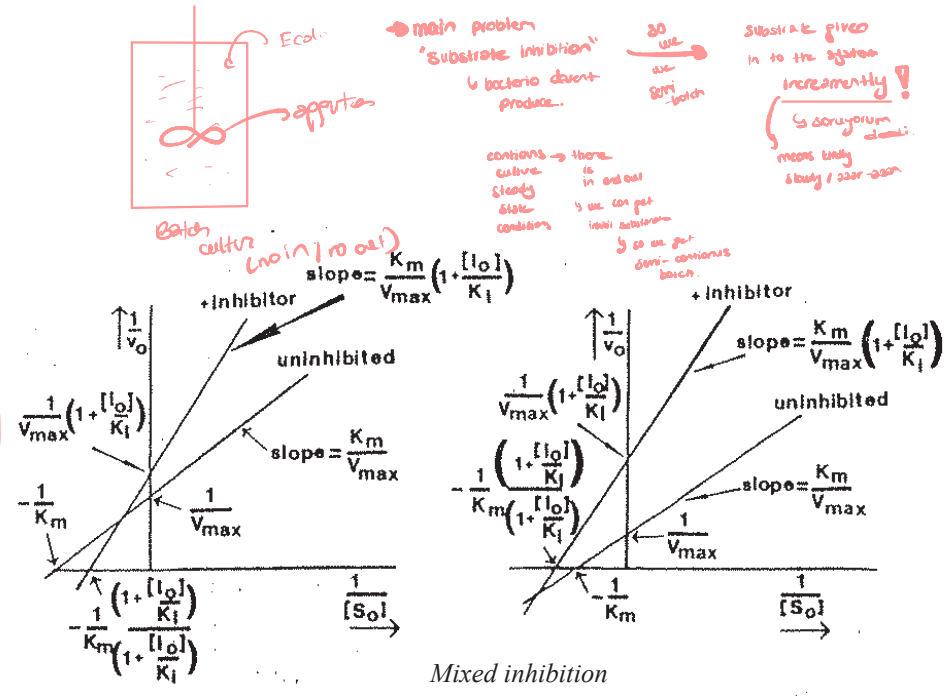
$$V_{max}^{app} = V_{max}/(1 + \tfrac{[I]}{K_{Iu}}) \qquad K_M^{app} = K_M/(1 + \tfrac{[I]}{K_{Iu}})$$

$$V_{max}/K_M \text{ unaffected}$$

Inhibitor binds only to ES complex, and not to E alone.

Substrate Inhibition & Mixed Inhibition

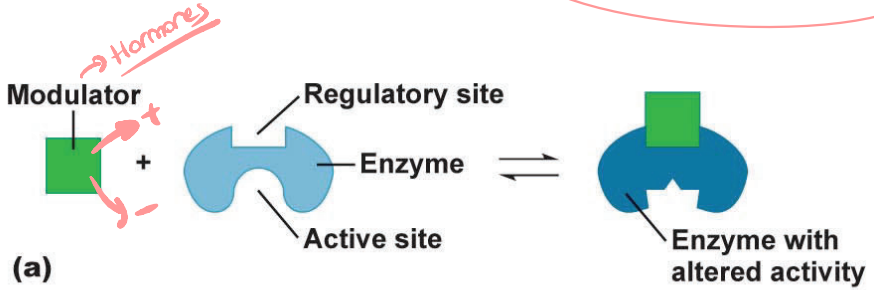


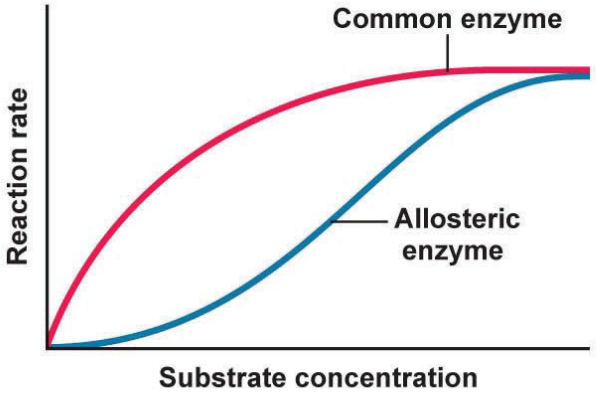


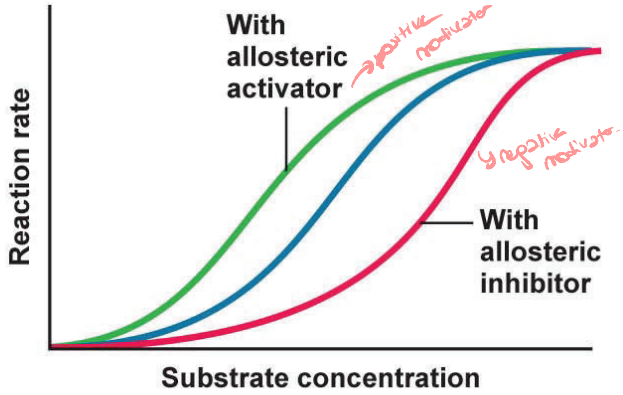
Mixed inhibition

Allosteric Enzymes

Shape of rate curve is sigmoidal rather than hyperbolic





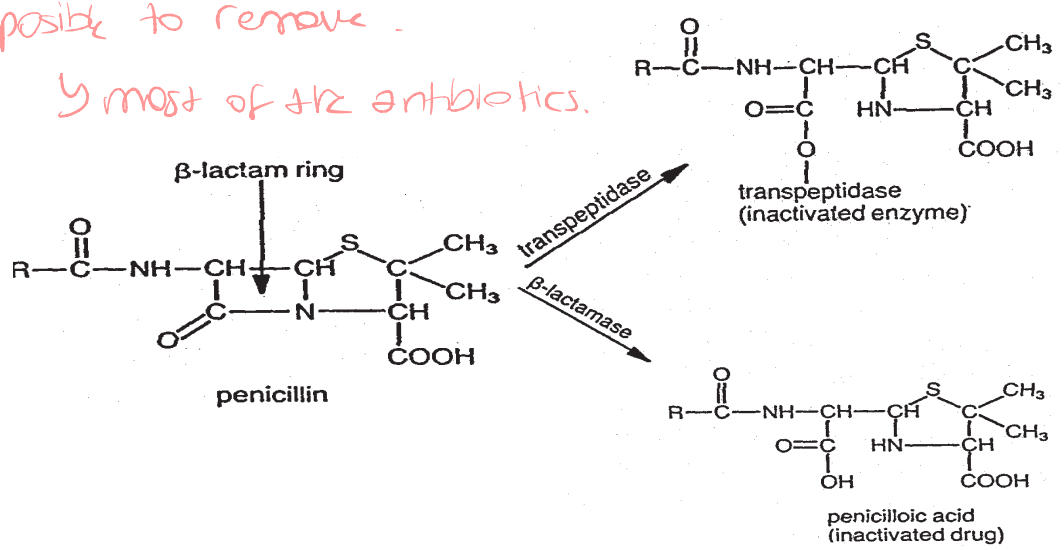


Irreversible Inhibitors

Irreversible inhibitors generally result in the destruction or modification of an essential amino acid required for enzyme activity. Frequently, this is due to some type of covalent link between enzyme and inhibitor. These types of inhibitors range from fairly simple, broadly reacting chemical modifying reagents to complex inhibitors that interact specifically and irreversibly with active site amino acids (termed suicide inhibitors). These inhibitors are designed to mimic the natural substrate in recognition and binding to an enzyme active site. Upon binding and some catalytic modification, a highly reactive inhibitor product is formed that binds irreversibly and inactivates the enzyme. Use of suicide inhibitors have proven to be very clinically effective

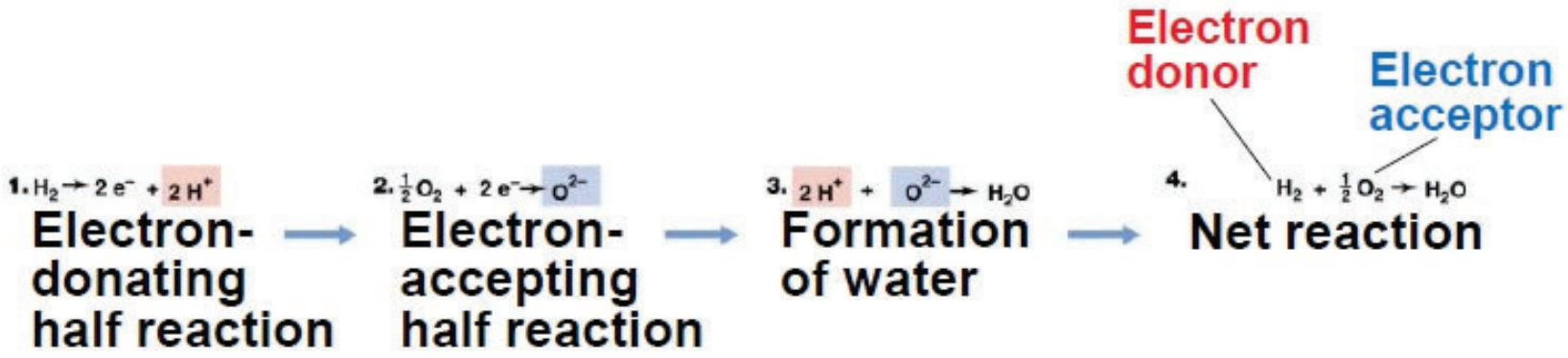
imposible to remove.

y most of the antibiotics.



Oxidation–Reduction and Energy-Rich Compounds

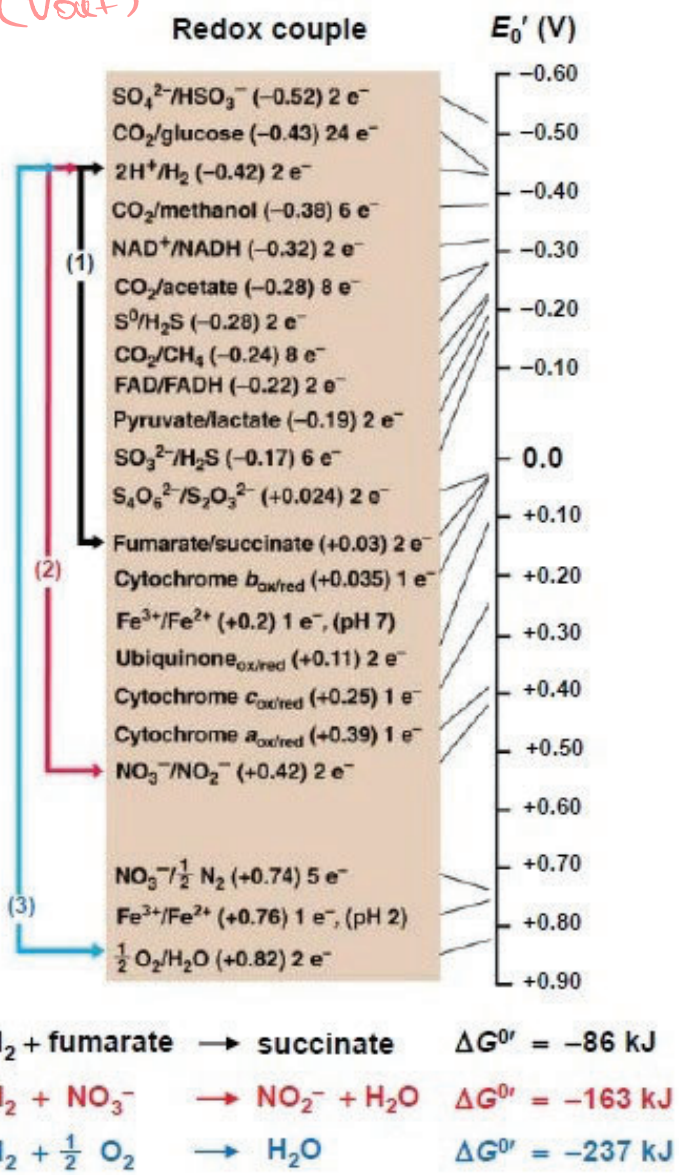
- Energy from oxidation–reduction (redox) reactions is used in synthesis of energy-rich compounds (e.g., ATP)
- Redox reactions occur in pairs (two half reactions)
- Electron donor: the substance oxidized in a redox reaction
- Electron acceptor: the substance reduced in a redox reaction



Oxidation–Reduction and Energy-Rich Compounds

- Reduction potential (E_0'): tendency to donate electrons
- Expressed as volts (V)
- Substances can be either electron donors or acceptors under different circumstances (redox couple)
- Reduced substance of a redox couple with a more negative E_0' donates electrons to the oxidized substance of a redox couple with a more positive E_0'
- The redox tower represents the range of possible reduction potentials
- The reduced substance at the top of the tower donates electrons
- The oxidized substance at the bottom of the tower accepts electrons
- The farther the electrons "drop," the greater the amount of energy released

E_0' → (Volt)



(1) H_2 + fumarate → succinate $\Delta G^{0\prime} = -86$ kJ

(2) $H_2 + NO_3^- \rightarrow NO_2^- + H_2O$ $\Delta G^{0\prime} = -163$ kJ

(3) $H_2 + \frac{1}{2}\ O_2 \rightarrow H_2O$ $\Delta G^{0\prime} = -237$ kJ

Electron Donors and Electron Acceptors

Redox reactions usually involve reactions between intermediates (carriers)

- •Electron carriers are divided into two classes
- Prosthetic groups (attached to enzymes)
- Coenzymes (diffusible)

Examples: NAD^+, $NADP^+$

(NAD^+: nicotinamide adeninedinucleotide)

NAD+and NADH facilitate redox reactions without being consumed; they are recycled

Energy-Rich Compounds and Energy Storage

Chemical energy released in redox reactions is primarily stored in certain phosphorylated compounds

- ATP; the prime energy currency
- Phosphoenolpyruvate
- Glucose 6-phosphate

Chemical energy also stored in coenzyme A

Long-term energy storage involves insoluble polymers that can be oxidized to generate ATP

Examples in prokaryotes

- Glycogen
- Poly-β-hydroxybutyrate and other polyhydroxyalkanoates
- Elemental sulfur

Examples in eukaryotes

- Starch
- Lipids (simple fats)