Chapter 8
Metabolism: Energy, Enzymes, and Regulation

Energy:
- Capacity to do work or cause a particular change.
- Thus, all physical and chemical processes are the result of the application or movement of energy.

Living cells carry out Three major types of work:
1. Chemical work
2. Transport work
3. Mechanical work

1. Chemical Work
- Synthesis of complex biological molecules required by cells from much simpler precursors.
- Energy is needed to increase the molecular complexity of the cell.

2. Transport Work
- Molecules and ions must be transported across cell membranes against a chemical gradient.
- Requires energy input in order to take up nutrients, eliminate wastes, and maintain ion balance.
- Example: molecules move into a cell even when the concentration is higher internally.
- Active transport

3. Mechanical Work
- Energy is required to change the physical location of organisms, cells, and structures within cells.

Sunlight:
- The ultimate source of most biological energy
- Photosynthesis: light energy is absorbed by chlorophyll and other pigment, and converted to chemical energy.

Chemical energy from photosynthesis and chemolithotrophy can be used by photolithoautotrophs and chemolithoautotrophs to transform CO$_2$ into biological molecules.

- Complex molecules derived from autotrophic organism (can use CO$_2$ as their principal source of carbon) serve as a carbon source of chemoheterotrophs (use organic cmpds as a source of energy) and other consumers that use complex organic molecules as a source of material and energy for building their own cellular structures.
**Aerobic Respiration:**
- \( O_2 \) acts as the final electron acceptor and is reduced to water.
- Much energy is released during process.
- Chemoheterotrophs often employ \( O_2 \) as an electron acceptor when oxidizing glucose and other organic molecules.

**Ecosystem Scheme:**
- Light and chemical energy is used by organisms to produce organic compounds which produce CO2 during aerobic respiration. The CO2 can then be used to make complex organic molecules during photosynthesis and chemolithautotrophy.

Cells must have a practical form of energy currency.

**ATP – adenosine 5’-triphosphate**
- High-energy molecule (high phosphate group transfer potential)- readily transfers its phosphate to water
- Hydrolyzes almost completely to ADP and \( P_i \)
- Picture

**ADP:**
- adenosine diphosphate (ADP) and orthophosphate (\( P_i \))
  \[
  ATP + H_2O \quad \rightarrow \quad ADP + P_i
  \]
- energy is made available for useful work

**Figure 8.3**

**The Laws of Thermodynamics**

- Chemical reactions are governed by the laws of
  Thermodynamics
- **Thermodynamics:** analyzes energy change in a collection of matter called a system.
- Focuses on energy differences between the initial state and the final state of a system, not with the rate of the process.
  - Example: Pan of heated boiling water – only the condition of water at start and at boiling is thermodynamics.

**First Law of Thermodynamics:**
- Matter or energy can neither be created nor destroyed, it can only be converted from one form or another or be redistributed.
- Therefore the total amount of energy in a reaction remains constant
Basics of understanding how energy is trapped or generated and how ATP functions as an energy currency.

Second Law of Thermodynamics:
Physical and chemical processes proceed in such a way that randomness or disorder of the universe (the system and its surroundings) increase to the maximum possible.

- **Entropy**: a measure of the randomness or disorder of a system. The greater the disorder, the greater the entropy.
  - **Example**: the expansion of gas into an empty cylinder redistributes the gas molecules until equilibrium is reached.

Free energy and Reactions:

Equation combining the 1 and 2 LT, relating the change in energy that can occur during a reaction.

\[ \Delta G = \Delta H - T \cdot \Delta S \]

\(\Delta G\) = change in free energy
- Energy release that is available to do useful work at constant \(T\) and \(V\)
- The difference in energy levels that exist between the reactants and products in a chemical reaction

\(\Delta H\) = change in enthalpy (change in heat content)
\(T\) = temperature in Kelvin (°C + 273)
\(\Delta S\) = change in entropy occurring during reaction

**\(\Delta G\) is negative**: the reaction will proceed with the release of free energy, energy that the cell may be able to conserve in the form of ATP.
- **Exergonic**: Energy-yielding reaction
- These reactions are generally irreversible, in that they may spontaneously occur in the forward direction but will not proceed in the reverse direction without a significant input of energy.

**\(\Delta G\) is positive**: the reaction requires energy in order to proceed.
- **Endergonic**: require energy.
- Many reactions require a significant input of energy in order to initiate the reaction.
This energy required is called the \textbf{activation energy}, this is simply an energy barrier which must be overcome in order for the reaction to proceed in the forward direction.

All compounds have an inherent energy level which is called the \textbf{potential energy}, which can be followed through the course of a chemical reactions.

\textbf{Oxidation- Reduction Reactions and Electron Carriers:}

The release of energy normally involves oxidation-reduction reactions where electrons are moved from a donor to an acceptor.

- For any oxidation to occur, a subsequent reduction must occur, because electrons cannot exist alone in solution.

\[
H_2 \rightarrow 2e^- + 2 H^+
\]

\textbf{Hydrogen gas} \quad \text{2 electrons} \quad \text{2 hydrogen ions (protons)}

Hydrogen is therefore oxidized.

\textbf{Oxidation is defined as the removal of an electron}
- Oxidized –electron donor

\textbf{Reduction is defined as the addition of an electron}
- Reduced –electron acceptor

\textbf{Carriers:}

In the cell, the transfer of electron in an oxidation-reduction reaction from donor to acceptor usually involves one or more intermediates referred to a \textbf{carriers}.

- Primary electron donor: initial donor
- Terminal electron acceptor: final acceptor

\textbf{Important electron carriers in the cell are:}

- \textbf{NAD+} Nicotinamide adenine dinucleotide
- \textbf{NADP+} Nicotinamide adenine dinucleotide phosphate
- \textbf{FAD} Flavin adenine dinucleotide
- \textbf{FMN} Flavin mononucleotide
- \textbf{Coenzyme Q (CoQ) or ubiquinone}
- \textbf{Cytochromes}
- \textbf{Nonheme iron proteins}

\textbf{Enzymes:}

- Protein catalysts that have great specificity for the reaction catalyzed and the molecules acted on.
Catalyst:
- A substance that increases the rate of a chemical reaction without being permanently altered itself and lowering the activation energy.

Therefore An enzyme is a biological catalyst; that is it increases the rate in which a chemical reaction proceeds without being consumed in that reaction

- The activity as catalysts is associated with their three dimensional shape, which creates a site for binding the substrates, this site is called the active site.

- The active site of an enzyme consists of a group of amino acids that are involved in making contact with the substrate(s)

- The active site is easily accessible to the substrate, but once the substrate is bound, the enzyme undergoes conformational changes which promotes greater interaction of the catalytically important residues with the substrate and ultimately promotes the molecular rearrangement which result in the formation of the product.

- It has been estimated that the bacterial cell contains more than 2000 different enzymes which function in a concerted manner within the physiological ranges of temperature and pH to carry out the day to day functions of the cell.

Enzymes speed up cellular reactions.
- **Substrates:** reacting molecules
- **Products:** substances formed

- Some enzymes consist of proteins only

- When an enzyme requires a cofactor, the enzyme unit by itself is called an Apoenzyme.

- When the enzyme is completed with its cofactor, it is called a Holoenzyme.

Cofactors:
- May require additional inorganic or organic (non-protein) components in order to exhibit catalytic activity.
- The simplest cofactors are metal ions, such as Mg²⁺, and Zn²⁺
- The most important and complex cofactors are the organic compounds called Coenzymes, without which, no catalytic activity will occur.
- **Coenzyme**: cofactor is loosely attached to the apoenzyme and can dissociate from the enzyme protein after products have been formed and carry one of these products to another enzyme. (figure 8.13).

  **Example**: NAD+ is a coenzyme that carries electrons within the cell.

- Most Coenzymes are loosely attached to the enzyme, and can separate when necessary.

- However some cofactors are firmly attached to the enzyme by covalent bonds. Covalently attached cofactors are called **Prosthetic groups**.

Many vitamins serve as coenzyme precursors.
- **Riboflavin** (B2) is used to synthesize Flavin Adenine Dinucleotide (FAD)
- **Thiamin** (B1) is used to synthesize Thiamin pyrophosphatase (TPP)

**Enzymes**
- Enzymes are classified by the general reactions that they catalyze. They are systematically named by the substrate + reaction type-ASE
  - Lactate dehydrogenase (LDH) removes hydrogens from lactate:
    \[ \text{LDH} \quad \text{Lactate} + \text{NAD}^+ \rightarrow \text{pyruvate} + \text{NADH} + \text{H}^+ \]
  - Cellulase is an enzyme that attack cellulose

**Six General Classes of Enzymes**:

1. **Oxidoreductases**, are involved in oxidation or reduction reactions. Some of the enzymes within this group catalyze the transfer of hydrogen from one reactant to another. These are called **dehydrogenases**
   i.e. D-lactate:NAD oxidoreductase

   Other enzymes in this group incorporate molecular oxygen into one of the reactants

2. **Transferases**. These enzymes transfer a group of atoms, or functional group from one reactant to another. These may be an amino group (amino transferase), a phosphate group (phosphotransferase), or an acetyl group (acetyl transferase)
   ATP: acetate phosphotransferase (ATP + Ace → ADP + Acetyl phosphate)
3. **Hydrolases** bring about the hydrolysis of molecules such as proteins, carbohydrates and lipids into their monomeric subunits.
   - Protein hydrolases for example cleave peptide bonds and release amino acids. This bond breaking activity comes at the expense of HOH, hence the name (hydro- lysis)

4. **Lyases** break covalent bonds and remove functional groups by a mechanisms other than water hydrolyses. The removal of a carboxyl group or water molecule from a compound are examples of lyase activity
   - oxalate carboxylase (oxylate $\rightarrow$ CO$_2$ + Formate)
   - Urea deaminase (urease) (urea $\rightarrow$ CO$_2$ + NH$_3$)

5. **Isomerases.** Isomerases are a group of enzymes that catalyze the re-arrangement of groups of atoms within a molecule. The conversion of an amino acid from a L form to a D form is an example of an isomerase
   - Alanine racemase (L-alanine $\longrightarrow$ D alanine)

6. **Ligases** are enzymes that join two molecules together, and during the reaction there is the breakdown of ATP or related energy molecule.
   - T4 DNA ligase (joins two nucleotide together by creating a phosphodiester bond between the 5′ phosphate of one nucleotide, and the 3′ hydroxyl of the other)

**Mechanism of Enzyme Rxns:**

- Enzymes increase the rates of reactions
- If rxn is endergonic- the presence of the enzyme will not shift its equilibrium, so that more products can be formed.
- Enzymes speed up the rate at which a rxn proceeds towards its final equilibrium.

**Activation Energy:** is required to bring the reacting molecules together in the correct way to reach transition state.

Enzymes accelerate reactions by lowering the activation energy.
- Enzymes bring substrates together at a special place on their surface called the active site or catalytic site.
  To form an enzyme-substrate complex.

**Enzyme interact with a substrate in two ways:**
1. **Lock and Key Model:**
Rigid and shaped precisely fit the substrate so that the correct substrate binds specifically and is positioned properly for reaction.

2. **Induced fit Model:**
   - An enzyme may change shape when it binds the substrate so that the active site surrounds and precisely fits the substrate.
   - Used by hexokinase

The formation of an enzyme-substrate complex can lower the activation energy in many ways:

- Bringing the substrates together at the active site, the enzyme is concentrating them and speeding up the reaction.
- The enzyme also binds substrates so they are correctly oriented with respect to each other in order to form a transition-state complex, which lowers the amount of energy that the substrates require to reach the transition state.
- Catalytic sites speed up the reaction hundreds of thousands of times.

**Effect of Environment on Enzyme Activity:**

1. **Substrate Concentration**
   - Substrate conc. is usually low inside the cell.
     - At low substrate conc., an enzyme makes a product slowly bc of seldom contacts with a substrate molecule.
     - The more substrate present, enzyme binds more, therefore, the reaction velocity is greater than at lower substrate conc.
     - The rate of an enzyme-catalyzed rxn increases with substrate conc.
     - Resulting in Maximal Velocity: enzyme is saturated with substrate and operating at MV.
     - Resulting in substrate concentration curve of the hyperbola Figure 8.17

**Michaelis Constant (K\textsubscript{m}):**

- Substrate concentration required for the enzyme to achieve half maximal velocity
- Is used as a measure of the apparent affinity of an enzyme for its substrate.
- The lower the K\textsubscript{m} value, the lower the substrate concentration at which an enzyme catalyzes its reaction.

2. **pH**
   - Enzymes also change activity with alterations in pH
   - Each enzyme functions most rapidly at a specific pH optimum.
   - When pH deviates too greatly from an enzyme’s optimum, activity slows and the enzyme may be damaged.

3. **Temperature**
   - Enzymes have a temp optimum for maximum activity.
   - If temp rises too much above the optimum, Denaturation occurs: an enzymes structure will be
disrupted and its activity lost (ph and temp).

**Enzyme Inhibition:**
- Poison to mos by serving as enzyme inhibitors.

**Competitive Inhibitors:**
- Directly competes with the substrate at an enzyme’s catalytic site and prevents the enzyme from forming product.
- Succinate dehydrogenase: catalyzes the oxidation of succinate to fumarate in the TCA.
- Malonic acid is an effective competitive inhibitor of succinate dehydrogenase bc it is closely resembles succinate, the normal substrate.

**Know the structure of Succinic Acid and Malonic Acid.**

After malonate binds to the enzyme, it cannot be oxidized and the formation of fumarate is blocked. Competitive inhibitors usually resemble normal substrates, but they cannot be converted to products.

**Noncompetitive Inhibitors:**
- Do not directly compete with the substrate
- Inhibitors bind to the enzyme at some location other than the active site, altering the enzyme’s shape, rendering it inactive or less active.
  
  Example: heavy metal - mercury

**Three ways to regulate the flow of carbon through a pathway:**

**Metabolic Channeling:**
- Localization of metabolites and enzymes in different parts of a cell that influence pathway activity.

**Compartmentation:**
- One of the most common mechanisms of metabolic channeling.
- Differential distribution of enzymes and metabolites among separate cell structures or organelles.
- Makes possible the simultaneous, but separate, operation and regulation of similar pathways

**Example:**
- Fatty acid oxidation located within the mitochondrion
- Fatty acid synthesis occurs in the cytoplasmic matrix

If two pathways in different compartments require NAD for activity, the pathway with access to the most NAD will be favored.