Question 11: (a) Explain the following:

- **(i) Many thermodynamically favorable reactions like hydrolysis of ATP do not occur readily at room temperature.**
    - **Thermodynamic favorability (**$\Delta G < 0$**)** means a reaction *can* happen spontaneously, but it doesn't say anything about *how fast* it will happen.
    - Reactions need to overcome an **activation energy barrier (**E_a**)** to proceed. This is the minimum energy required to reach a transition state where bonds can be broken and formed.
    - At **room temperature**, molecules typically don't have enough kinetic energy to consistently overcome this activation energy barrier. Most collisions between reactant molecules aren't energetic enough to lead to a reaction.
    - In biological systems, **enzymes** solve this problem. They are biological catalysts that significantly **lower the activation energy** of a reaction, allowing even thermodynamically favorable reactions like ATP hydrolysis to proceed very rapidly at physiological temperatures without changing the overall ΔG.
- **(ii) Accessory pigments tunnel light energy to the reaction center.**
    - **Accessory pigments** (like chlorophyll b, carotenoids, and phycobilins) absorb light at wavelengths that are not efficiently absorbed by the primary reaction center chlorophyll (e.g., chlorophyll a in plants). This allows the organism to capture a **broader spectrum of light energy**.
    - When an accessory pigment absorbs a photon, its electron gets excited. This excitation energy is then efficiently transferred from one pigment molecule to an adjacent one through **resonance energy transfer** (also known as Förster Resonance Energy Transfer or FRET).

- This energy is "tunneled" or "funneled" sequentially from higher-energy (shorter wavelength) absorbing pigments to lower-energy (longer wavelength) absorbing pigments.
- Ultimately, the energy reaches the **reaction center chlorophylls** (e.g., P680 or P700 in plants), which have the lowest excitation energy. This ensures that the captured light energy is concentrated and delivered to the site where the actual photochemical reaction (charge separation) of photosynthesis begins.

- **(iii) Oxygen is not evolved in photophosphorylation.**
  - **Photophosphorylation** refers to the light-driven synthesis of ATP.
  - There are two main types:
    - **Non-cyclic photophosphorylation:** Involves both Photosystem II (PSII) and Photosystem I (PSI). Here, electrons are extracted from water, leading to the **evolution of oxygen** as a byproduct ($2H_2O \rightarrow 4H^+ + 4e^- + O_2$). This pathway produces both ATP and NADPH.
    - **Cyclic photophosphorylation:** Involves only Photosystem I (PSI). Electrons are cycled back from ferredoxin to the cytochrome b_6f complex and then back to PSI. In this pathway, **water is not split**, and therefore, **oxygen is not evolved**. This process exclusively generates ATP.
  - The statement is specifically true for **cyclic photophosphorylation**, where the electron flow is confined to PSI and components that do not involve water as an electron source.

- **(iv) An inhibitory protein inhibits ATP hydrolysis during ischemia.**

- **Ischemia** is a condition of inadequate blood supply, leading to oxygen and nutrient deprivation in tissues.
- Under normal aerobic conditions, **ATP synthase** synthesizes ATP using the energy from a proton gradient. However, if oxygen is scarce (ischemia), the electron transport chain cannot function, the proton gradient collapses, and ATP synthase can reverse its action.
- In its reverse mode, ATP synthase acts as an ATPase, **hydrolyzing ATP** to pump protons out of the mitochondrial matrix. This futile cycle would rapidly deplete the cell's already limited ATP reserves, which is detrimental.
- To prevent this wasteful ATP consumption, an **inhibitory protein called IF1 (Inhibitor of F_1-ATPase)** becomes active. IF1 binds to the F_1 subunit of ATP synthase, specifically under the low pH conditions that typically arise during ischemia (due to increased anaerobic metabolism and lactic acid production).
- By inhibiting ATP hydrolysis, IF1 **conserves the cell's remaining ATP**, providing a crucial protective mechanism against severe energy depletion and damage during ischemic events.

- **(v) Each cytochrome present in the ETC has a different reduction potential.**
  - The **electron transport chain (ETC)** is a series of electron carriers arranged to facilitate a stepwise transfer of electrons.
  - **Reduction potential ($E_{0'}$)** is a measure of a molecule's tendency to gain electrons. A more positive $E_{0'}$ means a greater affinity for electrons.
  - For electrons to flow spontaneously and unidirectionally down the ETC, they must move from a carrier with a **lower (more**

negative) reduction potential to one with a **higher (more positive) reduction potential**.

- **Cytochromes** are heme-containing proteins that serve as electron carriers in the ETC. Each distinct cytochrome (e.g., cytochrome b, c_1, c, a, a_3) has a unique protein environment around its heme group, which subtly alters the redox properties of the iron within the heme.
- This ensures that each subsequent cytochrome in the chain has a slightly higher reduction potential than the previous one. This **gradual increase in reduction potential** allows for the controlled release of energy as electrons move, which is then harnessed to pump protons across the membrane. If all cytochromes had the same potential, electron flow would not be spontaneous or directed.

- **(vi) Bioluminescence is the reverse of photosynthesis.**
  - This statement is **incorrect**. Bioluminescence is fundamentally different from photosynthesis.
  - **Photosynthesis** is an **endergonic (energy-requiring)** process where light energy is captured and converted into chemical energy (glucose). It's about building complex molecules from simpler ones.
  - **Bioluminescence** is an **exergonic (energy-releasing)** process where chemical energy (from the oxidation of a substrate, often luciferin, catalyzed by an enzyme, luciferase, with ATP and oxygen) is converted into light energy. It's about breaking down molecules to release light.
  - While both involve light, the direction of energy conversion is opposite: light to chemical in photosynthesis, and chemical to light in bioluminescence. Therefore, they are not reverses of each other.

Question 11: (b) Define the following terms:

- **(i) Standard reduction potential ($E_{0'}$).**
    - The **standard reduction potential ($E_{0'}$)** is a quantitative measure, expressed in volts (V), of a chemical species' tendency to be reduced (gain electrons) under a defined set of **standard biochemical conditions**. These conditions typically include a temperature of 25°C (298 K), 1 atmosphere pressure, and a pH of 7.0 (denoted by the prime symbol '). It is measured relative to a standard hydrogen electrode, which is arbitrarily assigned a potential of 0.0 V. A more positive $E_{0'}$ indicates a stronger tendency to accept electrons, while a more negative $E_{0'}$ indicates a stronger tendency to donate electrons.
- **(ii) Action spectrum.**
    - An **action spectrum** is a graphical representation that plots the relative effectiveness of different wavelengths of light in driving a specific biological process (e.g., photosynthesis, phototropism, vision). It is determined by measuring the rate of the process at various wavelengths. The shape of an action spectrum often closely correlates with the absorption spectrum of the specific pigment(s) responsible for mediating that process, thereby helping to identify the pigments involved.
- **(iii) Uncoupler.**
    - An **uncoupler** is a chemical agent that disrupts the coupling between electron transport and ATP synthesis in processes like oxidative phosphorylation or photophosphorylation. These molecules, often lipophilic weak acids (protonophores) like 2,4-dinitrophenol (DNP), embed in the membrane (e.g., inner mitochondrial membrane). They allow protons to leak back across the membrane, dissipating the proton gradient that is essential for ATP synthase activity. This leads to continued

electron transport and oxygen consumption but without ATP production, with the energy being released as heat. Naturally occurring uncouplers like thermogenin in brown fat are used for non-shivering thermogenesis.

Question 23: (a) Give the role of the following:

- **(i) Isoprenoid chain in ubiquinone.**
    - The **isoprenoid chain** in ubiquinone (Coenzyme Q) is a long, hydrophobic tail that firmly anchors the ubiquinone molecule within the **hydrophobic lipid bilayer** of the inner mitochondrial membrane. This anchoring allows ubiquinone to diffuse laterally within the membrane, enabling its function as a **mobile electron shuttle** between the large, membrane-bound protein complexes (Complex I or II, and Complex III) of the electron transport chain. Its lipid solubility is essential for efficient electron transfer in the membrane environment.
- **(ii) Brown fat in newborn mammals.**
    - **Brown fat (brown adipose tissue, BAT)** in newborn mammals plays a crucial role in **non-shivering thermogenesis**, which is the production of heat without muscle contractions. Newborns have a high surface area-to-volume ratio and limited ability to shiver, making them vulnerable to cold. Brown fat mitochondria contain a specialized protein called **thermogenin (UCP1)**, which acts as an **uncoupling protein**. Thermogenin allows protons to re-enter the mitochondrial matrix without passing through ATP synthase, dissipating the energy from the proton gradient as heat, thereby helping the newborn maintain a stable body temperature.
- **(iii) ANT in mitochondria.**

- **ANT (Adenine Nucleotide Translocase)**, also known as the ADP/ATP carrier, is an integral membrane protein in the inner mitochondrial membrane. Its essential role is to facilitate the **antiport exchange of ADP and ATP** across this membrane. It transports one molecule of ADP from the mitochondrial intermembrane space (and cytosol) into the matrix, in exchange for one molecule of ATP from the matrix to the intermembrane space/cytosol. This transport is crucial for delivering the ADP substrate to ATP synthase within the matrix and for distributing the newly synthesized ATP from the mitochondria to the rest of the cell where energy is needed.

Question 23: (b) Describe the structure and binding change mechanism of the enzyme ATP synthase.

- **Structure of ATP Synthase:**
  - ATP synthase is a complex molecular machine composed of two main domains:
    - F_0 **(F-zero) Domain:** This domain is **embedded within the inner mitochondrial membrane** (or thylakoid/plasma membrane). It acts as a **proton channel and a rotary motor**. It consists of a ring of 'c' subunits that rotates as protons pass through a channel formed by the 'a' subunit. Subunits 'b' and 'δ' form a **stator arm** that connects F_0 to F_1, preventing the entire F_1 unit from rotating with the central shaft.
    - F_1 **(F-one) Domain:** This domain **protrudes into the mitochondrial matrix** (or chloroplast stroma/bacterial cytoplasm). It contains the **catalytic sites** for ATP synthesis. It is composed of a hexameric ring of three α and three β subunits ($\alpha_3\beta_3$). The β subunits contain the catalytic sites. A central asymmetric γ **subunit** (along with

the ε subunit) forms a rotating shaft that extends from F_0 into the center of the $\alpha_3\beta_3$ hexamer.

- **Binding Change Mechanism (Rotational Catalysis):**
    - This mechanism, proposed by Paul Boyer, explains how the mechanical rotation of the γ subunit is coupled to the chemical synthesis and release of ATP. The three catalytic β subunits in the F_1 domain exist in three distinct conformational states, which interconvert sequentially as the γ subunit rotates.
    - **The Three Conformational States of β Subunits:**
        i. **Open (O) State:** Has a very **low affinity** for substrates (ADP + P_i) and products (ATP). This is the state from which ATP is released.
        ii. **Loose (L) State:** Binds ADP and P_i **loosely**.
        iii. **Tight (T) State:** Binds ATP (and ADP + P_i) **very tightly**. In this state, the chemical reaction of ATP synthesis (ADP + $P_i \rightleftharpoons$ ATP + H_2O) proceeds spontaneously to form ATP because the binding energy significantly stabilizes the product.
    - **The Cycle of ATP Synthesis:**
        iv. **Proton Flow and Rotation:** Protons flow from the intermembrane space back into the matrix through the F_0 proton channel. This proton flow drives the rotation of the 'c' ring and the central γ subunit in discrete steps (typically 120 degrees for each ATP produced).
        v. **Sequential Conformational Changes:** As the γ subunit rotates, its asymmetric shape interacts with the $\alpha_3\beta_3$ hexamer, causing each of the three β subunits to sequentially cycle through the three conformations:
            - An **L-state** subunit binds ADP and P_i.

- Rotation then forces it into the **T-state**, where ATP is synthesized from the bound ADP and P_i.
- Further rotation forces it into the **O-state**, which causes the newly synthesized ATP molecule to be **released** due to the lowered binding affinity.

vi. **Continuous ATP Production:** This cycle repeats for each β subunit. For every full 360-degree rotation of the γ subunit (driven by the passage of a certain number of protons, typically 3-4), three molecules of ATP are synthesized and released, one from each β subunit.

- In essence, the energy from the proton gradient is converted into mechanical rotational energy, which in turn induces conformational changes in the catalytic sites, facilitating the binding of substrates, the formation of product, and the release of ATP.

Question 23: (c) Plant mitochondria have an alternate rotenone insensitive NADH dehydrogenase and a cyanide resistant oxidase that constitutes an alternate pathway to oxidize NADH. Comment on the role of this alternate pathway in plants.

- Plants possess a unique, branched mitochondrial electron transport chain (mETC) that includes the conventional complexes (I-IV) as well as alternative enzymes. The existence of a **rotenone-insensitive NADH dehydrogenase** (bypassing Complex I) and a **cyanide-resistant Alternative Oxidase (AOX)** (bypassing Complex IV) forms an alternative pathway for NADH oxidation.
- **Role of this Alternate Pathway in Plants:**
  - **1. Thermogenesis (Heat Production):** This is one of the most prominent roles. When electrons flow through AOX, the energy released from the redox reactions is dissipated primarily as

heat rather than being conserved in ATP. AOX does not pump protons across the membrane. This non-phosphorylating electron transport allows certain plants (e.g., skunk cabbage, Arum lilies) to generate significant heat, which can melt snow around them, attracting insect pollinators by emitting volatile scents. It also plays a role in cold acclimation in some plants.

- **2. Overflow and Redox Balancing:** Plants, especially under conditions of high carbohydrate availability (e.g., during active photosynthesis) or when ATP demand is low, can accumulate excess reducing equivalents (NADH, $FADH_2$). The alternative pathway acts as an **"overflow valve" or "safety valve"** to dissipate this excess reducing power. This prevents the over-reduction of the conventional electron transport chain, which can lead to the production of harmful **reactive oxygen species (ROS)** that cause oxidative stress and cellular damage.
- **3. Stress Tolerance:** Under various environmental stresses (e.g., drought, cold, high light, pathogen attack, nutrient deficiency), the balance between carbon metabolism and energy demand can be disrupted. The alternative pathway provides flexibility by allowing continued electron flow and NADH oxidation, thereby maintaining mitochondrial redox homeostasis and potentially mitigating ROS production, even if the conventional ATP-producing pathway is partially inhibited or overwhelmed.
- **4. Metabolic Flexibility and Regulation:** The presence of both conventional and alternative pathways allows plants to fine-tune the balance between ATP production and heat dissipation. This metabolic flexibility is crucial for adapting to constantly changing environmental conditions (e.g., fluctuating light intensity or temperature) and varying metabolic needs throughout the day-night cycle or different developmental stages. It provides a means to maintain respiration even when

ATP demand is low or when the conventional pathway is compromised.

- **5. Resistance to Inhibitors:** The alternative pathway provides a bypass around specific inhibitors that target the conventional pathway (e.g., rotenone for Complex I, cyanide for Complex IV). This ensures that plants can maintain some level of respiration even in the presence of such compounds, which might be produced by pathogens or be present in the environment.

Question 34: (a) What is the role of cytochrome bf complex in the photosynthesis carried out by plants. Depict the flow of electrons and protons through this complex.

- **Role of Cytochrome b_6f Complex in Photosynthesis:**
  - The **cytochrome b_6f complex** (also known as cytochrome bf) is a large protein complex embedded within the **thylakoid membrane** of chloroplasts (and in cyanobacteria).
  - Its primary role is to serve as a crucial intermediate electron carrier and a **proton pump** in the photosynthetic electron transport chain, bridging the gap between Photosystem II (PSII) and Photosystem I (PSI).
  - **Key functions include:**
    vii. **Electron Transfer:** It accepts electrons from reduced plastoquinone (PQH_2) that comes from PSII, and then transfers these electrons to plastocyanin (Pc), which subsequently delivers them to PSI.

    viii. **Proton Pumping (Chemiosmosis):** While transferring electrons, the cytochrome b_6f complex actively translocates protons (H^+) from the **stroma** (low proton concentration) to the **thylakoid lumen** (high proton concentration). This proton pumping significantly

contributes to the **proton motive force (**ΔpH**)** across the thylakoid membrane, which is essential for driving ATP synthesis by ATP synthase.

- **Flow of Electrons and Protons through Cytochrome** b_6f **Complex (Q-Cycle):** The cytochrome b_6f complex operates via a mechanism called the **Q-Cycle**, which allows it to pump four protons into the lumen for every two electrons passed through the complex to plastocyanin and eventually to PSI.

  b. **Oxidation of Plastoquinol (**PQH_2**) at the** Q_o **Site:**

  - A molecule of **plastoquinol (**PQH_2**)** (from PSII) binds to the Q_o site of the b_6f complex, facing the lumen side.
  - PQH_2 is oxidized back to plastoquinone (PQ), releasing **two protons (2** H^+**) directly into the thylakoid lumen**. This directly contributes to the proton gradient.
  - The two electrons from PQH_2 take different paths:
    - **Path 1 (to PSI):** One electron is transferred via the **Rieske iron-sulfur cluster (Fe-S)** to **cytochrome** f, and then to soluble **plastocyanin (**Pc**)** in the lumen. Pc will then carry this electron to PSI.
    - **Path 2 (recycled):** The second electron is transferred via **cytochrome** b_L to **cytochrome** b_H, moving across the membrane to the Q_i site (stroma side).

  c. **Reduction of Plastoquinone (PQ) at the** Q_i **Site:**

  - An oxidized **plastoquinone (PQ)** molecule binds to the Q_i site (stromal side). It accepts the electron from cytochrome b_H, becoming a **semiquinone radical (**$PQ^{\cdot -}$**)**.

  d. **Second Round of Q-Cycle (Another** PQH_2**):**

- A second molecule of PQH_2 arrives at the Q_o site and is oxidized, again releasing **two protons (2 H^+) into the thylakoid lumen**.
- One electron goes to plastocyanin (and then to PSI) via the Fe-S cluster and cytochrome f.
- The second electron goes via cytochrome b_L and b_H to the Q_i site.

e. **Completion of PQ Reduction at the Q_i Site:**

- The semiquinone radical ($PQ\cdot^-$) at the Q_i site accepts this second electron. It then takes up **two protons (2 H^+) from the stroma**, regenerating a full molecule of **plastoquinol (PQH_2)**, which can then re-enter the mobile PQ pool.

- **Net Proton Translocation:** For every two PQH_2 molecules oxidized at the Q_o site (which delivers 2 electrons to PSI), a total of **four protons ($4H^+$)** are translocated from the stroma to the lumen (2 from each PQH_2 at Q_o). The two protons consumed at Q_i are taken from the stroma, contributing to the proton depletion on that side and increasing the proton gradient.

Question 34: (b) A thermodynamically unfavorable reaction can be driven in forward direction by coupling it to hydrolysis of ATP.

- This statement highlights the crucial biological strategy of **reaction coupling** to enable cellular processes.
- A **thermodynamically unfavorable (endergonic) reaction** is one with a **positive standard free-energy change ($\Delta G > 0$)**. Such reactions require an input of energy to proceed spontaneously in the forward direction.

- **ATP hydrolysis** (e.g., ATP → ADP + P_i) is a highly **thermodynamically favorable (exergonic) reaction** with a large **negative** ΔG. This energy release is due to factors like electrostatic repulsion relief, resonance stabilization of products, and increased solvation.
- **Coupling Mechanism:** Cells overcome the energy barrier of endergonic reactions by **"coupling"** them with the exergonic hydrolysis of ATP. This is typically achieved through a shared intermediate.
    - Often, a phosphate group from ATP is transferred to one of the reactants of the endergonic reaction, forming a **phosphorylated intermediate**. This intermediate is higher in energy and more reactive than the original reactant.
    - The subsequent reaction of this activated intermediate to form the final product is then thermodynamically favorable (even though the overall uncatalyzed endergonic reaction was not).
- **Overall Favorable Process:** For the coupled reaction to proceed spontaneously, the **sum of the free-energy changes** for the individual (endergonic) reaction and the (exergonic) ATP hydrolysis must result in a **net negative** ΔG **for the overall coupled reaction**. The energy released from ATP hydrolysis must be greater than the energy required by the endergonic reaction ($|\Delta G_{ATPhydrolysis}| > |\Delta G_{endergonic}|$).
- **Biological Significance:** This principle is fundamental to almost all life processes, enabling cells to perform essential tasks such as:
    - **Anabolic synthesis:** Building complex molecules (proteins, nucleic acids, carbohydrates, lipids) from simpler precursors.
    - **Active transport:** Pumping ions and molecules against their concentration gradients across membranes.

- **Mechanical work:** Muscle contraction, flagellar movement. ATP acts as the universal energy currency, mediating these coupled reactions by transiently transferring its chemical energy.

Question 34: (c) Differentiate between the universal electron carriers and the mitochondrial electron carriers.

Feature	Universal Electron Carriers	Mitochondrial Electron Carriers
Location	Found throughout the cell (cytosol, mitochondrial matrix, chloroplast stroma)	Primarily located within the **inner mitochondrial membrane** and intermembrane space
Nature	**Soluble, diffusible** coenzymes	Mostly **membrane-bound proteins** or prosthetic groups (except Ubiquinone and Cytochrome c)
Role/Function	**Shuttle electrons** between various metabolic pathways; provide reducing power for anabolic or catabolic reactions; acts as coenzymes for dehydrogenases.	**Sequential transfer of electrons** within a fixed pathway (ETC) to ultimately reduce oxygen; contribute to **proton pumping** across the membrane.
Examples	**NAD^+/NADH**, **$NADP^+$/NADPH**, **FAD/$FADH_2$**	**Flavoproteins** (FMN, FAD-containing), **Iron-Sulfur (Fe-S) clusters**, **Ubiquinone (CoQ)**,

Feature	Universal Electron Carriers	Mitochondrial Electron Carriers
		Cytochromes, **Copper centers**
Electron Transfer	Typically transfer **2 electrons** (and often protons as hydrides) in a single step.	Can transfer **1 or 2 electrons** at a time, depending on the specific carrier in the chain.
Energy Conservation	Do not directly conserve energy from electron transfer as ATP; their reduced forms (NADH, $FADH_2$) *carry* energy for later use in ETC.	Their sequential electron transfers are **directly coupled to proton pumping**, generating the proton motive force used for ATP synthesis.
Mobility	Highly mobile, diffuse freely to interact with various enzymes.	Generally fixed components of large protein complexes; Ubiquinone and Cytochrome c are mobile within/between complexes.
Pathways Involved	Glycolysis, TCA cycle, fatty acid oxidation, pentose phosphate pathway, biosynthesis.	Oxidative phosphorylation (electron transport chain and ATP synthesis).

Question 445: (a) With the help of a diagram explain the Z-scheme of photosynthesis.

- **The Z-Scheme of Photosynthesis:**

- The Z-scheme is a model that illustrates the **non-cyclic (linear) flow of electrons** through the light-dependent reactions of oxygenic photosynthesis (in plants, algae, and cyanobacteria). It depicts the energetic pathway of electrons from water to $NADP^+$, along with the simultaneous generation of a proton gradient that drives ATP synthesis.
- The "Z" shape reflects the changes in the standard reduction potential (energy level) of the electron carriers as electrons are moved from low energy to high energy by light absorption, then "fall" to lower energy levels through electron transport, and are re-energized by a second light absorption.

- **Explanation of Electron Flow:**

f. **Photosystem II (PSII, P680):**

- Light energy is absorbed by antenna pigments and funneled to the reaction center chlorophyll of PSII, known as **P680**.
- When P680 absorbs light, an electron is excited to a much higher energy level (lower reduction potential), forming **$P680^*$**.
- $P680^*$ is a very strong reducing agent and immediately donates its excited electron to the primary electron acceptor, **Pheophytin (Pheo)**, becoming oxidized to **$P680^+$**.
- To replace the electron lost by $P680^+$, the **Oxygen-Evolving Complex (OEC)** associated with PSII **splits water molecules (photolysis)**: $2H_2O \rightarrow 4H^+ + 4e^- + O_2$. The electrons go to $P680^+$, neutralizing it. The protons (H^+) are released into the thylakoid lumen, and **oxygen (O_2) is evolved as a byproduct**.

g. **Electron Transport from PSII to Cytochrome b_6f Complex:**

- The electron from Pheophytin is passed sequentially to:
    - **Plastoquinone (PQ):** A mobile, lipid-soluble carrier that also picks up protons from the stroma, becoming reduced to plastohydroquinone (PQH_2).
    - **Cytochrome b_6f Complex:** PQH_2 delivers its electrons here. This complex operates via a **Q-cycle**, which passes electrons to plastocyanin and simultaneously pumps **additional protons from the stroma into the thylakoid lumen**. This significantly contributes to the proton gradient for ATP synthesis.

h. **Electron Transport from Cytochrome b_6f Complex to PSI:**

- Electrons from the cytochrome b_6f complex are transferred to **Plastocyanin (Pc)**, a small, water-soluble protein in the thylakoid lumen. Pc then carries these electrons to Photosystem I.

i. **Photosystem I (PSI, P700):**

- Simultaneously, PSI (P700) absorbs light energy (or receives it via resonance transfer from its antenna complex).
- An electron in P700 is excited to a very high energy level (lower reduction potential), forming **P700***.
- P700* donates its electron to its primary electron acceptor. The oxidized **P700**$^+$ then accepts an electron from plastocyanin, replacing the lost electron.

j. **Electron Transport from PSI to NADP$^+$:**

- The excited electron from PSI is passed through a series of carriers: **Ferredoxin (Fd)** (a soluble iron-sulfur protein in the stroma).

    - Finally, Fd transfers the electrons to the enzyme **Ferredoxin-NADP$^+$ Reductase (FNR)**. FNR uses these electrons (two electrons) and a proton from the stroma to reduce **NADP$^+$ to NADPH**.

- **Overall Products of Non-Cyclic Electron Flow:**
    - **ATP:** Synthesized by ATP synthase as protons flow back from the thylakoid lumen to the stroma, driven by the proton gradient.
    - **NADPH:** Provides reducing power for carbon fixation in the Calvin cycle.
    - O_2**:** Released as a byproduct of water photolysis.
- **Conceptual Z-Scheme:**
    - (Low Energy/High Reduction Potential)
    - **Water (H_2O)**
    - ↓ (Oxidation / Electron Donation)
    - **P680** (PSII Reaction Center)
    - ↑ (Light Absorption & Excitation)
    - **P680*** (Excited P680 - High Energy/Low Reduction Potential)
    - ↓ (Electron Transfer)
    - **Pheophytin (Pheo)**
    - ↓ (Electron Transfer)
    - **Plastoquinone (PQ) Pool**
    - ↓ (Electron Transfer, coupled to **H$^+$ pumping** to Lumen)
    - **Cytochrome b_6f Complex**
    - ↓ (Electron Transfer)

- **Plastocyanin (Pc)**
- ↓ (Electron Transfer)
- **P700** (PSI Reaction Center)
- ↑ (Light Absorption & Excitation)
- **P700*** (Excited P700 - Very High Energy/Very Low Reduction Potential)
- ↓ (Electron Transfer)
- **Ferredoxin (Fd)**
- ↓ (Electron Transfer)
- **NADP$^+$ Reductase**
- ↓ (Electron Transfer & Reduction)
- **NADPH** (Low Energy/Higher Reduction Potential than water)

Question 445: (b) Calculate the standard free-energy change for the reaction at oxidation-reduction pH 7.0: Acetaldehyde + NADH + H^+ → Ethanol + NAD^+ Given that the standard reduction potential of acetaldehyde/ethanol redox pair = -0.197V and the standard reduction potential of NAD^+/NADH redox pair = -0.320V.

- **The Reaction:** Acetaldehyde + NADH + H^+ → Ethanol + NAD^+
- **Identify Half-Reactions and their Potentials:**
  k. **Reduction Half-Reaction (Electron Acceptor):** Acetaldehyde + $2H^+$ + $2e^-$ → Ethanol Given: E_0'_{\text{Acetaldehyde/Ethanol}} = -0.197 \text{ V}
  l. **Oxidation Half-Reaction (Electron Donor):** NADH → NAD^+ + H^+ + $2e^-$ The given potential is for the reduction: NAD^+ + H^+ + $2e^-$ → NADH, which is E_0'_{\text{NAD^+/NADH}} = -0.320

\text{ V}. To get the oxidation potential, we reverse the sign: E_0'_{\text{NADH/NAD^+}} = +0.320 \text{ V}.

- **Calculate the Standard Change in Reduction Potential ($\Delta E_{0'}$):** \Delta E_0' = E_0'_{\text{electron acceptor}} - E_0'_{\text{electron donor}} \Delta E_0' = E_0'_{\text{reduction (Acetaldehyde)}} - E_0'_{\text{reduction (NAD^+)}} $\Delta E_{0'} = (-0.197 \text{ V}) - (-0.320 \text{ V})$ $\Delta E_{0'} = -0.197 \text{ V} + 0.320 \text{ V}$ $\Delta E_{0'} = +0.123 \text{ V}$
- **Calculate the Standard Free Energy Change ($\Delta G_{0'}$):** The relationship between $\Delta G_{0'}$ and $\Delta E_{0'}$ is: $\Delta G_{0'} = -nF\Delta E_{0'}$ Where:
  - n = number of electrons transferred in the reaction = 2 (from the half-reactions).
  - F = Faraday's constant = 96.485 kJ/mol·V (or 96485 J/mol·V).
  - $\Delta E_{0'}$ = standard cell potential = +0.123 V.

  $\Delta G_{0'} = -(2 \text{ mol electrons}) \times (96.485 \text{ kJ/mol·V}) \times (+0.123 \text{ V})$ $\Delta G_{0'} = -192.97 \times 0.123 \text{ kJ/mol}$ $\Delta G_{0'} = -23.73531 \text{ kJ/mol}$
- **Result:** The standard free-energy change ($\Delta G_{0'}$) for the reaction is approximately **-23.74 kJ/mol**.

Question 445: (c) Give the mode of action of the following inhibitors:

- **(i) Amytal:**
  - **Mode of Action:** Amytal (Amobarbital) is an inhibitor of **Complex I (NADH-ubiquinone oxidoreductase)** of the mitochondrial electron transport chain. It binds to a specific site within Complex I, blocking the transfer of electrons from the flavin mononucleotide (FMN) and iron-sulfur clusters to ubiquinone (CoQ).
  - **Consequences:** This prevents electron flow from NADH into the ETC, leading to a buildup of reduced NADH, inhibition of

proton pumping by Complex I, and ultimately a halt in oxygen consumption and ATP synthesis via oxidative phosphorylation.

- **(ii) Dinitrophenol (DNP):**
    - **Mode of Action:** DNP is an **uncoupler** of oxidative phosphorylation. It is a lipophilic weak acid that acts as a **protonophore**. It can passively shuttle protons across the inner mitochondrial membrane, bypassing ATP synthase.
    - **Consequences:** By dissipating the proton gradient (ΔpH and $\Delta\Psi$) across the membrane, DNP uncouples electron transport from ATP synthesis. Electron transport and oxygen consumption can even increase rapidly, but the energy released is dissipated as heat rather than being used to produce ATP, leading to severe hyperthermia if uncontrolled.
- **(iii) Atractyloside:**
    - **Mode of Action:** Atractyloside is a specific inhibitor of the **Adenine Nucleotide Translocase (ANT)**, also known as the ADP/ATP carrier, located in the inner mitochondrial membrane. It binds to ANT on the cytosolic (outer) side of the inner membrane.
    - **Consequences:** This binding traps the ADP/ATP carrier in a conformation that prevents the exchange of cytosolic ADP for mitochondrial ATP. As a result, ADP cannot enter the mitochondrial matrix to be phosphorylated by ATP synthase, and newly synthesized ATP cannot exit to the cytosol, effectively halting ATP synthesis even if electron transport and proton pumping are functional.
- **(iv) Cyanide:**
    - **Mode of Action:** Cyanide (CN^-) is a potent and rapid-acting inhibitor of **Complex IV (cytochrome c oxidase)** of the mitochondrial electron transport chain. It binds tightly and

irreversibly to the ferric iron (Fe^{3+}) within the heme a_3 of Complex IV.

- **Consequences:** This binding prevents the final transfer of electrons from cytochrome *c* to molecular oxygen. Electron flow is completely blocked at this terminal step, leading to an immediate cessation of oxygen consumption, a backup of electrons throughout the entire ETC, a collapse of the proton gradient, and a complete inhibition of ATP synthesis via oxidative phosphorylation, which is lethal.

- **(v) DCMU (Diuron):**
  - **Mode of Action:** DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea) is a herbicide that specifically inhibits **Photosystem II (PSII)** in the light-dependent reactions of photosynthesis. It binds to the Q_B binding site on the D1 protein of PSII.
  - **Consequences:** This binding displaces plastoquinone, thereby blocking the transfer of electrons from PSII to the plastoquinone pool. This interruption of electron flow from PSII halts the splitting of water (and thus oxygen evolution), prevents the formation of a proton gradient (and thus ATP synthesis), and also stops the reduction of $NADP^+$ to NADPH, ultimately inhibiting the entire process of non-cyclic photosynthesis.

Question 556: (a) Explain the chemiosmotic theory and discuss two evidences in support of the theory.

- **Chemiosmotic Theory (Mitchell's Hypothesis):**
  - Proposed by Peter Mitchell in 1961, the chemiosmotic theory explains how the energy released from electron transport (during cellular respiration in mitochondria or photosynthesis in chloroplasts) is used to drive the synthesis of ATP.

- It posits that the energy from the exergonic flow of electrons through an electron transport chain is not directly used to make ATP, but rather it is first used to **pump protons (H^+)** across a biological membrane (e.g., inner mitochondrial membrane or thylakoid membrane).
- This pumping creates an **electrochemical proton gradient**, also known as the **proton motive force (PMF)**, across the membrane. The PMF has two components: a chemical potential (difference in pH) and an electrical potential (difference in charge across the membrane).
- This stored potential energy in the PMF is then harnessed when protons flow back down their electrochemical gradient, from the side of high concentration to the side of low concentration, through a specialized enzyme complex called **ATP synthase**. The flow of protons through ATP synthase drives its rotary mechanism, leading to the conformational changes necessary for the phosphorylation of ADP to ATP.
- In essence, electron transport and ATP synthesis are "coupled" by an intermediate proton gradient.

- **Two Evidences in Support of the Theory:**
  - **1. Requirement for an Intact Membrane and Proton Gradient Integrity:**
    - **Evidence:** Experiments have consistently shown that for ATP synthesis to occur, the inner mitochondrial membrane (or thylakoid membrane in chloroplasts) must be **intact and impermeable to protons**. If the membrane is disrupted or made "leaky" to protons by adding **uncoupling agents** (like 2,4-dinitrophenol, DNP), electron transport continues (or even accelerates), but ATP synthesis completely stops.

- **Explanation:** Uncouplers directly dissipate the proton gradient by allowing protons to bypass ATP synthase and re-enter the matrix/stroma. This demonstrates that the proton gradient itself, not just electron transport, is the indispensable intermediate for ATP production. If ATP were formed directly from electron carriers, uncouplers would not halt ATP synthesis.

- **2. Artificial Induction of ATP Synthesis by a pH Gradient (Jagendorf's Experiment):**
  - **Evidence:** In a landmark experiment in 1966, André Jagendorf and his colleagues demonstrated that an artificially imposed proton gradient, **in the absence of any electron transport or light**, was sufficient to drive ATP synthesis in isolated chloroplast thylakoids.
  - **Experimental Setup:** Thylakoids were first soaked in an acidic solution (e.g., pH 4) in the dark, allowing the lumen to acidify. They were then rapidly transferred to an alkaline solution (e.g., pH 8) containing ADP and inorganic phosphate (P_i).
  - **Observation:** This sudden pH differential created a proton gradient (acidic lumen, alkaline stroma), and ATP was spontaneously synthesized.
  - **Explanation:** This experiment provided strong and direct proof for the chemiosmotic theory, showing that the proton motive force alone, regardless of its origin (whether from electron transport or artificially imposed), is the driving force for ATP synthesis. It definitively established the central role of the proton gradient as the coupling agent.

Question 556: (b) Describe the photosynthetic machinery of purple bacteria.

- **Introduction:** Purple bacteria are a group of anoxygenic phototrophic bacteria, meaning they perform photosynthesis without producing oxygen. Unlike plants, they don't use water as an electron donor, and their photosynthetic machinery is typically simpler, primarily focused on cyclic electron flow for ATP generation.
- **Location of Machinery:**
    - Their photosynthetic components are housed in specialized invaginations of the plasma membrane called **chromatophores**, or directly within the plasma membrane itself. These membranes provide the necessary environment for electron transport and proton gradient formation.
- **Key Components of Photosynthetic Machinery:**
    - **1. Reaction Center (RC):**
        - Purple bacteria possess a single type of reaction center, which is structurally and functionally homologous to Photosystem II (PSII) in plants, but it **lacks the water-splitting complex**.
        - The core of the reaction center is a specialized pair of **bacteriochlorophylls** (e.g., P870 or P960, referring to their absorption maxima in nanometers). This special pair serves as the primary electron donor.
        - Upon light absorption, the excited bacteriochlorophyll (e.g., P870*) undergoes charge separation, donating an electron to a primary electron acceptor, usually **bacteriopheophytin**, and then to a series of quinones (Q_A and Q_B).
    - **2. Light-Harvesting Complexes (LHCs):**

- Surrounding the reaction centers are antenna complexes composed of various **bacteriochlorophylls** (different types from the reaction center) and **carotenoids**.
- These LHCs efficiently capture light energy, often in the near-infrared and visible regions, and transfer this excitation energy via **resonance energy transfer** to the reaction center, maximizing light harvesting.

- **3. Cytochrome bc_1 Complex:**
  - This complex is an integral membrane protein analogous to Complex III in mitochondrial respiration and the cytochrome b_6f complex in plant photosynthesis.
  - It accepts electrons from reduced ubiquinone (QH_2) (which receives electrons from the reaction center Q_B site).
  - The cytochrome bc_1 complex uses a **Q-cycle mechanism** to pass electrons to cytochrome c_2 while simultaneously pumping **protons** from the cytoplasm to the periplasmic space (establishing a proton gradient).
- **4. Cytochrome c_2:**
  - A small, soluble cytochrome located in the periplasmic space.
  - It acts as a **mobile electron carrier**, accepting electrons from the cytochrome bc_1 complex and returning them to the oxidized bacteriochlorophyll special pair (e.g., $P870^+$) in the reaction center, thus completing a cyclic electron flow.
- **5. ATP Synthase:**
  - Embedded in the chromatophore membrane, similar to mitochondrial ATP synthase.

    - It utilizes the proton motive force (proton gradient) generated by the cytochrome bc_1 complex to synthesize **ATP** from ADP and P_i.

- **Electron Flow (Cyclic Photophosphorylation):**
    - The primary mode of electron flow in purple bacteria is **cyclic**.
    - Light excites the reaction center bacteriochlorophyll (e.g., P870).
    - The excited electron travels from P870 to bacteriopheophytin, then to Q_A and Q_B (quinones).
    - Q_B becomes reduced to QH_2 (ubiquinol).
    - QH_2 donates electrons to the cytochrome bc_1 complex, which pumps protons and passes electrons to cytochrome c_2.
    - Cytochrome c_2 returns the electrons to the oxidized $P870^+$ in the reaction center, completing the cycle.
    - This cyclic flow generates a proton gradient, driving **ATP synthesis** (cyclic photophosphorylation).
    - **No oxygen is produced** because water is not used as an electron source. For NADPH production (required for carbon fixation), purple bacteria typically perform **reverse electron flow**, where electrons are forcibly pushed to NAD^+ using energy from the proton gradient, or they obtain reducing power from external electron donors like H_2S or organic acids.

Question 556: (c) Calculate the Standard Free Energy change of the following reaction: Glucose-1-phosphate → Glucose-6-phosphate Given that starting with 20 mM of Glucose-1-phosphate and no Glucose-6-phosphate, the final equilibrium mixture at 25°C and at pH = 7.0, contains

1.0 mM Glucose-1-phosphate and 19 mM Glucose-6-phosphate. Does the reaction proceed with a loss or gain of energy?

- **Reaction:** Glucose-1-phosphate (G1P) ⇌ Glucose-6-phosphate (G6P)
- **Equilibrium Concentrations:**
  - $[G1P]_{\text{eq}} = 1.0$ mM
  - $[G6P]_{\text{eq}} = 19$ mM
- **Temperature (T):** $25°C = 25 + 273.15 = 298.15$ K
- **Gas Constant (R):** 8.314 J/mol·K
- **Step 1: Calculate the Equilibrium Constant (K_{eq})** At equilibrium, the equilibrium constant is the ratio of product concentration to reactant concentration: $K_{eq} = \frac{[G6P]_{\text{eq}}}{[G1P]_{\text{eq}}}$ $K_{eq} = \frac{19\text{ mM}}{1.0\text{ mM}}$ $K_{eq} = 19$
- **Step 2: Calculate the Standard Free Energy Change ($\Delta G_{0'}$):** The relationship between standard free energy change and the equilibrium constant is given by the equation: $\Delta G_{0'} = -RT\ln K_{eq}$

  Substitute the values: $\Delta G_{0'} = -(8.314\text{ J/mol·K}) \times (298.15\text{ K}) \times \ln(19)$

  First, calculate $\ln(19)$: $\ln(19) \approx 2.9444$

  Now, substitute this value back into the equation: $\Delta G_{0'} = -(8.314\text{ J/mol·K}) \times (298.15\text{ K}) \times (2.9444)$ $\Delta G_{0'} = -2478.8 \times 2.9444$ J/mol $\Delta G_{0'} = -7296.8$ J/mol

  Convert to kilojoules per mole (kJ/mol): $\Delta G_{0'} = -7.2968$ kJ/mol
- **Result:** The standard free-energy change ($\Delta G_{0'}$) for the reaction Glucose-1-phosphate → Glucose-6-phosphate is approximately **-7.30 kJ/mol**.
- **Does the reaction proceed with a loss or gain of energy?**

- Since the calculated $\Delta G_{0'}$ is a **negative value** (-7.30 kJ/mol), the reaction proceeds with a **loss of energy**. This indicates that the conversion of Glucose-1-phosphate to Glucose-6-phosphate is **thermodynamically favorable (exergonic)** under standard biochemical conditions and will proceed spontaneously in the forward direction.

Question 66: Write short notes on the following:

- **(a) Cyclic photophosphorylation**
  - **Cyclic photophosphorylation** is a light-dependent reaction in photosynthesis that generates ATP without producing NADPH or evolving oxygen. It involves only **Photosystem I (PSI)**. Electrons, excited by light in PSI, are transferred to ferredoxin, then controversially back to the cytochrome b_6f complex, and finally via plastocyanin, they return to PSI, completing a cycle. This electron flow drives the pumping of protons from the stroma into the thylakoid lumen by the cytochrome b_6f complex, establishing a proton gradient. This gradient then powers ATP synthase to produce ATP. This pathway is important for balancing the ATP/NADPH ratio to meet the specific energy demands of the cell, especially when more ATP than NADPH is required for processes like carbon fixation, or under certain stress conditions.
- **(b) Complex II of ETC**
  - **Complex II** (also known as **Succinate Dehydrogenase**) is one of the five major protein complexes of the mitochondrial electron transport chain (ETC), embedded in the inner mitochondrial membrane. Uniquely, it is also an enzyme of the citric acid cycle (TCA cycle). Complex II catalyzes the oxidation of **succinate to fumarate**, directly transferring the two electrons and two protons removed to its covalently bound **FAD**

prosthetic group, forming $FADH_2$. These electrons are then passed, via iron-sulfur clusters within Complex II, to ubiquinone (CoQ), reducing it to ubiquinol (QH_2). A key characteristic of Complex II is that, unlike Complexes I, III, and IV, it **does not pump protons** across the inner mitochondrial membrane. Therefore, it contributes less directly to the proton gradient for ATP synthesis compared to NADH-derived electrons, but it provides a direct link for electrons from the TCA cycle to enter the ETC.

- **(c) Q-Cycle**
    - The **Q-Cycle** is a biochemical mechanism that occurs within **Complex III (cytochrome bc_1 complex)** of the mitochondrial electron transport chain and the **cytochrome b_6f complex** in photosynthesis. Its purpose is to efficiently transfer electrons from a two-electron carrier (ubiquinol, QH_2, or plastoquinol, PQH_2) to a one-electron carrier (cytochrome *c* or plastocyanin), while simultaneously maximizing the **pumping of protons** across the membrane. In the Q-cycle, one molecule of QH_2 is oxidized at the Q_o site, releasing two protons and two electrons. One electron goes directly to the next carrier (e.g., cytochrome *c*), while the other electron is recycled back through cytochrome *b* subunits to the Q_i site, where it partially reduces another quinone molecule. A second QH_2 then similarly oxidizes, with its second electron completing the reduction of the quinone at Q_i. This mechanism effectively leads to the translocation of four protons for every two electrons passed through the complex, significantly contributing to the proton motive force used for ATP synthesis.