Photophosphorylation
The processes of photosynthesis

• Light capture
• Charge separation
• Synthesis of high-energy compounds
• Incorporation of CO$_2$ into organic compounds
In the bacterial and plant photosynthetic reactions,

\[ \text{CO}_2 + 2 \text{H}_2\text{D} \rightarrow \text{C(H}_2\text{O}) + 2 \text{D} + \text{H}_2\text{O} \]

The separate reactions are,

\[ 2 \text{H}_2\text{D} \rightarrow \text{light} \rightarrow 2 \text{D} \]

\[ 2 \text{NADP}^+ + 3 \text{ADP} + 3 \text{Pi} \rightarrow 2 \text{NADPH} + \text{H}^+ + 3 \text{ATP} \]

\[ \text{C(H}_2\text{O}) + \text{H}_2\text{O} \rightarrow \text{(dark)} \rightarrow \text{CO}_2 \]

The light reactions produce H\(^*\) (reductant) and energy.

Notice the need for ATP as well as NADPH.
The “Z-scheme”, showing $E_m$ values of electron carriers. The synthesis of ATP perhaps explains the need for additional energy.
“Chemiosmotic hypothesis” (Peter Mitchell): H+ passage across membrane powers ATP synthesis

How does it work?

Clues:

1. Reversed coupling: ATP hydrolysis powers H+ flow.

• Membrane vesicle containing ATP synthase (outside)
• Solution of acrydine orange (AO) (membrane-permeable fluorescent dye)
• Add ATP, vesicle interior becomes acidic
• AO → AO+, trapped inside vesicle
• High concentration of AO quenches fluorescence
2. $\text{H}^+$ flow in artificial vesicle powers ATP synthesis.
3. **Uncouplers** — compounds that permeabilize the mitochondrial inner membrane (or thylakoid membranes) to H\(^+\) inhibit ATP synthesis

(FCCP: carbonylcyanide p-trifluoromethoxyphenylhydrazone)
Non-cyclic photosynthetic electron transport moves $H^+$ into the lumen of thylakoids

4 $h_\nu$: 2 $e^-$: 4 $H^+$ pumped by PQ, 2 $H^+$ released by $H_2O$, 1 $H^+$ bound in NADPH
Cyclic photosynthetic electron transport also moves H\(^+\) into the lumen of thylakoids

\[ 1 \text{ hv: 1 e}^-: 2 \text{ H}^+ \text{ pumped by PQ in cytochrome } b_{6f} \text{ complex} \]
The net concentration difference of H+ across the thylakoid membrane represents stored energy: “proton motive force” (Δp, Mitchell)

\[ \Delta p = \Delta E - 2.3 \left( \frac{RT}{F} \right) \Delta pH \]

\( \Delta E \) = voltage across the membrane
\( \Delta pH \) = pH difference across the membrane
2.3 \( \frac{RT}{F} \) = 59 mV per pH unit at 25°C

Chloroplast: \( \Delta E \) is small and \( \Delta p \) is mostly from \( \Delta pH \)
(Mg\(^{2+}\) flow out of thylakoids reduces \( \Delta E \))

Mitochondria: \( \Delta p \) is mostly from \( \Delta E \)
(buffers reduce \( \Delta pH \))
Flow of H+ out of thylakoids through ATP synthase powers ATP synthesis

(Other names: ATPase, CF₀-CF₁, coupling factor)
From the text:

CF\textsubscript{0} rotates

CF\textsubscript{1} doesn’t rotate

CF\textsubscript{0} rotates

Stroma

ADP + P\textsubscript{i}

ATP

Thylakoid membrane

Thylakoid lumen

PLANT PHYSIOLOGY, 5e, Figure 7.32 (Part 1)
Note the rotor (base) and stator (head) and rotation

Hypothesis: \[ \text{ADP} + \text{Pi} \rightarrow \text{ATP} + \text{H}_2\text{O} \] occurs on head; \( \text{H}^+ \) flow turns rotor; rotation stimulates ATP release
Model for H+-induced rotation: flow of one H+ rotates the CF$_0$ one subunit forward.
Rotor rotation

ADP + P

ATP

ADP + P

H₂O

ATP

ADP + P

H₂O

ATP
The number of H+ needed per ATP depends on the number of CF₀ subunits.

EM imaging of the CF₀ shows 14 subunits, indicating a ratio of 14 H+/3 ATP.

At 6 H+ pumped/NADPH or 12 H+/2 NADPH (12 H+/O₂), we need 2 more H+ from cyclic photophosphorylation for 3 ATP/2 NADPH.

The generation of 2 NADPH and one O₂ requires 8 photons; cyclic photophosphorylation needs one more photon, explaining the quantum yield of 1 O₂/ 9-10 quanta (photons) mentioned in the first photosynthesis lecture.
Summary

• ATP is needed for carbon fixation

• Chemiosmosis represents an energy store for ATP synthesis

• The ATP synthase converts proton motive force into ATP

Using the concepts described in this lecture, explain how the addition of ATP to vesicles containing ATP synthase can form a pH gradient across the vesicle membrane. Will the pH inside the vesicle be higher or lower than outside?