Photosynthesis

Chloroplasts

Light Reactions
(photons $\rightarrow$ NADPH + ATP)

Dark Reactions
($\text{CO}_2 + \text{H}_2\text{O} \rightarrow$ carbohydrate)

$$\text{CO}_2 + \text{H}_2\text{O} \xrightarrow{\text{light}} \xrightarrow{hv} (\text{CH}_2\text{O}) + \text{O}_2$$
Chloroplasts

Site of photosynthesis in algae and higher plants
1 to 1000 per cell
typically ~5-μm long ellipsoids

**Outer membrane**
permeable

**Intermembrane space**

**Inner membrane**
impermeable

**Stroma**
similar to mitochondrial matrix
**Dark reaction enzymes** ([Calvin cycle or reductive pentose phosphate cycle](#)), DNA, RNA, ribosomes

**Thylakoid**
highly folded vesicle arising from invaginations of inner membrane (similar to mitochondrial cristae)
electron transport chain proteins
10 to 100 [grana](#) (disc-like sacs) interconnected by [stroma lamellae](#)
10% phospholipids
80% uncharged mono- and digalactosyl diacylglycerols
10% sulfoquinovosyl diacylglycerols (sulfolipids)
Light Reactions

Absorption of light

**Chlorophyll** (Chl and bacteriochlorophyll, BChl)

differs from heme:

Mg$^{2+}$
cyclopentanone ring V fused to pyrrole ring III
pyrrole ring IV partially reduced in Chl $a$ and Chl $b$ (rings II and IV partially reduced in BChl $a$ and BChl $b$)
propionyl side chain of ring IV esterified
Light Reactions

Absorption of light

Plank's law:

\[ E = h \nu = \frac{hc}{\lambda} \]

\( h = \) Plank's constant = 6.626 x 10\(^{-34}\) J \( \cdot \) s
\( c = \) speed of light = 2.998 x 10\(^8\) m \( \cdot \) s\(^{-1}\) (vacuum)
\( \lambda = \) wavelength

Molecules absorb photons whose energy match the energy difference between ground and excited states

Beer-Lambert law:

\[ A = \log \frac{I_0}{I} = \varepsilon c l \]

\( A = \) absorbance
\( I_0 \) and \( I = \) incident and transmitted intensities
\( \varepsilon = \) molar extinction coefficient
\( c = \) molar concentration
\( l = \) sample pathlength in cm
Light Reactions

Absorption of light

**Internal conversion** - electronic energy converted to heat, time frame $<10^{-11}$ s

**Fluorescence** - excited state decays to ground state by emitting photon, time frame $\sim 10^{-8}$ s

**Exciton transfer** (resonance energy transfer) - excited molecule transfers its excitation energy to nearby unexcited molecules, important in funneling light energy to photosynthetic reaction centers

**Photooxidation** - light-excited donor molecule transfers an electron to an acceptor molecule, the oxidized donor relaxes to ground state by oxidizing some other molecule
Light Reactions

Absorption of light

Chlorophylls (light-harvesting antennas) function to gather photons and transfer energy by exciton transfer to a photosynthetic reaction center, time frame <10^{-10} \text{s} with 90\% efficiency

Light-harvesting complex-II (LHC-II)

most abundant membrane protein in chloroplasts of green plants
232-residue transmembrane protein
at least 7 Chl \textit{a}, 5 Chl \textit{b} and 2 carotenoids
function to gather light energy and prevent energy transfer to O_2

Phycobilisomes (algae and cyanobacteria)
phycobiliproteins
phycocyanobilin pigment
phycoerythrobilin pigment
bound to outer face of photosynthetic membrane
Light Reactions

Electron transport in photosynthetic bacteria

**Reaction center composition:**

transmembrane protein, 3 subunits (H, L, M)

**P870 (BChl a)**

4 **BChl b** (two form a "special pair")

**bacteriopheophytin b (BPheo b, 2H⁺ replace Mg²⁺)**

**nonheme/non-Fe-S Fe²⁺**

**ubiquinone**

**menaquinone**

Photooxidation of P870 "special pair" \( \rightarrow \) BPheo \( a \rightarrow \)

menaquinone \( \rightarrow \) ubiquinone \( \rightarrow \) Q cycle \( \rightarrow \) cyt \( bc_1 \) (similar to Complex III) \( \rightarrow \) cyt \( c_2 \) \( \rightarrow \) 4 heme cyt \( c \) \( \rightarrow \) P870⁺

Sequence of electron transfers has a **quantum yield** of

\(~100\%\)!

No net oxidation-reduction, functions to translocate H⁺s across plasma membrane and photophosphorylation produces ATP, both processes similar to those discussed in oxidative phosphorylation
Light Reactions

Two-center electron transport (plants and cyanobacteria)

Reducing power of photooxidation of H₂O drives NADPH production

\[ \text{O}_2 + 4e^- + 4H^+ \rightleftharpoons 2\text{H}_2\text{O} \quad \varepsilon^\circ' = +0.815 \text{ V} \]

\[ \text{NADP}^+ + \text{H}^+ + 2e^- \rightleftharpoons \text{NADPH} \quad \varepsilon^\circ'' = -0.320 \text{ V} \]

\[ 2\text{NADP}^+ + 2\text{H}_2\text{O} \rightleftharpoons 2\text{NADPH} + \text{O}_2 + 2\text{H}^+ \]

\[ \Delta\varepsilon^\circ'' = -1.135 \text{ V} \]

\[ \Delta G^\circ' = +438 \text{ kJ mol}^{-1} \]

(requires 1 einstein of 223-nm photons)

This means that more than one photon of visible light is required for production of one O₂ molecule (8 to 10 photons)
Light Reactions

Two-center electron transport

Photosystems I and II (Z-scheme)

Photosystem I (PSI) - generates strong reductant (weak oxidant)

\[ \text{NADP}^+ + H^+ + 2e^- \rightarrow \text{NADPH} \]

Photosystem II (PSII) - generates strong oxidant (weak reductant)

\[ 2H_2O \rightarrow O_2 + 4H^+ + 4e^- \]

Cytochrome \( b_6f \) connects PSII and PSI
Light Reactions

Two-center electron transport

Three thylakoid transmembrane protein complexes

1. **PSII** - P680 (Chl *a*), Pheo *a*, QA, QB (herbicides complete with Q)

2. **Cytochrome b₆f complex** - (resembles cyt bc₁ and Complex III) cyt f, cyt b₆ (2 hemes), [2Fe-2S] protein, bound plastoquinol, transport H⁺ from outside to inside of thylakoid membrane using Q cycle
   Generates much of the H⁺ gradient that drives ATP synthesis

3. **PSI** - 7 subunits (2 large, 5 small), P700, 100 to 200 Chl *a*, 12 to 16 β-carotene, 3 [4Fe-4S] clusters, 2 phylloquinone (vit K₁)

Intercomplex electron transfer between PSII and cyt b₆f by plastoquinone/plastoquinol (Q/QH₂)

Intercomplex electron transfer between cyt b₆f and PSI by plastocyanin (PC, a peripheral membrane Cu metalloprotein)

**Oxygen evolving complex** (OEC) - protein complex with 4 Mn (III/IV)-O²⁻ clusters, 2 or 3 Ca²⁺, and 4 or 5 Cl⁻ ions

**Z** - tyrosine radical-containing (macro)molecule
Light Reactions

Two-center electron transport

Electrons from PSI may follow two routes:

1. **Noncyclic pathway** to 11-kDa, [2Fe-2S] soluble ferredoxin (Fd, 1e⁻ donor/acceptor) located in stroma, where *FAD*-containing ferredoxin-NADP⁺ reductase (FNR) reduces NAPD⁺

2. **Cyclic pathway** to return to plastoquinone (Q) pool, translocates H⁺s across thylakoid membrane, independent of PSII
   Functions to increase ATP production relative to that of NADPH

PSI resembles (functionally and genetically) bacterial photosystems
Light Reactions

Two-center electron transport

Distribution of photosynthetic complexes

**PSI** occurs *mainly in unstacked stroma lamellae*, in contact with stroma, where it has access to NADP⁺

**PSII** occurs *almost exclusively between stacked grana*, out of direct contact with stroma

Cytochrome $b_{6}f$ is *uniformly distributed throughout membrane*

Why?

Need to separate PSII from PSI so that exciton transfer *does not occur*

Allows response to low (long wavelength) and high (short wavelength) light illumination (light-activated protein kinase-dependent feedback mechanism)
Light Reactions

Photophosphorylation

Chloroplasts couple dissipation of $\text{H}^+$ gradient to synthesis of ATP (resembles mitochondrial system)

$\text{CF}_1\text{CF}_0$ complex

1. $\text{CF}_0$ is hydrophobic transmembrane protein, $\text{H}^+$ translocating channel

2. $\text{CF}_1$ is hydrophilic peripheral membrane protein, $\alpha_3\beta_3\gamma\delta\epsilon$ composition, $\beta$ is reversible ATPase, $\gamma$ controls $\text{H}^+$ flow from $\text{CF}_0$ to $\text{CF}_1$

3. Inhibited by oligomycin and dicyclohexylcarbodiimide (DCCD)

Chloroplast ATP synthase is located in unstacked portions of thylakoid membrane in contact with stroma

Translocates $\text{H}^+$ out of thylakoid space

How does that compare to the mitochondrial ATP synthase?
Light Reactions

Photophosphorylation

At saturating light intensities, chloroplasts generate proton gradient of ~3.5 pH units, which arise from two sources

1. Evolution of $O_2$ releases $4H^+$ (from stroma by way of NADPH synthesis) into thylakoid space
2. Transport of e’s through cyt $b_{6}f$ translocates $8H^+$ (from stroma to thylakoid space)

~12 $H^+$ translocated per $O_2$ produced by noncyclic electron transport

Thylakoid membrane allows passage of $Mg^{2+}$ and $Cl^-$, which results in elimination of membrane potential ($\Delta\Psi$)

Electrochemical gradient is almost entirely pH gradient

**ATP production:**

**Noncyclic electron transport**

4 ATP per $O_2$ evolved (and 2 NADPH $\rightarrow$ 6 ATP)
0.5 ATP per photon absorbed
so 1.25 ATP per photon absorbed!

**Cyclic electron transport**

0.67 ATP per photon absorbed
Dark Reactions

The Calvin cycle (reductive pentose phosphate cycle)

\[ 3\text{CO}_2 + 9\text{ATP} + 6\text{NADPH} \rightarrow \]
\[ \text{glyceraldehyde-3-phosphate} + 9\text{ADP} + 8\text{P}_i + 6\text{NADP}^+ \]

Two-stage process:

1. **Production phase**

\[ 3\text{Ribulose-5-phosphate} + 3\text{CO}_2 + 9\text{ATP} + 6\text{NADPH} \rightarrow \]
\[ 6\text{glyceraldehyde-3-phosphate} +9\text{ADP} + 6\text{P}_i + 6\text{NADP}^+ \]

   one glyceraldehyde-3-phosphate → biosynthesis

2. **Recovery phase**

\[ 5\text{Glyceraldehyde-3-phosphate} \rightarrow 3\text{ribulose-5-phosphate} \]

\[ \text{C}_3 + \text{C}_3 \rightarrow \text{C}_6 \]
\[ \text{C}_3 + \text{C}_6 \rightarrow \text{C}_4 + \text{C}_5 \]
\[ \text{C}_3 + \text{C}_4 \rightarrow \text{C}_7 \]
\[ \text{C}_3 + \text{C}_7 \rightarrow \text{C}_5 + \text{C}_5 \]
\[ 5\text{C}_3 \rightarrow 3\text{C}_5 \]
Dark Reactions

The Calvin cycle

Uses enzymes from glycolytic, gluconeogenic, and pentose phosphate pathways

Three unique enzymes:

- phosphoribulokinase
- ribulose bisphosphate carboxylase (RuBP carboxylase)
- sedoheptulose bisphosphatase (SBPase)
**Dark Reactions**

**Ribulose bisphosphate carboxylase (RuBP carboxylase)**

The most abundant protein in the biosphere!

$\text{k}_{\text{cat}} \sim 3 \text{ s}^{-1}$

Eight large (L) subunits (477 residues, encoded by chloroplast DNA) - catalytic site

Eight small subunits (123 residues, specified by nuclear gene) - unknown function

$L_8S_8$ composition

rate-determining step is $\text{C3 H}^+$ abstraction to generate enediolate

requires $\text{Mg}^{2+}$

$$\Delta G^\circ = -35.1 \text{ kJ mol}^{-1}$$

**ribulose-bisphosphate carboxylase activase** - catalyzes carbamate formation from $\text{CO}_2$ and $\varepsilon$-amino of Lys residue
Dark Reactions

The Calvin cycle

Recall stoichiometry of Calvin cycle:

\[ 3\text{CO}_2 + 9\text{ATP} + 6\text{NADPH} \rightarrow \]
\[ \text{glyceraldehyde-3-phosphate} + 9\text{ADP} + 8\text{P}_i + 6\text{NADP}^+ \]

Glyceraldehyde-3-phosphate may be converted to glucose-1-phosphate

Precursor of higher carbohydrates (through nucleotide sugars):

- sucrose (major transport sugar)
- starch (major storage polysaccharide)
- cellulose (primary structural polysaccharide)

precursor to fatty acids and amino acids
Dark Reactions

Control of Calvin cycle

During the day, plants use photosynthesis to produce ATP and NADPH for use in Calvin cycle
At night, plants process nutritional stores to produce ATP and NADPH

Must have light activate Calvin cycle and deactivate glycolysis (prevent futile cycle)

Regulation of RuBP carboxylase, FBPase, and SBPase by light-dependent factors

**RuBP carboxylase:**

- photons increase stroma pH from 7 to 8 (pH optimum for enzyme)
- photons translocate H\(^+\) to thylakoid, which drives Mg\(^{2+}\) efflux to stroma
- dark reaction intermediate (2-carboxyarabinitol-1-phosphate, CA1P) inhibits RuBP carboxylase
Dark Reactions

Control of Calvin cycle

**FBPase and SBPase:**

activated by increased pH, Mg$^{2+}$, and NADPH

activated by reduced thioredoxin (12 kDa redox sensitive protein), which is regulated by ferredoxin-thioredoxin reductase

Light stimulates Calvin cycle and deactivates glycolysis

Absence of light stimulates glycolysis and deactivates Calvin cycle
Dark Reactions

Photorespiration and the C$_4$ cycle

*Photorespiration* occurs at high [O$_2$] with production of CO$_2$

RuBP carboxylase has two substrates - CO$_2$ and O$_2$!

*Ribulose bisphosphate carboxylase-oxygenase (Rubisco)*

Ribulose-5-phosphate + O$_2$ $\rightarrow$

3-phosphoglycerate + 2-phosphoglycolate
Dark Reactions

Photorespiration and the $C_4$ cycle

$CO_2$ results from series of peroxisomal and mitochondrial reactions:

- phosphoglycolate phosphatase
- glycolate oxygenase
- catalase - heme enzyme takes $H_2O_2 \rightarrow O_2 + H_2O$
- transamination reactions
- hydroxypyruvate reductase
- glycerate kinase

Net result is that some photosynthetically generated ATP and NADPH is dissipated!

May be useful in protecting cells when $CO_2$ levels are low
Dark Reactions

Photorespiration and the C₄ cycle

**CO₂ compensation point** - [CO₂] at which rates of photosynthesis and photorespiration are equal

For many plants, CO₂ compensation point is ~40 to 70 ppm (normal atmospheric [CO₂] is 330 ppm)

so, *photosynthesis > photorespiration* rates

But this is temperature dependent!

On **hot sunny** days, CO₂ is depleted and O₂ is elevated at the chloroplast, *photosynthesis ~ photorespiration*!

Lessening oxygenase activity of Rubisco would be potentially beneficial
Dark Reactions

Photorespiration and the $C_4$ cycle

$C_4$ plants (sugar cane, corn, weeds) concentrate CO$_2$
CO$_2$ compensation point drops to ~ 2 to 5 ppm

$C_4$ cycle:

characteristic anatomy (*bundle-sheath cells, mesophyll cells*)
2 ATP to concentrate CO$_2$ in bundle-sheath cells

Photosynthesis in $C_4$ plants consumes 5 ATP per CO$_2$ fixed (compare to 3 ATP for Calvin cycle alone)

$C_4$ plants occur largely in tropical areas (high temperatures and light intensity)
$C_3$ plants occur largely in cooler areas (lower light intensity)

Desert plants use *Crassulacean acid metabolism (CAM)* to minimize H$_2$O loss during the day and maximize CO$_2$ absorption at night

Large amounts of phosphoenolpyruvate are required from starch breakdown and glycolysis

Malate is used to produce CO$_2$ for the Calvin cycle
Pyruvate is used to resynthesize starch