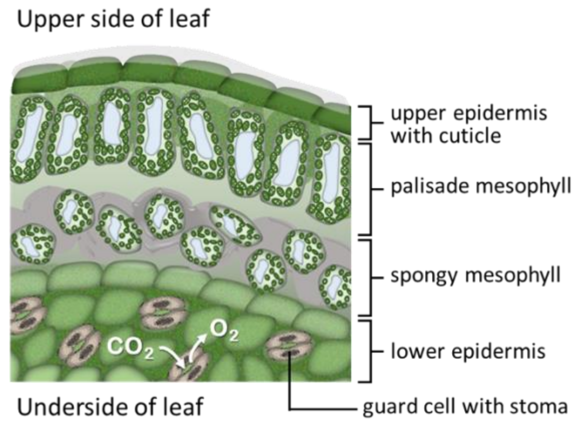
# The Light Dependent Stage of Photosynthesis in Plants

## Photosynthesis in Plants

Photosynthesis is the process by which plants use photons (packets of light energy) to create:

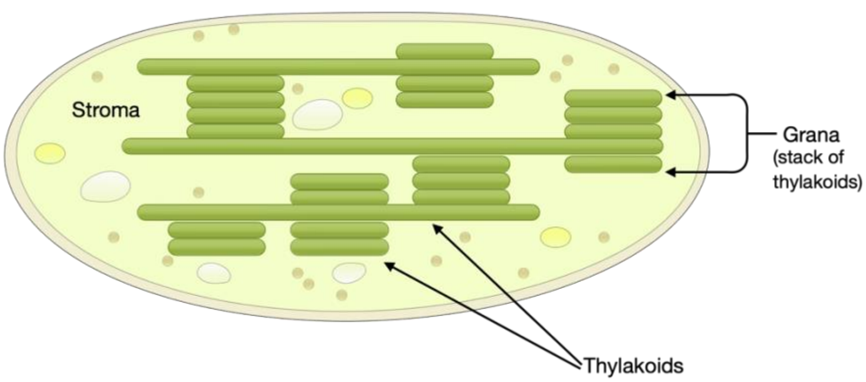
- oxygen (via the light dependent stage)
- the precursors for the creation of sugars and starch, fats and oils, and amino acids and proteins (via the light independent stage)




Cross section of a leaf. Wikimedia Commons

Photosynthesis takes place in the chloroplasts of plants. The plant cells richest in chloroplasts are in the mesophyll of its leaves; mesophyll cells contain about 50-100 chloroplasts each.

Below is a simplified diagram of a chloroplast showing its stroma[1] (the aqueous fluid inside it), and thylakoids (membrane-bound sac-like structures) within it.




The light-dependent stage of photosynthesis takes place in the thylakoids of the chloroplast. The light-independent stage takes place in the chloroplast's stroma.

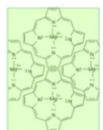
[1] The stroma is the matrix of aqueous fluid and dissolved enzymes, starch granules, lipids, and ribosomes found inside the chloroplast. The stroma surrounds the thylakoids. The stroma also includes the chloroplast's own DNA and RNA.

## The Light Dependent Stage of Photosynthesis

The light dependent stage of photosynthesis is the first step of photosynthesis. This phase produces:

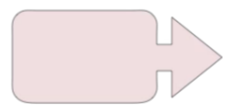
- oxygen gas which is released to the atmosphere
- ATP the energy-carrying molecule needed for biochemical reactions
- NADPH the reducing agent needed for the Calvin cycle and the biosynthesis of many biomolecules

There are several interconnected reaction centres needed for the light-dependent stage. They are all embedded in the thylakoid membrane.

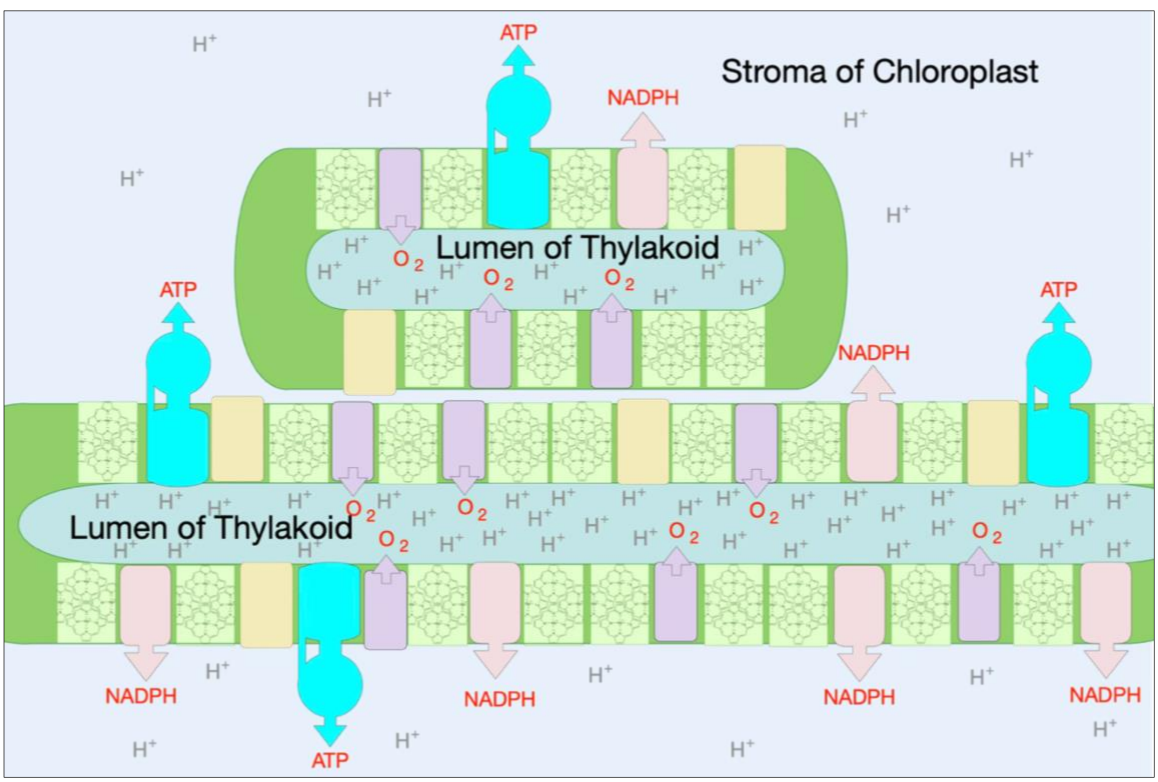
Light Harvesting Complex 

Cytochrome $b_6f$ Complex 

Photosystem II 

Photosystem I 

ATP synthase 

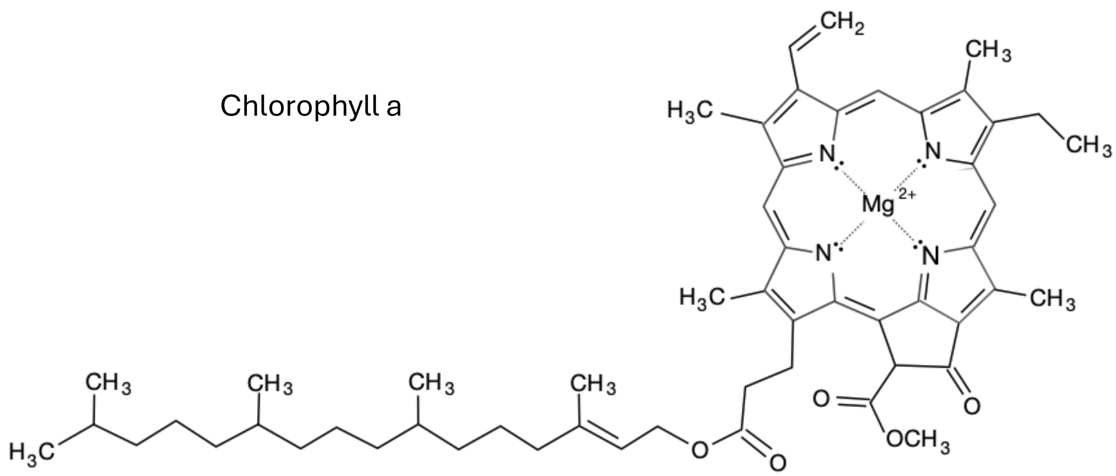


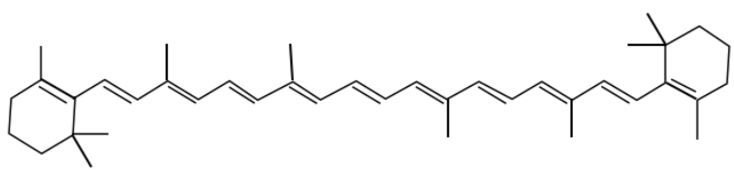
## The Five Reaction Centres for the Light-Dependant Stage of Photosynthesis

### 1. *The Light Harvesting Complex*:

The light harvesting complex (LHC) is a group of pigments - chlorophyll (a and b); and carotenoids (carotenes and xanthophylls). The pigments capture light energy. Having different types of pigment enables the plant to absorb a wide range of light wavelengths. Proteins hold the pigments in place within the thylakoid membrane and angle them towards the sun.

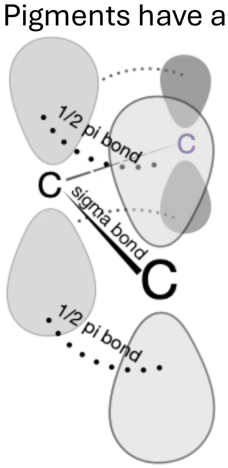
Chlorophyll a



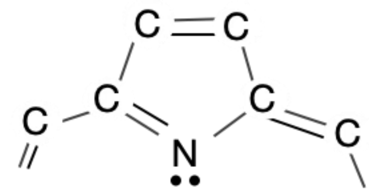
β-carotene

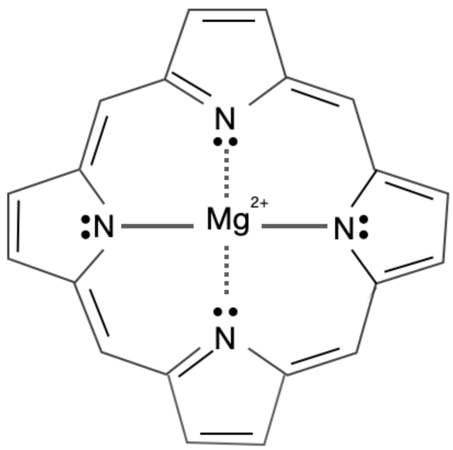
Pigments have alternating single and double bonds. Double bonds are formed from two bonds: the first bond is a sigma bond; the second is a pi bond. The pi bond is formed by overlapping p-orbitals above and below the sigma bond.




When there are alternating single and double bonds in a molecule, the pi bond can move from between carbon-1 and carbon-2, to between carbon-2 and carbon-3. This means the electrons in that double bond are moving (are delocalized). This freer movement allows electrons to more easily reach higher energy states. The more alternating double bonds there are in a molecule, the easier it is for an electron to move to a higher energy state. The electron can move to a higher energy state by aborbing a photon.

The pigment β-carotene has 11 alternating double bonds. Chlorophyll a has 11 double bonds in its ring alone. The ring structure puts the overlapping pi-bonds even closer together which provides stability for a high energy electron. Chlorophyll a is the dominant pigment in the chloroplast.




The rings are held close together. Because they are close together, the high energy from an excited electron in one ring can easily be shared among neighboring rings of chlorophyll molecules. In this way energy (not the electron itself) gained by an electron from a photon of light can be transferred from chlorophyll molecule to chlorophyll molecule. This type of transfer is called "resonance energy transfer."

Carotenoid pigments also pass their energy to chlorophyll molecules. Carotenoids are very good at capturing photons, but not as good at passing on energy. This works out well to protect a plant because a plant can receive too much sunlight which in turn creates too much energy for the plant cells to handle. When this happens, the carotenoids will release their captured energy as heat. On sunny days, most plants carry out photosynthesis in mid-morning and late afternoon. They shut down photosynthesis during noon when sunlight is at its most intense[2].

The chlorophyll and carotenoids function together as a 'light harvesting complex.' The energy collected from the pigments within the light harvesting complex is transferred on to the photosystems - Photosystem I and Photosystem II. Each photosystem has a pair of special chlorophyll molecules. The proteins attached to this special pair of chlorophyll molecules make them more reactive than the light-harvesting chlorophyll[3]. When an electron in one of those special chlorophylls absorbs energy, it is easier for that electron to to energize to the point of leaving the molecule altogether (ionizing). A nearby molecule immediately takes in that electron, making the event irreversible. It's this event that starts the 'electron transport chain' that is critical for the light dependent stage of photosynthesis to occur.

Note also that these pigment molecules are non-polar (they do not have partial electric charges) and so bond well within the non-polar lipid membrane of the thylakoid. The proteins that keep them in place are typically 200-250 amino acids long and most of these amino acids are hydrophobic (cannot bond with water), so they also bond well within the

[2] Biblical Biology 101. Robert W. Carter. Creation Book Publishers. Powder Springs, GA. 2024

[3] The type of proteins bound to a chlorophyll molecule influence is reactivity. The proteins bound to the special pair of chlorophyll molecules keeps them closer together, allowing them to function as a single unit.

hydrophobic lipid membrane. These proteins span the membrane, and each protein holds multiple pigments in place.

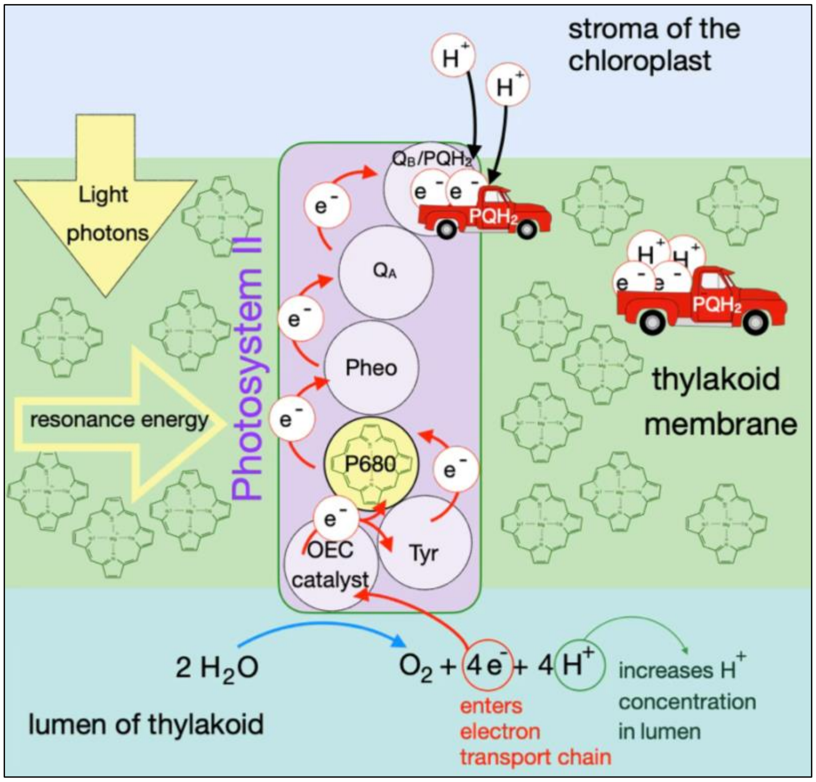
The Biosynthesis of Chlorophyll

Chlorophyll is the main pigment used by plants in photosynthesis. The biosynthesis of chlorophyll requires about 15 different enzymes to construct the molecule, and a few more to maintain it. All these enzymes need to be present and working in the same place at the same time. The place they are working in must be within a certain pH range or the enzymes will denature and no longer work. And the place must be neither too hot (high temperatures will denature them) nor too cold (low temperatures will inactivate them).

Each enzyme must be coded for in the leaf cell's DNA. The code determines how the enzyme will fold (what shape it will have). If the enzyme does not fold correctly, it does not work. The DNA in leaf cells is exposed to UV light - and UV light damages DNA. Therefore, DNA as well codes for another set of enzymes needed to repair itself. Generally, over 100 different enzymes and proteins are involved in repairing DNA. In leaf cells, this repair is continuous throughout the day. Repair can be activated in as little as two to ten minutes following damage. The DNA must recognize that it is damaged, so it codes for yet another set of enzymes and proteins to monitor itself with.

So, before a chlorophyll molecule is made, there needs to already be complex DNA in place. Creationists say this complexity requires a conscious, intelligent designer. Evolutionists say that given billions of years chemistry can produce a process of increasing complexity on its own - but we cannot carry out a scientific trial to test this theory since each trial would take billions of years.

## Photosystem II




| |
|---|
| OEC: oxygen evolving catalyst |
| Tyr: tyrosine site of a protein |
| P680: reactive pair of chlorophyll |
| Pheo: pheophytin |
| $Q_A$: plastoquinone A |
| $Q_B$: plastoquinone B |
| $PQH_2$: plastohydroquinone |

Photosystem II is actually the first photosystem used in photosynthesis. It's called "II" because it was the second photosystem to be discovered by researchers.

The special pair of chlorophyll molecules in Photosystem II[4] are named 'P680.' They are named P680 because the peak of their light absorption curve is that of wavelength 680 nm. They can absorb light directly as well as take in resonance energy from the light harvesting complex. The proteins attached to them hold them very close together, so close that they act as one unit. This allows their electrons to go up much higher in energy – to the point of one electron being released- and picked up by a nearby pheophytin molecule. So, while the chlorophyll in the light harvesting complex transfers energy, the P680 chlorophyll transfers an electron.

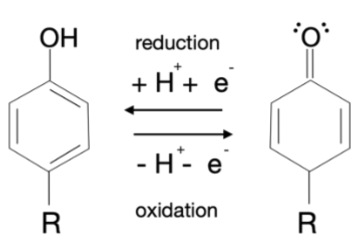
As said before, P680 can either directly take in a photon itself, or it can take in resonance energy from the light harvesting complex (LHC). The LHC can take in the energy of a wide range of wavelengths and combine those energies to meet P680's ionization energy. This is a good strategy in low light conditions. The LHC can also protect the plant from too much light energy (e.g. the intense sunlight at high noon) by dissipating unneeded light energy as heat.

[4] The first reaction centre is called Photosystem II (PSII or P680). It was the second reaction centre to be discovered and that is why it is called Photosystem II. It contains a $Mn_4CaO_5$-cluster that acts as a catalyst in the splitting of the water.

When P680 loses an electron, it becomes P680+. P680+ has such a high affinity for an electron that it can oxidize water (pull a replacement electron from a water molecule). This causes the water molecule to split apart. But P680+ won't take this electron directly because the energy release would be too great if done in a single step. Instead, it will do this in controlled steps in conjunction with a conveniently located tyrosine site of a nearby protein molecule and a powerful four-step catalyst (called the Oxygen-Evolving Complex (OEC)).

The transfer of electrons from water to P680+ happens this way:

1. The P680+ pulls an electron from (oxidizes) a tyrosine site of a nearby protein.

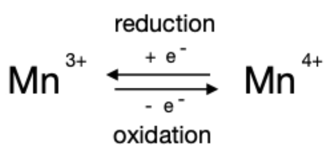



active site of a tyrosine residue

Tyrosine loses both the electron and an $H^+$ from its alcohol site (C-OH) to form a ketone (C=O).

This step is irreversible because P680+ has a higher reduction potential (greater ability to pull and hold an electron) than tyrosine.

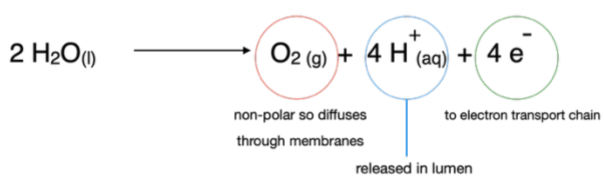
2. The oxidized tyrosine will then pull a replacement electron for itself from an Mn (manganese) ion in the catalyst (the oxygen-evolving complex (OEC)). This further oxidizes the Mn ion.




This step is irreversible because the oxidized tyrosine has a greater reduction potential (greater ability to pull and hold an electron) than the manganese ion.

The manganese catalyst (OEC) contains four manganese ions in a metal cluster (a $Mn_4CaO_5$ cluster). The Mn ions are oxidized (lose an $e^-$) in stages. This builds up the positive charge on the catalyst (OEC) in a slow and controlled way.

3. The metal cluster catalyst is held in place by several proteins. These proteins bind to the lumen side (the inside fluid) of the thylakoid membrane. When the Mn cluster is fully oxidized, it can then pull an electron from a water molecule in the lumen. The loss of this electron causes the water molecule to split into oxygen gas, free electrons and protons.

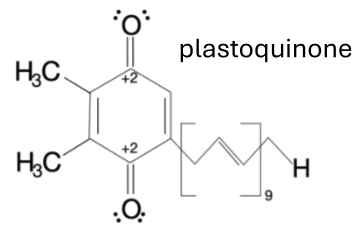


Because $O_2$ is a non-polar molecule, it easily diffuses out through the thylakoid membrane, the chloroplast membrane, the cell membrane, etc. until it passes out of the plant and into our atmosphere. Photosynthesis produces most of earth's oxygen.

The four electrons released by the two water molecules will all be taken on, one by one, by the oxidized OEC catalyst.

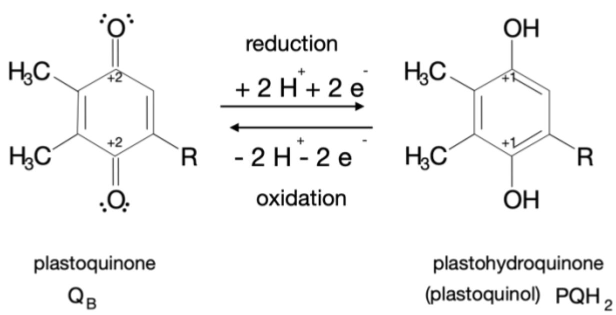
Meanwhile the ionized electron lost from P680 is passed from molecule to molecule within Photosystem II. The passage of the electron through these molecules happens this way:

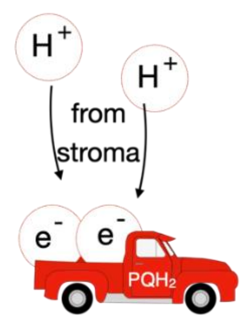
- The special chlorophyll molecule, P680, loses an electron: P680 → P680+ + $e^-$
- The ionized electron moves spontaneously to a nearby pheophytin molecule. (Pheophytin has a similar structure to chlorophyll.)
- When an atom or molecule gets an extra electron, you say it is reduced. The reduced pheophytin molecule then loses the electron to a nearby plastoquinone molecule (Plastoquinone A ($Q_A$)). Plastoquinone has a higher electron affinity (and therefore reduction potential) than pheophytin. A plastoquinone molecule will take on two electrons, one at a time – its two ketone sites (C=O) are reduced to alcohol sites (C-OH).




- The (now reduced) plastoquinone A molecule loses those two electrons to another nearby plastoquinone molecule, plastoquinone B ($Q_B$)). This happens because when $Q_B$ takes on the extra electron, it then takes it away from Photosystem II by leaving and diffusing through the thylakoid membrane.

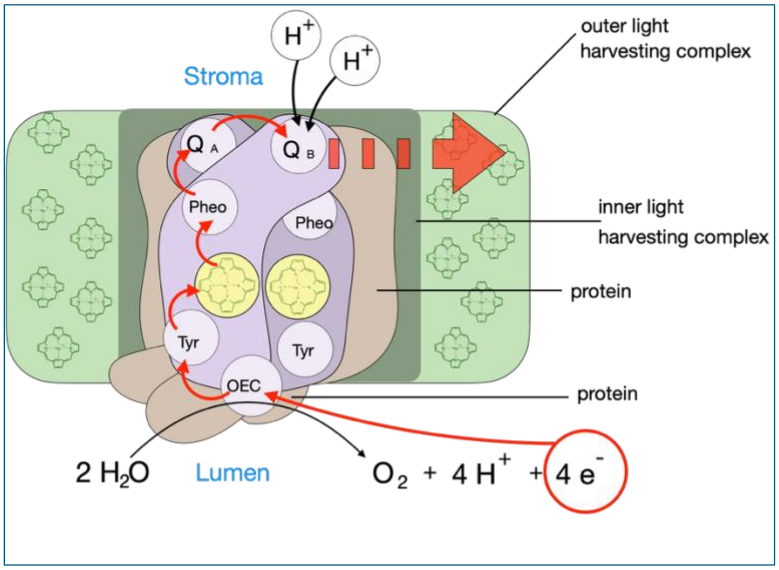
When $Q_B$ took on the two electrons (and two $H^+$ ions), its two ketone sites (C=O), became two alcohol sites (C-OH). $Q_B$ took two the $H^+$ ions from the stroma. $Q_B$ is now a mobile electron carrier with a new name; plastohyroquinone ($PQH_2$) (aka plastoquinol). Its destination is a complex called the cytochrome $b_6f$ complex.




Note also that when the plastoquinone loses its ketone groups (C=O) to alcohol (C-OH) groups, it also gains a stable aromatic ring (3 double bonds within a six-carbon ring).

More Detail on Photosystem II

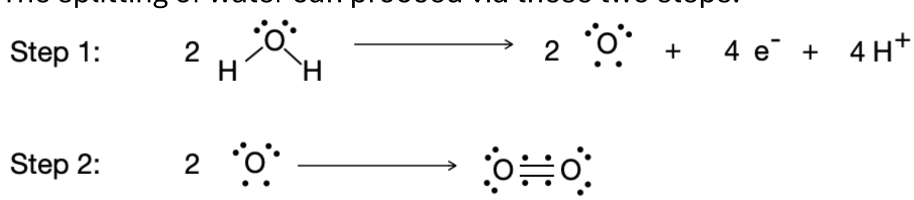



Photosystem II has two proteins in its centre. Each protein binds a copy of the electron transport chain (the tyrosine site of a protein, one of the special pairs of chlorophyll, pheophytin, and plastoquinone). There is only one copy of the OEC (4 Mn) catalyst; it's shared by both proteins.

Photosystem II is a multi-subunit enzyme and the only enzyme known in nature that can split water and release molecular oxygen[5].

## A Radical Problem for Photosynthesis

The splitting of water can proceed via these two steps:




Notice that a free oxygen atom (an oxygen radical) is produced in step 1. An oxygen radical is so reactive that it can do much damage to the protein molecules in Photosystem II (it can grab $e^-$ from the protein molecule to form $O^-$ and $O^{2-}$). The core protein in Photosynthesis II has a half-life of 8 hours because of this - and in less time when there is strong sunlight. There are enzymes present to repair and rebuild this protein (and others). But the radicals can also attack these enzymes. So, the leaf cells constantly repair or replace enzymes throughout the day. This repair is coded for by DNA, but the radicals also attack the DNA. The DNA in leaf cells is continuously repaired because of the radical damage (and also because of the damage from the UV rays of the sun).

The plant can expend the energy needed to keep up with all this repair and to keep photosynthesis under control. The oxidation of the Mn ions in the catalyst is done in

[5] Photosystem II. John Whitmarsh. Govindjee. Encyclopedia of Life Sciences. Macmillan Publishers. 2002

stages which helps slow down the production of oxygen radicals. This helps keep the oxygen radicals contained within the catalyst until $O_2$ is formed[6].

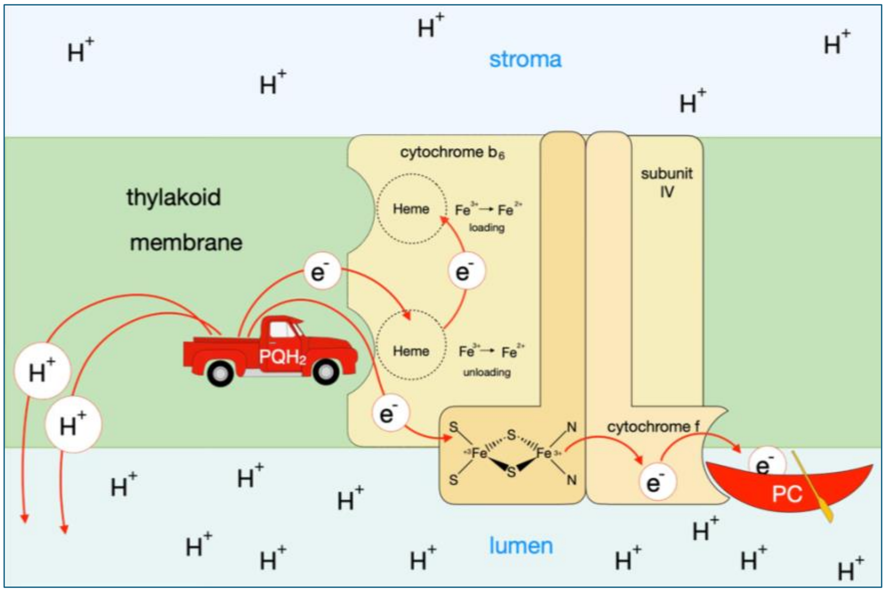
As well, there are carotenoid pigments in Photosystem II, and carotenoid pigments can bond to oxygen radicals and so keep them from reacting with other species.

On top of this, there are multiple feed-back loops in the chloroplast that shut down photosynthesis when it proceeds to quickly.

All these systems, and more, need to be in place for photosynthesis to work. If one part isn't working, photosynthesis cannot proceed. There's no model to explain how, then, plants were able to carry out photosynthesis before all this was place. An evolutionist would have to say it's a brute fact that it must have found a way. A brute fact is an unexplained scientific point that cannot be supported by any scientific explanation. Critics argue that brute facts make science and reason difficult to justify; others that there is no other rational choice but to accept their existence. The Bible supports the existence of brute facts, for example it says that God exists and created all this.

[6] For a deeper look at this, see the article, Green power (photosynthesis) by Dr. Jonathan Sarfati, published 05 July 2006 in Creation.com. https://creation.com/en/articles/gods-solar-power-plants-amaze-chemists

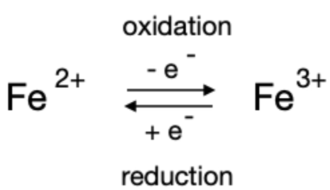
## The Cytochrome $b_6f$ Complex




The cytochrome $b_6f$ (cyt $b_6f$) complex is another multi-unit enzyme which connects Photosystem II and Photosystem I.

It has three reactive sites:

- a heme site (an iron (Fe) ion containing protein) near its lumen side (an unloading site)
- a heme (an iron (Fe) ion containing protein) near its stroma side (a loading site)
- an iron-sulfur (2Fe-2S) (the Rieske protein) cluster that juts into the lumen




The iron ions of all three sites can undergo a redox reaction between their $Fe^{3+}$ and $Fe^{2+}$ states.

### *Transfer of electrons within the cytochrome $b_6f$ complex*

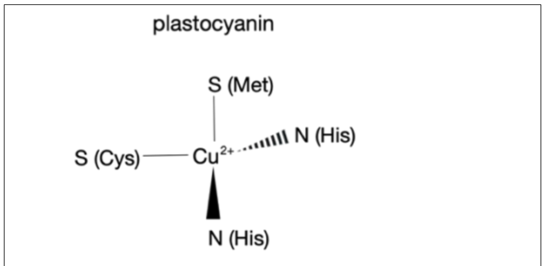
When the mobile electron transport molecule plastohydroquinone ($PQH_2$) arrives at the cyto $b_6f$ unit complex with its two electrons (which it carried as two O-H bonds), it attaches to the complex near the lumen side of the membrane. Within the $b_6f$ complex, the electrons are then transported as follows:

- The journey of the first electron from the plastohydroquinone carrier:
  - This electron transfers from the plastohydroquinone carrier ($PQH_2$) to the 2Fe-2S Rieske protein of the cyt $b_6f$ complex (that juts into the lumen) ($Fe^{3+} + e^- \rightarrow Fe^{2+}$)
  - The 2 Fe-2S Rieske protein transfers the $e^-$ to the f-unit of the cyt $b_6f$ complex ($Fe^{2+} \rightarrow Fe^{3+} + e^-$)
  - The f-unit transfers the $e^-$ to the mobile carrier plastocyanin (PC)

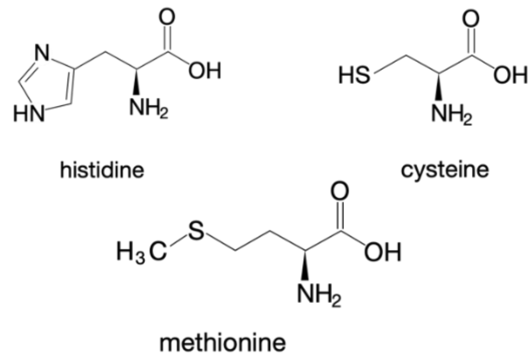
- the mobile carrier plastocyanin (PC) carries electrons away, one at a time.


Plastocyanin has a copper ion reactive site. Copper ions have two stable charges, $Cu^{+}$ (the reduced state) and $Cu^{2+}$ (the oxidized state). When the mobile carrier plastocyanin picks up an electron, its copper ion is reduced: $Cu^{2+} + e^{-} \rightarrow Cu^{+}$

The mobile carrier plastocyanin (PC) is also a small protein that is bound to polar amino acids:




Plastocyanin has a Cu ion bonded to four amino acids: two histidines, one cysteine, and one methionine.




Therefore, plastocyanin is hydrophilic (bonds with water) and because of this the carrier diffuses through the lumen.

- The journey of the second electron from the plastohydroquinone carrier:
  - It travels from the mobile carrier plastohydroquinone ($PQH_2$) to the lumen heme site of cyt $b_6$ unit ($Fe^{3+} + e^{-} \rightarrow Fe^{2+}$)
  - from the lumen heme site, it goes to the stroma heme site for further pickup

The mobile electron carrier, plastohydroquinone ($PQH_2$) also carries two $H^+$ ions (as O-H bonds). It had picked up these two $H^+$ ions from the stroma at Photosystem II. The $PQH_2$ will now unload these two $H^+$ ions into the lumen.

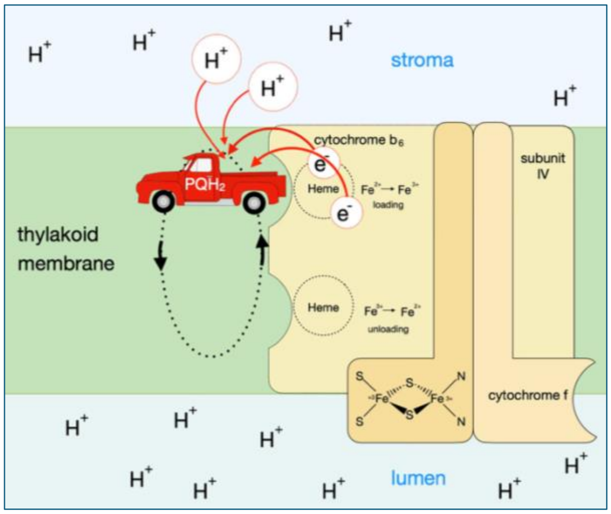
The now ‘empty’ plastohydroquinone mobile transporter (now demoted in name from plastohydroquinone to just plain plastoquinone) can:

1. diffuse back to Photosystem II to pick up another two electrons (and another two $H^+$ ions from the stroma)

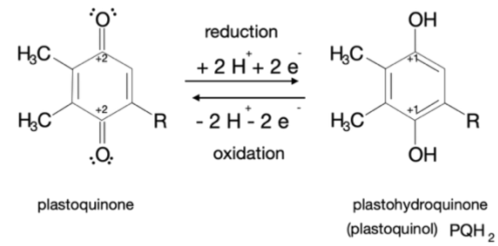
or

2. diffuse up to the stroma heme site of the cyto $b_6f$ complex to pick up that electron it so recently dropped off (and so enter the Q-cycle of the cyto $b_6f$ complex)

### *The Q-Cycle*




When the 'empty' mobile transporter (plastoquinone) diffuses upwards to the stroma (loading) heme site of the cyt $b_6f$ complex, it will take two electrons[7] from cyt $b_6f$. It will also take two $H^+$ ions from the stroma. It again combines the two electrons and two $H^+$ ions into an O-H bond:




And so, the carrier is reduced again to plastohydroquinone (plastoquinol). The mobile carrier plastohydroquinone ($PQH_2$) can then diffuse back down to the two lumen reactive sites. There it will again unload two electrons to cyt $b_6f$ and release the two new stroma $H^+$ ions into the lumen.

In this way, the cyt $b_6f$ centre acts as a proton pump moving $H^+$ ions from the stroma to the lumen. This creates a greater concentration of $H^+$ ions in the lumen. And this creates an $H^+$ concentration gradient across the thylakoid membrane. Subunit IV of the cyt $b_6f$ complex is a catalyst in this reaction.

The Proton Motive Force

The mobile electron carrier, plastohydroquinone ($PQH_2$), picks up two electrons and two $H^+$ ions by reducing its two ketone (C=O) sites into two alcohol (C-OH) sites. It first did this at Photosystem II and then does it again in the Q-cycle of cyt $b_6f$. Both times it picked up $H^+$ ions from the stroma and released them into the lumen. (The two electrons it picked up from Photosystem II (from the splitting of water) it deposits in the cyt $b_6f$ complex; the two electrons it picked up in the Q-cycle of cyt $b_6f$ it returns to cyt $b_6f$.)

In this way for every full cycle of the Q-cycle, four $H^+$ ions are carried from the stroma to the lumen. This happens in milliseconds. In this way a significant difference in pH (a measure of $H^+$ concentration) is built up between the stroma and the lumen. During the light-dependent reactions of photosynthesis, typical values for pH are ~8.0 in the stroma and ~5.0 in the lumen. This creates a charge difference called an electrochemical

[7] Each $PQH_2$ carrier deposits two electrons to the cytochrome $b_6f$ complex. These two electrons take different paths: one goes to the 2Fe-2S site and on to plastocyanin; the other goes up to the stroma heme site. Therefore, it takes two $PQH_2$ carriers to provide two electrons to the stroma heme site. And therefore, it takes two $PQH_2$ carriers to complete one full Q-cycle.

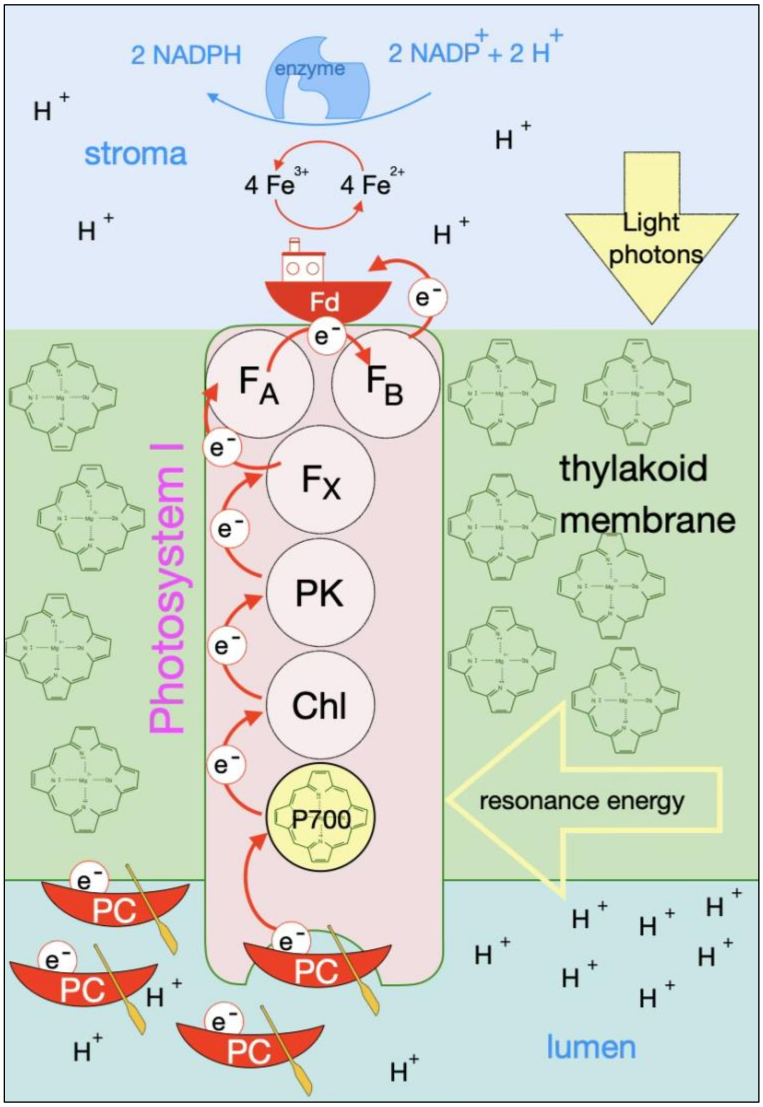
gradient. Because the gradient is created by a difference in $H^+$ ions, it is called the proton motive force.

The voltage (electric potential difference) across the thylakoid membrane in land plants typically ranges between 20 and 100 mV during active photosynthesis[8]. The electric potential energy across the thylakoid membrane, then, is a storage battery capable of doing energy-requiring biological work. The work that this proton-motive force accomplishes is the formation of ATP (see section on ATP synthase).

Therefore, the Q-cycle is essentially an engine for the synthesis of ATP. ATP is the universal 'energy currency' that powers functions essential to life.

[8] Modeling the light-induced electric potential difference ΔΨ across the thylakoid membrane based on transition state rate theory. Hui Lyu, Dušan Lazár. Biochimica et Biophysica Acta (BBA) bioenergetics. Volume 1858, Issue 3, March 2017, Pages 239-248

## Photosystem I [9] (and the formation of NADPH):




| | |
|---|---|
| PC: plastocyanin | |
| P700: reactive pair of chlorophyll molecule | |
| Chl: modified chlorophyll molecule | |
| PK: phylloquinone | |
| $F_X$: 1st iron-sulfur complex | |
| $F_A$: 2nd iron-sulfur complex | |
| $F_B$: 3rd iron-sulfur complex | |
| Fd: ferredoxin | |
| Enzyme: ferredoxin-NADP$^+$ -reductase | |

The mobile electron carrier, plastocyanin, which arrived via the thylakoid's fluid lumen, docks at a positively charged binding site on the lumen side of Photosystem I. After unloading the electron ($Cu^+ \rightarrow Cu^{2+} + e^-$), the 'empty' plastocyanin molecule (now oxidized to $Cu^{2+}$) can then diffuse back to the (cyt $b_6f$) complex and pick up another electron (and so be reduced again to $Cu^+$).

Photosystem I is much like Photosystem II. Comparing them:

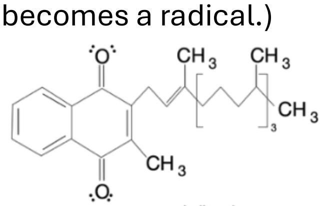
| Photosystem II | Photosystem I |
|---|---|
| is surrounded by a light-harvesting complex of pigments (chlorophyll and carotenoids) bound to proteins | is surrounded by a light-harvesting complex of pigments (chlorophyll and carotenoids) bound to proteins |

[9] The second reaction centre is called Photosystem I (PSI or P700). It was the first reaction centre to be discovered and that is why it is called Photosystem I.

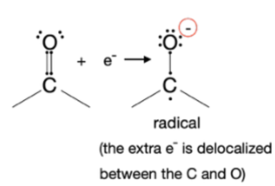
| | |
|---|---|
| has resonance energy from the pigments transferred to its special pair of chlorophyll molecules | has resonance energy from the pigments transferred to its special pair of chlorophyll molecules |
| its special pair of chlorophyll can also absorb light directly | its special pair of chlorophyll can also absorb light directly |
| Its special pair of chlorophyll is called P680 because it best absorbs light wavelengths of 680 nm | Its special pair of chlorophyll is called P700 because it best absorbs light wavelengths of 700 nm |
| The absorbed energy causes an electron in one of the special pairs to ionize (leave the molecule) P680 → P680+ + $e^-$ | The absorbed energy causes an electron in one of the special pairs to ionize (leave the molecule) P700 → P700+ + $e^-$ |
| P680+ replaces its lost electron with one pulled (along an electron transfer chain) from water. This resulted in the splitting of the water molecule. | P700+, replaces its lost electron with the one from the mobile electron carrier plastocyanin. |
| The ionized electron from P680+ is passed on through a chain of molecules (electron transport chain) within the photosystem to the mobile electron carrier, plastohydroquinone ($PQH_2$). | The ionized electron from P700+ is passed on through a chain of molecules (electron transport chain) within the photosystem to the mobile electron carrier, ferredoxin Fd. |
| The mobile carrier $PQH_2$ is lipophilic (bonds with lipids) and diffuses via the thylakoid membrane. | The mobile carrier Fd is hydrophilic (bonds with water) and diffuses via the stroma. |

*The Electron Transport Chain within Photosystem I* is as follows:

- Light provides the energy to ionize an electron from P700. This oxidizes it to P700+: P700 → P700+ + $e^-$
- The electron from the oxidized P700+ molecule is taken by a nearby modified chlorophyll molecule. This reduces the chlorophyll molecule. (P700+ takes its replacement $e^-$ from the mobile carrier PC)
- The reduced modified chlorophyll molecule then loses an electron to another molecule (with a higher reduction potential), phylloquinone. Phylloquinone (PK) is now reduced. (Unlike plastoquinone, phylloquinone will only take on one $e^-$ and becomes a radical.)



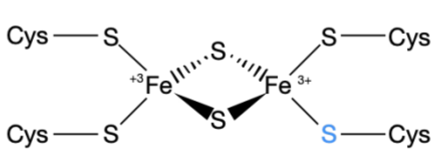

phylloquinone



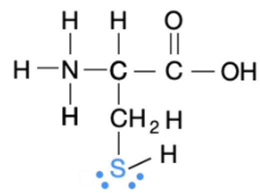

- The reduced phylloquinone molecule ($PK^-$) loses an electron to a protein containing a 4Fe-4S iron-sulfur cluster ($F_X$) ($Fe^{3+}$ + $e^-$ → $Fe^{2+}$); $F_X$ loses an electron to a second protein with an iron-sulfur cluster ($F_A$); and $F_A$ loses an electron to a third protein with an iron-sulfur cluster ($F_B$). Each of these three iron-sulfur cluster proteins has a higher reduction potential than the one before it.

- The final iron-sulfur cluster protein passes the electron to the mobile electron carrier ferredoxin (Fd). Ferredoxin docks on the stroma side of the thylakoid membrane.

  Ferredoxin is a small protein with a 2Fe-2S cluster. One of its $Fe^{3+}$ ions is reduced to an $Fe^{2+}$ ion when it accepts an electron. The cluster is attached to four cysteine amino acids. Since cysteine is polar, ferredoxin is soluble in the stroma and diffuses through the stroma.

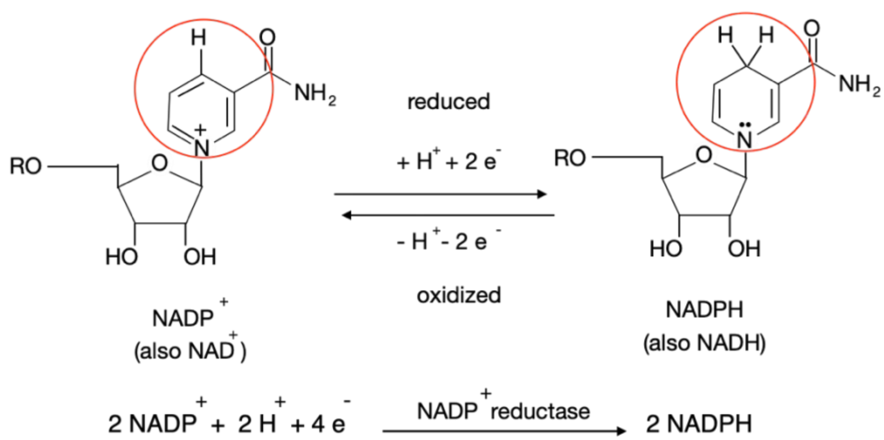



ferredoxin




cysteine

The electrons are now through Photosystem I. The mobile electron carrier ferredoxin can carry an electron to the enzyme ferredoxin-NADP$^+$-reductase[10] which is also in the stroma. This enzyme catalyzes molecules of NADP$^+$(which are present in the stroma) to pick up 2 $e^-$ (one at a time) and be reduced to NADPH.




NADPH is in turn a good reducing agent. It has a low reduction potential, so it will easily lose its electron to another molecule with a higher reduction potential. Its ability to reduce

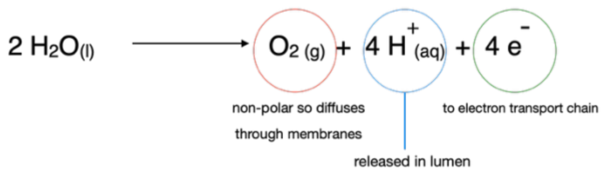
[10] In the dark the enzyme ferredoxin-NADP+-reductase binds to the thylakoid membrane via specific proteins. In the light the enzyme may leave the membrane and float in the stroma to operate as a soluble enzyme. Reference: Chloroplast-targeted ferredoxin-NADP+ oxidoreductase (FNR): Structure, function and location. Paula Mulo. Biochimica et Biophysica Acta (BBA) – Bioenergetics. Volume 1807, Issue 8, Pages 927-934

other biomolecules is needed in hundreds of biosynthesis reactions that proceed by reduction-oxidation. Notice that the NADPH is released into the stroma of the chloroplast.

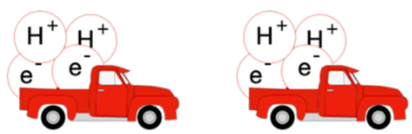
### *The Linear Electron Transport Chain*

The linear electron transport chain follows electrons that travel linearly from the splitting of water in Photosystem II to the formation of NADPH in Photosystem I (without going through the Q-cycle). To summarize it:

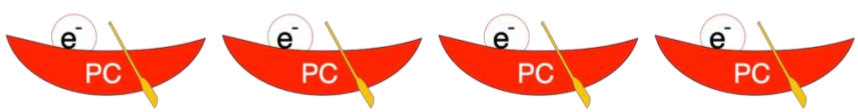
- In Photosystem II two molecules of water are split, releasing four electrons.



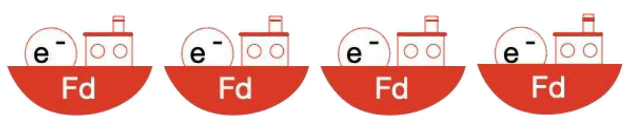

  The 4 $e^-$ are transported through a chain of molecules in Photosystem II to the mobile electron carrier, plastohyroquinone ($PQH_2$). $PQH_2$ carries the electrons two at a time (along with 2 $H^+$ from the stroma) to the cytochrome $b_6f$ (cyt $b_6f$) complex. Two $PQH_2$ molecules are needed to transport all 4 $e^-$ from water.




- At the cyt $b_6f$ complex, the $e^-$ are transported within the complex to the mobile electron transporter plastocyanin. Plastocyanin carries the electrons one at a time to Photosystem I (with no need of an $H^+$ ion).




- At Photosystem I, the electrons are transported within a chain of molecules to the mobile carrier, ferredoxin.




Ferredoxin carries the electrons to the enzyme ferredoxin-$NADP^+$ reductase which uses two electrons to reduce $NADP^+$ to NADPH. Using all four electrons will require two molecules of $NADP^+$.

$$2\ NADP^+ + 2\ H^+ + 4\ e^- \xrightarrow{NADP^+\ reductase} 2\ NADPH$$

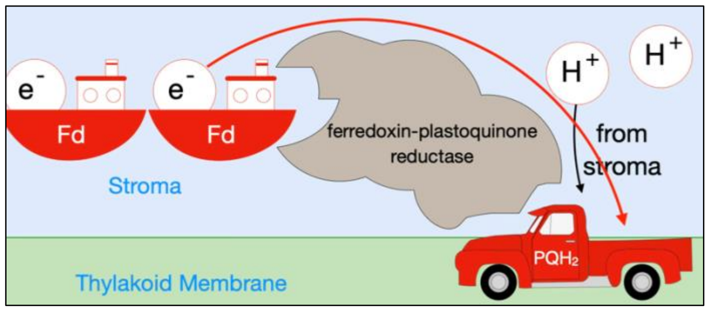
Therefore, for the linear electron flow from Photosystem II to the cytochrome $b_6f$ complex to Photosystem I, the stoichiometry would be:

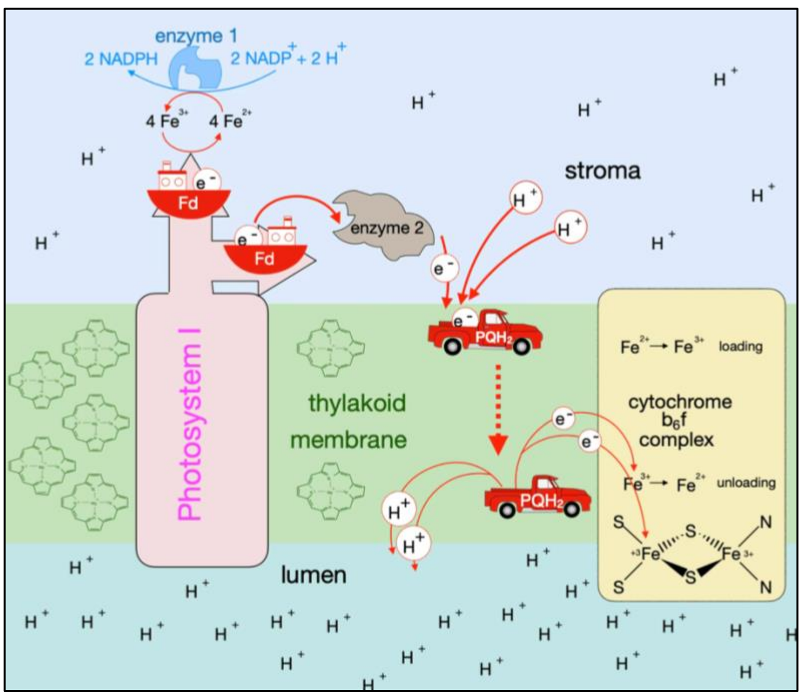
$$2\ H_2O_{(l)} + 2\ NADP^+_{(aq)} \longrightarrow O_{2(g)} + 2\ NADPH_{(aq)} + 2\ H^+_{(aq)}$$

However, during the light-dependent reaction, the plant is actually simultaneously carrying out more than just the linear electron transport chain. Remember that the cytochrome $b_6f$ complex also diverts electrons in its Q-cycle to increase the hydrogen gradient across the thylakoid membrane. Another diversion of electrons takes place via Cyclic Electron Flow.

### *Cyclic Electron Flow*

The mobile electron carrier ferredoxin can also carry an electron from Photosystem I to different enzyme, ferredoxin-plastoquinone reductase. The enzyme ferredoxin-plastoquinone reductase (enzyme 2) is also found in the stroma. This enzyme will transfer electrons from the mobile carrier ferredoxin (Fd) (in the stroma) to the mobile carrier plastoquinone (in the thylakoid membrane.




The mobile carrier plastoquinone takes on two electrons from the enzyme (one at a time) as well as two $H^+$ ions from the stroma to again become the carrier plastohydroquinone ($PQH_2$).

The now fully loaded carrier $PQH_2$ diffuses to the cytochrome $b_6f$ complex which takes the two electrons; and releases the two stromal $H^+$ ions into the lumen. (The electrons can be cycled back to Photosystem I.) Thus the cyclic electron flow also increases the concentration of $H^+$ in the lumen, thus increasing the electrical potential difference across the thylakoid membrane.

## The Indispensable Phylloquinone

Phylloquinone is a crucial electron carrier within Photosystem I. Its synthesis is coded for in a plant's nuclear DNA via eight or more enzymes[11]. Research shows that plants that have mutations in this code, and therefore have decreased phylloquinone synthesis, will have severely reduced rates of photosynthesis. Plants lacking phylloquinone die shortly after germination. Therefore, it's believed that the very first plants must have already had phylloquinone.

Evolution claims plants evolved from green algae which already had phylloquinone. But the algae also need phylloquinone for photosynthesis. How did the DNA of green algae come to code for all 8 enzymes and possibly more needed to synthesize phylloquinone? The probability that a random sequence of DNA will code for one functional average-sized enzyme is estimated[12] to be around 1 in $10^{77}$. The odds are this low because proteins must fold in a particular pattern to be functional and the code for folding is highly specific. Because of that, enzymes are statistically unlikely to exist.

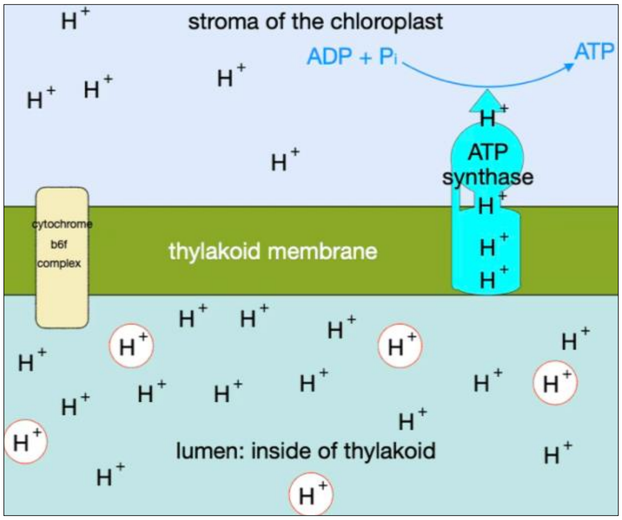
As said before, it's a brute fact that enzymes do exist. But why is it considered more intelligent to believe that time and chemistry as the brute force, rather than God? If we see a car, we automatically posit a manufacturer; but whereas man is thought capable of design and manufacture, God is not.

[11] Phylloquinone biosynthesis. Regulation of plant primary metabolism. Phylloquinone. Science Direct. https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/phylloquinone

[12] Estimating the prevalence of protein sequences adopting functional enzyme folds. Douglas D Axe. J Mol Biol. 2004 Aug 27. https://pubmed.ncbi.nlm.nih.gov/15321723/

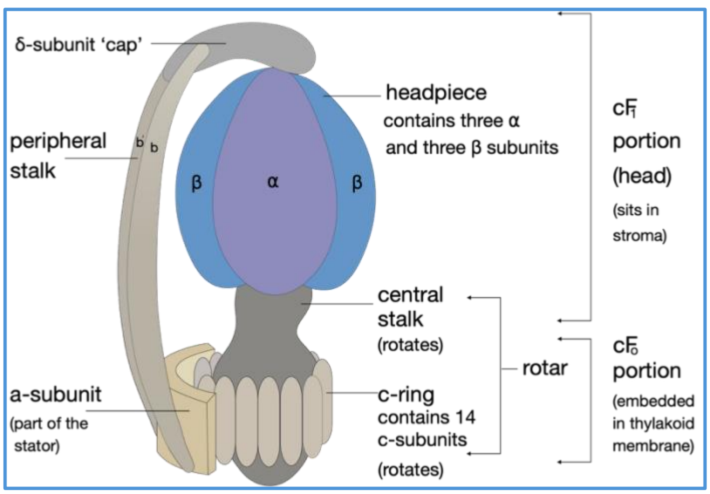
## Chloroplast ATP Synthase

We've already seen that the light-dependent steps create an $H^+$ gradient – and therefore an electrochemical gradient– across the thylakoid membrane. Because of this gradient, $H^+$ ions will spontaneously flow from the lumen back to the stroma, but they cannot diffuse through the non-polar lipid thylakoid membrane on their own. If there were an open channel through that membrane, they could rush through it.



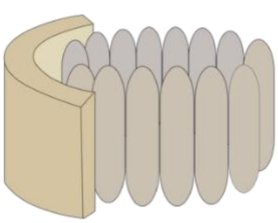

Sitting within the thylakoid membrane is the enzyme ATP synthase. Its 'bottom' half reaches the lumen and its 'top' half reaches the stroma. The enzyme contains two half-channels.

To understand how ATP synthase works will take the more complex diagram on the left. (This is still a simplified diagram of the actual ATP synthase.)



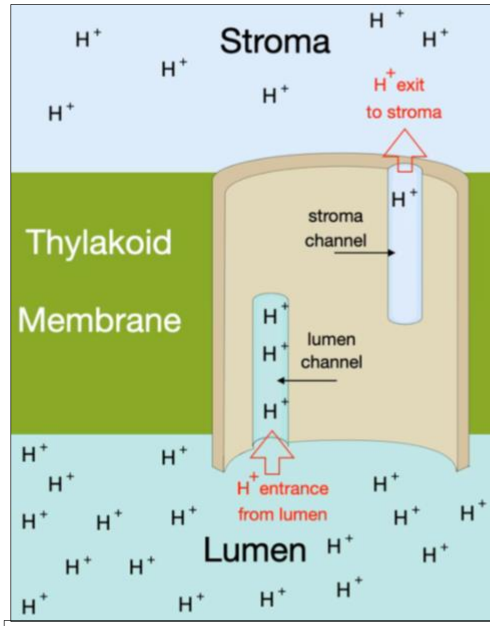

The portion of the enzyme that sits within the thylakoid membrane and down into the lumen is called the $F_o$ portion. The portion that sits up in the stroma is the $F_1$ portion.

<u>How the c-Ring Rotates</u>



In the $F_o$ portion are two units that rotate, the c-ring and the central stock. The rest of the units in ATP synthase remain stationary. Notice how the rotating c-ring is positioned against the stationary a-subunit. There is a chemical interaction between the c-ring and the a-subunit that will cause the c-ring to rotate.

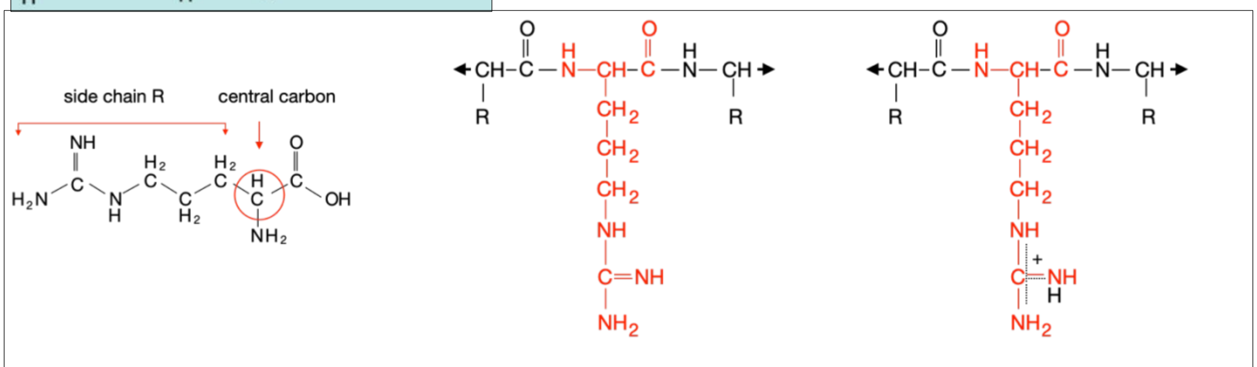
The a-subunit spans the thylakoid membrane; it touches both the lumen and the stroma.




At the lumen there is a half-channel that goes partway up; lumen enters this channel. At the stroma there is a second half-channel that goes partway down; stroma enters this channel.

Notice that $H^+$ ions enter the a-subunit from the lumen into the lumenal half-channel; $H^+$ ions exit the a-subunit into the stroma from the stromal half-channel. But the two half-channels do not connect with each other.

Sitting on the inside of the a-subunit, between the two half-channels, is a positively charged arginine residue (a residue is what you call an amino acid that has been incorporated into a protein chain).

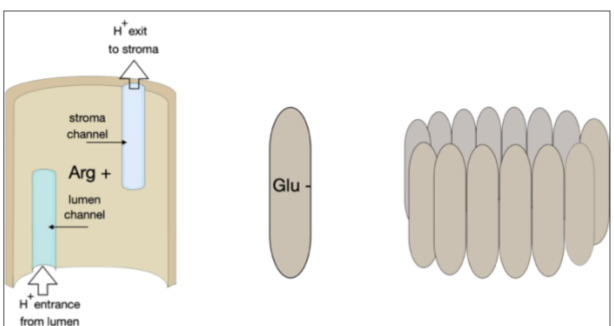



the amino acid arginine

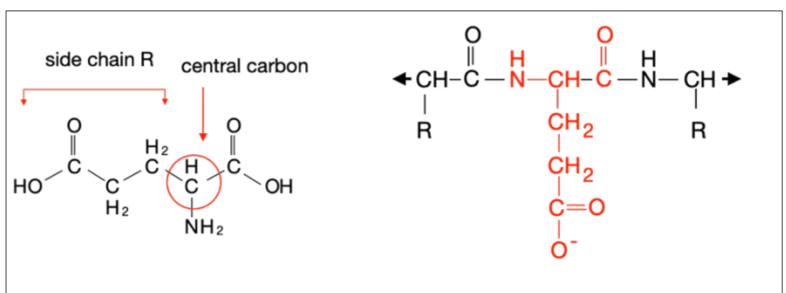
an arginine residue within a protein chain
The residue is neutral.

the addtion of $H^+$ to the arginine residue
The positive charge is shared among three nitrogens.

At the same time, sitting in the middle of each of the 14 c-subunits of the c-ring is a negatively charged glutamate residue:

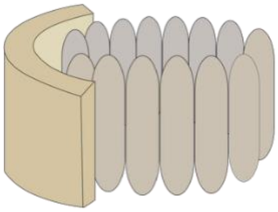



a-subunit c-subunit c-ring




the amino acid glutamate

a glutamate residue within a protein chain
This is what is in the middle of each c-subunit
The residue has a negative charge.



The a-subunit wraps around only a portion of the c-ring. The c-ring fits into the a-subunit in such a way that only some of its c-subunits (with their negative glutamate residue) are in direct contact with the positive arginine residue of the a-subunit as well as the positive charge (from the H+ ions) in the lumenal half-channel.

The sub-units of the c-ring do not have a membrane. That means an $H^+$ ion from the lumenal half-channel is free to bond with the negative glutamate site on the c-subunit that is directly in front of it. It does so, and the negative glutamate site now becomes neutral.

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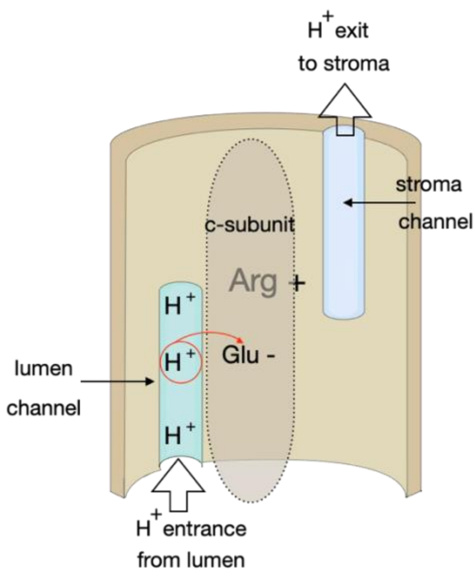



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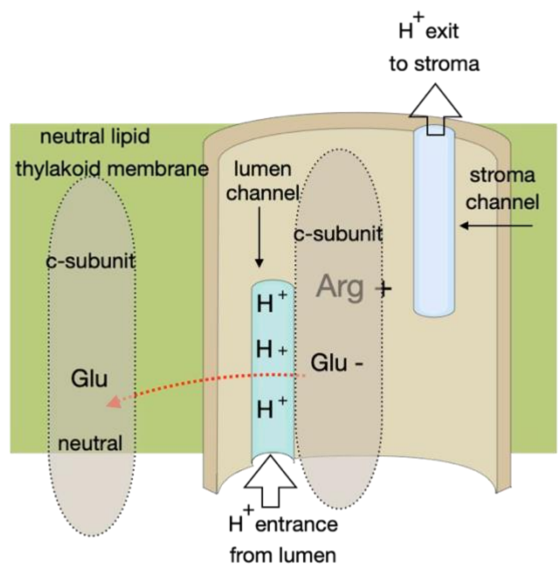



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The c-subunit that is in front of the lumenal half-channel contains a negatively charged glutamate residue. The lumen has a high concentration of $H^+$ ions. Since the c-subunit does not have its own membrane around it, an $H^+$ ion can freely move from the lumen half-channel to bond to the negative glutamate residue:

$$\text{Glu-COO}^- + H^+ \rightarrow \text{Glu-COOH}$$

The glutamate residue is now neutral, and so this c-subunit – which we will name 'Charlie' is now neutral.

</td>
<td>

Once Charlie is neutral, 'he' is no longer strongly attracted to the $H^+$ surplus in the lumen channel.

But the c-subunit to Charlie's right still has its negatively charged glutamate residue, and so is being pulled to its left by the attraction of the high number of $H^+$ ions in the lumenal half-channel.

At the same time, neutral Charlie is being pulled to 'his' left by attraction to the neutral lipid thylakoid membrane.

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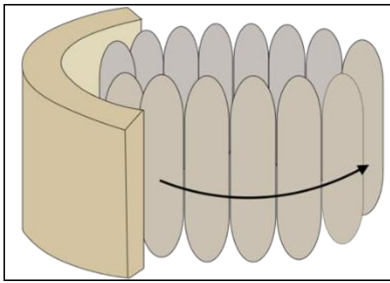
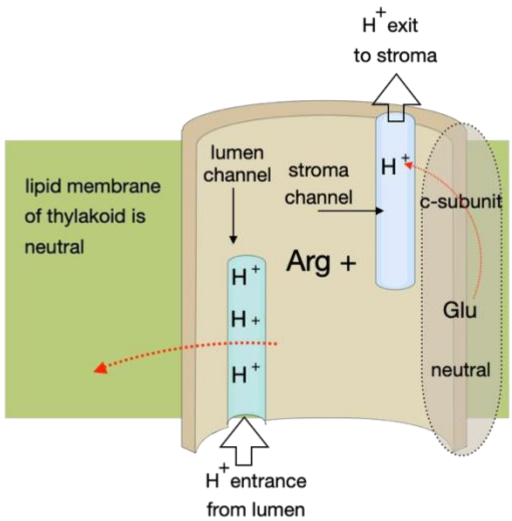
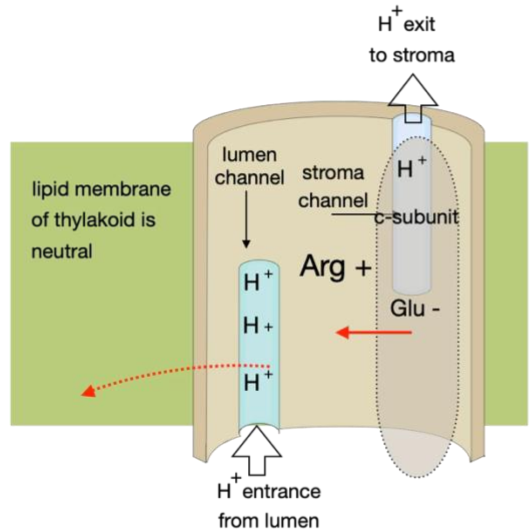


Because all the c-units are bound together and are free to move, the ring rotates left (counterclockwise).




The counterclockwise rotation of the c-ring will bring neutral Charlie back to the stationary a-subunit. Here neutral Charlie will come in front of the stromal half-channel. The stroma has so few $H^+$ ions in it that its pH is alkaline.

The alkaline stroma will pull the $H^+$ ion from the neutral glutamate residue. The free $H^+$ ion will then diffuse up through the stromal channel and into the stroma.

The glutamate residue has a low $k_a$ (it only weakly dissociates (breaks apart) to release the $H^+$. But the pull of the alkaline stroma and the immediate loss of that $H^+$

Once neutral Charlie's glutamate residue has lost its $H^+$ ion, that glutamate residue returns to being negatively charged.

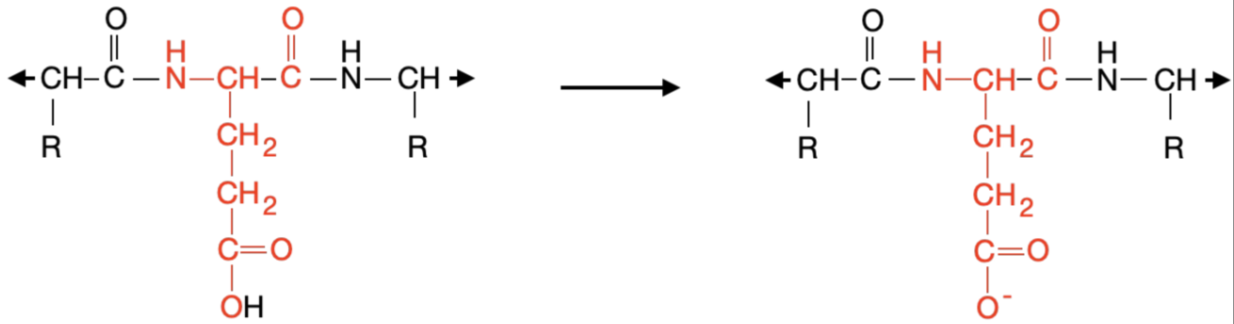
Negative Charlie is now attracted to the positively charged arginine residue ($Arg^+$)[13] in the center of the a-subunit. It is also attracted to the positive charge of the high $H^+$ concentration of the lumenal half-channel. So, Charlie moves left.

And so, the ring goes round and round proceeding counterclockwise at 160 revolutions[14] per second.

[13] The positively charge arginine residue has another function as well. Its positive charge repels the $H^+$ ions in the lumen half-channel. This keeps the $H^+$ ions from sliding along the inner surface of the a-subunit from the lumen half-channel to the stroma half-channel and so short-circuiting the system.

[14] Plant biochemistry Sixth Edition. Birgit Piechulla, Hans-Walter Heldt. Academic Press. 2026. P. 121

| | |
|---|---|
| ion to the stroma drives the reaction to the right.<br><br>Glu-COOH ⇌ Glu-COO$^-$ + H$^+$ | |

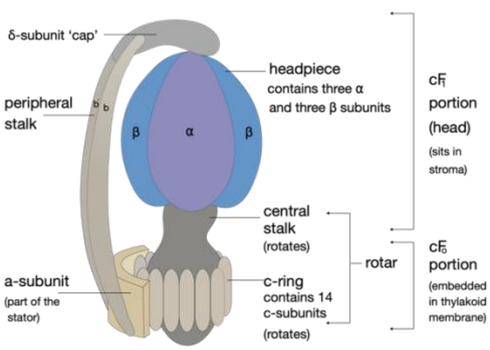


the addtion of H$^+$ to the glutamate residue at the lumenal half-channel

the loss of H$^+$ from the glutamate residue at the stromal half-channel

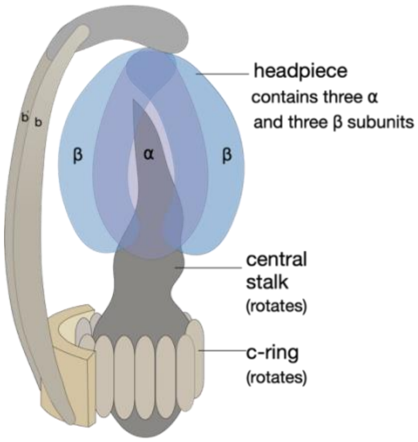
<u>How the Headpiece Works</u>

The rotating c-ring turns the central stock within it. While the c-ring and central stalk rotate (counterclockwise), the a-subunit, peripheral stalk and δ-subunit cap remain stationary. This keeps the headpiece from rotating as well.




But the rotational energy of the c-ring and stalk cannot just disappear. As the central stalk rotates, it puts pressure on the α- and β-subunits of the stationary headpiece – and that pressure changes the shape of those subunits. Now those subunits are enzymes.

Note that the central stalk has an asymmetric shape. As it rotates, it therefore puts asymmetric pressure on the subunits of the headpiece.




The rotation of the asymmetric stalk causes the shape of the α- and β-subunit enzymes to undergo three different shapes during each central stalk rotation. (Talk about shape shifters!) Their first shape change causes the enzyme subunits to bond to both ADP and Pi (inorganic phosphate); the second shape change causes them to squish ADP and Pi together; the third shape change causes them to release the ATP into the stroma.

One complete rotation of the c-ring produces 3 ATP molecules. The ATP is released[15] into the stroma of the chloroplast.

$$ADP + Pi \xrightarrow{\text{ATP synthase}} ATP$$

Feedback Loops

ATP synthase functions as a nano-turbine driven by the flow of $H^+$ ions. Since that $H^+$ gradient is produced by light-dependent reactions, at night when the $H^+$ gradient across the thylakoid membrane drops, the nano-turbine will stop. But if the $H^+$ gradient becomes too low, there's a possibility that $H^+$ ions could flow backwards from the stroma to the lumen. That would turn the stalk and c-ring clockwise. And that would drive the synthesis of ATP in reverse. To prevent this, a drop in $H^+$ concentration in the lumen will trigger a set of reactions that deactivate the α- and β-subunit enzymes of the headpiece.

As well, the plant has several other mechanisms in place to match the rate of photosynthesis to changing light conditions. These mechanisms can slow down or speed up the rate of synthesis within seconds to minutes[16].

The plant also can switch the tracks of the electron transport chain to adjust its output ratio of ATP to NADPH. The output ratio from the linear electron flow would be about 1.5

[15] The headpiece subunits continuously go through three alternating shape changes that cause them to trap the ADP and Pi, squish them together to form ATP, and open up to release the ATP to the stroma.

[16] Allen Derks, Kristin Schaven, Doug Bruce,
Diverse mechanisms for photoprotection in photosynthesis. Dynamic regulation of photosystem II excitation in response to rapid environmental change, Biochimica et Biophysica Acta (BBA) - Bioenergetics, Volume 1847, Issues 4–5,2015, Pages 468-485,

molecules of ATP to every 1 molecule of NADPH[17]. But in the second phase of photosynthesis, the Calvin cycle, 3 ATP are needed for every two NADPH. To meet this need, the photosynthetic pathway includes the Q-cycle provided by the cytochrome $b_6f$ complex and cyclic electron flow provided by Photosystem I.

We can appreciate that the bioengineering involved in the light-dependent stage of photosynthesis is ingenious.

Many people, when drawing a cartoon diagram of ATP synthase like to change the stalk to a more pleasing symmetrical cylinder. Yet the production of ATP depends on the stalk's asymmetric shape. So, you can trust in the same designer when life doesn't shape up according to your sense of aesthetics.

This is the end of the light-dependent stage of photosynthesis. The ATP and NADPH produced by it will now be used in the biosynthesis of many biomolecules.

[17] Plant Biochemistry. Sixth Edition. Birgit Piechulla. Hans-Watler Heldt. Academic Press. P. 66
Also: The Importance of Energy Balance in Improving Photosynthetic Productivity. David M. Kramer, John R. Evans. National Library of Medicine. Plant Physiol. 2010 Nov 15; 155(1):70-78