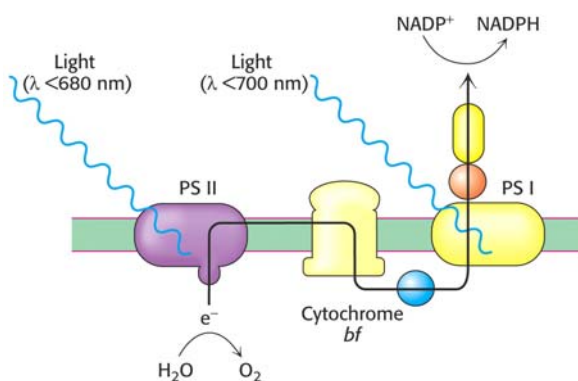


Photosystems I and II

March 17, 2003

Bryant Miles



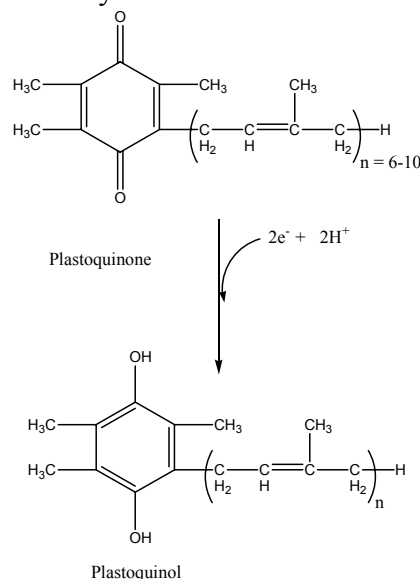
Within the thylakoid membranes of the chloroplast, are two photosystems. Photosystem I optimally absorbs photons of a wavelength of 700 nm. Photosystem II optimally absorbs photons of a wavelength of 680 nm. The numbers indicate the order in which the photosystems were discovered, not the order of electron transfer. Under normal conditions electrons flow from PSII through cytochrome *bf* (a membrane bound protein analogous to Complex III of the mitochondrial electron transport chain) to PSI.

Photosystem II uses light energy to oxidize two molecules of water into one molecule of molecular

oxygen. The 4 electrons removed from the water molecules are transferred by an electron transport chain to ultimately reduce 2NADP^+ to 2NADPH . During the electron transport process a proton gradient is generated across the thylakoid membrane. This proton motive force is then used to drive the synthesis of ATP. This process requires PSII, cytochrome *bf*, ferredoxin- NADP^+ reductase and chloroplast ATP synthase.

I. Photosystem II

Photosystem II transfers electrons from water to plastoquinone and in the process generates a pH gradient.

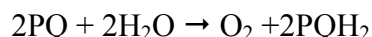


Plastoquinone (PQ) carries the electrons from PSII to the cytochrome *bf* complex. Plastoquinone is an analog of Coenzyme Q. The only differences are the methyl groups replacing the methoxy groups of Q and a variable isoprenoid tail. Plastoquinone can function as a one or two electron acceptor and donor. When it is fully reduced to PQH_2 it is called plastoquinol. Like CoQ, PQ is a lipophilic mobile electron carrier carrying electrons from PSII to cytochrome *bf*.

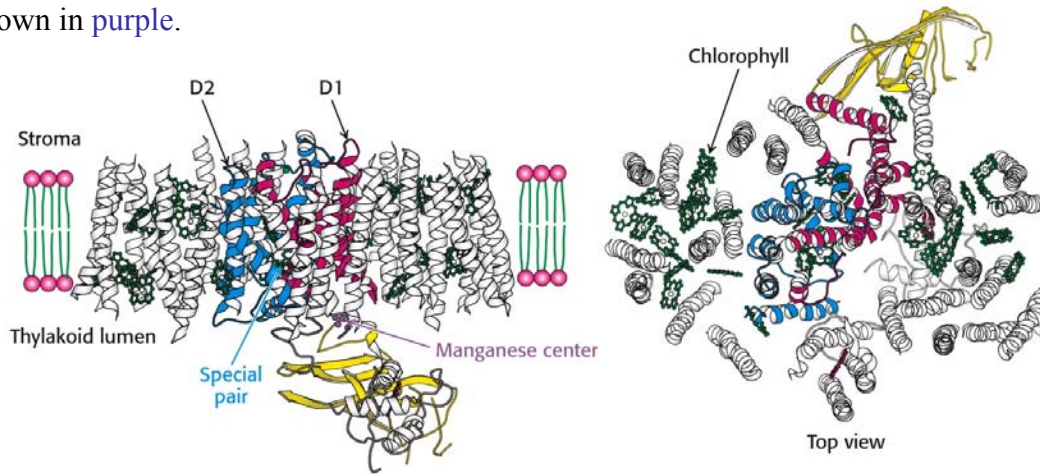
Photosystem II is homologous to the purple bacterial photoreaction center we talked about previously. PSII is an integral membrane protein. The core of this membrane protein is formed by two subunits D1 and D2. These two subunits span the membrane and are homologous to subunits L and M of the bacterial photosystem. Of course PSII is more complicated than its prokaryotic counterpart. PSII contains a lot more subunits and additional chlorophylls to achieve a lot

higher efficiency than bacterial systems.

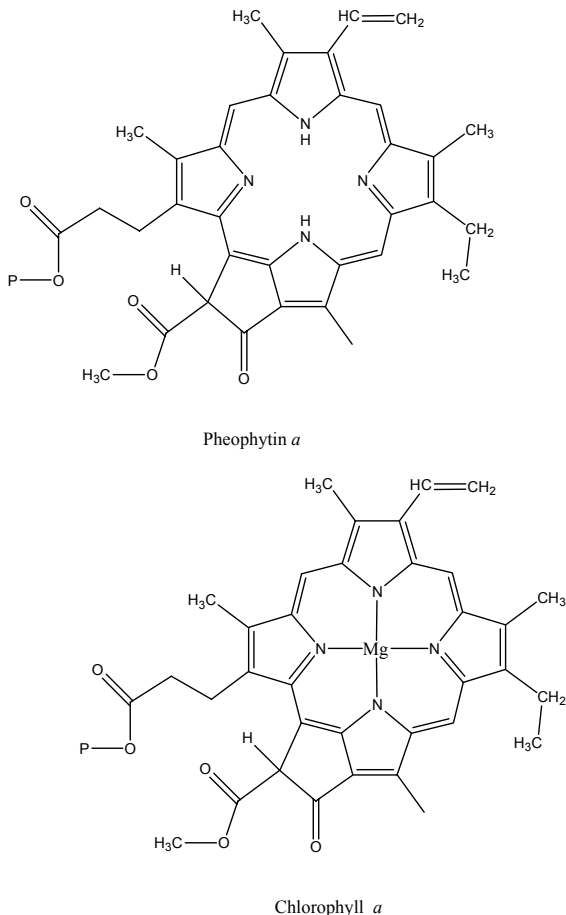
The overall reaction of PSII is shown below.



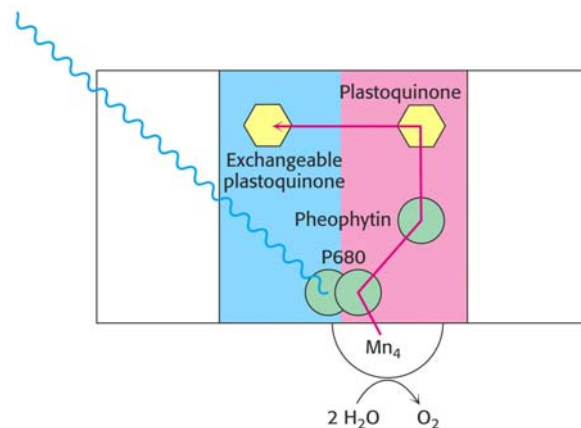
The ribbon diagram of the crystal structure of PSII is shown below. The D1 subunit is shown in red and is homologous to the L subunit of the bacterial photosystem. The D2 subunit is shown in blue and is homologous to the M subunit of the bacterial photosystem. The structure of a bound cytochrome molecule is shown in yellow. The chlorophyll molecules are shown in green. The manganese center is shown in purple.



Just as in the bacterial photosystem, there is a special pair of chlorophylls in PSII bound by D1 and D2 that are in close proximity of each other. This special pair is analogous to the special pair of bacteriochlorophylls in the bacterial photosystem. The PSII special pair consists of 2 chlorophyll *a* molecules that absorb light at an optimal wavelength of 680 nm. This special pair of chlorophylls is called P680.

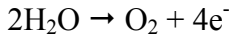


On excitation—either by the absorption of a photon or exciton transfer—P680* rapidly transfers an electron to a nearby pheophytin *a*. Pheophytin *a* is a chlorophyll *a* molecule with the Magnesium replaced by two protons. The electron is then transferred to a tightly bound plastoquinone at the Q_A site. The electron is then transferred to an exchangeable plastoquinone located at the Q_B site of the D2 subunit. The arrival of a second electron to the Q_B site with the uptake of two protons from the stroma produces plastoquinol, PQH₂.

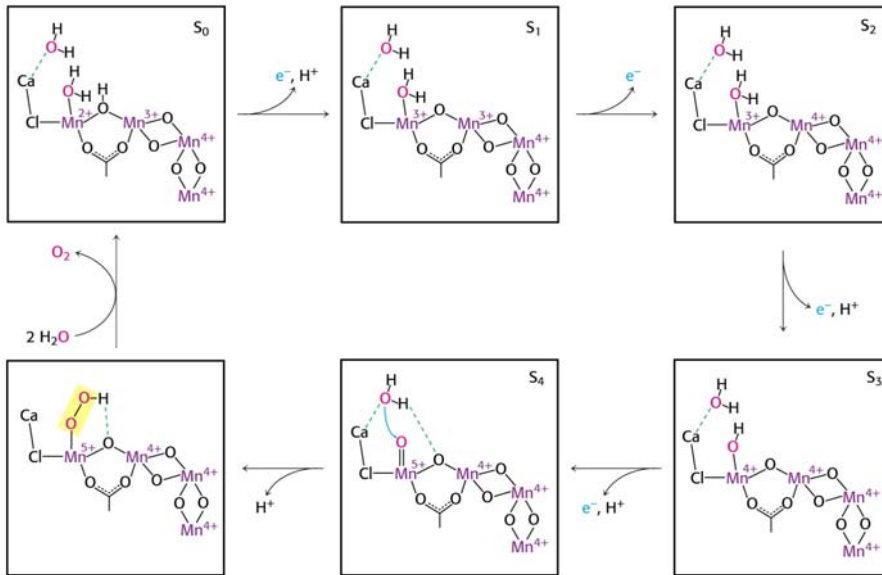


When the electron is rapidly transferred from P680* to pheophytin *a*, a positive charge is formed on the special pair, P680⁺. P680⁺ is an incredibly strong oxidant which

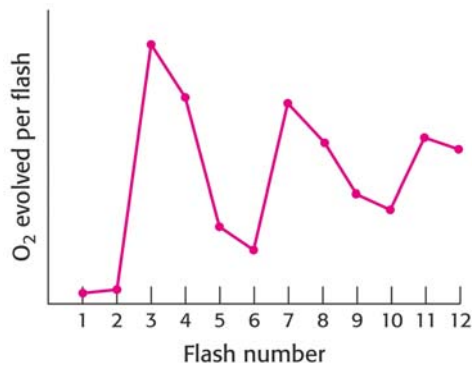
extracts electrons from water molecules bound at the manganese center. The structure of this manganese center includes 4 Manganese ions, a calcium ion, a chloride ion, and a tyrosine radical. Manganese is the core of this redox center because it has four stable oxidation states (Mn^{2+} , Mn^{3+} , Mn^{4+} and Mn^{5+}) and coordinates tightly to oxygen containing species. Each time the P680 is excited and an electron is kicked out, the positively charged special pair extracts an electron from the manganese center.



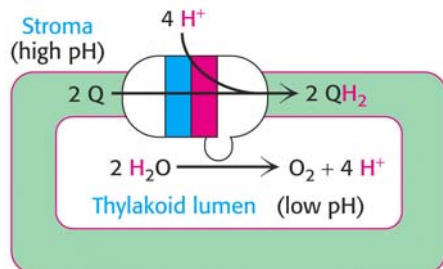
4 electrons must be transferred to 2 molecules of plastoquinone in order to oxidize H_2O to molecular oxygen. This requires 4 photochemical steps.



The Manganese center is oxidized one electron at a time, until two molecules of H_2O are linked to form O_2 which is then released from the center. A tyrosine residue not shown participates in the proton electron transfers. The structures are designated S_0 through S_4 to indicate the number of electrons removed.



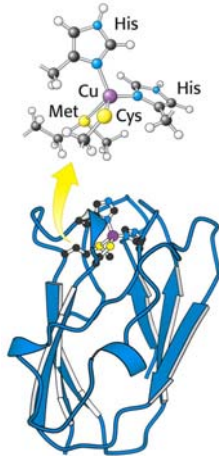
When isolated chloroplasts that have been held in the dark are illuminated with very brief flashes of light, O_2 evolution reaches a peak on the third flash and every fourth flash thereafter as shown to the left. The oscillation in O_2 evolution dampens over repeated flashes and converges to an average value. We know the manganese center exists in five different oxidation states numbered for S_0 to S_4 as shown above. One electron and a proton are removed during each photochemical step. When S_4 is attained, an O_2 molecule is released and two new molecules of water bind. The reason the third pulse of light produces O_2 is because the resting state of the PSII in the chloroplast is S_1 not S_0 .



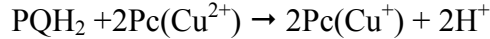
PSII spans the thylakoid membrane. The site of plastoquinone reduction is on the stroma side of the membrane. The manganese complex is on the thylakoid lumen side of the membrane. For every four electrons harvested from H_2O , 2 molecules of PQH_2 are formed extracting four protons from the stroma. The four protons formed during the oxidation of water are released into the thylakoid lumen. This distribution of protons across the thylakoid membrane generate a pH gradient with a low pH in the lumen and a high pH in the stroma.

II. Cytochrome *bf*

The plastoquinol formed by PSII contributes its electrons through an electron transport chain that terminates at PSI. The intermediary electron transfer complex between PSII and PSI is cytochrome *bf* also known as cytochrome *b₆f*. In this electron transfer complex electrons are passed one at a time from plastoquinol to plastocyanin (Pc) a copper protein of the thylakoid lumen.



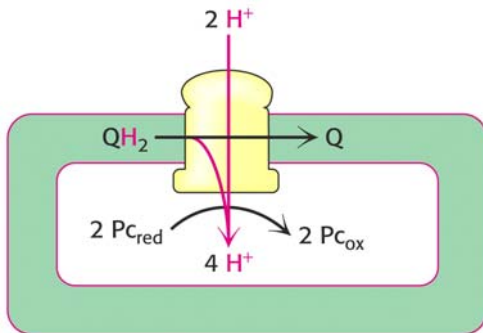
The reaction is shown below:



The protons are released into the thylakoid lumen.

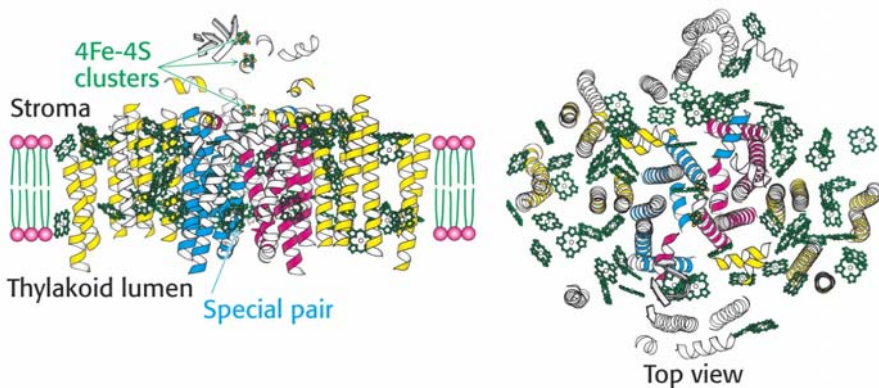
Plastocyanin is a water soluble electron carrier found in the thylakoid lumen of chloroplasts. It contains a single Copper atom coordinated to two histidine residues and a cysteine residue in a distorted tetrahedron. The molecule is intensely blue in the cupric form. This mobile electron carrier carries electrons from cytochrome *bf* to PSI.

The electron transfer of cytochrome *bf* is very similar to the electron transfer catalyzed by Complex III of the mitochondrion. The subunit components of cytochrome *bf* are homologous to the subunits of Complex III, cytochrome *c* reductase. The cytochrome *bf* contains two *b*-type heme cytochromes, a Rieske protein-type Fe-S protein, and a *c*-type cytochrome similar to cytochrome *c₁*. This enzyme transfers electrons from plastoquinol through the same Q cycle as Complex III. The net result is two protons are picked up from the stroma side of the thylakoid membrane and 4 protons are released into the lumen contributing to the pH gradient.

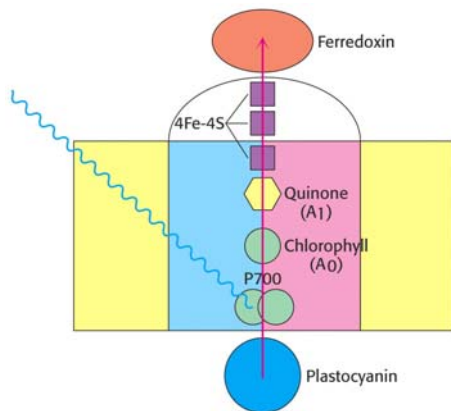


III. Photosystem I.

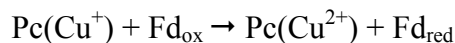
The final stage of the light reactions is catalyzed by PSI. This protein has two main components forming its core, *psaA* and *psaB*. These two subunits are quite a bit larger than the core components of PSII and the bacterial photosystem. Nonetheless, the subunits are all homologous.



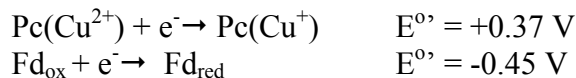
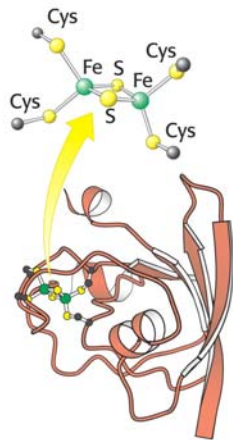
The *psaA* and *psaB* subunits are shown in yellow with the regions homologous to the core of PSII shown in red and blue. Chlorophyll molecules are shown in green and the 3 4Fe-4S clusters are also shown in green.



A special pair of chlorophyll *a* molecules lies at the center of the structure which absorbs light maximally at 700 nm. This special pair is denoted P700. Upon excitation-either by direct absorption of a photon or exciton transfer- P700* transfers an electron through a chlorophyll and a bound quinone (Q_A) to a set of 4Fe-4S clusters. From these clusters the electron is transferred to ferredoxin (Fd) a water soluble mobile electron carrier located in the stroma which contains a 2Fe-2S cluster coordinated to 4 cysteine residues. The electron transfer produces a positive charge on the special pair which is neutralized by the transfer of an electron from a reduced plastocyanin. The overall reaction is shown below.

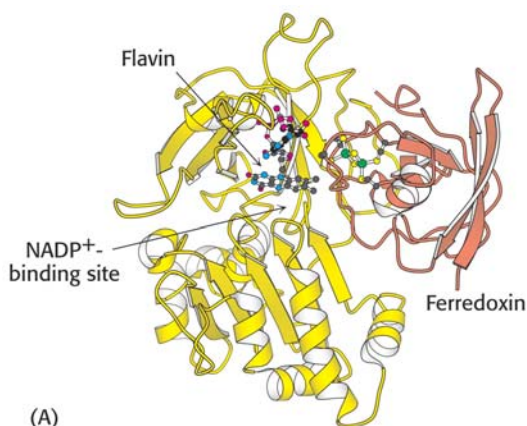
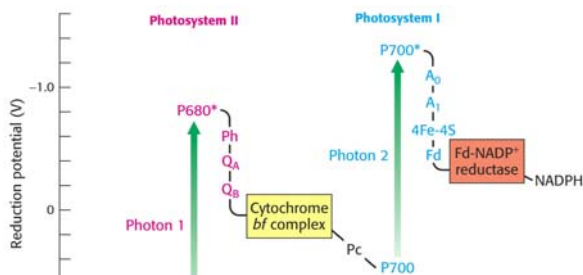


The structure of ferredoxin is shown to the left. Ferredoxin contains a 2Fe-2S cluster which accepts electrons from PSI and carries them to ferredoxin-NADP⁺ reductase.



The electron acceptor in the overall reaction shown above is the oxidized ferredoxin, the electron donor is the reduced plastocyanin. From the reduction potentials listed above, the change in reduction potential is: $\Delta E^{\circ} = -0.45 - 0.37 = -0.82 \text{ V}$ which corresponds to a $\Delta G^{\circ} = 79.1 \text{ kJ/mol}$, very endergonic.

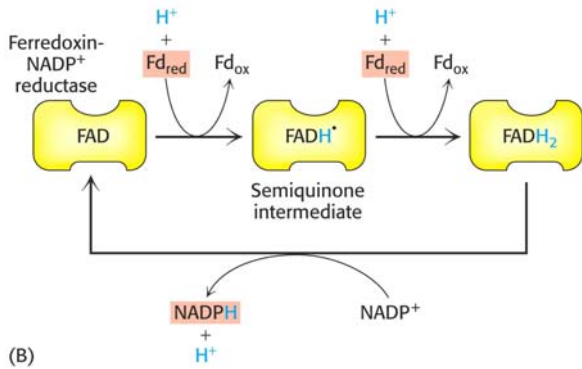
This uphill electron transfer is driven the by absorption of a 700-nm photon of light which has an energy of 171 kJ/mol. The electron transport pathway between PSII and PSI is called the Z-scheme because the redox diagram looks like a sideways letter Z.



IV. Ferredoxin-NADP⁺ Reductase

Ferredoxin is a strong reductant but can only function in one electron reductions. NADP⁺ can only accept 2 electrons in the form of a hydride. Clearly we need an intermediary to facilitate the electron transfer. The transfer of electrons from reduced ferredoxin to NADP⁺ is catalyzed by ferredoxin-NADP⁺ reductase which is a flavoprotein. This complex contains a tightly bound FAD which accepts the electrons one at a time

(A)

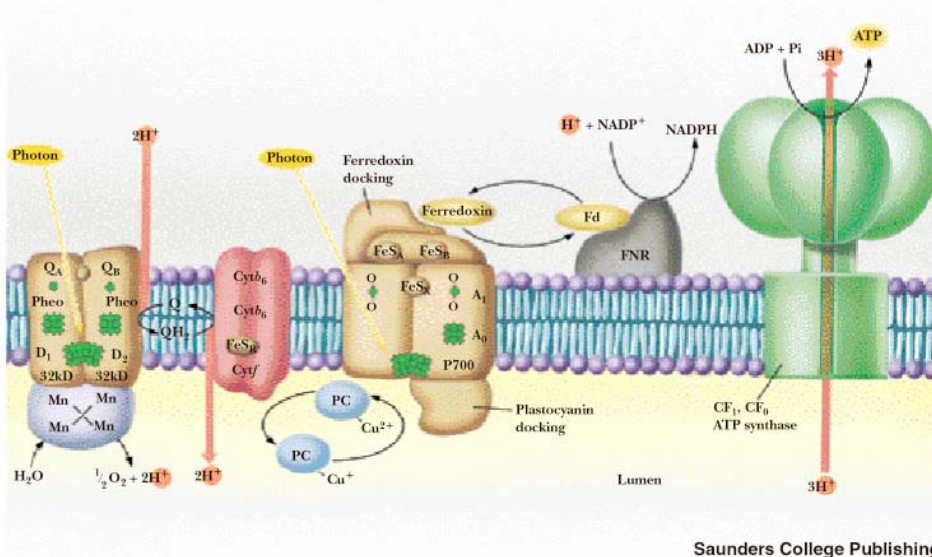


from ferredoxin. The FADH_2 then transfers a hydride to NADP^+ to form NADPH . This reaction takes place on the stromal side of the thylakoid membrane. The uptake of a proton by NADP^+ further contributes to the pH gradient across the thylakoid membrane.

V. Chloroplast ATP Synthase

The transport of electrons from water to NADP^+ generated a pH gradient across the thylakoid membrane. This proton motive force is used to drive the synthesis of ATP. The synthesis of ATP in the chloroplast is nearly identical with ATP synthesis in the mitochondrion. The pH gradient generated between the stroma and the thylakoid lumen is possible because the thylakoid membrane is impermeable to protons. When the chloroplast is illuminated the thylakoid lumen becomes markedly acidic, $\text{pH} \approx 4$. The pH of the stroma is around 7.5. The light induced pH gradient is about 3.5 pH units. The transmembrane electrical potential is not a significant factor in the proton motive force in the chloroplast because the thylakoid membrane is permeable to Cl^- and Mg^{2+} . Because of this permeability, the thylakoid lumen remains electrically neutral while the pH gradient is generated. A pH gradient of 3.5 pH units thus corresponds to a proton motive force of -20 kJ/mol .

Garrett & Grisham: Biochemistry, 2/e
Figure 22.21



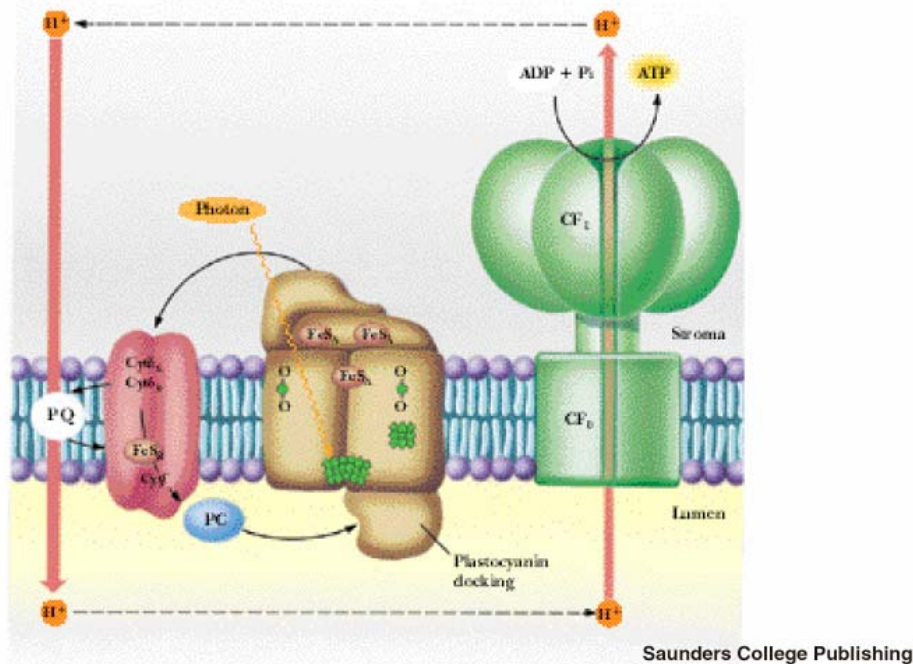
The ATP synthase of the chloroplast is called the $\text{CF}_1\text{-CF}_0$ complex where C stands for chloroplast and F_1 and F_0 relate to the homologous ATP synthase of the mitochondria. The mitochondrial and the

chloroplast ATP synthase are essentially identical with similar subunits and subunit stoichiometries. The catalytic subunit is the β subunit of CF_1 . The CF_1 complex lies in the stroma. The CF_0 complex channels protons from the thylakoid lumen to the stroma driving rotation of the 12 c subunits which in turn drives ATP synthesis. The ATP formed is released into the stroma where it is needed for the dark reactions of photosynthesis.

VI. Cyclic Photophosphorylation

There is an alternative pathway for the electrons arising from PSI giving photosynthesis versatility. The electrons carried in reduced ferredoxin can be transferred to the cytochrome *bf* complex rather than the ferredoxin-NADP⁺ reductase complex as shown below. The electrons then flow back through cytochrome *bf* to reduce plastocyanin, which then reduces the P700⁺ to complete the cycle. The net outcome of this cyclic flow of electrons is the pumping of protons across the thylakoid membrane by the cytochrome *bf* complex, producing a pH gradient which then drives the synthesis of ATP. This process produces ATP without NADPH generation. In addition PSII does not participate in cyclic photophosphorylation, so O₂ is not generated during this process. Cyclic photophosphorylation only occurs when the NADP⁺ concentration becomes limiting, such is the case when there is a very high ratio of NADPH/NADP⁺.

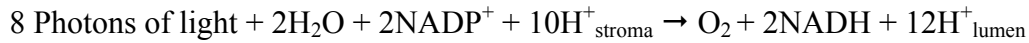
Garrett & Grisham: Biochemistry, 2/e
Figure 22.22



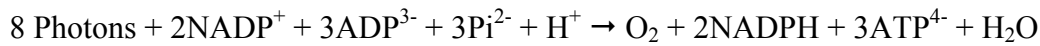
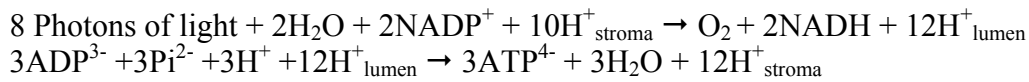
VII. Overall Stoichiometries

4 photons of light were required to oxidize 2H₂O into O₂ + 4H⁺. The four electrons were transferred to 2 molecules of plastoquinone to form 2 molecules of plastoquinol. These 2 molecules of PQH₂ transferred their electrons to 4 molecules of plastocyanin releasing 8 more protons into the thylakoid lumen. Finally the 4 molecules of reduced plastocyanin were transferred to 4 molecules of ferredoxin by the absorption

of 4 more photons of light by PSI. The 4 molecules of reduced ferredoxin generated 2 molecules of NADPH. The overall reaction is:



The F_0 complex has 12 c-subunits, so it takes 12 H^+ to produce one complete rotation. Each complete rotation produces 3 ATP molecules. Therefore, it takes 4 H^+ to synthesize one ATP.



This is 2.667 photons per ATP.

Cyclic photophosphorylation is more productive in regards to ATP synthesis.

4 Photons absorbed by PSI result in 8 protons released into the lumen by the cytochrome *bf* complex. These protons drive the synthesis of 2 ATP molecules. Thus 2 photons per ATP.