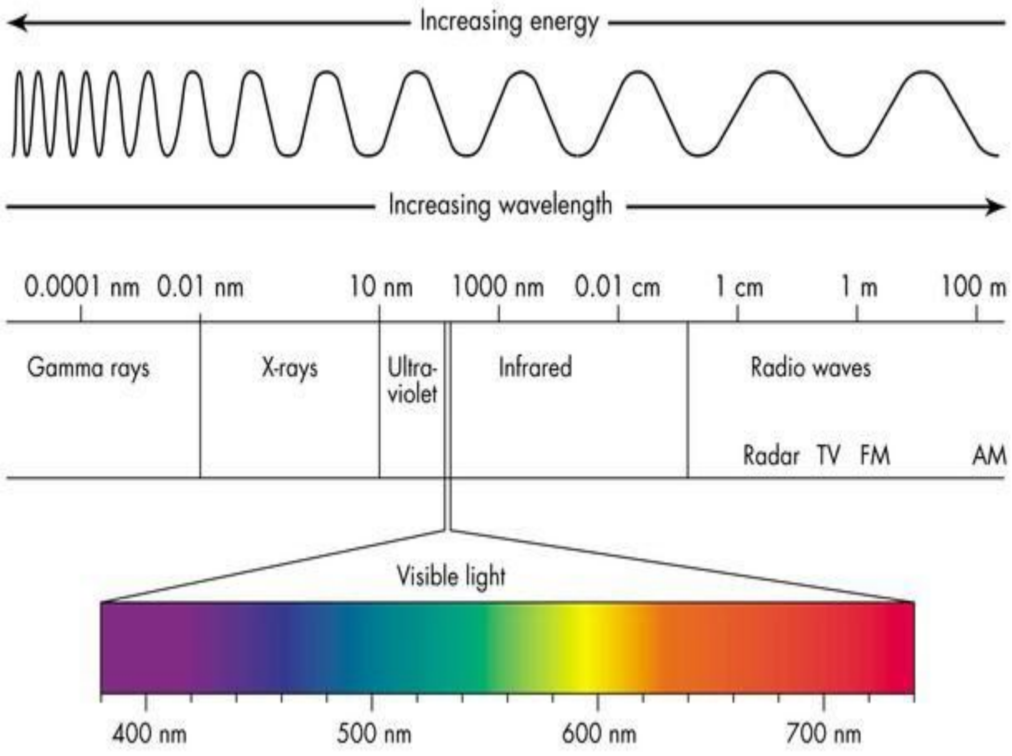
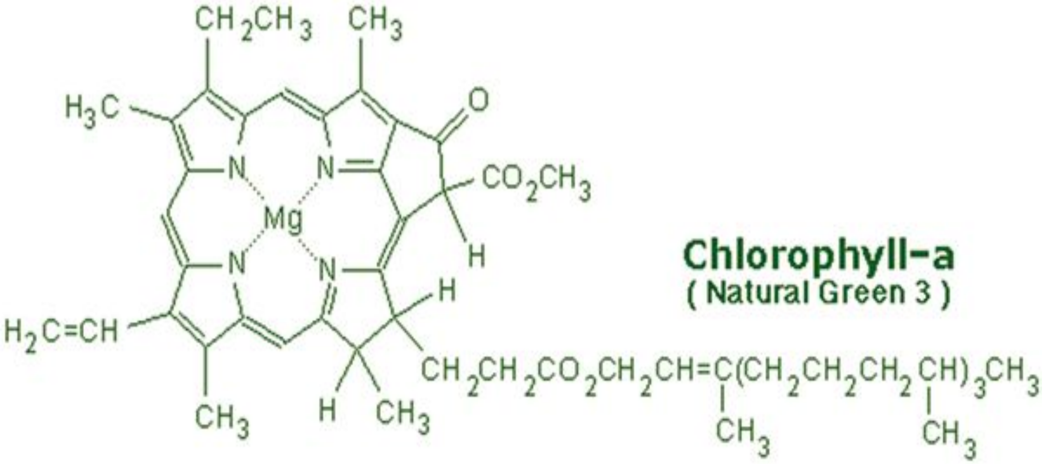
Photosynthesis

- **Photosynthesis is the process by which light from the sun is converted into the energy necessary for the vital functions of living things**
- Light is unique in having both particulate and wave properties
- Wave is characterised by wavelengths (λ) and frequencies (ν)
- Light is also a particle called photon and each photon contains an amount of energy called a quantum (pl. quanta)
- Sunlight is like a rain of photons of different frequencies

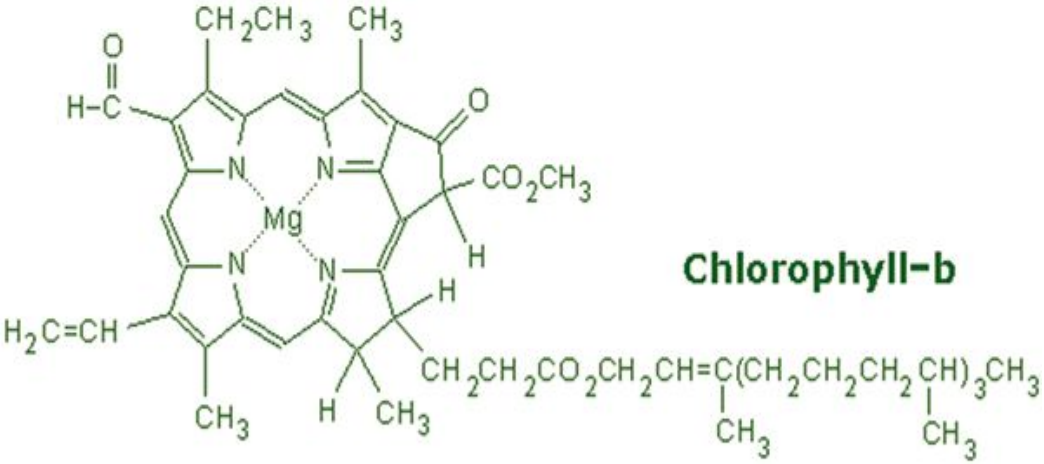


Plant pigments:

- Three major classes of pigments have been found to occur in photosynthetic organisms- I) Chlorophylls, II) Carotenoids and III) Phycobilins
- **Chlorophylls-** Different types of chlorophylls found in nature are chl a, chl b, chl c, chl d, chl e, and bacteriochlorophyll
- Chl a and chl b are present in photosynthesizing cells of all land plants
- Chl a usually appear bluish green while chl b is yellow green in colour
- Chlorophyll molecule has a cyclic tetrapyrrolic structure with an isocyclic ring containing Mg atom at its centre
- The four pyrrole rings are lined together by CH bridges
- With third pyrrole ring a cyclopentanone ring is present
- In chl b the Ch_3 group at 3C position is replaced by an aldehyde (CHO) group



Chlorophyll–a
(Natural Green 3)

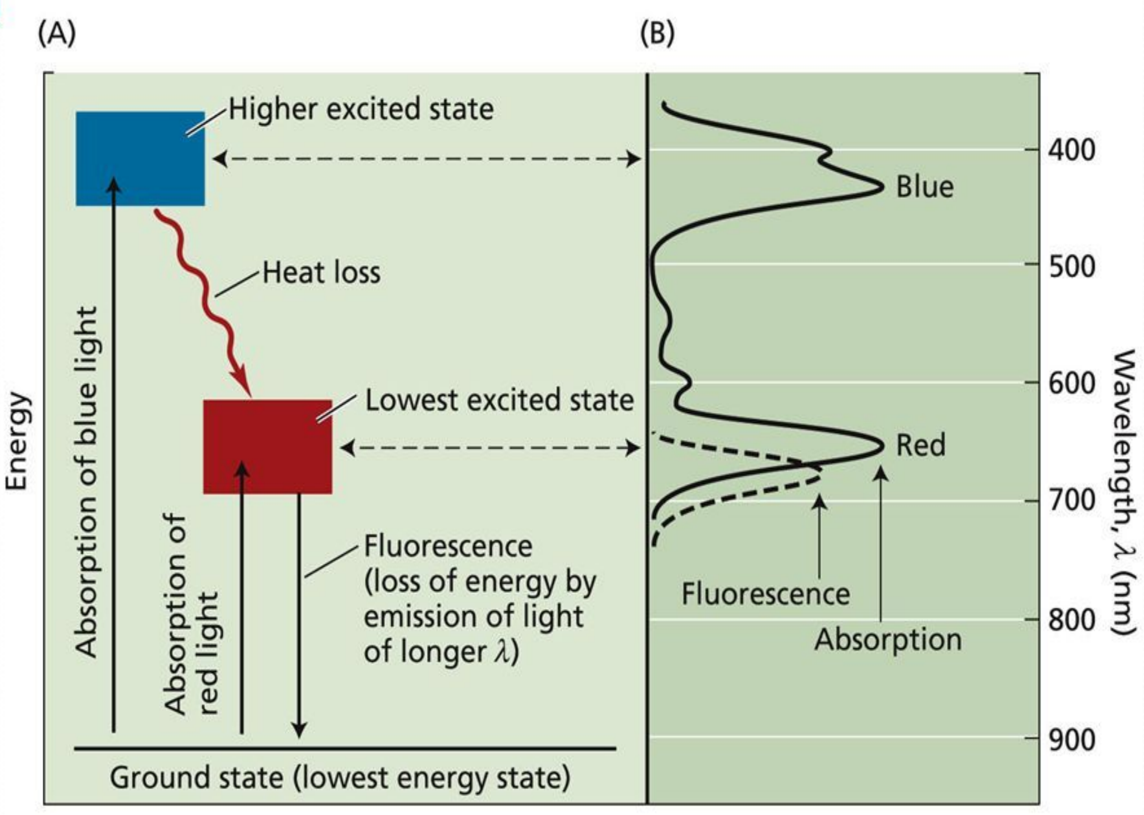


Chlorophyll–b

Properties of Chlorophyll:

- Chlorophyll is insoluble in water but soluble in alcohol, chloroform, acetone, ether, benzene etc.
- Chlorophyll appear green as they absorbs light in the red and blue parts of the spectrum
- Whenever chlorophyll in its lowest energy absorbs a photon it gets transmitted to a higher energy state or excited state
- Absorption of blue light excites chlorophyll to higher energy state than absorption of red light
- This state is very unstable and chlorophyll rapidly releases some of its energy as heat and enters the lowest excited state
- In the lowest excited state chlorophyll can take four different pathways to dissipate its available energy
- It can reemit a photon and return to ground state (fluorescence)
- It can return to ground state by directly converting its excitation energy into heat
- It can transfer its energy to another molecule
- Lastly, the energy of the excited state causes chemical reactions to occur (photochemistry)
- Photochemical process is the fastest and hence the most likely process to deactivate excited chlorophyll

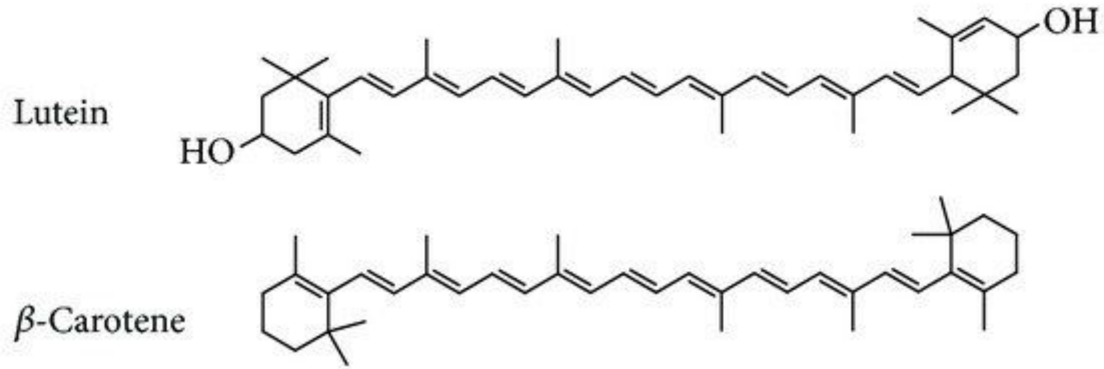
Light absorption and emission by chlorophyll

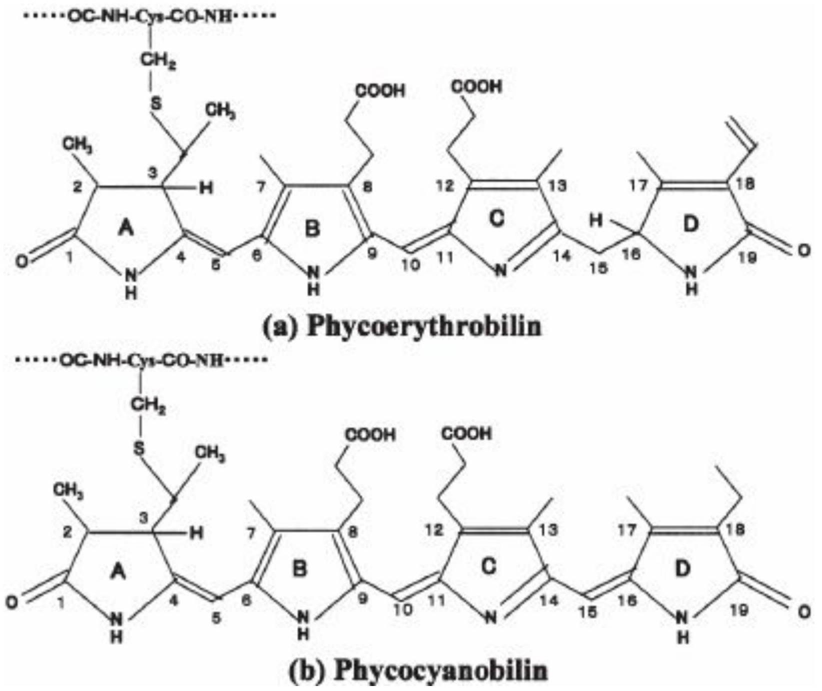


PLANT PHYSIOLOGY, 5e, Figure 7.5

Other pigments:

- **Carotinoids:** Present in all photosynthesizing cells
- They are terpenoid group of compounds divided into two classes= carotenes (β carotene) and xanthophylls (lutein, violaxanthin etc.)
- Carotenes are simple hydrocarbons while xanthophyls are their oxygen derivatives
- **Phycobilins:** They are common only in blue green alga and red alga as phycocyanin (blue) and phycoerythrin (red)
- It is an open-chain tetrapyrrole that remain conjugated with a protein moiety and hence called phycobiliproteins

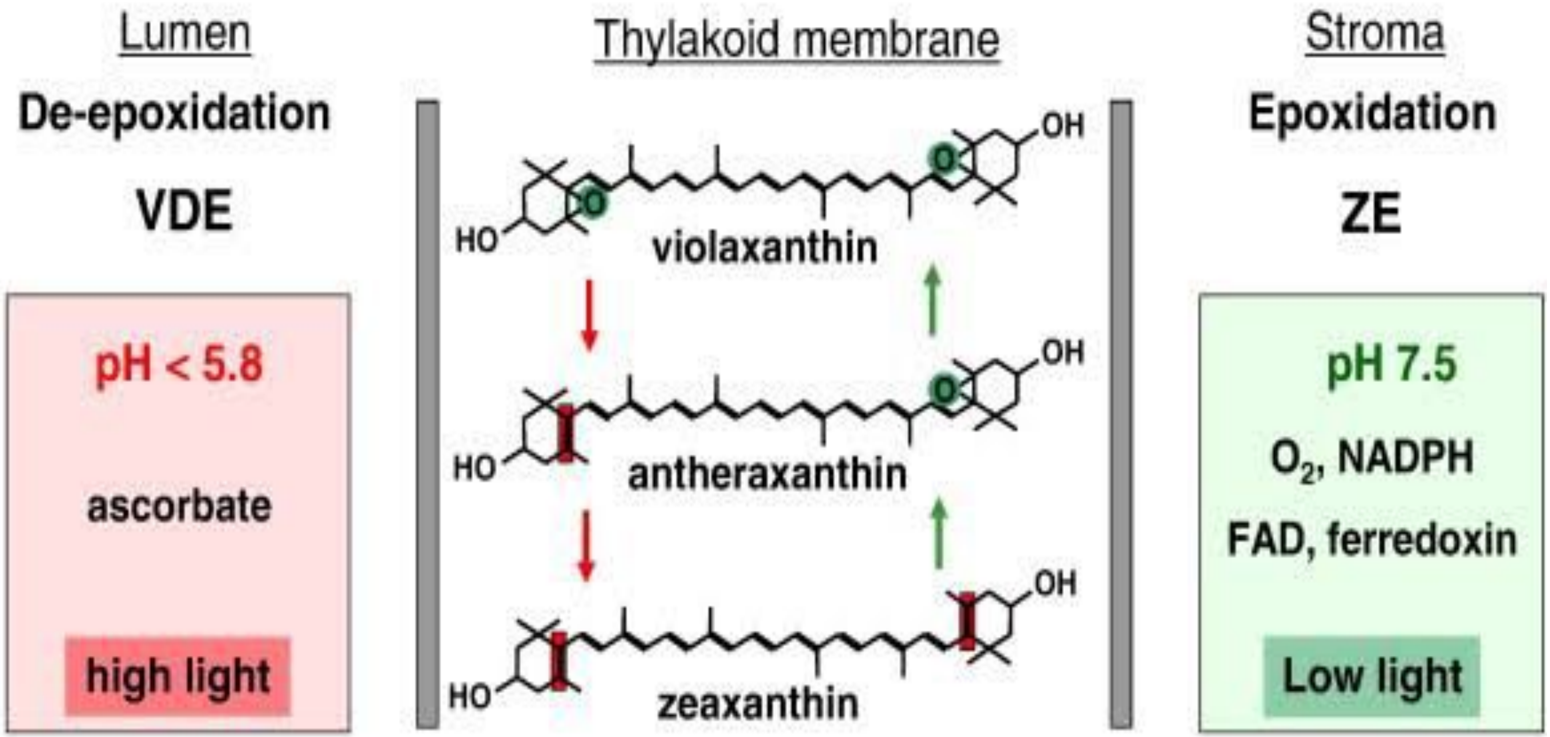




Role of Carotinoids and Phycobilins in Photosynthesys:

- The carotenoids act as accessory pigment in photosynthesis as the liCarotenoids protect the chlorophylls against the photo oxidation
- ght energy absorbed is not directly utilised but transferred to chlorophyll a
- Phycocyanin pigment absorb light at 630nm while phycoerythrin absorb light at 500 -570nm
- Both these pigments are also considered as accessory pigments as they do not utilise the light energy directly but transfer it to chlorophyll a
- Photo oxidation of chlorophyll and other constituents of chloroplast occur due to imbalance between the number of photon absorbed and utilised in photosynthesis
- The excess absorbed light energy if not dissipated will induce the reduced ferredoxin of PSI to react with molecular oxygen to produce highly reactive and toxic superoxide radicals
- Photo oxidation can be prevented either by removing the superoxide or by preventing its formation
- Enzyme superoxide dismutase, formed in chloroplast, can remove the superoxide by scavenging it, but produces H_2O_2 in the process which is itself toxic
- Production of superoxide can be prevented by trapping and dissipating excess excitation energy before it reaches the reaction centre
- It has been observed in xanthophyll cycle occurring in chloroplast that under excess light and / or low pH de-epoxidation of violaxanthin to zeaxanthin occurs; the reverse happens under low light
- It has been suggested that zeaxanthin present in antenna complex of chloroplast can accept the downhill transfer of excess energy directly from excited chlorophyll and dissipate it harmlessly as heat, thereby photoprotecting the chloroplast

The Violaxanthin Cycle



Quantum Yield

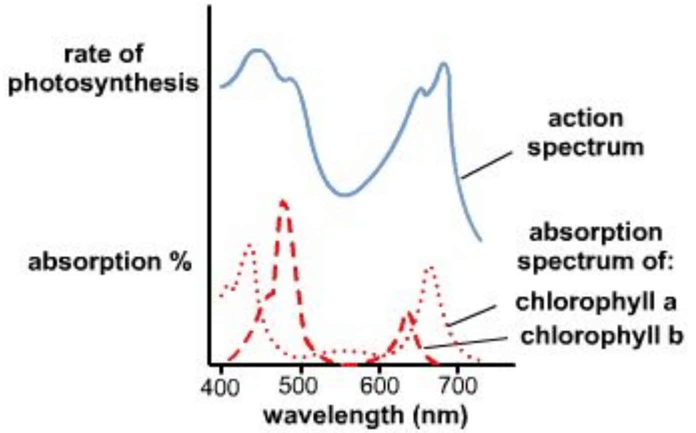
- A property relevant to most photo physical and photochemical processes
- A measure of efficiency with which absorbed radiation causes the molecule to undergo a specified change
- It is the number of product molecules formed for each quantum of light absorbed

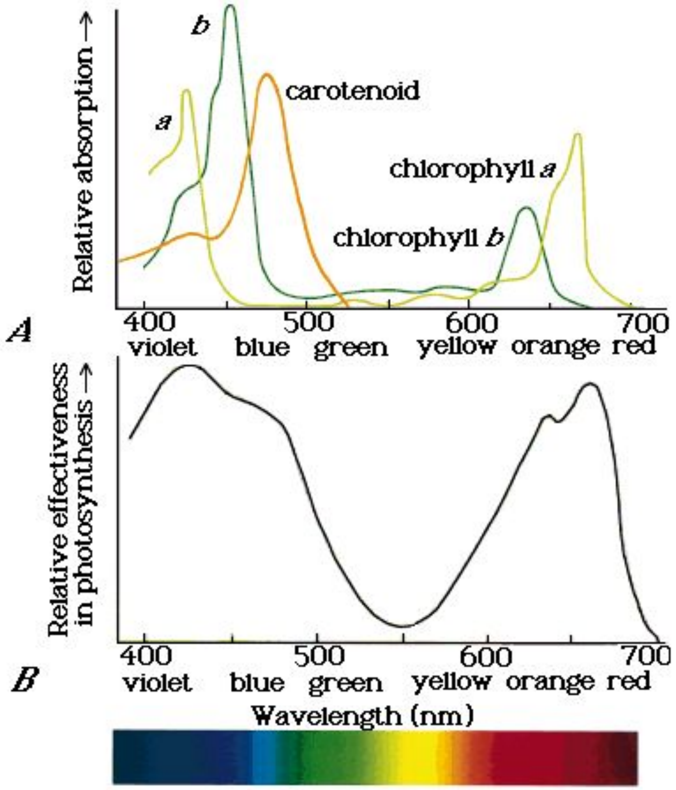
$$\text{Quantum yield for fluoresence} = \frac{\text{no: of photons emitted}}{\text{no:of photons absorbed}}$$

- The value of φ for a particular process ranges from 0 (if the process never involved in decay of the excited state) to 1.0 (if that process always deactivates the excited state)
- In functional chloroplasts in light the quantum yield of photochemistry is 0.95, while that of other processes is negligible
- It concludes that majority of the excited chlorophylls lead to photochemistry

Absorption spectrum and action spectrum:

- An absorption spectrum is the measure of the extent to which a given substance absorbs the light of different wavelengths
- An action spectrum is the measure of the efficiency of a light mediated process induced light of different wavelengths but of same intensity
- A comparison between the absorption spectrum and action spectrum of a pigment indicate whether or not the pigment is involved in the response
- Absorption spectrum of carotenoids and Phycobilins overlap with the action spectrum of photosynthesis in the fluorescence peak suggestir transfer of energy to chlorophylls
- Duysens (1950) showed with red algae that energy was transferred from pigments absorbing blue light (carotenoids) to those absorbing green (phycoerythrin), then to those absorbing orange (phycocyanin) and finally to those absorbing red (chlorophyll a)
- It was also shown that action spectrum f photosynthesis is not exactl identical to the absorption spectrum of chlorophyll, indicating involvement of other pigments also





Photosynthetic unit:

- A photosynthetic unit is the smallest group of pigment molecules where a light quantum is absorbed and migrated causing the release of an electron
- Emerson and Arnold (1932) showed that 2500 chlorophyll molecules are needed to release one molecule of O_2
- Again about 8 quanta of light absorption is required by chlorophyll to release one molecule of O_2 and reduce one molecule of CO_2
- Thus the size of a photosynthetic unit should be 300 chlorophyll molecules
- It is now established that components of light reaction (cytochrome, P700 and ferredoxin) are present one molecule each per 300 molecules of chlorophyll
- Currently a photosynthetic unit consists of about 600 chlorophyll molecules (300 each per reaction centre) along with an electron transport chain

Red Drop and Emerson Effect:

- Emersion and his colleagues measured the quantum yield of photosynthesis for wavelengths at which chloroplasts absorbs light and found a sharp drop (red drop) at wavelengths greater than 680nm
- Again Emerson measured the rate of photosynthesis with light beams of two different wave lengths simultaneously, one of which was in far red region
- The rate of photosynthesis with combined wavelengths was found much greater than the sum of the individual rates, known as Emerson enhancement effect
- Emerson and Rabinowitch (1960) observed that the action spectra of enhancement effect and absorption spectra of one of the accessory pigments were same
- Thus first proposed the involvement of two photosystems in the process-one controlled by chlorophyll and other by accessory pigment
- Later Govindjee and Rabinowitch showed another peak at 670nm within main absorption band of chl a
- It was concluded that enhancement effect was due to absorption of another quantum of light in another form of the same pigment, chl a
- It is now known that in the red region of the spectrum one of the photoreactions (PSI) absorbs preferentially far red light (›680nm), while the second (PSII) absorbs red light (680nm)

Emerson:

Red drop effect

Far-red light alone is insufficient in driving photosynthesis

Enhancement effect

Accessory pigments

> 680 nm

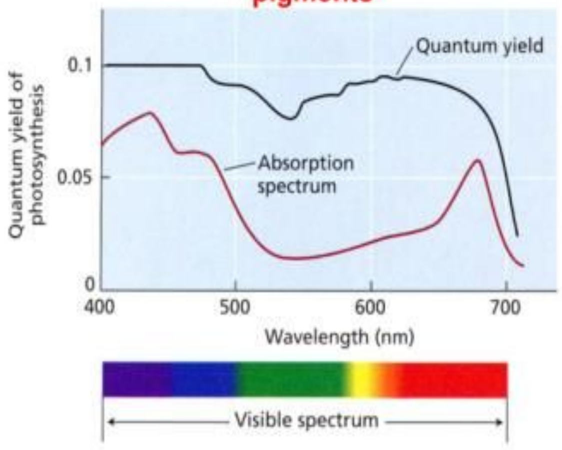


FIGURE 7.12

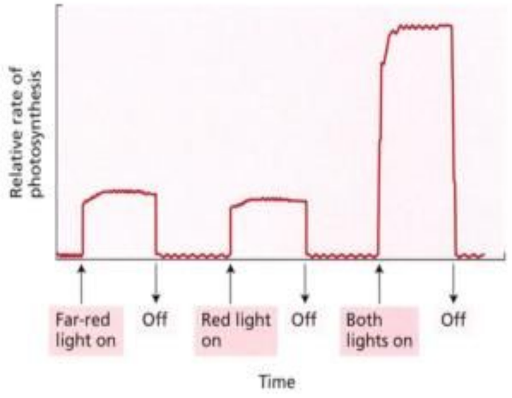
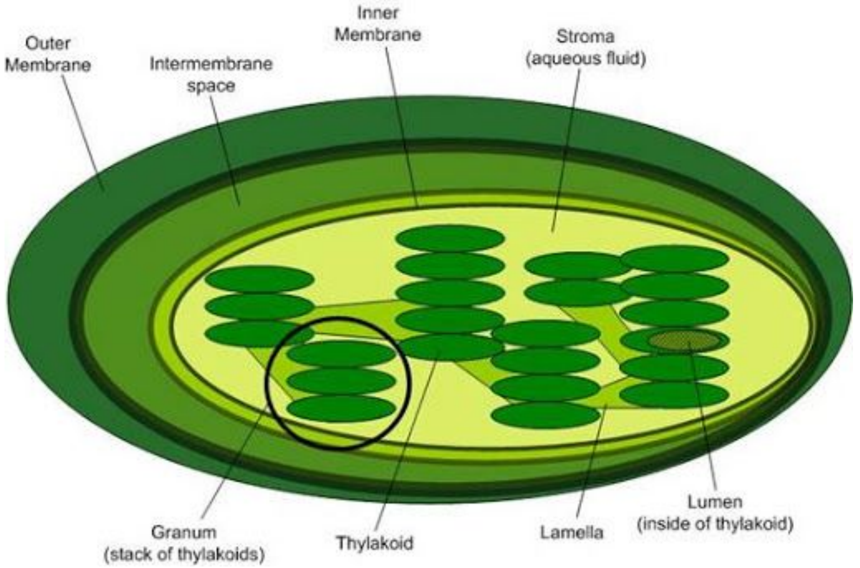


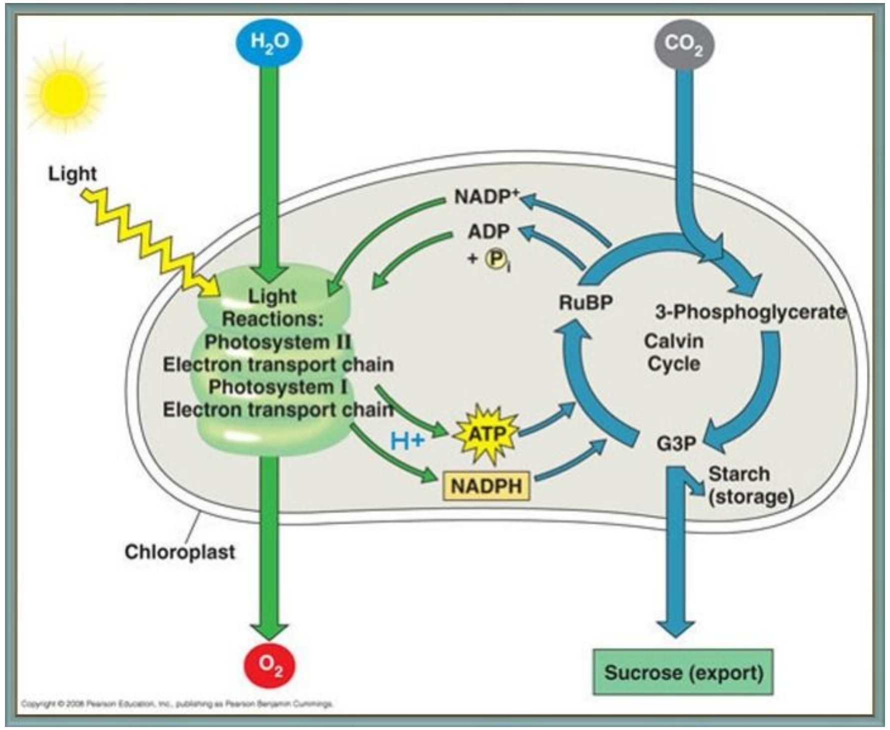
FIGURE 7.13

⇒ Two photochemical complexes

Site Of photosynthesis:

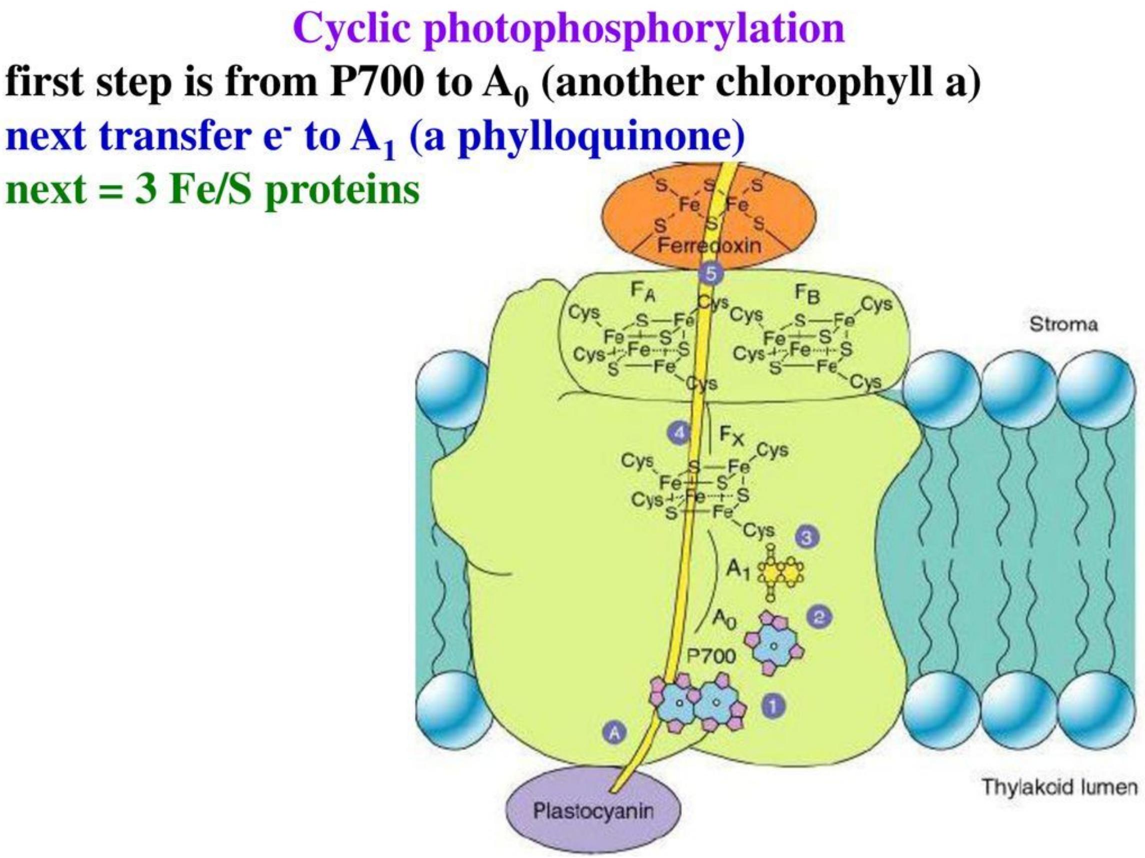
- Photosynthesis takes place in the thylakoid membrane of chloroplasts, which again remain closely stacked as Grana lamellae
- The proteins essential for photosynthesis remain embedded in thylakoid membrane
- The reaction centres, the antenna complex and most of the electron transport enzymes are integral membrane proteins
- The chlorophylls and accessory light gathering pigments in the thylakoid membrane are always associated in a non-covalent but highly specific way with proteins forming chlorophyll proteins
- Both antenna and reaction centre chlorophylls are associated with chlorophyll proteins that are inserted into the membrane
- The PS-II reaction centre along with its antenna chlorophylls and associated electron transport proteins, is located predominantly in the grana lamellae
- The PS-I reaction centre, associated antenna pigments and electron transfer proteins as well as the CF enzymes are found in the stroma lamellae
- Thus, the two photochemical events are spatially separated
- The ratio of PS-II to PS-I is about 1.5:1



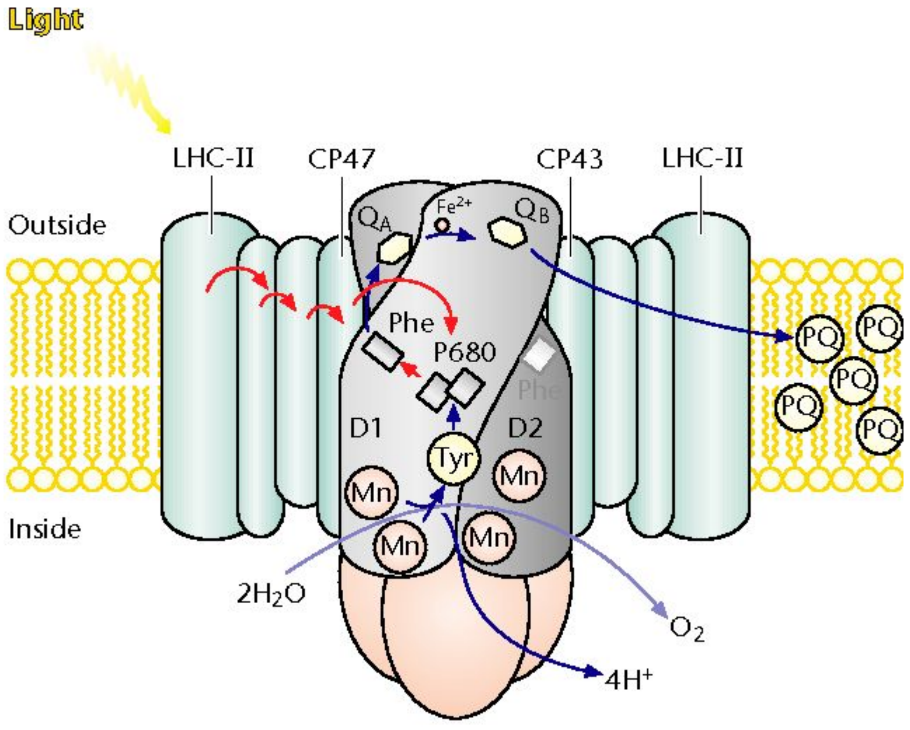


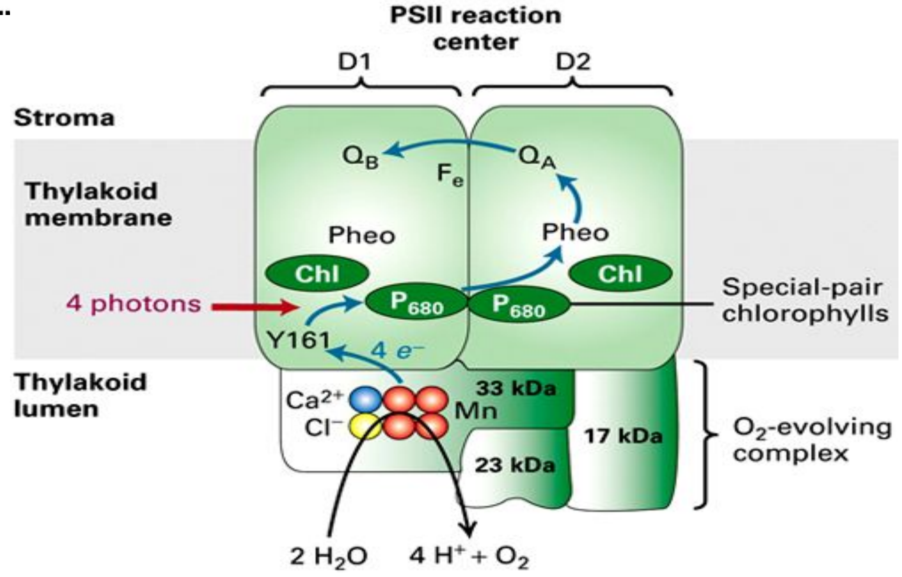
Components of Photosystem:

- Emerson and Rabinowitch postulated the existence of two photosystems (I and II)
- PS-I contains chl a, small amount of chl b and some β-carotenes attached to several proteins
- One of the chl a molecules absorb light near 700nm (P700) and forms the reaction centre for PS-I
- All other chlorophylls and carotenoids transfer their energy to the P700
- A number of electron acceptors (A_0, A_1, F_X, F_A, F_B)remain associated with the reaction centre
- A_0 is the primary electron acceptor while A_1, F_X, F_a and F_B are Fe-S proteins
- Final electron acceptor is ferredoxin
- P700 is associated with a 70kda tetramer which in addition to P700 binds about 130 chl a and 16 carotenoids
- It forms the core antenna of PS-I the accessory light harvesting antenna of PS-I (LHC-I) 60-80 chl a and chl b molecules
- Thus total functional antenna chl of PS-I contains about 200-210 chl molecules

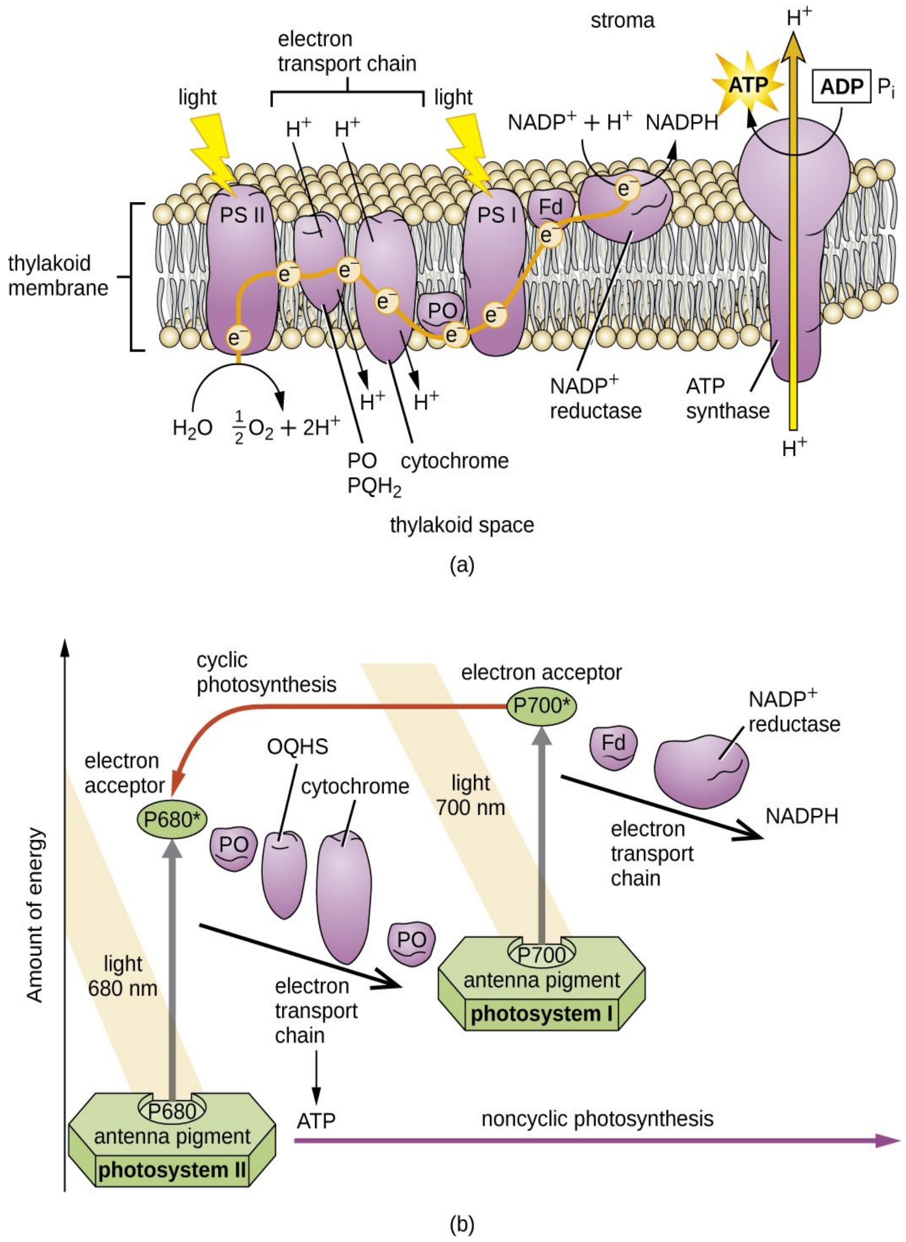


- PS-II also contains chl a, a little of chl b and β-carotene
- Reaction centre is another special chl a molecule (P680)
- Primary electron acceptor is phaeophytin (Pheo)
- Secondary electron acceptor is Quinone (Q_A and Q_B)
- PS-II contains mangano-proteins, four Mn^{2+} ions bound to one or more proteins and a Cl^- ion bridges two Mn^{2+}ions
- P680 is reduced by a tyrosine residue in D_1 protein
- Mangano protein lies in the inner side of the thylakoid membrane and directly involved in H_2O oxidation
- The accessory light harvesting antenna of PS-II contain chl a and chl b organised into two distinct light harvesting complexes (LHC II)
- CP43 and CP47 are two chl a binding proteins



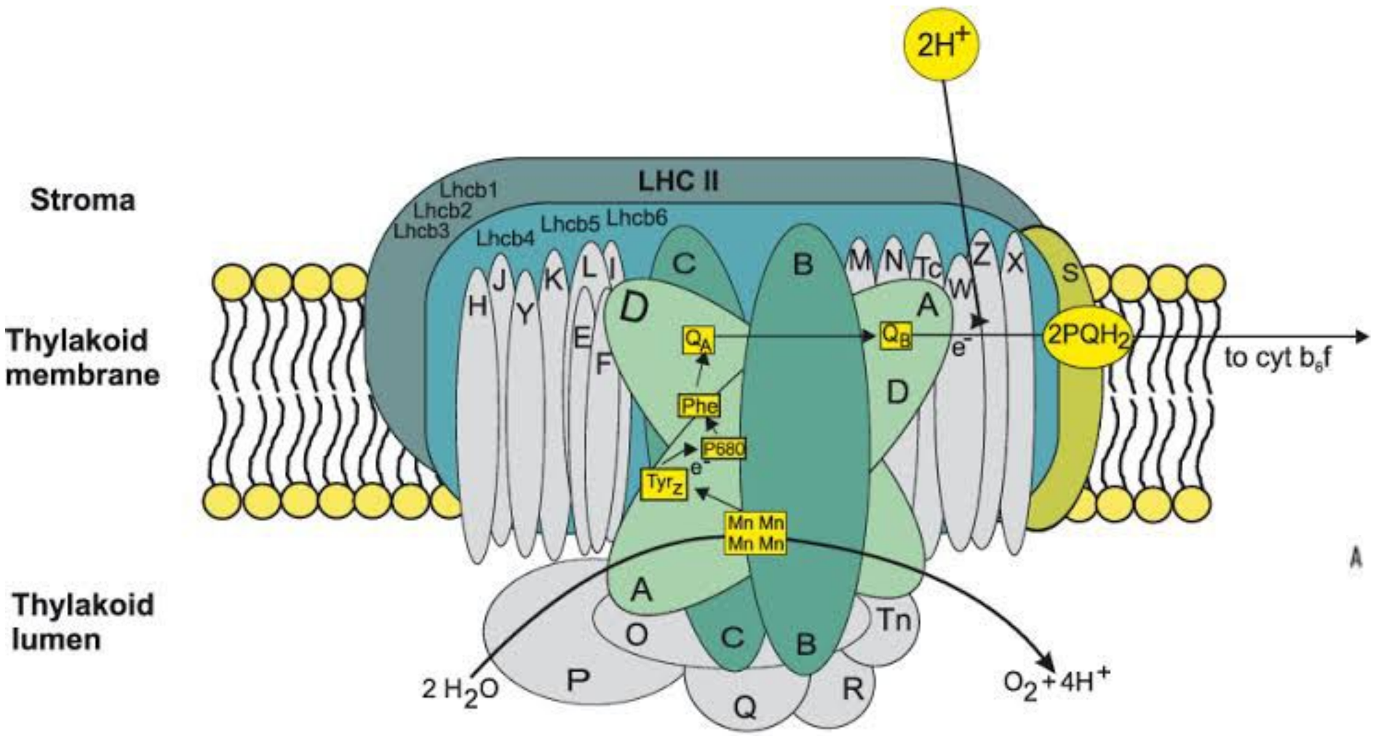


- PS I and PSII together function to transfer electrons from H_2O to NADP
- Two electron carriers, copper containing plastocyanin and another plastoquinone, carry electrons from PS II to PS I
- Reduced plastocyanin (cuprous,Cu^{1+}) move along the membrane and carry electron to PS I and reoxidised to Cu^{2+} (cupric)
- Plastoquinone carry two electrons and two H^+ from PS II to PS I
- Other electron transport component are cytochrome b6 and cytochrome f along with another Fe-S protein
- Cytochrome b_3 and another Fe-S protein (ferredoxin) also take part in electron transport
- Finally, F_1-ATPase complex or coupling factor (CF) complex is necessary for photophosphorylation

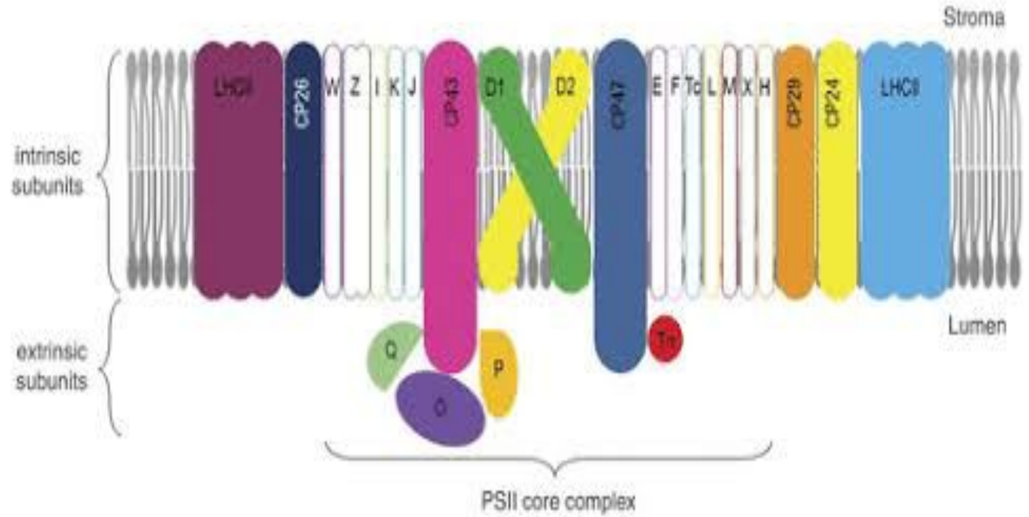


Organisation of light absorbing antenna system:

- Antenna system of different classes of photosynthetic organisms are different
- Pigments with some antenna proteins form Light Harvesting Complexes (LHCII and LHCI)
- Antenna systems function to deliver energy efficiently to the associated reaction centres
- The size of antenna systems varies from a low of 20-30 bacteriochlorophyll to 200-300 chlorophylls in higher plants
- Mechanism of transfer of excitation energy from the chlorophyll that absorb light to the reaction centre is done by a nonradiative process analogous to resonance transfer
- Approximately 95-99% of the photons absorbed by the antenna pigments are transferred to the reaction centre
- The energy absorbed in antenna pigments is funnelled towards the reaction centre by a sequence of pigments with absorption maxima progressively shifting towards the longer red wavelengths
- The energy of the excited state is lower nearer the reaction centre and the difference of energy is lost to the environment as heat

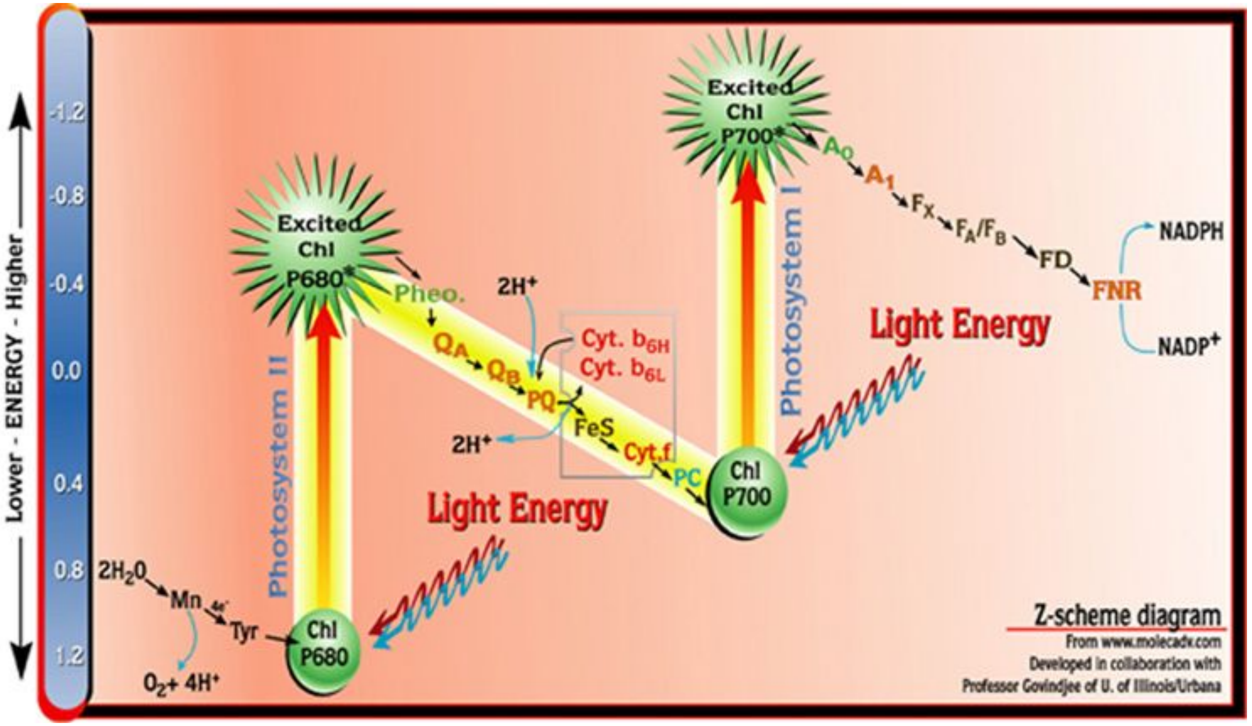

2H+
Stroma
LHC II
Lhcb1
Lhcb2
Lhcb3
Lhcb4
Lhcb5
Lhcb6
H
J
Y
K
L
I
E
F
C
B
D
M
N
Tc
W
Z
X
S
A
D
QA
QB
e-
2PQH2
to cyt b6f
Thylakoid membrane
Phe
P680
Tyrz
e-
Mn Mn
Mn Mn
Thylakoid lumen
A
O
C
B
Tn
2 H2O
P
Q
R
O2 + 4H+

A

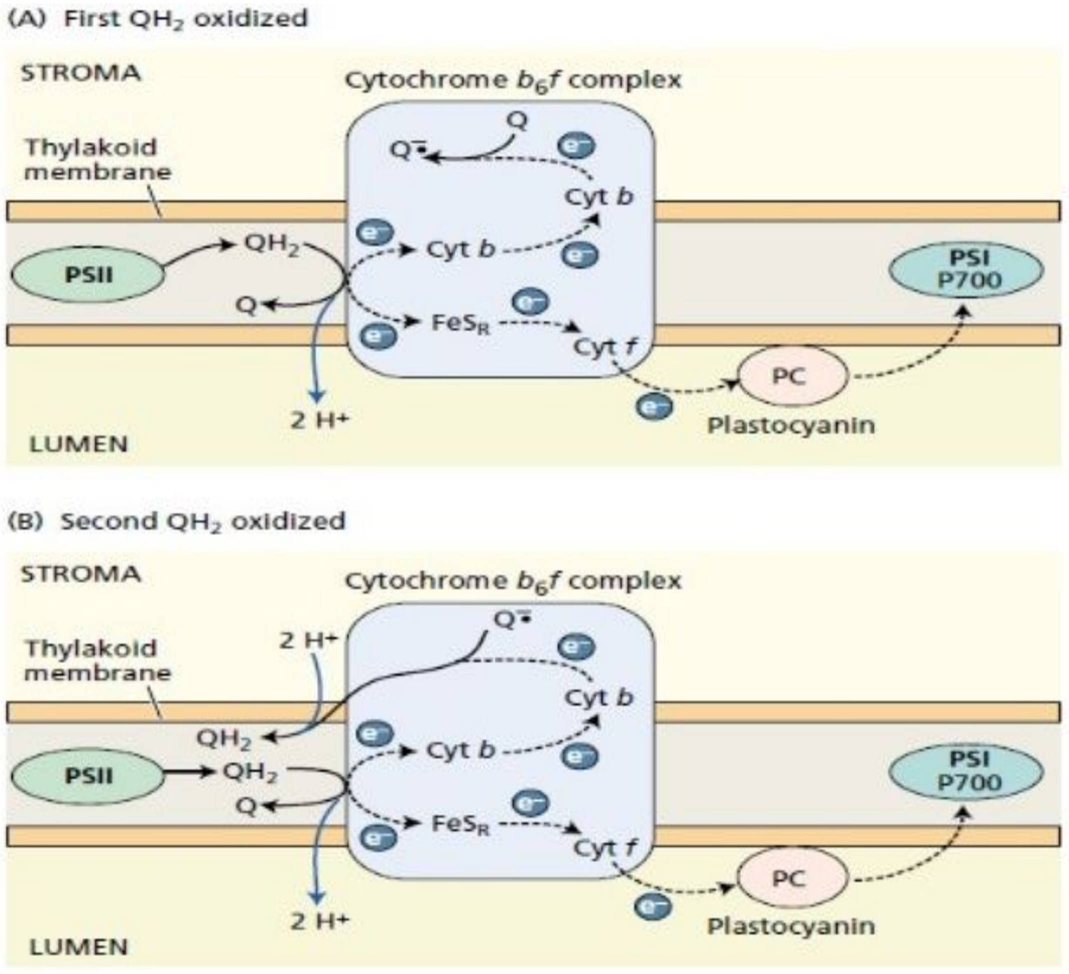

Stroma
LHCII
CP26
W
Z
I
K
J
CP43
D1
D2
CP47
E
F
Tc
L
M
X
H
CP29
CP24
LHCII
intrinsic subunits
Lumen
extrinsic subunits
Q
O
P
Tn
PSII core complex

Noncyclic Electron Transport

- The mechanism of electron transport in oxygenic photosynthetic organisms can be explained by the Z- scheme (zigzag)
- Photon absorbed by LHCII is transferred to P680
- Phaeophytin acts as an early electron acceptor in PS II
- Pheo is a chlorophyll where the central Mg atom is replaced by two hydrogen atoms
- Phaeophytin is followed by two plastoquinone in close proximity to an iron atom
- The PQ bound to the reaction centre acts as a two electron gate
- One electron is transferred from pheo to the first Quinone and then rapidly to the second Quinone
- By this time, oxidised P680 regains an electron from Yz (tyrosine) and return to a photochemically competent state
- A second electron is transferred from pheo to the two quinones sequentially
- Two protons are picked up from the medium, producing a fully reduced QH_2
- QH_2 dissociates from the complex and enters the hydrocarbon portion of the membrane where it transfers its electrons to the cytochrome b_6f complex

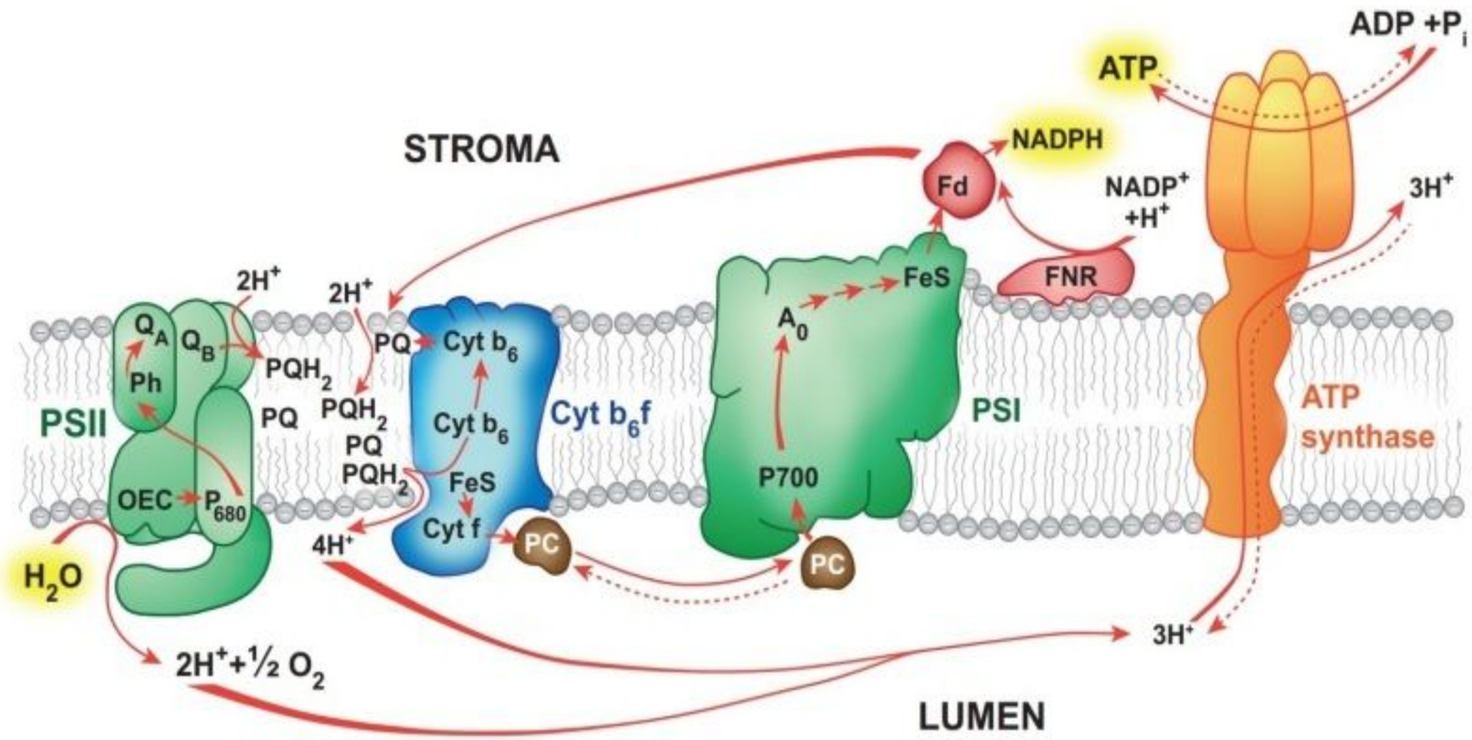


- Cytochrome b_6f is a large multisubunit protein with two b-type heme and one c-type heme
- In addition the complex contains a Rieske Fe-S protein where two iron atoms are bridged by two sulphur atoms
- A mechanism known as Q cycle accounts for the electron and proton flow through the Cytochrome b_6f
- A QH_2 is oxidised near the luminal side of the complex, transferring two electrons to the Rieske Fe-S protein and one of the b-type cytochromes, simultaneously expelling two protons to the lumen
- The electron from Fe-S is Passed to Cyt f and then to plastocyanin (PC), Cu containing protein, which reduces P700 of PS I
- The reduced cyt b transfers electron to other type of cytb, which reduces a quinone to semiquinone
- A second QH_2 is oxidised in the next step with one electron going from Fe-S to Pc to P700 and the other electron passes through two cyt b and finally reducing the semiquinone to QH_2
- At the same time two protons are picked from the stroma



- The net result of two turnovers of the complex is that 2 electrons are transferred to P700, 2 PQH_2 are oxidised to quinones, 1 oxidised PQ is reduced to PQH_2 and 4 protons are transferred from stromal to luminal side of the membrane
- Electron flow connects the acceptor side of the PS II reaction centre to donor side of the PS I and an electrochemical gradient is generated across the membrane due to concentration differences in protons on two sides of the membrane which is utilised to synthesise ATP

- PC is the electron donor to P700 only when P700 has already lost one electron by light excitation
- The electron acceptors like X,A, B remain bound to Fe-S rich centres
- Electrons pass through ferredoxin reducing substance (FRS) to ferredoxin, reducing its Fe^{3+} to Fe^{2+}
- Terminal electron acceptor is NADP which is reduced by FD by giving 2 electrons one at a time
- This reaction takes place in stroma catalysed by ferredoxin-NADP reductase (FNR)



Water Splitting Mechanism

- Water is oxidised according to following chemical raction

$$2\,H_2O \xrightarrow{\text{catalyst}} 2\,H_2 + O_2$$

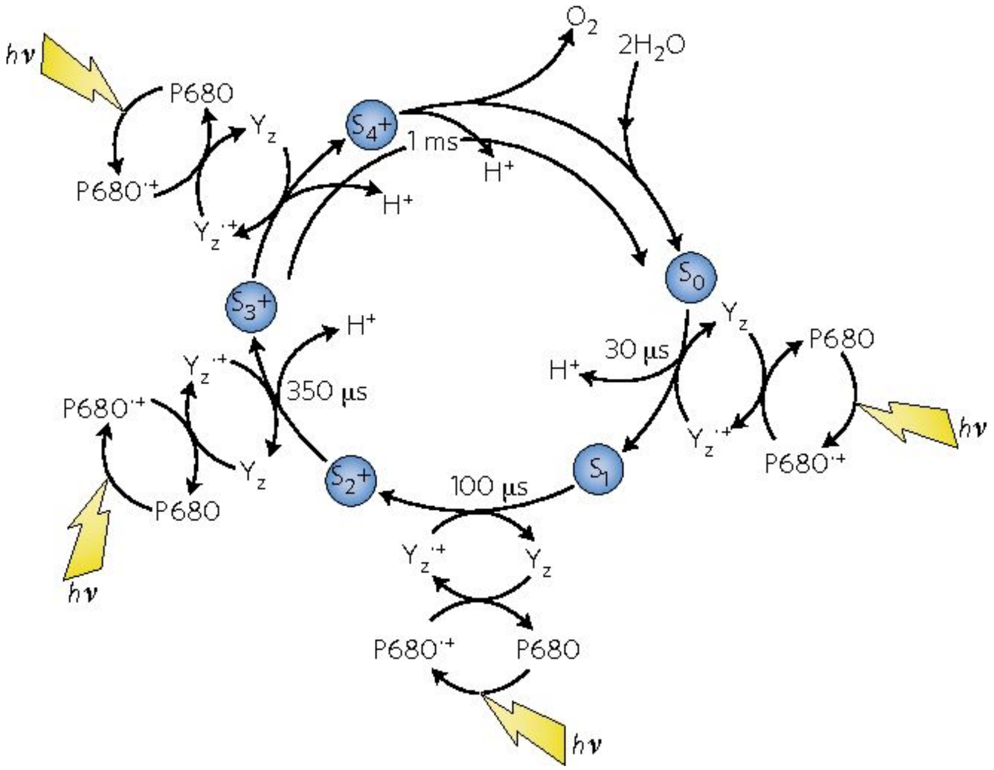
Oxidation: $2\,H_2O \longrightarrow O_2 + 4\,H^+ + 4\,e^-$

Reduction: $4\,H^+ + 4e^- \longrightarrow 2\,H_2$

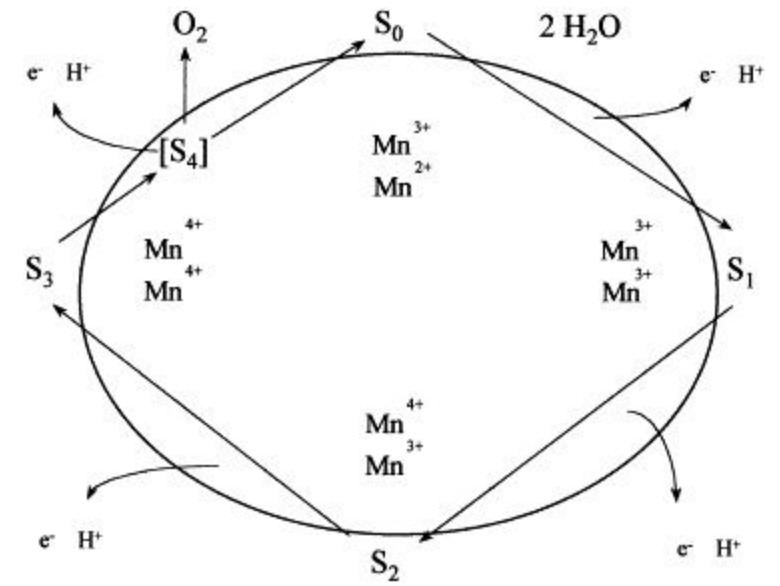
Scheme 1. Overall water splitting reaction and the two constituent half reactions.

- Four electrons are removed from two water molecules generating one oxygen molecule and four hydrogen ions
- Oxidation of water is a very difficult reaction which occurs only in photochemical reactions
- It is the source of almost all oxygen on earth
- When a dark adopted photosynthetic membrane is exposed to sequential, brief, intense flashes; no oxygen is produced in the first two flashes
- Maximum oxygen is released on the third flash and every fourth flash thereafter until it reaches a constant value

- Photo oxidation of water takes place by S state mechanism consisting of five states S_0,S_1,S_2,$S_3$and S_4 in a cyclic manner called **kok clock** (Kok *et al*, 1970)
- It is the water oxidising enzyme system or oxygen evolving complex (OEC)
- The light flashes advances the system from one S state to the next, until S_4 is reached
- S_4 is unstable and reacts with two water molecules to produces O_2 and returns the system to S_0
- Hydrogen are also a product of water oxidation
- Protons are released before O_2 and are generated from ionisable protein groups in the region of OEC
- Protons are released in the lumen side of the thylakoid as the OEC is located on the interior surface of the thylakoid
- These protons are eventually released from the lumen to the stroma through ATP synthesis



- Manganese is an essential cofactor in the water oxidation process
- X-ray absorption and ESR studies indicate that 4 Mn ions together with 2-3 Ca^+ ions and 4-5 Cl^- ions remain associated with each OEC
- OEC remains bound to the D1 and D2 proteins of the PS II reaction centre
- The four electrons extracted from water pass to P680 one at a time
- Tyrosine residue (Tyr_Z) attached to D1 protein is the immediate donor of electron to P680
- Tyr_Z regains its lost electron by oxidising the Mn cluster
- With the loss of each electron the cluster becomes more oxidised and ultimately reach the 4+ state
- Mn-complex^{4+} can extract 4 electrons from two water molecules and finally release proton (H^+) and O_2



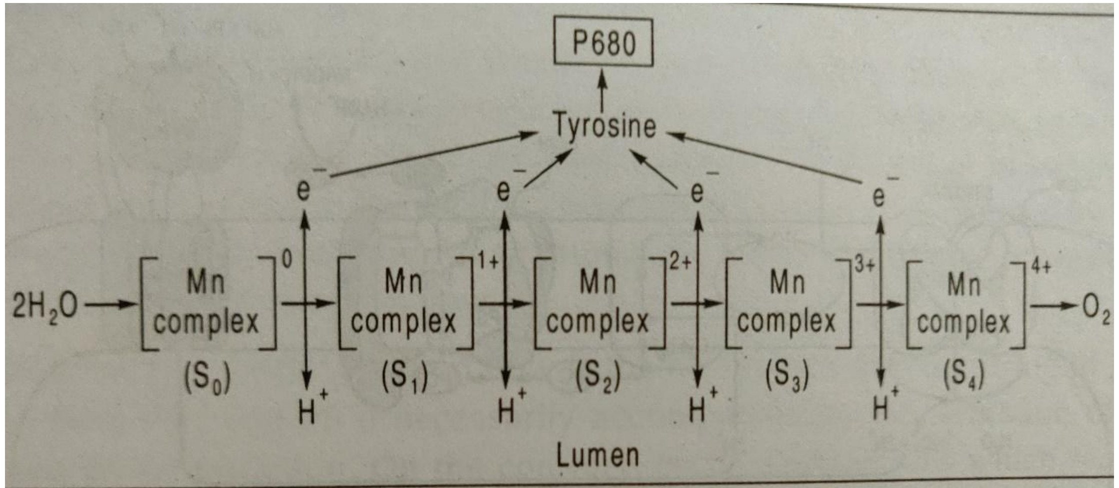
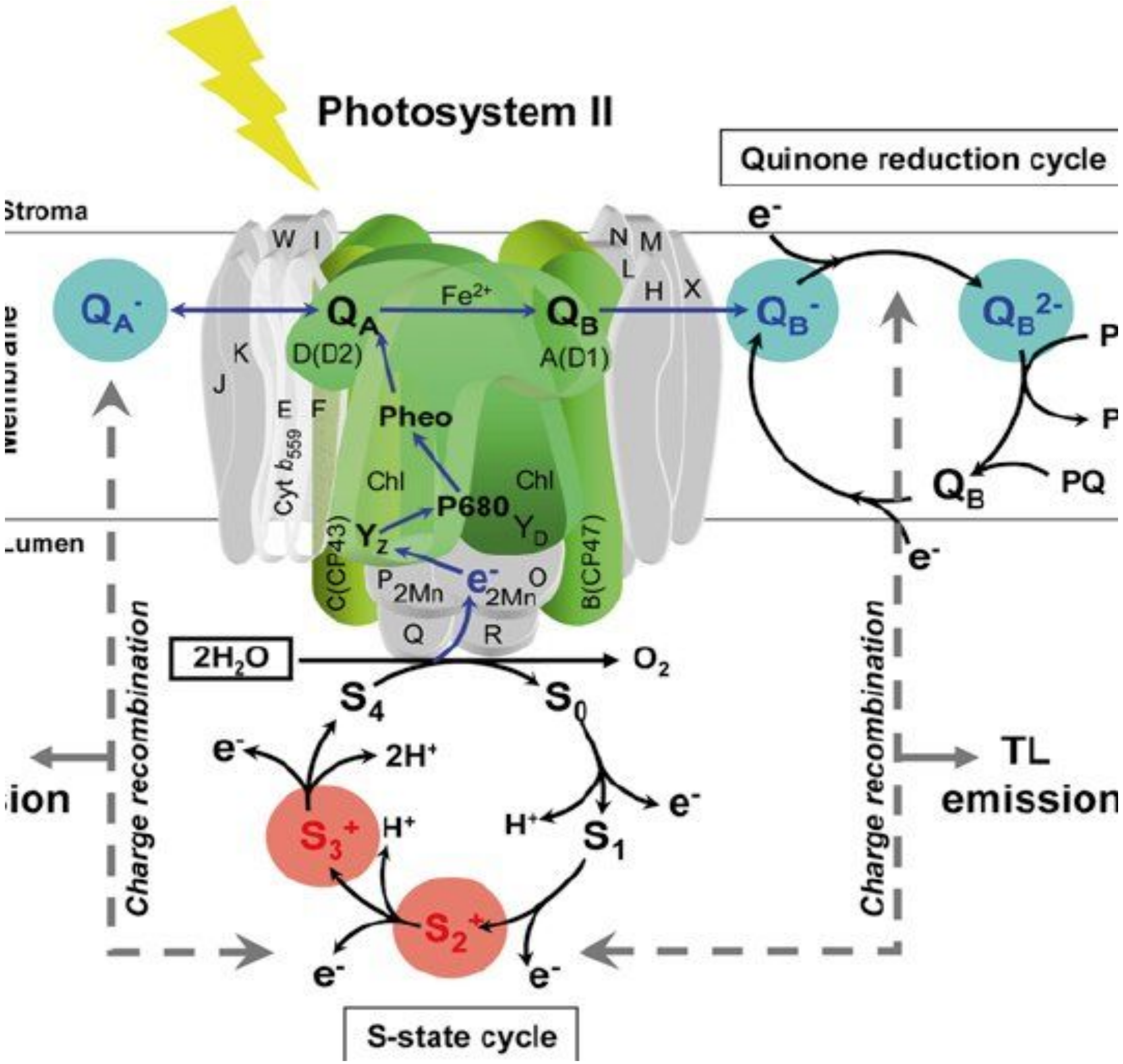


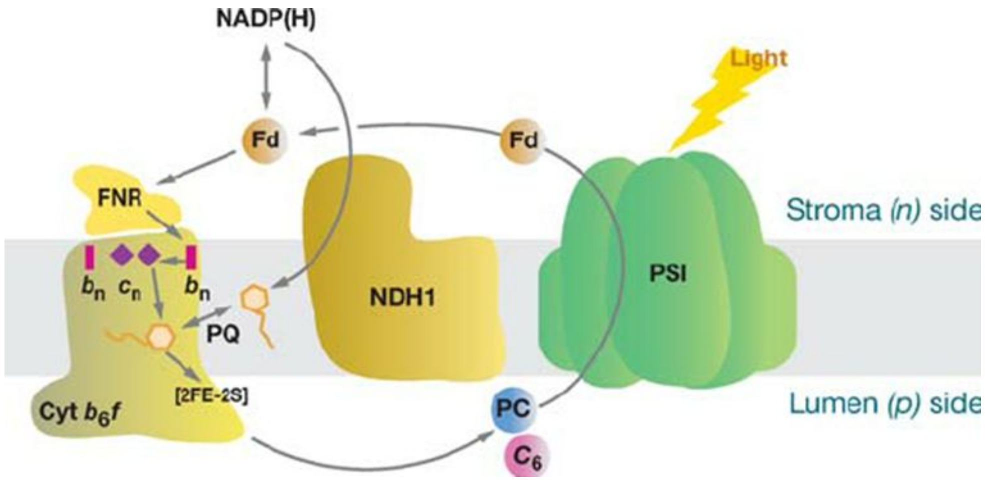
Fig. 8.16 : Water-splitting activity of the oxygen-evolving complex. The oxygen-evolving complex pass $4e^-$, one at a time to $P680^+$ through tyrosine residue which oxidize a cluster of 4 Mn ions. Four single electron transfers, each corresponding to the absorption of one photon, produce a charge of 4+ on the Mn-complex. Mn-complex can take $4e^-$ from a pair of water molecules, releasing $4H^+$ and O_2



Photosystem II
Quinone reduction cycle
Stroma
Lumen
W I
N M
L
H X
K
J
E F
Cyt b_{559}
Q_A^-
Q_A
Fe^{2+}
Q_B
D(D2)
A(D1)
Q_B^-
Q_B^{2-}
Pheo
Chl
Chl
P680
Y_Z
Y_D
C(CP43)
B(CP47)
P
O
2Mn
e^-
2Mn
Q
R
$2H_2O$
O_2
S_4
S_0
S_1
S_2^+
S_3^+
e^-
$2H^+$
H^+
H^+
e^-
e^-
e^-
Q_B
PQ
Charge recombination
Charge recombination
TL
emission
S-state cycle

Cyclic Electron Transport

- Cyclic electron transport neither involve oxidation of water, nor reduction of $NADP^+$, require only the excitation of P700
- It is a shunt or bypass when NADP+ is not available to act as an electron acceptor
- It is not inhibited by DCMU (dichlorophenyldimethyl urea),an inhibitor for non-cyclic electron flow, but by DBMIB (dibromo methyl isopropyl benzoquinone)
- P700 looses one electron by light excitation which passes through acceptors like X, A, B and finally reduces ferredoxin
- Fd_{red}, instead of transferring electron to $NADP^+$, interact with a Fd-plastoquinone oxidoreductase that transfers electron into PQ pool
- Plastoquinol is oxidised by cytochrome b_6f complex
- Protons are translocated across the membrane via PQ cycle while electrons are passed to the electron hole in PS I
- Coupled to this electron flow phosphorylation of ADP to ATP occurs (cyclic photophosphorylation)



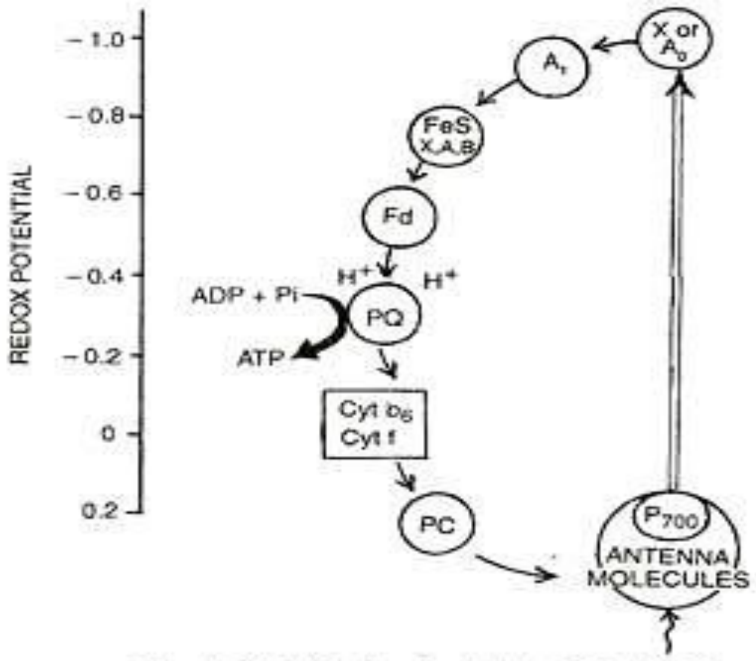


Fig. 13.17. Cyclic photophosphorylation.

S. No.	Photosystem I	Photosystem II
1.	PS I lies on the outer surface of the thylakoids.	PS II lies on the inner surface of the thylakoids.
2.	In this system, molecular oxygen is not evolved.	As the result of photolysis of water, molecular oxygen is evolved.
3.	Its reaction center is P700.	Its reaction center is P680.
4.	NADPH is formed in this reaction.	NADPH is not formed in this reaction.
5.	It participates both in cyclic and non-cyclic photophosphorylation.	It participates only in non-cyclic photophos-phorylation.
6.	It receives electrons from photosystem II.	It receives electrons from photolytic dissociation of water.
7.	It is not related with photolysis of water.	It is related with photolysis of water.

Table 13.3 Differences between Cyclic Photophosphorylation and Non-Cyclic Photophosphorylation

Cyclic Photophosphorylation	Non-Cyclic Photophosphorylation
1. PS I only involved	1. PS I and PS II involved
2. Reaction centre is P700	2. Reaction centre is P680
3. Electrons released are cycled back	3. Electron released are not cycled back
4. Photolysis of water does not take place	4. Photolysis of water takes place
5. Only ATP synthesized	5. ATP and NADPH + H^+ are synthesized
6. Phosphorylation takes place at two places	6. Phosphorylation takes place at only one place
7. It does not require an external electron donor	7. Requires external electron donor like H_2O or H_2S
8. It is not sensitive to di chloro di methyl urea (DCMI)	8. It is sensitive to DCMI and inhibits electron flow