

Inti dari sintesis NADPH dan ATP dalam proses fotosintesis adalah aliran elektron ( $e^-$ ) dan proton ( $H^+$ ) yang melibatkan dua fotosistem (Photosystem I dan II) dan sejumlah molekul.

### LECTURE OUTCOMES

After the completion of this lecture and mastering the lecture materials, students should be able to

1. Explain the conversion mechanism of light energy to chemical energy (NADPH & ATP) in the process of photosynthesis
2. Explain electron ( $e^-$ ) excitation and flow in the conversion mechanism of light energy to chemical energy
3. Explain the function of photosystem I and II in the synthesis of NADPH and ATP
4. To explain proton ( $H^+$ ) transport in relation to ATP synthesis

### LECTURE OUTLINE

#### 1. NADPH SYNTHESIS

##### 1.1 Light Absorption

##### 1.2 Electron Excitation

*Fluorescence*

*Phosphorescence*

##### 1.3 Electron Transfer & NADPH Synthesis

#### 2. ATP SYNTHESIS

##### 2.1 Introduction

##### 2.2 Chemiosmosis

##### 2.3 Enzymen ATP Synthase

##### 2.4 Stoichiometry of ATP Synthesis

##### 2.5 Effect of Herbicides

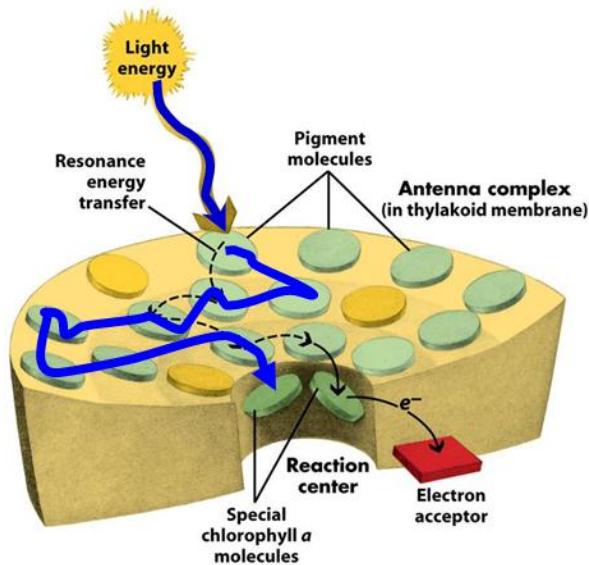
## MODUL 03



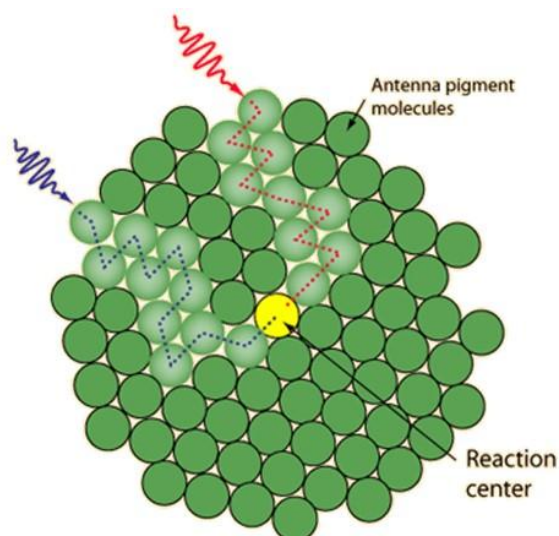
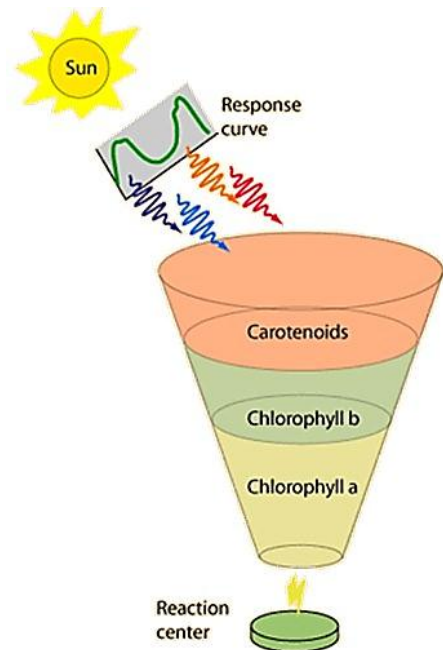
SELF-PROPAGATING ENTREPRENEURIAL EDUCATION DEVELOPMENT

# 1. NADPH SYNTHESIS

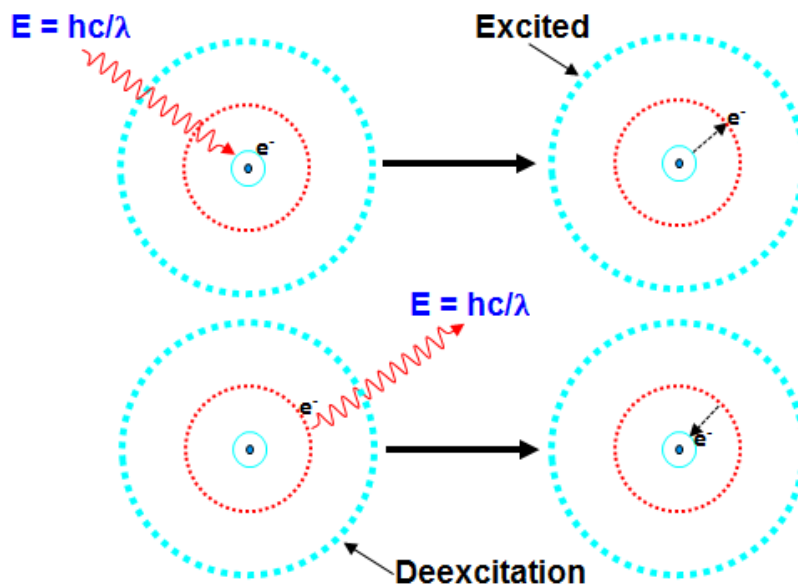
## 1.1 Light Absorption



1. The capture of light energy for photosynthesis is enhanced by networks of pigments.
2. Robert Emerson and William Arnold in 1932 found that some 2500 molecules of chlorophyll was required to produce one molecule of  $O_2$ , and that a minimum of eight photons of light must be absorbed in the process.
3. The model that emerges is that of some 300 chlorophyll molecules and 40 or so beta carotenes and other accessory pigments acting as a light harvesting antenna surrounding one chlorophyll a molecule that is a part of a reaction center.

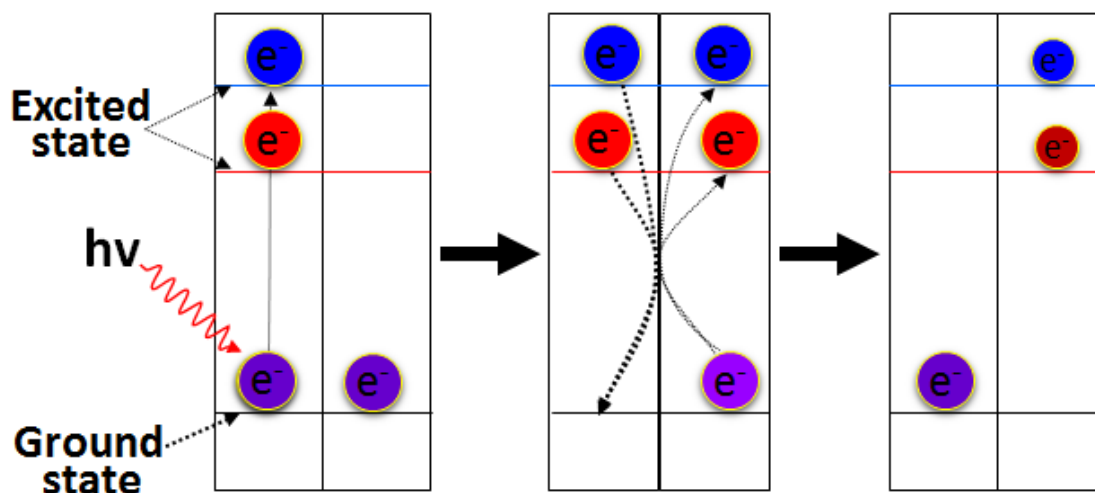


4. A photon is absorbed by one of the pigment molecules then transferred by successive fluorescence events to neighboring molecules until it reaches the action center where the energy is used to transfer an energetic electron to an electron acceptor.



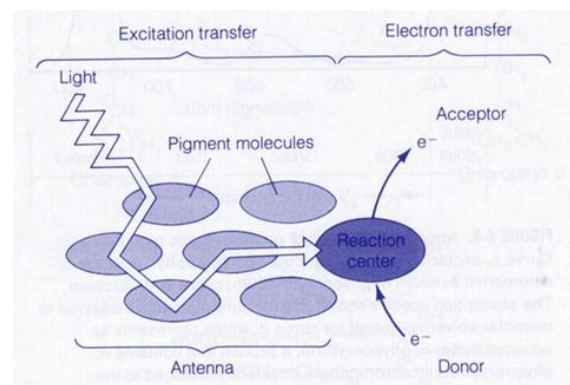
**Basic principle of electron excitation**

5. Resonance Energy Transfer (**RET**) is also known as Förster resonance energy transfer (**FRET**), fluorescence resonance energy transfer (**FRET**), or electronic energy transfer (**EET**).
6. The fluorescence model would suggest that each transferred photon has a longer wavelength and lower quantum energy with some energy being lost to heat.



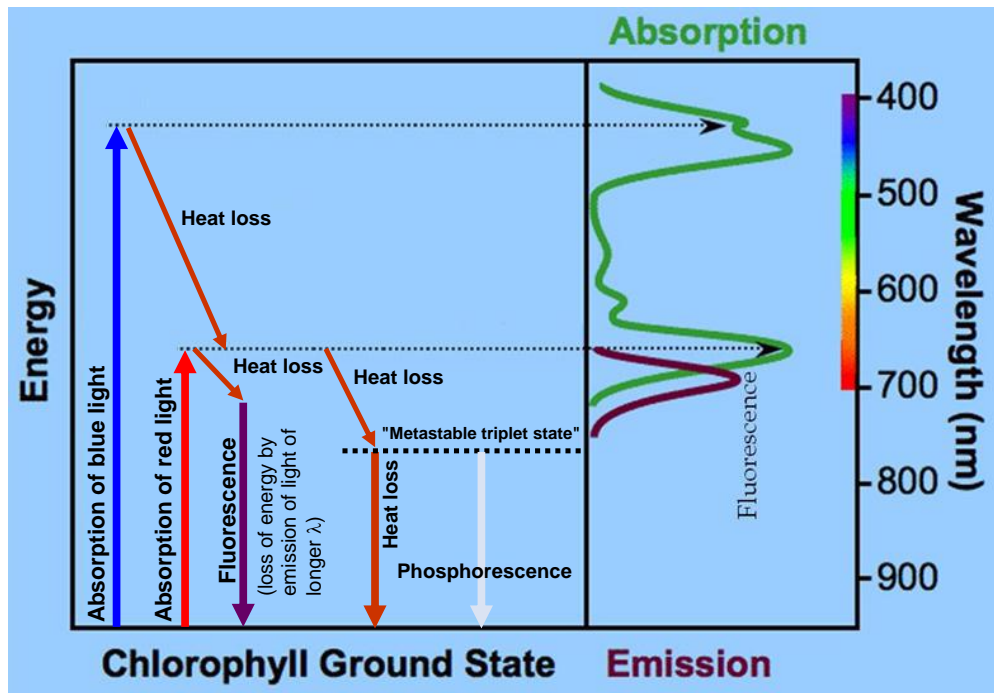
## 1.2 Electron Excitation

1. Absorpsi foton mengakibatkan pengaturan elektron intramolekul pada pusat reaksi yang diikuti dengan tranfer elektron antar molekul.
2. Pada mulanya, elektron khlorofil pada pusat reaksi tereksitasi pada orbit yang menjauhi inti atom dan molekul dengan absorpsi foton langsung atau lebih mungkin melalui transfer energi



foton dari antenna pigmen.

- When a photon reaches the chlorophyll a in the reaction center, that chlorophyll can receive the energy because it absorbs photons of longer wavelengths than the other pigments.



- Red light absorbed by photosystem II (PSII) produces a strong oxidant and a weak reductant.
- Far-red light absorbed by photosystem I (PSI) produces a weak oxidant and a strong reductant.
- The strong oxidant generated by PSII oxidizes water, while the strong reductant produced by PSI reduces NADP.

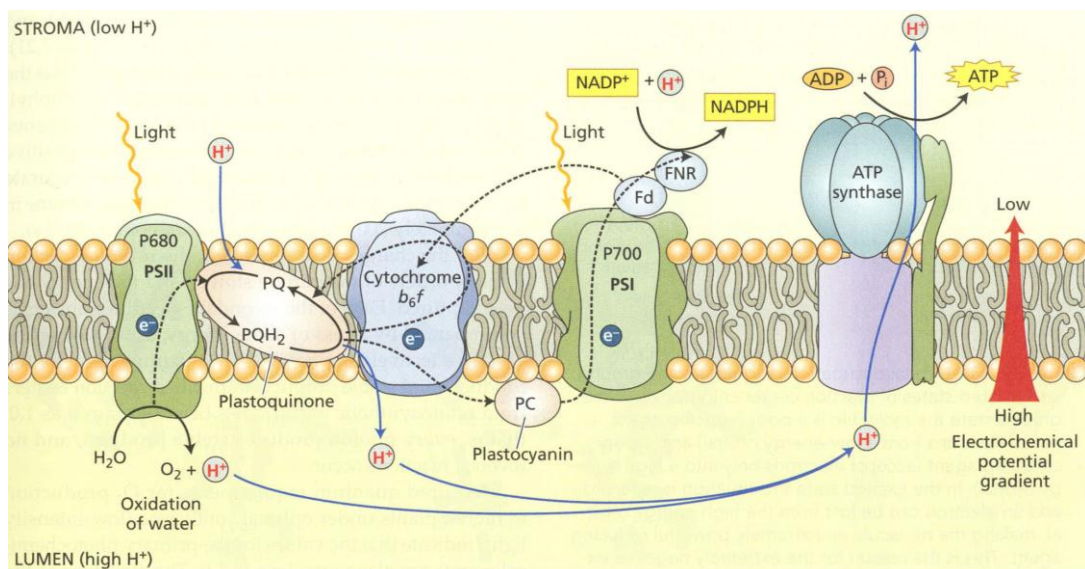


Fig. 7.22 The transfer of electrons and protons in the thylakoid membrane is carried out vectorially by four protein complexes. Water is oxidized and protons are released in the lumen by PSII.



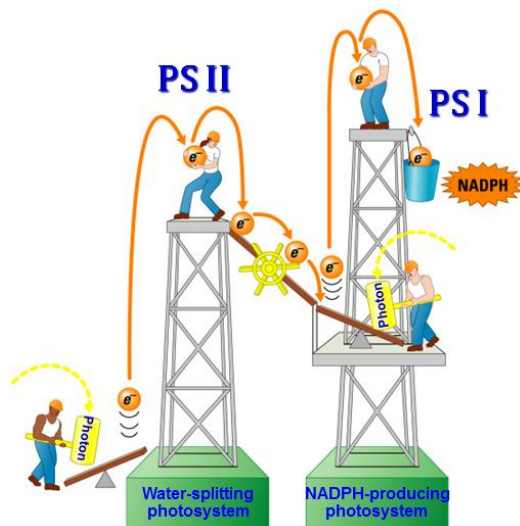


Illustration of two photosystems (PS) that cooperate in the light reactions

### Electron transport in the thylakoid membrane

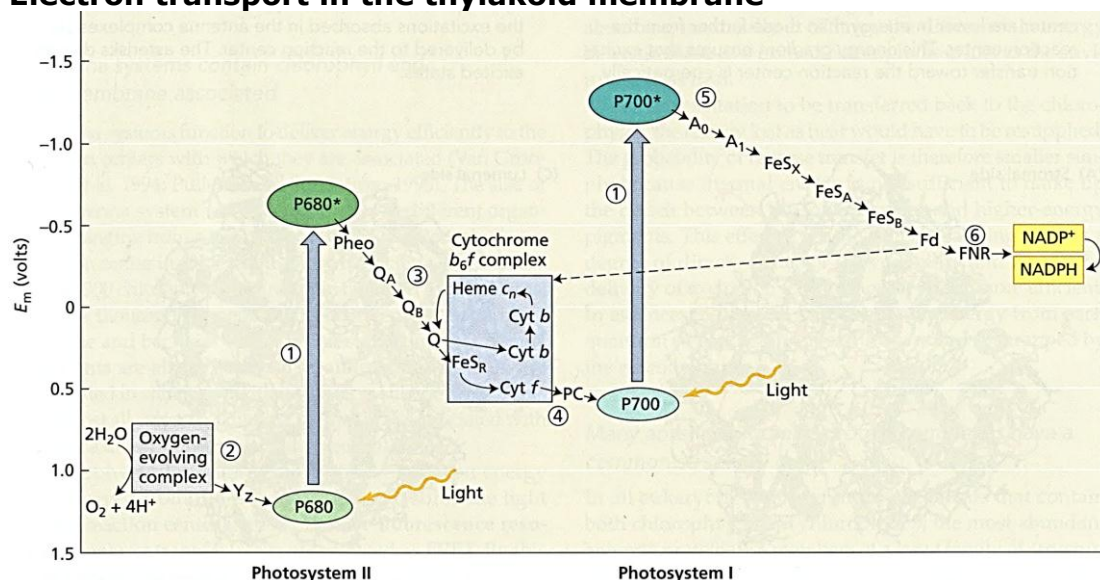


Fig. 7.21 Detailed Z scheme for  $O_2$ -evolving photosynthetic organisms. The redox carriers are placed at their midpoint redox potentials (at pH 7).

### 1.3 Electron Transfer & NADPH Synthesis

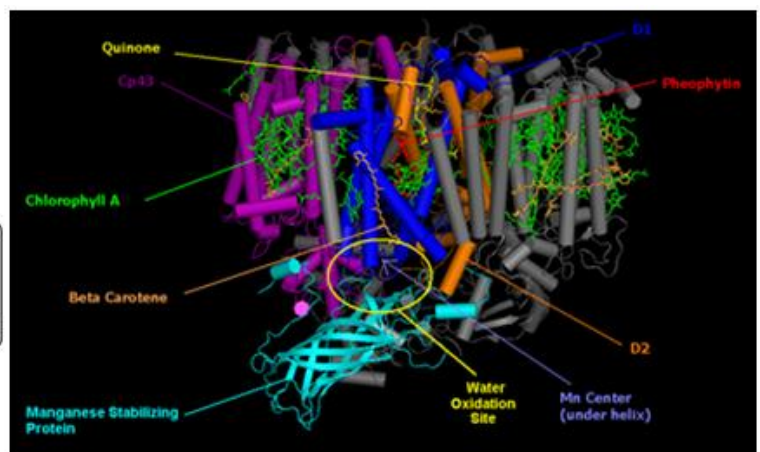
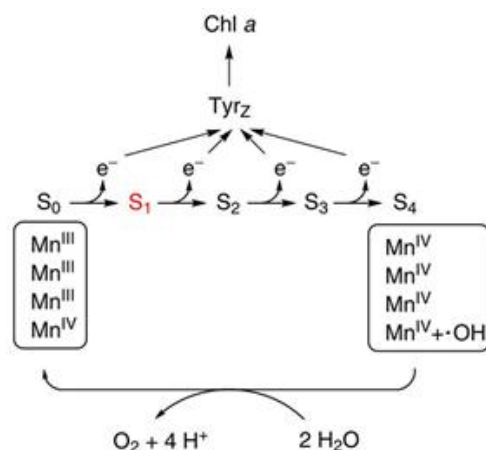
1. $H_2O$	8. $P700^+$ (PSI reaction center chlorophyll)
2. <b>Z (PSII)</b>	9. $A_0$ (chlorophyll ?)
3. <b><math>P680^*</math> (PSII reaction center chlorophyll)</b>	10. $A_1$ (quinone?)
4. <b>Pheo (pheophytin)</b>	11. <b><math>FeS_x</math>, <math>FeS_B</math>, &amp; <math>FeS_A</math></b> (membrane-bound iron-sulfur proteins)
5. <b><math>Q_A</math> and <math>Q_B</math> (plastoquinone acceptors)</b>	12. <b>Fd (soluble ferredoxin)</b>
6. <b>Cytochrome <i>b.-f</i> complex</b>	13. <b>Fp (flavoprotein ferredoxin-NADP reductase)</b>
7. <b>PC (plastocyanin)</b>	14. <b>NADP</b>

## 1.4 Fluorescence and Phosphorescence

1. **Fluorescence** ad. Emisi cahaya dari molekul yang sedang diiradiasi sebagai akibat dari penurunan elektron dari orbit 1 ke orbit dasar. Proses ini tidak tergantung suhu dan berlangsung cepat (lifetime  $<10^{-8}$  detik). Panjang gelombang lebih besar dari panjang gelombang yang diabsorpsi (chlorophyll a mengabsorpsi cahaya pada 430 & 630 nm, dan mememisi cahaya pada 668 nm).
2. **Phosphorescence** ad. Emisi cahaya dari molekul sebagai akibat penurunan elektron dari "triplet state" ke orbit dasar. Cahaya yang dihasilkan berlangsung relatif perlahan ( $10^{-4} - 2$  detik), dan panjang gelombang relatif sangat panjang.

## 1.5 Electron Transfer

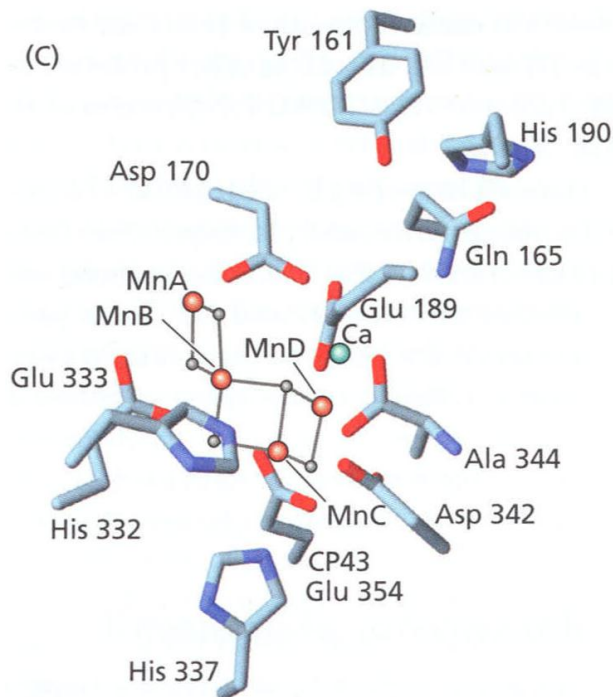
1. Water ( $H_2O$ ) is the source of electrons extracted by **the oxygen-evolving complex (OEC)** that are used to rereduce  $Z^+$ , **a tyrosine** side chain on the reaction center protein D1



[https://en.wikipedia.org/wiki/File:Photosystem-II\\_2AXT.PNG](https://en.wikipedia.org/wiki/File:Photosystem-II_2AXT.PNG)

2. On the oxidizing side of PSII, to the left of the arrow joining P680 with P680\* (Fig. 7.21), P680<sup>+</sup> is rereduced by Z, the immediate donor to PSII.
3. The excited PSII reaction center chlorophyll, (P680\*) transfers an electron to **pheophytin** (Pheo).
4. On the reducing side of PSII (to the right of the arrow joining P680 with P680\*), the pheophytin transfers electrons to the **plastoquinone** acceptors Q<sub>A</sub> and Q<sub>B</sub>.
5. The cytochrome *b*.-*f* complex, reduced by plastoquinone, transfers electrons to **plastocyanin** (PC), which in turn reduces **P700<sup>+</sup>**.
6. The *b*.-*f* complex contains a **Rieske iron-sulfur protein** (FeSR), two *b*-type cytochromes (**cyt b**), and cytochrome *f* (**cyt f**).
7. The acceptor of electrons from P700\* (A<sub>0</sub>) is thought to be a **chlorophyll**, and the next acceptor (A<sub>1</sub>) may be a **quinone**.
8. A series of membrane-bound iron-sulfur proteins (FeS<sub>x</sub>, FeS<sub>B</sub>, and FeS<sub>A</sub>) transfer electrons to soluble ferredoxin (Fd).
9. The flavoprotein ferredoxin-NADP reductase (Fp) serves to reduce **NADP**, which is used in the Calvin cycle to reduce CO<sub>2</sub>.
10. The dashed line indicates cyclic electron flow around PSI.
11. PSII produces electrons that reduce the cytochrome *b*.-*f* complex, while PSI produces an oxidant that oxidizes the cytochrome *b*.-*f* complex.

12. *P680* and *P700* refer to the wavelengths of maximum absorption of the reaction center chlorophylls in PSII and PSI



Detail of the Mn-containing water-splitting complex

### QUESTIONS

1. How is the light energy transferred from antenna pigments to chlorophyll reaction centers?
2. What is the compound that receives electrons from OEC (the oxygen-evolving complex)?
3. What is the first chemical compound that receives electrons from the excited reaction center of PS II?
4. What is fluorescence?
5. What is the function of plastoquinone?
6. What is the compound that reduces plastocyanin?
7. What is the enzyme that functions to reduce NADP to NADPH?
8. What is the first chemical compound that receives electrons from the excited reaction center of PS I?

### Brain Exercise

Eksitasi 1 mol  $e^-$  pada setiap pusat reaksi (PSII & PSI) membutuhkan 1 kuantum cahaya. Reduksi 1 mol  $NADP \rightarrow 1$  mol  $NADPH$  membutuhkan 2 mol  $e^-$ .

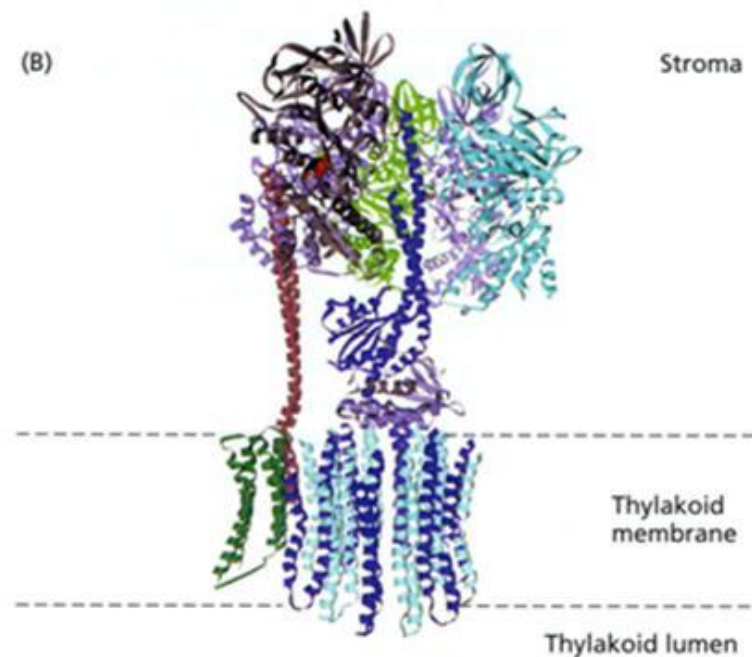
1. Berapa kuantum cahaya dibutuhkan untuk pembentukan 1 mol  $NADPH$  ?
2. Berapa  $NADPH$  dihasil dari hasil fotolisis air ?
3. Tingkat Cahaya di Malang sekitar  $1 \text{ mmol} \cdot \text{s}^{-1}$ , berapa  $NADPH$  yang dihasilkan dengan tingkat cahaya demikian ?

### Tugas

1. Where does the light reaction happen ?

2. What is the function of water in photosynthesis ?
3. What is the event to happen after the light interception by pigments
4. What is the first molecule receiving electrons from the pigments (chlorophyll) excited at PS I
5. What is the first molecule receiving electrons from the pigments (chlorophyll) excited at PS II

## 2. ATP SYNTHESIS



Compiled crystal structure of chloroplast  $F_1F_0$  ATP synthase

### • Introduction

1. A portion of the photon's energy is captured as the high-energy phosphate bond in **ATP**.
2. Electron flow must occur for the formation of ATP (photophosphorylation), although under some conditions electron flow may not be accompanied by phosphorylation.
3. It is now widely accepted that photophosphorylation works via the chemiosmotic mechanism, first proposed in the 1960s by Peter Mitchell.

### • Chemiosmosis

1. The basic principle of chemiosmosis is that **ion concentration differences** and **electrical potential differences** across membranes are **Chemiosmosis energy** that can be utilized by the cell.

### • Photophosphorylation

1. The synthesis of ATP in chloroplasts occurs when the energy of a proton gradient is coupled to phosphorylate ADP to ATP by an F-type ATPase.
2. Protons are accumulated on the inside of the thylakoid lumen and depleted in the stroma compartment.
3. As described by the second law of thermodynamics, any nonuniform distribution of matter or energy represents a source of energy.



4. Chemical potential differences of any molecular species whose concentrations are not the same on opposite sides of a membrane provide such a source of energy.

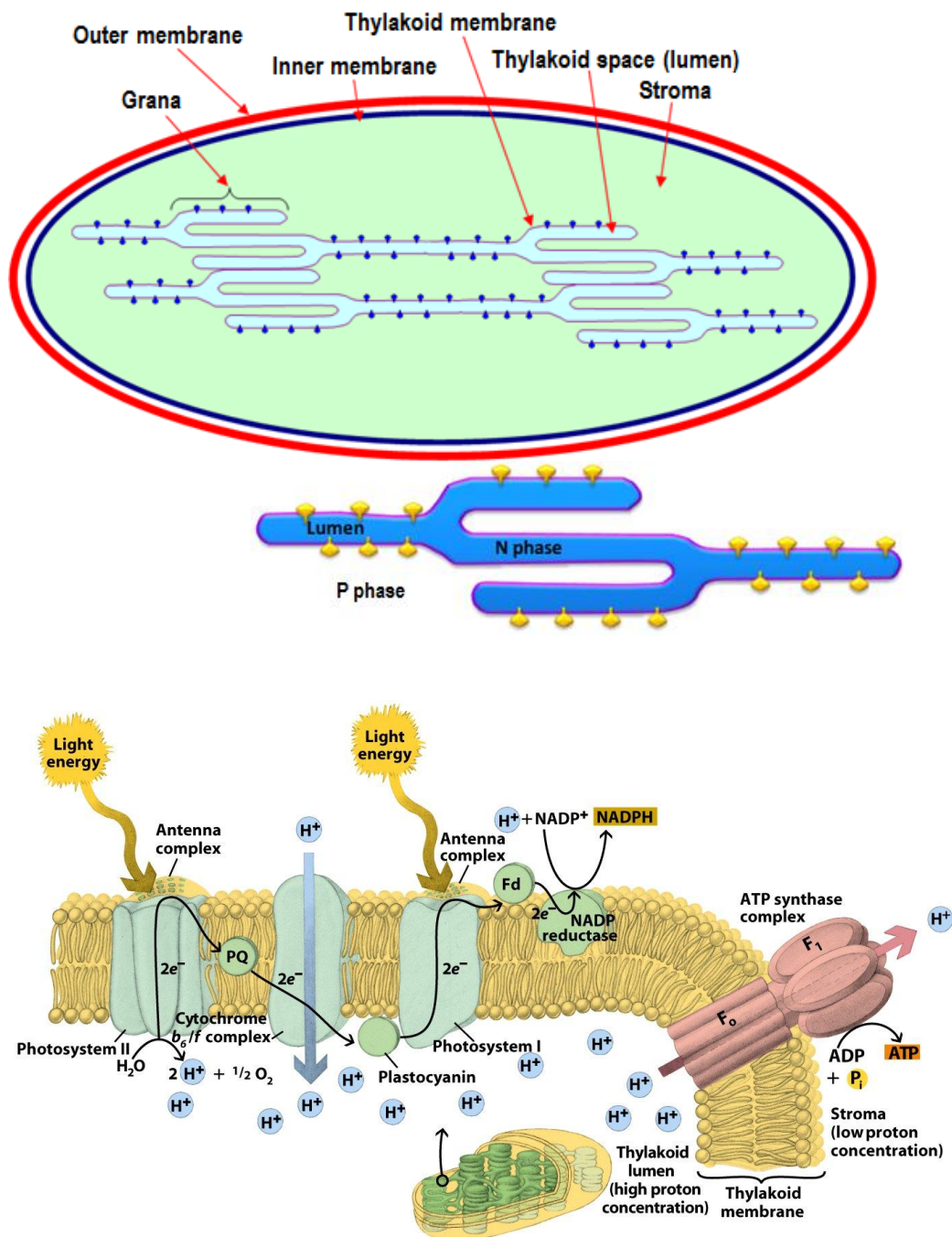


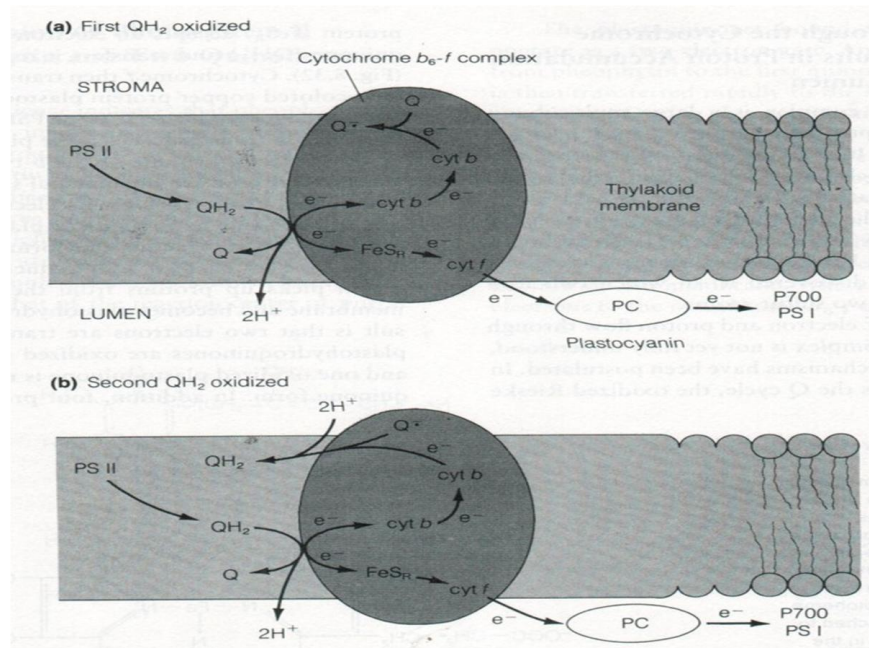
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The production of ATP by chemiosmosis in photosynthesis

5. Proton flow from one side of the membrane to the other accompanies electron flow in addition to the asymmetric nature of the photosynthetic membrane.
6. The direction of proton translocation is such that the STROMA BECAMES MORE ALKALINE and the LUMEN BECAMES MORE ACIDIC when electron transport occurs.

Condition	pH of chloroplast compartment	
	Thylakoid lumen	Stroma
Dark	7.0	7.0
Light	5.2	8.2

Transport of proton from stroma to lumen following the transfer of electron from plastoquinone, through cytochrome  $b_6-f$ , to plastocyanine.



7. Some of the early evidence in support of a chemiosmotic mechanism was done by Andre Jagendorf and co-workers.
  - They then rapidly injected the suspension into a pH 8 buffer solution thereby creating a pH difference of 4 units across the thylakoid membrane, with the inside acidic relative to the outside.
  - They found that large amounts of ATP were formed from ADP and Pi by this process, without any light input or electron transport.

### ATP Experiment: Jagendorf and co-workers

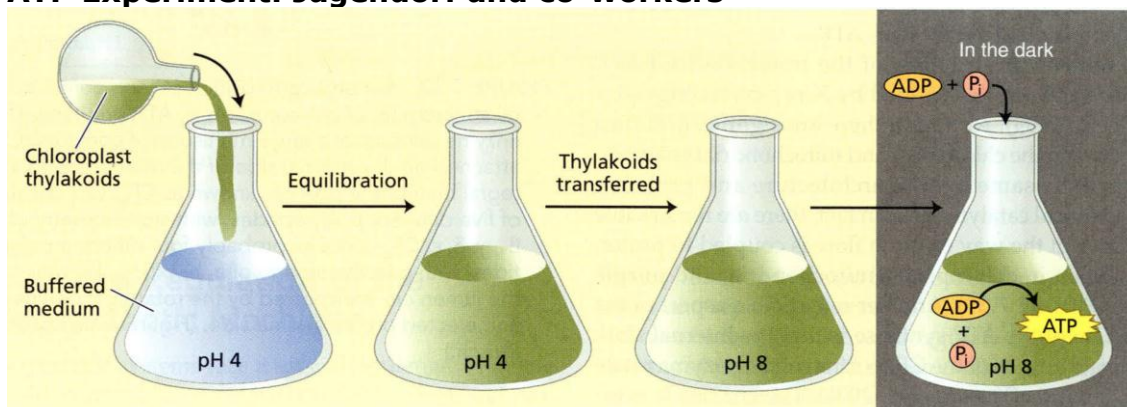
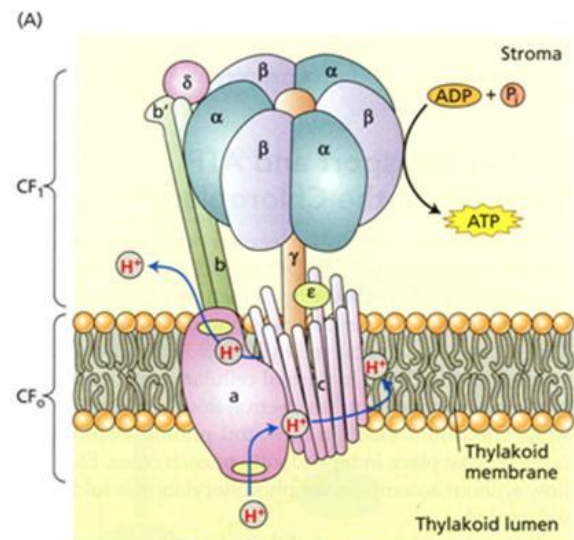


Fig. 7.31 Summary of the experiment carried out by Jagendorf and co-workers. Isolated chloroplast thylakoids kept previously at pH 8 were equilibrated in an acid medium at pH 4. The thylakoids were then transferred to a buffer at pH 8 contained ADP and Pi.

### • Enzyme ATP Synthase

1. The ATP is synthesized by a large (400-kDa) enzyme complex known as the coupling factor, ATPase, ATP synthase or CF<sub>0</sub>-CF<sub>1</sub>.

2. This enzyme consists of two parts: a hydrophobic membrane bound portion called  $CF_0$  and a portion that sticks out into the stroma called  $CF_1$ .
3.  $CF_0$  appears to be a channel across the membrane through which protons can pass, while  $CF_1$  is the portion of the complex that actually synthesizes ATP.
4. Protons from the lumen are transported by the rotating c polypeptide and ejected on the stroma side. (Figure courtesy of W. Frasch in Taiz & Zeiger, 2010).
- 5.

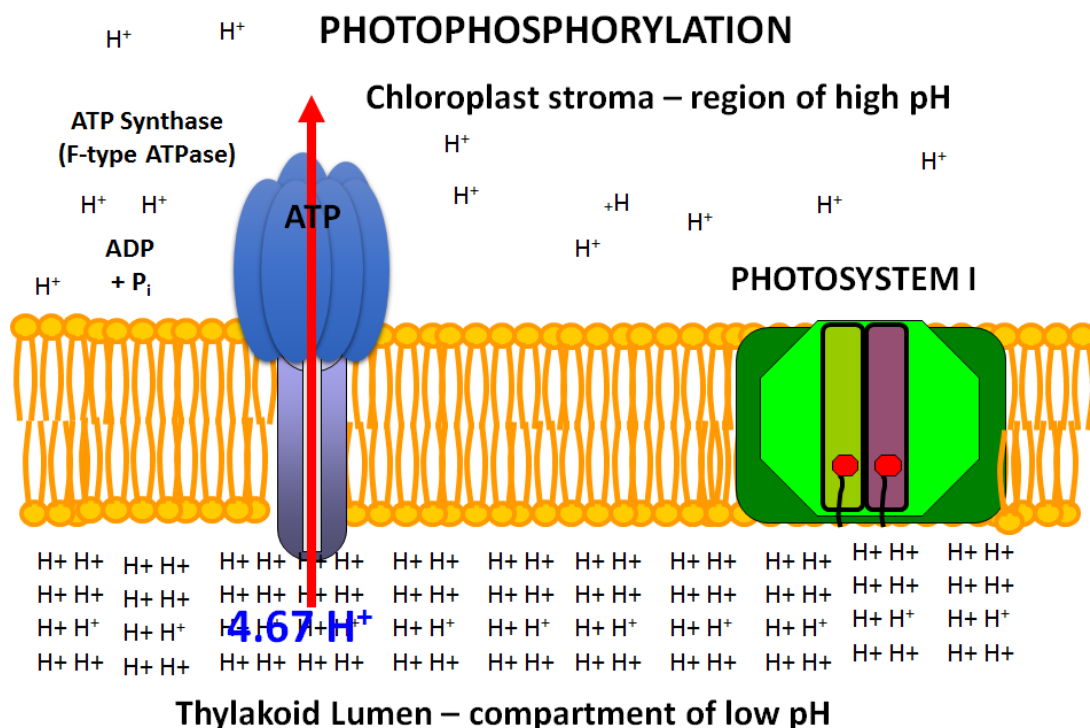


### • Stoichiometry of ATP Synthesis

1. Total energy available for ATP synthesis, proton motive force ( $\Delta p$ ), is the sum of a proton chemical potential and a transmembrane electrical potential and
2. These two components of the proton motive force from the outside of the membrane to the inside are given by

$$\Delta p = \Delta E_m - 59(pH_i - pH_o) \quad (mV)$$

- $\Delta E_m$  (or  $\Delta \psi$ ) is the transmembrane potential and
- $pH_i - pH_o$  (or  $\Delta pH$ ) is the pH difference across the membrane.



3. Based on the equation, a transmembrane pH difference of one unit is equivalent to a membrane potential of 59 mV.



- Under conditions of steady state electron transport in chloroplasts, the membrane electrical potential is quite small, so  $\Delta p$  is due almost entirely to  $\Delta pH$ .
- The stoichiometry of protons translocated to ATP synthesis has been to be **(14/3)**  $H^+/ATP \rightarrow 4.67 H^+/ATP$  before  $3H^+/ATP$ .

## QUESTIONS

- What is the enzyme that catalyze the phosphorylation of ADP to be ATP?
- What is the function of each component of the enzyme (question No. 1)?
- What is the basic principle of chemiosmosis?
- What is the early evidence that support chemiosmotic mechanism?
- What is the stoichiometry of protons translocated to ATP synthesis?

## • Effect of Herbicides

- Herbicides are xenobiotics (foreign chemicals to plants) that are designed to kill plants but are not supposed to harm humans and animals.
- Ideally, herbicides will only kill weeds and various noxious plants, leaving crops unharmed.
- Major types of herbicides
  - Hormone mimics: e.g. 2,4-D mimics the plant hormone auxin. (trade-name of Weed-B-Gone)
  - Inhibitors of biosynthetic pathways unique to plants: e.g. glyphosphate (Round-up)
  - Inhibitors of photosynthetic electron transport: e.g. methyl viologen (Paraquat) and atrazine

## Inhibitor of Photosynthetic Electron Transport

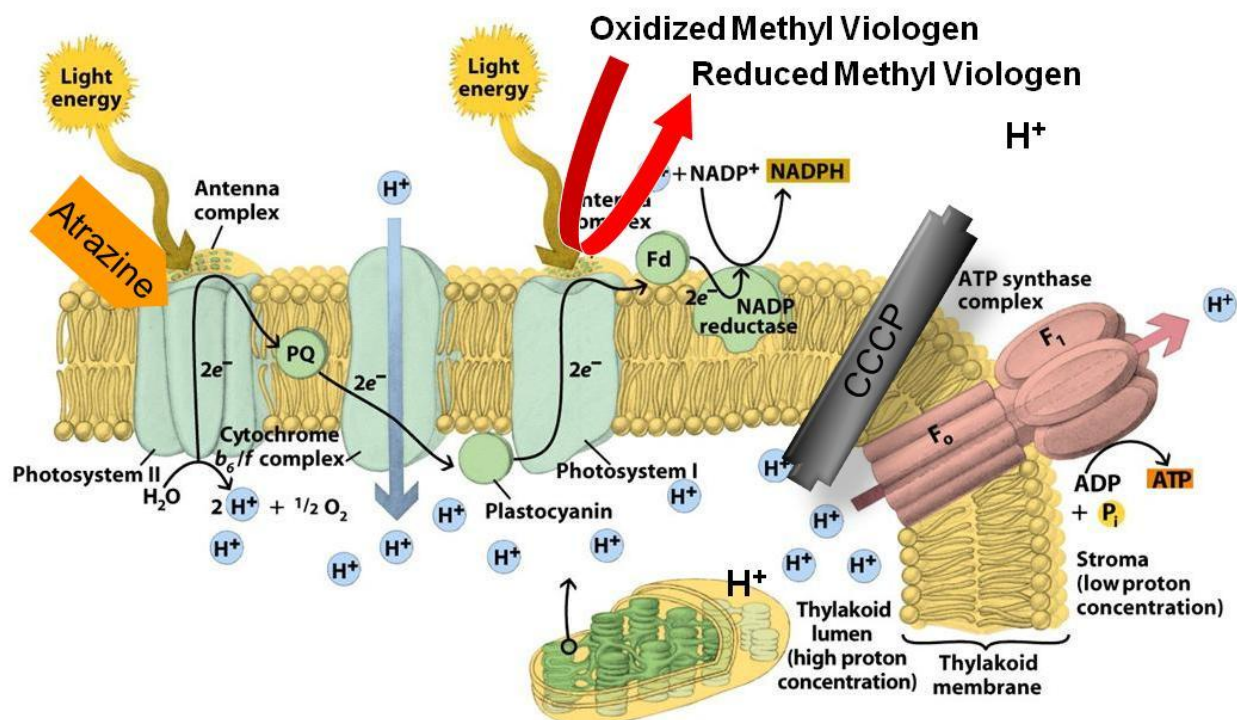
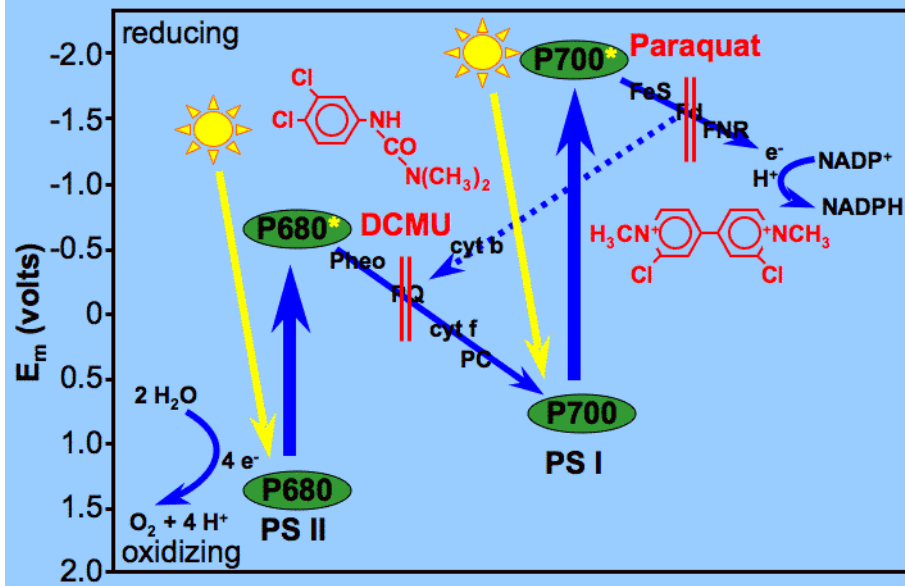


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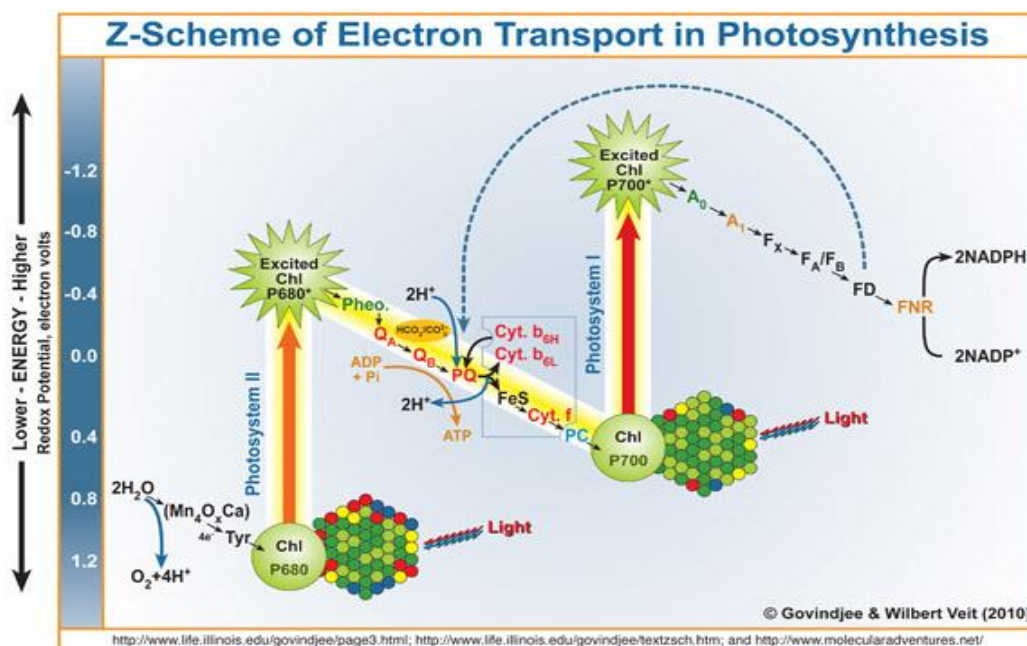
### The Z-scheme of the Light Reactions: An Energy Diagram



**DCMU** (3-(3,4-dichlorophenyl)-1,1-dimethylurea) blocks the plastoquinone binding site of photosystem II, disallowing the electron flow from where it is generated, in photosystem II, to plastoquinone

### • Cyclic Photophosphorylation

1. Electron in Photosystem I is excited and transferred to **ferredoxin** (FD) that shuttles the electron to the **cytochrome complex**.
2. The electron then travels down the electron chain and re-enters photosystem I.



Process for ATP generation associated with some Photosynthetic Bacteria

3. In sulfur bacteria, only one photosystem is used and ATP is generated via electron transport

4. In anoxygenic photosynthesis, excited electron passed to electron transport chain that generates a proton gradient for ATP synthesis

### **Tugas**

1. How many is  $H^+$  transported across membrane from lumen to stroma for the transfer of each  $1e^-$  to  $NADP^+$
2. How many is ATP produced for each formation of 1 NADPH
3. How much is the light quanta required for each formation of ATP
4. What does it mean by proton motive force
5. What is the enzyme catalyzing the synthesis of ATP from ADP

### **REFERENCE**

Taiz, L. and Zeiger, E., 2010. Plant Physiology Chapter 7: Light Reactions. Benjamin/Cummings, Company, Inc., Redwood City, California