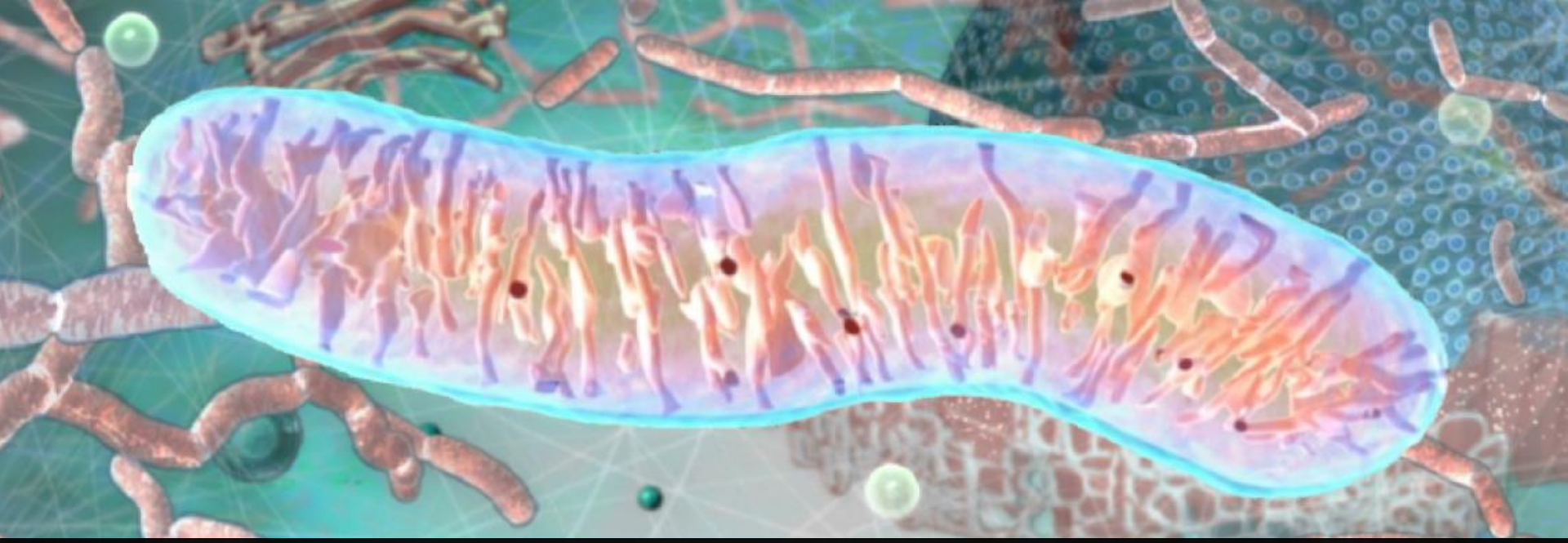


The enzymatic basis of energy-generation

Lecture 4: Photosynthesis light reactions

Dr Chris Greening

Lecturer / Group Leader

Monash University

April 18 2016

Lecture 4: Photosynthesis light reactions

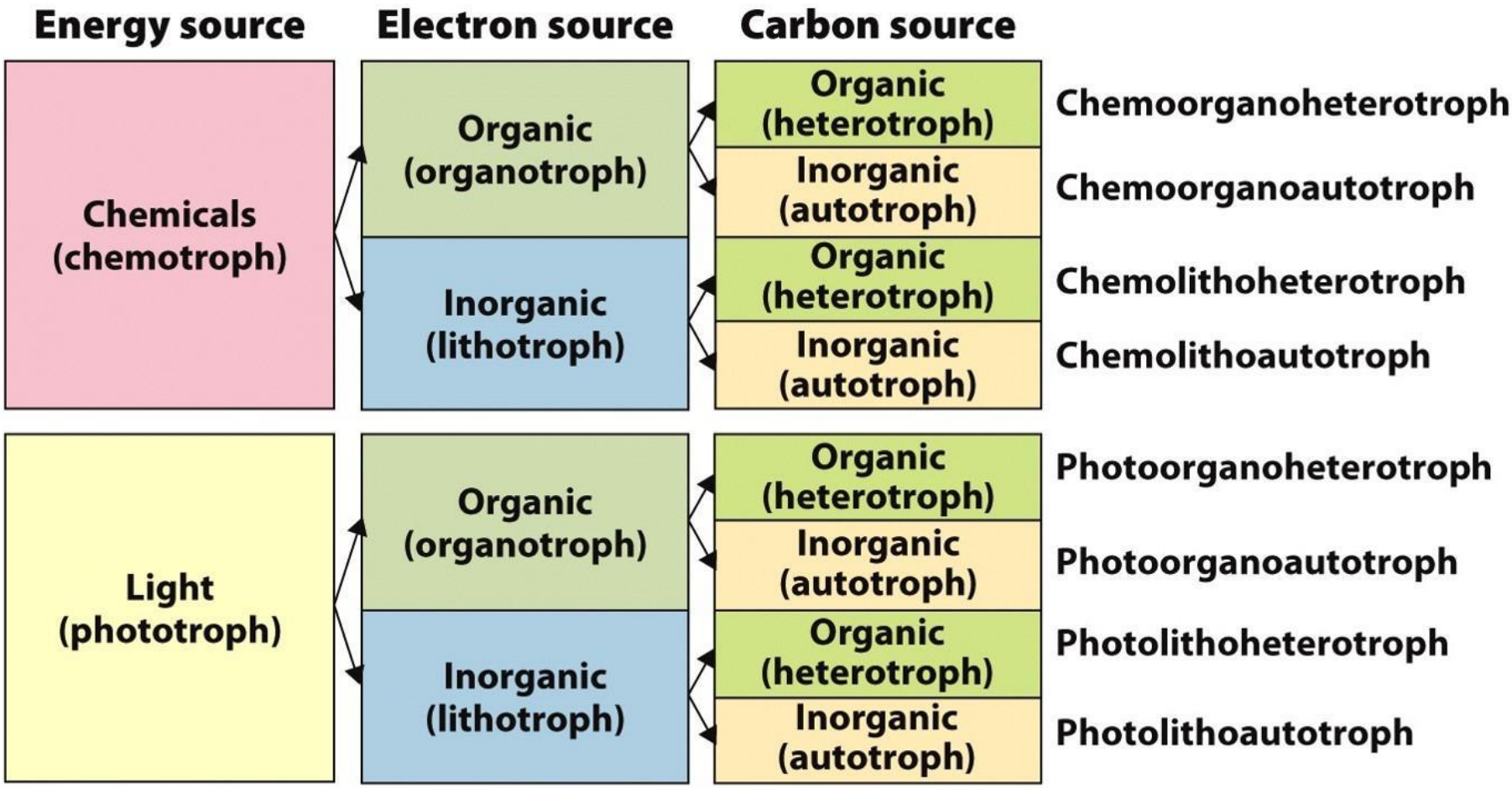
I. Principles of photosynthesis

II. Oxygenic photosynthesis

III. Cyclic and anoxygenic photosynthesis

IV. Redox-independent energy conservation

Classification of the trophic groups



Energetic principles of photosynthesis

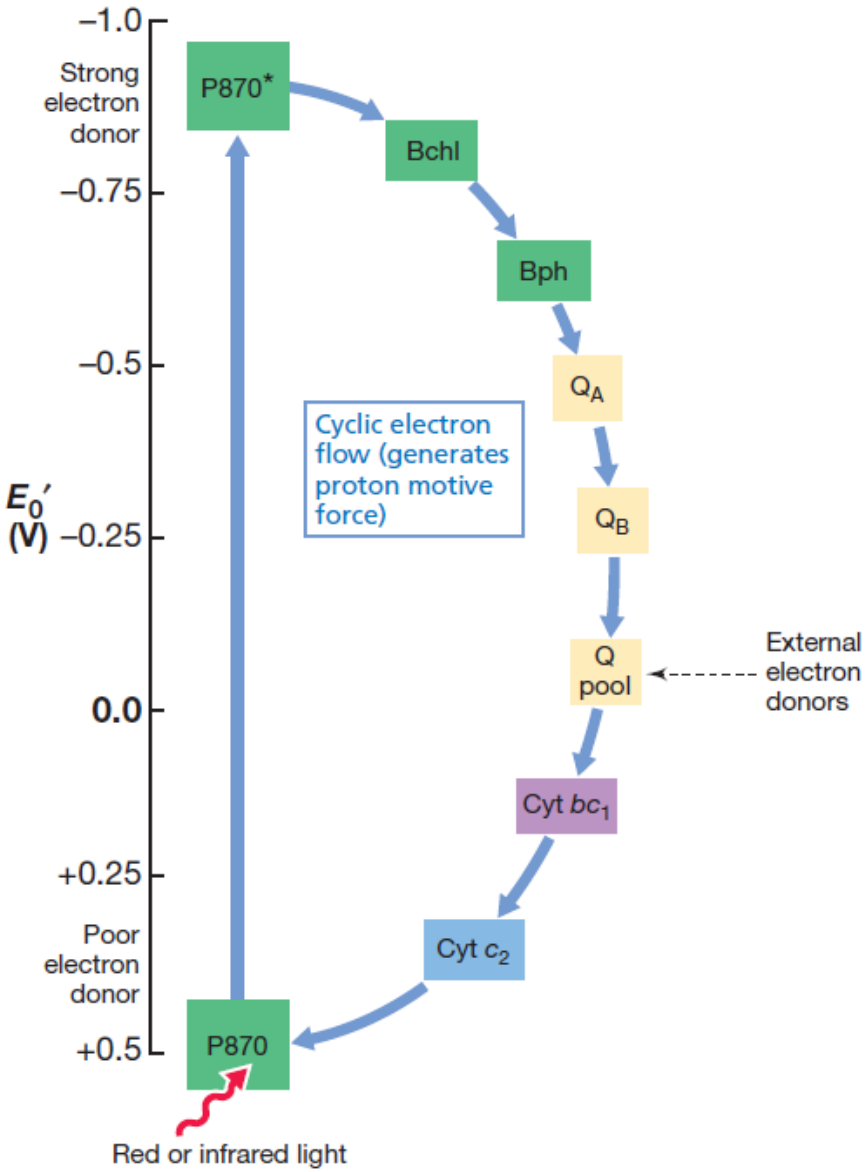
- Photosynthesis uses the energy of light to pump protons across energy-transducing membranes. The Δ*p* generated fuels ATP synthase just as it does in respiration.

- Photosynthesis is employed by organisms across the three domains of life: all plants and algae, seven bacterial phyla, and one archaeal phylum.

- Most photosynthetic organisms can use cyclic schemes to generate Δ*p* independently of reductant. Cyanobacteria, plants, and algae can use non-cyclic schemes that use water-derived electrons to reduce $NADP^+$ to sustain photosynthetic dark reactions.

Light-energised electrons fuel ETC

- Light is captured by a chlorophyll-containing reaction centre (e.g. P870). This results in an electron being excited and becoming a low-potential electron donor for a ETC.
- Electron transfer through photosystem and ETC generates Δp through specific complexes (e.g. cytochrome bc_1). Electrons can also be used to generate reductant.
- Up to 1 V of the energy absorbed is lost as a result of downhill electron flow. However, this ensures that the photosynthesis operates at quantum unity and the photosystems are irreversible.



Energetics of photon capture

- In photosynthetic systems, the primary energy source is the photon that is absorbed by the photosynthetic reaction centre. ΔG from the absorption of 1 einstein (mole) of photons is:

$$\Delta G = \frac{Nhc}{\lambda} = \frac{120{,}000}{\lambda}$$

ΔG is the free energy change (in kJ Einstein^{-1}) λ is wavelength (in nm), *N* is Avogadro's constant, *h* is Planck's constant, and *c* is the velocity of light.

- The energy release from the absorption of one einstein of photons at 700 nm is:

$$\Delta G = \frac{120{,}000}{\lambda} = \frac{120{,}000}{700} = +171 \text{ kJ einstein}^{-1}$$

$$\Delta E = -\frac{\Delta G}{nF} = -\frac{171}{1 \times 96} = -1.78 \text{ eV}$$

Antennae increase efficiency of light capture

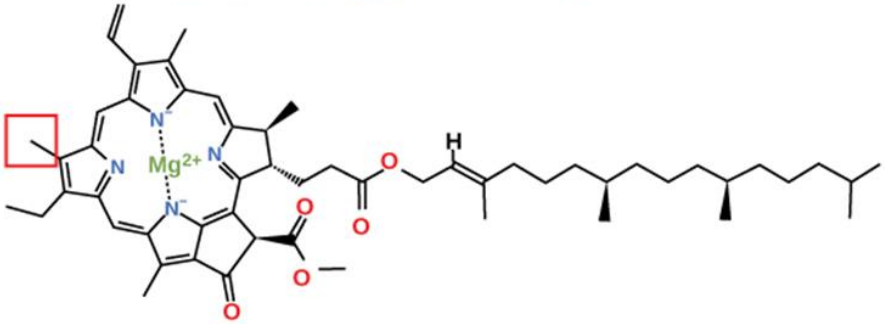
- Photons hit photosynthetic reaction centres relatively infrequently (1 s^{-1}) and are usually at the wrong wavelength to be efficiently absorbed by the pigments.

- To achieve more efficient photon capture, photosynthetic reaction centres are surrounded protein-bound photosynthetic pigment molecules. These capture light at shorter wavelengths and transfer down energy gradient to the reaction centres.

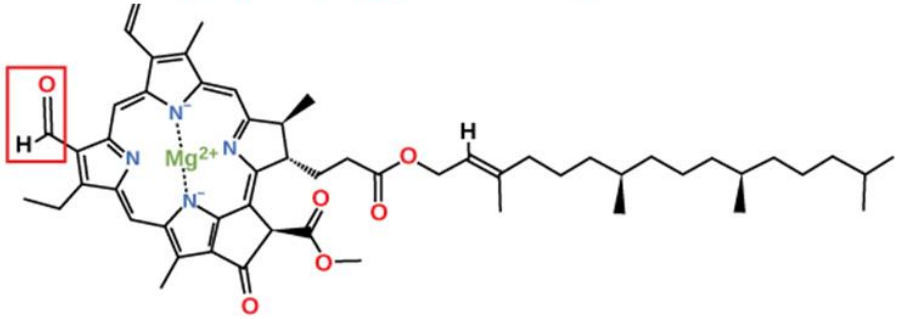
- Light transferred from an excited to an unexcited chromophore in two ways: delocalised exciton coupling (DEC, at < 15 Å) and Förster resonance energy transfer (FRET, at > 15 Å).

Antennae increase efficiency of light capture

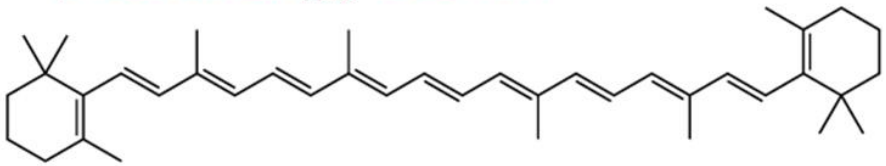
Chlorophyll *a* (λ_{max} = 660 nm)

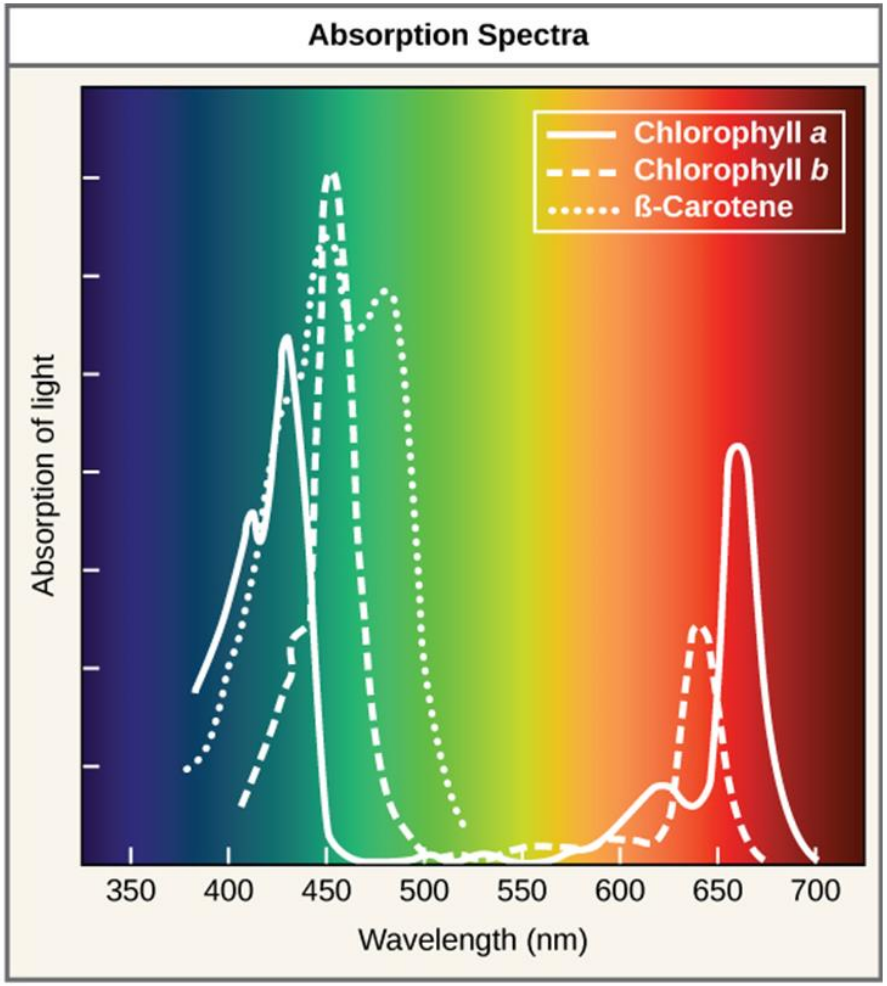


Chlorophyll *b* (λ_{max} = 640 nm)



β-carotene (λ_{max} = 520 nm)





Lecture 4: Photosynthesis light reactions

I. Principles of photosynthesis

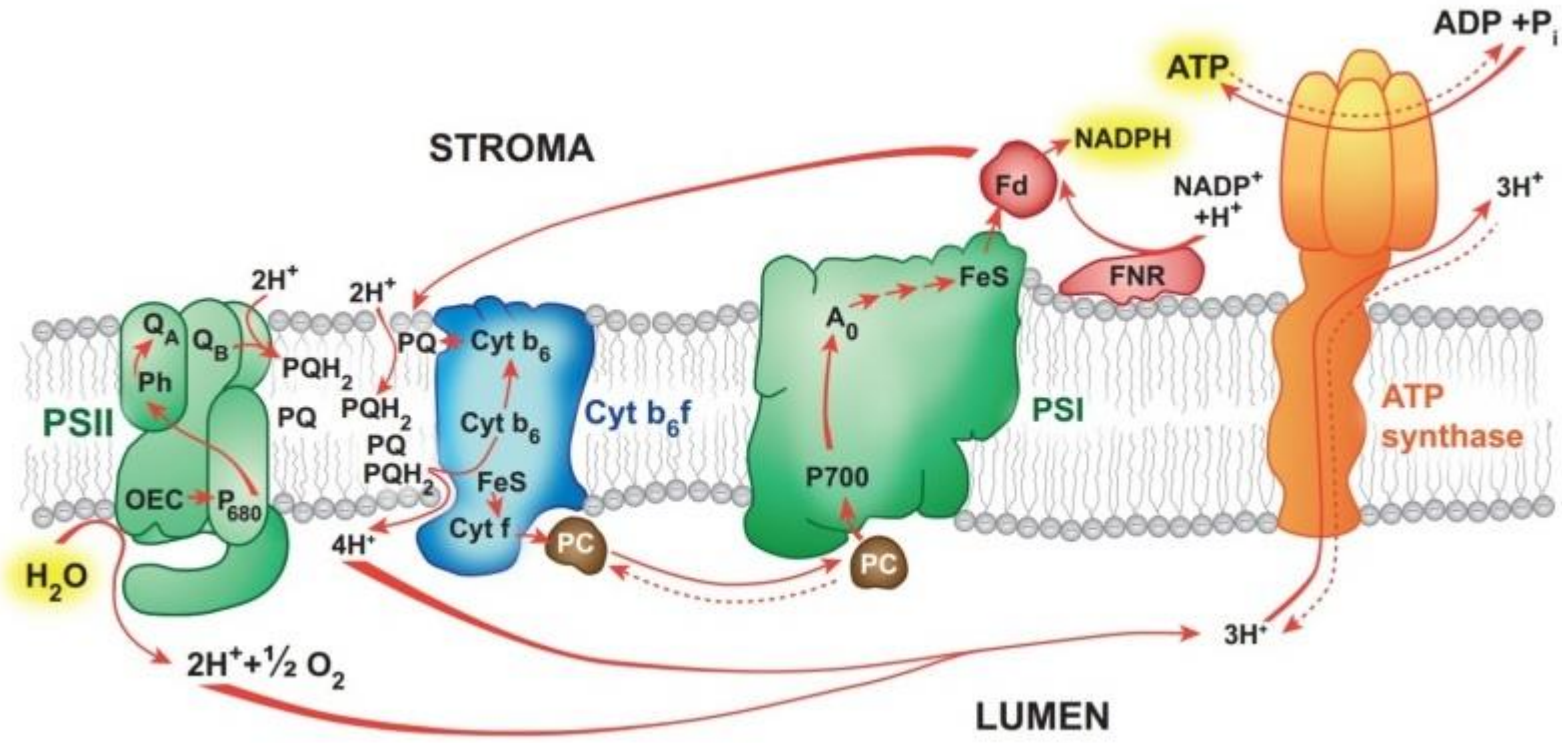
II. Oxygenic photosynthesis

III. Cyclic and anoxygenic photosynthesis

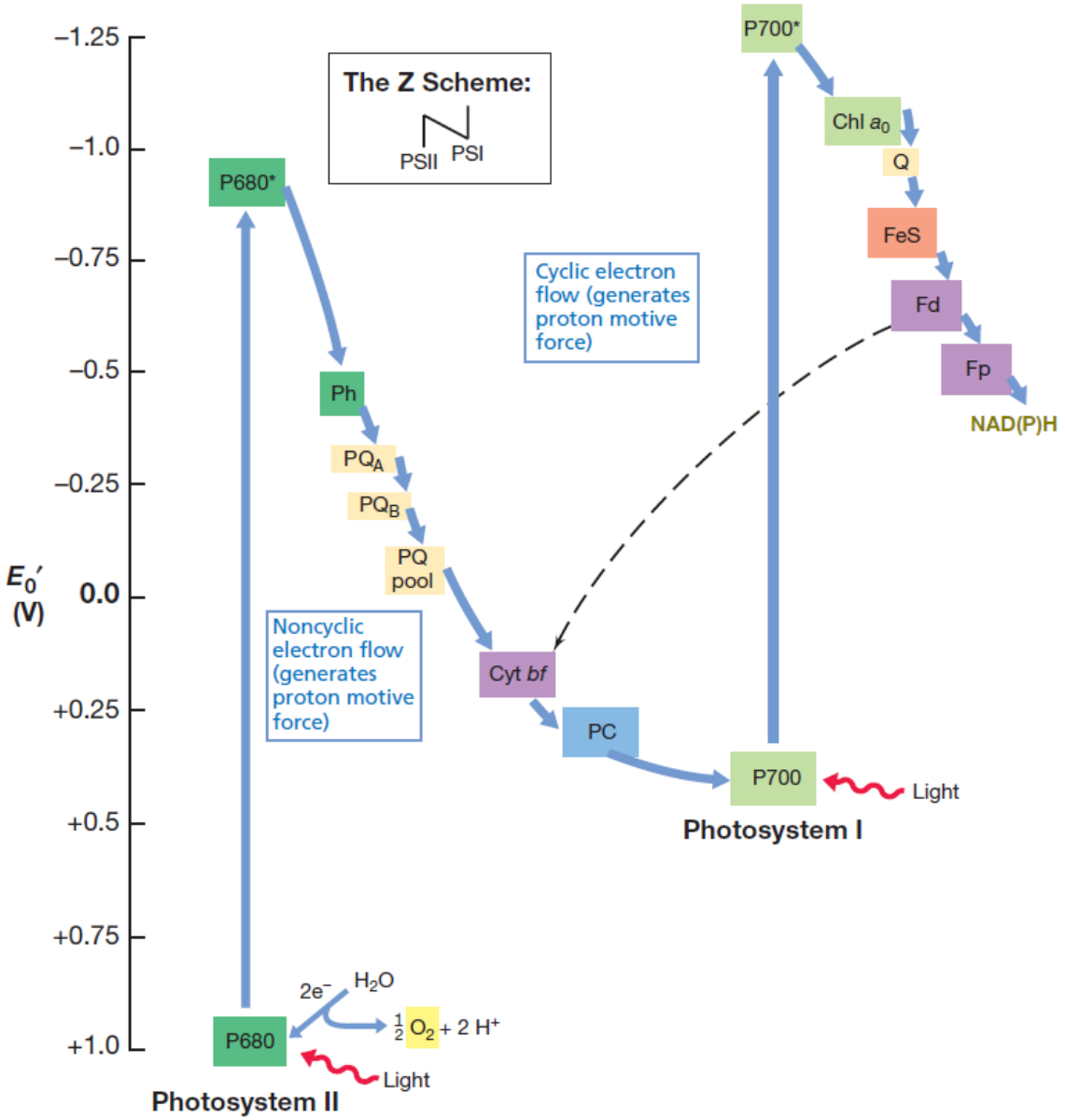
IV. Redox-independent energy conservation

Photosynthetic electron transport chains

1. Electrons derived from H_2O oxidation are transferred to photosystem II, excited with light, and flow downhill through the cytochrome *bf* complex. This generates Δp.
2. The electron is subsequently transferred to photosystem I, where it is excited with light and flows downhill to ferredoxin and $NADP^+$. This generates reductant.

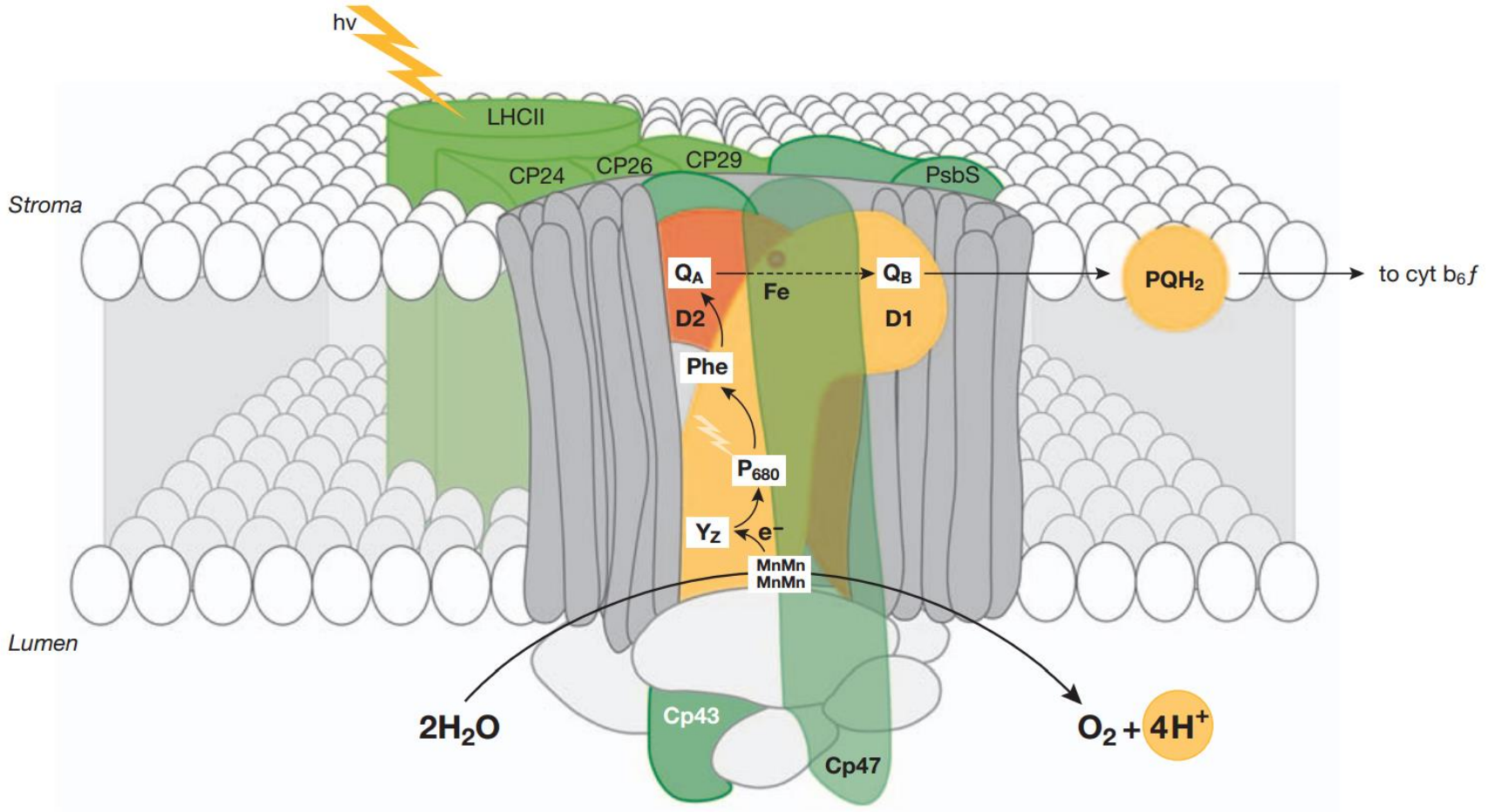


Energetics of oxygenic photosynthesis



Architecture of Photosystem II

- At the core of Photosystem II is a photosynthetic reaction centre made by the subunits D1 and D2. They are surrounded by multiple accessory subunits involved in photoprotection and light-harvesting complexes responsible for light capture.

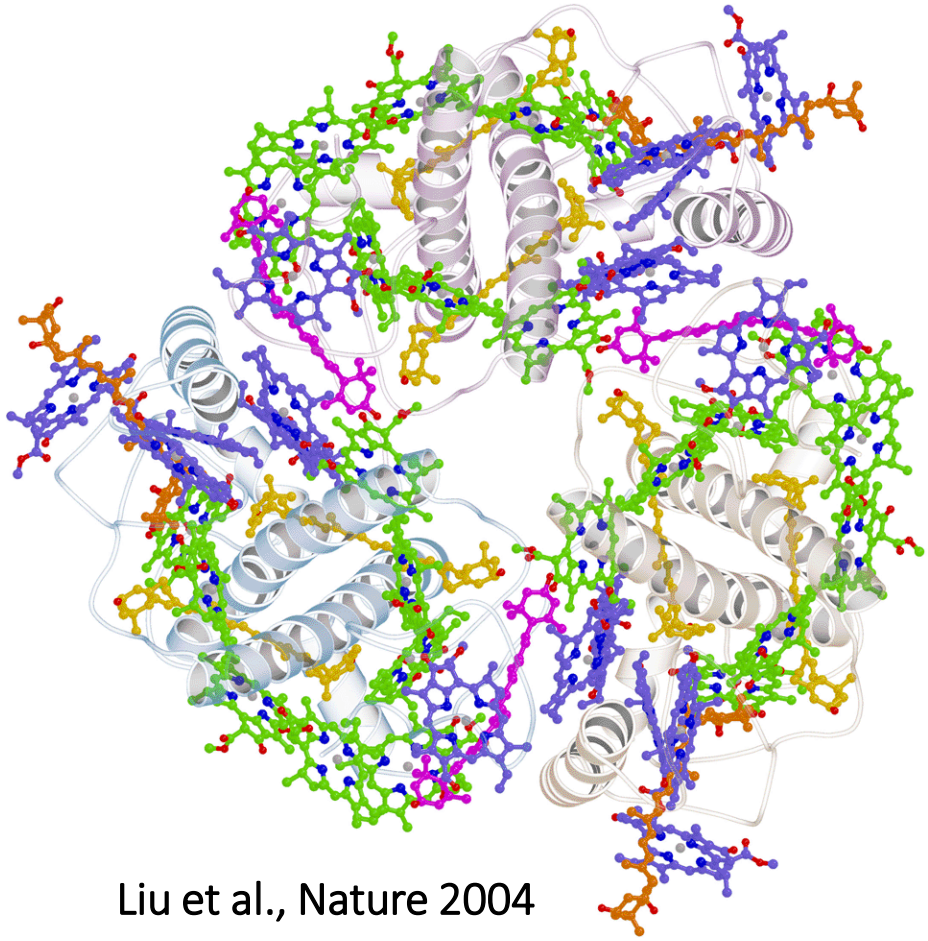


LHCII is packed with chromophores

- Photosystem II surrounded by multiple light-harvesting complexes that capture light < 680 nm and transfer to P680 reaction centre. Light-harvesting complex II (LHCII) most important.

- LHCII is a trimer. Each monomer made of three transmembrane α-helices that bind 8 chlorophyll *a* (blue), 6 chlorophyll *b* (yellow), and 4 carotenoids (orange/magenta). Chromophores arranged in two layers, one close to N side, other at P side.

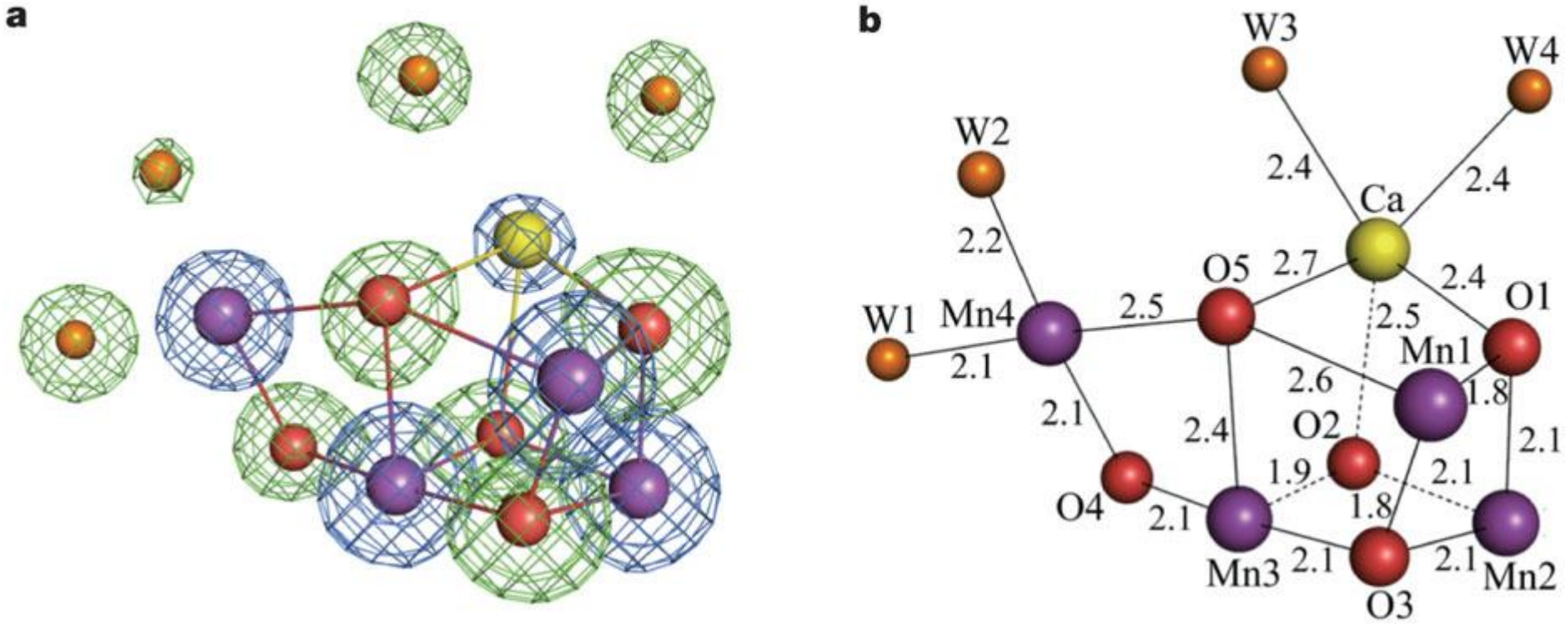
- Close proximity of chlorophylls enables rapid energy transfer. Carotenoids enhance light capture and protect from photodamage by quenching ROS.



Liu et al., Nature 2004

Oxygen-evolving complex

- High-resolution structure of cyanobacterial Photosystem II confirmed structure of oxygen-evolving complex. It is a unique Mn_4CaO_5 complex. One of the Mn atoms has two free coordination sites. This allows it to bind H_2O molecules destined for reaction to O_2.

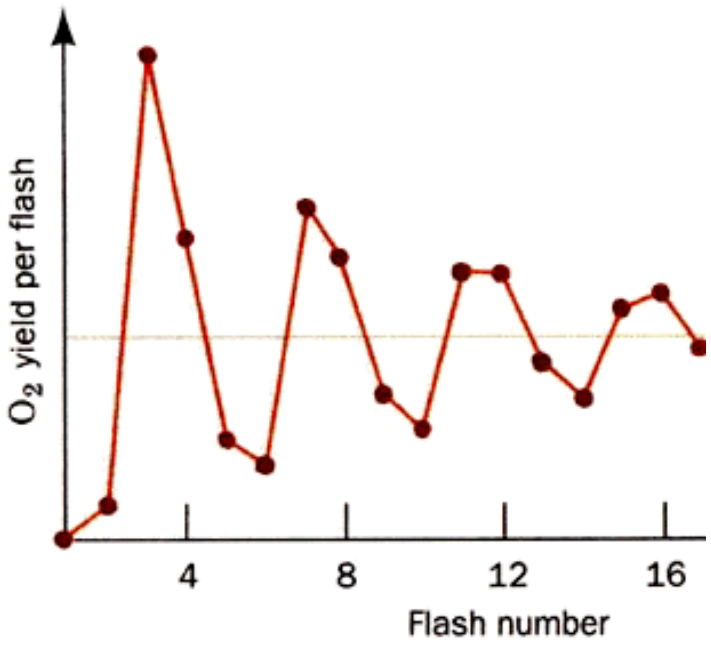


Umena et al., Nature 2011

Mechanism of oxygen-evolution

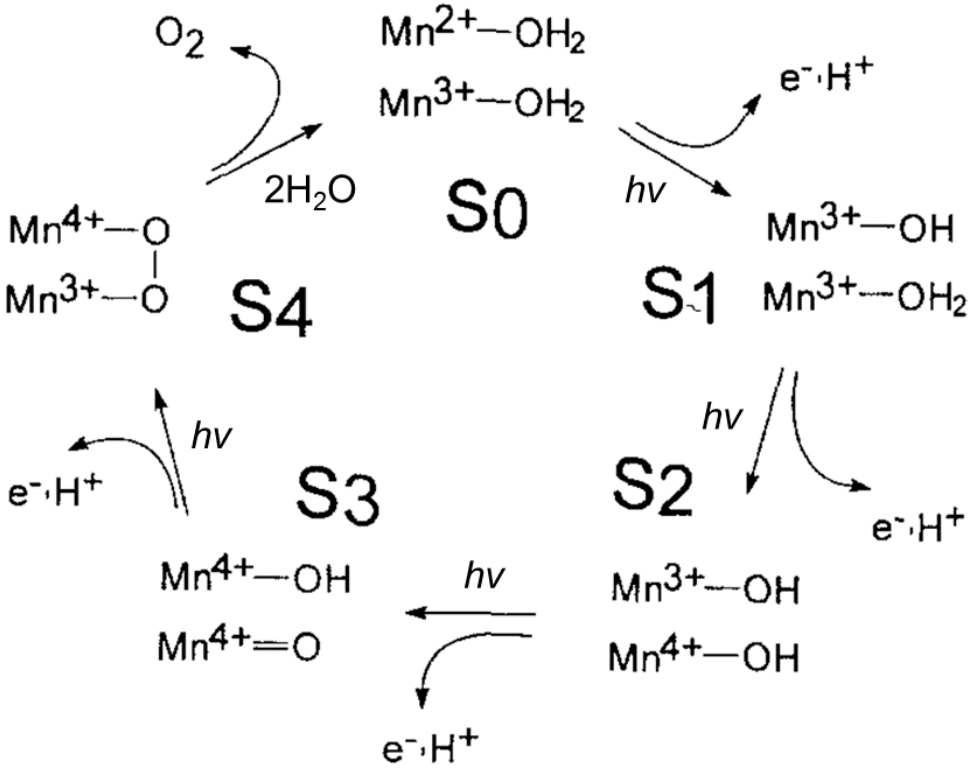
The evidence:

Classical experiments showed that O_2 release depended on how many times light was flashed on a chloroplast. Indicated the existence of five intermediates, S0 to S4. Spectroscopic studies have shown Mn is redox-active.



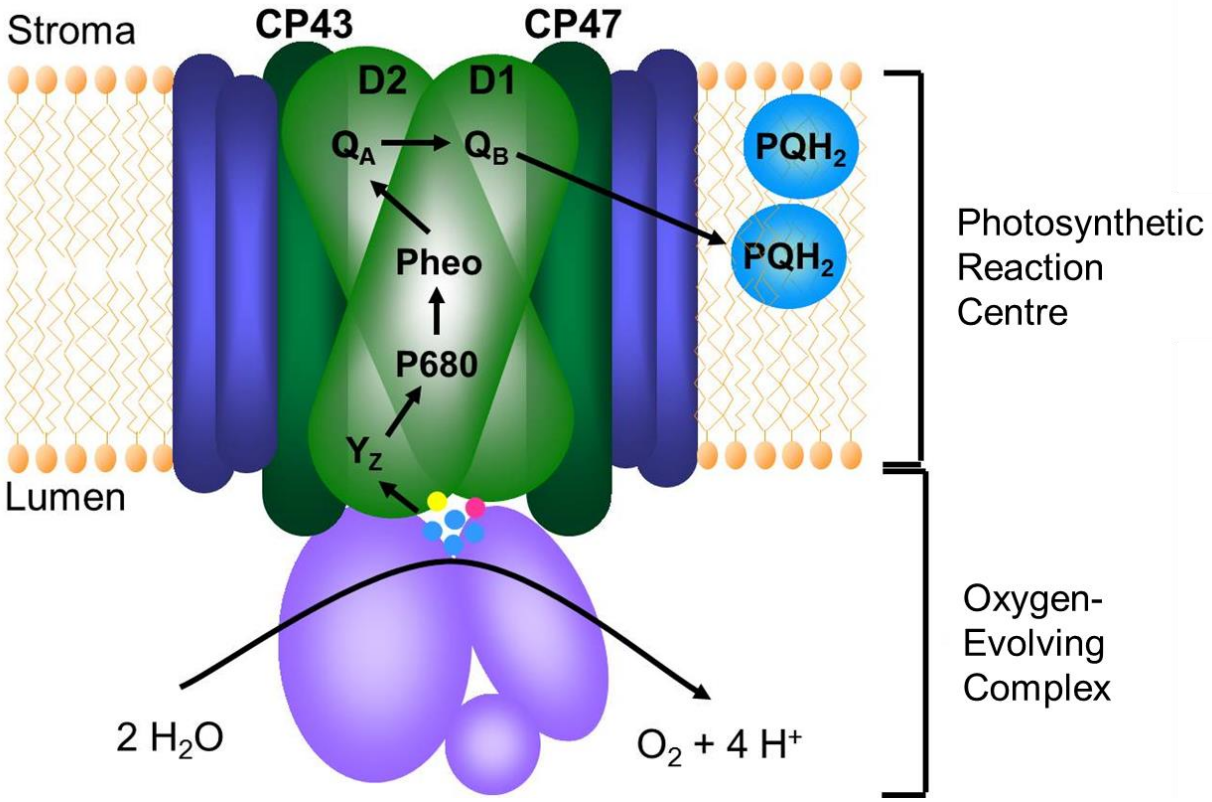
The mechanism:

2 H_2O molecules are oxidised one e^- at a time following photon absorbance. Four photons absorbed for complete $4e^-$ oxidation. Protons accumulate at P-side. e^- transferred via tyrosine to P680 at N-side. Results in effective translocation of $2H^+$ per $2e^-$.



Electron flow through D1 and D2

- P680 is a reaction centre containing a ‘special pair’ of photochemically active chlorophylls at interface of D1 and D2. H_2O-derived electrons are transferred by tunnelling to P680. Light captured by LHCII is transferred to P680 by DEC. Results in excitation of P680 electron.

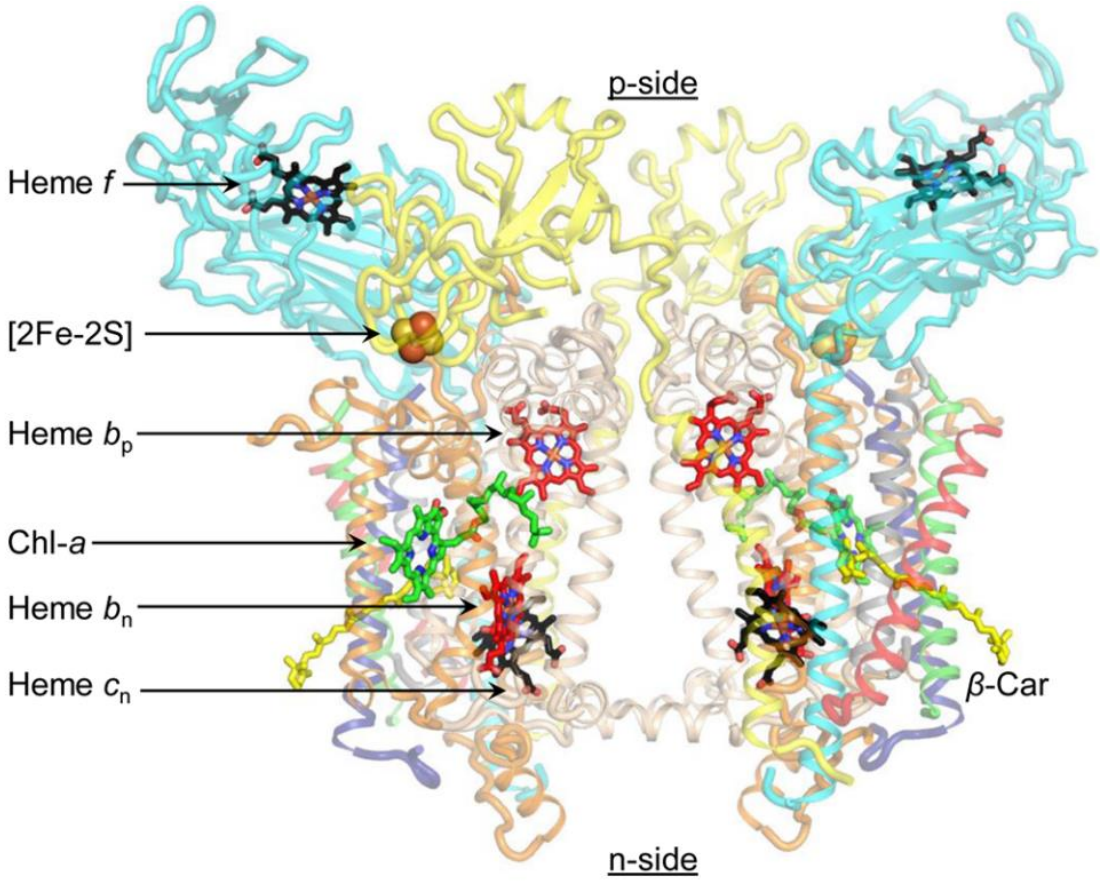


- The excited electron is transferred downhill to pheophytin (chlorophyll without Mg^{2+}), Q_A and Q_B (covalently bound plastoquinones), and a membrane-diffusible plastoquinone.

Cytochrome *bf* is the main generator of Δ*p*

- Photosynthesis proceeds with the electrons from plastoquinol being oxidised by the cytochrome *bf* complex leading to scalar proton translocation. Plastocyanin, a copper protein plastocyanin with $1e^-$ redox chemistry, serves as the e^- acceptor.

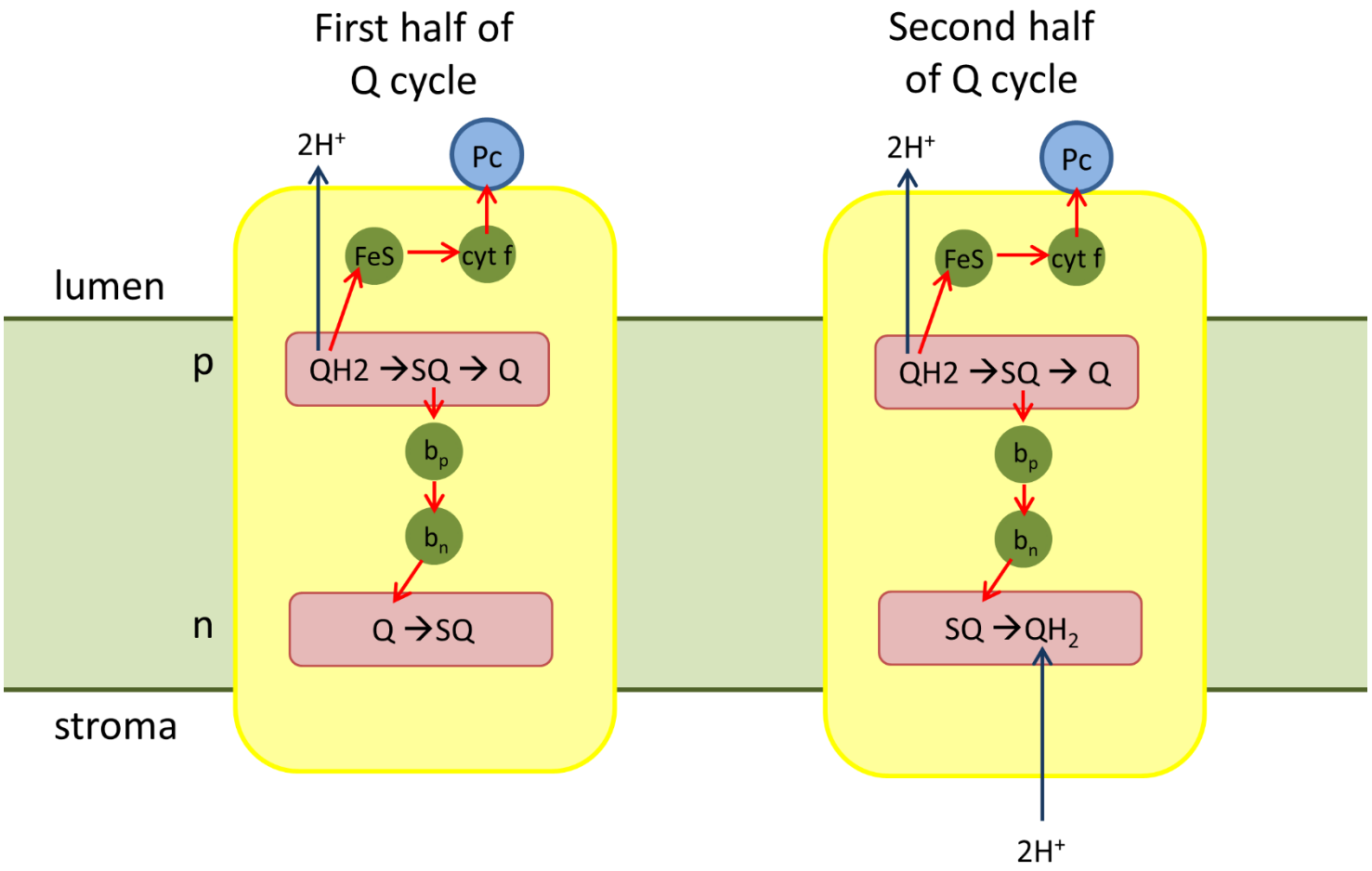
- The complex is structurally and functionally equivalent to Complex III (cytochrome bc_1). Its cofactor composition is similar, except modified heme *f* replaces heme c_1 and an additional heme of controversial function (heme c_i) is present.



Stroebel et al., Nature 2003

Cytochrome *bf* operates a Q-cycle

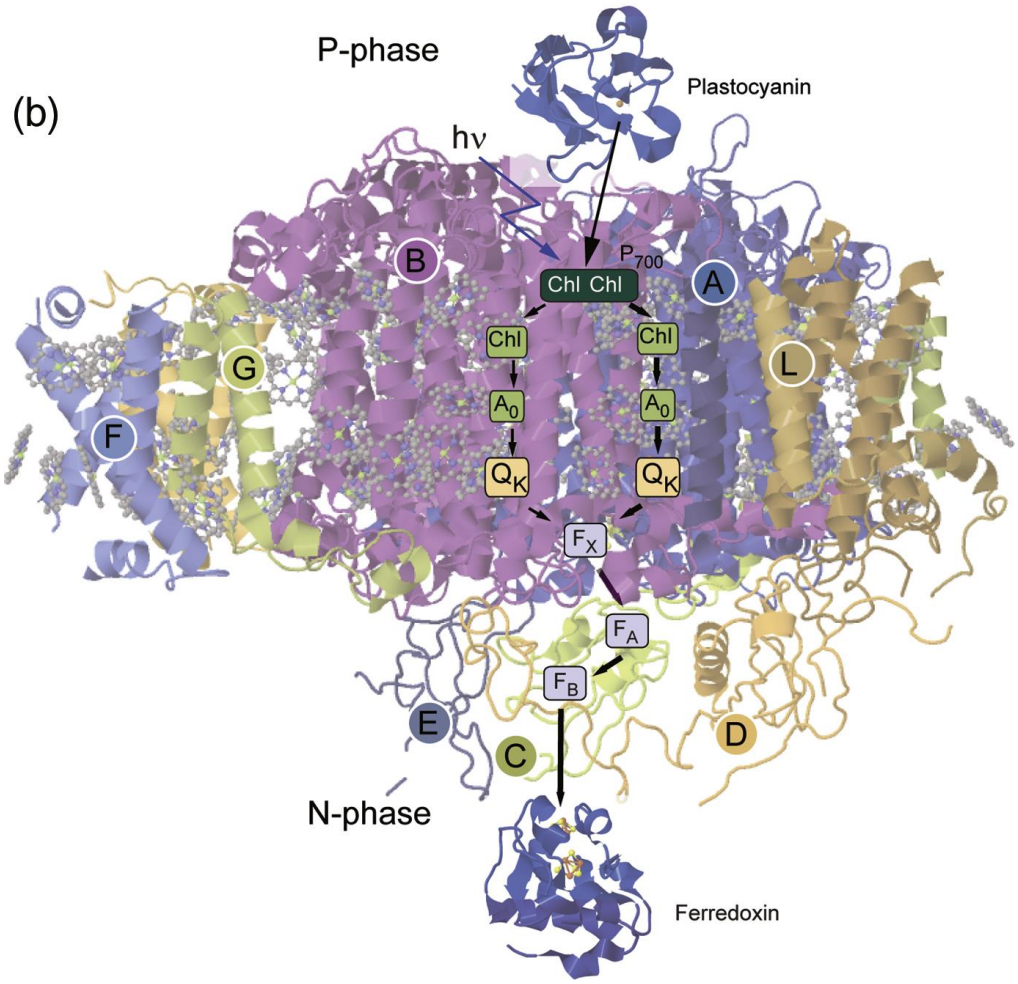
- Cytochrome *bf* complex displaces charge across membrane via a Q-cycle. Operates through electron-bifurcation, mechanistically near-identical to Q-cycle of Complex III.



Moving to Photosystem I

- High-resolution structures show Photosystem I is a supercomplex formed of at least 12 subunits. The P700 reaction centre is formed at the interface of the main PsbA and PsbB subunits.

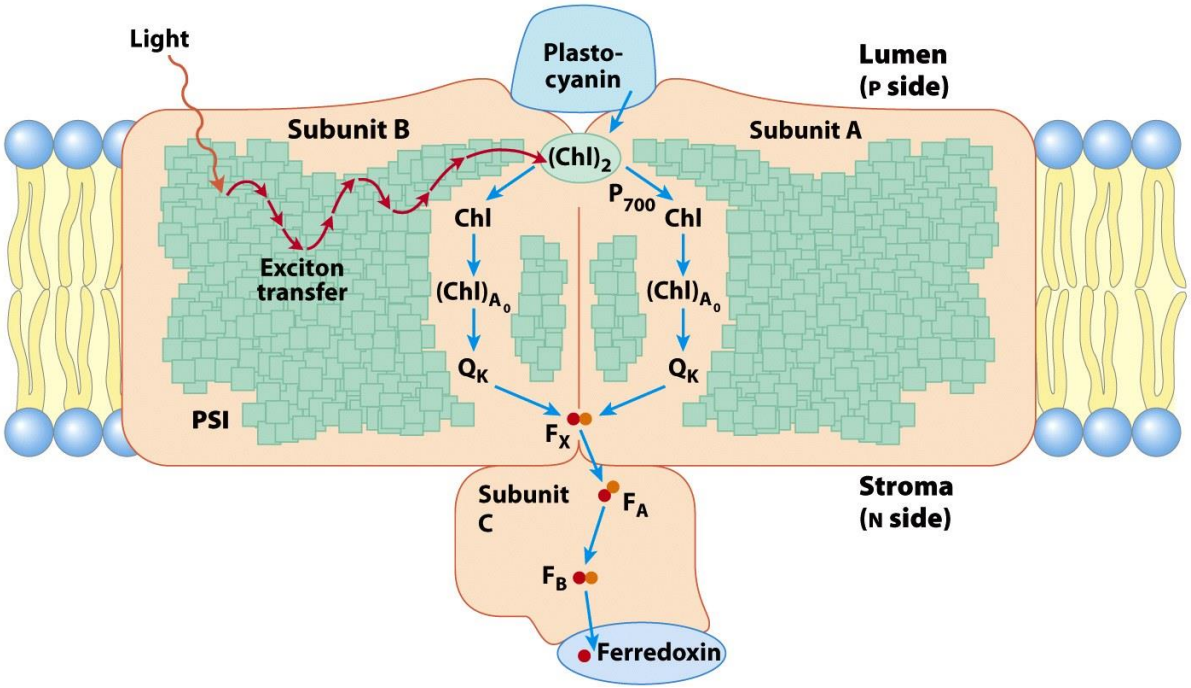
- Whereas Photosystem II is associated with dedicated light-harvesting complexes, light capture in Photosystem I is mainly provided by core antennal system formed by at least 90 chlorophylls and 22 carotenoids surrounding the reaction centre.



Electron transfer through Photosystem I

- Electrons are transferred from plastocyanin to P700 reaction centre at interface of the core PsbA and PsbB. Analogous to photosystem I, absorbance of light at 700 nm by the special pair of chlorophylls caused excitation of the electron.

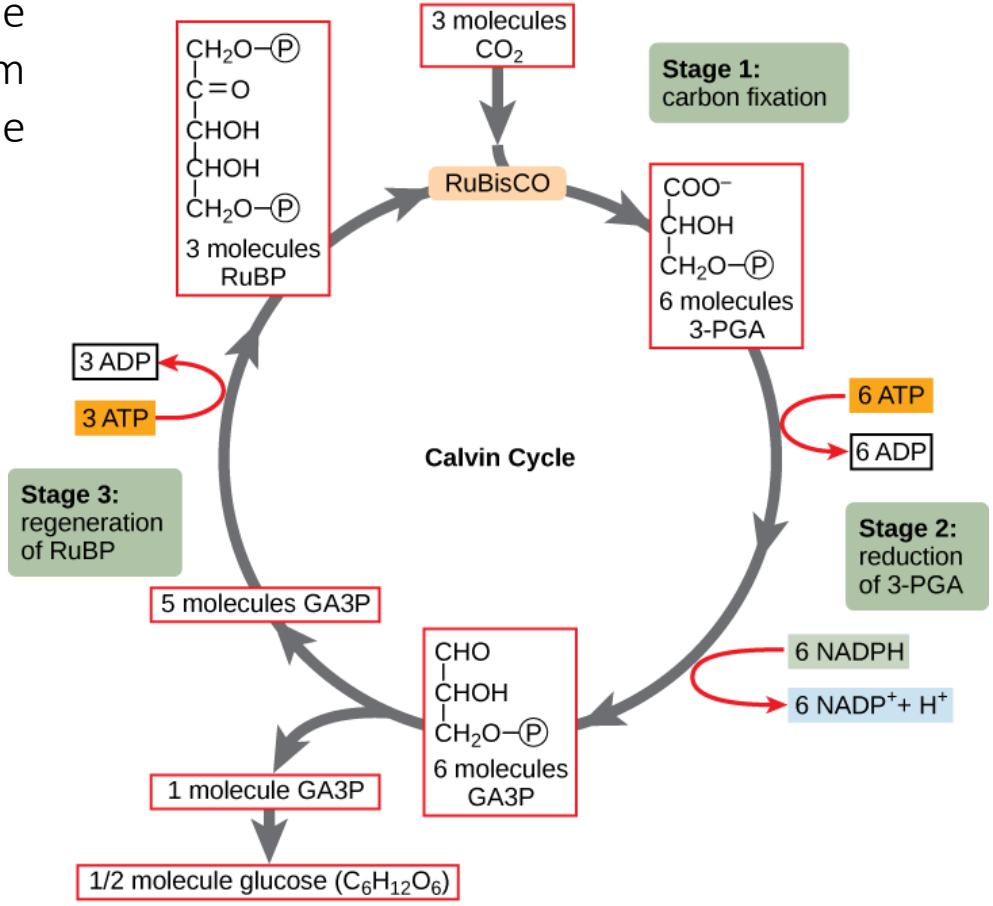
- Electrons subsequently flow downhill through two additional chlorophylls, a phylloquinone, and three iron-sulfur clusters to ferredoxin. As ferredoxin is so electronegative (-0.5 V), this reaction is only possible with the eV released from the absorbance of light.



The fate of ferredoxin and NADPH

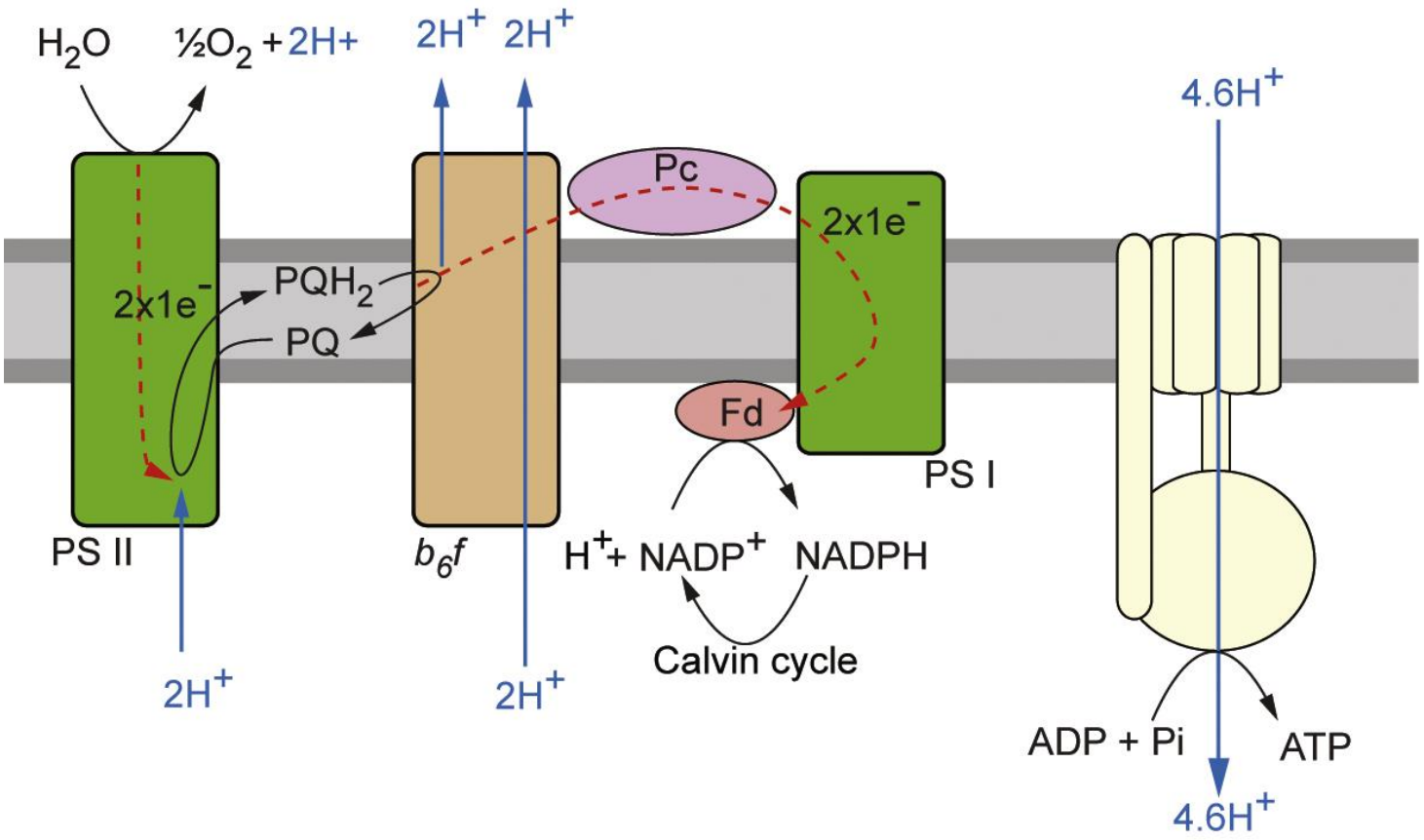
- Reduced ferredoxin supports a range of redox functions in photosynthetic organisms. Most notably, ferredoxin-NADP oxidoreductase couples electron transfer from Fd_{red} to $NADP^+$

- Oxygenic phototrophs primarily use the ATP and NADPH derived from photosynthesis to fix CO_2 through the Calvin cycle for assimilation into biomass.



Counting up the protons one last time

- In total, the equivalent of six protons are translocated for every two electrons transduced through photosynthetic ETC ($6H^+/2e^-$). Two generated directly from the photolysis of water. Charge displacement at cytochrome *bf* in combination with PSI leads to four protons pumped.



Lecture 4: Photosynthesis light reactions

I. Principles of photosynthesis

II. Oxygenic photosynthesis

III. Cyclic and anoxygenic photosynthesis

IV. Redox-independent energy conservation

Modes of photosynthesis

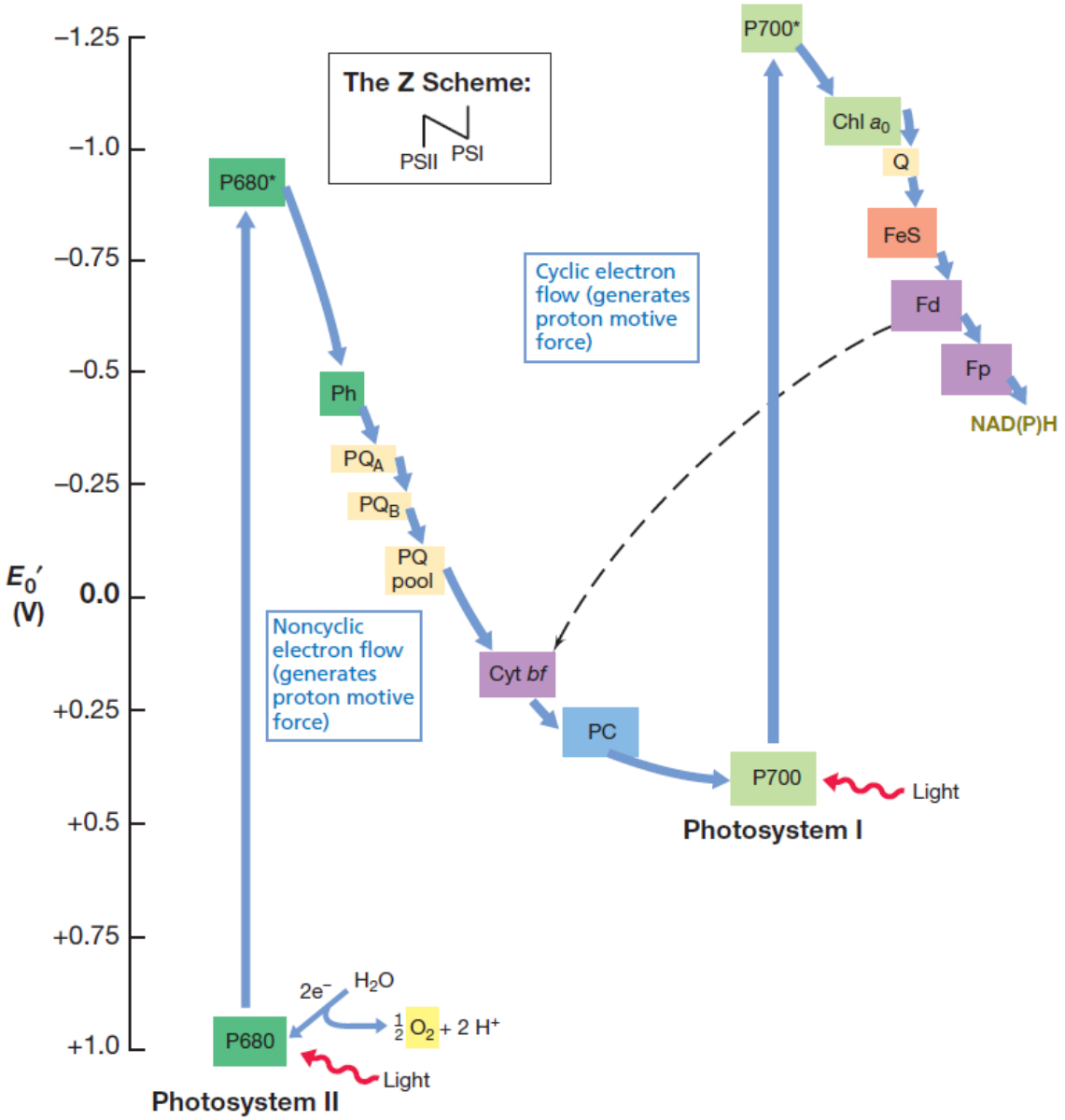
Scheme	Energy flow	Yield	Organisms
Oxygenic photosynthesis	Photon excites e^- derived from H_2O e^- transduced through photosystems e^- either recycled or donated to $NADP^+$	Δp (NADPH)	Plants Algae Cyanobacteria
Anoxygenic photosynthesis	Photon excites e^- derived from organic, H_2, H_2S, S e^- transduced through electron transport chain e^- either recycled or donated to NAD or Fd	Δp (NADH / Fd_{rd})	Purple bacteria Green bacteria Heliobacteria
Redox-independent photosynthesis	Catalysed by bacteriorhodopsin / proteorhodopsin Photon isomerises retinal to excited state Relaxation coupled to proton translocation	Δp	Halobacteria Proteobacteria Protists

Cyclic photosynthesis generates Δ*p*

- Photosynthesis fulfils two demands for oxygenic phototrophs: it generates ATP (via Δ*p* production) and it provides reductant (via ferredoxin and $NADP^+$ reduction). However, under many conditions, the need for ATP exceeds that of reductant.

- In such circumstances, organisms operate cyclic photosynthesis schemes in which there is no net electron input or output. Instead, continual cycles of electron excitation, transfer, and return to the ground state is coupled to Δ*p* production by the cytochrome *bf* complex.

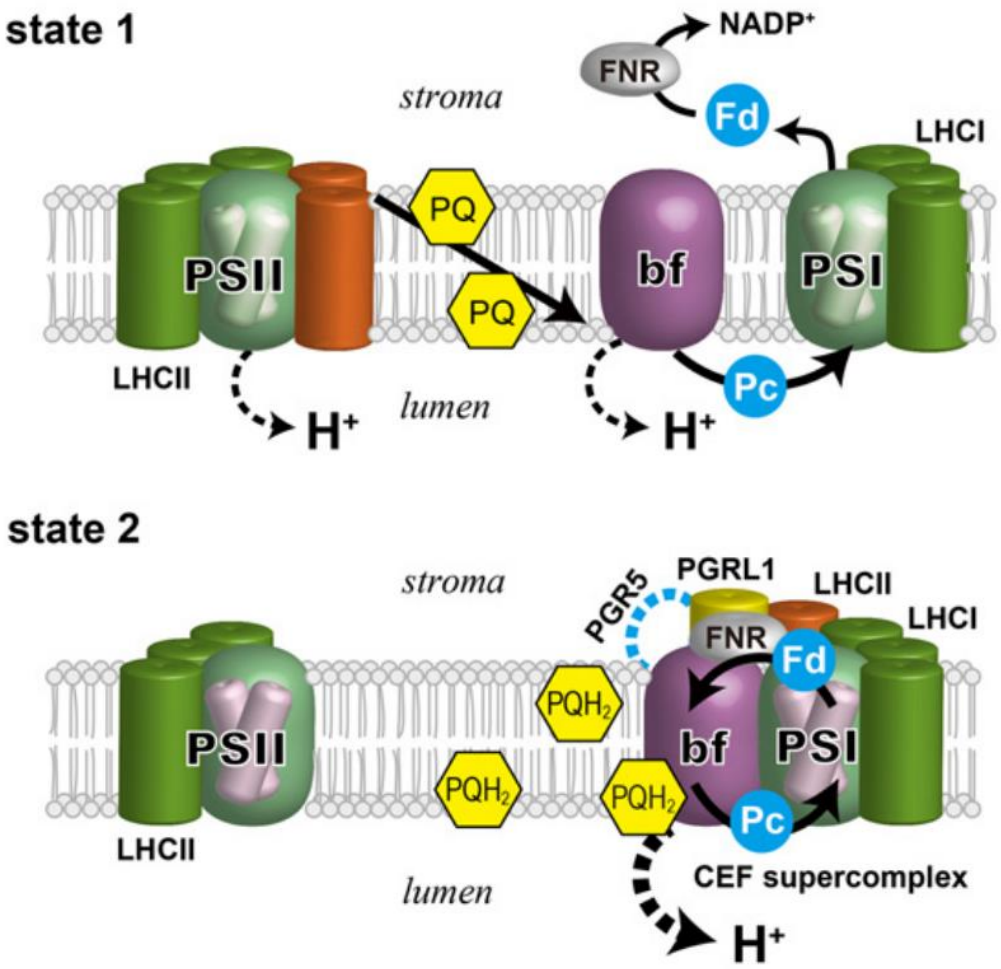
Energetics of cyclic photosynthesis



A cyclic photosynthesis supercomplex

- It was recently discovered that algae form a CEF supercomplex (Nature 2010) comprising Photosystem I, cytochrome *bf*, ferredoxin reductase, and dedicated light-harvesting complexes.

- It is formed when quinols accumulate, which is a signal that demand for ATP exceeds demand for reductant.



Anoxygenic photosynthetic bacteria

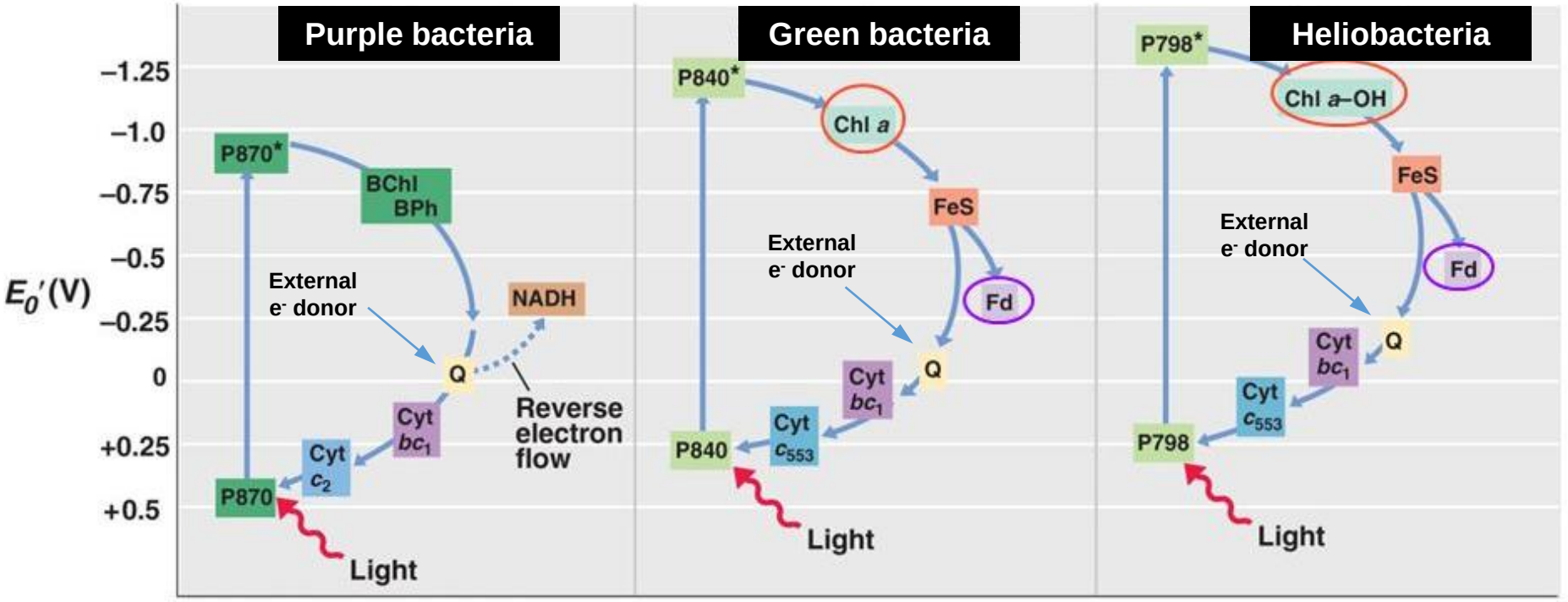
- Beyond cyanobacteria, there are at least six phyla of bacteria that catalyse anoxygenic photosynthesis. The best studied are the Proteobacteria (purple bacteria), Chlorobi (green bacteria), and Heliobacteria.

- Potential electrons for anoxygenic phototrophs include organic compounds, H_2, H_2S, and S_0. These electrons can be used in cyclic schemes to generate Δp *via* the cytochrome bc_1 complex. Alternatively, they can be used in noncyclic schemes to generate reductant.

- All three excite these electrons by absorbing light at high wavelengths (> 750 nm) using modified chlorophylls (bacteriochlorophyll *a* or *g*) at photosystems resembling either Photosystem II (purple bacteria) or Photosystem I (green bacteria, Heliobacteria).

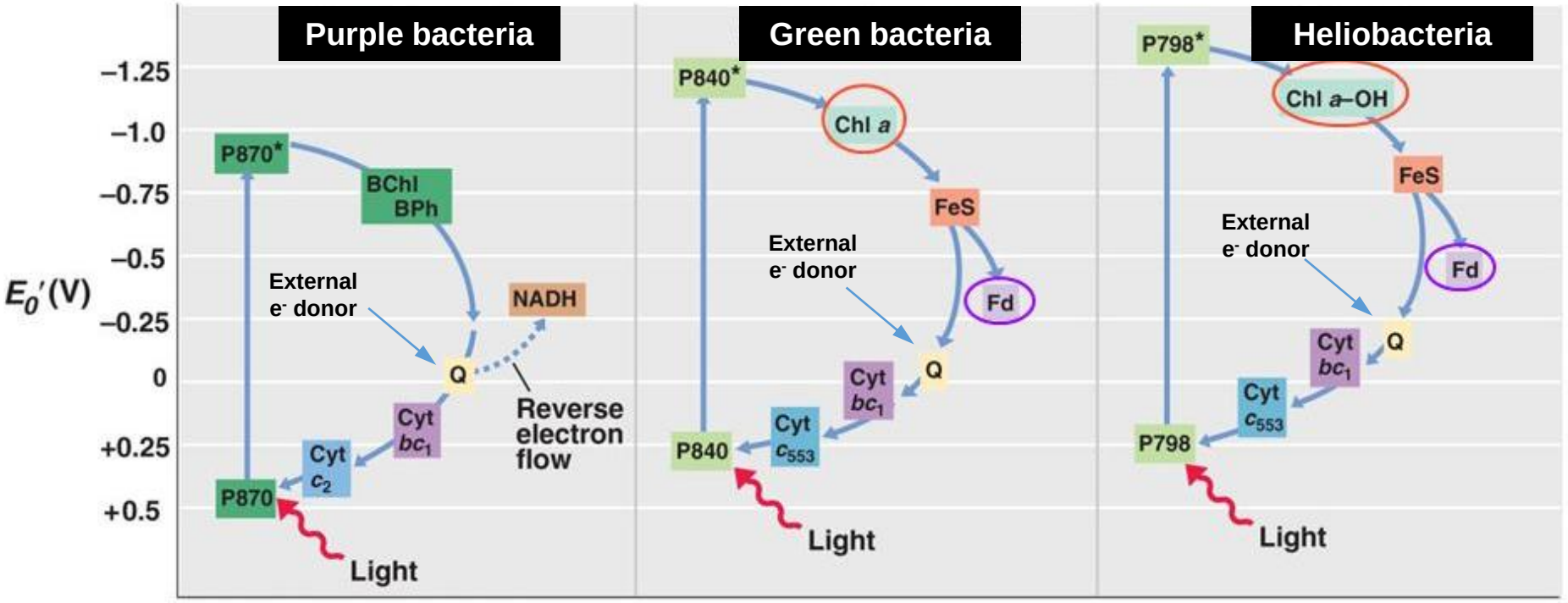
Similar cyclic photosynthesis schemes

- In all three cases, photosynthetic reaction centres excite an electron derived from an external donor, excited electron flows through a cytochrome bc_1 complex generating Δp by redox loop mechanism, and electron returns to the ground state via a *c*-type cytochrome.



Different noncyclic photosynthesis schemes

- Reductant generation differs in anoxygenic phototrophs. As with cyanobacteria, green bacteria and Heliobacteria reduce ferredoxin using light-excited low-potential electron. Purple bacteria only generate reductant by Δ*p*-dependent reversed electron flow to NAD^{+}.

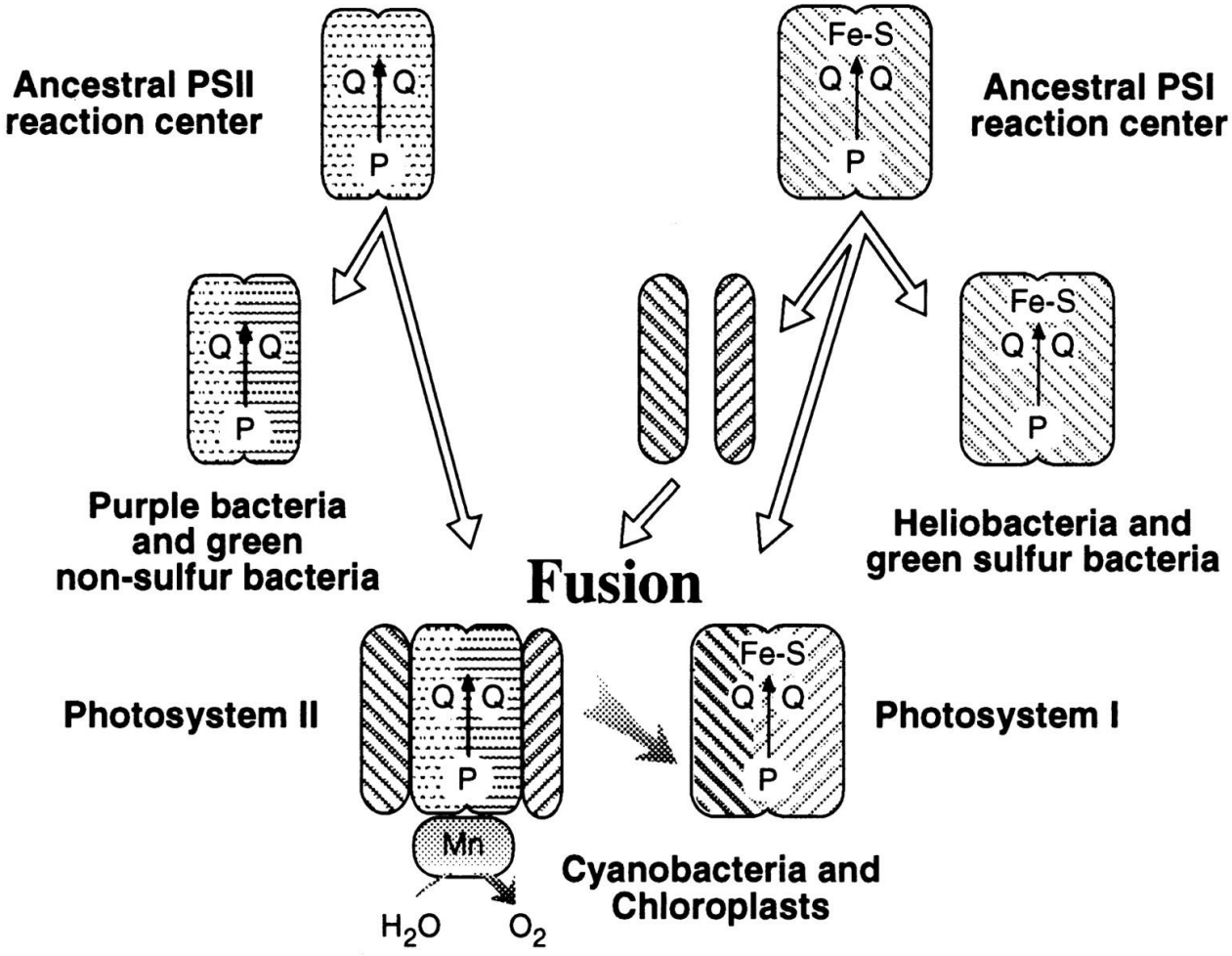


Summary of anoxygenic photosynthesis

Bacterium	Purple bacteria	Green bacteria	Heliobacteria
Wavelength absorbed	870 nm	840 nm	798 nm
Reaction centre pigment	Bacteriochlorophyll *a*	Bacteriochlorophyll *a*	Bacterochlorophyll *g*
Photosystem structure	Similar to photosystem II	Similar to photosystem I	Similar to photosystem I
Electron source	H_2S, S_0, H_2 (sulfur bacteria) Organic, H_2 (nonsulfur bacteria)	H_2, H_2S, S	Organic
Terminal acceptor (noncyclic)	NAD (reversed electron flow)	Ferredoxin	Ferredoxin
Δp source (cyclic)	Cytochrome bc_1	Cytochrome bc_1	Cytochrome bc_1

Evolution of photosynthesis

- Strong evidence that photosynthesis evolved in anoxygenic phototrophic bacteria. Fusion of ancestral photosystem I and II resulted in photosynthesis in cyanobacteria. Water-splitting complex evolved in Photosystem II. Cytochrome bc_1 duplicated to produce cytochrome *bf*.



Lecture 4: Photosynthesis light reactions

I. Principles of photosynthesis

II. Oxygenic photosynthesis

III. Cyclic and anoxygenic photosynthesis

IV. Redox-independent energy conservation

Redox-independent energy-conservation

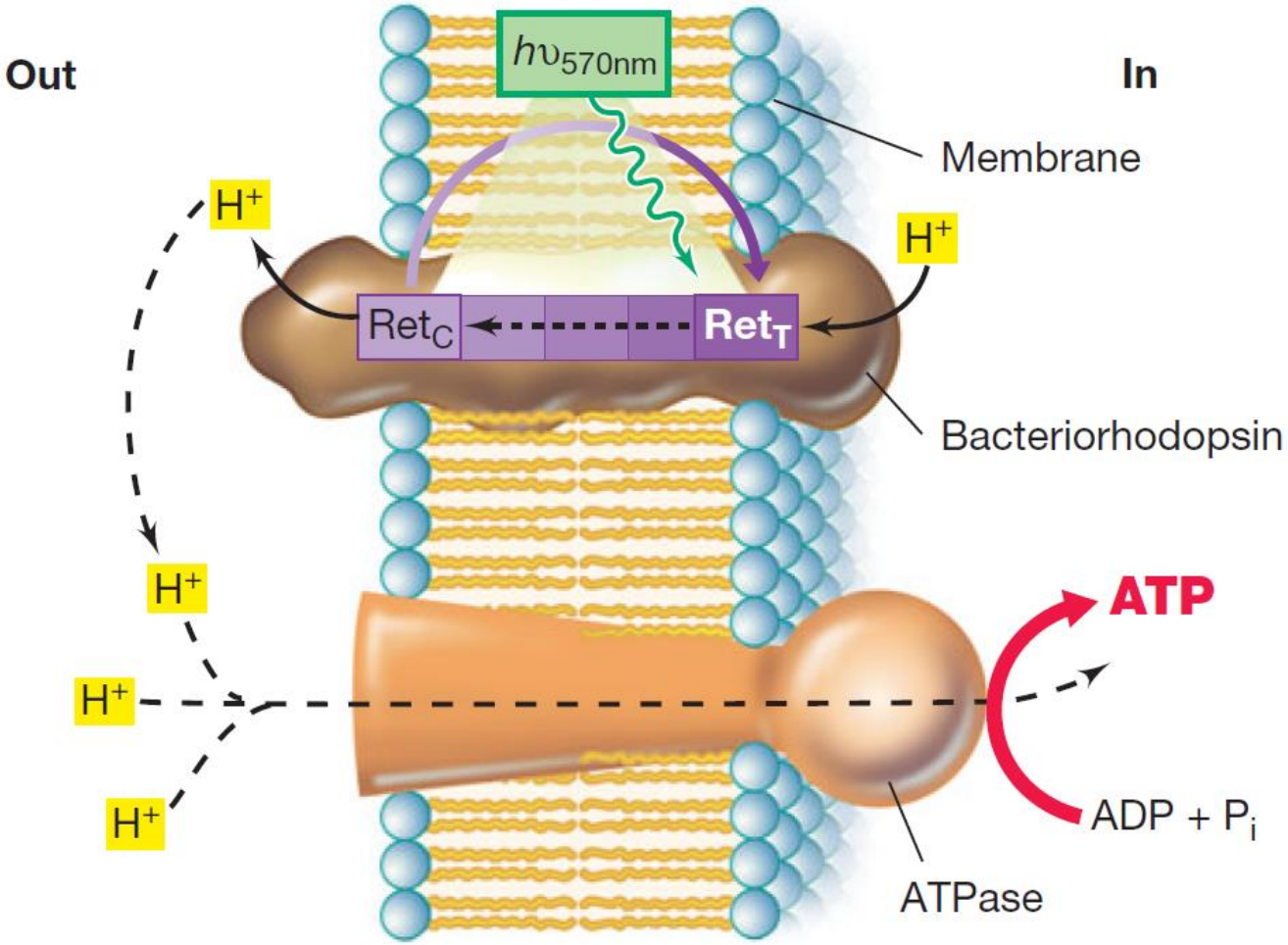
- The majority of energy-conservation processes couple exergonic redox reactions to translocation of protons (Δp) or or sodium (ΔNa^+). There are two prominent exceptions:

1. Many environmental bacteria and archaea use light-sensitive proton pumps (bacteriorhodopsin, proteorhodopsin) to directly couple light capture to proton pumping.

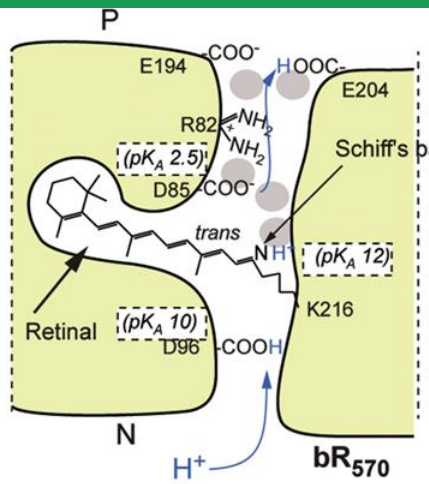
2. Some anaerobic bacteria (e.g. the periodontal pathogen Fusobacteria) directly couple decarboxylation of organic compounds (e.g. succinate) to Na^+ pumping.

Bacteriorhodopsin is a light-driven proton pump

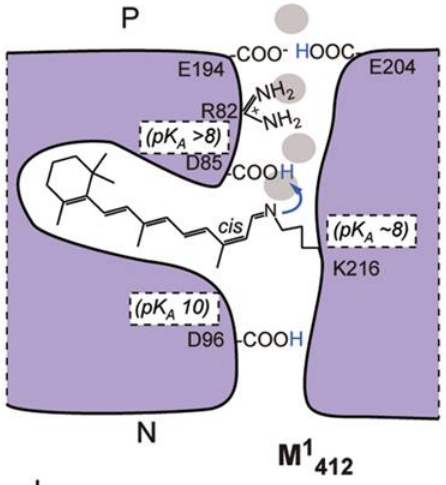
- Bacteriorhodopsin is a 7TM (seven transmembrane helix) membrane protein containing a central retinal chromophore. Light capture by retinal causes a transition from a ground *trans* isomer to a high-energy *cis* isomer. Relaxation to ground state drives proton pumping.



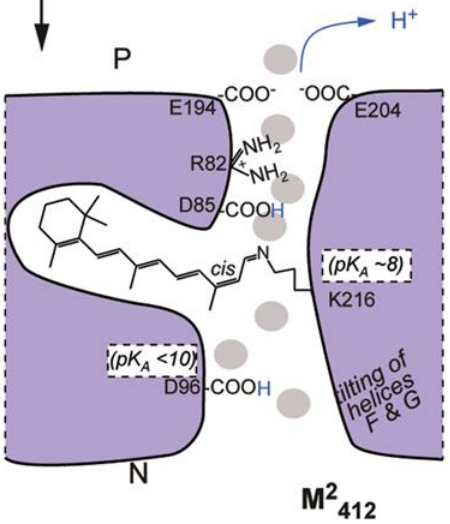
Bacteriorhodopsin is a light-driven proton pump



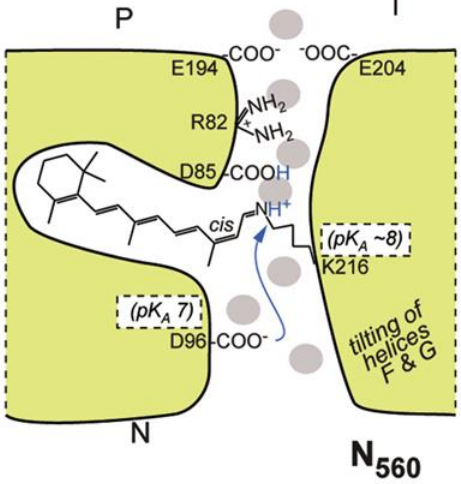
1. Light absorption at 570 nm causes isomerisation of retinal from *trans* to *cis*.



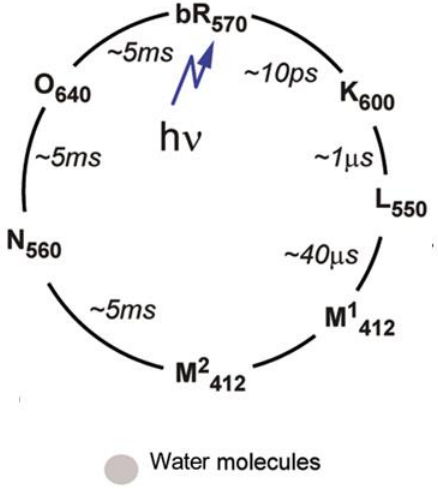
2. Proton released to P-side by P-surface Glu. Resultant pK_a shifts cause proton transfer from Schiff base to central Glu.



3. Schiff base relaxation and entrance of water at N-side propagates further pK_a shifts. Proton from N-surface Glu donated to Schiff base.

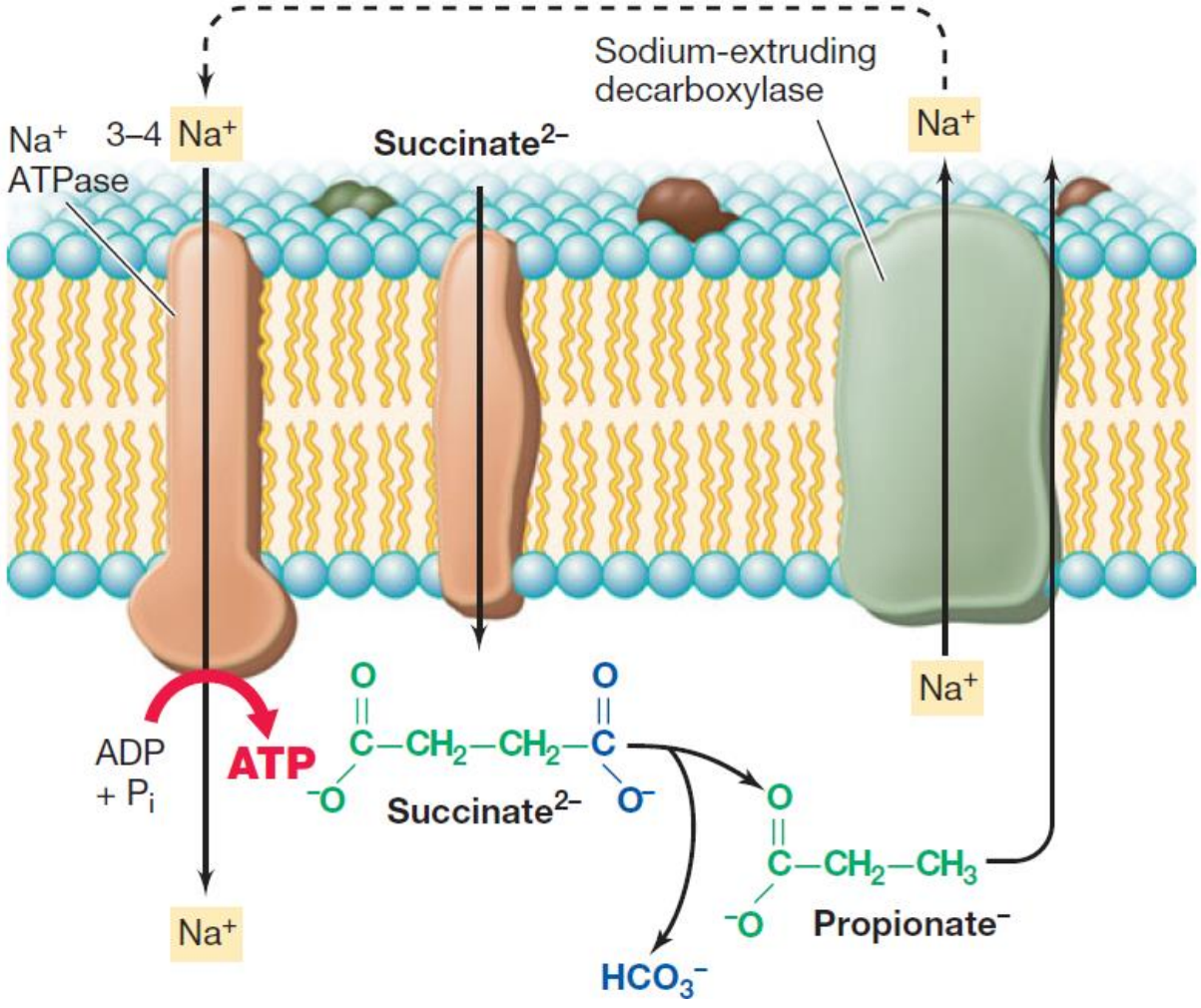


4. Schiff base isomerises back to *trans* state. Proton from central Glu residue donated to P-surface Glu.



Succinate decarboxylase is a sodium pump

- In Fusobacteria, decarboxylation of succinate to propionate releases a large amount of free energy (ΔG = -20.5 kJ mol^{-1}). This can be coupled to Na^+ extrusion at the succinate decarboxylase through biotin-dependent transformations. The Na^+ pumped drives ATP synthase.



Lecture summary

- Redox-dependent photosynthesis uses light energy to generate low-potential electrons at chlorophyll-containing photosynthetic reaction centres.

- Electron flow through cyclic schemes generate Δp through Q-cycle mechanisms. Electron output in noncyclic schemes can additionally be used to reduce ferredoxin and $NADP^+$.

- Oxygenic phototrophs use Mn_4CaO_5 centre to mediate the $4e^-$ oxidation of $2H_2O$ to O_2. Derived electrons flow through two reaction centres generating ATP and NADPH.

- Anoxygenic phototrophs use electrons derived from organic or inorganic donors in cyclic and noncyclic schemes. There is diversity in the way light is harvested and e^- are transferred.

- In redox-independent photosynthesis, light capture is used to isomerise a retinal chromophore in bacteriorhodopsin. Relaxation back to ground state causes proton pumping.

Recommended reading

Recommended reading:

Nicholls DG & Ferguson SJ (2015). Bioenergetics 4. Elsevier Press.

Comprehensive, up-to-date textbook on bioenergetics.

All available for download at greeninglab.com