The Far-Red Limit of Photosynthesis

FREDRIK MOKVIST
Abstract

The photosynthetic process has the unique ability to capture energy from sunlight and accumulate that energy in sugars and starch. This thesis deals with the light driven part of photosynthesis. The aim has been to investigate how the light-absorbing protein complexes Photosystem I (PS I) and Photosystem II (PS II), react upon illumination of light with lower energy (far-red light; 700-850 nm) than the absorption peak at respective primary donor, \( \text{P}_{700} \) and \( \text{P}_{680} \). The results were unexpected. At 295 K, we showed that both PS I and PS II were able to perform photochemistry with light up to 130 nm above its respective primary donor absorption maxima. As such, it was found that the primary donors’ action spectra extended approximately 80 nm further out into the red-region of the spectrum than previously reported. The ability to perform photochemistry with far-red light was conserved at cryogenic temperatures (< 77 K) in both photosystems. By performing EPR measurements on various photosystem preparations, under different illumination conditions the origin of the effect was localized to their respective reaction center. It is also likely that underlying mechanism is analogous for PS I and PS II, given the similarities in spatial coordination of the reaction center pigments. For PS II, the results obtained allowed us to suggest a model involving a previously unknown electron transfer pathway. This model is based upon the conclusion that the primary cation from primary charge separation induced by far-red light resides primarily on Chl\(_{D1}\) in \( \text{P}_{680} \). This is in contrast to the cation being located on \( \text{P}_{680} \), as has been suggested as for visible light illumination.

The property to drive photochemistry with far-red wavelengths implies a hitherto unknown absorption band, probably originating from the pigments that compose \( \text{P}_{700} \) and \( \text{P}_{680} \). The results presented here might clarify how the pigments inside \( \text{P}_{680} \) are coupled and also how the complex charge separation processes within the first picoseconds that initiate photosynthetic reactions occur.

Keywords: Far-red light, Photosystem I, Photosystem II, P700, P680, EPR, Charge Separation

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I  Performed three of the presented experiments and its analysis. Contributed during discussions to the final manuscript.

II  Contributed in planning and performed all experiments and its analysis, significantly contributed to writing of the manuscript.

III  Contributed in planning, performed all experiments and its analysis, wrote most of the manuscript.

IV  Performed all of the experiments and its analysis, contributed significantly to writing of the manuscript.
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


II  Mokvist, F., Mamedov, F., Styring, S. (2013). The photochemistry in photosystem II is different in visible and far-red light. *Submitted manuscript*

III Mokvist, F., Mamedov, F., Styring, S., (2014) Defining the far-red limit of photosystem I in spinach. The primary charge separation is functional up to 840 nm. *Submitted Manuscript*


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Abbreviations

ATP  Adenosine triphosphate
A₀   The primary chlorophyll acceptor in PS I
A₁   The secondary phylloquinone acceptor in PS I
CarD₂ β-CaroteneD₂
Chl  Chlorophyll
CT-band Charge transfer band
Cyt b₅₅₉ Cytochrome b₅₅₉
EPR  Electron paramagnetic resonance
EXAFS Extended X-ray absorption fine structure
Far-red light Light between 700-850 nm
Fd   Ferredoxin
FNR  FAD-containing ferredoxin-NADP⁺ reductase
LHC  Light harvesting complex
NADPH Nicotiamide adenine dinucleotide phosphate
PC   Plastocyanin
Pheo Pheophytin
PQ   Plastoquinone
PS I Photosystem I
PS II Photosystem II
QA   Primary quinone acceptor in PS II
QB   Secondary quinone acceptor in PS II
YD   Tyrosine 160 on the D2 protein
YZ   Tyrosine 161 on the D1 protein
1 Photosynthesis –from an energy point of view

The sun provides the earth with enormous amounts of energy every day and has done so since the dawn of time. The ability to take advantage of the abundant energy from the incident sunlight appeared 2.5 billion years ago, when the concept of photosynthesis was born. This took place in ancestral cyanobacteria. They mastered the task to capture the energy in the incident sunlight, via the oxidation of water and to store it as carbohydrates by carbon fixation. While photosynthesis has provided life with energy in the past, the modern civilization has eagerly harvested the remains of the earth’s fossil forests, now processed to coal and oil, and used it as cheap and abounded energy sources.

Our prominent combustion of fossil fuels and the release of carbon dioxide (CO$_2$) into the atmosphere has however had its consequences. It has recently been concluded by the U.N climate committee (IPCC) that the elevated levels of CO$_2$ into the atmosphere caused by human activity is responsible for the current global warming. It is of course of great importance that we try to inhibit this climate change that potentially can cause future environmental disasters. An interesting aspect that is not discussed much in this context is that the amount of CO$_2$ that can be released into the atmosphere is strictly depending on how much coal and oil there is left to exploit. The fossil fuels are without doubt limited resources. The question is how limited? Based on recent reports (see (7) and references there-in) the oil findings and more surprisingly also the coal resources are about to end. In other words the demand for a new clean energy resource is growing for every day.

Perhaps the mechanism of photosynthesis is one solution to our future energy problems. Therefore deep understanding of photosynthesis at all levels is crucial. This idea is one major factor behind the basic research carried out in this thesis.

1.1 The aim of the thesis

This thesis aims to explain how two of the key enzymes involved in photosynthesis are able to utilize light of much lower energy than previously demonstrated. This knowledge can further clarify how the very complex
charge separation process that initiate the photosynthetic reaction takes place within the first picoseconds.
2 An historical overview of photosynthesis – evolution and science

The photosynthesis process is indeed vital for life on earth, it produces the oxygen we breath, fixates the inorganic carbon we eat and are built of and has provided mankind with the energy that we currently use through oil and coal combustion.

Already in elementary school Swedish school kids are taught the general formula that describes the basic steps in the photosynthetic process.

Water + Carbon dioxide + sunlight $\rightarrow$ Oxygen + Sugars

This chapter will present a slightly more detailed view about photosynthesis, how it evolved and also some groundbreaking discoveries that has made it possible to reach our current detailed understanding.

2.1 Evolution

The very first signs of life on earth dates back to 3.5 billion years ago and was found in stomatolites and microfossils (8, 9). 700 million years later (or 2.8 billion years ago) is the first signs of early cyanobacteria found (10), this ancestral cyanobacteria was remarkably similar to the present species. Therefore it has been argued that this was one of the first species able to perform oxygenic photosynthesis. This is of course impossible to know, but geological evidence strongly indicates that the atmosphere contained significant amounts of oxygen (O$_2$) 2.2 billion years ago. The build up of atmospheric oxygen is however quite complex and depend on several geological variables, therefore it is possible that the oxygenic photosynthesis started much earlier than the first sign of atmospheric oxygen.

Exactly how oxygenic photosynthesis was evolved is a question that probably will remain unsolved. It is though likely that early bacteria carried out the first photosynthetic metabolism in an anoxygenic fashion, using oxidizable compounds such as H$_2$, Fe$^{2+}$ and H$_2$S as a source of electrons. As these compounds were slowly consumed, the development of oxygenic photosynthesis started to take place in ancestral cyanobacteria. Abundant water was then used as source of electrons and protons and highly reactive oxygen
was evolved as a byproduct and released into the atmosphere. The electrons extracted from water were used to reduce carbon dioxide \((\text{CO}_2)\) and store energy as carbohydrates. The conversion of inorganic carbon into organic compounds was also beneficial for the non-photosynthetic organisms on the earth. The organic compounds were able to be oxidized, thereby was the stored energy recaptured by non-photosynthetic organisms. The early life on earth took advantage of this and since then has photosynthesis been crucial.

2.2 Scientific landmarks

From the 17th century up to present day has many great scientists contributed to the present knowledge about the photosynthetic process. It is here impossible to mention all of them; this section will remark some of groundbreaking discoveries with the earliest dating back to 1640.

In 1640 did a Belgian scientist by the name van Helmont J.B. conclude that the materia that made up a growing tree originates from water and not from the soil. This was done by careful weighting the total mass of the tree and soil before and after harvesting a couple of years later, finding that the soil was almost equally heavy while the tree had gained a substantial mass. This was of course only partly correct but significantly ahead of its time. The law of conservation of matter was formulated 100 years later by the French scientist Lavoiser.

More than 100 years later found an English scientist by the name Priestly J. that plants were able recover polluted air. At that time it was believed that a burning candle or a breathing mouse/human produced something called phlogiston. A candle or mouse put in a sealed container would produce phlogiston and eventually extinguish them. Priestly discovered that if a plant was placed together with the burning candle or breathing mouse in the sealed container, the candle could be lit again after some time and the mouse survived (at least longer). He later presented his experiments for the above mentioned Lavoiser, who used this information in combination his own experiments to formulate the matter conservation law and thereby formed the modern study of chemistry.

Already in 1804 was the first chemical formula for photosynthesis written which strongly resembles formula (a), but at that time it was not clear what was formed and the term “organic matter” was used instead of sugar.

The chlorophyll (chl) pigments was described around the same time period by Pelletier and Caventou in 1818, Willsäter was later rewarded the nobel prize (1915) for determining major features on the chl molecule.

In 1864 managed Boussignault T.T. and von Sachs J. to prove that the carbon dioxide to oxygen reaction quotient was 1. From this could the still valid balanced chemical formula for photosynthesis be written (b). Where \([\text{CH}_2\text{O}]\) represents a carbohydrate.
Almost 100 years later in 1930 found Hill R. that oxygen production and carbohydrate production could be separated into the following redox equation.

\[
2\text{H}_2\text{O} \rightarrow \text{O}_2 + 4e^- + 4\text{H}^+ \quad \text{(c)}
\]
\[
\text{CO}_2 + 4e^- + 4\text{H}^+ \rightarrow [\text{CH}_2\text{O}] + \text{H}_2\text{O} \quad \text{(d)}
\]

At the same time period was the model of photosynthetic units (psu) developed by Emerson R. and Arnold W. They discovered that ~2000 chl pigments was needed to evolve one molecule of oxygen (c). This was a big surprise at the time since it was generally considered that only one chl molecule was needed to evolve one molecule of oxygen \(^{(11)}\).

Sequent to these experiments Emerson R. showed that the photosynthetic activity varied with the applied excitation wavelength \(^{(12)}\). Eventually, did that experiment lead to the suggestion of two photosystems working in synergy.

Those who finally developed a model for two photosystems were Hill and Bendall. They studied two cytochromes connected to the photosynthesis chain (now identified as the Cyt \(b_f\) complex) where one was reduced with visible light and the other oxidized with far-red light. The model is now referred to as the Z-scheme and is displayed in Figure 1. This scheme summarizes the energetics of the light driven electron transport. Two charge separation events drive water oxidation in Photosystem II and NADPH reduction in Photosystem I.

\[
\text{CO}_2 + \text{H}_2\text{O} + \text{light} \rightarrow [\text{CH}_2\text{O}] + \text{O}_2 \quad \text{(b)}
\]

\[\text{Figure 1. The Z-scheme of photosynthesis. The electron transport is driven by the incident sunlight that excites } P_{680} \text{ and } P_{700}. \text{ The electron transport is driven by the power of the incident photons.}\]
The photosynthetic membrane

The light driven part of photosynthesis are carried out by four major protein complexes; Photosystem II (PS II), Photosystem I (PS I), Cytochrome \(b_{6f}\) (Cyt \(b_{6f}\)) and F-ATPase. This thesis will exclusively deal with PS I and PS II in the following chapters, but in order to get a more comprehensive view of the light driven part, it is important to be aware of their existence. All four major complexes are located in the thylakoid membrane of plants and cyanobacteria. The membrane consists of a lipid bilayer which separates the lumen (inside of thylakoid) from the stroma (outside of thylakoid). By having a non-permeable membrane, the thylakoid can build up chemical gradients and drive chemical reactions.

Photosynthesis is initiated at PS II, which reduces a quinone to plastoquinol by extracting electrons from water by utilizing the energy of incident sunlight. After plastoquinol formation, it is released from PS II into the membrane and form the plastoquinone pool, indicated in Figure 2 as PQH\(_2\). The plastoquinol is then oxidized by the Cyt \(b_{6f}\) complex, which then reduces the soluble Plastocyanin protein on the luminal side. Cyt \(b_{6f}\) also functions a regulator as it senses the redox state of the plastoquinone pool and helps to balance the two photosystems. The release of protons into the lumen occurs through the Q-cycle in Cyt \(b_{6f}\) (reviewed in \(^{(13)}\)). PS I reduces Ferredoxin (Fd) in the stroma and the oxidized PS I is then reduced by the electron mediating Plastocyanin. Fd reduces in turn FNR that subsequently produces the high energy intermediate NADPH. NADPH is later used in the Calvin-Benson cycle that reduces CO\(_2\) and through a quite complex process produces carbohydrates. Ultimately are those carbohydrates converted into sucrose or starch which is more beneficial for long time storage. This is also referred to as the dark reactions of photosynthesis. Under certain conditions can Fd also reduce Cyt \(b_{6f}\) possibly supported by FNR, promoting cyclic electron transfer and NADPH production without carbon assimilation \(^{(13)}\).

Figure 2. A simplified presentation of the compartments in the thylakoid membrane, they perform light driven electron transport and initiates photosynthesis. The stoichiometry is also given. Two molecules of water produce two molecules of NADPH and three molecules of ATP. Figure was adapted from \(^{(14)}\).
The proton gradient that is built up during the light driven electron transfer by PS II and Cyt b_{6f} is used to drive the formation of ATP by F-ATPase. ATP is also a high energy intermediate absolutely vital for the cell and here used in for example the Calvin-Benson cycle.
3 The photosynthetic pigments

The first step in the photosynthetic process is the absorption of incident sunlight. This essential task is in all photosynthetic organisms taken care of by the photosynthetic pigments located in the protein sub-units associated to the two photosystems. This chapter will deal with the pigments that are able to transform the incident photons into energy, transfer that energy to the reaction center and perform charge separation.

3.1 Light harvesting in oxygenic photosynthesis

Oxygenic photosynthesis mainly utilizes the chlorophyll (chl) molecule for light absorption, presented schematically in figure 3. There are at least 5 different chlorophyll variants found in the pamphlet of oxygen evolving photosynthetic organisms; chl a, present in all oxygen evolving photosynthetic organisms, chl b, present in plants, chl c, found in groups of marine algae and chl d and f, at present found exclusively in Acaryochloris marina and Halomicronema hongdechloris, respectively.

Figure 3 displays a schematic presentation of the chl a molecule which is present in all oxygen evolving photosynthetic organisms. It is therefore chosen to present the common features for all chlorophylls presently known. The shape is square like and consists of a 10 Å wide chlorin ring structure numbered a-d (IUPAC) and an additional ring denoted e next to the c ring. In the center of the ring structure a Mg ion is coordinated by four nitrogens. This centrally coordinated Mg ion is present in all chls (and also the bacterio chlorophylls that
are not discussed in detail in this thesis). A characteristic long hydrocarbon tail is attached to the d-ring. This tail facilitates anchoring of the chlorophyll molecule to other hydrophobic parts of the protein.

Out of the 5 different known variants of the chl molecule, chl \(a\) and chl \(d\) is the only taking part in both light harvesting and charge separation processes in both PS I and PS II, described in detail in later chapters (15-17). All other chls are contributing to the light harvesting and are located exclusively in the antenna sub-units, out-side of the reaction center. The variations between the chl molecules are relatively small from a structural point of view, with one or two of the groups encircled in Figure 3 exchanged to formyl or vinyl side chains. These quite small changes generate different absorption abilities to the molecule which is discussed below.

### 3.1.1 Light absorption

The interaction between light and matter will only occur if the incident energy (wavelength) matches the energy gap between a populated and an unpopulated state (denoted \(\Delta E\)). This holds for all types of electromagnetic radiation.

Visible light induces valence electron orbital transitions in for example the chlorophyll molecule. In photosynthesis research, different types of light absorption spectroscopy are therefore very important tools for studying different aspects of photosynthesis. The absorption of light is described by Lambert-beers law (Eq. 1).

\[
\text{Abs}_\lambda = \log \left( \frac{I_{0\lambda}}{I_{\lambda}} \right) = \epsilon_{\lambda,\chi} c_{\chi} l
\]

Eq. 1

This equation relates the absorption \((\text{Abs}_\lambda)\) to the logarithmic ratio of the incoming \((I_{0\lambda})\) and transmitted \((I_{\lambda})\) light, which depends on the sample concentration \((c_{\chi})\), light path length \((l)\) and the extinction coefficient \((\epsilon_{\lambda,\chi})\).

Figure 4 displays a typical absorption spectrum of a chl a/b mixture in their monomeric forms dissolved in acetone. In the spectrum three different absorption bands can be identified for each chlorophyll species and they are also marked in the spectrum. The transition of the highest energy is commonly denoted the soret band and the lowest in energy is denoted the Q band. The Q band can be split into two bands depending on molecular orientation in respect to the excitation light, as indicated (x and y) for the schematically drawn chlorophyll molecule (Figure 4 middle). The bands derive from \(\pi \rightarrow \pi^*\) transitions of the conjugated electrons in the chlorine macrocycle seen in Figure 3. The non-identical absorption profiles for the two chl molecules are explained by the side-chain differences which cause different “disruptions” in the conjugation. This leads to changes in the absorption profile.
3.1.2 Light absorption in Photosystem II

In this and the following section I will discuss how chl interaction with its surroundings can effect the absorption profile compared to the monomeric form presented in the previous section.

A straightforward yet a bit simplified example of how the absorption properties are changed due to the surroundings of the absorbing pigments is shown in Figure 5. Presented here are the absorption spectrum in the $Q_y$ region (see Figure 4) of a mixture of monomeric chl $a$ and chl $b$ in aceton (red) together with the absorption spectrum of PS II membranes in water, both recorded at 295 K. In the PS II membranes the chl pigments are coordinated by the surrounding protein. Investigation of the $Q_y$ peak reveals a 15 nm redshift for chl $a$ between the monomeric chls in solution and the PS II membranes. The weak shoulder for monomeric chls at 650 nm belonging to chl $b$ has been much more pronounced in the PS II membranes since it is no longer shielded behind the chl $a$ peak. It is also slightly red-shifted by 1-2 nm.

The absorption of a single pigment inside a protein matrix is depending of a number variables, for example phonon couplings, site energies and pigment-pigment interactions\(^{(18)}\).

The observed red-shift for chl $a$ is therefore a combination effect but is partly caused by pigment interactions that occur in the primary donor of PS II denoted $P_{680}$. 

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*Figure 4. Left* Absorption spectra of chl $a$ and $b$ in aceton, the markings indicate the three main transitions for respective pigment. *Middle* a schematically drawn chl molecule with $Q_y$ and $Q_x$ transition marked. *Right* schematic description of how an electron is absorbed.
Excitonic couplings occur when two or more pigments are physically very close (< 10 Å), and therefore interact (so called coupling) by their individual charges. When one of these pigments absorbs a photon the excited electron is delocalized over the interacting pigments creating a so called exciton. The absorption properties of two interacting pigments are also changed, schematically displayed in Figure 6 as a dimer. The magnitude of the excitonic splitting (two different excited states) in the dimer depends on the distance and relative orientations of the transition dipole moments \(^{(17)}\).

If the molecular orbitals of two or more pigments are overlapping, a so called charge transfer (CT) state can be formed (a theoretical description can be found in \(^{(19)}\)). A mixed exciton CT state has been observed in the antenna of PS I and also in the reaction center of PS I, PS II and the bacterial reaction center\(^{(20-22)}\). These bands can have the ability to absorb light in the far-red regions, but are in PS I and PS II very weak and difficult to detect by normal absorption spectroscopy. They can however be detected with so called stark spectroscopy which have been used in the above referred examples.

---

**Figure 5.** Comparison of monomeric Chl \(a\) and Chl \(b\) in aceton (red) and PS II enriched membranes (black) in water, recorded at 295 K.

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**Figure 6.** Energy level diagram, represents the ground and excited states of a monomer and an exciton-split dimer.
3.2 Energy transfer in the photosystems

After excitation of a chl pigment somewhere in the antenna, the formed excited state can be transferred to the reaction center via excitation energy transfer. The basic theory to describe energy transfer between molecules is called Förster theory\(^{(23, 24)}\). In this theory the excited state is assumed to be localized on one pigment at the time. The excitation is then transferred from an excited donor to an acceptor in the ground state via dipole-dipole resonance coupling, where the excitation of the acceptor molecule is coupled to the de-excitation of the donor, schematically described in Figure 7.

![Figure 7. Schematic description of Förster energy transfer.](image)

This theory is excellent in describing the energy transfer between non-coupled pigments with a relatively large spatial distance, such as separated chromophores in a protein complex\(^{(25)}\). Förster theory describes an excited state that is “hopping” from one pigment to another, until it reaches the reaction center. The chls have no interaction between each other in the ground state in this theory. This is unfortunately not the situation for the antenna chls where many of them are interacting and therefore does this theory not describe energy transfer in the photosystems accurately\(^{(26-29)}\).

A number of different theories exists in order to model the energy transfer that occurs in the antennas of the photosystems. Apart from the already mentioned Förster theory which doesn’t take any coupling into account there are Redfield, modified Redfield and generalized Förster theory that all have different couplings included in their formula\(^{(18)}\), such as pigment-phonon couplings pigment-pigment couplings etc. However, no theory so far manage to take all different interactions into account, and it seem like different types of antennas also requires different types of considerations\(^{(17)}\).
Photosystem I is a multisubunit protein complex located in the thylakoid membrane of plants and cyanobacteria. Its primary role is to reduce stromal Ferredoxin that produces NADPH, an intermediate electron carrier vital in the carbohydrate synthesis performed by the Calvin-Benson cycle. This chapter aims to highlight the proteins, co-factors and processes that lead to charge separation in the reaction center, necessary to perform any type of photochemistry.

**Figure 8.** Photosystem I and the reaction center co-factors adapted from (30, 31).

### 4.1 Pigment-protein composition

In order to understand the complicated process that leads up to the reduction of the [4Fe-4S]-clusters in PS I and the subsequent Ferredoxin reduction, it is necessary to get a complete view of the protein-pigment organization.

#### 4.1.1 In vivo arrangement

**4.1.1.1 Protein organization**

PS I and its outer antenna complexes are located in or associated to the thylakoid membrane as displayed in Figure 8. In plants, PS I operates as a monomer with the outer antenna subunits (LHC I) located at specific binding sites on the PS I core (Figure 8 A). Four polypeptides Lhca1, Lhca2, Lhca3 and Lhca4 make up the LHC I, all with slightly different pigment organization (32). *In vivo*, the four polypeptides are associated as two hetero dimers (Lhca1/4 and Lhca2/3) (32). Also, it has been shown that LHC II, normally associated to PS II, under certain conditions can be associated to PS I (33).
This is coupled to regulation and redistribution of excitation energy between the two photosystems. A similar phenomenon, where the phycobilisomes sometimes can distribute energy, has been seen in cyanobacteria.

The PS I core in cyanobacteria operates as a monomer or trimers \textit{in vivo} depending on species and environmental conditions \cite{34}. While the PS I core is highly conserved throughout billions of years of evolution the outer antenna is not. Cyanobacteria lack membrane bound outer antenna subunits completely. Instead they use soluble antenna complexes called phycobilisomes. These antenna complexes can migrate between PS I and PS II and redistribute the captured energy depending on environmental conditions \cite{35}. The PS I preparations from cyanobacteria presented in this thesis have no phycobilisomes present, therefore these complexes will not be discussed in detail.

\subsection*{4.1.1.2 Pigment organization}

The PS I core (the reaction center and inner antenna) contains only chl \textit{a} in both cyanobacteria and plants. This is similar to the PS II core. The LHC I antenna complexes have both chl \textit{a} and \textit{b} with a ratio ranging from two to six \cite{36}. A detailed structure for LHC I in plants has not yet been obtained, and the exact number of pigments coordinated by LHC I is therefore not conclusive. However by comparison to the very similar, and structurally resolved (2.72 Å \cite{37}) outer antenna protein complex LHC II in PS II, it is reasonable to assume that the polypeptides coordinates 12-14 chlorophylls each. Thus approximately 50 chlorophylls are present in the outer antenna. The total number of chlorophylls that are associated to the PS I reaction center in plants are approximately 170 \textsuperscript{30} (when LHC II is not associated to PS I). Meaning that the majority of chlorophyll pigments are located in the PS I core.

\subsection*{4.1.2 Photosystem I}

The core of Photosystem I (Figure 8 B) is composed of 16 different subunits in plants and 12 in cyanobacteria, and has a combined molecular weight of approximately 356 kDa. The co-factor harboring protein subunits for both plants and cyanobacteria are the same, PsaA and PsaB. They coordinate most of the electron transfer co-factors in the reaction center as well as most of the inner antenna chlorophylls in total ~100 chls (Figure 7 C). PsaA and PsaB consists of 11 transmembrane helices each. PsaC is located on the stromal side of PsaA and PsaB and coordinate the terminal acceptors F\textsubscript{A} and F\textsubscript{B} [4Fe-4S]-complexes (F\textsubscript{X} is coordinated by PsaA and PsaB \cite{31}). The remaining sub-units are small and located in the periphery of PsaA and PsaB. Their roles are diverse, from stabilization of the protein complex to provide Ferredoxin docking sites on the stromal side \cite{34}. 24
4.1.3 The reaction center -the light induced electron transfer and redox active cofactors.

PS I is a type I reaction center using Fe-S clusters as terminal acceptors similar to reaction centers in Green sulfur bacteria and Heliobacteria \(^{(17)}\). Electron transfer is mediated by the redox active co-factors displayed in Figure 8 C. PS I has one electron donor, which is a chl \(\alpha\)-chl \(\alpha'\) heterodimer that make up the special pair \(P_{700}^{(31)}\). After its oxidation, \(P_{700}^{+}\) is reduced in the ms time regime by the electron mediating Plastocyanin protein located in the stroma (Figure 2 and 9) \(^{(4)}\). The acceptors, located towards the stromal side relative to \(P_{700}\), are symmetrically coordinated in two branches, the A and B-branch.

The electron transfer is occurring through both \(^{(38, 39)}\). This ability stands in contrast to the bacterial reaction center and PS II where electron transfer is occurring in only one specific branch (Chapter 5).

4.1.3.1 Primary and secondary electron transfer

The electron transfer in the reaction center of PS I is divided in primary and secondary electron transfer \(^{(40)}\). Figure 9 presents the approximate kinetic half-times of the different electron transfer steps. The primary electron transfer involves the initial very fast steps from the primary donor \(P_{700}\) to the phylloquinones (A\(_1\)) (Figure 9), the accessory Chl A shown in Figure 8 C is here excluded since it is not conclusively participating in the electron transfer. After excitation of \(P_{700}\) is instead the primary acceptor \(A_0\) reduced in 2 ps, which subsequently reduces \(A_1\) in 10-50 ps forming the first stable charge pair \(P_{700}^{+}A_1^-\). The secondary electron transfer involves the slow electron transfer rates from \(A_1\) up to \(F_B\). Reduction of \(F_X\) from \(A_1\) occurs in 20 and 200 ns, and is probably branch dependent \(^{(39)}\). Reduction of \(F_A\) and \(F_B\) occurs in ~500 ns per reduction.

\[\text{Figure 9. PS I reaction center oxidation time scales, half-time rates 1-5 from }^{(2)} \text{ and 6 from }^{(4)}\]
4.2 Properties of the primary donor, $P_{700}$ – its excitation, oxidation and reduction.

This section highlights the interesting and important primary donor in PS I, $P_{700}$. Figure 10 displays $P_{700}$ ($P_A$ and $P_B$) together with its accessory chlorophylls, denoted A. The $P_{700}$ chlorophylls exhibit a weak coupling of ca. 138 cm$^{-1}$ (41) compared to the special pair in the bacterial reaction center with a coupling of 500-1000 cm$^{-1}$ (42). Yet it appears that this coupling might be very important for the far-red light absorption discussed in chapters 6 and 7.

Figure 10. $P_{700}$ ($P_A$ and $P_B$) with the accessory chlorophylls. The Figure is adapted from PDB ID: 1JB0.

4.2.1 The excited states and primary charge separation in PS I

Photosystem I is extremely efficient in converting an absorbed photon into photochemistry, it manages to do so in 99.97% of the cases at 295 K (34). The primary donor $P_{700}$ (displayed in Figure 9), are within excitation energy transfer distance of approximately nine antenna chlorophylls (31). After excitation of $P_{700}$ is the exciton is located at $P_{700}$ and probably also at its accessory chl pigments (43). The exact location where the primary charge separation is initiated is a matter of debate. The general view has been that charge separation is initiated at $P_{700}$ and one of the accessory chl acts as transient acceptor before the first stable charge pair $P_{700}^+A_0^-$ is formed (40, 44). This has however been challenged by Holzwarth and co-workers (45). They suggest that primary
charge separation is initiated at the accessory one of the accessory chls (here denoted Chl A). The cation is then transferred to $P_{700}$, forming the stable radical $P_{700}^+(40)$. Further spectroscopic evidence is however needed to clarify this.

4.3 $P_{700}^+$ reduction

Reduction of the formed $P_{700}^+$ radical after primary charge separation in PS I is handled by the soluble Plastocyanin protein in plants or cytochrome $c_6$ in some cyanobacteria and green algae. The latter occurs upon copper deprivation (46, 47). The reduction occurs in the ms time regime at physiological conditions (4). Plastocyanin mediates electrons between the Cyt $b_6f$ complex where it becomes reduced and PS I where it’s oxidized. It binds to luminal loops of PsaA and PsaB in PS I, and also to recognition site located at the luminal PsaF protein subunit.

4.4 Probing electron transfer in PS I

Photosystem I has several species that are detectable via cw-EPR, including the oxidized primary donor $P_{700}$ and the reduced iron sulfur acceptor clusters ($F_A$ and $F_B$), also denoted [4Fe-4S]. The $P_{700}^+$ radical EPR signal is easily detected during illumination at both 295 K and cryogenic temperatures (< 20 K) (Figure 11 left). It can also be chemically oxidized with ferricyanide ($Fe(CN)_6^{3-}$) in the dark. The EPR signal has a $g$-value of 2.0026 and a width of 7 G. The narrow width is important as it is 3-4 G narrower than an oxidized monomeric chlorophyll (10-11 G). This indicates that the $P_{700}^+$ originates from a coupled radical species. This coupling involves the close lying $P_A$ and $P_B$ which together constitute $P_{700}$.

The [4Fe-4S]-clusters are located on the stromal side and mediates electrons to Ferredoxin. At cryogenic temperatures the reduction of Ferredoxin and also the recombination with $P_{700}^+$ are strongly inhibited which make the detection of reduced $F_A$ and/or $F_B$ possible with cw-EPR. The EPR signals appears around the $g=2$ region. They are 650 G wide and often appear as a mix of the EPR signals from the distal clusters $F_A$ and $F_B$.  

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Figure 11. The $P_{700}^+$ radical EPR signal and the $F_A$ and $F_B$ [4Fe-4S] clusters. EPR conditions left: temperature 295 K, microwave power 8 mW, modulation amplitude 5 G and microwave frequency, 9.82 GHz. Right: temperature 15 K, microwave power 10 mW, modulation amplitude 10 G and microwave frequency, 9.46 GHz.
5 Photosystem II

Photosystem II is a multi-competent protein. It has the ability to combine three very challenging tasks. 1; Perform charge separation by utilizing the energy of the incident photons. 2; Reduce $\text{P}_{680}^-$ after charge separation with electrons via oxidation of water at the CaMn$_4$-cluster.3; Store the captured energy as plastoquinol, which drives the Cyt $b_6f$ complex and the formation of a proton gradient. All of these processes are absolutely vital for photosynthesis and will be addressed in this chapter.

Figure 12. The PS II, organization and reaction center co-factors. The structures are adapted from, PDB ID: 3ARC (PS II) and PDB ID: 2BHW (LHC II)

5.1 Pigment-protein composition

In order to understand the complexity of PS II it is necessary to grasp the complete picture, from the protein organization facilitating light harvesting to the individual electron transfer co-factors in the reaction center. Figure 12 presents an overview of this which will be discussed in the subsequent sections.

5.1.1 In vivo arrangement

5.1.1.1 Protein organization

Figure 12 A presents a schematic overview of the protein organization surrounding PS II in the grana stack in the thylakoid membrane of plants. In vivo, PS II is functional as a dimer surrounded by the outer antenna protein complexes LHC II, CP 29, CP 26 and CP 24. This dimer is also referred to as the PS II supercomplex. LHC II is the most abundant and the most studied of the outer antenna complexes and the structure has been resolved.
to 2.72 Å \(^{(37)}\). Its main role is to harvest light and transfer it through excitation energy transfer (eet) to the PS II reactions center. The minor antenna complexes (CP 29, CP 26 and CP 24) are much less studied. Their locations are between the LHC II and PS II and it has been suggested that their role is to absorb and mediate the energy flow between the outer antenna LHC II and the PS II reaction center \(^{(49)}\).

5.1.1.2 Pigment organization

The numbers of chlorophylls that are associated to each PS II reaction center are approximately 200, depending on the light conditions which control the exact size of the LHC II antenna \(^{(50)}\). The absolute majority of those are located in the outer antenna protein subunits; the presence of the outer antenna enhances the number of photons that are being absorbed per reaction center. The outer antenna also increases the number of excitations that reaches the PS II reaction center where photochemistry takes place. In vivo LHC II, is in contrast to its analog in PS I LHC I, active as a trimer (Figure 12 A), each trimer coordinates 26-32 chl \(a\) and \(b\) pigments depending on species. LHC II also has a number of carotenoids that participate in energy transfer, structure stabilization and photo protection against damaging triplet species \(^{(51)}\).

5.1.2 Photosystem II

The PS II monomer is displayed in Figure 12 B (adapted from \(^{(52)}\)), it has a molecular weight of approximately 350 kDa and consists of up 20 protein subunits. There are in addition to this over 20 proteins loosely associated that binds to the PS II complex during certain conditions \(^{(2)}\). The reaction center core of PS II is composed of the heterodimeric protein subunits D1 and D2 that each has 5 transmembrane helices spanning through the thylakoid membrane. Adjoining the PS II core is the two inner antenna protein subunits CP 43 and CP 47 that has 6 transmembrane helices each coordinating 13 and 16 chl \(a\) respectively. At least three extrinsic protein subunits are located on the luminal side (PsbO, PsbP and PsbQ) \(^{(53)}\), they shields the CaMn\(_4\)-cluster and PsbO also provides it with water through specific channels \(^{(54)}\). The PS II monomer contains 35 chl \(a\) pigments and 11 \(\beta\)-Carotenones that participates in light harvesting, charge separation and photo protection \(^{(17)}\).
5.1.3 The reaction center redox active cofactors and plastoquinol formation

Electron transfer in the PS II reaction center (Figure 12 B) occurs through the redox active co-factors displayed in Figure 12 C. These co-factors are spatially divided into donors and acceptors separated by $P_{680}$ (Chl$_{D1}$, P$_D$, P$_D$ and Chl$_{D2}$). $P_{680}$ is the primary donor, on the luminal side of $P_{680}$ the secondary donors Y$_Z$ and the CaMn$_4$-cluster are mostly associated to the D$_1$ protein. The auxiliary donors Car$_{D2}$, Chl$_{ZD}$ and Cyt $b_{559}$ are located on the D$_2$ protein. The acceptors are located towards the stroma relative to $P_{680}$.

PS II is a type II reaction center using quinones as terminal acceptors similar to the purple bacteria (17). After oxidation of $P_{680}$, reduction of the acceptors occurs exclusively via the D1 branch, indicated with an arrow in Figure 12 C. This is analogous to the bacterial reaction center and in contrast to the two active branches of electron transfer previously described for PS I (chapter 4).

After primary charge separation at $P_{680}$ the transient acceptor Pheo$_{D1}$ is reduced within 2 pico seconds (ps) (see Figure 13 and references therein). The Pheophytin molecules resembles the chl $a$ molecule but lack the central Mg ion. Subsequently the primary quinone acceptor (QA) is reduced in 350 ps creating the first stable primary charge pair $P_{680}^{+}\text{QA}^{-}$. Thus formation of QA$^{-}$ occurs before $P_{680}^{+}$ is reduced (Figure 13). Electron transfer from QA$^{-}$ is fairly slow though and the electron is transferred to the secondary quinone (QB) in 150 or 400 µs depending on its present oxidation state (QB or QB$^{-}$). QB is exchangeable. After double reduction and subsequent protonation it leaves PS II as plastoquinol (Q$_{B}$H$_{2}$) which is used to drive the Cyt $b_{56}$ complex. A new oxidized quinone thereafter takes its place in the QB-site.

On the luminal side of the reaction center the redox active tyrosine residues Y$_Z$ and Y$_D$ are located in the D$_1$ and D$_2$ protein respectively. Y$_Z$ takes an

\[ \begin{align*}
& \text{(1)} \\
& \text{Pheo} \\
& \text{(2)} \\
& P_{680}^{+} \quad \text{h}_v \times 4 \\
& \text{QA}^{+} \\
& \text{QB}^{-} \\
& \text{(3)} \\
& \text{Y}_{Z} \\
& \text{CaMn}_{4} \\
& \text{S}_{0} \quad \text{S}_{1} \\
& \text{S}_{2} \quad \text{S}_{3} \\
& \text{(4)} \\
& \text{Car}_{D2}/\text{Chl}_{Z} /\text{Cyt} \text{ b}_{559} \\
& \text{(5)} \\
& 150 \mu s \\
& 400 \mu s \\
& (5) \\
& \text{(6)} \\
& 20 \text{ ns - } 35 \text{ ps} \\
& 1-2 \text{ ms} \\
& 1-2 \text{ ms} \\
& \text{(6)} \\
& \text{(5)} \\
& \text{(4)} \\
& \text{(3)} \\
& \text{(2)} \\
& \text{(1)} \\
\end{align*} \]

Figure 13. PS II reaction center oxidation time scales, half-time rates. Reaction 1-2 and 5 from (1), 3 from (3), 5 from (5) and 6 from (6), and references therein.
active part in the electron transfer between $P_{680}$ and the CaMn$_4$-cluster and will be discussed in detail later. YD has been much studied (reviewed in (55)). It does not take an active part in the charge separation events nor in linear electron transfer but is entirely conserved and fulfill several different roles in PS II (55). It is easily oxidized by $P_{680}$ and the absence of a nearby oxidizable species makes it a very stable radical ($YD^*$). This radical is very important from an EPR perspective since it is detectable both at 295 and 5 K and is present as 1 radical per PS II. It has therefore been used as an internal standard in all the measurements presented in this thesis (see section 5.4).

The donors, involved in reduction of $P_{680}^+$, are TyrZ, the CaMn4-cluster, CarD2, ChlZ and Cyt b$_{559}$. The oxidation of these donors, and the reduction of $P_{680}$ are the focus of the remaining part of this chapter.

5.2 Properties of the primary donor, $P_{680}$ –its excitation, oxidation and concomitant reduction.

$P_{680}$ consists of the four central chlorophyll pigments in the PS II reaction center, ChlD1, P$_{D1}$, P$_{D2}$ and ChlD2. The pigments in the reaction center are considered to be a multimere with quite weak excitonic couplings ranging from 85-150 cm$^{-1}$ (42, 56, 57). All pigments involved in $P_{680}$ contributes to a functional reaction center (58), surprisingly also the PheD2 located on the inactive D2 branch. Through site directed mutagenesis it has been shown that mutants of this residue are not able to evolve oxygen to any significant extent (59).

![Figure 14. $P_{680}$ with its pigments, ChlD1, P$_{D1}$, P$_{D2}$, and ChlD2, their accessory pigments PheD1 and PheD2 are also included in this figure. Figure adapted from PDB ID 3ARC](image)

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5.2.1 The excited states and primary charge separation in PS II.

Visible light (< 680 nm) is absorbed in the antenna. Subsequently the excitation energy is transferred to the reaction center where the primary donor is excited to \( \text{P}_{680}^* \). The immediate excitation of \( \text{P}_{680} \) is likely to occur from either of the nearby Chl\(Z\) pigments and is also possibly mediated by the Carotenoids located between Chl\(Z\) and \( \text{P}_{680} \)\(^{(60)} \). The exact nature of the excited states that initiate primary charge separation has been and still is a very challenging task to resolve. The dominating spectroscopic technique to investigate this is time resolved absorption spectroscopy. However the pigments in the PS II reaction center all have very similar absorption profiles in the \( \text{Q}_Y \) region. What further complicates the analysis is that the constituent pigments of \( \text{P}_{680} \) have excitonic interactions between all or some of them \(^{(57)}\). One of the latest models however presents the possibility that there are three different excited states that can initiate charge separation \(^{(20)}\). All of these were suggested to be of a mixed exciton CT character. The presence of charge transfer bands among the pigments of \( \text{P}_{680} \) is in line with what we and others have suggested to be responsible for the far-red light absorption (paper II and \(^{(62, 63)}\)).

The primary charge separation was, by Romero and co-workers \(^{(20)}\) suggested to be initiated from either Chl\(D_1\) followed by hole migration to \( \text{P}_{D1} \) or on \( \text{P}_{D1} \) directly after visible light absorption, depending on what exited state that was initially formed. The presence of several excited states giving rise to different primary charge separation pathways \(^{(20)}\) suggested to be caused by different protein configurations. The experiments in \(^{(20)}\) were mainly carried out in reaction center preparations and are not conclusive and different models for the very early events in the primary charge separation exists \(^{(64)}\).

5.2.2 \( \text{P}_{680}^+ \) reduction

Establishment of a stable \( \text{P}_{680}^+ \) occurs after ~350 ps in PS II (Figure 13 and references there-in). This is very fast compared to its subsequent reduction discussed in the next section. Several experimental studies indicate that the first stable primary cation primarily resides on \( \text{P}_{D1} \) after the primary charge separation events \(^{(20, 42, 65-68)}\). It can potentially also be shared with \( \text{P}_{D2} \) to a small degree at physiological temperatures \(^{(65)}\). The reduction potential of the formed \( \text{P}_{680}^+ \) is strong, in fact very strong. It is one of the strongest oxidants that exist in nature and has been estimated to as high as ~1.4 V \(^{(42, 69)}\). This high reduction potential is also needed to oxidize the nearby Tyr\(Z\) residue, with an oxidation potential of 1.2 V.
5.2.2.1 The YZ/CaMn4-pathway

The primary donor \( P_{680}^+ \) is in intact PS II centers almost always reduced by electrons from the water oxidizing CaMn4-cluster under physiological conditions. The immediate reductant of \( P_{680}^+ \) is Tyr-161 on the D1 protein (YZ) which in turn oxidizes the CaMn4-cluster (Figure 8 and 9). This electron transfer pathway is in this thesis denoted the YZ/CaMn4-pathway.

Reduction of \( P_{680}^+ \) is fairly slow compared to its formation (Figure 9), at 295 K \( YZ \) is oxidized with three distinct kinetic phases, ranging from tens of ns to tens of \( \mu s \) \(^{(3, 70)} \). These phases reflect the relaxation process between \( P_{680}^+YZ \) and \( P_{680}YZ^\bullet \), the protonation state of \( YZ \) and the S-state of the CaMn4-cluster. Nevertheless, \( P_{680}^+ \) reduction from \( YZ \) is fast enough to out-compete recombination of \( P_{680}^+Q_A^\bullet \) and also faster than the competing donation from CarD2 discussed in the next section.

Reduction of \( YZ^\bullet \) occurs from the water oxidizing CaMn4-cluster. The rate of the CaMn4-cluster electron transfer depends on its present oxidation state, called S-state \(^{(71)} \). The rates are ranging from tens of \( \mu s \) to ms, where the fastest oxidation occurs in the \( S_1 \rightarrow S_2 \) transition \(^{(72)} \). This probably reflects that this transition only involves an electron transfer from the CaMn4-cluster without major structural rearrangement or proton transfer reactions occurring. Accordingly, the slowest oxidation is coupled to the water oxidizing chemistry occurring in the \( S_3 \rightarrow \left[S_4\right] \rightarrow S_0 \) transition. This involves bond formation, deprotonations and major protein structure rearrangements \(^{(73)} \).

Under non physiological conditions, the reduction of \( P_{680}^+ \) is more complex. At temperatures below 77 K electron donation from the CaMn4 cluster is strongly inhibited \(^{(74)} \). At first, it was believed that also \( YZ \) was unable to donate electrons to \( P_{680}^+ \) in this temperature range\(^{(75)} \). This however changed with the discovery and assignment of the so called Split EPR signals that represent an interaction between \( YZ^\bullet \) and the CaMn4-cluster \(^{(76-79)} \). These signals proved that \( YZ \) indeed could be oxidized despite the inhibited CaMn4-cluster at < 77 K. It was later demonstrated that up to ~40 % of the PS II centers in the S0 and S1 state were able to efficiently oxidize \( YZ \) \(^{(79, 80)} \) at 5-10 K, thus outcompeting other electron donors in PS II (see below). The lower yield of the \( YZ \) oxidation at these temperatures is commonly explained by a frozen-in heterogeneity around \( YZ \). This heterogeneity has been coupled to the phenolic proton of \( YZ \) that binds to the nearby His D1-190 residue and is reflected in the multiphasic kinetics of the \( YZ^\bullet \) oxidation \(^{(80, 81)} \). The proton moves in this hydrogen bond upon \( YZ \) oxidation (to yield \( YZ^\bullet \)). At low temperatures it is vital that the hydrogen bond is in the right configuration in order for the proton to move. Only in those centers will oxidation of \( YZ \) be possible at for example 5 K.
5.2.2.2 The Car/ChlZ/Cyt \( b_{559} \)-pathway

Photoinduced oxidation of cytochrome and chlorophyll species in PS II was first discovered more than 40 years ago \(^{(82-85)}\) and have since been extensively studied \(^{(86)}\). The donors were eventually identified as Cyt \( b_{559} \) located in the PsbF protein subunit and the Chloroohyll\( \text{ZD2} \) located in the D\( _2 \) subunit. The exact identity of the chlorophyll donor has however been questioned and a recent report has indicated several different oxidized chlorophyll species upon illumination \(^{(87)}\). For the present discussion this is not vital as all oxidized chlorophyll species report on photo induced electron transfer to \( P_{680}^+ \). In this thesis all chlorophyll donors will collectively be referred to as ChlZ.

In addition to ChlZ and Cyt \( b_{559} \) it was found that a Carotenoid species was involved as an intermediate electron carrier in the reduction of \( P_{680}^+ \) \(^{(84, 85, 88-91)}\). The current view is that the pathway is branched, where CarD2 always functions as the “primary” electron donor and is subsequently oxidized by either Cyt \( b_{559} \) or ChlZ. This electron transfer pathway is in this thesis collectively called the Car/ChlZ/Cyt \( b_{559} \)-pathway. The role of this pathway is despite a lot of research still unclear \(^{(86)}\). It is most likely related to photo protection and/or cyclic electron transfer around PS II, but no conclusive evidence have so far been presented \(^{(86, 87, 92)}\).

Before it was discovered that \( Y_Z \) could be oxidized at 5-10 K, it was believed that the Car/ChlZ/Cyt \( b_{559} \)-pathway was the only functional electron transfer pathway to \( P_{680}^+ \) at ultra-low temperatures. At higher temperatures however (77-295 K) reduction of \( P_{680}^+ \) was known to occur from both the \( Y_Z/\text{CaMn}_4 \)- and the Car/ChlZ/Cyt \( b_{559} \)-pathway, first studied by \(^{(93)}\). The electron transfer rate from CarD2 is slow (1-2 ms \(^{(6)}\)) compared to the electron transfer from its opponent \( Y_Z \) and as it seems only at temperatures where the \( Y_Z/\text{CaMn}_4 \)-pathway is inhibited the species in the Car/ChlZ/Cyt \( b_{559} \)-pathway are oxidized instead. In this thesis (paper II and IV), new light will be shed on the competition between the two pathways when using far-red light.

5.3 Water oxidation

The supply of electrons that reduce the formed cation \( P_{D1}^+ \) after charge separation in the reaction center ultimately derives from water oxidized at the \( \text{CaMn}_4 \)-cluster (Indicated in Figure 12 B). The \( \text{CaMn}_4 \)-cluster is deeply embedded inside the protein (Figure 12 B and C) and is supplied with water through channels connected to the lumenal surface \(^{(45)}\). The \( \text{CaMn}_4 \)-cluster consists of one calcium ion, four manganese ions and five oxygen atoms \(^{(52)}\). They are coordinated by amino acid residues from the D1 protein with one exception which is provided by the CP 47 protein \(^{(52)}\).
The CaMn\textsubscript{4}-cluster oscillates via stepwise oxidation by Y\textsubscript{Z} through four metastable oxidation states \cite{71} (denoted S-states, Figure 15) and has the unique ability to accumulate redox equivalents during the cycle. At the fourth electron oxidation water is “split” into four electrons, four protons and two molecules of oxygen. The oxidation of the two water molecules puts the CaMn\textsubscript{4}-cluster in its most reduced state (S\textsubscript{0}) and the cycle can be repeated. Every transition except the S\textsubscript{1}→S\textsubscript{2} transition involves in addition to an electron release also a proton release. The structure of the CaMn\textsubscript{4}-cluster in the individual S-state has not yet been solved, in spite of the high resolution structure of PS II by Chen and co-workers \cite{52} and detailed EXAFS measurements \cite{75, 94}. This will however be vital in order to explain the water oxidation that occurs in the S\textsubscript{3}→[S\textsubscript{4}]=S\textsubscript{0} transition. Siegbahn has presented a theoretical model on how the water oxidation occurs \cite{39, 56}. This model remains to be convincingly tested by various forms of spectroscopy.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure15.png}
\caption{The S-cycle, displaying the different S-states (S\textsubscript{0} to S\textsubscript{4}). The release of electrons and protons are also indicated. Adapted from \cite{14}.}
\end{figure}

\subsection*{5.4 Probing electron transfer in PS II}

Photosystem II chemistry involves a wide variety of light induced species that are possible to detect by different advanced EPR measurements. This section will describe all species presented in this thesis.
5.4.1 Detection of donor side electron transfer

Figure 16. Presentation of the EPR signals from the donor side investigated in this thesis, recorded at temperatures below 15 K. All signals except the Tyrosine_D radical is presented as light–dark differences spectrum. EPR conditions; \textit{S}_2 multiline EPR signal: microwave frequency 9.38 GHz, microwave power 10 mW, modulation amplitude 20 G and temperature 7 K. \textit{Split }S_1 \textit{EPR signal: temperature 5 K, microwave power 25 mW, modulation amplitude 10 G and microwave frequency, 9.28 GHz. Y}_D \textit{and Chl}_D \textit{radical EPR signals: temperature 15 K, microwave power 1.2 }\mu\text{W, modulation amplitude 3.5 G and microwave frequency, 9.28 GHz. Cyt }b_{559} \textit{EPR signals: temperature 15 K, microwave power 5 mW, modulation amplitude 15 G and microwave frequency, 9.28 GHz.}

5.4.1.1 The CaMn$_4$-cluster

All S-states except the transient S$_4$ state can be detected by cw-EPR spectroscopy at cryogenic temperatures. In this thesis, a signal from the S$_2$ state of the OEC has been used to identify electron transfer originating from the CaMn4-cluster. The S$_2$ state multiline signal originates from a S=1/2 ground state that gives rise to a characteristic 1800-2000 G wide so called multiline EPR signal, centered around g=2 (presented in Figure 16).

5.4.1.2 Y$_D$/Y$_Z$

The two redox active tyrosines Y$_Z$ and Y$_D$ can be detected by EPR at room temperature and at cryogenic temperatures. The dark stable Y$_D$ radical EPR
signal is always oxidized after illumination at physiological conditions and represents one radical/PS II center. It has a width of 19 G and a g-value of 2.0046 (Figure 16). The TyrD radical is often used as a probe during EPR measurements.

The split EPR signals derive from an interaction between $Y_Z^\cdot$ and the CaMn$_4$-cluster and can detected in all S-states around the $g=2.00$ region with various induction techniques, reviewed in (95, 96). In this thesis have the Split S$_1$ EPR signal been used as probe for charge separation. It is inducible by exposing S$_1$ synchronized PS II membranes to light while the PS II membranes is poised at cryogenic temperatures (< 15 K). It is detected as a peak at $g=2.05$ and has a half time of approximately 3 minutes.

The formation of the normally, by the CaMn$_4$-cluster very rapidly reduced at 295 K, $Y_Z^\cdot$ can be studied in tris-washed PS II enriched membranes and thylakoid membranes. The tris-washing procedure removes the CaMn$_4$-cluster, slows down the reduction of $Y_Z^\cdot$ significantly and makes it detectable by EPR at 295 K (97-99). The signal induction is detectable as an amplitude increase at the field position indicated with a star in the YD radical spectrum.

5.4.1.3 Cytochrome b$_{559}$ and Chl$Z$

Cyt b$_{559}$ and Chl$Z$ donates an electron to P$_{680}^+$ in conditions where electron donation from $Y_Z$ is inhibited at for example low temperatures (described in section 5.4.4.1 and 5.4.4.2). The oxidized Cytochrome b$_{559}$ EPR signal has three observable resonances, $g_z$, $g_y$ and $g_x$. This thesis however only reports on the quite easily observed and quantified $g_z$ signal. It appears in the $g=3$ region and has 3 peaks, denoted the high, intermediate and low potential peak. Upon illumination appears the high potential peak at 3.06, presented in Figure 16 as the light–dark difference spectrum. The Cyt b$_{559}$ is present in one/PS II center. The amount of oxidized Cyt b$_{559}$ in a specific experiment is quantified by comparing it to a fully oxidized Cyt b$_{559}$ achieved with low temperature illumination.

The Chl$Z$ radical EPR signal has a g-value of 2.0026 and has a width of 10-11 G (Figure 16), indicating that it is a monomeric chl species. The donation from Chl$Z$ is also dependent on the oxidized state of Cyt b$_{559}$ which is usually more efficiently oxidized by Car$_{D2}$. 
5.4.2 Detection of acceptor side electron transfer

![EPR signal plot]

*Figure 17.* Presentation of the Pheo⁻ radical EPR signal from the acceptor side recorded at room temperature. EPR conditions: temperature 15 K, microwave power 1.3 µW, modulation amplitude 3.5 G and microwave frequency, 9.28 GHz

5.4.2.1 Pheophytin⁻

Addition of dithionite to PS II enriched membranes makes it possible to accumulate and detect the primary acceptor pheophytin anion (Pheo⁻) under intense illumination at 295 K \(^{(100-102)}\). This is possible since forward electron transfer from pheophytin to the doubly reduced QA is blocked. The Pheophytin radical EPR signal has a g-value of 2.0035 and is 12-13 G wide (Figure 17).

5.4.2.2 QA⁻

Reduction of the primary quinone acceptor QA was monitored by a technique called variable fluorescence \(^{(103-106)}\). The fluorescence from PS II membranes was followed by very weak excitation light pulses before and after a saturating flash that induces charge separation in all available PS II centers. Formation of QA⁻ in a PS II reaction center prevents further photochemistry to occur in that specific PS center. The center is “closed” and fluorescence increased. This increase is denoted Fv. By varying the wavelength and power of the saturating flash provided with a laser, was QA⁻ formation followed in the far-red region in this thesis (Chapter 6, Figures 18 and 19).
6 Far-red light induced photochemistry in photosystem I and II

In now classical experiments, Emerson and co-workers found that the quantum yield of photosynthesis dropped significantly for excitation light above 700 nm (12, 107, 108). This drop is now referred to as the red-drop of photosynthesis or the Emerson effect. The Emerson effect combined with the vanishingly small absorption above 700 nm (see Figure 5 for PS II), lead to the general conclusion that light above 700 nm was not able to perform light driven photosynthesis. This chapter will show the contrary. The results in the papers included in this thesis will demonstrate that PS I and II can utilize light up to 840 and 810 nm respectively at 295 K. This is ~130 nm above their primary donor absorption maximum. In addition, this ability is not dependent on the thermal energy of the surrounding protein. An in common mechanism for this unexpected property will be suggested. To explain our results obtained in PS II we also suggest an alternative charge separation pathway, responsible for the reduction of P680 after primary charge separation following far-red illumination at 5 K.

6.1 Far-red light induced photochemistry in Photosystem II at 295 K

The scientific literature dealing with far-red light induced photochemistry in PS II is very limited, and in this respect my thesis is somewhat pioneering. Over the years a few reports have been published but without having any greater impact on the understanding of PS II chemistry (109-111). One of the reasons for the skepticism to the observed far-red effects has been that the preparations used were intact cells or leaves. This made it difficult to establish the origin of the absorbed light and sometimes also hard to prove that the activity derived from PS II. It was suggested that the excitation instead came from the uphill reversed energy spill-over from the far-red absorbing PS I.

The first publications on far-red light induced photochemistry in PS II made in preparations without PS I were presented by Krausz and co-workers in 2005 (62, 112). They demonstrated reduction of the acceptor side components in PS II by optical measurements. From this it was concluded that PS
II indeed was able to induce photochemistry even at 1.7 K up to at least 730 nm.

The following section will describe how we extended the action spectrum for intact PS II almost 70 nm into the red region at physiological conditions (295 K). In addition, this previously unknown ability of PS II is explained in an energetic model adopted for 295 K \(^{(113)}\). This work was published in \(^{(113)}\), paper I in this thesis.

6.1.1 Discovery of the far-red effect

This section presents the normalized action spectra of every species involved in the electron transfer chain in PS II after primary charge separation at \(P_{680}\) (Figure 18, left), and how the far-red limit was defined by monitoring the \(S_2\) multiline formation after flash illumination (Figure 18, right).

In papers I and III, photochemistry in PS II was induced at 295 K with wavelengths ranging from 730 to 810 nm. Reduction of the primary acceptor Pheophytin (Figure 18, pink triangles) was followed by EPR, whereas the secondary acceptor \(Q_A\) was detected and quantified by variable fluorescence (Figure 18 blue triangles). Oxidation of the primary donor \(Y_Z\) was followed both during illumination (Figure 18 red triangles, paper III) and as a kinetic EPR trace after one flash (Figure 18 green triangles, paper I).

Oxidation of the CaMn4-cluster was followed by the formation of the \(S_2\) state multiline EPR signal after one flash (Figure 18, black triangles). In the figure, the wavelength dependencies of the electron transfer co-factors are compared to \(P_{680}^{*}\) fluorescence excitation spectra measured at 684 nm and excited between 725-780 nm.

![Figure 18](image-url)

**Figure 18 Left:** The action spectra of PS II induced photochemistry at 295 K. **Right:** careful definition of the far-red limit by flash illumination. Figure adapted from \(^{(113)}\)

Surprisingly, the action spectra showed that PS II was able to drive photochemistry up to 790 nm. The formation of the charge separation products displayed strong wavelength dependence and started to decrease immediate-
ly after 730 nm. The major factor for this decline is probably the decreased absorption of PS II in this region.

To further define the far-red limit of the photochemistry in PS II, PS II enriched membranes were exposed to 100 flashes at 295 K at 790 and 810 nm. After freezing the sample, the stable S2 multiline EPR signal was monitored as a probe for charge separation. From this it was clear that flashing at 790 nm rendered a significant signal while no S2 multiline EPR signal was detected after flash illumination at 810 nm (Figure 18, right). It was therefore concluded that the far-red limit was 810 nm. Thus, the action spectrum for PS II induced photochemistry was by these measurements extended approximately 70 nm further out into the red region of the spectrum than previously reported in PS II preparations (62).

By measuring the uphill energy transfer to P680 via the fluorescence emission at 684 nm we found that the it decreased more rapidly with excitation wavelength than the formation of the charge separation products (QA−, Pheo−, YZ• and S2 multiline). This suggests that the excitation of P680 via uphill energy transfer is not sufficient to explain the wavelength dependencies of the far-red light induced charge separation. This is interesting, and the challenging question about thermally populated states as an alternative explanation will be addressed further in later sections.

6.1.2 A first attempt to localize the absorbing species

The results described in detail in this section had a fundamental impact on our understanding of the underlying mechanism of the far-red light induced charge separation in PS II (113). This lead us to develop a new energetic model to describe the photochemistry at longer wavelengths (see Figure 20). However, first it was necessary to more precisely localize the absorbing species.

The determination of the limit of photochemistry was made in PS II membranes with intact reaction centers with all outer antenna components present (Figure 18, right). To narrow the possibilities of where the absorbing species were localized we tried to induce charge separation in PS II core particles, where the outer antenna components has been washed away. Figure 19 A presents a comparison of the S2 multiline EPR signal formed after giving one flash at 750 nm to PS II cores (top spectrum) and PS II membranes (bottom spectrum) synchronized in the S1 state. It was clear from the PS II core preparation that it was possible to induce charge separation in 10-15 % of the PS II centers (113). This was a very important result and showed that the outer antennas were not necessary to induce far-red light photochemistry in PS II. Instead the phenomena must be connected to either an absorption by an inner antenna chlorophyll followed by energy transfer to the reaction center, or to
the properties of the reaction center pigment assembly itself, as partly suggested in (62).

Figure 19. A: S2 multiline formation after 1 flash at 750 nm to PS II cores (top) and PS II enriched membranes (bottom) synchronized in the S1 state. B: Power dependence of Fv induction at different far-red wavelengths. Figure adapted from (113)

The experiment displayed in figure 19 A clearly demonstrated that the absorption of far-red light was not by necessity coupled to the outer antenna subunits. However, it was still possible that some kind of two-photon process in the inner antenna was responsible for the absorption. This process would make it possible for any chlorophyll to absorb far-red light and hence would the absorption not be coupled to any specific chlorophyll or its location. A two-photon process would in addition be able to create an excited state of the absorbing chlorophyll similar to the absorption band at 680 nm. This would define the far-red light induced photochemistry as an entirely antenna induced phenomena. If a two-photon process were to be involved the power dependence of the formed charge separation species would be cubic. To address a possible two-photon process, we followed the power dependence of Fv (i.e. QA reduction) as a function of excitation wavelength and light power (Figure 19 B). Two important observations were made; first, the initial slope for each trace is clearly linear. Second, wavelengths above 730 nm cannot induce QA to the same extent as 532 and 730 nm despite of the higher flash powers. Clearly, the formation trend is not cubic. In addition, as full induction can’t be reached with one flash above 730 nm no matter how many photons you apply, these wavelengths must have a different intrinsic yield.

Taken together, it is quite clear that only one photon is responsible for the absorption process and thus a two-photon process can be ruled out.
6.1.3 Energetic model for far-red light induced charge separation at 295 K

Our experiments performed at 295 K indicated that the far-red light induced mechanism was associated with the PS II reaction center without any significant involvement of uphill energy transfer. Further the far-red effect remained after exclusion of the outer antenna (figure 19 A) and the power dependence of the reduction of the secondary acceptor \( Q_A \) (figure 19 B) displayed different intrinsic yields excluding two-photon effects. This lead us to develop a new energetic model to explain how charge separation can occur after induction with far-red light.

![Figure 20](image)

*Figure 20.* The model to energetically describe far-red light induced charge separation.

This model is summarized in Figure 20. \( X \) represents a previously unknown state that is able to absorb far-red light to become an excited species \( X^* \). \( X^* \) can then either by itself drive reduction of Pheophytin leading to the state \( X^+ \text{Phe}^- \) or reduce pheophytin by populating \( P_{680}^+ \) by charge equilibrium. In both cases this leads to the final charge pair \( Y_Z^+Q_A^- \) which is the same state as obtained with \( P_{680} \) excitation and charge separation.

This model was based on the formation of charge separation products obtained at 295 K. At this temperature the formed primary cation (\( X^* \) or \( P_{680}^+ \)) is always reduced by \( Y_Z \). However, at cryogenic temperature this is not the case. This property of PS II is interesting and gave us information to further develop our model, which will be discussed in section 6.4.
6.2 Far-red light induced photochemistry in Photosystem I at 295 K

The discovery of the extended action spectrum for PS II in the far-red region seen in paper I, raised the question if PS I had the same analogous ability. It was known that PS I was able to induce photochemistry further out into the red region of the spectrum than PS II. The limit has been reported to 760 nm \(^{(114)}\) and light at 720 nm is used to “exclusively” oxidize PS I when PS II is present in the preparation \(^{(115)}\). This ability is often attributed to far-red light absorbing red chlorophylls in PS I, coupled to an uphill energy transfer event. \(^{(32, 116)}\).

The results in paper III show that PS I, similar to PS II, can perform photochemistry more than 130 nm above its primary donor absorption maximum. This ability also seems to be preserved between higher plants and cyanobacteria.

6.2.1 Defining the far-red limit of PS I at 295 K

Figure 21 shows the maximum inducible EPR signal from P\(_{700}\) (P\(_{700}^+\)) as a function of excitation wavelength, measured in thylakoid membranes from spinach (black dots) and cyanobacteria (red dots). Clearly, it was possible to induce photochemical oxidation of P\(_{700}\) up to almost 840 nm. This result extended the action spectrum 80 nm further out into the red region of the spectrum than previously reported \(^{(114)}\). Interestingly, the action spectra for spinach and cyanobacteria are close to identical. Since cyanobacteria lack the outer antenna sub-units that possess most of the red chlorophylls, these can hardly play an important role in this process. Further, it can also be noted that as much as 50 % of the PS I centers are able to perform photochemistry using 800 nm light in both species.
6.3 Summary of the far-red light induced photochemistry at 295 K

By comparing the normalized action spectra for PS I and PS II in figure 22, a few conclusions can be made. The far-red limit for PS II was set to 810 nm after flash illumination, while the far-red limit for PS I is clearly even more red-shifted and ends around 840 nm. Thus, the far-red limit for PS I and PS II is 135 and 130 nm above their primary donor absorption maximum respectively. This is remarkably similar. The decrease of formed charge separation products in PS II starts immediately after 730 nm while for PS I the induction is over 80% up to 780 nm. The PS II related species are not easily accumulated over time (due to fast recombination at physiological temperatures). The induction was therefore almost exclusively done with only one flash.

Normally the incident visible sunlight (< 680 nm) is absorbed by a chlorophyll in the antennae and transferred by excitation energy transfer (eet) to the reaction center. However, the antennae components of PS I and PS II are very different (31, 117). Given the similar behavior of the photosystems in the far-red region it seems unlikely that the different antennae would have an almost identical effect on the charge separation. Instead, the similar behavior suggests that the far-red absorption is a property ascribed to the reaction centers. This seems reasonable since the reaction centers pigments are not too different in PS I and PS II. The pigment assemblies in both reaction centers are densely packed and show excitonic cou-
pling combined with the presence of charge transfer states \(^{20, 118, 119}\). Coupled pigments hold the ability to absorb far-red light \(^{62, 63}\). It is therefore not unlikely that a state with charge transfer character is excited and by itself drives the photochemistry i.e. reduction of the primary acceptor. Given the very similar outer edge of activity for the photosystems it seems likely that the same process is involved for both photosystems.

*Figure 22.* The far-red limit of PS I and PS II normalized at 730 nm

In all the experiments presented so far the induction of photochemistry was studied at 295 K. This is not the optimal experimental conditions in order to exclude thermal energy as the cause of the far-red effect. Thermal energy (also denoted \(kT\) or phonon coupling) of the surroundings can at 295 K potentially populate a state of higher energy than the applied photon energy. This means that charge separation can potentially occur at \(P_{680}\) and \(P_{700}\) with far-red light in the same way as after absorption of a higher energy photon in the antenna. In fact, uphill energy transfer and thermally populated sublevels of \(P_{700}\) or \(P_{680}\) are two processes that do occur at 295 K (Mokvist, Styring and Mamedov, unpublished results and \(^{29, 113, 120}\)). However, calculations assuming a Boltzmann distribution reveal that this processes can only populate \(<1\%\) of the reaction centers (paper I). The concern about the thermal effects in our system was the major remark to the results in paper I. Therefore the project continued by trying to induce charge separation in PS II at cryogenic temperatures using far-red light. At these temperatures the thermal energy is strongly inhibited. The outcome of this is presented in following sections and gave us a far better understanding of the far-red light induced charge separation mechanism.
6.4 Photochemistry at cryogenic temperatures with far-red light

At cryogenic temperatures (in our case below 15 K) only minor atomic movements will occur in the protein and all molecular properties in the reaction centre that have been frozen in will remain until the sample is thawed. In addition all molecules are in their vibrational ground state. This is also valid for the photosynthetic pigments. Basically no thermally activated sublevels can exist due to the extremely small thermal energy of the system. The distances between the antenna chlorophylls in PS II do not allow any orbital overlap that could lead to a CT-state (52, 120, 121). These chlorophylls are therefore, as already discussed in previous sections, depending on thermal energy to absorb light in the far-red region. In PS II this is also supported by the fact that the longest wavelength absorption band at 5 K of the outer antenna LHC II is at 678 nm (122) and the inner antenna CP 47 at 703 nm (62, 123).

The antenna composition of PS I is different. The outer antenna of PS I in plants LHC I, possesses tightly coupled chlorophylls denoted red chlorophylls. The red chlorophylls have an orbital overlap that renders a charge transfer character to the excited state and can therefore absorb light in the far-red region also at cryogenic temperatures (124). However, recall from figure 21 that photochemistry from far-red light was also detected in a cyanobacterium that lack LHC I. This shows that the far-red light photochemistry in PS I functions independently of the red chlorophylls present in the LHC I of plants. Furthermore, at cryogenic temperatures the red chlorophylls function as energetic traps. They are therefore easily detected with fluorescence emission measurements at 77 K, as a band at 735 nm (120). Such a fluorescence emission band does definitely not exist in PS II and this thus excludes the possibility of having red chlorophylls present in the outer or inner antennas (120, 125).

Given the above mentioned remarks about the known pigment behaviour at cryogenic temperatures we were prompted to investigate whether the far-red effects observed in PS I and PS II could be detected at these lower temperatures as well.

This was done in papers II and III and the data is presented in figure 23.
In this Figure, PS I (dots) and PS II (triangles) were exposed to flash illumination at 15 and 5 K respectively. In PS I, reduction of the [4Fe-4S]-clusters on the acceptor side was monitored by EPR and the plotted data represents the total amount of centres that has performed photochemistry. In PS II, the oxidation of all potential donors; YZ, ChlZ and Cyt $b_{559}$ was monitored independently from each other and then summarized to represent the total amount of centres that has performed photochemistry. YZ oxidation was monitored via the Split S1 EPR signal which under these conditions is a clear sign of YZ oxidation. Oxidation of ChlZ and Cyt $b_{559}$ was also monitored by EPR and both have well-established signatures in the recorded EPR spectra.

The outcome was clear; charge separation in the far-red region of the spectrum does occur in both PS I and II and to a larger extent than we imagined. The amount of observed photochemistry performing centres and the trend of the action spectra between the photosystems did however differ.

In PS I ~75 % of the centres performed photochemistry after application of flashes at 730 nm. The decrease started immediately after 730 nm, but photochemistry was detected up to at least 830 nm. This was very similar to the action spectra in Figure 21 obtained at 295 K with spinach and cyanobacteria. This means that PS I was able to drive far-red light induced photochemistry independent of temperature.

In PS II, 42 % of the centres performed charge separation at 730 nm and the amount was more or less equal at 740 nm (37 %). At 750 nm the yield had dropped significantly to 11 % of the centres and it seems unlikely that any charge separation occurs after 760-765 nm. This means that the outer edge for photochemistry at 5 K is approximately 50 nm below the outer edge obtained at 295 K.

These results show that the far-red photochemistry performing abilities are preserved for both PS I and II at cryogenic temperatures. Since no uphill energy transfer can occur, and thermally populated sublevels are annihilated...
at these temperatures, it leads to the conclusion that the antenna chlorophylls are not involved in this process. As a consequence it is safe to claim that the far-red light is absorbed by the coupled pigments in the reaction centre core of respective photosystem. It also shows that while the yield is almost equal with 532 nm light illumination, PS I has a much higher yield with 730 nm illumination than PS II. The reason for this was to be exposed upon careful analysis of the oxidation of the different donors in PS II.

6.4.1 The biased P$_{680}^+$ reduction in PS II

The reduction of the oxidized primary donor P$_{680}^+$ in PS II occurs through two different electron transfer pathways at 5 K. This is due to a frozen in heterogeneity that occurs mainly around Y$_Z$. In one protein configuration Y$_Z$ is able to efficiently reduce P$_{680}^+$ while it is unable to do so in another. This probably reflects the position of the phenolic proton coupled to D1-His-190 (Johannes ref). In addition to the Y$_Z$/CaMn$_4$-pathway, the Car/Chl$_Z$/Cyt $b_{559}$-pathway can also reduce P$_{680}^+$. The two pathways are described in detail in sections 5.2.2.1. and 5.2.2.2. Illumination of PS II with visible light (<680 nm) at 5 K renders a partition ratio$^1$ of approximately 1$^{79, 80, 132}$ between the two pathways.

In Figures 24 and 25, oxidation of electron donors in PS II enriched membranes synchronized in the S$_1$ state were quantified after flash illumination at 5 and 77 K respectively (paper II and IV). Figure 24 A shows the donor oxidation from the Y$_Z$/CaMn$_4$-pathway quantified via the Split S$_1$ EPR signal (grey). This is to our knowledge the first time the Split S$_1$ EPR signal was induced with light of wavelengths up to 750 nm. A further investigation was therefore made to confirm these results (see paper II). The amount of Split S$_1$ EPR Signal was similar between 532 nm (40 %) and 740 nm (27 %). Thereafter the yield dropped significantly at 750 nm (8 %).

Oxidation from the Car/Chl$_Z$/Cyt $b_{559}$-pathway was monitored by the formation of the Chl$_Z$ radical (dark grey) and oxidation of Cyt $b_{559}$ (white). Together these signals amounted to 55 % of the centers after the 532 nm flash illumination. In contrast, the 730 nm light oxidized the Car/Chl$_Z$/Cyt $b_{559}$-pathway in only 15 % of PS II, the 740 nm light in 10 % and the 750 nm light in < 3 %.

Figure 24 B summarizes the donor oxidations as % of charge separation to get a clearer view of the partition ratios, which were 0.8 at 532 nm, 1.8 at 730 nm, 2.7 at 740 nm and >2.7 at 750 nm. The Y$_Z$/CaMn$_4$-pathway clearly dominated over the Car/Chl$_Z$/Cyt $b_{559}$-pathway after far-red light illumination at 5 K and the partition ratios increased with increasing wavelengths. Obviously, P$_{680}^+$ reduction after far-red light illumination at 5 K almost ex-

---

$^1$ We define in this work a partition ratio between the electron donor pathways in PS II. The partition ratio equals (yield of Y$_Z$/CaMn$_4$ oxidation):(yield of Cyt$b_{559}$/Chl$_Z$/Cair$_{192}$ oxidation).
clusively occurred from the YZ/CaMn4-pathway. The results are also summarized in table 1.

![Figure 24. Oxidation of electron donors by P680+ induced with flash illumination at 5 K. A: The staples represent formation of the Split S1 signal (grey), oxidation of ChlZ (dark grey) and Cyt b559 (white) in percent of PS II. B: Oxidation of the different electron donors as a fraction of the total stable charge separation achieved at the specific excitation wavelength. YZ oxidation (grey staples), ChlZ oxidation (white staples) and Cyt b559 oxidation (dark grey staples). The partition ratio was indicated above each wavelength. (Figure adapted from paper II).](image)

At 77 K, the frozen-in heterogeneity is different compared to at 5 K, movements of the protein and single atoms are possible to some extent. White light illumination causes reduction of P680+ almost entirely through the Car/ChlZ/Cyt b559-pathway at 77 K (80, 131). The reasons for this is not entirely clear, but is probably caused by a frozen-in heterogeneity in combination with electron transfer kinetics that favors the Car/ChlZ/Cyt b559-pathway (92).

The Split S1 EPR signal is not inducible at 77 K; YZ oxidation was therefore instead followed indirectly via the formation of the S2 multiline EPR signal.

In paper IV PS II membranes synchronized in the S1 state were exposed to flash illumination at 77 K. Below 200 K all S-state transitions except the S1→S2 transition are inhibited by the temperature (133). During flash illumination will the PS II membranes will therefore accumulate in the S2 state, which is detectable via the S2 multiline EPR signal.

Figure 25 A displays the donor oxidations after flash illumination for 10 minutes at 77 K. The S2 multiline formation (black) represents oxidation of the YZ/CaMn4-pathway whereas formation of the ChlZ radical (dark grey) and Cyt b559 (white) oxidation represents oxidation from the Car/ChlZ/Cyt b559-pathway. The S2 multiline EPR signal was present in ~30 % of the PS II centers irrespective of excitation wavelength. This means that P680+ was reduced to the same extent by the YZ/CaMn4-pathway independent of excitation wavelength.

Oxidation of the Car/ChlZ/Cyt b559-pathway amounted to ~80 % of the PS II centers after flash illumination at 532 nm. At 730 nm, the Car/ChlZ/Cyt
$b_{559}$-pathway was oxidized in ~60% of the centers, somewhat less than after 532 nm illumination but clearly a lot more than after illumination at 730 nm at 5 K.

Figure 25 B shows the donor oxidations as % of charge separation and indicates the partition ratios. The partition ratios were also here increasing with increasing wavelength from 0.3 at 532 nm to 0.6 for 730 nm and 1.6 for 750 nm. The data is also summarized in table 1.

Seemingly, the reduction of $P_{680}^+$ was dominated by the Car/Chl$_z$/Cyt $b_{559}$-pathway after both 532 and 730 nm light induction. At 750 nm light, the reduction was preferentially done via the Y$_z$/CaMn$_4$-pathway although the partition ratio was significantly lower compared to the situation at 5 K.

The reduction pattern is clearly different between 5 K and 77 K and will have impact on our understanding of this mechanism.

![Figure 25: Oxidation of electron donors to $P_{680}^+$ by extensive flash illumination at 77 K with different wavelengths A: Formation of the $S_2$-multiline EPR signal (black), oxidation of Cyt$b_{559}$ (white) and Chl$_z$ radical (dark grey). B: Oxidation of the various donors as fraction of total charge separation achieved with a specific wavelength. The partition ratio between the Y$_z$/CaMn$_4$ and the Cyt$b_{559}$/Chl$_z$/Car$_D2$ pathways is also shown, above respective wavelength. (Figure adapted from paper IV) ]

6.4.2 Temperature dependence of the far-red photochemistry in PS II

As already mentioned in the previous section, it is possible to indirectly detect Y$_z$ oxidation above 77 K via the formation of the $S_2$ multiline EPR signal. Formation of the $S_2$ multiline EPR signal show that the Y$_z$/CaMn$_4$-pathway has supplied $P_{680}^+$ with an electron. Likewise, formation of the Chl$_z$ $^\ast$ radical or oxidation of Cyt $b_{559}$ indicates that the Car/Chl$_z$/Cyt $b_{559}$-pathway has supplied $P_{680}^+$ with an electron. Induction using a single flash forms not enough amounts of oxidized Cyt $b_{559}$ to allow quantification in my experiments, similar as found earlier (74). Thus, in paper IV the
temperature dependence of $P_{680}^+$ reduction from the two pathways was followed by the $S_2$ multiline EPR signal and Chl$Z^\bullet$ signal formation. After application of one laser flash at 532, 730 or 750 nm to PS II membranes poised at various temperatures (flash temperature) the sample was rapidly frozen to 77 K. The EPR spectra were then recorded within 24 hours. Before the flash the PS II membranes were synchronized in the $S_1$ state.\(^{(134)}\)

The flash temperatures ranged from 77 to 295 K and the formation of the $S_2$ multiline EPR signal is summarized in Figure 26 A. It was clear that the formation was both temperature and wavelength dependent. Photochemistry generated by a 532 nm flash (dots) rendered a maximal induction of the $S_2$ multiline EPR signal at 273 K (100 %). A decrease in the yield was observed below 250 K and complete loss of amplitude occurred below 77 K.

The far-red flashes displayed very different trends in the obtained $S_2$ multiline yield. After one flash at 730 nm (open triangles) the maximal $S_2$ multiline EPR signal induction was observed at 295 K (83 %). The decrease in yield started immediately below this temperature. At 200 K the amplitude of the $S_2$ multiline EPR signal was significantly reduced (20 % of maximum) compared to a 532 nm flash at the same flash temperature (61 % of maximum). Similar to the 532 nm flash no $S_2$ multiline EPR signal was observed at 77 K. Also the 750 nm flash (squares) displayed a maximal yield of the $S_2$ multiline EPR signal at 295 K (27 %). However, decrease of the signal amplitude was here initiated at 250 K and complete inhibition occurred below 160 K.

From the data in Figure 26 A the half inhibition temperature ($T_{0.5}$) was determined. This parameter was defined in previous publications \(^{(74)}\) as the temperature where 50 % of the maximally inducible $S_2$ multiline EPR signal was achieved. The $T_{0.5}$ values for the different excitation wavelengths were determined to $185 \pm 5$ K at 532 nm, $255 \pm 5$ K at 730 nm and $240 \pm 5$ K at 750 nm. Due to recombination kinetics at 295 K $T_{0.5}$ for the 730 nm was flash carefully analyzed and adjusted to this known fact (paper IV and \(^{(134)}\)).

After a close examination of other radical species formed synchronously (paper IV) it was concluded that no Chl$Z^\bullet$ radical was formed after application of a 730 or 750 nm flash independently of flash temperature (dots and open triangles, Figure 24 B). In contrast, after application of a 532 nm flash, a 10-11 G wide EPR radical could be deconvoluted and assigned to Chl$Z$ (paper IV), at flash temperatures below 250 K \(^{(131)}\). By comparing the temperature dependence of the $S_2$ multiline signal and the signal from Chl$Z^\bullet$ (inset, Figure 24 B) after the 532 nm flash, it is clear that the $P_{680}^+$ reduction from the two pathways are complementary.
Figure 26. Temperature dependence of the S₂ state formation measured as the S₂-multiline EPR signal formation and of ChlZ* formation after 1 flash at 750 nm (squares), 730 nm (triangles) or 532 nm (dots). A: The fraction of PS II centres in the S₂ state after 1 flash

The main results obtained in PS II and described in the preceding sections are here summarized in Table 1.

Table 1. Partition ratios\(^a\) and \(T_{0.5}\)\(^b\) after visible and far-red light induced photochemistry in PS II.

<table>
<thead>
<tr>
<th>Excitation wavelength (nm)</th>
<th>Partition ratio</th>
<th>(T_{0.5})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 K</td>
<td>77 K</td>
</tr>
<tr>
<td>532</td>
<td>0.8</td>
<td>0.3</td>
</tr>
<tr>
<td>730</td>
<td>1.8</td>
<td>0.6</td>
</tr>
<tr>
<td>740</td>
<td>2.7</td>
<td></td>
</tr>
<tr>
<td>750</td>
<td>&gt; 2.7</td>
<td>1.6</td>
</tr>
</tbody>
</table>

\(^a\) The partition ratio equals (yield of Y\(_Z\)/CaMn\(_4\) oxidation):(yield of Cyt\(_b\)/Chl\(_Z\)/Car\(_D\)_2 oxidation).

\(^b\) The temperature where 50 % of the S₂ multilne signal is formed compared to the maximum achieved signal at respective induction wavelength\(^{74}\).
7 The nature and consequences of far-red light absorption

This chapter will present some interpretations of the results obtained and discussed in the preceding chapters. I will firstly suggest a location for the far-red absorbing pigments in PS I and PS II and second display the electron transfer that will follow in PS II upon such an event.

7.1 Identity of the far-red absorbing pigments

![Figure 27. A comparison of the co-factors in the primary donors P_700 (left) and P_680 (right) with its respective accessory pigments. Adapted from PDB ID 1JB0 and 3ARC.](image)

It has from the earlier chapters been concluded that the far-red light absorbing abilities in both PS I and PS II are connected to respective reaction center. This observation is very interesting since the primary donors P_700 and P_680 are in many ways analogous. Structurally they consists of densely packed chlorin pigments and in addition they both have excited states with charge transfer character \(^{(20, 22)}\). A weak charge transfer band could explain how light of far-red wavelengths can be absorbed and has already been observed and studied in the red chls of PS I \(^{(135, 136)}\).
As can be seen in Figure 27 the primary donors of the photosystem are far from identical. $P_{700}$ (Figure 27 left) is a chl a-chl a’ heterodimer ($P_A$ and $P_B$) (31), while $P_{680}$ is often defined as all of the displayed chl pigments Figure 27 right. The $P_{700}$ entity has the strongest coupling in the reaction center of 138 cm$^{-1}$ (41) and is often referred to as “the special pair”. The distance between the chlorophylls is probably not the most important property in order to form a charge transfer state even though it is necessary to be close enough to interact. In the bacterial reaction center is the special pair tilted and thus allow strong overlap of ring a (indicated with an arrow in Figure 27). This overlap is probably of major importance in order to explain the red shifted absorbance maximum seen for the bacterial reaction centers (42). However, in PS I is the $P_{700}$ dimer the strongest candidate for forming a charge transfer band, which needs orbital overlap to be formed. Also the temperature independent ability to perform photochemistry with far-red light indicates a similar primary cation location as with visible light.

The situation is more complex in $P_{680}$. Here a weak coupling exists between all pigments, and the term special pair for the $P_{D1}$-$P_{D2}$ pigments is therefore not appropriate (57). However it has been suggested that charge transfer bands appears in interactions between $P_{D1}$ and $P_{D2}$ and Chl $D_1$ and PheD1 (20). Our results strongly suggest that the far-red light absorbing pigment/s in PS II are an interaction between the latter and our proposal will be discussed in the following sections.

7.1.1 Indications of the existence of an alternative charge separation pathway in PS II

Excitation with visible light creates a cation that is presently considered to resides on $P_{D1}$ after the primary charge separation (20, 42, 65-68). The first formed stable charge pair is therefore $P_{D1}^+Q_A^-$. The reduction of $P_{D1}^+$ occurs at 5 K either from the $Y_Z$/CaMn$_4$-pathway or the Car/Chl$Z$/Cyt $b_{559}$-pathway, depending on a heterogeneity around $Y_Z$ (section 5.2.2.1). It is safe to say that reduction of $P_{D1}$ gives rise to a donor partition ratio of ~1 in intact PS II centers using visible light (79, 80). In papers II and IV an alternative reduction pathway to $P_{680}^+$ was suggested in order to explain the observed oxidation bias towards the $Y_Z$/CaMn$_4$-pathway after excitation with far-red light. In our experimental protocol all samples are treated in the same way (paper II and IV) before applying the flash/es. The heterogeneity around $Y_Z$ will therefore be identical independent of excitation wavelength. Thus, if $P_{D1}^+Q_A^-$ was formed after far-red light excitation the partition ratios would be identical. This is clearly not the case (Figure 24 B and 25 B).

Instead we suggest that far-red light excites a different component at 5 K denoted $P_X$ (denoted X in (113) and section 6.1). $P_X$ is by itself capable of reducing the primary acceptor Pheophytin and this leads to the stable charge pair $P_X^+Q_A^-$. $P_X^+$ have to be spatially and/or energetically different from $P_{D1}^+$.
hence the skewed partition ratios. It is obvious that $P_X^+$ is able to oxidize $Y_Z$ with its relatively high oxidation potential of 1.2 V \(^{(137)}\). If $P_X$ is located in close vicinity of Car$_{D2}$ it is reasonable to assume that it would have been able to oxidize this species as well, since it has a much lower oxidation potential (~785 mV \textit{in vitro} \(^{(138)}\)). Therefore we suggested that $P_X^+$ resides on another chlorophyll in the reaction centre assembly further away from Car$_{D2}$. The most likely candidate is Chl$_{D1}$. Chl$_{D1}$ is located farthest away from Car$_{D2}$ of the chlorophylls in P$_{680}$, 28.8 Å (edge to edge), but only 11.3 Å away from $Y_Z$. The distance is comparable to the distance from P$_{D1}$, 9.4 Å. Thus this distance should allow direct reduction from $Y_Z$ \(^{(3)}\).

In the assignment of $P_X$ to Chl$_{D1}$ an important issue to bring up is the potential redox equilibria or hole migration that occurs between the reaction center chlorophylls \(^{(42)}\). We suggest that Chl$_{D1}$ is directly reduced by $Y_Z$ and in order to do this the formed cation have to reside on Chl$_{D1}$ long enough to allow its reduction. This would indicate that, if existent, the redox equilibrium between Chl$_{D1}$ and P$_{D1}$ (Chl$_{D1}^+P_{D1}=Chl_{D1}P_{D1}^-$) is strongly inhibited or biased to Chl$_{D1}$ at 5 K.

In paper IV the $T_{0.5}$ parameter was determined for 750, 730 and 532 nm. This had previously been determined for visible light (532 nm) to 135 K by means of EPR \(^{(74)}\) and FTIR \(^{(139)}\). It was there concluded that the S$_2$ multiline EPR signal inhibition was related to the CaMn$_4$-cluster. At low temperatures the cluster is frozen-in and is therefore unable to perform the small structural rearrangements needed for the S$_1$$\rightarrow$$S_2$ transition. Our $T_{0.5}$ value for the 532 nm flash was slightly higher than previously observed \(^{(74)}\), 185 K. The reasons for this is discussed in detail in paper IV and depends on the nature of the S$_2$ state combined with small experimental procedure differences between the measurements. The obtained results for the 730 and 750 nm flash were interesting. The far-red flashes displayed significantly higher $T_{0.5}$ values, 255 K and 240 K for 730 and 750 nm respectively. The applied excitation flash only effects the pigment chlorophylls, not the redox active cofactors. The higher $T_{0.5}$ cannot be related to the CaMn$_4$-cluster since the S$_1$$\rightarrow$$S_2$ transition functions very well after a 532 nm flash (Figure 26).

Instead we propose that the higher $T_{0.5}$ reveals different temperature dependence in the equilibrium between the oxidized donor (Chl$_{D1}^+$ or P$_{D1}^+$) and $Y_Z$.

Another important observation is that it clearly seem like far-red light was not able to oxidize the Car/ChlZ/Cyt b$_{559}$-pathway to any extent with one flash. This is identical to the results obtained after flash illumination at 5 K and very similar at 77 K discussed below.
7.1.2 Cation steered donor oxidations in PS II

The observed partition ratios at 5 and 77 K combined with the $T_{0.5}$ differences obtained using visible and far-red light in Figures 24, 25 and 26 (papers II and IV) allowed us to suggest a model to explain our results. This was presented in paper IV. In this model the different partition ratios between the two pathways with visible and far-red light were proposed to reflect different sets of electron transfer equilibria between the secondary donors and the primary donors, dependent on where the latter was formed (on Chl$_{D1}$ or P$_{D1}$).

Figure 28 shows the essential features in this model and compares the electron transfer equilibria that occurs after visible and far-red light excitation at different temperatures. Figure 28 A shows the situation after visible light (532 nm) induced charge separation. These photons are absorbed in the antenna and then the excitation is transferred via excitation energy transfer to the reaction centre where photochemistry occur (section 5.2.1). Giving rise to the oxidized primary donor located to P$_{D1}$.

After visible light induced charge separation (here 532 nm) the stable cation is located at P$_{D1}$ (see section 5.2.1) which is 9.4 Å away from the redox active Y$_Z$. This close distance allows that P$_{D1}^+$ is fast and efficiently reduced by Y$_Z$ in the ns time regime, this electron transfer is illustrated in equilibrium (eq.) I that is shifted far towards oxidation of Y$_Z$. The $T_{0.5}$ value obtained in paper IV with visible light (532 nm) is reflected in equilibrium II. This electron transfer equilibrium is S-state dependent (74) and thus depends on the oxidation state of the CaMn$_4$-cluster. When Y$_Z$ or the CaMn$_4$-cluster is unable to donate an electron to P$_{D1}^+$ this will be delivered from the Car/Chl$Z$/Cyt$559$-pathway instead (see section 5.2.2.1 and 5.2.2.2), this is indicated in the equilibrium III.

From the preceding discussion in this chapter it is clear that equilibrium II is blocked somewhere below 77 K, equilibria I and III on the other hand are clearly functional down to at least 5 K. In paper II and IV the results points towards a strong temperature dependence for the equilibrium between Chl$_{D1}$P$_{D1}$ ⇌ Chl$_{D1}$P$_{D1}^+$, here indicated as eq. IV. This equilibrium appears to be almost completely inhibited at 5 K and very slow at 77 K.

Our results obtained with far-red light are explained in two models in Figures 28 B (from paper IV) and C. Figure 28 B displays the situation after far-red light (750 nm) induced charge separation. Here is the absorbing species is located inside P$_{680}$ and no antenna light harvesting is involved. Therefore, the absorption is very low.

Our results obtained with far-red light are explained in Figures 28 B (from paper IV) and 28 C. Here, the absorbing species is located inside P$_{680}$ and no antenna light harvesting is involved. Therefore the absorption is very low. Figure 28 B displays the new equilibria proposed to occur after formation of P$_X^+$ induced with 750 nm light. In paper II we argue that P$_X^+$ is...
located on Chl\textsubscript{D1} after illumination with 750, 740 and 730 nm light at 5 K. The data in paper IV further support this hypothesis (Figure 26). An important observation is that the observed T\textsubscript{0.5} the S\textsubscript{1}→S\textsubscript{2} transition after far-red light induced charge separation is not dependent on eq. II, since electron donation from the CaMn\textsubscript{4}-cluster to Y\textsubscript{Z} clearly is operational at lower temperatures after visible light induced photochemistry. Instead we propose that the dominating equilibrium after 750 nm light induced charge separation is the new equilibrium V between Chl\textsubscript{D1}−Y\textsubscript{Z}=Chl\textsubscript{D1}Y\textsubscript{Z}\textsuperscript{*} which control the fraction of formed Y\textsubscript{Z}\textsuperscript{*} and also its lifetime (indirectly via recombination with Chl\textsubscript{D1} and the acceptor side electron). Chl\textsubscript{D1} is probably less oxidizing than P\textsubscript{D1} \textsuperscript{(42)}, which we hypothesize is reflected in the different temperature dependence and equilibrium position of V as compared to I (Figure 28 A). Thus, we suggest that the observed high T\textsubscript{0.5} value after 750 nm light depends on eq V.

\textit{Figure 28. A:} The electron transfer equilibria after visible light induced photochemistry. B: The previously unknown set of equilibria obtained after 750 nm induced photochemistry. C: A model which explain the observed partition ratios at 77 K in terms of the formed excited state.

On the basis of the model described in paper IV and displayed in Figure 28 B, a further extended model was developed, to explain the partition ratios at 77 K (Figure 25). This is shown in Figure 28 C. Here both equilibria I (Figure 28 A) and V (Figure 28 B) are included, representing the equilibria Chl\textsubscript{D1}−Y\textsubscript{Z}=Chl\textsubscript{D1}Y\textsubscript{Z}\textsuperscript{*} and P\textsubscript{D1}−Y\textsubscript{Z}=P\textsubscript{D1}Y\textsubscript{Z}\textsuperscript{*} respectively. In addition a new
factor denoted IV is introduced. This represents the temperature dependent location of \( P_x^+ \) and will be discussed below.

Our data showed that the oxidation pattern of the electron donors was different between 77 and 5 K. At 77 K, the 750 nm was light able to induce approximately the same amount of \( S_2 \) multiline EPR signal as 730 and 532 nm light. Also, the 750 nm light still preferably oxidized the \( Y_Z/\text{CaMn}_4 \) -pathway at 77 K with a partition ratio of 1.6 which is lower than the almost total preferences at 5 K (partition ratio was lowered from \( > 2.7 \) at 5 K).

The partition ratio was significantly lowered at 730 nm, from 1.8 to 0.6 at 5 and 77 K respectively. The 730 nm induced partition ratio was thereby not too different from that obtained with 532 nm flash illumination (0.3) at 77 K, thus this might imply that \( P_{D1}^+ \) was formed as the primary cation. This is indicated in eq. I in Figure 28 C. In contrast at 5 K, our data suggests domination of eq V. The question is however how \( P_{D1} \) is formed after 730 nm illumination at 77 K?

The effect observed after flash illumination at 730 nm light is a lowering of the partition ratio by 3 times as a consequence of a temperature increase from 5 to 77 K. By raising the temperature, more energy is available in a system (in our case the PS II preparation), i.e., \( kT \) is increased. This energy will put molecules in thermally populated states, speed up reactions and can potentially alter redox equilibria. The observed increase in the oxidation of the Car/\( \text{Chl}_2/\text{Cyt} \) \( b_{559} \)-pathway upon raising the temperature could be related to a temperature dependent shift of the redox equilibrium between \( \text{Chl}_{D1} \) and \( P_{D1} \) towards \( P_{D1}^+ \).

However, if the increased oxidation of the Car/\( \text{Chl}_2/\text{Cyt} \) \( b_{559} \)-pathway observed at 730 nm and 77 K was exclusively dependent on the shifted redox equilibrium between \( \text{Chl}_{D1} \) and \( P_{D1} \) the redox equilibrium would change in the same way when we used 750 nm light. This is because the same fraction of the \( S_2 \) multiline signal was formed irrespective of wavelengths. However, this was not the case and the yield of Car/\( \text{Chl}_2/\text{Cyt} \) \( b_{559} \)-pathway components clearly decreased when going from 730 to 750 nm light. Thus we conclude that, the effect we study is probably not solely dependent on a shift in the redox equilibrium between \( \text{Chl}_{D1}^+P_{D1} = \text{Chl}_{D1}P_{D1}^+ \) (eq. IV in Figure 28 B).

The excited states inside \( P_{680} \) are of a mixed exciton CT-character \(^{(20)}\). Therefore we suggest that the 730 nm light might excite two different states at 5 and 77 K. In this hypothesis the primary donor cation becomes localized at \( \text{Chl}_{D1} \) at 5 K and at \( P_{D1} \) at 77 K. At 5 K our idea is that, 730 nm light excites a band with charge transfer character (\( P_x^+ \)) formed between \( \text{Chl}_{D1} \) and \( \text{Phe}_{D1} \). Thereby \( P_x^+ \) becomes localized at \( \text{Chl}_{D1} \) with preferred secondary electron donation being dominated by the \( Y_Z/\text{CaMn}_4 \)-pathway, indicated in equilibrium V.

As the temperature is elevated to 77 K, we instead propose that the charge transfer band becomes more mixed with a exciton state. It can therefore be shared even over three pigments, likely alternative for these are the close
lying Phe_{D1}, Chl_{D1} and P_{D1} on the D1 protein side of the reaction center. From this excitonic state charge separation would be initiated at P_{D1}, indicated in equilibrium VI (potentially VI can involve several equilibria). Subsequently this leads to the formation of P_{D1}^{+} which is able to oxidize the Car/ChlZ/Cyt_{b559}-pathway in addition to the Y_{Z}/CaMn_{4}-pathway, with a partition ratio close to 1.

The same effect is also seen with 750 nm light at 77 K but to a smaller extent. This might be explained by a more inhibited mixing at 750 nm. This would lead to P_{X}^{+} remaining at Chl_{D1} which subsequently followed by the formation of Chl_{D1}^{+}. This will preferentially oxidize the Y_{Z}/CaMn_{4}-pathway.

The two above described models for far-red light induced electron transfer in PS II are not mutually excluding or contradictory to each other. Figure 28 B describes the situation after 750 nm flash/es where the primary cation is suggested to end up at Chl_{D1} (papers II and IV). Figure 28 C is more speculative and we will need further experiments to test critical details. Here we try to explain how the cation at some light regime appear to end up at P_{D1} based on the partition ratios achieved at 77 K. The true situation in the PS II reaction center might very well be a mix or combination of the two models. The partial models would then appear to different extents at different temperatures, probably in a wavelength dependent way.
8 Svensk sammanfattning

Fotosyntesen är och har varit en av de viktigaste kemiska processerna för livet på Jorden. Den producerar syret vi andas och maten vi äter. Under årmiljonerna har den dessutom fixerat den koldioxid som människan nu med stor iver förbränner som kol och olja, genom detta har den starkt bidragit till dagens industrialiserade värld. Fotosyntesens betydelse för livet här på jorden sen den uppkom för ca 2.5 miljarder år sedan i en förfader till dagens cyanobakterier kan inte överdrivas.

Fotosyntesen är unik. Den har förmågan att fånga in energin i solljuset och lagra den energin i socker och stärkelse. Fotosyntes processen sammanfattas som nedan.

\[ \text{Vatten + Koldioxid + ljus} \rightarrow \text{Syre + Socker} \]

Den kemiska processen beskriven ovan brukar delas in ljus och mörker reaktioner. Ljus reaktionerna fångar in solljuset, spjälkar vatten till elektroner och protoner, producerar syrgas och förser sedan mörker reaktionerna med elektroner som används till att fixera koldioxid och produktion av socker. Vattenspjälkningen sker i proteinet Fotosystem II, där också syrgas bildas som en biprodukt. Överlämnandet av elektronerna till mörkerreaktionerna sker vid proteinet Fotosystem I via ett antal intermediärer. Båda dessa proteins aktiviteter är beroende av infångandet av ljus som de omvandlar till kemisk energi, Fotosystem II producerar plastoquinol och Fotosystem I produc-
er NADPH via Fd och FNR. Nedan är en något förenklad bild av de proteiner som deltar i den ljusdrivna delen av fotosyntesen. Den ljusdrivna delen av fotosyntesen sker i membranen hos thylakoider i växter och cyanobakterier (grönt, Figur 29). Thylakoiderna i sin tur finns i kloroplasterna som är en av organellerna i enväxtcell.

Detta membran avskiljer stroma (utsidan på Thylakoiderna) och lumen (insidan på Thylakoiderna). Thylakoid membranet innehåller förutom Fotosystem I (PS I) och II (PS II) också ett antal andra protein komplex. De transporterar elektroner (plastoquinone (PQH₂) och Plastocyanin), bygger upp kemiska gradienter (Cyt b₆f) samt tillverkar ATP (F-ATPase).
Figur 29. En förenklad bild av proteinerna som är inblandade i den ljus drivna delen av fotosyntesen (14).

Den här avhandlingen handlar enbart om Fotosystem I och II. När fotosystemen absorberar ljus transporterar energin det till dess reaktions center (Figur 30), där laddningsseparation (fotokemi) sker. Den primära donatorn (inringade pigment i Figur 30) reducerar ett närliggande pigment och blir då positivt laddad och kallas då för den primära katjonen. Elektronen från den primära donatorn överförs enligt pilarna i respektive reaktions center. För att skapa en stabil laddnings separation måste den primära donatorn snabbt bli reducerad igen (dvs få en elektron). PS I blir reducerad av Plastocyanin (Figur 29), medan PS II blir reducerad av Tyrosin Z (YZ Figur 30).

Resultaten som erhölls hos Fotosystem II gjorde att vi kunde föreslå en modell som innehöll en hitintills okänd väg för elektronerna att reducera $P_{680}^+$ (paper II samt figur 28). Modellen byggde på att katjonen som bildas efter laddnings separation i $P_{680}$ hamnar på Chl$_{D1}$ vid belysning med ljus som nämns ovan. Till skillnad från belysning med synligt ljus $< 680$ nm där katjonen hamnar på $P_{D1}$.

Egenskapen att kunna driva fotokemi med våglängder mellan 730-850 nm indikerar närvaro av ett hittills okänt absorptionsband, fotosystemens likartade beteende gör det troligt att det härstammar från interaktioner mellan pigmenten i respektives reaktions center. Denna kunskap kommer ligga till grund för en djupare förståelse för processerna som sker innan bildandet av den primära katjonen samt hur de olika pigmenten interagerar med varandra.
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