UPPSALA
UNIVERSITET

Purification of photosystem II for future spectroscopic characterization

Henrik Lagerqvist

Supervisor: Prof. Fikret Mamedov
Department of chemistry – Ångström
Bachelor programme in chemistry
Degree project C in chemistry, 1KB010
May 2019

Abstract

In this project, the aim was to purify a PSII-LHCII supercomplex from spinach. By applying thylakoid membranes onto a sucrose gradient containing the detergent dodecyl-β-D-maltoside and a high concentration of betaine and then centrifugation for 12 h, a band showing high oxygen evolution and low contamination of photosystem I was formed. This indicates that a PSII-LCHII supercomplex has been purified by this method. The yield needs to be optimized. More biochemical and EPR experiments are also needed to further characterize the purified PSII.

Abbreviations

ATP	Adenosine triphosphate
Chlorophyll a	Chl a
Chlorophyll b	Chl b
DM	Dodecyl-β-D-maltoside
EPR	Electron paramagnetic resonance spectroscopy
LCHII	Light-harvesting complex II
NADPH	Nicotinamide adenine dinucleotide phosphate
PPBQ	2-phenyl-p-benzoquinone
OEC	Oxygen evolving complex
PSI	Photosystem I
PSII	Photosystem II

Contents

Introduction

Electron transport chain in photosynthesis

If photosynthesis would not exist, we would not have the oxygen we breathe today. In photosynthesis, light induces a series of electron transfer reactions that result in the oxidation of water to dioxygen and synthesis of ATP and NADPH. There are several protein complexes that catalyze these redox processes. The photosynthetic reactions can be divided into the light reactions and carbon-assimilation reactions. There are seven major protein components that carry out the light induced reactions. These are, photosystem II (PSII), cytochrome b_6f complex, plastocyanin, photosystem I (PSI), ferredoxin, ferredoxin: $NADP^+$ oxidoreductase and ATP synthase. The carbon-assimilation reactions are the processes when carbohydrates are formed from CO_2, with the help of NADPH and ATP which are produced in the light reactions. Depending on organism, photosynthesis can occur without the production of oxygen (anoxygenic photosynthesis) or with the production of oxygen (oxygenic photosynthesis)[1]. In this report, the focus will be on the light reactions in oxygenic organisms.
All photosynthetic reactions are dependent on when light hits the first protein complex of the light reactions, photosystem II. The antenna complexes of PSII absorb photons via the bound antenna molecules (mainly chlorophyll (Chl), but also carotenoids) and are then transferred via so called exciton transfer to other antenna molecules until they reach and excite P680, the special pair of chlorophylls, where the redox reactions are started. P680 will then be oxidized to $P680^+$, and as a result reducing pheophytin. Then electrons are transferred to two different plastoquinones (PQ). First to PQ_a and then to PQ_b. Before PQ_b gains its electrons, $P680^+$ gets reduced by one of the electrons that comes from the oxidation of water into O_2. Then, the PQ_b will gain two electrons and at the same time bind two protons from the stromal side. PQ_bH_2, then carries the electrons to the next major component of photosynthesis, the cytochrome b_6f complex. By the cytochrome *f* part of the complex, the electrons are transferred to the protein plastocyanin which then reduces photosystem I (PSI). PSI contains light harvesting proteins and chlorophyll molecules and they absorb photons, which again are transferred between several Chls and finally reach the special pair of chlorophylls called P700. P700 then reduces the electron acceptor A_0, which is a chlorophyll. The reduced A_0 then oxidizes phylloquinone (A_1) and then transfers the electron via three different iron-sulfur (Fe-S) centers to the Fe-S protein, ferredoxin. This in turn oxidizes the next component, ferredoxin: $NADP^+$ oxidoreductase. This protein then transfers its gained electrons to $NADP^+$, which with H^+ forms NADPH[2].

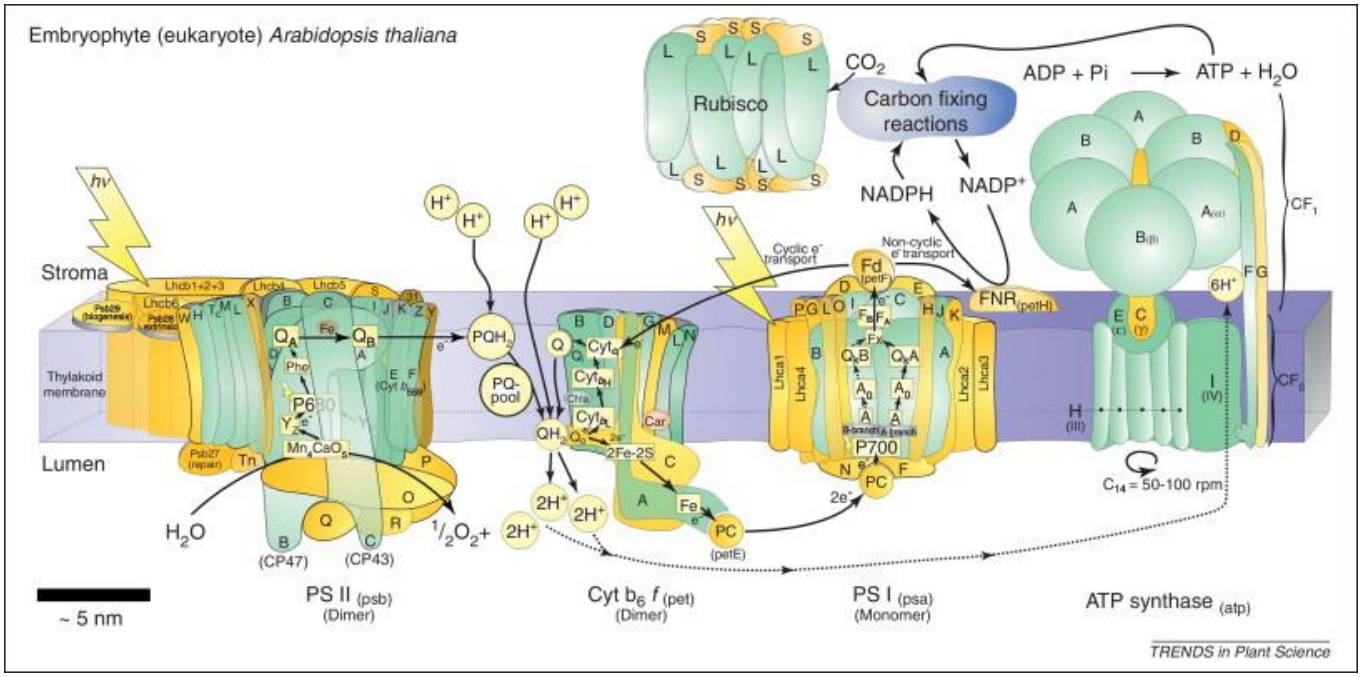

Figure 1. The components of photosynthesis. PSII = photosystem II, PQ/Q = plastoquinone, Cytb_6f = cytochrome b_6f complex, PC = plastocyanin, PSI = photosystem I, Fd = ferredoxin, FNR = ferredoxin: $NADP^+$ oxidoreductase. Reprinted from Trends in Plant Science, Vol 16, Allen et al, *A structural phylogenetic map for chloroplast photosynthesis*, Pages 645-655., 2011, with permission from Elsevier.

Proton gradient and ATP synthesis

The other main purpose, except forming NADPH from the light reactions, are the synthesis of ATP. This is made possible due to the formation of a proton gradient across the thylakoid membrane from the luminal side to the stromal side during the redox reactions. The sites in the electron transport chain where the protons originate from is at the oxygen evolving complex (OEC) where water is split into oxygen and protons in the lumen. The other site is at the cytochrome b_6f complex, where protons are pumped from stroma to lumen with the aid of PQ_bH_2. The protons from the plastoquinol dissociate at the luminal side while the electrons obtained from PSII is transferred to the cytochrome b_6f complex and then further transported through the electron transport chain[1,2].

Chloroplasts and thylakoids

All photosynthetic components reside in the chloroplasts. These membrane enclosed organelles have two different compartments. The first one facing the outer membrane, called stroma, is where carbohydrates are synthesized. The other compartment, called the lumen is enclosed by the membranes of the thylakoids within the chloroplasts. It is in these membranes the light induced reactions occur. In spinach, the thylakoid membranes can arrange in so called grana lamellae which is where PSII almost exclusively are found. Non-stacked membranes are called stroma lamellae and hold the place for photosystem I (PSI)[2].

A common preparation that has high oxygen evolution is to prepare BBY membranes. These are also called PSII-enriched membranes because most of the regions that contain very little active PSII such as the stroma lamellae, have been removed and left is the core region of the grana stacks which are rich in active PSII[3,4].

Photosystem II

Only one particular protein complex can be attributed with the production of oxygen, and that is photosystem II (PSII). It is PSII that initiates all the subsequent reactions. The electrons transported have their origin from the splitting of water by the oxygen evolving complex (OEC).

In vivo PSII forms dimers but each monomer seems to work on its own. Each monomer contains a core consisting of the heavy D1, D2, CP43 and CP47 subunits and twelve small subunits where all small subunits consist of a single membrane spanning alpha helix except one which contains two transmembrane helices. D1, D2 CP43 and CP47 in spinach have a high amino acid sequence resemblance to the corresponding subunits of photosystem II in cyanobacteria. The core complex does also contain 4 polypeptides facing the lumen, namely PsbO, PsbP, PsbQ and PsbTn, also called the extrinsic subunits. The function of PsbTn is yet to be investigated, but the other three interact with D1 and CP43 on the luminal side. These two core subunits bind to OEC. PSbO, PsbP and PsbQ play an important role with stabilizing the OEC by this interaction. All organisms having the Mn-cluster contain PsbO. On the other hand, PsbP and PsbQ that exist in spinach are replaced by PsbU and PsbV in cyanobacteria. Surrounding the core, are three different antenna proteins, namely, CP26, CP29 and an LHCII trimer. Chlorophyll are bound to all these. One PSII monomer contains more than 100 chlorophyll molecules. Both chlorophyll a and b can be found in the peripheral antenna complexes, while the PSII core only has Chl a. The PSII-LHCII supercomplex consists of the core complex (D1, D2, CP43, CP47) and all the peripheral antenna complexes (LCHII, CP26 and CP29)[2,5]. The structures of the PSII-LCHII supercomplex and its subunits can be seen in figure 2.

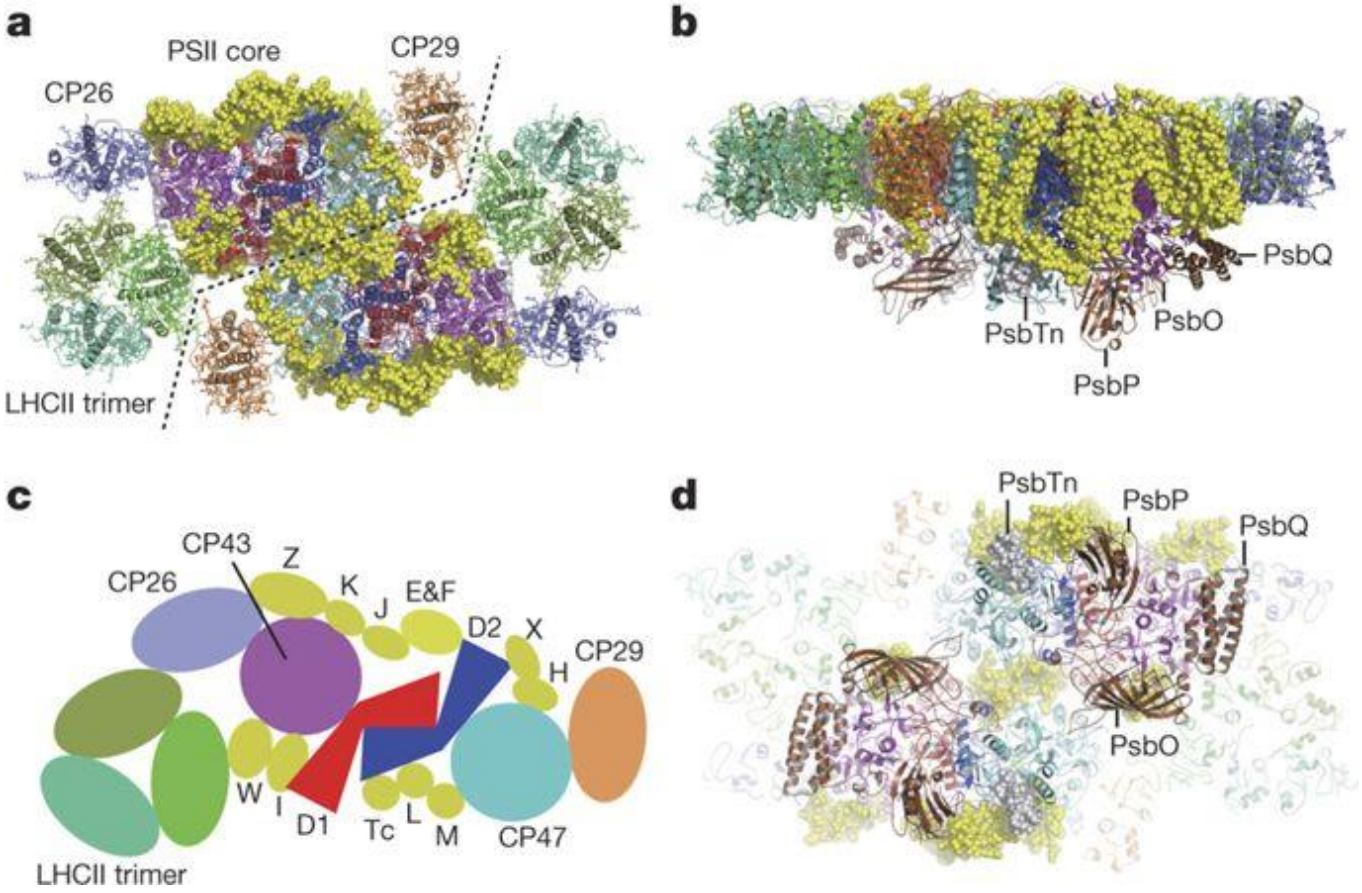

Figure 2. The structure of the dimeric PSII-LHCII supercomplex from spinach, obtained with single-particle cryo-electron microscopy. a) The dashed line shows the interface between the monomers. The view is from the stroma. b) Side-view of the dimer where the lumen is at the bottom. C) Schematic structure of one monomer. The colors correspond to the ones shown in a. d) The dimer from the luminal side is shown. The extrinsic proteins are highlighted.
Reprinted by permission from Nature/Springer. Nature. Structure of spinach photosystem II–LHCII supercomplex at 3.2 Å resolution, Wei et al, 2016[5].

The oxygen evolving complex

One of the most interesting properties of photosystem II is its ability to oxidize water into O_2 and H^+. This is catalyzed by the oxygen evolving complex (figure 3), which resides on the luminal side of the membrane. The chemical formula is Mn_4CaO_5. To split water, one needs a high redox potential since oxygen is a great oxidant. What makes it possible is the oxidation of P680 as described above. $P680^+$ is very oxidizing. The OEC has a distorted cubane structure containing three manganese, 4 oxygen and one calcium, with the fourth Mn and fifth oxygen outside of the cubane structure. Four water molecules are bound to the Mn cluster. Two of them binds to Mn4 and the other two to the calcium. A proposed mechanism for the oxidation of water by the cluster is the so-called S cycle, composed of five different states, S_0-S_4 (figure 3). Each time a photon is absorbed, the cluster goes to the next S state. Each state with a higher number of the subscript corresponds to a more oxidized state of the Mn cluster. Dioxygen is formed when the S_4 state goes back to S_0. The electrons formed from the water splitting are used to reduce $P680^+$ to P680. This is mediated by a certain tyrosine called Tyr-Z or Y_Z, which resides in D1. Tyr-Z reduces $P680^+$ forming a tyrosine radical which then regains an electron from the oxygen evolving complex[1,2]. There is, however, another redox active tyrosine in the PSII core, but in the D2 subunit. This

one, called Tyr-D or Y_D is not involved in the splitting of water, but it can both reduce and oxidize the OEC. In the dark, Y_D gets reduced by the Mn-cluster, which takes several hours. Then when light hits PSII, it will be oxidized and it will remain oxidized the whole time PSII is illuminated. It has been shown that mutated PSII lacking Tyr-D can still evolve O_2. However, all organisms that have PSII, do also have a Tyr-D. When Tyr-D is oxidized it is also deprotonated. One suggested reason for having this tyrosine is that this positively charged proton formed during oxidation will electrostatically interact with $P680^+$ which thus will lead to a higher potential energy of the special pair of chlorophylls. This can for example lead to a faster oxidation of Y_Z[6].

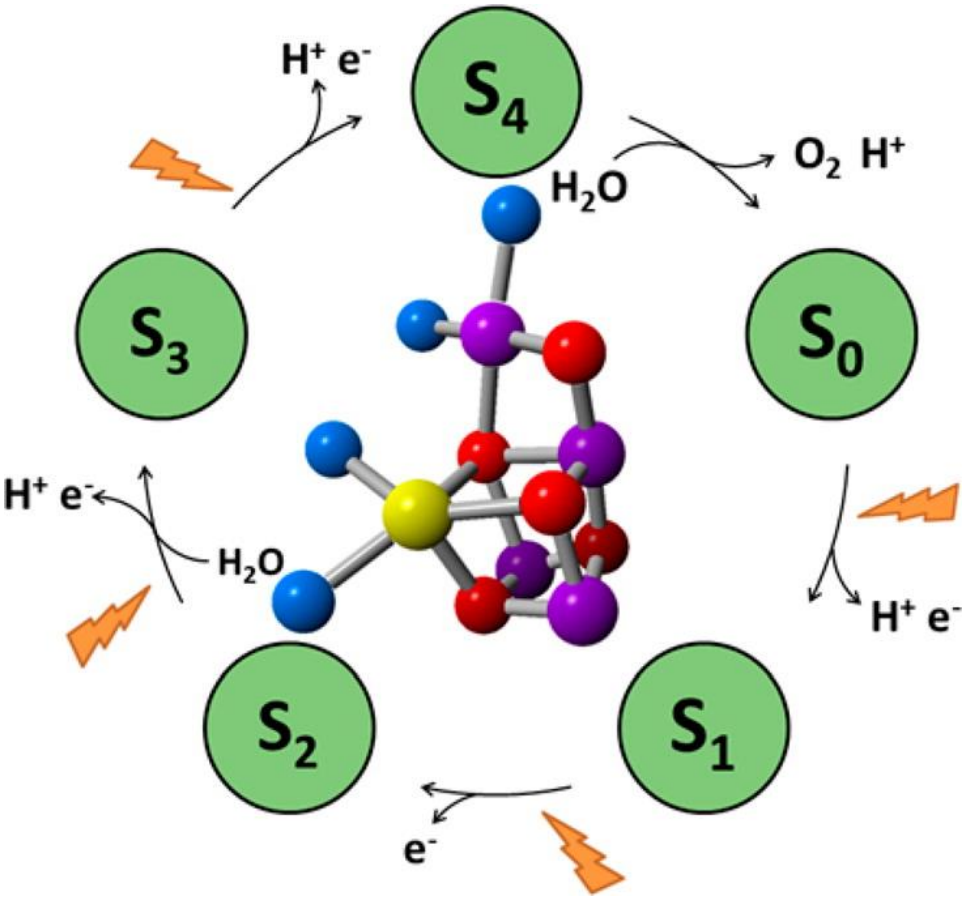

Figure 3. The S-cycle and the oxygen evolving complex. Purple is manganese, red is oxygen, yellow is calcium and blue is water. Reprinted (adapted) with permission from (Young, K.J., Brennan, B.J., Tagore, R., Brudvig, G.W. 2015. *Photosynthetic Water Oxidation: Insights from Manganese Model Chemistry*. Acc. Chem. Res. 48, 567–574.). Copright (2015) American chemical society.

Clark-type O_2 electrode

A common way of measuring the activity of PSII is by measuring the O_2 evolution upon illumination with a Clark-type O_2 electrode. For the OEC to be able to split water into oxygen, artificial electron acceptors need to be present. Two common acceptors that work well together is PPBQ and ferricyanide. PPBQ gets reduced by PSII while ferricyanide is reduced by the reduced PPBQ, keeping PPBQ in an oxidized state. The electrode works by reducing the produced oxygen at the platina electrode. The result is an electrical current that is related to the O_2 concentration[7].

Electron paramagnetic resonance

Electron paramagnetic resonance spectroscopy (EPR) is a very useful spectroscopic technique for detecting and studying paramagnetic systems, that is, molecules with unpaired electrons[8].
Since, the electron transfer in photosynthesis usually means that the reduced species have unpaired electrons, EPR can be used to study these reactions[9].

Fluorescence emission spectroscopy

Both photosystem I and photosystem II give rise to signals in fluorescence emission spectroscopy. The reason for this is the chlorophylls bound to the protein complexes. PSI and PSII have characteristic signals since they contain different antenna complexes. The fluorescence experiment in this degree project will be done at 77 K, since the fluorescence of PSII and PSI are easier to detect at lower temperatures[10].

Aim

The aim of this degree project was to purify a highly active PSII-LCHII supercomplex from spinach by carrying out a sucrose density gradient centrifugation containing the detergent dodecyl-β-D maltoside.

Methods

Thylakoids preparation

The steps were carried out in the cold room, with dim green light.
Buffers used in all preparations:
Buffer 1: 50 mM HEPES (pH 7.5), 300 mM sucrose and 5 mM $MgCl_2$
Buffer 2: 50 mM MES pH 6.0, 400 mM sucrose, 15 mM NaCl and 5 mM $MgCl_2$
Buffer 3: 25 mM MES pH 6.0, 300 mM sucrose, 10 mM NaCl and 5 mM $MgCl_2$

First thylakoid preparation

Spinach was grinded in buffer 1 and then filtered. The filtrate was centrifugated (1000 x g, 3 min, 4 °C). The supernatant was saved for later analysis. Buffer 1 was added to the pellet and centrifugated at 1000 x g, 3 min, 4 °C. The resulting pellet was homogenized with a glass homogenizer in 5 mM $MgCl_2$ (aq). The homogenate was diluted 1:1 with buffer 2 and then centrifugated for 10 min at 3000 x g, 4 °C. The pellet was washed by adding buffer 3 and centrifugated (3000 x g, 10 min, 4 °C). The resulting pellet was resuspended with a small volume of buffer 3 and then added dropwise to liquid nitrogen, creating frozen droplets of thylakoids. These were then stored in – 80 °C.

Second thylakoid preparation

This was carried out in the same way as the first preparation but with a few changes: after the first centrifugation, the pellet was saved for later analysis and the supernatant was centrifugated at 3000 x g, 5 min, 4 °C. The formed pellet was homogenized in the magnesium chloride solution and the following steps were the same as described above.

Sucrose density gradient centrifugation

All work was carried out in the cold room. The steps with thylakoids or BBYs were carried out under dim green light.
For the sucrose gradients, two solutions containing DM and different concentrations of sucrose were added to a gradient mixer connected to a peristaltic pump. A stirrer was present in the chamber containing the higher concentration of sucrose. The gradients were collected in ultracentrifuge tubes.
During the solubilization step, either BBY or thylakoid membranes were homogenized with a glass homogenizer by six strokes, in the presence of a buffer containing DM. Then the solubilized PSII solution was added carefully on top of the gradients, which were then centrifugated in a Beckman ultracentrifuge using a Sw-32 Ti at 28000 rpm and 4 °C at different times. After centrifugation, the formed bands were collected with a syringe and stored at – 80 °C

All sucrose gradient centrifugations except the last one, used the following buffers:
Buffer $_{gradient}$: 25 mM MES pH 6.0, 10 mM NaCl, 5 mM $CaCl_2$
Buffer $_{solubilization}$: 300 mM sucrose, 25 mM MES pH 6.0, 10 mM NaCl, 5 mM $MgCl_2$

For the last sucrose gradient centrifugation, the following buffers were used:
Buffer $_{gradient}$: 2 M betaine, 25 mM MES pH 6.0, 10 mM NaCl, 5 mM $CaCl_2$
Buffer $_{solubilization}$:2 M betaine, 25 mM MES pH 6.0, 10 mM NaCl, 5 mM $MgCl_2$

Chlorophyll absorption measurements

Samples were diluted with 80 % (v/v) acetone, vortexed and left in the dark for 10-15 min to extract the chlorophyll. Then, they were vortexed and centrifugated, 12000 rpm, 2 min. The absorption of chlorophyll in the supernatant was measured by a spectrophotometer at 663 nm (chlorophyll a) and 645 nm (chlorophyll b). If needed the solution was further diluted by 80 % acetone.

Oxygen evolution

The chamber of a clark-O_2 electrode contained a buffer (25 mM MES pH 6.0, 300 mM sucrose, 10 mM NaCl, 5 mM $CaCl_2$), 0.5 mM Ferricyanide, 2 mM PPBQ and the PSII sample. This solution was illuminated and the production of oxygen was measured.

Fluorescence emission spectroscopy

This was carried out at 77 K with an excitation wavelength of 440 nm, slit = 2 nm. Emission was measured from 600 nm – 800 nm, slit = 5 nm.

Results

The first sucrose gradient centrifugation (table 1) was carried out during 4 h and with three different sucrose gradients. The DM concentrations in the gradient and solubilization were constant in all three tubes. BBYs were used at a concentration of 0.5 mg Chl/ml in the solubilization step, which had a rate of 232 µmol O_2 (mg Chl)$^{-1}$ h^{-1}. As can be seen in the table, the bottom bands of two tubes showed activities of approximately 84 – 86 µmol O_2 (mg Chl)$^{-1}$ h^{-1} which was a decrease compared to the initial BBYs. No bands could be seen in the tube containing 5-15 % sucrose.

Table 1. First sucrose gradient centrifugation, prepared from BBY membranes.

Centrifugation (h)	% sucrose	%DM in gradient	Conc. DM in solub. (mM)	Bands	Activity (µmol O_2 (mg Chl)$^{-1}$ h^{-1})	Chl a/b
4	0.5-20	0.03	20	1st 2nd	0 87	1.5 1.9
4	5-15	0.03	20	0	-	-
4	10-20	0.03	20	1st 2nd	0 84	1.5 1.9

The next PSII supercomplex preparation, table 2, was done with a centrifugation of 12 h. All tubes contained 0.5-20 % sucrose and the DM concentrations were the same as last time. BBYs were used for the first two tubes with concentrations of 0.5 mg Chl/ml and 1.0 mg Chl/ml in the solubilization. The third tube contained thylakoids, 0.5 mg Chl/ml. In figure 4, one can see the resulting bands. In tubes 1 and 2, only one band can be seen, where the band in tube 2 is stronger due to the higher concentration of BBYs added. The third tube prepared from thylakoids, shows two distinct bands. However, none of the tubes had any activity.

Table 2. Second sucrose gradient centrifugation

Centrifugation (h)	% sucrose	%DM in gradient	Conc. DM in solub. (mM)	Bands	Activity (µmol O_2 (mg Chl)$^{-1}$ h^{-1})	Chl a/b	Membranes used
12	0.5-20	0.03	20	1 band	0	1.4	BBYs (0.5 mg Chl/ml)
12	0.5-20	0.03	20	1 band	0	1.6	BBYs (1 mg Chl/ml)
12	0.5-20	0.03	20	1st 2nd	0 0	1.5 2.0	Thylakoids (0.5 mg Chl/ml)

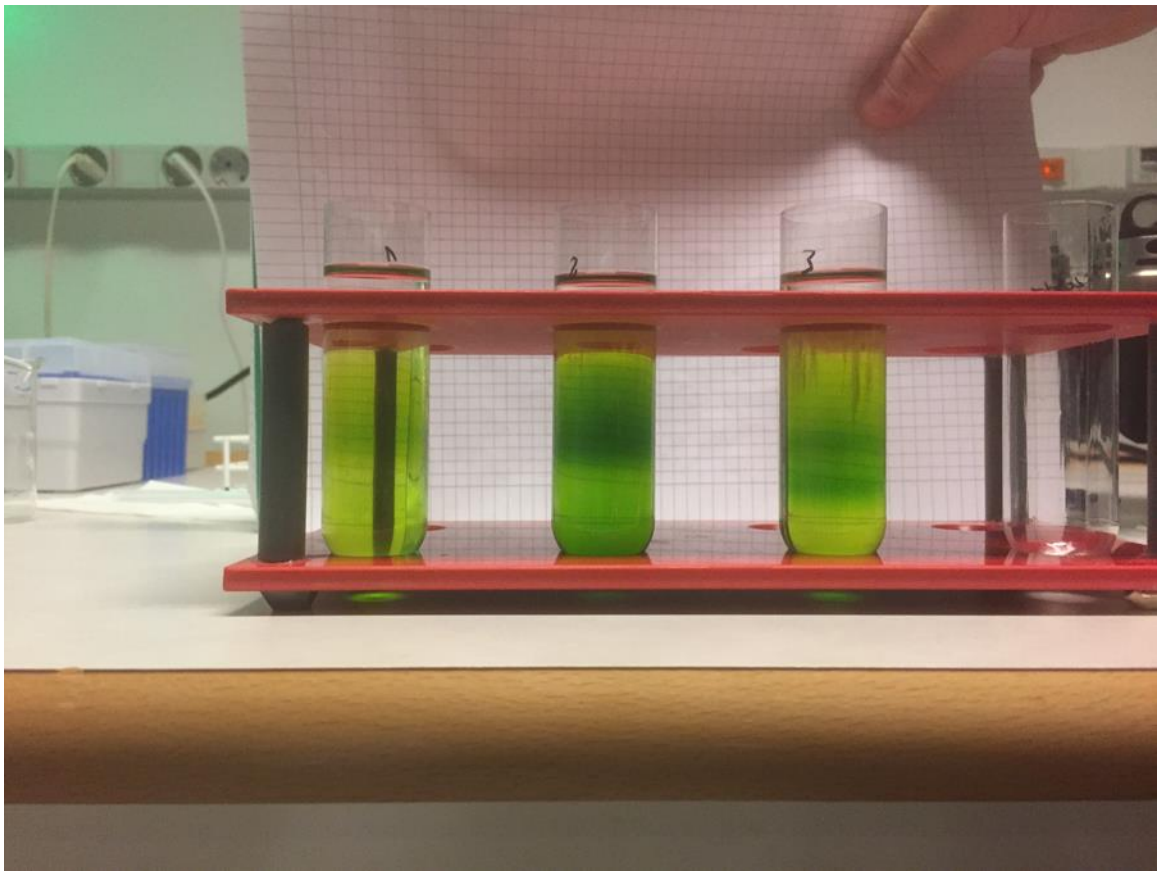

Figure 4. Resulting bands after sucrose gradient centrifugation in table 2.

In table 3, another PSII supercomplex preparation can be seen. The conditions were the same as for table 2, but thylakoids and supernatant from one thylakoids preparation were used this time, at a concentration of 0.5 mg Chl/ml. The supernatant was used in one tube since it showed a higher oxygen evolution activity than the final thylakoids. The tubes after centrifugation can be seen in figure 5. In this picture it is, however, very difficult to see the bands, but they were there, but very weak. The lowest bands in tube 2 and 3, showed low oxygen evolving activities, which were lower than the initial thylakoids which had activities in the range of approximately 60 – 100 µmol O_2 (mg Chl)$^{-1}$ h^{-1}. Also, the oxygen evolution traces showed that these bands had some oxygen consumption, which was caused by a high concentration of PSI, which is known to be able to reduce oxygen[11].

Table 3. Third sucrose gradient centrifugation. Thylakoids 1 = thylakoids prepared before my project, Thylakoids 2 = thylakoids from the first thylakoids preparation and Thylakoids 3 = the supernatant collected from the first thylakoids preparation

Centrifugation (h)	**% sucrose**	**%DM in gradient**	**Conc. DM in solub. (mM)**	**Bands**	**Activity (µmol O_2 (mg Chl)$^{-1}$ h^{-1})**	**Chl a/b**	**Membranes used**
12	0.5-20	0.03	20	1 band	12	2.8	Thylakoids 1 (0.5 mg Chl/ml)
12	0.5-20	0.03	20	1st 2nd	7.3 34	2.4 3.4	Thylakoids 2 (0.5 mg Chl/ml)
12	0.5-20	0.03	20	1st 2nd	0 26	2.5 3.4	Thylakoids 3 (0.5 mg Chl/ml)

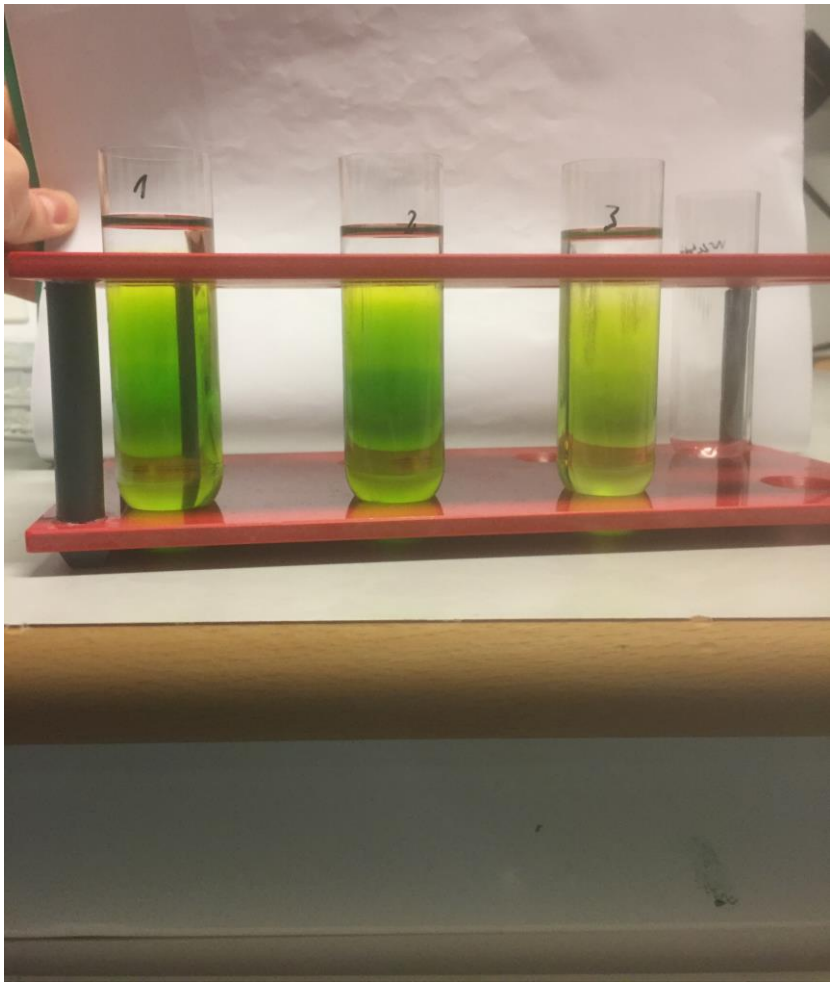

Figure 5. Resulting bands from centrifugation in table 3 (they are difficult to see in the picture).

In table 4 the conditions and results for the last sucrose gradient centrifugation can be found. The same thylakoids were used in all tubes. This time, tube 1 contained half the concentration of DM in the solubilization compared to what had previously been used. In tube 2 the DM concentration was reduced

in both the gradient and solubilization. In the third tube, the amount of DM was not changed compared to the previous experiments. As mentioned in the methods part above, this experiment used buffers that contained 2 M betaine, which is known to stabilize the oxygen evolving complex[12].

In figure 6, one can see that tube 1 and 2 contained 2 distinct bands. The third tube, for the first time contained 3 bands, where the third bottom band was quite weak. This band evolved oxygen at a high rate (table 4) at 670 µmol O_2 (mg Chl)$^{-1}$ h^{-1} compared to the initial thylakoids with a rate of 75.7 (µmol O_2 (mg Chl)$^{-1}$ h^{-1}). The band had a very low chlorophyll concentration of 14 µg Chl/ml while the thylakoids had a concentration of 3820 µg Chl/ml.

Table 4. The fourth sucrose gradient centrifugation. "-" means, not measured.

Centrifugation (h)	**% sucrose**	**%DM in gradient**	**Conc. DM in solub. (mM)**	**Bands**	**Activity (µmol O_2 (mg Chl)$^{-1}$ h^{-1})**	**Chl a/b**	**Conc. (µg Chl/ml)**
12	0.5-20 (tube 1)	0.03	10	1st 2nd	- -	- -	- -
12	0.5-20 (tube 2)	0.02	10	1st 2nd	- -	- -	- -
12	0.5-20 (tube 3)	0.03	20	1st 2nd 3rd	- - 670	1.9 3.1 2.7	28 35 14

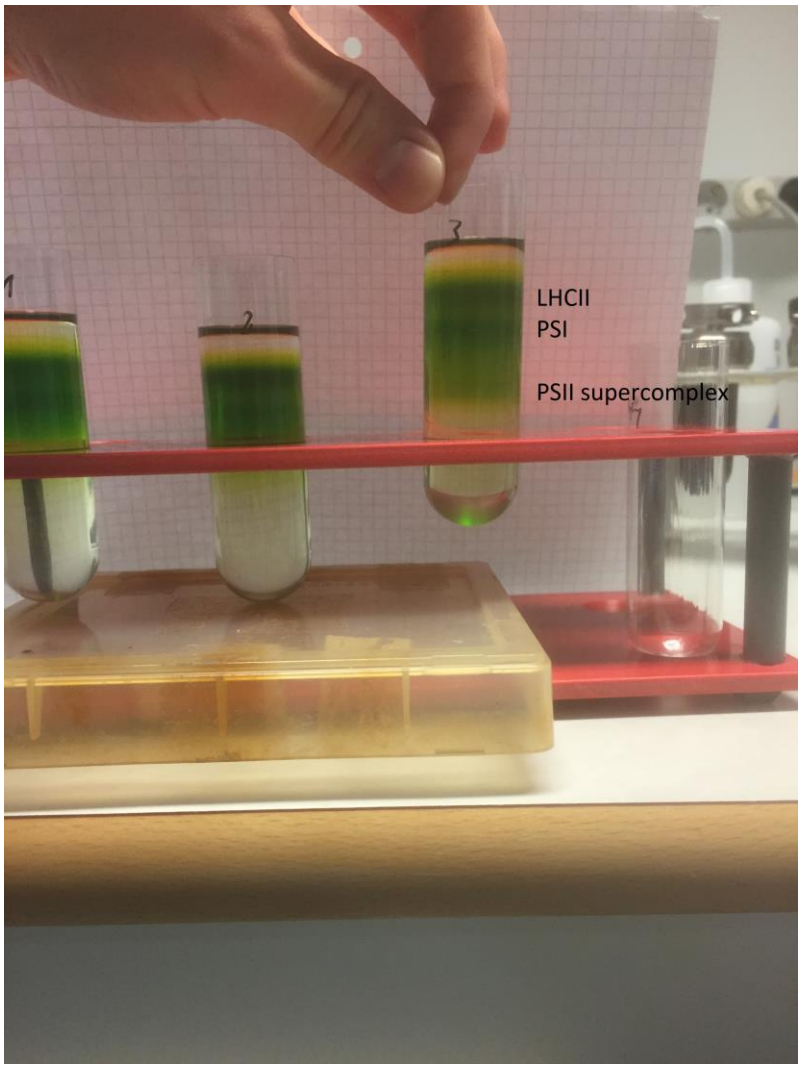

Figure 6. Resulting bands from centrifugation in table 4. The identity next to the bands in tube 3 are what the bands have been reported to contain[12].

Figure 7 compares the different rates measured for thylakoids, BBYs and the third band from figure 6, at the same chlorophyll concentrations. There has been a substantial increase in activity with the sucrose density gradient step. In this figure, illumination starts at 60 s and stops at 180 s. Table 5 shows the rates for thylakoids, BBYs and the possible PSII supercomplex.

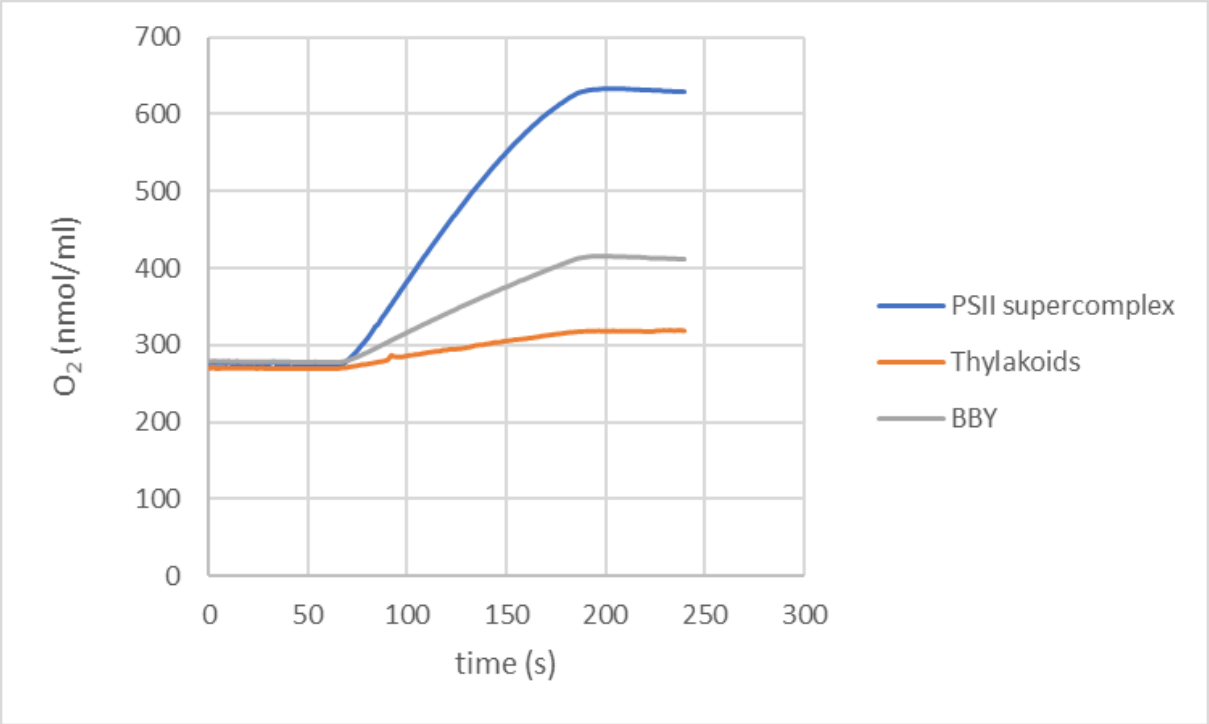

Figure 7. Comparison between oxygen evolution traces of PSII supercomplex, thylakoids and BBYs at Chl concentrations of 20 µg/ml.

Table 5. The rates of three different PSII preparations.

PSII Preparation	**Activity (µmol O_2 (mg Chl)$^{-1}$ h^{-1})**
Thylakoids	76
BBYs	230
PSII supercomplex	670

A fluorescence emission experiment at 77 K was carried out for thylakoids and all three bands in tube 3, figure 6. Figure 8 shows the results.

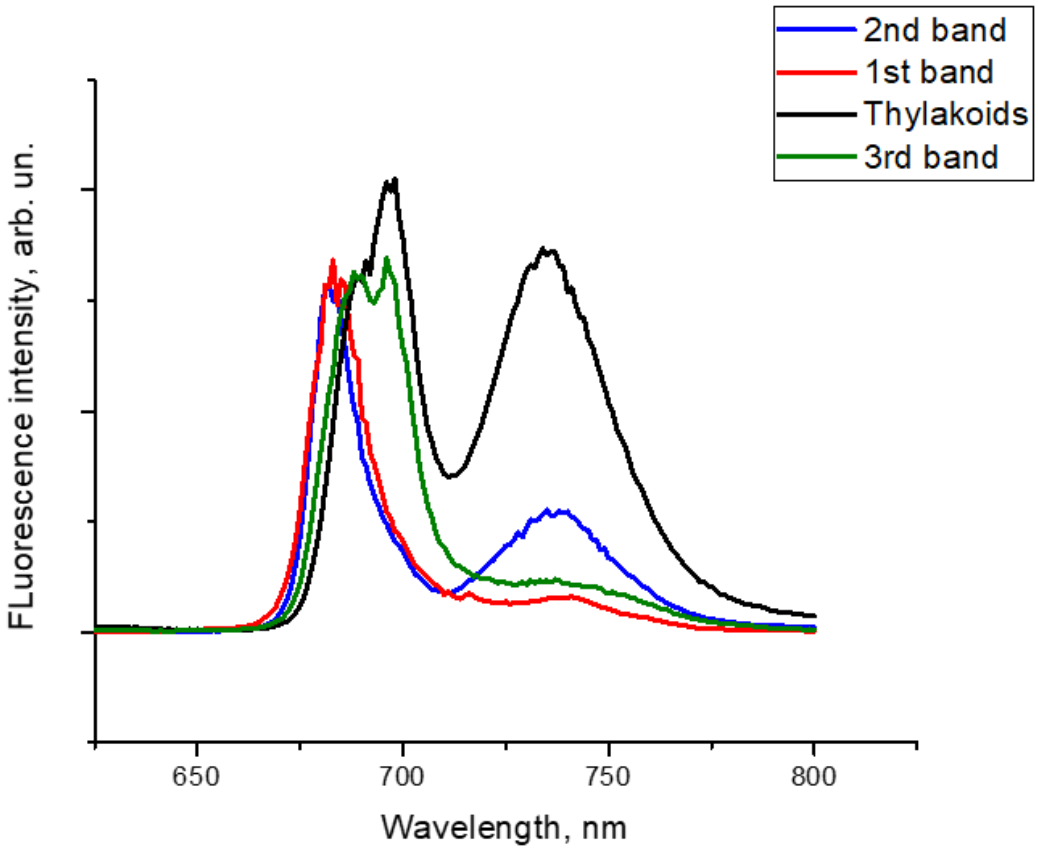

Figure 8. Fluorescence emission spectrum of thylakoids and the three bands from the tube containing the supposed PSII-LHCII supercomplex at 77 K.

Discussion

The third band of the sucrose gradient is supposed to be the PSII supercomplex according to literature, with a high rate of oxygen evolution and an activity increase of approximately 7.5 times compared to the initial thylakoids that were used[12]. In our case, the third band in tube 3, figure 6, has a high oxygen evolution which is almost 9 times higher than for the thylakoids. According to the same article, the second band should have the highest Chl a/b ratio of 6.5-7.1. In this experiment, the second band had the highest Chl a/b but with a value of 3.1 it was much lower than in the paper. This might be because the band is contaminated with constituents from the other bands which are supposed to have a much lower Chl a/b ratio. The first and third band do not have the exact same values as in the article but they are roughly in the same range.

An EPR experiment was carried out, trying to detect Tyr-D in the third band, but the signal was too low to be of any use. This is probably due to the very low concentration of chlorophyll in the sample, 14 µg/ml. The reason why this experiment was carried out was to compare the EPR spectrum of the PSII supercomplex and the corresponding EPR spectra of thylakoids and BBYs. Then one would have expected the signal to be higher for the PSII supercomplex due to a higher concentration of PSII. Tyr-D was chosen over Tyr-Z because the radical of the latter is difficult to detect.

The fluorescence spectrum of band 2 shows the characteristic peak for PSI at around 740 nm. For band 3, the two peaks that lie within approximately 680-690 nm are most likely caused by the two core light harvesting complexes CP43 and CP47 of PSII. The region of the spectrum at approximately 740 nm belongs probably to PSII and not PSI since it is known that PSII overlaps with PSI in that region. Therefore, the fluorescence experiment indicates that the purified PSII contains a low concentration of PSI[10].

Conclusion

Photosystem II has been purified from spinach thylakoids by centrifuging a sucrose gradient containing dodecyl-β-D-maltoside. The formation of three bands, where the bottom band showed a great increase in activity compared to the initial thylakoids, indicates that the band contained a PSII-LHCII supercomplex. According to the fluorescence experiment, it also seems like the contamination of PSI is low. The yield, however, is very low which is something that needs to be optimized for further studies. What seems to be critical for the formation of the third band containing the PSII supercomplex, is the presence of a high concentration of the OEC stabilizing betaine. BBY membranes were used in the earlier experiments without success. But since they were not added onto the gradients containing 2 M betaine, it is difficult to make a conclusion if they are also possible to purify to get an increase in oxygen evolution. However, if the increase in activity and purity would not be substantially higher than for the purification of thylakoids, there is no point in using the BBYs in this purification step since the preparation of BBY membranes would include another step in the protocol.

Even though the purified PSII seems to exhibit some of the characteristics of the previously reported PSII-LCHII supercomplex, further biochemical and spectroscopical studies are needed to verify that it really is the PSII supercomplex. The spectroscopical experiments should include EPR characterization of the different intermediates of the S-cycle. For the purified PSII supercomplex it is expected to be no interference from PSI components such as $P700^+$ and Fe-S clusters.

Acknowledgements

Firstly, I really want to thank Prof. Fikret Mamedov for making this project possible and for all help he has provided. I have learned a lot during this degree project.

Secondly, I would like to thank all people at Molecular Biomimetics, for helping me when I have had questions regarding the lab, especially Casper de Lichtenberg, who introduced me to the oxygen evolution and chlorophyll measurements.

Finally, I would like to thank my family for all their support.

References

1. Nelson, D.L., Cox, M.M. 2013. *Lehninger Principles of Biochemistry*, sixth edition, W. H. FREEMAN AND COMPANY.

2. Blankenship, R.E. 2014. *Molecular mechanisms of photosynthesis*, second edition, WILEY Blackwell.

3. Dunahay, T.G., Staehelin, L.A., Seibert, M., Ogilvie, P.D., Berg, S.P. 1984. *Structural, biochemical and biophysical characterization of four oxygen-evolving Photosystem II preparations from spinach.* Biochim. Biophys. Acta BBA - Bioenerg. 764, 179–193.

4. Mamedov, F., Stefansson, H., Albertsson, P.-Å., Styring, S., 2000. *Photosystem II in Different Parts of the Thylakoid Membrane: A Functional Comparison between Different Domains.* Biochemistry 39, 10478–10486.

5. Wei, X., Su, X., Cao, P., Liu, X., Chang, W., Li, M., Zhang, X., Liu, Z. 2016. *Structure of spinach photosystem II–LHCII supercomplex at 3.2 Å resolution*. Nature 534, 69.

6. Styring, S., Sjöholm, J., Mamedov, F., 2012. *Two tyrosines that changed the world: Interfacing the oxidizing power of photochemistry to water splitting in photosystem II*. Biochim. Biophys. Acta BBA - Bioenerg., Photosystem II 1817, 76–87.

7. Shevela D., Schröder W.P., Messinger J. (2018) *Liquid-Phase Measurements of Photosynthetic Oxygen Evolution*. In: Covshoff S. (eds) Photosynthesis. Methods in Molecular Biology, vol 1770. Humana Press, New York, NY

8. Chechik, V., Carter, E., Murphy, D. 2016. *Electron paramagnetic resonance*. Oxford university press.

9. Miller, A.-F., Brudvig, G.W. 1991. *A guide to electron paramagnetic resonance spectroscopy of Photosystem II membranes*. Biochim. Biophys. Acta BBA - Bioenerg. 1056, 1–18.

10. Lamb, J.J., Røkke, G., Hohmann-Marriott, M.F. 2018. *Chlorophyll fluorescence emission spectroscopy of oxygenic organisms at 77 K*. Photosynthetica 56, 105–124.

11. Kozuleva, M.A., Petrova, A.A., Mamedov, M.D., Semenov, A.Yu., Ivanov, B.N. 2014. *O_2 reduction by photosystem I involves phylloquinone under steady-state illumination*. FEBS Lett. 588, 4364–4368.

12. Eshaghi, S., Andersson, B., Barber, J. 1999. *Isolation of a highly active PSII-LHCII supercomplex from thylakoid membranes by a direct method*. FEBS Lett. 446, 23–26.