

# DOCTORAL THESIS

## *The influence of endogenous and exogenous factors on the activity of photosystem II and its heterogeneity*

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## Abstract

The direction of life on Earth was determined by oxygenic photosynthesis. The first organisms capable of carrying out this process appeared more than 3 billion years ago. Cyanobacteria, algae and higher plants are able to use sunlight to extract electrons from water, as they have photosystem II (PSII). A side-product of this process is O<sub>2</sub>. PSII is not without reason called the 'heart' of photosynthesis. The following steps which occur during photosynthesis depend on the activity of the oxygen-evolving complex (OEC). Understanding the structure and mechanism of action of the OEC remains the greatest challenge of modern science, despite the enormous progress made in recent years thanks to the combined efforts of many research groups from different disciplines and using different research approaches. The difficulty in understanding the functioning of the OEC, which is formed from the Mn<sub>4</sub>CaO<sub>5</sub> complex, lies in obtaining experimental data that would allow it to be unambiguously assigned to intermediate catalytic states. There is still no consistent model of the OEC that can be constructed from the available data. The main problem is that the Mn<sub>4</sub>CaO<sub>5</sub> complex cannot be isolated from PSII; it degrades. Only when bound to the PSII protein matrix, does it exist as a highly active compound capable of accumulating four positive charges and extracting electrons from water. Questions that remain open are: (i) how do the states of the Mn<sub>4</sub>CaO<sub>5</sub> complex change in the Kok cycle, (ii) what are the pathways of proton and oxygen release and water molecule binding, (iii) what are the regulatory mechanisms responsible for the high efficiency of the OEC, and finally (iv) which stage in the Kok cycle is the bottleneck and what is the cause of this.

The mechanism of action of the OEC is fascinating, and its understanding will have a massive impact on the design of efficient fuel cells. Currently, the most widespread systems are semiconductor compounds capable of oxidising water. These include tungsten compounds. Unfortunately, compared to a natural PSII system equipped with the Mn<sub>4</sub>CaO<sub>5</sub> complex, their efficiency in O<sub>2</sub> production is usually about five orders of magnitude lower.



Another problem is the narrow range of wavelengths that activate them. PSII supercomplexes absorb light in a wide spectral range (visible light range) with high efficiency due to the presence of antenna complexes. This is why attempts are increasingly made to create bio-nano-hybrid systems in combination with isolated or entire structures of the photosynthetic apparatus. For this reason, more and more attempts are being made to produce bio-nano-hybrid systems by combining semiconductors with isolated or whole structures of a photosynthetic apparatus.

This work focuses on studying endogenous and exogenous factors in the efficiency and heterogeneity of oxygen production by PSII BBY (in PSII-enriched thylakoids). In the first case, the influence of external proteins (PsbO, PsbP and PsbQ) was investigated. These proteins are known to stabilise the  $Mn_4CaO_5$  complex and may be responsible for forming channels through which water is supplied near the manganese complex and  $H^+$  and  $O_2$  are extracted.  $WO_3$  nanoparticles (NPs) were chosen as the exogenous agent because of their known ability to oxidise water and their resistance to aquatic environments. This makes them an attractive candidate for the PSII BBY -  $WO_3$  NPs hybrid system, which could exhibit enhanced oxygen release stimulated by interactions with PSII compared to a pure semiconductor system. The size of the nanoparticles is about 1-3 nm. It was expected that they would be able to incorporate into the membranes and interact with both the donor and acceptor side of PSII. A new measurement protocol using fast three-electrode polarography (Joliot-type electrodes) was developed to directly study the contribution and kinetics of oxygen release in PSII via the slow and fast pathways. Experiments have been carried out using PSII BBY isolated from fresh tobacco leaves (*Nicotiana tabacum* var. *John William's Broadleaf* (JWB)) to study the effect of external proteins on PSII activity and from fresh spinach leaves (*Spinacia oleracea*) to study PSII BBY -  $WO_3$  NPs hybrid systems. No external acceptors were added in any of the experiments, which could alter the acceptor side of the PSII and thus alter how it works.

There are two main achievements of this work. One is related to direct measurements of oxygen release heterogeneity using fast polarography and identifying factors influencing PSII switching between fast and slow phases of oxygen production. The other is related to a new approach to developing biohybrid systems using the PSII mechanisms necessary to optimise hybrid performance under near-natural, i.e. environmental, conditions.

Using the heterogeneous 5S – state extended Kok model, which considers the existence of a metastable  $S_4$  state, an analysis of oxygen evolution oscillations under the influence of short saturating flashes of white light has been carried out. The data were obtained for untreated PSII BBY and depleted of external proteins surrounding the  $Mn_4CaO_5$  complex. All transitions between the  $S_i \rightarrow S_{i+1}$  states are highly efficient in the control sample, except the transition from the  $S_2$  to the  $S_3$  state, proving its unique nature. Moreover, the probability of this transition is independent of the presence of external proteins. It was observed that the removal of the two proteins PsbQ and PsbP caused a decrease in the transition probability between the states  $S_0 \rightarrow S_1$ ,  $S_1 \rightarrow S_2$  and  $S_3 \rightarrow S_0$  to different degrees. The most considerable changes were observed for the  $S_0 \rightarrow S_1$  transition. However, the

contribution of the fast phase of oxygen evolution of more than 80% in control PSII BBYs decreased almost 4-fold in PSII depleted of PsbP/PsbQ. The elution of all three proteins almost entirely abolished the fast phase of O<sub>2</sub> yield. A similar disappearance of the fast phase of O<sub>2</sub> release in the samples studied was also confirmed by the results from measurements of the dependence of the changes in the amplitude of oxygen release under the third flash as a function of the distance between the 1<sup>st</sup> and 2<sup>nd</sup> flashes and between the 2<sup>nd</sup> and 3<sup>rd</sup> flashes. At the same time, its independence from the measurement protocol adopted confirms the sequential nature of the Kok cycle. The time constant of the fast oxygen evolution pathway determined for the control sample increased from about 4 ms to about 6 ms in PSII BBY depleted of the two external proteins. The change in the slow time constant is surprising, which is about 60 ms in the control sample and then accelerates almost 2 and 4 times in PSII-PsbQ, PsbP and PSII-PsbQ, PsbP, and PsbO, respectively. All of the observed changes were discussed in relation to the accepted knowledge of the structure of the Mn<sub>4</sub>CaO<sub>5</sub> complex and the functioning of the PSII, as well as existing models describing the transitions between the S<sub>i</sub> → S<sub>i+1</sub> states. To explain these phenomena, it was necessary to take into account both the organisational changes in the Mn<sub>4</sub>CaO<sub>5</sub> complex, including other coordination spheres of manganese and calcium besides the immediate ones, as well as changes in the functioning of the identified O1, O4 and Cl1 channels and their controlling role in the supply of H<sub>2</sub>O to and removal of H<sup>+</sup> and O<sub>2</sub> from the OEC.

This work also provides new insights into the design of nano-biohybrid systems using photosystem II for O<sub>2</sub> production. Commonly studied 2D/3D semiconductor nanostructures require a large active surface showing a highly specific junction with PSII in order to obtain correctly oriented PSII systems with high packing density. This requires, on one hand, the functionalisation of these meso-nano surfaces and, on the other hand, genetic modification of the PSII itself. Usually, electron mediators are introduced to ensure electron cycling. Nevertheless, the highest rate of oxygen release recorded for these systems, which they achieved in their initial phase of operation, converted per g of catalyst, i.e. PSII supercomplex, remains 2-3 orders of magnitude lower than that exhibited by isolated PSII systems under their native conditions. This work demonstrated a new approach to constructing hybrid systems based on natural PSII BBY, which displays the highest oxygen evolution capacity observed so far. Superfine WO<sub>3</sub> nanoparticles were introduced in PSII BBY. They have been shown to have a concentration-dependent effect on the organisation of these membranes and the functioning of PSII. The most remarkable NP-induced changes were observed for O<sub>2</sub> evolution. The maximum O<sub>2</sub> yield of 70% was detected for the control sample and for concentrations of 0.02-0.03 μg NPs/μg Chl and 0.15-0.3 μg NPs/μg Chl. This correlated with a slight decrease in photochemical yield, which made it possible to identify the mechanism responsible for this phenomenon. Namely, the highly specific action of WO<sub>3</sub> NPs on the donor side, their ability to take up electrons from the OEC, and the formation of conducting chains reaching the acceptor side of PSII enabled them to transfer electrons to quinones bound at the Q<sub>A</sub> and Q<sub>B</sub> sites. The effect of NPs on the compactness of PSII supercomplexes is of great importance here. Thus, NPs can modify the redox potentials of Q<sub>A</sub> and Q<sub>B</sub> and regulate PQ's access from the external



quinone pool to the  $Q_B$  binding site. This approach can benefit the design of biohybrid systems that convert solar energy into fuel. It allows the use of the entire spectrum of wavelengths emitted by the sun in the UV-VIS range with a high quantum efficiency of water-splitting reactions and oxygen production at the level of native PSII activity. The system operates at pH 6.5 and requires no additional electron acceptors. Of course, the stability of such hybrid systems is crucial. It depends, among other things, on the rate of  $O_2$  uptake (lower  $O_2$  uptake = lower ROS generation) and the ability to regenerate  $PQ_{ox}$ , which may require an external electron mediator. In the latter case, it requires the interaction of two systems; the second could be a system capable of producing  $H_2$  (this would require their spatial separation) or electricity using a suitable electrode capable of extracting electrons from  $PQH_2$ .

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