Final report on the NKFIH-OTKA (NN-110960) project entitled „Exploration of alternative electron transport pathways in cyanobacteria by computer modeling and experimental approaches: From regulation of light reactions to biosolar fuel production”

The aim of the project was to develop *in silico* photosynthesis models, which will serve as important tools to study the interaction of photosynthetic, metabolic, and respiratory electron transport components by performing *in silico* experiments. The *in silico* experimentation provides a very fast and efficient approach to screen a large number of experimental conditions, from which the most important predictions can be verified by real experiments. The work has concentrated on the following main areas:

Development of computer models for photosynthetic and connected respiratory electron transport

We have used the scheme of electron transport processes shown in Fig. 1. for creating the model, which consists of over 500 coupled differential equations. The model runs practically

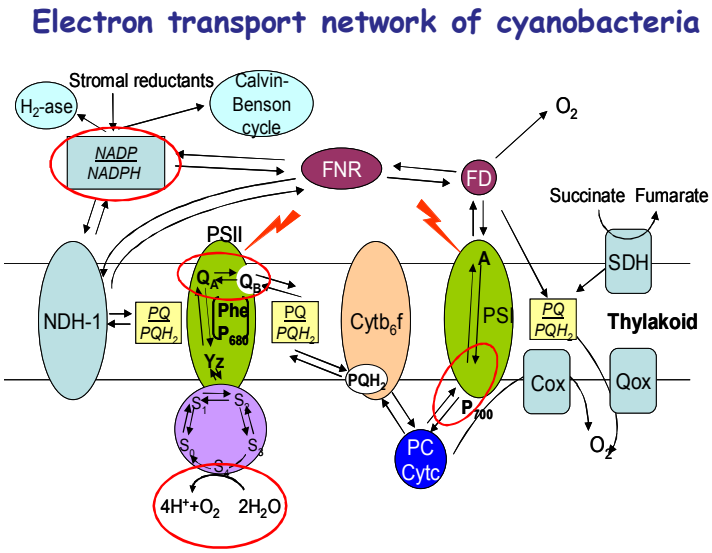

Figure 1. Scheme of the electron transport network used for the modeling.

real time on a notebook computer and results in the kinetic changes of all included electron transport components from the ns to hours time scale, which can be visualized for any of the selected components (Fig. 2).

One particular aim of the modeling was to describe the characteristic period-four oscillation of the yield of oxygen evolution when initiated by single turnover flashes. In our model the dampening of the oscillation pattern arises from charge recombination processes between the oxidized components (S-states) of the Mn cluster of the water oxidizing complex and the reduced electron carriers on the acceptor side of Photosystem II (PSII) and in the PQ pool.

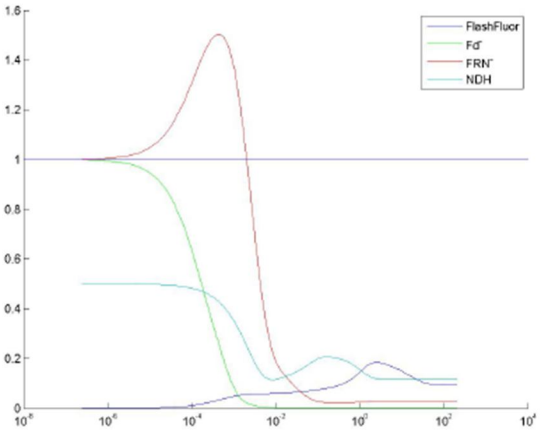

Figure 2. Representative kinetic changes of selected electron transport components

The scheme of the S-state turnovers and the corresponding electron transport processes are shown in Fig. 3, left and right panels, respectively.

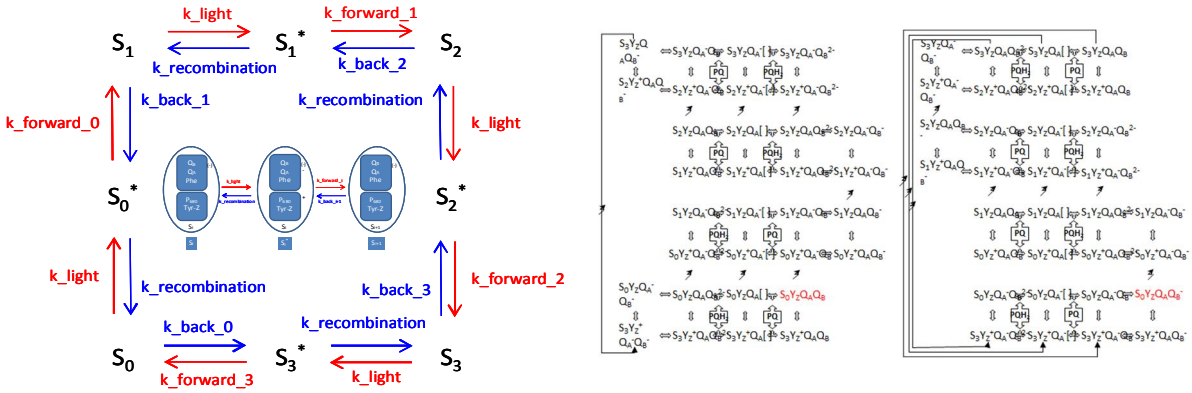

Figure 3. Scheme of S-state turnovers (left panel) and the corresponding electron transport processes (right panel)

The resulting simulated O_2 flash pattern in comparison to an actual measurement is shown in Fig. 4.

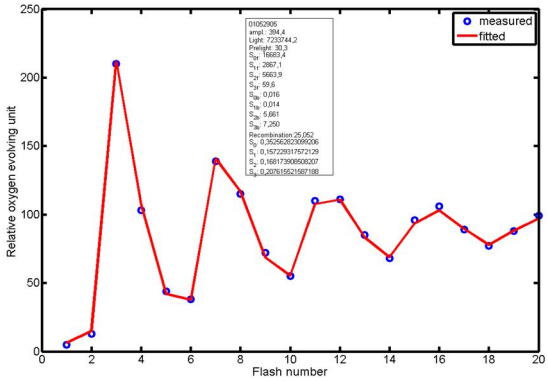

Figure 4. Simulated (red line) and measured (blue circles) O_2 pattern.

Another important target was the description of PSII acceptor side electron transport in relation to the kinetic changes of Chl fluorescence yield changes. The scheme of the acceptor side electron transport, as well as simulated traces of Chl fluorescence yield changes after flash excitation are shown in Fig .5 (left and right panels, respectively).

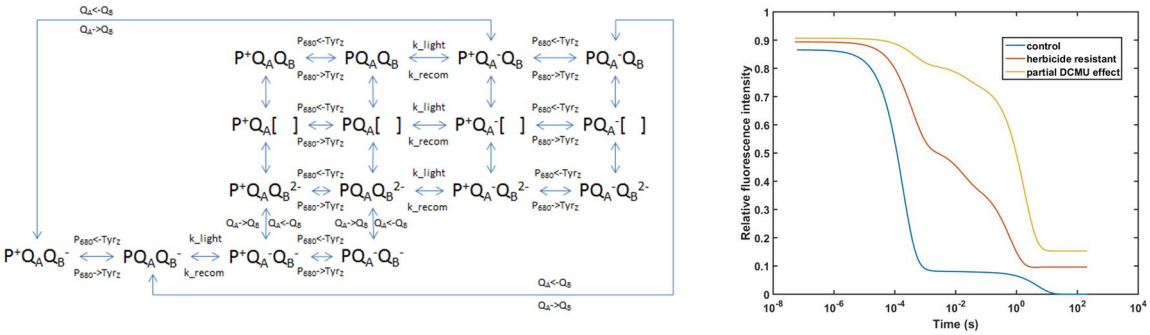

Figure 5. Scheme of electron transfer at the acceptor side of Photosystem II (left panel) and simulated changes of Q_A^- kinetics corresponding to Chl fluorescence yield changes.

The results related to the development of the *in silico* photosynthetic system were presented in various conferences [1-3] and a summarizing publication is under preparation.

Studies on the role of flavodiiron proteins in mediating alternative electron transport from PSII

The Flv2 and Flv4 flavodiiron proteins mediate a highly important alternative electron transport pathway from PSII or the PQ pool to so far unidentified electron acceptors. The flv4-2 operon the Flv2 and Flv4 protein together with a small protein Sll0218. The separate function of the components was studied in cooperation with the collaborating Finnish group (Prof. Eva-Aro, Turku University) by using specific deletion mutants. It was concluded that the Flv2/Flv4 heterodimer supports PSII functionality by contributing to photoprotection via directing excess electrons away from the PSII acceptor side, while the Sll0218 protein assists PSII assembly and stabilization. These results were published [4].

Characterization of electron transport at PSI and PSII in Symbiodinium dinoflagellates

The *in silico* model, which was developed during the project allows to study electron transport between PSII and PSI as well as through PSI. This useful feature was utilized in the investigation of PSI electron transport in the *Symbiodinium* dinoflagellate alga, that acts as photosynthetic partner in coral symbiosis. The experiments were performed in intact corals that contain host embedded *Symbiodinium* cells. We have developed a non-intrusive

measurement method to examine photochemistry of intact corals, based on redox kinetics of the primary electron donor in Photosystem I (P700) and chlorophyll fluorescence kinetics. Design and analysis of experiments were assisted by the modeling of PSI ($P700^+$) kinetics. Since the redox state of P700 depends on the operation of both PSI and PSII, important information can be obtained on the PSII-PSI intersystem electron transfer kinetics. Based on the coordinated response of P700 parameters and PSII-PSI electron transport properties, the simple P700 kinetics parameters serve as important indicators of the integrity of PSII-PSI electron transfer dynamics in corals. These results were published [5].

Exploration of a novel alternative electron transport pathway from the inorganic electron donor H_2S to the PQ pool

Ancient photosynthetic organisms were able to utilize energy rich inorganic electron donors (e.g. H_2S and arsenic) for the production of hydrocarbons. With the evolutionary invention of oxygenic photosynthesis the water oxidation served as the main electron donor. However, in some cyanobacteria the ability to use H_2S and arsenic as alternative electron donors was retained. We have demonstrated for the first time that in the photosynthetic model organism *Synechocystis* sp. strain PCC6803 the two metabolic pathways are linked by coregulated genes that are involved in arsenic transport, sulfide oxidation, and probably in sulfide-based alternative photosynthesis. Although *Synechocystis* sp. strain PCC6803 is an obligate photoautotrophic cyanobacterium that grows via oxygenic photosynthesis, we discovered that specific genes are activated in the presence of sulfide or arsenite to exploit the energy potentials of these chemicals. These genes form an operon that we termed *suoRSCT*, located on a transposable element of type IS*4* on the plasmid pSYSM of the cyanobacterium. *suoS* (sll5036) encodes a light-dependent, type I sulfide:quinone oxidoreductase. The suoR (sll5035) gene downstream of suoS encodes a regulatory protein that belongs to the ArsR-type repressors that are normally involved in arsenic resistance. We found that this repressor has dual specificity, resulting in 200-fold induction of the operon upon either arsenite or sulfide exposure. The suoT gene encodes a transmembrane protein similar to chromate transporters but in fact functioning as an arsenite importer at permissive concentrations. We propose that the proteins encoded by the suoRSCT operon might have played an important role under anaerobic, reducing conditions on primordial Earth and that the operon was acquired by the cyanobacterium via horizontal gene transfer. Modeling data show that the H_2S dependent electron flow contributes to regulation of the redox equilibrium of the PQ pool. These results were published [6].

Exploration of a novel alternative electron transport pathway from the acceptor side of Photosystem II to molecular oxygen

Electron transport from Photosystem I (PSI) to molecular oxygen is an important alternative pathway which provides protection against photooxidative damage under conditions of limited CO_2 fixation. In prokaryotic organisms this, so called Mehler reaction, proceeds via

O_2 reduction, i.e. superoxide production. Superoxide is a harmful reactive oxygen species, which is eliminated via enzymatic reactions in intact organisms. However, in cyanobacteria the electrons are transferred from PSI to O_2 via the Flv1/Flv3 flavidiiron proteins, without superoxide production. Therefore cyanobacteria are not equipped with the superoxide detoxifying enzyme systems, which makes them vulnerable to superoxide production. During photoinhibition studies protein synthesis inhibitors are applied to separate photodamage from the continuous repair. One of these inhibitors is chloramphenicol, which has been reported to mediate electron transfer from PSI to O_2, resulting in superoxide production. In spite of this knowledge chloramphenicol is still used in photoinhibition studies in cyanobacteria, which can not detoxify this harmful reactive oxygen form, leading to confusing experimental data. We aimed at a deeper understanding of the chloramphenicol mediated alternative electron transport pathway. We demonstrated for the first time that chloramphenicol interacts not only with PSI, but also with PSII and induces superoxide dependent photodamage to the PSII complex. These data indicate that in contrast to the generally accepted view the acceptor side components of PSII are able to transfer electron to exogenous acceptors at very negative (ca. -500 mV) redox potentials. This part of our results has been published [7]. In the further step of these studies we have also shown that chloramphenicol dependent superoxide production, as well as superoxide dependent photodamage in intact cells of the cyanobacterium *Synechocystis* 6803, which has only PSII, but no PSI. This demonstrates that chloramphenicol mediates superoxide production from both PSI and PSII, which enchances photodamage in intact cyanobacteria. Therefore the usage of chloramphenicol as protein synthesis inhibitor in photoinhibition studies should be avoided. Publication of these results is under preparation.

Publications

[1] Imre Vass, László Sass, Zsuzsanna Deák(2015): In silico modelling of photosynthetic electron transport, Abstract book of the 1st Solar Fuel Conference, Uppsala, 2015, April 26-May 1, 2015

[2] Imre Vass, László Sass, Zsuzsanna Deák (2015) : In silico photosynthetic electron transport, Abstract book of: Phototech-2015 Towards a photosynthesis-biobased economy, 2015

[3] Imre Vass, László Sass (2017) Kinetic modelling of photosynthetic electron transport. Gordon Research Conference on Photosynthesis, Sunday River, USA, July 16-21, 2017.

[4] Luca Bersanini, Yagut Allahverdiyeva, Natalia Battchikova, Steffen Heinz, Maija Lespinasse, Essi Ruohisto, Henna Mustila, Jörg Nickelsen, Imre Vass, Eva-Mari Aro (2017): Dissecting the Photoprotective Mechanism Encoded by the flv4-2 Operon: a Distinct Contribution of Sll0218 in Photosystem II Stabilization. Plant, Cell and Environment (2017) 40, 378–389

[5] Szabó, M., Larkum, A.W.D., Suggett, D.J., Vass, I., Sass, L., Osmond, B., Zavafer, Al., Ralph, P.J, Chow, W.S. (2017) Non-intrusive assessment of photosystem II and photosystem I in whole coral tissues, Frontiers in Marine Science**,** 4, Art. 269, doi: 10.3389

[6] Csaba I. Nagy, Imre Vass, Gábor Rákhely, István Zoltán Vass, András Tóth, Ágnes Duzs, Loredana Peca, Jerzy Kruk, Péter B. Kós (2014): Coregulated Genes Link Sulfide:Quinone Oxidoreductase and Arsenic Metabolism in Synechocystis sp. Strain PCC6803, J. Bacteriology, 196: 3430-3440.

[7] Ateeq Ur Rehman, Sandeesha Kodru and Imre Vass (2016), Chloramphenicol Mediates Superoxide Production in Photosystem II and Enhances Its Photodamage in Isolated Membrane Particles, Frontiers in Plant Science, 7: Article 749

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