# Ph.D. projects in progress

**1.**

**Mentor:** Imre Vass

**Doctoral School:** University of Szeged, Faculty of Science and Informatics, Doctoral School of Biology

**Ph.D. Student**: Sandeesha Kodru

**Title of the research topic:** Characterization of alternative photosynthetic electron transport pathways and their role in photodamage and photoprotection

**Description of the research topic:** Photosynthesis is a process in which green plants, algae, cyanobacteria utilize energy from sunlight to produce carbohydrates from $CO_2$ and water. It is the main source of energy for all life on Earth. The photosynthetic apparatus contains four protein complexes in the thylakoid membrane namely PSI, PSII, cytochrome b/6f and ATP synthase. Too much light reaching the photosynthetic apparatus causes decline of photosynthetic activity, which process is known as photoinhibition. The major site of photodamage is the PSII complex, which can be repaired via de novo synthesis of the damaged reaction centre protein subunits. Light stress to PSII becomes a problem when the rate of photodamage exceeds the capacity of repair process. Separation of photodamage and repair requires the addition of protein synthesis inhibitors. One of the frequently used compounds is chloramphenicol, which is also known to act as electron acceptor in PSI and to result in superoxide production, which is a reactive oxygen species that can damage its environment.

The aim of the work is to characterize the chloramphenicol-mediated electron transport pathways as well as its possible side effects in photoinhibition studies.

**2.**

**Mentors:** Péter Kós, Imre Vass

**Doctoral School:** University of Szeged, Faculty of Science and Informatics, Doctoral School of Biology

**Ph.D. Student**: Gábor Patyi

**Title of the research topic:** Assessment of intracellular singlet oxygen by GFP fluorescence in *Synechocystis* PCC6803 cyanobacteria

**Description of the research topic:** Singlet oxygen ($^1O_2$) is a very important reactive oxygen species (ROS), it can damage a wide range of macromolecules, like lipids, carotenoids and proteins. Its formation takes place in the second photosynthetic reaction centre (PSII), during the photosynthetic reactions. In high light condition the generated triplet state chlorophyll can interact with the molecular oxygen leading to $^1O_2$ formation via energy transfer.

This highly reactive ROS, besides its degradation effects can take part in signal transduction mechanisms and other intracellular reactions. This importance is the reason why we investigate the $^1O_2$ intracellular mechanisms. There are various $^1O_2$ detection methods, such as His mediated $O_2$ uptake, which allows to calculate the rate of $^1O_2$ generation by the rate of $O_2$ consumption. However, still there is a lack in detection methods, that could be used to detect the spatial distribution of $^1O_2$ generation inside intact cyanobacterial cells.

The Green Fluorescent Protein (GFP) is a very commonly used reporter protein in biological research nowadays. $^1O_2$ can damage this protein, hence quenching its fluorescence. We treated GFP producing *Synechocystis* PCC6803 cyanobacterial mutant cells with the $^1O_2$ sensitizer Rose bengal (Rb) and Methylene blue (Mb) dyes under high light conditions. We observed that the GFP fluorescence decreased suggesting that $^1O_2$ mediated degradation of GFP can be utilized for *in vivo* $^1O_2$ detection.

We investigated the specificity and sensitivity of the quenching reaction and established experimental parameters for a widely applicable *in vivo* $^1O_2$ assessment method protocol.

**3.**

**Mentors:** Zoltán Gombos, László Kovács and Gábor Galiba

**Doctoral School:** University of Szeged, Faculty of Science and Informatics, Doctoral School of Biology

**Ph.D. student**: Terézia Kovács

**Title of the research topic:** Lipid composition changes of photosynthetic membranes

**Description of the research topic:** Thylakoid membrane composition plays a key role in regulating the function of the photosynthetic electron transport chain in photosynthesizing autotrophic organisms. In our work, we study the changes of lipid composition of photosynthetic membranes induced by environmental factors (heat and light). We are also looking for an answer to the question of what differences or similarities can be detected between cyanobacteria, algae, and barley plants during acclimation processes. In addition, we study the role of the two rate-determining components of the photosynthetic electron transport chain, PSI and the cytochrome $b_6$/f complex in state transition using mutant cyanobacterial strains.

**4.**

**Mentors:** Vass Imre, Szabó Milán

**Doctoral School:** University of Szeged, Faculty of Science and Informatics, Doctoral School of Biology

**Ph.D. student**: Faiza Bashir

**Title of the research topic:** Protoplast technology as an experimental platform for characterizing oxidative stress in Symbiodinium sp. and other microalgae

**Description of the research topic:** Singlet oxygen ($^1O_2$) is a highly reactive damaging agent, which is produced by the interaction of the triplet excited state pigment molecules with molecular oxygen. $^1O_2$ is produced primarily in chlorophyll containing photosynthetic complexes in cells of photosynthetic organisms, such as plants, algae and cyanobacteria and damage pigments, lipids, proteins and other cellular constituents in their environment. Via this damaging effect $^1O_2$ is one of the main mediators of photo-oxidative damage in photosynthetic systems. Besides $^3$Chl-dependent intracellular $^1O_2$ production, it has recently been observed that certain unicellular microalgae (e.g. *Synechocystis* sp. PCC 6803 or the coral endosymbiont alga *Symbiodinium* sp.) excrete so far unidentified metabolites, which sensitize $^1O_2$ outside the cells. The physiological role of this extracellular $^1O_2$ production is so far an unresolved question, and it is also unknown, how does external $^1O_2$ interfere with cellular processes especially with the photosynthetic functions. In the current project, we characterize the effect of externally produced $^1O_2$ on the photosynthetic activity of isolated thylakoid membranes and intact *Synechocystis* cells. Another aim of the project is to isolate protoplast and characterize the intracellular $^1O_2$ production in *Symbiodinium* sp., by using the Single Oxygen Sensor Green (SOSG) dye. Moreover, we aim to optimize the protoplast isolation and regeneration in microfluidic system, which is an efficient technology for the analysis of single cell under precisely controlled conditions.

**5.**

**Mentors:** Péter Kós, Imre Vass

**Doctoral School:** University of Szeged, Faculty of Science and Informatics, Doctoral School of Biology

**Ph.D. Student:** Barbara Hódi

**Title of the research topic:** Investigation of singlet oxygen specific gene expression in *Synechocystis* PCC 6803, a cianobactérium

**Description of the research topic:** $^1O_2$ is one of the most harmful type of ROS, which is produced as a byproduct during photosynthesis. Under high light conditions triplet chlorophyll is formed, which is able to interact with molecular oxygen and gives them energy to form singlet oxygen.

$^1O_2$ has very well-known intracellular effects, it causes DNA damages, lipid peroxidation and inhibits the function of PSII in photosynthetic microorganisms through D1 protein degradation.

Nowadays it is proved that singlet oxygen has an effect on gene expression and it might have a signal transduction pathway. In plants and higher eukaryotic organisms genes that are involved in singlet oxygen generated intracellular signal transduction pathway have been identified, but are not known in cyanobacteria.

So our goal is to find and identify the elements of singlet oxygen specific signal transduction pathway in cyanobacteria.

**6.**

**Mentors:** Szabó Milán, Vass Imre

**Doctoral School:** University of Szeged, Faculty of Science and Informatics, Doctoral School of Biology

**Ph.D. student**: Sabit Mohammad Aslam

**Title of the research topic:** The role and significance of cyclic electron transport in microalgae

**Description of the research topic:** Photosynthesis is a series of redox reactions, in which several electron transport processes operate to provide the energetic balance of light harvesting. Besides linear electron flow, which ensures the basic functions of photosynthetic productivity and carbon fixation, alternative electron transport pathways operate, i.e. the cyclic electron transport, which play a role in fine-tuning of photosynthesis and balancing the ATP/NADPH ratio under stress conditions. The enzymes NAD(P)H dehydrogenases (NDHs) play important role in the regulation of cyclic electron transport by mediating the electron flow from stromal NAD(P)H to the plastoquinone molecules located in the lipid phase of the thylakoid membranes. The type I NDH is well characterized in several microalgae species, however some species possess only type II NDH dehydrogenase (NDH-2), the role of which in cyclic electron flow remains uncharacterized so far.

The aim of the project is the characterization cyclic electron flow in microalgae that play crucial role in aquatic ecosystems (the coral endosymbiont Symbiodinium sp.), and in species that have high relevance in bioenergy (e.g. biofuel) and valuable compounds (e.g. carotenoid) production. Various biophysical methods (variable chlorophyll fluorescence, NADPH fluorescence, absorption kinetics) will be applied to investigate the impact of various stress conditions on the rate of cyclic electron flow. The components of cyclic electron transport chain will be assayed using inhibitors and mutants. Thus we aim to understand the species-specific characteristics and the role of cyclic electron flow in mediating stress responses.