

Biochemical and molecular-genetic methods of the study of photosynthetic membrane complexes, namely Photosystem II, using a model cyanobacterium *Synechocystis* PCC 6803

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Background:

The crucial components of the photosynthetic apparatus of plants, algae and cyanobacteria are thylakoid membrane embedded pigment-protein complexes, so called photosystems. They capture light energy and mediate its conversion into the energy of chemical bonds. The key component of this intricate machinery is Photosystem II (PSII), an enzyme composed of many protein subunits, pigments, lipids and further cofactors, utilising captured light energy for energetically extremely demanding reaction of water splitting. This reaction provides the driving force for photosynthetic electron transport and formation of the transmembrane proton gradient used to generate ATP and NADPH for carbon dioxide (CO₂) fixation into the organic matter. The by-product of water splitting is molecular oxygen (O₂) whose presence in the atmosphere is a prerequisite of life on the Earth.

Within the last decade the detailed structure of Photosystem II and the roles of its key subunits have been described. We have also fundamental knowledge of the principles of PSII biogenesis, i.e. *de novo* synthesis of PSII in the course of life and growth of the cell. Given the extraordinary complexity of Photosystem II its biogenesis requires highly orchestrated synthesis and incorporation of proteins, pigments and other cofactors and precise assembly of multiple components into the functional unit. This process is facilitated by many auxiliary proteins whose specific functions have not been clarified yet.

One of the interests of our laboratory is to identify these so far enigmatic proteins, to study their interactions with the arising photosystem and to clarify their roles. As a model organism

we employ a cyanobacterium *Synechocystis* PCC 6803. The cyanobacteria are prokaryotic photoautotrophic organisms supposed to be ancestors of chloroplasts. The photosynthetic apparatus of cyanobacteria, algae and plants are therefore very similar. A general advantage of cyanobacteria as experimental models is the relative simplicity of their cellular structure and organisation of the genetic apparatus. The *Synechocystis* PCC 6803 is a popular model as it can be easily cultivated, it grows quickly, its genome has been fully sequenced, and it can be genetically manipulated which enables targeted mutations in genes encoding proteins of our interest.

Description of student's work:

The student will take part in currently running experiments. In the course of the work she/he will be introduced to the fundamentals of microbiological methods, methods of cultivation of cyanobacteria, construction of mutant strains, physiological methods of the assessment of mutant phenotype, biochemical methods of separation and detection of photosynthetic membrane complexes and their components using electrophoretic gel separation, Western blotting and immunodetection, and the isolation of proteins and revealing protein-protein interactions by affinity chromatography.

Requirements on student:

- We require a student of a bachelor program in biology or biochemistry; however, the project can be adjusted also for a motivated student of a high school (Gymnasium) or, on the other hand, a MSc. student.
- Recommended literature (not required in case of a high school student):

Alberts et al., Essential Cell Biology (Garland Publishers, 1998): Chapters dealing with membrane structure and photosynthesis

- Advanced scientific literature (papers) can be provided on request