Prof. Roman Sobotka's group
Laboratory of Photosynthesis

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Title of the project: Isolation and characterization of the chlorophyll-binding ferrochelatase enzyme from the model cyanobacterium *Synechocystis* PCC 6803

For how many student(s): 1-2

Description of the Project

Chlorophyll (Chl) is the most abundant pigment on Earth, and its unique photochemical properties enable oxygenic photosynthesis, which powers virtually all forms of life on our planet. However, for cyanobacteria, algae and plants, dealing with Chl is not an easy task. If the light energy absorbed by Chl is not rapidly utilized for photosynthesis, excited pigments can form damaging oxygen radicals. The combination of Chl molecules, light, and oxygen can be a deadly cocktail. The synthesis of Chl is therefore under strict control. Although the status of Chl as the pigment of life is almost iconic, our understanding of how Chl biosynthesis is regulated is poor.

Chl is a product of a long-branched tetrapyrrole pathway together with other tetrapyrroles such as hemes and bilins. Interestingly, the enzyme producing heme (ferrochelatase) can bind Chl and probably serves as a safety valve arresting the production of Chl molecules if their production is too high. How Chl binding regulates the ferrochelatase activity remains, however, a mystery.

In this course, students will purify and analyse the ferrochelatase enzyme from the model cyanobacterium *Synechocystis* PCC 6803 using our recently developed approach. The aim is to isolate this enzyme associated with Chl molecules as a potential sample for cryo-electron microscopy and for determination of its atomic structure. Students will learn a range of state-of-the-art biochemical methods, specifically:

a) Isolation of thylakoid membranes from cyanobacteria (biochemical scale), working with biological buffers and detergents
b) Protein isolation using immune-affinity chromatography (anti-FLAG)
c) One-dimensional and two-dimensional electrophoresis (clear-native/SDS)
d) Immunodetection of proteins using specific antibodies (western blotting)
e) Analysis of pigments using HPLC chromatography and spectrometry
f) Preparation of samples for protein mass spectrometry
g) Interpretation of mass-spectrometry data

Requirements:
Enthusiasm and a dream to make a scientific discovery are essential together with proficiency in spoken and written English. Previous laboratory experience is not required but is advantageous, as the project is predominantly wet-lab based.