



Supervisor's evaluation on PhD student Mahendra Kumar Shukla

Mahendra's PhD studies started in March 2013 and his project was focused on the family of small, single-helix proteins called Hlips that are essential for the surviving of cyanobacteria even under mild stress conditions. Before Mahendra came, we have already observed that Hlips can bind chlorophyll and carotenoid molecules; however we lack any information about individual carotenoid species, pigment stoichiometries or about a potential quenching of absorbed energy. Even more urgent was to clarify the biological role of Hlips, there were no mechanistic explanation of how these proteins protect the cell against a damage. Mahendra's thesis had therefore two ambition goals: a) to establish a solid biochemistry and photochemistry of isolated Hlips and, b) to explore what these proteins are doing.

Solid biochemistry requires a good amount of pure protein, which is a challenging task for low-abundant, small and transmembrane proteins like Hlips. At the beginning, Mahendra had to learn all work with *Synechocystis* and quite a lot of laboratory techniques. It was a bit complicated by Mahendra's specific language, which people around called Hindu with English accent, and his shy nature did not make the situation easier. This communication however progressively vanished and Mahendra finally mastered a broad spectrum of methods. Mahendra was always working hard and together with his discipline, motivation and persistence, these were important qualities for the first goal of the thesis. Mahendra grew litres and litres of cells, testing buffers, membrane solubilization, chromatographic techniques. This effort was crown with success. The quality and quantity of isolated protein was good enough even for ultrafast (femtosecond) spectroscopy and for resonance Raman spectroscopy. Together with our collaborators from České Budějovice and from Paris we published two strong papers describing pigment binding and the dissipation of absorbed energy in HliD protein; Mahendra is a co-author on both these publications. Later, we developed a completely

new approach for the isolation of a different Hlip (HliC). Mahendra has characterized the isolated HliC protein in his first-author paper published in *Photosyn Res* and he also prepared a sample for our co-workers in Amsterdam, who analyzed energy dissipation by femtosecond Raman spectroscopy. This second work is under review in *J. Phys Chem Lett*.

In the second half of Mahendra studies we were mostly focused on the question what is the role of Hlips in the cell. Mahendra conducted a plenty of experiments with various *Synechocystis* mutants, partly in UK during his stay on Sheffield University. The obtained results revealed a new and interesting mechanism of how Hlips regulate the formation of a protein complex that is crucial for the protoprotection of Photosystem II. In my opinion, these data, already summarized in a Mahendra's first-author manuscript, have quality for a top journal. Mahendra also contributed to a manuscript that is currently re-submitted to PNAS and one more manuscript dealing with the chlorophyll synthase enzyme is under preparation.

Mahendra is a nice, friendly person dealing easily with his colleagues, patient and willing to learn. He is hard working, skilful and reliable. On the other hand, I lacked creativity and originality in ideas and the ability to design experiments providing clear-cut results. Mahendra also struggled with writing, with too much of my effort needed to produce a standard scientific text. However, regarding the last point, I saw a big progress during the work on his PhD thesis. In my opinion this is a very good thesis and the list of Mahendra's achievements is surely long enough to meet the criteria for the PhD degree. As algae and plant Hlip homologues have very likely similar functions like their cyanobacterial counterparts, the results obtained during this project mean an important step in the understanding of how oxygenic phototrophs acclimate to stressful conditions.

Třeboň, March 2nd, 2018

Roman Sobotka, PhD

