



## Ph.D. Thesis Defence

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### **“Spectroscopic Studies of Novel Microbial Rhodopsins from Fungi and Bacteria”**

#### **ABSTRACT**

Microbial rhodopsins are widespread bacteriorhodopsin-like proteins found in many prokaryotes and lower eukaryotic groups. They serve as photosensors, light-driven ion pumps, and light-gated channels. The main goal of this thesis was to spectroscopically characterize a new subgroup of fungal rhodopsins (so-called auxiliary group) and a new type of rhodopsin found in flavobacteria.

Towards the first goal, products of two known rhodopsin genes from the fungal wheat pathogen, *Phaeosphaeria nodorum*, were investigated. The two yeast-expressed *Phaeosphaeria* rhodopsins were spectroscopically characterized by Raman, time-resolved visible and Fourier transform infrared (FTIR) spectroscopy and shown many similarities: absorption spectra, conformation of the retinal chromophore, fast photocycling, and carboxylic acid protonation changes. It is likely that both *Phaeosphaeria* rhodopsins are proton-pumping, at least in vitro. We suggest that auxiliary rhodopsins have separated from their ancestors fairly recently and have acquired the ability to interact with as yet unidentified transducers, performing a photosensory function without changing their spectral properties and basic photochemistry.

In the second project, we studied a flavobacterial rhodopsin with highly unusual sequence, which was functionally expressed in *E.coli* by our collaborators and produced light-induced pH changes in the spheroplast suspensions, suggesting an inward H<sup>+</sup> transport. Using time-resolved visible spectroscopy, we revealed that its photochemical reaction cycle was fast and strongly dependent on cations. Analysis of the sequence alignments, combined with visible and FTIR spectroscopic studies of the wild-type and mutant flavobacterial rhodopsin, provided the clues for the mechanism of binding metal ions and origin of the observed light-induced pH changes.

Finally, isotope labeling protocol, previously used for soluble secreted proteins, was successfully implemented and optimized to produce homogeneous samples of eukaryotic rhodopsin from *Leptosphaeria* in methylotrophic yeast. Isotope-labeling extent and functionality were verified by FTIR spectroscopy, and obtained samples gave high-resolution ssNMR spectra suitable for structural studies. This protocol for overexpression of isotope-labeled multi-spanning eukaryotic membrane proteins in *Pichia pastoris* can be adopted for challenging mammalian targets, which often resist characterization by other structural methods.

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