Spectroscopic analysis of the red-shifted channelrhodopsin-1 from *Chlamydomonas augustae*

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Channelrhodopsins are photoreceptors which control phototaxis in green algae. Electrophysiological experiments showed that they act as light-gated ion channels when heterologously expressed in oocytes or HEK cells. Due to this function these cation channels are meanwhile used in the new field of optogenetics where specific nerve cells are depolarized by light. Although the channelrhodopsins are already widely-used in neurophysiological applications, the mechanism how these proteins transfer ions, is still not clarified in detail. Most algae containing light-gated ion channels exhibit two different types of channelrhodopsins (ChR1 and 2) with apparent mechanistic differences.

In this study we analysed the red-shifted channelrhodopsin-1 from Chlamydomonas augustae (CaChR1). We want to understand the processes leading to the opening of this channel, which include isomerization of the retinal after light excitation and proton transfer reactions from the Schiff base which is protonated in the ground state. Therefore, we apply time-resolved spectroscopic methods to determine and compare the intermediate states on a time scale from 100 ns to 5 s. Resonance Raman spectroscopy as well as retinal extraction with HPLC detection determined an overall ratio of 70% all-trans and 30% 13-cis retinal in the ground state of solubilized CaChR1. By FTIR difference spectroscopy we identified large changes in the amide I region and characteristic bands for carboxylic groups, cysteines and dangling water molecules inside the protein.