Sequential all-trans to 13-cis to 11-cis photoisomerization in bestrhodopsin, an unusual red-absorbing microbial light-gated anion channel as revealed by femtosecond stimulated Raman spectroscopy

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Bestrhodopsins are a newly discovered class of light-regulated ion channels that consist of two rhodopsins in tandem fused with a bestrophin ion channel domain, together forming a giant pentameric ion channel structure. Bestrhodopsin of the marina alga Phaeocystis antarctica covalently bind all-trans retinal as Schiff-base (RSB) chromophores uniquely absorbing in the red at 660 nm, which conveys an excellent potential for optogenetic applications. Upon red light absorption, the rhodopsins switch to a metastable state absorbing green light at 540 nm denoted as P540. The P540 state corresponds to an unusual 11-cis RSB isomer, rather than the canonical 13-cis isomer found in nearly all other microbial rhodopsin photoreactions. To assess the reaction and isomerization dynamics of bestrhodopsin, we performed femtosecond – submillisecond transient absorption (TA) and femtosecond stimulated Raman spectroscopy (FSRS). Steady-state stimulated Raman spectroscopy of the bestrhodopsin dark state (D661) showed that it is very similar to the bacteriorhodopsin O intermediate, with a highly distorted all-trans RSB and protonated counterion, partly explaining the unusual red absorption of bestrhodopsin.

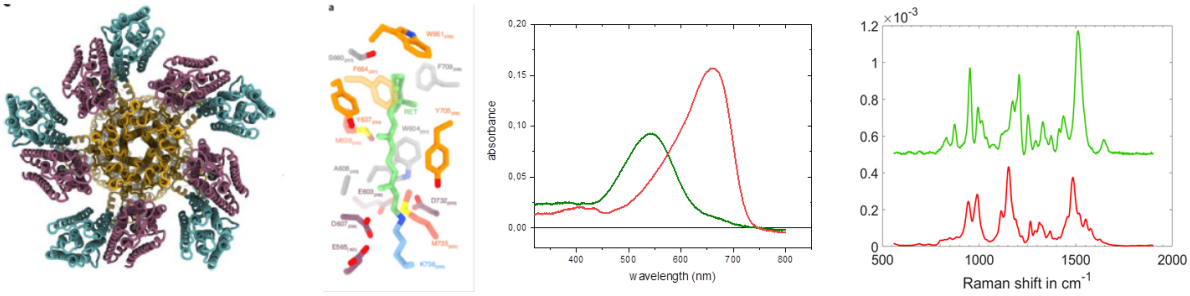

Figure 1. Structure of bestrhodopsin oligomer, configuration of retinal in the binding domain and electronic absorption and Raman vibration spectra of the Red and Green state.