Study on the dynamics of channel opening and closing of cation channelrhodopsin, C1C2

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Channelrhodopsins (ChR) are light-gated cation channels that non-specifically transport Na^+, H^+, Ca^{2+}, and other cations. ChR consists of heptahelical transmembrane helices covalently binding retinal Schiff base chromophore at a lysine residue in the seventh helix. Despite its extensive application in optogenetics, the detail of the mechanism of channel opening and closing of ChR remains unclear. Recently, it was proposed by high-level QM/MM calculation[1] and time-resolved serial femtosecond crystallography[2] that twisting and lateral movement toward TM3 of the retinal occur in the pre-open state.

To obtain the structural information about the retinal in the open state of ChR and to clarify the role played by the retinal governing the channel opening and closing, we conducted the time-resolved resonance Raman spectroscopy, a measurement of kinetic isotope effect (KIE) by the laser flash photolysis spectroscopy, and the laser patch-clamp for ChR C1C2. As the result, a gradual increase in Raman bands of hydrogen-out-of-plane modes, indicating enhancement of retinal twisting was observed after photo-excitation and it was maximized in the open state (P_{2b}) (Fig. 1). Furthermore, while the channel opening exhibited no KIE between in H_2O and D_2O, the channel closing of C1C2 became about 3-fold slower, indicating the channel closing is regulated by a proton transfer to the retinal from the protein moiety.

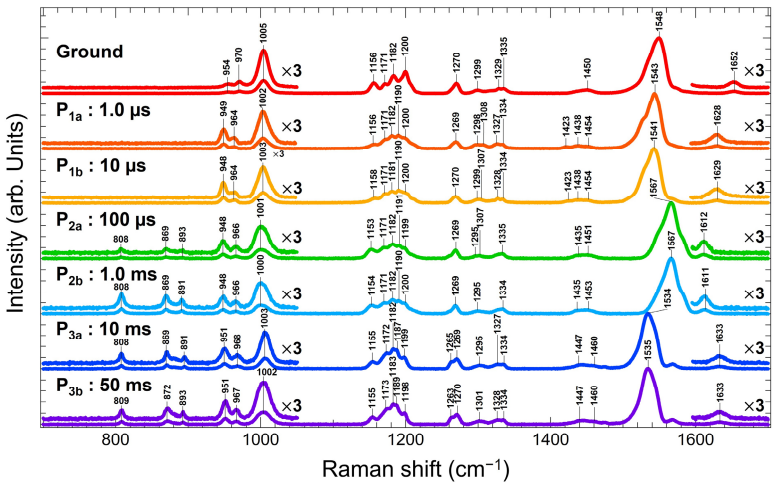

Figure 1. Resonance Raman spectra of C1C2

1) C. Cheng, *Biophys. J.* **2018**, *115*, 1281. 2) K. Oda, *eLife*. **2021**, *10*, e62389.