Electronic Supporting Information

Light-Induced Structural Changes during Early Photo-Intermediates of the Eubacterial Cl^- Pump *Fulvimarina* Rhodopsin Observed by FTIR Difference Spectroscopy

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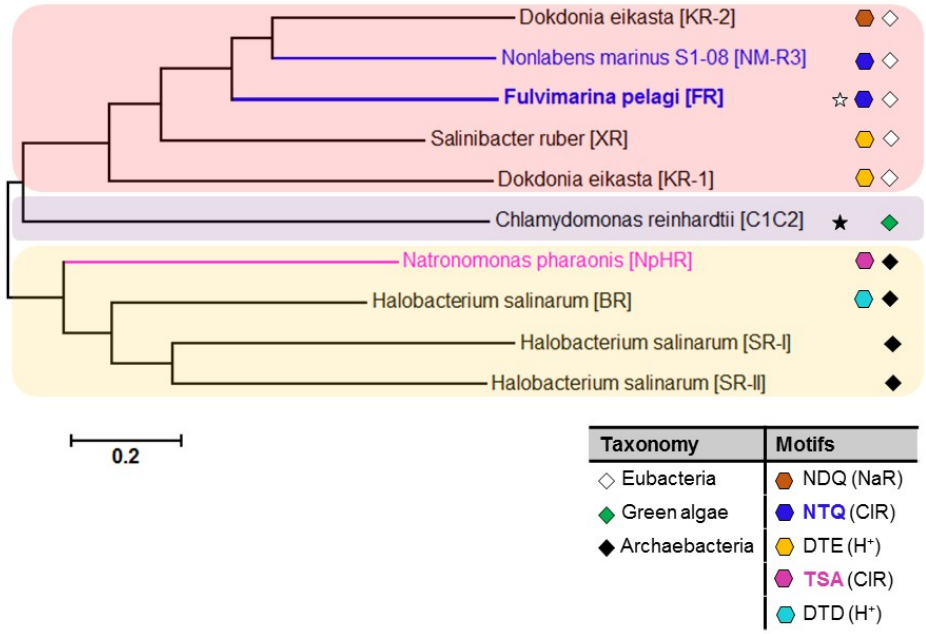

Fig. S1. Phylogenetic analysis of FR against different types of microbial rhodopsins namely, bacteriorhodopsin (BR), halorhodopsin (N*p*HR), xanthorhodopsin (XR), Sodium ion pump (KR2), Chloride ion pump (NM-R3 and FR), sensory rhodopsins (SR-I and SR-II), and chimeric channel rhodopsin (*C*1*C*2).

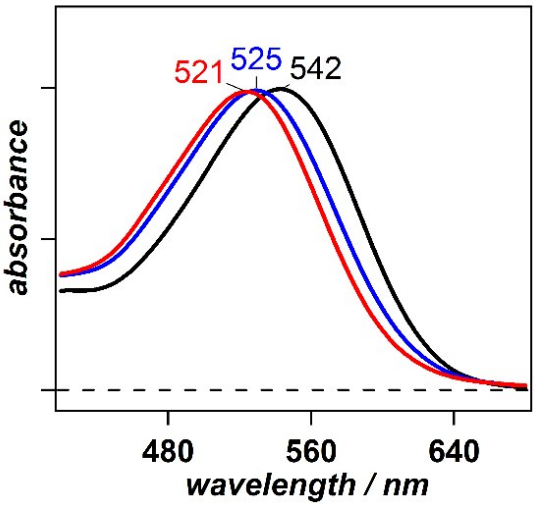

Fig. S2. UV-vis absorption spectra of β-DDM solubilized FR solution recorded at ambient temperature using a UV-vis spectrophotometer connected with integrating sphere for salt-free (black-line), 250 mM NaCl (blue-line) and 600 mM NaCl (red-line) containing-buffers, which clearly indicate the salt-dependency (blueshift) of FR as we reported previously.[1]

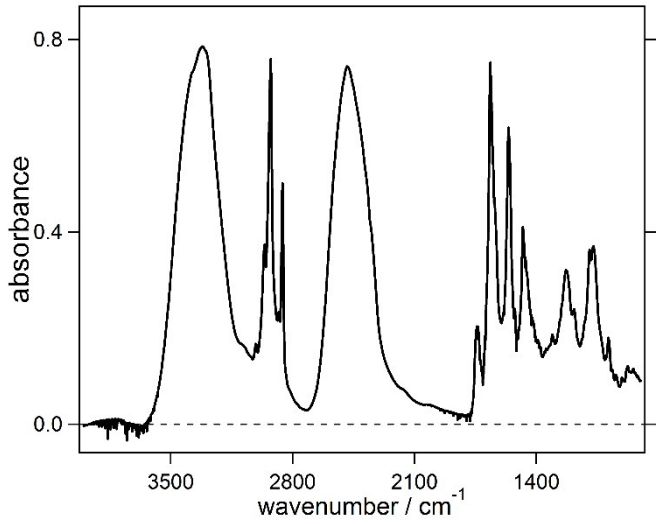

Fig. S3. A typical IR absorption spectrum of deuterium oxide hydrated film measured at 77 K of FR-liposomes. The FR-films are normally deuterated with ~1.5 µl D_2O divided into two drops and quickly assembled before mounting into a cryostat holder connected with the FTIR spectrometer.

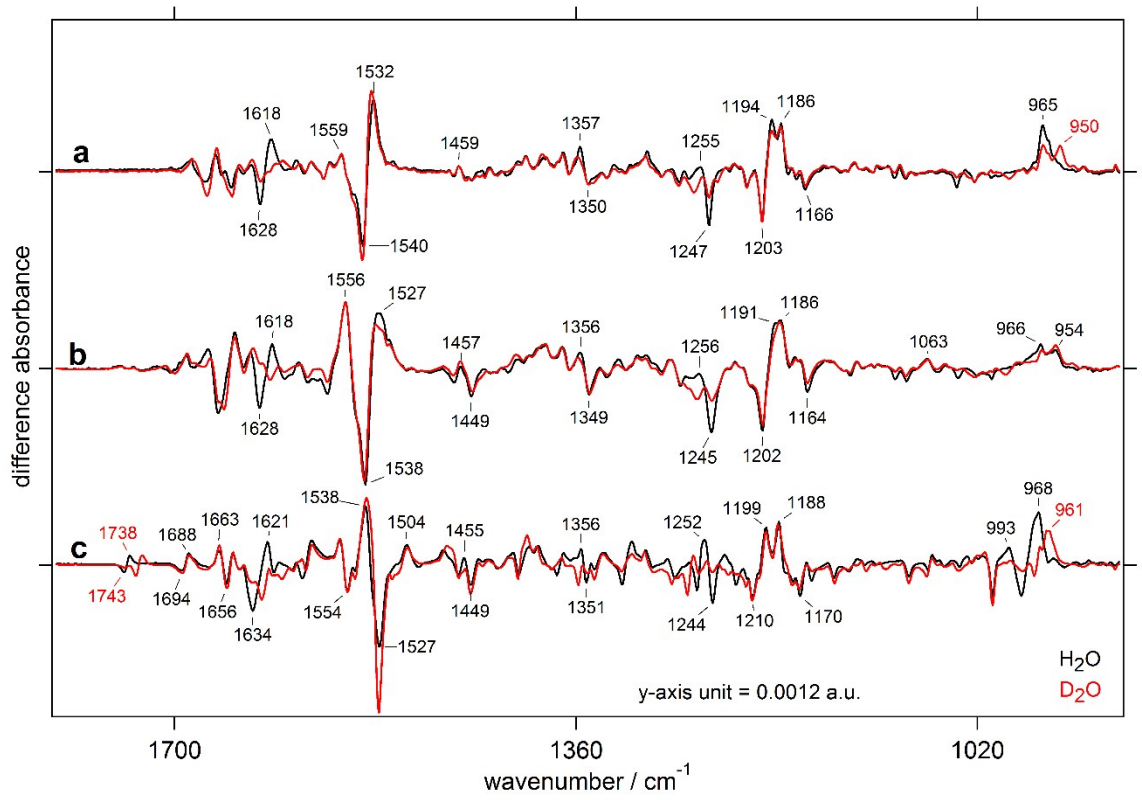

Fig. S4. Typical light-induced difference FTIR spectra of FR_K-minus-FR recorded at 77K (a), FR_L-minus-FR recorded at 220 K (b), and the archaeal halorhodopsin N*p*HR spectra of pHR_K-minus-*p*HR recorded at 77 K (c). Spectra at 220 K in (b) were normalized to the spectrum at 1186 cm^{-1} of the 77 K FTIR spectra and multiplied by a factor of 2.1 for H_2O and 1.4 for D_2O spectra.

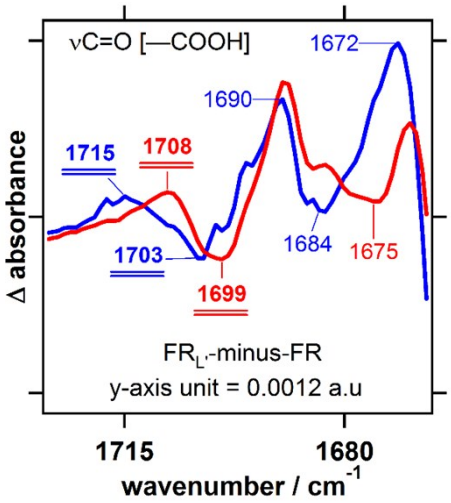

Fig. S5. Protonated carboxylic group (C=O stretching vibrations) at the 1728–1668 cm^{-1} frequency of likely an Asp residue(s) trapped at 220 K as a new component implying a re-protonation of a carboxylate residue nearby the retinal chromophore.