

Photochemical & Photobiological Sciences

Supporting Information

Characterization of photo-intermediates in the photo-reaction pathways of
in a bacteriorhodopsin Y185F mutant using *in situ* photo-irradiation solid-
state NMR spectroscopy

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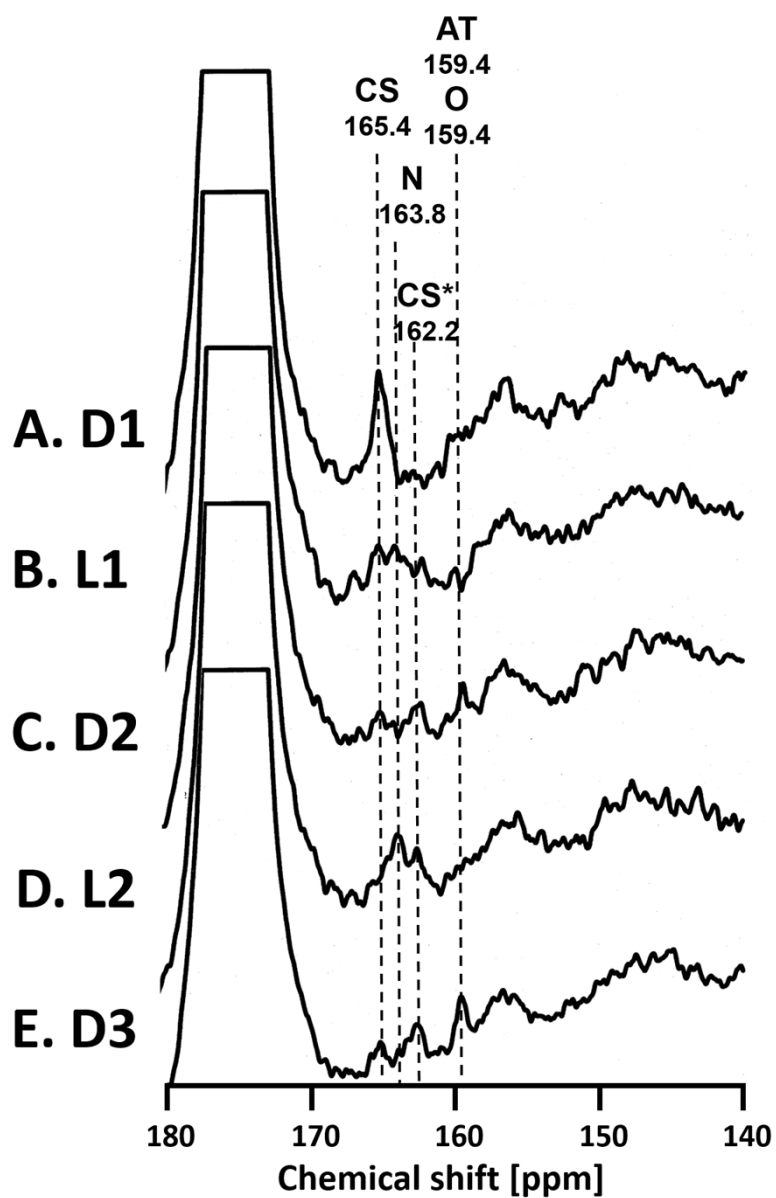


Figure S1. ^{13}C CP-MAS NMR spectra of $[15\text{-}^{13}\text{C}]\text{Ret-Y185F-bR}$ at $-40\text{ }^\circ\text{C}$ and 4 kHz spinning frequency under various conditions. NMR spectra were collected (A. D1) in the dark, (B. L1) under irradiation with 520 nm light, (C. D2) in the dark, and (D. L2) under irradiation with 520 nm light.

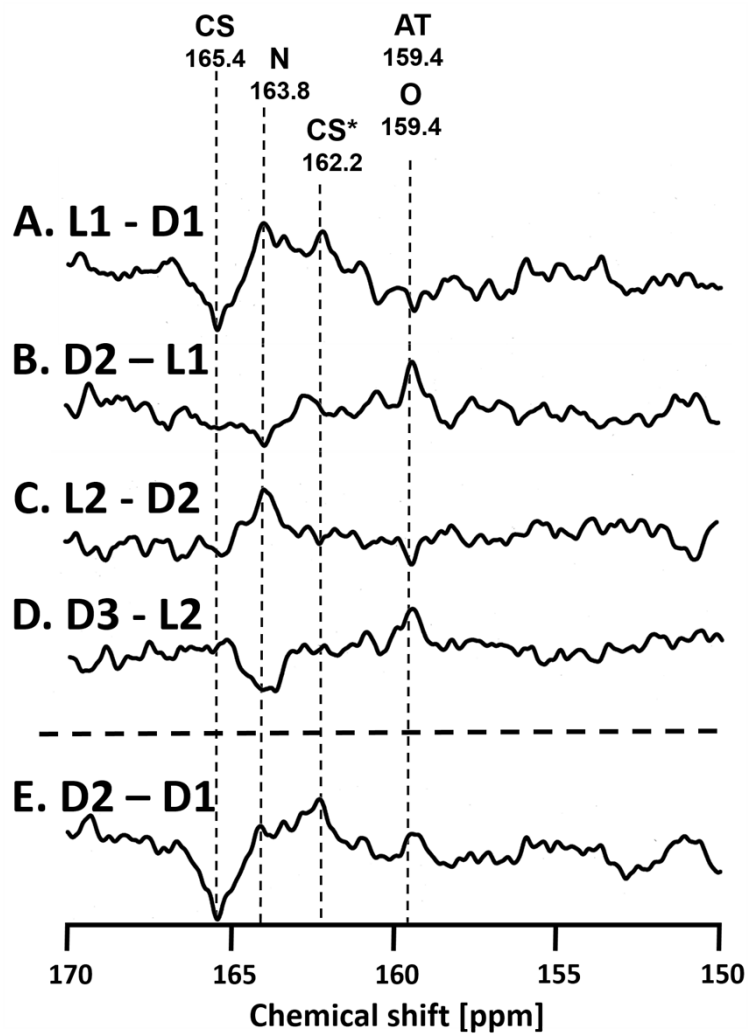


Figure S2. Difference spectra of $[15-^{13}\text{C}]$ Ret-Y185F at $-40\text{ }^\circ\text{C}$ and 4 kHz spinning frequency in the pathway from (A. L1-D1) D1 to L1, (B. D2-L1) L1 to D2, (C. L2-D2) D2 to L2, (D. D3-L2) L2 to D3, and (E. D2-D1) D1 to D2.

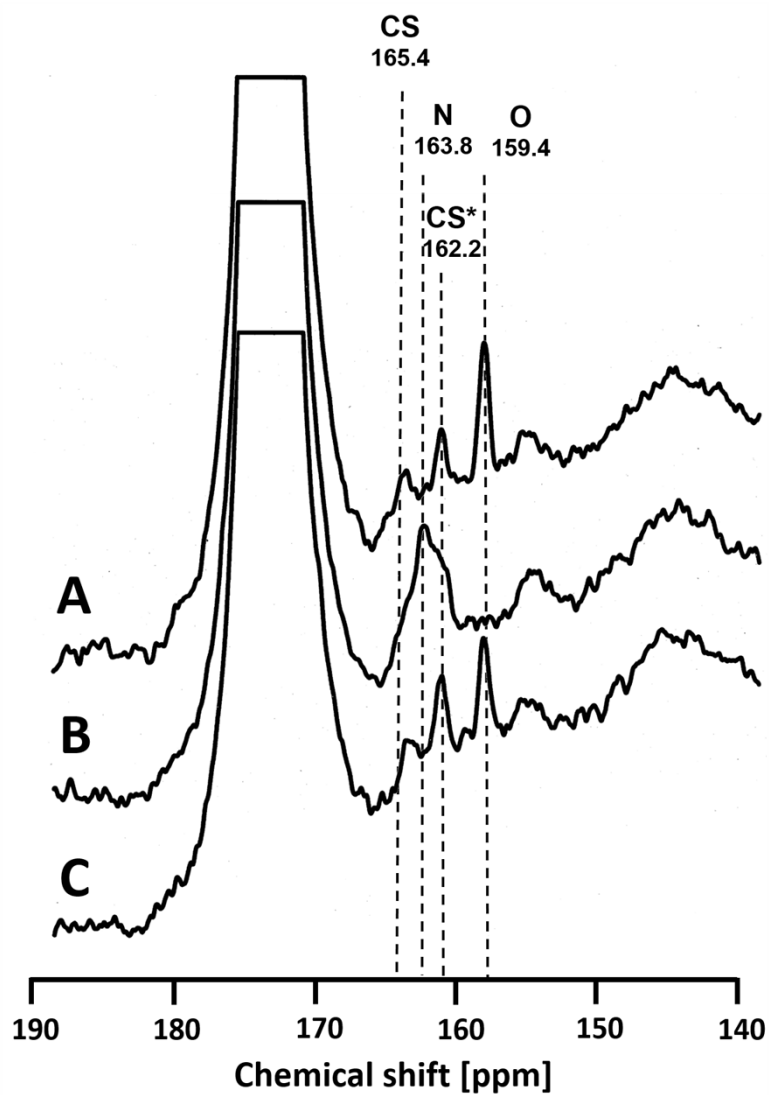


Figure S3. ^{13}C CP-MAS NMR spectra of $[15\text{-}^{13}\text{C}]\text{Ret-Y185F-bR}$ at $-40\text{ }^\circ\text{C}$ and 6 kHz spinning frequency under various conditions. NMR spectra were measured (A. D2) in the dark after irradiation with 520 nm light, (B. L2) under irradiation with 520 nm light, and (C. D3) in the dark.

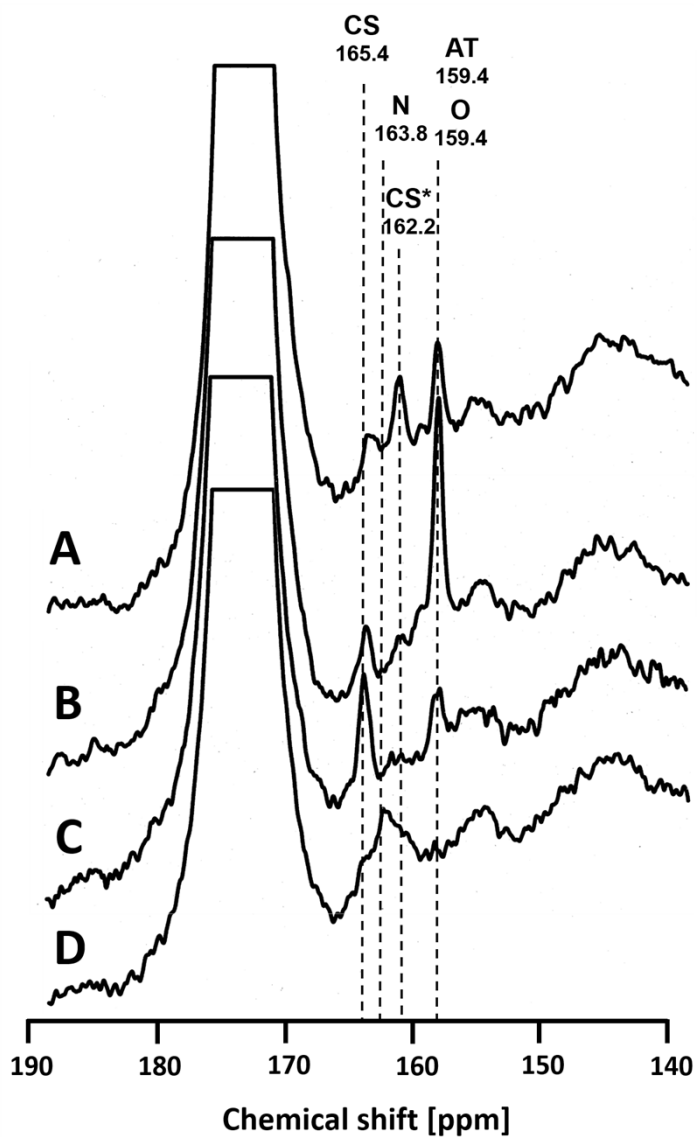


Figure S4. ^{13}C CP-MAS NMR spectra of $[15\text{-}^{13}\text{C}]\text{Ret-Y185F-bR}$ at 6 kHz spinning frequency under various conditions. (A) Collected at $-40\text{ }^\circ\text{C}$ in the dark after irradiation with 520 nm light. (B) After increasing the temperature to $-20\text{ }^\circ\text{C}$, and collected at $-40\text{ }^\circ\text{C}$. (C) After increasing the temperature to $20\text{ }^\circ\text{C}$, and collected at $-40\text{ }^\circ\text{C}$. (D) Measured the NMR spectrum at $-40\text{ }^\circ\text{C}$ under irradiation with 520 nm light.

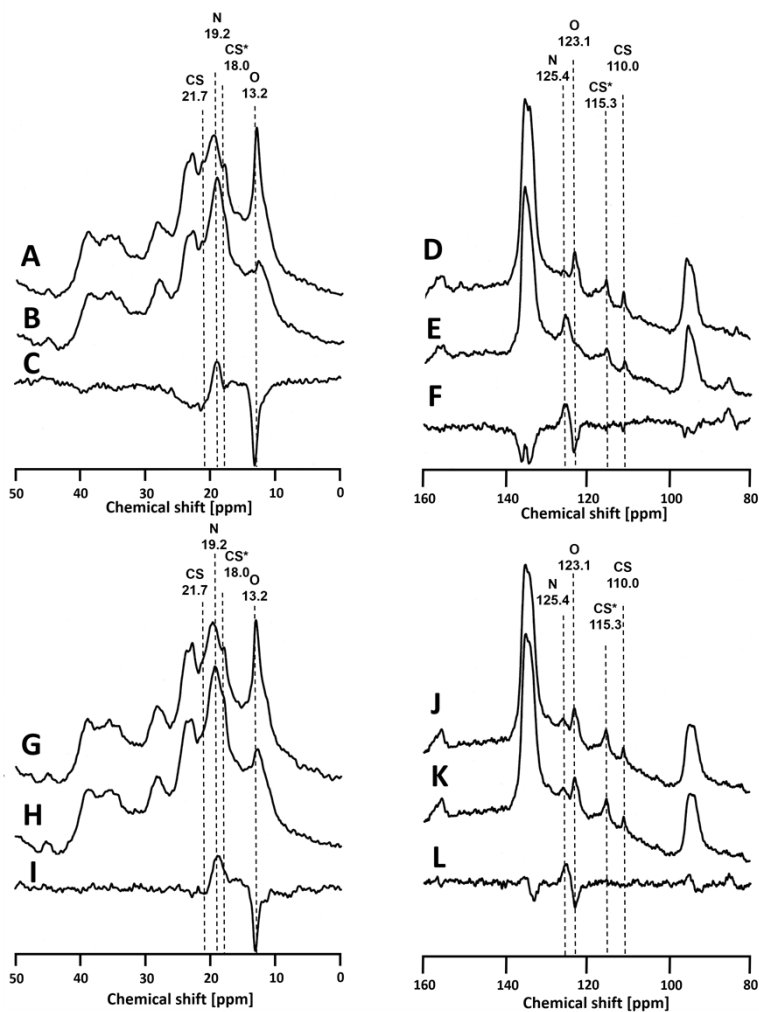


Figure S5. ^{13}C CP-MAS NMR spectra of $[20\text{-}^{13}\text{C}]\text{Ret-Y185F-bR}$ at $-40\text{ }^{\circ}\text{C}$ (A. D) in the dark after irradiation with 520 nm light, (B. E) under irradiation with 520 nm light, and (C, F) difference spectra (B - A) and (E - D), respectively. ^{13}C CP-MAS NMR spectra of $[20\text{-}^{13}\text{C}]\text{Ret-Y185F-bR}$ at $-40\text{ }^{\circ}\text{C}$ (G. J) in the dark after irradiation with 520 nm light, (H. K) under irradiation with 595 nm (orange) light, (I, L) difference spectra (H - G) and (K - J), respectively.

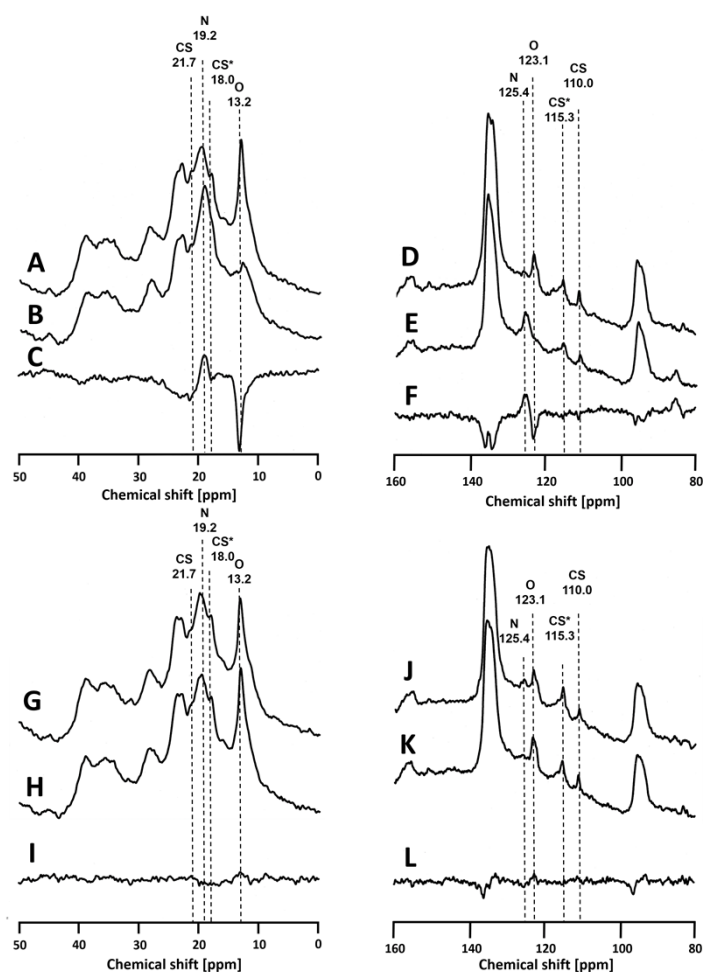


Figure S6. ^{13}C CP-MAS NMR spectra of $[20\text{-}^{13}\text{C}]\text{Ret-Y185F-bR}$ at $-40\text{ }^{\circ}\text{C}$ (A. D) in the dark after irradiation with 520 nm light, (B. E) under irradiation with 520 nm light, and (C, F) difference spectra (B - A) and (E - D), respectively. ^{13}C CP-MAS NMR spectra of $[20\text{-}^{13}\text{C}]\text{Ret-Y185F-bR}$ at $-40\text{ }^{\circ}\text{C}$ (G. J) in the dark after irradiation with 595 nm light, (H. K) under irradiation with 365 nm (blue) light, (I, L) difference spectra (H - G) and (K - J), respectively.