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Low-temperature Raman spectroscopy of sodium-pump rhodopsin from *Indibacter alkaliphilus*: Insight of Na^+ binding for active Na^+ transport

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Supporting Information

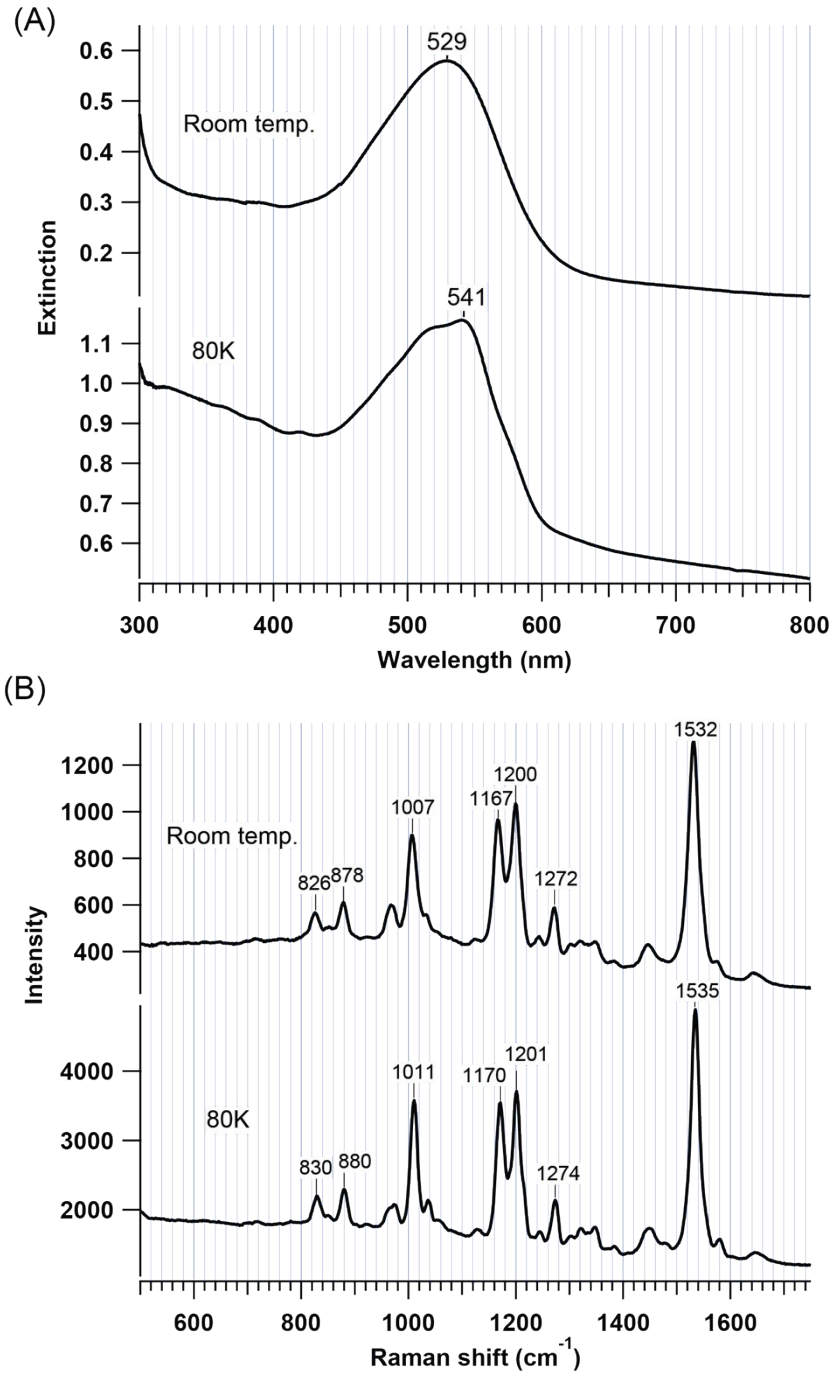

Figure S1. (A) Extinction and (B) Raman spectra of wild-type *Ia*NaR with NaCl at room temperature and 80K

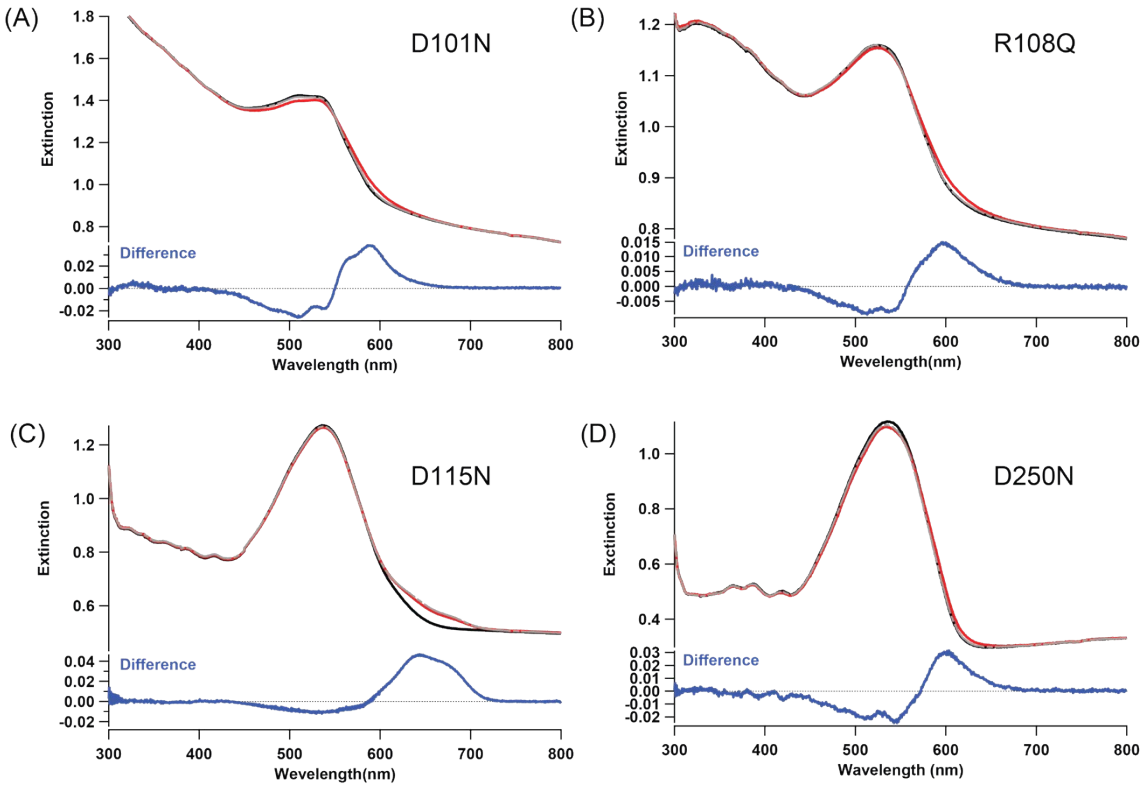

Figure S2. Extinction spectra of *Ia*NaR mutants of (A) D101N, (B) R108Q, (C) D115N, and (D) D250N at 80K. The samples were prepared at pH 8.0 with NaCl. Black: unphotolyzed state, red: after green-LED excitation, grey: after subsequent red-LED excitation, blue: difference between the spectrum of unphotolyzed state and that after green-light excitation.

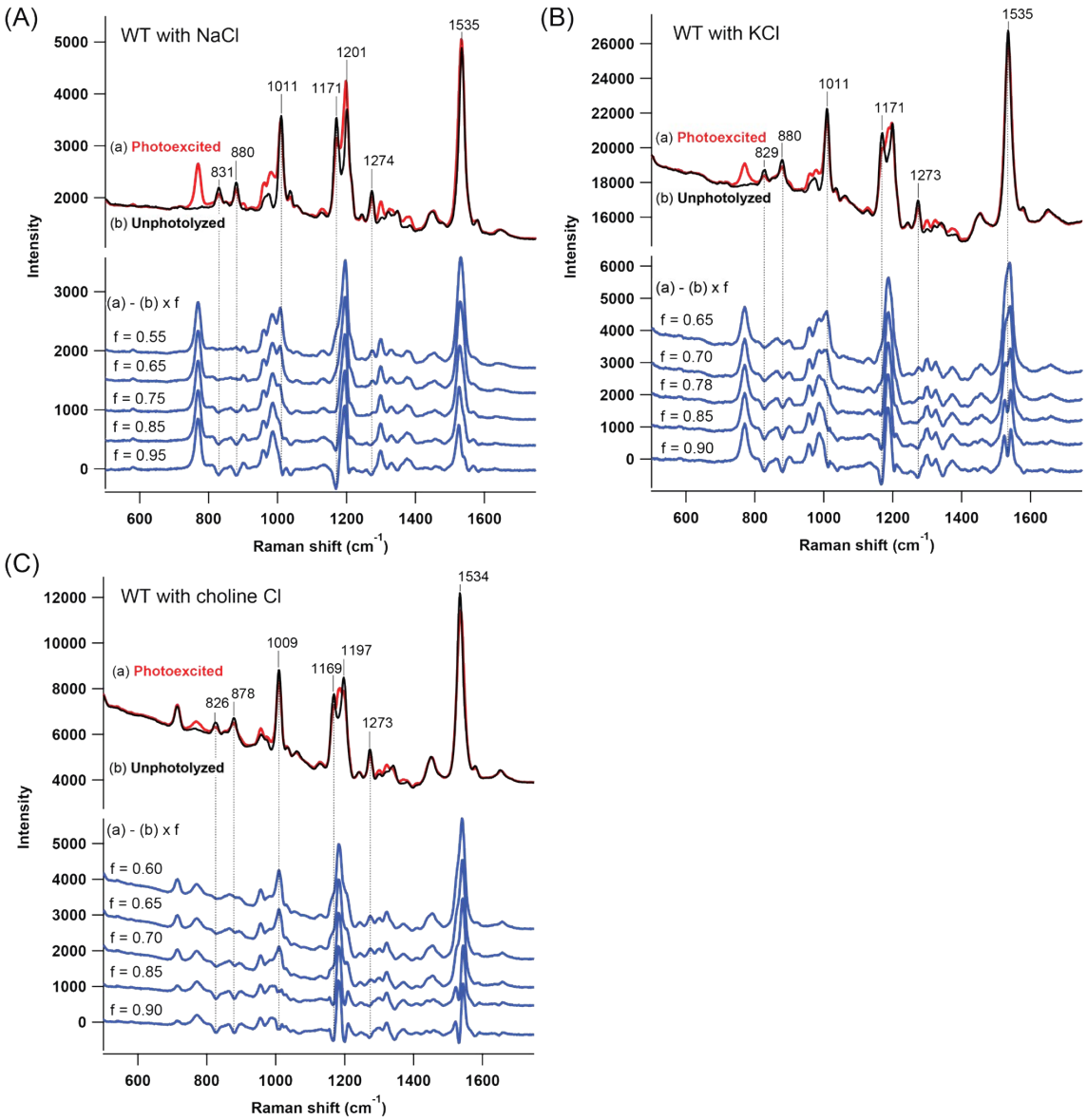

Figure S3. Raman spectra of wild-type *Ia*NaR at 80K in the presence of (A) NaCl, (B) KCl, and (C) choline Cl. (a) after green-LED excitation, (b) unphotolyzed state. Difference spectra are obtained as (a) – (b)×f with f being the scaling factor, and shown in blue. f=0.65, 0.70, and 0.70 are employed for the K-intermediate spectra with NaCl, KCl, and choline Cl, respectively.

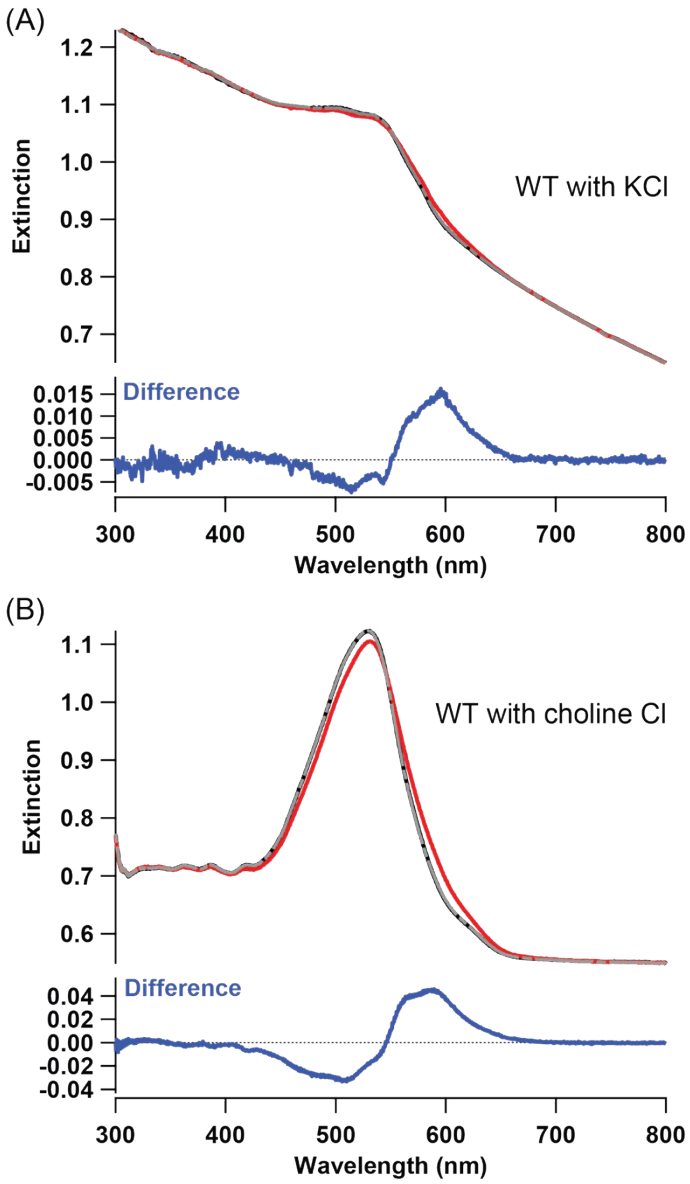

Figure S4. Extinction spectra of wild-type *Ia*NaR at 80K in presence of (A) KCl and (B) choline Cl. The samples were prepared at pH 8.0. Black: unphotolyzed state, red: after green-LED excitation, grey: after subsequent red-LED excitation, blue: difference between the spectrum of unphotolyzed state and that after green-light excitation.

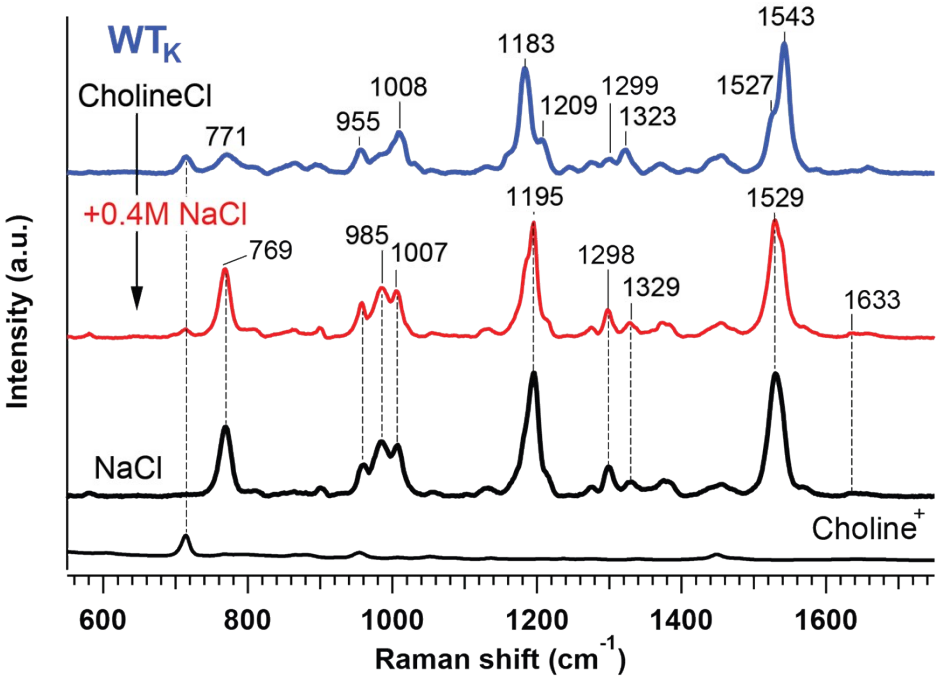

Figure S5. Raman spectrum of K intermediate of wild-type *Ia*NaR (WT_K) when adding NaCl to the sample prepared with choline Cl. Blue: obtained from sample film prepared with choline Cl, red: after adding a few drops of 0.4M NaCl solution to the sample film. For comparison, Raman spectrum of WT_K obtained from the sample prepared with NaCl, and Raman spectrum of choline Cl are shown in black.

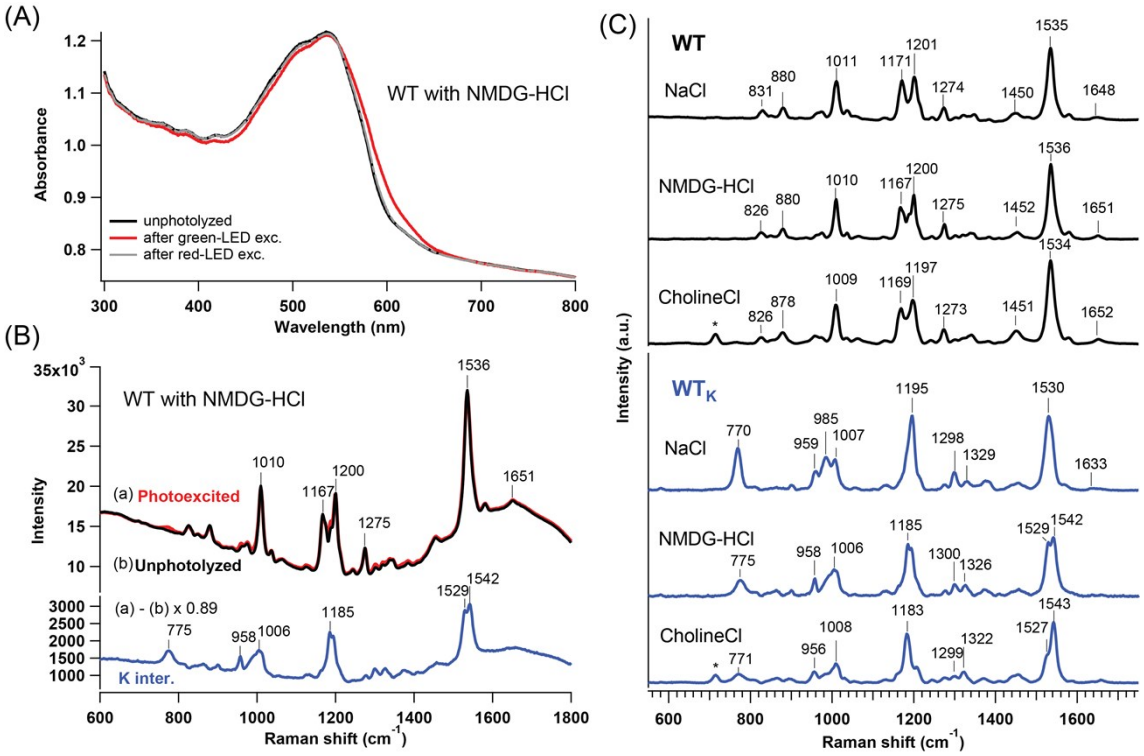

Figure S6. (A) Absorption spectra of wild-type *Ia*NaR (WT) at 80K in the presence of N-methyl-D-glucamine hydrochloride (NMDG-HCl); (black) unphotolyzed state, (red) after green-LED excitation, (gray) after subsequent red-LED excitation. (B) Raman spectra of WT at 80K in the presence of NMDG-HCl, (black) unphotolyzed state, (red) after green-LED excitation, (blue) K intermediate. (C) Comparison of Raman spectra of WT in the presence of NMDG-HCl, NaCl and choline Cl; (black) unphotolyzed state, (blue) K intermediate.

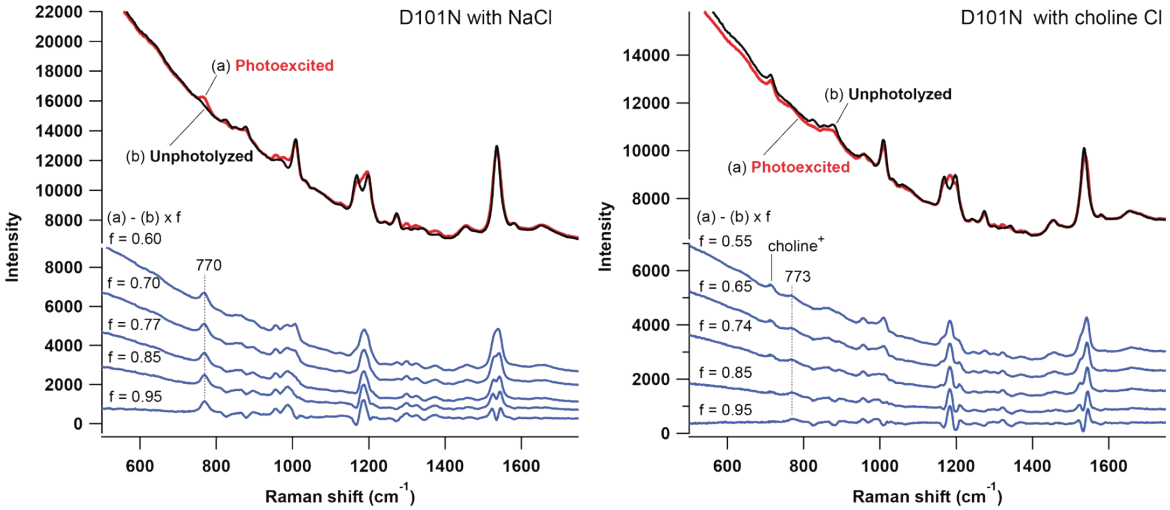

Figure S7. Raman spectra of D101N at 80K in the presence of (left) NaCl and (right) choline Cl. (a) after green-LED excitation, (b) unphotolyzed state. Difference spectra are obtained as (a) – (b)×f with f being the scaling factor, and shown in blue.

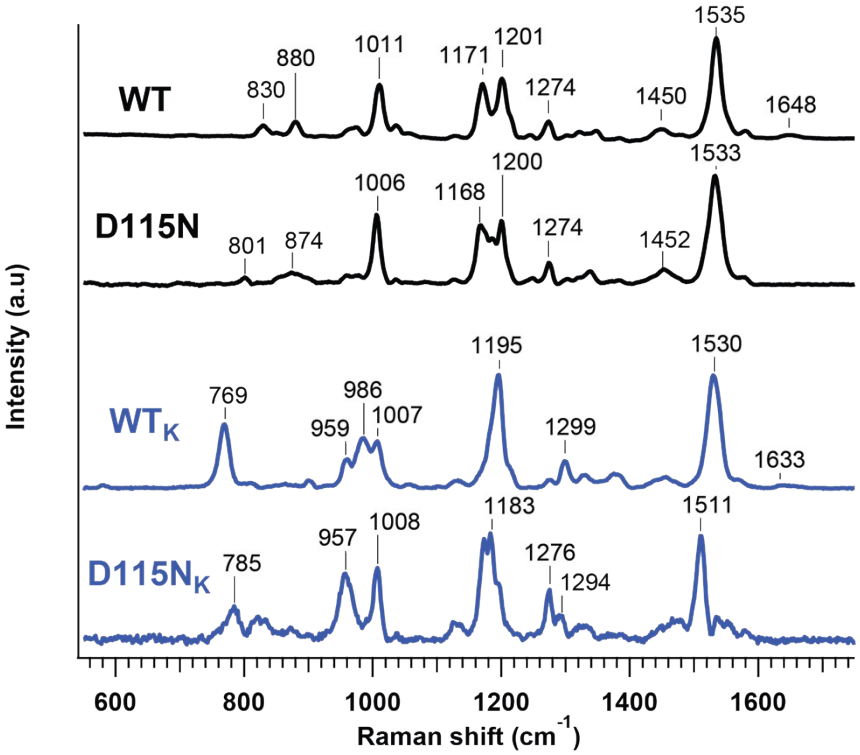

Figure S8. Raman spectra of D115N mutant of *Ia*NaR in the presence of NaCl. Black: unphotolyzed state, blue: K intermediate. The spectra of wild-type *Ia*NaR (WT) and the K intermediate (WT_K) are shown for comparison.

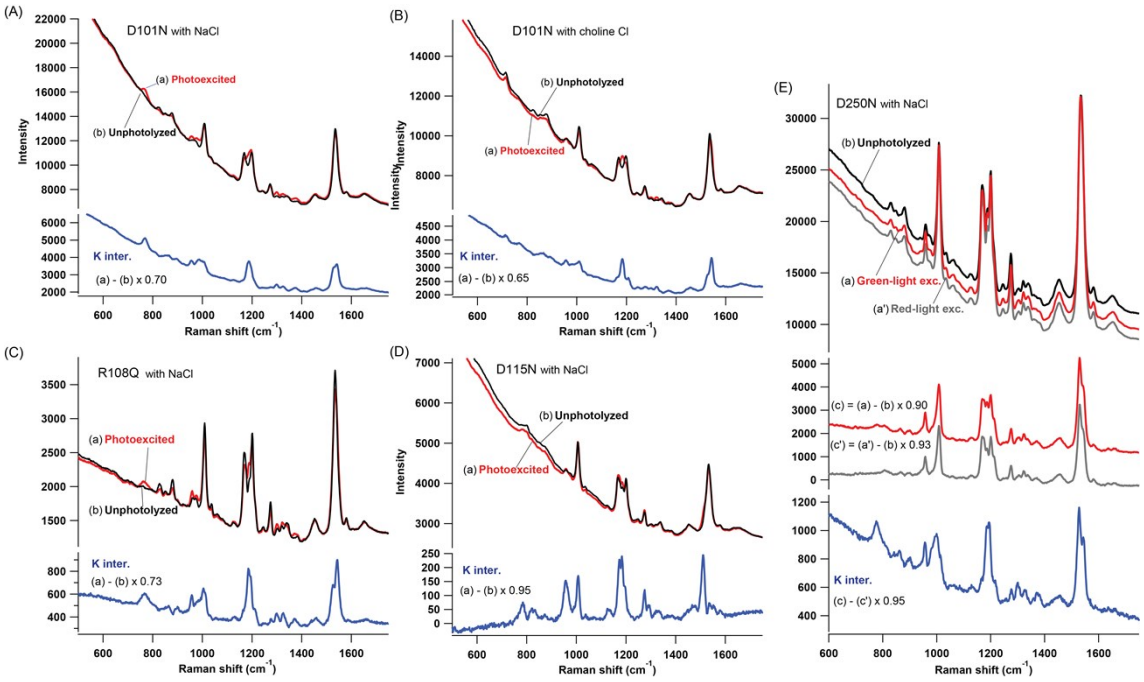

Figure S9. Raman spectra of (A) D101N with NaCl, (B) D101N with choline Cl, (C) R108Q with NaCl, (D) D115N with NaCl, and (E) D250N with NaCl, at 80K. For A-D, (a) spectra after photoexcitation by green-light excitation and (b) spectra of unphotolyzed states are shown in red and black, respectively. The spectra of K intermediates are obtained as (a) – (b) × f with f being the scaling factor, and shown in blue. For E, (a) spectrum after photoexcitation by green-light excitation and (a') spectrum after subsequent red-light excitation are shown in red and grey, respectively. The spectra of photoproducts produced by green light and red light are obtained as (c) = (a) – (b) × 0.90 and (c') = (a') – (b) × 0.93, respectively. The spectrum of K intermediate is obtained as (c) – (c') × 0.95 with f being the scaling factor, and shown in blue.

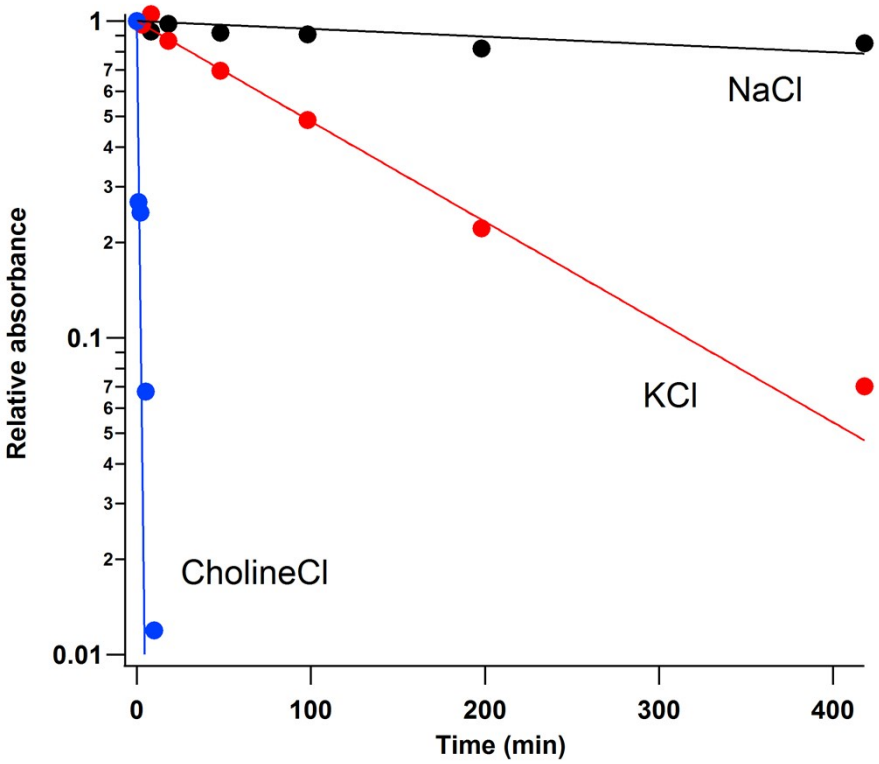

Figure S10. Thermal stability measurement of the detergent-solubilized *Ia*NaR in the presence of NaCl (1.4 M), KCl (1.4 M), and choline Cl (0.4 M). The thermal bleaching of absorption band at 348 K is plotted as a function of time. The time constants of thermal bleaching are 1800 min, 137 min, and 1 min in the presence of NaCl, KCl, and choline Cl, respectively.