Figure S1

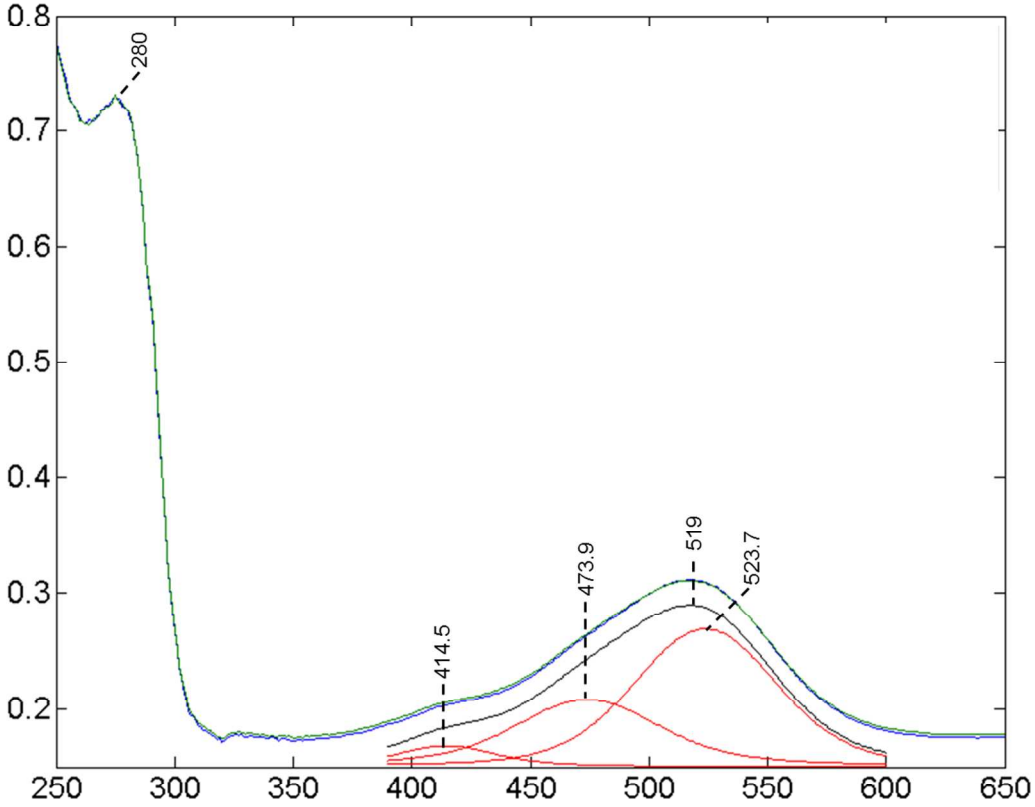

Figure S1. Visible Absorption of Light and Dark-adapted CaChR1. The light and dark-adapted spectra are very similar and shown as green and blue plots, respectively. The subcomponent bands for the light-adapted spectrum are also shown (red traces). The black trace is the sum of the three fitted bands. After base-line correction the peak of the band in the UV region appears at 280 nm. See Materials and Methods for additional information.

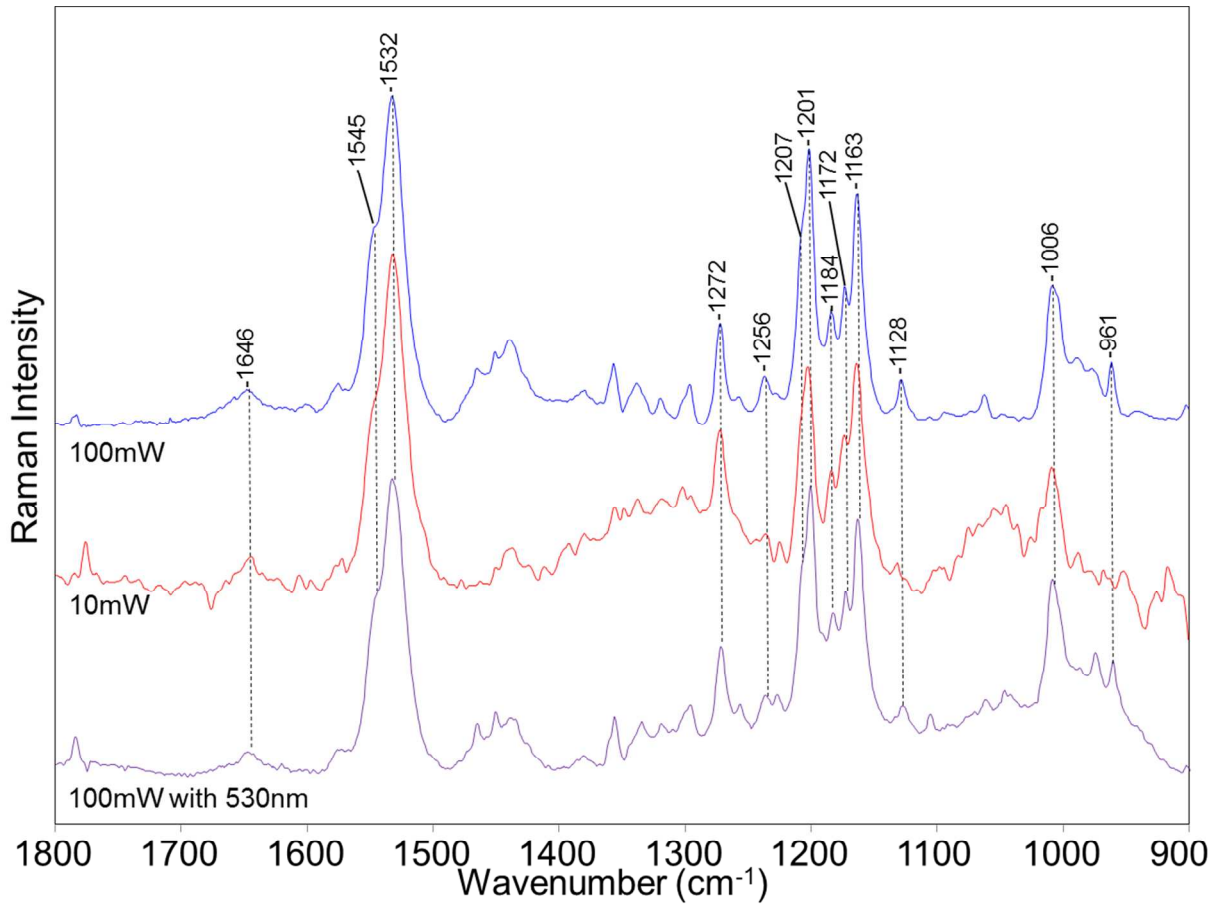

Figure S2. Comparison of *Ca*ChR1 resonance Raman spectra recorded under different conditions. *Top*: RRS recorded at pH 7 with 100mW 785-nm laser excitation power. *Middle*: pH 7, 10mW. *Bottom:* same conditions as top except with 20mW/cm^2 continuous illumination from a 530-nm emitting LED.

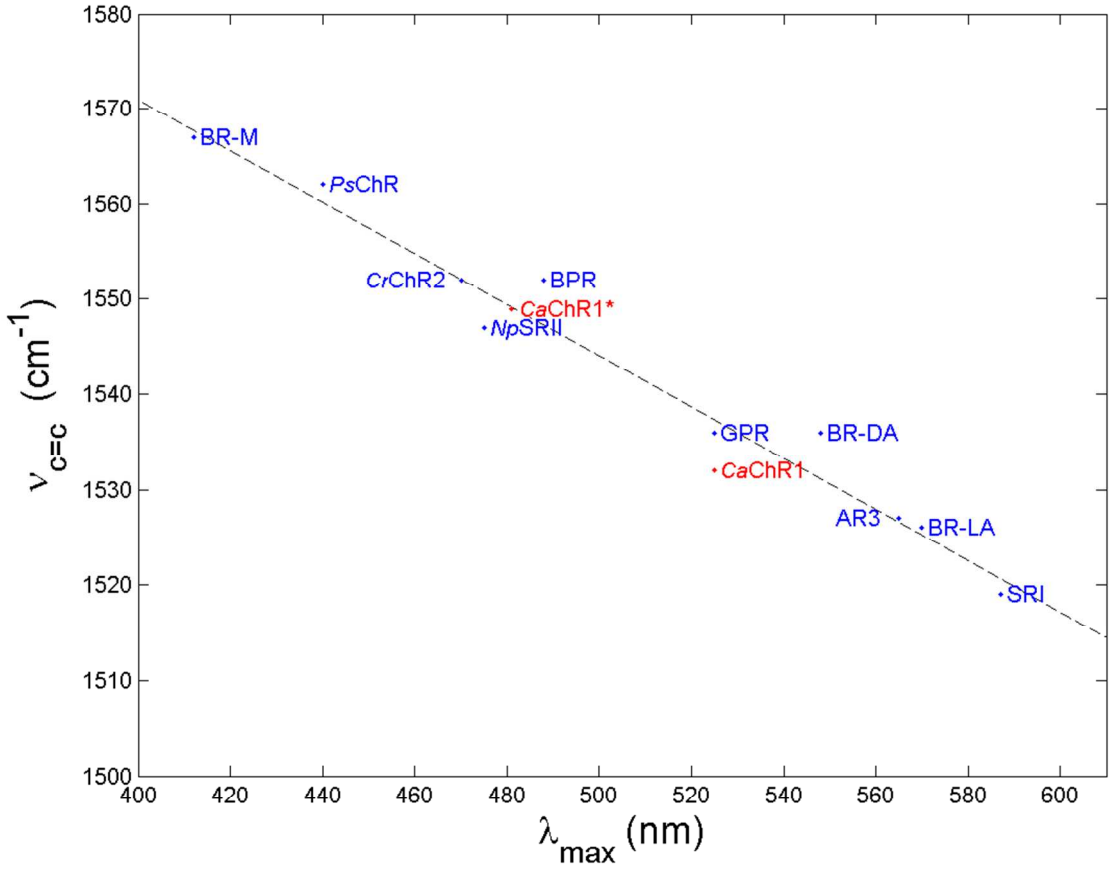

Figure S3. Inverse linear correlation between ethylenic frequency and visible absorption wavelength maximum of several microbial rhodopsins including *Ca*ChR1. All wavelength and frequency values are from this paper (*Ca*ChR1, *Cr*ChR2, NpSRII, light-adapted BR) or from previously published data: light-adapted AR3 (*1*); BR dark-adapted (*2*); BR M-intermediate (*3,4*); BPR and GPR (*5*); SRI (*6*).

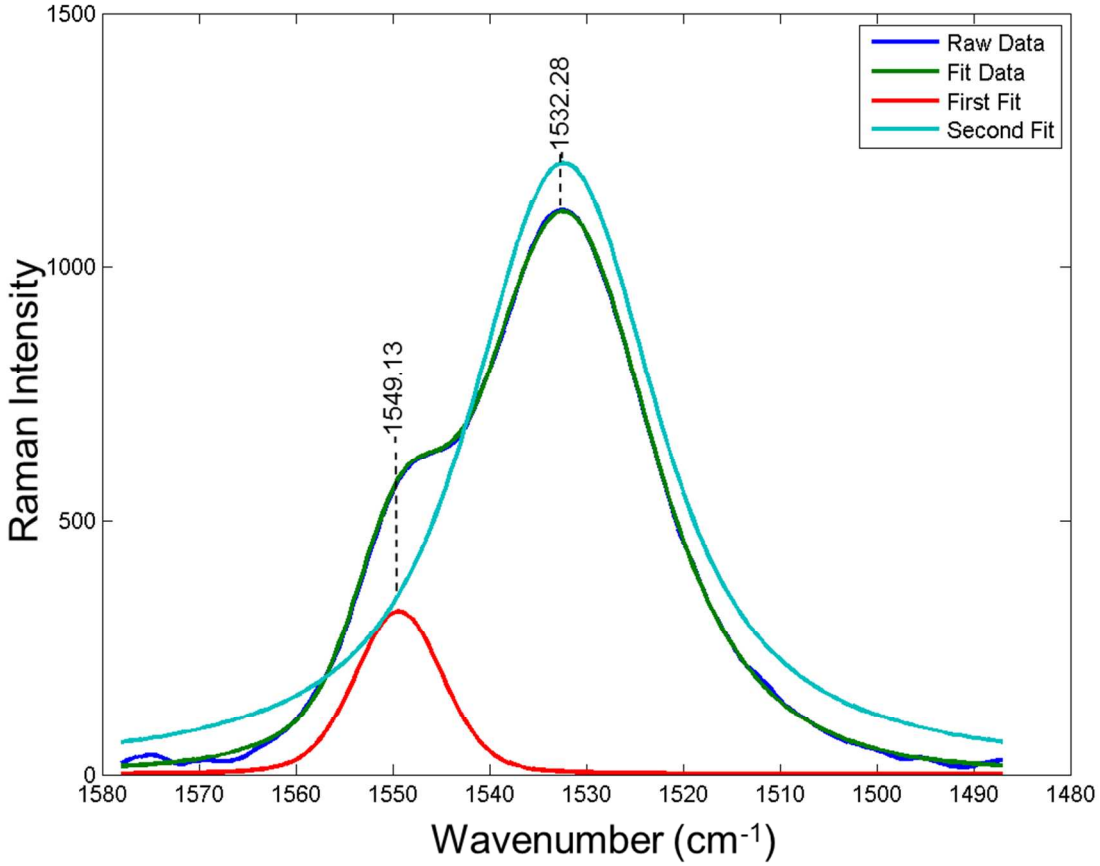

Figure S4. Curve fit of ethylenic region of the RRS of *Ca*ChR1 at pH 7. Fitted data is same as shown in Figure 2. Curve fitting was performed using the GRAMS spectroscopy suite (Thermo-Fischer Scientific) as described in Materials and Methods. Two Voigtian components (red and light blue) plus a baseline (not shown) resulted in a close fit (green curve) to the raw data (blue curve). A similar analysis was used for RRS at other pHs and also for the two mutants E169Q and D299N in order to determine the frequency, intensity and full-width at half-maximum of each of the component bands.

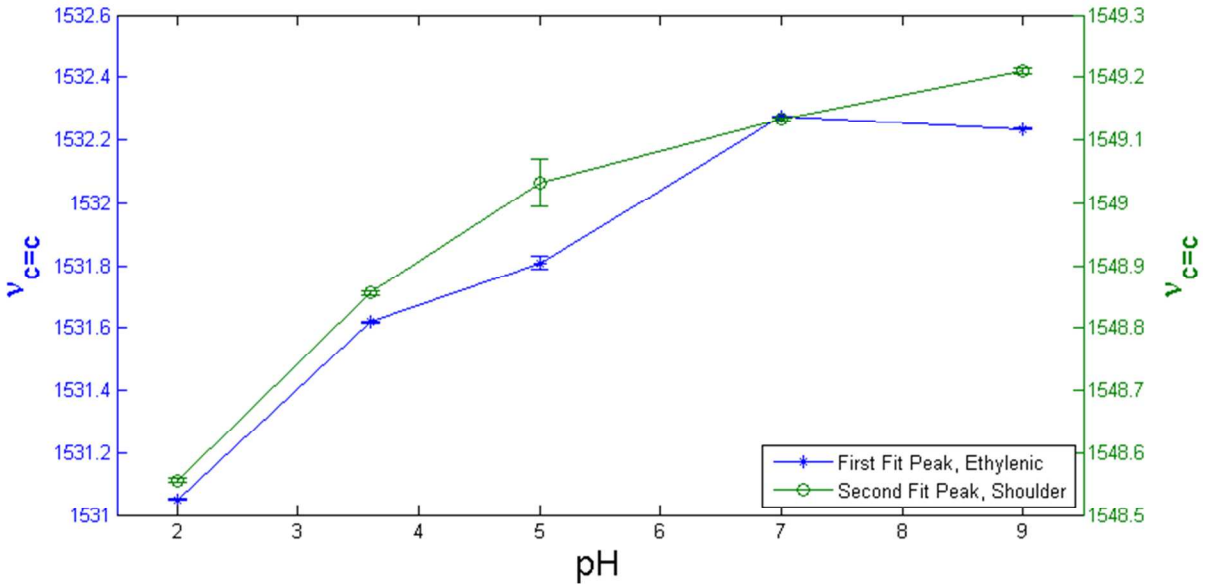

Figure S5. Peak frequency of two fitted components in ethylenic region of RRS of *Ca*ChR1 as a function of pH. The two component bands of the ethylenic region (main peak in blue and shoulder in green) were fit using GRAMS for each of the pH values reported in figure 4 and plotted as a function of pH. An example of a single fit is provided in Figure S4. Error bars shown indicate error in the fitted frequency from a single spectrum.

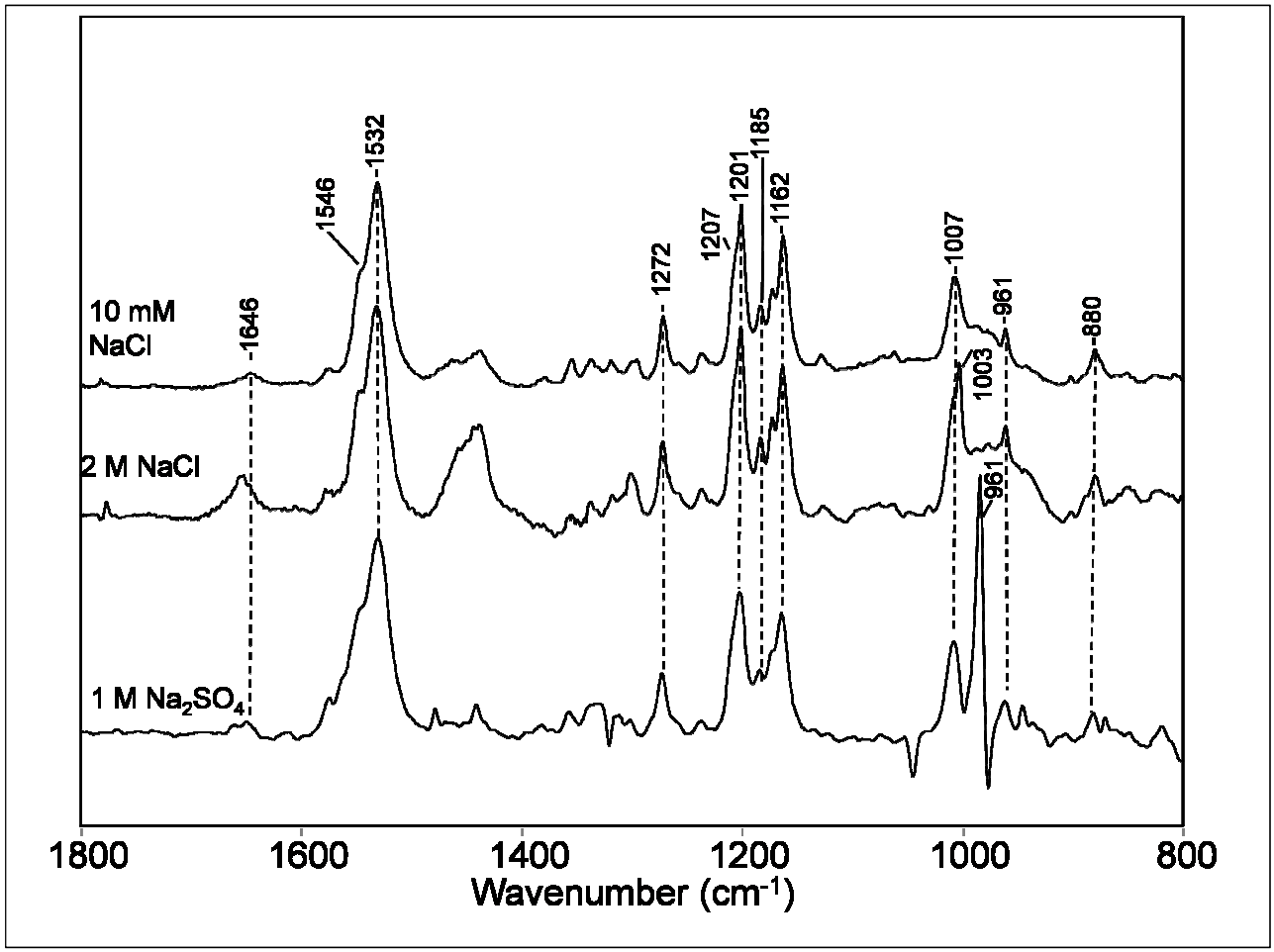

Figure S6. Effects of salt concentration and anion species on the RRS of *Ca*ChR1 at pH3. All conditions used for RRS were the same as described in Figure 2 of paper and as described in Materials and Methods. Top trace is pH3.6 spectrum shown in Figure 4. Buffer used to record bottom two traces are described in Materials and Methods.

References for Supplementary Material

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