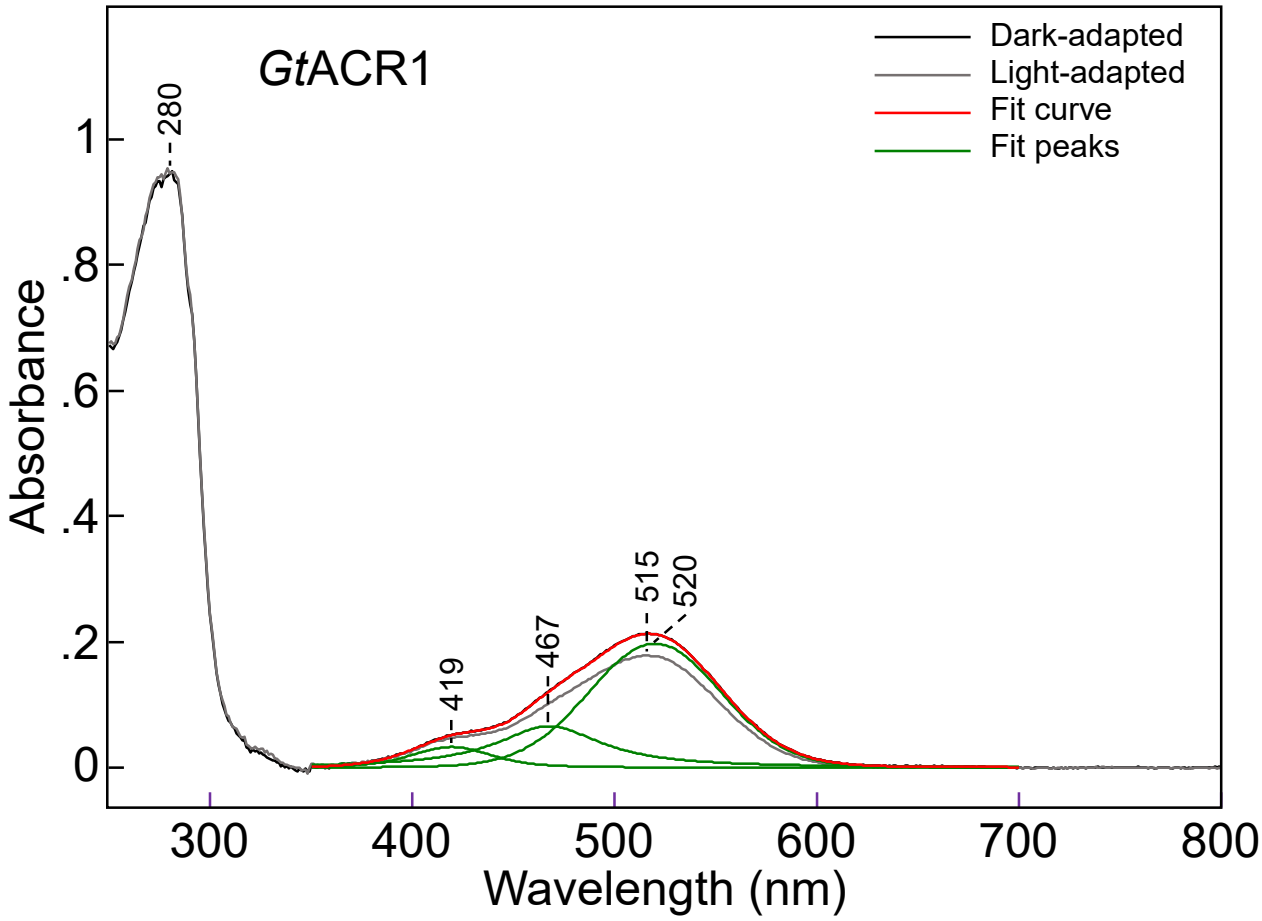
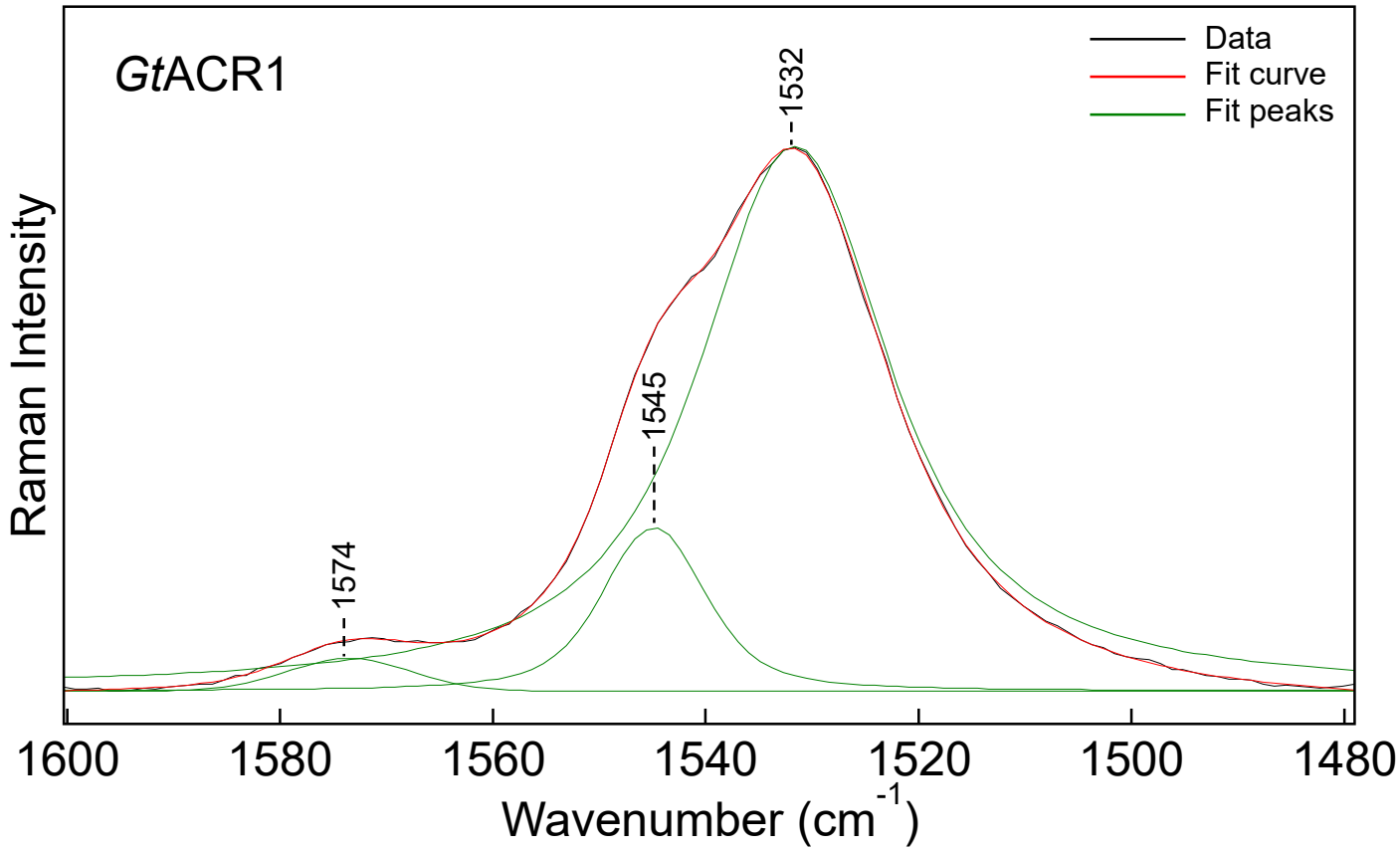
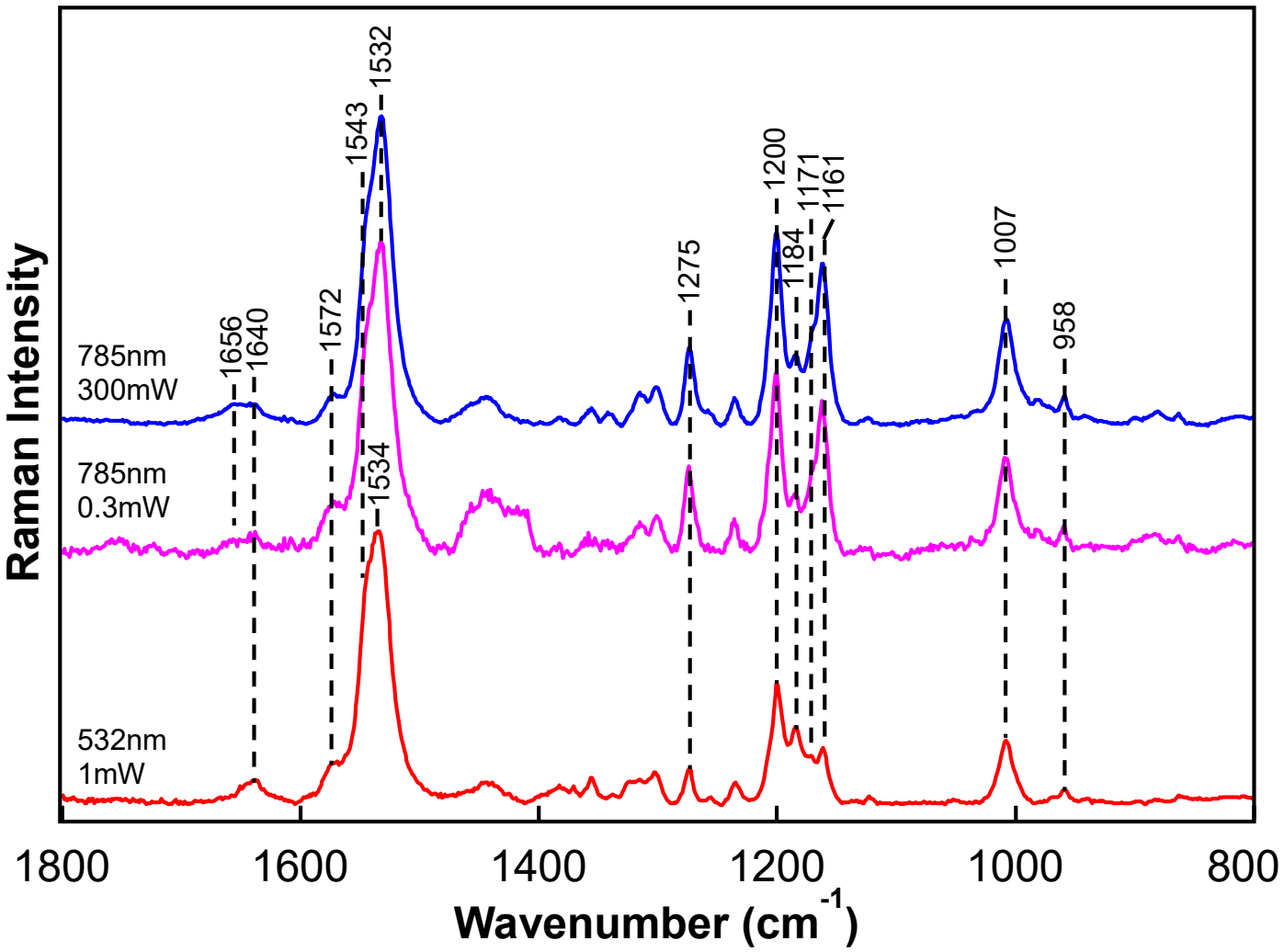
Supplementary Figures



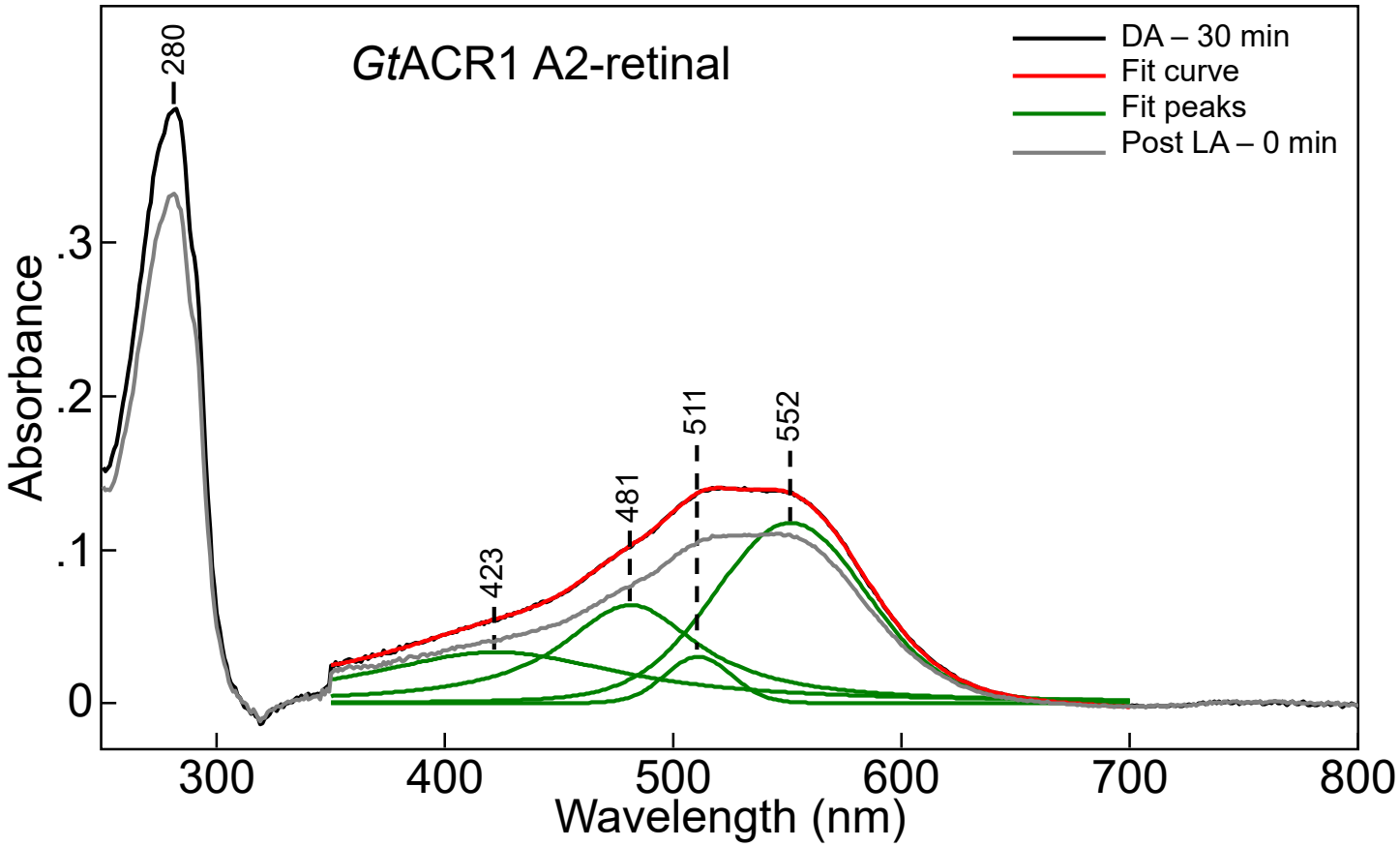
Supplementary Figure 1. Curve fit of λ_{max} region of the UV/Vis spectrum of *Gt*ACR1 at pH 7. Spectra were acquired using a Cary 6000 (Agilent Technologies) using steps of 1 nm. Curve fitting was performed using the GRAMS spectroscopy suite (Thermo-Fischer Scientific). Three Voigtian components (green curves) plus a linear baseline (not shown) resulted in a close fit (red curve) to the raw data (black curve).



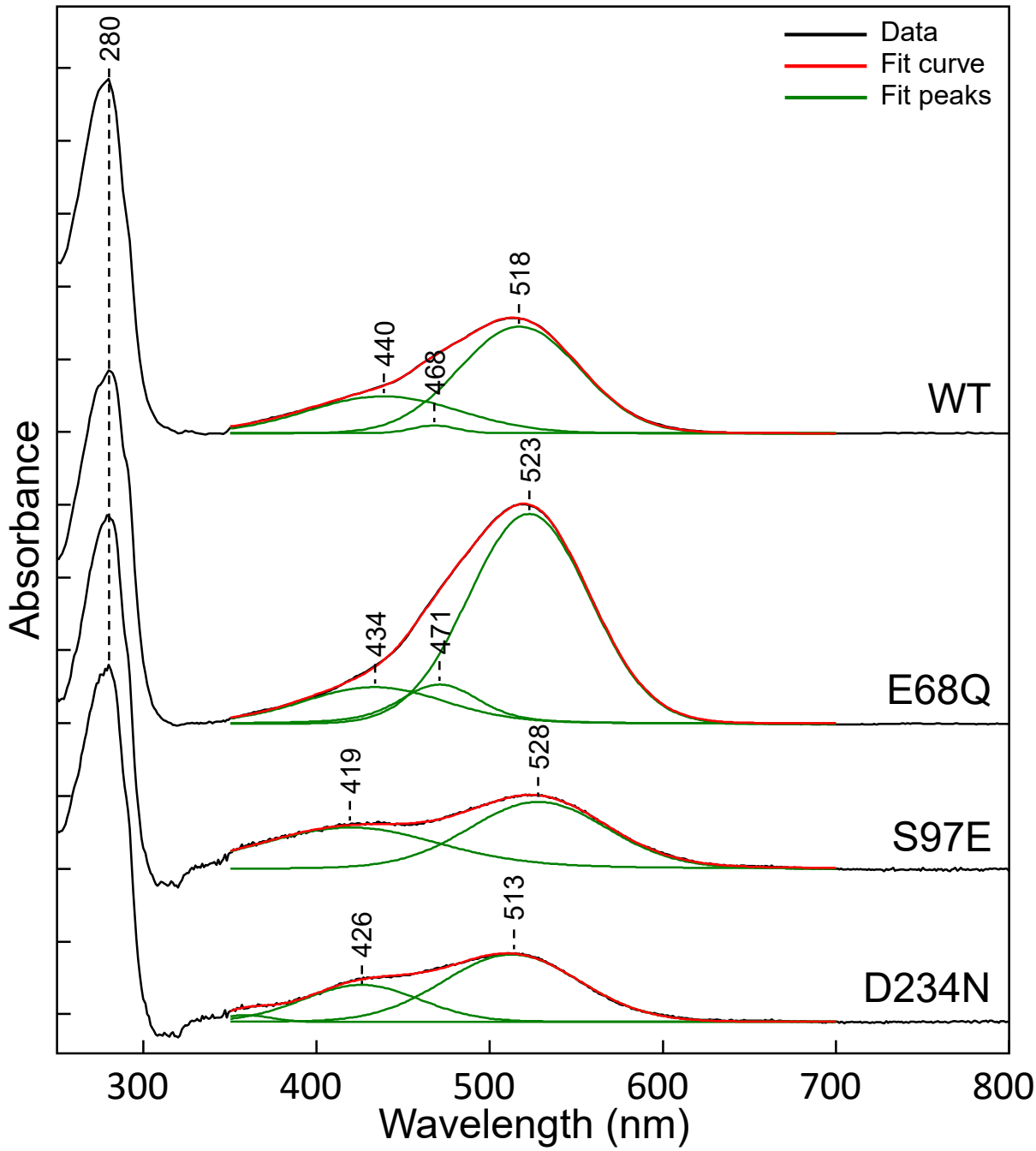
Supplementary Figure 2. Curve fit of ethylenic region of the RRS of *Gt*ACR1 at pH 7. Fitted data is same as shown in Figure 1. Curve fitting was performed using the GRAMS spectroscopy suite (Thermo-Fischer Scientific). Three Voigtian components (green curves) plus a linear baseline (not shown) resulted in a close fit (red curve) to the raw data (black curve).



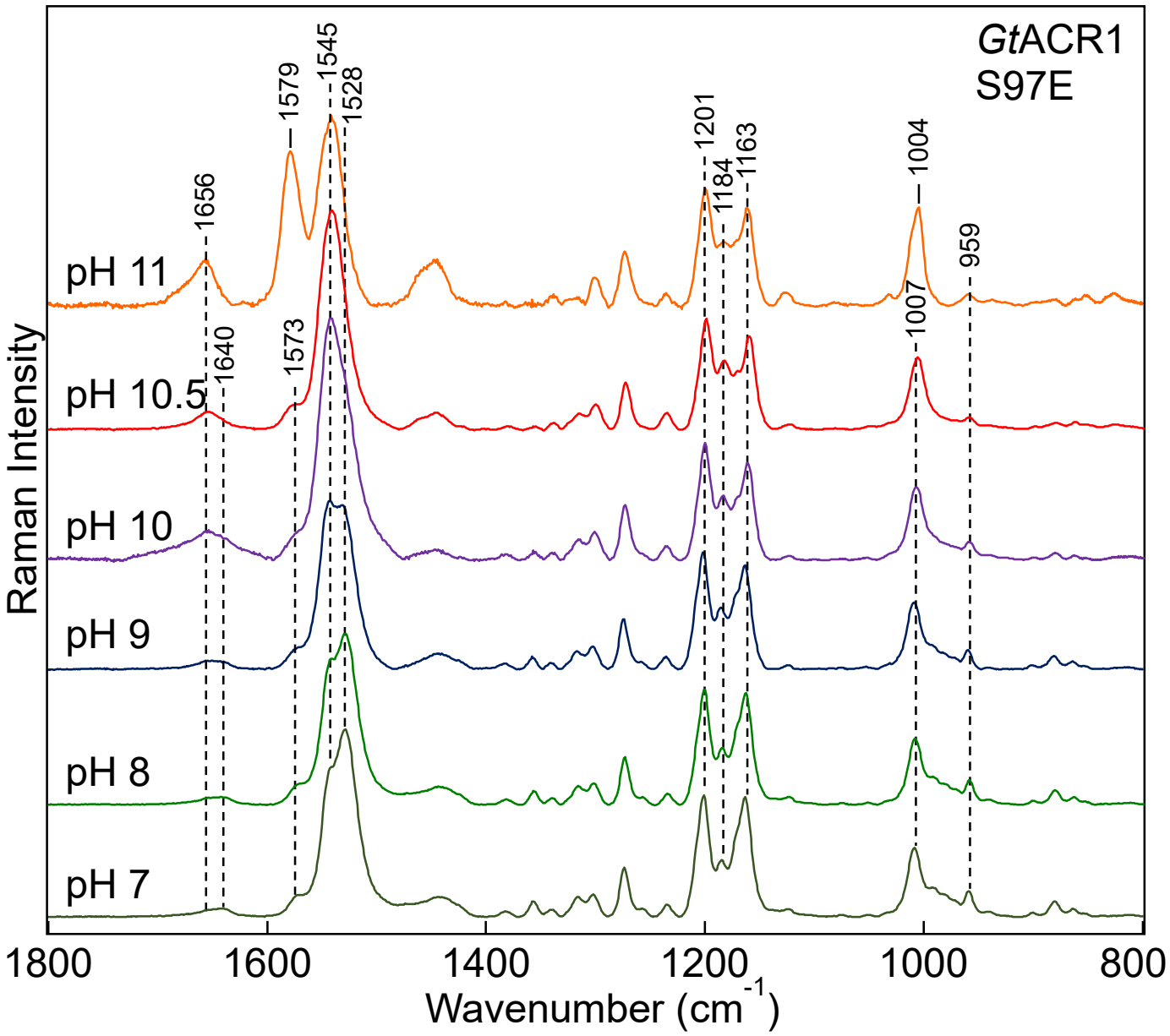
Supplementary Figure 3. Comparison of *Gt*ACR1 resonance Raman spectra recorded under different conditions. Data was recorded at room temperature using a laser wavelength and power source as labeled. Spectra were not smoothed and were scaled using the intensity of the peak near 1532 cm^{-1}. A background spectrum of the borosilicate capillary and buffer was subtracted from the sample.



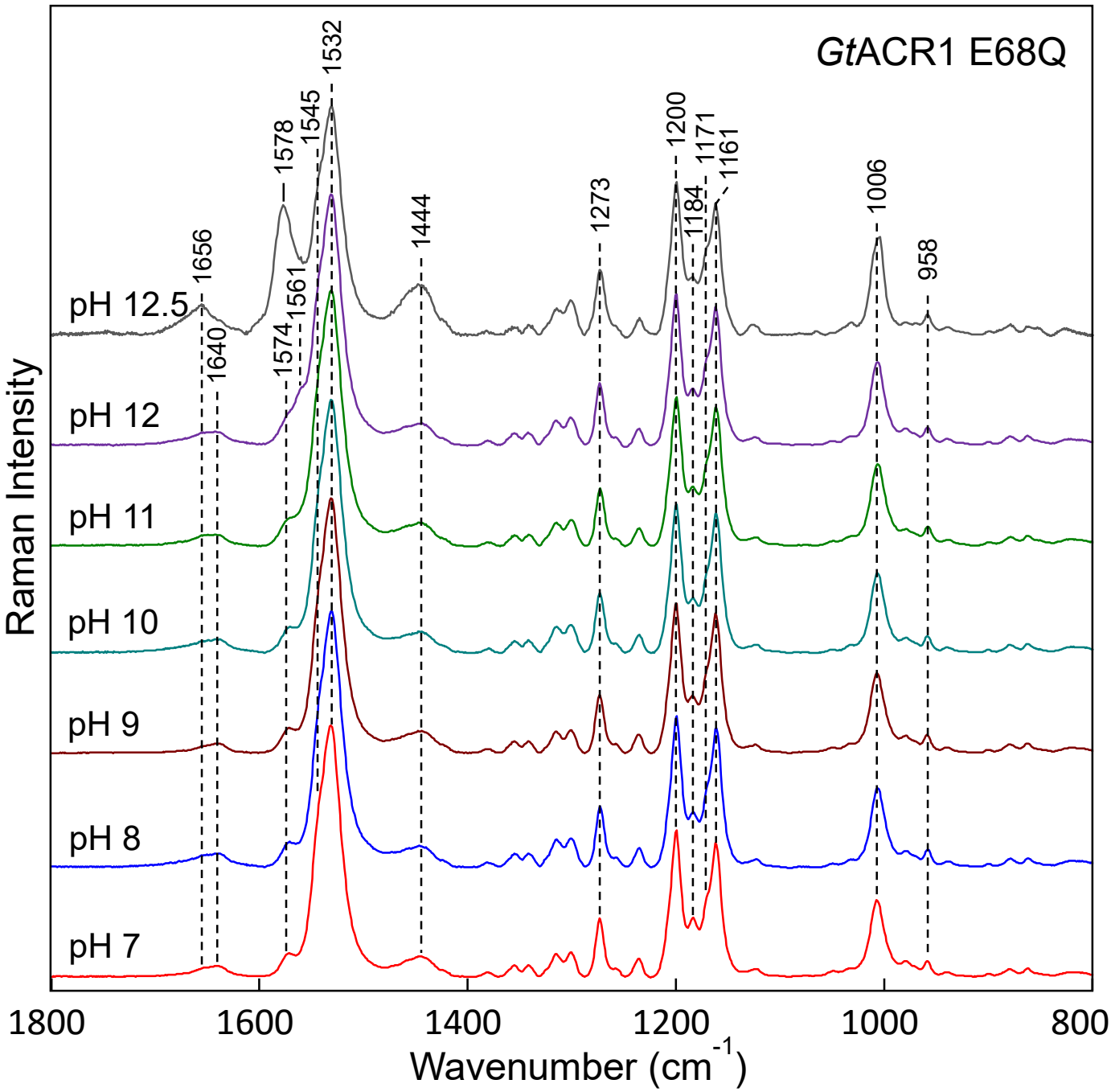
Supplementary Figure 4. Curve fit of λ_{max} region of the UV/Vis spectrum of *Gt*ACR1 WT with A2 retinal at pH 7. Spectrum were acquired using a Cary 6000 (Agilent Technologies) using steps of 1nm. Curve fitting was performed using the GRAMS spectroscopy suite (Thermo-Fischer Scientific). Three Voigtian components (green curves) plus a linear baseline (not shown) resulted in a close fit (red curve) to the raw data (black curve). DA, dark-adapted; LA, light-adapted.



Supplementary Figure 5. Comparison of λ_{max} curve fits of the UV/Vis spectra of *Gt*ACR1 WT and its mutants at pH 7. Spectra were acquired using a Cary 6000 (Agilent Technologies) using steps of 1nm. Curve fitting was performed using the GRAMS spectroscopy suite (Thermo-Fischer Scientific). Two or three Voigtian components (green curves) plus a linear baseline (not shown) resulted in a close fit (red curve) to the raw data (black curve). Spectra were scaled using the protein band at 280nm. Y-axis tick-marks correspond to 0.15, 0.2, 0.05, and 0.06 OD for WT, E68Q, S97E, and D234N, respectively.



Supplementary Figure 6. Resonance Raman spectra of *Gt*ACR1 mutant S97E recorded at various pH values ranging from 7 to 11. Data was recorded at room temperature using a 785-nm probe laser with 300mW (70mW measured at the sample) power.



Supplementary Figure 7. Resonance Raman spectra of *Gt*ACR1 mutant E68Q recorded at various pH values ranging from 7 to 12.5. Data was recorded at room temperature using a 785-nm probe laser with 300mW (70mW measured at the sample) power.