Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supporting Information

The Reductive Phase of *Rhodobacter sphaeroides* Cytochrome *c* Oxidase Disentangled by CO Ligation

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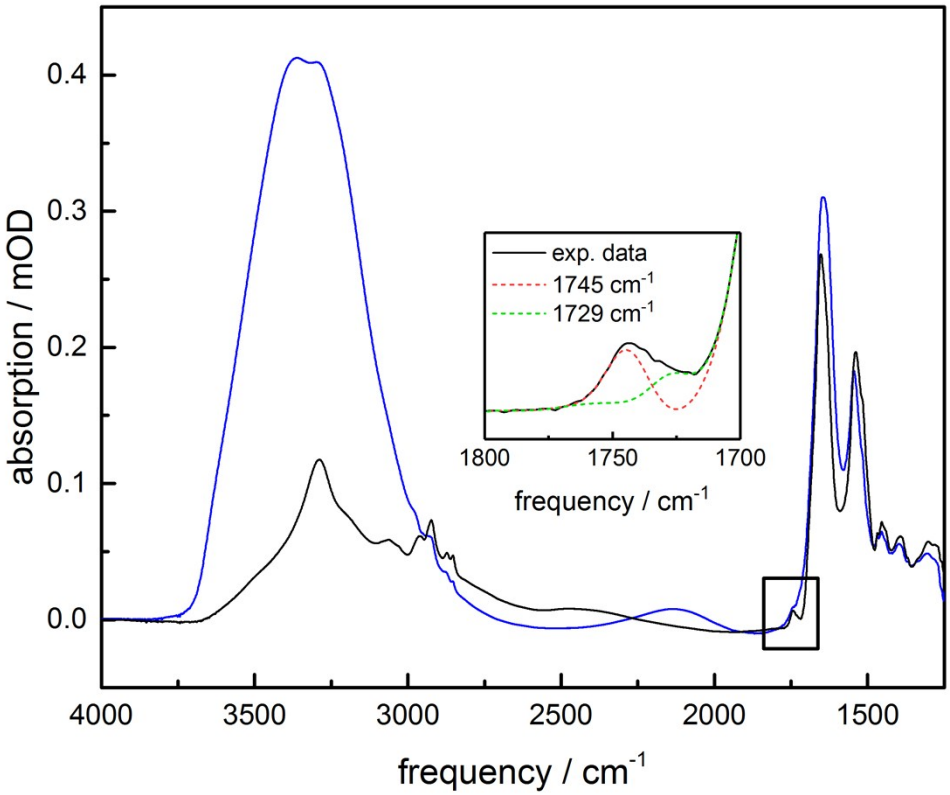

Figure S1. Hydration of the C*c*O protein film as recorded by FTIR spectroscopy (black, dry film; blue, hydrated film). The amide II band (1540 cm^{-1}) remained at ~80% peak height for dry C*c*O. The DMPC C=O band (inset) showed a remarkable contribution at 1745 cm^{-1} (red trace) which overlays a potential change in the carbonyl vibration of E286 during redox titration.

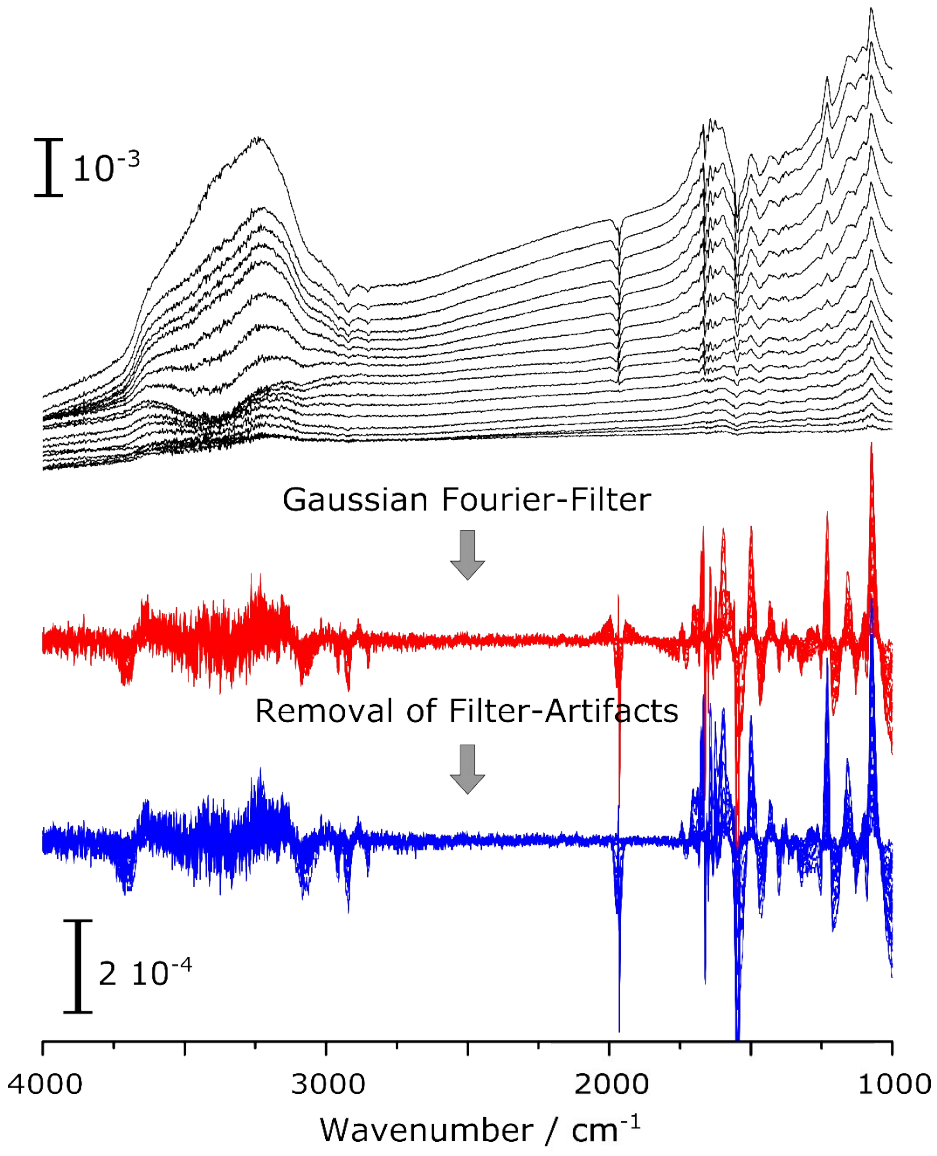

Figure S2. The baseline drift due to changes of film hydration during the measurement was corrected by calculation of individual baselines through Fourier-Filtering. For this purpose, the spectra were multiplied by a Gaussian shaped window centered at the center burst in the Fourier domain. In order to remove filtering artifacts in the CO region (see inset), the baseline was restored manually.

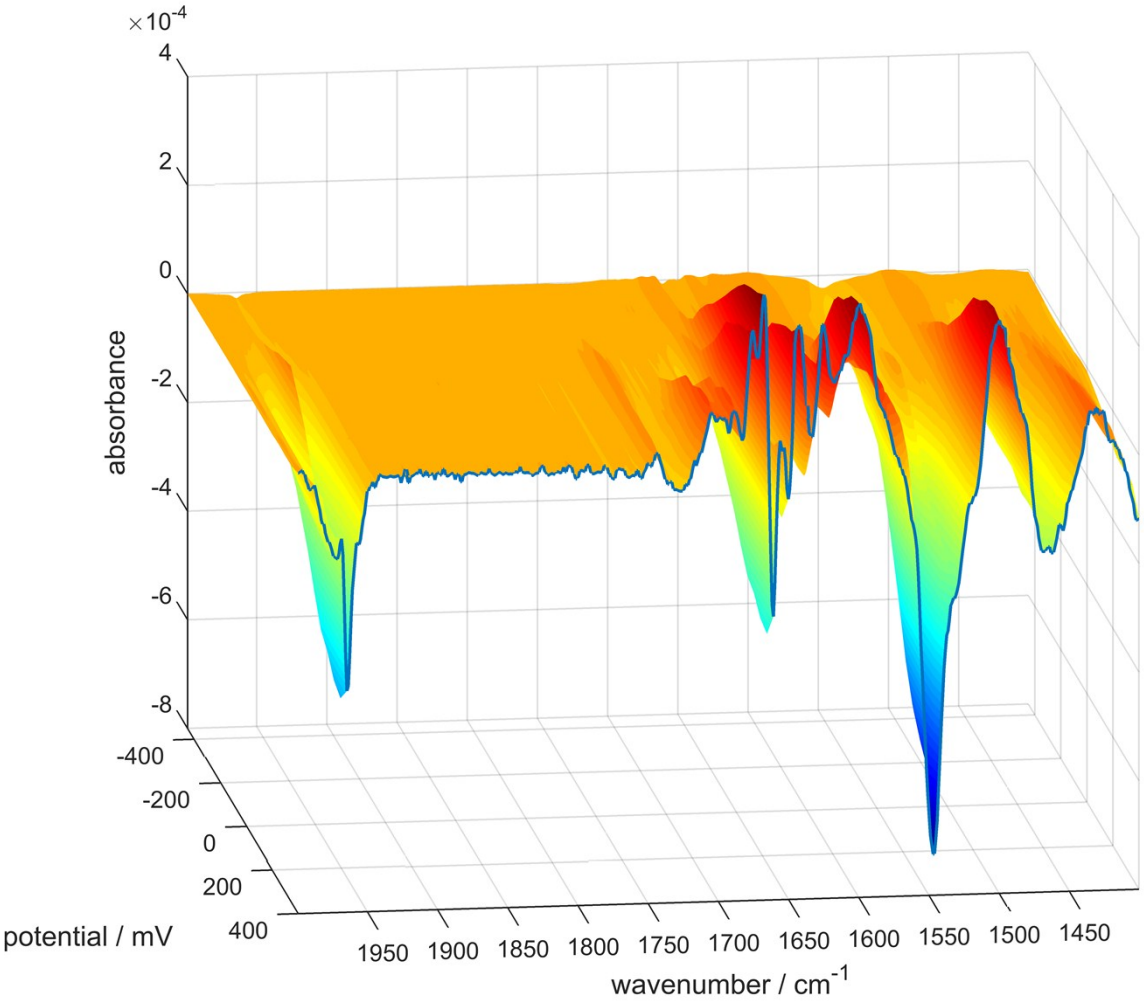

Figure S3. Full recorded data set with a potential resolution of 50 mV and a spectral resolution of 2 cm^{-1}.

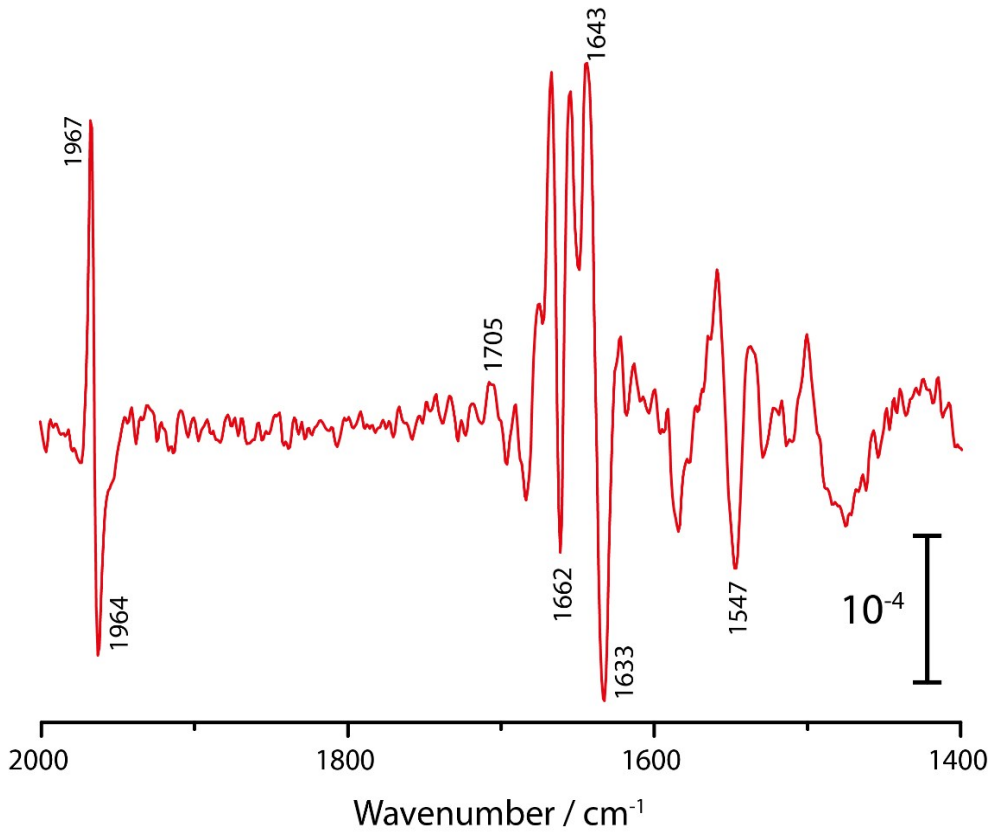

Figure S4. Redox spectrum of C*c*O in bacteriorhodopsin polar lipids that lack the C=O vibration of the ester group. This spectrum does not show a band at 1745 cm^{-1} (compare Fig. S1) upon the CO blue shift from 1964 cm^{-1} to 1967 cm^{-1}. The positive band at 1705 cm^{-1} remains though.

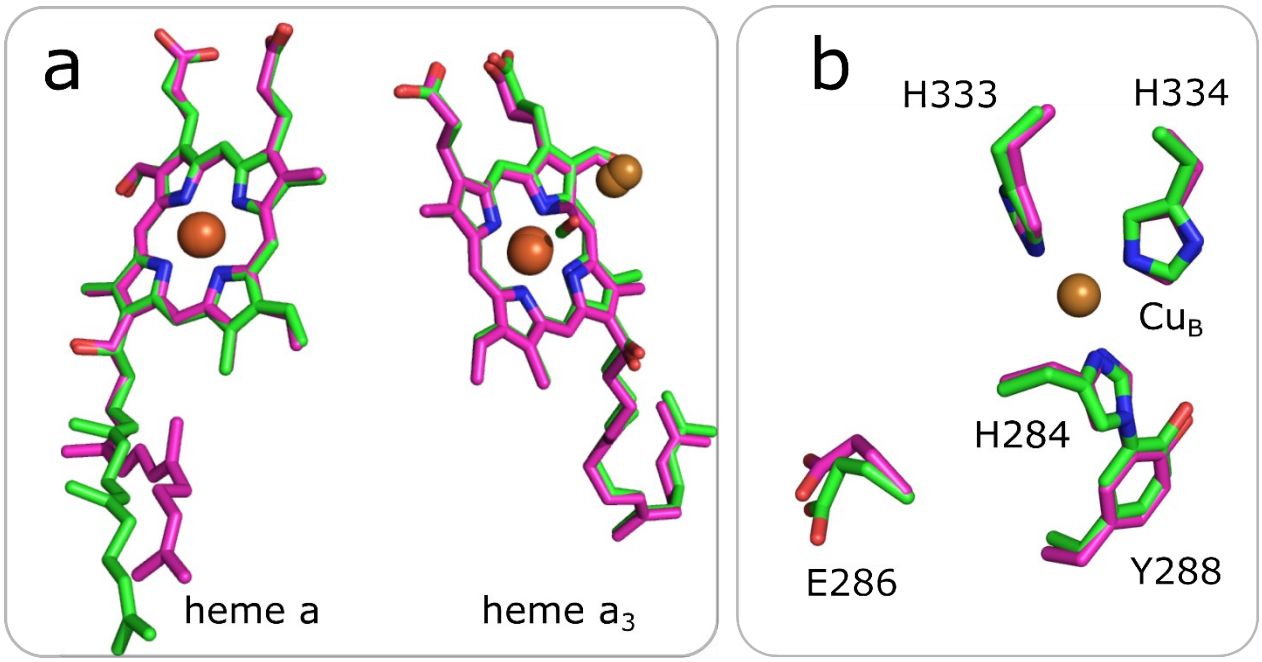

Figure S5. C*c*O crystal structures from *bovine heart* (PDB code: 3AG1, green) and *Rhodobacter sphaeroides* PDB code: 2GSM, magenta). The overlay shows heme a and heme a_3 **(a)** as well as Cu_B and relevant amino acid sidechains **(b)**. Histidines H333, H334, and H284 coordinate Cu_B while tyrosine Y288 and glutamate E286 were suggested to serve as proton donors / acceptors (*R. sphaeroides* nomenclature).

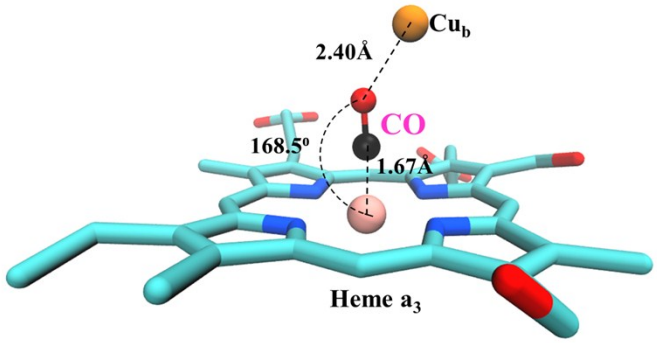

Figure S6. Orientation and geometry of CO molecule relative to heme a_3.

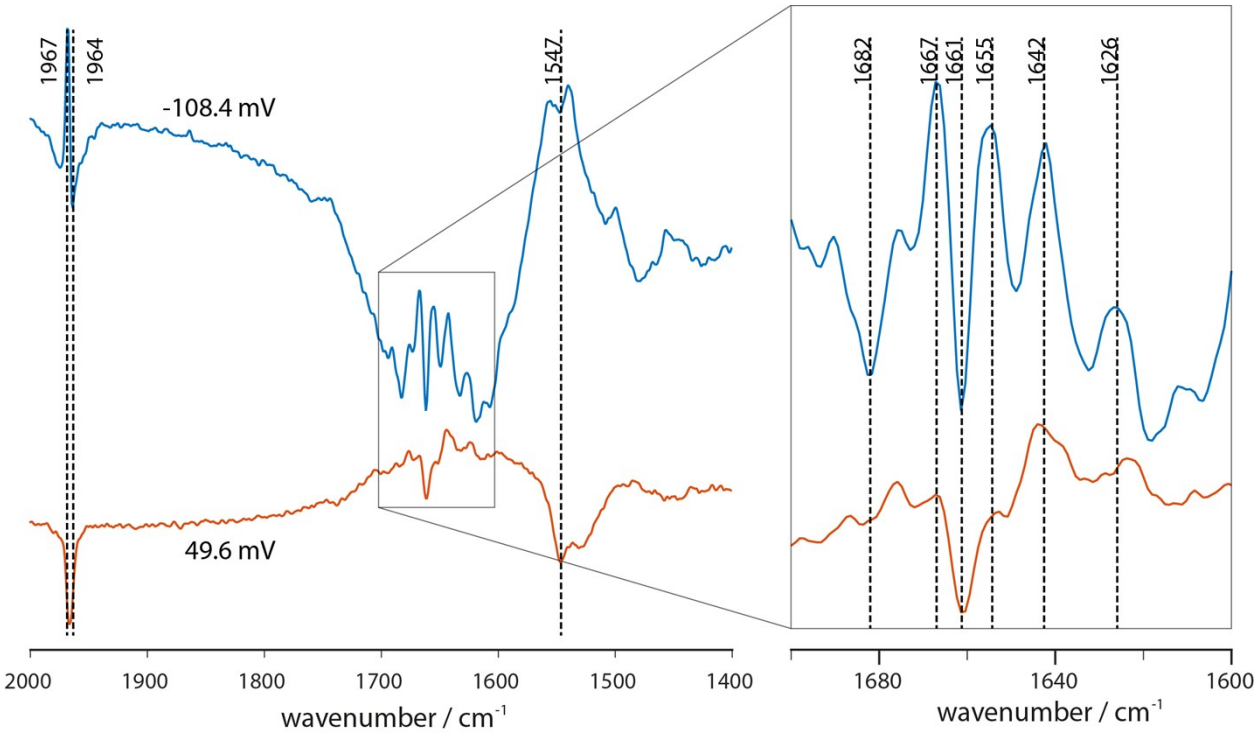

Figure S7. Cross correlation spectra of the original data set (no corrections performed) with the two sigmoidal transitions that result from the fit of the CO band. The similarity to the decay associated spectra after global fit of the baseline corrected data (Fig. 3B) indicates that no major artifacts are created that change the transitions of the protein bands.

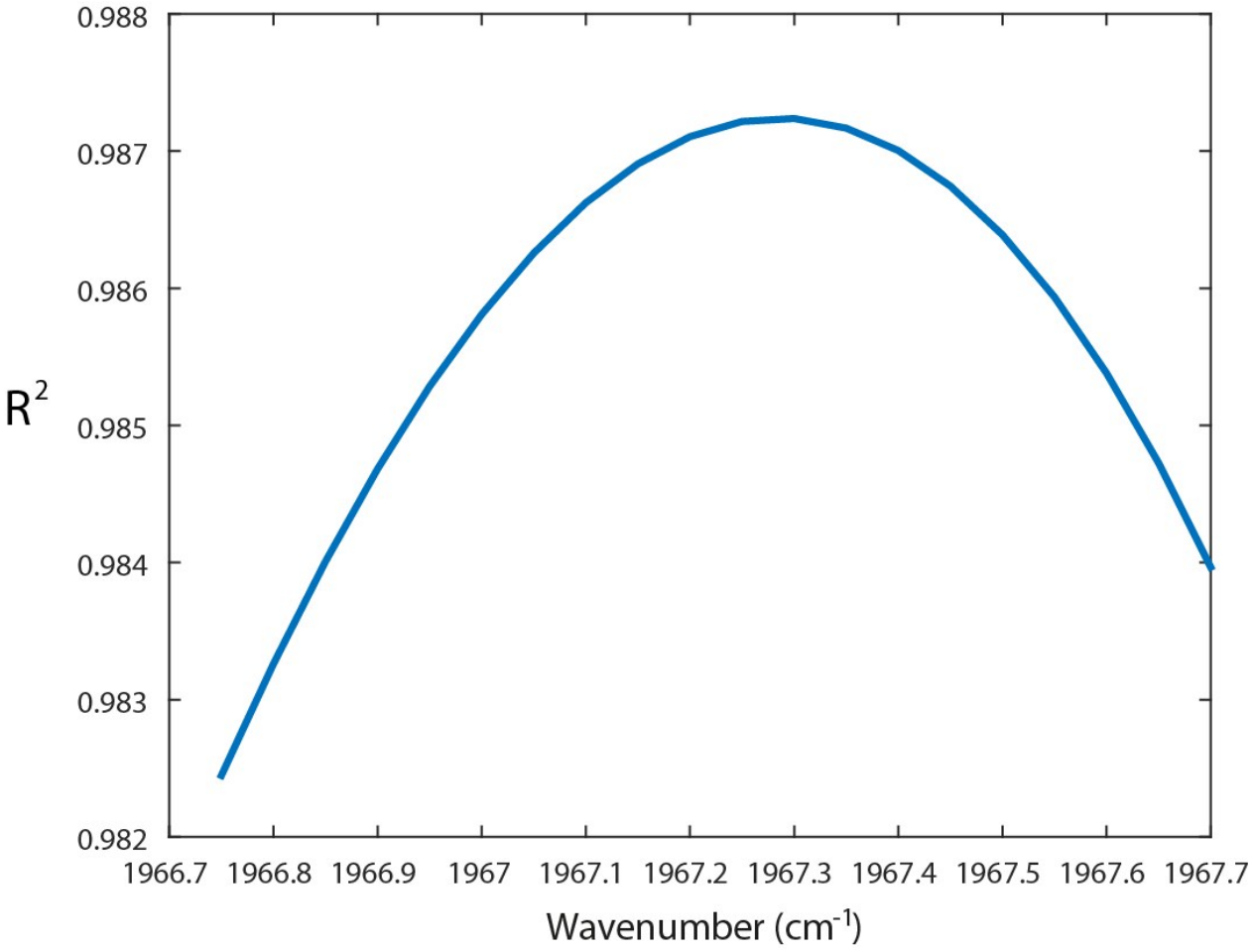

Figure S8. The difference spectrum at +20 mV was fitted using four Gaussian components, accounting for the two negative β-bands, the negative α-band (from reduced C*c*O) and the positive α-band corresponding to the up-shift through heme *a* oxidation. The positions and width of the negative bands were fixed according to the absorbance spectrum with an α-band position of 1964.2 cm^{-1}. Thus, the only fitting parameter were the four amplitudes of the bands. The spectra was then fitted for positions of the up-shifted α-band increased in steps of 0.1 cm^{-1}. The plot shows the goodness of the fit in dependence of the up-shifted peak position. The maximum was reached at 1967.3 cm^{-1} which results in an up-shift of 3.1 cm^{-1}.