Resonance Raman, EPR and MCD Spectroscopic Investigation of Diheme Cytochrome *c* Peroxidases from *Nitrosomonas europaea* and *Shewanella oneidensis*

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Supporting Information

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Table S1

Bacterial CcPs that require reduction	Bacterial CcPs that are constitutively active
Paracoccus denitrificans[6]	*Nitrosomonas europaea*[3]
Pseudomonas aeruginosa[7]	*Methyloccocus casulatus*[18]
Paracoccus pantrophus[8]	
Marinobacter hydrocarbonoclasticus[9]	
Rhodobacter capsulatus[10]	
Pseudomonas stutzeri[11]	
Geobacter sulfurreducens[12]	
Shewanella Oneidensis[13]	

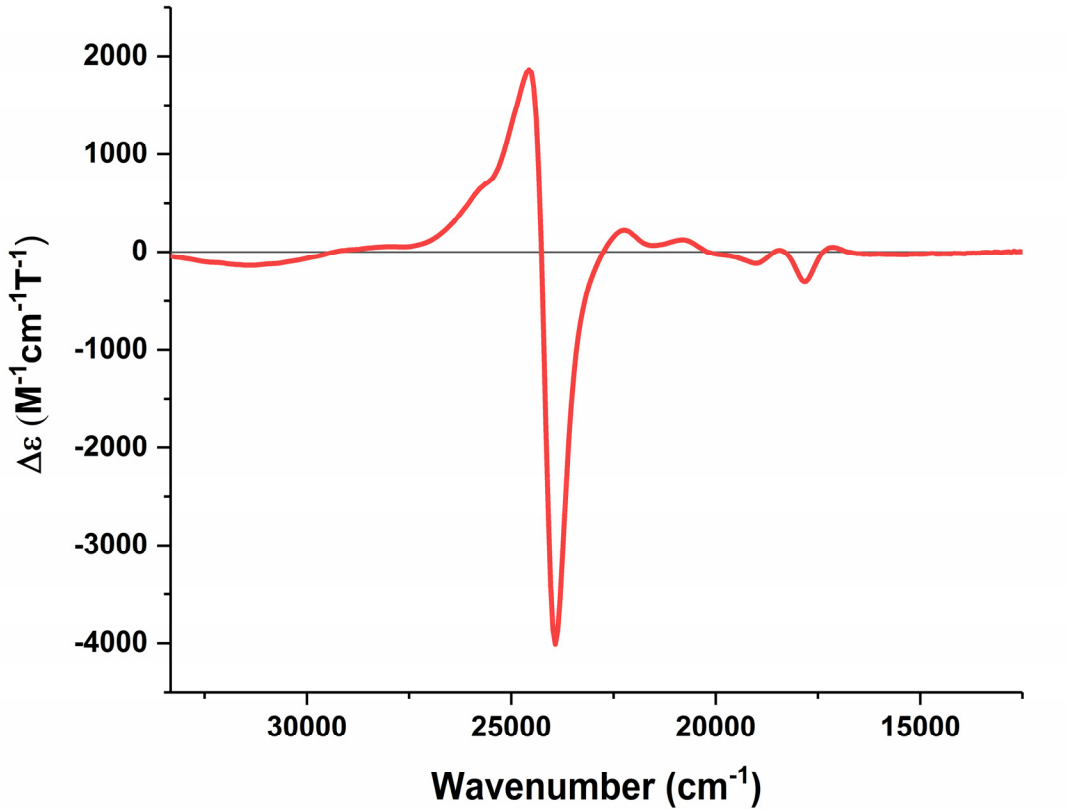

Figure S1. Near-UV/visible-region MCD spectrum **of low-spin ferric** Cytochrome b_5. The sample was 3.7 μM with ~50% glycerol in a TIP7 buffer at pH 7. This spectrum was recorded at 2 K.

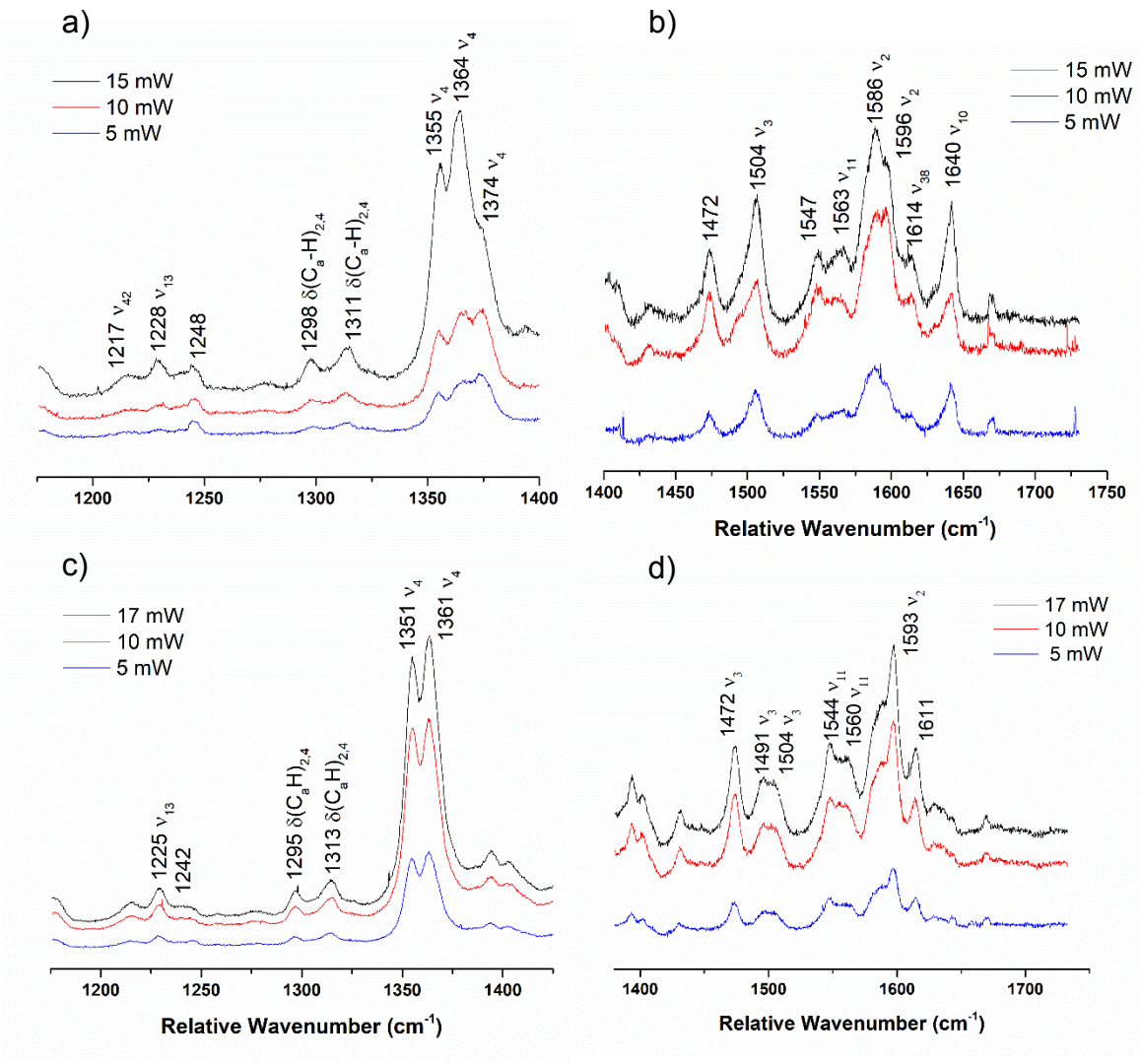

Figure S2. Power dependence resonance Raman spectra of **diferric *Ne*CcP** from a) 1150 to 1400 cm^{-1}, and b) 1400 to 1750 cm^{-1} and the power dependence spectra of **semireduced *Ne*CcP** from c) 1150 to 1400 cm^{-1}, and d) 1400 to 1750 cm^{-1}. The diferric sample was 420 μM and the semireduced sample was 490 μM in protein. both with 30% glycerol in pH 7 TIP7 buffer. The sample was excited at 413.1 nm using a Krypton ion gas laser at 77 K.

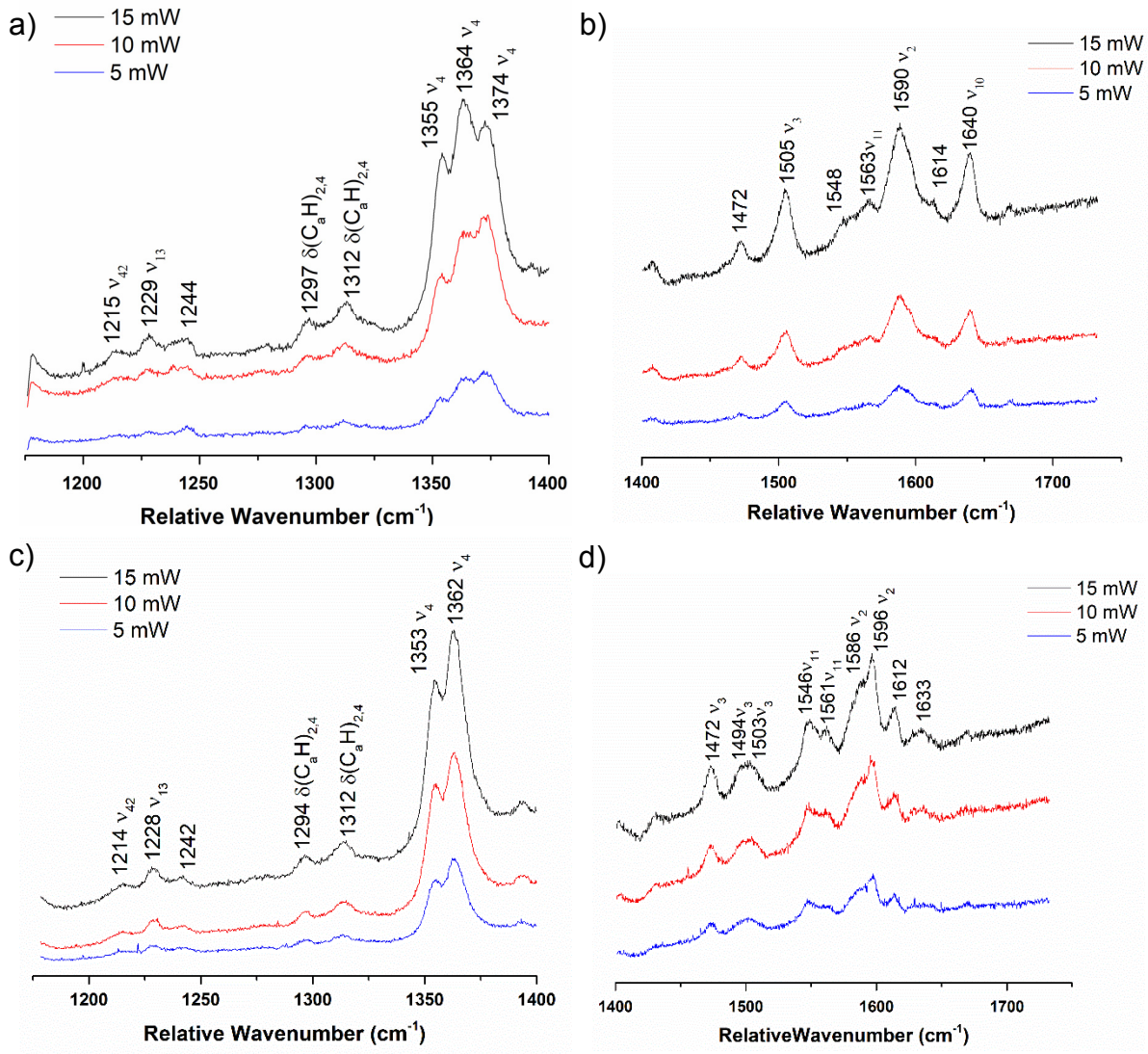

Figure S3. Power dependence resonance Raman spectra of **diferric *Ne*H59G CcP** from a) 1150 to 1400 cm^{-1}, and b) 1400 to 1750 cm^{-1} and the power dependence spectra of **semireduced *Ne*H59G CcP** from c) 1150 to 1400 cm^{-1}, and d) 1400 to 1750 cm^{-1}. The diferric sample was 419 μM and the semireduced sample was 460 μM in protein, both with 30% glycerol in pH 7 TIP7 buffer. The sample was excited at 413.1 nm using a Krypton ion gas laser at 77 K.

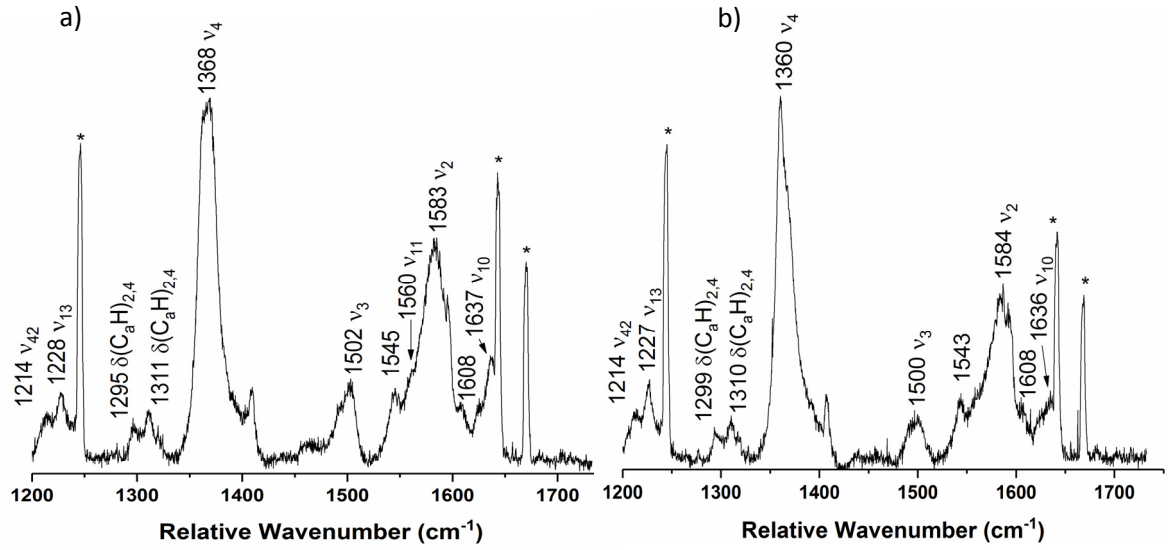

Figure S4. Room temperature resonance Raman spectrum of a) **diferric *Ne*CcP** and b) **diferric *Ne*H59G CcP**. The samples were ~420 μM in protein with 30% glycerol in pH 7 TIP7 buffer. The samples were rotated and excited at 413.1 nm using a Krypton ion gas laser. Plasma lines are indicated with an asterisk.

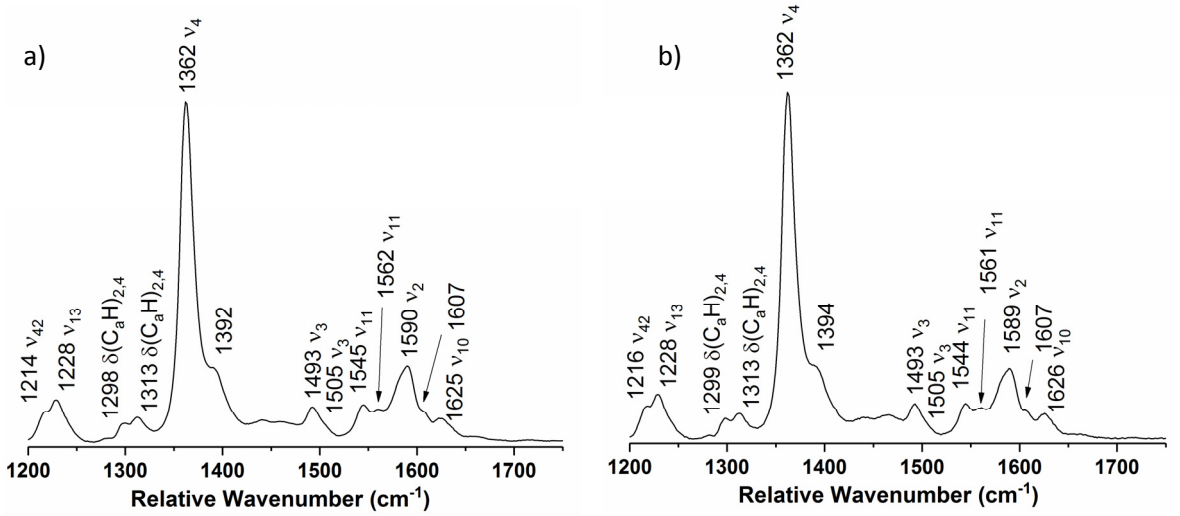

Figure S5. Room temperature resonance Raman spectrum of a) **semireduced *Ne*CcP** and b) **semireduced *Ne*H59G CcP**. The samples were ~200 μM in protein with 30% glycerol in pH 7 TIP7 buffer. The samples were excited at 406.7 nm using a tunable titanium:sapphire laser.

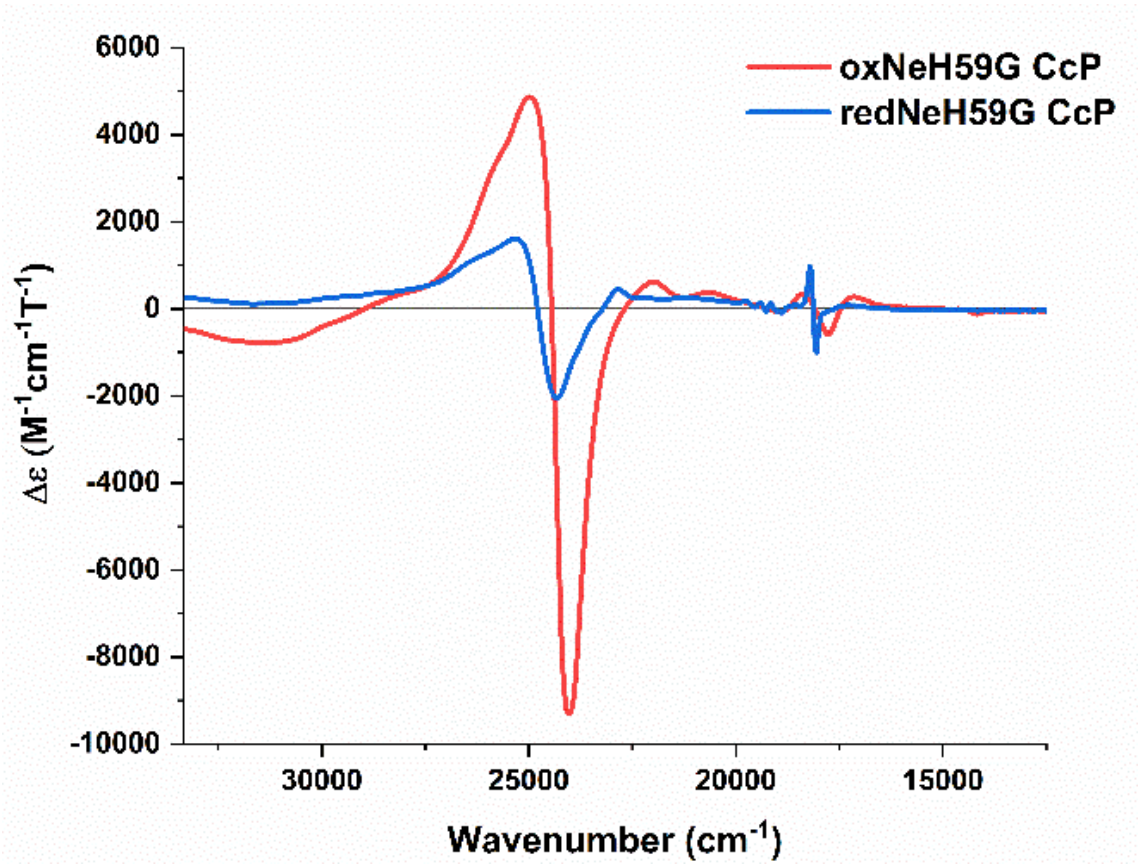

Figure S6. Near-UV/visible-region MCD spectra of the diferric and semi-reduced states of *Ne*H59G CcP. Samples were 2-2.6 μM with ~50% glycerol in a TIP7 buffer at pH 7. Spectra were recorded at 2 K.at pH 7. Spectra were recorded at 2 K.

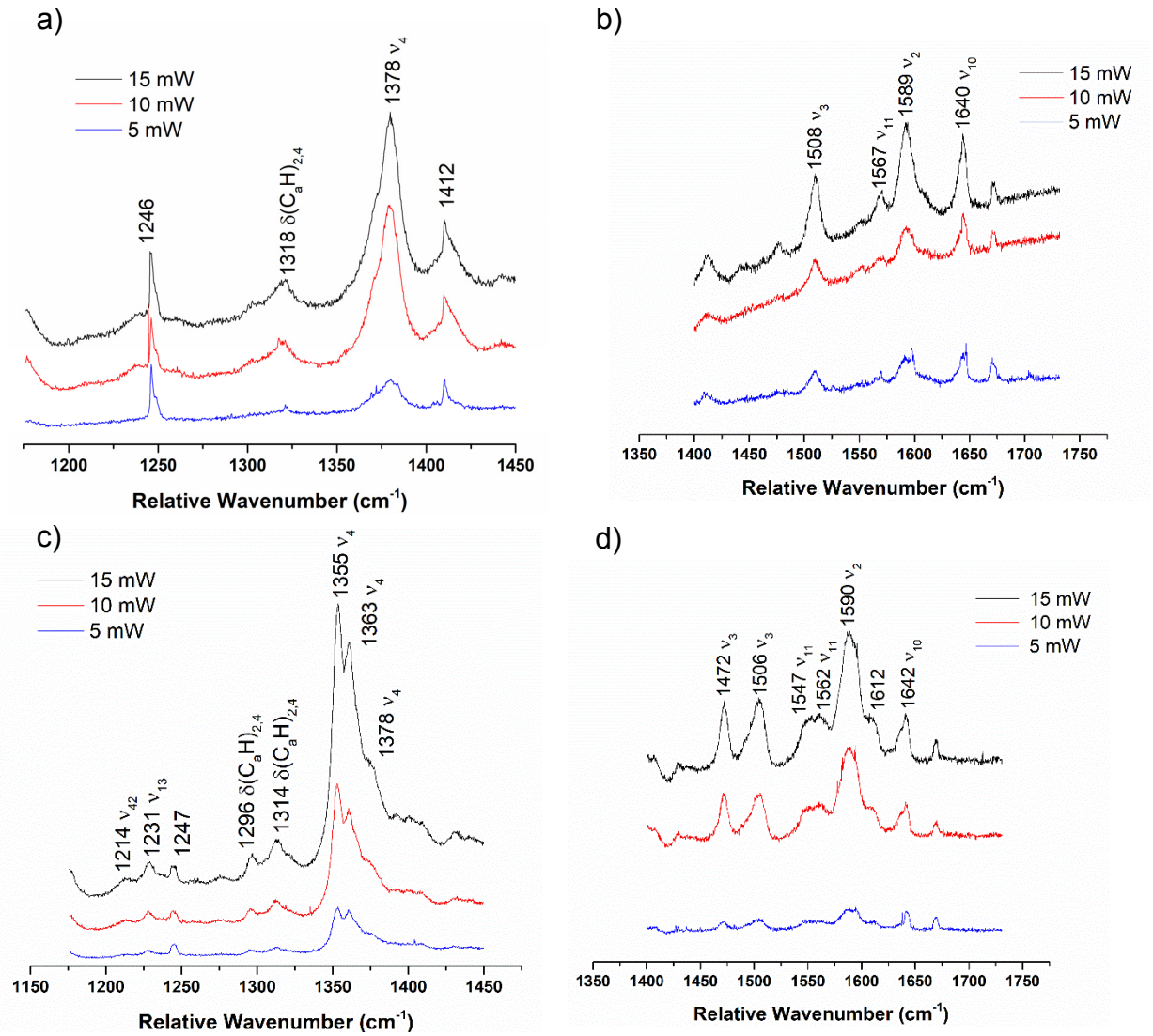

Figure S7. Power dependence resonance Raman spectra of **diferric *So*CcP** from a) 1150 to 1400 cm^{-1}, and b) 1400 to 1750 cm^{-1} and the power dependence spectra of **semireduced *So*CcP** from c) 1150 to 1400 cm^{-1}, and d) 1400 to 1750 cm^{-1}. The diferric sample was 330 μM and the semi-reduced sample was 230 μM in protein, both with 30% glycerol in pH 7 TIP7 buffer. The sample was excited at 413.1 nm using a Krypton ion gas laser at 77 K.

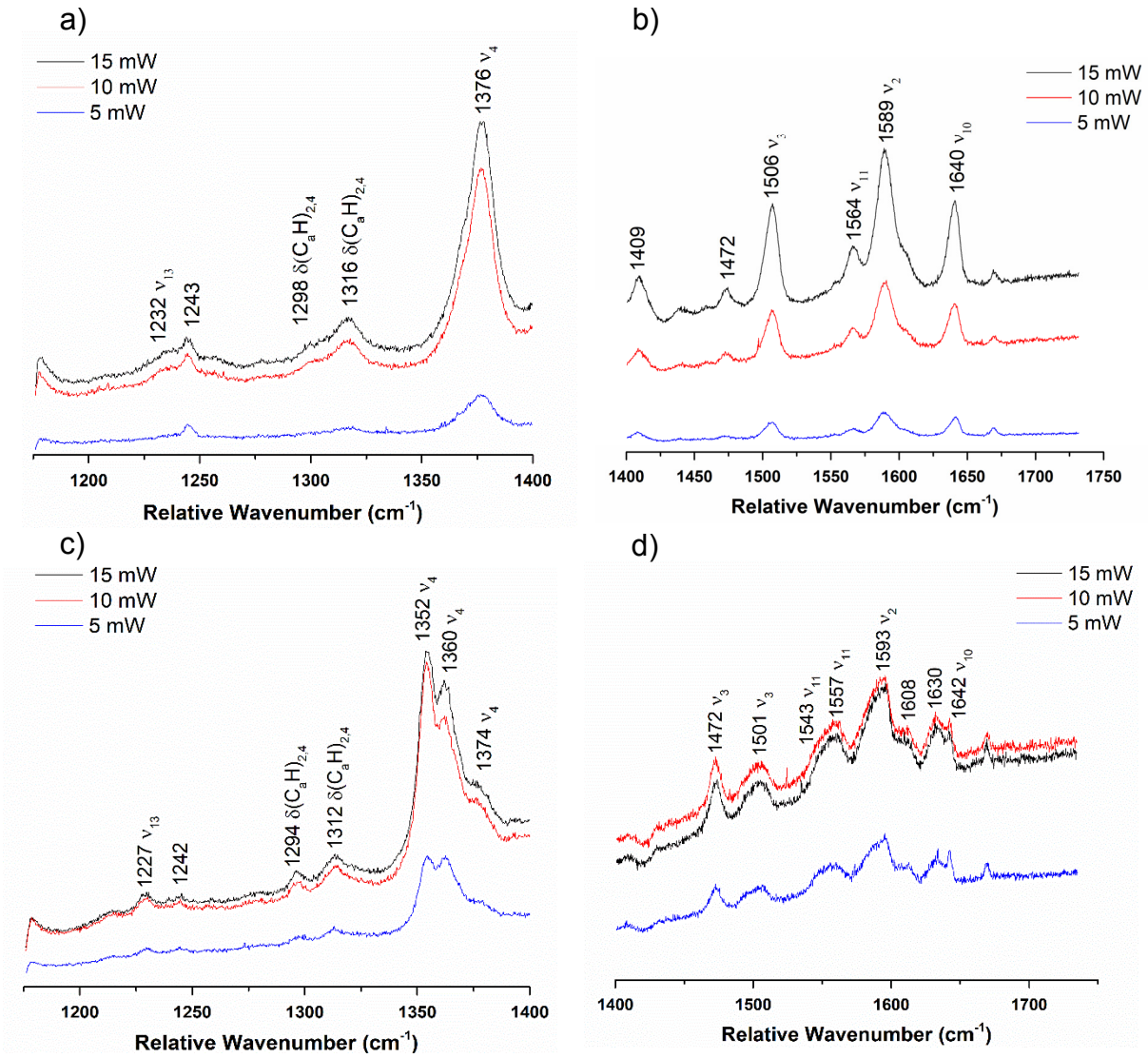

Figure S8. Power dependence resonance Raman spectra of **diferric *So*H80G CcP** from a) 1150 to 1400 cm^{-1}, and b) 1400 to 1750 cm^{-1} and the power dependence spectra of **semireduced *So*H80G CcP** from c) 1150 to 1400 cm^{-1}, and d) 1400 to 1750 cm^{-1}. The diferric sample was 280 μM and the semi-reduced sample was 340 μM in protein, both with 30% glycerol in pH 7 TIP7 buffer. The sample was excited at 413.1 nm using a Krypton ion gas laser at 77 K.

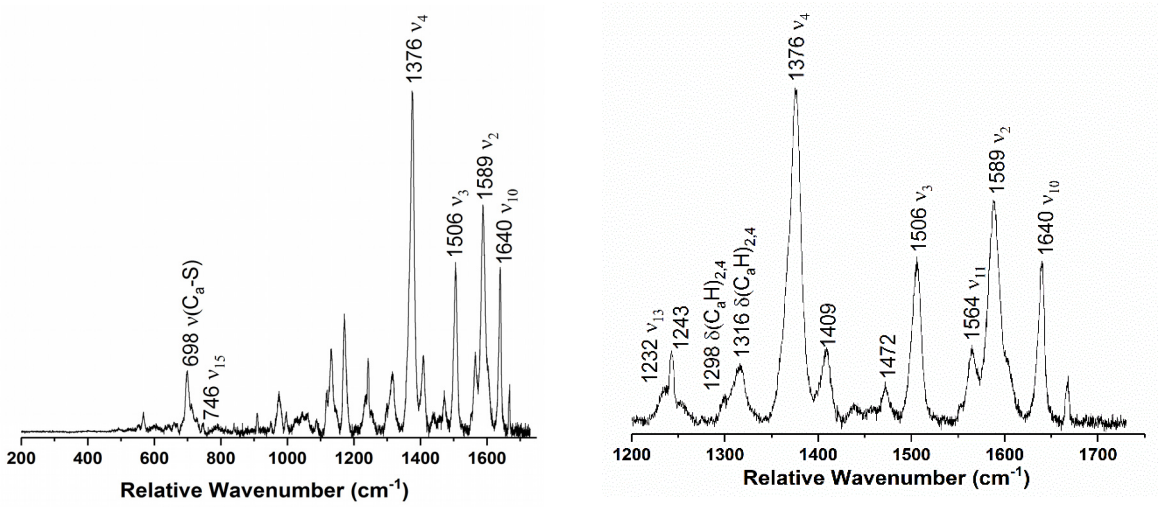

Figure S9. Resonance Raman spectrum of **diferric *So*H80G CcP** from a) 200 to 1750 cm^{-1}, and b) enlarged view of the 1200 to 1750 cm^{-1} region. The sample was 280 μM in protein with 30% glycerol in pH 7 TIP7 buffer. The sample was excited at 413.1 nm using a Krypton ion gas laser at 77 K.

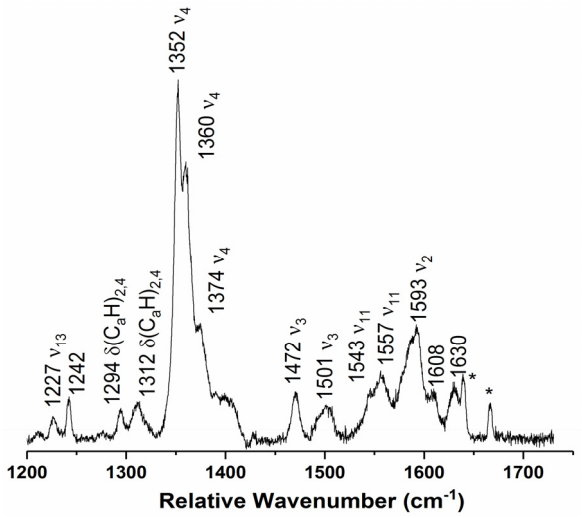

Figure S10. Resonance Raman spectrum of **semi-reduced *So*H80G CcP** from 1200 to 1750 cm^{-1} region. The sample was 280 μM in protein with 30% glycerol in pH 7 TIP7 buffer. The sample was excited at 413.1 nm using a Krypton ion gas laser at 77 K.

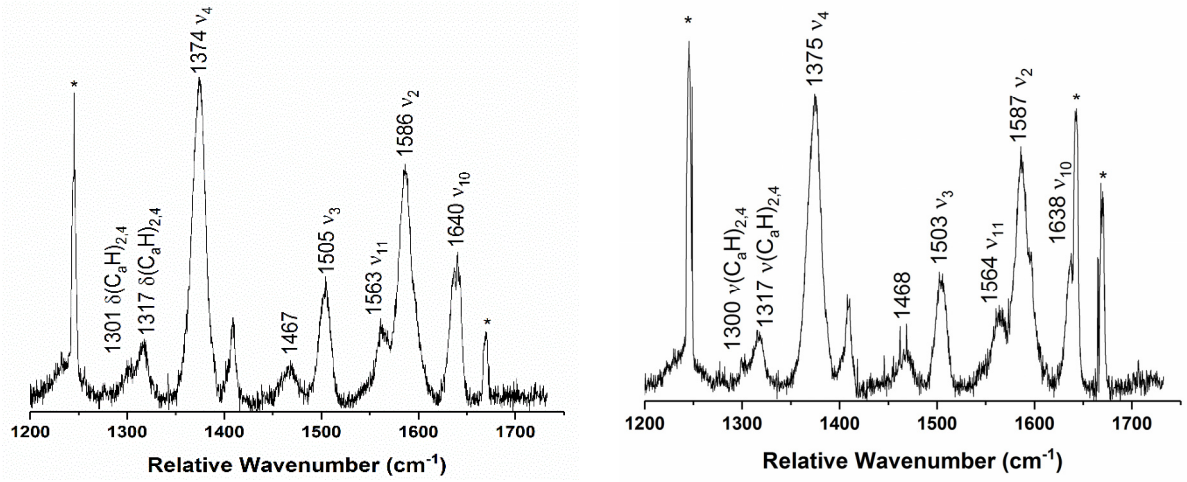

Figure S11. Room temperature resonance Raman spectrum of a) **diferric *So*CcP** and b) **diferric *So*H80G CcP**. The samples were 280-330 μM in protein with 30% glycerol in pH 7 TIP7 buffer. The samples were rotated and excited at 413.1 nm using a Krypton ion gas laser. Plasma lines are indicated with an asterisk.

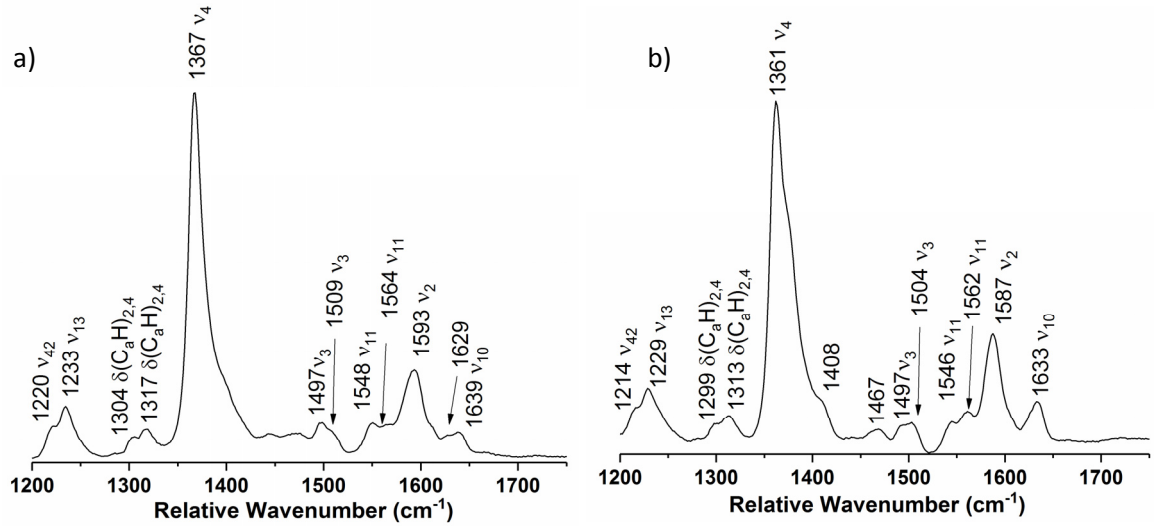

Figure S12. Room temperature resonance Raman spectrum of a) **semireduced *So*CcP** and b) **semireduced *So*H80G CcP**. The samples were ~100 μM in protein with 30% glycerol in pH 7 TIP7 buffer. The samples were excited at 406.7 nm using a tunable titanium:sapphire laser.

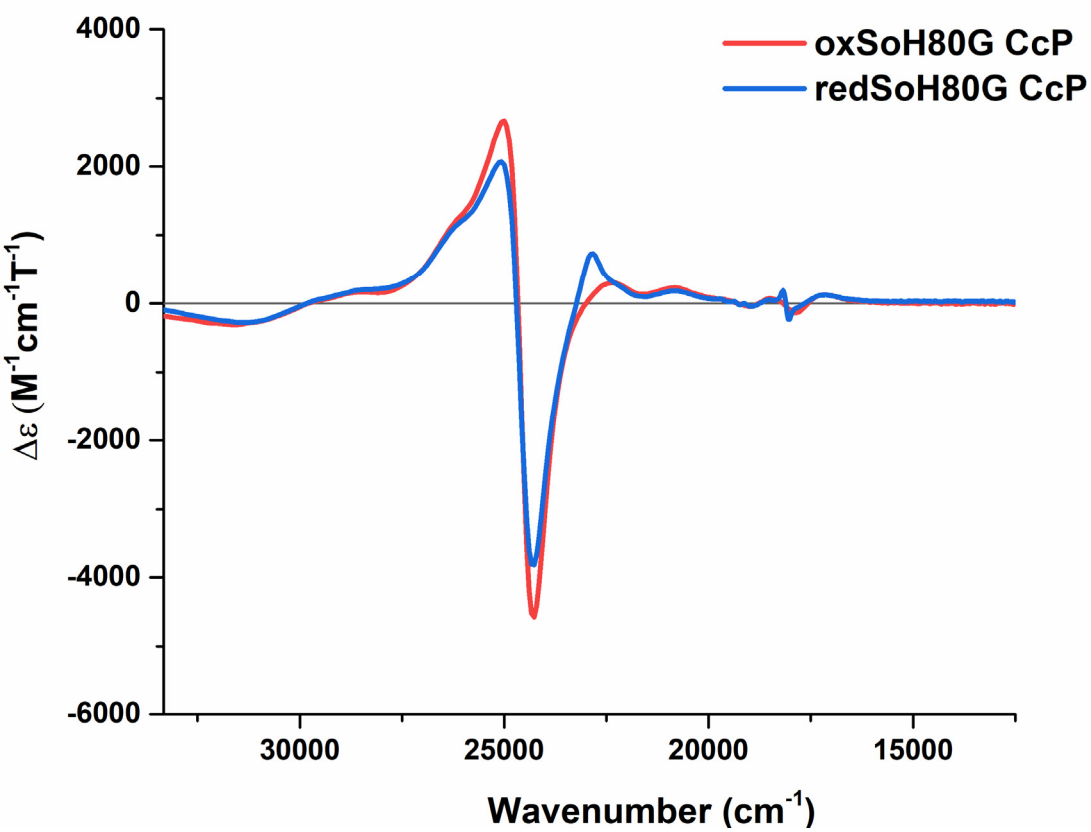

Figure S13. Near-UV/visible-region MCD spectra of the diferric and semi-reduced states of *So*H80G CcP. Not all of the H-heme was reduced in the semi-reduced sample, some H-heme remained ferric. The samples were 1.2-1.4 μM with ~50% glycerol in a TIP7 buffer at pH 7. Spectra were recorded at 2K.

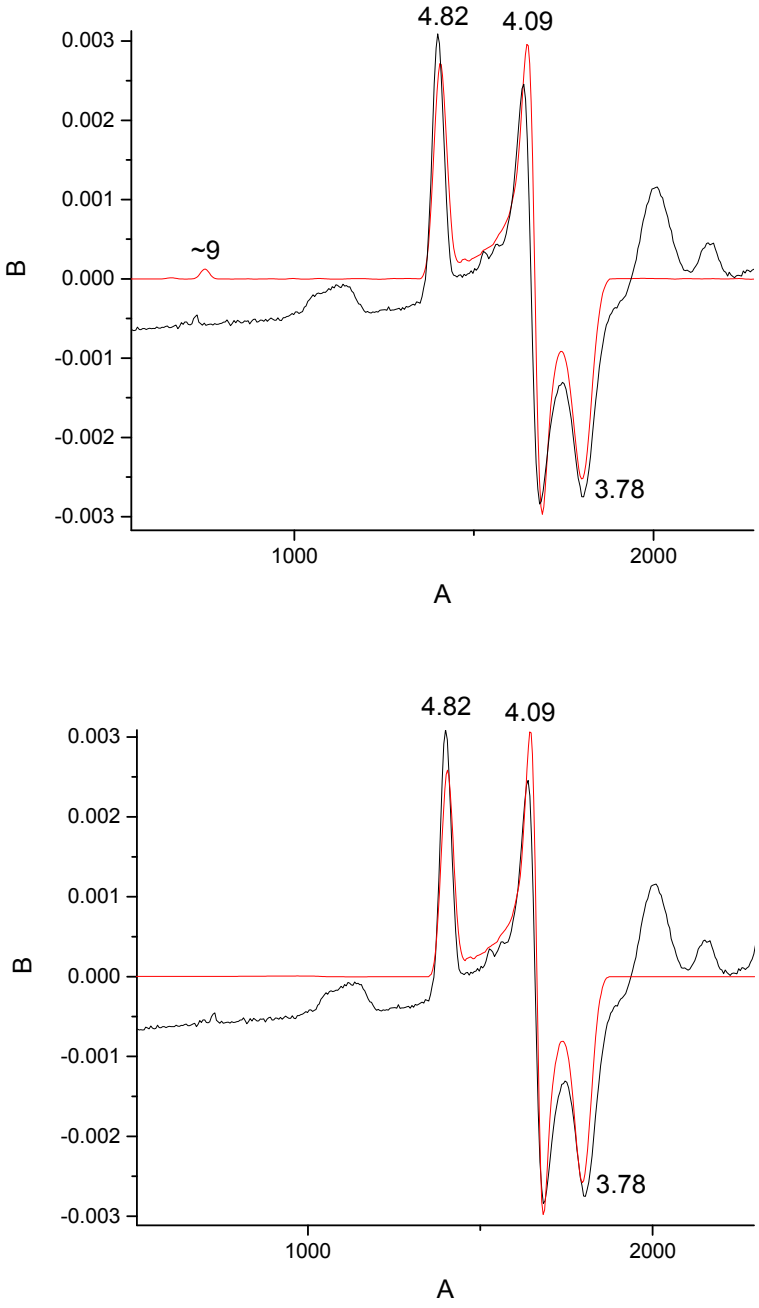

Figure S14. Fit of the highly rhombic high-spin ferric signal in the EPR spectrum of diferric *Ne*H59G CcP (see Figure 3C). Top: fit using g_x = 1.94, g_y = 1.90, g_z = 2.11, *D* = +1 cm^{-1}, E/D = 0.28, T = 12 K. Bottom: fit with the same parameters, except that D = -2.5 cm^{-1}. Only the fit with a small, positive *D* value is able to reproduce the small signal around g ~ 9. In addition, using a larger, positive *D* increases the intensity of this feature, which is not in agreement with experiment. It can therefore be concluded that the highly rhombic species responsible for this signal must have a *D* of about +1 cm^{-1}.

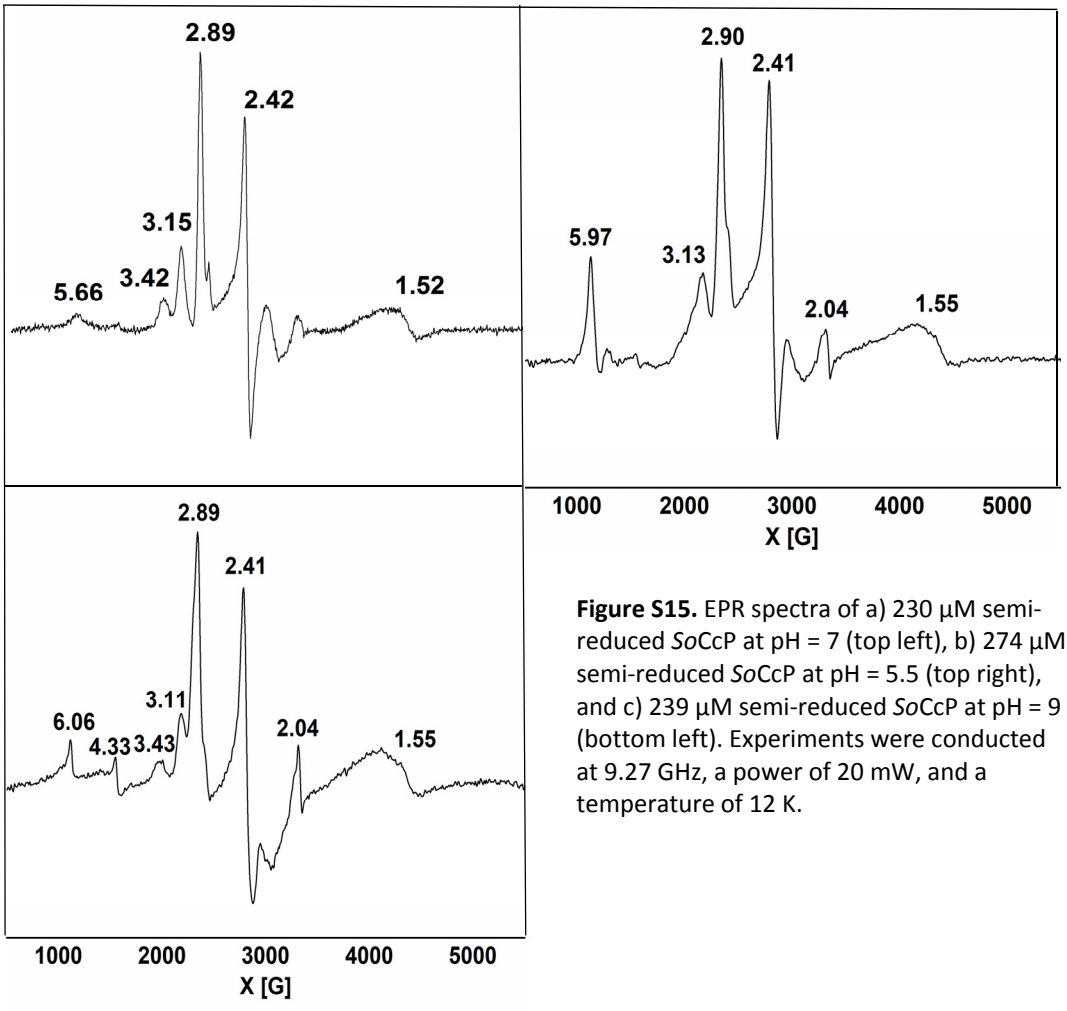

Figure S15. EPR spectra of a) 230 µM semi-reduced *So*CcP at pH = 7 (top left), b) 274 µM semi-reduced *So*CcP at pH = 5.5 (top right), and c) 239 µM semi-reduced *So*CcP at pH = 9 (bottom left). Experiments were conducted at 9.27 GHz, a power of 20 mW, and a temperature of 12 K.