Supplementary Materials

Reactivity of canonical bacterial cytochrome c peroxidases: insights into the electronic structure of Compound I

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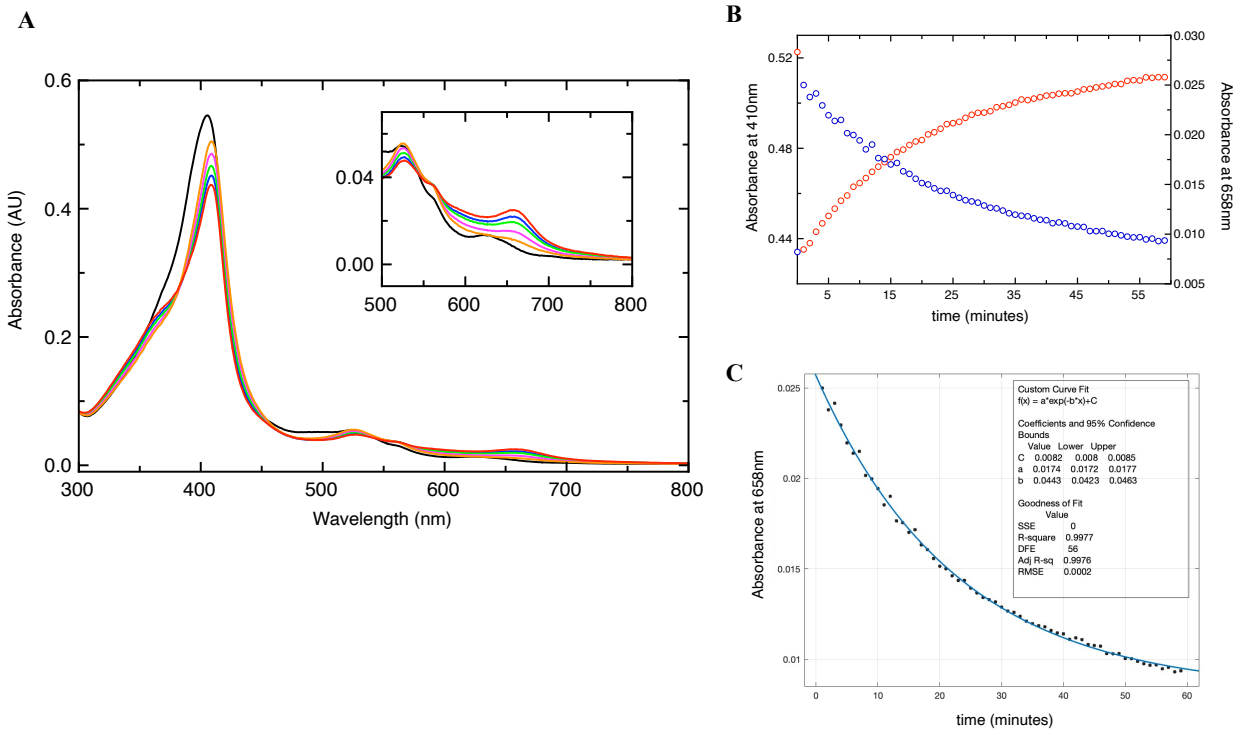

Figure S1: Decay of *Ne* bCcP compound 1 in the absence of reducing substrates at pH 7.5. A) spectra of selected timepoints: Diferric *Ne* bCcP (black), 1 minute post H_2O_2 addition (red), 5 minutes (blue), 10 minutes (green), 30 minutes (pink), and 40 minutes (orange). B) $A_{410\ nm}$ (red circles) and $A_{658\ nm}$ (blue circles) collected over 60 minutes (H_2O_2 was added after timepoint 0). C) exponential fit of the decrease in absorbance at 658nm vs time. The decay rate of the 658nm absorbance is 0.0443 min^{-1} at pH 7.5

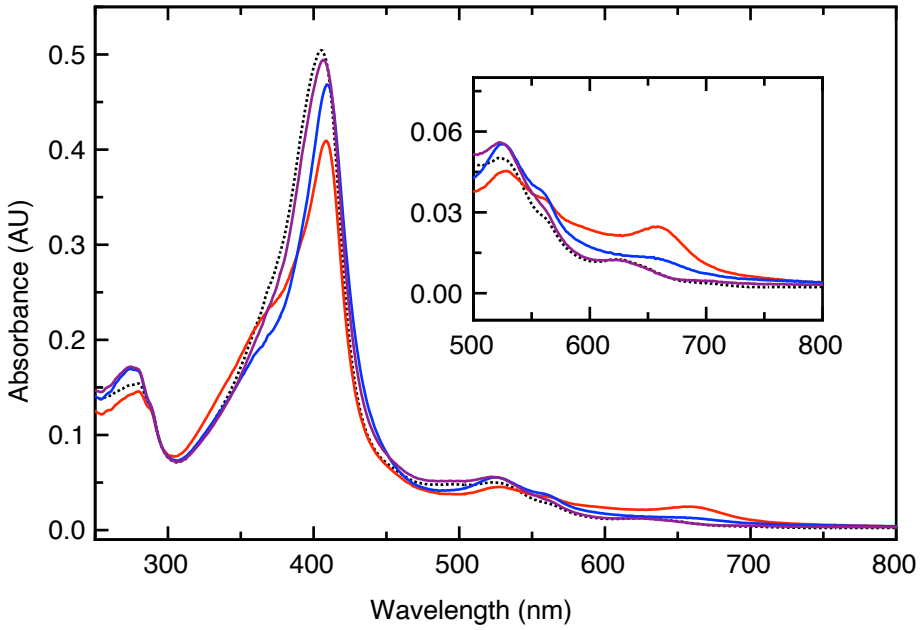

Figure S2: Addition of guiacol to *Ne* bCcP compound 1. Starting material of the diferric enzyme shown as the black dotted line. 1 equivalent of H_2O_2 was added to form compound 1 (red), which was quickly converted into compound 2 upon the addition of 2.5 equivalents guiacol (blue). The spectra taken 7 minutes post-guiacol addition (purple) resembles that of diferric *Ne* bCcP.

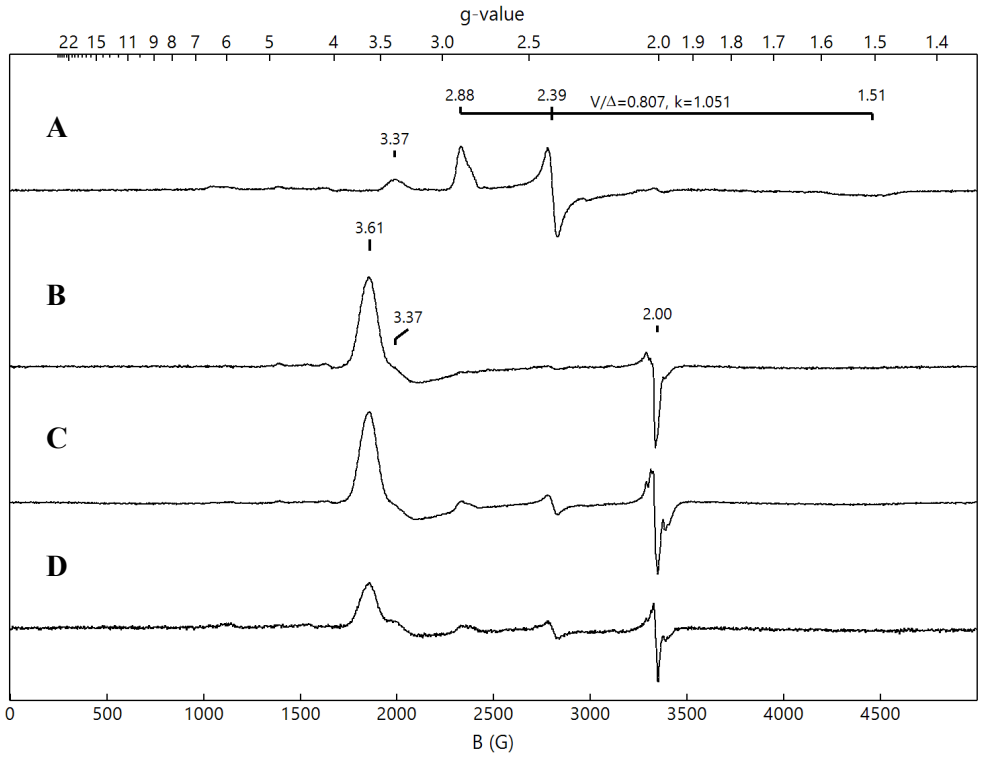

Figure S3: Decay of the g=3.6, 2 species over 30 minutes. Three separate diferric *Ne* bCcP samples were reacted with 5 equivalents peroxide and frozen at varying timepoints. Spectra of starting material were scaled to match LP heme signal intensity, and respective scale factors were used to scale the peroxide-reacted spectra of each sample. A) Diferric *Ne* bCcP. B) 2 minutes post-H_2O_2 addition. C) 4 minutes post-H_2O_2 addition. D) 30 minutes post-H_2O_2 addition. All spectra shown were collected at 0.2mW between 11-12K.

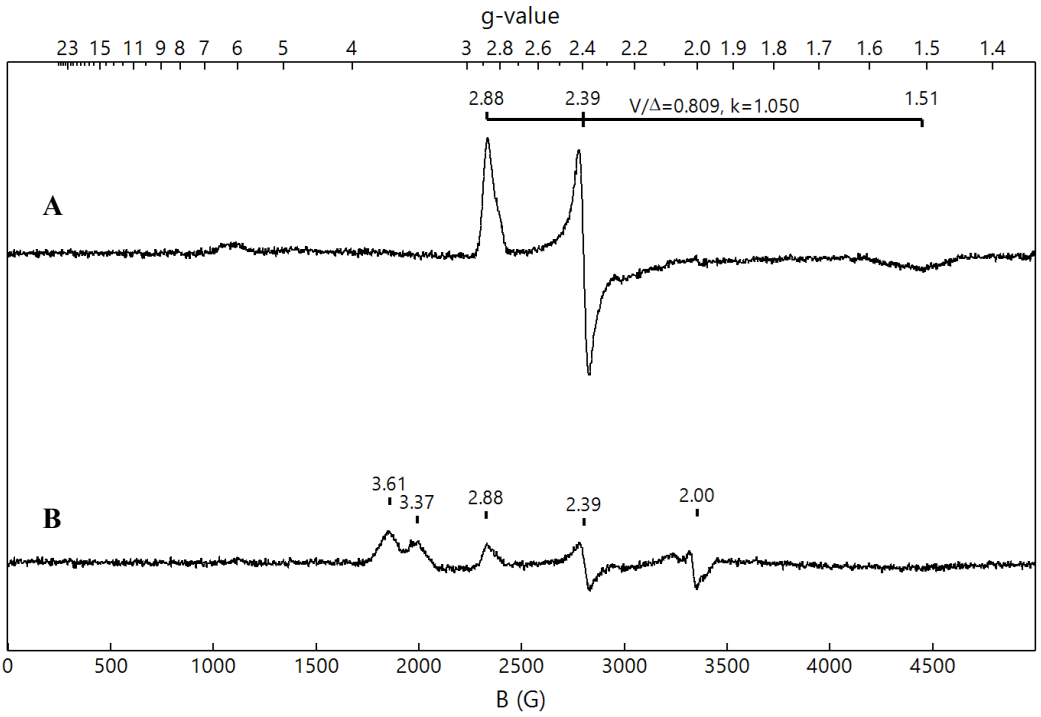

Figure S4: EPR spectra of H_2O_2 addition to semi-reduced *Ne* bCcP with a 5-minute incubation time at pH 7.5. A) Semi-reduced *Ne* bCcP. B) sample from A plus 5 equivalents peroxide, frozen ~5 minutes after peroxide addition. Both spectra were measured with 0.2mW power at 11.6K and 11.9K respectively.

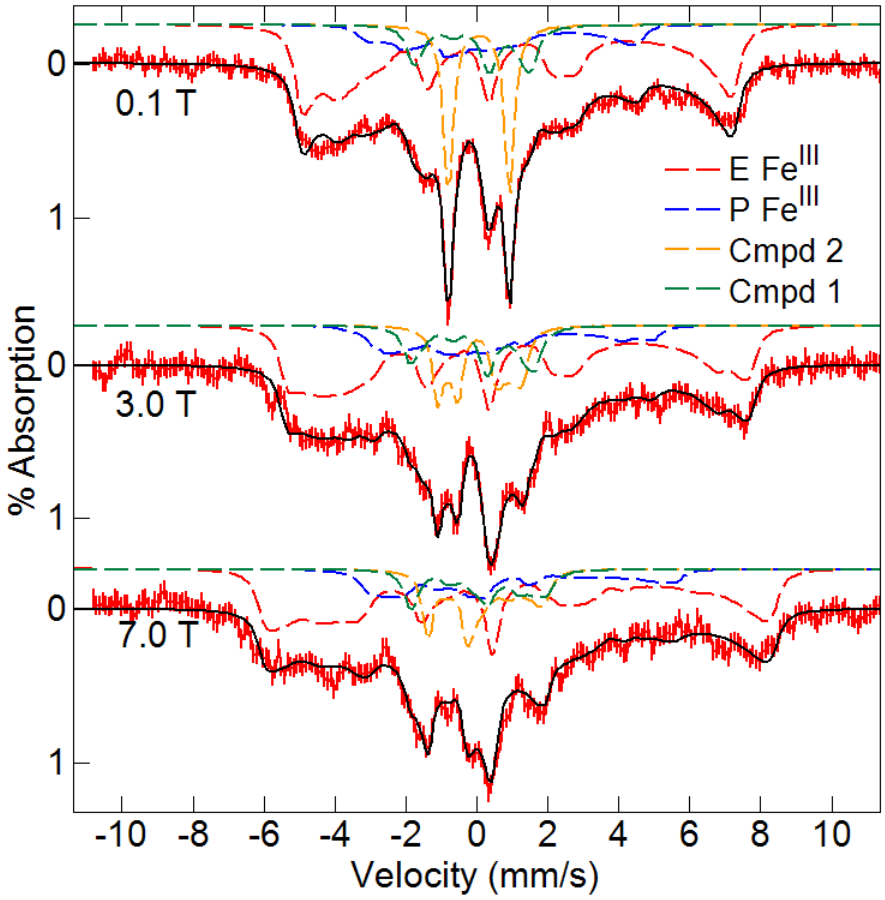

Figure S5: Mössbauer spectra (red vertical bars) of oxidized *Ne* bCcP at pH 7.5 reacted with H_2O_2 recorded at 4 K in the magnetic fields listed.

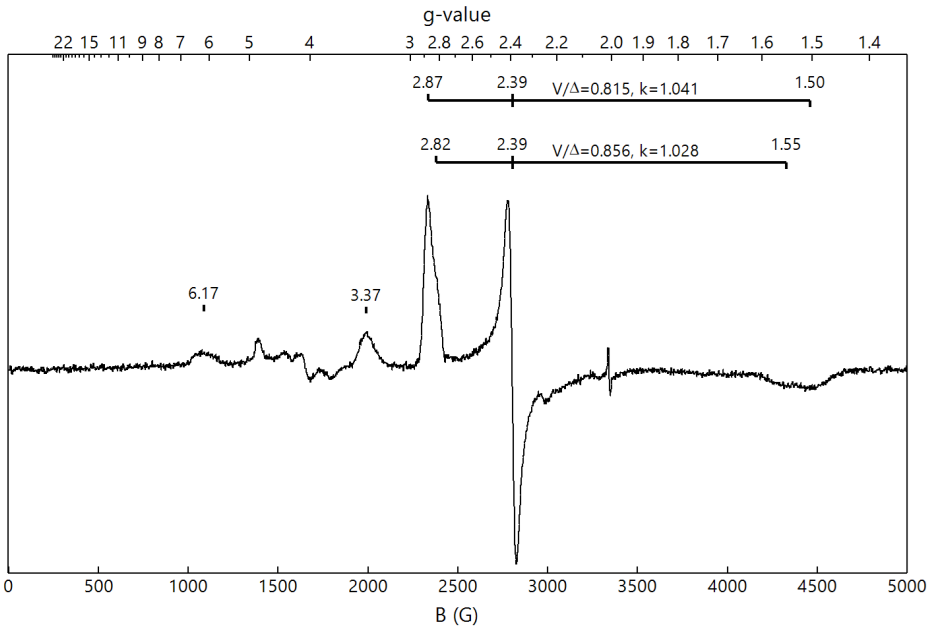

Figure S6: EPR Spectra of *Ne* bCcP at pH 8.6. An EPR sample of *Ne* bCcP was prepared in 20mM TAPS, 10% w/v glycerol, pH 8.6. Spectra shown was measured at 11K, 0.2mW power.

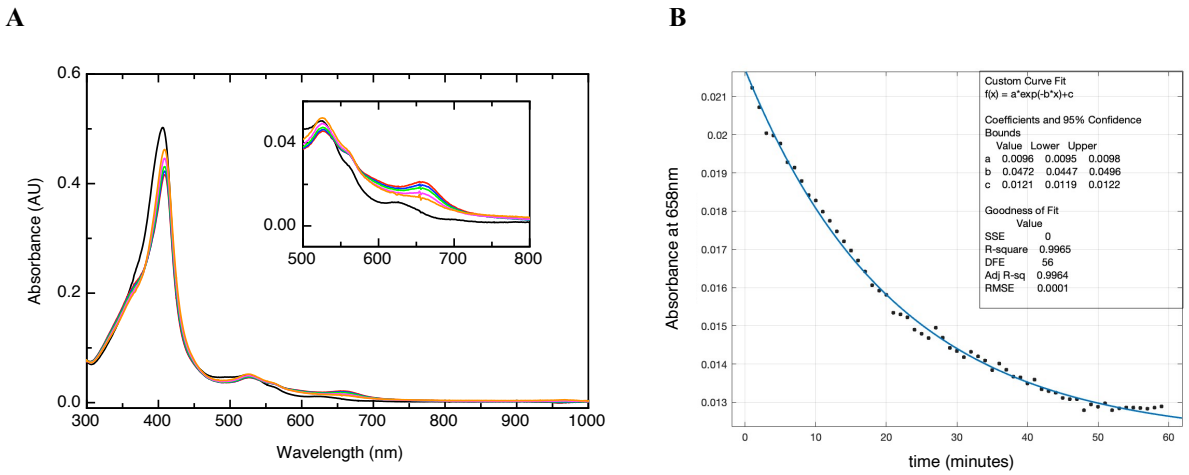

Figure S7: Decay of compound 1 at pH 8.6. A) Diferric *Ne* bCcP (black). 2 equivalents H_2O_2 added to form compound 1. The resulting decay of compound 1 is shown at 1 minute (red), 5 minutes (blue), 10 minutes (green), 20 minutes (pink), and 40 minutes (orange). B) Exponential fit of decrease of 658nm absorbance vs time. The decay rate of the 658nm absorbance is 0.0472 min^{-1} at pH 8.6

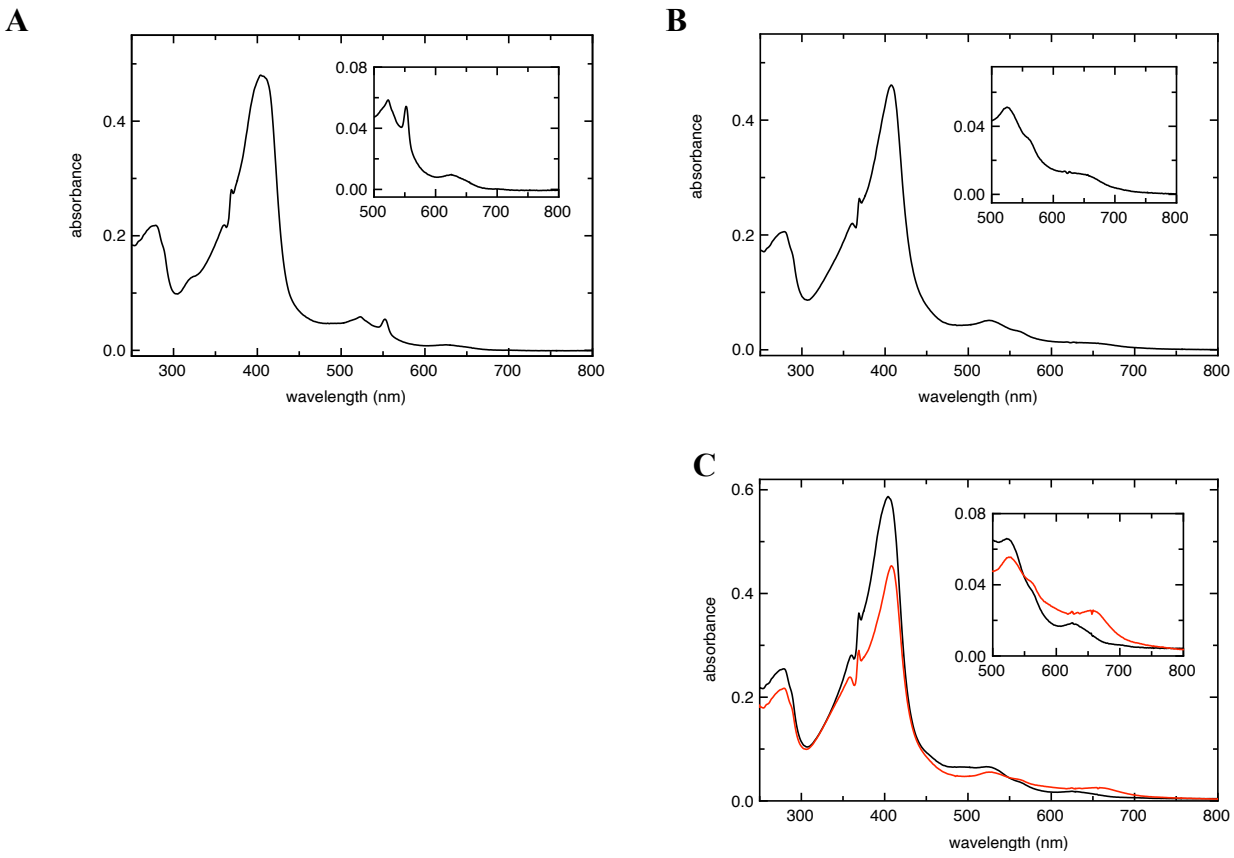

Figure S8: Representative UV-Vis spectra of Mössbauer samples. Before final freezing of the Mössbauer samples, a small a volume of the sample was taken from the cup and diluted in buffer for UV-Vis measurement. **A)** The *Ne* bCcP sample with a fraction of the E heme reduced (Mössbauer spectra in figure 4 in main text). **B)** the low compound 1 yield pH 7.5 sample, where 20 equivalents of H_2O_2 were added to *Ne* bCcP that had a fraction of the E heme reduced. Samples in A and B were prepared side by side from the same stock of concentrated protein. **C)** The high yield compound 1 sample at pH 8.6 (Mössbauer spectra in figure 5 in main text). Spectra were taken from this sample before (black) and after (red) addition of 15 equivalents of H_2O_2.