Electronic Supplementary Information

Structure of a ferrous heme-nitro species in the binuclear heme a_3/Cu_B center of ba_3-cytochrome *c* oxidase as determined by resonance Raman spectroscopy

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Experimental Methods

The ba_3-oxidase was isolated from *T. thermophilus* HB8 cells according to the published procedures.[16] The purified enzyme was concentrated to 200-400 μM, frozen in liquid nitrogen and stored at -80 °C until use. Chemicals were purchased from Sigma Aldrich and were of analytical grade. The ba_3-oxidase samples used for optical absorption and RR measurements were diluted to 5 μM and 50 μM, respectively, using the desired buffers (pH 6.0, MES; pH 7.5, Tris-HCl; pH 9.0, CHES). The pD of solutions prepared in D_2O buffers were measured by using a pH meter and were reported by assuming pD = pH (observed) + 0.4. The nitrite adducts of ba_3-oxidase were prepared by incubation of the oxidized enzyme with a 500-fold excess of sodium nitrite for 5 h and the subsequent addition of a 300-fold excess of cysteine under anaerobic conditions that were attained by cycling the samples 4-6 times between vacuum and nitrogen in a vacuum line. The isotopically labeled $Na^{15}NO_2$ (98% ^{15}N, 95% CP) and $Na^{15}N^{18}O_2$ (98% ^{15}N, 90% ^{18}O, 95% CP) were obtained from Sigma Aldrich.

Optical absorption spectra were collected with a Shimadzu UV1700 UV-Vis spectrometer. All samples were prepared under anaerobic conditions in quartz cells. Optical absorption spectra were collected upon the addition of 15 mM cysteine to nitrite solution (25 mM) to control if nitrite is reduced by cysteine under our experimental conditions. The absorbance of the nitrite band at 354 nm (ε_{354}=22.7 M^{-1} cm^{-1}) remained constant confirming that nitrite is not reduced by cysteine in agreement with previous report.[9] Resonance Raman spectra were acquired at room temperature (20 ^{o}C) using a 640mm focal length Czerny-

Turner spectrograph (Horiba, T64000 system operated in single stage), equipped with a 1800 g/mm holographic grating and a Horiba Symphony BIUV1024x256 CCD detector. Samples were placed anaerobically in a quartz spinning tube to minimize local heating, and scattering was collected in a 90° geometry. The excitation wavelength at 441.6 nm was provided by a Kimmon HeCd laser, and a Semrock 442 nm long-pass edge filter was used to reject Rayleigh scattering. The power incident on the sample was 4-5 mW and the total accumulation time for each spectrum was 20-40 min. An Ondax SureLock LM-405 laser with an integrated CleanLine ASE filter was used to provide the excitation wavelength at 405 nm, and a Semrock StopLine 405 nm single-notch filter was used to reject Rayleigh scattering. The power incident on the sample was 4 mW and total accumulation time for each spectrum was 10-20 min. The Raman shifts were calibrated using toluene. Origin software (OriginLabs) was used for spectra processing and analysis. Optical absorption spectra before and after the Raman measurements were collected to ensure the formation and stability of the ba_3-NO_2^- adducts.

Supplementary Figures

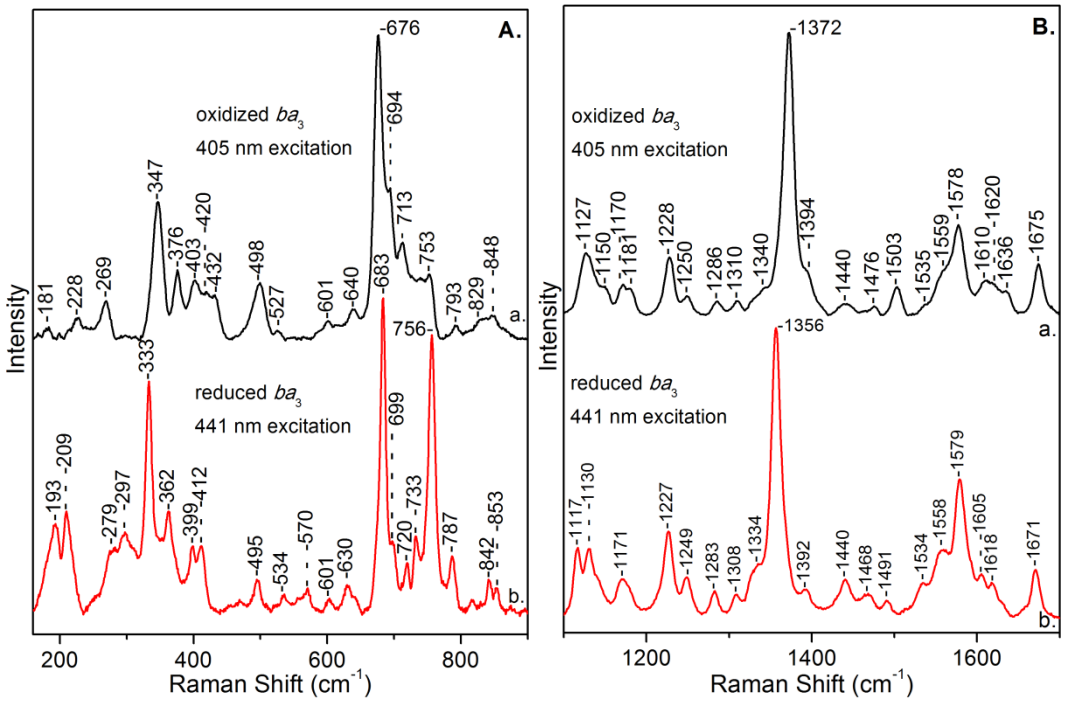

Fig. S1 Resonance Raman spectra of ba_3-oxidase in the oxidized (trace a) and fully-reduced (trace b) forms in the low frequency (panel A) and high frequency (panel B) regions. The excitation wavelengths were 405 nm and 441 nm for the oxidized and reduced forms of ba_3-oxidase, respectively.

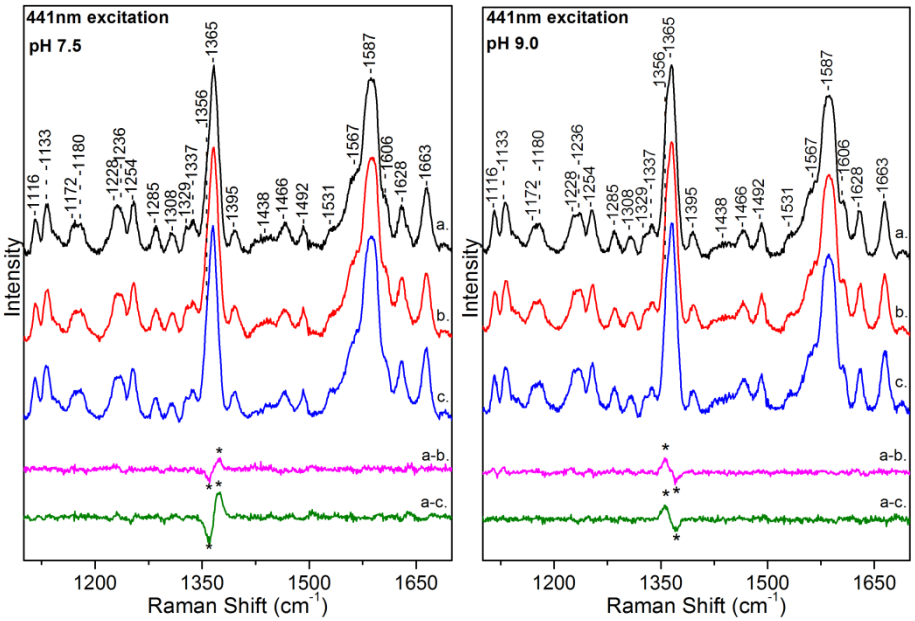

Fig. S2 High frequency resonance Raman spectra of the cytochrome ba_3-NO_2 adducts formed after cysteine addition to $^{14}N^{16}O_2^-$ (trace a), $^{15}N^{16}O_2^-$ (trace b) and $^{15}N^{18}O_2^-$ incubated enzyme at pH 7.5 (left panel) and pH 9.0 (right panel). The difference a-b (ba_3-$^{14}N^{16}O_2$ *minus* ba_3-$^{15}N^{16}O_2$) and a-c (ba_3-$^{14}N^{16}O_2$ *minus* ba_3-$^{15}N^{18}O_2$) resonance Raman spectra are included in each panel. The excitation wavelength was 441 nm and the incident power on the samples 4-5 mW.