

The Rate-Limiting Step in O₂ Reduction by Cytochrome *ba*₃ from *Thermus thermophilus*

Tsuyoshi Egawa[§], Ying Chen^{||}, James A. Fee^{||}, Syun-Ru Yeh[§] and Denis L. Rousseau^{§*}.

[§]Department of Physiology and Biophysics, Albert Einstein College of Medicine, Bronx, NY

^{||}Department of Molecular Biology, The Scripps Research Institute, La Jolla, 92037 CA.

Supplementary Data

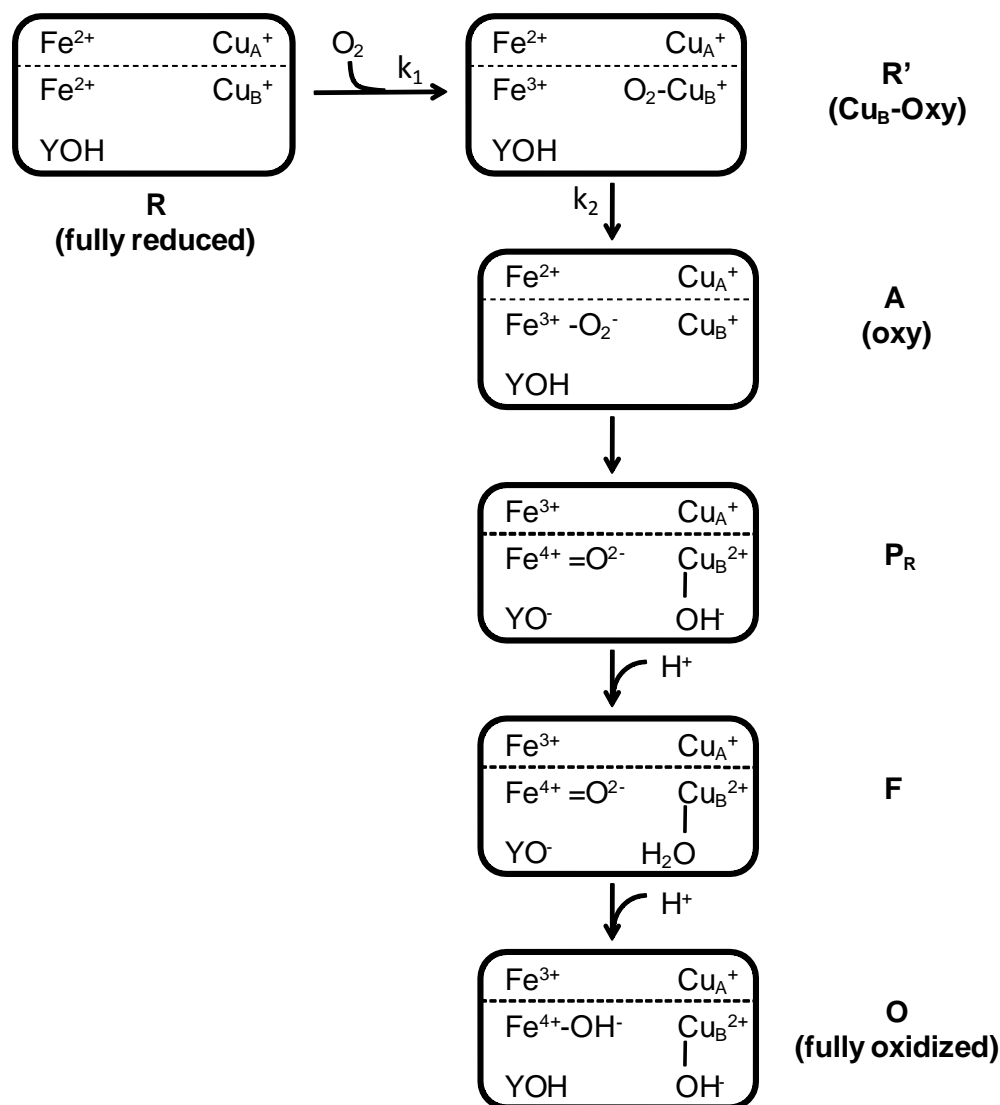


Fig. S1: Proposed reaction scheme for the 4 electron reduction of dioxygen catalyzed by heme-copper oxidases. The upper row in each rectangle represents the electron storage metal centers, Cu_A and the iron corresponding to either heme *a* in the *aa*₃ oxidases or heme *b* in the *ba*₃

protein. The lower row shows the Cu_B -heme a_3 binuclear center together with the nearby tyrosine residue. In the case of the ba_3 reaction, the structure of the intermediates may differ from those proposed for the aa_3 oxidases shown here.

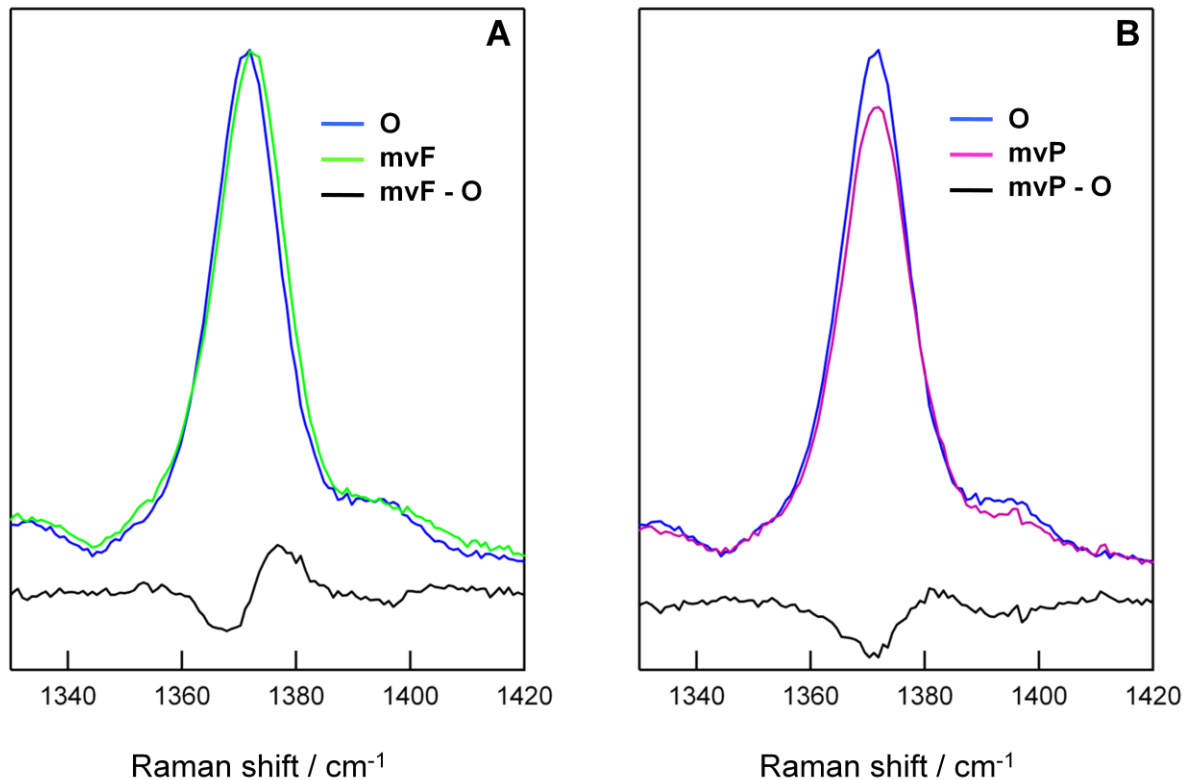


Fig. S2. A comparison of the ν_4 resonance Raman (RR) bands of bovine CcO from the oxidized state, **O**, the mixed valence **F** (mvF) and mixed valence **P** (mvP) states. In A the ν_4 resonance Raman bands of the **O** (blue trace) and the mvF (green trace) states are compared. The apparent frequency shift is small because both heme a and heme a_3 contributed to the RR intensity. In both the **O** and mvF states, heme a is in its ferric oxidation state (a^{3+}); therefore, the 1:1 subtraction between the recorded spectra yields a difference spectrum (black trace) that corresponds to the difference between the ferryl and the ferric derivatives of heme a_3 . The derivative-like shape in the difference spectrum in the 1370 cm^{-1} region demonstrates an up-shift of the ν_4 of heme a_3 upon the conversion of the ferric to ferryl state. In B the ν_4 resonance Raman bands of the **O** (blue trace) and the mvP (red trace) states are compared and a result similar to that of mvF was obtained. These data serve as a reference for the analysis of the ν_4 RR bands of our ba_3 samples to determine if a ferryl was formed in the reaction of the enzyme with O_2 .

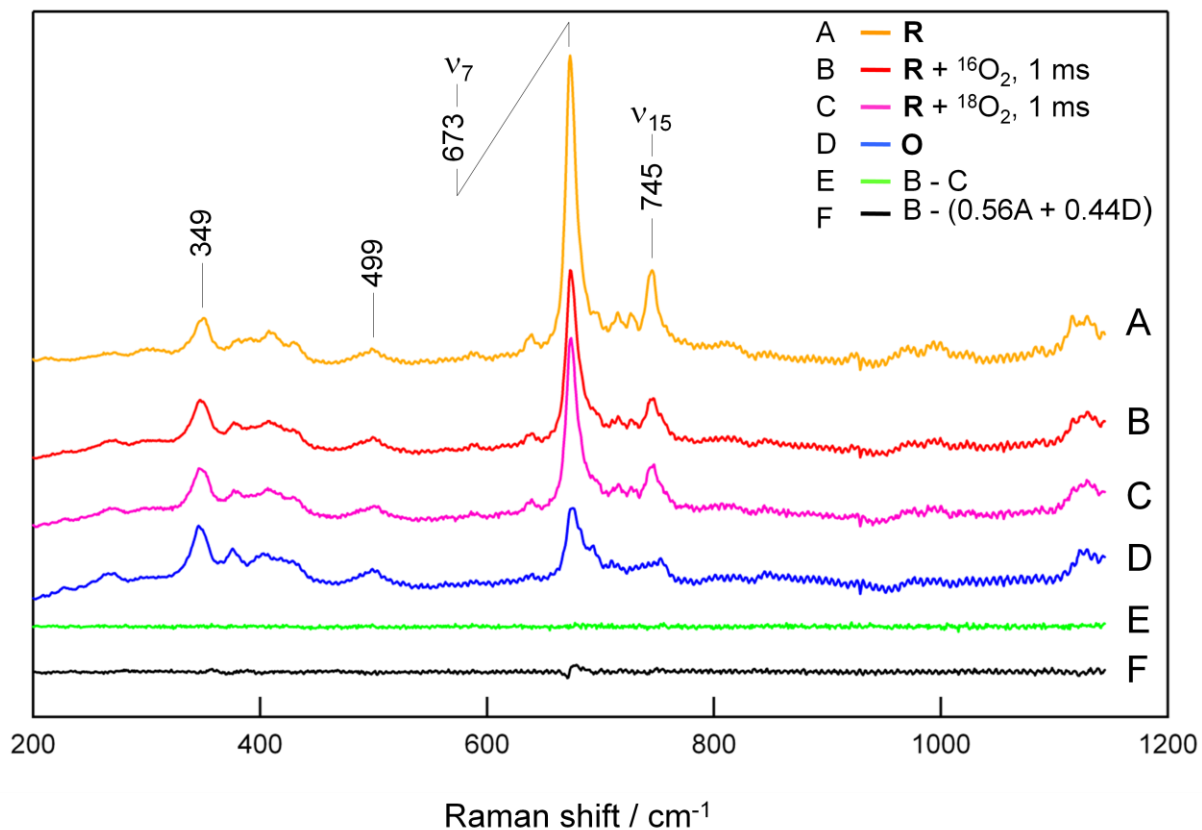


Fig. S3. Low frequency RR spectra of the ba_3 samples. The RR spectra were measured for the **R** and **O** state ba_3 , and for the reaction of **R** state ba_3 with ${}^{16}\text{O}_2$ and ${}^{18}\text{O}_2$ at 1ms. All spectra were recorded for the samples in the continuous-flow cell. Differences among the spectra were calculated as indicated. The observed and difference spectra are on the same scale.