# Stable Cu(II) and Cu(I) Mononuclear Intermediates in the Assembly of the CuA center of *Thermus thermophilus* Cytochrome Oxidase

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## Supplementary Information

Fig S1. Fourier transforms and EXAFS (insets) for experimental (black) and simulated (red) spectra of the fully metalated dicopper forms of M160SeM *T. thermophilus* soluble CuA. Plots on the left are Cu EXAFS, while plots on the right are Se EXAFS. Top two panels are the mixed-valence spectra while the bottom two panels are the di-Cu(I) spectra.

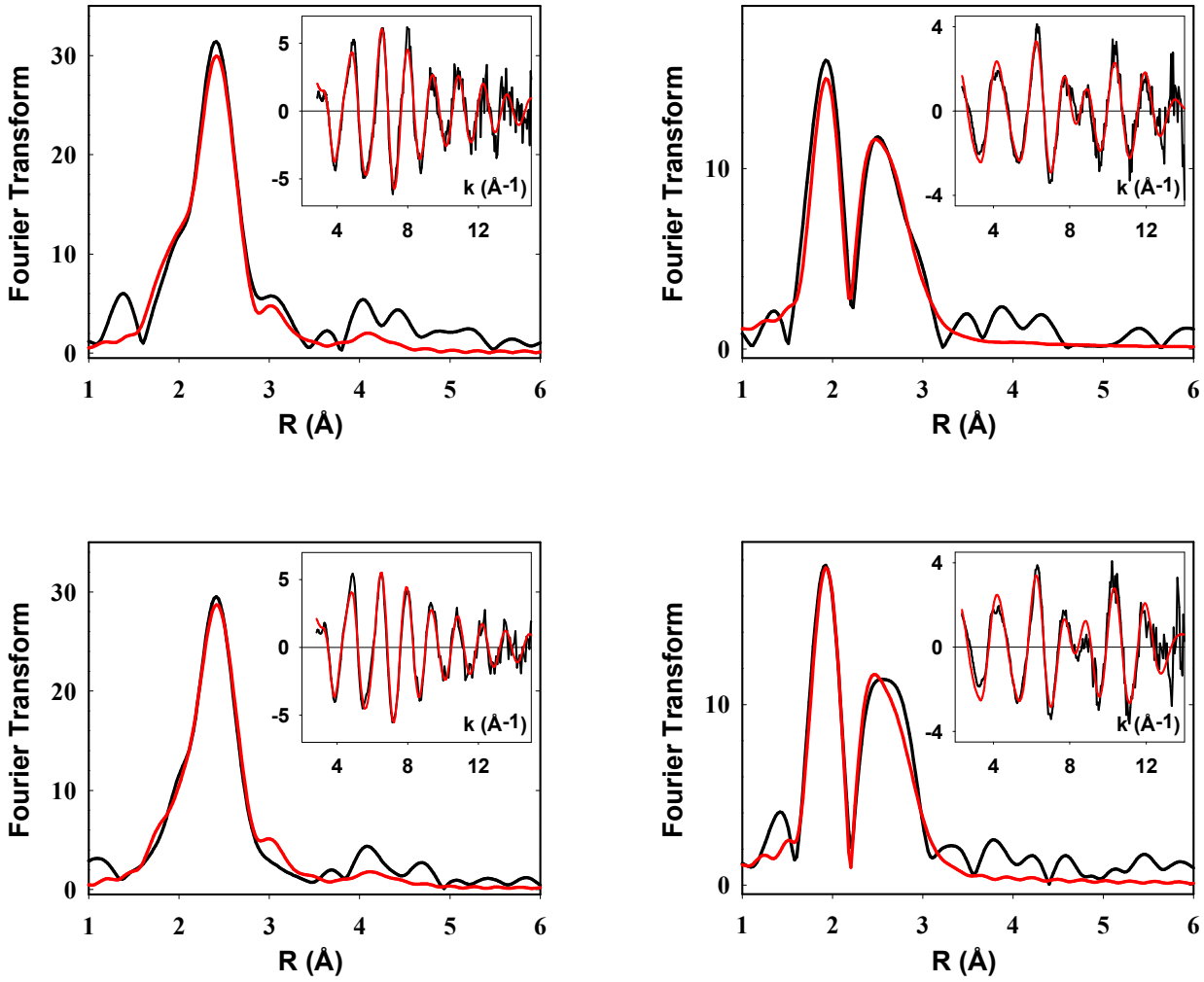

Figure S2. Multiwavelength fits to the kinetic data for the reaction of 175 μM apo M160SeM CuA with 500 μM Cu(II) at 23 $^{0}$C. Fits were based on the mechanism and rate constants listed in Table 2.

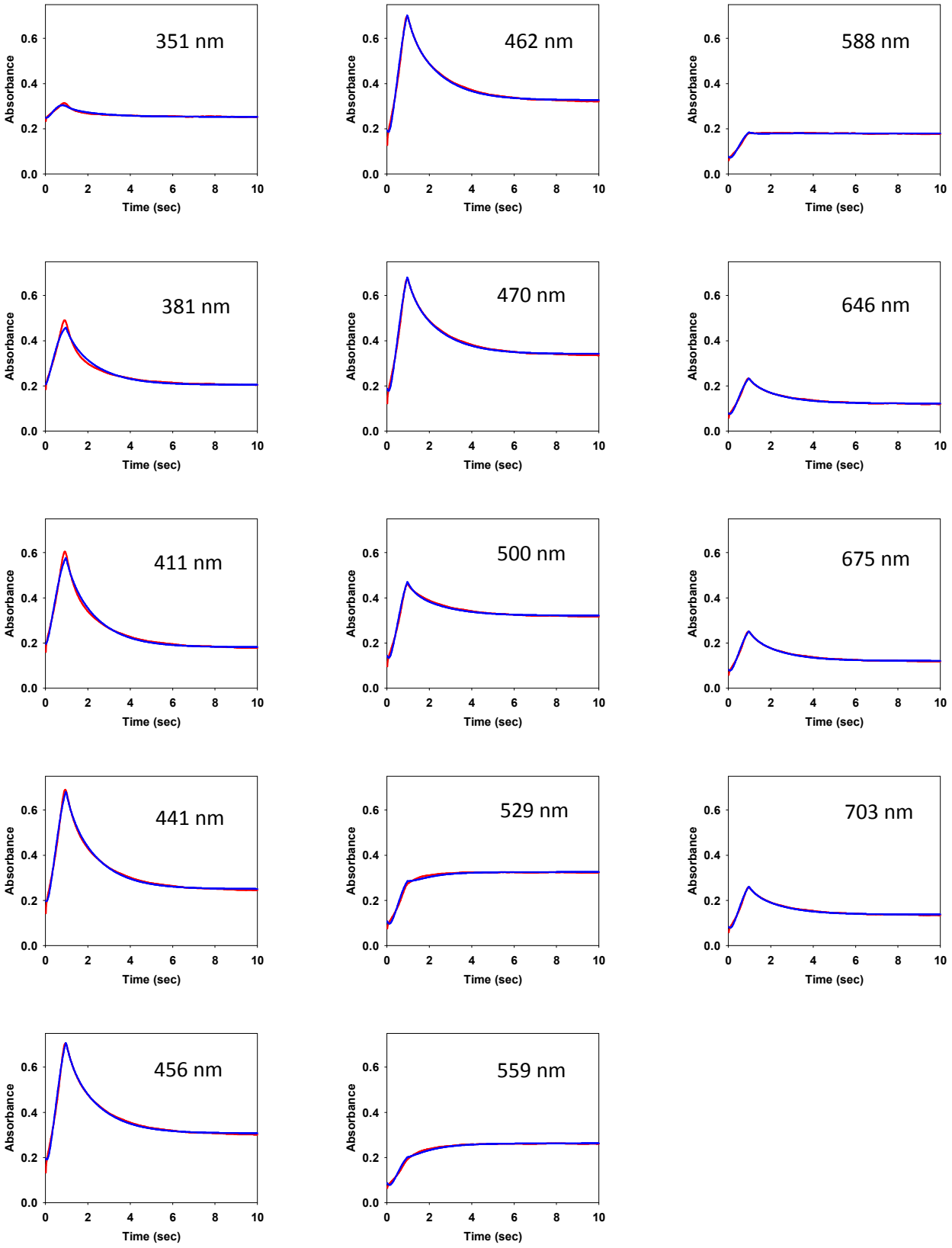

Table S1. Parameters used to fit the Cu and Se EXAFS spectra of the dinuclear forms of M160SeM *T. thermophilus* CuA at the Cu and Se edges. The mixed valence form was prepared by addition of an excess of aqueous Cu(II) sulfate to the bis-thiol apo protein, followed by dialysis to remove excess Cu(II). The reduced di-Cu(I) forms were prepared either by dithionite reduction of the mixed-valence derivative, or by addition of a 3-fold excess of $[Cu(I)(CH_3CN)_4]PF_6$ followed by removal of excess Cu(I) using a desalting spin column.

| Cu edge | F[a] | C-N(His)[b] | | | Cu-Se | | | Cu-S | | | Cu-Cu | | | $E_0$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | No[c] | R (Å)[d] | DW (Å$^2$) | No[c] | R (Å)[d] | DW (Å$^2$) | No[c] | R (Å)[d] | DW (Å$^2$) | No[c] | R (Å)[d] | DW (Å$^2$) | |
| Mixed-Valence | 0.541 | 1 | 1.93 | 0.009 | 0.5 | 2.49 | 0.012 | 2 | 2.23 | 0.016 | 1 | 2.46 | 0.006 | 3.2 |
| di-Cu(I)-dithio | 0.561 | 1 | 1.93 | 0.013 | 0.5 | 2.51 | 0.012 | 2 | 2.24 | 0.016 | 1 | 2.47 | 0.006 | 2.5 |
| di-Cu(I)-ACN | 0.297 | 1 | 1.93 | 0.020 | 0.5 | 2.52 | 0.011 | 2 | 2.25 | 0.016 | 1 | 2.48 | 0.007 | 0.6 |

| Se Edge | F[a] | Se-C(met) | | | Se-Cu[f] | | | Se-S | | | -$E_0$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | No[c] | R (Å)[d] | DW (Å$^2$) | No[c] | R (Å)[d] | DW (Å$^2$) | No[c] | R (Å)[d] | DW (Å$^2$) | |
| Mixed_Valence | 0.949 | 2 | 1.96 | 0.005 | 1 | 2.48 | 0.011 | 1 | 3.06 | 0.025 | 4.7 |
| di-Cu(I)-dithio | 0.914 | 2 | 1.95 | 0.002 | 1 | 2.49 | 0.012 | 1 | 3.06 | 0.023 | 4.5 |
| di-Cu(I)-ACN | 0.734 | 2 | 1.95 | 0.005 | 1 | 2.51 | 0.010 | 1 | 3.05 | 0.025 | 4.7 |

[a] F is a least-squares fitting parameter defined as $F^2 = \frac{1}{N}\sum_{i=1}^{N} k^6 ( Data - Model )^2$

[b] Fits modeled histidine coordination by an imidazole ring, which included single and multiple scattering contributions from the second shell (C2/C5) and third shell (C3/N4) atoms respectively. The Cu-N-$C_x$ angles were Cu-N-C2 126°, Cu-N-C3 -126°, Cu-N-N4 163°, Cu-N-C5 -163°.

[c] Coordination numbers are generally considered accurate to ± 25%

[d] In any one fit, the statistical error in bond-lengths is ±0.005 Å. However, when errors due to imperfect background subtraction, phase-shift calculations, and noise in the data are compounded, the actual error is probably closer to ±0.02 Å.