Supplementary Information

Sequential oxidations of thiolates and cobalt metallocenter in a synthetic metallopeptide: Implications for the biosynthesis of nitrile hydratase

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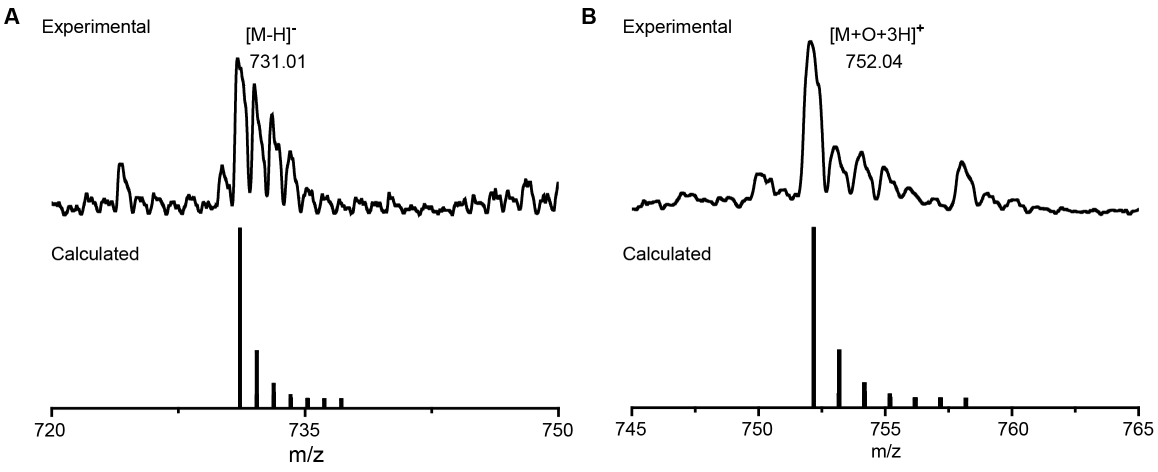


Figure S1. Experimental and calculated ESI-MS spectra from (A) Co-SODA (reduced; negative ion mode) and (B) Co-SODA (oxidized; positive ion mode).

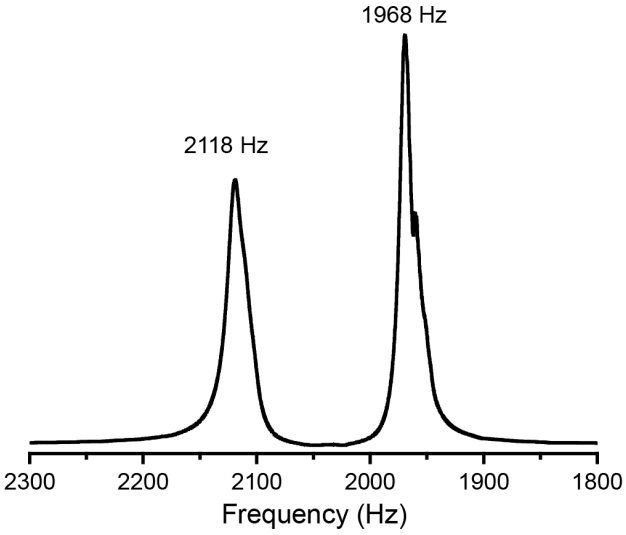


Figure S2. ^{1}H NMR resonances for water in a partially oxidized Co-SODA sample. The sample was placed within another NMR tube (O.D. ~ 5 mm) containing pure H_2O as described in the Methods section.

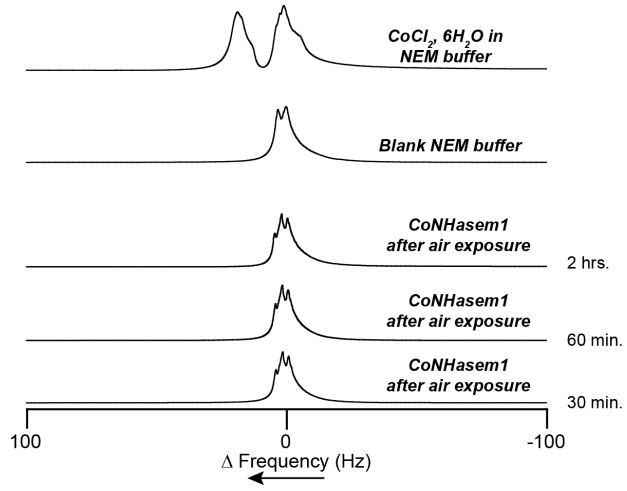


Figure S3.Solvent ^{1}H NMR resonance signal shifts of $CoCl_2$ sample, blank NEM buffer, and reduced Co-NHasem1. All samples are in aqueous buffer (50 mM NEM, pH 7.5) and the time since exposure to air is indicated in the figure. The shift in the solvent resonance frequency is plotted relative to a blank (aq. NEM buffer solution without any sample) solution (Δ Frequency (Hz) = Solvent NMR Frequency$_{(Sample)}$ − Solvent NMR Frequency$_{(Blank)}$). CoNHasem1 showed rapid formation of diamagnetic Co(III) species in the presence of air.

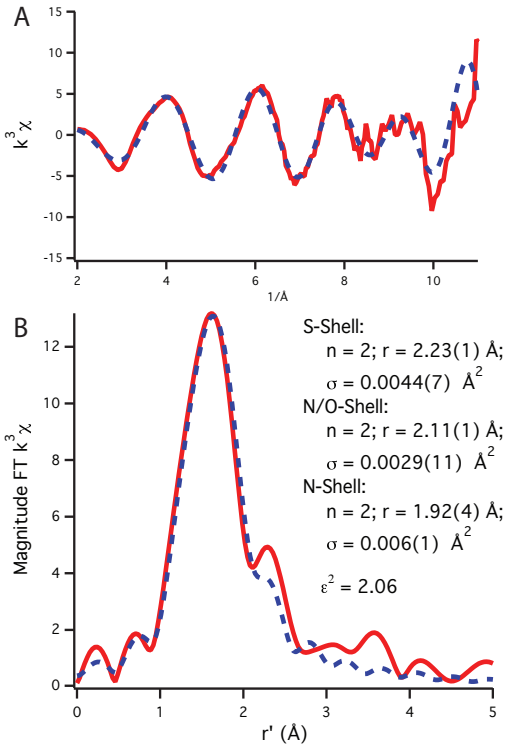


Figure S4. EXAFS region of the X-ray absorption spectrum of partially oxidized Co-SODA. (A) Unfiltered k^3 data and (B) FT k^3 data (experimental data and simulated data are represented as the red solid line and the blue dotted line, respectively).

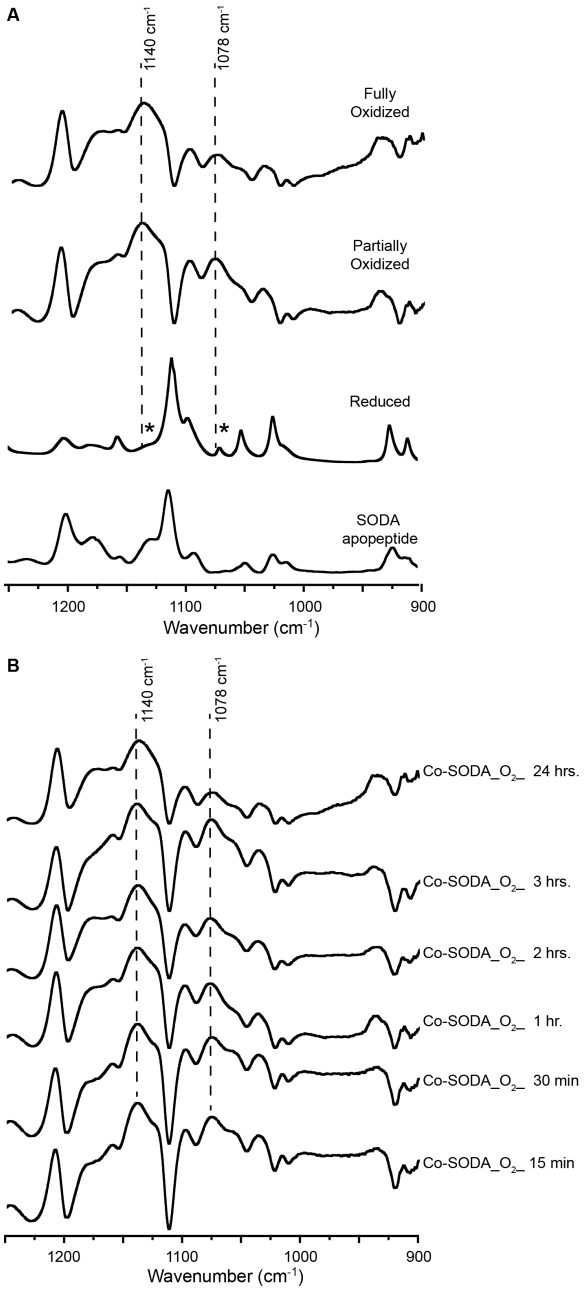


Figure S5. (A) FTIR spectra of SODA apopeptide (dry film), reduced Co-SODA (KBr pellet sample, asterisks showed slight oxidation of the sample during the experiment), partially oxidized Co-SODA (dry film) and fully oxidized CoSODA (dry film). (B) Time course FTIR measurement of Co-SODA sample following different times after air exposure (dry film prepared from metallopeptide in aqueous 50 mM NEM buffer at pH 7.5).

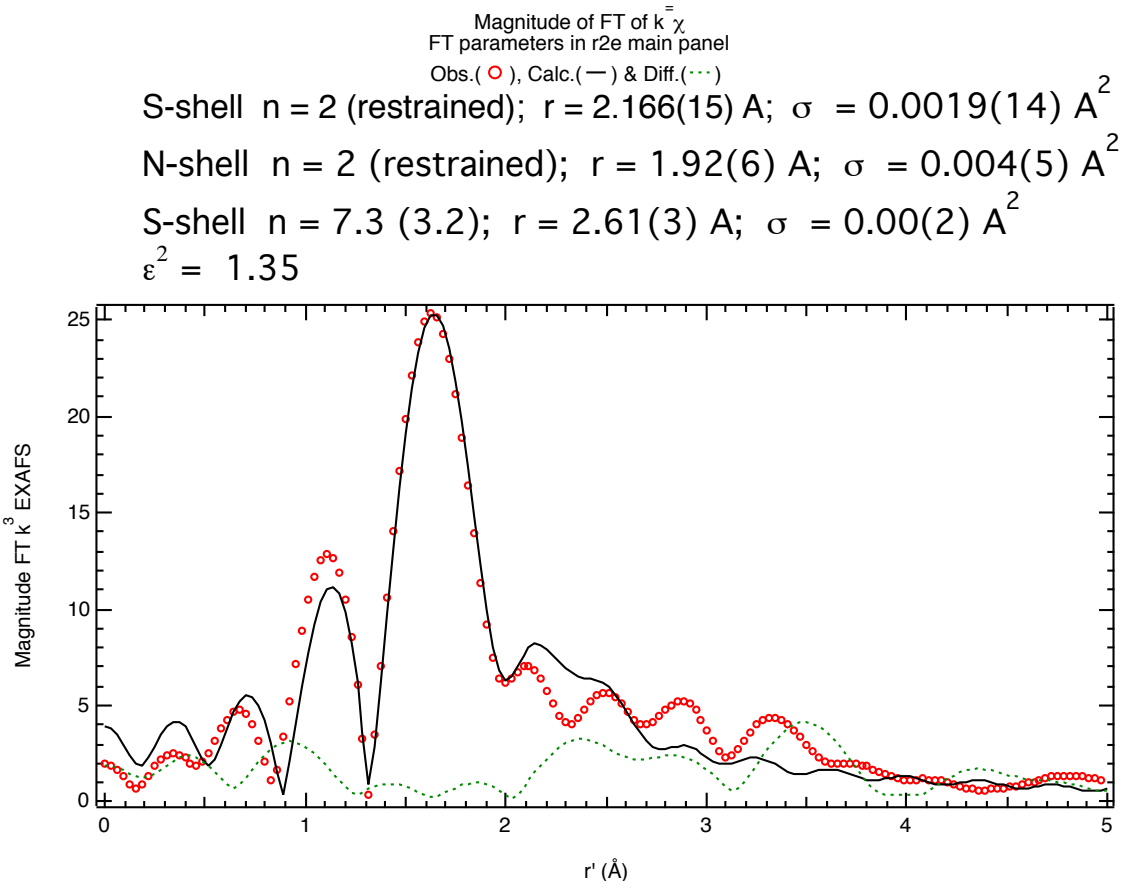


Figure S6. Magnitude Fourier Transformed $k^3(\chi)$ (open circles: raw data; black solid line simulated spectrum; dotted green line difference spectrum) for fully reduced CoSODA. Fourier transform from k = 2.2 - 11.0 $Å^{-1}$. Simulation parameters are displayed in the figure.

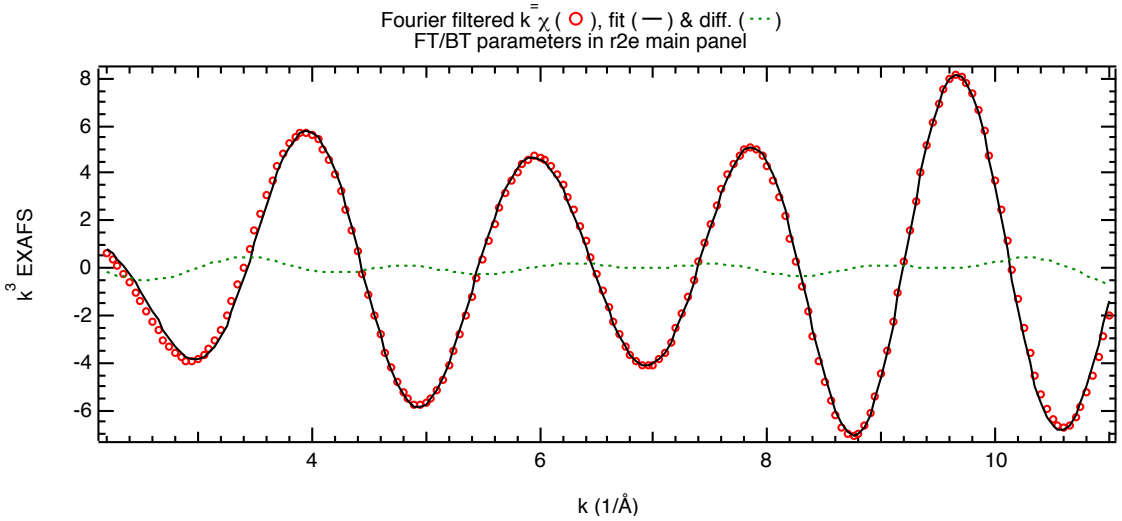
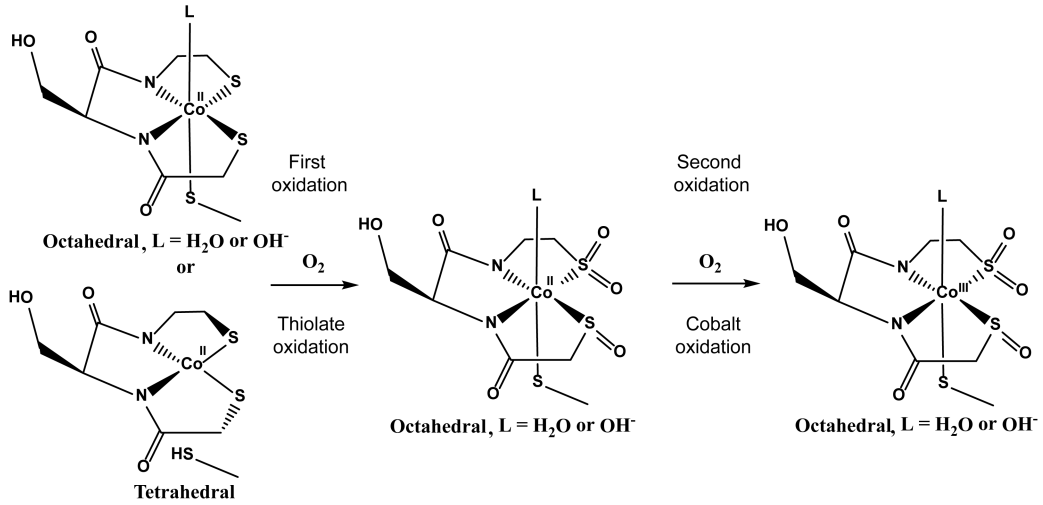


Figure S7. Fourier Filtered $k^3(\chi)$ (open circles: raw data; black solid line simulated spectrum; dotted green line difference spectrum). Fourier transform from k = 2.2-11.0 $Å^{-1}$; Back transformed from 1.0 to 2.5 Å. Simulation parameters are displayed in the FT EXAFS figure from above.

Scheme S1. Hypothetical sequence for *in vivo* oxidation of Co-NHase.



Scheme S2. Energy diagrams for the d orbitals of Co for various geometries and oxidation states.