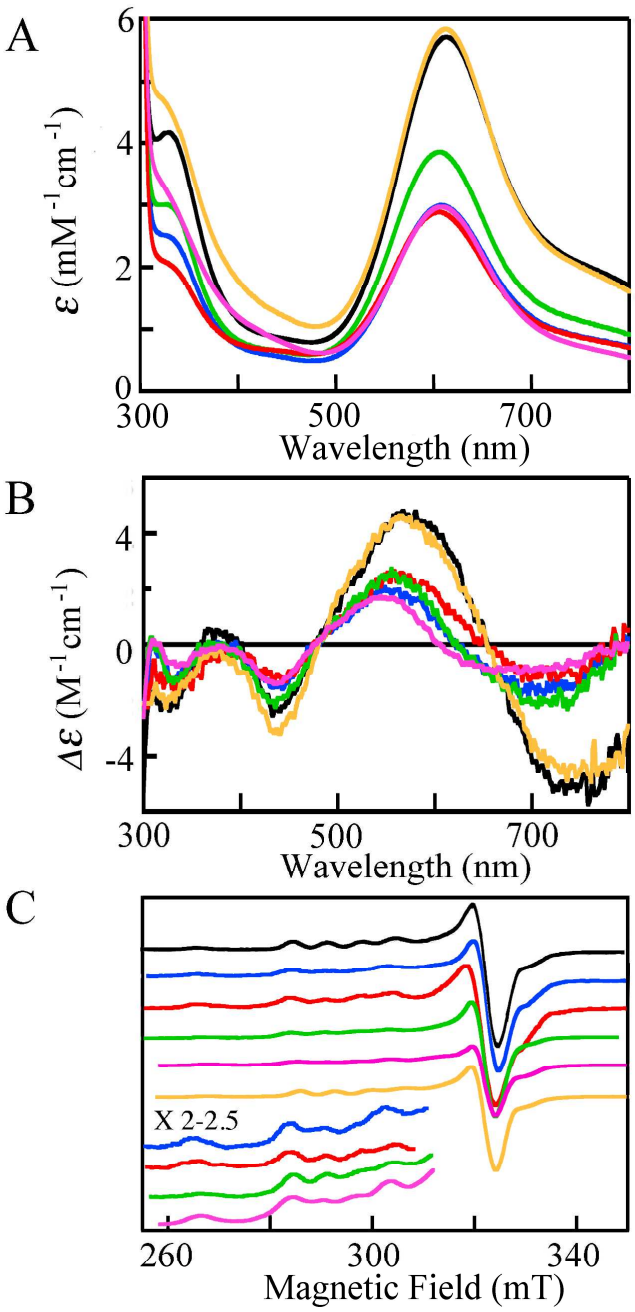
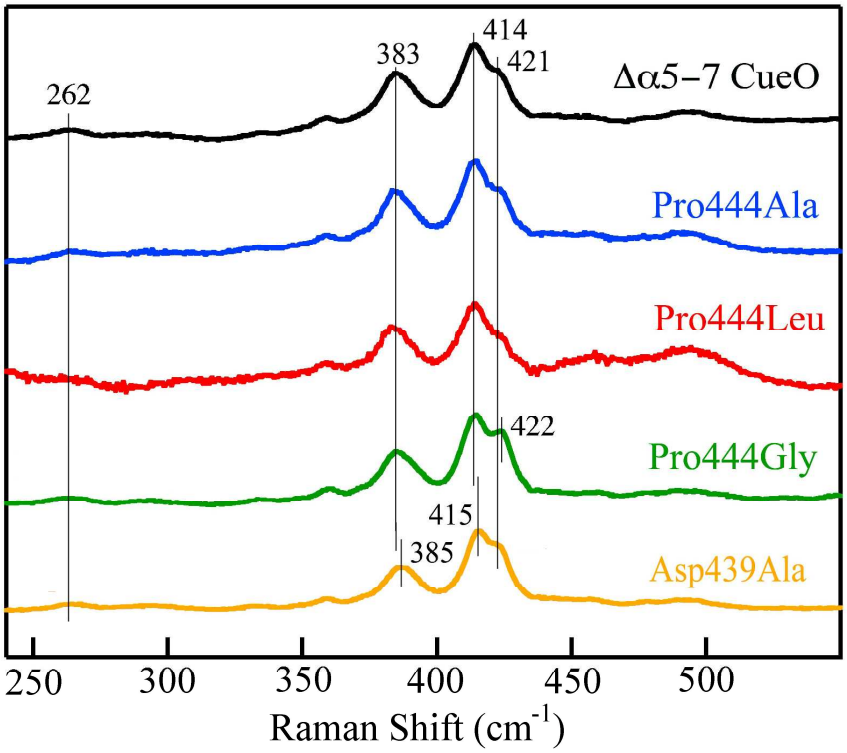
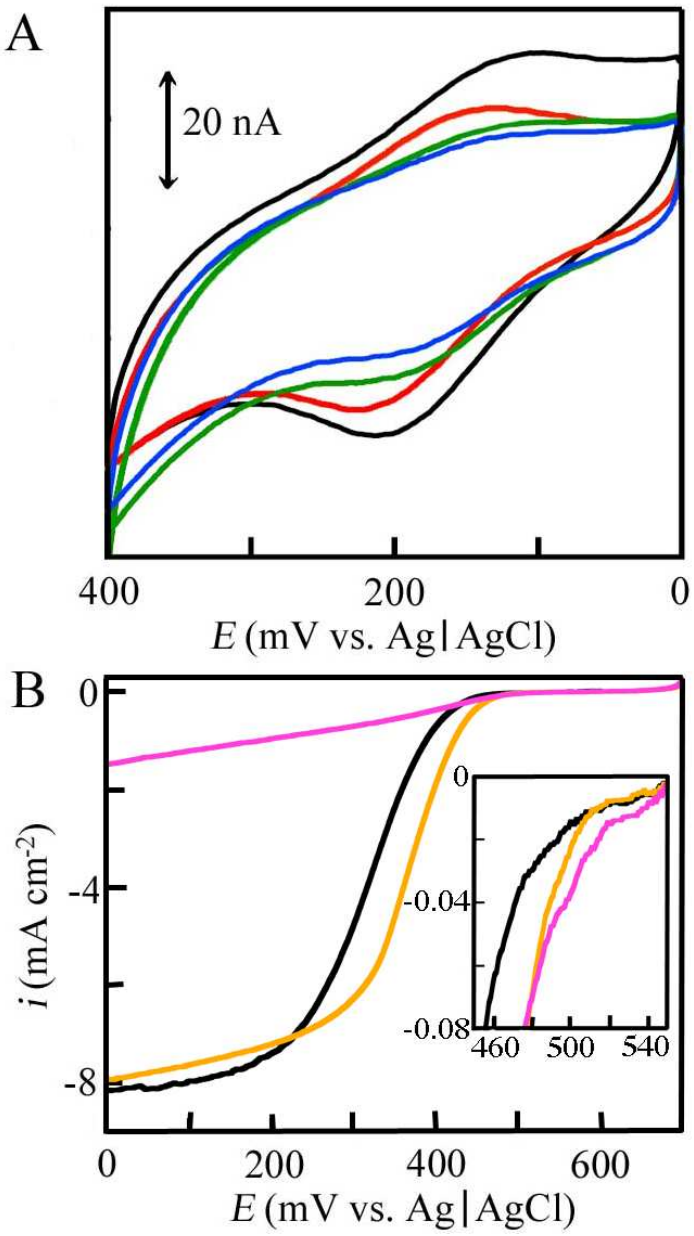


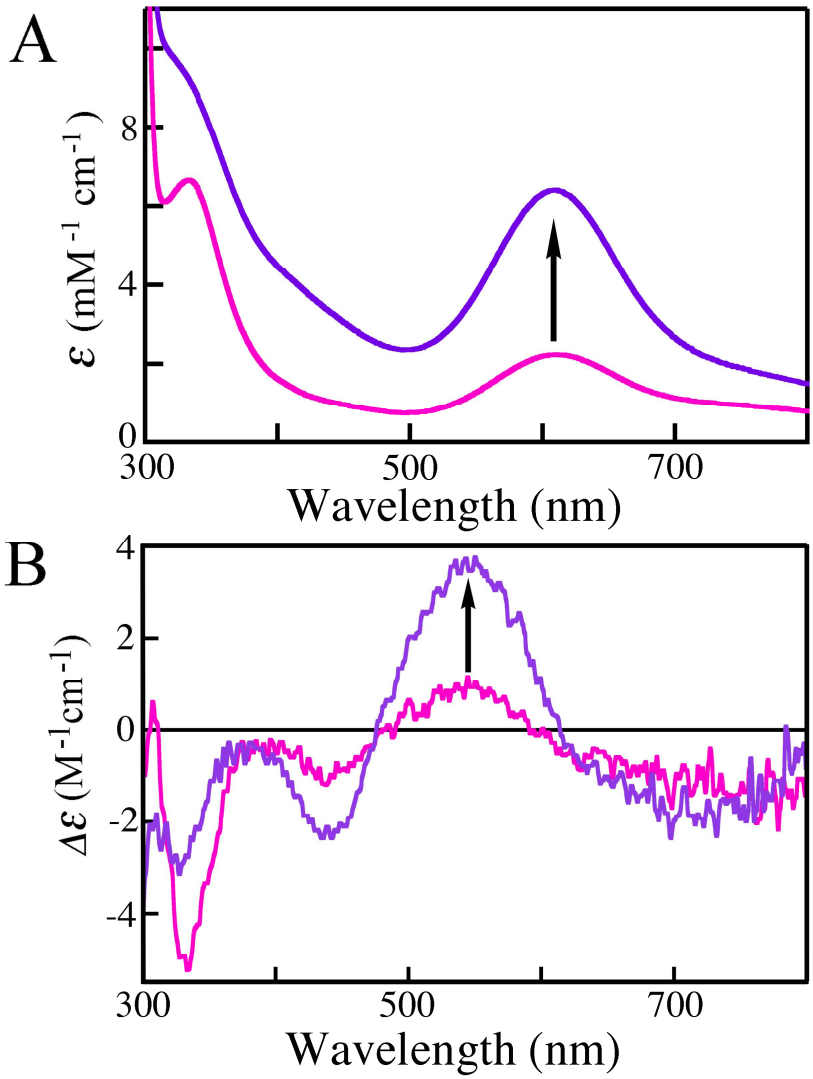
Supplemental FIGURE S1: Absorption (A), CD (B), and EPR (C) spectra of Δα5-7 CueO (black), and the mutants Pro444Gly (green), Pro444Ala (blue), Pro444Leu (red), Asp439Ala/Pro444Ala (magenta), and Asp439Ala (ocher). Absorption and CD spectra of ~100 μM proteins in 0.1 M phosphate buffer, pH 7, were measured at room temperature using a 1-cm path length quartz cell. The units of the ordinate are based on the protein molecule. EPR spectra were measured at 77 K with a frequency of 9.2 GHz, microwave power of 4 mW, modulation of 1 mT at 100 kHz, filter of 0.3 s, sweep time of 4 or 8 min, and amplitude of 200-400.



Supplemental FIGURE S2: Resonance Raman spectra of Δα5-7 CueO (black), Pro444Ala (blue), Pro444Leu (red), Pro444Gly (green), and Asp439Ala (ocher). Measurement conditions are given in the experimental section.



Supplemental FIGURE S3: Cyclic (A) and rotating disk (B) voltammograms of Δα5-7 CueO (black), and the mutants Pro444Gly (green), Pro444Ala (blue), Pro444Leu (red), Asp439Ala (ocher), and Asp439Ala/Pro444Ala (magenta). For cyclic and rotating disk voltammetries, an Au electrode modified with thioglycolate and a carbon cryogel electrode were used, respectively. Concentrations of mutants were about 0.4 μM. For other measurement conditions see the caption to FIGURE 5.



Supplemental FIGURE S4: Absorption (A) and (B) CD spectra of Asp439Ala/Pro444Ala mutant in rCueO as isolated (magenta) and as reacted with a slight excess of hexachloroirridate(IV) (purple). The increase in absorption spectrum at < 500 nm was due to hexachloroirridate(IV).