Supporting Information

On the Effects of Reversing the Protein Positive Charge in Proximity of the N(1)-Flavin Locus of Choline Oxidase

Mahmoud Ghanem[§], and Giovanni Gadda [§, #, +]

Departments of [§]Chemistry and [#]Biology, and [+]The Center for Biotechnology and Drug Design,

Georgia State University, Atlanta, GA 30302-4098

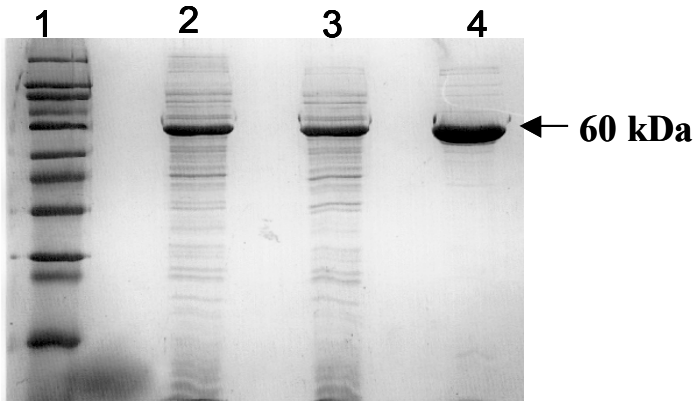

FIGURE S1: Purification of CHO-H466D. Lane 1, molecular weight marker proteins; lane 2, cell-free extract of *Escherichia coli* strain Rosetta(DE3)pLysS harboring plasmid pET/*codA*-H466D induced with 0.05 mM IPTG for 5 h at 23 °C; lane 3, sample treated with 30 and 65% ammonium sulfate saturation; lane 4, purified CHO-H466D in 20 mM Tris-Cl and 10% glycerol, pH 8, after DEAE-Sepharose column chromatography. The molecular mass of the purified CHO-H466D is indicated.

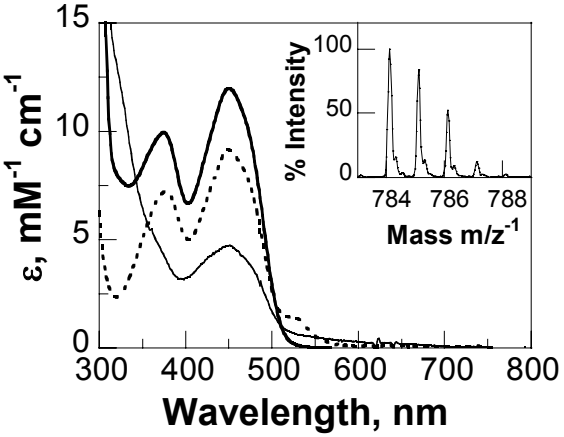

FIGURE S2: Stoichiometry and flavin content of CHO-H466D. Thick solid curve, UV-visible absorbance spectrum of CHO-H466D in 20 mM Tris-Cl and 10% glycerol, pH 8; dotted curve, UV-visible absorbance spectrum of the soluble fraction after treatment of CHO-H466D with 10% of trichloroacetic acid for 30 min on ice and centrifugation to remove denatured protein; thin solid curve, UV-visible absorbance spectrum of the denatured precipitated fraction after solubilization in 4 M urea. Inset, MALDI-TOF mass spectrometric analysis of the soluble fraction after treatment of CHO-H466D with 10% of trichloroacetic acid. The spectrum was recorded in negative ion mode with a 50:50 methanol/acetonitrile matrix.

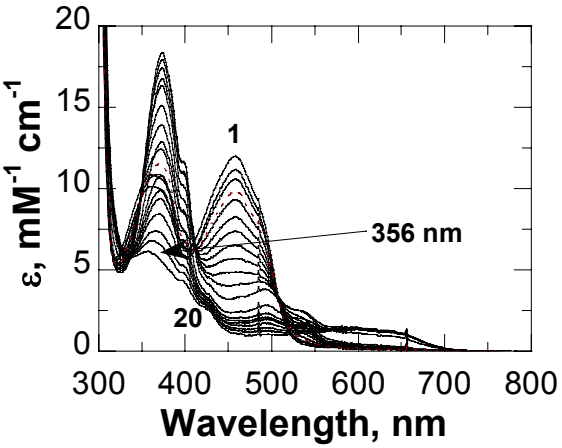

FIGURE S3: Anaerobic reduction of CHO-H466A with 300 μM xanthine and ~0.5 μM xanthine oxidase in 50 mM sodium phosphate, 50 mM sodium pyrophosphate and 10% glycerol, at pH 8 and 15 °C. Curve 1: UV-visible absorbance spectrum of the fully oxidized species of the enzyme. Unnumbered curves: selected intermediate spectra recorded during the reduction of the enzyme showing the formation of anionic flavin semiquinone. Curve 20: UV-visible absorbance spectrum of the hydroquinone form of enzyme.

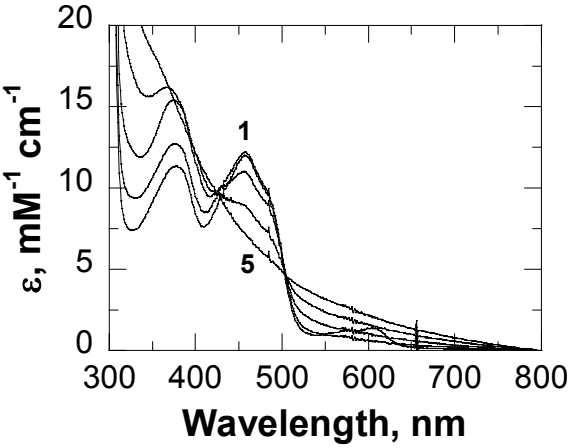

FIGURE S4: Potentiometric reduction titration of CHO-H466D in complex with choline. Curve 1: UV-visible absorbance spectrum of the fully oxidized CHO-H466D at an ambient redox potential of +267 mV at a concentration of ~20 μM in the presence of 10 mM choline, in 20 mM Tris-Cl and 10% glycerol, pH 7, at 15 °C. Curves 2-4: selected intermediate spectra recorded during reduction of the ligand-bound CHO-H466D after each addition of sodium dithionite at ambient redox potentials of +40, –77, and –130 mV, respectively. Curve 5: UV-visible absorbance spectrum of the fully reduced ligand-bound CHO-H466D at ambient redox potential of -217 mV, showing significant turbidity of the reduced enzyme solution.