Supporting Information for

Guanidine Hydrochloride-Induced Unfolding of the Three Heme Coordination States of the CO-Sensing Transcription Factor, CooA†

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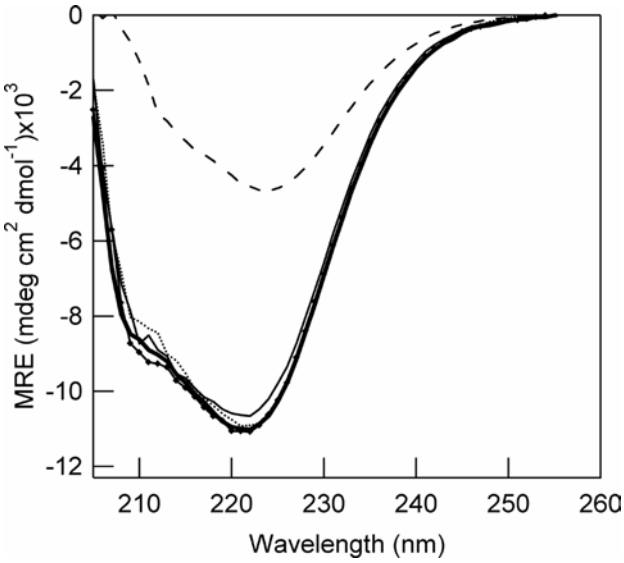
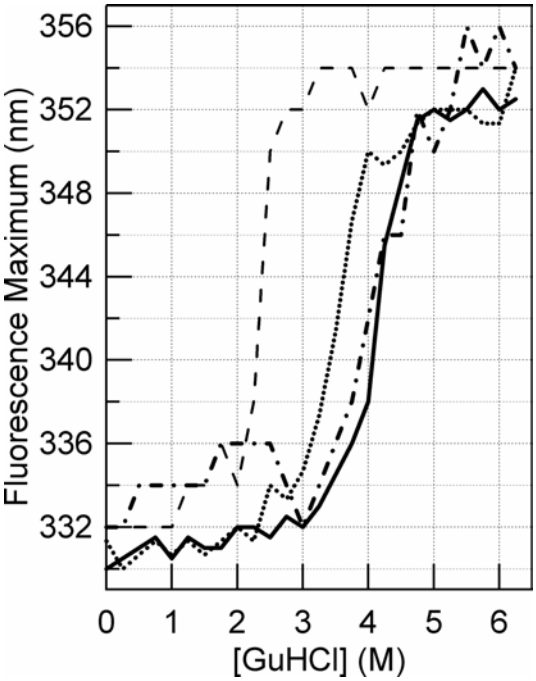
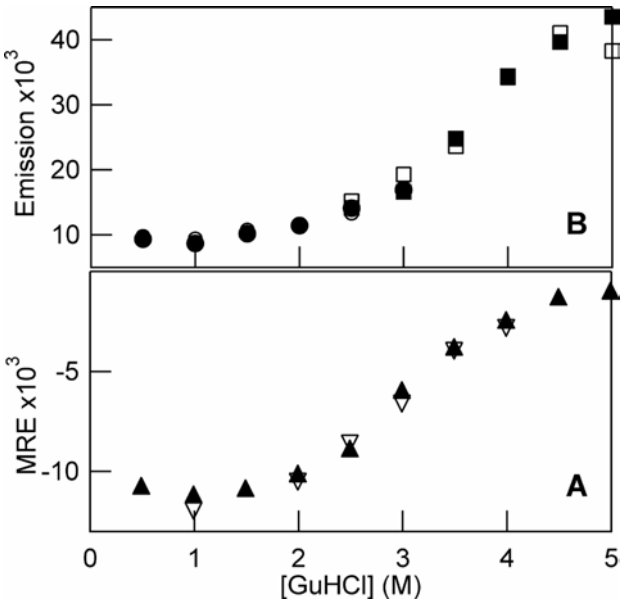


Supplemental Figure 1. Comparison of CD spectra of native CooA. The difference in CD spectra between Fe(III) WT (bold solid), Fe(II) WT (fine solid), Fe(II)-CO WT (solid with points), and Fe(III) F164W (fine dotted) is minimal, while Fe(III) truncated CooA (dashed) has greatly decreased CD intensity. For all proteins, samples at a fixed protein concentration (2 μM) were prepared in 25 mM MOPS buffer, 100 mM NaCl, pH 7.4. CD spectra were measured in a 2 mm pathlength cuvette with a 10 s averaging time.



Supplemental Figure 2. Comparison of the GuHCl dependence of the tryptophan fluorescence maximum of CooA. An increase in the fluorescence peak maximum wavelength with increasing concentrations of GuHCl indicates that the tryptophan at the dimer interface of CooA has been exposed to solvent. A plot of these changes is shown for the Fe(III) WT (solid), Fe(III) F164W (dotted), Fe(III) truncate (dash-dot), and Fe(II)-CO WT (dashed) CooA proteins. The comparison shows the tryptophan of Fe(II)-CO WT CooA is exposed at much lower concentrations of GuHCl than in the Fe(III) WT CooA curve. This may indicate a significant difference in the unfolding mechanism for the two protein conformational states, specifically in the GuHCl concentration that corresponds to exposure of the dimer interface. The Fe(III) truncated CooA shows an elevated fluorescence maximum at low GuHCl concentrations, but the major change in environment occurs between 3 and 4 M GuHCl as is observed for the Fe(III) WT protein. Fe(III) F164W CooA shows a change in peak maximum at lower concentrations of GuHCl, likely due to fluorescence changes of the extra tryptophan residue in the DNA-

binding domain as it unfolds around 2 M. The data plotted are averages of the peak maximum of fluorescence intensity for several repetitions of unfolding for each protein.



Supplemental Figure 3. Reversibility of unfolding of WT CooA in CD (A) and fluorescence spectroscopies (B). The alpha-helical structure of CooA is completely recovered upon refolding from a fully denatured state (A). A discontinuity in fluorescence reversibility (B) was observed for dilution from 5 M GuHCl to below 3 M GuHCl, so a separate dilution was carried out from 3 M to 0.5 M GuHCl (circles). The data for refolded samples (open markers) were then overlaid with samples unfolded in the forward direction (closed markers). For both methods, WT CooA (50 μM) was denatured for two hours at a concentration of 5 M GuHCl at room temperature. The protein was then diluted to 2 μM and the final GuHCl concentration desired, and allowed to incubate for 2 h at room temperature.