

## Supporting Information

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**Title:** Probing protein folding with substitution-inert metal ions. Folding kinetics of cobalt(III)-cytochrome *c*.

**Journal:** Journal of the American Chemical Society

**S1.** Denaturation curves of yeast-, horse- and tuna Co-cyt *c* and comparison of their stabilities with the Fe(III)-cyt *c* species.

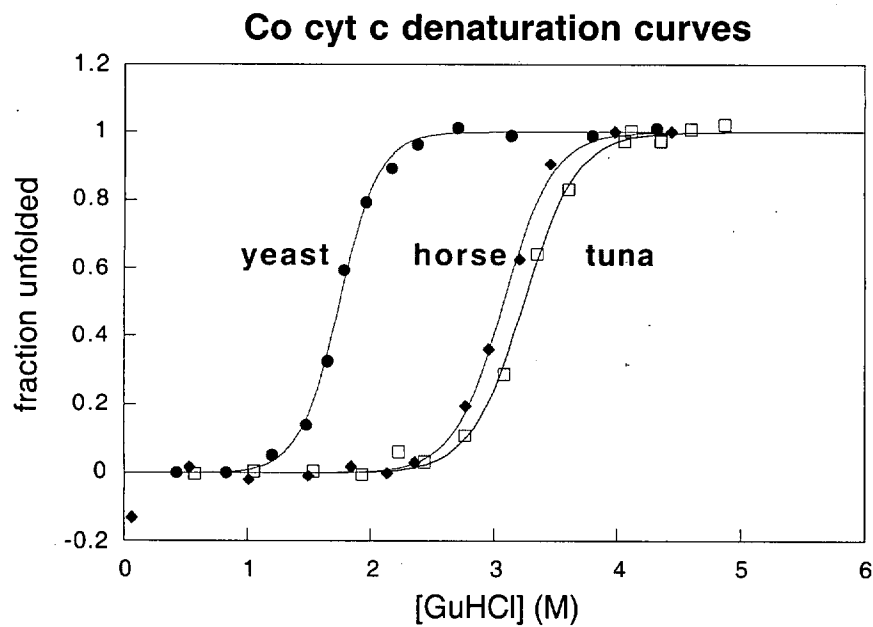
**S2.** Differences between the far-UV CD, Trp-fluorescence and UV-vis spectra of folded (no GuHCl) and unfolded Co-cyt *c* ([GuHCl]  $\geq$  4.0 M).

**S3.** Changes in the absorption spectrum of Co cyt *c* upon cyanide binding, and kinetics of cyanide binding to unfolded Co-cyt *c* (12 h of unfolding, [GuHCl] = 4.0 M, [Co-cyt *c*]  $\sim$  5  $\mu$ M, [CN<sup>-</sup>]  $\sim$  50 mM).

**S4.** pH-dependent changes in the Soret band of tuna Co-cyt *c*<sub>u</sub> ([GuHCl] = 4.0 M).

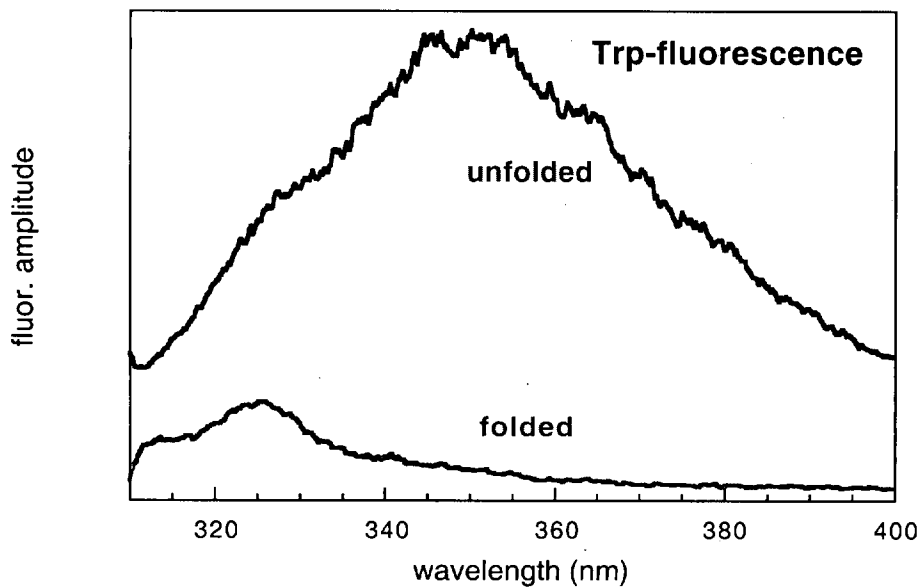
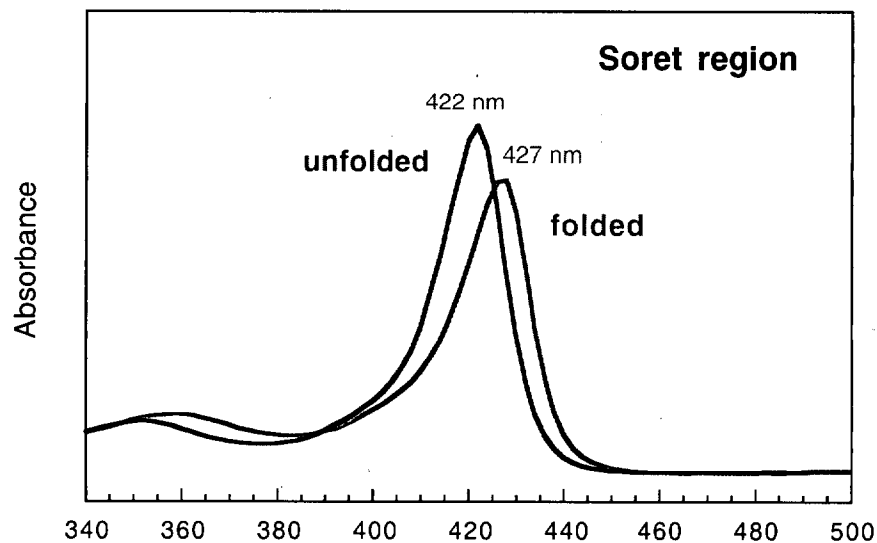
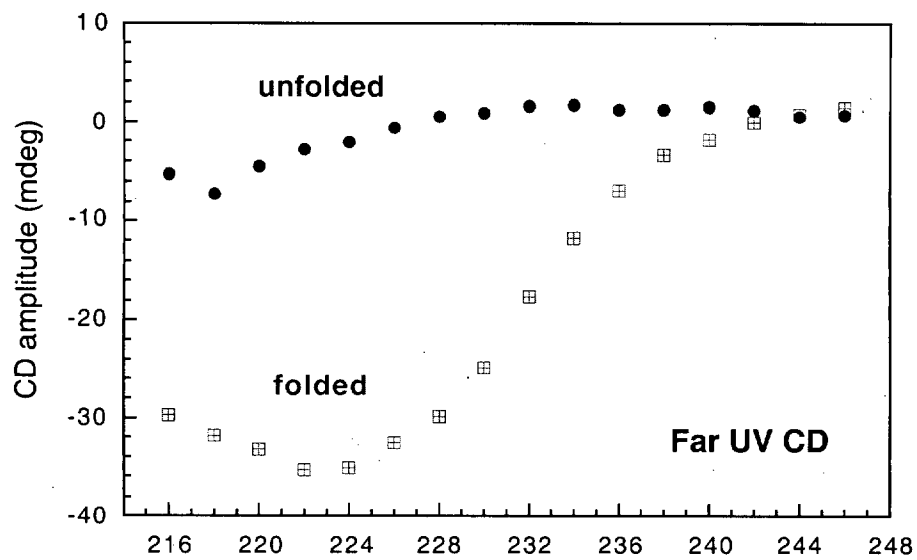
**S5.** Reaction scheme and parameters used for the simulation of horse Co-cyt *c* unfolding kinetics. The species in parentheses indicate the ligand and "+" indicates protonated species. The forward and backward rate constants for reactions 1 and 2 were set high ( $\geq 10^{6.5}$ ) in order to accommodate fast (de)protonation equilibria and to temporally separate them from reactions 3 and 4. The rate constants for reactions 3 and 4 were best guesses to fit observed unfolding kinetics.

**S6.** Simulation of horse Co-cyt *c* unfolding kinetics.

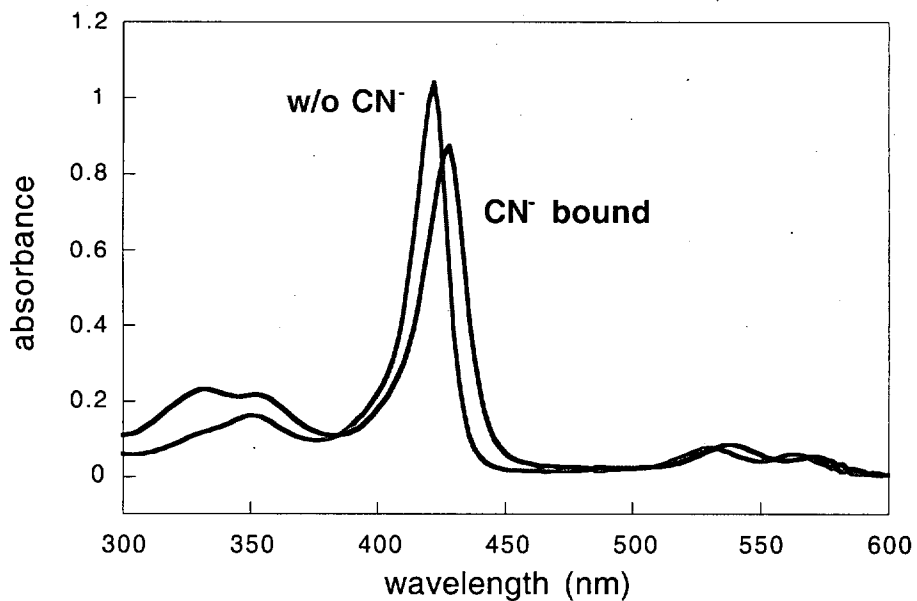


**Comparison of folding free energies (denaturation midpoint  
([GuHCl], M))**

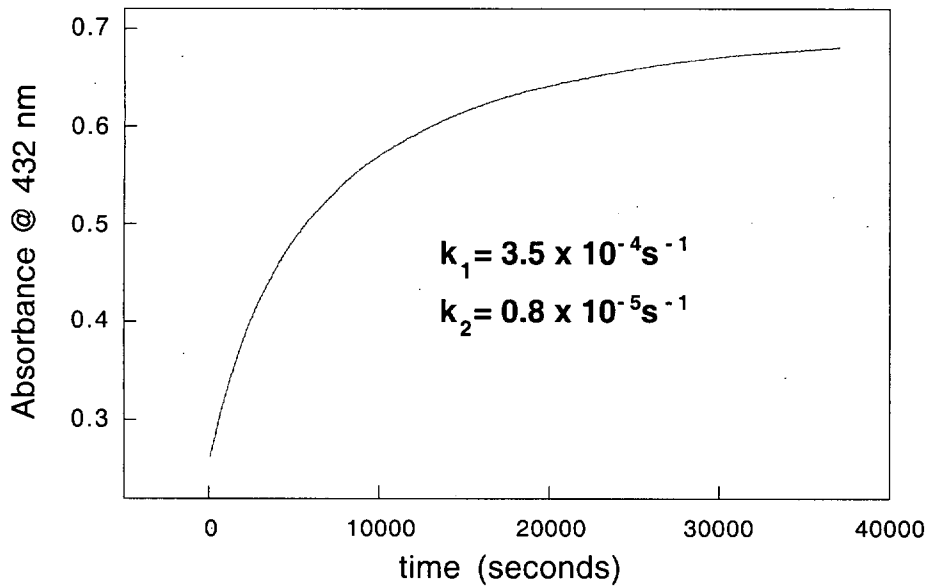
	yeast	horse	tuna
<b>Co(III) cyt c</b>	6.1 kcal/mol (1.75 M)	9.9 kcal/mol (3.0 M)	9.8 kcal/mol (3.25 M)
<b>Fe(III) cyt c</b>	4.6 kcal/mol (1.3 M)	9.0 kcal/mol (2.8 M)	9.4 kcal/mol (2.9 M)

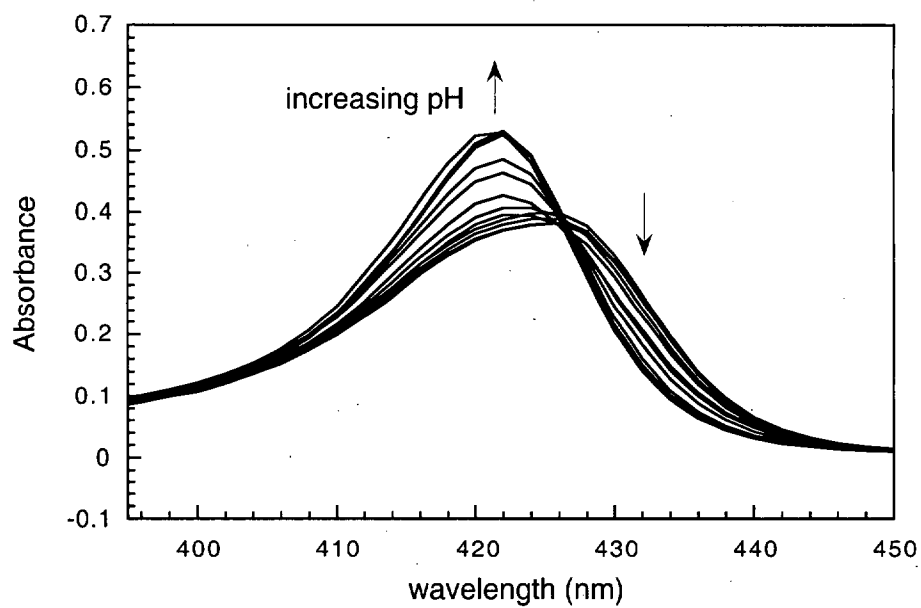


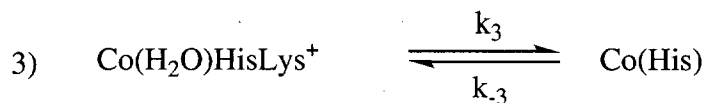
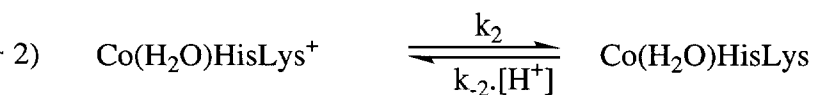
### Spectral changes upon CN<sup>-</sup> binding to unfolded Co cyt c



### Kinetics of CN<sup>-</sup> binding to unfolded Co cyt c







$$-\log(k_1/k_{-1}) = 6.5 = \text{pK}_a \text{ of free histidine}$$

$$-\log(k_2/k_{-2}) = 10 = \text{pK}_a \text{ of free lysine}$$

$$-\log(k_3/k_{-3}) = -\log(10^{-2} / 10^{-5}) = -3 = \text{pK of histidine binding to Co(III)}$$

$$-\log(k_4/k_{-4}) = -\log(9.5 / 3 \times 10^{-5}) = -5.5 = \text{pK of lysine binding to Co(III)}$$

$$[\text{Co(H}_2\text{O)His}^+\text{Lys}^+]_0 = 2.4 \times 10^{-6} \text{ M}$$

$$[\text{Co(H}_2\text{O)HisLys}^+]_0 = 7.6 \times 10^{-6} \text{ M}$$

$$[\text{Co(H}_2\text{O)HisLys}]_0 = 1 \times 10^{-8} \text{ M}$$

$$[\text{Co(His)}]_0 = 0 \text{ M}$$

$$[\text{Co(Lys)}]_0 = 0 \text{ M}$$

$$[\text{H}^+]_{\text{const.}} = 10^{-7} \text{ M}$$

$$\left. \begin{array}{l} [\text{Co(H}_2\text{O)His}^+\text{Lys}^+]_0 = 2.4 \times 10^{-6} \text{ M} \\ [\text{Co(H}_2\text{O)HisLys}^+]_0 = 7.6 \times 10^{-6} \text{ M} \\ [\text{Co(H}_2\text{O)HisLys}]_0 = 1 \times 10^{-8} \text{ M} \\ [\text{Co(His)}]_0 = 0 \text{ M} \\ [\text{Co(Lys)}]_0 = 0 \text{ M} \end{array} \right\} \text{total concentration} = 10 \times 10^{-6} \text{ M}$$

