SUPPORTING INFORMATION

Effect of motional restriction on the unfolding properties of a cytochrome *c* featuring a His/Met-His/His ligation switch

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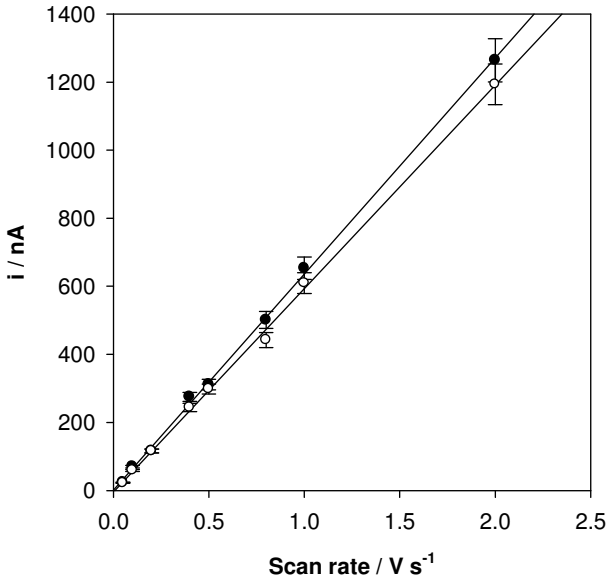

Figure S1. Cathodic current intensity as a function of the scan rate for the HP_{pH5} (●) and LP_{pH5} (○) signals for the K72A/K73H/K79A variant of yeast iso-1-cytochrome *c* adsorbed on polycrystalline gold electrode coated with a SAM of MUA/MU at pH 5 in the presence of 1M and 8M urea, respectively. Working solution contained 10 mM acetate buffer and 10 mM sodium perchlorate, pH 5. T = 20 °C.

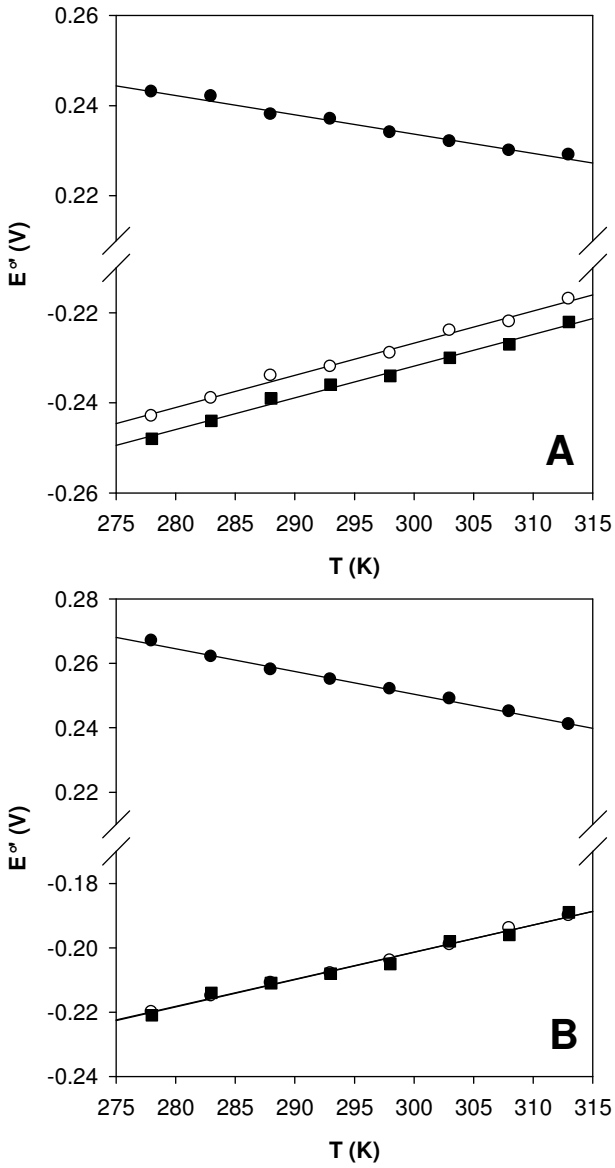

Figure S2. Typical plots of E°' versus temperature for the CV signals of the K72A/K73H/K79A variant of yeast iso-1-cytochrome *c* adsorbed on polycrystalline gold electrode coated with a SAM of MUA/MU (A) and freely diffusing (B), at varying urea concentrations. HP_{pH5}, 1M urea (●); LP_{pH5}, 8 M urea (○), $LP_{pH7.4}$, 8 M urea (■). Working solution: 10 mM phosphate (pH 7.4) or acetate (pH 5) buffer, plus 10 mM sodium perchlorate. Sweep rate: 0.05 V s^{-1}, T = 20 °C.

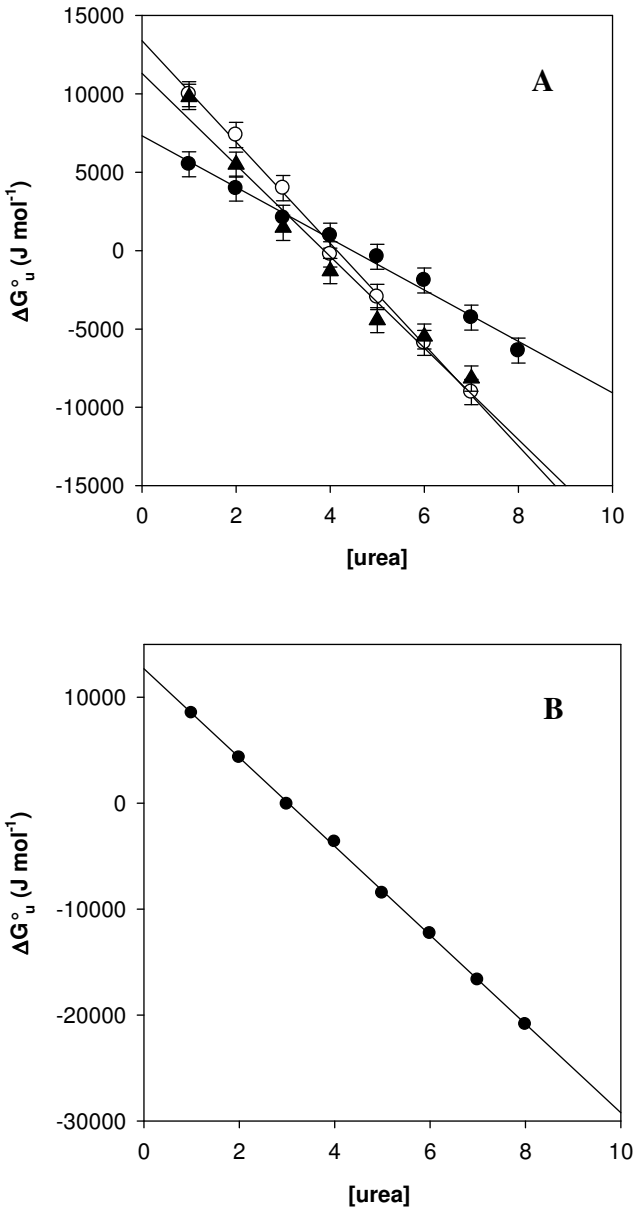

Figure S3. Plot of $\Delta G°_u$ versus [urea] for (A) wt (●), the K72A/K73H/K79A (○) and the K72A/K73A/K79A (▲) variants of yeast iso-1-cytochrome *c* adsorbed on polycrystalline gold electrode coated with a SAM of MUA/MU and (B) the K72A/K73H/K79A variant of yeast iso-1-cytochrome *c* under diffusing conditions. $\Delta G°_u$ was calculated from the current ratio of the signals corresponding to the His/His and His/Met axial heme ligation, according to eq. (3) and (4). Working solution: 10 mM acetate buffer and 10 mM sodium perchlorate at pH 5. Sweep rate: 0.05 V s^{-1}, T = 5 °C. Solid lines are least-squares fits to the data points.

Kinetics of His to Met axial ligand switching for the immobilized urea-unfolded reduced His/His form of the K72A/K73H/K79A variant of yeast iso-1 cytochrome *c*. The kinetics of His to Met ligand switching for the reduced His/His form were determined form using the Laviron model for an electrochemical reaction followed by a first order chemical reaction (see ref. 78 main text), according to the scheme:

$$cytc_{ox}(His/His) + e \rightarrow cytc_{red}(His/His)$$

$$cytc_{red}(His/His) \xrightarrow{k} cytc_{red}(His/Met)$$

The i_a/i_c current ratio for the signal of the His-His form increases with increasing scan rate, as expected, because less time is allowed to the reduced protein to evolve toward the reduced His/Met form. In particular, the scan rates which correspond to i_a/i_c ratios of 0.25, 0.5 and 0.75 at E_i-E_{pa} = -0.4 V (where E_i is the initial potential of the backward scan and E_{pa} is the anodic peak potential, , see Laviron paper) have been determined. Using equation 23 and the working curves in Fig.7 of ref. 78, the following data were obtained at T=293K:

i_a/i_c	l_e	v_e/Vs^{-1}	k/s^{-1}
0.25	0.0398	0.180	0.28
0.5	0.0218	0.330	0.29
0.75	0.0095	0.740	0.28