Imaging Metals in Proteins by Combining Electrophoresis with Rapid X-ray Fluorescence Mapping

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Supporting Information

Table S1. Results of multiple linear regression fittings of XANES spectra of Cr(III)-protein adducts separated by gel electrophoresis.

Protein band [a]	**Cr(III)-albumin** [b]	**$[Cr(asp)_2]^-$** [b]	**$[Cr(OH)_6]^{3-}$** [b]	R^2 [c]
Lane 5, ~65 kDa	0.65(2)	0.27(2)	0.08(1)	0.99954
Lane 7, ~65 kDa	0.65(2)	0.28(2)	0.07(1)	0.99971
Lane 7, ~130 kDa	0.84(2)	0.12(1)	0.04(1)	0.99957

[a] Designations correspond to Figure 5, main text. [b] Molar components of each of the contributing model complexes (asp = L-aspartato(2–)) [36], calculated by multiple linear regression analyses [35]. Errors in the last significant figures are shown in parentheses. [c] Correlation coefficient of the regression (see Figure S1 for the comparison of experimental and calculated spectra).

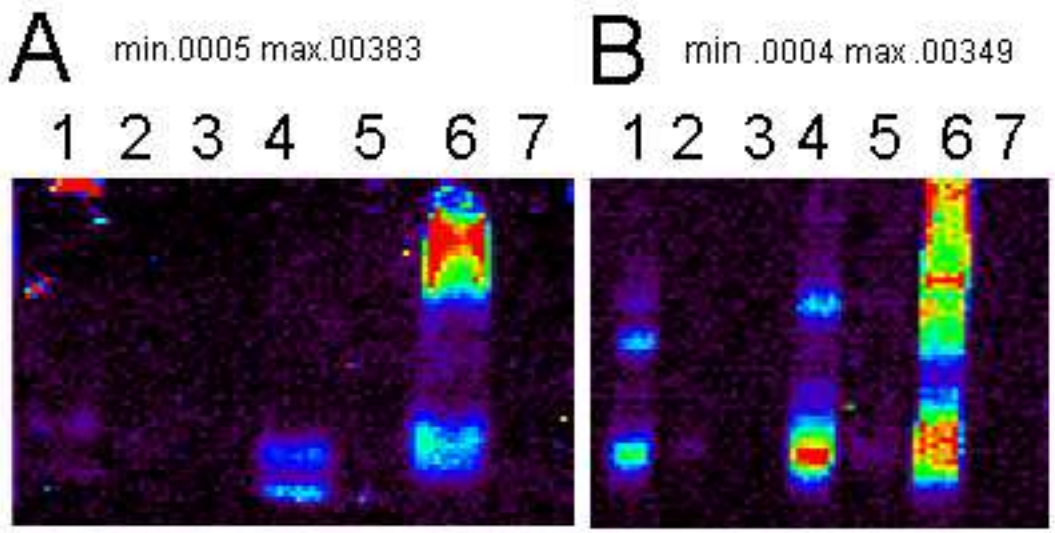

Figure S1. Addition of SDS Minimally Disrupts Chromium-Protein Interactions. Identical samples of chromium-treated BSA-depleted bovine serum, lanes 1-7, were run under two different conditions (only the >65 kDa areas of the gels are shown; compare with Figure 5 in the main text). Panel A, electrophoresis was run under completely native conditions. Panel B, electrophoresis was run as described in Materials and Methods, which included 0.1% SDS in the running buffer. Lane 1: $[Cr^{III}(OH_2)_6](NO_3)_3$ (1.0 mM). Lane 2: $Na_2Cr^{VI}O_4$ (1.0 mM). Lane 3: GSH (5.0 mM), no Cr added. Lane 4: $Na_2Cr^{VI}O_4$ (1.0 mM) + GSH (5.0 mM). Lane 5: Cr(III)-GSH complex (1.0 mM, generated by the reaction of 10 mM $Na_2Cr^{VI}O_4$ with 50 mM GSH in water at pH ~7 for 2 h at 310 K immediately prior to the addition to serum proteins) (*45, 46*); Lane 6: $[Cr_3^{III}O(OCOC_2H_5)_6(OH_2)_6](NO_3)$ (Cr(III)-propionate, 1.0 mM). Lane 7: no Cr compounds added. All the Cr compounds were incubated with serum proteins (1.0 mg protein per mL in 20 mM HEPES buffer, pH 7.4) for 2 h at 310 K prior to the separation by gel electrophoresis. Comparable amounts of chromium are present in each case, with the exception of lane 4, where we presume that some of the chromium protein did not

migrate into the gel in the native case. Minimum and maximum threshold values are displayed above each map. Maps displayed are quantified, with threshold values in $\mu g/cm^2$.

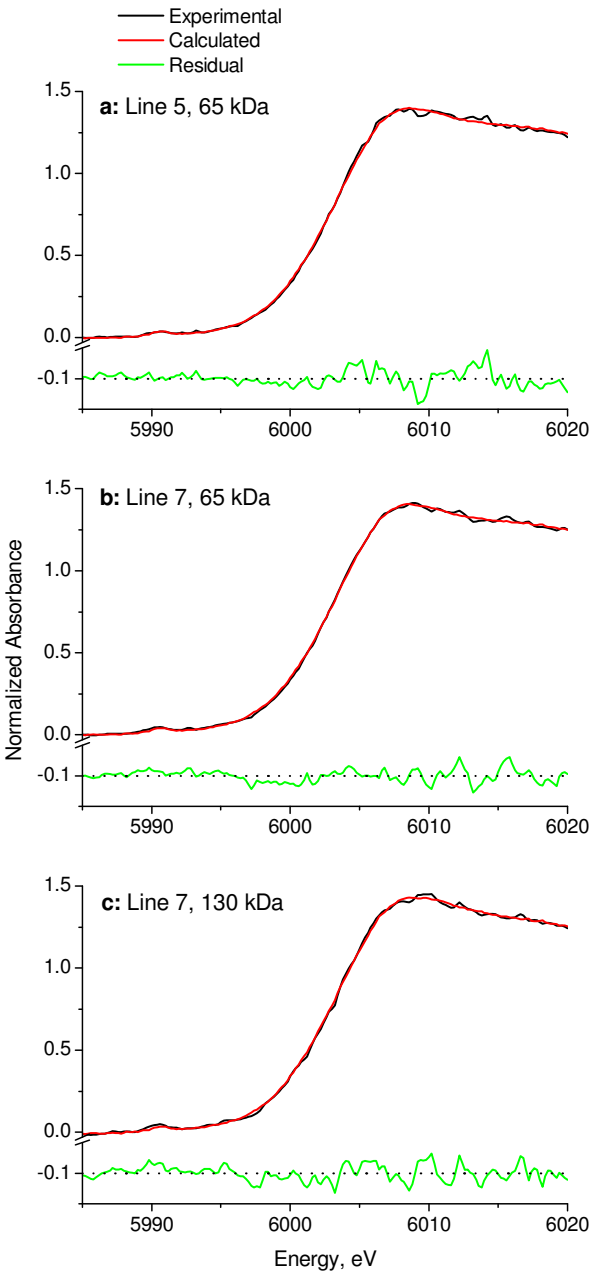

Figure S2. Results of multiple linear regression analyses (see Table S1) for XANES spectra of Cr(III)-protein adducts separated by gel electrophoresis.