Denaturing and non-denaturing microsolution isoelectric focussing to mine the metalloproteome

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Supporting information contains one figure (figure S-1) and additional discussion of it, and six tables of results (tables S-1 to S-6) and additional discussion of those results to underpin Figures 3 and 4 in the main text.

Results and Discussion.

We have concluded that the metal-protein complexes remain intact because in the presence of protein (we added it and it is detectable from its UV 254 nm absorbance), we observe Cu and Zn eluting with the protein peak, while in the absence of protein (no UV 254 nm peak) we observe no Cu or Zn peaks at the protein retention times. We have analysed SOD, CA and MYO separately (figure S-1 of overlaid metal SEC-ICP-MS chromatograms) and have shown the behaviour of individual metalloprotein standards on SEC-ICP-MS. It is clear that these match those of the proteins in our IEF fractions (tables 3 and 4).

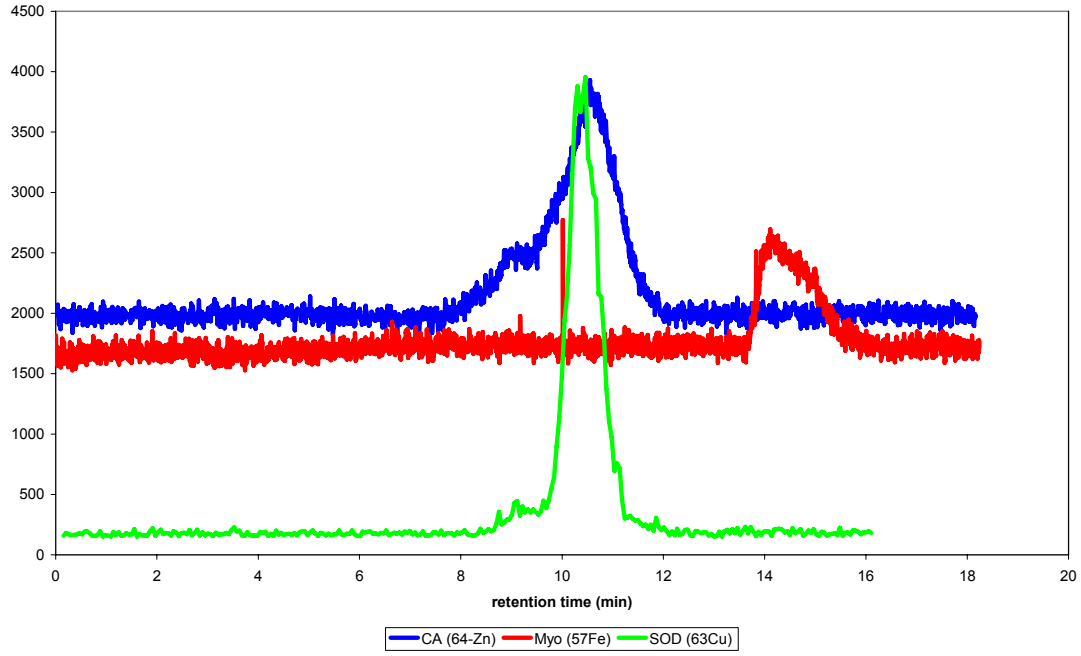

Figure S-1. Metalloprotein standard chromatograms using SEC-ICPMS; Tables 3 and 4 contain information about the t_Rs of metal peaks associated with metalloproteins and ampholytes. The column recovery in respect of the metal using Superdex 75 with 20 mM (for SOD) and 50 mM TRIS buffer (of CA and MYO) as eluent was for SOD 90+/-3 % Cu and 100 % +/-9 % Zn, for CA 85 % Zn and for MYO 95-100 % Fe.

Separation of intact metal-protein complexes under both denaturing and non-denaturing microsol IEF conditions has been accomplished. The simultaneous evaluation of protein and metal data allowed characterisation of which complexes were still intact after the fractionation procedure. For example, the exchange of metal observed under both experimental conditions suggested weaker binding of Zn and sometimes Fe than of Cu to the standard proteins under examination. This indicates that especially the detection of metals bound to protein always needs careful evaluation. Some fractions have been shown to contain metals in which no protein has been detected. This is the case for fractions F1 and F2 for copper (table S-1) and F1 in the case of iron under denaturing conditions (tables S-1 and S-3, respectively), which cannot be correlated with the presence of specific metal-protein complexes.

However, we need to keep in mind that the limits of detection of proteins visualised on the 1D gels using Coomassie Blue staining is higher than that of metal detection, which is able to determine the presence of metals bound to minor populations of proteins focussing into a different pH fraction because of a different conformation, but in which the protein is below the detection limit.

Table S-1: Amount of copper (in nanograms [ng]) associated with eluted standard proteins following SEC ICP-MS of fractions obtained following separation of a solution containing the proteins CA, BSA, MYO, Cytochrome C and SOD by sIEF under denaturing and non-denaturing conditions.

SD = standard deviation, RSD = relative standard deviation, t_R = retention time.

Copper (Cu)						
pH fraction	***denaturing***				***Non-denaturing***	
	t_R (min)	**ng ± SD (RSD)**	**t_R (min)**	**ng ± SD (RSD)**	**t_R (min)**	**ng ± SD (RSD)**
F1	10.5	1.2 ± 0.5 (44.3)	14.2	1.8 ± 0.8 (43.6)		
F2	10.3	3.0 ± 1.4 (47.1)	14.6	2.8 ± 1.2 (43.7)		
F3	10.2	13.5 ± 2.5 (18.3)	9.5	1.6 ± 1.2 (75.9)	12.6	16.5 ± 1.5 (9)
F4	10.6	3.9 ± 2.0 (51.2)	13.3	3.6 ± 0.9 (25.4)	13.1	22.2 ± 6.3 (28.4)
F5	10.3	0.8 ± 0.6 (72.3)			13.1	2.0 ± 0.6 (32.4)

Table S-2: Amount of zinc (in nanograms [ng]) associated with eluted standard proteins following SEC ICP-MS of pH fractions obtained following separation of a solution containing the proteins CA, BSA, MYO, Cytochrome C and SOD by sIEF under denaturing and non-denaturing conditions.

SD = standard deviation, RSD = relative standard deviation, t_R = retention time.

Zinc (Zn)								
pH fraction	***denaturing***				***Non-denaturing***			
	t_R (min)	**ng ± SD (RSD)**	**t_R (min)**	**ng ± SD (RSD)**	**t_R (min)**	**ng ± SD (RSD)**	**t_R (min)**	**ng ± SD (RSD)**
F1	3.4	0.0 ± 0.0 (141.4)						
F2							12.5	2.9 ± 2.9 (100.3)
F3	10.1	12.4 ± 2.4 (19.6)	13.5	4.9 ± 0.4 (7.8)	3.8	0.3 ± 0.4 (141.4)	13.1	22.0 ± 2.4 (11.1)
F4	10.8	0.6 ± 0.1 (21.3)	13.5	5.1 ± 0.8 (15.4)	11.4	1.8 ± 0.6 (31.2)	13.1	13.0 ± 3.8 (29.1)
F5			13.6	0.5 ± 0.2 (37.1)			13.1	1.1 ± 0.3 (31.3)

Table S-3: Amount of iron (in nanograms [ng]) associated with eluted standard proteins following SEC ICP-MS of pH fractions obtained following separation of a solution containing the proteins CA, BSA, MYO, Cytochrome C and SOD by sIEF under denaturing and non-denaturing conditions.

SD = standard deviation, RSD = relative standard deviation, t_R = retention time.

Iron (Fe)								
pH fraction	***denaturing***				***Non-denaturing***			
	t_R **(min)**	**ng ± SD (RSD)**	t_R **(min)**	**ng ± SD (RSD)**	t_R **(min)**	**ng ± SD (RSD)**	t_R **(min)**	ng ± SD (RSD)
F1	3.3	0.1 ± 0.2 (141.4)						
F2								
F3	10.4	0.5 ± 0.2 (30)						
F4			13.6	0.6 ± 0.1 (26.6)	14.6	1.6 ± 1.5 (92.0)		
F5					14.8	5.2 ± 0.7 (14.5)		

Table S-4: Amount of copper (in nanograms [ng]) associated with eluted proteins following SEC ICP-MS of pH fractions obtained following separation of a cytosolic fraction of sheep liver homogenate by sIEF under denaturing and non-denaturing conditions.

SD = standard deviation, RSD = relative standard deviation, t_R = retention time.

Copper (Cu)								
pH fraction	***denaturing***				***Non-denaturing***			
	t_R (min)	**ng ± SD (RSD)**	**t_R (min)**	**ng ± SD (RSD)**	**t_R (min)**	**ng ± SD (RSD)**	**t_R (min)**	**ng ± SD (RSD)**
F1	12.9	4.2 ± 3.3 (77.9)	18.8	3.2 ± 2.4 (73.8)	-		-	
F2	12.3	4.5 ± 2.6 (58.2)	18.9	5.8 ± 3.8 (65.7)	-		8.3	0.1 ± 0.1 (71)
F3	12.3	14.7 ± 11.8 (80.8)	18.9	1.7 ± 1.3 (79.1)	10.1	0.1 ± 0.0 (15)	12.7	1.3 ± 0.1 (6.5)
F4	12.5	13.1 ± 10.6 (81.0)	19.3	2.1 ± 1.2 (59.8)	10.8	0.1 ± 0.1 (33.0)	13.1	6.1 ± 0.5 (7.4)
F5	-	-			-	-	13.1	2.4 ± 0.2 (7.5)

Table S-5: Amount of zinc (in nanograms [ng]) associated with eluted proteins following SEC ICP-MS of pH fractions obtained following separation of a cytosolic fraction of sheep liver homogenate by sIEF under denaturing and non-denaturing conditions.

SD = standard deviation, RSD = relative standard deviation, t_R = retention time.

Zinc (Zn)								
pH fraction	***denaturing***		***Non-denaturing***					
	t_R (min)	**ng ± SD (RSD)**	**t_R (min)**	**ng ± SD (RSD)**	**t_R (min)**	**ng ± SD (RSD)**	**t_R (min)**	**ng ± SD (RSD)**
F1	17.7	3.1 ± 0.1 (2.8)	-	-	-	-	-	-
F2	17.9	4.2 ± 0.3 (7.3)	-	-	11.3	0.1 ± 0.0 (34.9)	-	-
F3	18.0	7.3 ± 2.2 (30.1)	9.9	0.1 ± 0.0 (33.0)	11.0	0.5 ± 0.1 (11.3)	12.9	0.4 ± 0.0 (4.4)
F4	18.0	4.9 ± 2.6 (53.5)	6.8	0.1 ± 0.1 (96.0)	11.3	0.4 ± 0.2 (52.2)	13.1	4.1 ± 0.4 (8.7)
F5	18.2	9.2 ± 2.9 (31.9)	10.1	0.2 ± 0.1 (34.1)	12.1	4.8 ± 0.3 (5.9)	-	-

Table S-6: Amount of iron (in nanograms [ng]) associated with eluted proteins following SEC ICP-MS of pH fractions obtained following separation of a cytosolic fraction of sheep liver homogenate by sIEF under denaturing and non-denaturing conditions.

SD = standard deviation, RSD = relative standard deviation, t_R = retention time.

Iron (Fe)						
pH fraction	***denaturing***		***Non-denaturing***			
	t_R (min)	**ng ± SD (RSD)**	**t_R (min)**	**ng ± SD (RSD)**	**t_R (min)**	**ng ± SD (RSD)**
F1	9.8	0.5 ± 0.0 (0.0)	-	-	-	
F2	9.7	1.8 ± 1.2 (68.7)	9.7	4.7 ± 2.6 (56.1)	-	
F3	9.7	15.8 ± 8.9 (56.1)	9.7	4.2 ± 2.0 (48.9)	-	
F4	9.7	1.4 ± 0.1 (10.6)	3.3	0.1 ± 0.1 (141.4)	4.3	0.3 ± 0.5 (141.4)
F5	-	-	-	-	12.9	1.6 ± 0.4 (22.4)