

Electronic Supplementary Information for McCormick *et al.* 2013

Table of Contents:

- Figure S1. Calibration curve for determining molecular masses.
- Table S1. Compounds used for calibrating the size-exclusion column.
- Table S2. Concentrations of LMM metal ions in the brain.
- Figure S2. Individual phosphorus chromatograms.
- Figure S3. Individual sulfur chromatograms.
- Figure S4. Individual cobalt chromatograms.
- Figure S5. Individual copper chromatograms.
- Figure S6. Individual zinc chromatograms.
- Figure S7. Individual iron chromatograms.
- Figure S8. Individual manganese chromatograms.
- Figure S9. Individual molybdenum chromatograms.
- Figure S10. Chromatograms obtained while testing for sample degradation.
- Figure S11. Chromatograms using different buffers.
- Table S3. Cellular metal ion limits of detection using the current instrument
- Figure S12. Closeup of minor chromatographic peaks

Figure S1. Calibration curve for determining molecular masses based on the migration through the size-exclusion column. Open circles represent the compounds listed in Table S1. The best-fit line through the data (solid line) has a slope of -1.3128 and intercept of 6.0148. The r^2 for the fit was 0.989, and V_0 was 14.79 mL.

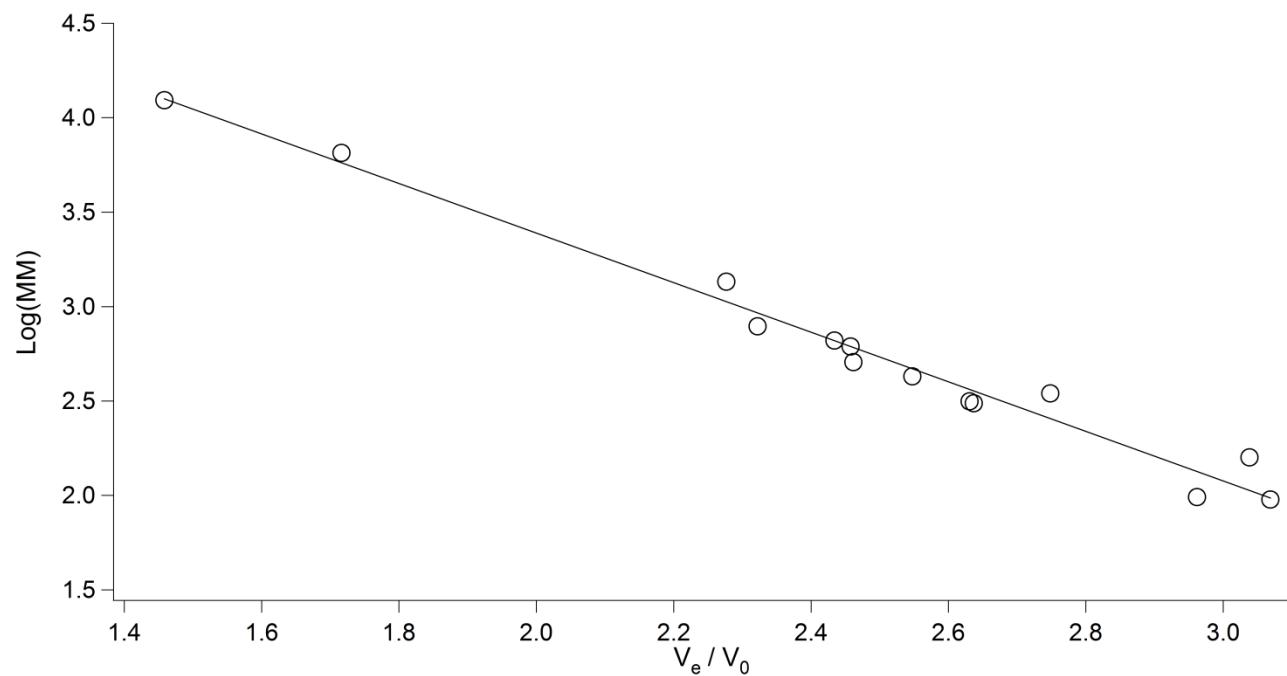


Table S1. Compounds used for calibrating the size-exclusion columns.

Compound	Source	Method of Detection	Molecular Weight (Da)	Log(MW)	Elution Volume (mL)	V_e/V_o
$P_i(PO_4^{3-})$	Fisher Sci.	ICP-MS (P)	95	1.978	45.39	3.069
Sulfate (SO_4^{2-})	Fisher Sci.	ICP-MS (P)	98	1.991	43.81	2.962
Molybdate (MoO_4^{3-})	Sigma Aldrich	ICP-MS (Mo)	159	2.201	44.94	3.039
Glutathione (GSH)	Sigma Aldrich	ICP-MS (S)	307.32	2.488	39	2.637
Molybdopterin from Xanthine Oxidase ^{**}	Purified	ICP-MS (Mo)	315 ¹	2.498	38.92	2.631
AMP	Fisher Sci.	ICP-MS (P)	347	2.540	40.65	2.748
ADP	Fisher Sci.	ICP-MS (P)	427.2	2.631	37.68	2.548
ATP	Fisher Sci.	ICP-MS (P)	507.18	2.705	36.41	2.462
Oxidized Glutathione (GSSG)	Sigma Aldrich	ICP-MS (S)	614.64	2.789	36.35	2.458
Inositol Hexaphosphate	Sigma Aldrich	ICP-MS (P)	660	2.820	36	2.434
Flavin Adenine Dinucleotide ^{***}	Purified	UV-VIS (450 nm)	785.55	2.895	34.35	2.322
Cyanocobalamin	Fisher Sci.	ICP-MS (Co)	1,355 ²	3.132	33.67	2.277
Aprotinin (bovine lung)	Fisher Sci.	UV (280 nm)	6,512 ³	3.814	25.39	1.716
Cytochrome C (<i>Saccharomyces cerevisiae</i>)	Sigma Aldrich	ICP-MS (Fe)	12,384 ⁴	4.093	21.57	1.459

^{*} V_o determined to 14.79mL using Blue Dextran (Fisher Sci)

^{**} Purified from xanthine oxidase.

^{***} Copurified during extraction of molybdopterin

¹ J. Deistung, R. C. Bray, *Biochem J* 1989, 263. 477-483.

² X. B. Luo, B. Chen, L. Ding, F. Tang, S. Z. Yao, *Anal Chim Acta* 2006, 562. 185-189.

³ B. Kassell, Radicevi.M, M. J. Ansfield, Laskowsk.M, *Biochem Bioph Res Co* 1965, 18. 255-&.

⁴ X. Zhang, D. A. Narcisse, K. K. Murray, *J Am Soc Mass Spectr* 2004, 15. 1471-1477.

Table S2. Concentrations of LMM metal ions in the brain.

Trace	Brain LMM Concentrations (μM)							
	P	S	Mn	Fe	Co	Cu	Zn	Mo
A	10117.78	1577.20	0.910	18.014	0.058	4.564	56.339	0.251
B	10228.06	1427.36	0.764	17.512	0.051	4.154	38.416	0.168
C	11644.15	1558.52	0.728	16.653	0.048	4.469	44.594	0.210
D	10385.55	1490.66	0.692	18.336	0.064	4.092	45.450	0.189
E	11303.93	1430.73	0.947	18.980	0.058	4.406	38.416	0.293
F	12312.71	1500.22	0.874	17.763	0.075	4.186	53.250	0.398
G	11904.76	1573.58	0.692	18.515	0.064	3.903	41.138	0.230
H	10063.02	1417.55	0.983	18.085	0.051	4.312	39.609	0.147
I	11363.47	1465.79	0.910	17.405	0.058	4.249	53.127	0.377
J	11968.04	1436.23	0.837	17.834	0.054	4.123	39.211	0.440
K	9448.96	1464.04	0.874	16.187	0.061	4.312	40.495	0.335
L	8771.42	1365.06	0.874	17.369	0.051	4.154	39.303	0.168
Average	10792.65	1475.58	0.840	17.721	0.058	4.244	44.112	0.267
Std. Dev	1112.10	66.98	0.099	0.774	0.008	0.181	6.540	0.100

Figure S2. Individual Phosphorus Chromatograms. Vertical dashed lines indicate the elution volume of species listed in Table 1.

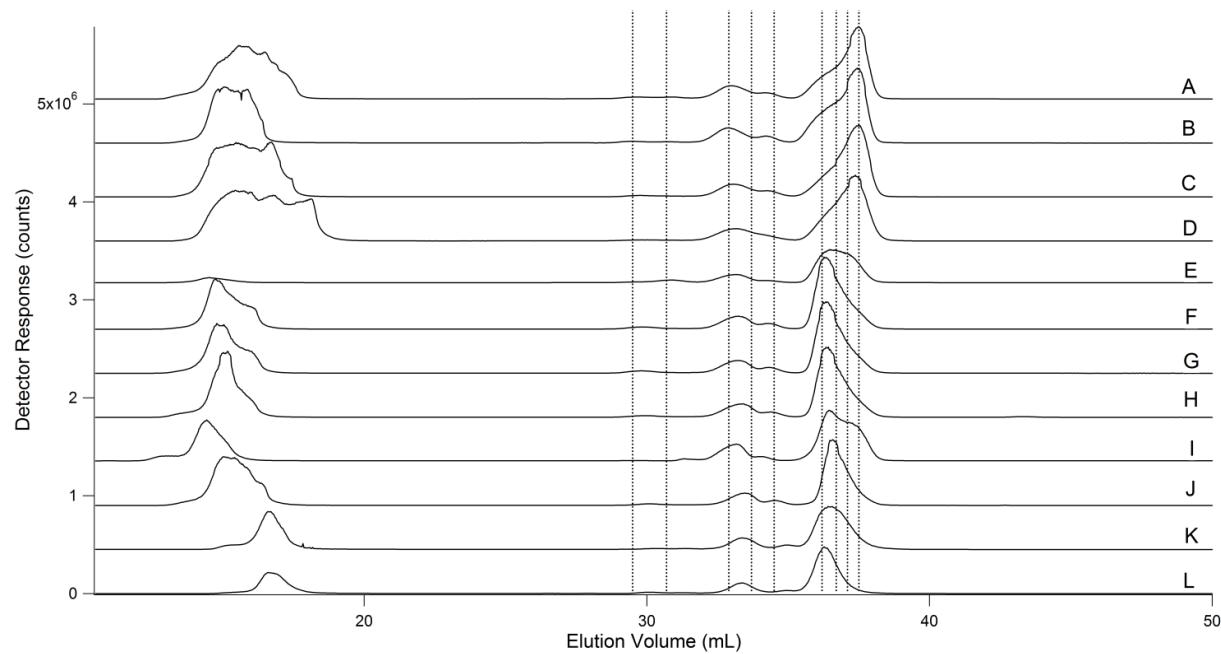


Figure S3. Individual Sulfur Chromatograms. Vertical dashed lines indicate the elution volume of species listed in Table 1.

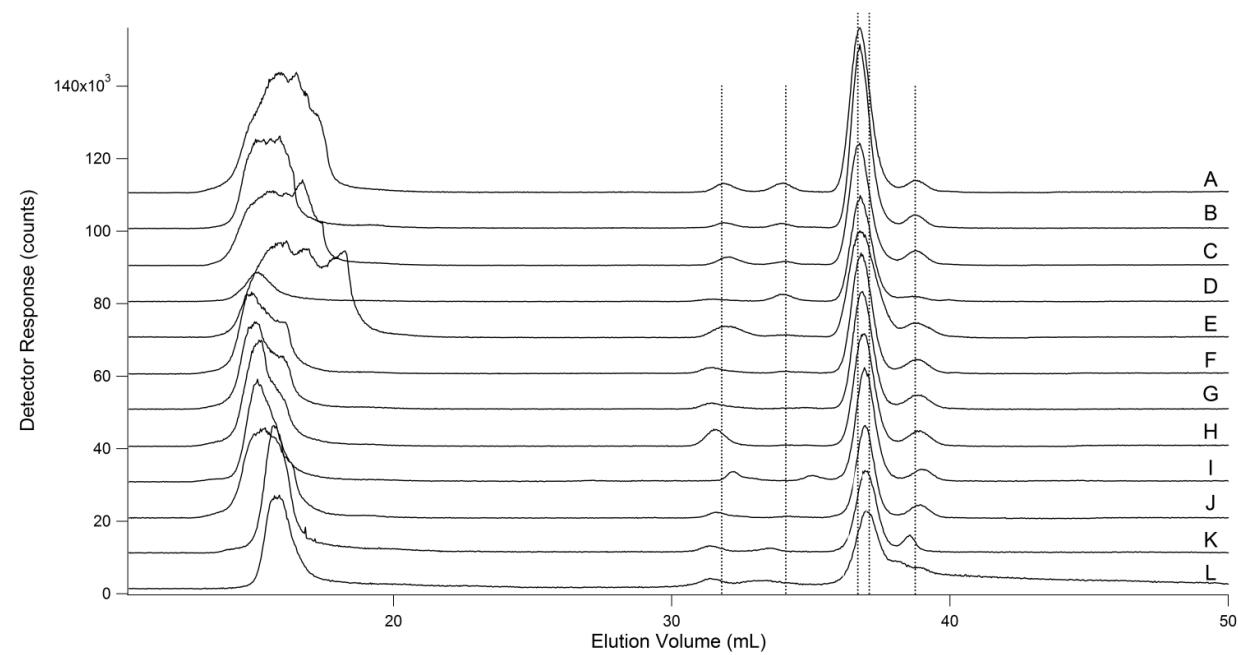


Figure S4. Individual Cobalt Chromatograms. Vertical dashed lines indicate the elution volume of species listed in Table 1.

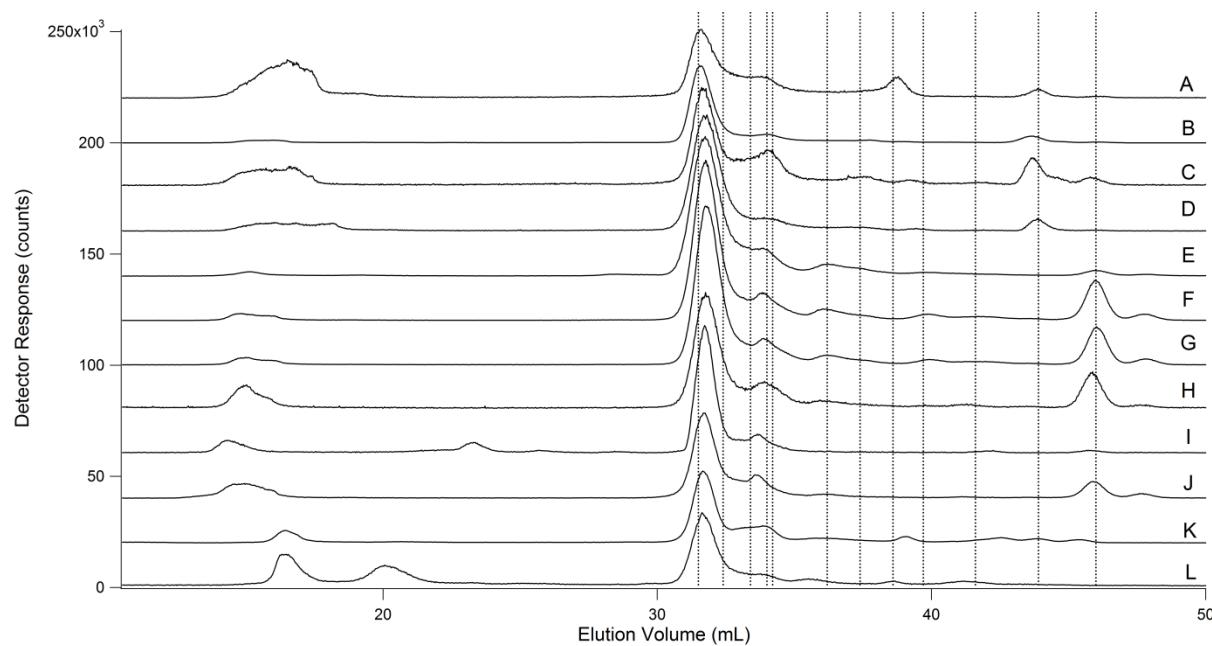


Figure S5. Individual Copper Chromatograms. Vertical dashed lines indicate the elution volume of species listed in Table 1.

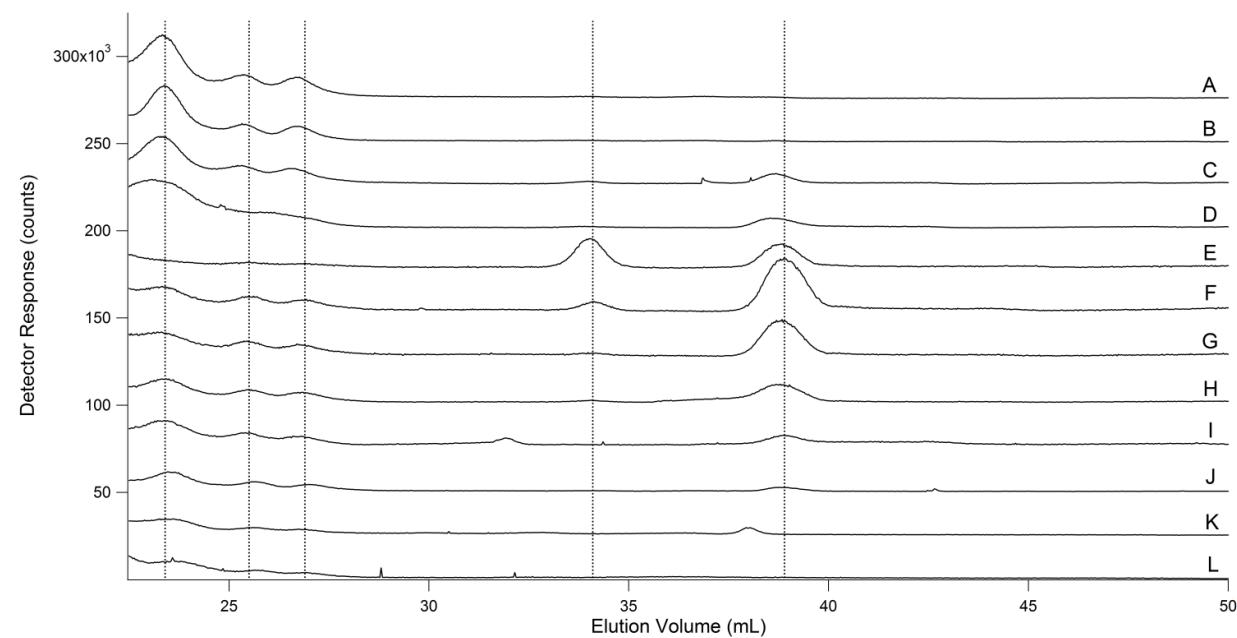


Figure S6. Individual Zinc Chromatograms. Vertical dashed lines indicate the elution volume of species listed in Table 1.

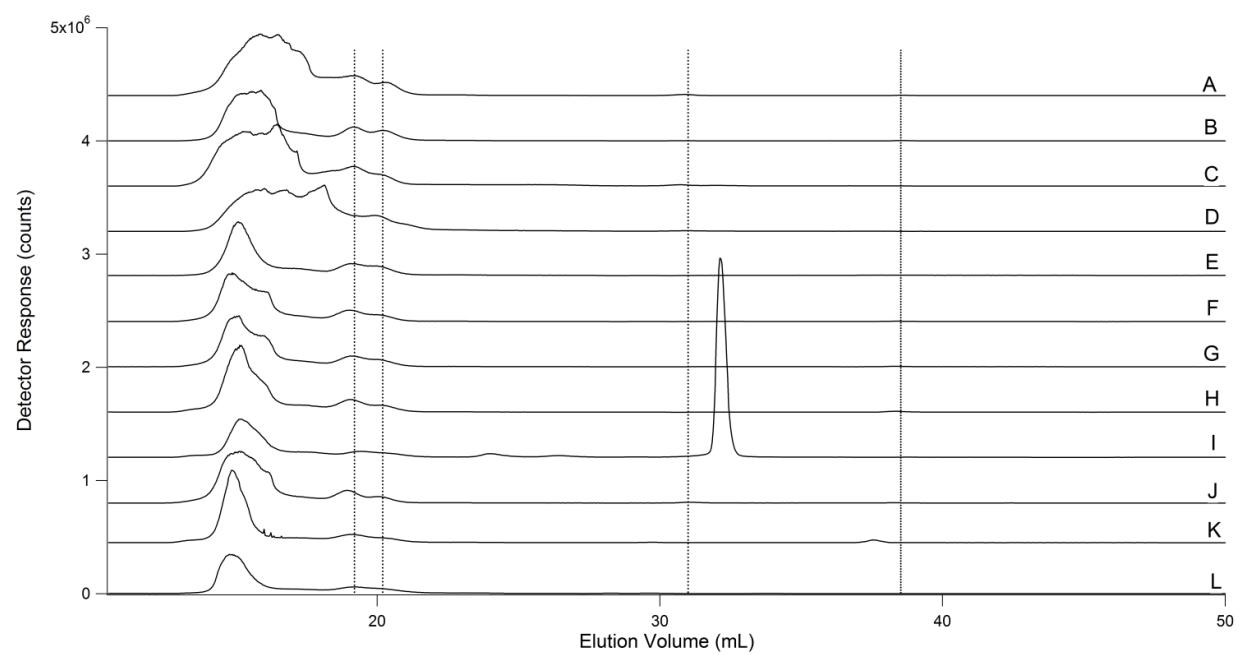


Figure S7. Individual Iron Chromatograms. Vertical dashed lines indicate the elution volume of species listed in Table 1.

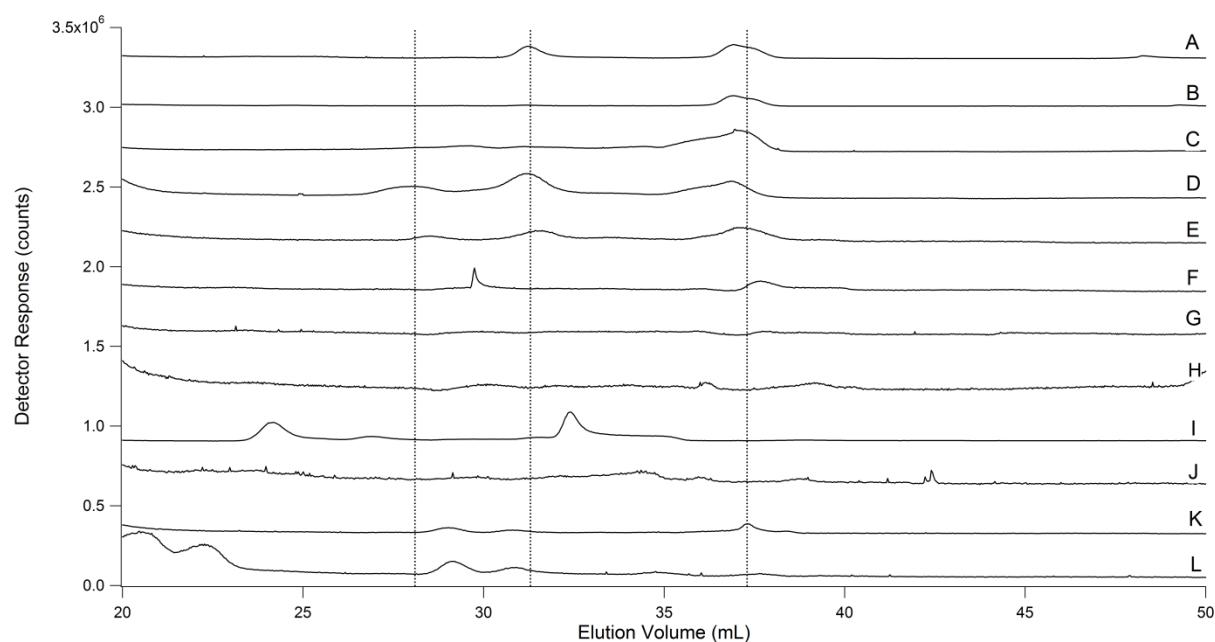


Figure S8. Individual Manganese Chromatograms. Vertical dashed lines indicate the elution volume of species listed in Table 1.

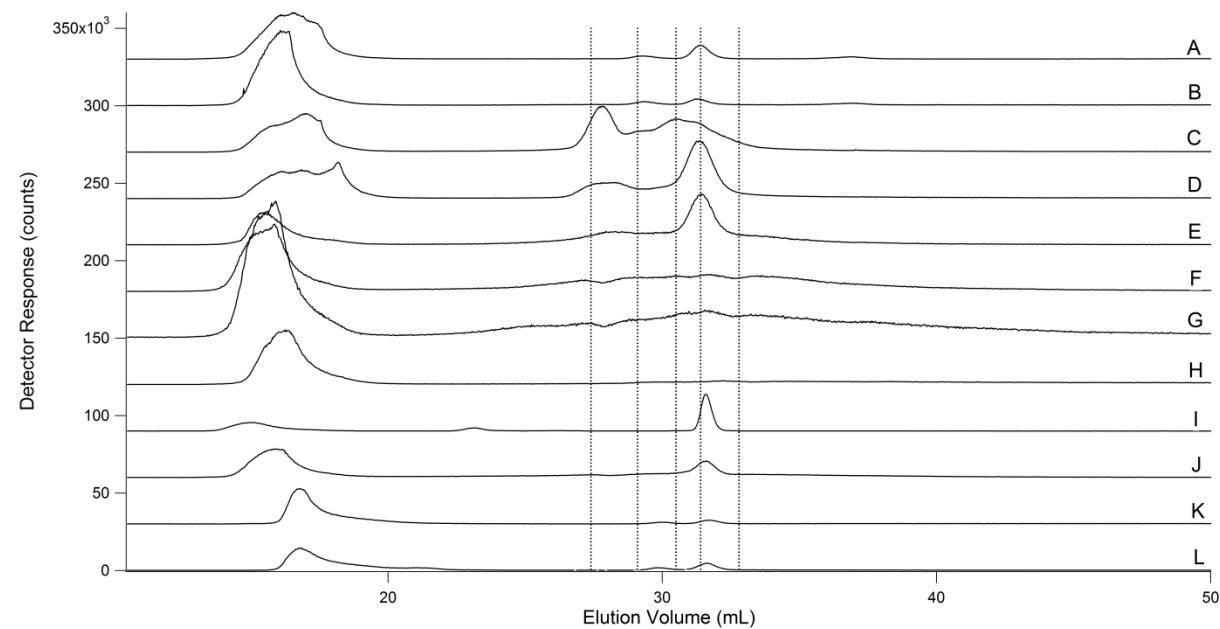


Figure S9. Individual Molybdenum Chromatograms. Vertical dashed lines indicate the elution volume of species listed in Table 1.

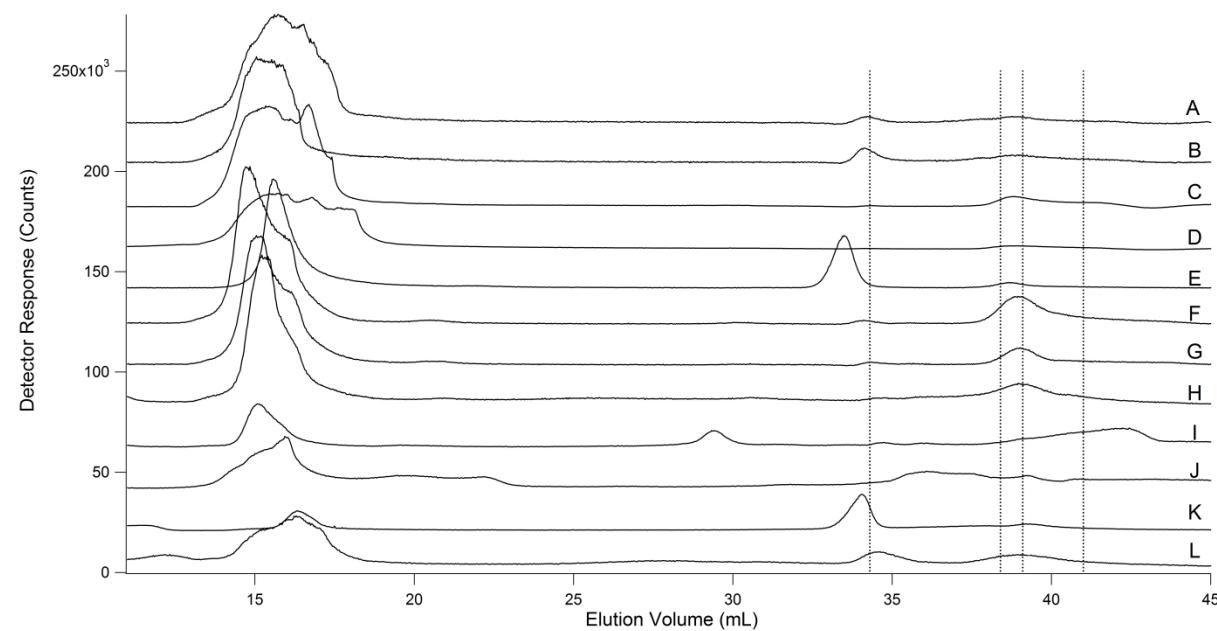


Figure S10. Chromatograms obtained testing for sample degradation. Fresh brain FTS (black) and 13 Day old (red) refrigerated and stored anaerobically were analysized as described in the text. Traces for P (A), S (B), Cu (C), Zn (D), Fe (E), Mn (F) are shown.

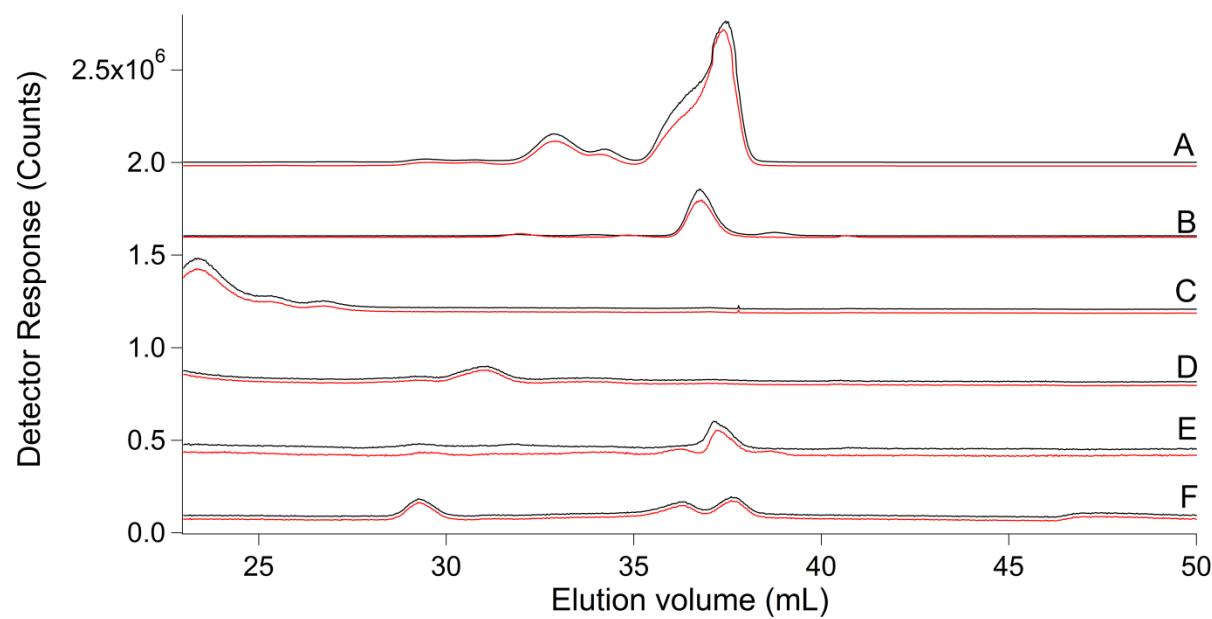


Figure S11. Chromatograms obtained using HEPES (red) and Tris (black).

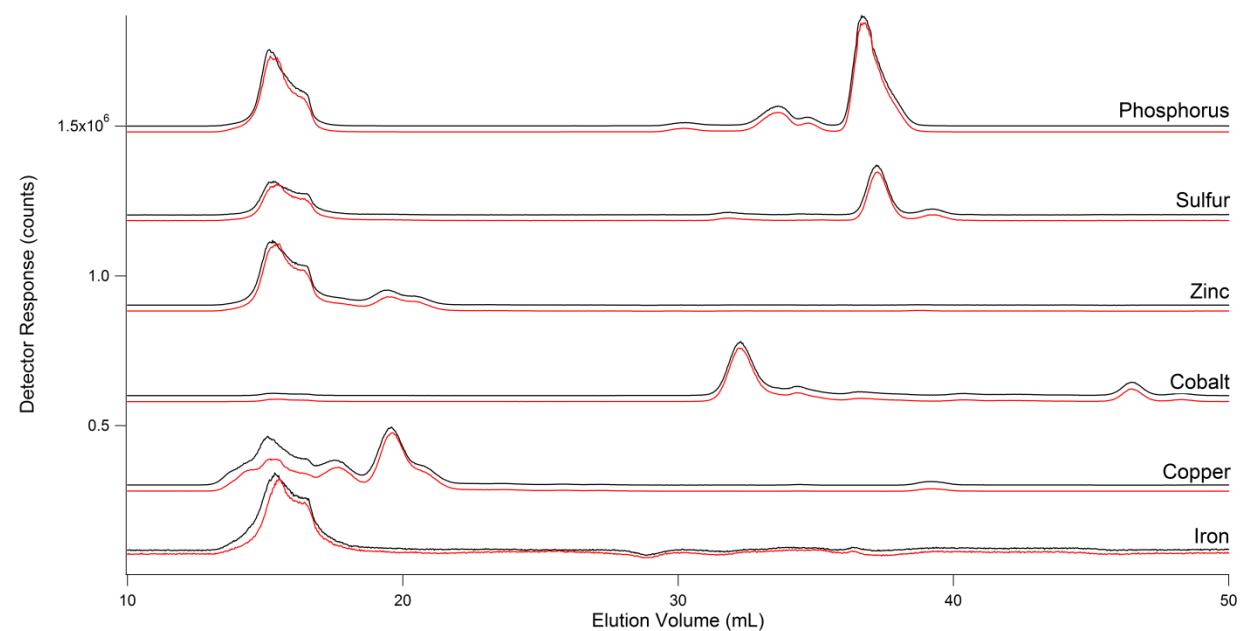


Table S3. Minimal Concentrations of Detectable Metals using the current instrument.

Element X	Cellular Limit of Detection, $[X]_{\text{cell}}$ (μM)	Cellular Limit of Detection (# of X Atoms per cell)
P	2.1 ± 0.5	$300,000 \pm 100,000$
S	3.1 ± 1	$580,000 \pm 20,000$
Co	0.0004 ± 0.0001	600 ± 150
Cu	0.002 ± 0.0003	300 ± 30
Zn	0.006 ± 0.0003	$1,000 \pm 200$
Fe	0.02 ± 0.001	$3,000 \pm 200$
Mn	0.03 ± 0.005	$6,200 \pm 100$
Mo	0.003 ± 0.001	800 ± 100

Figure S12. Zoom in of Minor Features

