Electronic Supplementary Information

Experimental

Chemicals

Phosphate-buffered saline (PBS) tablets, $CdCl_2$, diethylenetriaminepentaacetic acid (DTPA, ≥99%), zinc oxide (ZnO, 99%), sodium hydroxide (NaOH, ≥97%) and blue dextran were purchased from Sigma-Aldrich (St. Louis, MO, USA). PBS-buffer (10 mM phosphate, 2.7 mM KCl and 137 mM NaCl, pH 7.4) was prepared by dissolving PBS tablets in the appropriate volume of de-ionized water which was obtained from a Simplicity water purification system (Millipore, Billerica, MA, USA). If necessary, pH was adjusted to 7.4 by the addition of dilute HCl. The mobile phase was filtered through 0.45 mm nylon-filter membranes (Mandel Scientific, Guelph, ON, Canada) and degassed by purging N_2 gas for 30 min before use. A mixture of protein standards which contained thyroglobulin (670 kDa), γ-globulin (158 kDa), ovalbumin (44 kDa), myoglobin (17 kDa), and vitamin B_{12} (1.35 kDa) was purchased from Bio-Rad Laboratories (Hercules, CA, USA) to calibrate the Superdex 200 SEC column.

Solutions

A stock solution of $CdCl_2$ (500 mg Cd/L) was prepared by dissolving an appropriate amount in de-ionized water. A $ZnNa_3DTPA$ stock solution (10 mM, pH 7.2) was prepared by following a previously reported procedure [46]. In brief, DTPA (0.7867 g) was dissolved in 100 mL ~80 ˚C de-ionized water. Then, a stoichiometric amount of ZnO (0.1627 g) was added while stirring (magnetic stirrer). After cooling to room temperature, the pH was adjusted to 7.1 (1.0 M NaOH). The solution was diluted to 200 mL

(volumetric flask) with de-ionized water to give a $ZnNa_3DTPA$ concentration of 10 mM (pH 7.2). This solution was analyzed by electrospray ionization mass spectrometry (ESI-MS) using an Agilent 6520 Accurate Mass Q-TOF MS instrument in the positive ion mode (ESI source temperature: 200 ˚C; flow rate: 0.2 mL/min using methanol as solvent). An intense *m/z* peak at 543.987 displayed the characteristic Zn isotope pattern and was assigned to $[C_{14}H_{18}N_3O_{10}Na_4Zn]^+$, which is referred to as $ZnNa_3DTPA$ in the text.

SEC-ICP-AES system

The SEC-ICP-AES system which was employed in this study was described previously [29, 32] and consisted of a Smartline 1000 HPLC pump (Knauer, Berlin, Germany), a Rheodyne 9010 PEEK injection valve (Rheodyne, Rhonert Park, CA, USA) equipped with a PEEK injection loop (0.5 mL) and a pre-packed Superdex™ 200 10/300 GL Tricorn™ high performance size-exclusion chromatography column (30.0 × 1.0 cm I.D., separates globular proteins between ~600 and ~10 kDa; GE Healthcare, Piscataway, NJ, USA). The exit of the SEC column was connected to the Meinhard concentric glass tube nebulizer of the ICP-AES with FEP Teflon tubing (30 cm, I.D. 0.5 mm). The mobile phase flow rate was 1.0 mL/min (column temperature: 22˚ C). Simultaneous multielement-specific detection of C (193.091 nm), S (180.731 nm), P (213.618 nm), Cu (324.754 nm), Fe (259.940 nm), Zn (213.856 nm), Cd (226.502 nm), and Ca (393.366 nm) in the column effluent was achieved with a Prodigy, high-dispersion, radial-view ICP-AES (Teledyne Leeman Labs, Hudson, NH, USA) at an Ar gas-flow rate of 19 L/min, an RF power of 1.3 kW and a nebulizer gas pressure of 35 psi. Time scans were

performed using the time-resolved analysis (TRA) mode (Salsa software version 3.0) and a data acquisition rate of 1 data point per 2 s was used. According to the void volume of the Superdex 200 column [which was determined by the injection of blue dextran and C-specific detection (7.83 mL or 470 s)], a 7.0 min delay was implemented between injection and the beginning of data acquisition and a 1200 s acquisition window was used. The raw data were imported into Sigmaplot 12 and smoothed using the bisquare algorithm.

Analysis of Cd^{2+} spiked rabbit plasma by SEC-ICP-AES

The Animal Care Committee (University of Calgary) approved the procedure to collect blood from New Zealand white rabbits (Protocol AC12-0025). A male rabbit was purchased from Riemens Fur Ranches Ltd. (St. Agatha, ON, Canada), fed *ad libitum* on a "high fiber" diet (Lab Diet 5321, Canadian Lab Diets, Leduc, AB, Canada) and fasted 4.5 h before blood collection. The rabbit plasma stock was prepared by drawing an adequate amount of blood from a rabbit with a 60 mL syringe (heart puncture) into 6 mL heparinized trace metal testing blood collection tubes (Vacuette, Greiner bio-one North America, Monroe, NC, USA). In order to prevent blood coagulation in the syringe, the syringe was coated with 1.0 mL heparin solution (Hepalean-LOK, Organon Canada Ltd., Toronto, ON, Canada). The obtained blood was centrifuged at 3000 rpm (4 ˚C) for 10 min and the supernatant plasma was removed and gently mixed in a plastic test tube (magnetic stirring bar) under a N_2 atmosphere. Then plasma aliquots (2.0 mL) were transferred to cryovials and kept frozen at -30˚ C. Plasma aliquots were thawed at room temperature for 40 min, transferred to a thermostated incubator (37˚ C) and gently shaken

at 125 rpm under a N_2 atmosphere for 5 min. An appropriate volume (8 µL) of the $CdCl_2$ stock solution (500 mg Cd^{2+}/mL) was added to 2 mL plasma to obtain a final concentration of 2.0 µg Cd^{2+}/mL (this dose was chosen based on previous results [33]). 5 min later, each plasma aliquot (2.0 mL) was spiked with PBS-buffer (66 µL, control) or 66 µL $ZnNa_3DTPA$ (10 mM) to obtain final concentrations of 0.66 mM. The results for 0.22 mM and 0.99 mM of $ZnNa_3DTPA$ were similar to those of 0.66 mM presented herein. 5 min and 30 min after the addition of $ZnNa_3DTPA$, 500 µL of the obtained rabbit plasma mixtures were injected onto the SEC-ICP-AES system (Superdex 200 SEC column). All experiments were carried out in triplicate unless otherwise stated. SigmaPlot 12.0 was used to smooth the raw data using the bisquare algorithm. The number and relative intensity of the detected Ca, Cu, Fe and Zn-peaks at the 5 min time point (of Cd^{2+}-spiked rabbit plasma) were in accord with previous studies and essentially identical to those obtained at the 30 min time point [29].

Figure S1

Representative SEC-ICP-AES derived Fe-specific (A) and Cu-specific (B) chromatograms which were obtained for the analysis of Cd^{2+}-spiked rabbit plasma (2.0 µg of Cd^{2+}/mL plasma). To 1.0 mL of plasma either 33 µL of PBS-buffer (black lines) or $ZnNa_3DTPA$ was added (0.66 mM final concentration, red lines) and the obtained mixtures were analyzed 5 min later. Column: Superdex 200 10/300 GL (30 x 1.0 cm I.D., 13 µm particle size), Column temperature: 22°C, Mobile Phase: PBS-buffer (pH 7.4), Flow-rate: 1.0 mL/min, Injection volume: 500 µL, Detector: ICP-AES at 259.940 nm (Fe) and 324.754 nm nm (Cu). Retention times of the molecular weight markers are depicted on top.

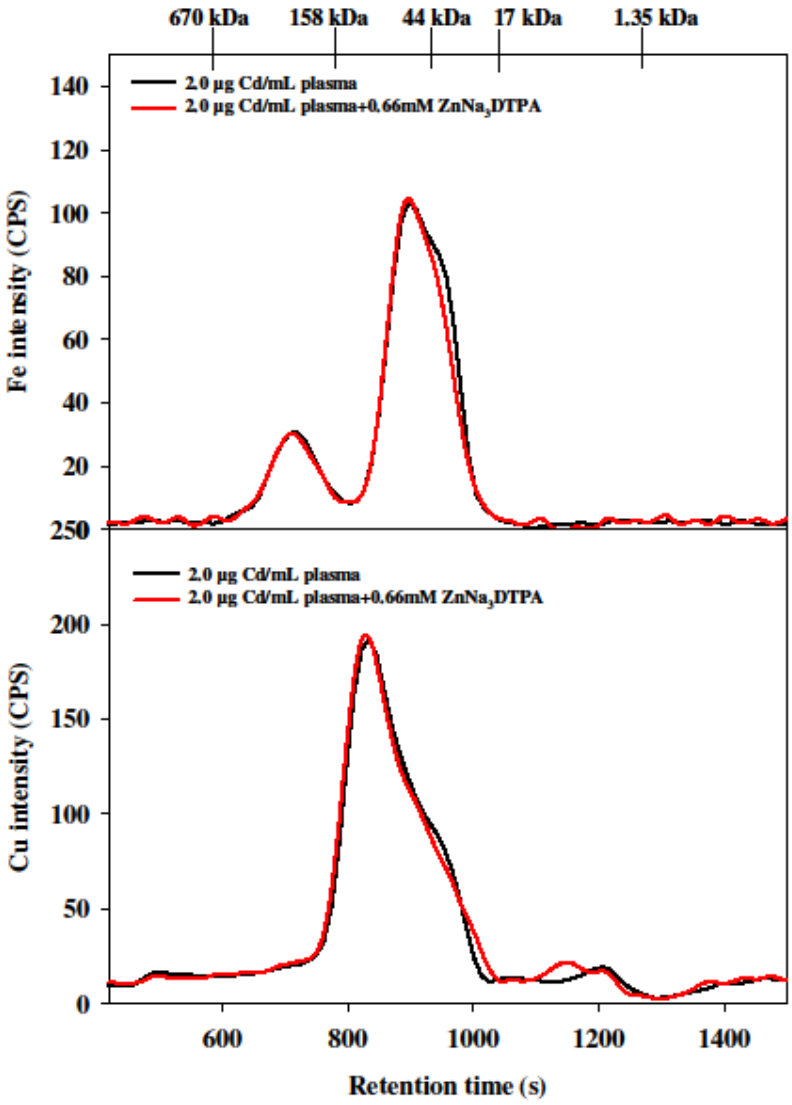

Figure S2

Representative SEC-ICP-AES derived Zn-specific chromatogram which was obtained for the analysis of $ZnNa_3DTPA$-spiked rabbit plasma (no Cd^{2+} added to plasma). To 1.0 mL of plasma $ZnNa_3DTPA$ was added (0.66 mM final concentration) and the obtained mixture was analyzed 5 min later. Column: Superdex 200 10/300 GL (30 x 1.0 cm I.D., 13 µm particle size), Column temperature: 22°C, Mobile Phase: PBS-buffer (pH 7.4), Flow-rate: 1.0 mL/min, Injection volume: 500 µL, Detector: ICP-AES at 213.856 nm (Zn). Retention times of the molecular weight markers are depicted on top.