SUPPLEMENTAL METHODS

SEC-ICP-MS Quality Control and Calibration

All solutions for SEC-ICP-MS analysis were prepared in 18MQ/cm double deionized water (Sybron Barnstead), in which no metal was detected. The mobile phase for SEC was made by dissolving Tris in double deionized water and adjusting the pH with hydrochloric acid. Before running the samples, the SEC column was calibrated with a UV detector (wavelength, 280 nm) by using a gel filtration standard mixture. The SEC standard (Bio-Rad Laboratories) is a lyophilized mixture of molecular weight markers ranging from 1300 to 670,000 Da. (molecular weight of thyroglobulin, 670kDa; molecular weight of g-globulin, 158kDa; molecular weight of ovalbumin, 44kDa; molecular weight of myoglobin, 17kDa; molecular weight of vitamin B12, 1.3kDa).

Protein In-Solution Tryptic Digestion

Fractions from SEC-ICP-MS were collected and freeze-dried. The pellet was re-suspended in 20µl of 50mM ammonium bicarbonate. After resuspension, 2µl of 100mM DTT was added as reducing buffer and the mixture was heated at 95°C for 5 minutes. After cooling the sample, an alkylation step was carried out to protect the thiol groups of the cysteine residues by adding 3µl of 100mM iodoacetamide. The mixture was then incubated in the dark at room temperature for 20 minutes. After the alkylation, 1µl of modified sequence grade trypsin solution was added and incubated at 37^{o}C for 2 hours and then 2µl of additional trypsin were added to complete the reaction, followed by incubation at 37^{o}C for 8 hours. At the end, 1µl of formic acid was added to stop the reaction, and the solution was ultra-filtered through a 10kDa filter to remove the undigested proteins and the unreacted trypsin.

Proteomics and MASCOT Search Parameters

An Agilent 6300 Series mass spectrometer system equipped with both capillary and Nano pumps was used for mass identification. The chip used for the analysis consists of a Zorbax 300SB C18 enrichment column (4mm x 75µm, 5µm) and a Zorbax 300SB C18 analytical column (150mm x 75µm, 5µm). Two microliters of sample were loaded *via* the capillary pump onto the on-chip enrichment column. Samples were loaded on to the enrichment column at a flow rate of 3 $\mu L.min^{-1}$ with a 97:3 ratio of solvent A (0.1% FA, formic acid (v/v), in water) and B (90% ACN (acetonitrile), 0.1% FA (v/v) in water). After the enrichment column was loaded, the on-chip microfluidics switched to the analytical column at a flow rate of $0.3\mu L.min^{-1}$. The following gradient conditions were used in the analysis: 0–5 min, 10% B; 5–85 min, 35%B; 85–90 min, 75% B; 90–95 min, 75% B; 95–98 min, 3% B; 98–105 min, 3% B. Full scan mass spectra were acquired over the m/z range 150–2200 in the positive ion mode (peptide charge: +1 to +3). For MS/MS experiments, experimental conditions consisted of: m/z range: 150–2200; isolation width: 2 m/z units, fragmentation energy: 30–200%, fragmentation time: 40ms. The ionization system utilized was the microfluidic chip, which is automatically loaded and positioned into the MS Nano spray chamber and also contains the electrospray tip.

The MS/MS data obtained from the above experiments were exported to the online MASCOT server (Matrix Science Inc.) database search engine, and submitted with the following parameters: Taxonomy, all; Enzyme, Trypsin; Missed Cleavages, Two; variable modifications, Carbamidomethylation of C; Peptide tolerance, 1.8Da; MS/MS tolerance, 0.8Da; Peptide charge, +1,+2,+3 and Instrument, ESI-TRAP. MASCOT then searched against Uniprot database and the reported hits were validated doing a blast analysis of the reported peptides.

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Representative LC-MS/MS Chromatogram. The upper panel shows a total ion chromatogram obtained on full scan mode, and the lower panel shows the extracted ion corresponding to the assigned peptide, corresponding the 42kDa sequence.

Figure S2. Representative MS/MS fragmentation spectra. (A) MS/MS spectra for the signal at m/z 894.42, assigned to the sequence ENIASFMESYVEQIK; **(B)** at m/z 1709.8493, assigned to the sequence ELSQNQPVYYQGVGK; and **(C)** at m/z 1370.6811, assigned to the sequence VGGHAFVIDGADGR as $[M+H]^{2+}$ are shown. The list of predicted b and y ions are shown and highlighted are the founded masses observed in the spectra.