Online Resource 3

In solution analysis (shotgun):

Protein identification by mass spectrometry analysis. The proteins lyophilized were resuspended in 100mM Ammonium Bicarbonate / 5 % Acetonitrile. The reduction and alkylation were performed using DTT (Sigma-Aldrich, Saint Louis, MO) at 65°C for 5 minutes, followed by Iodoacetamide (Sigma-Aldrich, Saint Louis, MO) at room temperature in dark condition for 30 minutes. Trypsin sequencing grade (Promega, Madison, WI) was used for the *in solution* digestion at 37°C overnight, and the reaction was stopped by changing the pH to 4 using TFA (Sigma-Aldrich, Saint Louis, MO) at a final concentration of 0.5 %. Finally, the tryptic digested peptides were concentrated at a centrifuge concentrator (SpeedVac, Thermo Savant). The peptides were in-line desalted through at the list. The trypsin digested peptides were dissolved in 100 mM Ammonium Formate, pH 10, and separated through 2D-nanoLC with dilution using a 2D-nanoAcquity UPLC (Waters, Milford, MA) according to Callegari (2016). The first dimension was performed in XBridge BEH130 C18, 5µm, 300µm×50mm NanoEase Column (Waters, Milford, MA) using solvent A1: 20 mM Ammonium Formate, pH=10 and B1: 100% Acetonitrile (Fisher Optima, LC-MS grade). The flow at 1st dimension was 2 µL/min and 10 different step gradients (dilution method) were performed for 20 minute separately. The second dimension included trapping and desalting online through 180µm×20mm, 5 µm Symmetry C18 nanoAcquity UPLC trap column (Waters, Milford, MA) at flow= 20 µL/min, 99% A2 (H_2O, 0.1% Formic Acid) and 1% B2 (100% Acetonitrile, 0.1% Formic Acid) for 20 minutes. After the peptides were desalted and concentrated, they were separated online in the second dimension through BEH130 C18 1.7um, 100 µm×100 mm nanoAcquity UPLC column (Waters, Milford, MA). The standard solvent gradient used was: 0–2 min, 3% B2 isocratic; 2–40 min, 3–85% B2 linear, at a flow rate of 400nL/min for 60 minutes. The eluted ions were analyzed by one full precursor MS scan (400-1500 m/z) followed by four MS/MS scans of the most abundant ions detected in the precursor MS scan while operating under dynamic exclusion or direct data acquisition system. Spectra obtained in the positive ion mode with nano ESI-Q-Tof Synapt G1 HDMS mass spectrometer (Waters, Milford, MA) were deconvoluted, and analyzed using the MassLynx software 4.1 (Micromass, UK), and exported to ProteinLynx Global Server v3.0 (PLGS v3.0) (Waters, Milford, MA) to creates the list of masses. A peak list (PKL format) was generated to identify +1 or multiple charged precursor ions from the mass spectrometry data file (**Online Resource 2**). The instrument was calibrated in MS/MS mode using 100 fmole of (Glu^1) -

Fibrinopeptide B human (Sigma, Saint Louis, MO) with a RMS residual of 3.857 e^{-4} amu or 6.9413 e^{-1} ppm. Parent mass (MS) and fragment mass (MS/MS) peak ranges were 400-2000 Da and 65-2000 Da, respectively.

- Callegari EA (2016) Shotgun Proteomics Analysis of Estrogen Effects in the Uterus Using Two-Dimensional Liquid Chromatography and Tandem Mass Spectrometry. Methods Mol Biol 1366:131-148. https://doi.org/10.1007/978-1-4939-3127-9_11

Intracellular proteomic analysis of *Streptomyces* sp. MC1 when exposed to Cr(VI) by gel-based and gel-free methods. Current Microbiology. José O. Bonilla, Eduardo A. Callegari, María C. Estevez, Liliana B. Villegas.

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