Supplementary Material 1

In solution analysis (shotgun)

Protein identification by mass spectrometry analysis.

Lyophilized samples containing intra and extracellular proteins were reconstituted in a solution to achieve a final concentration of 50mM Tris-HCl pH 8.0 and 1 µg µL^{-1} of final protein concentration. Protein reduction and alkylation were carried out using DTT (Sigma-Aldrich, Saint Louis, MO) at 65 °C for 5 min, followed by Iodoacetamide (Sigma-Aldrich, Saint Louis, MO) at room temperature and in the absence of light for 30 min. The samples were in solution-digested with Trypsin (Promega, Madison, WI) in 1:40 ratio (µg Trypsin: µg Protein) at 37 °C overnight. The reaction was stopped by changing to pH 4 with Trifluoroacetic Acid (Sigma-Aldrich, Saint Louis, MO) at 0.5% final concentration. The tryptic digested peptides were concentrated with a centrifugal concentrator (SpeedVac, Thermo Savant) and reconstituted in Water/Acetonitrile/Trifluoroacetic Acid (97/3/0.05%).

The digested peptides were separated by Ultra Performance Liquid Chromatography on a nano scale (nanoUHPLC) using Dionex Ultimate 3000 nanoRS UHPLC (Thermo Scientific, Bremen, Germany). The peptides were captured and in-line desalted using the column Acclaim PepMap 100, 75 µm diameter and 2 cm length, nanoViper C18, 3 µm, 100 A (Thermo Scientific) with a 6 µL min^{-1} flow for 5 min, using Water/Acetonitrile/Trifluoroacetic Acid (97/3/0.05%) as the mobile phase. Then, the analytical procedure consisted of using mobile phases A (Water/Acetonitrile/Formic Acid 98/2/0.05%) and B (Water/Acetonitrile/Formic Acid 20/80/0.05%), and the column Acclaim PepMap 100, 75 µm diameter and 150 mm length, nanoViper C18, 2 µm, 100 A (Thermo Scientific). The standard gradient was: 0-4 min, 1.2% B isocratic for 4 min, then 4-35 min 3-98% B linear for 40 min, followed by A:B (98/2%) to balance. The total run time was 50 min at 300 nL min^{-1} flow.

At the end of the separation, the peptides were analyzed using an Orbitrap FT QExactive Plus quadrupole mass spectrometer (Thermo Scientific, Bremen, Germany) with a nano-scale Electrospray ionization source (nanoESI). The data acquisition method was based on the full precursor search (Full-MS) followed by precursor-dependent data acquisition ddMS2 (DDA), for 50 min (MS/MS) (Kelstrup et al., 2012; Sun et al., 2013; Liu et al., 2020). Mascot Distiller v2.6.2.0 (www.matrixscience.com) and Proteome Discoverer v2.1 (Thermo Scientific) were used to generate the file or list of generic masses or peaks (MGF). The

considered ion precursor charge was +1 or multiple charges, generated from the RAW data folder. The assigned mass range for the acquisition of precursors (MS) and fragments (MS/MS) was 300-1800 Da (resolution @70000) and 65-2000 Da (resolution @17500), respectively.

1. Kelstrup CD, Young C, Lavallee R, Nielsen ML, Olsen JV (2012). Optimized Fast and Sensitive Acquisition Methods for Shotgun Proteomics on a Quadrupole Orbitrap Mass Spectrometer. J Proteome Res 11(6): 3487-3497

2. Liu Y, Min JW, Feng S, Subedi K, Qiao F, Mammenga E, et al. (2020). Therapeutic role of a cysteine precursor, OTC, in ischemic stroke is mediated by improved proteostasis in mice. Transl Stroke Res 11(1):147-160

3. Sun L, Zhu G, Dovichi NJ (2013). Comparison of LTQ-Orbitrap Velos and Q-Exactive for proteomic analysis of 1-1000 ng RAW 264.7 cells lysate digests. Rapid Commun Mass Spectrom 27(1): 157-162