Supplementary Experimental Section

An Nanoflow Low Pressure High Peak Capacity Single Dimension LC-MS/MS Platform for In-Depth Analysis of Mammalian Proteomes

Feng Zhou[‡], Yu Lu[‡], Scott B. Ficarro[†,‡], James T. Webber[†],

Jarrod A. Marto[†,‡,§]

Materials

Sequencing grade, modified trypsin was purchased from Promega (Madison, WI). All other chemicals were purchased from Sigma Aldrich (St. Louis, MO). Solid phase extraction cartridges and strong cation exchange embedded, 96-well plates were purchased from Waters (Milford, MA) and Supelco (Bellafonte, PA), respectively.

Cell Culture and preparation of digested lysate

Mouse embryonic stem cell line CCE was generously provided by the Harvard Stem Cell Institute Human Embryonic Stem Cell Facility. Initially, 10cm Nunclon tissue culture dishes (Nunc # 172958, Fisher Cat # 12-565-99) were coated with 0.1% Gelatin (Chemicon Cat # ES-006-B) at room temperature for approximately 30 minutes. Gelatin was aspirated and PMEFs (Chemicon cat# PMEF-N) were plated at a density of ~$6\times10^4/cm^2$ in Dulbecco's modified Eagle's medium with high glucose (DMEM+) (GIBCO cat# 11960) supplemented with L-glutamine (Gibco cat# 25030) and standard 10% fetal calf serum (FCS) (HyClone cat# SH 30070-02). CCE were seeded onto established MEFs at ~$1\times10^4/cm^2$ in DMEM+ supplemented with 15% embryonic stem cell validated FCS (Stem Cell Technologies cat # 06902) and 2-mercaptoethanol,

nucleosides (Chemicon cat# ES-008-D), nonessential amino acids (Gibco cat# 11140), and 10 ng/mL murine LIF 6 (Chemicon cat# ESG-1107). After 5 days, plates were washed with cold PBS to remove serum proteins and adherent cells were lysed by the addition of a boiling SDS solution (50mM Tris-HCl , pH 7.5, 7.5% SDS, 5% glycerol, 50mM DTT, 5mM EDTA). Lysed cells were centrifuged and the supernatant extract was stored at –80 ^{o}C. Proteins were precipitated by adding six volumes of cold (-20°C) acetone and resolubilized in a digestion buffer containing 8 M urea and 0.1 M NH_4HCO_3. Dithiothreitol (DTT) was added to a final concentration of 10 mM and incubated for 30 minutes at 60 ºC, followed by addition of iodoacetamide to 20 mM. After 30 min. incubation in the dark at room temperature, excess iodoacetamide was quenched by addition of DTT to a final concentration of 20 mM. Reduced and alkylated proteins were diluted to a final volume of 12 mL in 0.1M ammonium bicarbonate. Trypsin (150 µg) was added and digestion was performed at 37 ºC overnight with end-over-end rotation. The resulting peptide solution was acidified with 10% TFA, and desalted on a C_{18} solid phase extraction cartridge, followed by final clean-up on strong cation exchange embedded 96-well plates. Eluted peptides were lyophilized by vacuum centrifugation and stored at -80 ºC in 400 µg aliquots.

Mass Spectrometry

LTQ-Velos Orbitrap and LTQ-Orbitrap XL (ThermoFisher Scientific, Waltham, MA) mass spectrometers were used for LC-MS/MS in the course of this work. Both instruments were equipped with a Digital PicoView ESI source (New Objective, Woburn, MA) and operated in data dependent mode, such that the top 10 most abundant precursors in each MS scan were subjected to MS/MS CAD in the linear trap for Orbitrap XL and

top 20 were used for Velos-Orbitrap. The following parameters were used on both instruments for MS/MS: Normalized collision energy is set to 35%, precursor isolation width = 2.8 Da, intensity threshold for precursor selection = 5,000. Dynamic exclusion was enabled with a repeat count of 2 and exclusion duration set to 120 seconds. Electrospray voltage was 2.2 kV. Lock mass was enabled with *m/z* = 445.120025 ($[Si(CH_3)_2O]_6$) as the internal calibrant ion. The following parameters were used on the quadrupole time-of-flight mass spectrometer (TripleTof 5600, AB Sciex, Framingham, MA) equipped with a Digital PicoView ESI source (New Objective, Woburn, MA). The instrument was operated in IDA mode, with the top 50 precursors (charge state +2 to +5, >50 counts) in each MS scan (800 ms, scan range 350-1500 m/z) subjected to MS/MS (minimum time 100 ms, scan range 100-1800 m/z).