

Bindley Bioscience Center Global Proteomics methodology:

Instrument setup

XCT Plus ESI ion trap

NanoLC-Chip-MS. Trypsin digested proteins will be separated on a nanoLC-Chip system (1100 Series LC equipped with HPLC Chip interface, Agilent, Santa Clara, CA). After injection of 1 μg , the peptides will be concentrated on the on-chip 300SB-C18 enrichment column and washed with 5% acetonitrile (ACN); 0.01 % TFA at flow rate 4 $\mu\text{l}/\text{min}$ for 5 minutes. The enrichment column will be switched into the nano flow path and further separated with the on-chip C-18 reversed phase ZORBAX 300SB-C18 (0.75 μm x 150 mm; Agilent) analytical column coupled to the electrospray ionization (ESI) source of the ion trap mass spectrometer (XCT Plus, Agilent). The column will be eluted with a 55 min linear gradient from 5%-35% buffer B (100% acetonitrile, 0.01% TFA) at a rate of 300 nL/min, followed by a 10 minute gradient from 35%-100% buffer B. The column will be equilibrated with an isocratic flow (5% buffer B) at 300 nL/min. The system will be controlled by ChemStation software (Agilent). NanoLC-MS chromatograms will be acquired in positive ion mode under the following conditions: a capillary voltage of 1850 V and an end plate offset of 500 V. The dry temperature will be set at 300 $^{\circ}\text{C}$. Dry gas flow will be maintained 6 l/min. Acquisition range will be 350 - 2000 m/z with 0.15 s maximum accumulation time and scan speed of 8,100 m/z per second.

NanoLC-Chip-MS/MS and targeted MS/MS. Peptides will be separated on a nanoLC-Chip system (1100 Series LC equipped with HPLC Chip interface, Agilent, Santa Clara, CA) using the same platform as described above. Automated MS/MS spectra will be acquired during the run in the data-dependent acquisition mode with the selection of the three most abundant precursor ions (0.5 min active exclusion; 2+ ions preferred). If the peptides associated with our peaks of interest are not identified due to low abundance then the samples will be rerun using the same parameters except the specific mass associated with the peaks of interest will be selected for preferentially or targeted MS/MS analysis.