MS Analysis of Gel Bands from Partial Proteolysis of Huntingtin (SPARC BioCentre, The Hospital for Sick Children, Toronto)

The bands from the partial tryptic digest of Huntingtin were excised from the gel, the proteins reduced with DTT and the free cysteine residues were alkylated with iodoacetamide. The proteins were digested overnight with trypsin and the resulting peptides extracted. The peptides were loaded onto a 50 cm x 75 µm ID column with RSLC 2 µm C18packing material (EASY-Spray, Thermo-Fisher) with an integrated emitter. The peptides were eluted into a Q-Exactive hybrid mass spectrometer (Thermo-Fisher, San Jose, CA) using an Easy-Spray nLC 1000 chromatography system (Thermo-Fisher) with a 1 hr. gradient from 0% to 35% acetonitrile in 0.1% formic acid. The mass spectrometer was operated in a data dependent mode with 1 MS followed by 10 MS/MS spectra. The MS was acquired with a resolution of 70,000 FWHM, a target of 1 x 10^6 ions and a maximum scan time of 120 ms. The MS/MS scans were acquired with a resolution of 17,500 FWHM, a target of 1 X 10^6 ions and a maximum scan time of 120 ms using a relative collision energy of 27%. A fixed first mass of 80 Da and a dynamic exclusion time of 15 seconds was used for the MS/MS scans. The raw data file was acquired with XCaibur 2.2. The relative amount of each Huntingtin peptide was calculated by using extracted ion currents (XICs) generated using the Pinnacle software suite (Optys).