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Negative Ion Mode Proteomics: An MS/MS-Free Approach for Increased Proteome Coverage

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Negative ion mode proteomics has the potential to increase both sequence coverage and depth of proteome analysis. However, challenges such as corona discharge, spray stability and inefficient ionization need to be addressed by optimizing both chromatographic and mass spectrometric conditions. Moreover, despite demonstration of some MS/MS techniques for negatively charged peptides, their implementation in routine proteomics experiments is not yet standard. To address this, we have explored an alternative approach using MS1-only data in conjunction with predicted peptide chromatographic retention times.

Negative mode analyses were performed using an Orbitrap Eclipse mass spectrometer with alkaline mobile phases, and short 5-15 min. chromatographic gradients. For protein identification, Biosaur feature detection and ms1searchpy search engine based on accurate mass and retention time were applied.

The best results in terms of spray stability and signal intensity were achieved using mobile buffers at 2.5 mM imidazole and 3% isopropanol. The method was validated on a *HeLa* standard, showing comparable performance to the positive mode, with over 1,000 proteins identified in a single-shot 10-minute gradient. Results highlighted the complementarity of data for protein identification and sequence coverage, with 15% of proteins and 40% of peptide features detected only in the negative mode. Evaluating the proteins as a function of pI showed a preference for the acidic part of the proteome for negative mode data. When all replicates for the 10-minute gradients in both polarities were combined, it allowed to increase the number of identified proteins to more than 1,773. We also looked at how using four different proteases (LysC, GluC, AspN, and trypsin) affected the performance of our method, and found that trypsin and LysC performed best in terms of protein identification, indicating that the same lysis and digestion procedures as for positive mode proteomics can also be utilized for analysis in the negative ion mode.