IDENTIFICATION AND QUANTIFICATION OF PROTEINS FROM *METHYLOPHAGA THIOOXIDANS* AND *METHYLOCELLA SILVESTRIS* USING LABEL-FREE LC/MS

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An alternative scanning LCMS approach was used to conclusively and stringently identify *Methylophaga thiooxidans* and *Methylocella Silvestris* proteins from tryptic digests of whole cell lysates. The system comprised of an LC-MS^E acquisition mode and novel database search algorithm 'Ion Accounting' allowed the physiochemical characteristics of fragmented tryptic peptides to be used to aid stringent protein identification. Simultaneous absolute quantification of the identified proteins showed detection of proteins present over 3 orders of magnitude.

The novel data-independent analysis mode (MS^E) is employed on the SYNAPT MS mass spectrometer enabling precursor and fragment ions from the tryptic digest to be analyzed simultaneously. The inclusion of a spike of phosphorylase B tryptic digest of a known concentration allows the software not only to identify the components of the complex mixture but also to calculate the absolute amounts of identified proteins[1]. MS^E provides accurate mass measurements of all detectable precursor and product ions. Chromatographic alignment of precursor and product ion data reduces miss-assignment of product ions to parent ions of similar mass or retention time. Protein identifications are confirmed using the parent and product ion accurate mass and additional peptide physiochemical properties used by the Ion Accounting algorithm.

Quantitative measurement of low energy precursor ions is facilitated by the data independent analysis and an increase in dynamic range is observed as the limitations posed by conventional Data Directed Analysis MS/MS duty cycle are negated. In the example of *Methylophaga thiooxidans* tryptic digest, 3 orders of magnitude of protein concentration can be detected, ranging from 0.01ng to 74ng on column. The total column loading can also be calculated using this methodology – in this case a total column loading of 367µg allowed the confident identification of 309 proteins under stringent conditions. A false positive rate of 2.27% was calculated for this experiment. Previous MS analysis of the *Methylophaga thiooxidans*, which is currently considered to be a poorly characterized bacterium, using SCX/RP and conventional analysis had returned only 81 protein identifications. Approximately half of the identifications made using conventional MS methods were made with only 1 peptide per protein. In contrast

to this, on average 9 peptides per protein were identified using the data independent methodology combined with Ion Accounting. Triplicate injections were undertaken to allow filtering of protein identification on replication and to give statistical information on absolute quantification calculations. The result of this type of analysis in practical terms is the detection of higher numbers of proteins with additional peptides per protein identification, giving increased confidence in the protein assignment. The improvement in protein identification becomes more apparent with increasing complexity of analytes. Experiments are underway to investigate both *Methylophaga thiooxidans* and *Methylocella Silvestris* proteomes under differential growth conditions using label free quantification. Relative protein quantification obtained using this methodology will be compared to previous results obtained using iTRAQ labeled techniques.