

**A RAPID APPROACH TO THE IDENTIFICATION AND CHARACTERISATION OF TRYPTIC PEPTIDES USING HIGH LINEAR VELOCITY NANOBORE UPLC SEPARATIONS COUPLED WITH ESI MS/MS.**

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Mass spectrometry has now firmly established itself as the primary technique for identifying proteins due to its unparalleled speed, sensitivity and specificity. Strategies can involve either analysis of the intact protein, or more commonly digestion of the protein using a specific protease that cleaves at predictable residues along the peptide backbone. This provides smaller stretches of peptide sequence that are more amenable to analysis via mass spectrometry. When coupled with protein level pre-fractionation strategies, thus reducing the complexity of the protein mixture, this approach has proven highly successful in comprehensive protein identification and characterisation. A common approach to protein prefractionation is the use of 1 dimensional PAGE, coupled with LC-MS/MS. The downside of this approach is the number of gel samples, or fractions, to be analysed by the LC-MS/MS system. With typical analytical HPLC run times of 45 minutes to 1 hour the amount of time required to analyse one top level sample can be prohibitive.

In this presentation we describe the use of elevated flow rates combined with nanoscale columns packed with sub 2µm particles for rapid peptide based separations using a nanoUPLC system. Increasing the flow rate of the separation from approximately 300nL/min to 900nL/min and running a very rapid gradient over 8 minutes on a 75µm x 15cm column allows high quality peptide separations to be achieved for tryptic peptides with a sample to sample inject time of ten minutes. This combined with an orthogonal acceleration time-of-flight mass spectrometer, using a newly developed high speed data dependant MS/MS approach fragmenting up to 8 precursor ions per second, allows for the rapid characterisation of simple protein mixtures, such as those obtained from 1D gel bands.

We will present data from standard tryptic digests of known proteins and simple mixtures of protein digest used in the development of this method and data from in-gel digests of 1D gel bands. This will be compared and contrasted against data acquired using conventional separations and mass spectrometric analysis.