

**An investigation into the dynamic range of protein identification; MALDI vs electrospray on a hybrid quadrupole orthogonal acceleration time-of-flight (Q-ToF) mass spectrometer**

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Mass spectrometry has rapidly become the method of choice for the identification and characterisation of proteins. For organisms whose genomes are completely sequenced peptide mass fingerprinting using MALDI mass spectrometry is useful as a primary screen.

Electrospray ionisation tandem mass spectrometry (ESI-MS/MS) is an ideal complementary technique to MALDI since it can be used to provide high quality sequence data from individual peptides produced by the enzymatic digestion of protein. However in the case of endogenous complex protein mixtures and individual protein complexes, the dynamic range of protein identification becomes extremely important and the ionisation technique can become the limiting factor.

In this paper we describe a comparison between MALDI and ESI ionisation techniques on the Q-ToF mass spectrometer. We have compared HPLC separated standard mixtures of tryptic peptides obtained from protein mixtures over a wide dynamic range. These have been analysed by MALDI and ESI. In the MALDI case we have spotted the LC eluent onto a high density MALDI array such that each position contains simplified mixtures of tryptic peptides, whilst the ESI experiment has been performed on-line. In both the MALDI and ESI case we have used data dependant analysis to select potential precursor ions for analysis in the product ion MS/MS mode. These MS/MS spectra are then used to identify the parent proteins from a databank search .

Initial results have been obtained from the analysis of standard protein mixtures. Firstly, the detection limit has been determined for tryptic digests of a single protein by ESI LC MS/MS and the LC-MALDI approach. These results will be compared and contrasted against the detection limits of both techniques when high levels of a second protein digest are present. These experiments, thus far, have resulted in an effective dynamic range of protein identification of greater than 1000:1. The impact of the molecular weight of both the high and low-level proteins will be discussed, with not just the ability to identify the low-level protein but also the effect on the protein coverage obtained.

The effect of several analytical parameters on the dynamic range of protein identification will also be presented. These will include the gradient of the reverse phase separation and the use of a trapping column, prior to the analytical reverse phase separation.

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