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OVERVIEW

- Quantitative analysis of a complex ^{16}O : ^{18}O labelled tryptic digest sample**
- Robust coupling of 2D chromatography with MALDI MS and MS/MS analysis**
- Increased sample throughput using high repetition rate, 200Hz laser**
- Initial results from mouse serum samples comparing a control mouse with a tumour burdened animal**

INTRODUCTION

Mass spectrometry is now an accepted technique for the identification of proteins. In the qualitative analysis of digest mixtures from a cell lysate or sub-cellular fraction, the challenge is often the dynamic range of proteins present in the sample, as the detection and identification of these components is often biased towards the larger and most abundant species. This has resulted in the development of chromatographic techniques, such as two-dimensional chromatography. Recent developments in MALDI MS/MS and LC-MALDI spotting devices, allows the uncoupling of the chromatographic separation step from the subsequent MS analysis. This permits the use of targeted data acquisition schemes, resulting in additional information content compared to either an ESI LC-MS/MS, or MALDI experiment on the un-separated mixture. In addition, relative quantification of proteins from the MALDI analysis can be achieved through the incorporation of stable isotope labels, in combination with separation and subsequent analysis by mass spectrometry.

In this study an isotope labelling ^{18}O quantitation technique was used to analyze serum from a xenograph mouse model, and to compare this against a control mouse, to identify potential early detection markers in cancer.

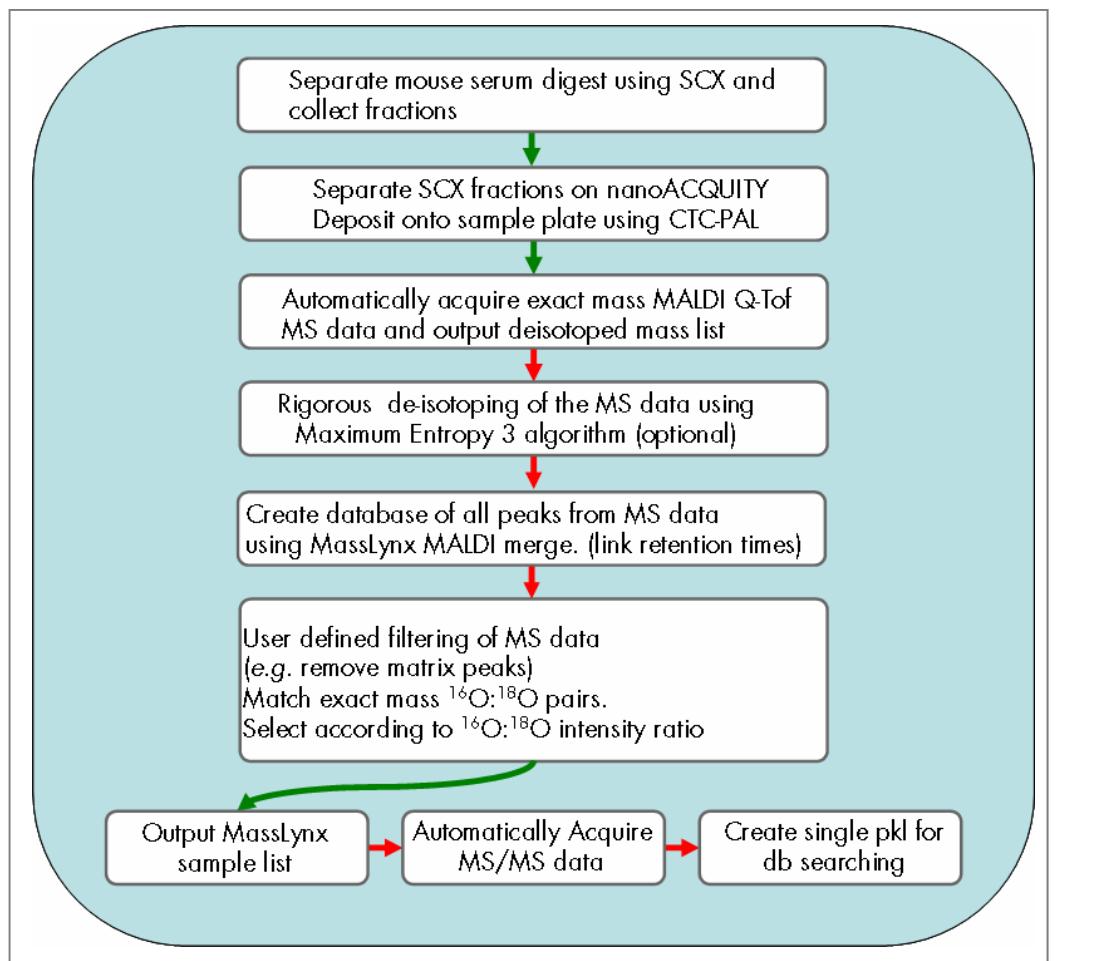


Figure 1. Flow diagram illustrating the analytical method used in this study.

METHODS

- Quantitative analysis of a complex ^{16}O : ^{18}O labelled tryptic digest sample**
- Robust coupling of 2D chromatography with MALDI MS and MS/MS analysis**
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OVERVIEW

A flow diagram providing an overview of the analytical methodology is shown in Figure 1.

Sample preparation

V12-Harvey ras-expressing NIH3T3 fibroblast cells were implanted into athymic nude mice to induce fibrosarcomas. 50 μL of serum from tumour-burdened mice and control mice was diluted in 30% trifluoroethanol, 200mM Tris pH 8 and 38mM NaCl. The sample was subsequently reduced with 10mM TCEP and alkylated (50mM Iodoacetamide) prior to digestion with Lys-C and trypsin. The resulting tryptic peptides from the tumour bearing mouse serum was labelled with ^{18}O whilst the normal mouse serum was digested in the presence of ^{16}O water. Equal amounts of serum from control and experimental states were then mixed.

Chromatographic separation

SCX fractionation of the ^{16}O : ^{18}O labelled mouse serum was performed on a 2.1 mm column flowing at 200 $\mu\text{l}/\text{min}$. Peptides were collected into 40x200 μl fractions. In the preliminary work presented here the five most intense fractions were analyzed, to establish a robust method.

SCX fractions were separated by reverse phase chromatography on a Waters nanoACQUITY™ system, under full Masslynx software control.

Column: Waters BEH™ C18 100mm x 15 μm , 1.7 μm particles

Mobile phase: A - 0.1% FA in water , B - MeCN

Gradient: 1 - 40 % B over 60mins

Flow rate: 1 $\mu\text{l}/\text{min}$; Injection volume: 2 μl

LC-MALDI fraction collection

The eluent from the HPLC was collected directly onto a 96 well MALDI target plate using the CTC-MALDI device

Collection time: 40secs/spot

Matrix: α -cyano-4-hydroxycinnamic acid (2mg/ml) added post-column at 1.0 $\mu\text{l}/\text{min}$ via the nanoACQUITY auxiliary solvent manager.

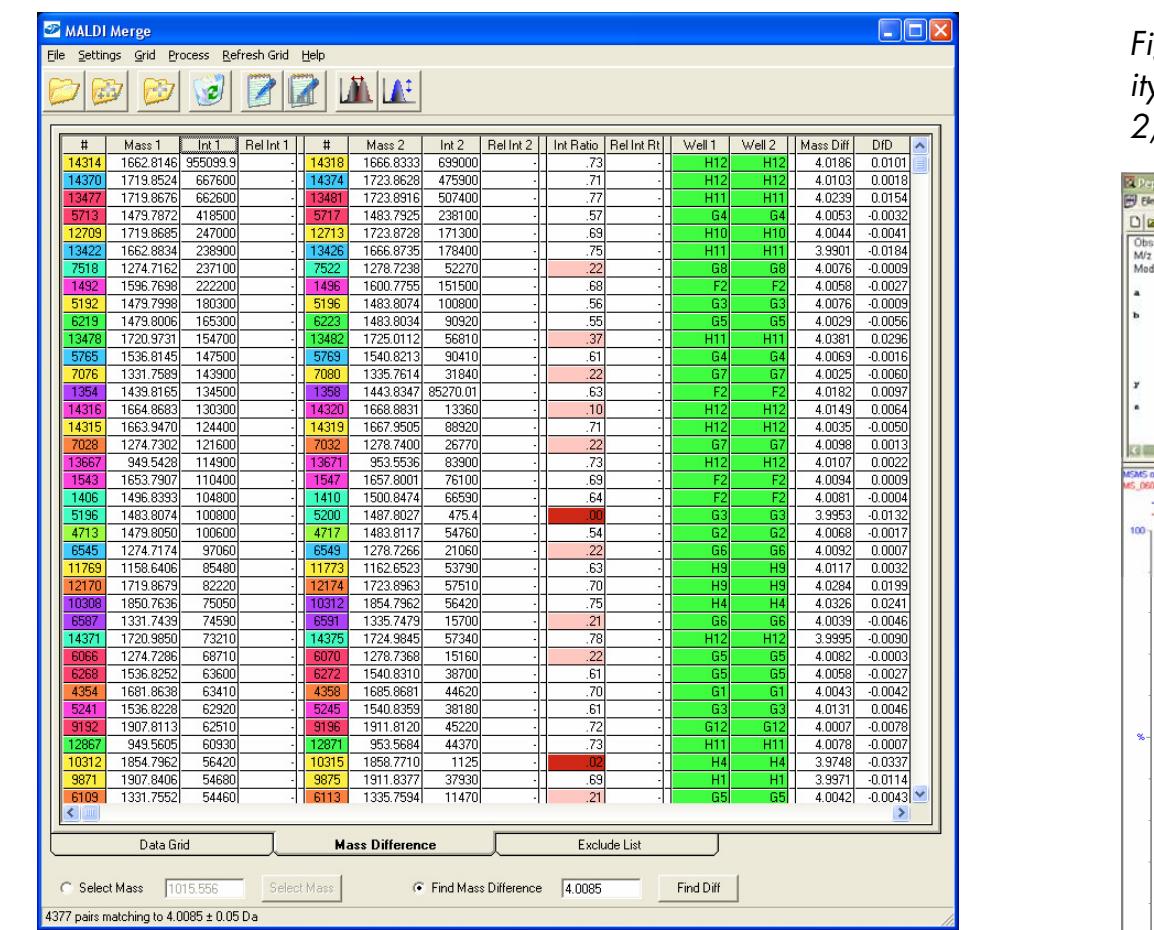


Figure 2. MALDImerge software used to identify ^{16}O : ^{18}O pairs, and to create include lists for MS/MS experiments.

Mass Spectrometry

MS analysis was performed on a Waters Q-ToF Premier MALDI mass spectrometer in positive ion mode. Each MALDI target position (40sec HPLC fraction) was analysed in MS mode over the m/z range 800 – 3,000. Following acquisition each spectrum was automatically de-isotoped and a list of monoisotopic mass and intensity pairs for each spot was imported into the Masslynx MALDI merge program to identify ^{16}O : ^{18}O doublets; (see Figure 2).

This was automatically performed by filtering for mass pairs differing by 4.0085Da within the data from each well. All ions above a user definable intensity, here 50 counts, were considered. The output from the MALDI merge program is a table of mass doublets with intensity, ^{16}O : ^{18}O ratio, and the MALDI well position (see Figure 2), as well as a separate list of un-paired masses. In the first experiments described here the un-paired peptides were selected for MS/MS analysis. The collision energy for each precursor mass was automatically selected from a collision energy profile. The quadrupole resolution was set to ensure that only one precursor ion was selected for fragmentation by CID, Figure 3.

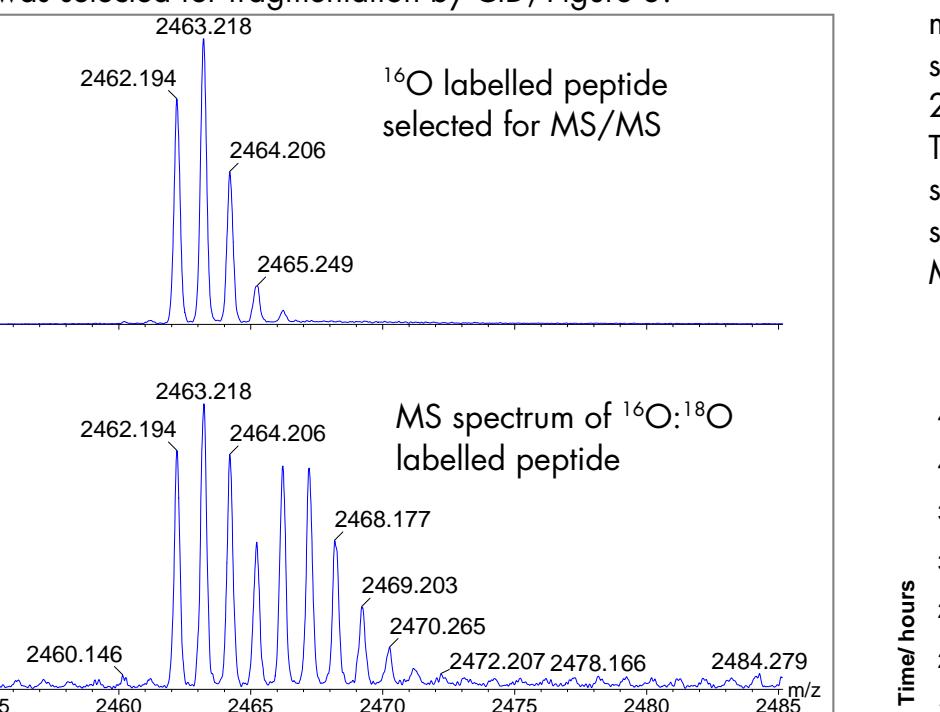


Figure 3. Illustration of the quadrupole resolution used, showing the ability to select only the ^{16}O peptide for CID from an ^{16}O : ^{18}O doublet at m/z 2,462.

