16O/18O Labelling Quantitative Analysis of Tryptically Digested Mouse Serum using LC MALDI MS and MS/MS

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OVERVIEW

- In this presentation we detail the quantitative analysis of Mouse serum by LC-MALDI MS
- Serum samples were obtained from tumour bearing and control mice, tryptically digested and then labelled with either ^{16}O or ^{18}O
- Labelled peptides were separated by off-line SCX fractionation and collected into X fractions
- Further fractionation was achieved via RP LC collecting onto 96 well MALDI target plates
- Each position was analysed first via MALDI MS to identify labelled peptide pairs via an automated computer algorithm
- Peptide pairs exhibiting a differential ^{16}O:^{18}O ratio were interrogated via MS/MS on the MALDI Q-Tof mass spectrometer to provide structural information to identified via databank searching

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 quantification 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.

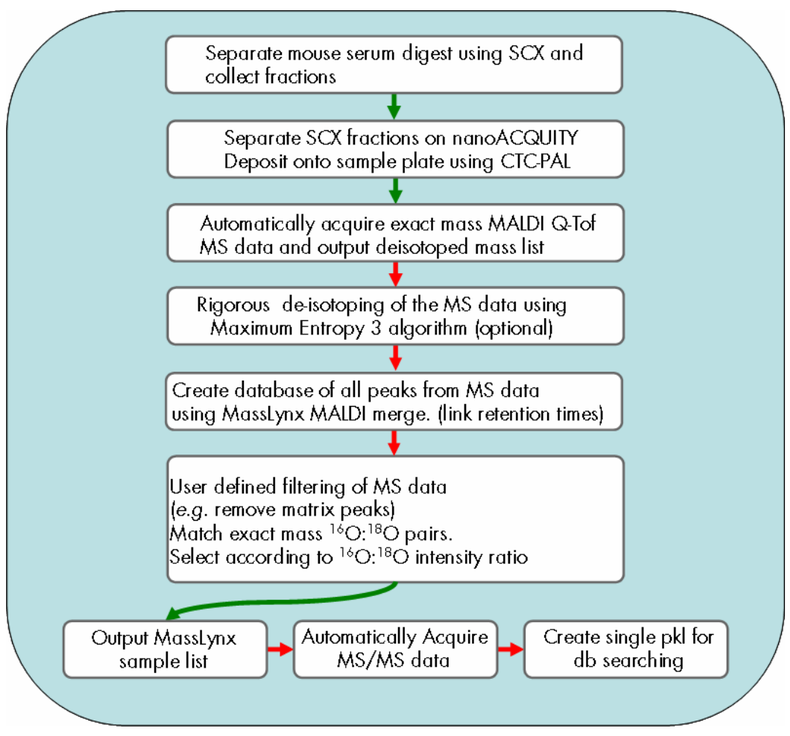

Figure 1. Flow diagram illustrating the analytical method used in this study.

METHODS

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, 200 mM Tris pH 8 and 38 mM NaCl. The sample was subsequently reduced with 10 mM TCEP and alkylated (50 mM Iodoacetamide) prior t o 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. SCX fractionation of the ^{16}O:^{18}O labelled Mouse serum was performed on a 2.1 mm column flowing at 200 µL/min. Peptides were collected into 40 x 200 µL fractions.

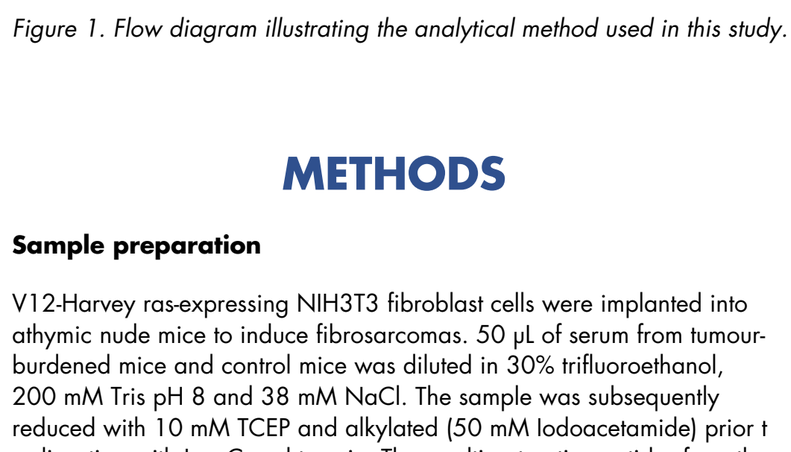

Figure 2. Base Peak Intensity plot from the reverse phase LC-MS analysis of SCX Fraction 17.

LC-MALDI

Samples were separated by reverse phase chromatography on a Waters® nanoACQUITY UPLC™ System, under full MassLynx software control.

Column:	Waters Atlantis® dC18 100 mm x 75 µm
Mobile phase:	A: 0.1% TFA in water , B: ACN
Gradient:	3–40 % B over 60 mins
Flow rate:	300 nL/min; Injection volume: 12 µL

LC-MALDI fraction collection
The eluent from the HPLC was collected directly onto a 96 well MALDI target plate using the CTC-MALDI fraction collector.

Collection time:	40 secs/spot
Matrix:	α-cyano-4-hydroxycinnamic acid (2 mg/mL) added post-column at 1.7 µL/min via nanoACQUITY™ UPLC™ auxiliary solvent manager
	A typical chromatogram obtained from the LC-separation is shown below for fraction 17

Mass Spectrometry

MS analysis was performed on a Waters MALDI Q-Tof™ Premier mass spectrometer in the positive ion mode. Each MALDI target position (40 sec HPLC fraction) was analyzed in MS mode on the 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™ (Waters Corp.) MALDI merge program to identify ^{16}O:^{18}O doublets; (see Figures 1 and 4).

RESULTS

From the first dimension SCX fractionation, five of the collected fractions were selected and analysed by LC-MALDI. For each HPLC separation 96 samples were analyzed by MALDI MS. A representative MALDI mass spectrum from fraction 17 is shown in Figure 3. Within the spectrum pairs with different ratios, presumably originating from different proteins, can clearly be seen. Interrogation of the peptides that exhibited a differential ratio allowed identification of the parent protein. The results are summarised in the corresponding Spotfire (Spotfire Inc.) plots (Figure 5 & 6) and the tabulated summary Figure 7.

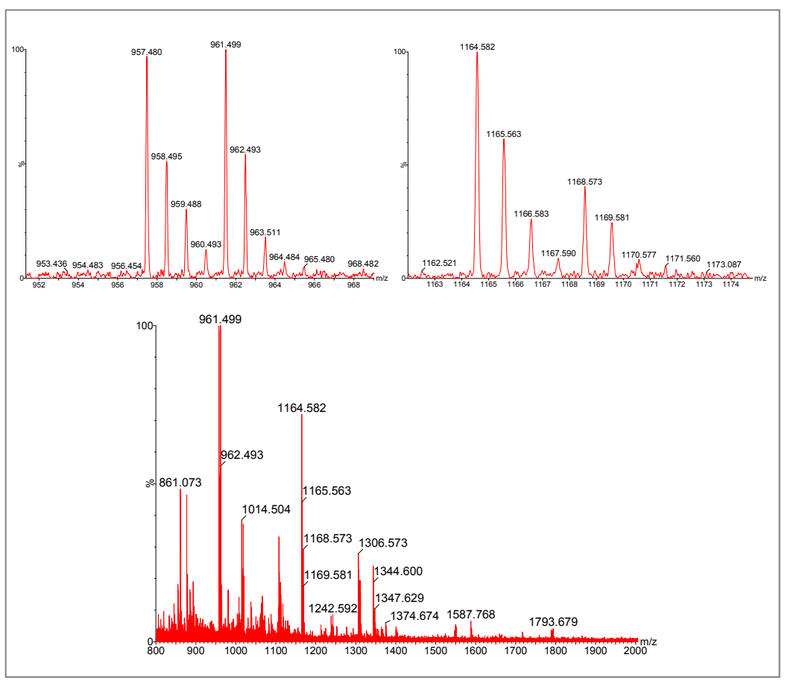

Figure 3. MALDI mass spectrum obtained from one 30 second HPLC fraction from SCX fraction 17, (bottom spectrum). The top spectra show expanded m/z regions around isotope doublets with differing ^{16}O:^{18}O ratios.

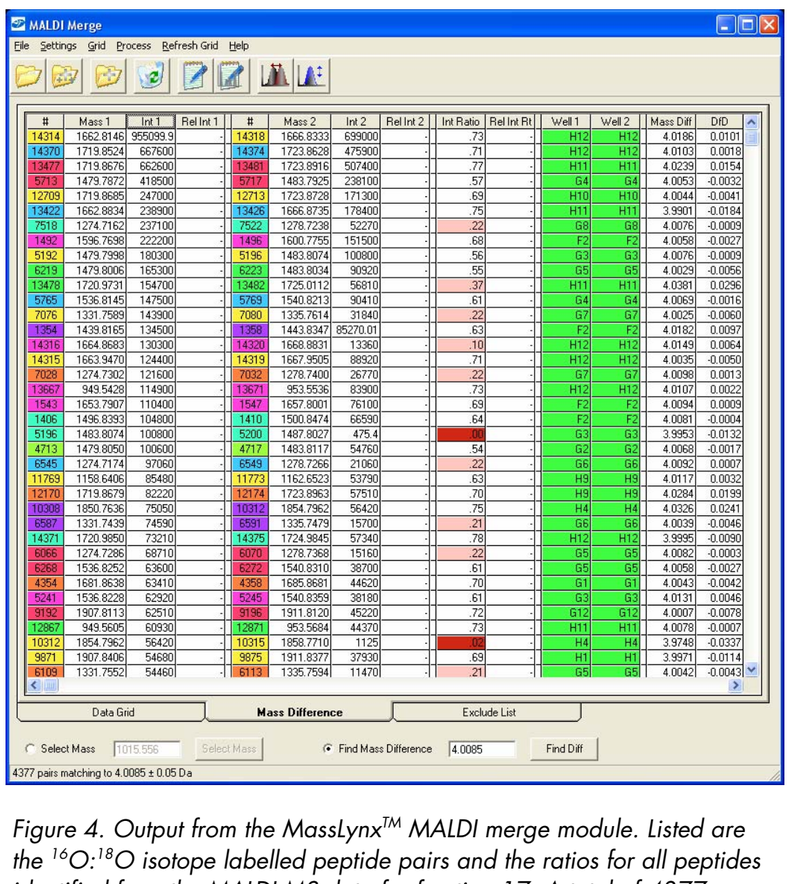

Figure 4. Output from the MassLynx™ MALDI merge module. Listed are the ^{16}O:^{18}O isotope labelled peptide pairs and the ratios for all peptides identified from the MALDI MS data for fraction 17. A total of 4377 peptide pairs were identified from this HPLC fraction.

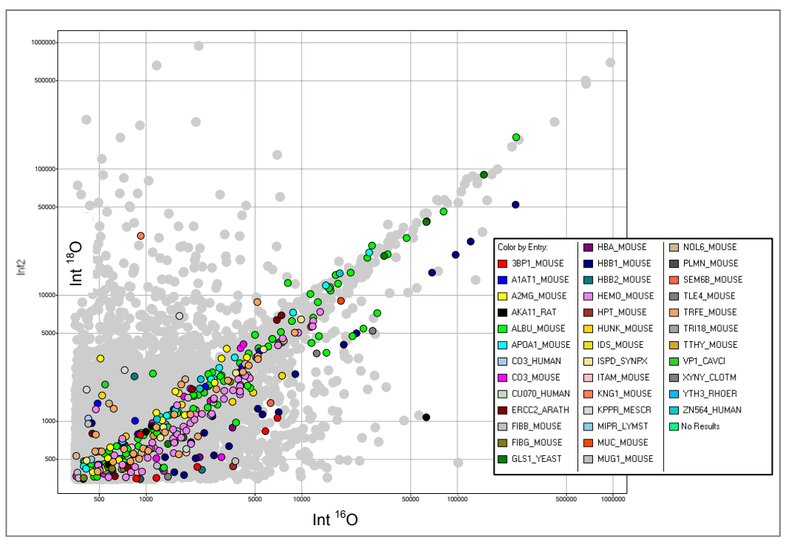

Figure 5. Spotfire plot of the ^{16}O peptide intensity vs the ^{18}O peptide intensity for all peptide pairs determined from the MS data. Highlighted in colour are those peptides identified as matching to a protein sequence from the MS/MS data.

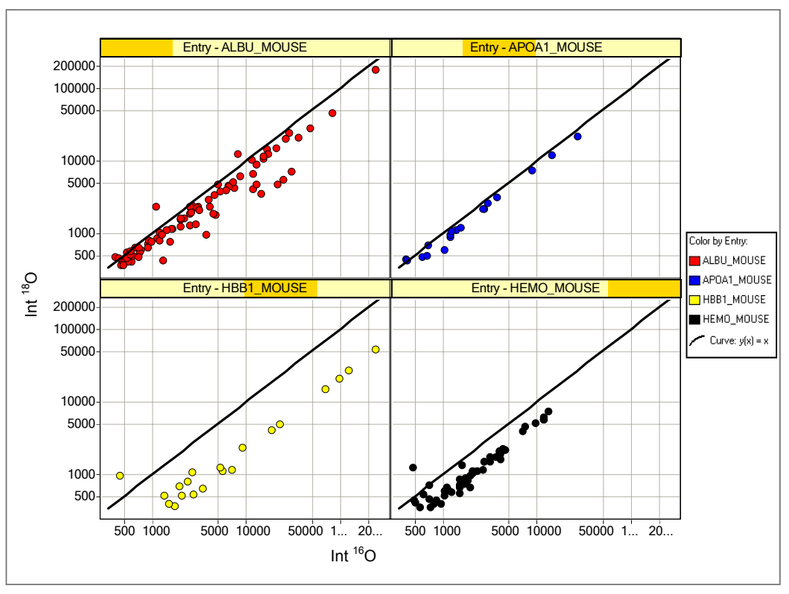

Figure 6. Spotfire trellis plots for selected proteins identified from the Mouse serum. Each plot displays the ^{16}O peptide intensity vs the ^{18}O peptide intensity from the MALDI MS data. The upper panes clearly show proteins not differentially expressed between the control and tumour bearing mice, whilst the lower two plots show proteins up regulated in the tumour bearing mice.

Protein ID	Accession	Description	Mass	Score	Peptides matched	$^{18}O/^{16}O$ ratio	Std dev.
ALBU_MOUSE	(P07724)	Serum albumin precursor	70700	767	35	0.74	0.29
HEMO_MOUSE	(Q91X72)	Hemopexin precursor	52049	260	16	0.60	0.36
TRFE_MOUSE	(Q921I1)	Serotransferrin precursor (Transferrin) (Siderophilin) (Beta-1-metal binding globulin)	78841	232	10	0.87	0.45
A1AT1_MOUSE	(P07758)	Alpha-1-antitrypsin 38718 precursor (Serine protease inhibitor 1-1) (Alpha-1 protease inhibi	46145	224	13	0.84	0.18
A2MG_MOUSE	(Q61838)	Alpha-2-macroglobulin precursor (Alpha-2-M) [Contains: Alpha-2-macroglobulin 165 kDa subun	167091	202	9	0.91	0.17
FIBB_MOUSE	(Q8K0E8)	Fibrinogen beta chain precursor [Contains: Fibrinopeptide B]	55402	160	11	^{16}O only	-
APOA1_MOUSE	(Q00623)	Apolipoprotein A-I precursor (Apo-AI) (ApoA-I)	30569	155	8	0.85	0.12
FIBG_MOUSE	(Q8VCM7)	Fibrinogen gamma chain precursor	50044	112	8	0.70	0.15
HBB1_MOUSE	(P02088)	Hemoglobin beta-1 subunit (Hemoglobin beta-1 chain) (Beta-1-globin) (Hemoglobin beta-major	15813	94	9	0.24	0.06
HPT_MOUSE	(Q61646)	Haptoglobin precursor [Contains: Haptoglobin alpha chain; Haptoglobin beta chain]	39241	94	4	^{16}O only	-
KNG1_MOUSE	(O08677)	Kininogen-1 precursor [Contains: Kininogen-1 heavy chain; Bradykinin; Kininogen-1 light ch	74140	89	2	1.30	0.05
CO3_MOUSE	(P01027)	Complement C3 precursor (HSE-MSF) [Contains: Complement C3 beta chain; Complement C3	187904	69	6	0.75	0.29
TTHY_MOUSE	(P07309)	Transthyretin precursor (Prealbumin)	15880	47	2	0.64	0.07
HBB2_MOUSE	(P02089)	Hemoglobin beta-2 subunit (Hemoglobin beta-2 chain) (Beta-2-globin) (Hemoglobin beta-minor	15851	45	6	1.07	1.41
FIBA_RAT	(P06399)	Fibrinogen alpha chain precursor [Contains: Fibrinopeptide A]	87373	34	3	^{16}O only	-
NOL6_MOUSE	(Q8R5K4)	Nucleolar protein 6 (Nucleolar RNA-associated protein) (Nrap)	128878	34	2	0.85	0.29
MUG1_MOUSE	(P28665)	Murinoglobulin-1 precursor (MuG1)	166460	33	3	0.64	0.27
PLMN_RAT	(Q01177)	Plasminogen precursor (EC 3.4.21.7) [Contains: Plasmin heavy chain A; Activation peptide;	93214	32	3	0.38	-
MUCM_MOUSE	(P01873)	Ig mu chain C region membrane-bound form	53121	30	2	0.70	0.24

Figure 7. Identified proteins quantified from their $^{16}O/^{18}O$ isotope labels, showing score, number of matched peptides, expression ratio and standard deviation (where appropriate).

CONCLUSION

- Here we present the use of an ^{18}O isotope labeling strategy in combination with LC-MALDI analysis for the quantitative analysis of Mouse serum, from control and tumor bearing mice.
- A new processing algorithm allows the interrogation of the LC-MALDI MS data to identify isotope labeled doublets and rank them by their intensity ratio, in this case $^{16}O/^{18}O$. This information can be used to supply precursor ion information for the MALDI Q-Tof mass spectrometer and in addition this can be exported for further statistical treatment or display.
- The analysis of serum from tumor-bearing mice showed changes in regulation of numerous previously well-characterized serum proteins.
- Further work will focus on the analysis of all fractions obtained from the SCX fractionation and comparison to data obtained from electrospray LC-MS experiments.

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