



ASMS Mascot User Meeting 30th May 2

Quantification by ^{15}N Metabolic Labelling: From Sample to Graph

Cambridge Centre for Proteomics
Department of Biochemistry
University of Cambridge

Matthew Russell

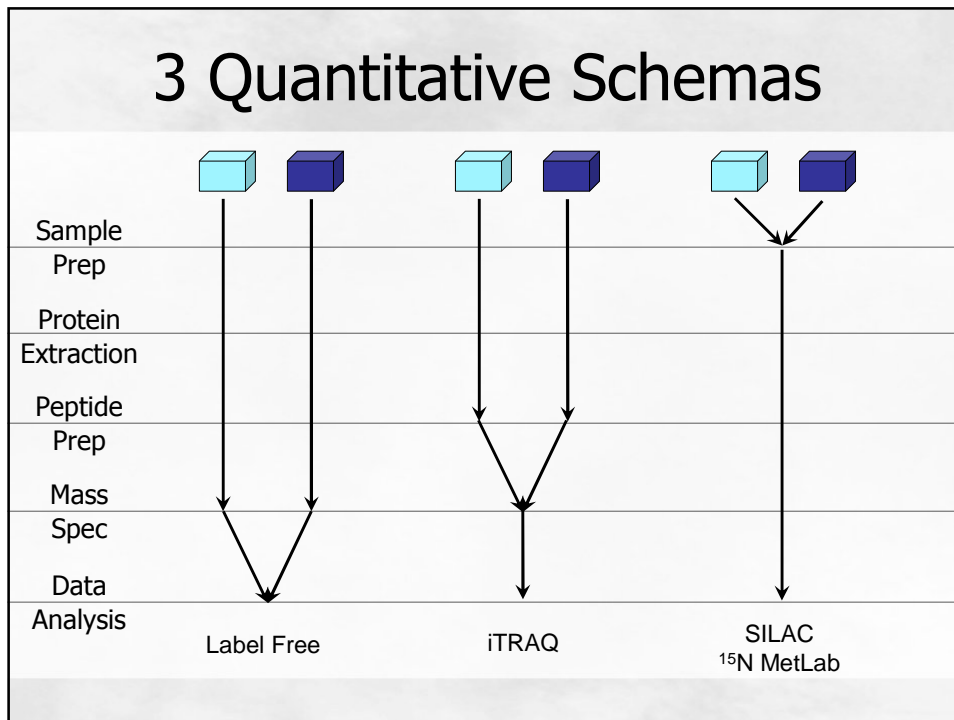
mr418@cam.ac.uk

www.bio.cam.ac.uk/proteomics

Outline

- Introduction
- Workflows
- Tips and Tricks
- Using Distiller
- Handling the Data

3 Quantitative Schemas



Why ¹⁵N Labelling

Amino acid - SILAC

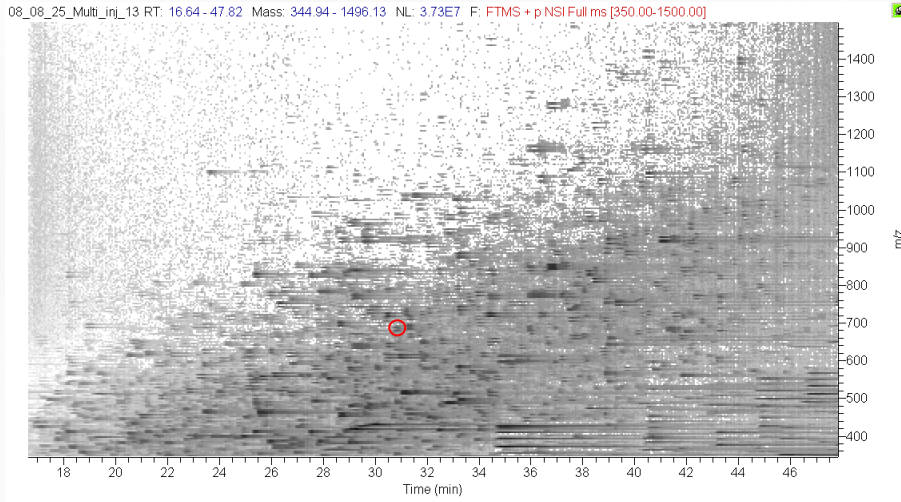
- Replacement of an amino acid with isotopologue.
- Typically ¹³C instead of ¹²C labelled K R or L.
- Known mass difference.
- Need to check for extent of incorporation.
- ✘ Need auxotrophic organisms
- ✘ Conversion of arginine to proline in eukaryotes.
- ✘ Plants difficult to fully label.

Elemental

- Replacement of an element with a heavy isotope in media.
- Typically ¹⁵N instead of ¹⁴N, or ¹³C instead of ¹²C
- Mass difference between light and heavy pairs unknown until sequence is deduced.
- Need to check for extent of incorporation
- ✓ Suitable for plants

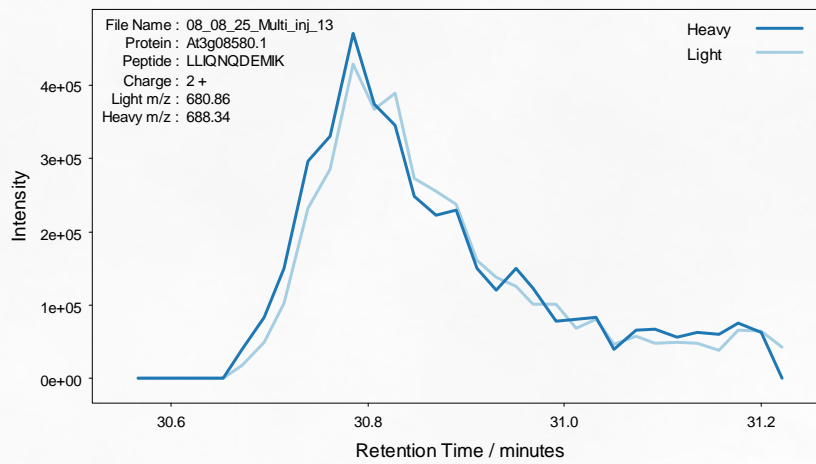
Ole N. Jensen, *Molecular and Cellular Proteomics* 2005, 4, 1697-1709

Raw Data



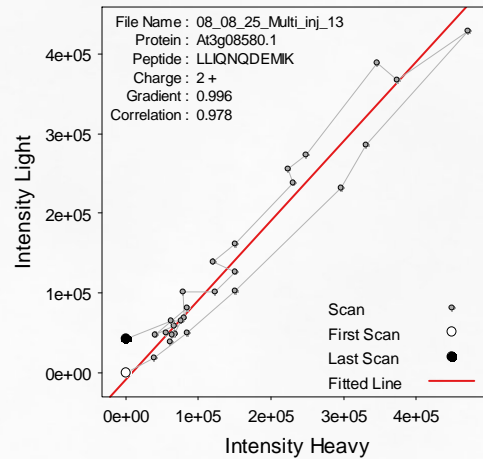
XIC

Extracted Ion Chromatogram



Quantification

Quantification Plot



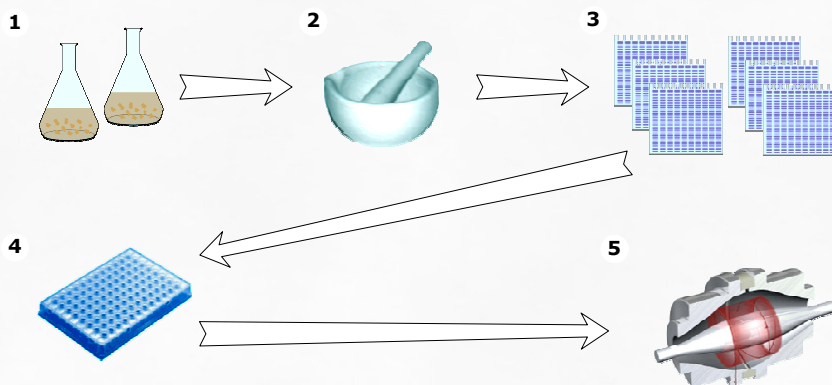
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Biological System

- *Arabidopsis* root derived callus
 - Grown in liquid culture
 - Fairly homogeneous
 - Loosely aggregated cells
 - Mostly free of chloroplasts (RUBISCO)

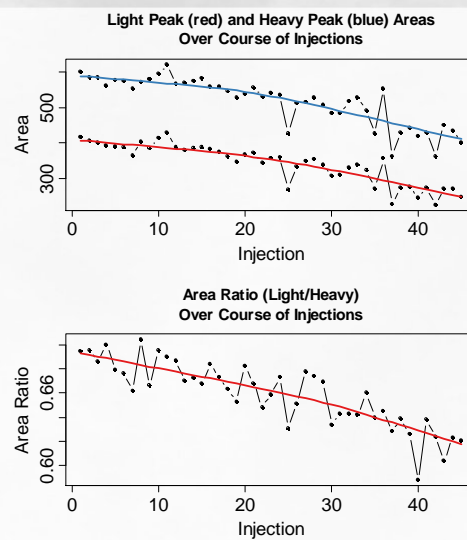
Workflow



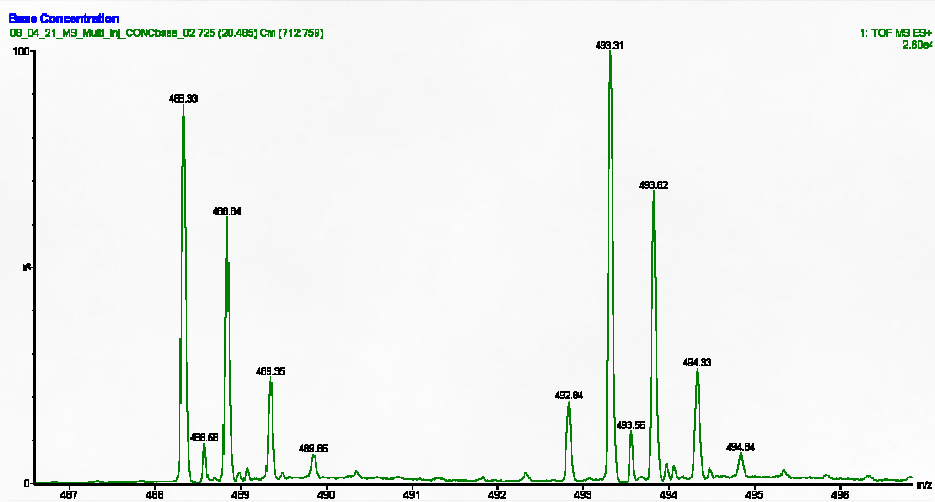
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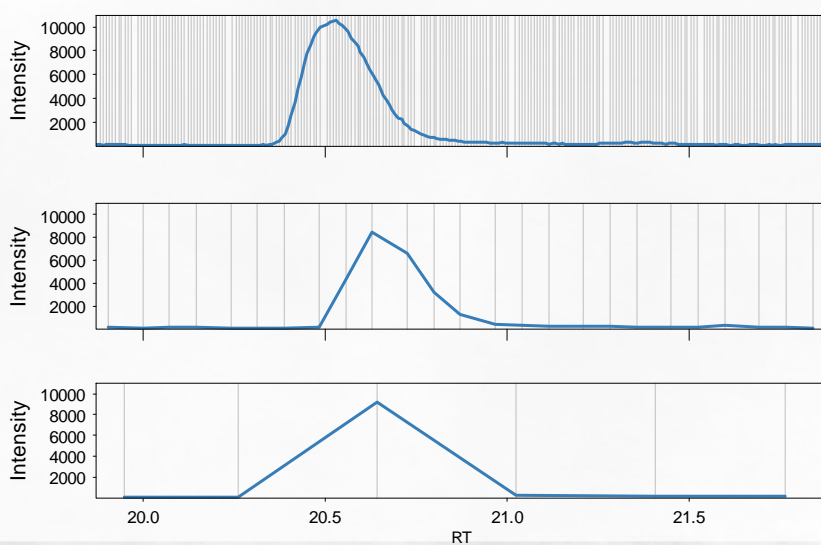
The Auto-sampler



Temporal Resolution 1.



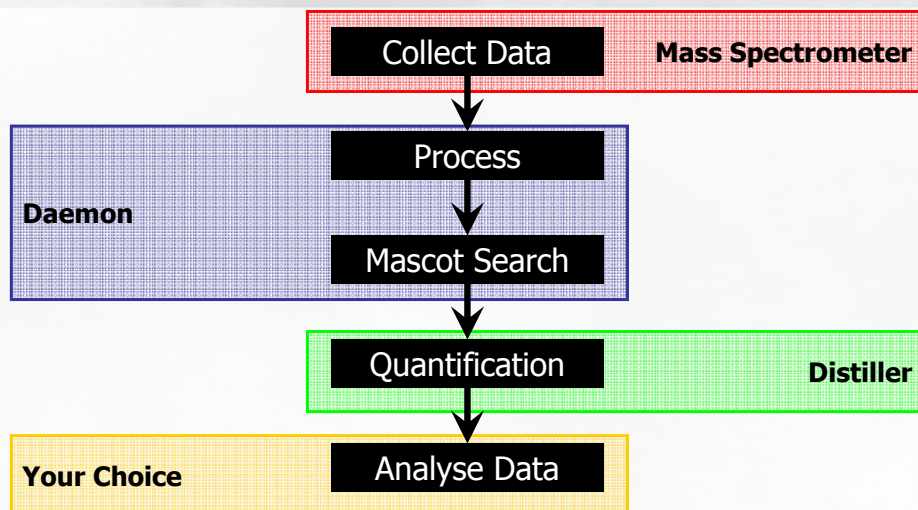
Temporal Resolution 2.



Outline

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Flowchart



Get to Config

(MATRIX) SCIENCE HOME | MASCOT:HELP Search

Welcome

This site features **Mascot**, a powerful search engine that uses Mascot forms a substantial knowledge base concerning protein id

If this is your first visit, please check for [browser compatibility](#) and or Electrophoresis, **20(18)** 3551-67 (1999) (abstract).

Security

Mascot incorporates a role based security model, which allows that you may be prompted to log in if you attempt to access a page or simply confirm this.

- [Log in](#)
- [Log out](#)
- [Change password](#)
- [Edit settings](#)
- [Current session information](#)

Whether or not Mascot security is enabled, in order to comply with the licensing conditions, access to this Mascot server must be restricted to authorised users. We strongly recommend that your server is protected by a secure firewall. For more information, start from the [WWW Security FAQ](#).

Mascot Utilities

Your system administrator may have restricted access to these utilities

[Database Status](#)
View the status of all the sequence databases. Links to database statistics, search status, log files, etc.

[Search Log](#)
View the status of the search log. Can be filtered to find specific search results.

[Configuration Editors](#)
View the configuration editors, including security administration and sequence database management.

Mascot Configuration

Elements	Element masses
Amino Acids	Amino Acid Data
Modifications	Modification definitions
Symbols	Symbols used in chemical formulae
Enzymes	Enzyme definitions
Instruments	Fragmentation Rules
Quantitation	Quantitation Methods
Database Maintenance	Sequence databases, Parse Rules and Options
Security	Security Administration Pages

Open Method

Mascot Configuration: Quantitation Methods

Name	Protocol			
None	null			
ITRAQ 4plex	reporter	Copy	Delete	Print
ITRAQ 8plex	reporter	Copy	Delete	Print
18O corrected multiplex	multiplex	Copy	Delete	Print
SILAC K+6 R+6 multiplex	multiplex	Copy	Delete	Print
TMT 6plex	reporter	Copy	Delete	Print
ICAT ABI Cleavable [MD]	precursor	Copy	Delete	Print
ICPL duplex pre-digest [MD]	precursor	Copy	Delete	Print
ICPL duplex post-digest [MD]	precursor	Copy	Delete	Print
SILAC K+6 R+10 [MD]	precursor	Copy	Delete	Print
18O corrected [MD]	precursor	Copy	Delete	Print
15N Metabolic [MD]	precursor	Copy	Delete	Print
Matt1 of 15N Metabolic [MD]	precursor	Copy	Delete	Print
Matt2 of 15N Metabolic [MD]	precursor	Copy	Delete	Print
Matt3 of 15N Metabolic [MD]	precursor	Copy	Delete	Print
Matt Multi_inj 15N	precursor	Copy	Delete	Print
15N Metabolic Matt4 [MD]	precursor	Copy	Delete	Print
15N Metabolic Matt5 [MD]	precursor	Copy	Delete	Print
ITRAQ 4plex Matt	reporter	Copy	Delete	Print
ITRAQ 4plex Matt2	reporter	Copy	Delete	Print
ITRAQ 4plex Arnoud	reporter	Copy	Delete	Print
12Nov08 15N	precursor	Copy	Delete	Print
12Nov08 15N-No Elute	precursor	Copy	Delete	Print
15N No-Elute 15Nmp	precursor	Copy	Delete	Print
[Phil] 15N Universal Label	precursor	Copy	Delete	Print

[New quantitation method](#) [Main menu](#)

Setting up the Method

Edit Quantitation Method:Matt's 15N Metabolic [MD]

Name Description

Method Protocol Component Report Ratio Integration Quality Outliers Normalisation

Method

Property	Value	Action
Constrain Search	<input type="checkbox"/>	
Protein Ratio Type	weighted	
Protein score Type	mudpit	<input type="button" value="Clear"/>
Report Detail	<input checked="" type="checkbox"/>	
Show subsets	0.5	<input type="button" value="Clear"/>
Require bold red	<input type="checkbox"/>	<input type="button" value="Clear"/>
Minimum Peptides	2	
Significance Threshold	0.05	<input type="button" value="Clear"/>
comp qualifier		<input type="button" value="Add Composition"/>
seq qualifier		<input type="button" value="Add Sequence"/>
Modification groups		<input type="button" value="Add Modification Group"/>

Help Window

Some Key Settings

Edit Quantitation Method:15N No-Elute 15Nimp

Name Description

Method Protocol Component Report Ratio Integration Quality Outliers Normalisation

Protocol

Property	Value
Protocol	precursor
Allow mass time match	<input checked="" type="checkbox"/>
Allow elution shift	<input type="checkbox"/>
All charge states	<input checked="" type="checkbox"/>

True to extends the mass and time matching to cover contiguous charge states

Incorporation Rate

Edit Quantitation Method: 15N No-Elute 15Nimp

Name: 15N No-Elute 15Nimp Description: 15N metabolic labelling

Method Protocol **Component** Report Ratio Integration Quality Outliers Normalisation

Component

Components: heavy New Copy Delete

Property	Value	Action
Component	heavy	

Modification groups Add Modification Group

Isotopes Old: N New: 15N Delete Add isotope

Corrections	Type	Shift	Action
	impurity		
	Element: N	1.1	Delete
	Type: impurity		
	Element: 15N	98.9	Delete Add correction

Save changes Cancel

Help Window

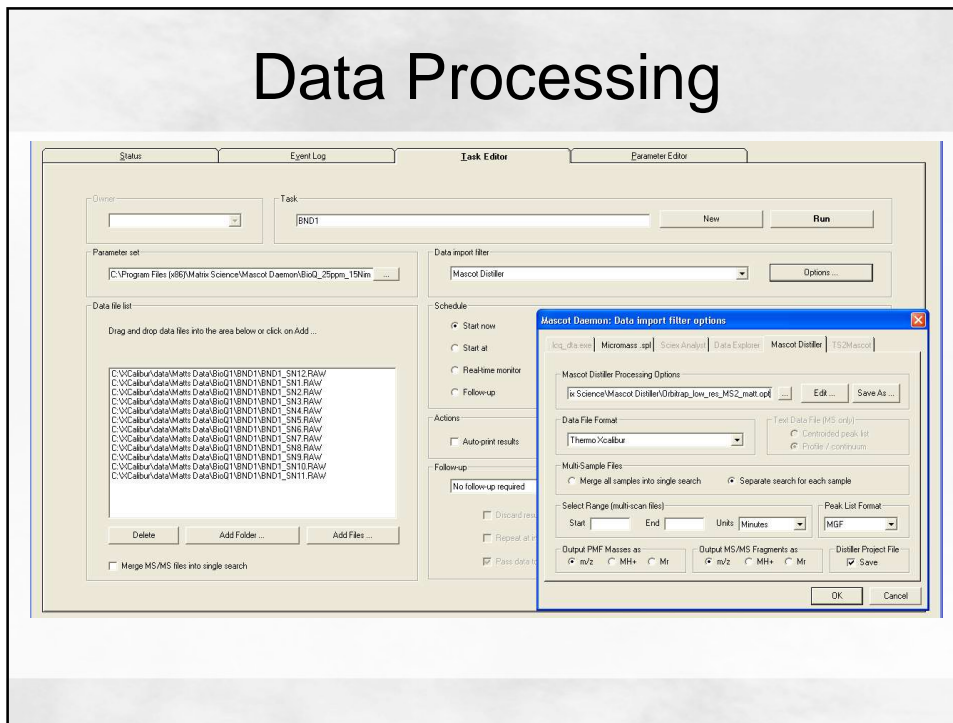
The Bug

```
547 <<method constrain_search="false"
548 <<<component name="light"/>
549 <<<component name="heavy">
550 <<<<isotope>
551 <<<<<old>N</old>
552 <<<<<new>15N</new>
553 <<<<</isotope>

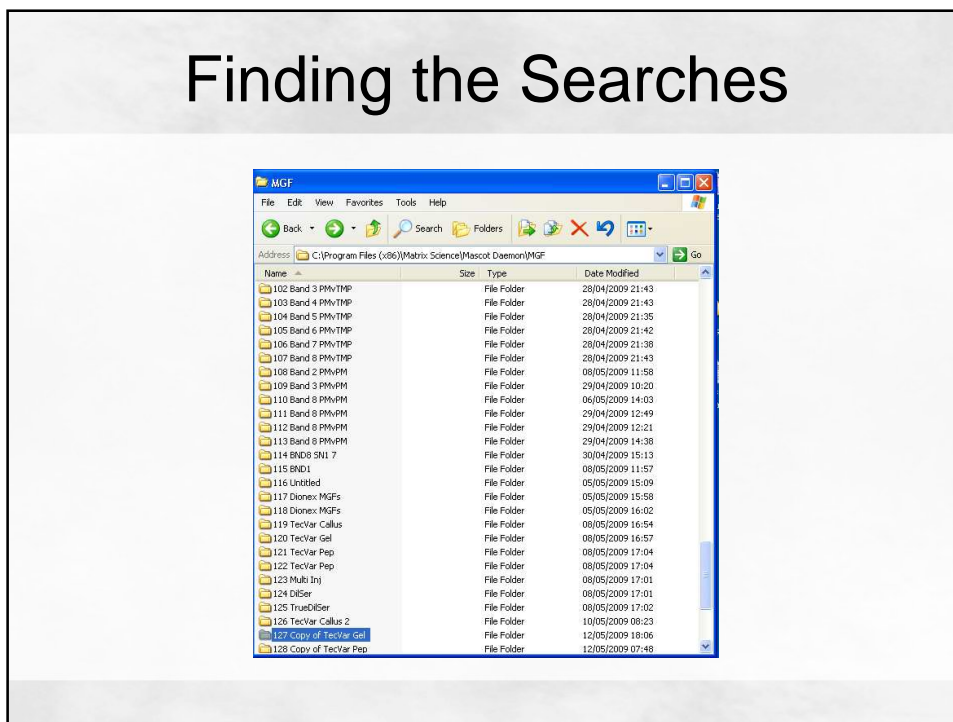
816 <<method constrain_search="false"
817 <<<component name="light">
818 <<<<isotope/>
819 <<<</component>
820 <<<component name="heavy">
821 <<<<isotope>
822 <<<<<old>N</old>
823 <<<<<new>15N</new>
824 <<<<</isotope>
```

“config” folder on mascot server.
quantitation.xml

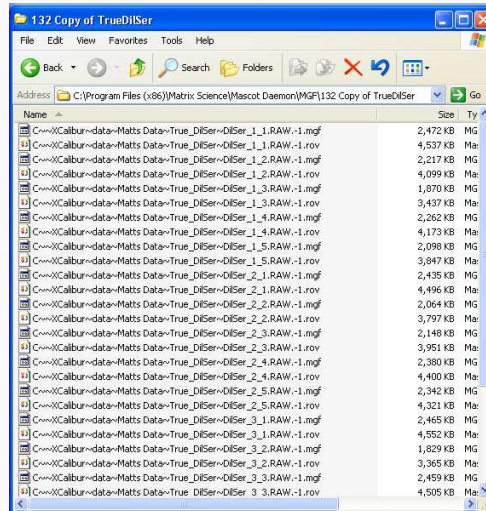
Data Processing



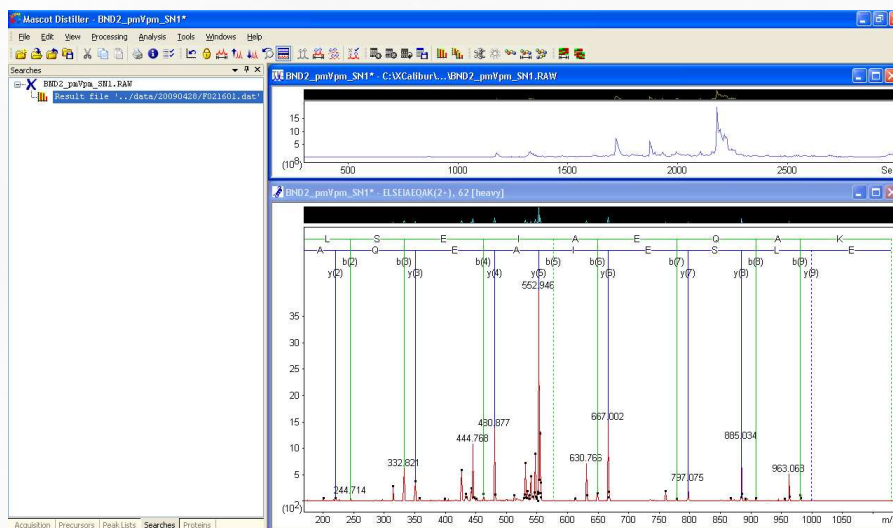
Finding the Searches



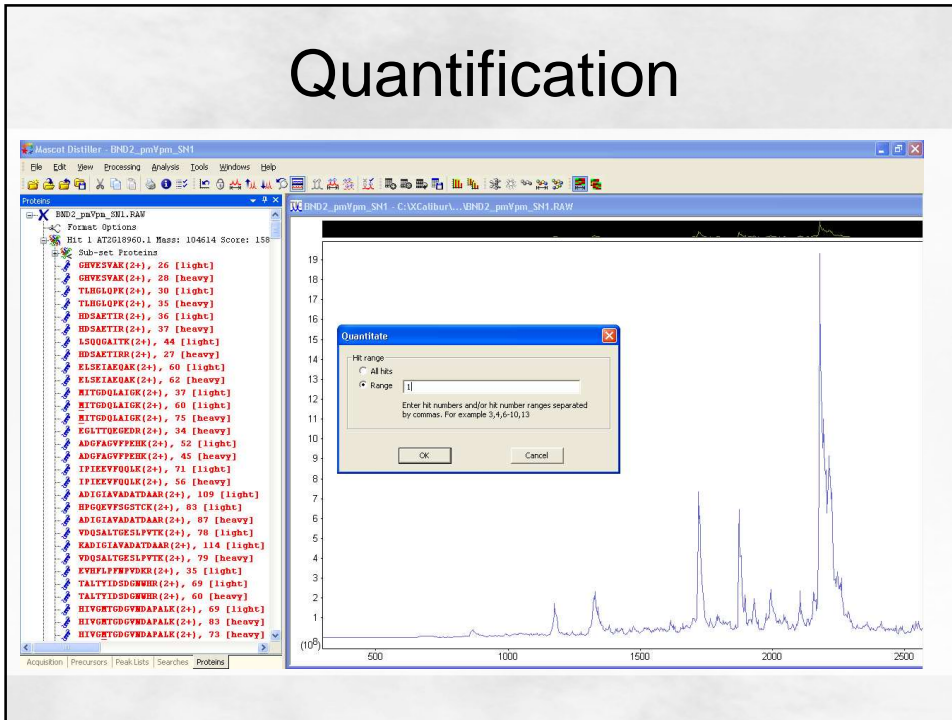
.ROV files



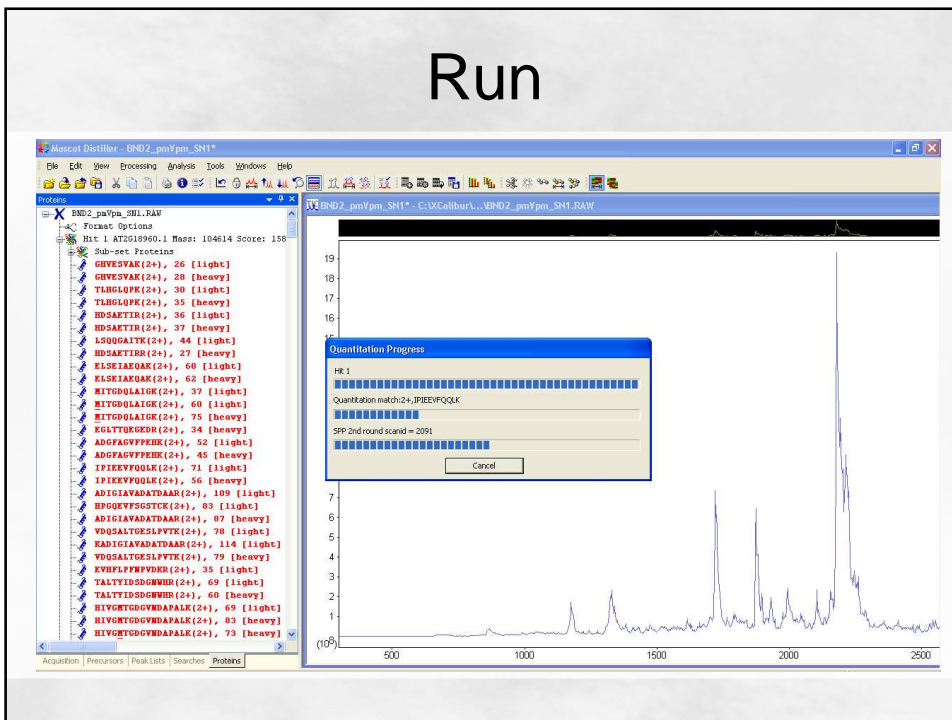
Access Search



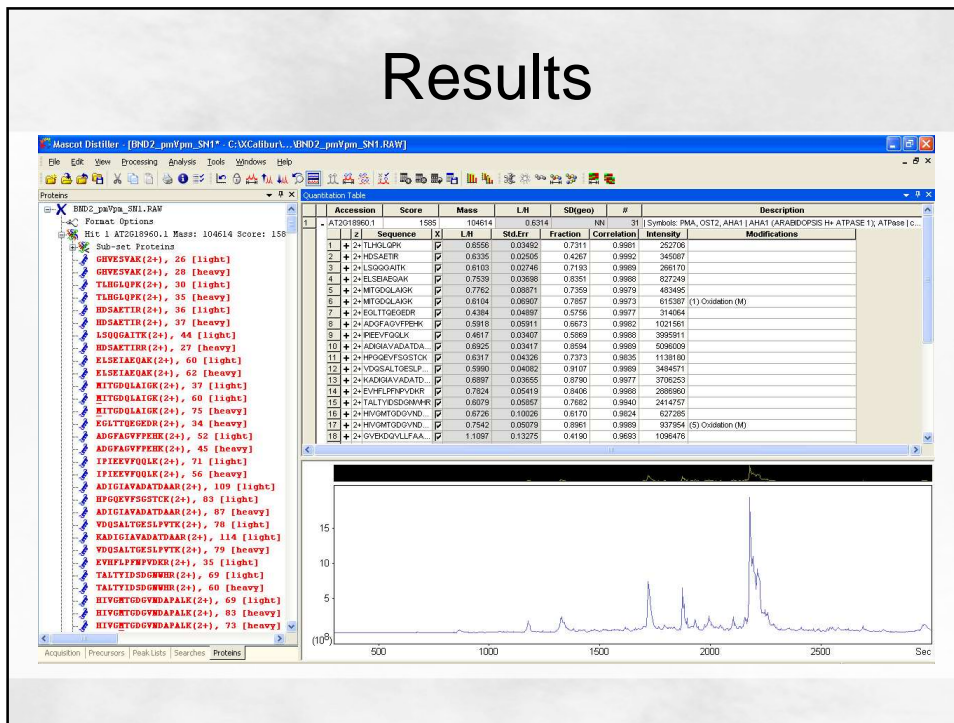
Quantification



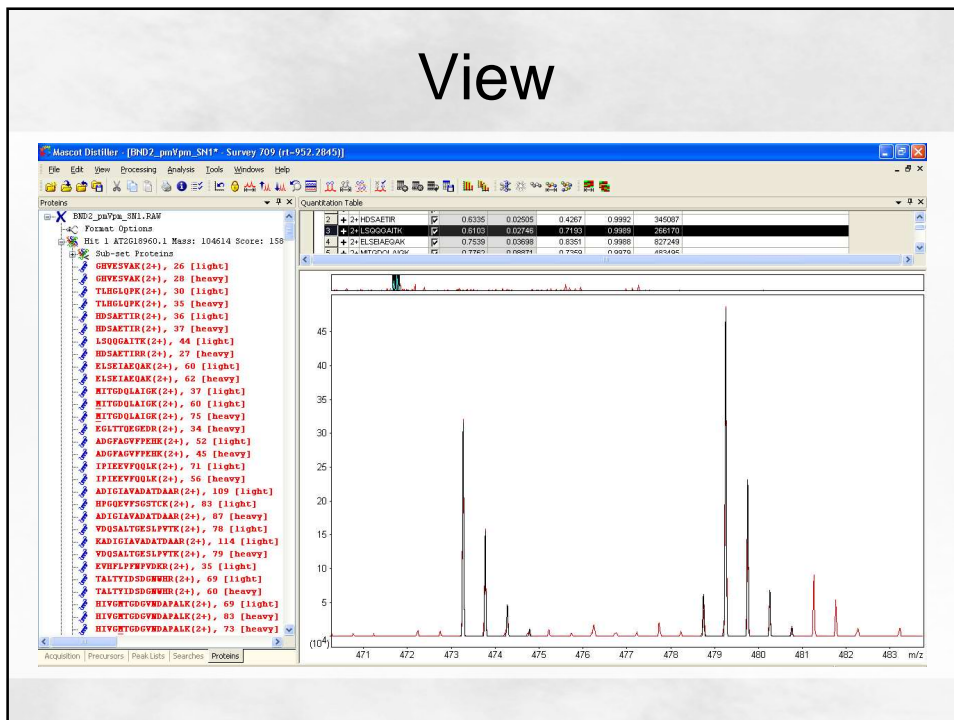
Run



Results



View



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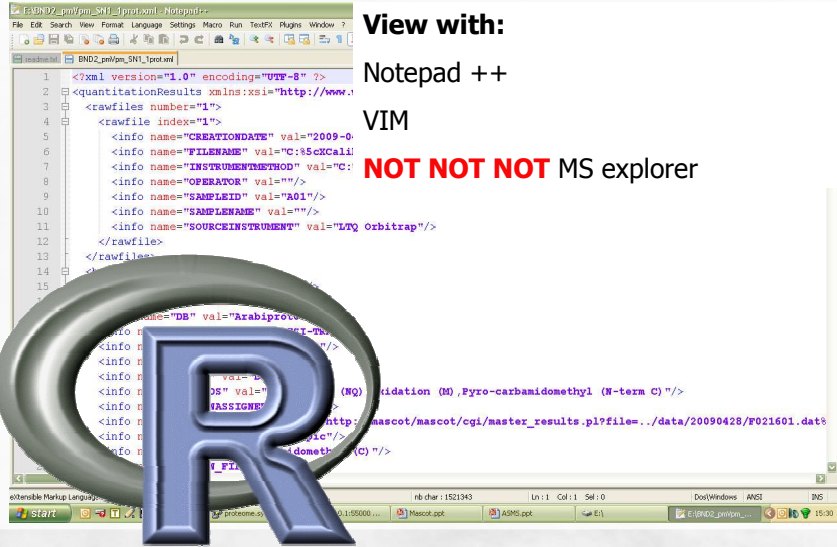
Get XML

The screenshot displays the Mascot Distiller software interface. The main window shows a list of peptides with columns for Accession, Score, Mass, L.M, S.Digmo, #, and Description. A red circle highlights the 'Save As XML...' button in the 'Quantitation Reports' section. Below the list, a mass spectrum plot is visible, showing relative intensity versus m/z. The plot is titled 'BND2_ppm_SNH1* - ELSEIATQAK(2+), 62 [heavy]'. The x-axis ranges from 500 to 2500 m/z, and the y-axis ranges from 0 to 15 (10⁵). The plot shows several peaks, with the most prominent one at approximately 1000 m/z. Below the mass spectrum, a protein sequence diagram is shown, with the peptide sequence ELSEIATQAK(2+) highlighted in red. The sequence is: L S E I A T Q A K. The peptide is shown as a fragment of the protein sequence, with the start and end positions indicated by brackets.

z	Sequence	X	LN	Std.Err	Fraction	Correlation	Intensity	Modifications
1	+2+ TLKGLQPK	0.6556	0.03492	0.7311	0.9861	252706		
2	+2+ HSGHETK	0.6335	0.02926	0.4207	0.9952	349397		
3	+2+ LSGDGLATK	0.6103	0.02746	0.7193	0.9989	266170		
4	+2+ ELSEIATQAK	0.7538	0.03689	0.9351	0.9998	827249		
5	+2+ MITDGLQALQK	0.7162	0.08071	0.7359	0.9979	483465		
6	+2+ MITDGLQALQK	0.6104	0.06907	0.7957	0.9973	615387 (1) Oxidation (M)		
7	peptides	ER	0.4384	0.04887	0.5756	0.9977	314064	
8	proteins	FEHK	0.5916	0.05911	0.6673	0.9962	1021561	

XML files

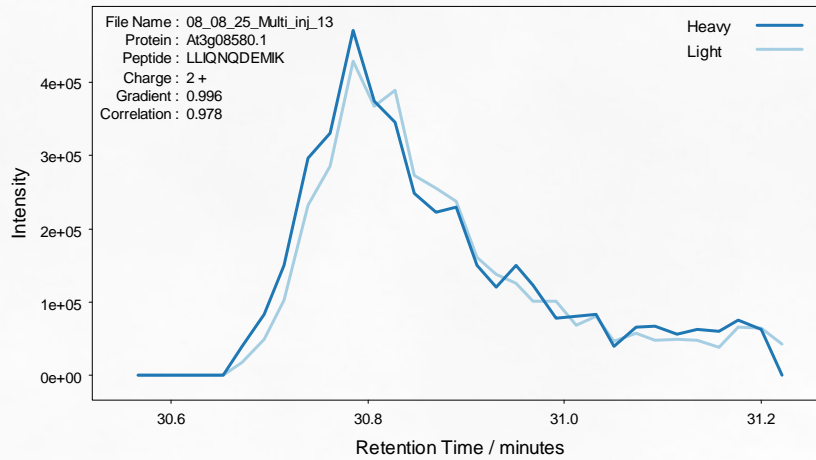
View with:
Notepad ++
VIM
NOT NOT NOT MS explorer



The screenshot shows a Notepad++ window displaying XML data. The XML content includes fields such as CREATIONDATE, FILENAME, INSTRUMENTMETHOD, OPERATOR, SAMPLEID, SAMPLENAME, and SOURCEINSTRUMENT. A large, stylized 'OR' watermark is overlaid on the text. The status bar at the bottom indicates the file path and encoding.

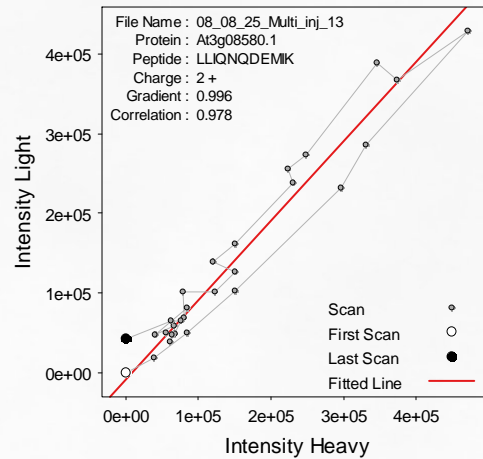
XIC

Extracted Ion Chromatogram



Quantification

Quantification Plot



Conclusion

- Great technique and software but beware:
 - The Auto-Sampler
 - Peak Temporal Resolution
 - Peak Symmetry
- To get the best out of the data learn R
 - Sorry!

NB: After the talk someone mentioned other analysis software which would work as well.

Acknowledgements

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