

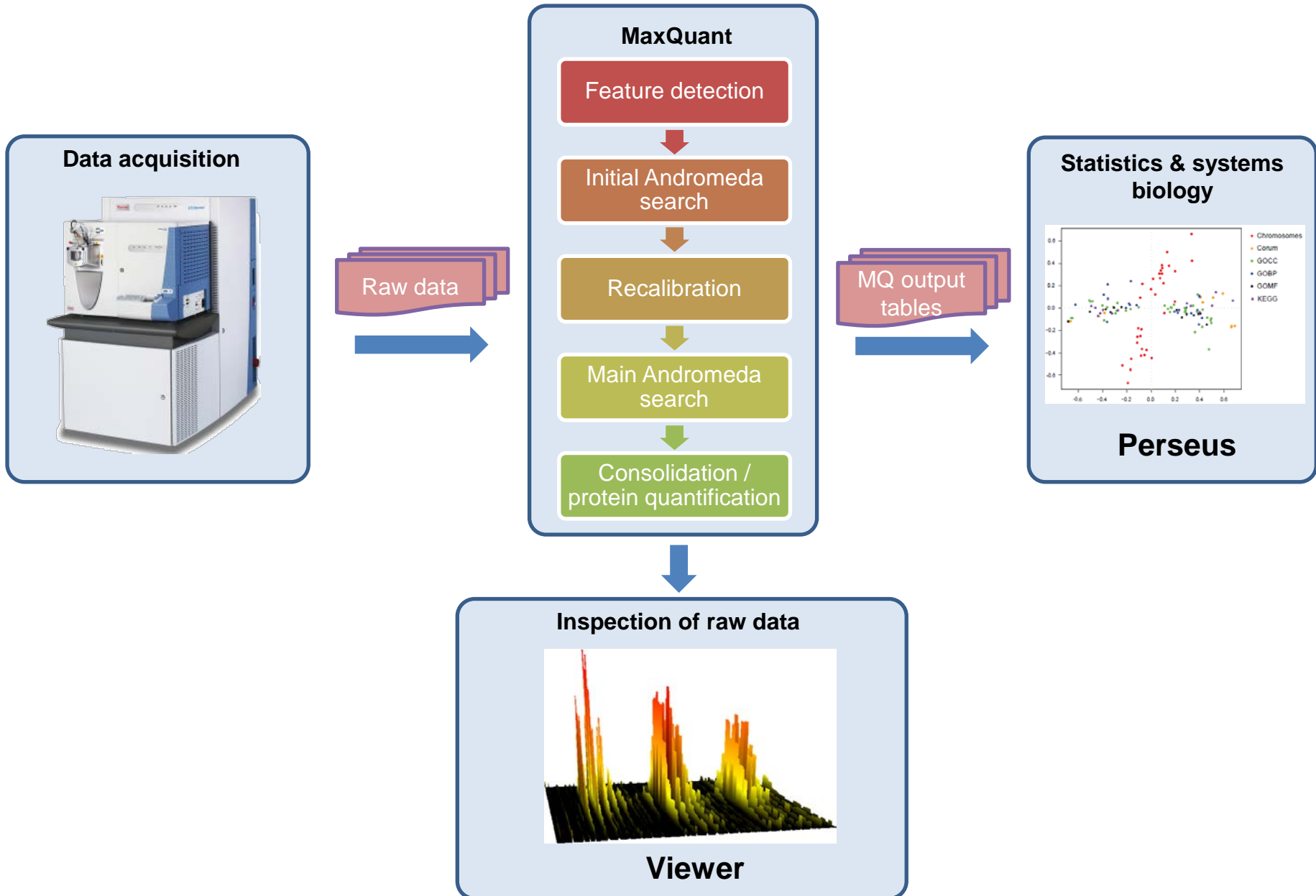


Proteome-wide label-free quantification with MaxQuant

Jürgen Cox
Max Planck Institute of Biochemistry
July 2011



MaxQuant



Supported input data

Labeling methods

- SILAC
- Label free
- Di-methyl
- ^{18}O
- ICAT
- ICPL

Work in progress:

- iTRAQ

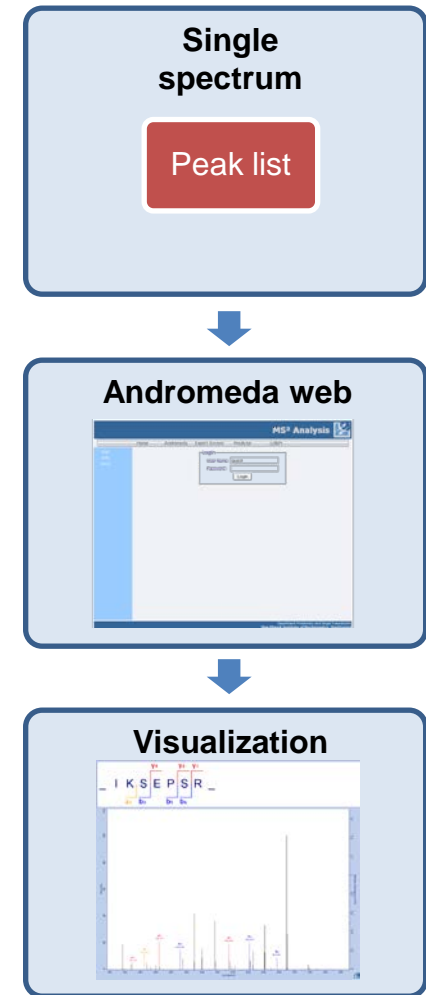
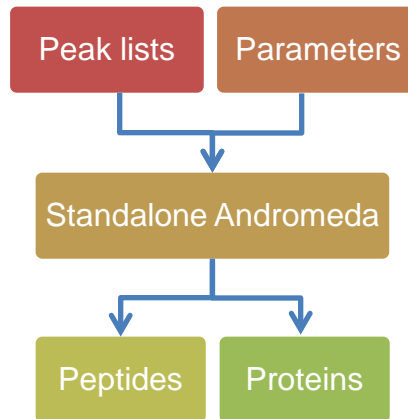
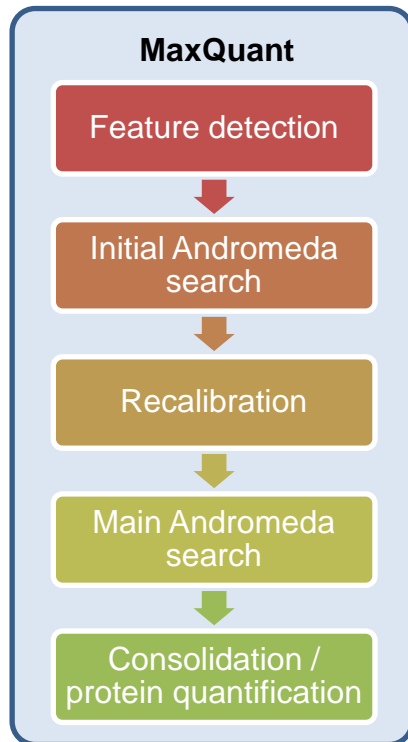
Mass spectrometers

- Thermo Fisher Orbitrap and FT

Work in progress:

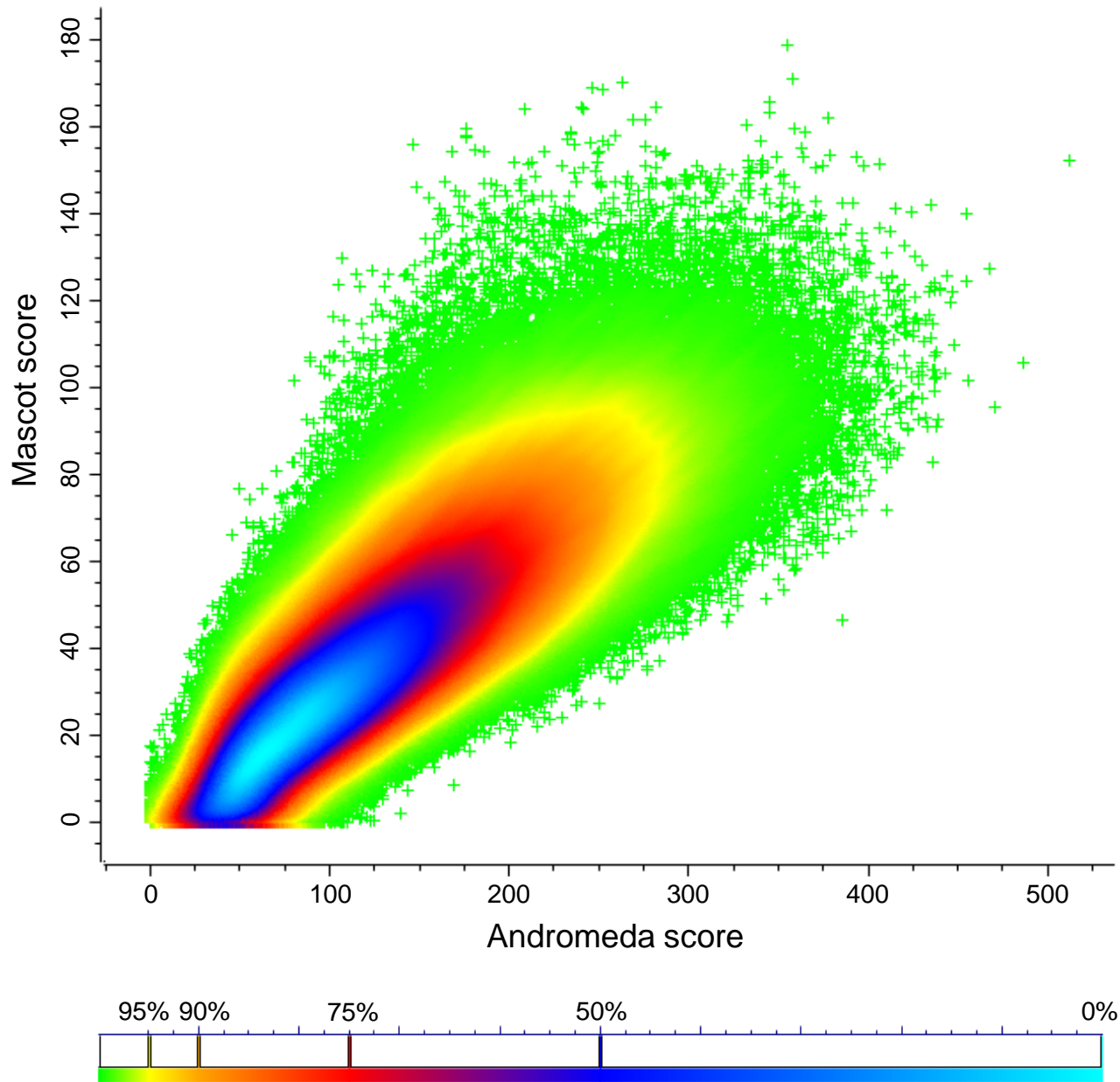
- SCIEX Triple TOF

Search engine

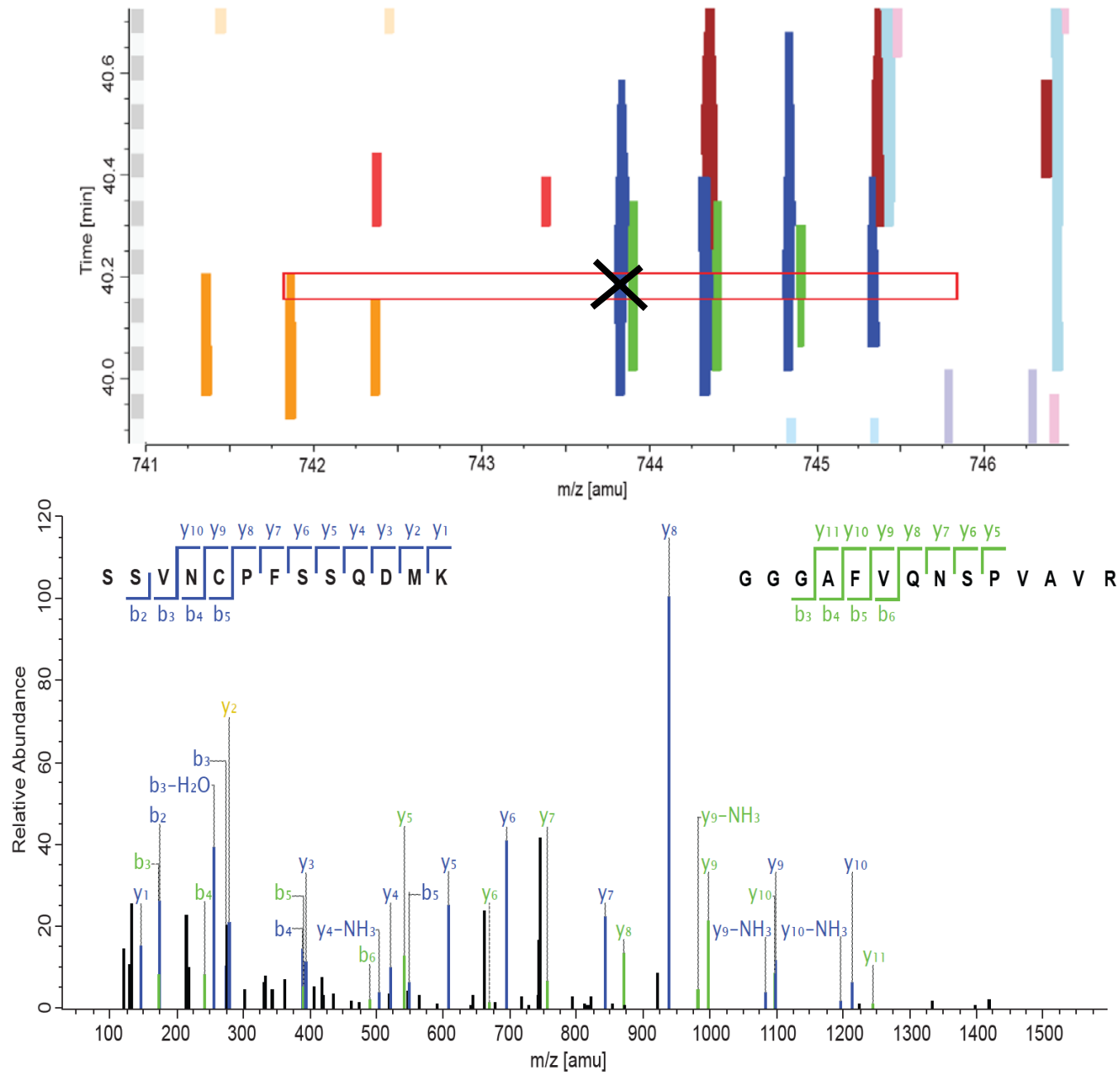


Cox et al, Andromeda – a peptide search engine integrated into the MaxQuant environment. JPR (2011)

Mascot vs. Andromeda score

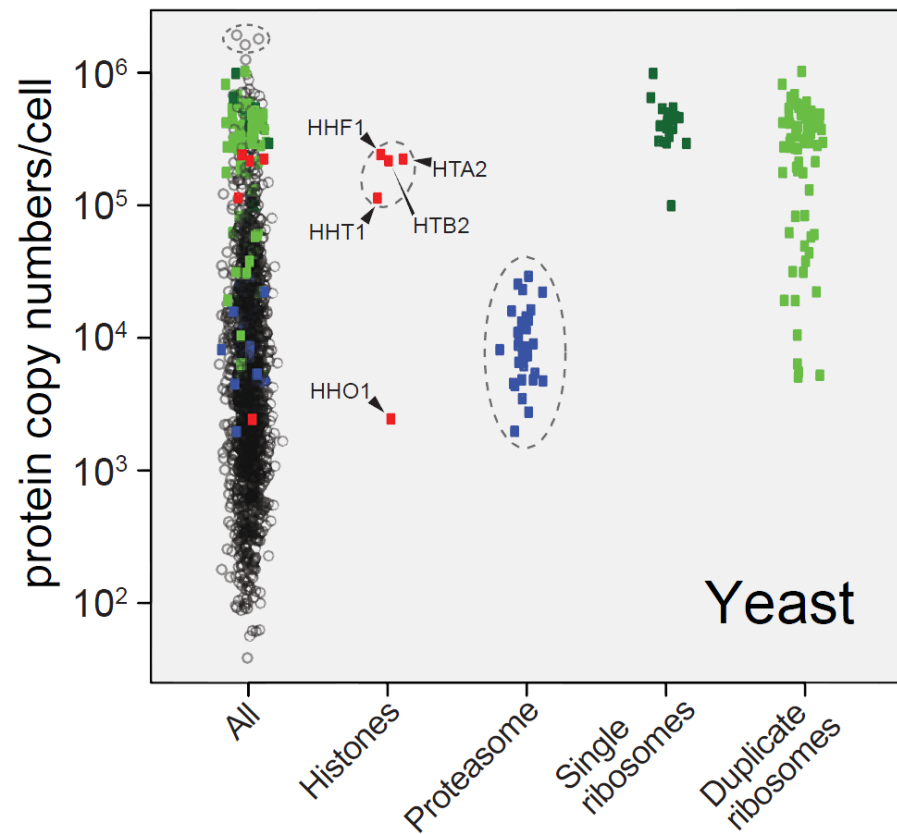


Identification of co-fragmented peptides

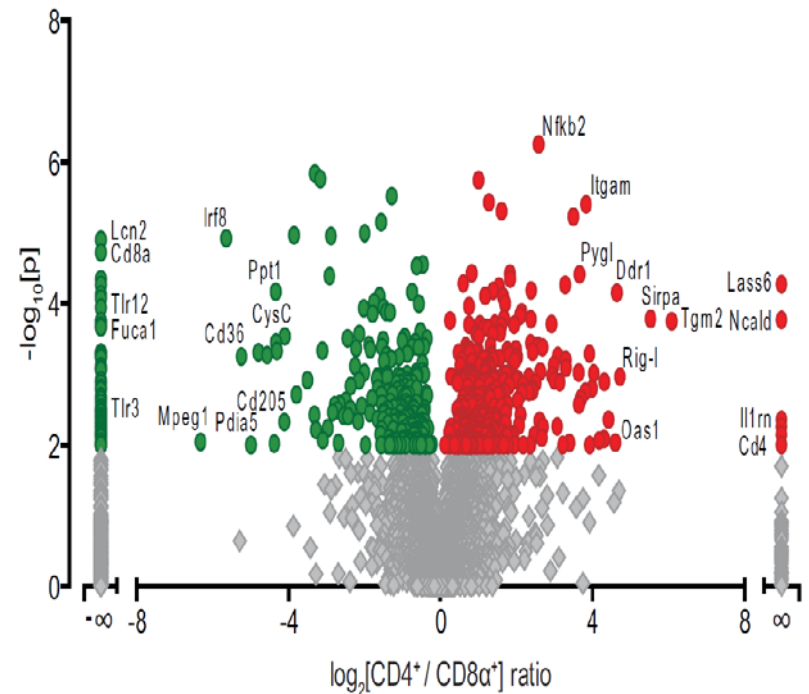


Absolute vs. relative quantification

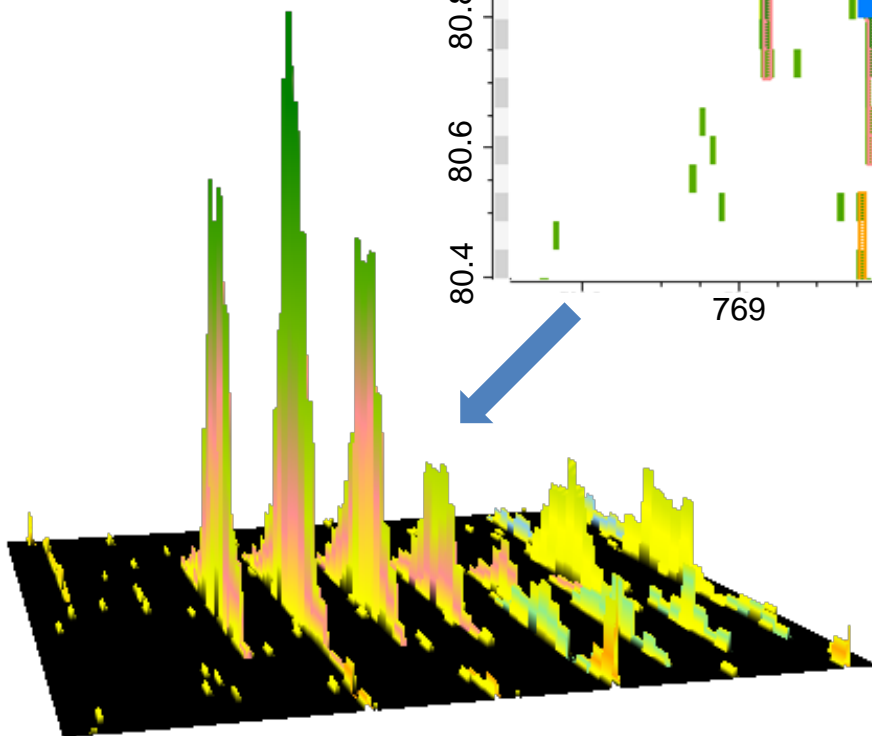
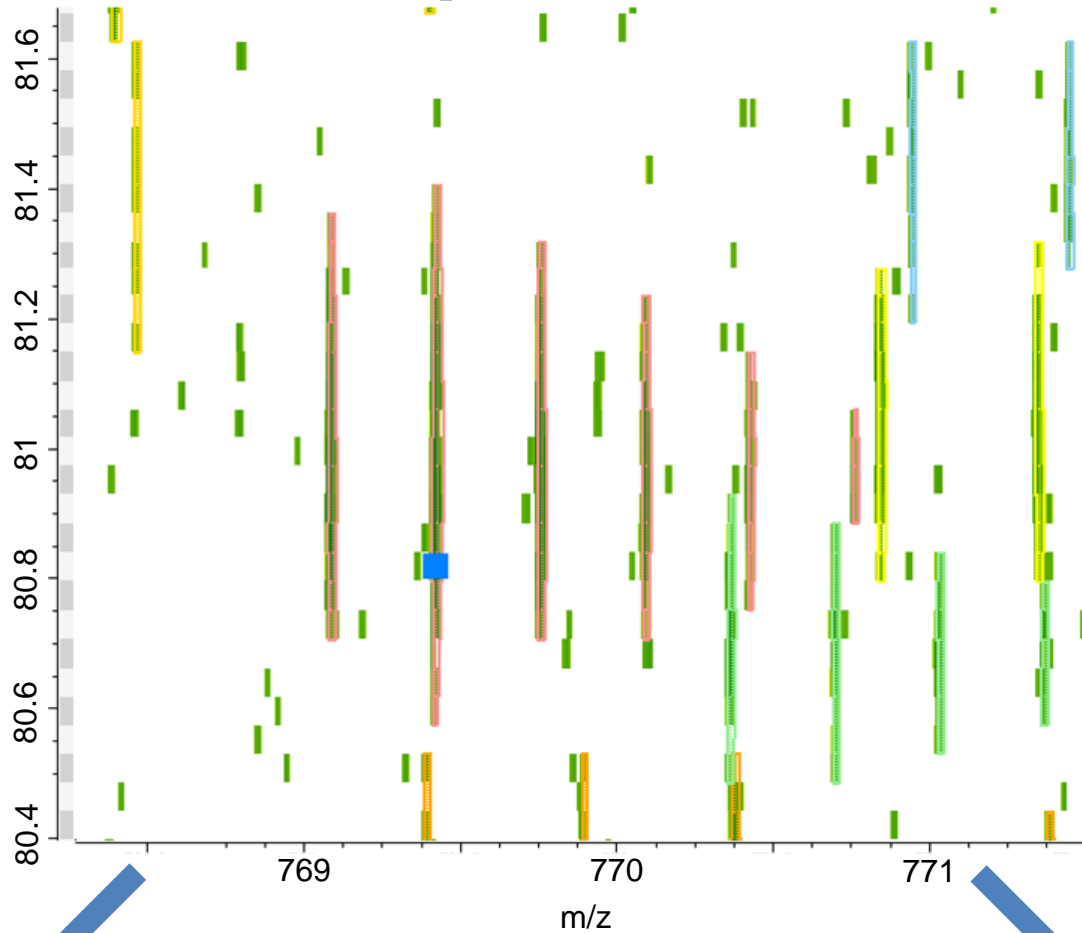
- Absolute quantification: copy numbers for each protein



- Relative quantification: compare same protein in different sample

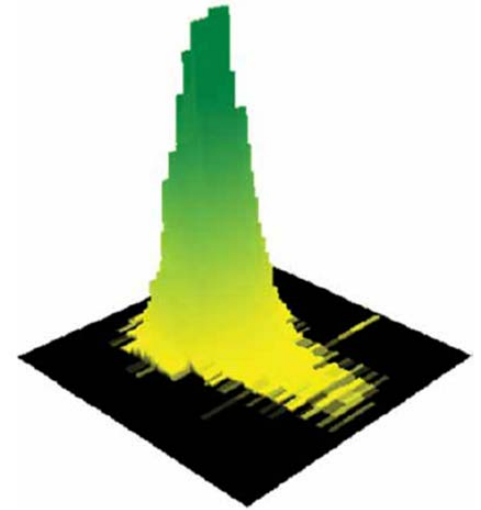
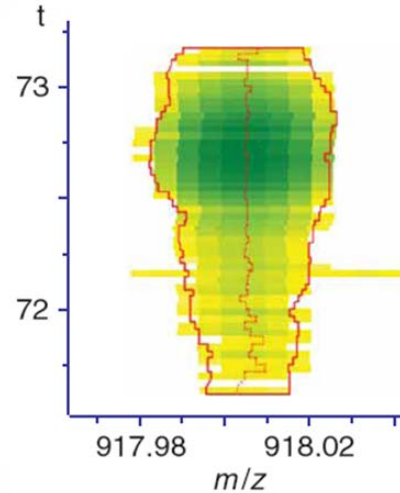
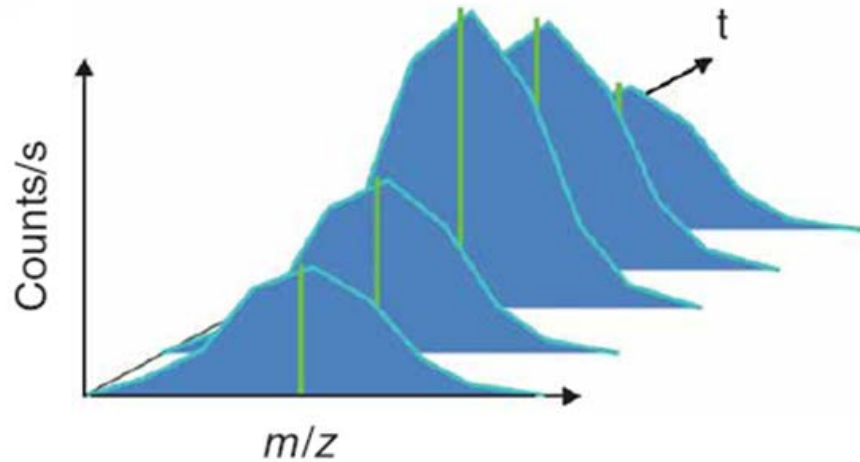


XIC vs. spectral count



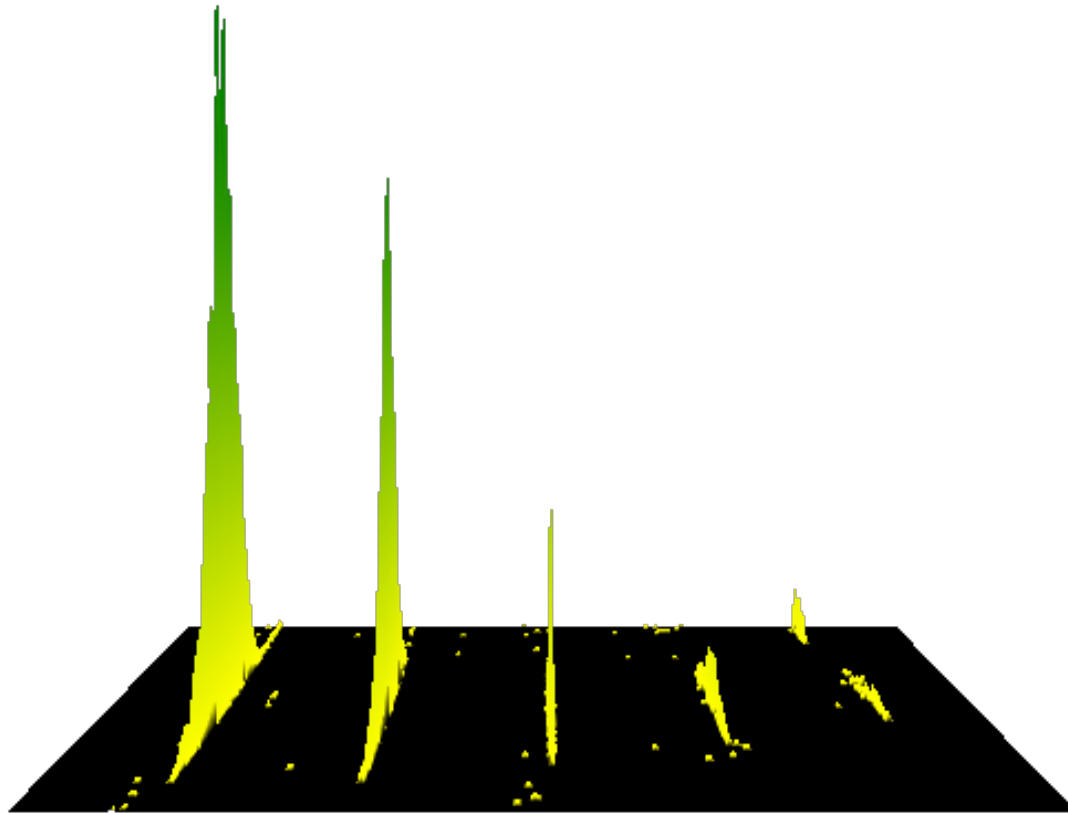
1

3D peak detection



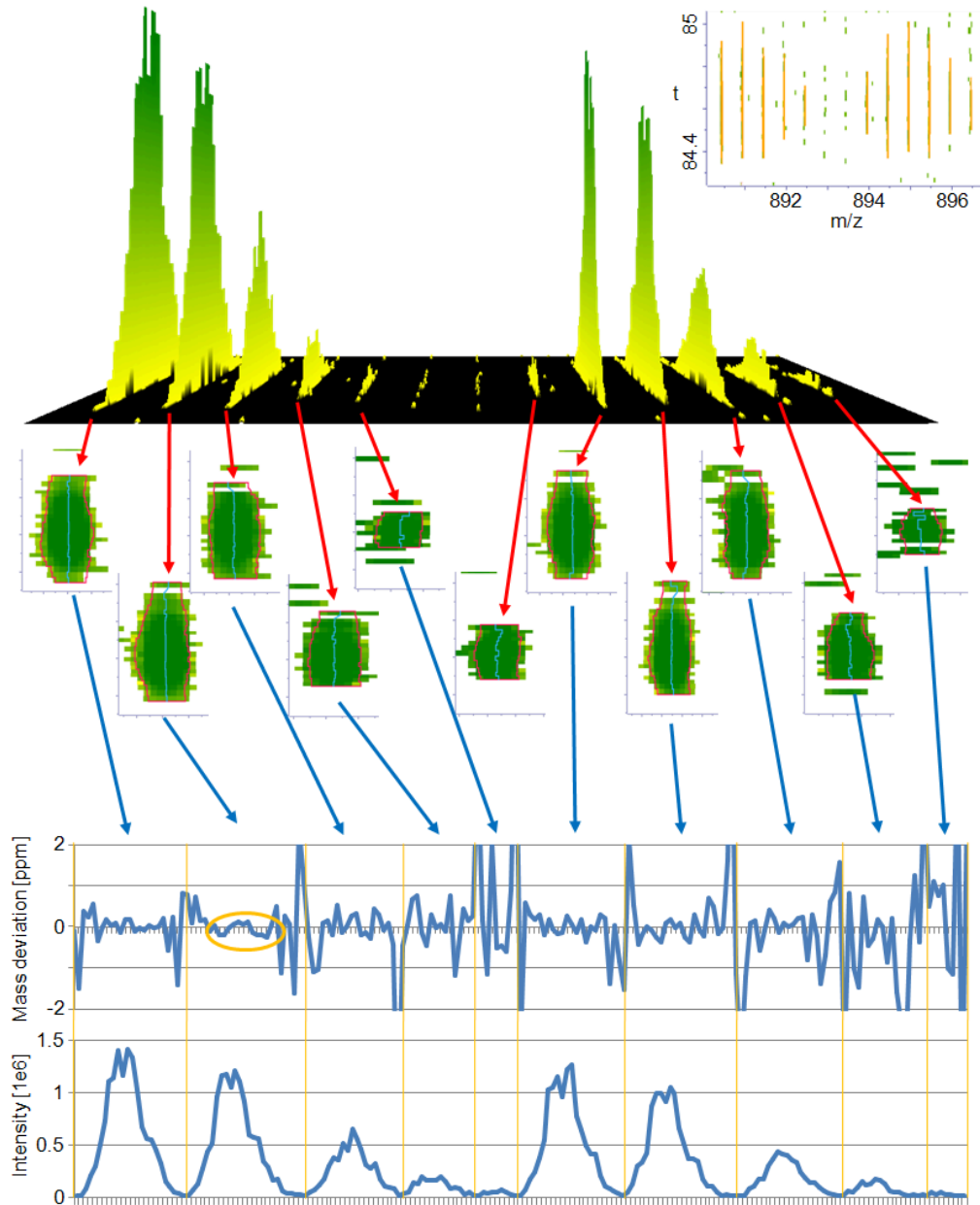
- 2D peaks are assembled into 3D peaks
- Two 2D peaks in adjacent scans are connected when $\Delta m < 7\text{ppm}$
- Also next to nearest scan is checked

De-isotoping

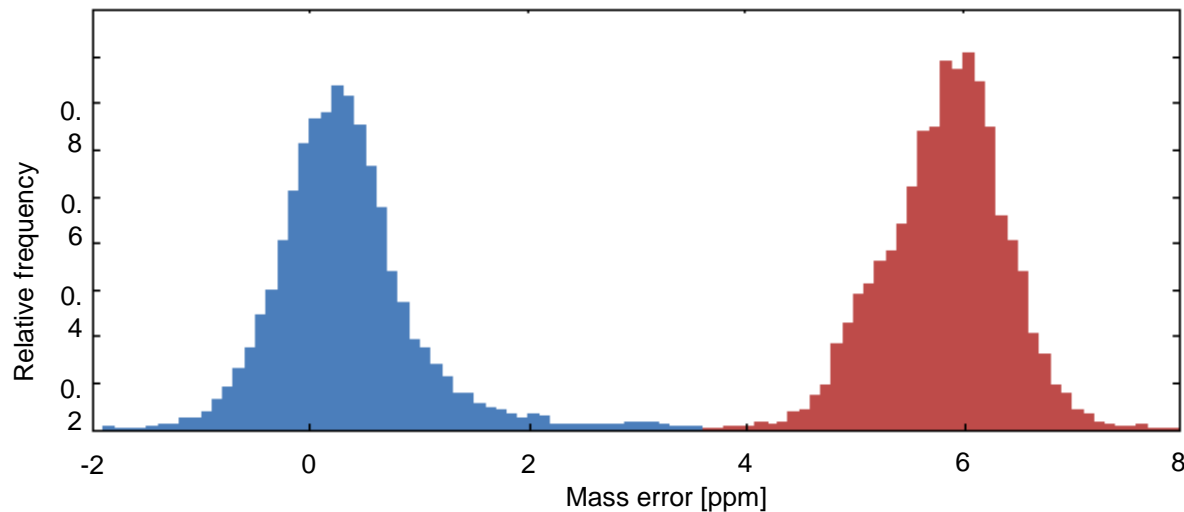
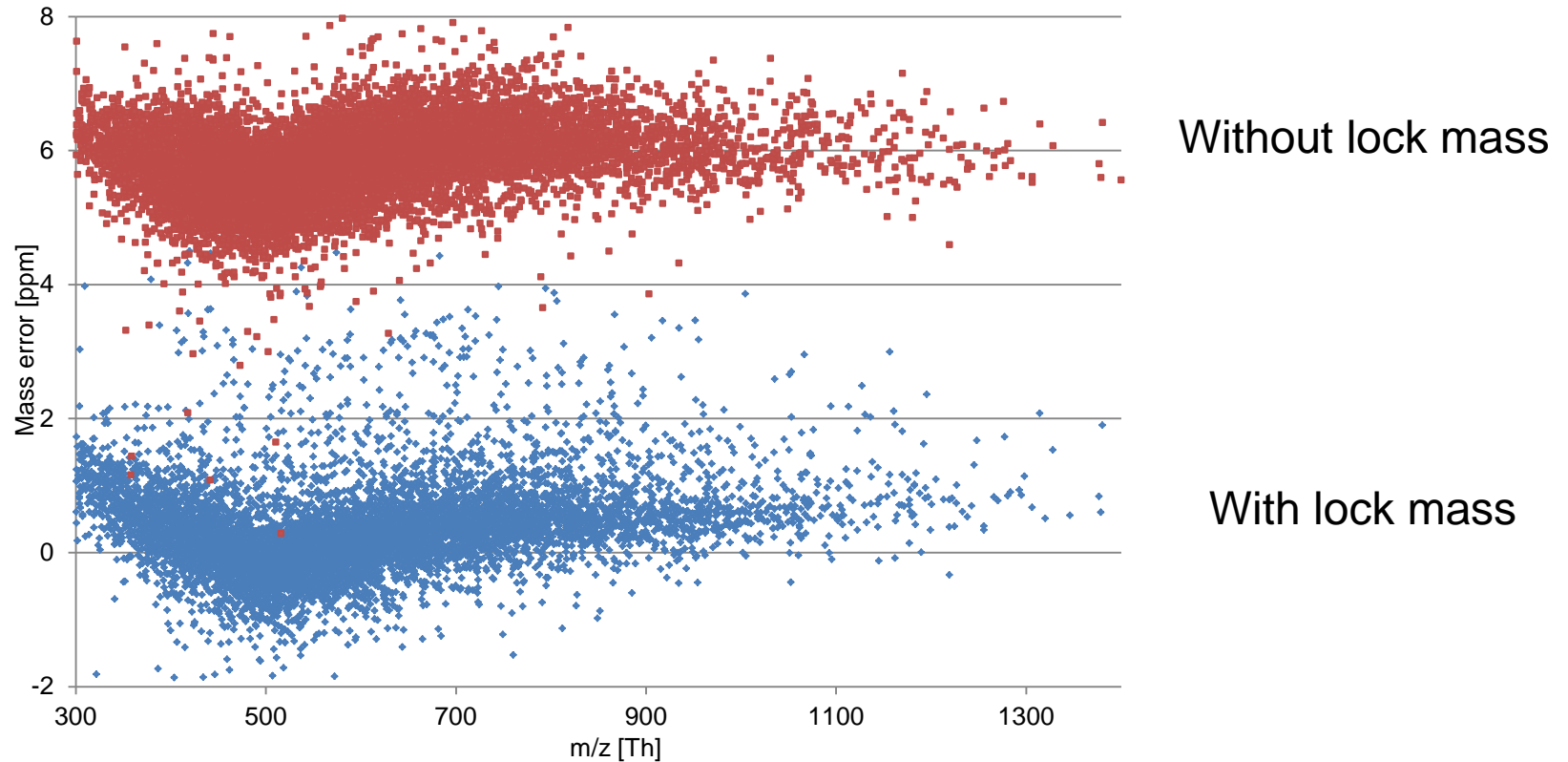


Calculation of precise peptide masses

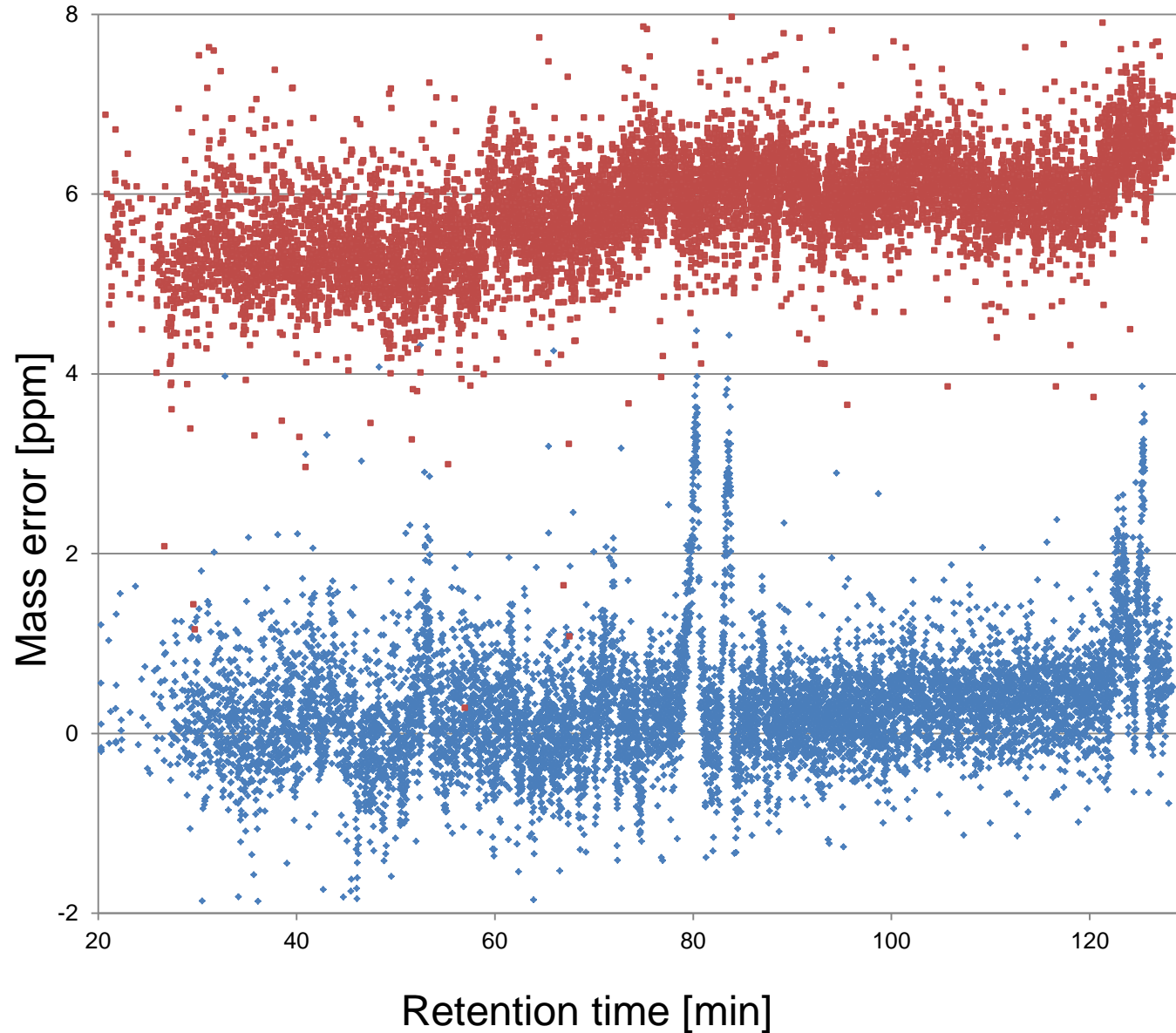
Calculate precise
mean and standard
deviation for each
peptide mass



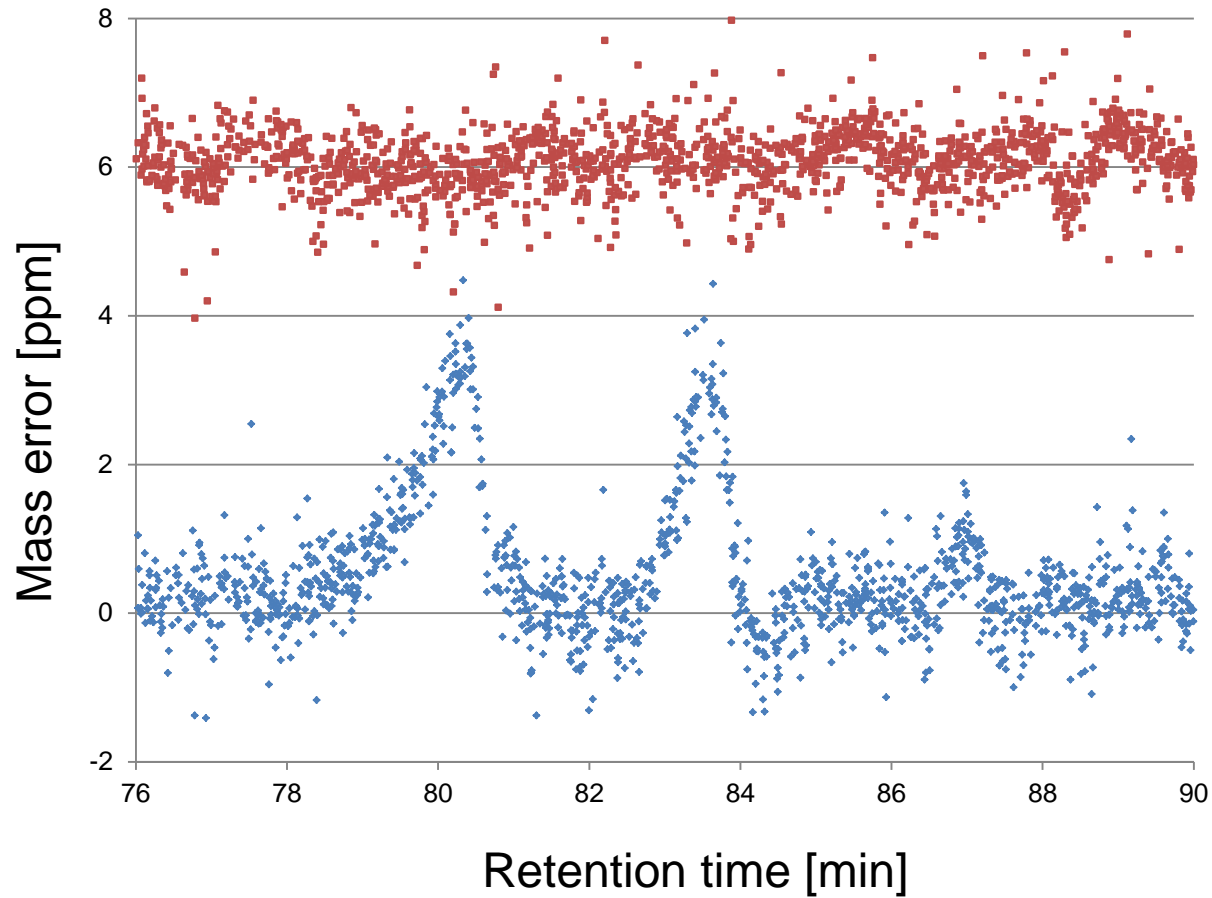
Nonlinear mass recalibration



Nonlinear mass recalibration

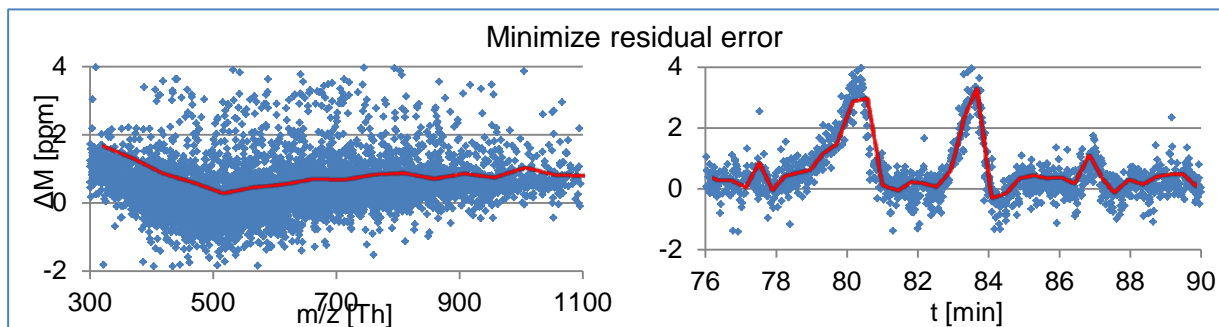
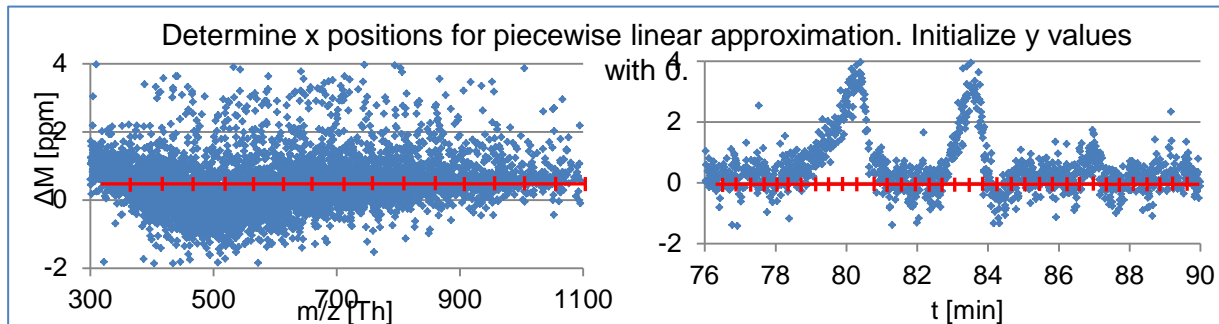
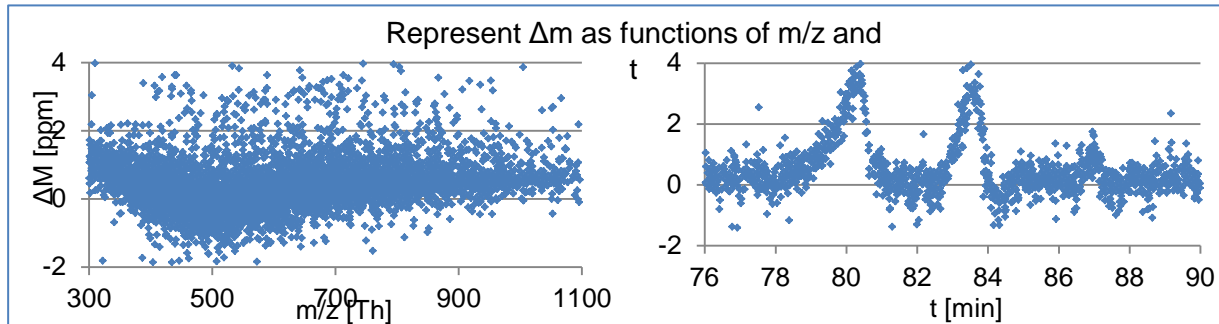


Nonlinear mass recalibration



Nonlinear mass recalibration

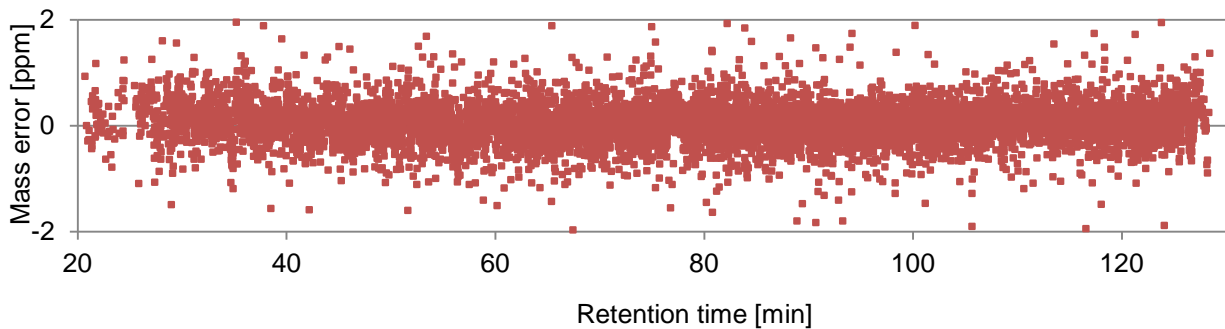
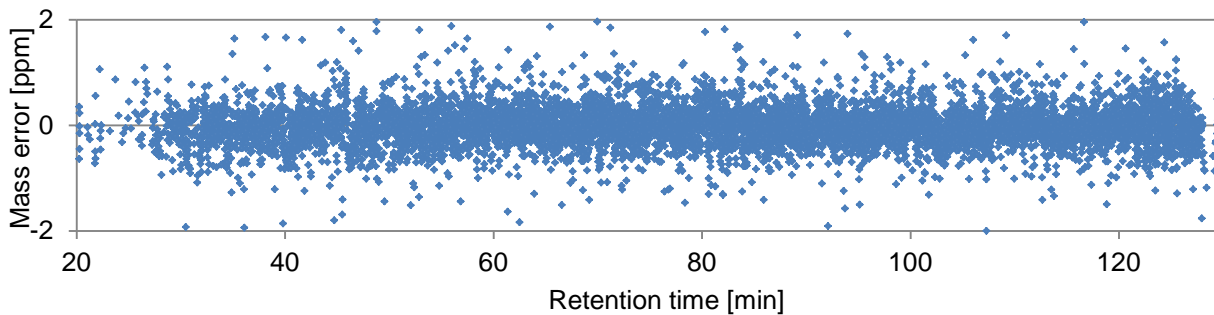
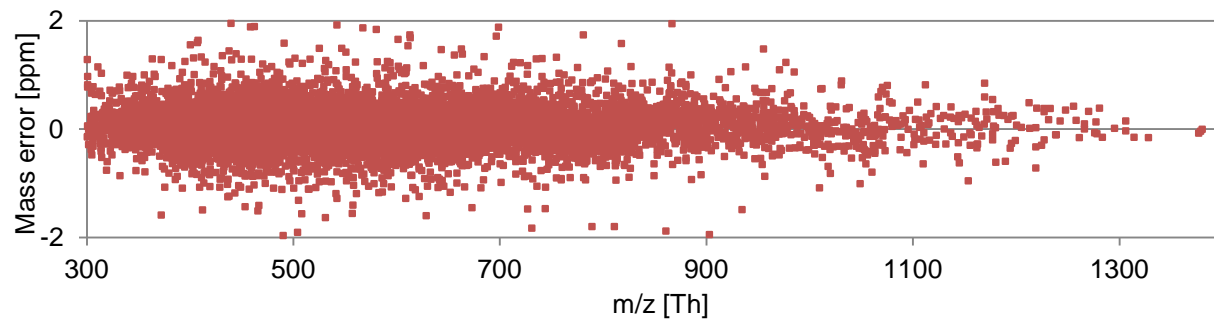
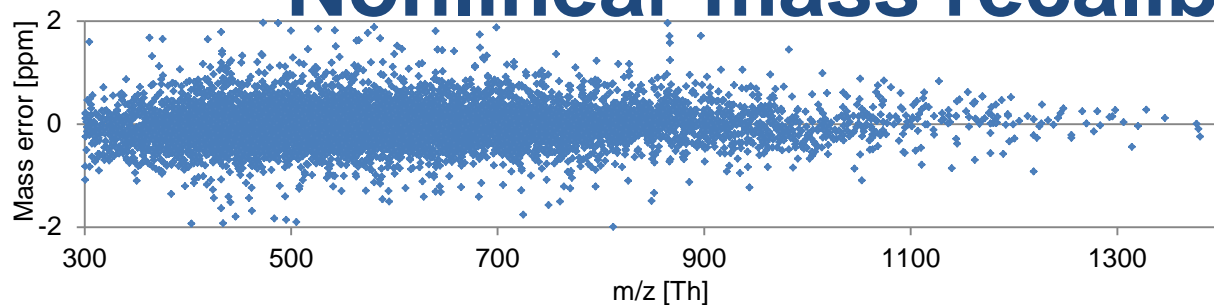
First Andromeda search with 20ppm mass tolerance and score threshold 80



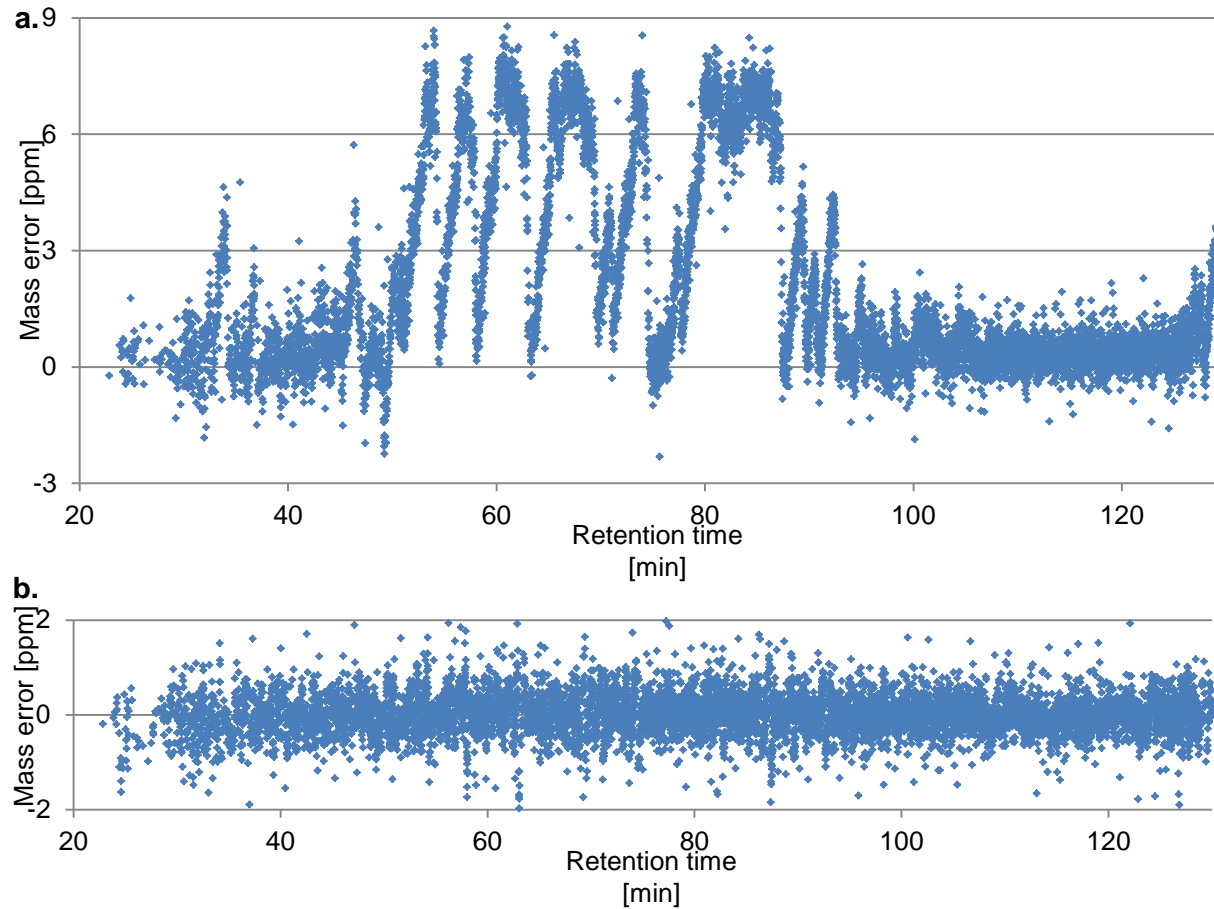
Subtract recalibration functions from all measured peptides

Perform the actual Andromeda search with small individualized mass tolerances

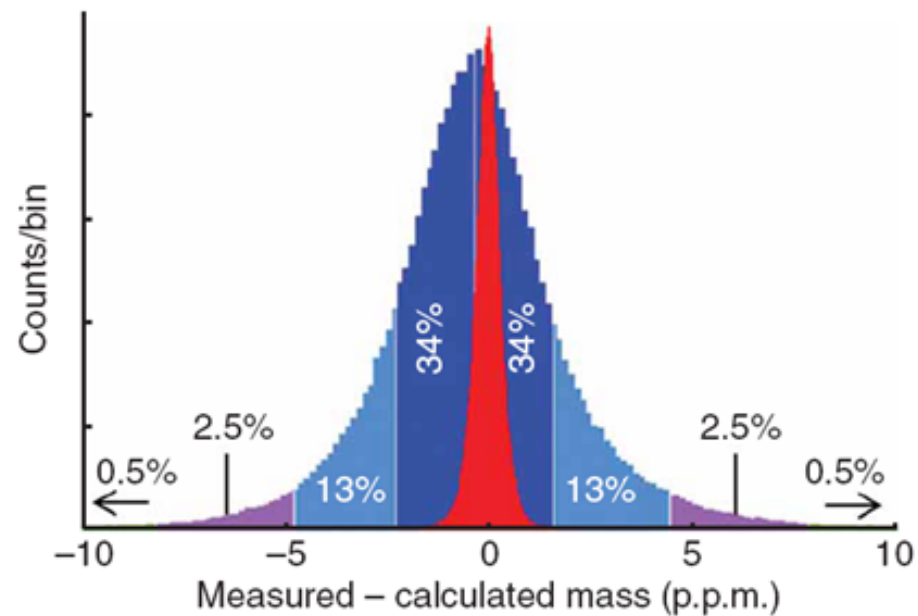
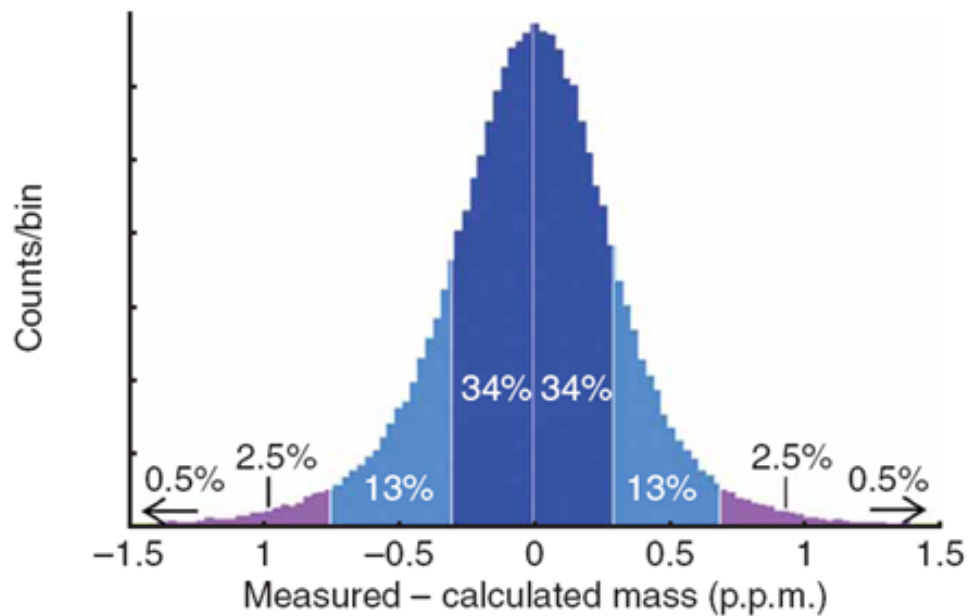
Nonlinear mass recalibration



Nonlinear mass recalibration



Improvement in mass accuracy

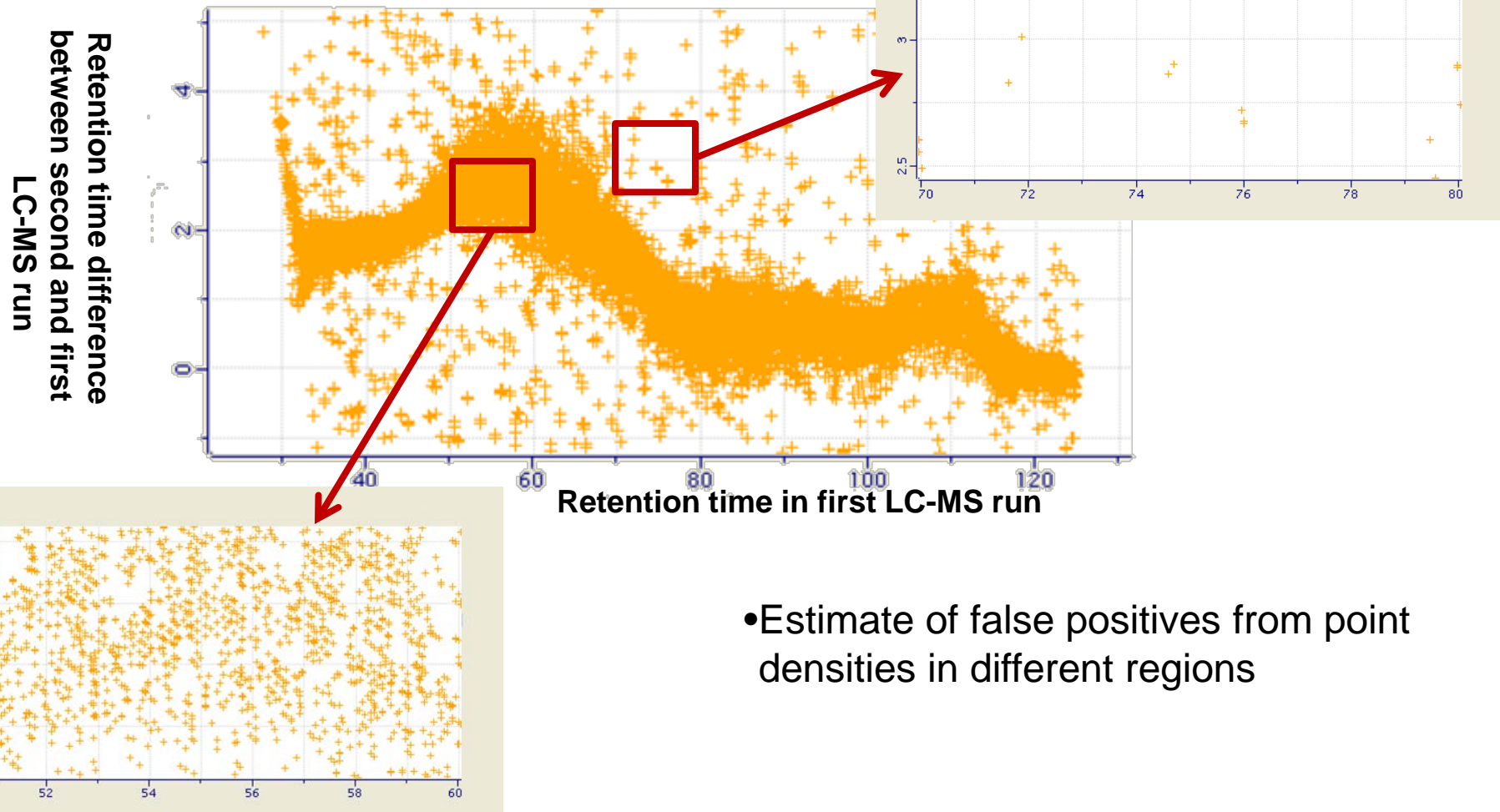


Problems in label free quantification

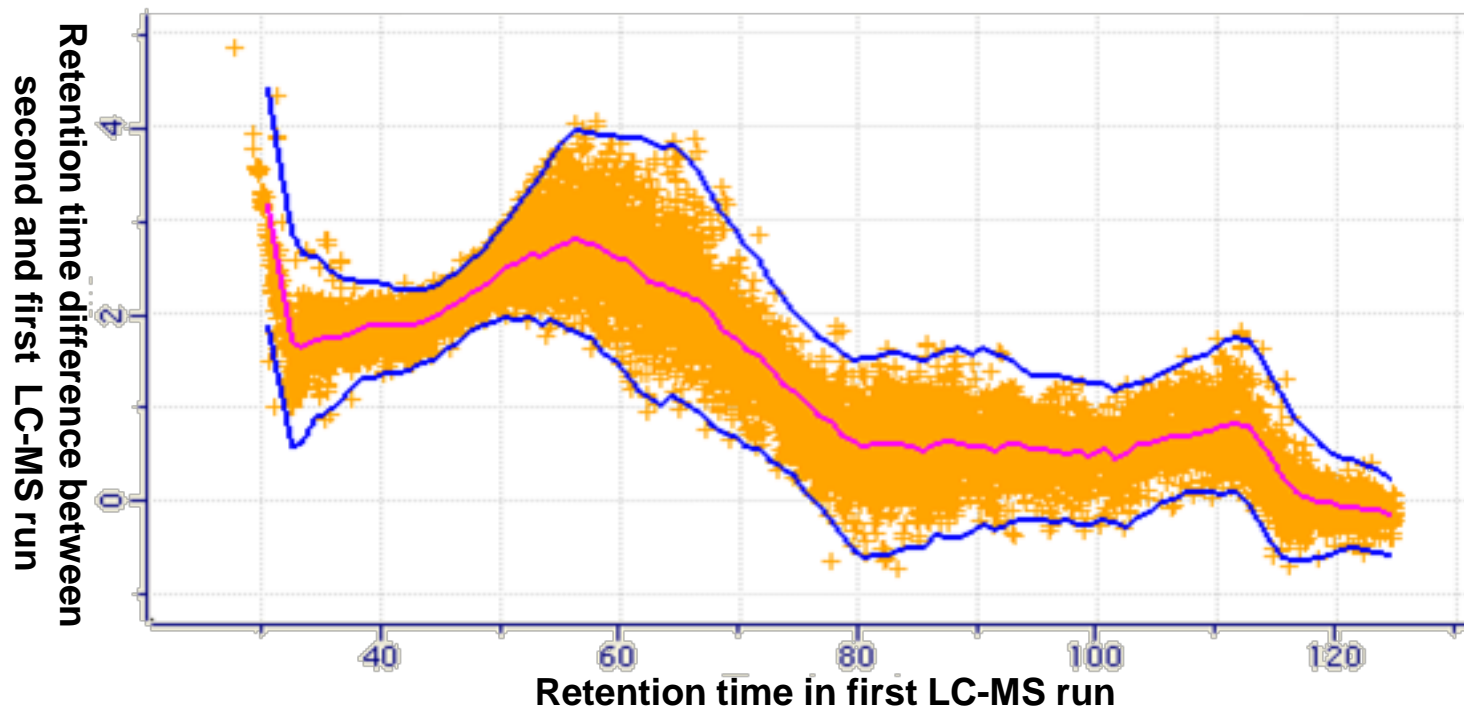
- Irreproducibility of retention time
- Incompatibility with pre-fractionation
- Quantification in a sample relies on MS/MS identification
- Identified peptides can be different in different samples

Retention time alignment

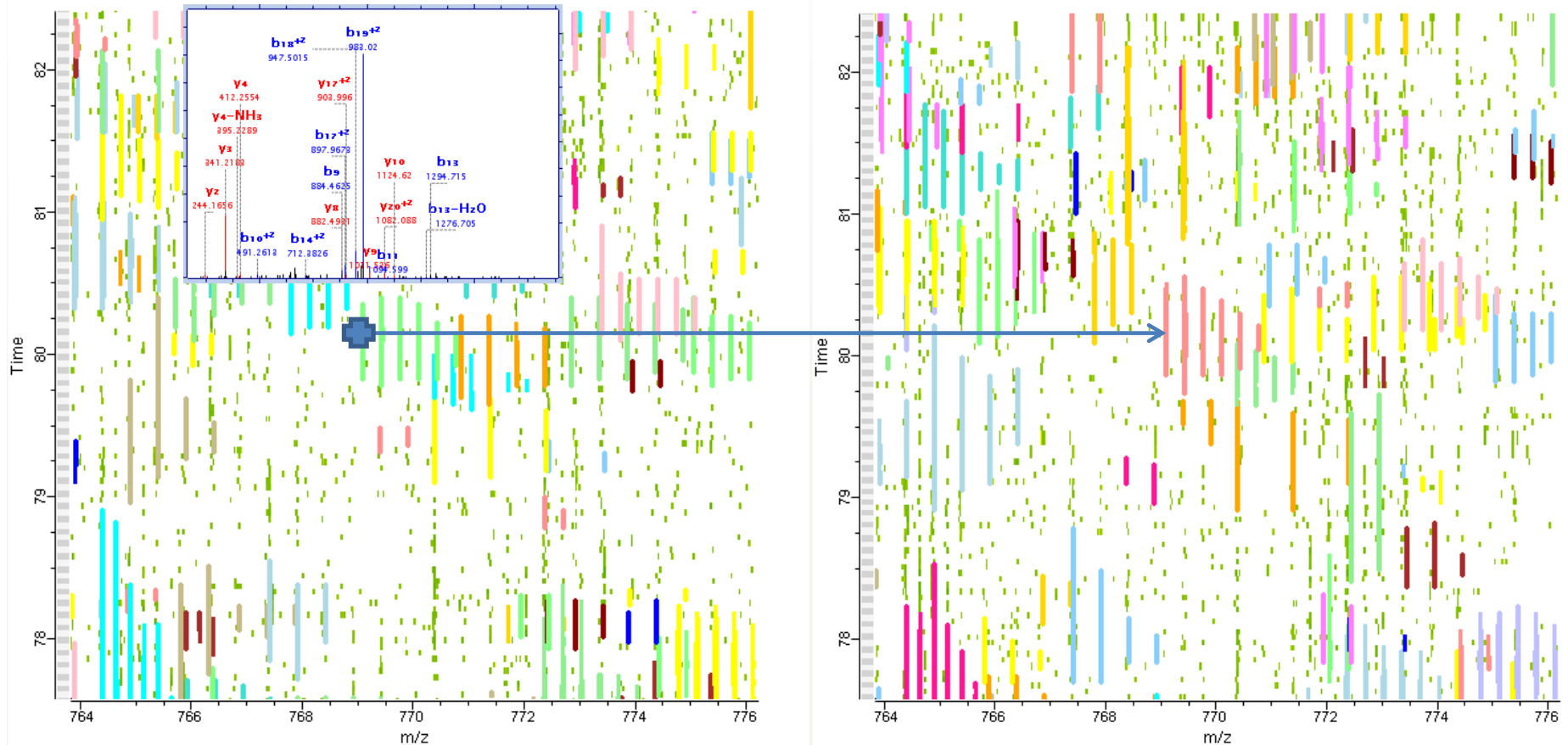
- Two LC-MS runs
- Peptides are matched by mass and retention time (only preliminary)



Retention time alignment



Matching between runs



- Identification transfer only between same or adjacent slices/fractions
- Transferring identifications after alignment increases base for quantitation by >100%

Label-free quantification: normalization

Fraction	A	B	C	D	E	F
:						
5		●		●		
6	●	●		●	●	
7	●	●	●	●	●	●
8	●	●	●			●
9			●			
:						
13		●	●			
14	●	●	●	●	●	●
15	●	●	●	●	●	●
16	●	●			●	
:						
19		●			●	
20		●	●	●	●	●
21	●	●	●	●	●	●
22	●		●			
:						

Peptide P:

$$\begin{aligned}
 I_{P,A}(N) &= N_{A,6} XIC_{A,6} + N_{A,7} XIC_{A,7} + N_{A,8} XIC_{A,8} \\
 I_{P,B}(N) &= N_{B,5} XIC_{B,5} + N_{B,6} XIC_{B,6} + N_{B,7} XIC_{B,7} + N_{B,8} XIC_{B,8} \\
 I_{P,C}(N) &= N_{C,7} XIC_{C,7} + N_{C,8} XIC_{C,8} + N_{C,9} XIC_{C,9} \\
 I_{P,D}(N) &= N_{D,5} XIC_{D,5} + N_{D,6} XIC_{D,6} + N_{D,7} XIC_{D,7} \\
 I_{P,E}(N) &= N_{E,6} XIC_{E,6} + N_{E,7} XIC_{E,7} \\
 I_{P,F}(N) &= N_{F,7} XIC_{F,7} + N_{F,8} XIC_{F,8}
 \end{aligned}$$

Peptide Q:

$$\begin{aligned}
 I_{Q,A}(N) &= N_{A,14} XIC_{A,14} + N_{A,15} XIC_{A,15} + N_{A,16} XIC_{A,16} \\
 I_{Q,B}(N) &= N_{B,13} XIC_{B,13} + N_{B,14} XIC_{B,14} + N_{B,15} XIC_{B,15} + N_{B,16} XIC_{B,16} \\
 I_{Q,C}(N) &= N_{C,13} XIC_{C,13} + N_{C,14} XIC_{C,14} + N_{C,15} XIC_{C,15} \\
 I_{Q,D}(N) &= N_{D,14} XIC_{D,14} + N_{D,15} XIC_{D,15} \\
 I_{Q,E}(N) &= N_{E,14} XIC_{E,14} + N_{E,15} XIC_{E,15} + N_{E,16} XIC_{E,16} \\
 I_{Q,F}(N) &= N_{F,14} XIC_{F,14} + N_{F,15} XIC_{F,15}
 \end{aligned}$$

Peptide R:

$$\begin{aligned}
 I_{R,A}(N) &= N_{A,21} XIC_{A,21} + N_{A,22} XIC_{A,22} \\
 I_{R,B}(N) &= N_{B,19} XIC_{B,19} + N_{B,20} XIC_{B,20} + N_{B,21} XIC_{B,21} \\
 I_{R,C}(N) &= N_{C,20} XIC_{C,20} + N_{C,21} XIC_{C,21} + N_{C,22} XIC_{C,22} \\
 I_{R,D}(N) &= N_{D,20} XIC_{D,20} + N_{D,21} XIC_{D,21} \\
 I_{R,E}(N) &= N_{E,19} XIC_{E,19} + N_{E,20} XIC_{E,20} + N_{E,21} XIC_{E,21} \\
 I_{R,F}(N) &= N_{F,20} XIC_{F,20} + N_{F,21} XIC_{F,21}
 \end{aligned}$$

$$H_P(N) = \left| \log \left(\frac{I_{P,A}(N)}{I_{P,B}(N)} \right) \right|^2 + \left| \log \left(\frac{I_{P,A}(N)}{I_{P,C}(N)} \right) \right|^2 + \left| \log \left(\frac{I_{P,A}(N)}{I_{P,D}(N)} \right) \right|^2 + \text{other sample pairs}$$

$$H_Q(N) = \left| \log \left(\frac{I_{Q,A}(N)}{I_{Q,B}(N)} \right) \right|^2 + \left| \log \left(\frac{I_{Q,A}(N)}{I_{Q,C}(N)} \right) \right|^2 + \left| \log \left(\frac{I_{Q,A}(N)}{I_{Q,D}(N)} \right) \right|^2 + \text{other sample pairs}$$

$$H_R(N) = \left| \log \left(\frac{I_{R,A}(N)}{I_{R,B}(N)} \right) \right|^2 + \left| \log \left(\frac{I_{R,A}(N)}{I_{R,C}(N)} \right) \right|^2 + \left| \log \left(\frac{I_{R,A}(N)}{I_{R,D}(N)} \right) \right|^2 + \text{other sample pairs}$$

$$H(N) = H_P(N) + H_Q(N) + H_R(N) + \text{other peptides}$$

Protein quantification

a.

>P63208
 MPSIKLQSSDGEIFEVDVEIAKQSVTIKTMLEDLGMKDEGDD
 DPVPLPNVNAAILKKVIQWCTHHKDDPPPPEDDENKEKRTDD
IPVWDQEFFLKVDQGTLLFELILAANYLDIKGLLDVTCKTVANM
IKGKTPEEIRKTFNIKNDFTEEEEAQVRKENQWCEEK

b.

Peptide	Sequence
P ₁	LQSSDGEIFEVDVEIAK
P ₂	TMLEDLGMK
P ₃	VIQWCTHHK
P ₄	RTDDIPVWDQEF
P ₅	TVANMIK
P ₆	TPEEIRK
P ₇	NDFTEEEEAQVR

c.

Sample	P ₁	P ₂	P ₃	P ₄	P ₅	P ₆	P ₇
A		+				+	
B		+	+			+	
C	+	+	+	+		+	+
D	+	+		+		+	+
E		+		+			+
F		+			+		

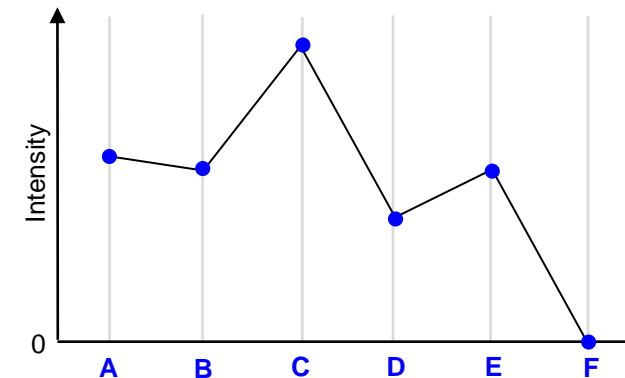
d.

A						
B	r_{BA}					
C	r_{CA}	r_{CB}				
D	r_{DA}	r_{DB}	r_{DC}			
E	r_{EA}	r_{EB}	r_{EC}	r_{ED}		
F	r_{FA}	r_{FB}	r_{FC}	r_{FD}	r_{FE}	
	A	B	C	D	E	F

e.

$r_{BA} = I_B / I_A$	$r_{CA} = I_C / I_A$	$r_{CB} = I_C / I_B$
$r_{DA} = I_D / I_A$	$r_{DB} = I_D / I_B$	$r_{DC} = I_D / I_C$
$r_{EC} = I_E / I_C$	$r_{ED} = I_E / I_D$	$I_F = 0$

f.



Label-free quantification

Benchmark dataset

- HeLa and E. coli cell lysates are mixed
- Proteins were digested with trypsin.
- In three replicates peptides were separated by isoelectric focusing in 24 fractions.
- This was repeated with the same amount of HeLa, but E. coli lysate tripled.
- This results in six samples for which all human proteins have constant protein profiles, while E. coli proteins have a ratio of three between replicate groups.
- LC-MS on an LTQ-Orbitrap mass spectrometer.

Identification results

1,234,125 MS isotope patterns identified by MS/MS

1,852,556 MS isotope patterns identified by matching between runs

3,086,681 MS isotope patterns in total

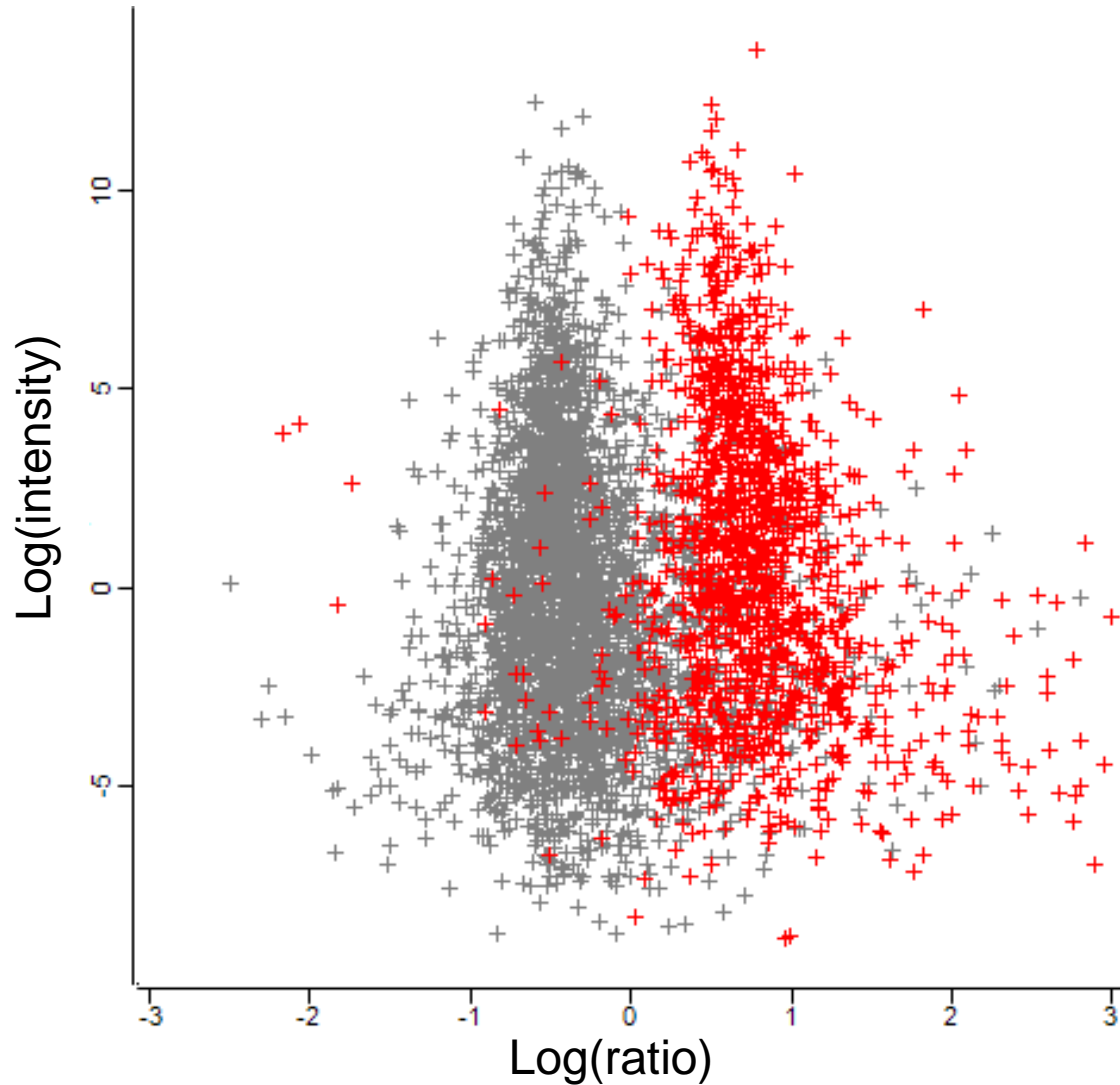
6,577 proteins

5,161 proteins in at least 3/6 samples

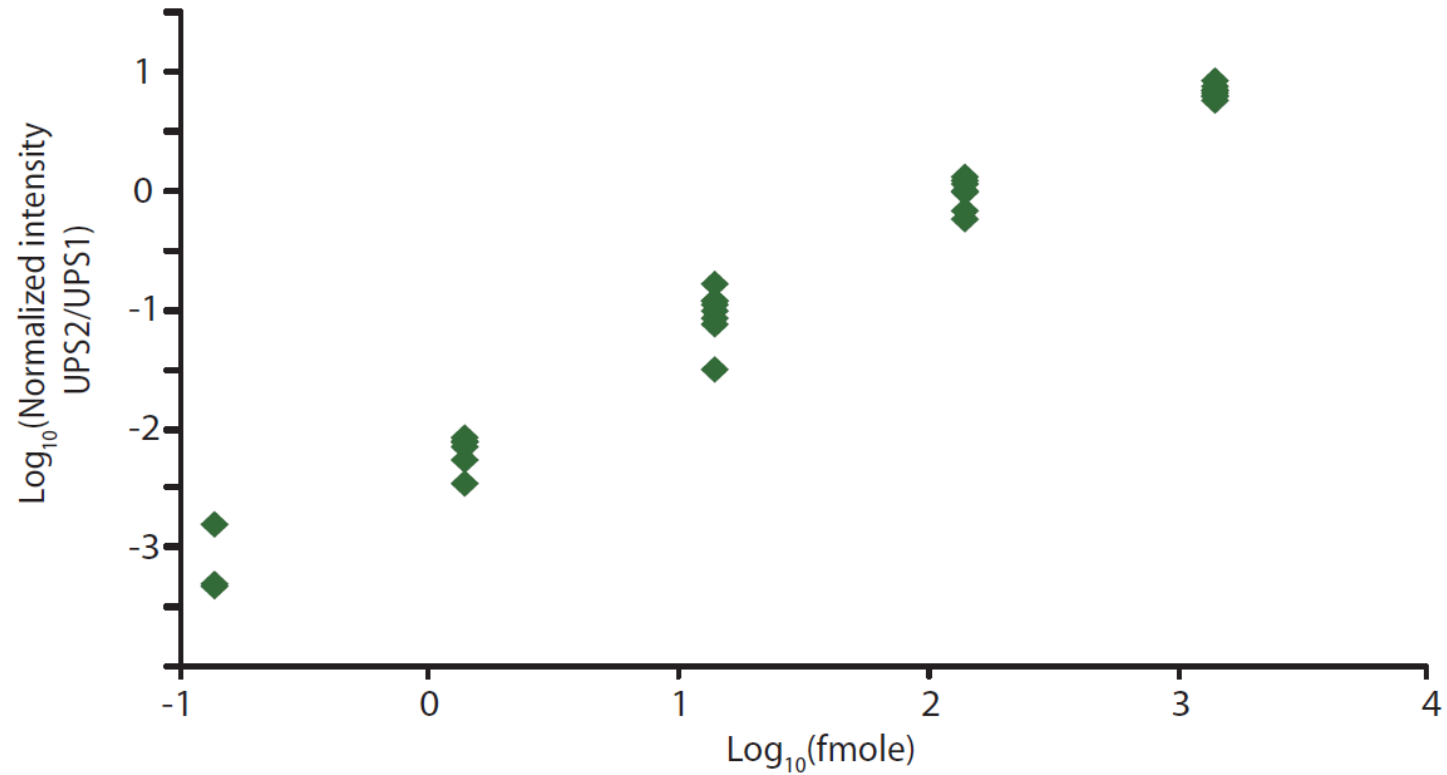
4,589 proteins in 6/6 samples

46,839 peptide sequences

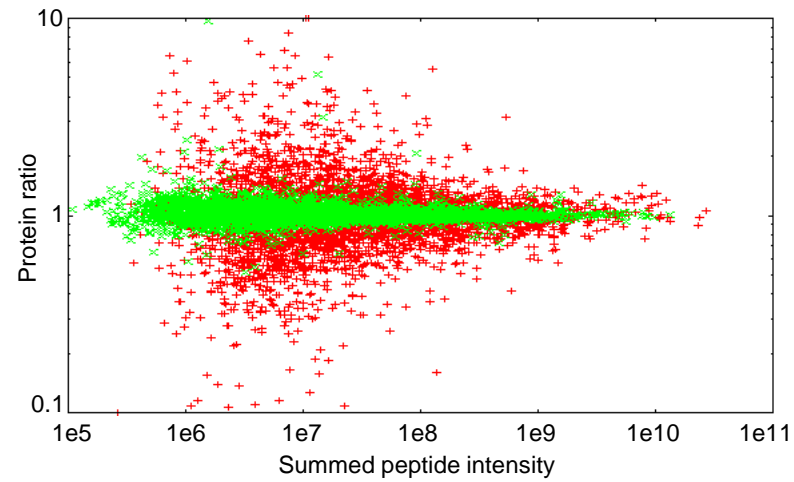
Label-free quantification results



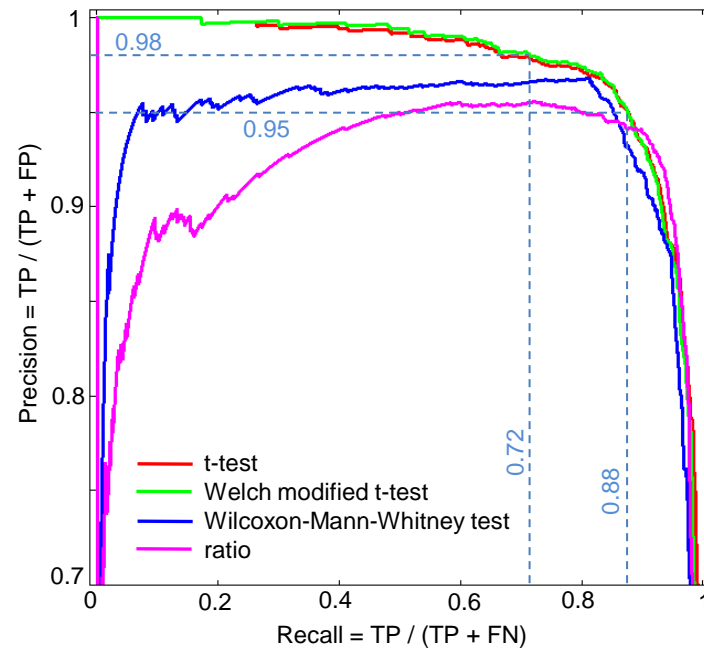
Dynamic range benchmark dataset



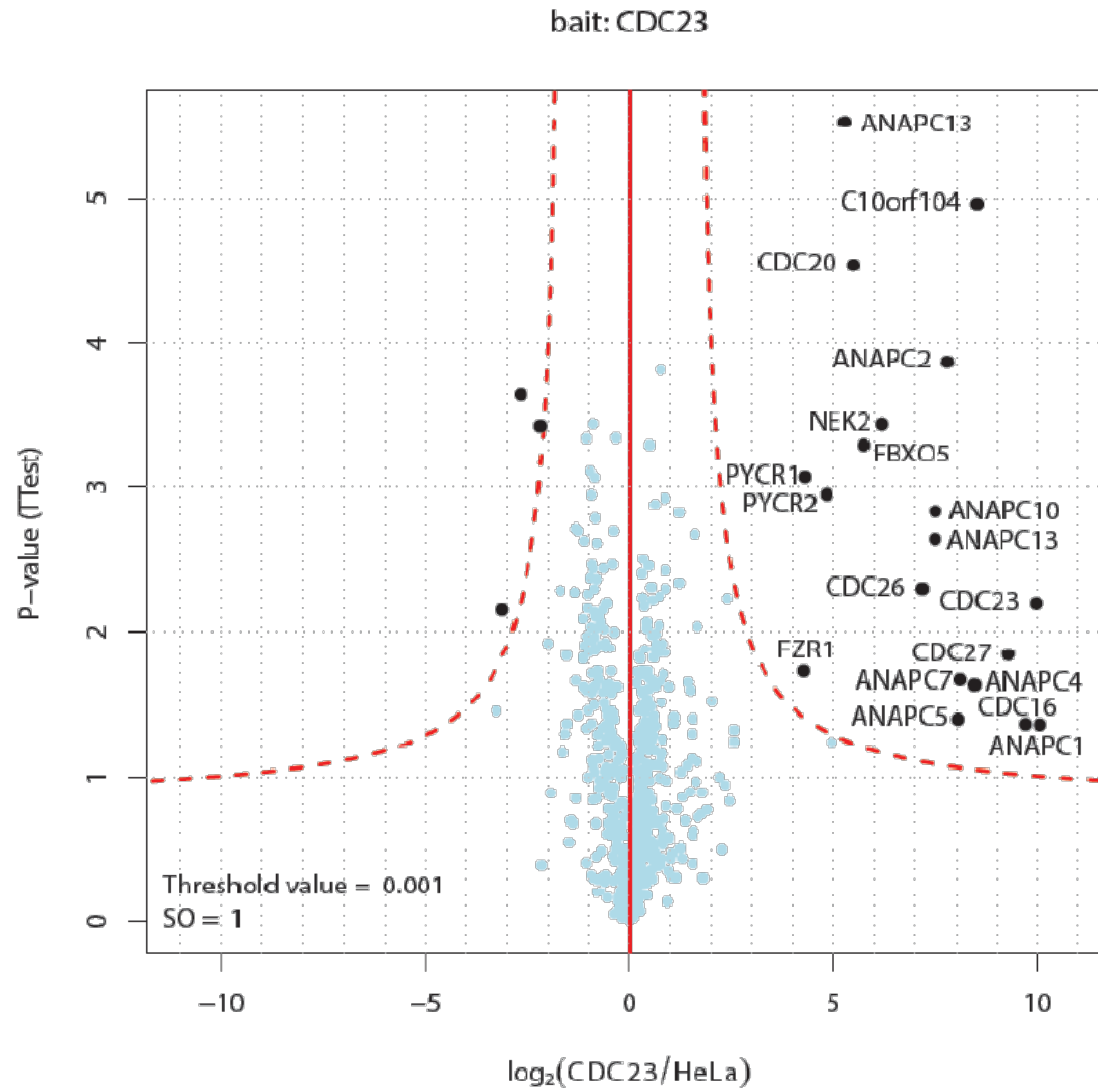
Comparison to SILAC



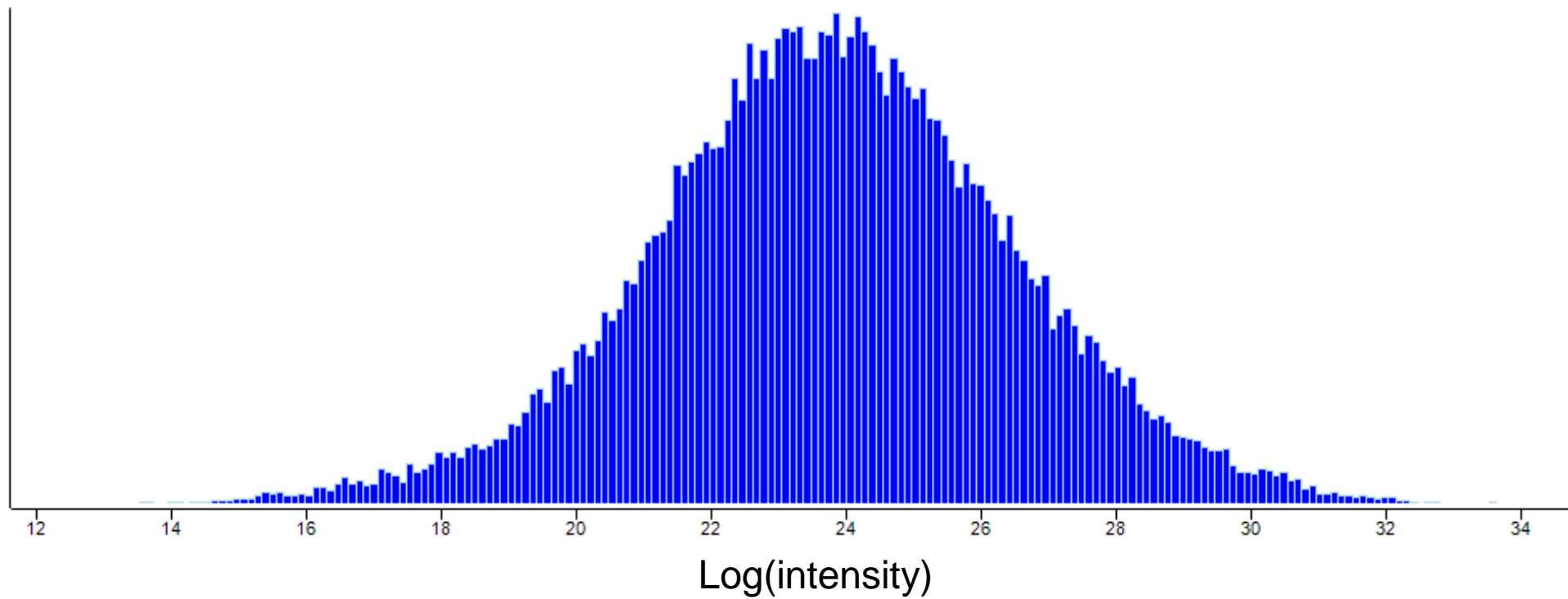
Precision vs. recall



Pulldowns



Imputation



MaxQuant homepage - Windows Internet Explorer

http://www.maxquant.org/

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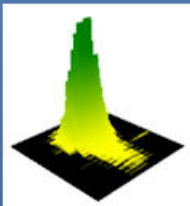
Downloads

System requirements

FAQ

Publications


Links



MaxQuant

Welcome

MaxQuant is a quantitative proteomics software package designed for analyzing large mass-spectrometric data sets. It is specifically aimed at high-resolution MS data. Several labeling techniques as well as label-free quantification are supported. MaxQuant is freely available and can be downloaded from this site. The download includes the search engine Andromeda which is integrated into MaxQuant as well as the Viewer application for inspection of raw data and identification and quantification results.



MaxQuant Help

[Visit this group](#)

News

Version 1.2.0.18

This is essentially the version as presented at the summer school. The bug with the re-quantified ratios has been fixed.

[Download.](#)

Getting started

Download the latest version in the 'Downloads' section. After registering you will receive an email with the download link. Unzip the folder containing the MaxQuant binaries. Make sure that your computer fulfils the hardware requirements and that all necessary software components are installed. You start MaxQuant by double-clicking on MaxQuant.exe. A getting started document you can get in the main menu of MaxQuant under Help -> Getting started. In case of problems you may find answers to your questions in the frequently asked questions (FAQ) section or you may consult the MaxQuant Google group. Your question may have been posted already which you can find out with the search functionality.

Reference

Cox, J. and Mann, M. (2008) MaxQuant enables high peptide identification rates, individualized p.p.b.-range mass accuracies and proteome-wide protein quantification. *Nat Biotechnol* 26, 1367-72.

Perseus

Perseus is designed to perform all downstream bioinformatics and statistics on the MaxQuant output tables.

[Go](#)

OKSTA files

Add file

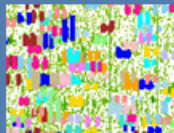
FASTA files

List of fasta

Multiple fast

registered v

☒ Include contaminants





b ₁	b ₆	y ₆
356.1928	427.22	609.181
y ₃ -NH ₂	y ₃ ²⁺	
327.1901	401.7498	
b ₄	y ₁	y ₅
31.1557	154.2227	510.2429

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By J. Cox - 12:29am - 2 authors - 2 replies

[\[maxquant-list:2764\] No results with MaxQuant 1.2.0.18](#)
By J. Cox - 12:25am - 2 authors - 1 reply

[Differences in ratios peptides.txt vs evidence.txt](#)
By Ilian A - Jul 11 - 2 authors - 2 replies

[Help with Label-free quantitation](#)
By parimal samir - Jul 11 - 2 authors - 2 replies

[\[maxquant-list:2637\] MQ 1.36 cannot find xxx.params file](#)
By J. Cox - Jul 10 - 2 authors - 1 reply

[\[maxquant-list:2738\] PEAK -SILAC state?](#)
By J. Cox - Jul 10 - 4 authors - 4 replies

[\[maxquant-list:2734\] Re: FDR filtering](#)
By J. Cox - Jul 10 - 3 authors - 5 replies

[\[maxquant-list:2718\] Re: MaxQuant for iTRAQ](#)
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[\[maxquant-list:2741\] MQ label-free](#)
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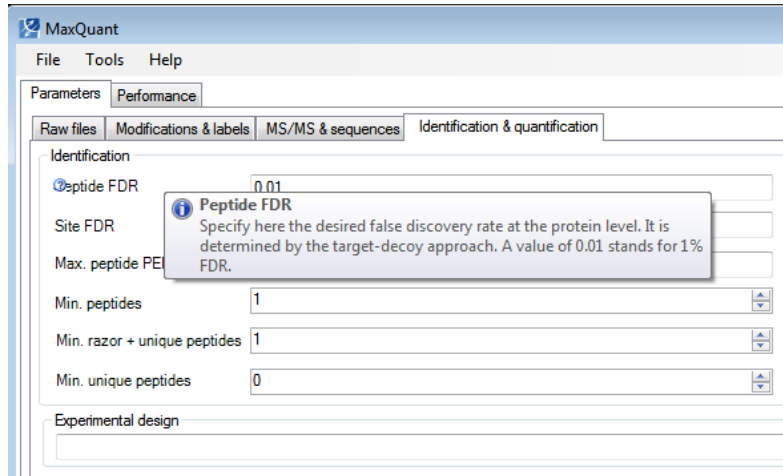
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Usability, documentation, software quality



Getting started with MaxQuant

Welcome to the MaxQuant computational proteomics workflow. We hope that you will enjoy your time you will be spending with processing your data and that MaxQuant delivers some helpful results. Currently we support the following instrument types:

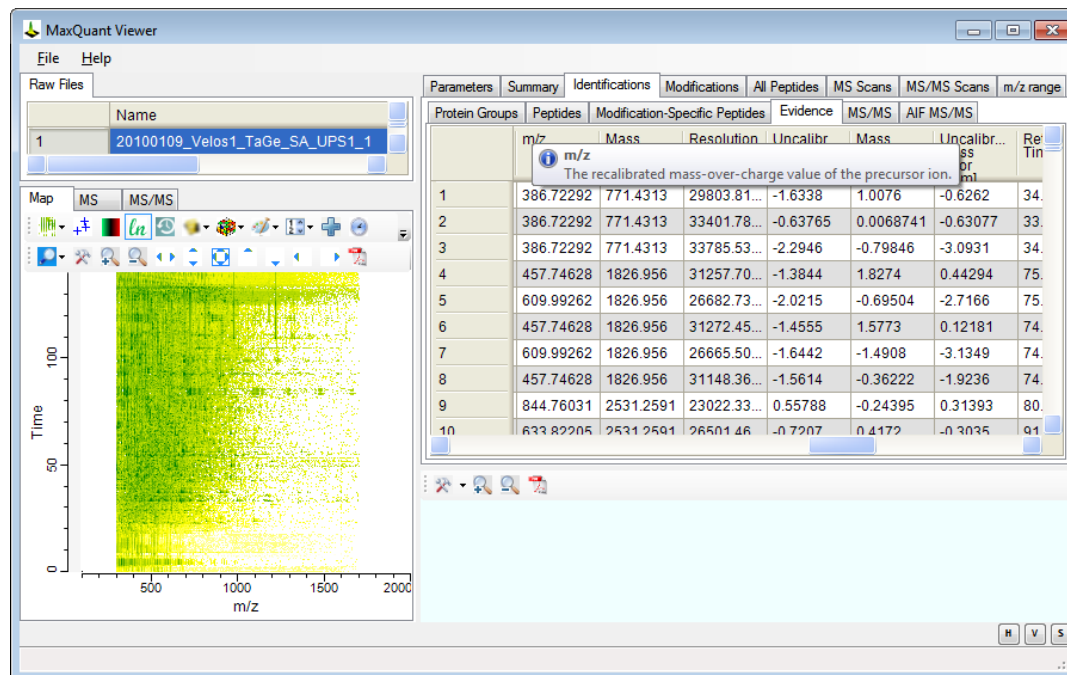
1. Thermo Velos
2. Thermo Orbitrap
3. Thermo FT
4. Thermo Exactive

Many labeling technologies are supported. In fact, MS-level quantification labels can be freely configured. Supported sample labeling quantification techniques include:

1. SILAC
2. Label-free quantification
3. Dimethyl
4. 18O
5. ICAT
6. ICPL
7. mTRAQ

In case you are a first time user you might be worried by the many options and parameters that one can set in the user interface. In that case we have good news for you. In almost all use cases the standard values of most parameters are fine and you only need to adjust a small number of factors. Typically there is only little information that you need to provide. Every parameter in the interface has context help which you obtain by moving the mouse pointer to the beginning of the text string for this parameter and clicking on the question mark that will appear. Here are seven steps that you typically have to go through before MaxQuant can process your data:

1. Specify the raw files that you want to process with MaxQuant.



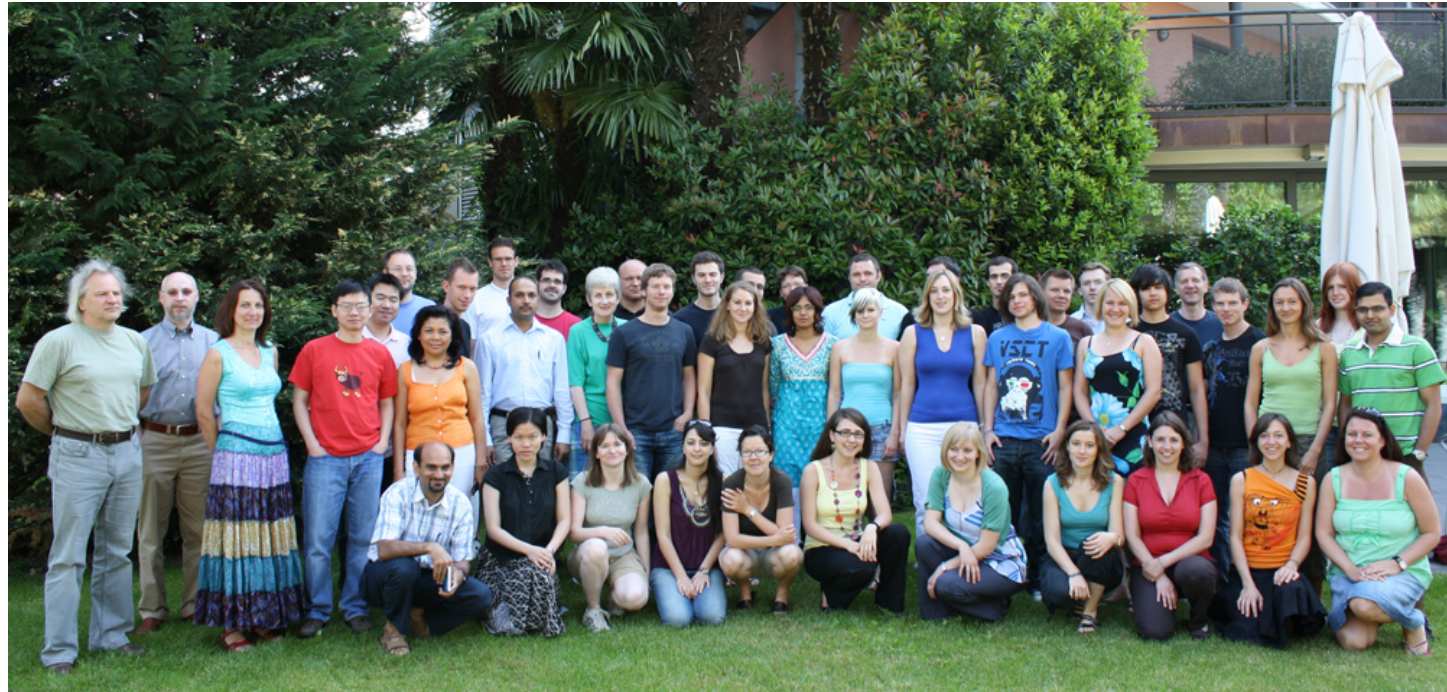
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Thank you for your attention

<http://www.maxquant.org>

<http://groups.google.com/group/maxquant-list>
<http://groups.google.com/group/andromeda-list>
<http://groups.google.com/group/perseus-list>

cox@biochem.mpg.de