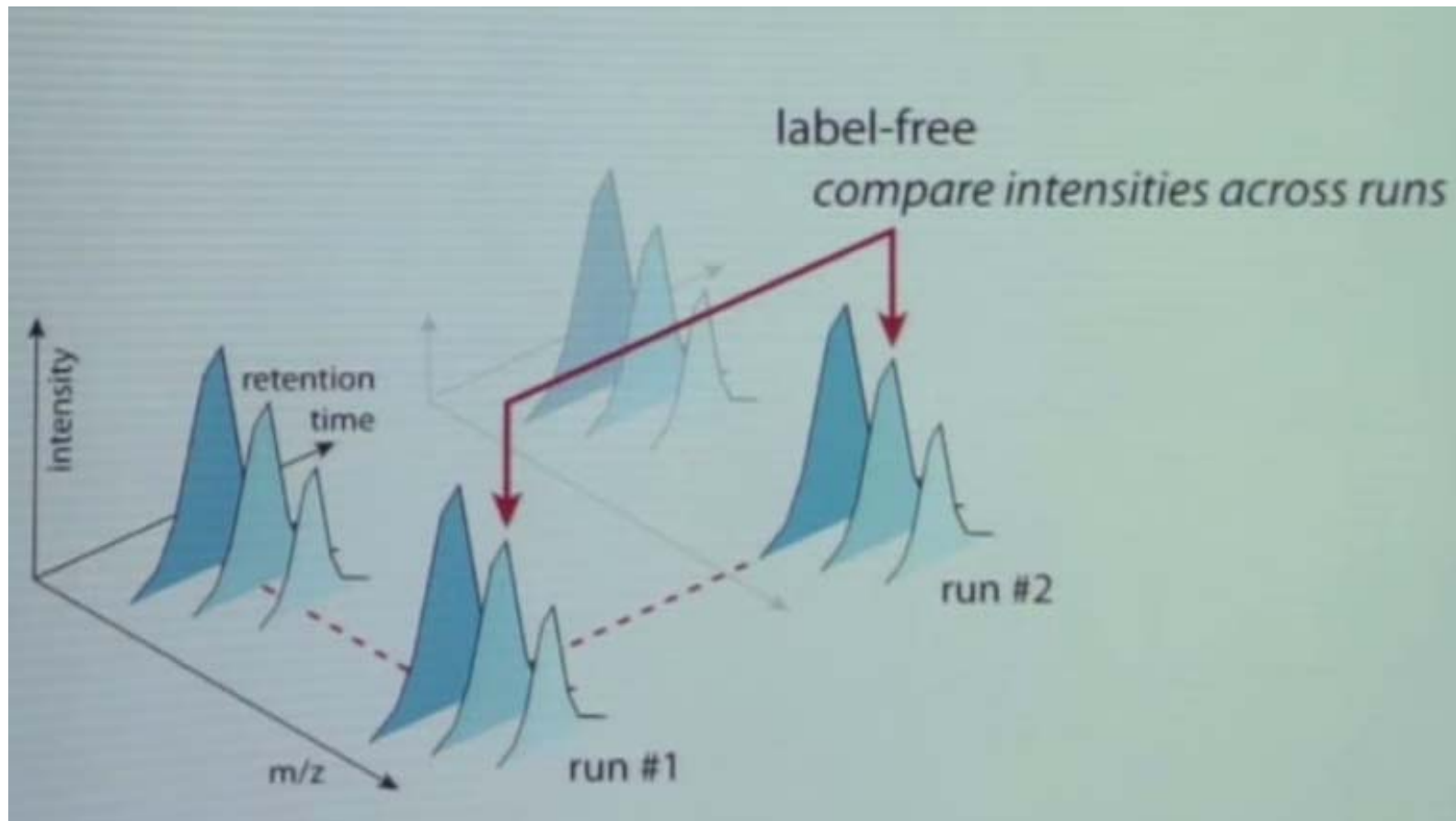


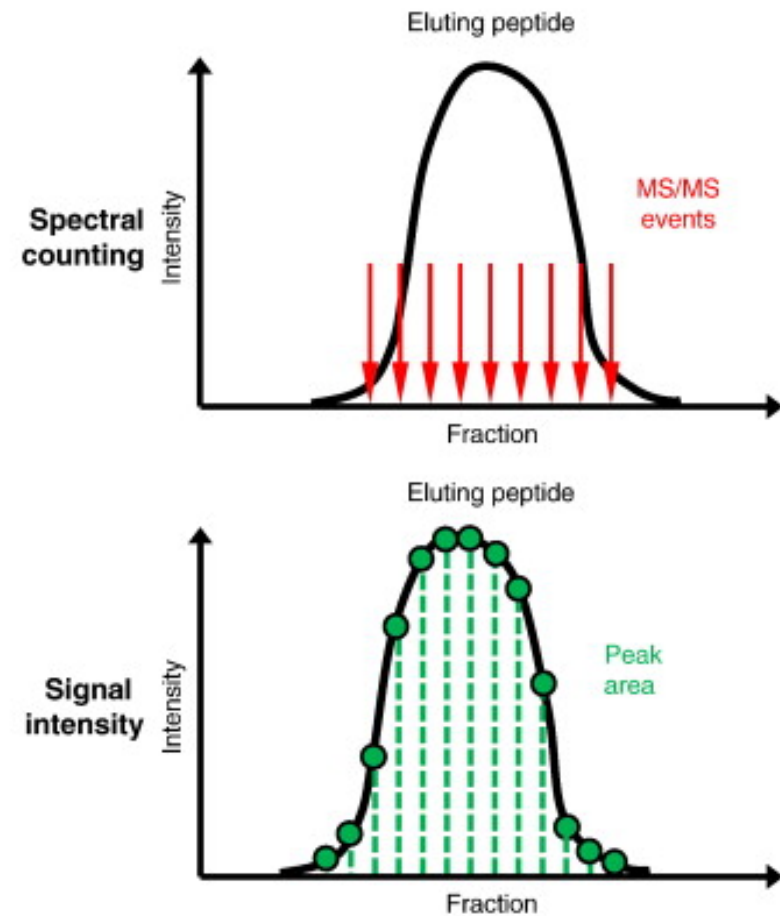
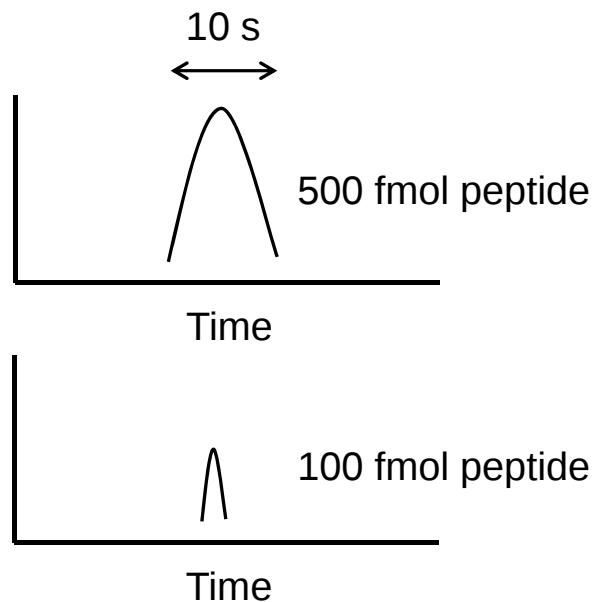
Label-free

Label-free quantification: principle



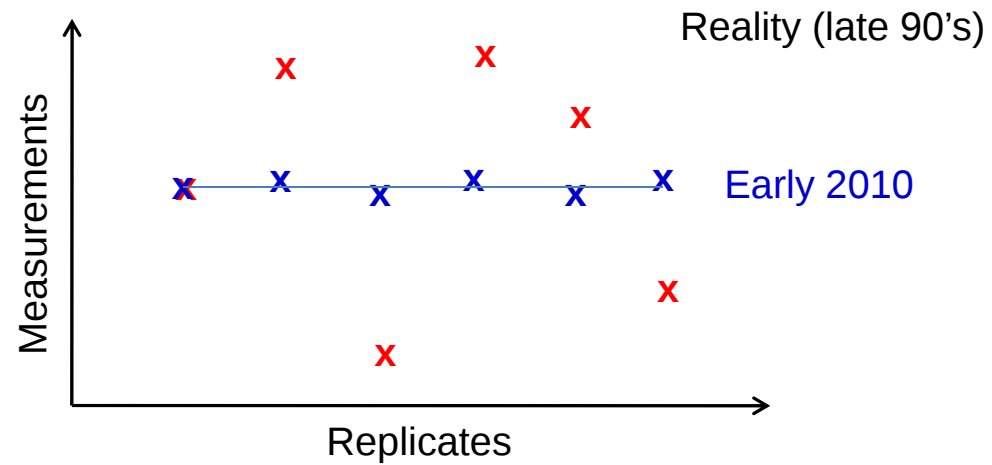
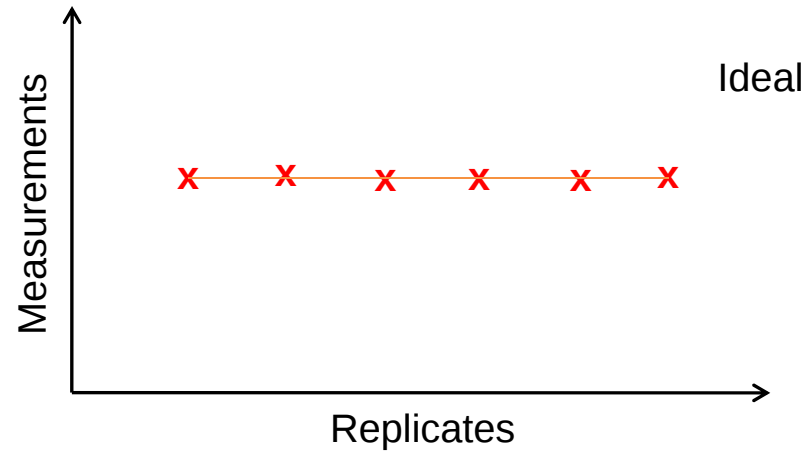
Label-free quantification

- **Spectral counting**
- **AUC (Area Under Chromatogram) or peak intensity**

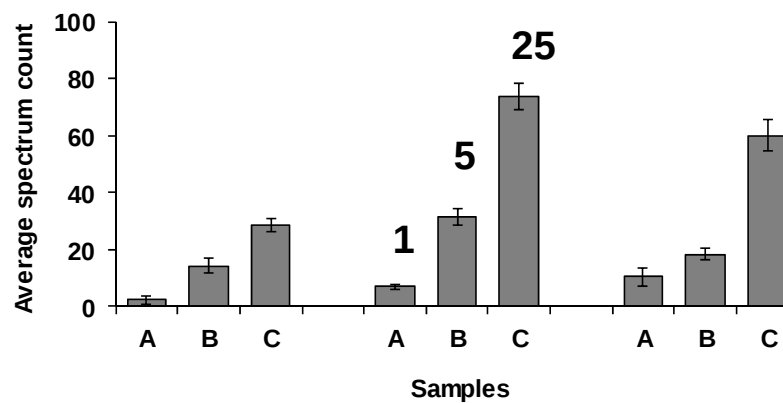
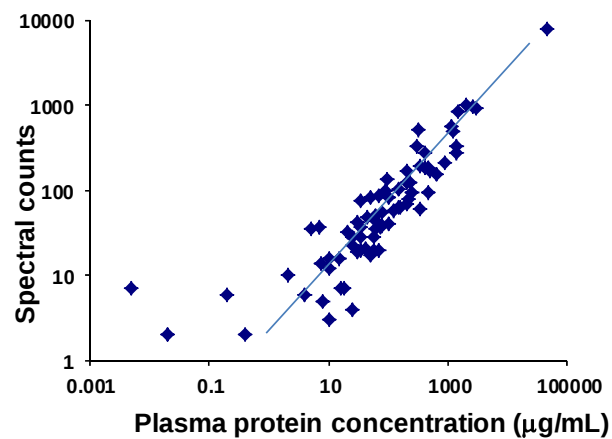
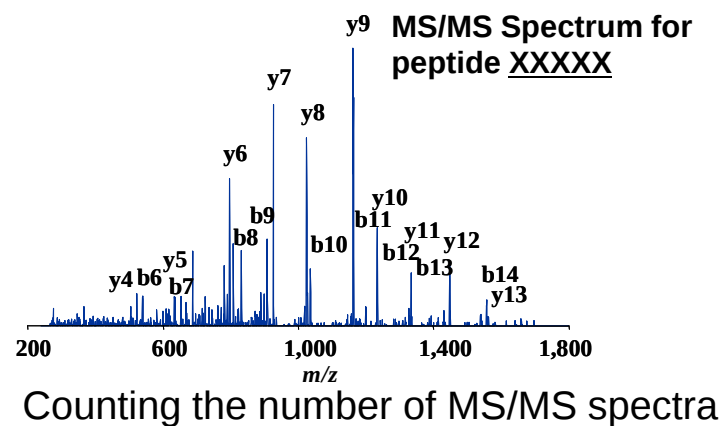
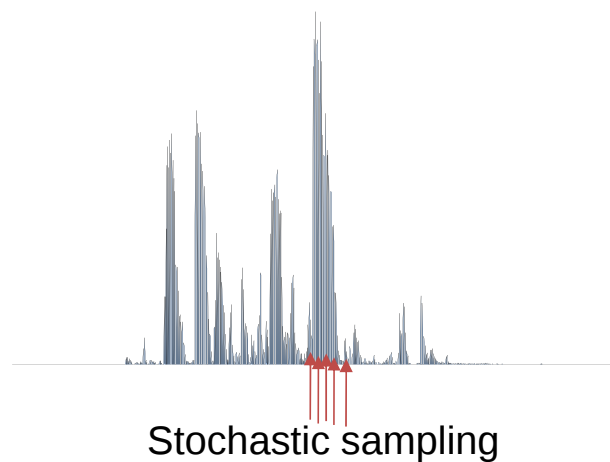


J Proteomics. 2010 Sep 10;73(10):1954-73

Label-free quantification

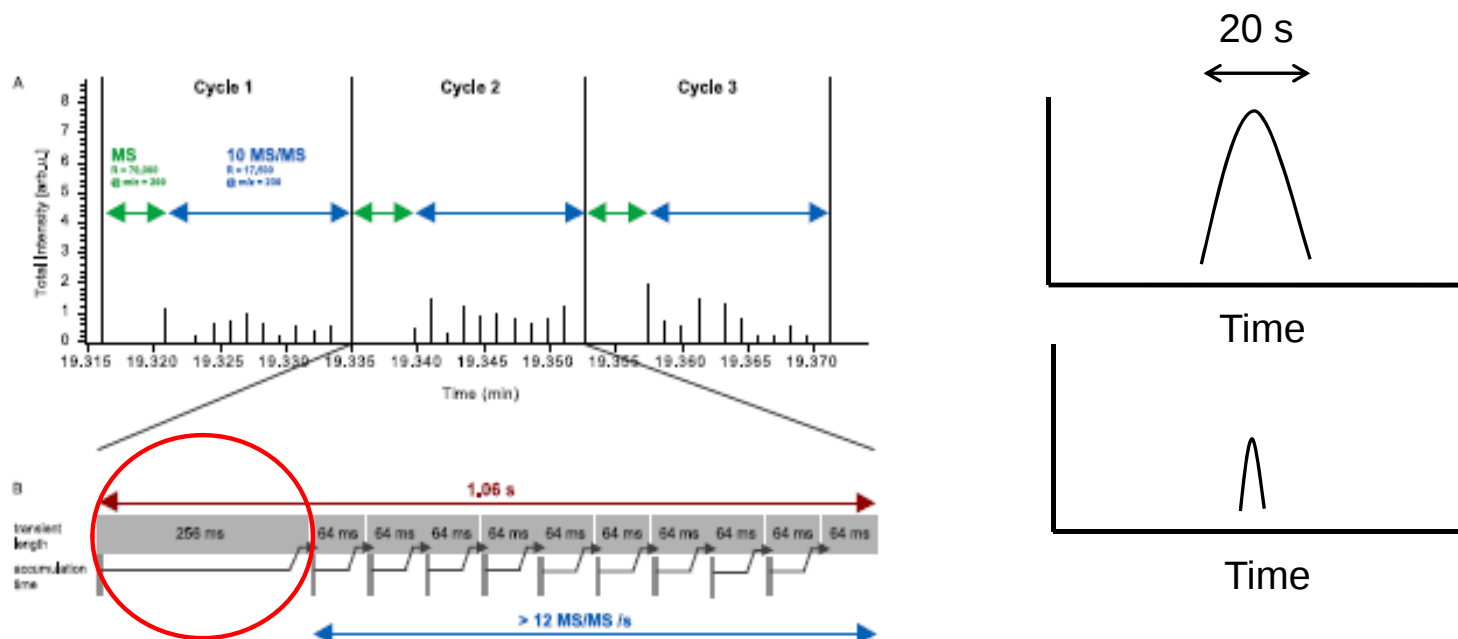


Spectral counting



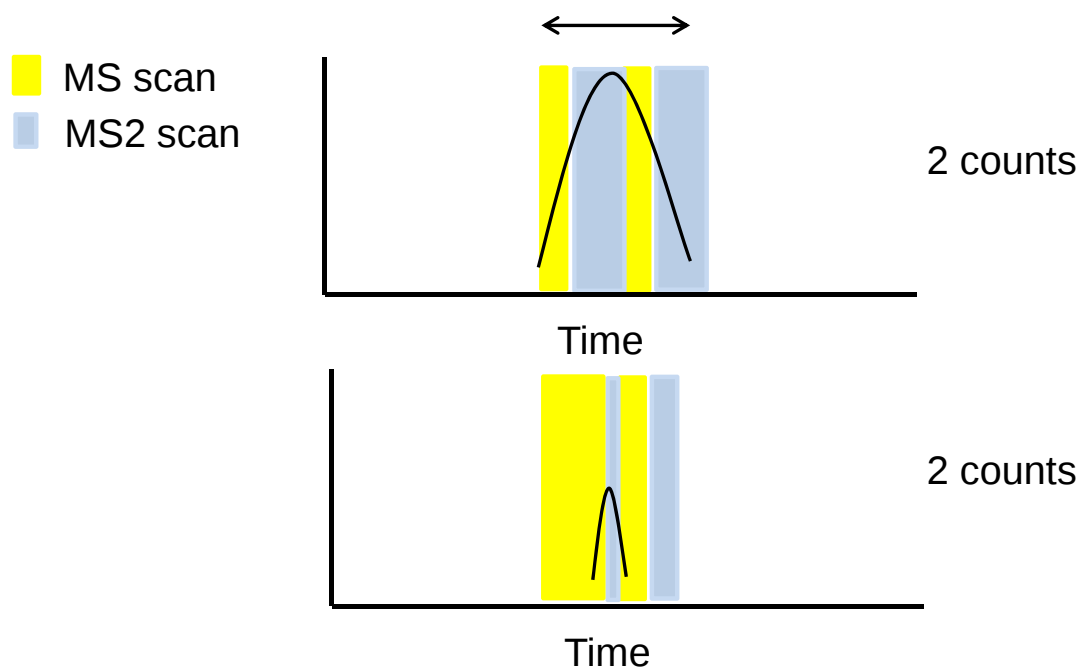
Spectral counting is semi-quantitative!

Spectral counting

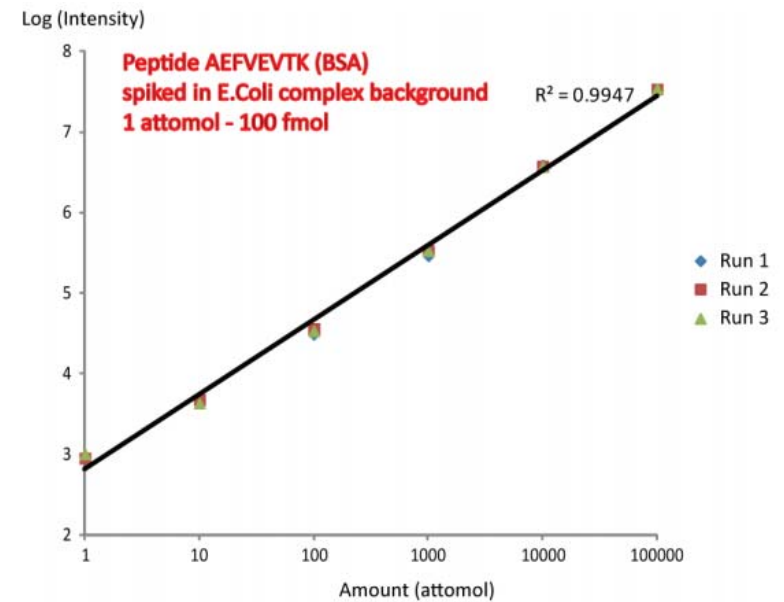
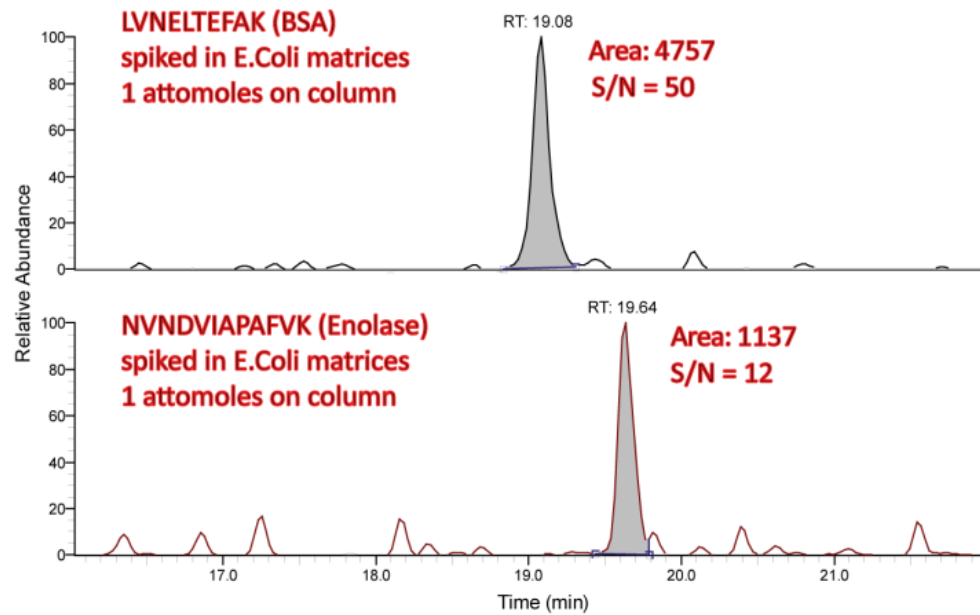


Depending on how complex the sample is at a specific retention time, the machine might be busy (i.e., doing many MS2) or idle (i.e., few or none MS2)

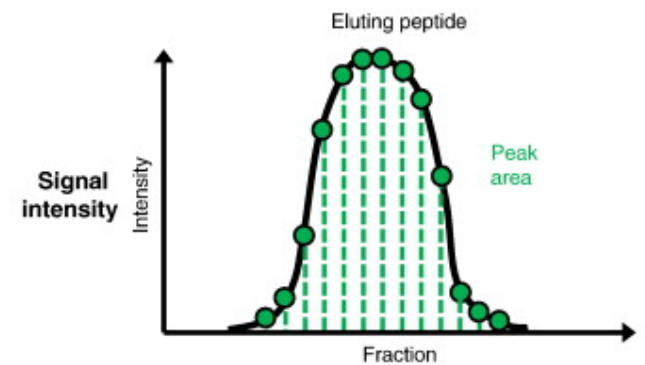
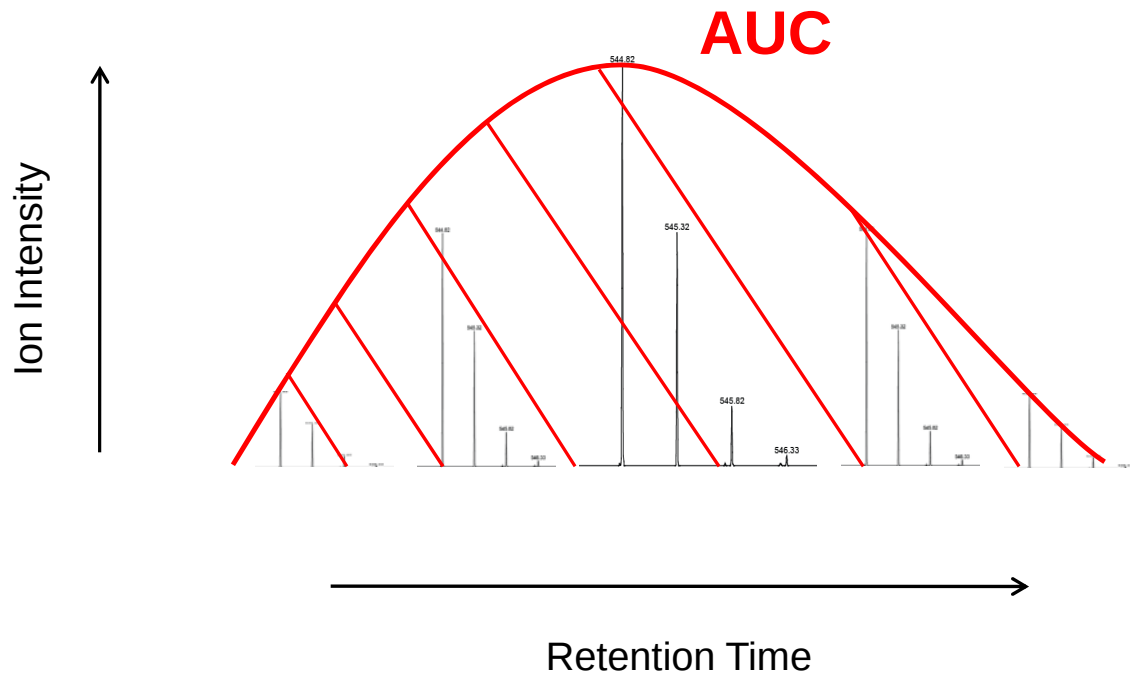
Spectral counting: limitation



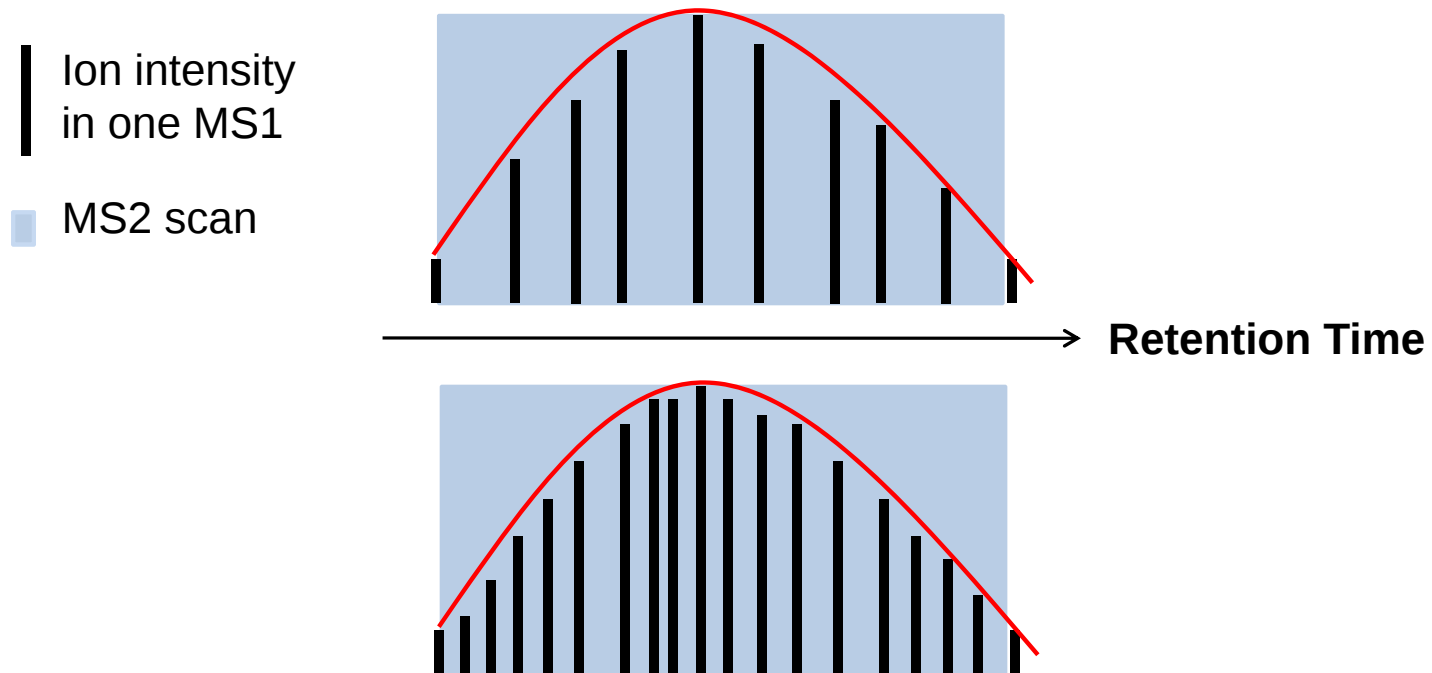
Label-free: peak area (QQQ MS)



Label-free: Area Under Curve measurement

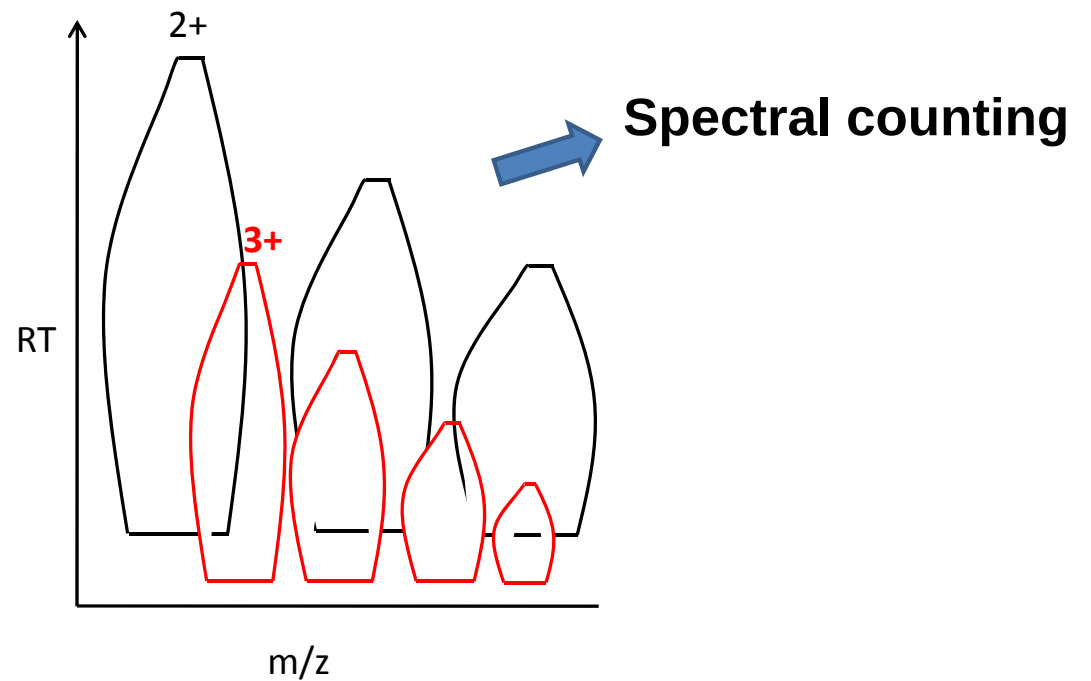
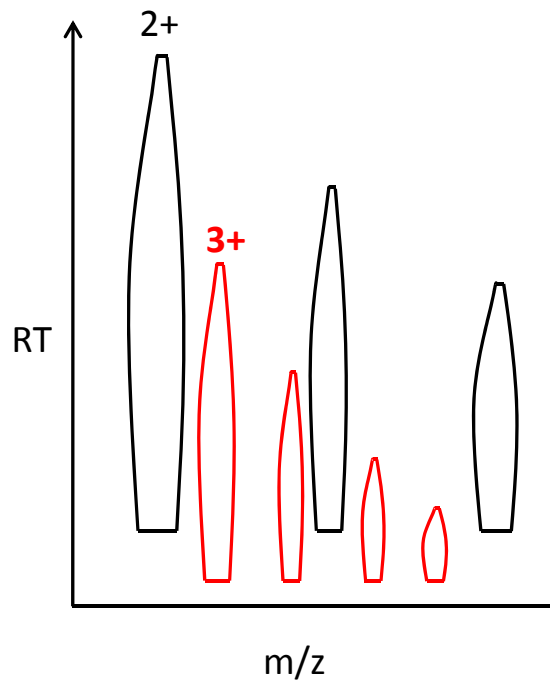


Label-free: Area Under Curve measurement



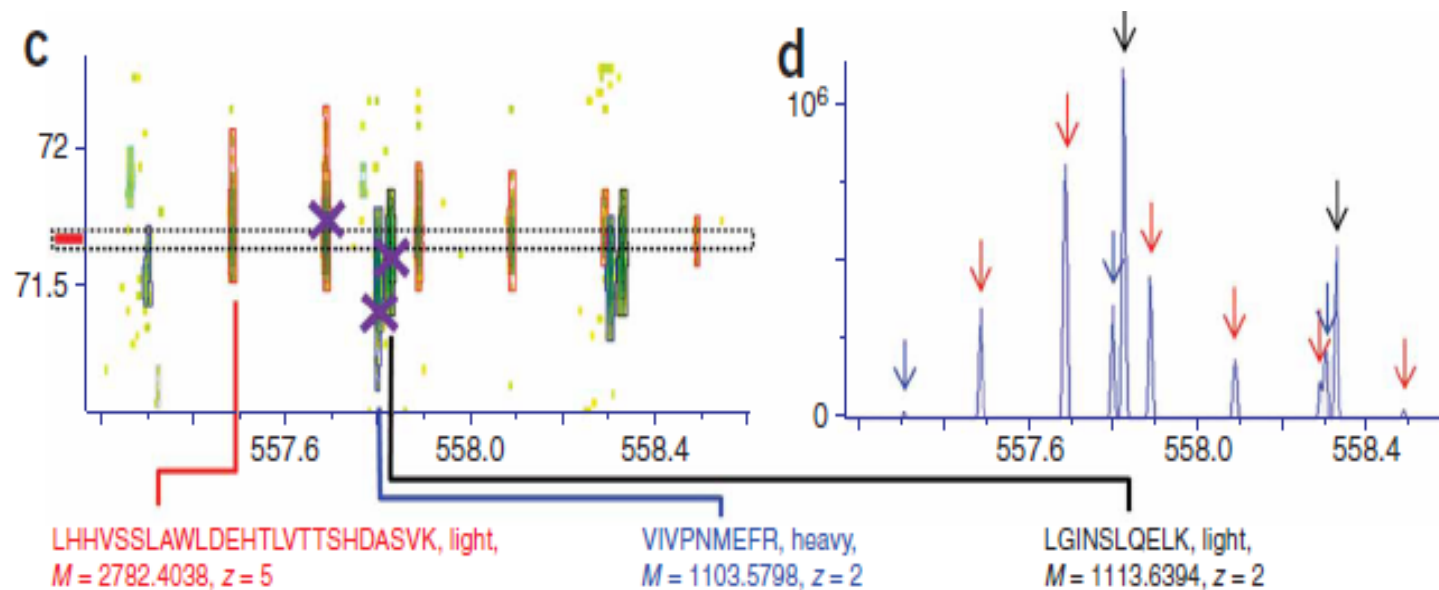
Importance of Resolution for label-free

: MS1 level feature detection

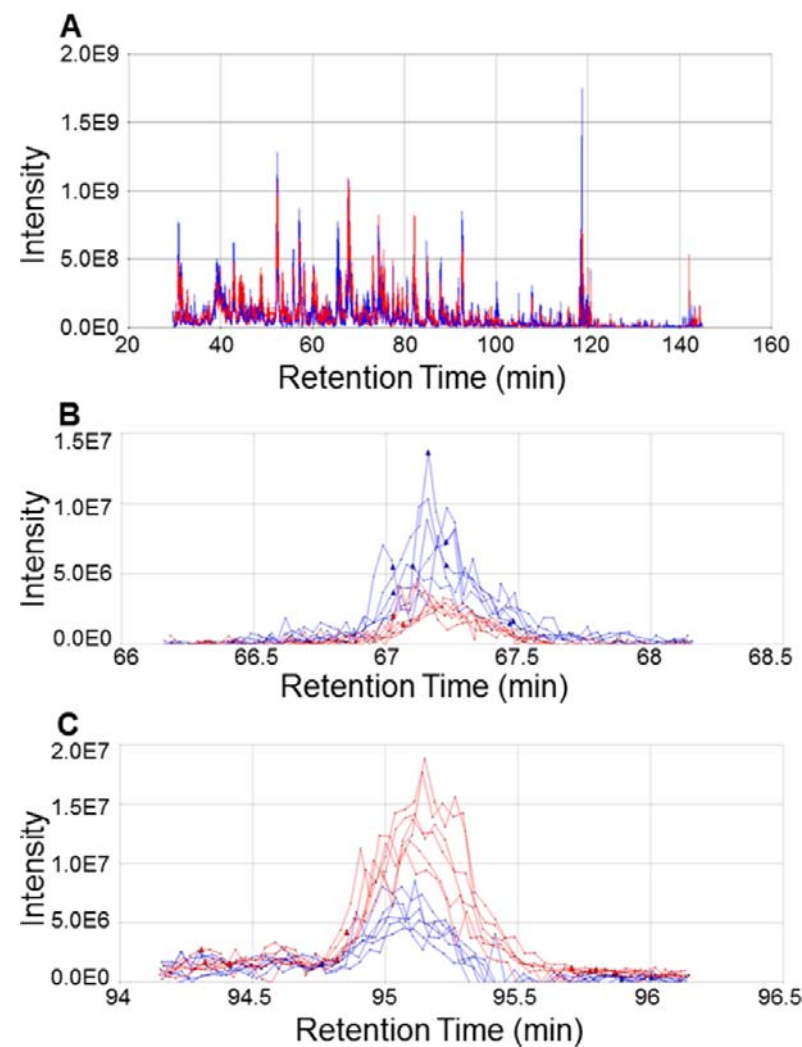
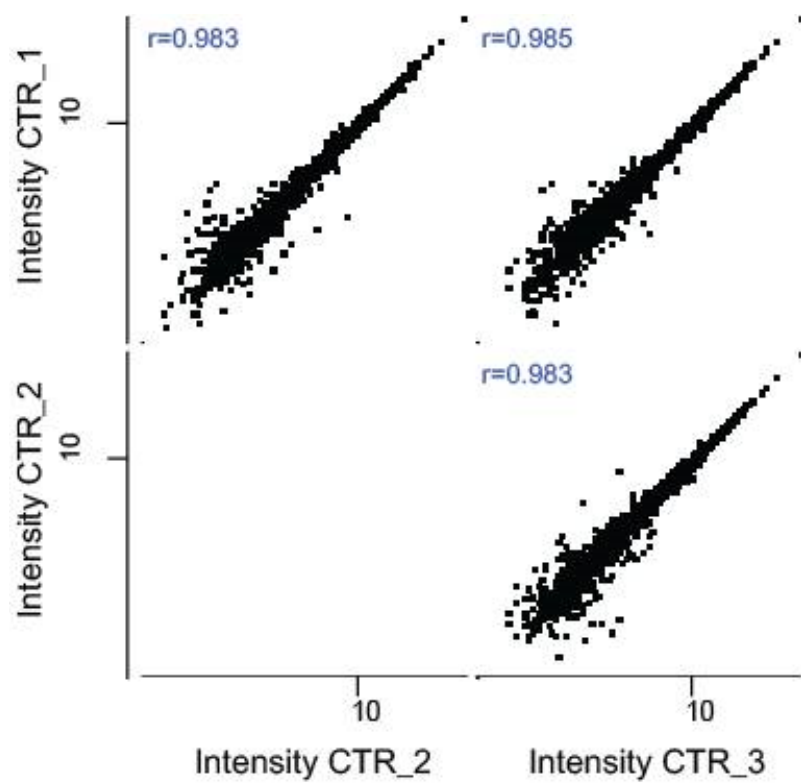


Importance of Resolution for label-free

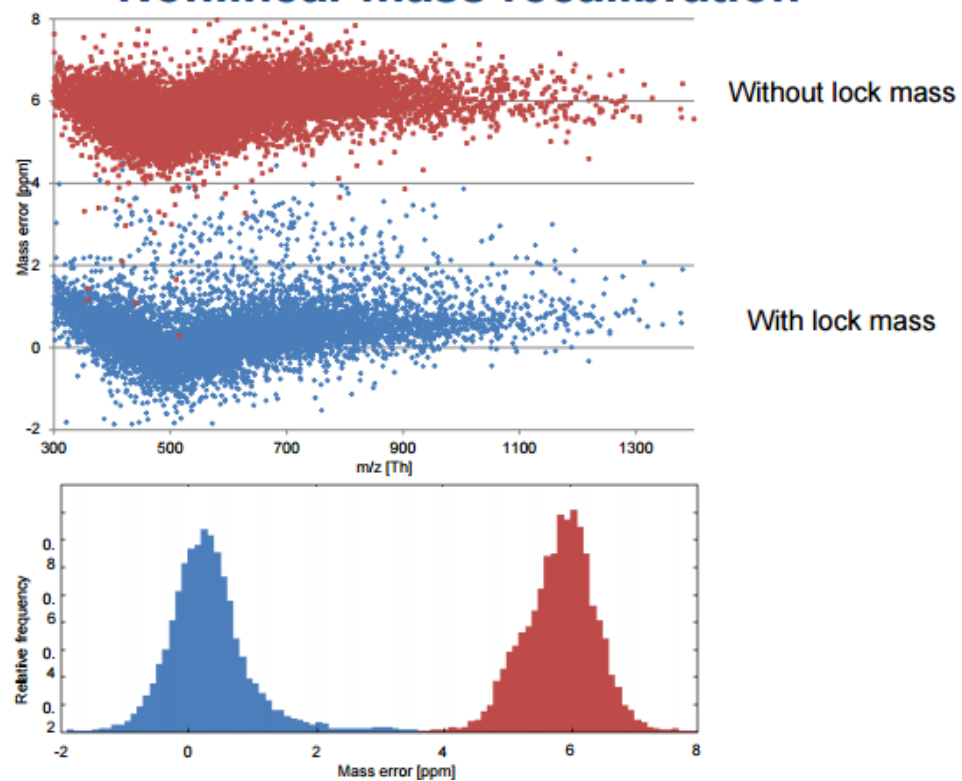
: MS1 level feature detection



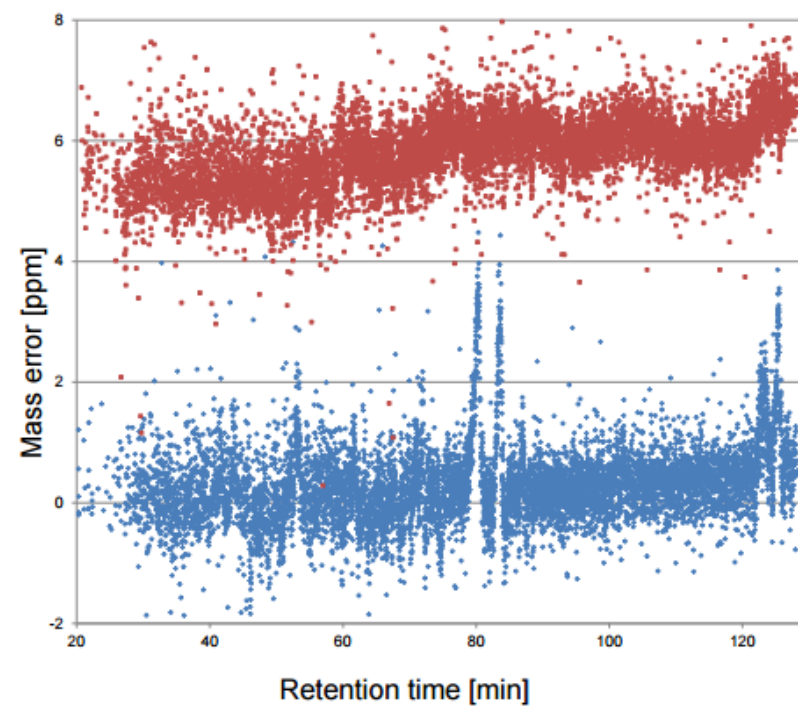
Label-free: Area Under Curve



Nonlinear mass recalibration

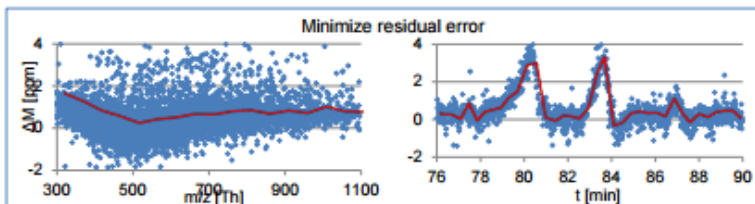
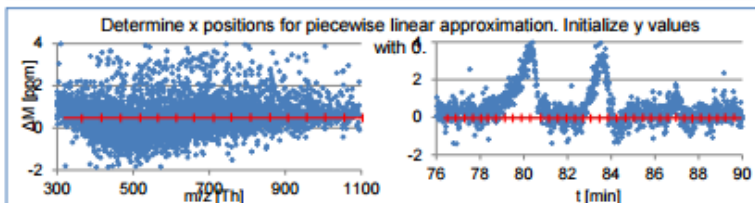
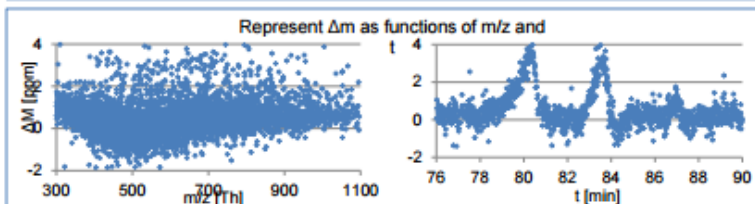


Nonlinear mass recalibration



Nonlinear mass recalibration

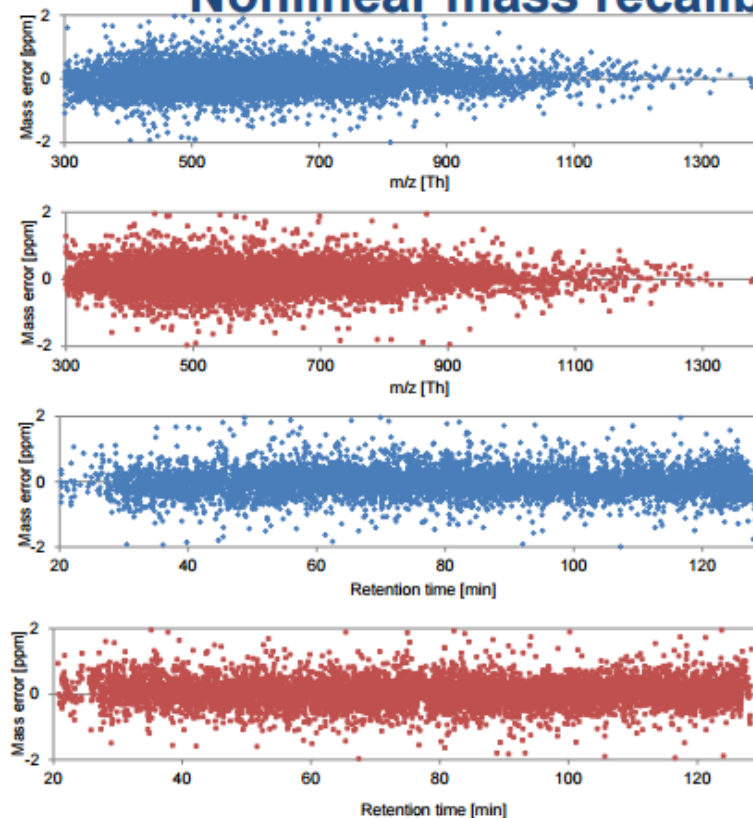
First Andromeda search with 20ppm mass tolerance and score threshold 80



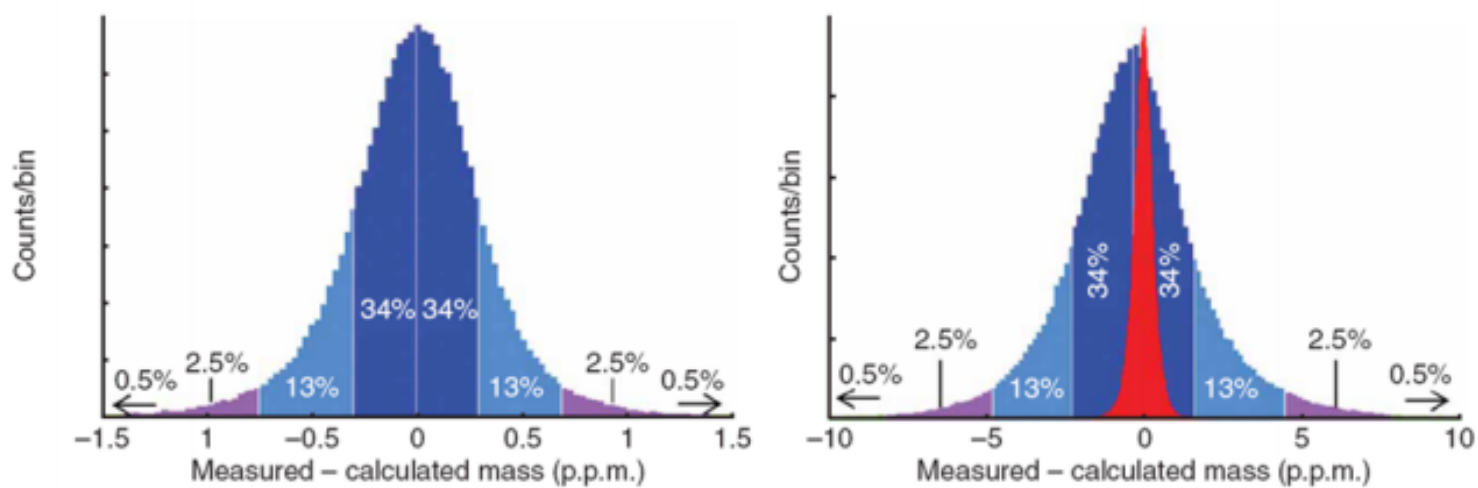
Subtract recalibration functions from all measured peptides

Perform the actual Andromeda search with small individualized mass tolerances

Nonlinear mass recalibration

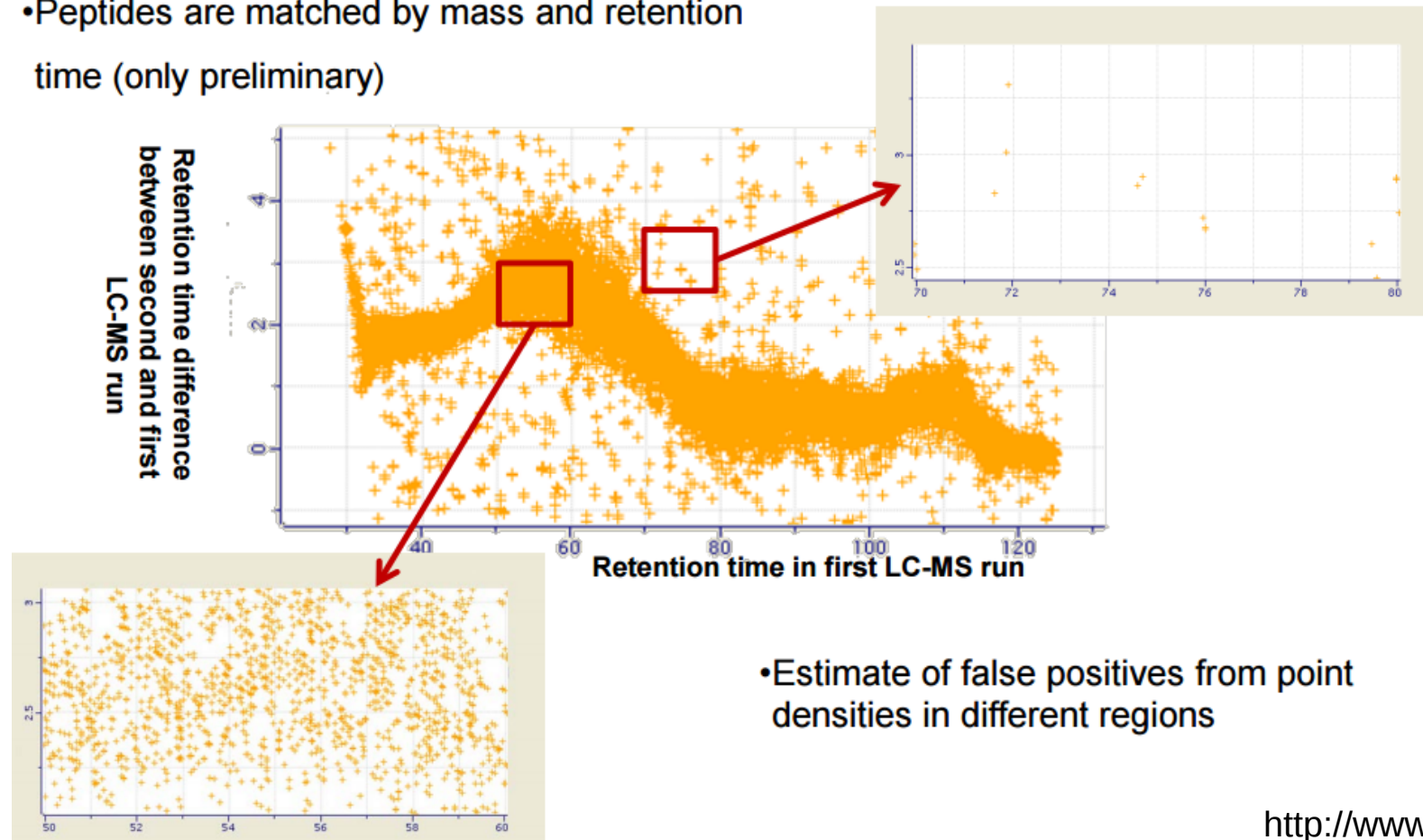


Improvement in mass accuracy

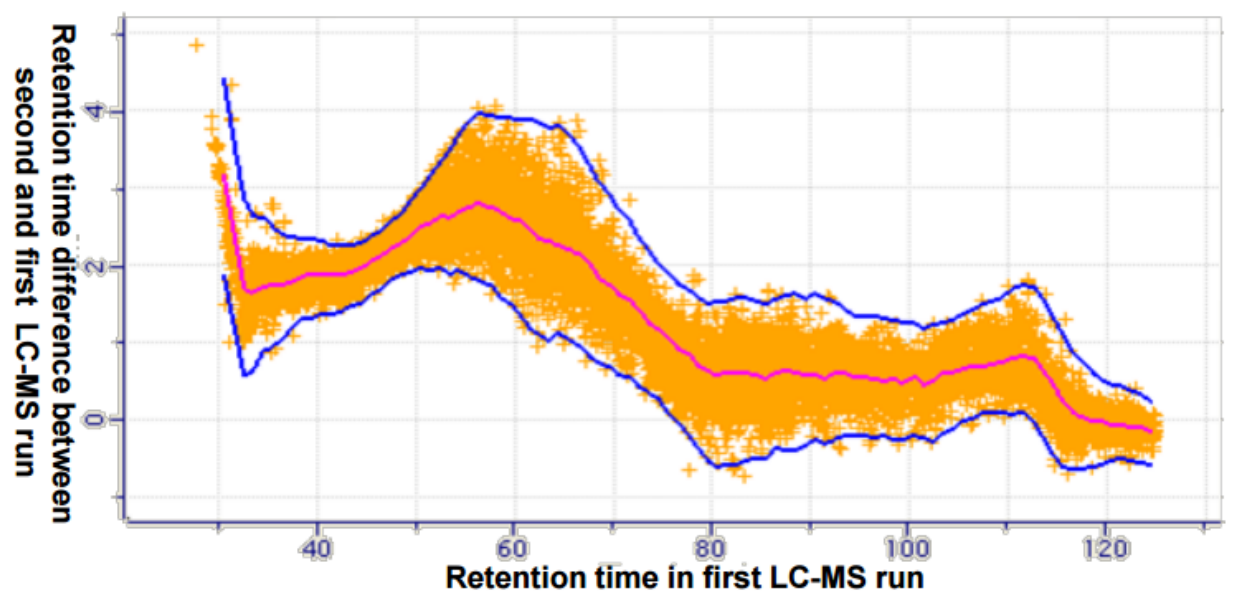


Retention time alignment

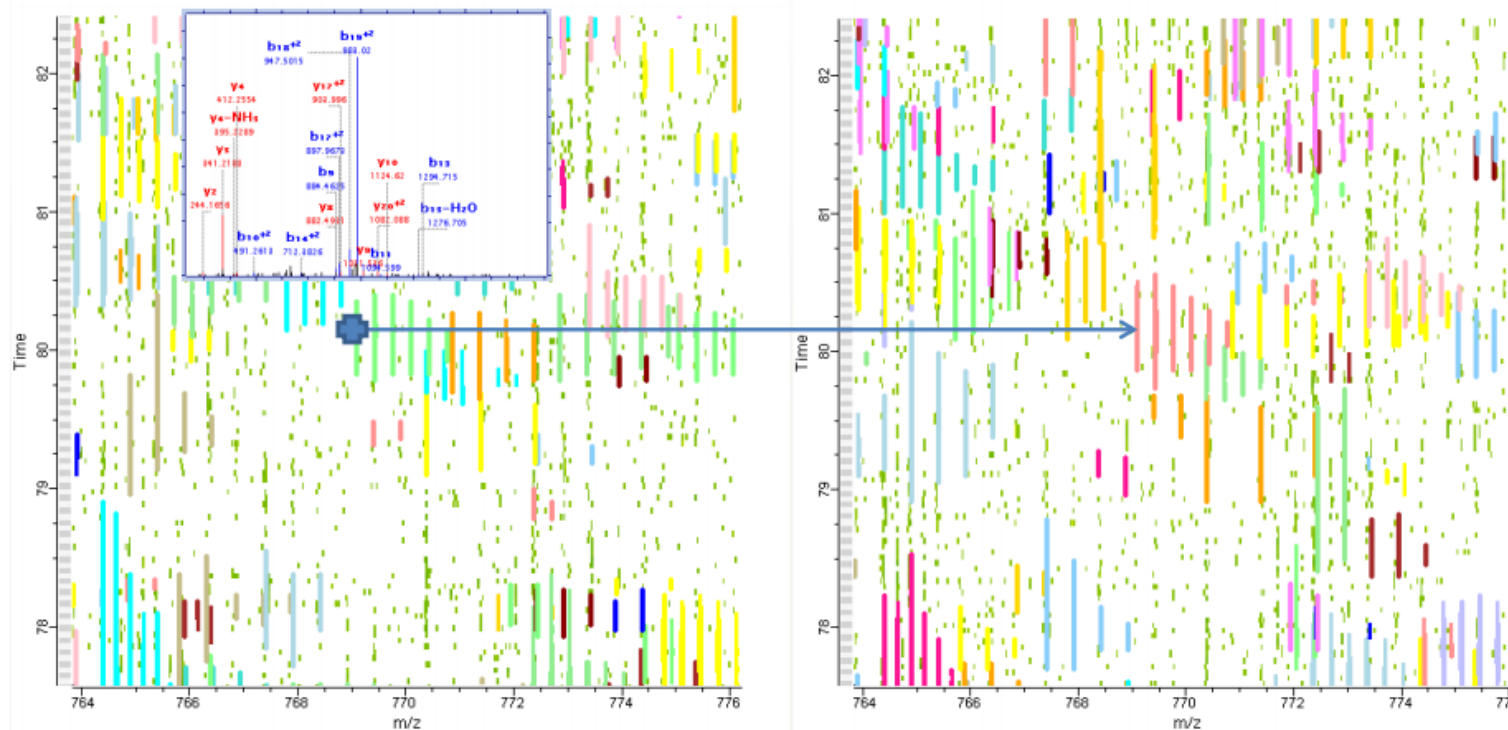
- Two LC-MS runs
- Peptides are matched by mass and retention time (only preliminary)



Retention time alignment

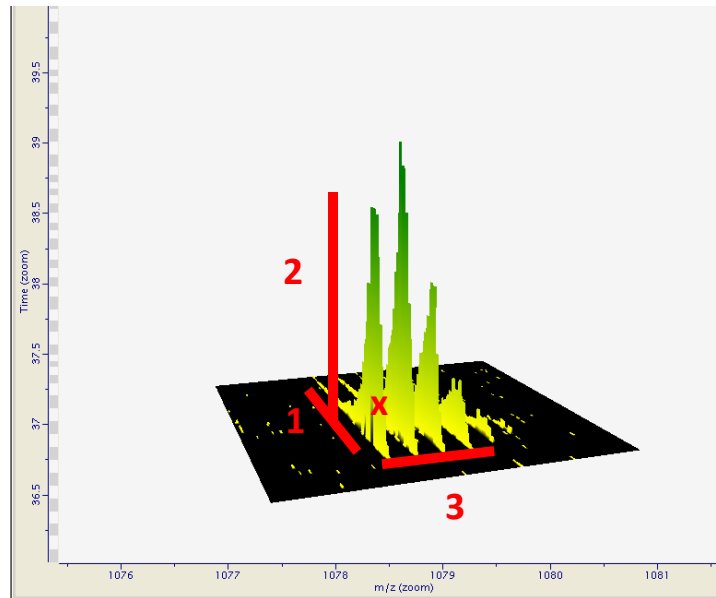
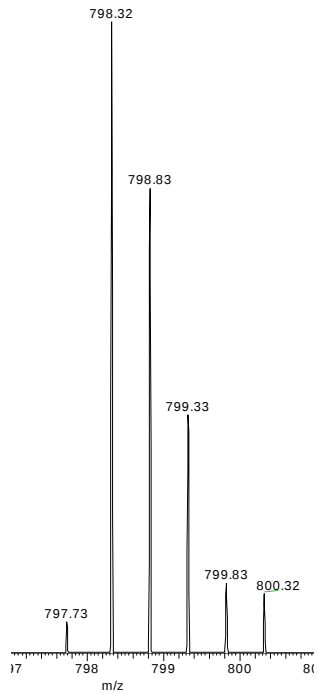


Matching between runs



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

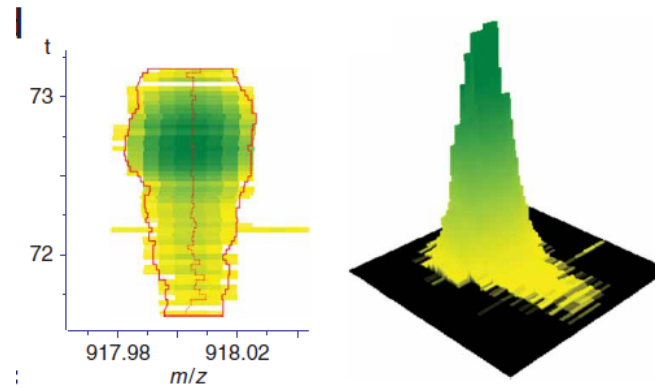
Area Under Curve measurement



1. Retention time
2. Peak intensity
3. Monoisotopic mass accuracy

Cox and Mann, Nature Biotechnol 26, 2008.

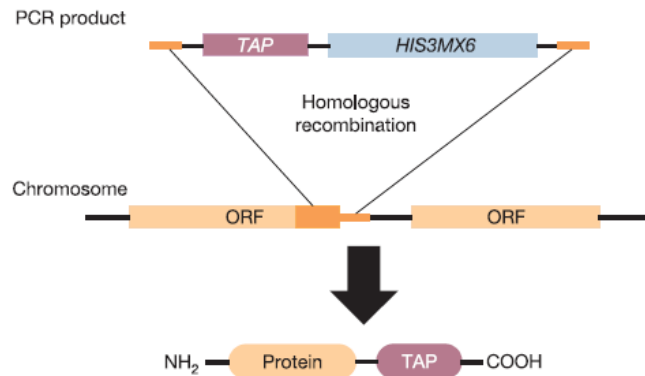
Regarding Label-free...



- Calculate individual peptide “Intensity”.
Protein Intensity = mean of peptides intensities

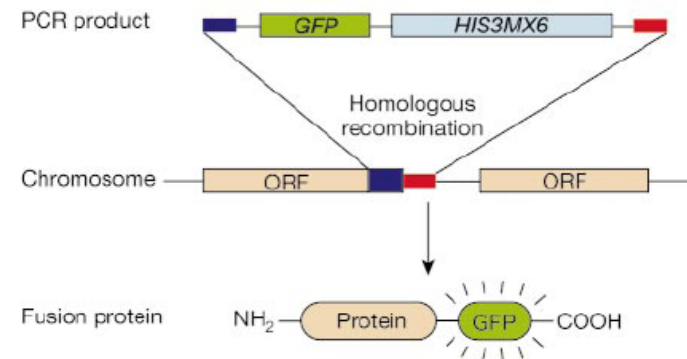
- LFQ **normalization**

Label-free is Reliable?



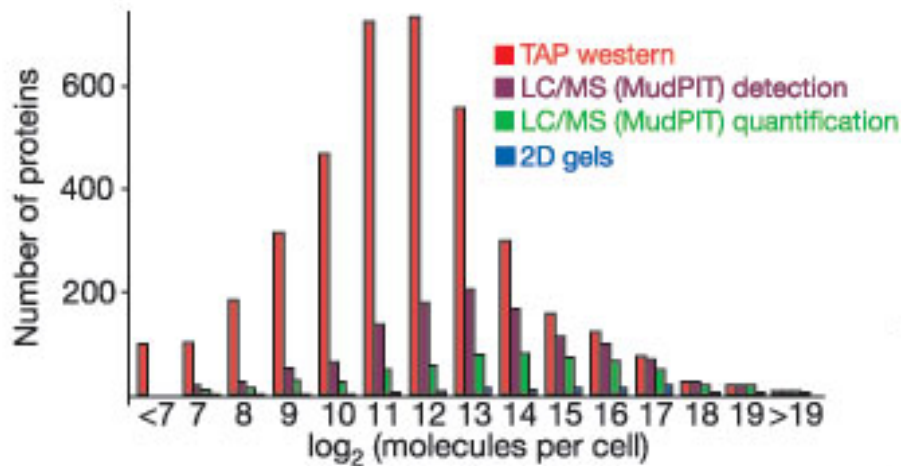
Ghaemmagami S. et al., Nature 425, 2003

Yeast model

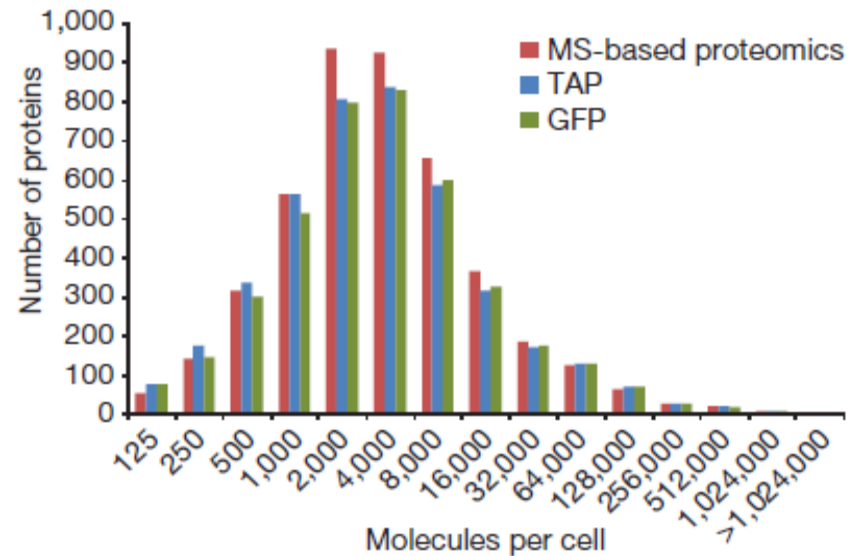


Huh WK. et al., Nature 425, 2003

Label-free is Reliable?



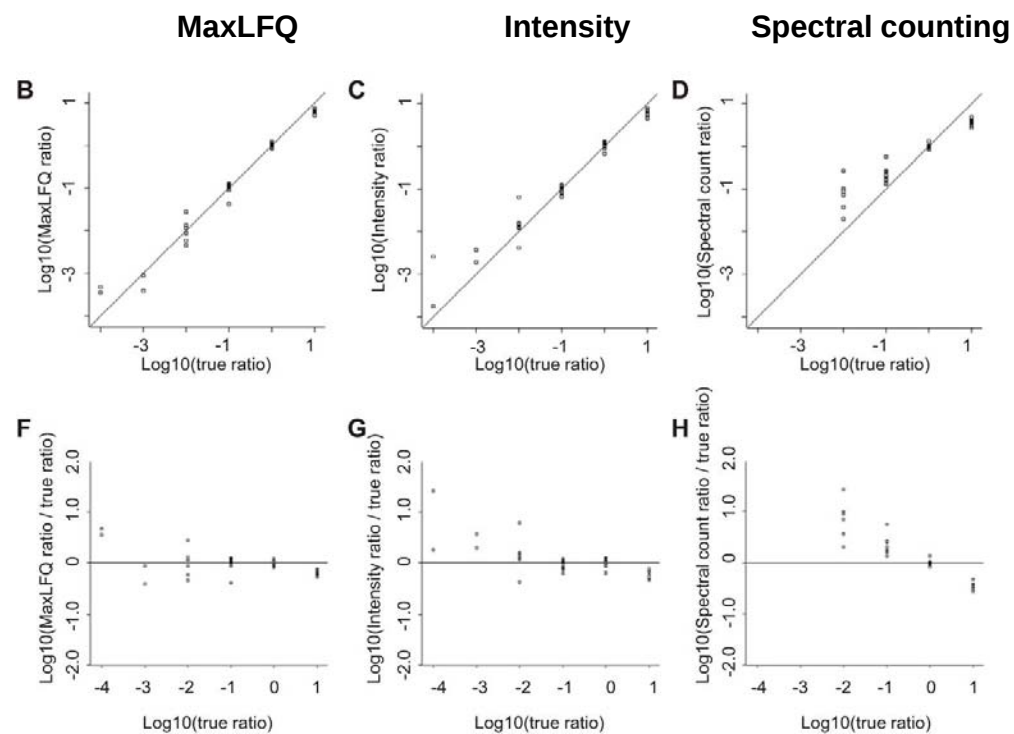
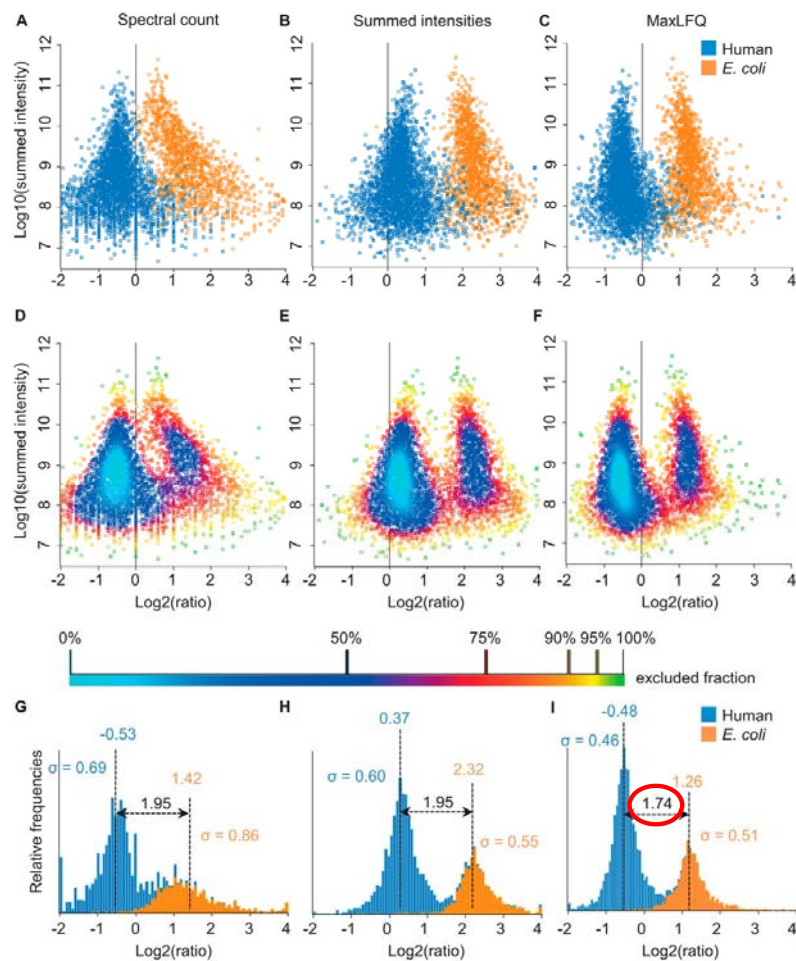
Ghaemmagami S. et al., Nature 425, **2003**



De Godoy LM. et al, Nature, **2008**.

-Label-free became reliable AND showed good correlation with a well-established model

Label-free is Reliable?



Quantitative Proteomics Reveals Subset-Specific Viral Recognition in Dendritic Cells

Christian A. Luber,^{1,7} Jürgen Cox,^{1,7} Henning Lauterbach,³ Ben Fancke,³ Matthias Selbach,⁴ Jurg Tschopp,⁵ Shizuo Akira,⁶ Marian Wiegand,^{2,8} Hubertus Hochrein,³ Meredith O'Keeffe,^{3,9,*} and Matthias Mann^{1,*}

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²Department of Molecular Virology

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³Research Immunology, Bavarian Nordic GmbH, Fraunhoferstr. 13, D-82152 Martinsried, Germany

⁴Intracellular Signalling and Mass Spectrometry, Max Delbrück Center for Molecular Medicine, Robert-Rössle Str. 10, D-13125 Berlin, Germany

⁵Department of Biochemistry, University of Lausanne, CH-1066 Epalinges, Switzerland

⁶Department of Host Defense, Research Institute for Microbial Diseases, Osaka University and ERATO, Osaka 565-0871, Japan

⁷These authors contributed equally to this work

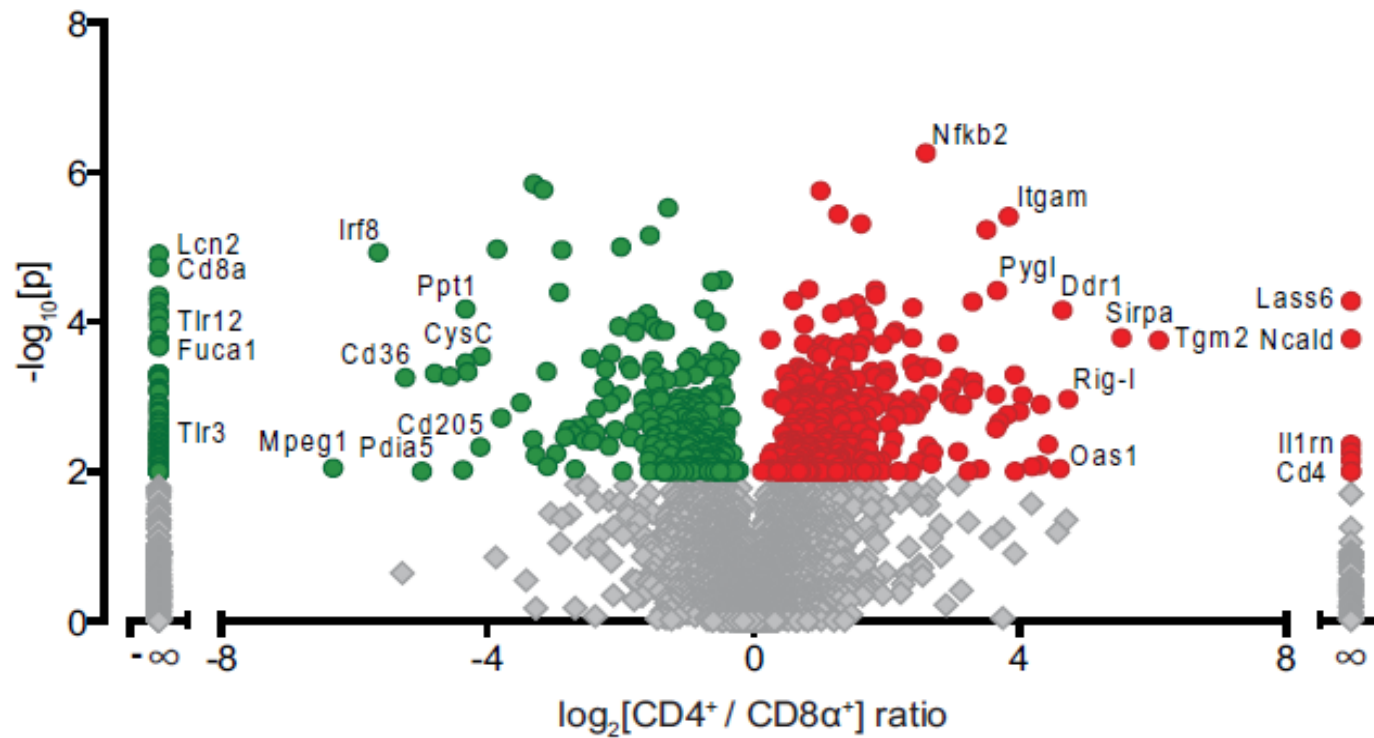
⁸Present Address: AmVac Research GmbH, Lochhamer Strasse 29, D-82152 Martinsried, Germany

⁹Present Address: Centre for Immunology, Burnet Institute, 85 Commercial Road, Melbourne Victoria, Australia 3004

*Correspondence: meredith@burnet.edu.au (M.O.), mmann@biochem.mpg.de (M.M.)

DOI 10.1016/j.immuni.2010.01.013

Label-free in primary cells

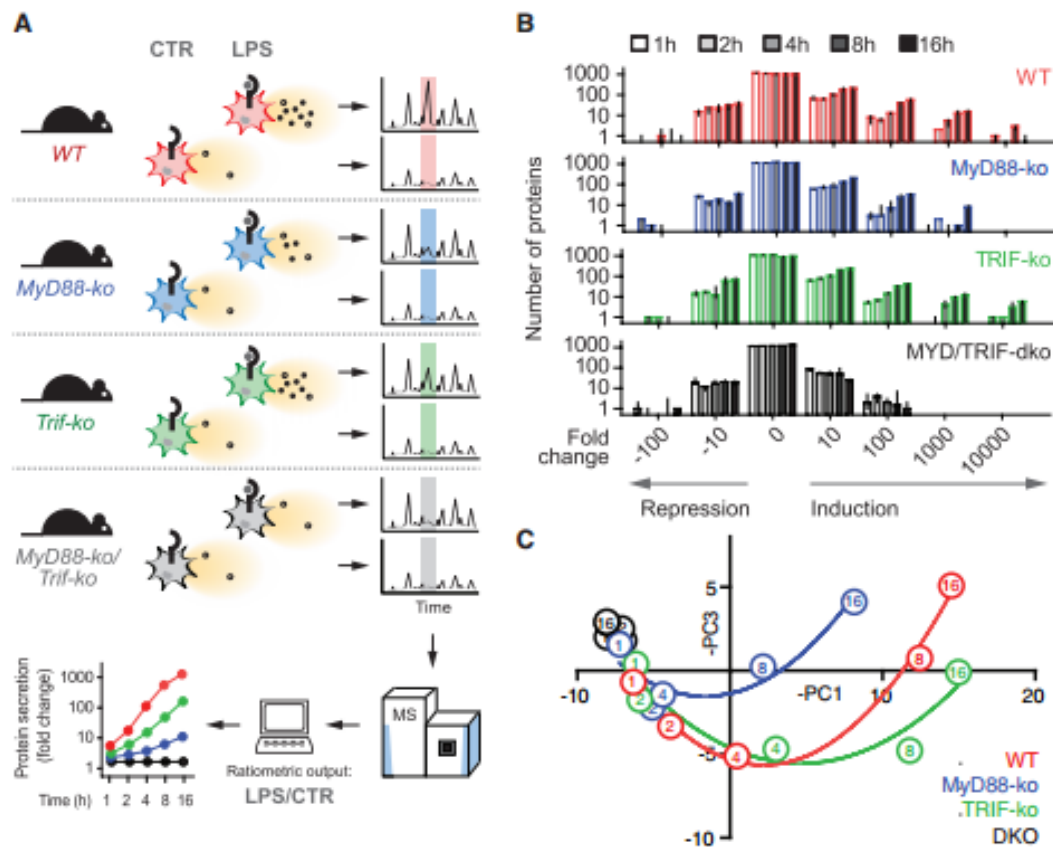


Direct Proteomic Quantification of the Secretome of Activated Immune Cells

Felix Meissner *et al.*

Science **340**, 475 (2013);

DOI: 10.1126/science.1232578



Cell-Type-Resolved Quantitative Proteomics of Murine Liver

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*Correspondence: mmann@biochem.mpg.de

<http://dx.doi.org/10.1016/j.cmet.2014.11.002>

Cell Metabolism Resource

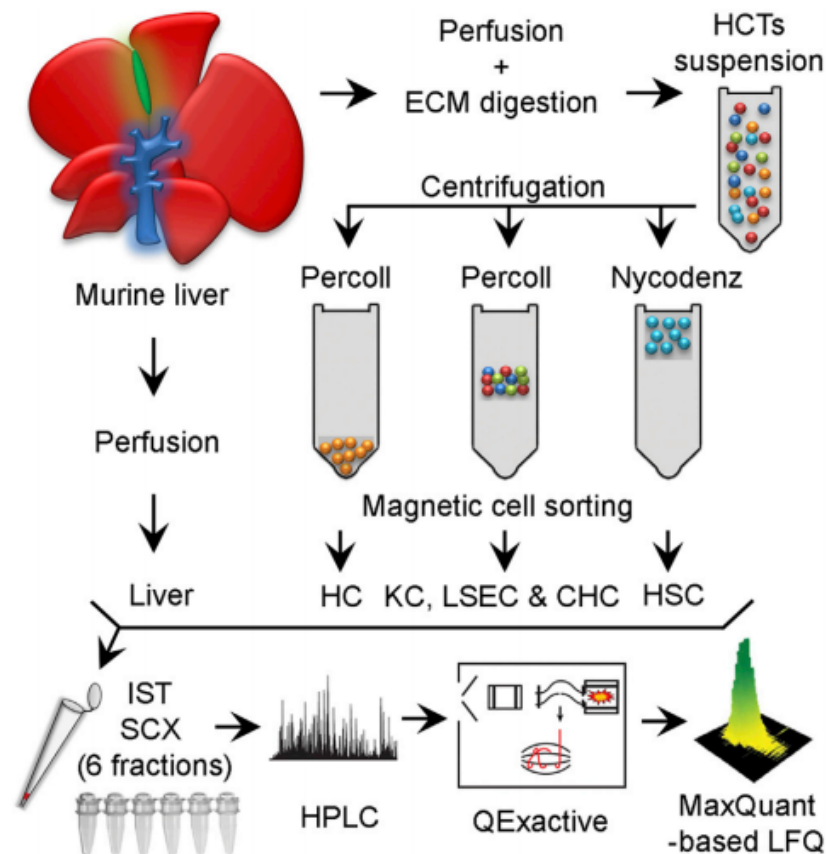


Figure 1. Proteomic Analysis of HCTs

Schematic workflow for the cell-type-resolved proteomics of murine liver.

A Map of General and Specialized Chromatin Readers in Mouse Tissues Generated by Label-free Interaction Proteomics

H. Christian Eberl,¹ Cornelia G. Spruijt,² Christian D. Kelstrup,³ Michiel Vermeulen,^{2,*} and Matthias Mann^{1,*}

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³Department for Proteomics, NNF Center for Protein Research, Faculty of Health Sciences, University of Copenhagen, Blegdamsvej 3b, DK-2200 Copenhagen, Denmark

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<http://dx.doi.org/10.1016/j.molcel.2012.10.026>

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Ultradeep Human Phosphoproteome Reveals a Distinct Regulatory Nature of Tyr and Ser/Thr-Based Signaling

Kirti Sharma,¹ Rochelle C.J. D'Souza,¹ Stefka Tyanova,¹ Christoph Schaab,¹ Jacek R. Wiśniewski,¹ Jürgen Cox,^{1,*} and Matthias Mann^{1,*}

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<http://dx.doi.org/10.1016/j.celrep.2014.07.036>

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