

Protein mass spectrometry and proteomics

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Spring Semester 2026

EPFL

Course outline

1. Proteomics and mass spectrometry

Introduction to protein analysis and proteomics; Reminders in mass spectrometry; Why proteomics and mass spectrometry?; Ionization sources, analysers, and detectors used in proteomics; Latest generation of mass spectrometers used in proteomics

2. Mass spectrometry-based proteomic strategy and workflows

Bottom-up versus top-down strategies; Data-dependent acquisition (DDA) and data-independent acquisition (DIA) approaches; Sample preparation

Lab visit of the Proteomics Laboratory at Nestlé Research (EPFL Innovation Park)

3. Quantitative proteomic workflows

Label-free methods; Labelling-based techniques; Other quantitative techniques

4. Proteomic bioinformatics

Databases; Identification of protein; Quantification of proteins; Bioinformatics tools; Practical examples

5. Applications to biology, clinical research, and beyond

What strategy?; Experimental design & randomization; Biomarker discovery; Industrialized and population proteomics; Forensics; Targeted mass spectrometry-based approaches; Other biological applications of mass spectrometry; Advanced innovations (single-cells, 4D proteomics, multi-omics) and emerging technologies; Limitations and ethical consideration

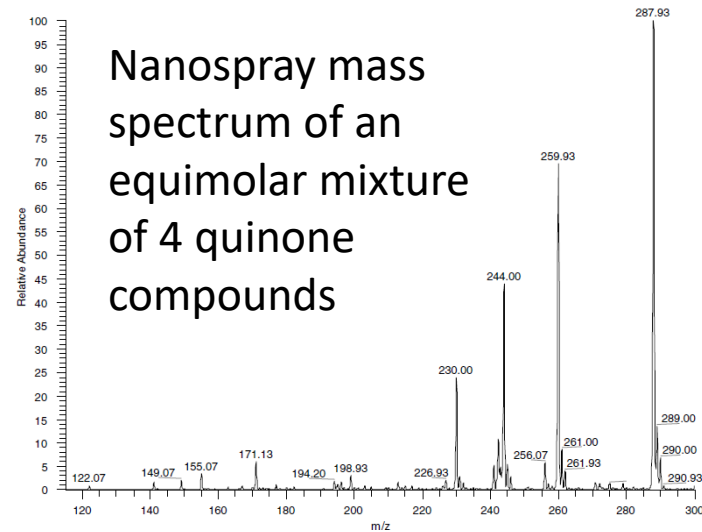
Additional support/information on [Separations techniques in proteomics](#)

Course outline

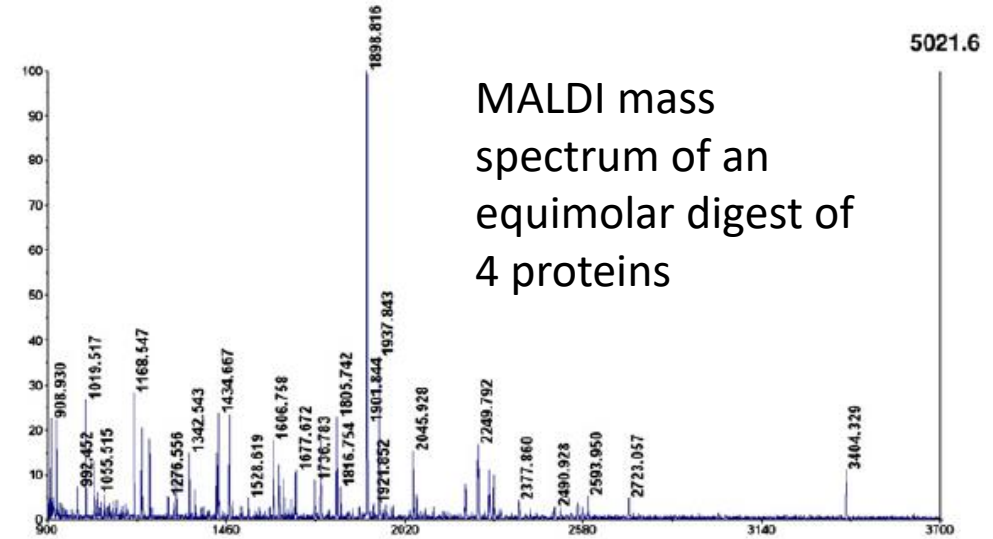
- 3. Quantitative proteomic workflows

Label-free methods; Labelling-based techniques; Other quantitative techniques

Mass spectrometry is not inherently quantitative



doi:10.1016/j.jelechem.2004.03.029

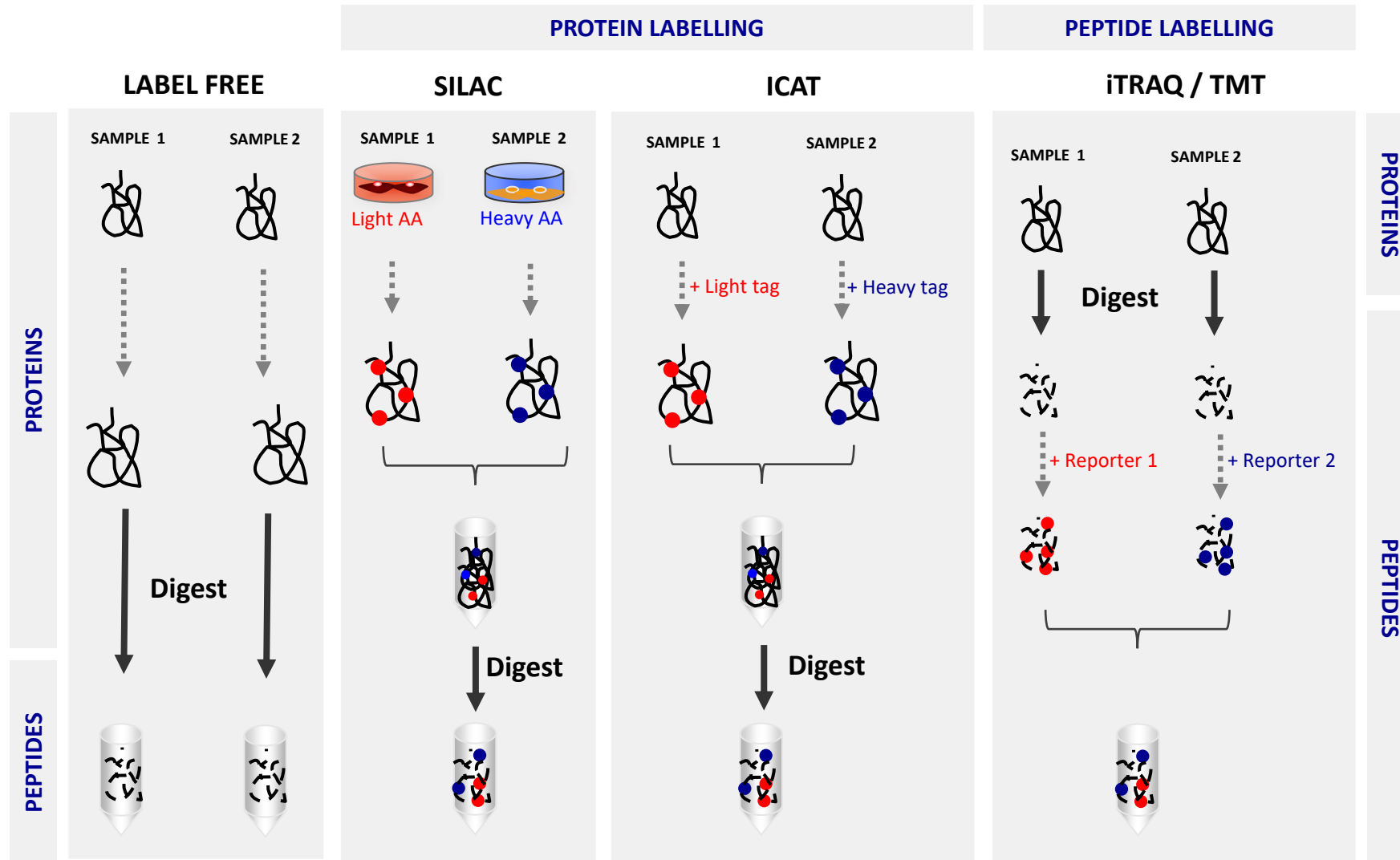


doi:10.1016/j.jprot.2008.11.009

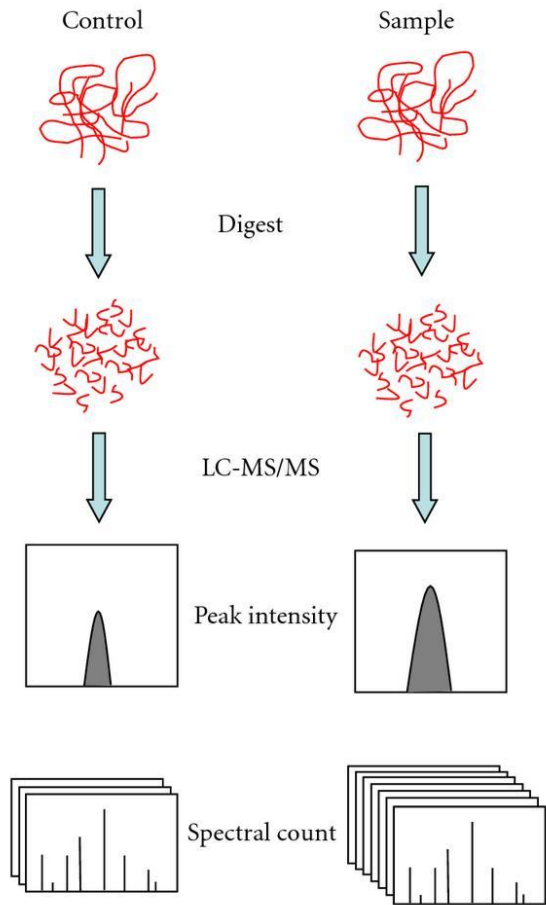
But we need to compare proteomes quantitatively and therefore, methodologies have been implemented to turn MS-based proteomics quantitative

doi:10.1038/nchembio736

Protein relative quantitation with mass spectrometry



3.1. Label-free methods



- Spectrum Counting, which counts and compares the number of fragment spectra identifying peptides of a given protein
- Total Ion Count (TIC), which considers peak intensities from MS/MS spectra combined with counting of the spectra
- Precursor Ion Intensity, which measures and compares the mass spectrometric signal intensity of peptide precursor ions belonging to a particular protein

Spectrum counting

spectral counting: quantification based on spectral counting and concomitant identification (MS/MS)
dynamic range with spectral counting: 3 orders of magnitude

Total spectra

This method uses the sum of all the spectra associated with a specific protein within a sample which includes also those spectra that are shared with other proteins and is referred to as the Total Spectrum Count

emPAI

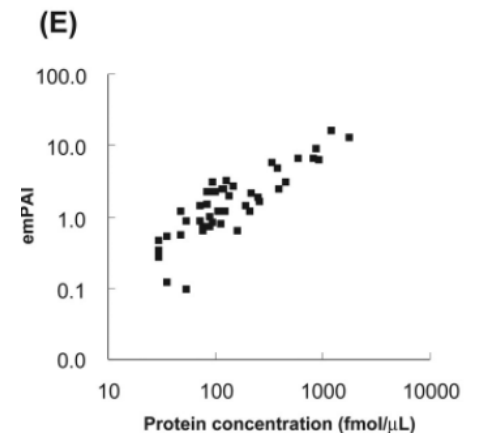
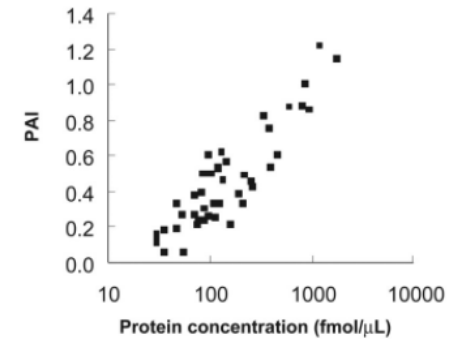
Spectrum Counting methods can also be used in the determination of absolute abundance of proteins. The Protein Abundance Index (PAI) is defined as the number of observed peptides divided by the number of all possible tryptic peptides from a particular protein, that are within the mass range of the employed mass spectrometer:

$$PAI = \frac{N_{observed}}{N_{observable}}$$

where $N_{observed}$ is the number of experimentally observed peptides and $N_{observable}$ is the calculated number of observable peptides for each protein.

In a subsequent refinement PAI was transformed into an exponential form called emPAI and defined as follows:

$$emPAI = 10^{PAI} - 1$$



Precursor ion intensity

extracted ion chromatogram (XIC): quantification based on peptide-ion intensity (MS) and subsequent identification (MS/MS)
dynamic range for intensities: 6 orders of magnitude

Average Precursor Intensity

This method takes the geometric mean of the peptide intensity values for a given protein

Total Precursor Intensity

The sum of all distinct intensity values for a protein

Top Three Precursor Intensities

The sum of the three highest peptide intensity values for a protein. If fewer than three peptides have intensity values, the intensities that are present are summed

iBAQ

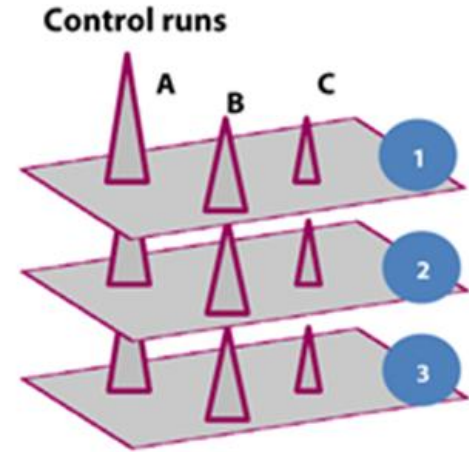
iBAQ (Intensity-Based Absolute Quantification) is a popular approach for absolute quantification of proteins.

It is similar in its approach to the emPAI method. iBAQ and similar algorithms are called intensity-based because they calculate the sum of parent or precursor ion intensities of identified peptides per protein.

iBAQ intensities provide an accurate determination of the relative abundance of all proteins identified in a sample:

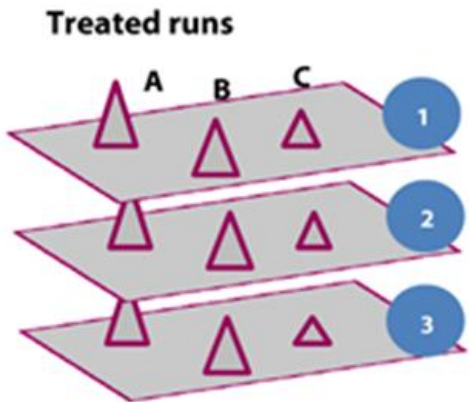
$$\text{iBAQ: } \Sigma \text{ intensity} / \# \text{theoretical peptides}$$

Precursor ion intensity



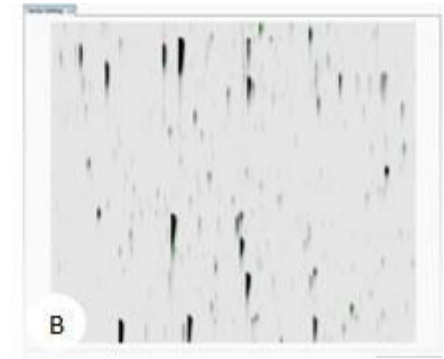
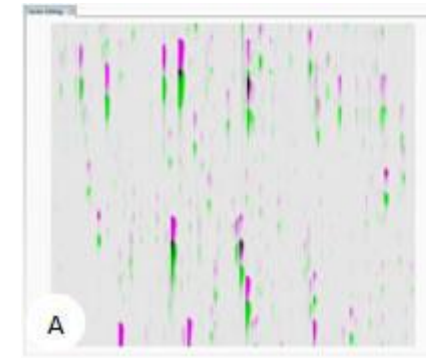
Control runs	
Peptides unique to Protein X	
Run 1	Σ peptide ion abundances A, B, C
Run 2	Σ peptide ion abundances A, B, C
Run 3	Σ peptide ion abundances A, B, C

ANOVA p-value & fold change calculated from **SUM of unique peptide ion abundances per run** for a specific protein

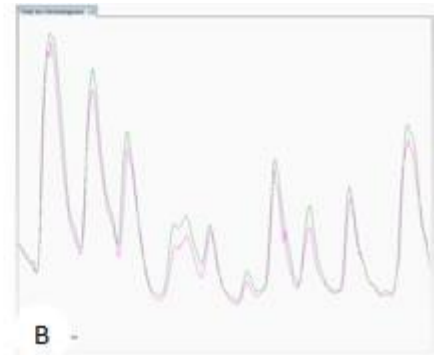
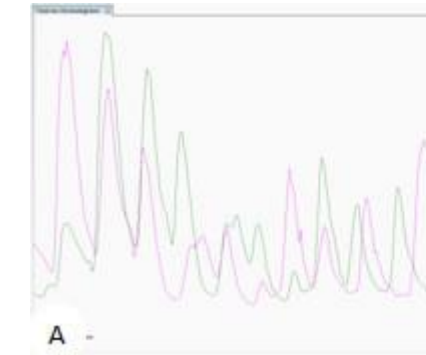


Treated runs	
Peptides unique to Protein X	
Run 1	Σ peptide ion abundances A, B, C
Run 2	Σ peptide ion abundances A, B, C
Run 3	Σ peptide ion abundances A, B, C

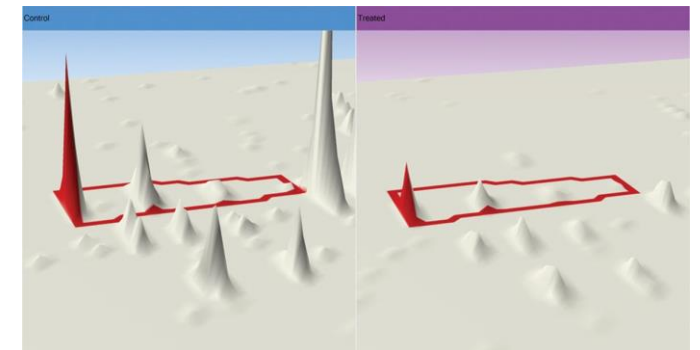
Relative expression difference of Protein X in Control vs. Treated groups



LC-MS runs represented as m/z vs. RT overlaid. (A) unaligned runs (B) aligned run



Total Ion Chromatogram view. (A) unaligned runs (B) aligned runs

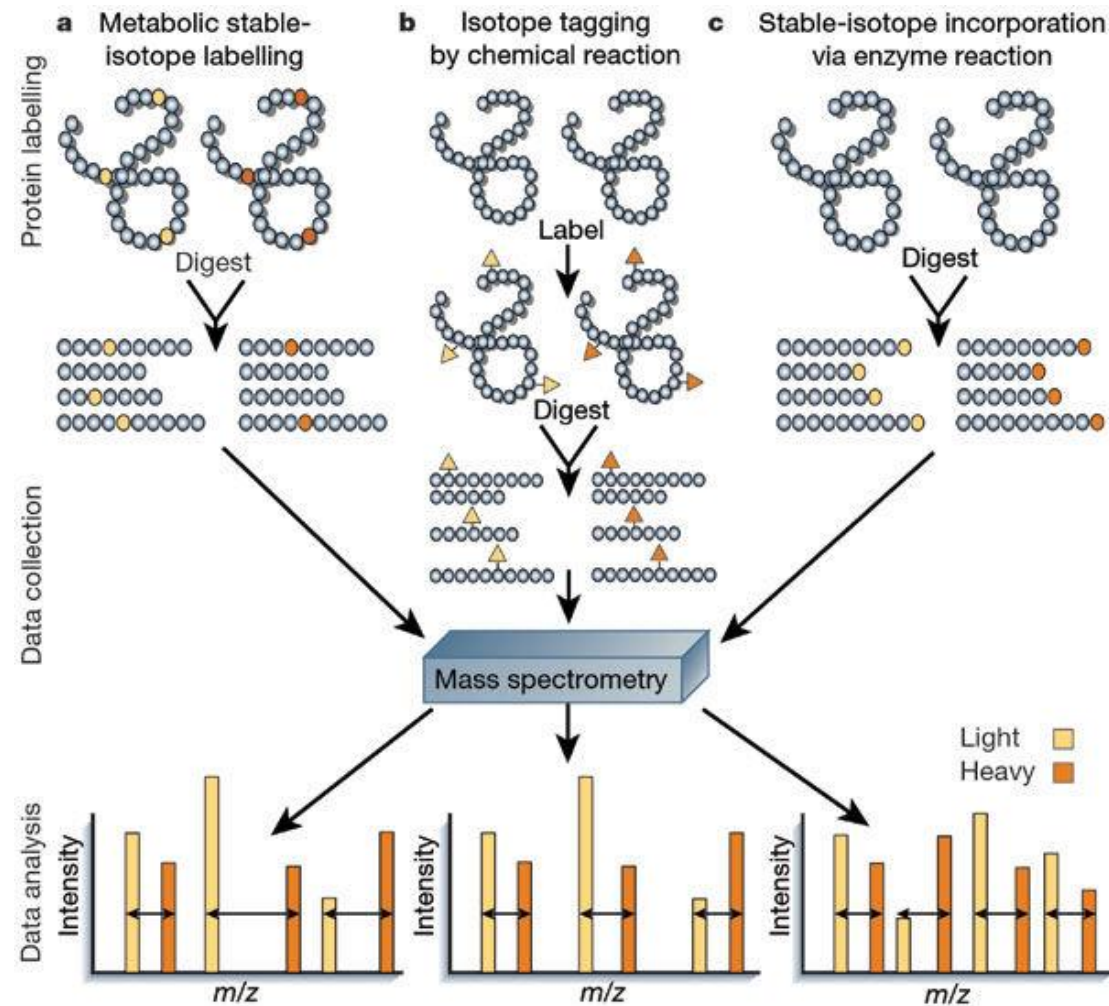


3.2. Labelling-based techniques

Metabolic labelling

Proteolytic labelling

Chemical labelling

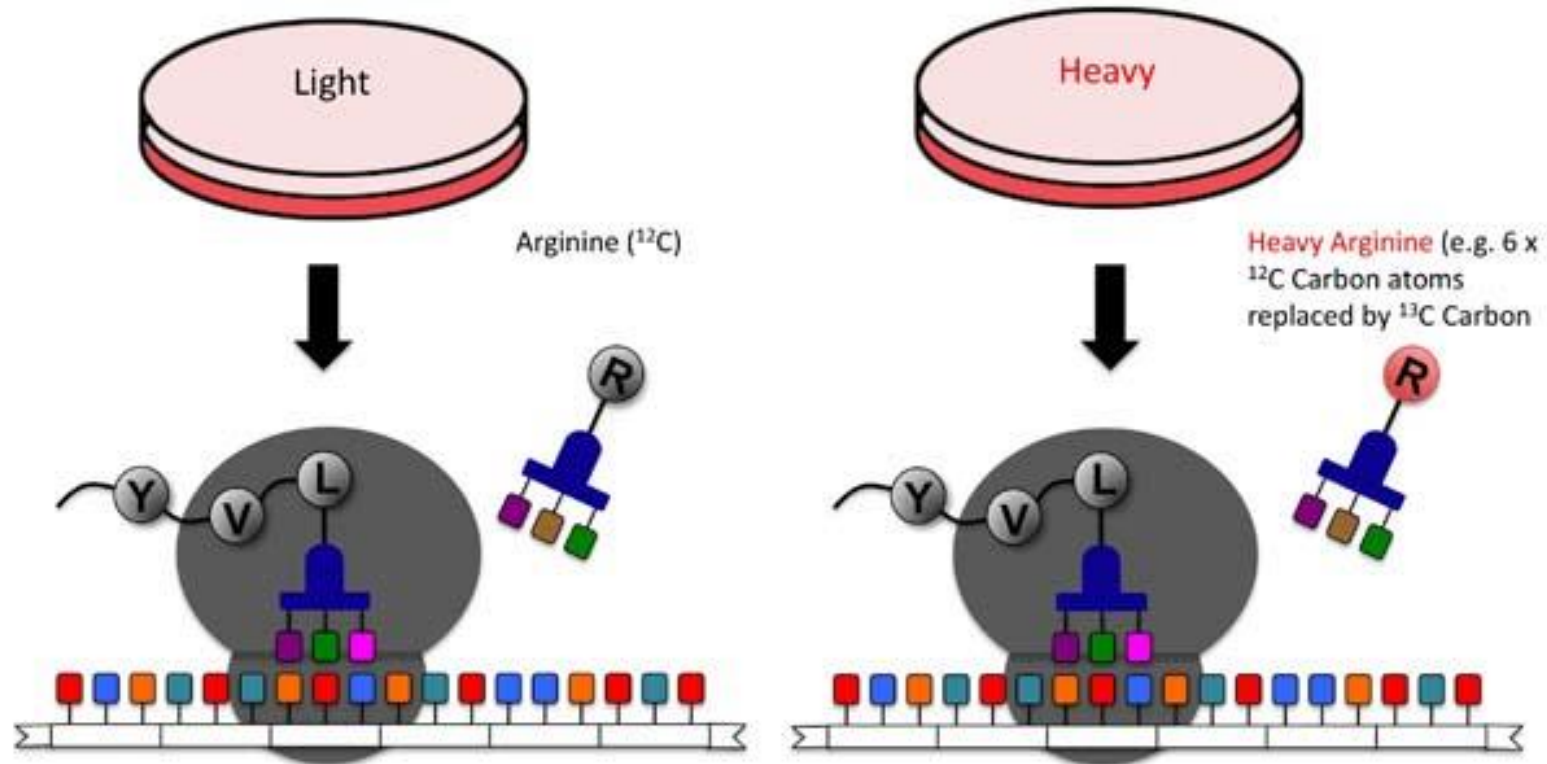


Metabolic labelling: SILAC

Stable Isotope Labeling with Amino Acids in Cell Culture (SILAC)

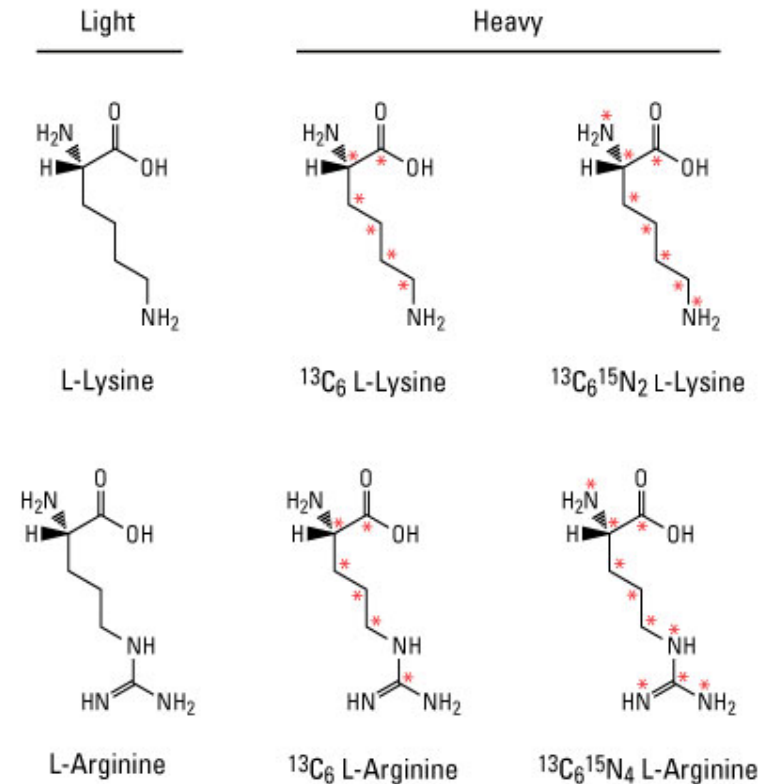
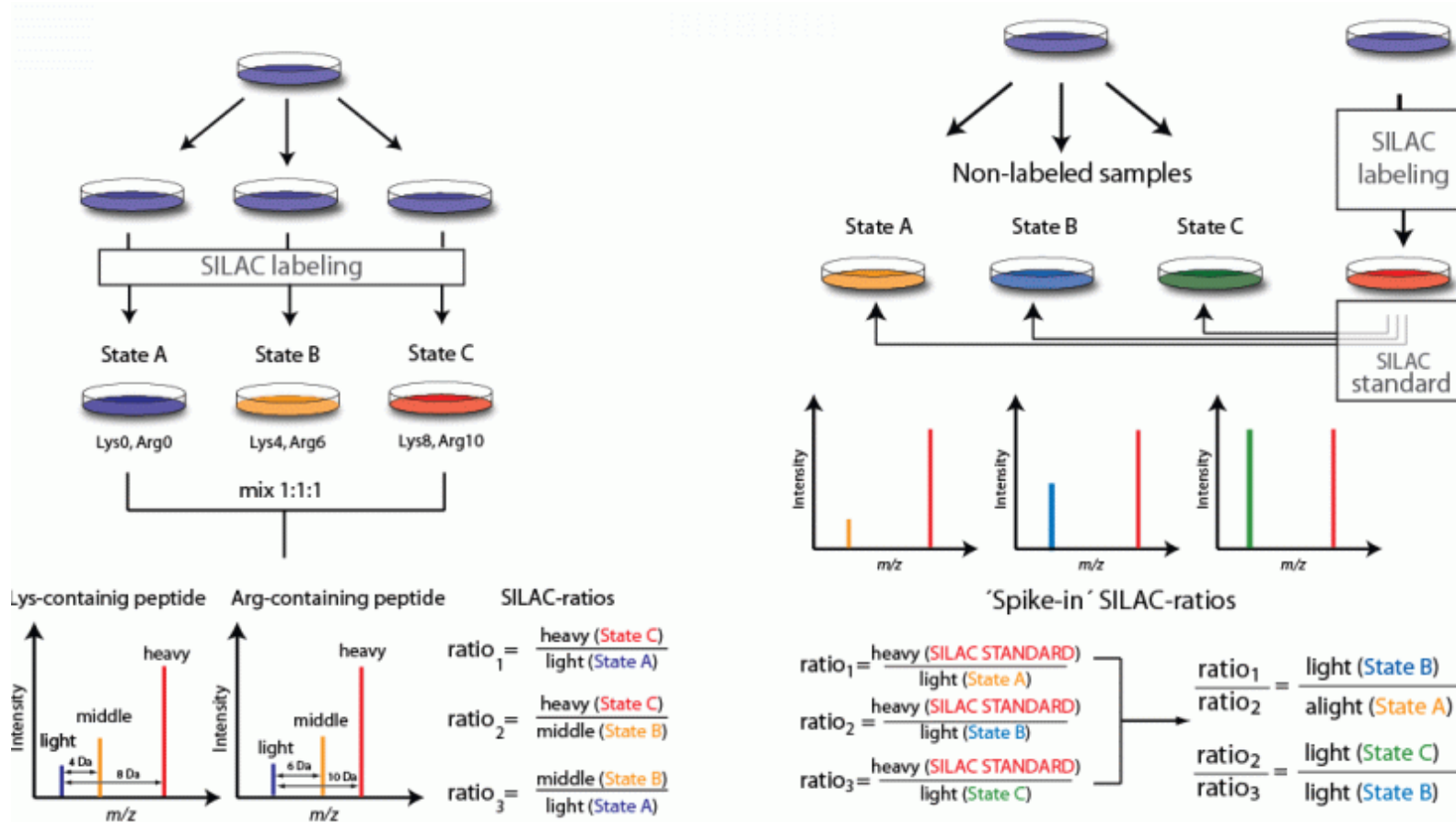


Matthias Mann



<http://www.trinklelab.com/proteomics.html>

SILAC

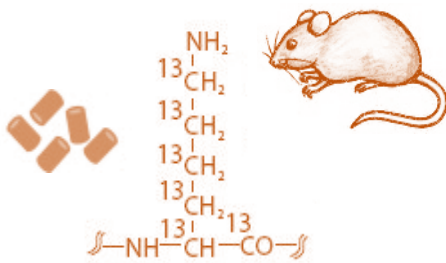
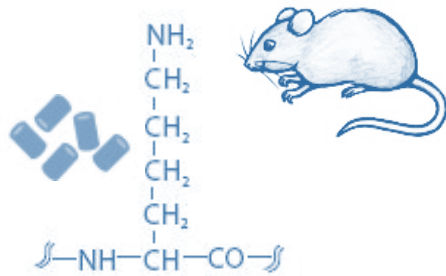


<http://www.biochem.mpg.de/221777/SILAC>

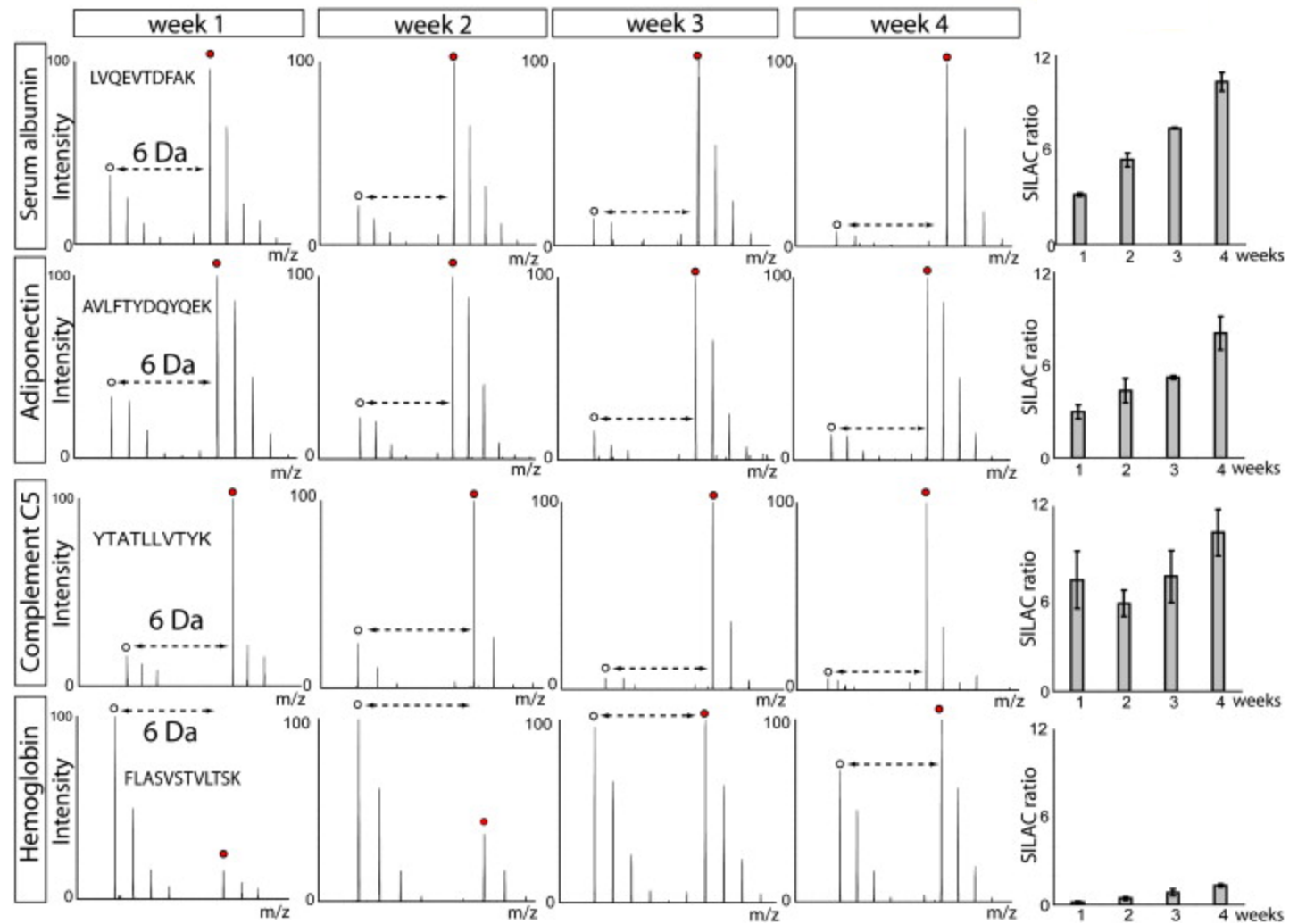
doi:10.1038/nprot.2010.192

<https://www.thermofisher.com>

In-vivo SILAC

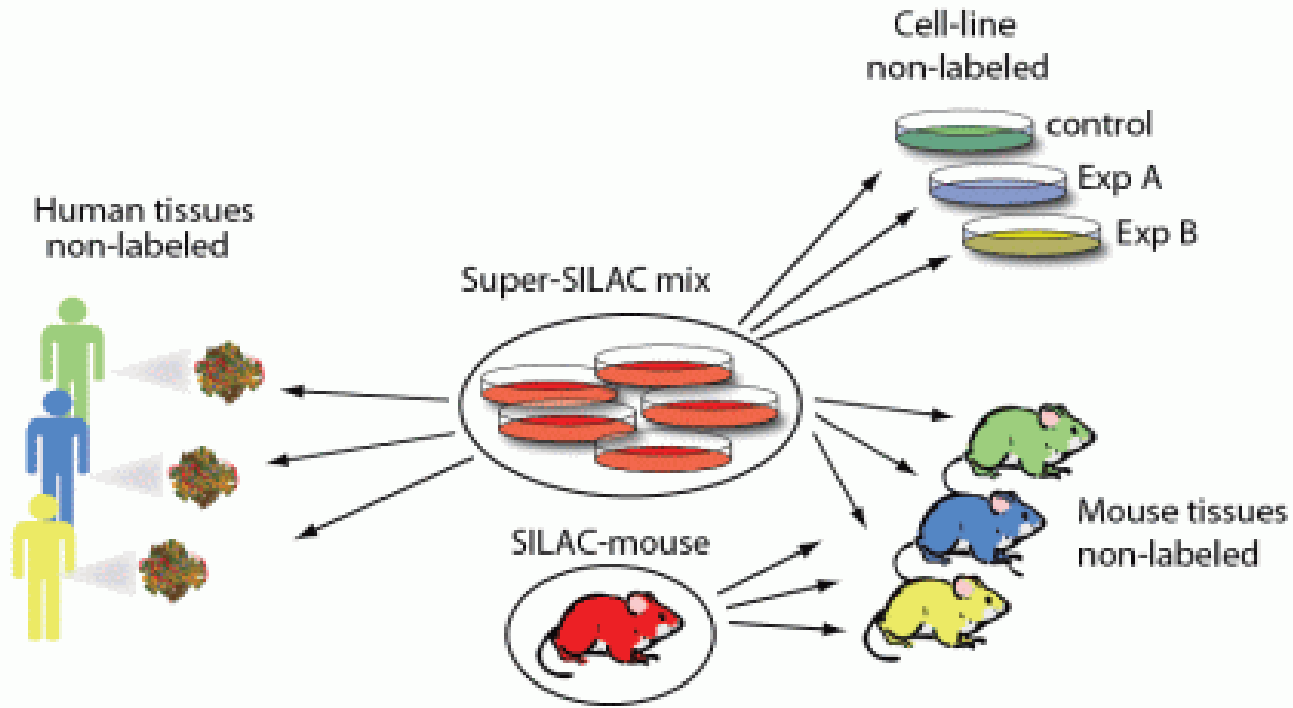


$^{13}\text{C}_6$ -Lysine

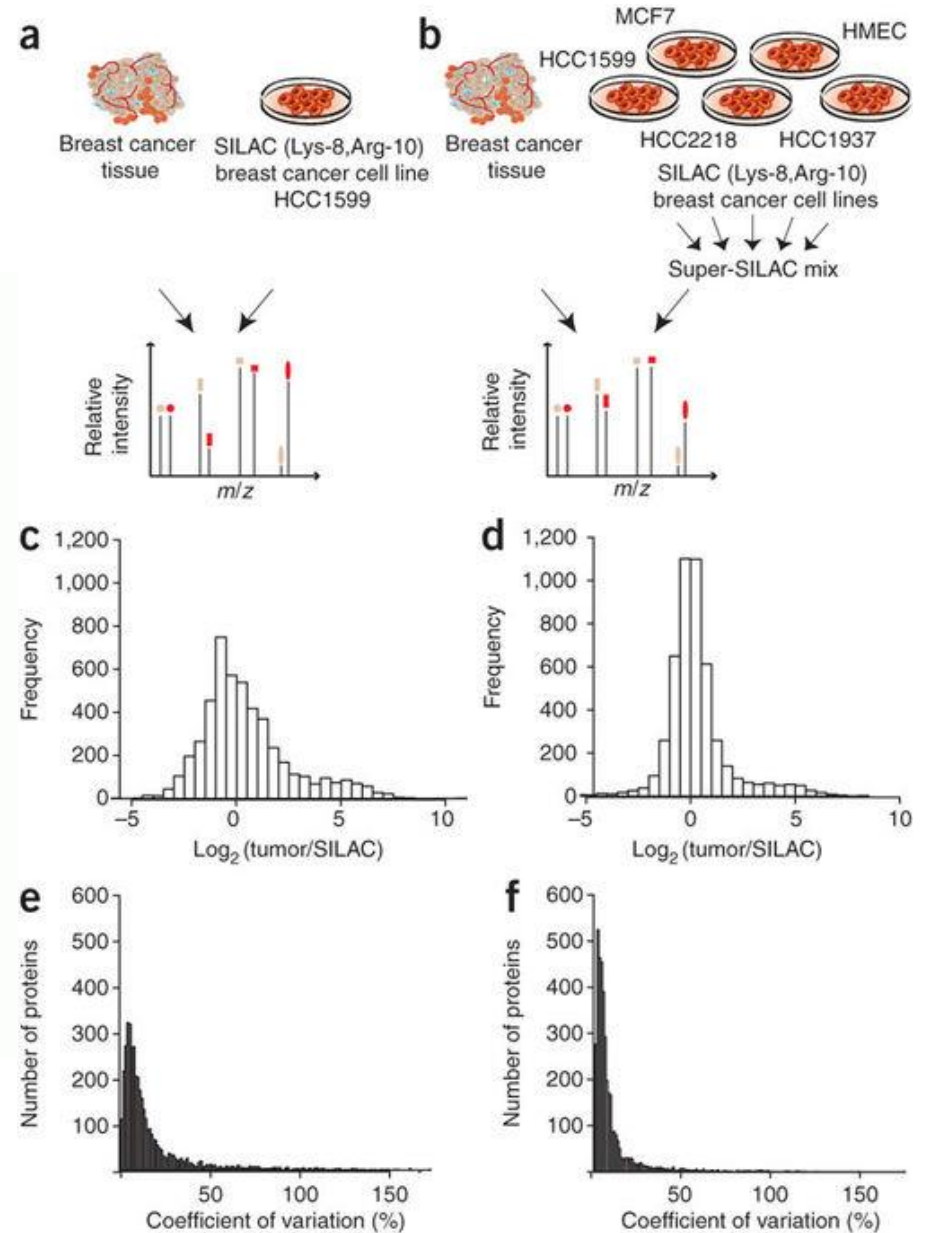


DOI: <https://doi.org/10.1016/j.cell.2008.05.033>

Super-SILAC

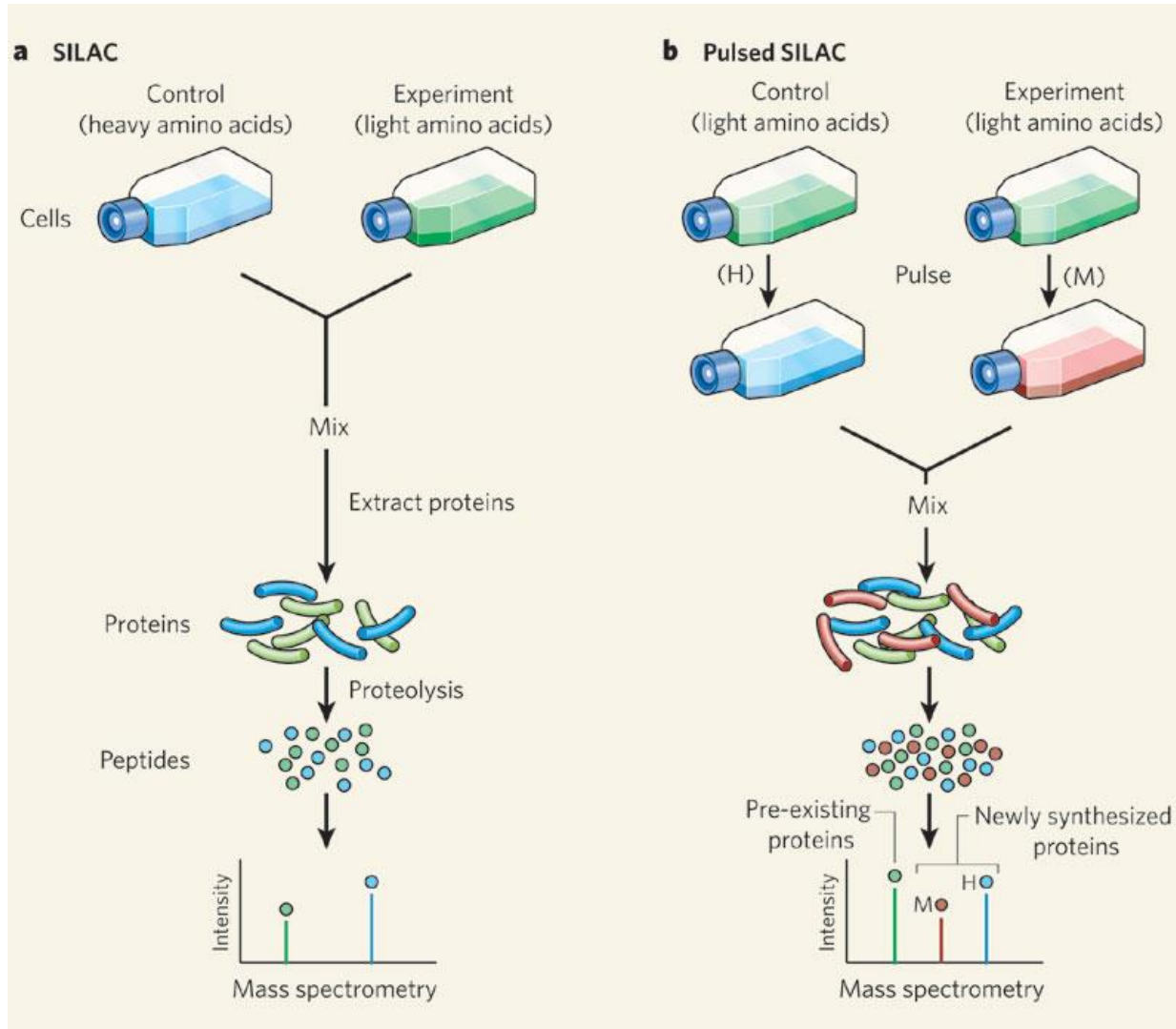


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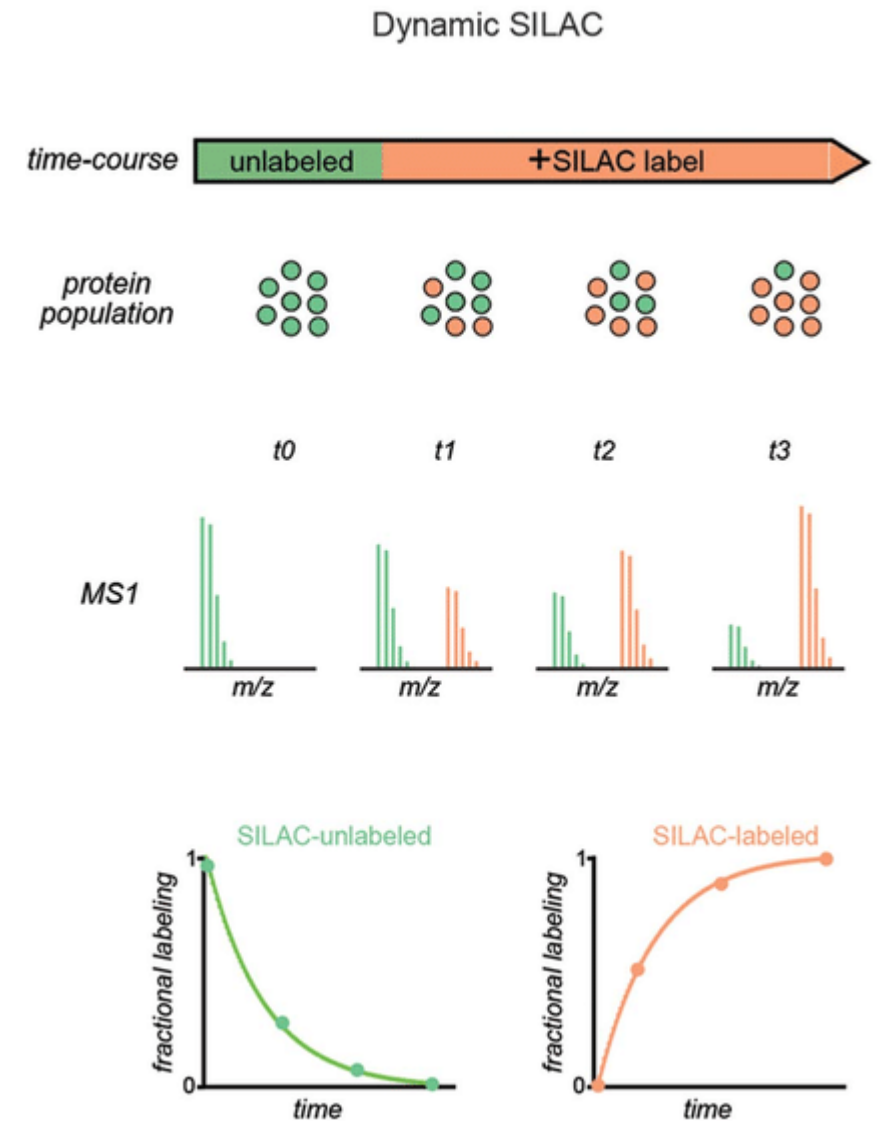


doi:10.1038/nmeth.1446

Pulsed and dynamic SILAC



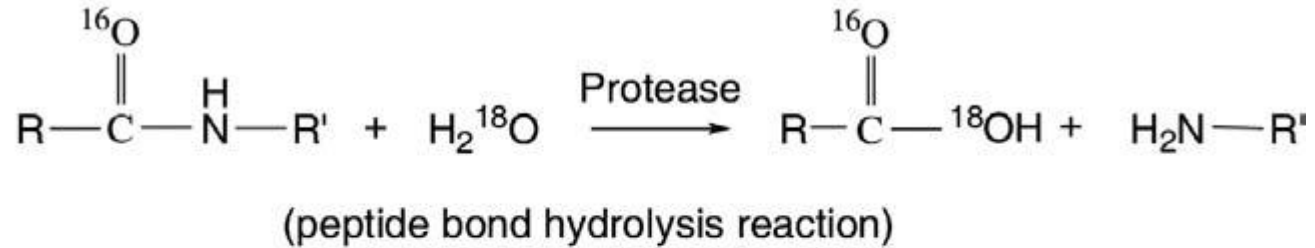
DOI: 10.1038/455044a



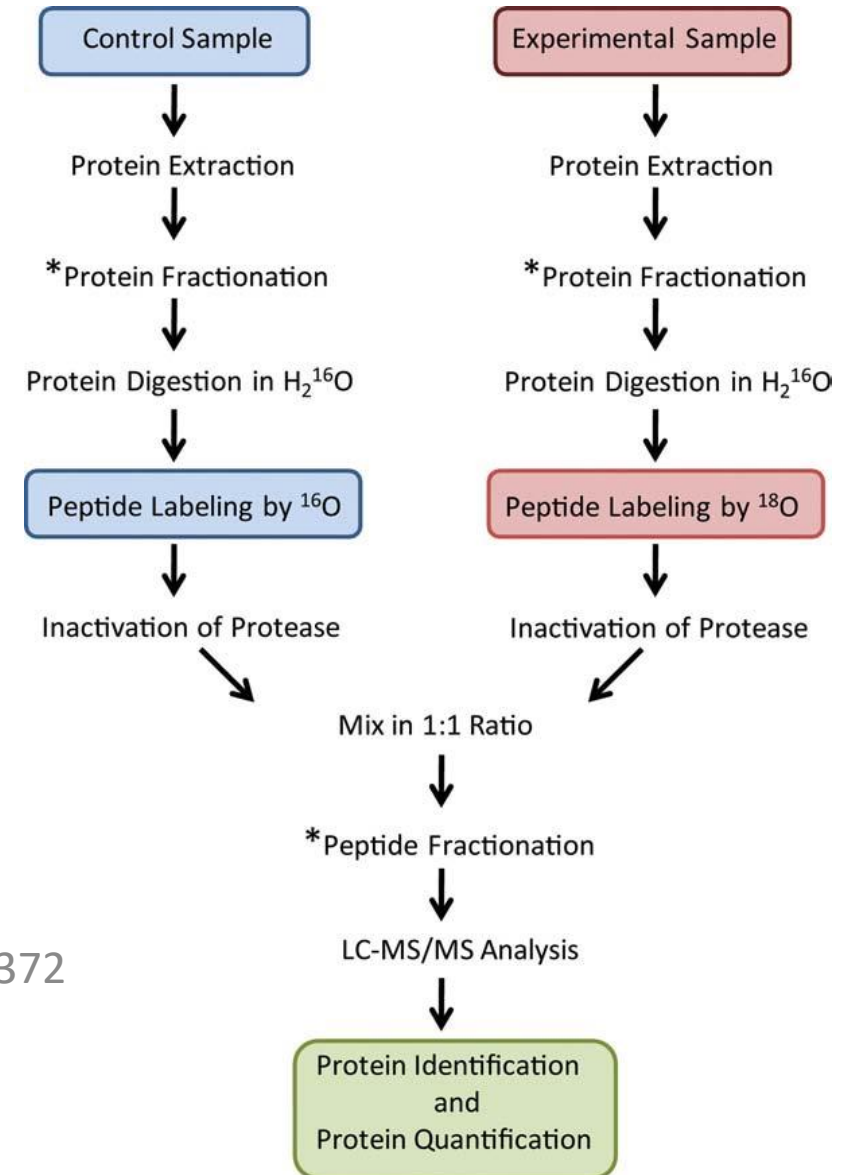
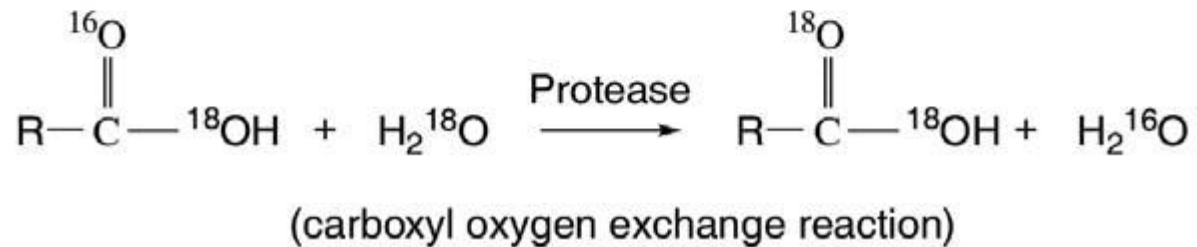
DOI: 10.1074/mcp.M116.063230

Proteolytic labelling

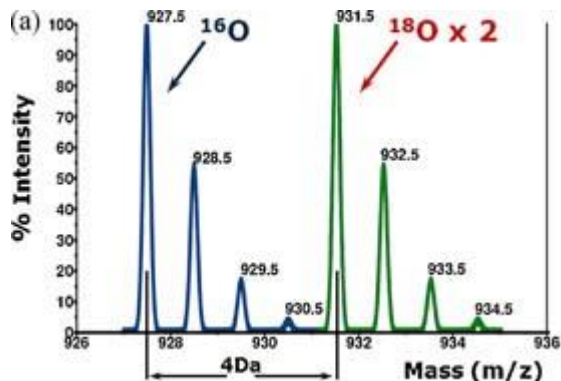
a)



b)

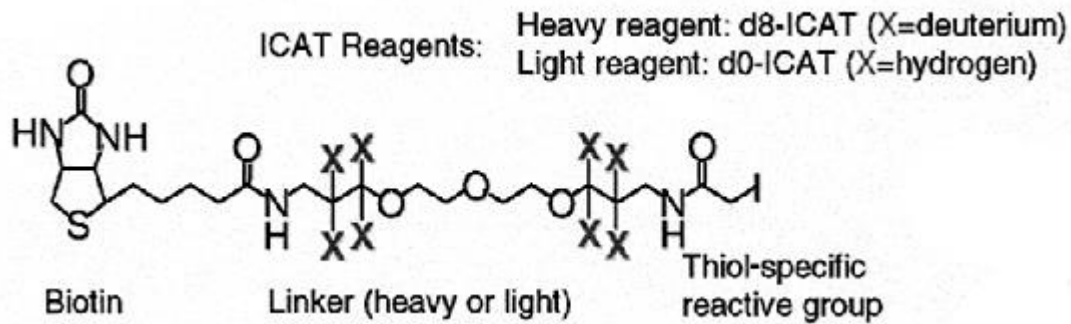


DOI: 10.2174/157016411794697372

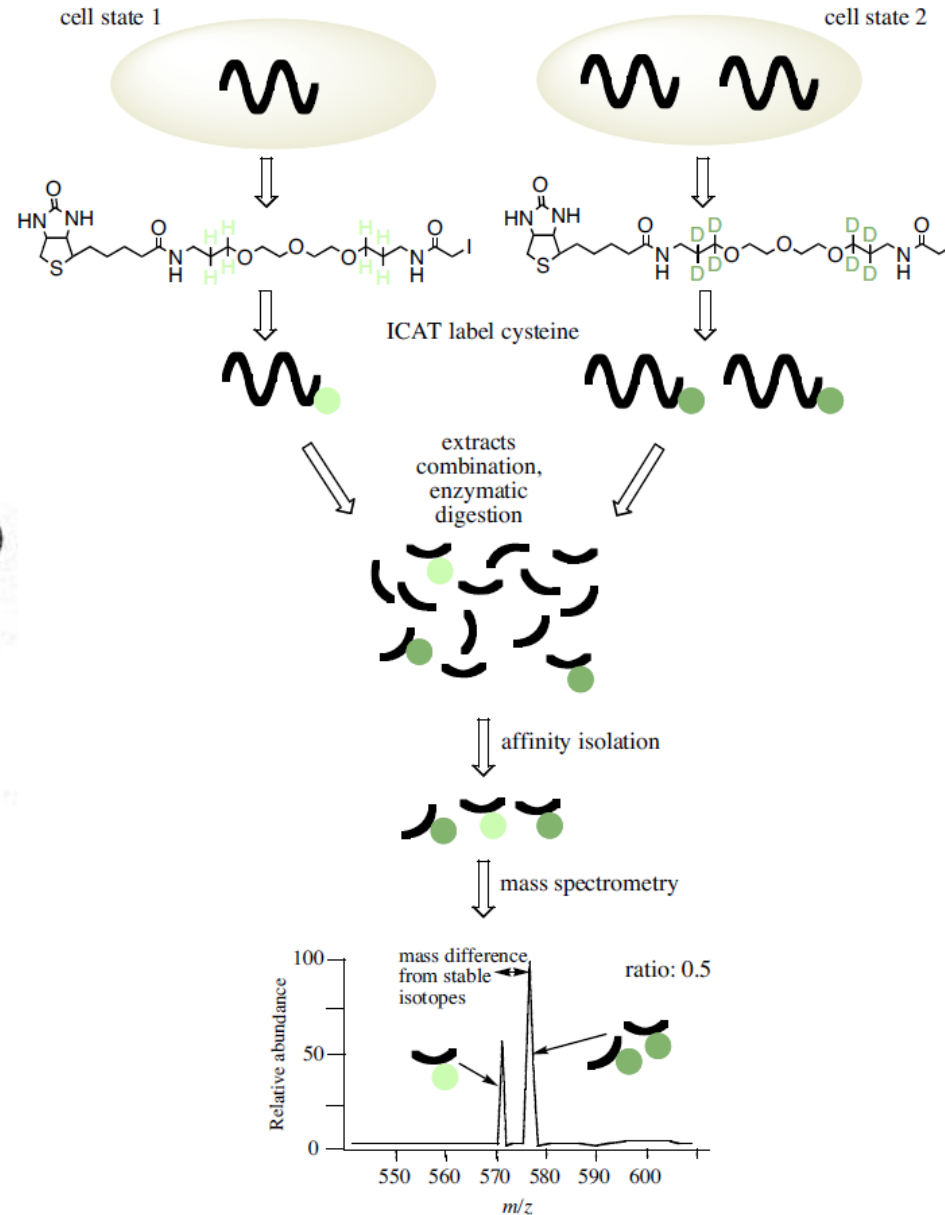


DOI: 10.1002/rcm.4816

Chemical labelling



DOI: 10.1038/13690



Ruedi Aebersold



Steven Gygi

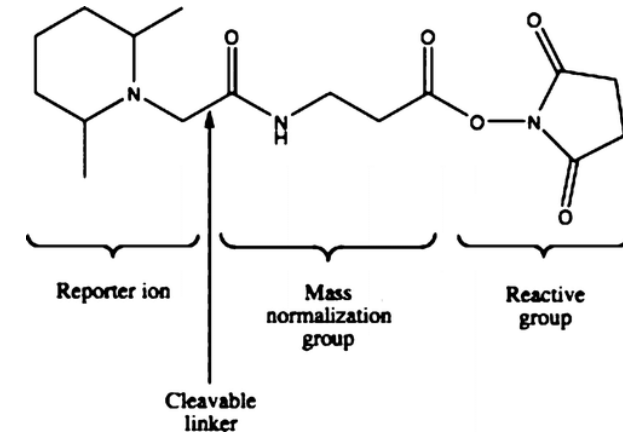
Chemical labelling: Isobaric tagging

Tandem mass tags (TMTs)

Tandem Mass Tags: A Novel Quantification Strategy for Comparative Analysis of Complex Protein Mixtures by MS/MS

Andrew Thompson,[†] Jürgen Schäfer,[‡] Karsten Kuhn,[‡] Stefan Kienle,[‡] Josef Schwarz,[‡] Günter Schmidt,[†] Thomas Neumann,[‡] and Christian Hamon^{*,‡}

DOI: 10.1021/ac0262560



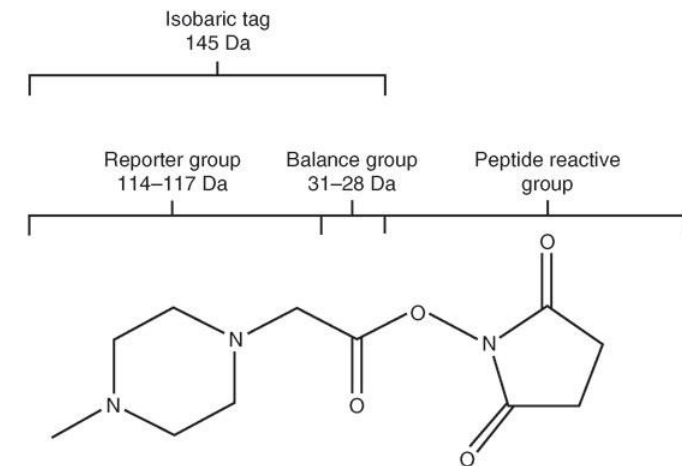
DOI: 10.1021/ac702422x

Isobaric tags for relative and absolute quantitation (iTRAQ)

Multiplexed Protein Quantitation in *Saccharomyces cerevisiae* Using Amine-reactive Isobaric Tagging Reagents[§]

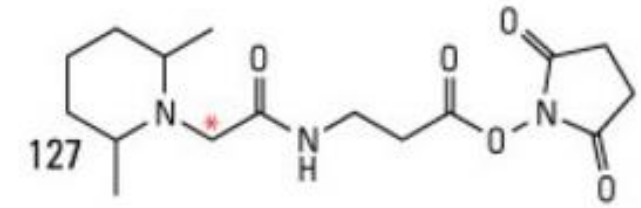
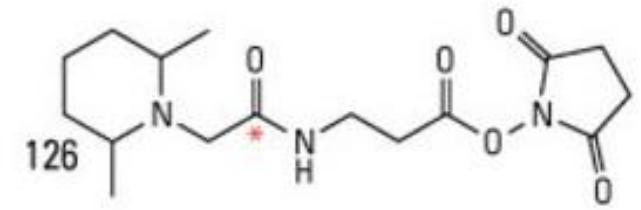
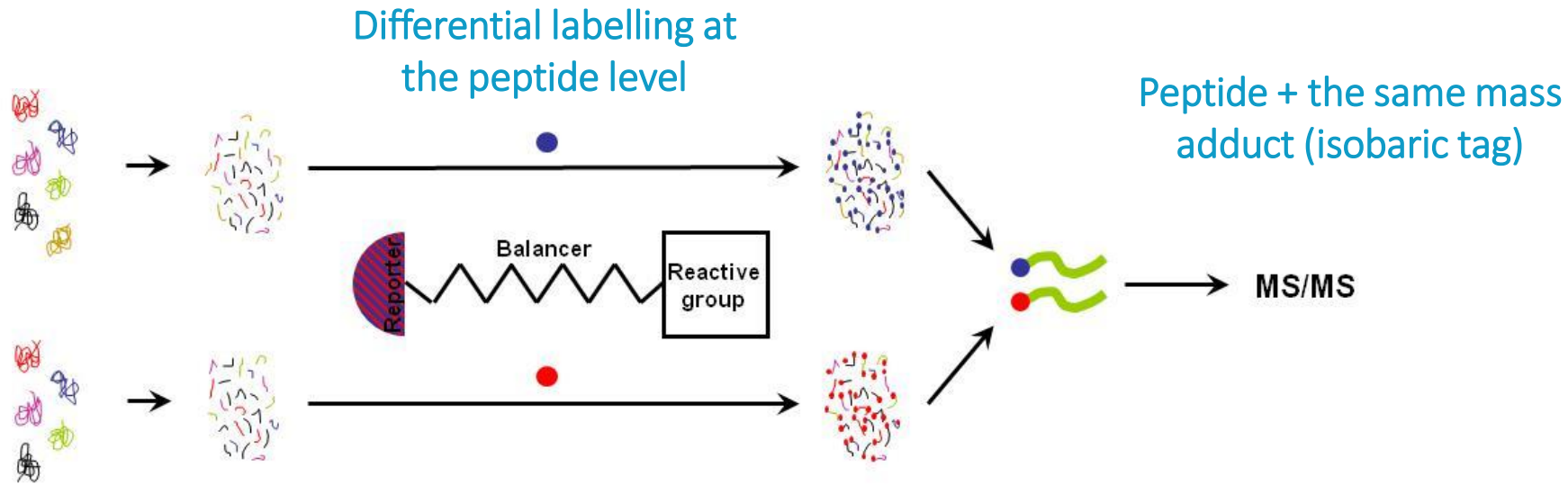
Philip L. Ross[‡], Yulin N. Huang[‡], Jason N. Marchese[‡], Brian Williamson[‡], Kenneth Parker[‡], Stephen Hattan[‡], Nikita Khainovski[‡], Sasi Pillai[‡], Subhakar Dey[‡], Scott Daniels[‡], Subhasish Purkayastha[‡], Peter Juhasz[‡], Stephen Martin[‡], Michael Bartlett-Jones[§], Feng He[¶], Allan Jacobson[¶], and Darryl J. Pappin^{‡||**}

DOI: 10.1074/mcp.M400129-MCP200

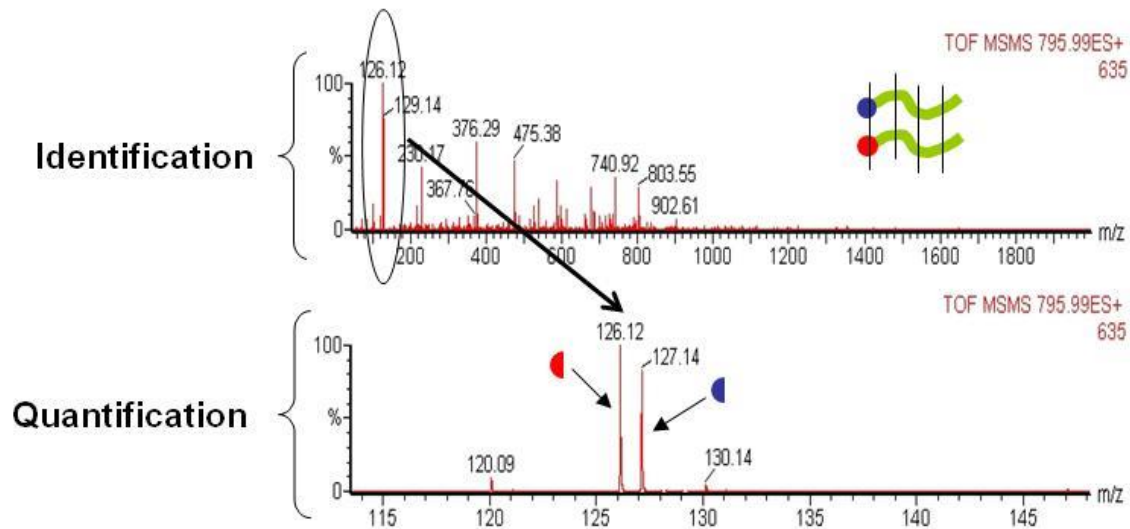


DOI: 10.1038/nprot.2006.254 18

Chemical labelling: Isobaric tagging



<https://www.thermofisher.com>

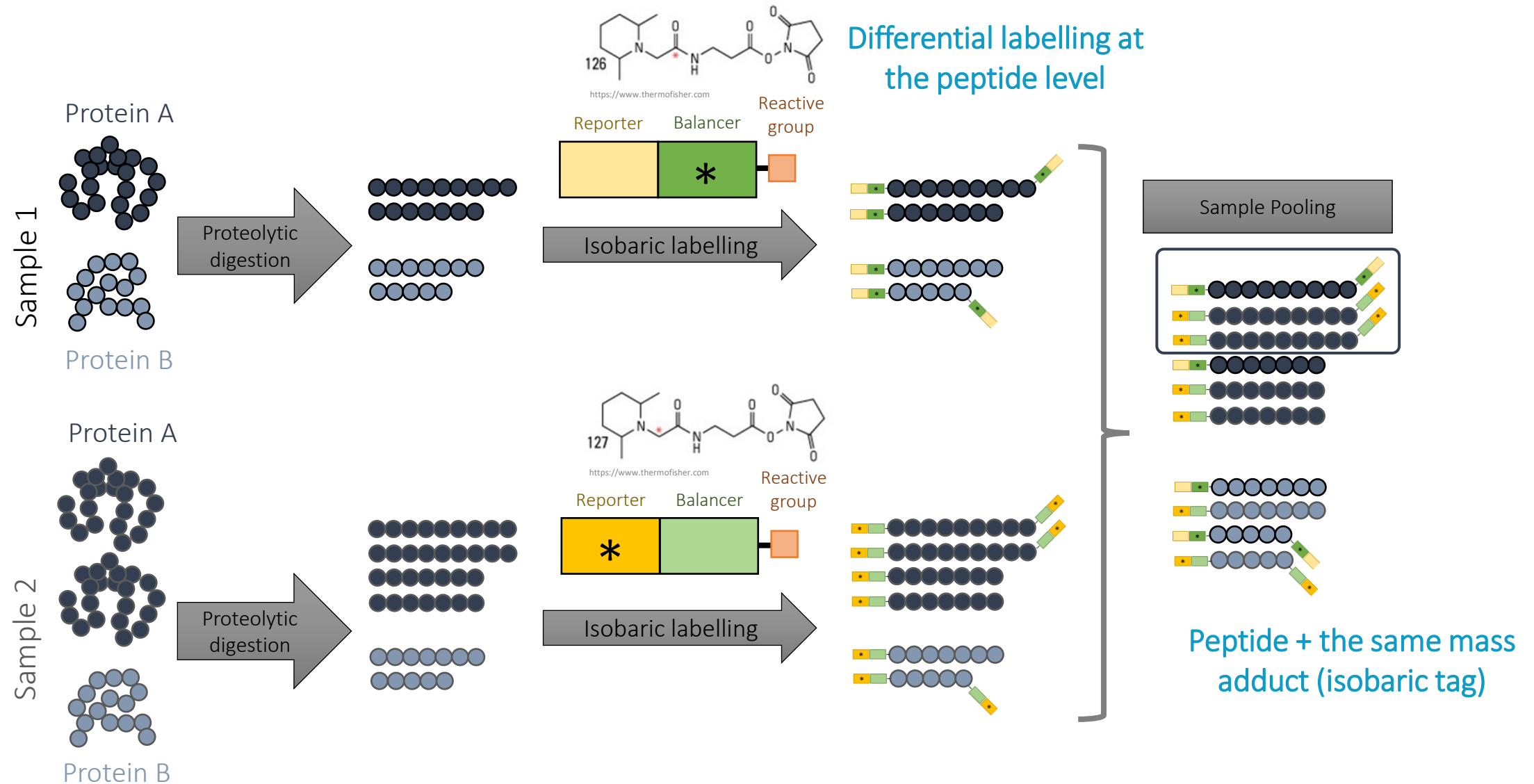


Peptide fragments EQUAL

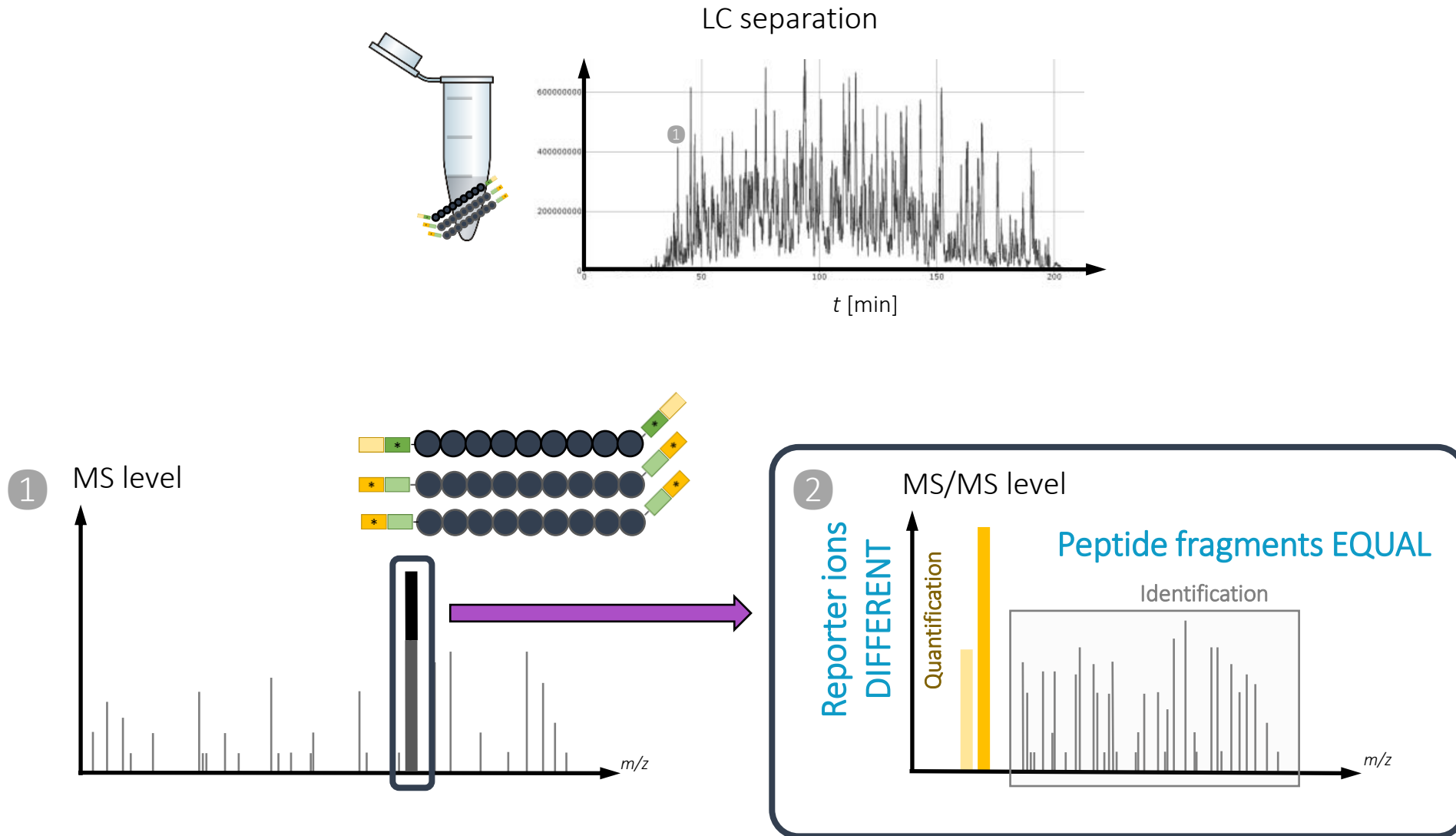
Tandem mass spectrum

Reporter ions DIFFERENT

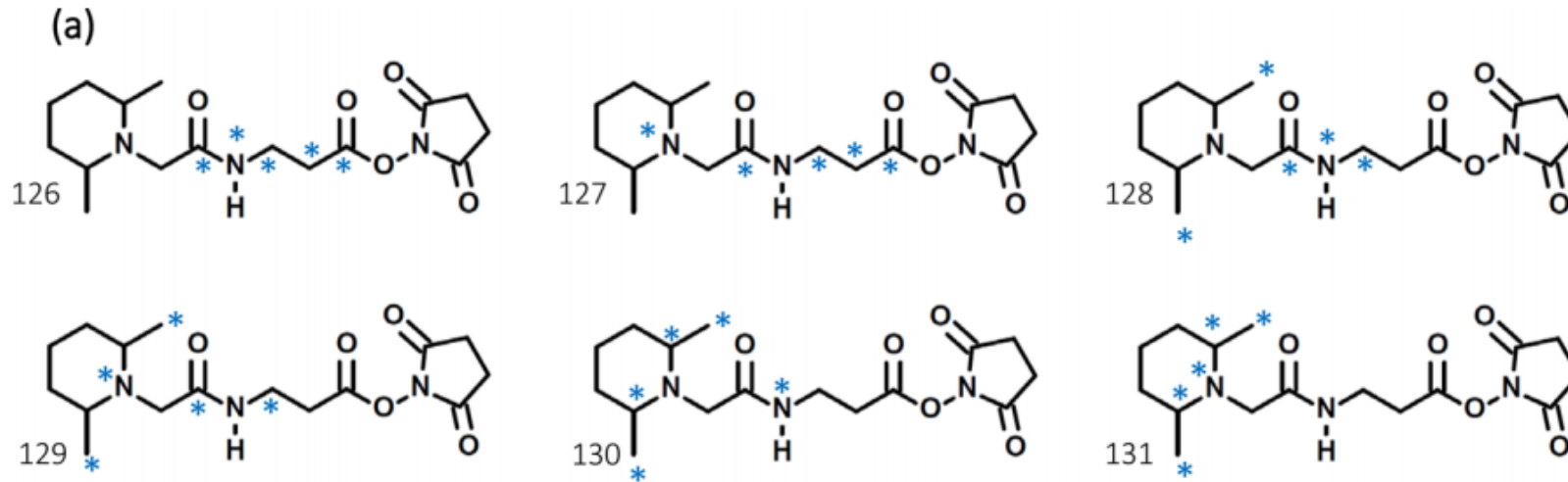
Chemical labelling: Isobaric tagging - Sample preparation



Chemical labelling: Isobaric tagging - LC-MS and MS/MS analysis



Tandem mass tags

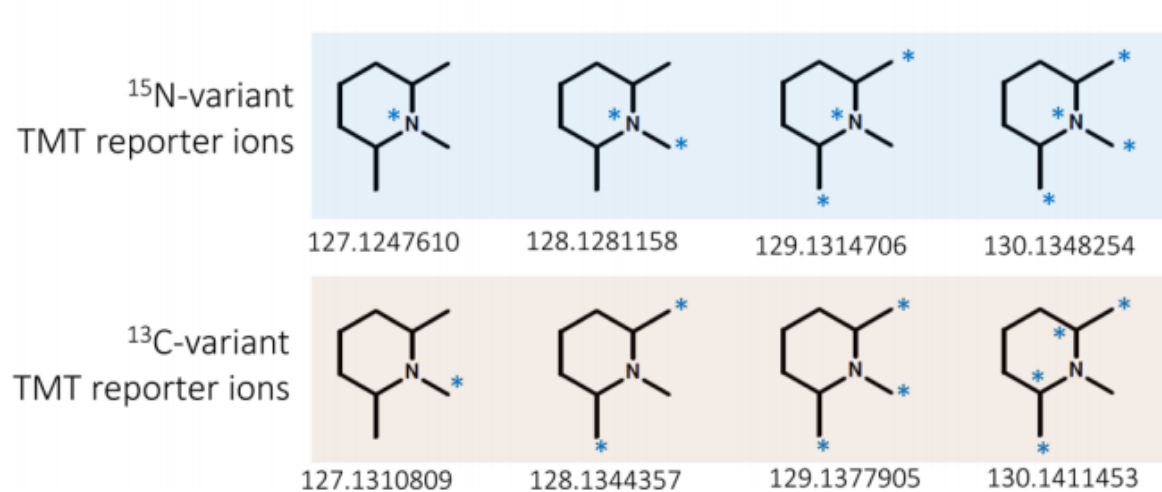


Targets:

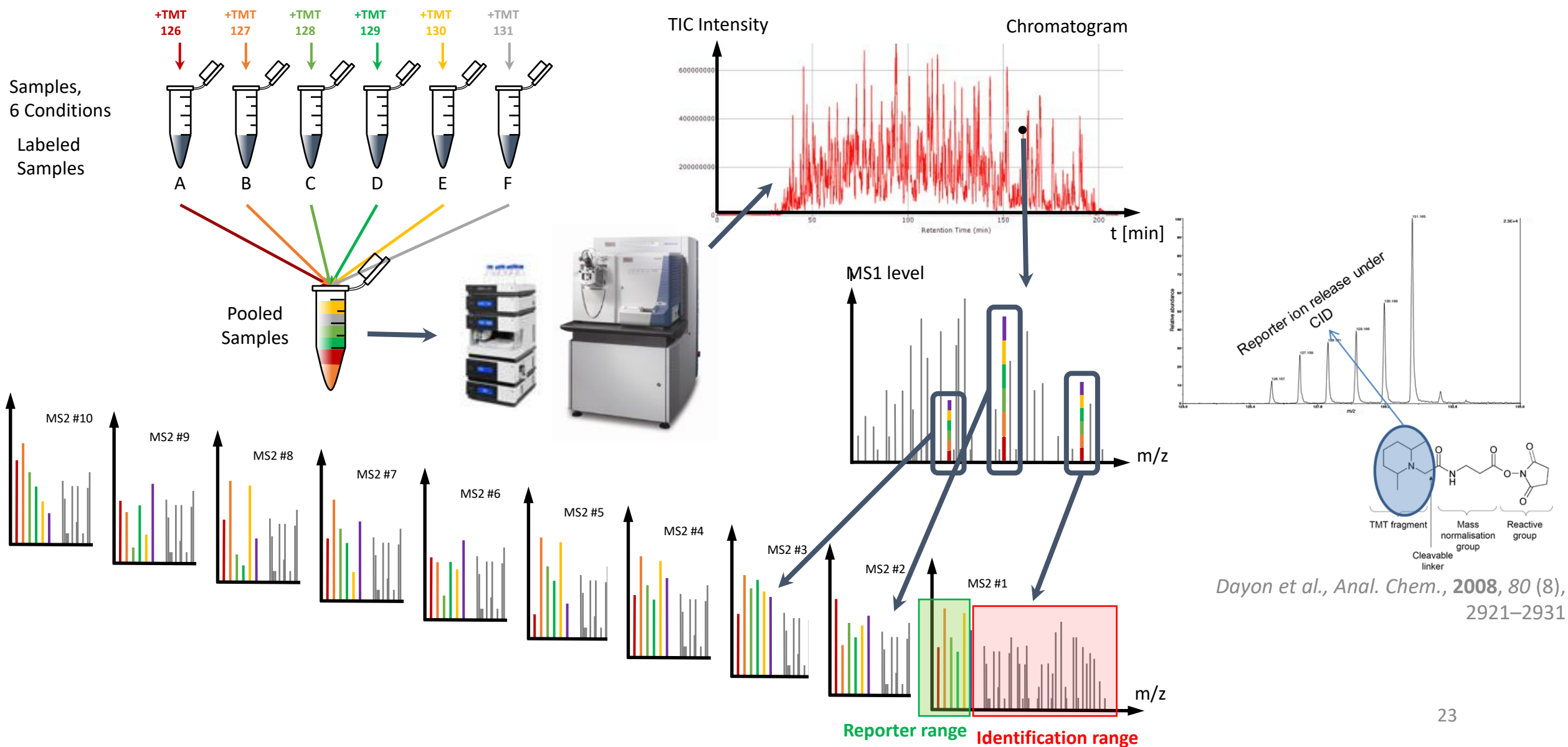
- N-terminus
- Lysine

Multiplexing:

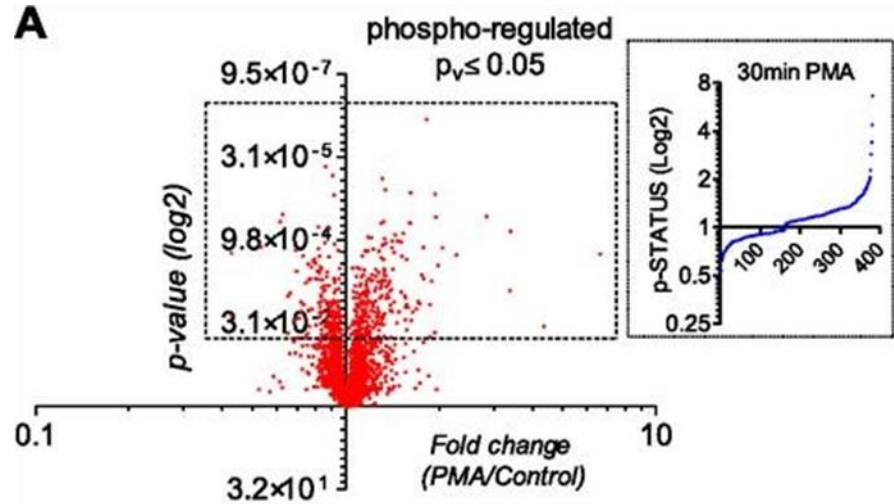
- 2-plex
- 6-plex
- 10-plex
- 11-plex
- 16-plex...



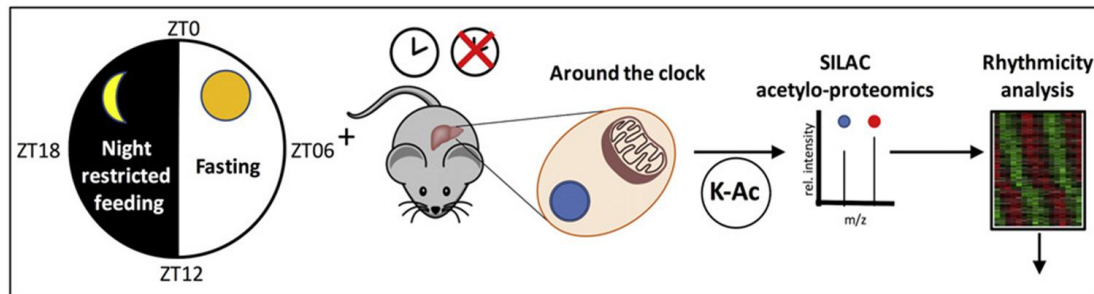
TMT technology



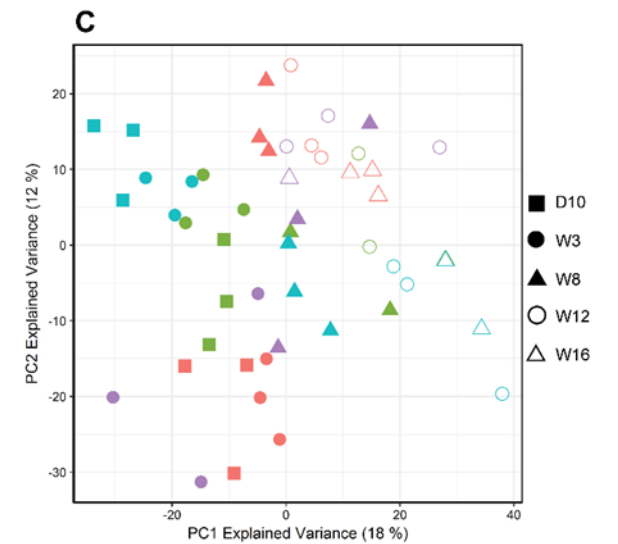
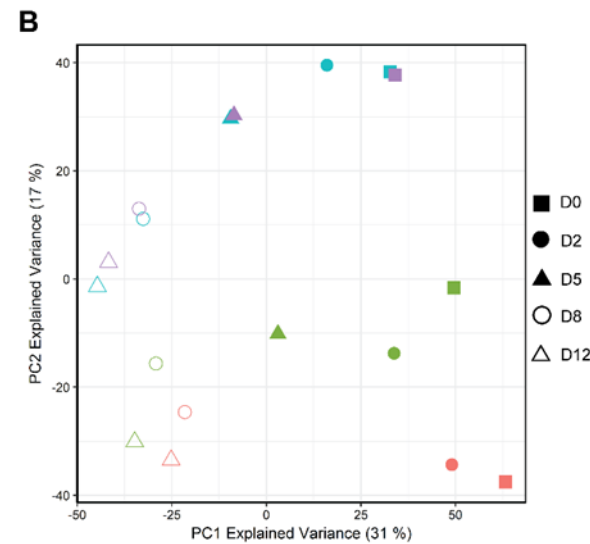
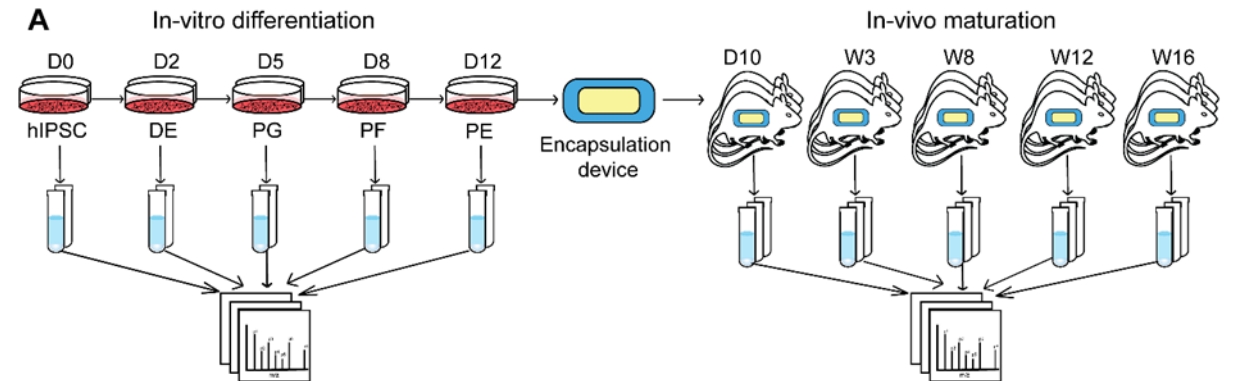
Main applications: Response to *stimuli*



DOI: 10.1096/fj.201600837R

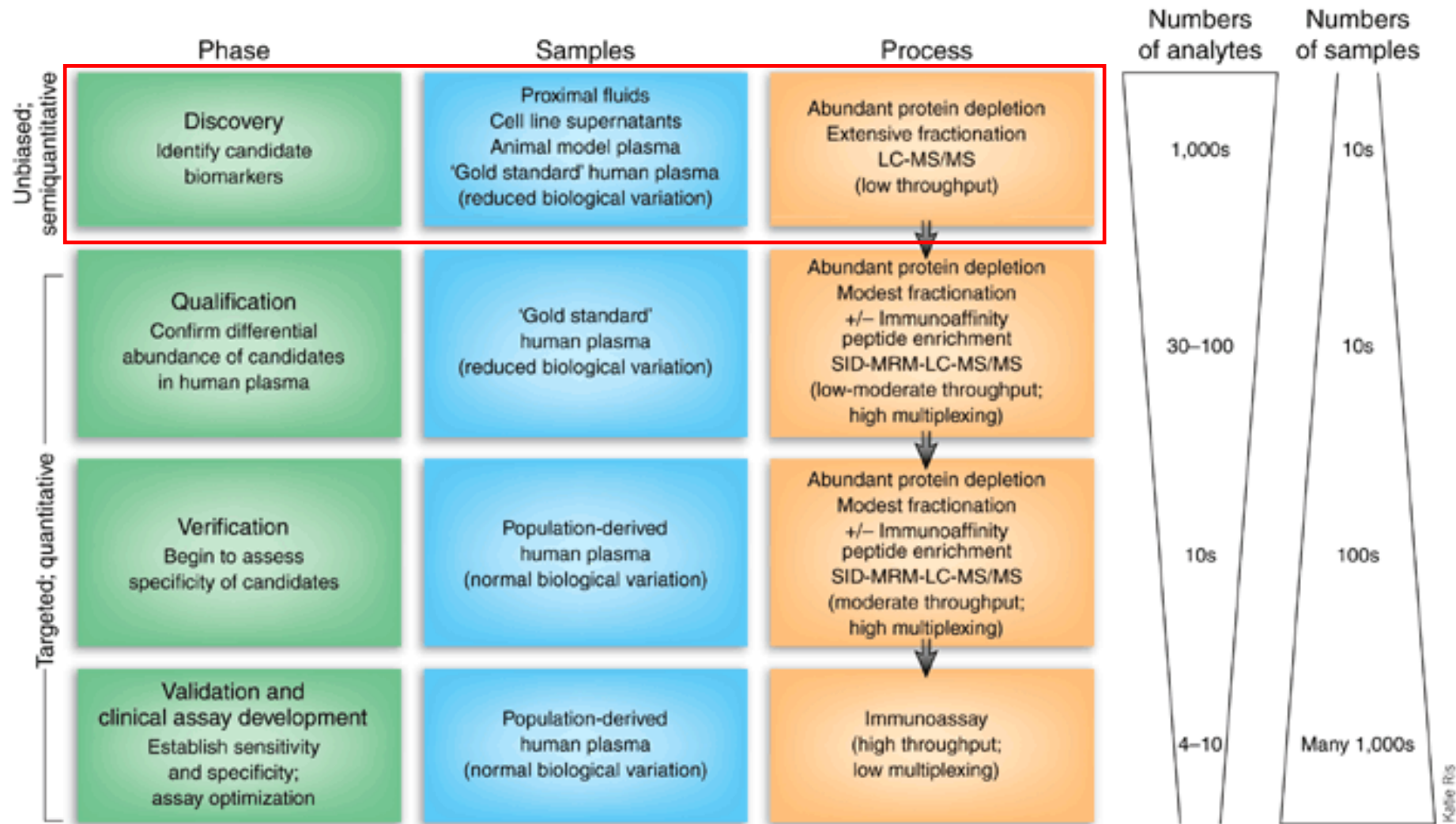


DOI: 10.1016/j.celrep.2017.07.065

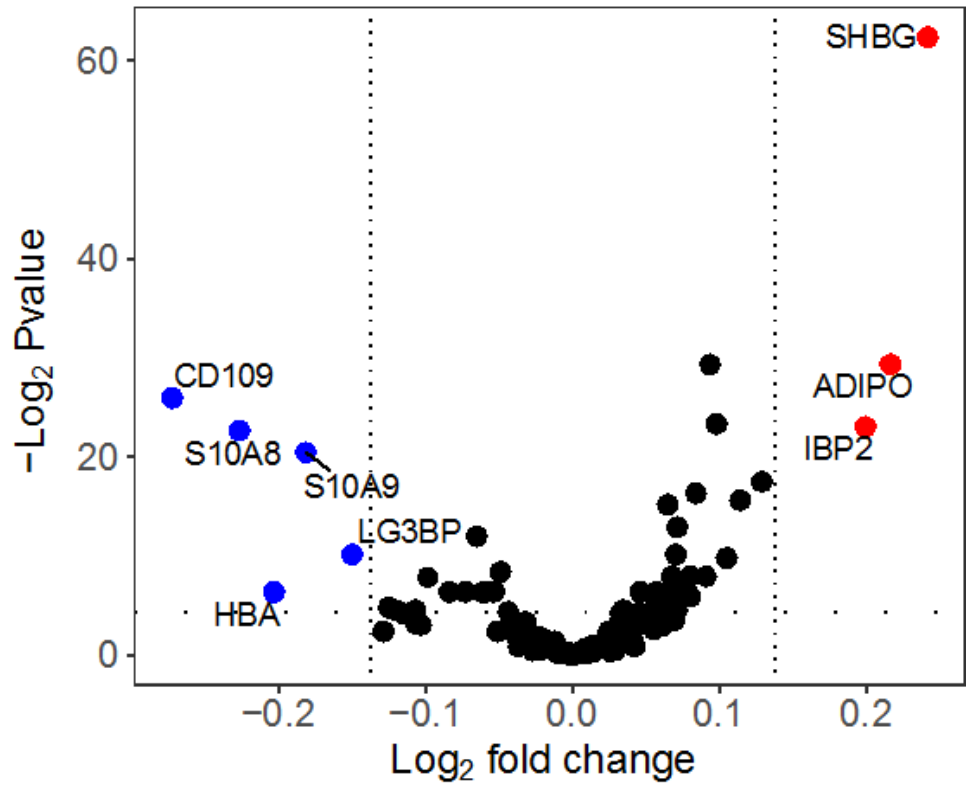


DOI: 10.1002/prca.201600173

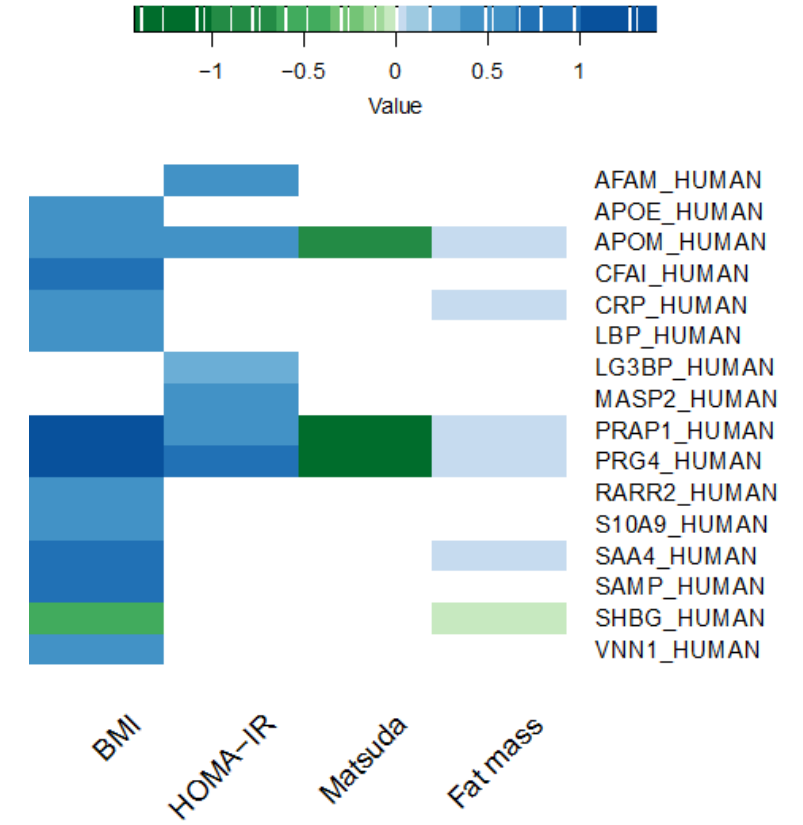
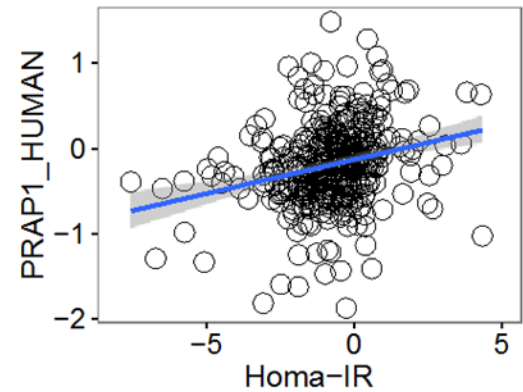
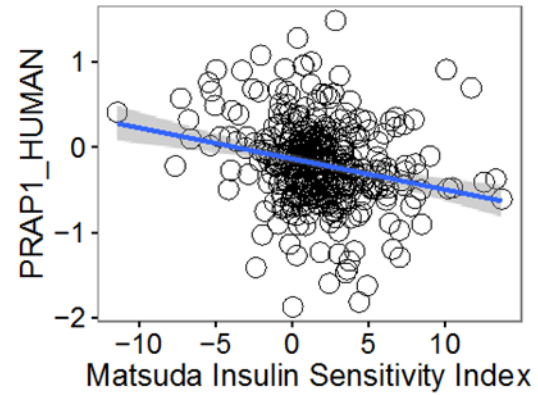
Main applications: Discovery of biomarkers



Discovery of biomarkers



● Up-regulated ● Down-regulated ● No regulation

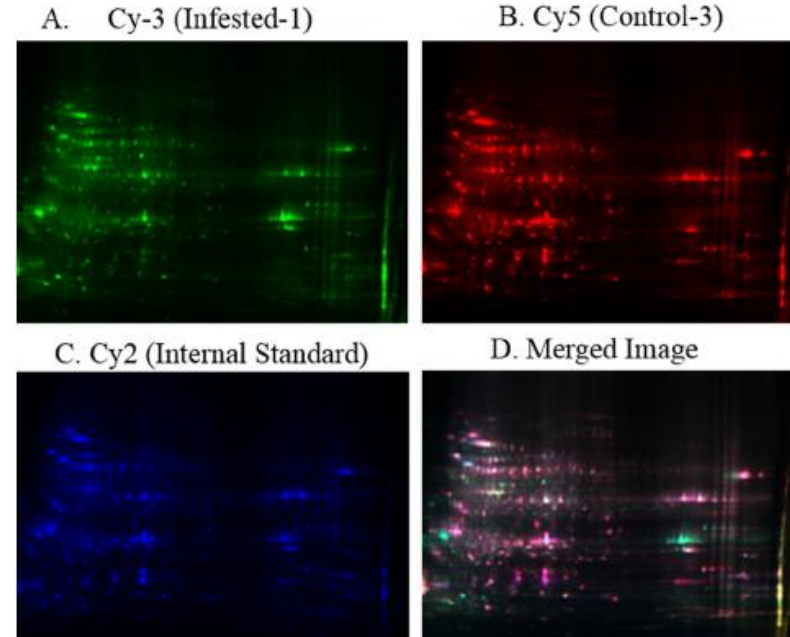
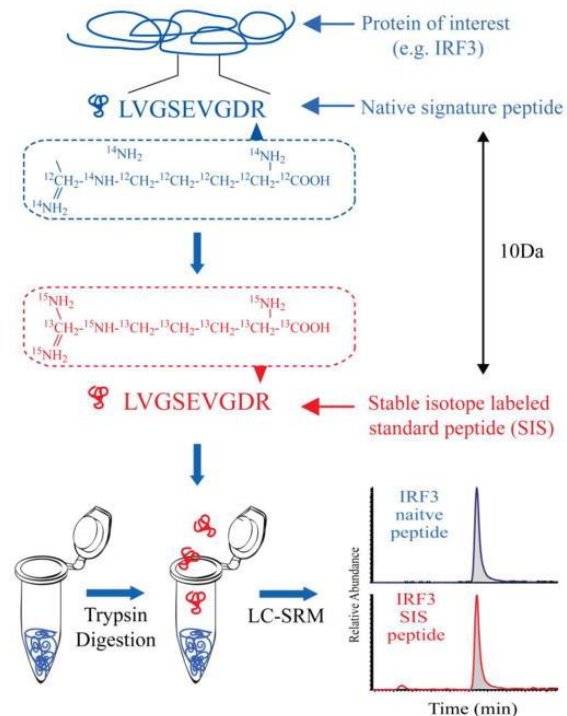


DOI: 10.1002/prca.201600150

3.3. Other quantitative techniques

“Absolute” (or precise relative quantification) with targeted MS

Other techniques (e.g., 2-dimensional differential gel electrophoresis (2D-DIGE))

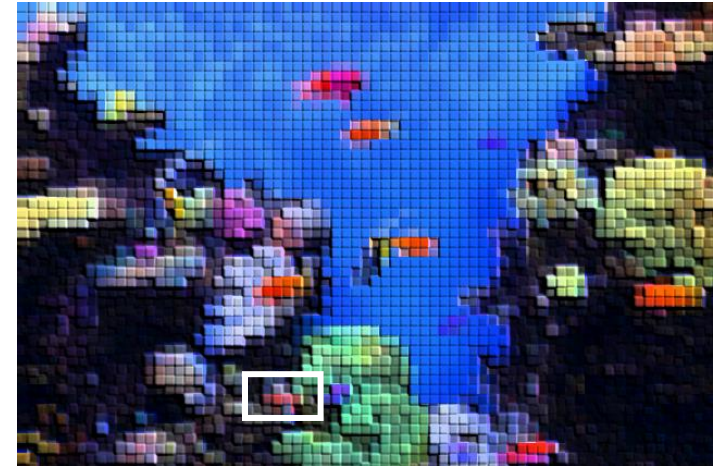


Quantitative “resolution”

Real “proteome” picture

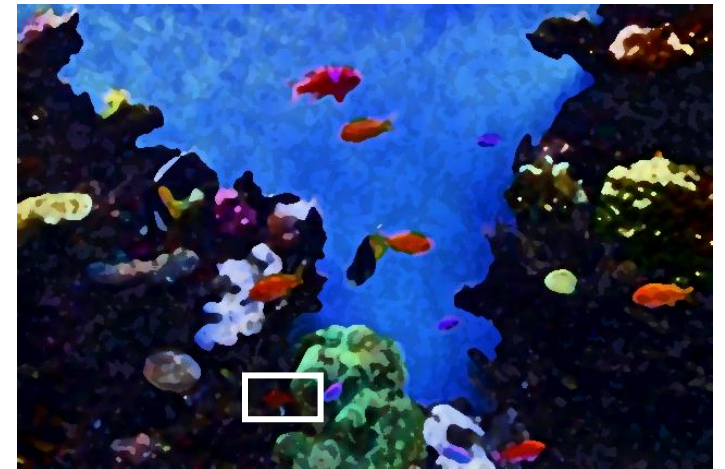


Semi/relative quantification



e.g., spectral counting

Discovery/untargeted



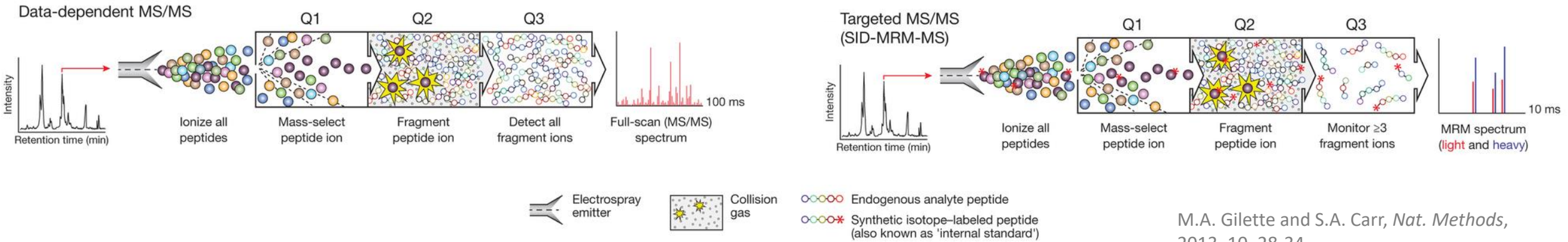
e.g., isobaric labelling

Targeted

Precise “absolute” quantification



Discovery and Targeted MS



M.A. Gillette and S.A. Carr, *Nat. Methods*, 2013, 10, 28-34

Discovery Mode



ThermoFisher
LTQ-Orbitrap



ThermoFisher
Orbitrap LUMOS

- Relative quantification/differential display
- 1'000-10'000 protein analytes
- Specific, accurate & robust
- Moderate throughput
- Data-driven/hypothesis generation
- Plasma, CSF, tissue, cell, organelle
- Proteins and their PTMs

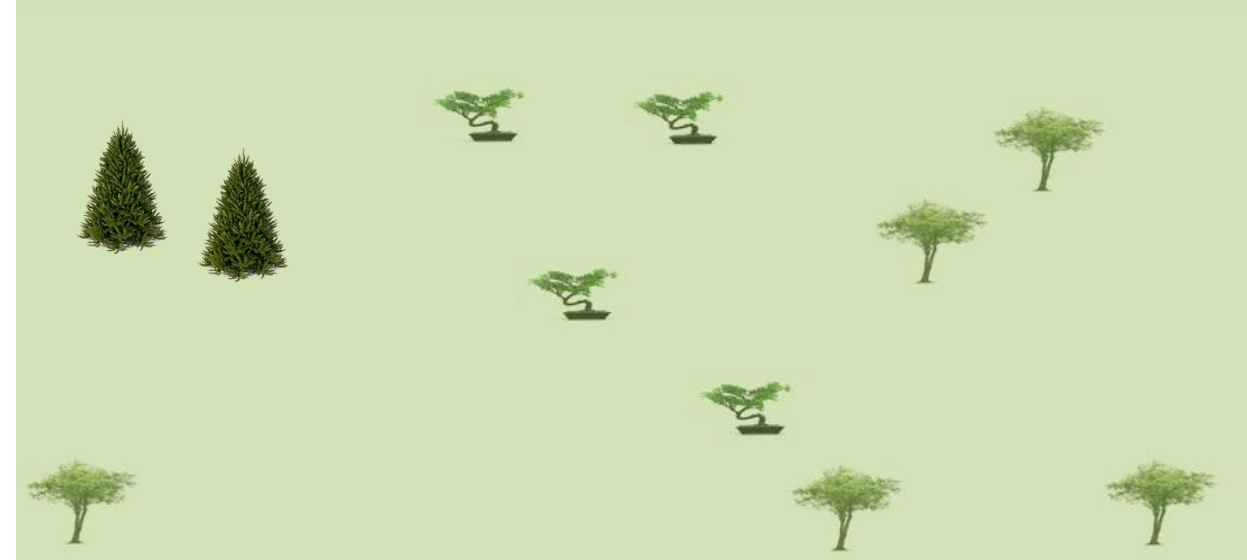


ThermoFisher
TSQ Vantage

Targeted Mode

- “Absolute” quantification
- 10-100 protein analytes
- Specific, sensitive, accurate & robust
- “High” throughput
- Hypothesis-driven/hypothesis validation
- Plasma, CSF, tissue, cell, organelle
- Proteins and their PTMs

Discovery and Targeted MS



Discovery Mode



ThermoFisher
LTQ-Orbitrap



ThermoFisher
Orbitrap LUMOS

- Relative quantification/differential display
- 1'000-10'000 protein analytes
- Specific, accurate & robust
- Moderate throughput
- Data-driven/hypothesis generation
- Plasma, CSF, tissue, cell, organelle
- Proteins and their PTMs

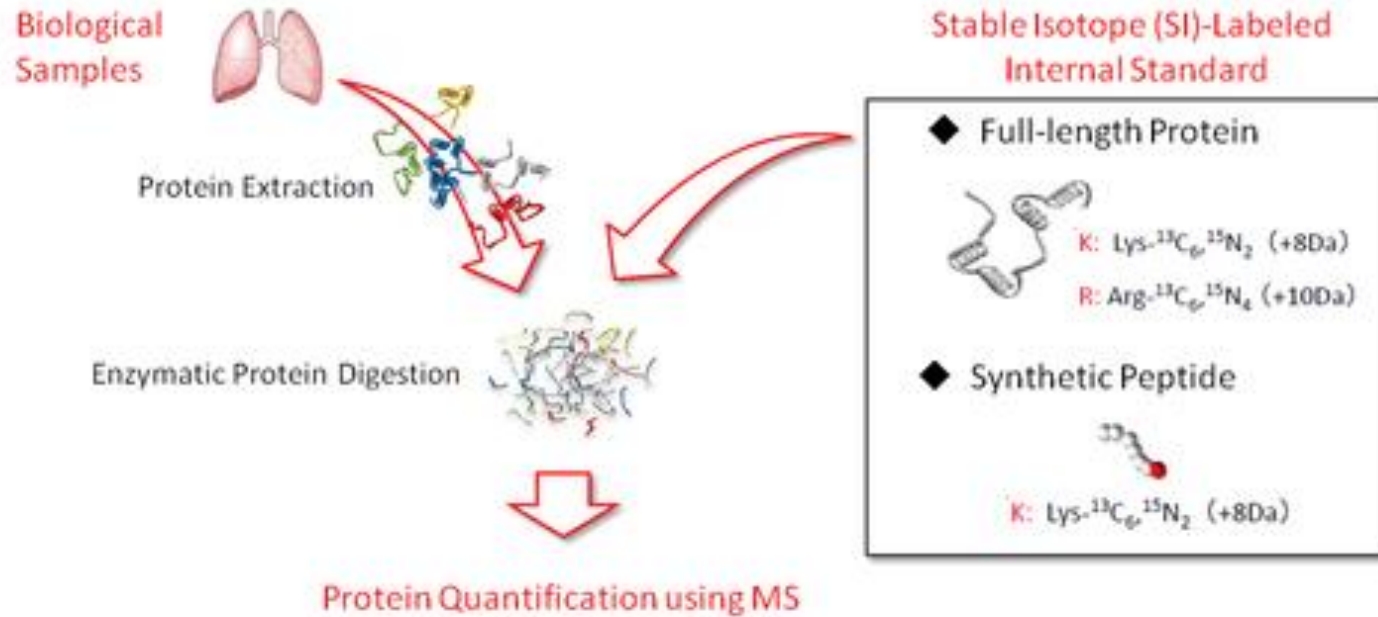


ThermoFisher
TSQ Vantage

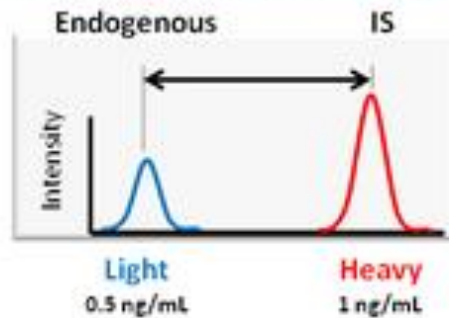
Targeted Mode

- “Absolute” quantification
- 10-100 protein analytes
- Specific, sensitive, accurate & robust
- “High” throughput
- Hypothesis-driven/hypothesis validation
- Plasma, CSF, tissue, cell, organelle
- Proteins and their PTMs

Stable isotope dilution(SID)

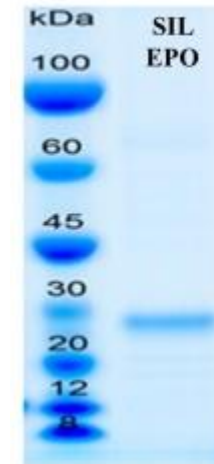


Protein Quantification using MS



<https://www.proteomicslaboratory.com/>

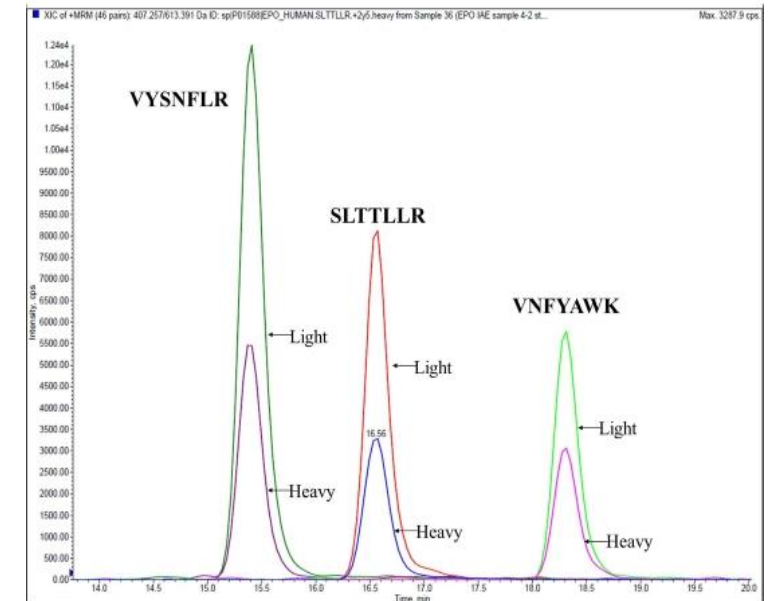
Purity and peptide target selection



Protein Sequence Coverage: SIL Erythropoietin (Human) P01588

APPRLICDSRVLERYLLEAKEAENITTG
 CAEHCSLNENITVPDTK**VNFYAWK**RMEV
 GQQAVEVWQGLALLSEAVLRGQALLVNS
 SQPWEPLQLHVDKAVSGLR**SLTTLLR**AL
 GAQKEAISPPDAASAAPLRTITADTFRK
 LFR**VYSNFLR**GKLKLYTGEACRTGDR

<https://www.sigmaaldrich.com>



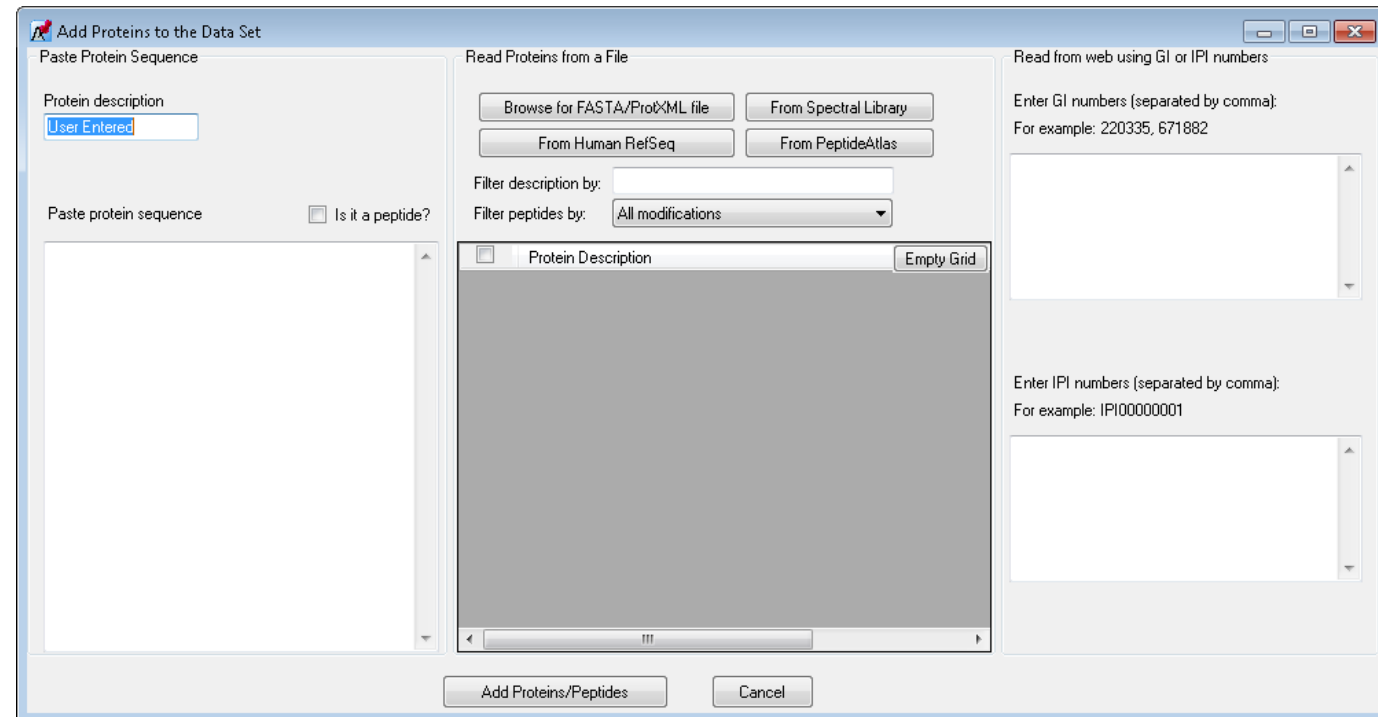
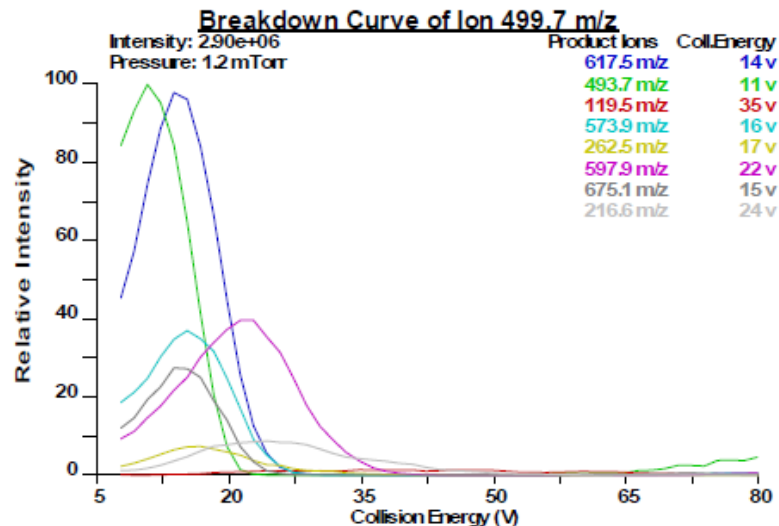
<https://www.sigmaaldrich.com>

Targeted method development (1)

- Choice of peptides (<http://www.uniprot.org/blast/>;
<https://www.nextprot.org/viewers/unicity-checker/app/index.html>)

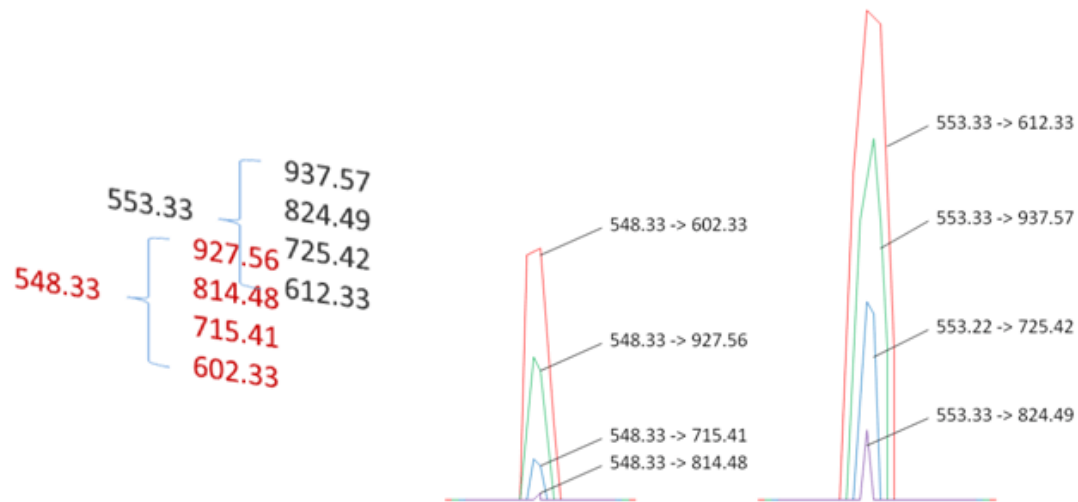
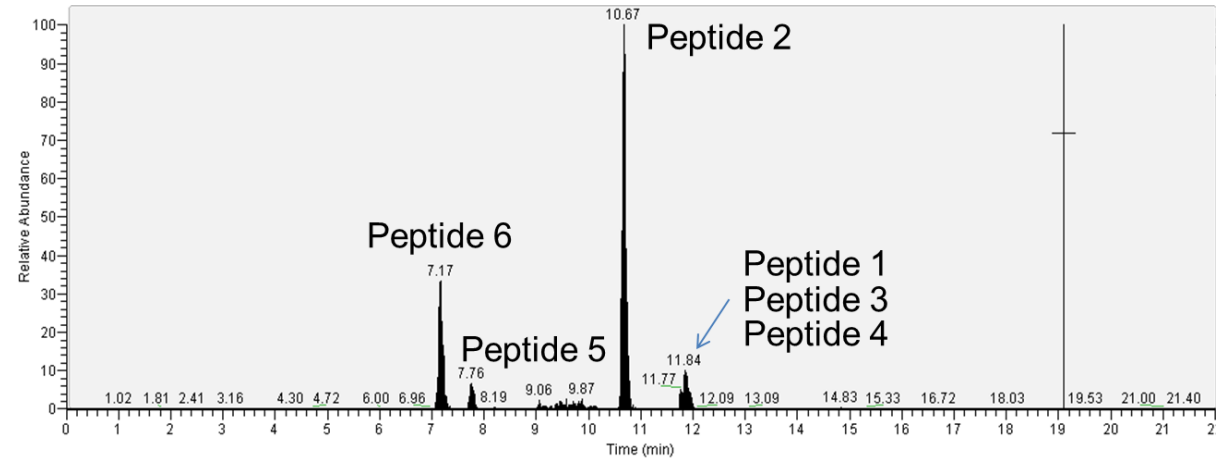


- Synthesis of light and heavy peptides
- Study with MS/MS (infusion)

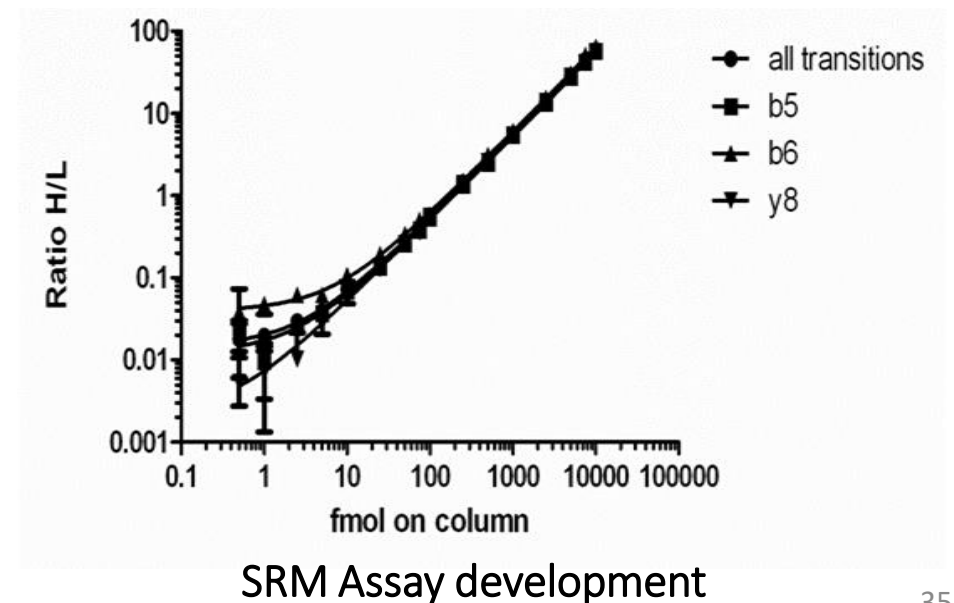


Targeted method development (2)

- Study with LC MS/MS (in buffer)
- Study with LC MS/MS (in sample matrix)
- Assay characterization (in sample matrix)

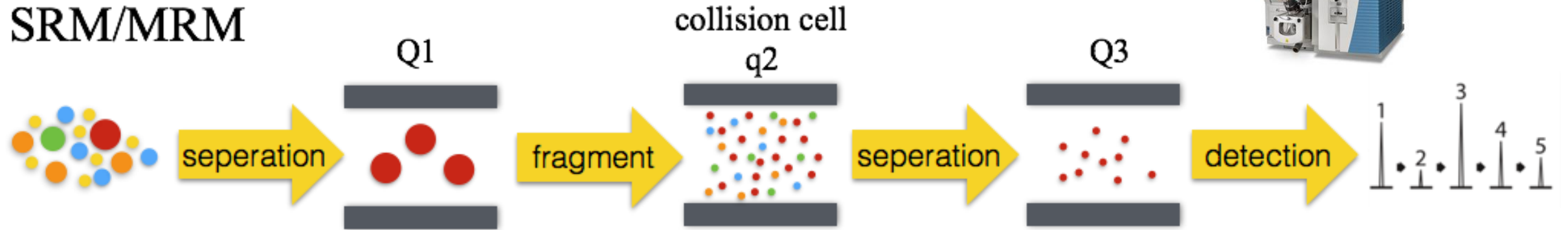


<https://moritz.systemsbiology.org/resources/proteomics-program/targeted-proteomics/>

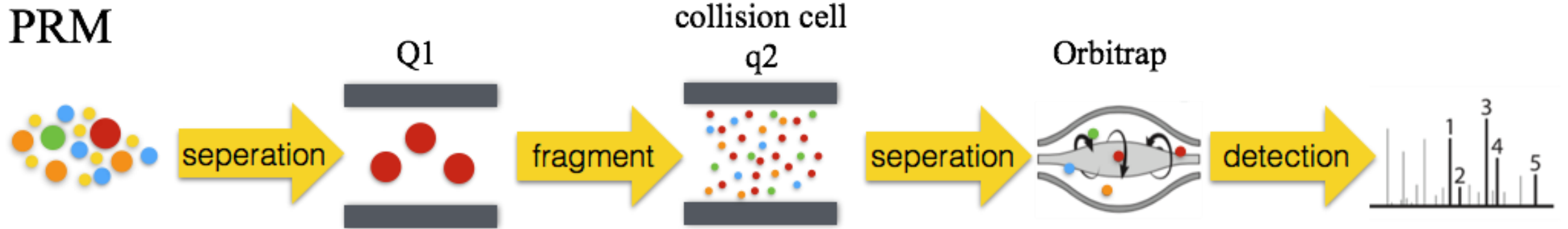


Parallel Reaction Monitoring (PRM)

SRM/MRM



PRM



Precursor ion

Fragment ions



<https://www.creative-proteomics.com>

Parallel Reaction Monitoring (PRM)

Peptide selection – Fragmentation – Fragment analysis



Proteins and peptides selection

Transitions selection

Collision Energy optimisation

Selectivity

Sensitivity

Higher Specificity

Sample preparation optimisation

LC-MS method development

Accuracy

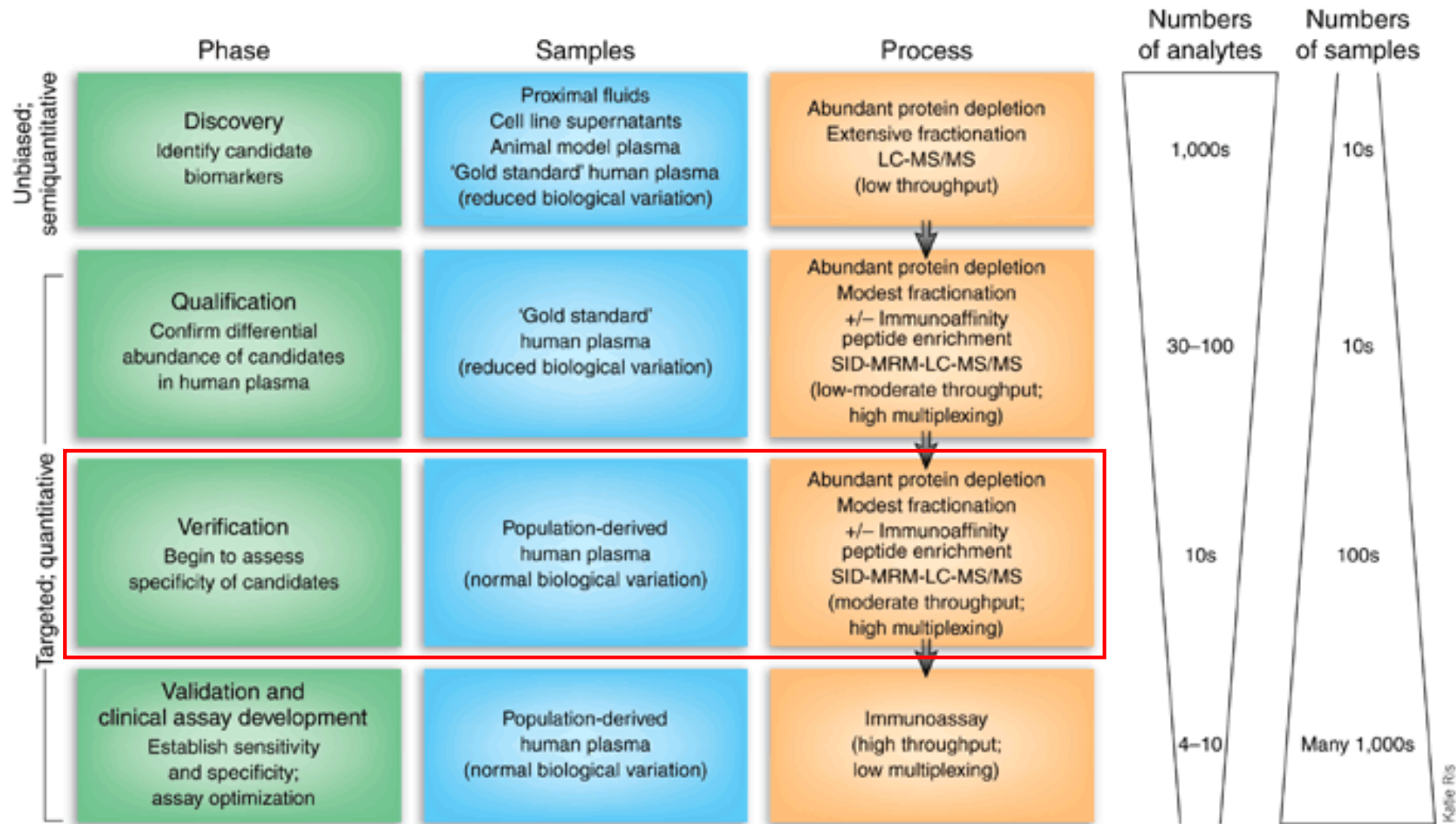
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Method development

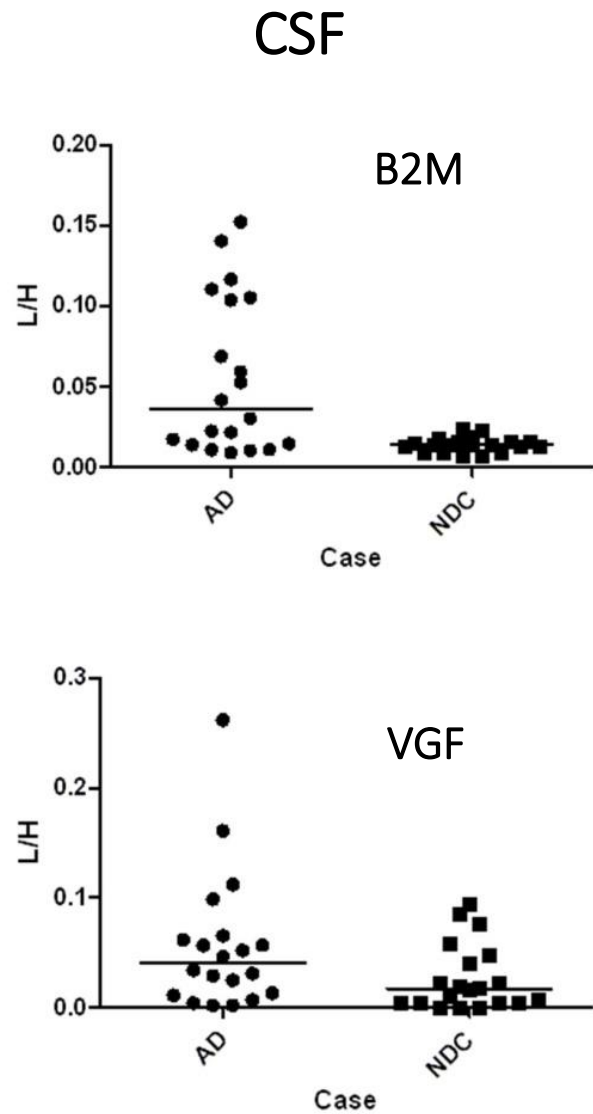
Method characteristic

Method development time divided by 3
Simplified workflow
More confident in quantification results

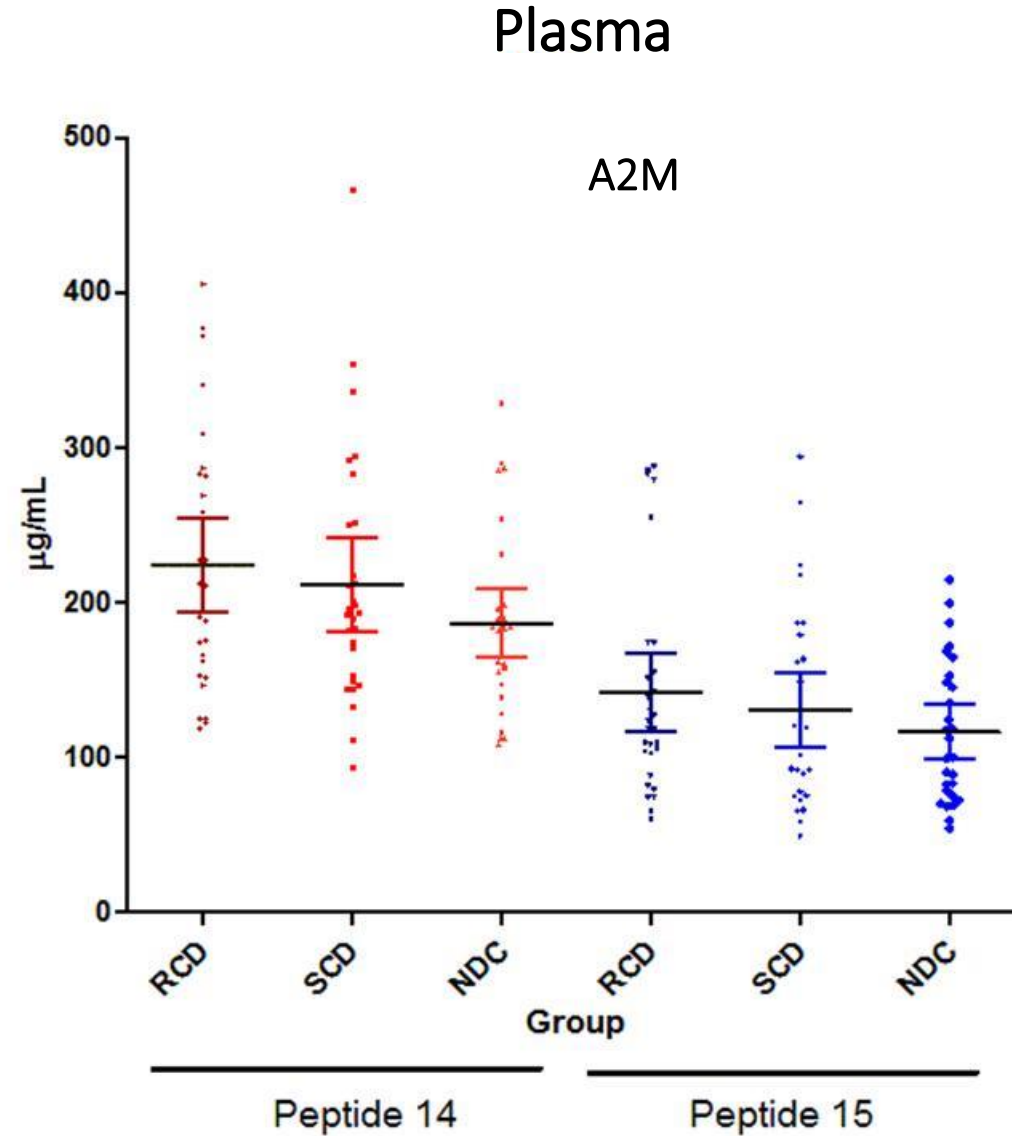
Main applications: Verification of biomarker discoveries



Verification of biomarker discoveries

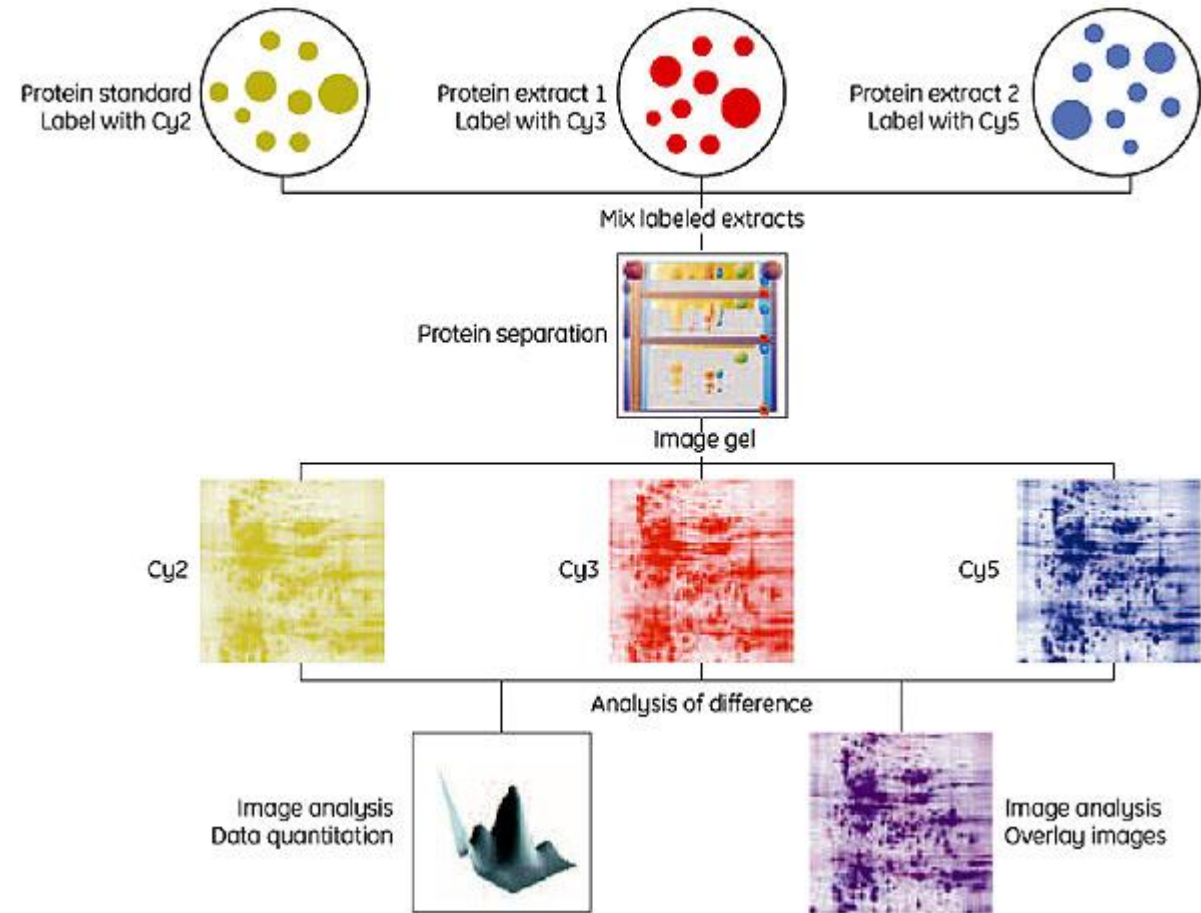
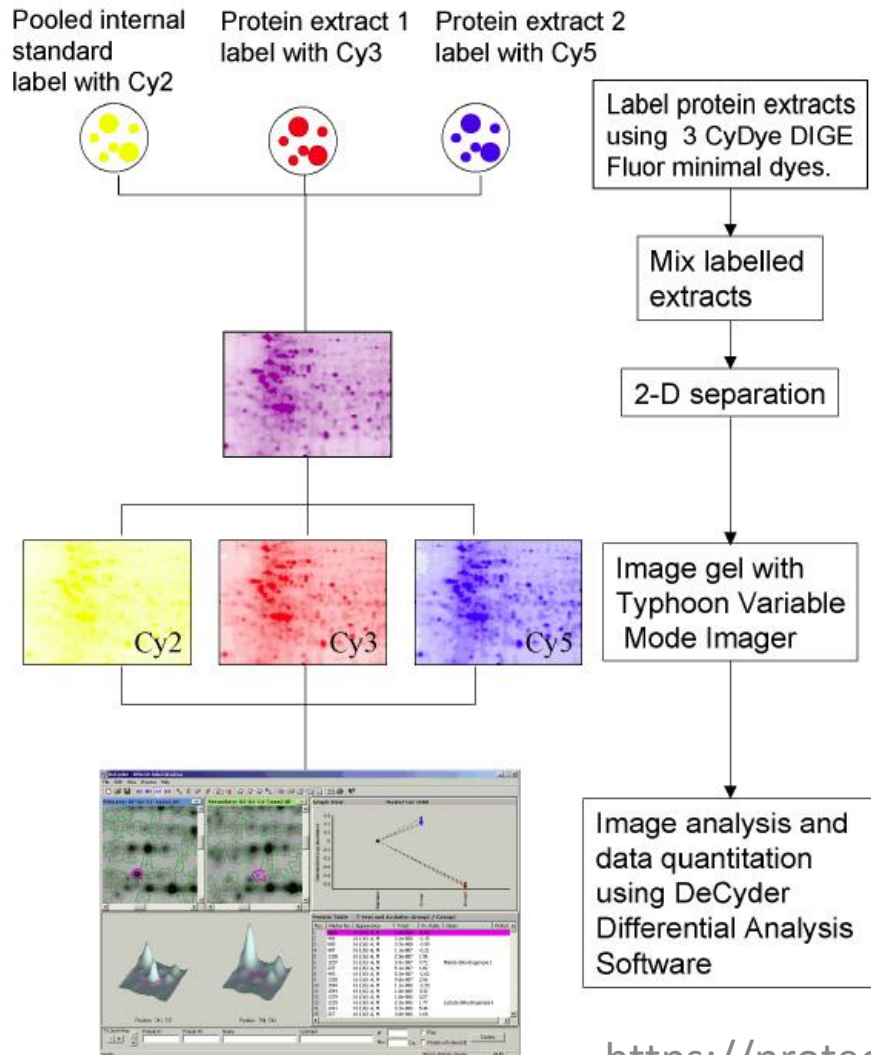


Alzheimer's and Dementia, Volume 7, Issue 4, Supplement, July 2011, Pages S150-S151



Alzheimer's and Dementia, Volume 7, Issue 4, Supplement, July 2011, Page S333

2-dimensional differential gel electrophoresis (2D-DIGE))



<http://pawatbioinformatics.exteen.com/>

<https://proteomics.cf.gu.se>

Summary

- In proteomics, quantification is key to compare different biological conditions
- Different quantitative techniques are used to obtain relative quantification or precise “absolute” quantification of peptides and proteins
- MS is an alternative to standard biochemical methods (*e.g.*, immuno-assays) that allows easily multiplexing of samples and/or analytes
- In the next chapters, we will see the bioinformatic tools used to process the MS data and some concrete applications of those MS-based workflows to study proteomes in biology and clinical research