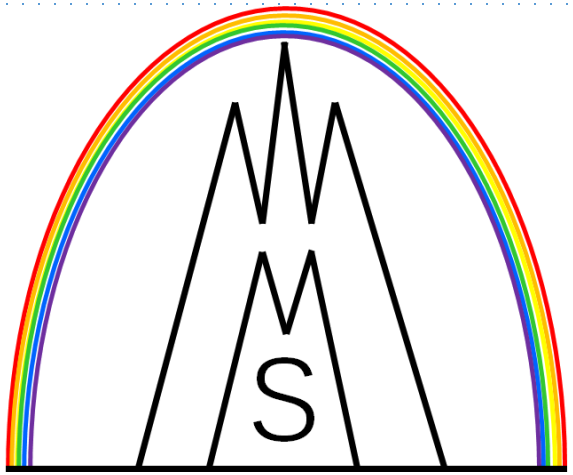
Quantification in Proteomics

5th Short Mass Spectrometry Courses

1/4/2016

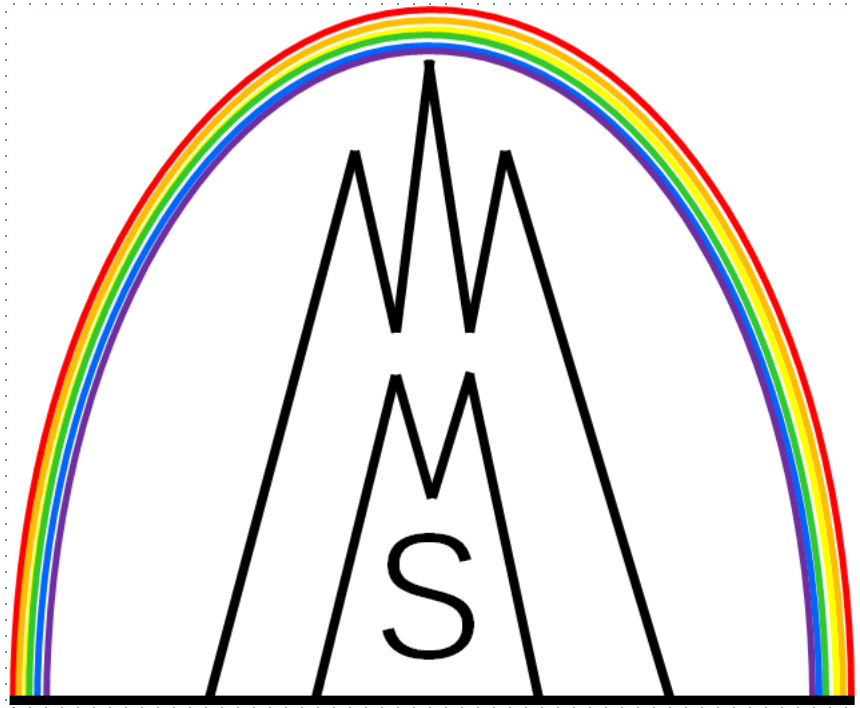
Jana Březinová

Mass Spectrometry IOCB AS CR



Presentation Outline

- Bottom-up approach in proteomics
- Quantification methods
  - Labelling techniques
    - Metabolic labelling – SILAC
    - Chemical labelling – dimethyl labelling
  - Label-free techniques
    - MS approach – MaxQuant LFQ
    - MS/MS^{all} approach –SWATH
    - Targeted approach – SRM
- How to design your own experiment?



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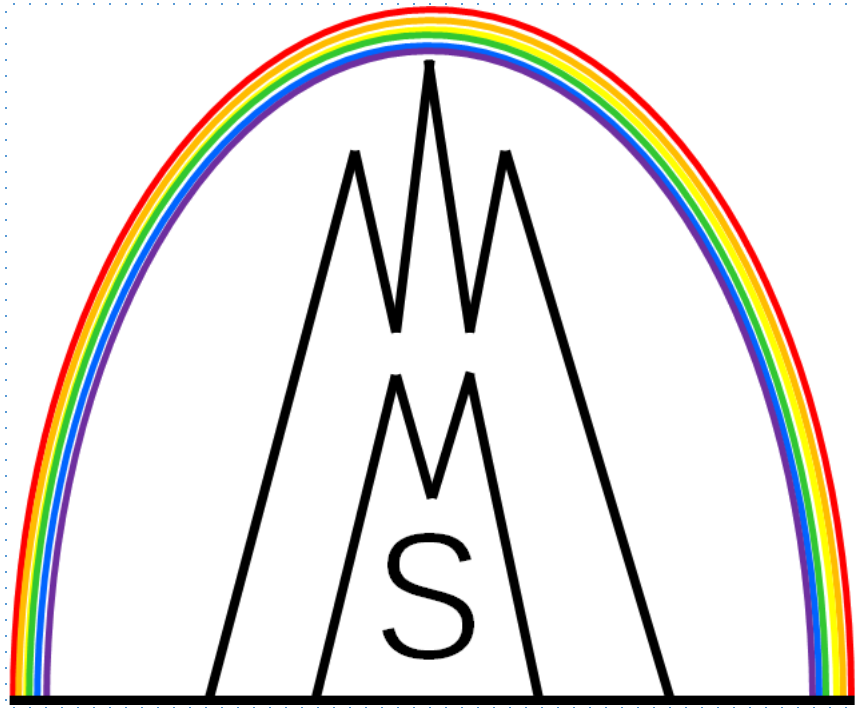
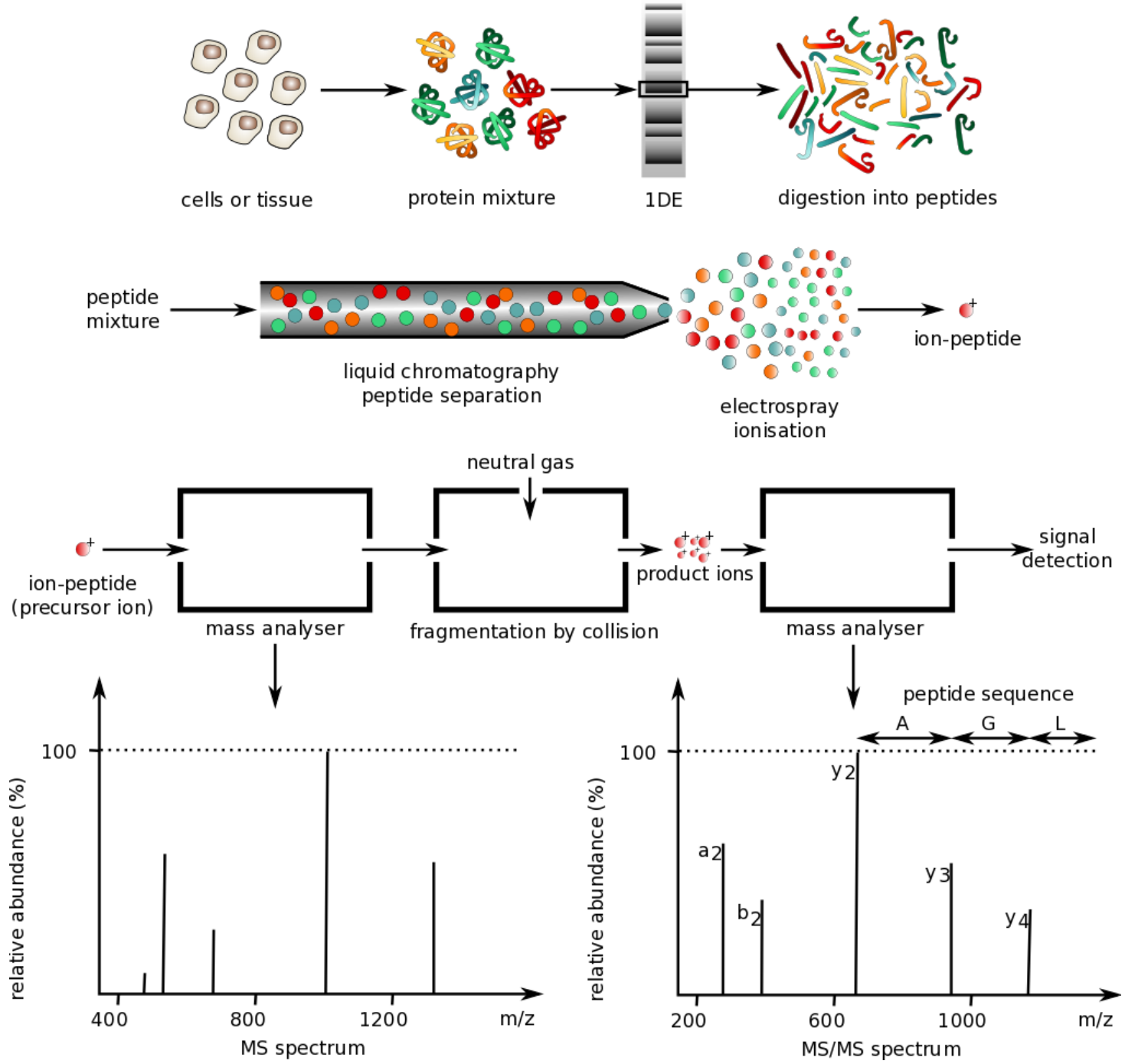


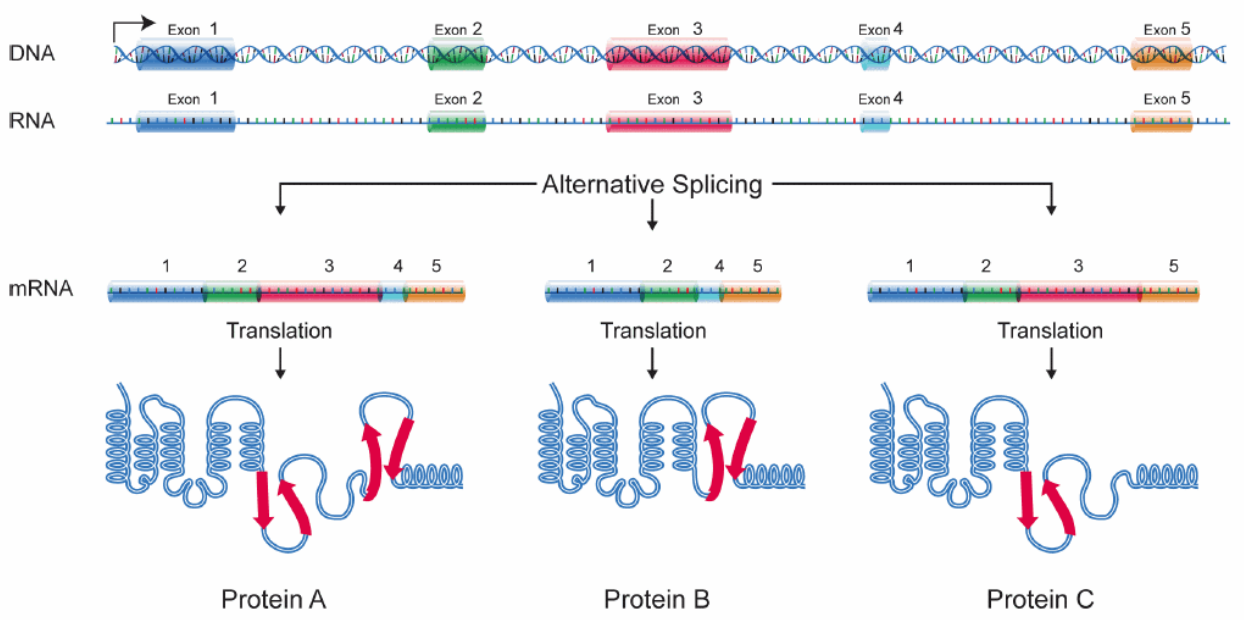
Bottom-up proteomics

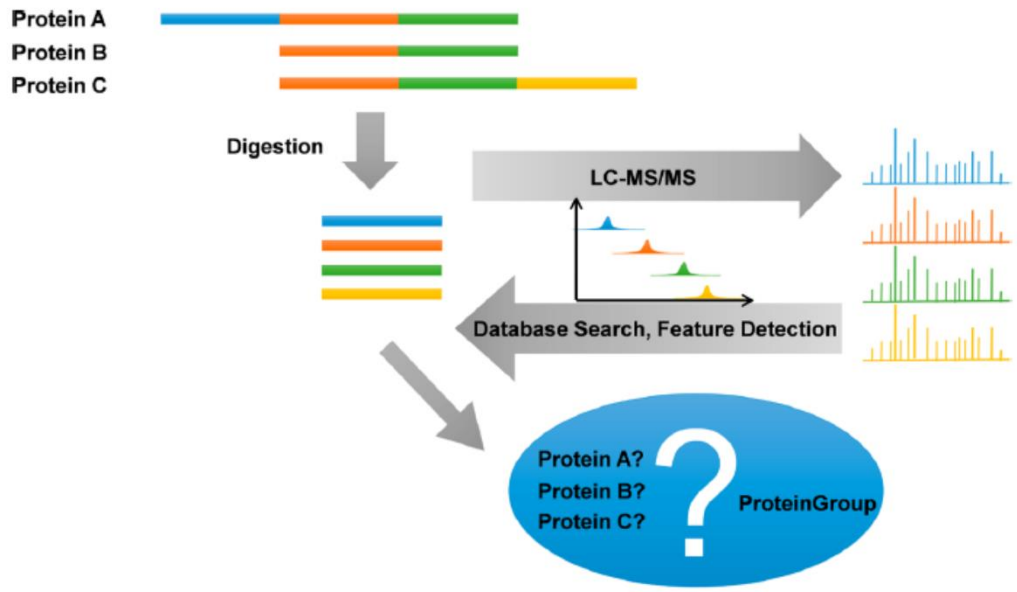


https://upload.wikimedia.org/wikipedia/commons/1/1f/Mass_spectrometry_protocol.png

Limitations of Bottom-up Approach

- Protein level information is inherently lost
  - **Proteins are quantified indirectly**, their ratios are inferred **from peptides** after digestion.
  - **Protein isoforms** impose a problem to protein quantitation. Close *inspection of data on peptide level* is required.





http://www.genome.gov/Images/EdKit/bio2j_large.gif

http://pubs.acs.org/doi/pdf/10.1021/acs.analchem.5b04148

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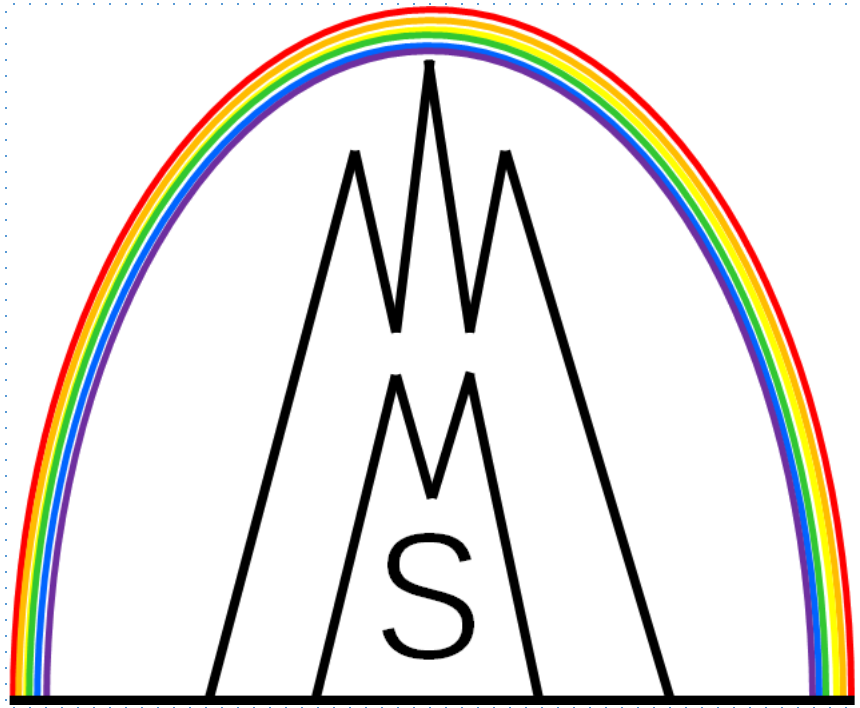
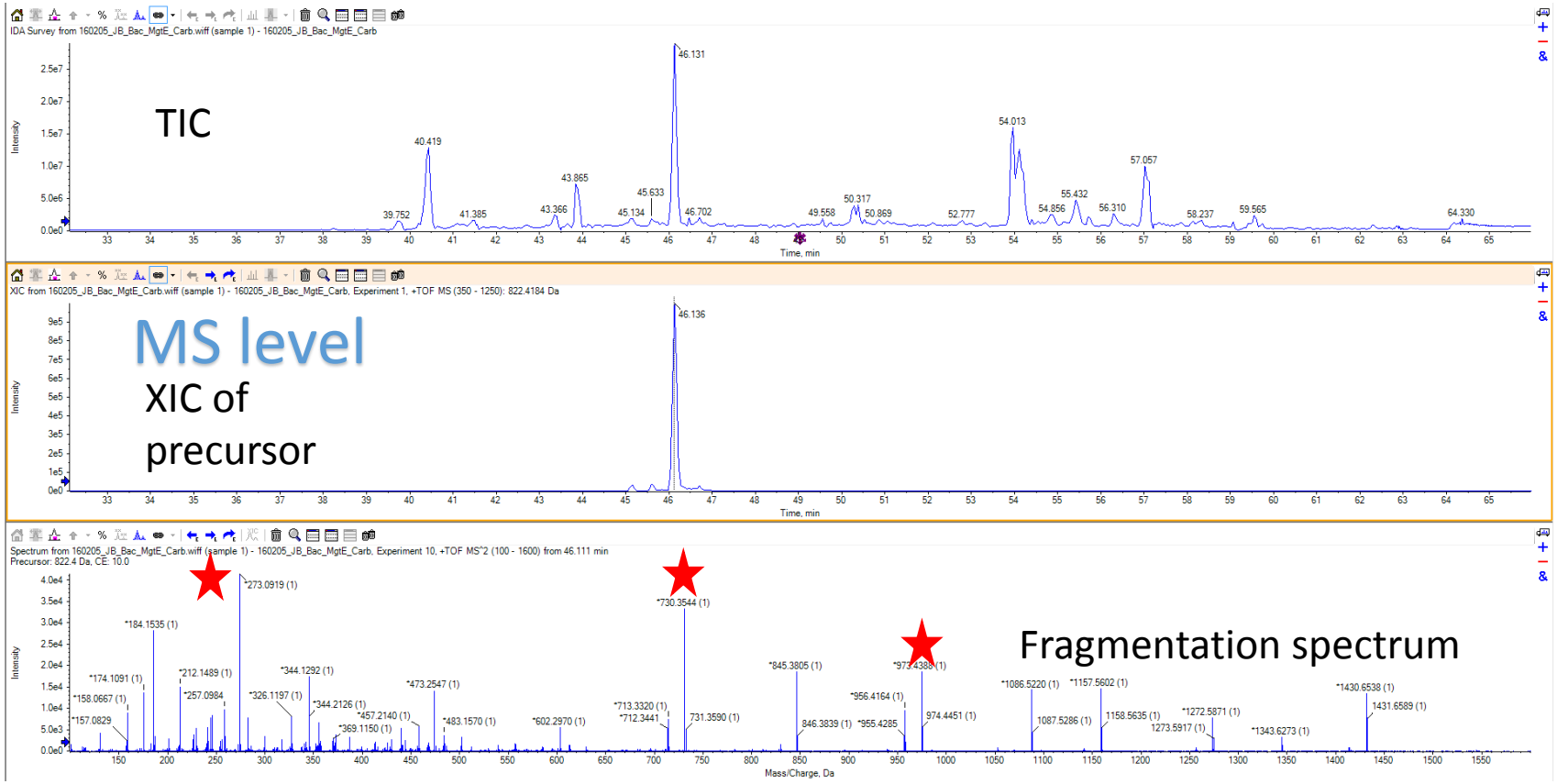


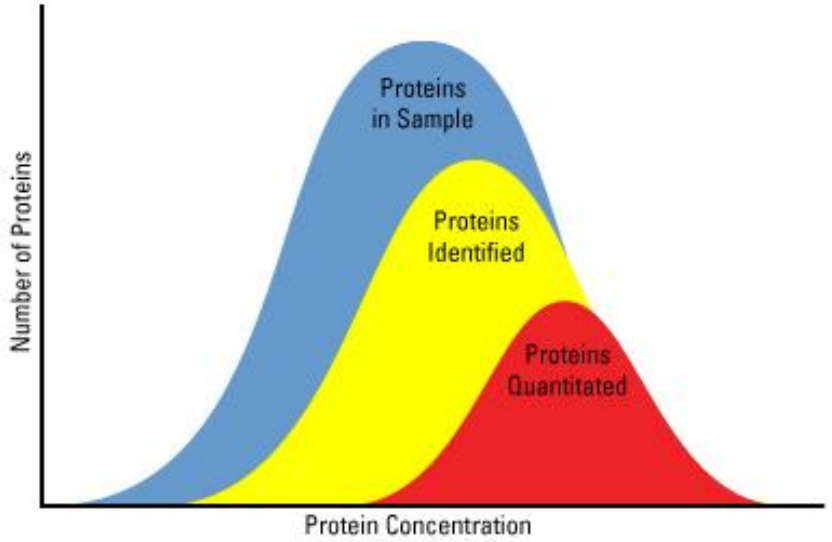
MS quantification

- Based on **peak heights or areas**
  - **MS level quantification**: XIC of precursor peptide(s) m/z
  - **MS/MS level quantification:** XIC Of fragment peptide ion(s) m/z



MS quantification in proteomics

- Often only **relative** determination of quantity
- **Labelling techniques**
  - A **mass tag (label)** is introduced into the protein or peptide. Compared **samples are mixed together and analyzed.** The introduced mass shift enables relative quantitation.
- **Label-free techniques**
  - The **mass** of the protein or peptide remains **unchanged**, samples are **analysed separately.**



https://www.thermofisher.com/cz/en/home/life-science/protein-biology/protein-biology-learning-center/protein-biology-resource-library/pierce-protein-methods/quantitative-proteomics.html

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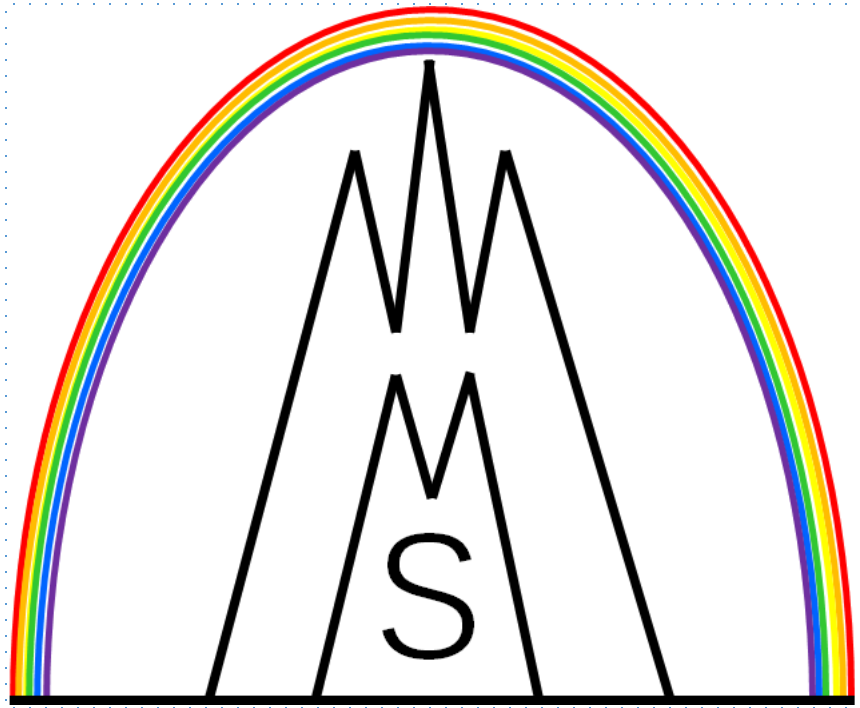


Labelling techniques

Stable isotopes

- **Differential mass labels**

  Heavy: ^{13}C, ^{15}N, ^{18}O, ^{2}H

  Light: ^{12}C, ^{14}N, ^{16}O, ^{1}H
- **Introduction of single elements**
  - Trypsin digestion in $H_2{}^{18}O$
  - ^{15}N labelling of cell cultures
- **Introduction of compounds labelled by multiple heavy isotopes**
  - Stable isotope labelling of amino acids in cell culture (SILAC)
  - Isobaric tag for relative and absolute quantitation (iTRAQ)

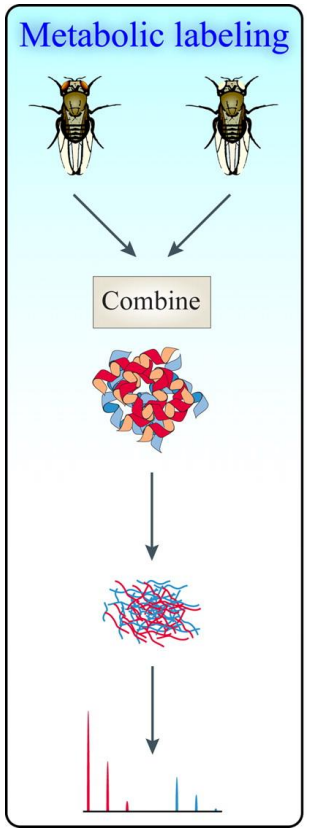
Presumptions

Equal behavior under **chromatographic conditions** – corresponding H/L labeled peptides elute at the same time

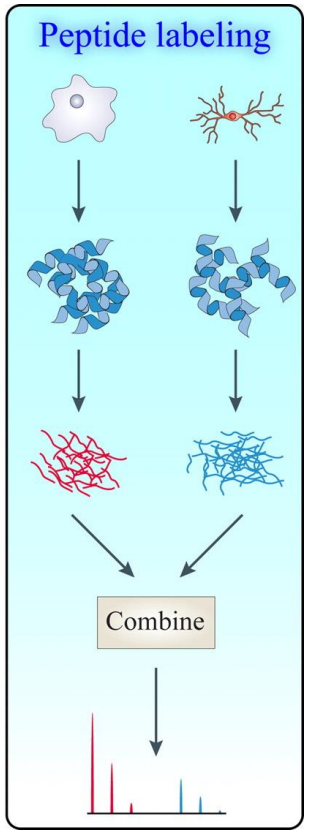
Equal **MS sampling probability** of the isotopes during their elution window

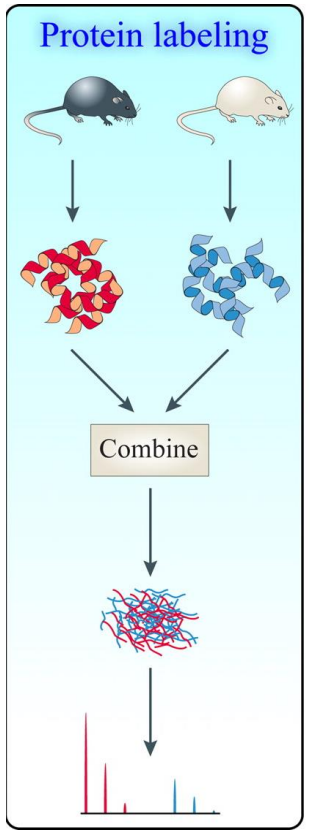
Labelling techniques

- Metabolical
  - Eg. SILAC



- Chemical
  - Eg. Dimethyl labelling





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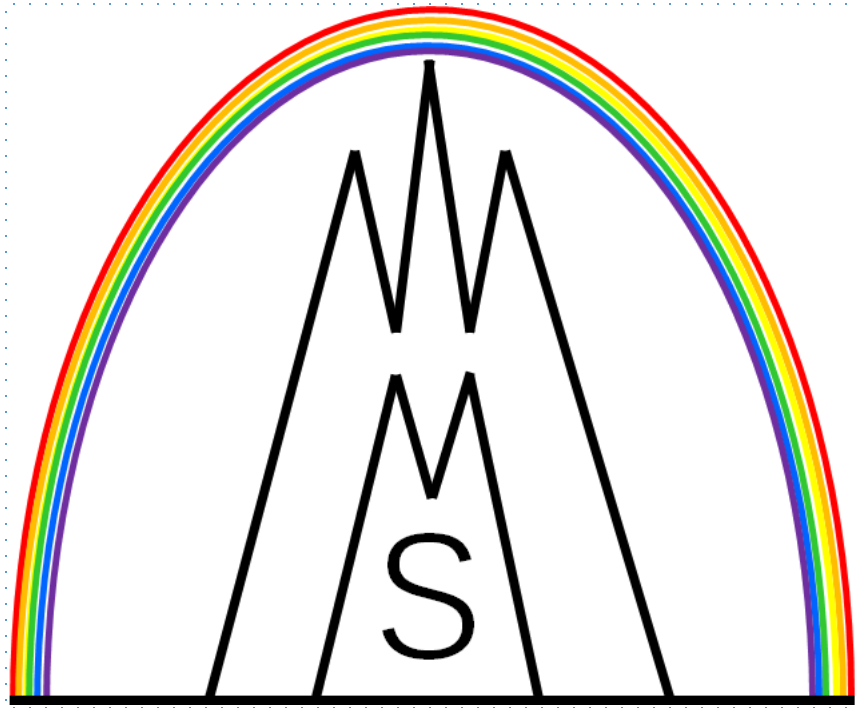


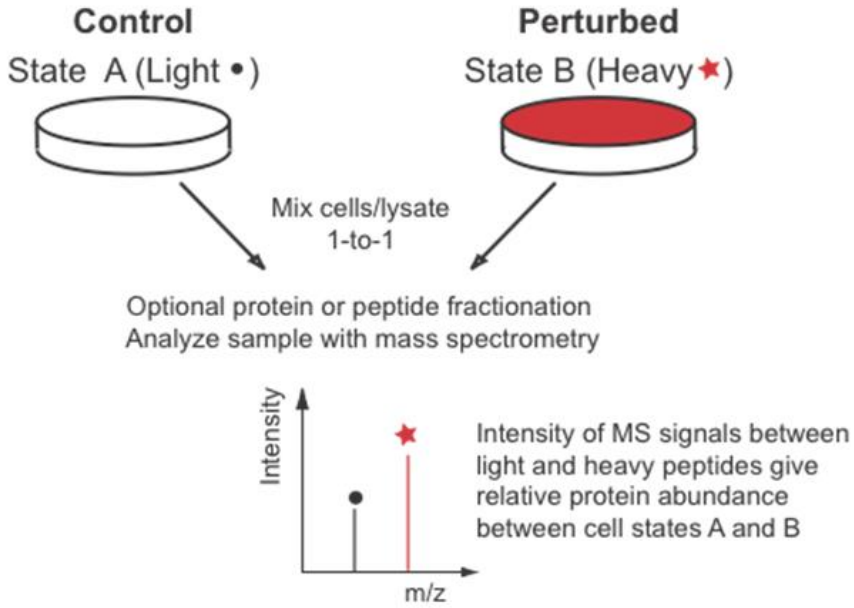
Metabolic Labelling – SILAC

Stable isotope labeling by amino acids in cell culture

- **Cell culture** is grown on a medium containing either only **heavy or light AAs** (Arg, Lys) – auxotrophy required
- **Labelled AAs** are used as protein **building blocks**
- After at least **5 cell cycles** a nearly full incorporation of the heavy AAs is achieved
- **Label incorporation** needs to be monitored
- Eliminated influence of

  sample preparation variations

  on quantification

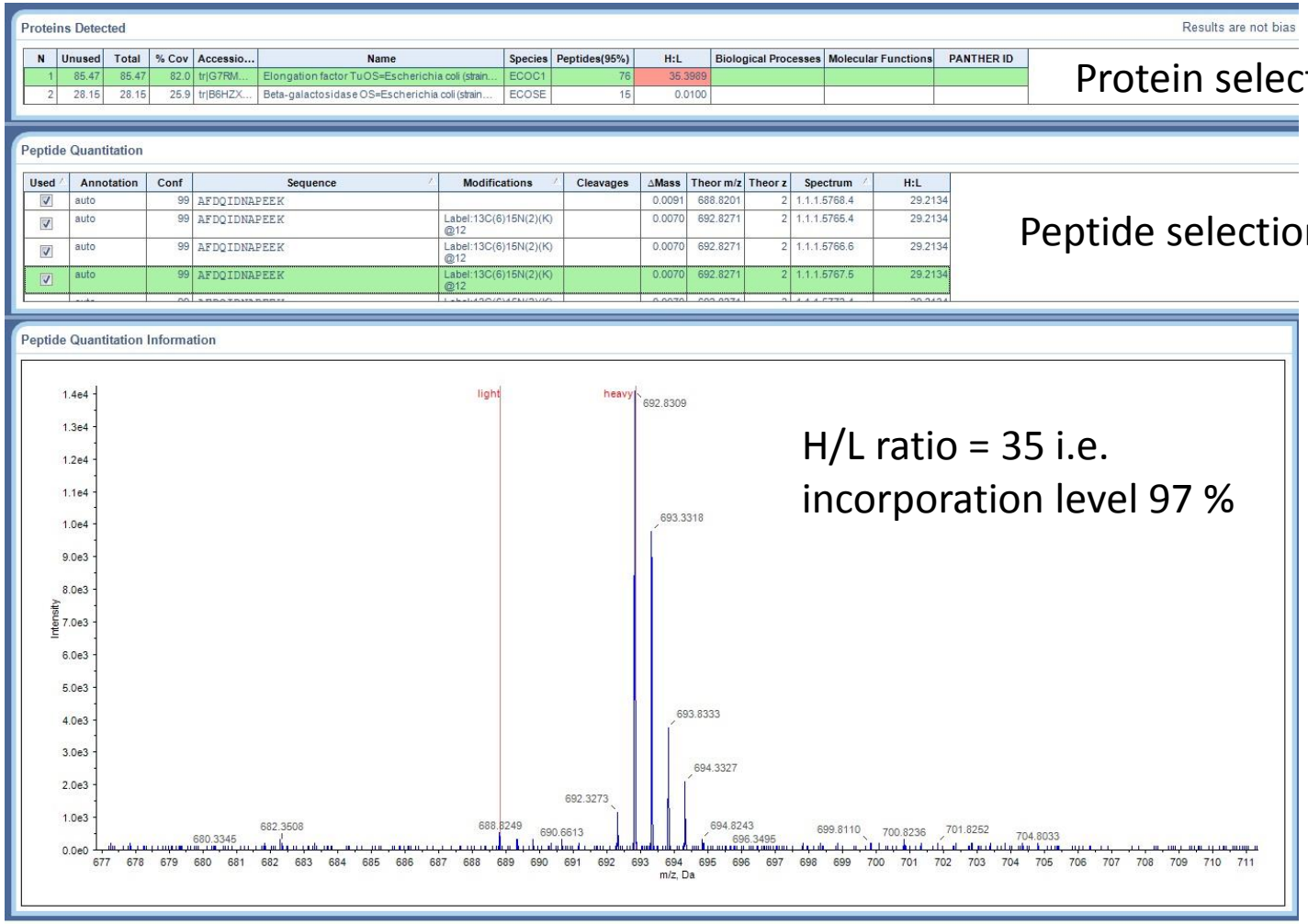


https://www.broadinstitute.org/scientific-community/science/platforms/proteomics/silac

SILAC Incorporation

LC-MS/MS quantitation result of a selected protein from a heavy labeled cell culture

- First step: Incorporation level of heavy AAs (^{13}C ^{15}N Arg, Lys) into the cell culture proteins



SILAC

Advantages

- Samples are **mixed early** in experiment – accounts for any sample losses
- Suited also when **extensive sample prep** is required
- Both **shotgun** and **targeted** approach

Disadvantages

- **Auxotrophy** for Lys, Arg
- Easily applicable only to **cell cultures**
- Metabolic conversion of Arg to Pro
- **Limited multiplicity**
- **Expensive**

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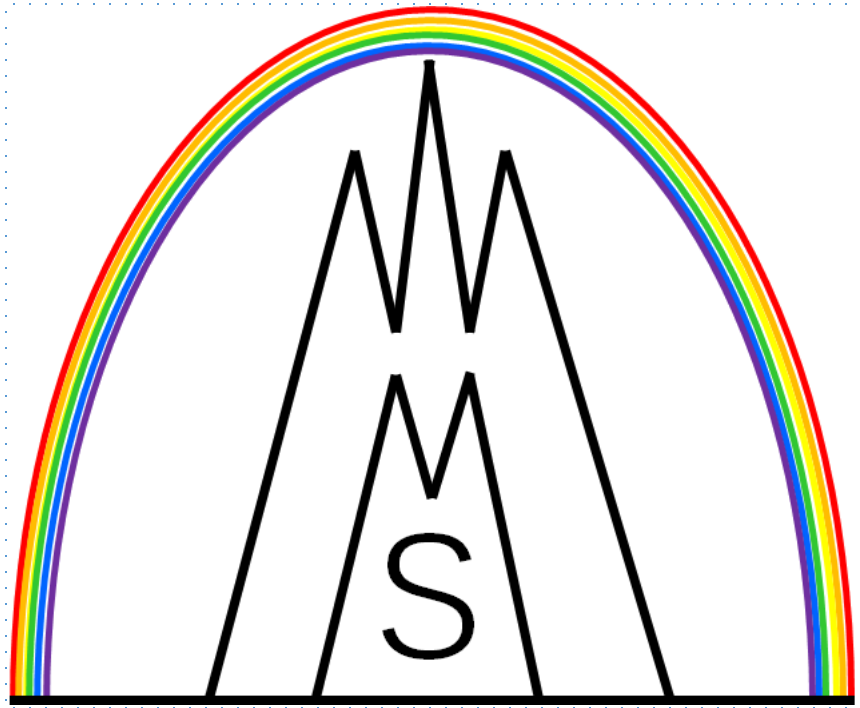
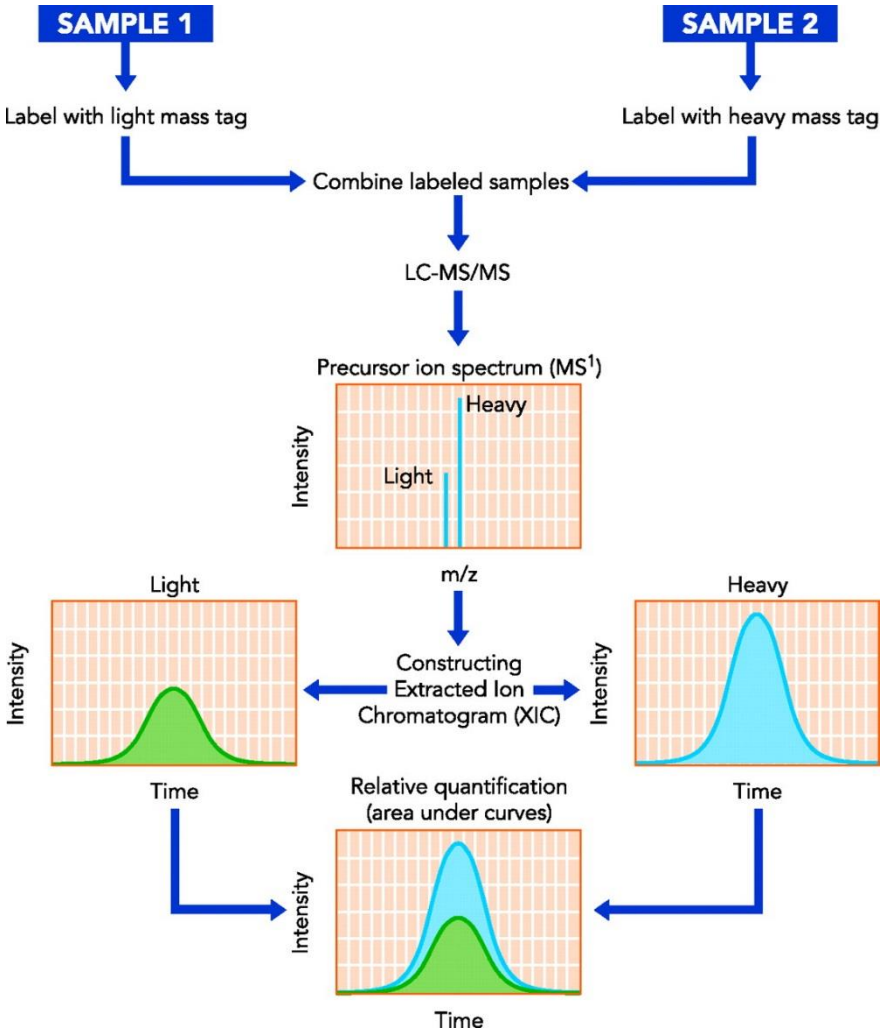


Chemical labelling

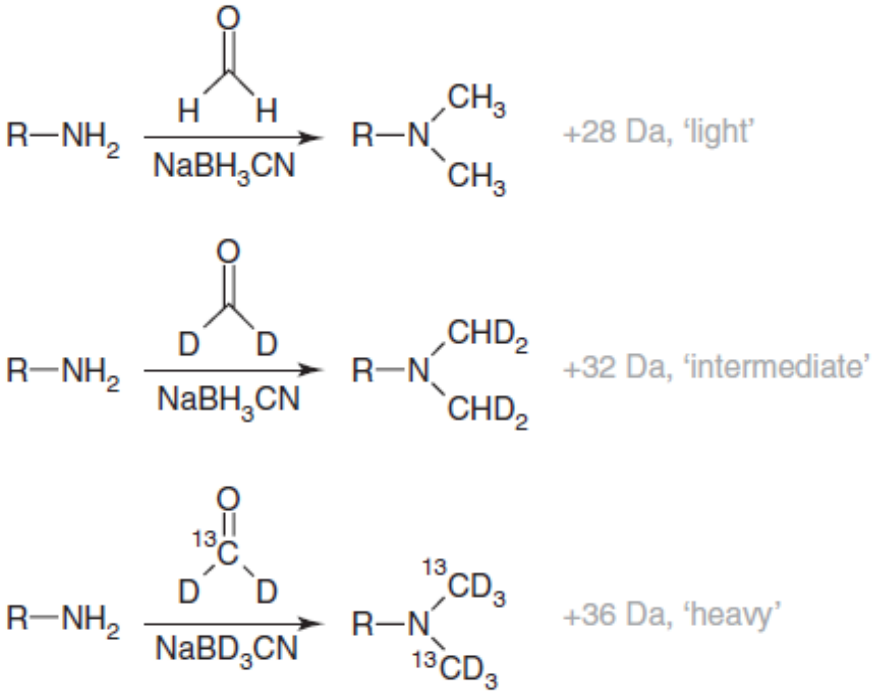
- Cysteine labelling techniques
  - ICAT – Isotope coded affinity tag
- Primary amine labelling techniques
  - Dimethyl labelling
  - iTRAQ (AB Sciex)
  - TMT (Thermo)

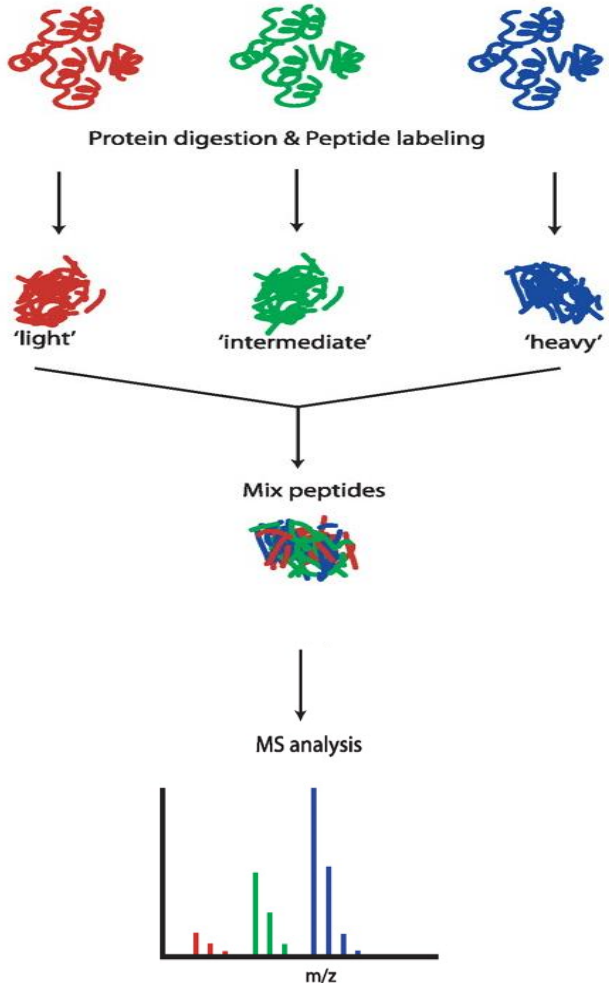


http://physiologyonline.physiology.org/content/22/6/390

Dimethyl labelling

- Reaction of N-termini and ε-amino group of lysine with formaldehyde followed by reduction with sodium cyanoborohydride





Boersema, P. J., et al. Triplex protein quantification based on stable isotope labeling by peptide dimethylation applied to cell and tissue lysates. Proteomics. 8, 2008, pp. 4624–4632.

Dimethyl labelling

Advantages

- **Cheap and easily accessible** reagents
- **Reaction**
  - Fast
  - In solution after digestion

Disadvantages

- Other **primary amines** may react with formaldehyde – avoid Tris, Am. Bic, use TEAB
- All steps prior mixing of samples may influence your results – **optimisation required**

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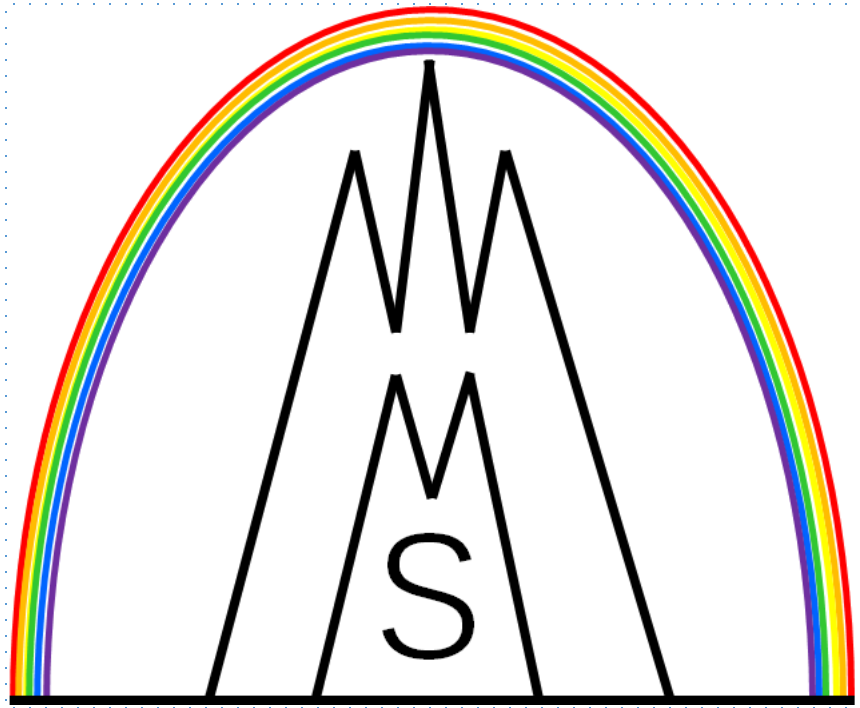
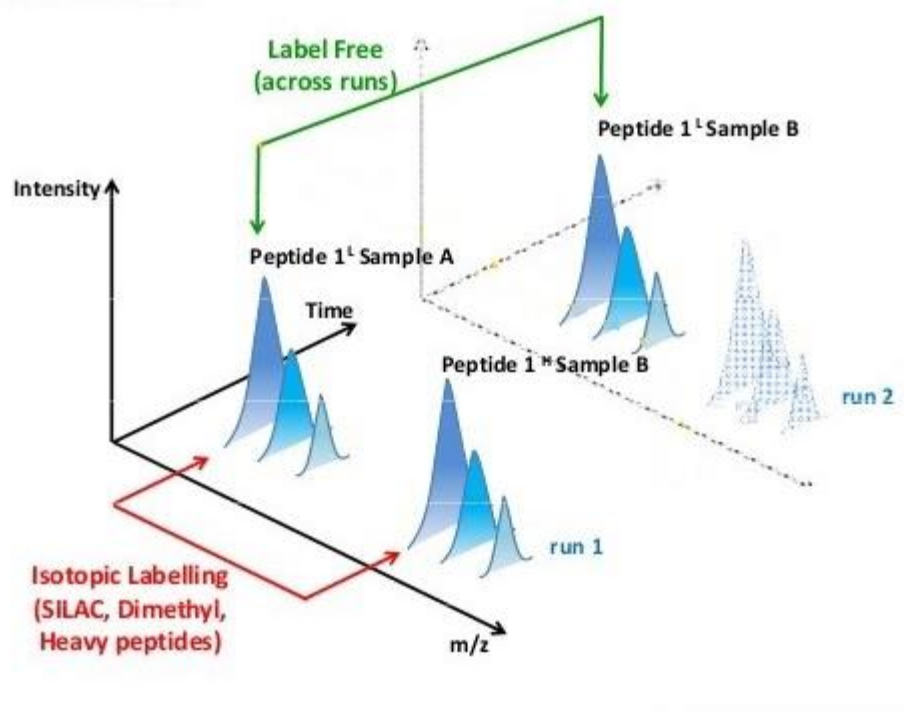


Label-free techniques

- **Less expensive** (no labels)
- Practical for **large** animal or biomarker **studies**
- **Unrestricted number of compared samples**
- **Sample prepation** needs to be **highly reproducible** – internal standards or house-keeping proteins

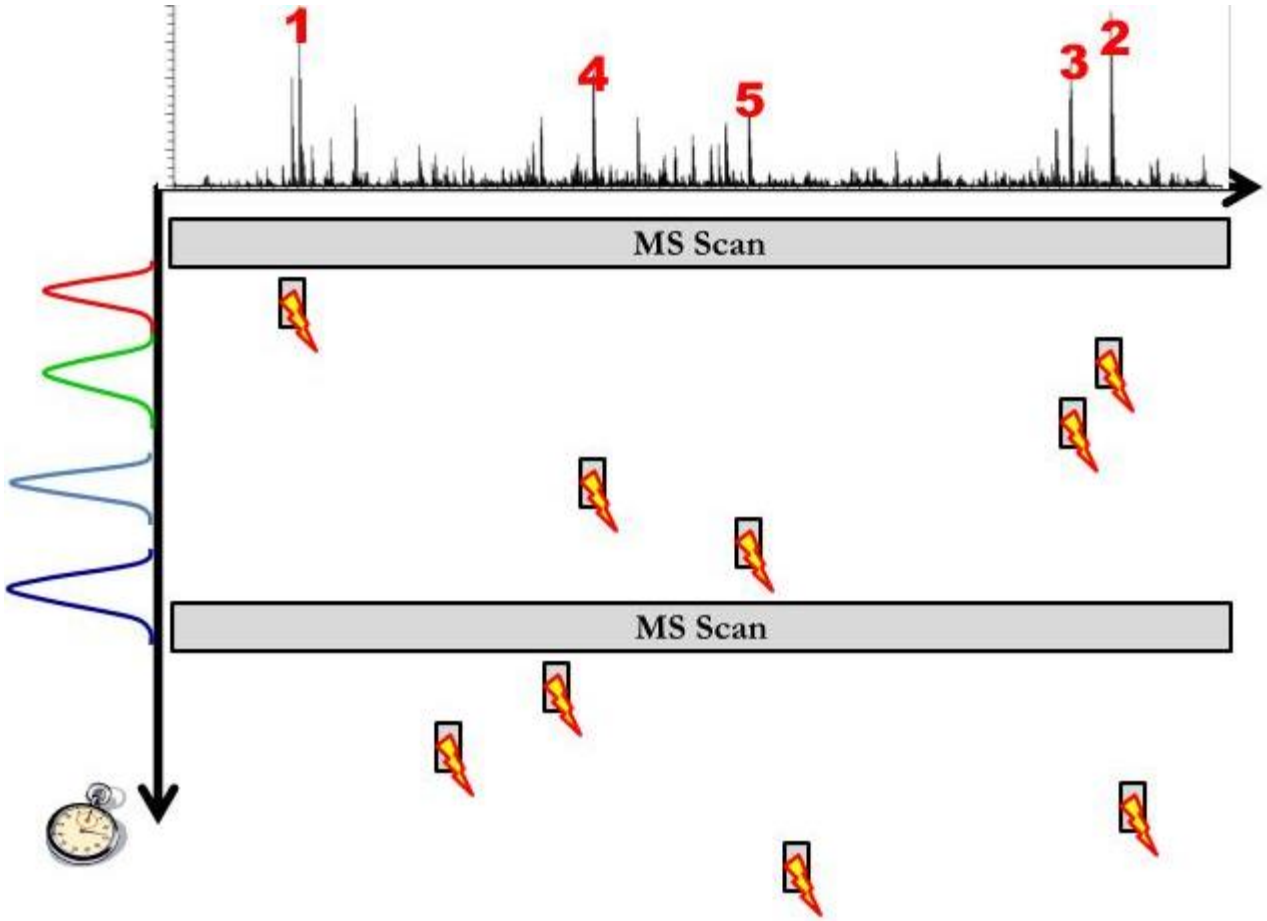


http://www.slideshare.net/COST-events/proteomics-analysis-basics-and-applications

MaxQuant Label-free Quantitation (MaxLFQ)

- Quantification on **MS-level**
- **Unidentified peptides** in one sample are matched to expectant m/z and retention time from another more intensive one
- **Advanced normalization** also for fractionated samples

Standard data-dependent acquisition



http://www.slideserve.com/claral/jarrett-d-egertson-ph-d-maccoss-lab-department-of-genome-sciences-university-of-washington

MS-level quantification

Advantages

- **Precursor ion intensity** > Fragment ion intensity

Disadvantages

- Instrument with **high mass resolution** is essential
- **Less selective** than MS/MS based quantification methods

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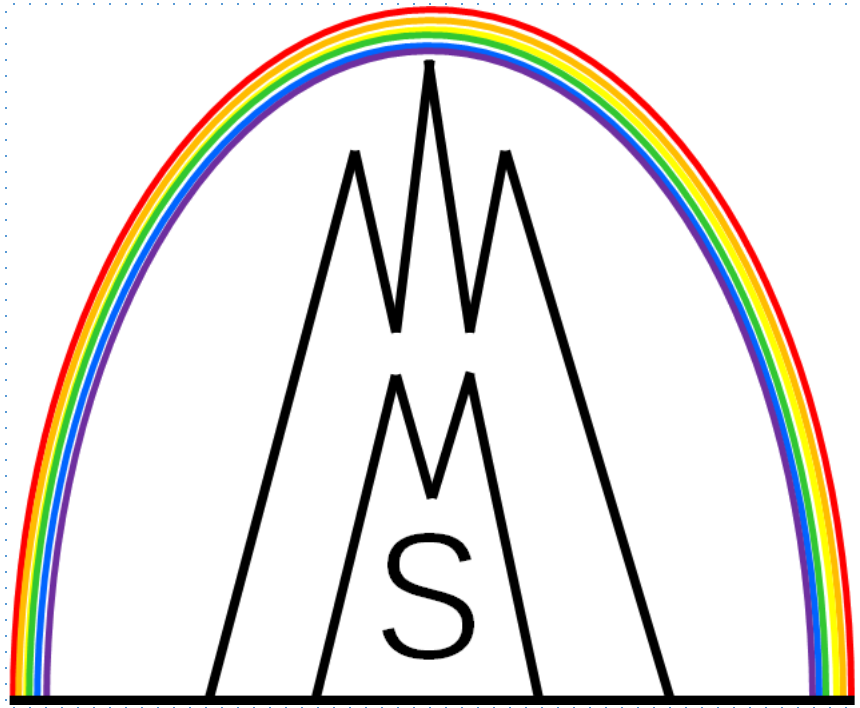
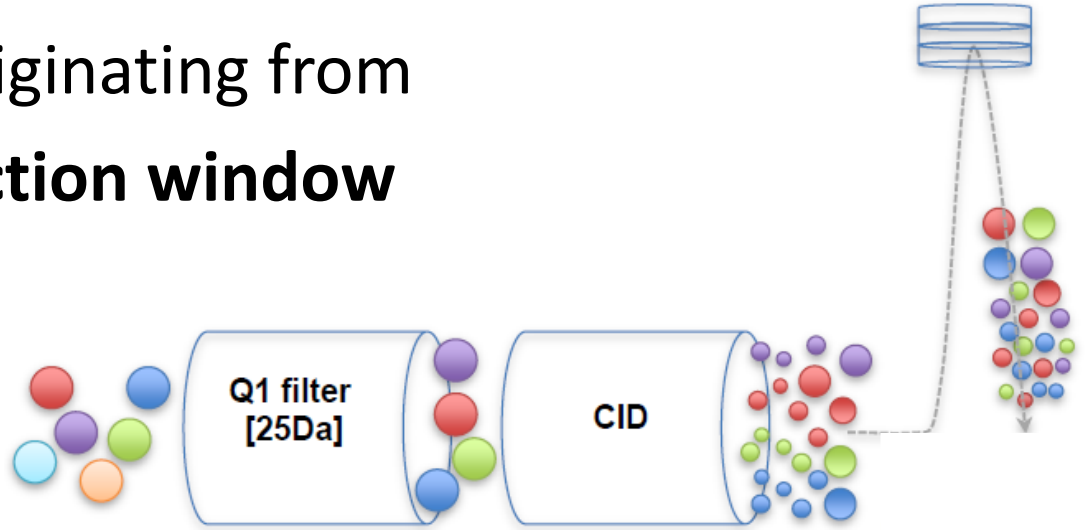


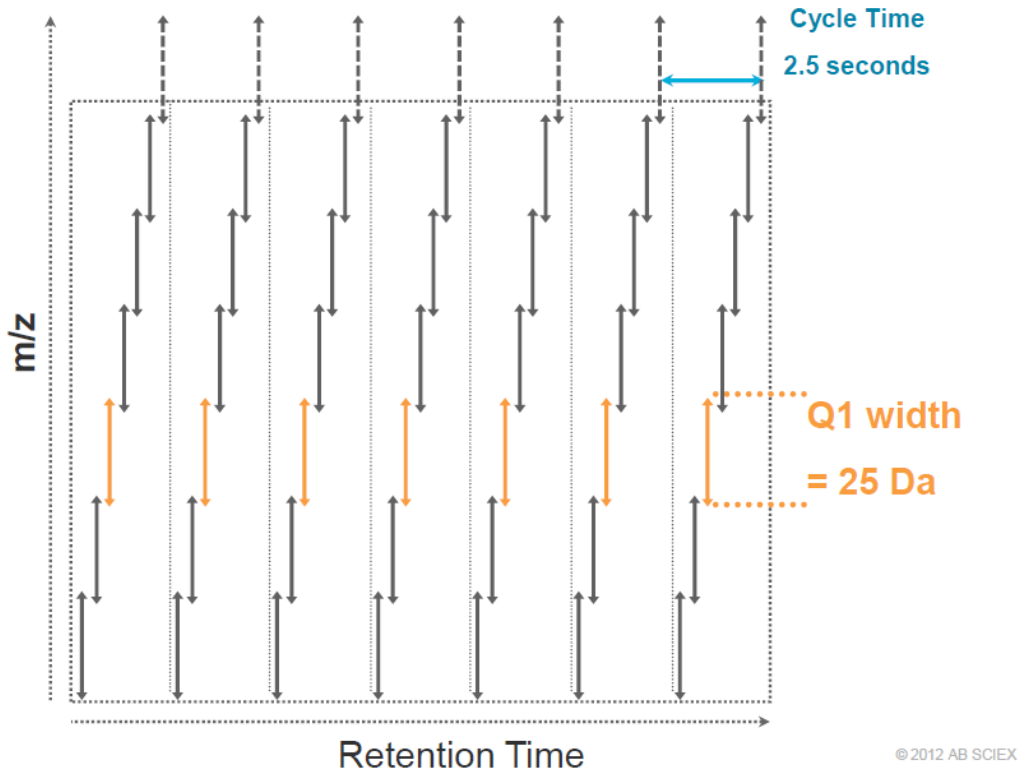
MS/MS-level quantitation - SWATH acquisition

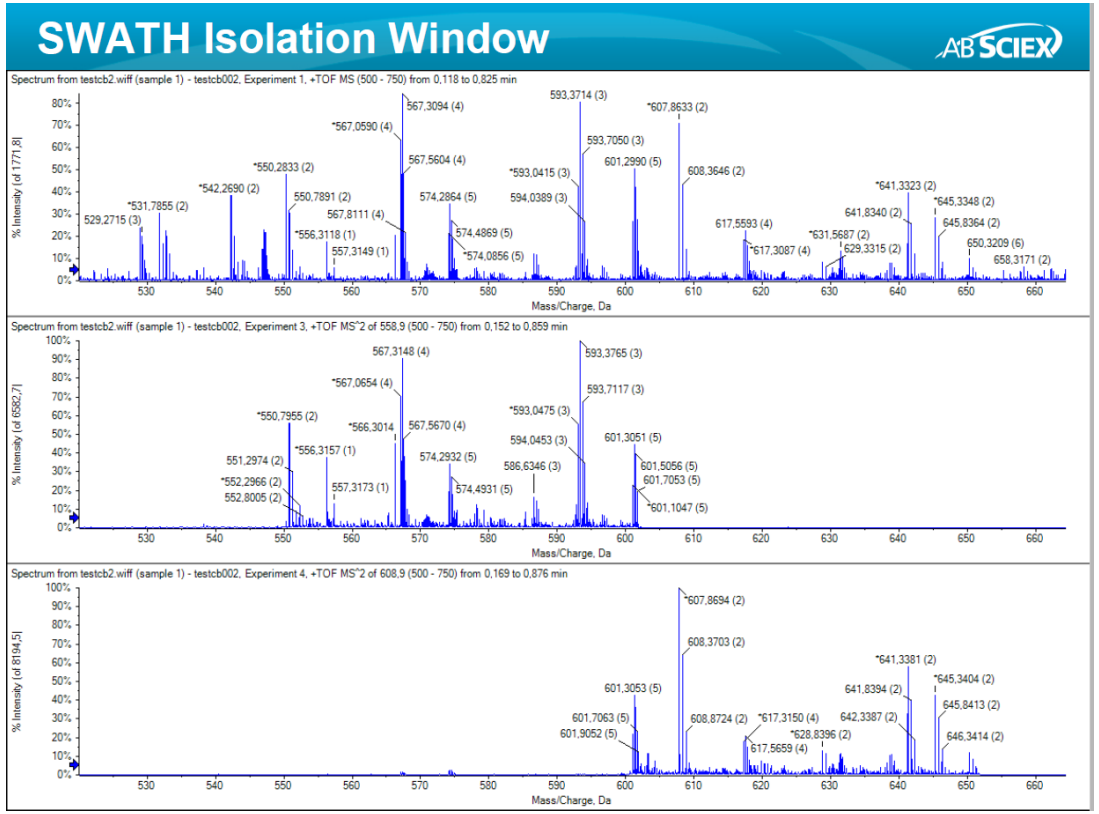
TripleTOF 5600

- Precursor selection **window** (in DDA single m/z)
- Fragmentation in the collision cell
- MS/MS scan of fragments originating from **all precursors from the selection window**



SWATH – MS/MS^{ALL} Acquisition





SWATH

Advantages

- Enables quantititation of previously not considered proteins
- Simplifies SRM method development – choice of precursor ions is less elaborate

Disadvantages

- Large and complex data files
- Spectral library needs to be generated in a separate acquisition run

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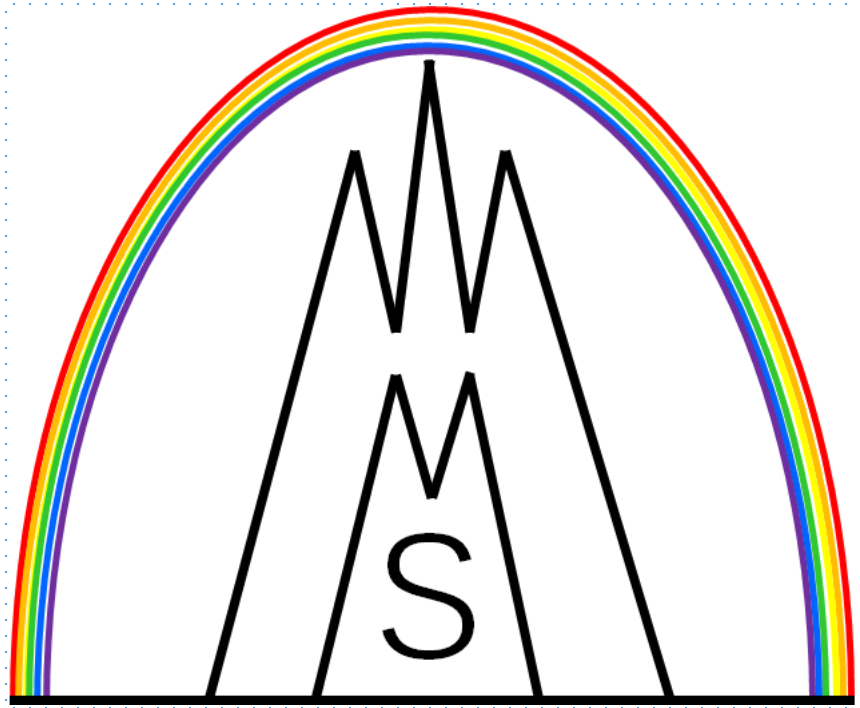
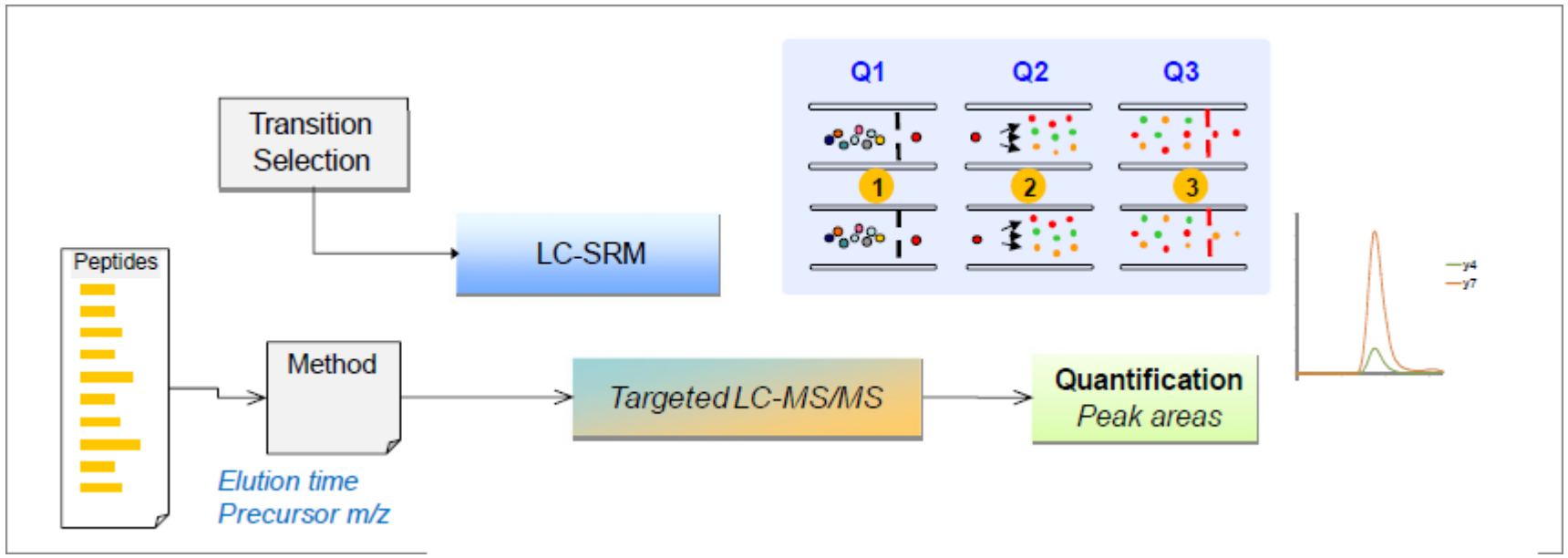
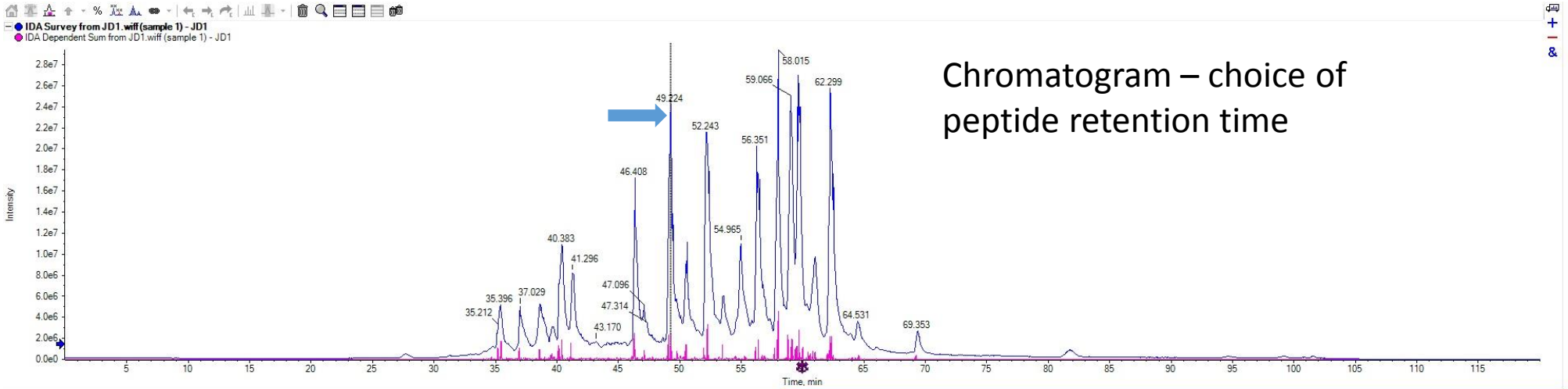


Targeted analysis

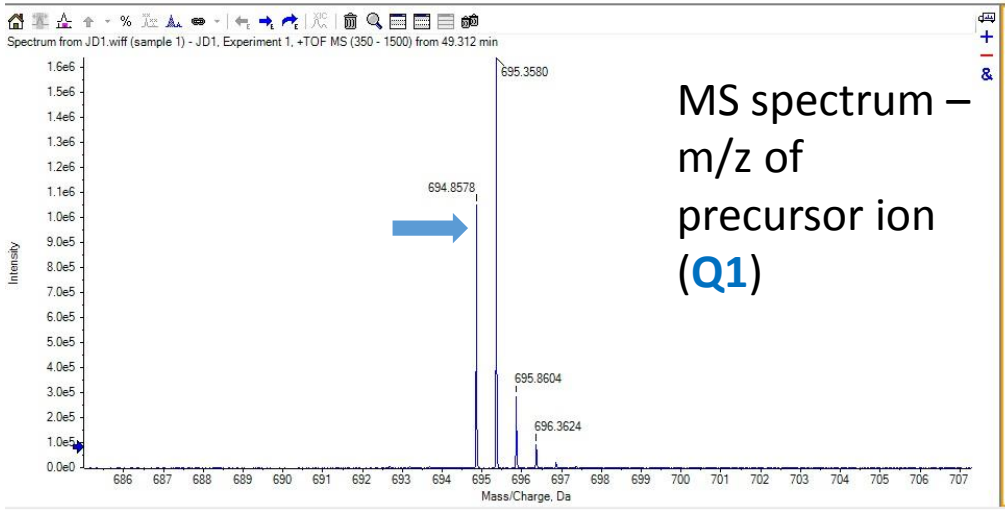
- Precursor selection (Peptide m/z) **Q1**
- Fragmentation in the collision cell **Q2**
- Fragment ion scan (Peptide fragment m/z) **Q3**
  - **Quantitation**



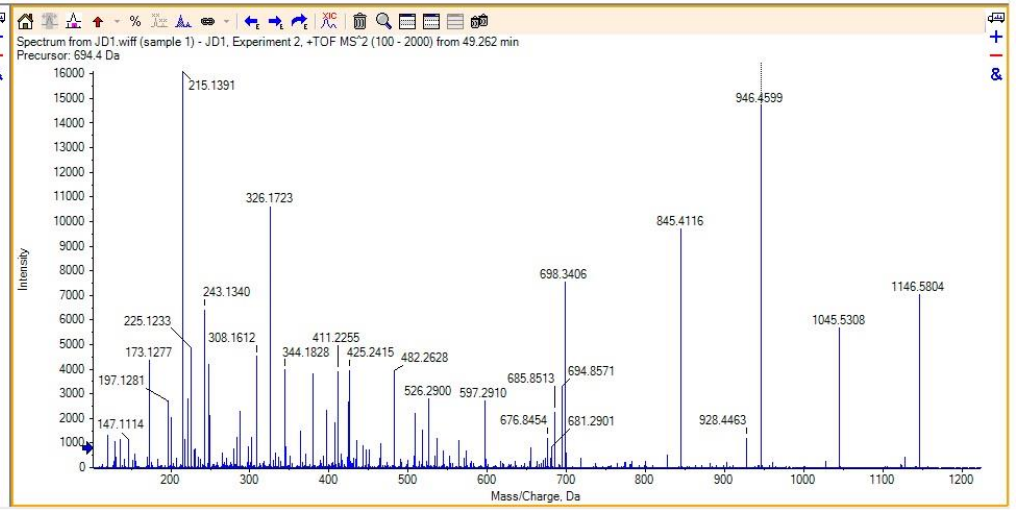
SRM principle



Chromatogram – choice of peptide retention time



MS spectrum – m/z of precursor ion (**Q1**)



MS/MS fragmentation spectrum (**Q3**)

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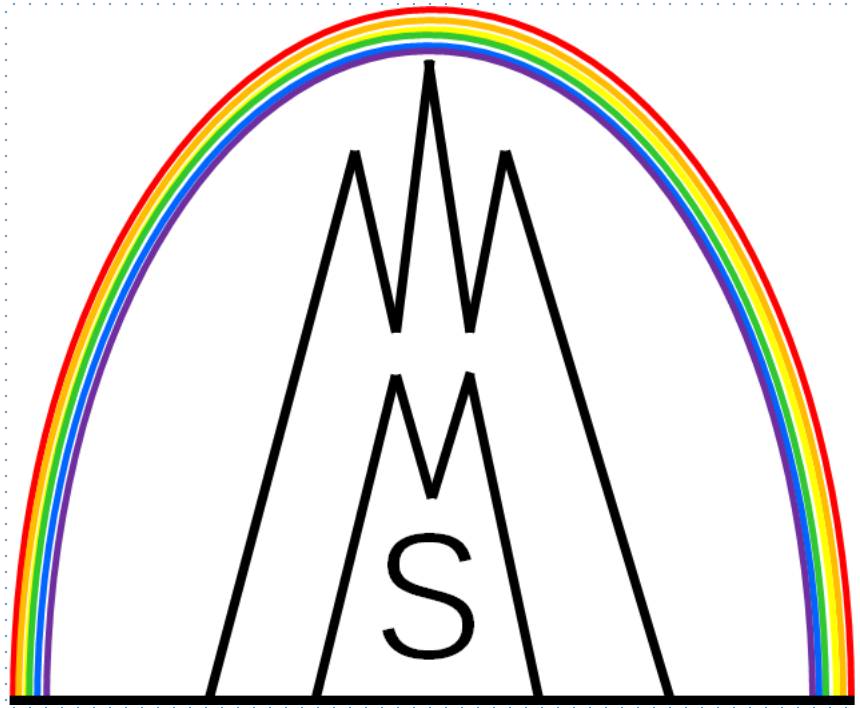

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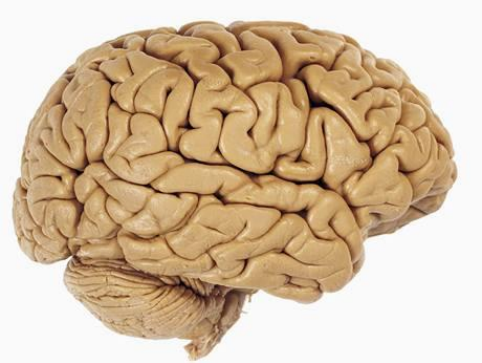


What do you want to quantify?

TARGETED vs. DISCOVERY analysis

- Quantification of **one protein** you **as many as possible?**
- What do you **know** about the proteins and the sample?
- Do you expect any **modifications**?
- Do you need **enrichment**?

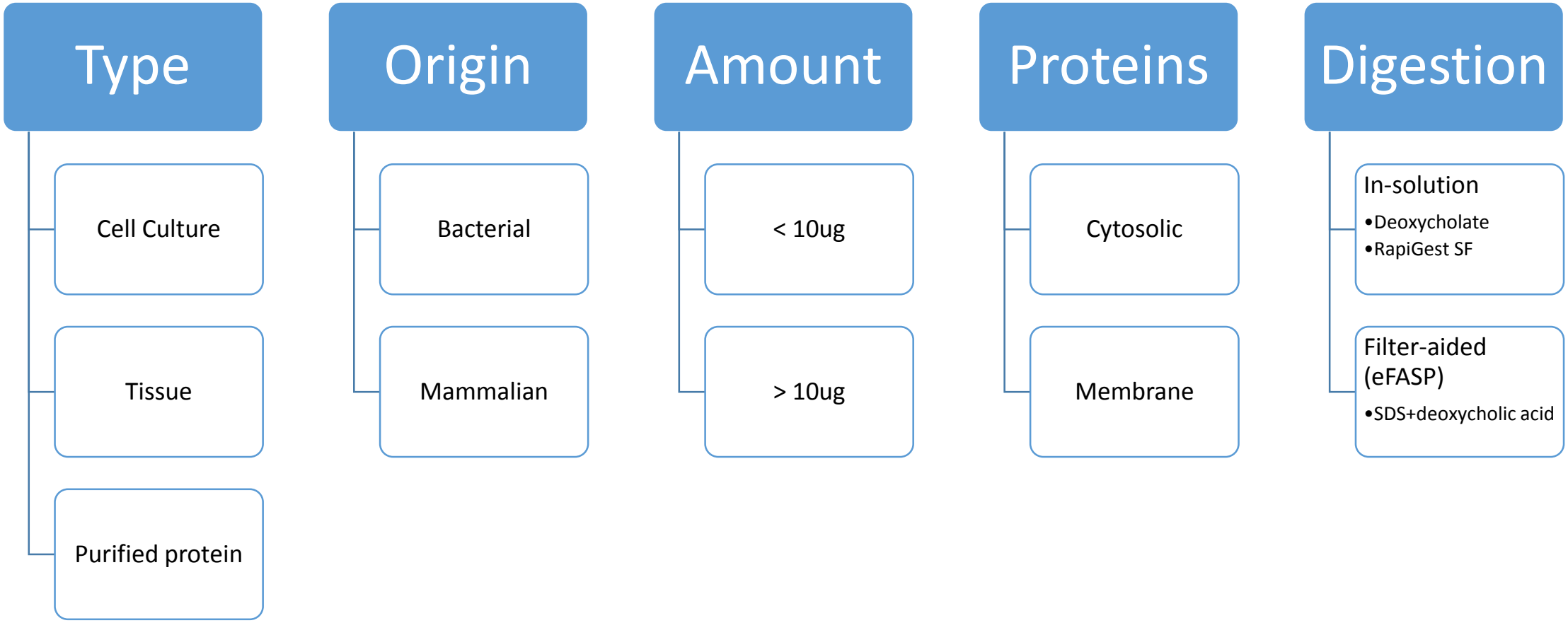
How precise and accurate need to be the results?

LABEL vs. LABEL-FREE techniques

- What is the **biological variation** you expect?
- Do you expect major **changes** in protein levels in your experiment?
- How many **replicates** are you able to perform?
- Can you **validate** the results using a complementary method?

Try to identify the critical steps in your sample preparation workflow

How to design your own quant. experiment?
Sample characterization



Pitfalls in protein quantification

- **Accuracy** of quantitation is limited by
  - Protein Isoforms: part of their protein sequence is shared but belongs to multiple proteins
  - Unwanted modifications occuring during sample preparation (oxidation of Met, incomplete labeling etc.)
  - Deuterium labelled peptides may behave differently in reversed-phase chromatography
- **Reproducibility** of quantitation is limited by
  - Variations in experimental conditions, sample preparation, sample complexity
  - DDA: Data dependent acquisition – interferences influence the m/z picking for MS/MS fragmentation

Try to identify the critical steps in your sample preparation workflow

Thank you for your attention

Do not hesitate to contact us

Martin Hubálek, Ph.D.
martin.hubalek@uochb.cas.cz

Jana Březinová
jana.brezinova@uochb.cas.cz

Karel Rücker
Lab assistant