

Peptide / Protein Quantification

Outline

Terminology

- MS Quantification
- Why variance matters
- Label-free / normalization
- Labelled
 - iTRAQ
- Peptides to proteins

- Absolute Quantification
- Relative Quantification
- Bias
- Variability / Variance
- Label-free
- Normalization
- Labelled
- iTRAQ
- Isobaric
- Reporter ions

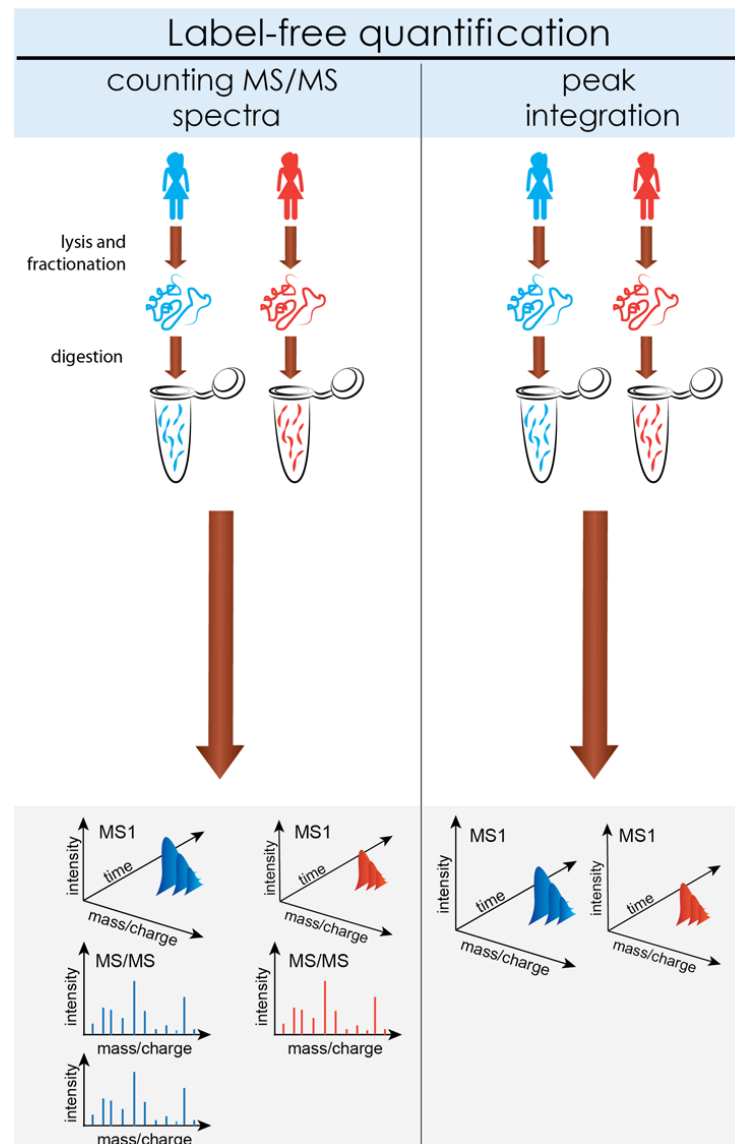
Peptide / Protein Quantification

- Absolute – Estimate the molar amount of protein / peptide in the biological sample
 - PTMs
 - Validation
- Relative – Fold change / statistically significant difference between 2 biological states
 - Biological variation
 - Biomarker studies



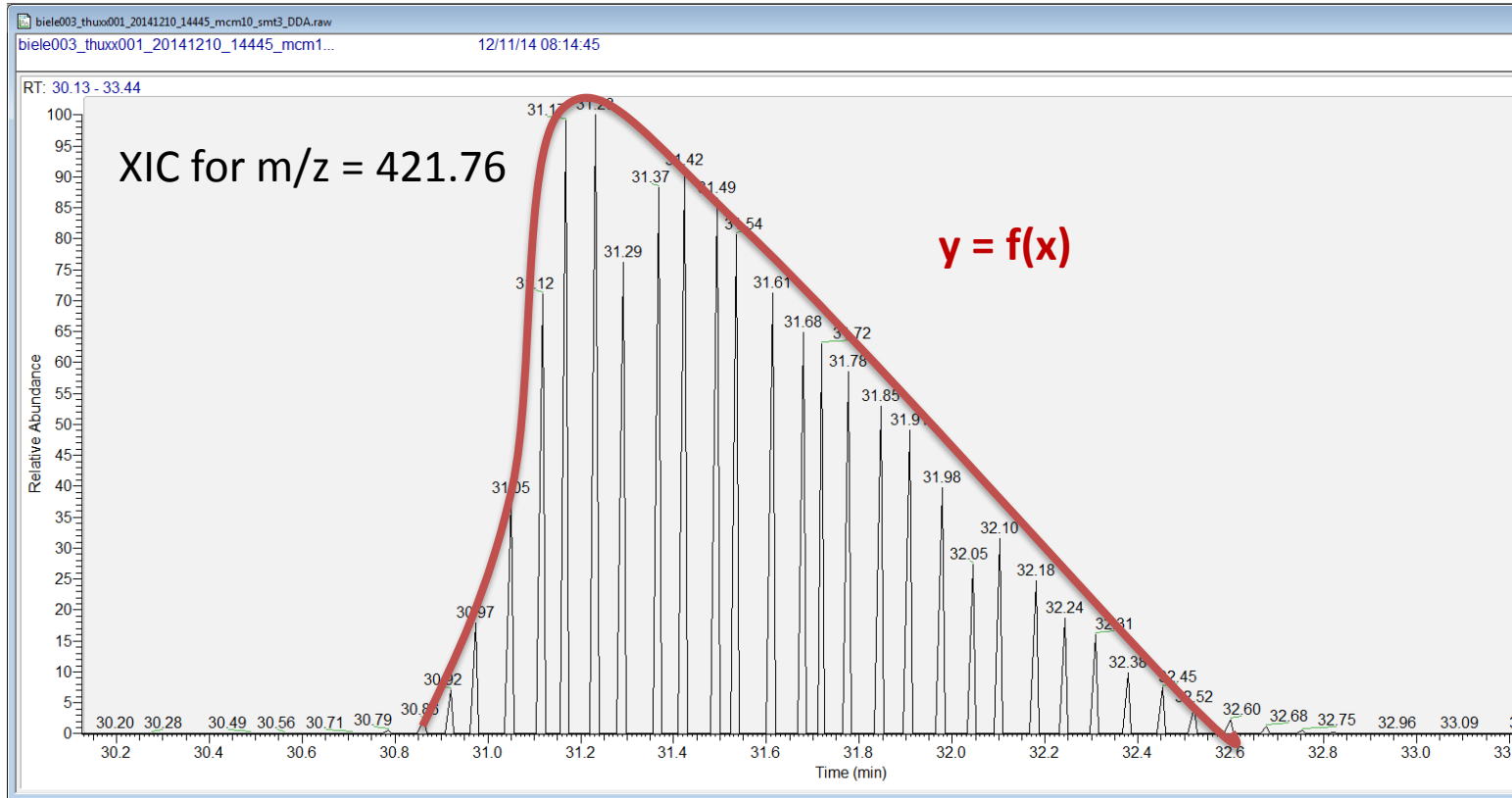
Label-free

- Area Under Curve
 - MS1
 - Integrate XIC
- Spectral Counting
 - MS2
 - High abundant proteins



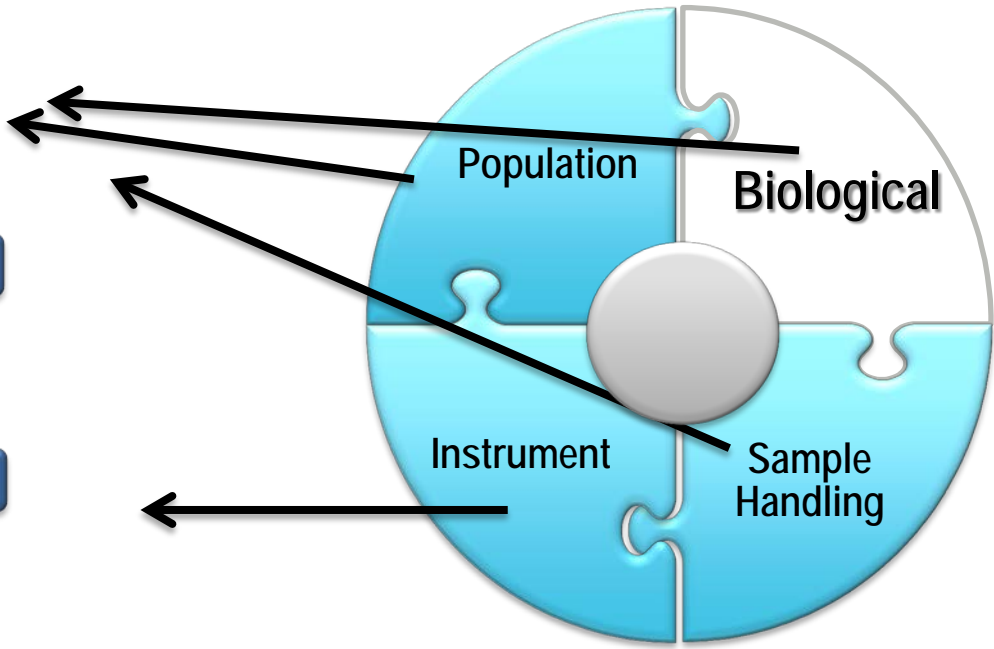
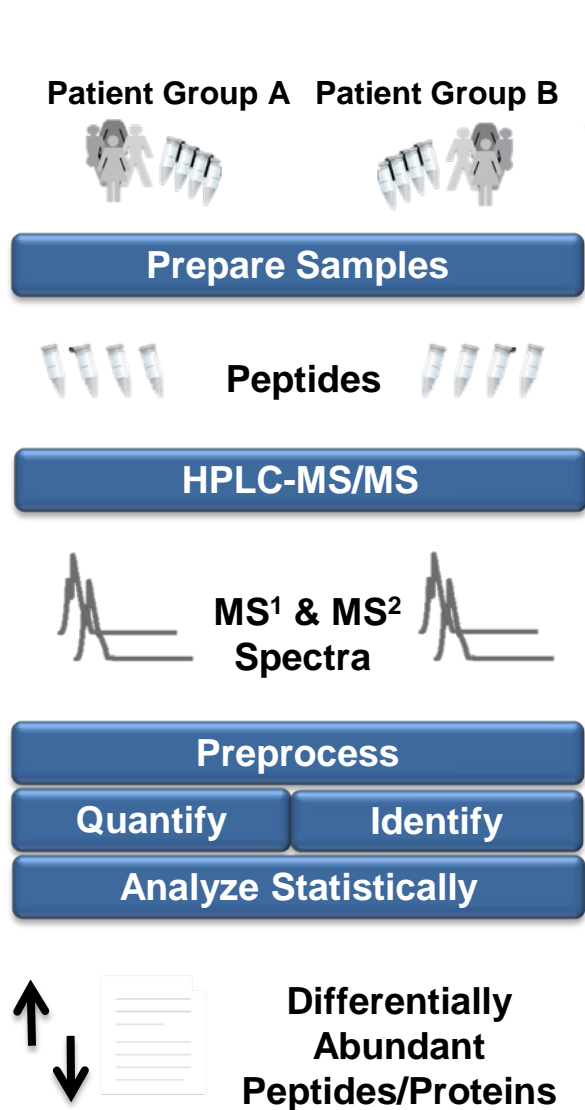
Käll L, Vitek O (2011) Computational Mass Spectrometry-Based Proteomics. PLoS Comput Biol 7(12): e1002277. doi:10.1371/journal.pcbi.1002277

MS1 AUC



$$\text{Area} = \int_a^b f(x)$$

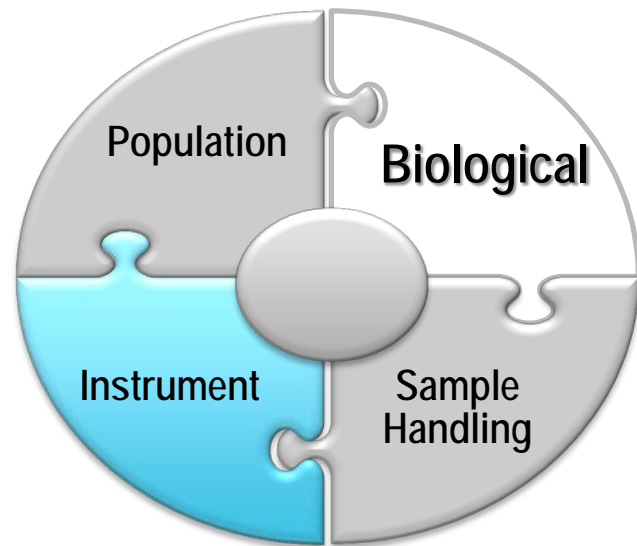
Typical Discovery Experiment



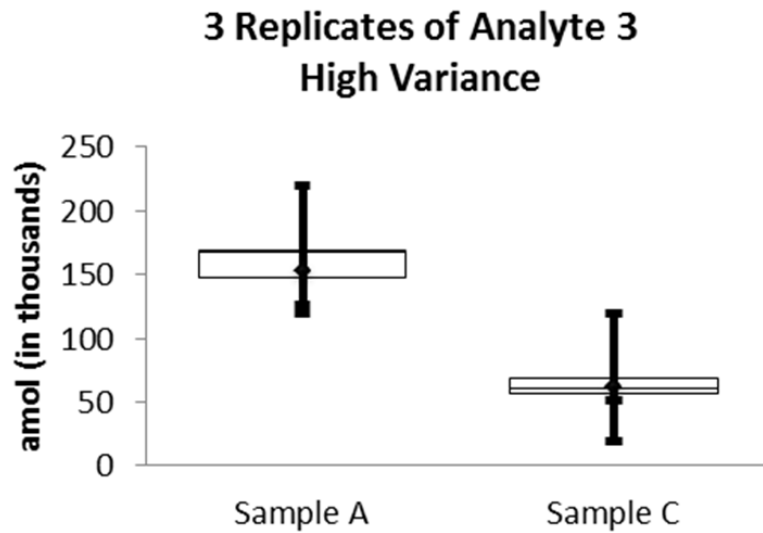
Sources of Bias and Variability

MS Quantification

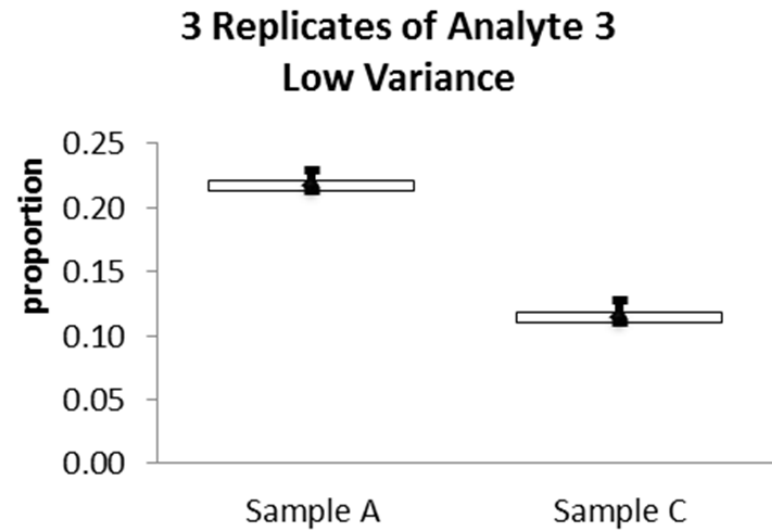
- MS not inherently quantitative
- Physiochemical properties invoke different MS responses
- MS only samples a small percentage of total peptides
- Bias and variability



Why Variance Matters



Mean Fold Change 2.09
t-test p-value 0.07



Mean Fold Change 1.89
t-test p-value 0.0001



Normalization

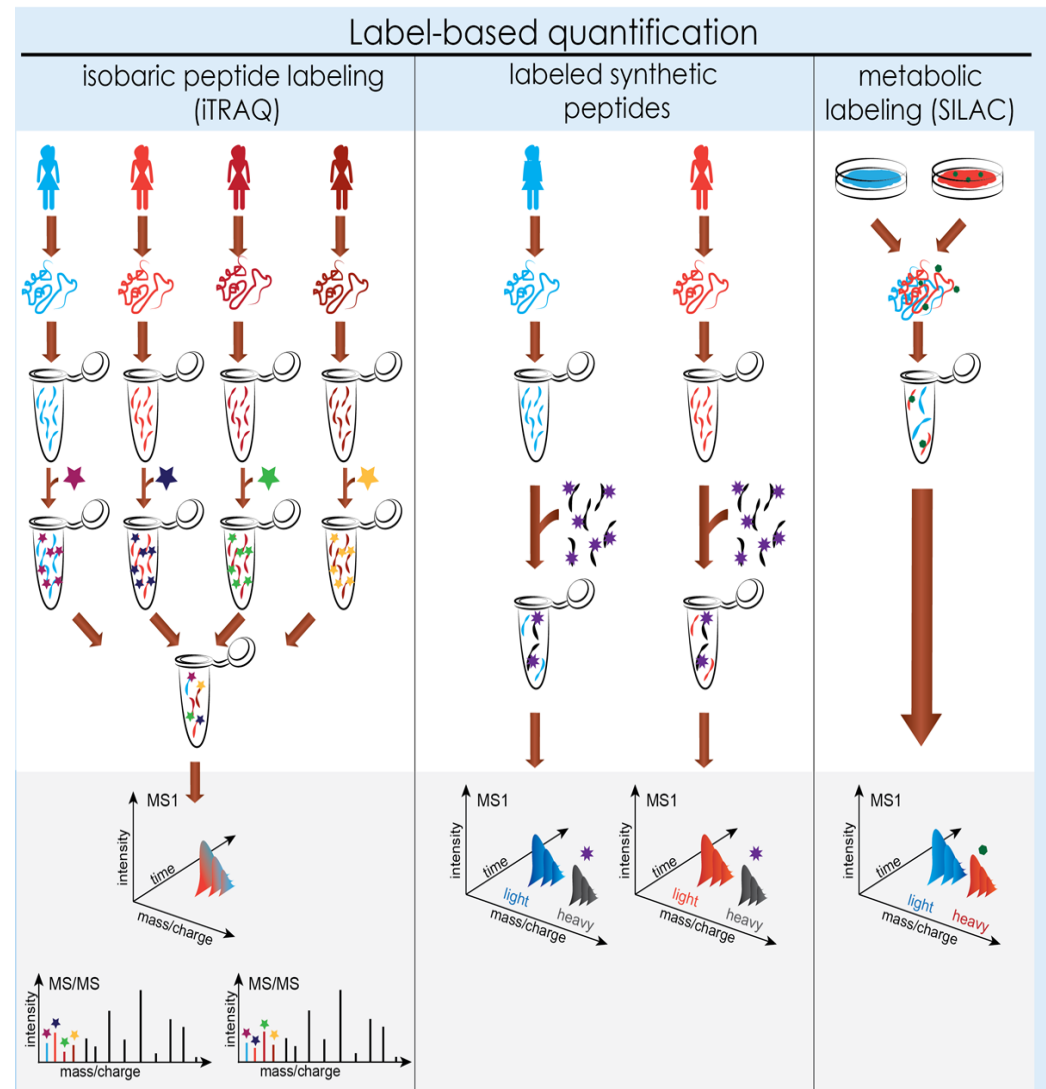
- Remove bias and variability *between* runs
- Global – commonly used
 - Median scale
 - Total ion current (TIC)
- Local – very recent development
 - Proximity-based intensity normalization (PIN)

Labeled Quantification

- Run samples simultaneously on in a single run
- Add label to samples
- Mix samples together
- Compute ratios / statistically significant diffs.

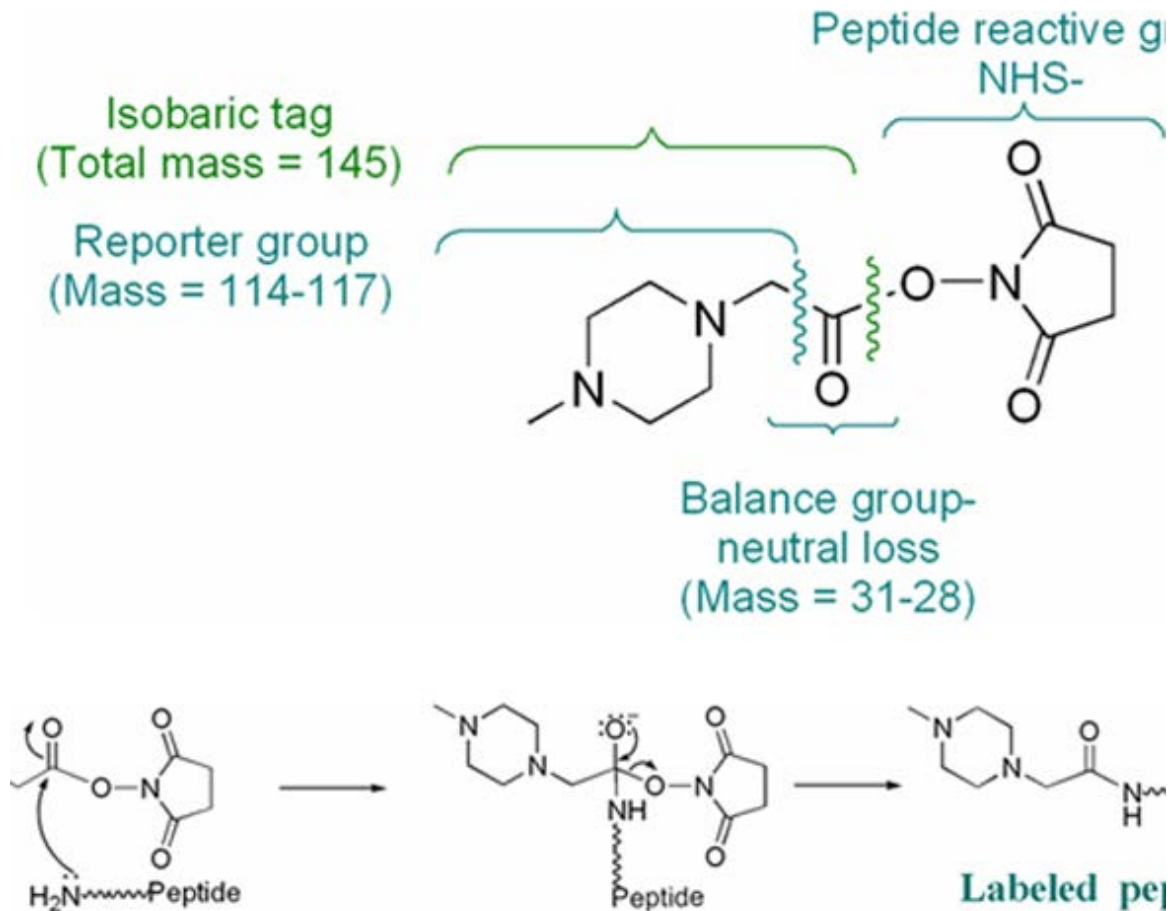
Labeled

- Isobaric
 - MS2, iTRAQ
 - Number of samples
- Synthetic Peptides
 - MS1
 - Absolute (AQUA)
- Metabolic
 - MS1, SILAC
 - Not higher life forms



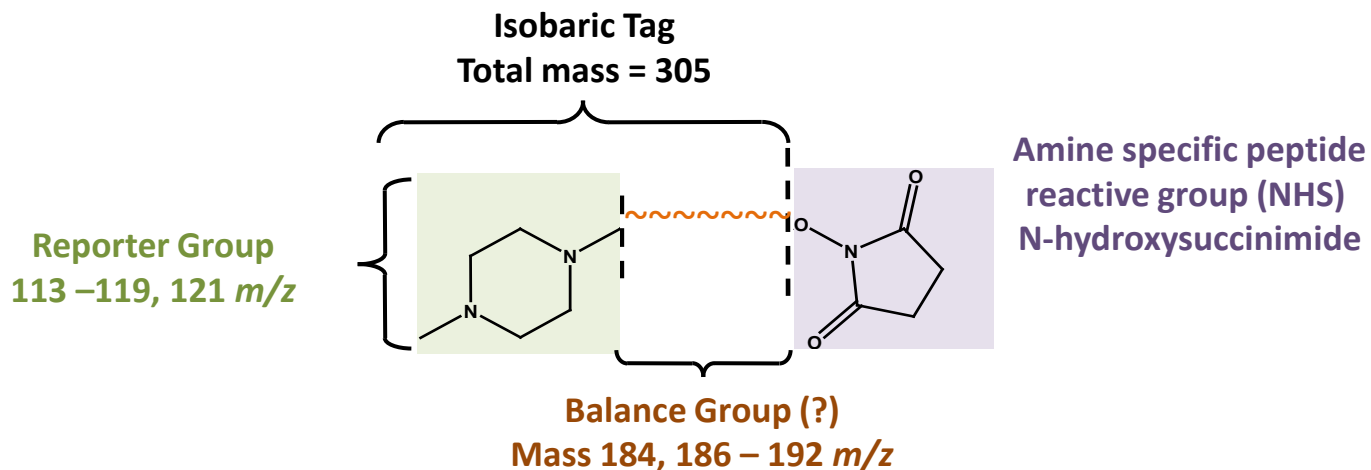
Käll L, Vitek O (2011) Computational Mass Spectrometry-Based Proteomics. PLoS Comput Biol 7(12): e1002277. doi:10.1371/journal.pcbi.1002277

Isobaric Example - iTRAQ

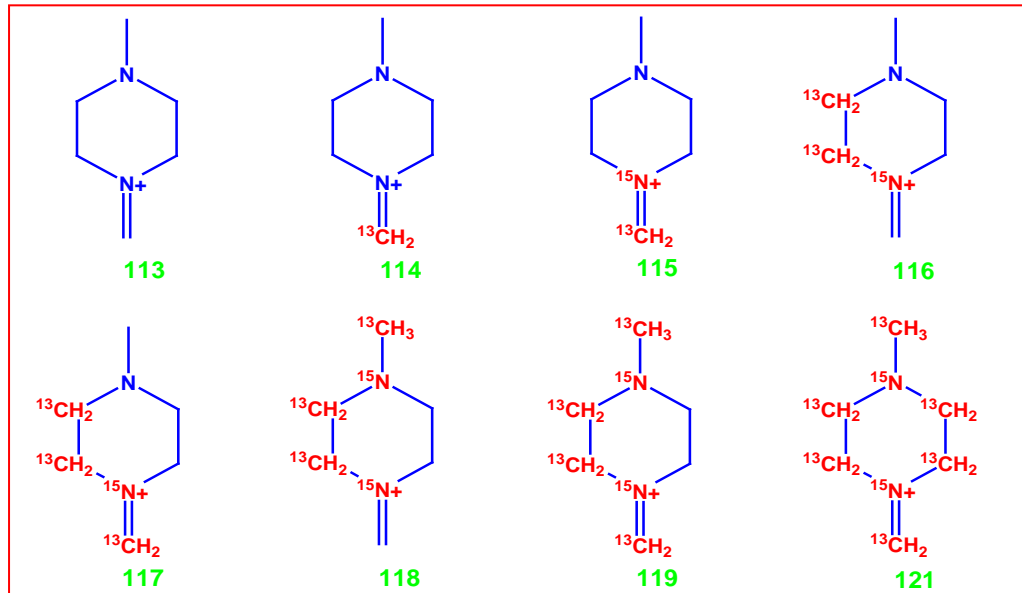


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iTRAQ[®] 8-Plex Reagent Chemical Structure



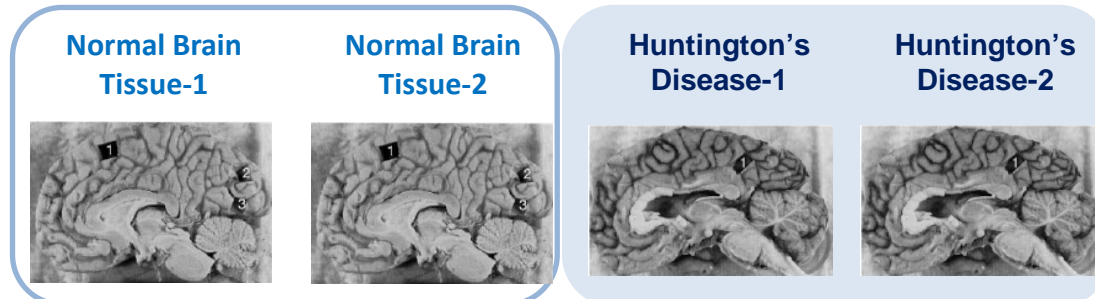
Isobaric Reporter Groups
113 – 119, 121 *m/z*



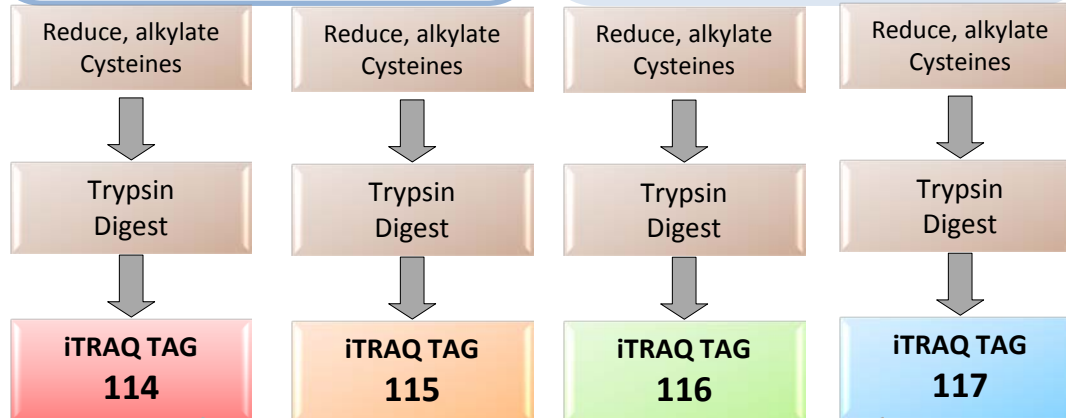
Applied Biosystems has granted permission to use this slide.

iTRAQ Experiment

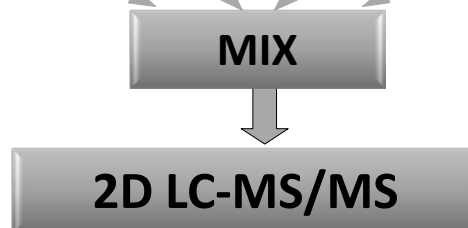
Obtain protein-containing sample, extract protein



Proteolytic Digestion

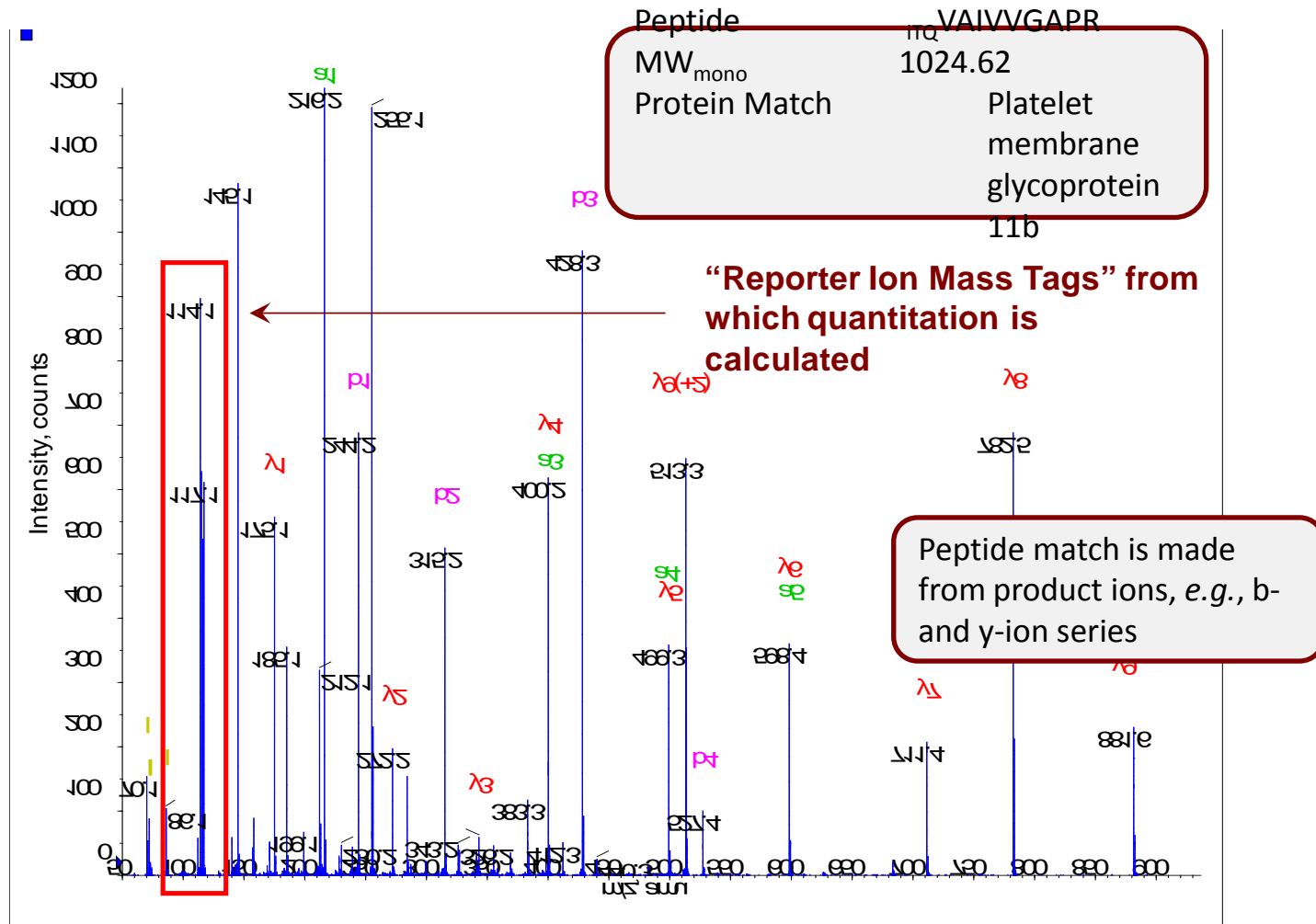


Label peptides with iTRAQ® Reagents



Tissue Images: Rosas HD et al, (2002) Neurology, 58, 695

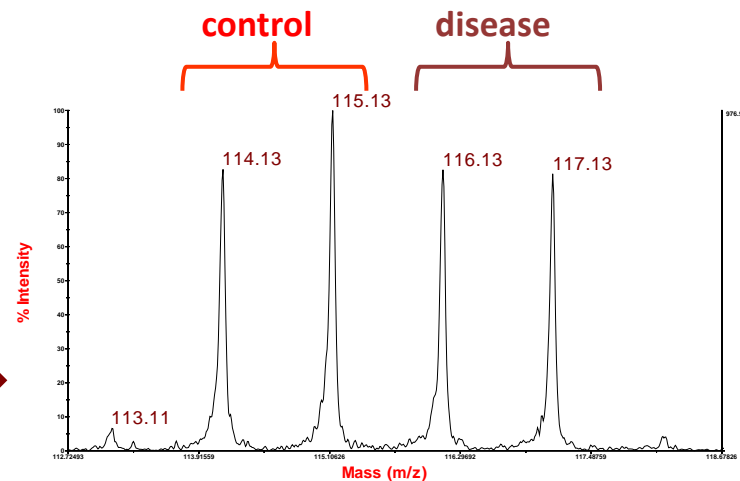
iTRAQ Experiment MS2 Spectrum



iTRAQ Results

- Reporter ion intensities reflect relative peptide amounts

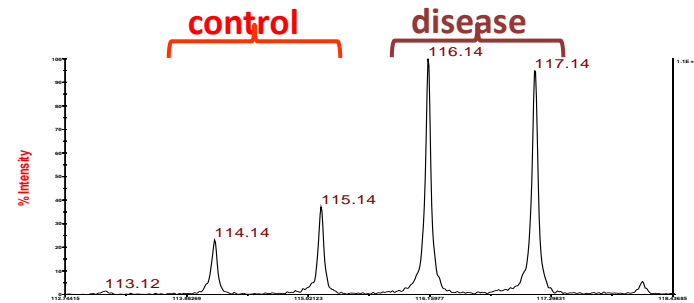
No change disease:control



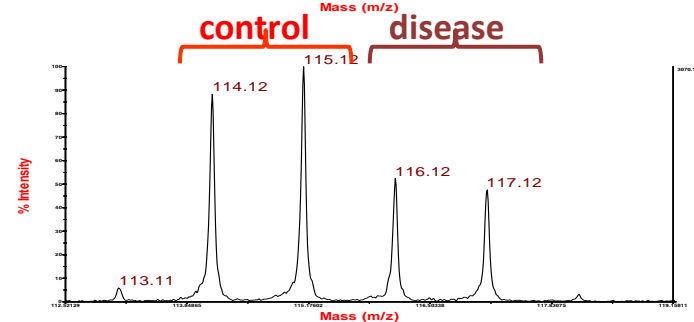
iTRAQ Results

- What fold changes are significant?
- Do they represent biological relevance as opposed to experimental variability?

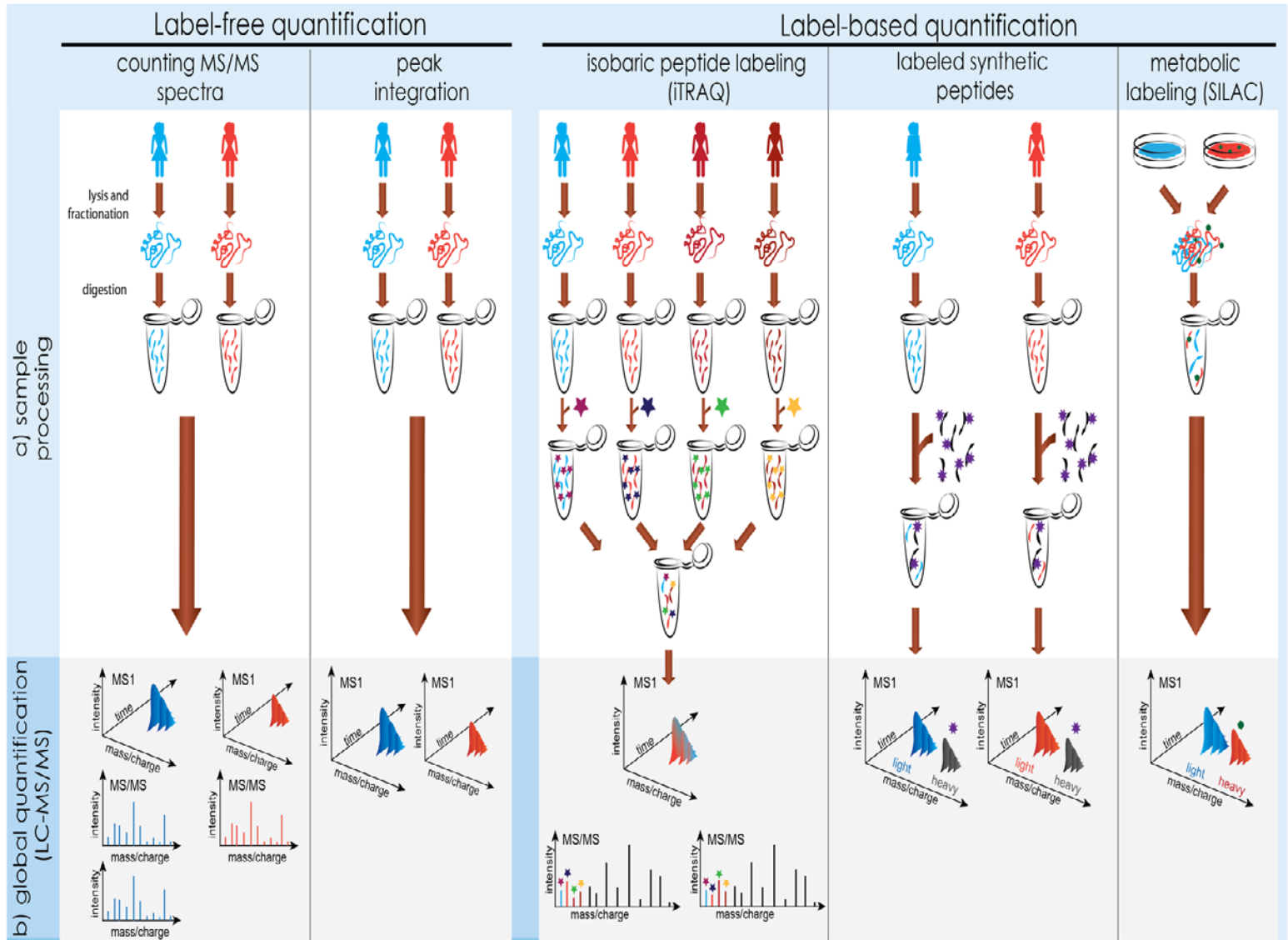
Increase disease:control



Decrease disease:control



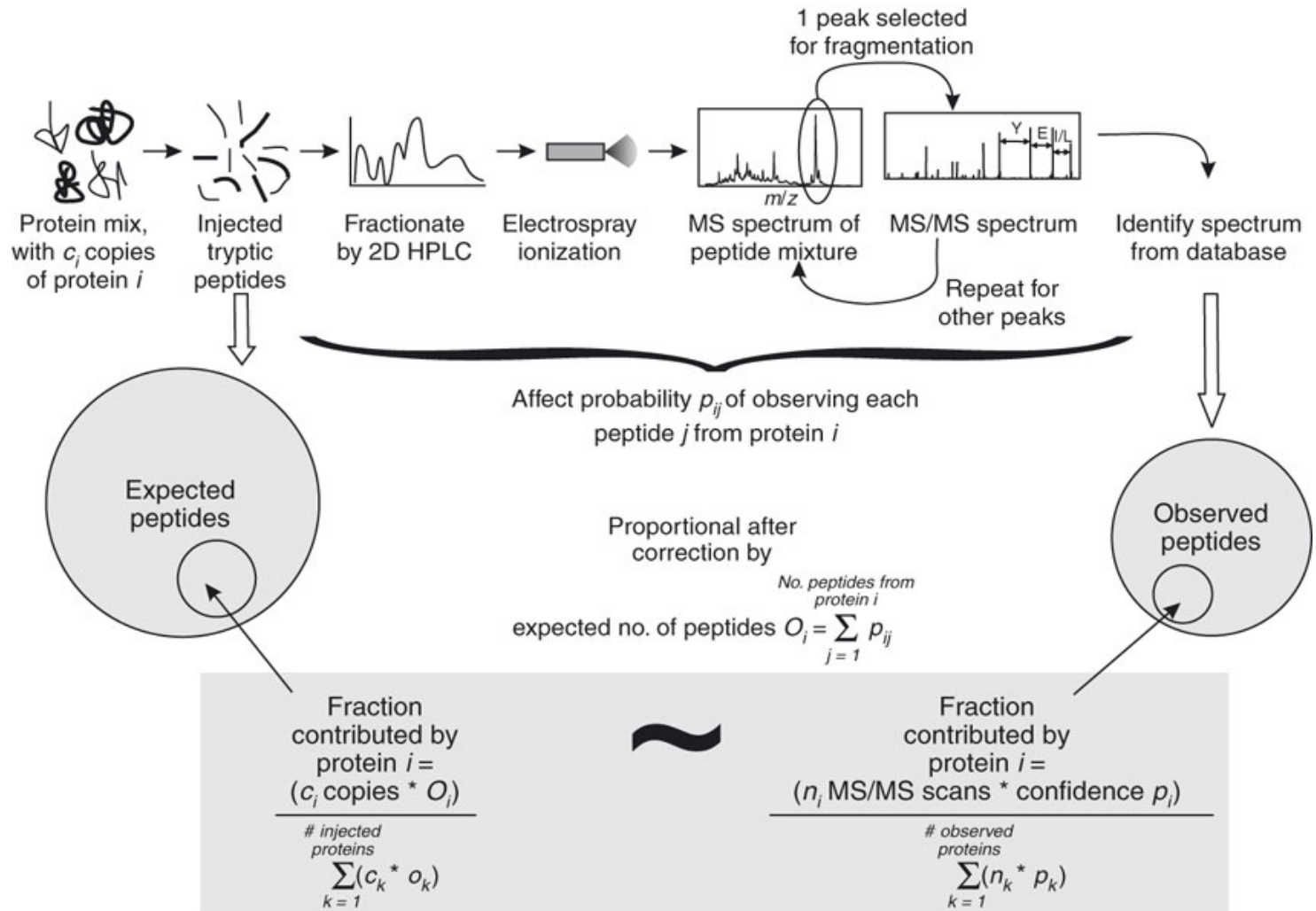
Labeled & Label-free



From Käll L, Vitek O (2011) Computational Mass Spectrometry-Based Proteomics. PLoS Comput Biol 7(12): e1002277. doi:10.1371/journal.pcbi.1002277

From Peptides to Proteins

APEX



Lu, et al., Nature Biotechnology 25, 117 - 124 (2007)