UNIVERSITEIT GENT

BIG N2N
Bioinformatics Institute Ghent
from nucleotides to networks

Statistical Methods for Quantitative MS-Based Proteomics
Quantification: Part I. Normalization and Summarization

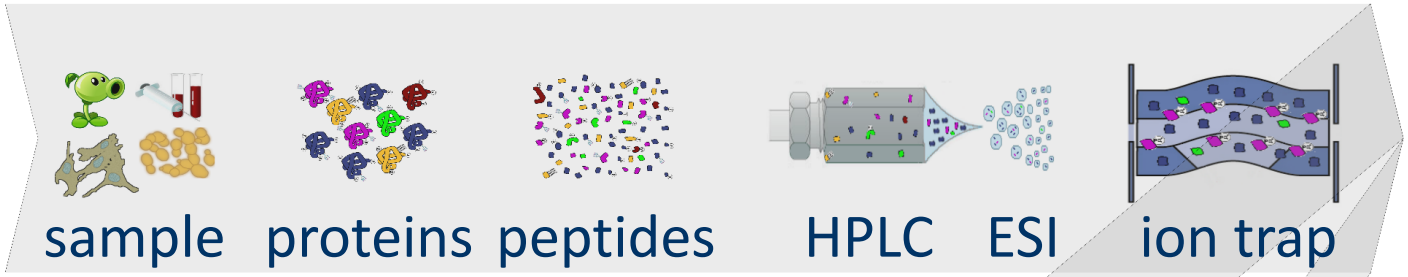
Lieven Clement

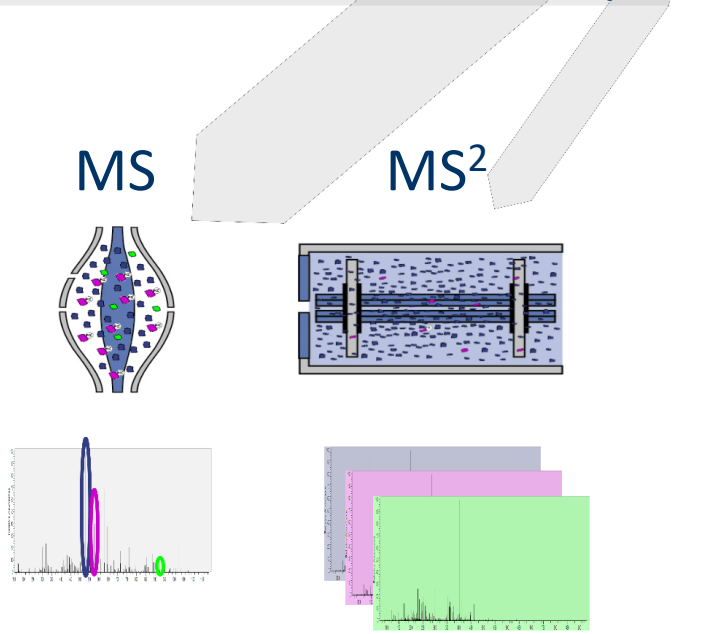
Proteomics Data Analysis Shortcourse

Outline

1. Introduction
    1. Label free MS based Quantitative Proteomics Workflow and Challenges
2. Preprocessing
    1. Filtering
    2. Log transformation
    3. Normalization
    4. Summarization

Challenges in Label Free Quantitative Proteomics





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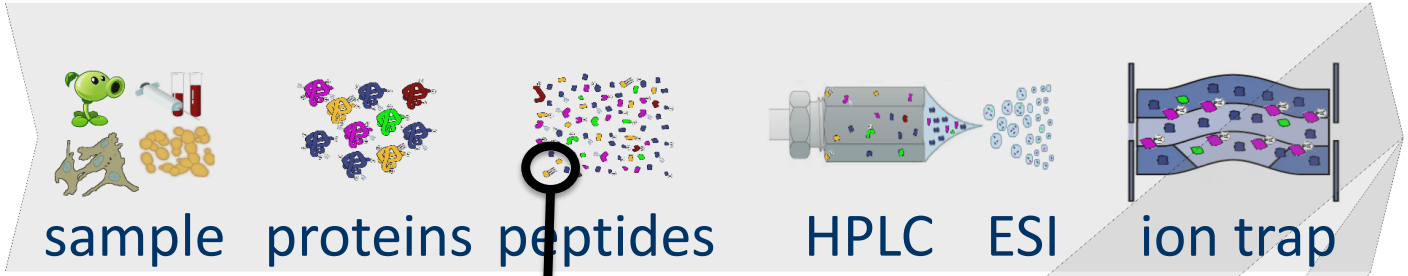
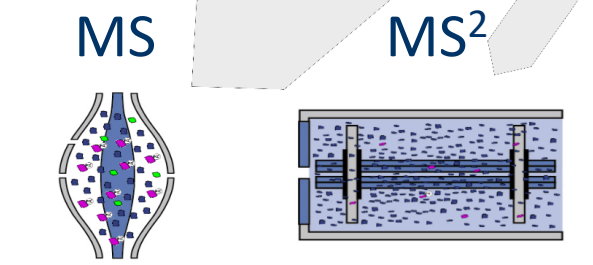
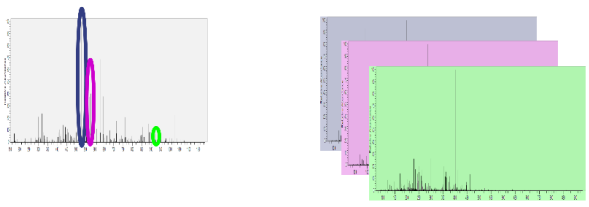


Peptide characteristics

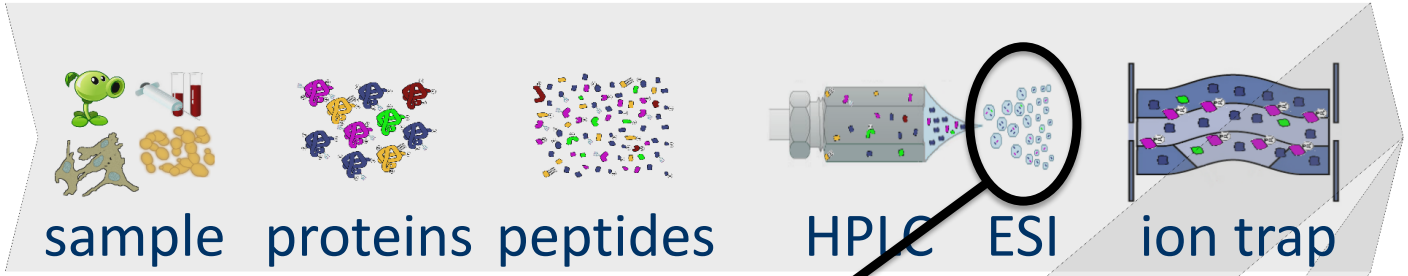
- Modifications





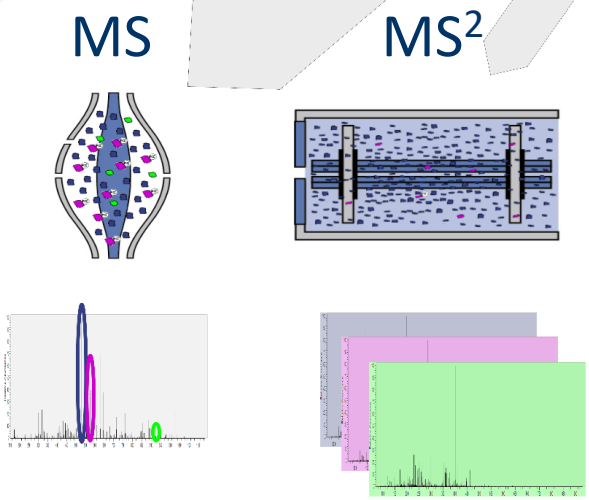
Quantification Identification

Challenges in Label Free Quantitative Proteomics

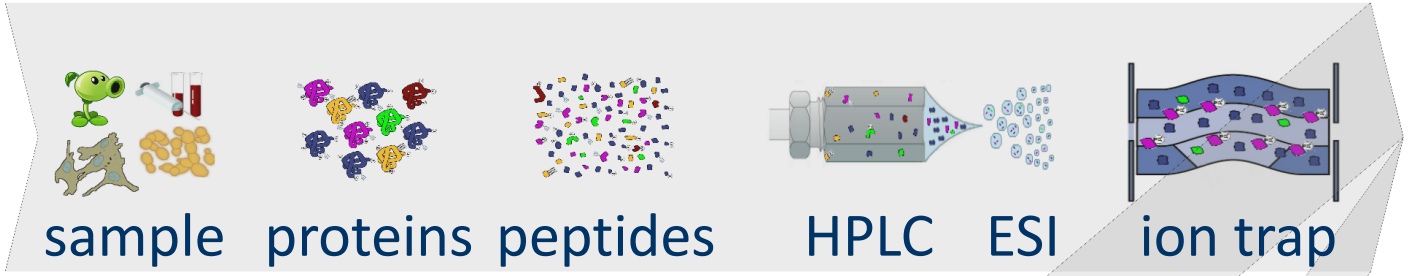


Peptide characteristics

- Modifications
- Ionisation efficiency
  - Outliers
  - Huge variability

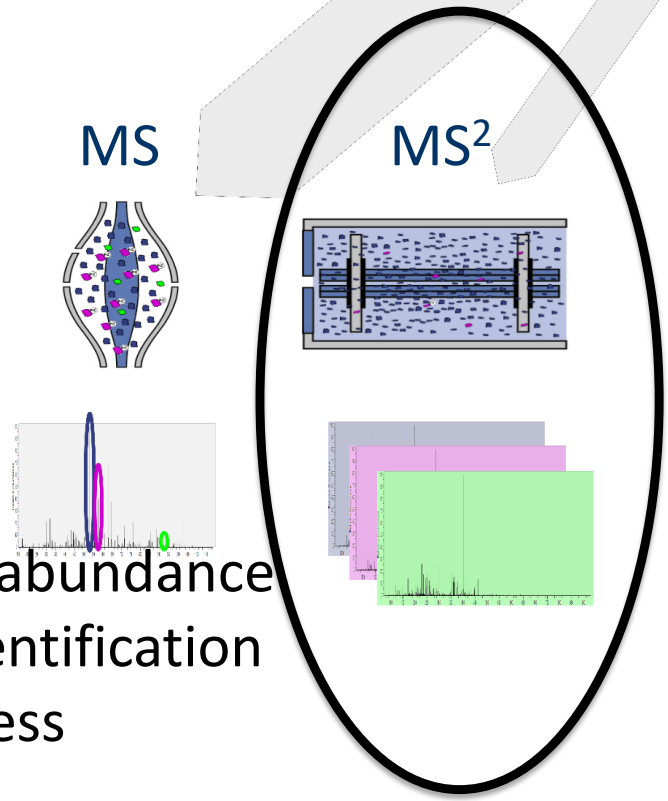


Challenges in Label Free Quantitative Proteomics

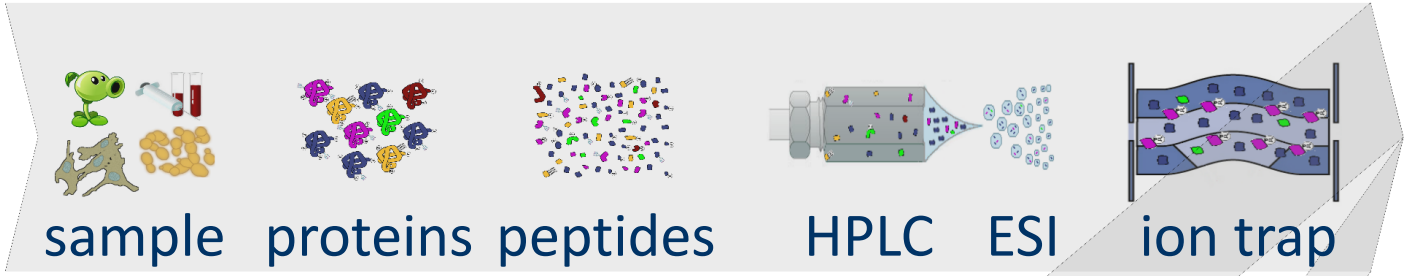


Peptide characteristics

- Modifications
- Ionisation efficiency
  - Outliers
  - Huge variability
- MS^2 selection on peptide abundance
  - Context dependent Identification
  - Non-random missingness



Challenges in Label Free Quantitative Proteomics



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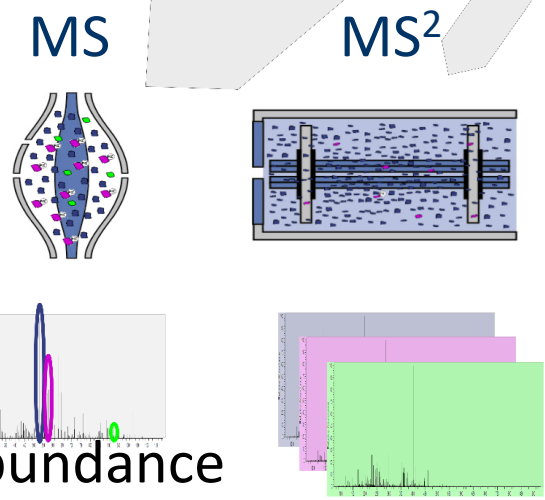
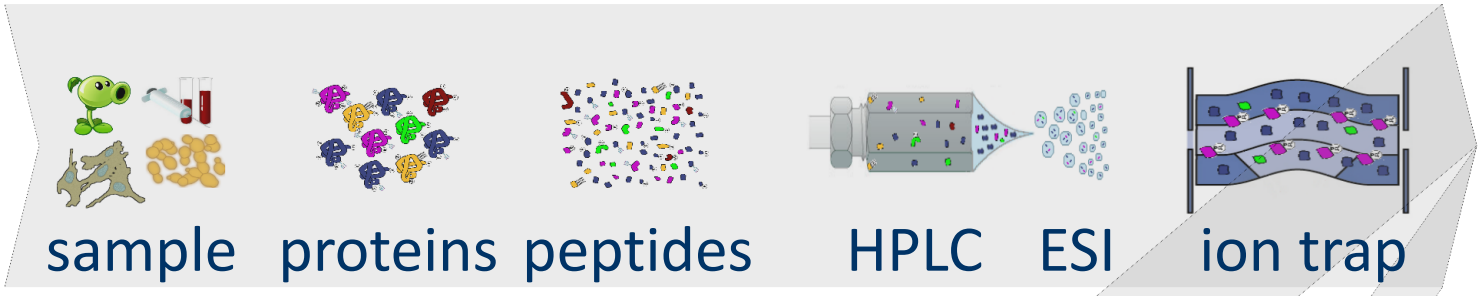
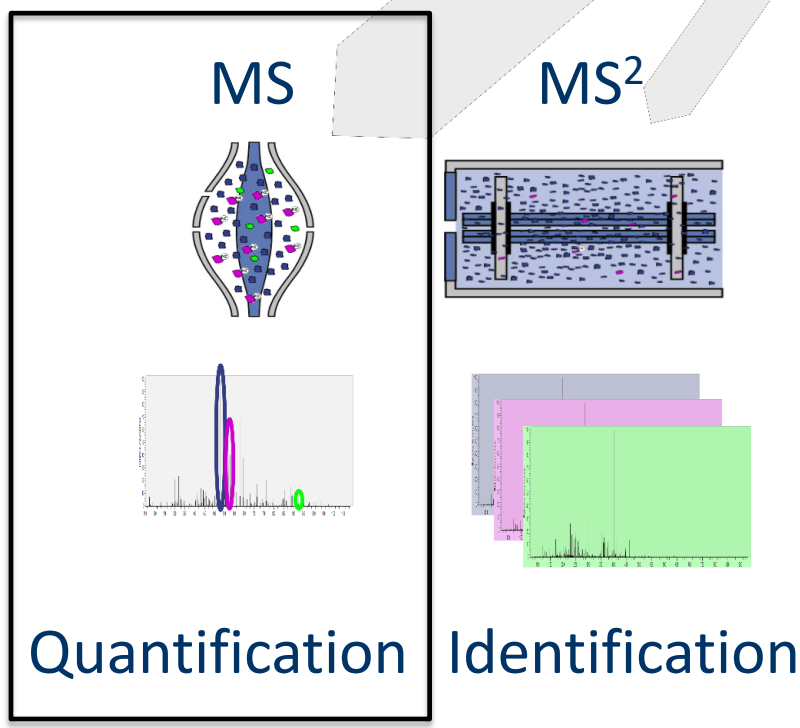


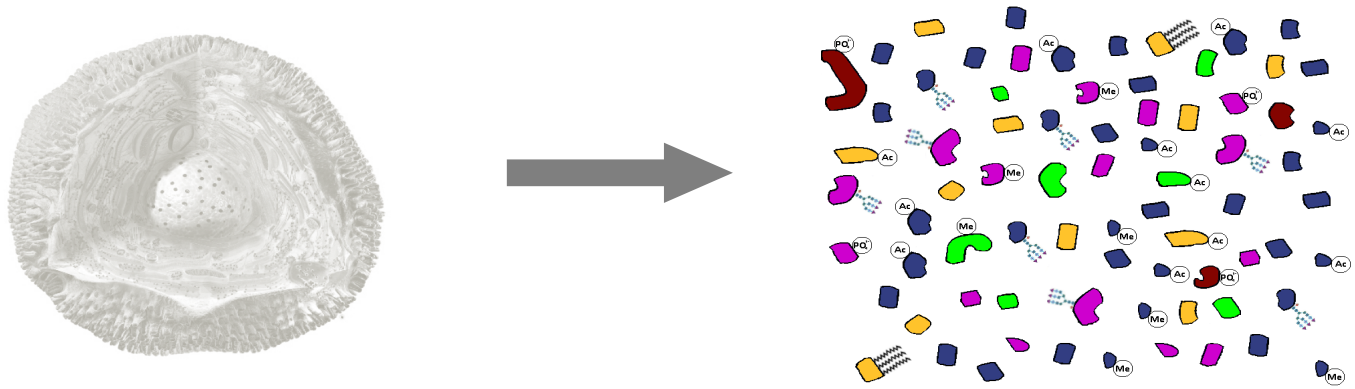
Unbalanced peptides identifications across samples and messy data

Challenges in Label Free MS-based Quatitative proteomics



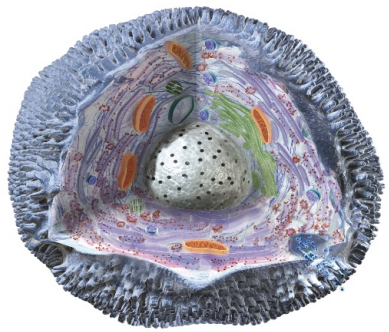


MS-based proteomics returns **peptides**: pieces of proteins

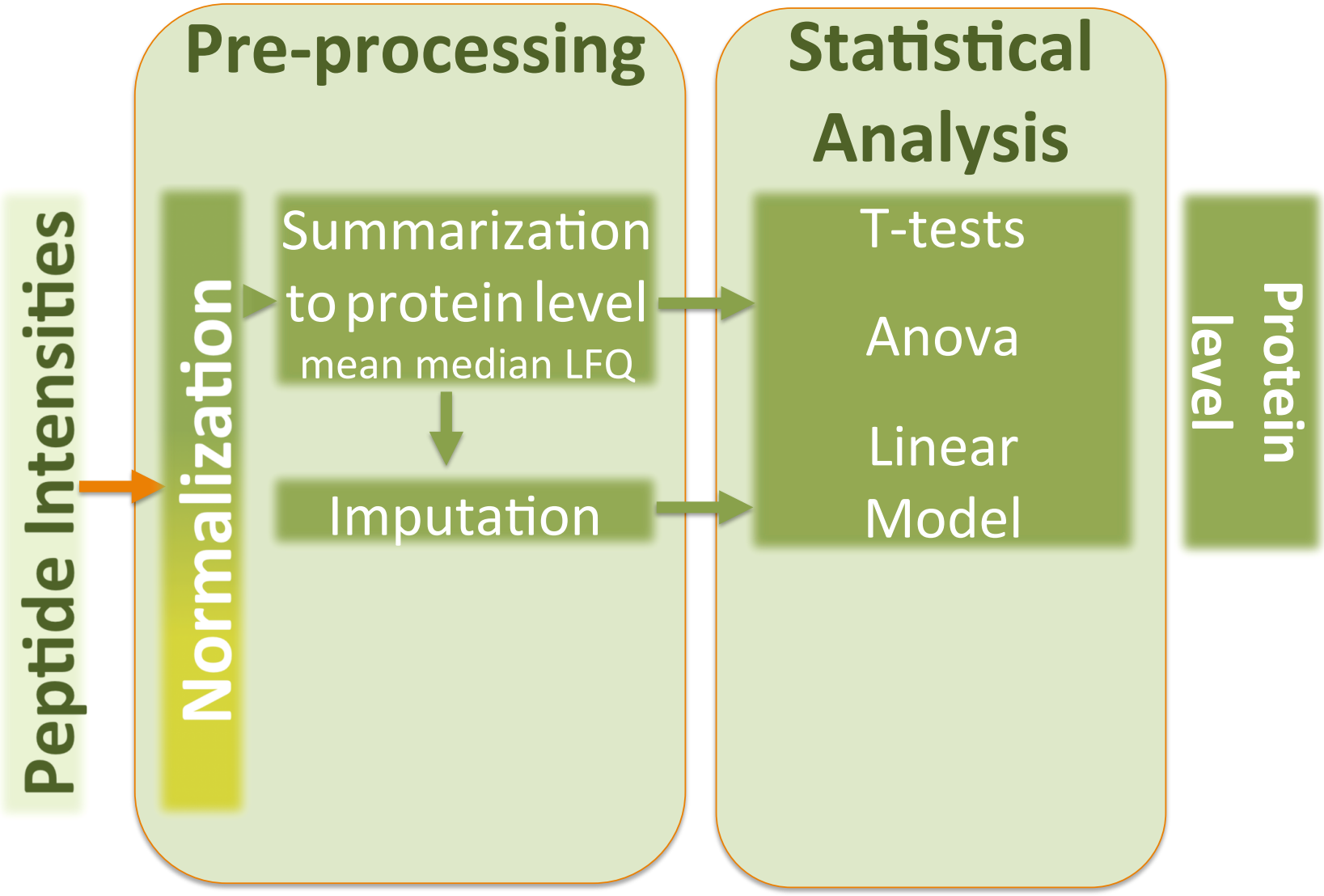


Challenges in Label Free MS-based Quatitative proteomics

We need information on protein level!



Label-free Quantitative Proteomics Data Analysis Pipelines



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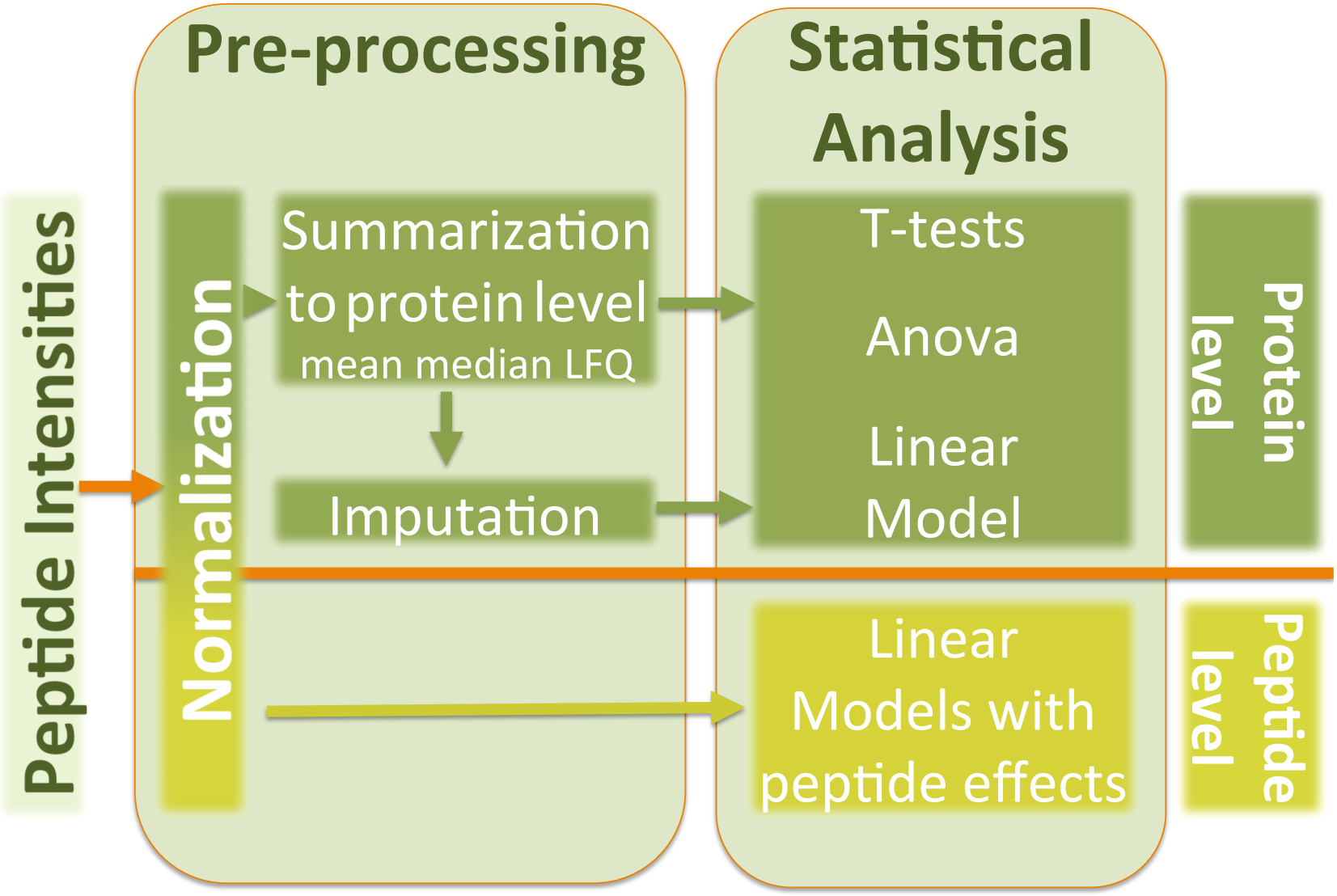
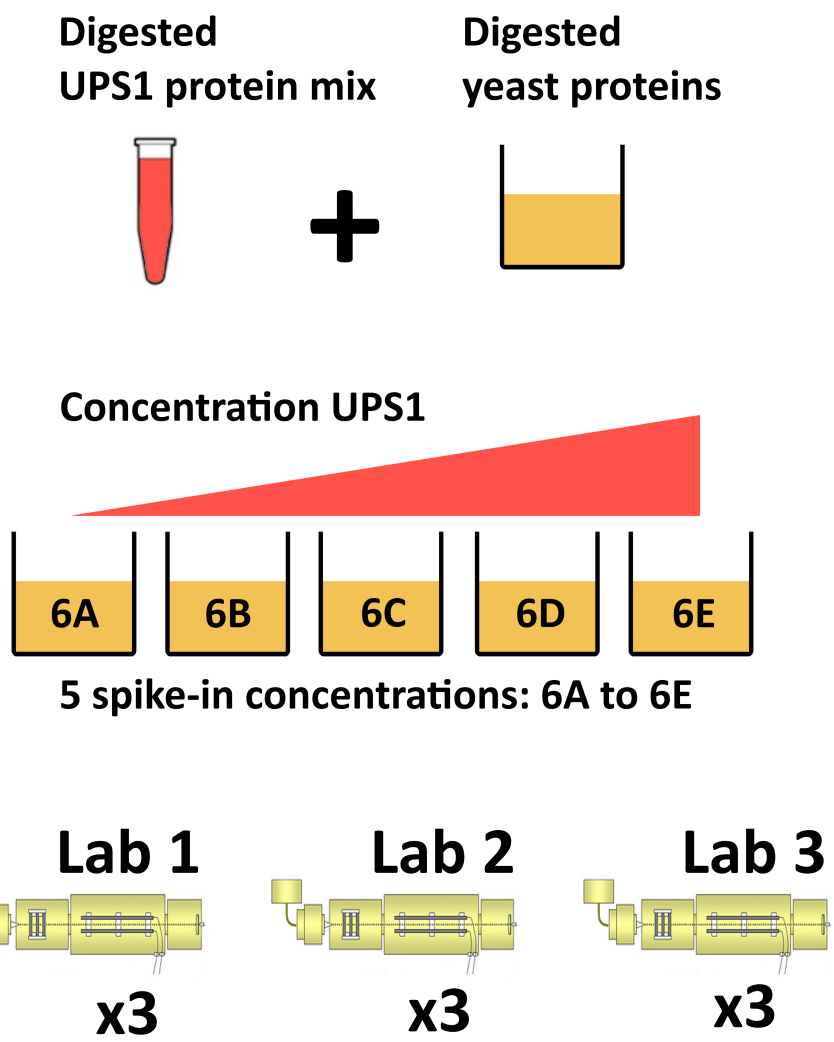


CPTAC Spike-in Study



- Same trypsin-digested yeast proteome background in each sample
- Trypsin-digested Sigma UPS1 standard: 48 different human proteins spiked in at 5 different concentrations (treatment A-E)
- Samples repeatedly run on different instruments in different labs
- After MaxQuant search with match between runs option
  - 41% of all proteins are quantified in all samples
  - 6.6% of all peptides are quantified in all samples

$\rightarrow$ **vast amount of missingness**

Preprocessing

- Typical preprocessing steps
  1. Filtering
  2. Log-transformation
  3. Normalization
  4. (Summarization)

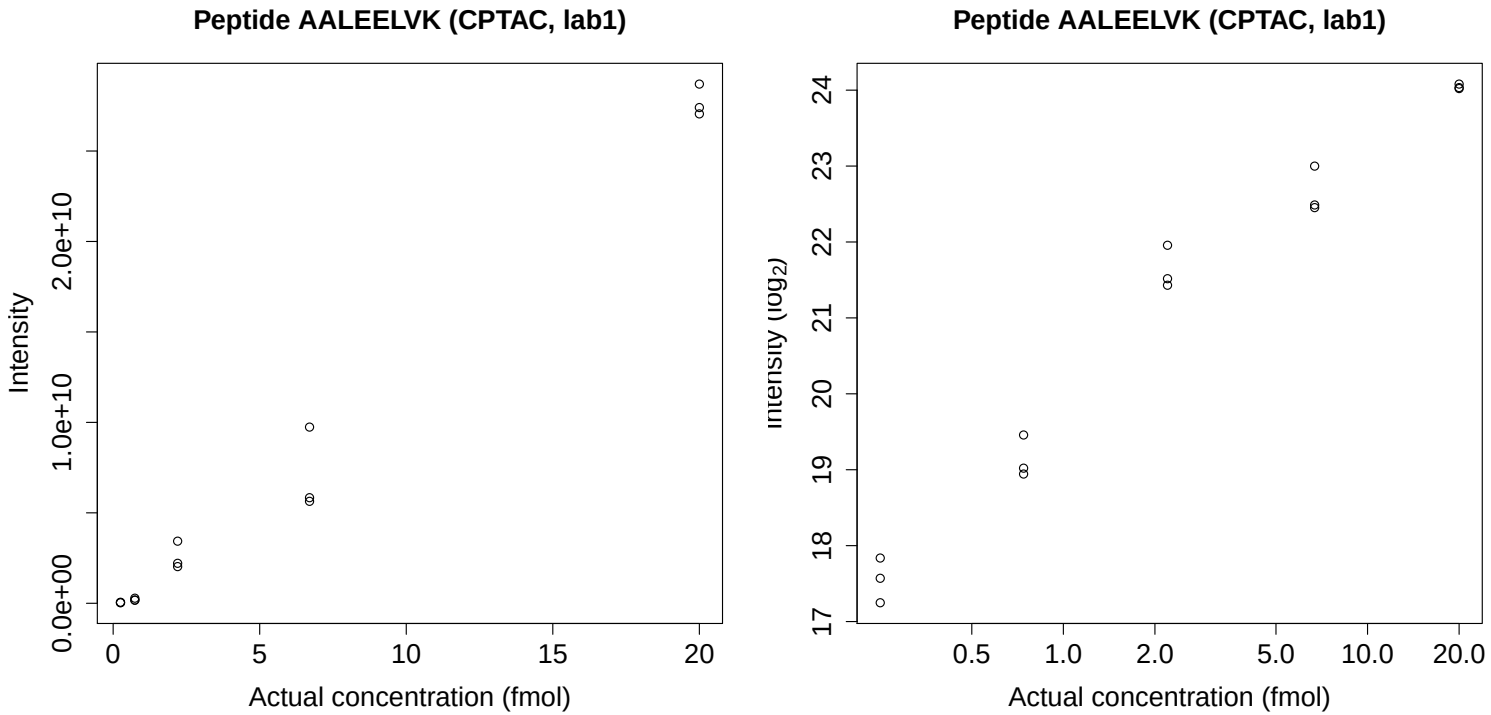
- Many methods exist

Filtering

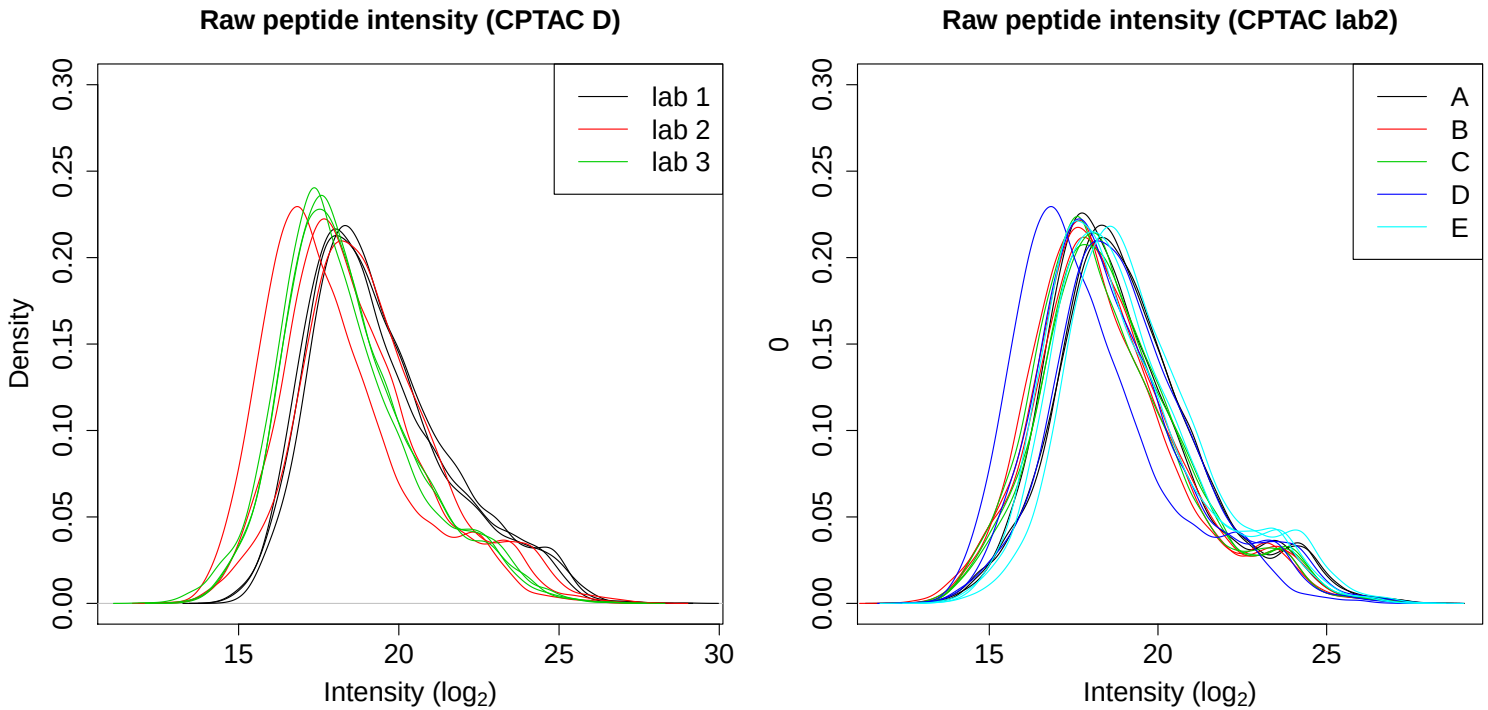
- Reverse sequences
- Only identified by modification site (only modified peptides detected)
- Razor peptides: non-unique peptides assigned to the protein group with the most other peptides
- Contaminants
- Peptides few identifications
- Proteins that are only identified with one or a few peptides

- Filtering does not induce bias if the criterion is independent from the downstream data analysis!

Log-transformation



Variability more equal upon log transformation: often multiplicative error structure of intensity-based read-outs



Even in very clean synthetic dataset (same background, only 48 UPS proteins can be different) the marginal peptide intensity distribution across samples can be quite distinct

- Considerable effects between and within labs for replicate samples
- Considerable effects between samples with different spike-in concentration

→ Normalization is needed

Mean or median?

- Over a period of 30 years males desire to have on average 64.3 partners and females 2.8. (Miller and Fishkin, 1997)

Mean or median?

- Over a period of 30 years males desire to have on average 64.3 partners and females 2.8. (Miller and Fishkin, 1997)
- Over a period of 30 years males, is the median of the number of desired partners is 1 for both males and females. (Miller and Fishkin, 1997)

Mean or median?

Mean is very sensitive to outliers!

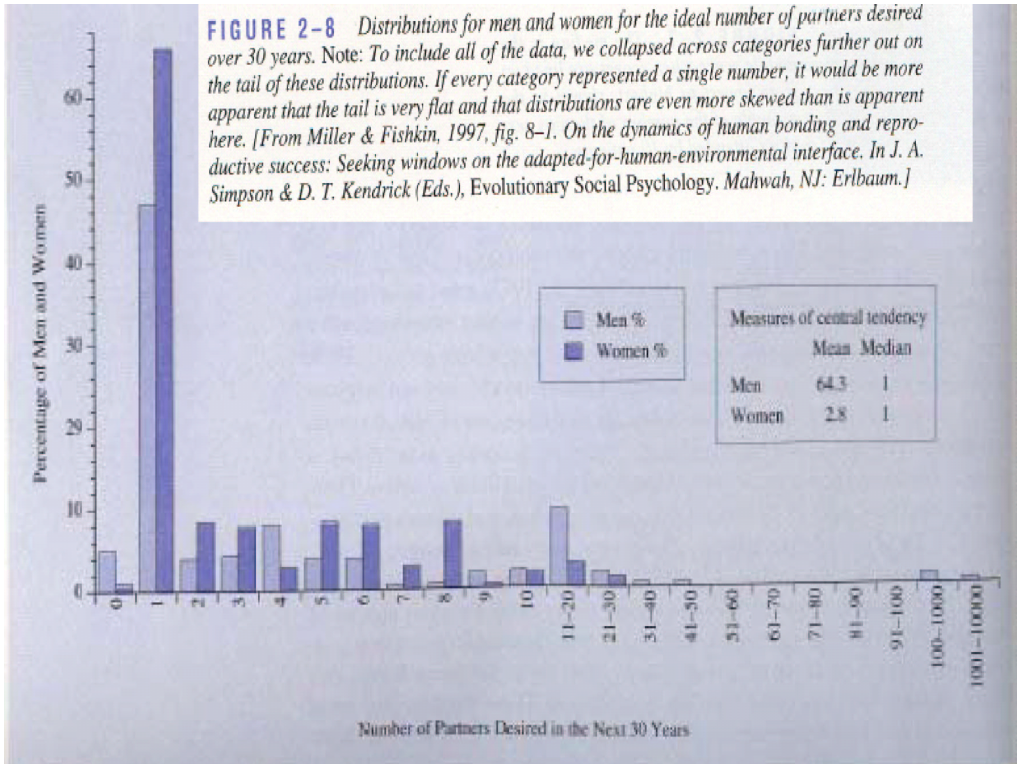
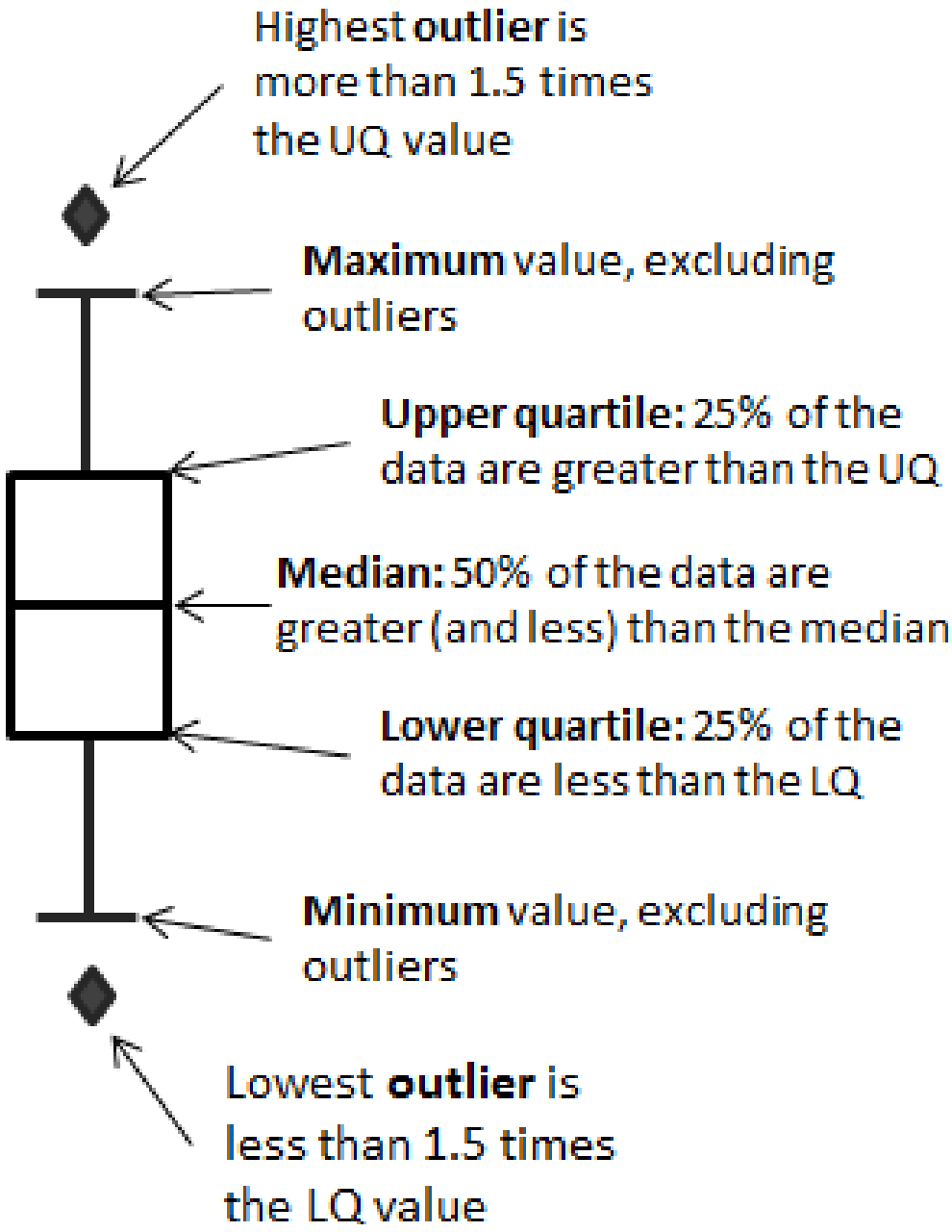
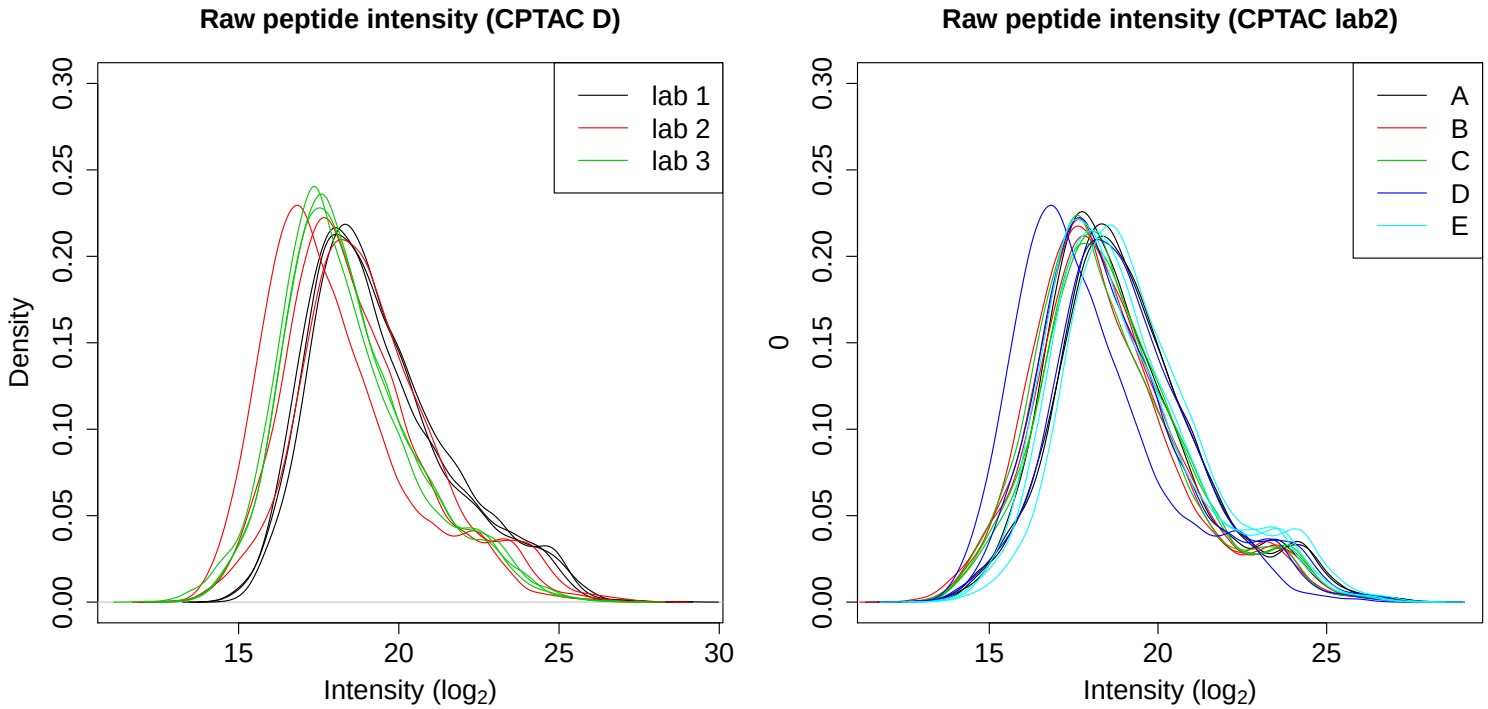


FIGURE 2–8 *Distributions for men and women for the ideal number of partners desired over 30 years.* Note: *To include all of the data, we collapsed across categories further out on the tail of these distributions. If every category represented a single number, it would be more apparent that the tail is very flat and that distributions are even more skewed than is apparent here. [From Miller & Fishkin, 1997, fig. 8–1. On the dynamics of human bonding and reproductive success: Seeking windows on the adapted-for-human-environmental interface. In J. A. Simpson & D. T. Kendrick (Eds.),* Evolutionary Social Psychology. *Mahwah, NJ: Erlbaum.]*


Highest **outlier** is more than 1.5 times the UQ value
Maximum value, excluding outliers
Upper quartile: 25% of the data are greater than the UQ
Median: 50% of the data are greater (and less) than the median
Lower quartile: 25% of the data are less than the LQ
Minimum value, excluding outliers
Lowest **outlier** is less than 1.5 times the LQ value



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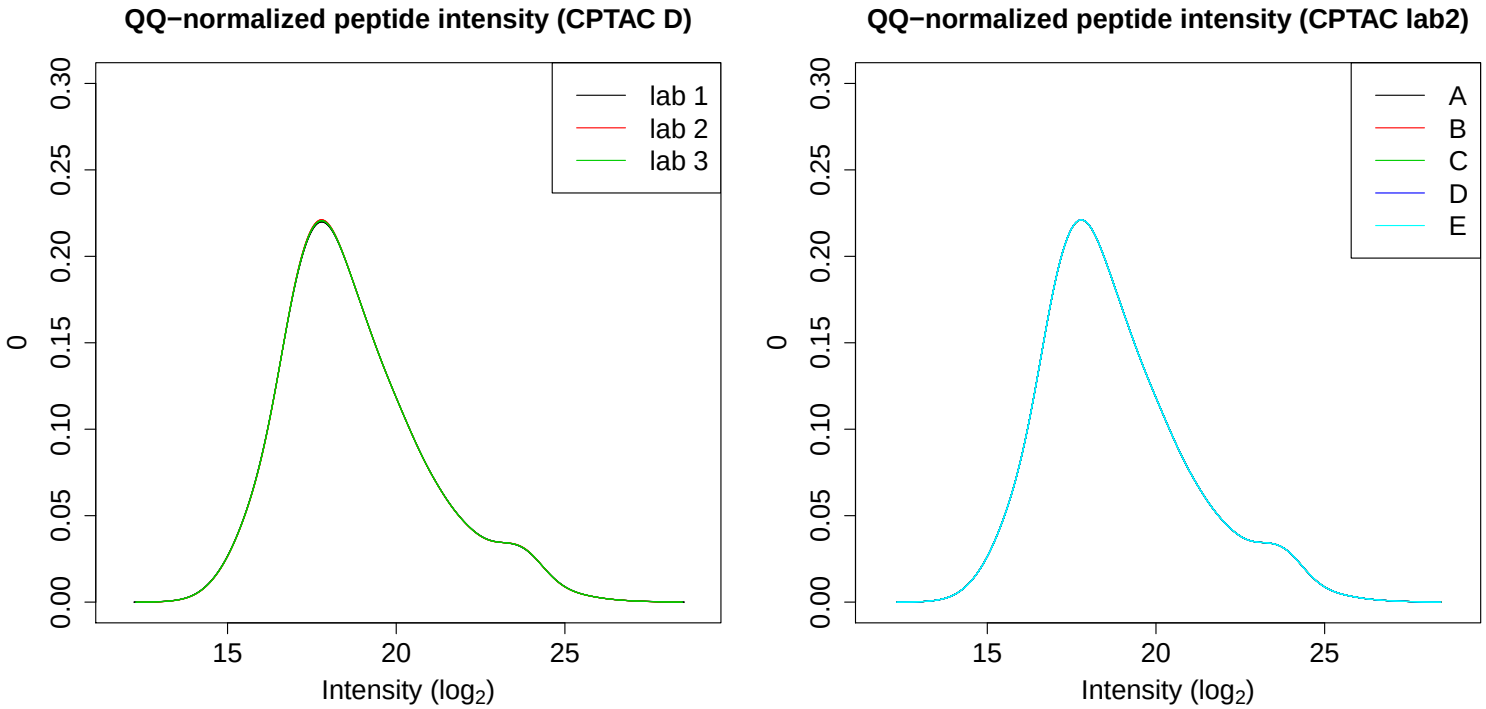
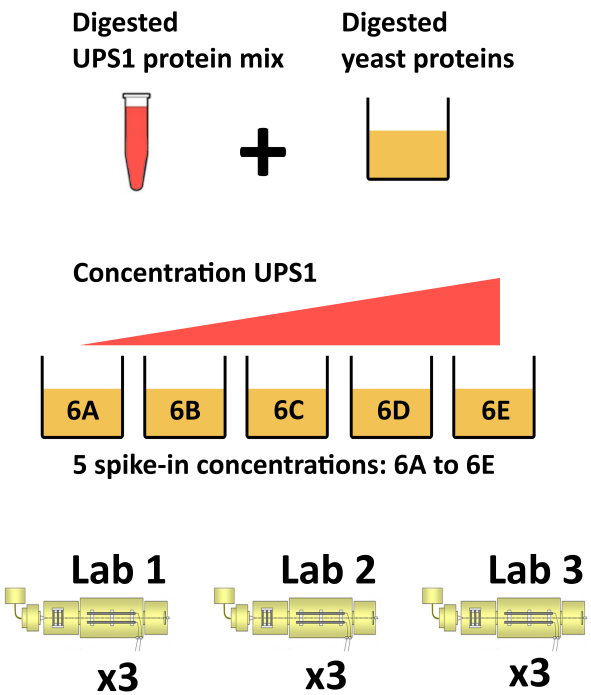


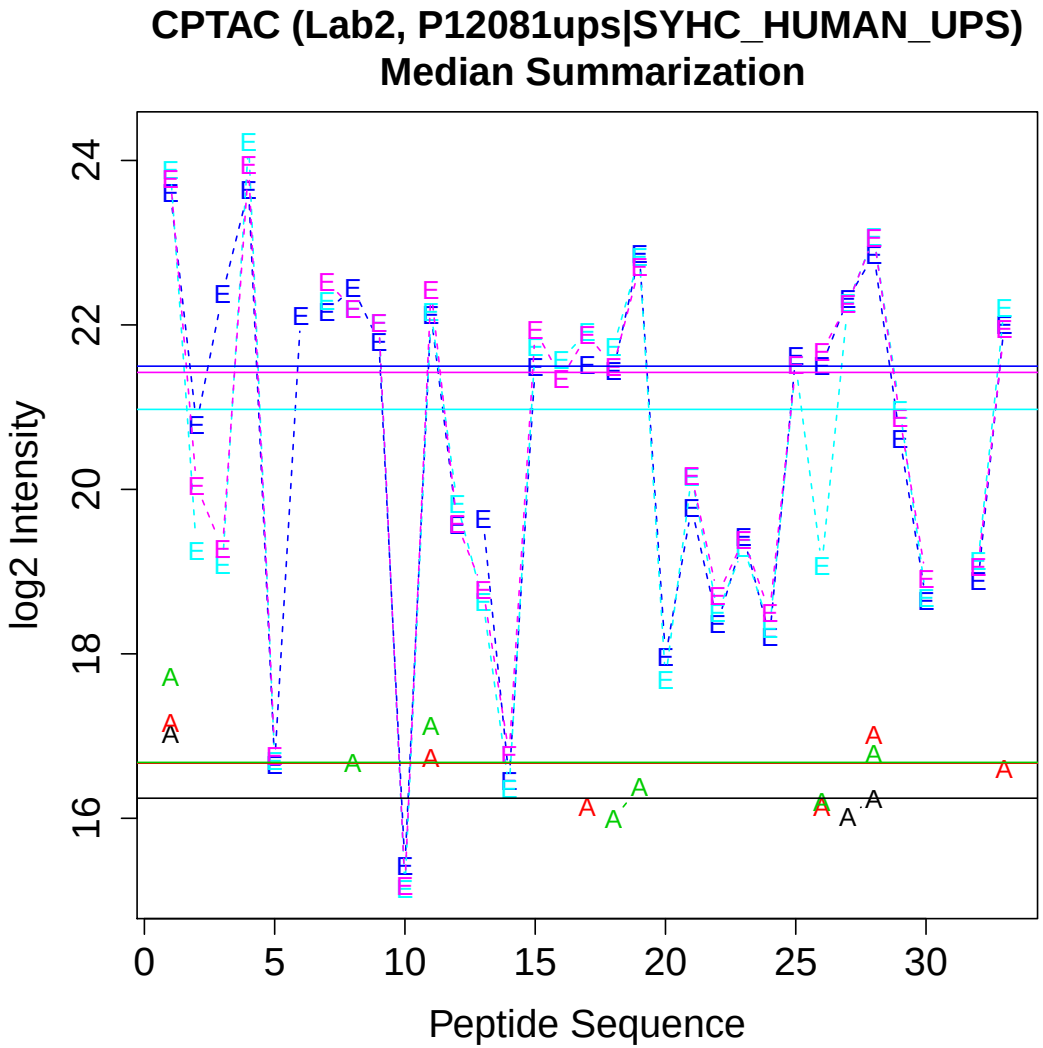
Even in very clean synthetic dataset (same background, only 48 UPS proteins can be different) the marginal peptide intensity distribution across samples can be quite distinct

- Considerable effects between and within labs for replicate samples
- Considerable effects between samples with different spike-in concentration

→ Normalization is needed, e.g. **quantile normalization**

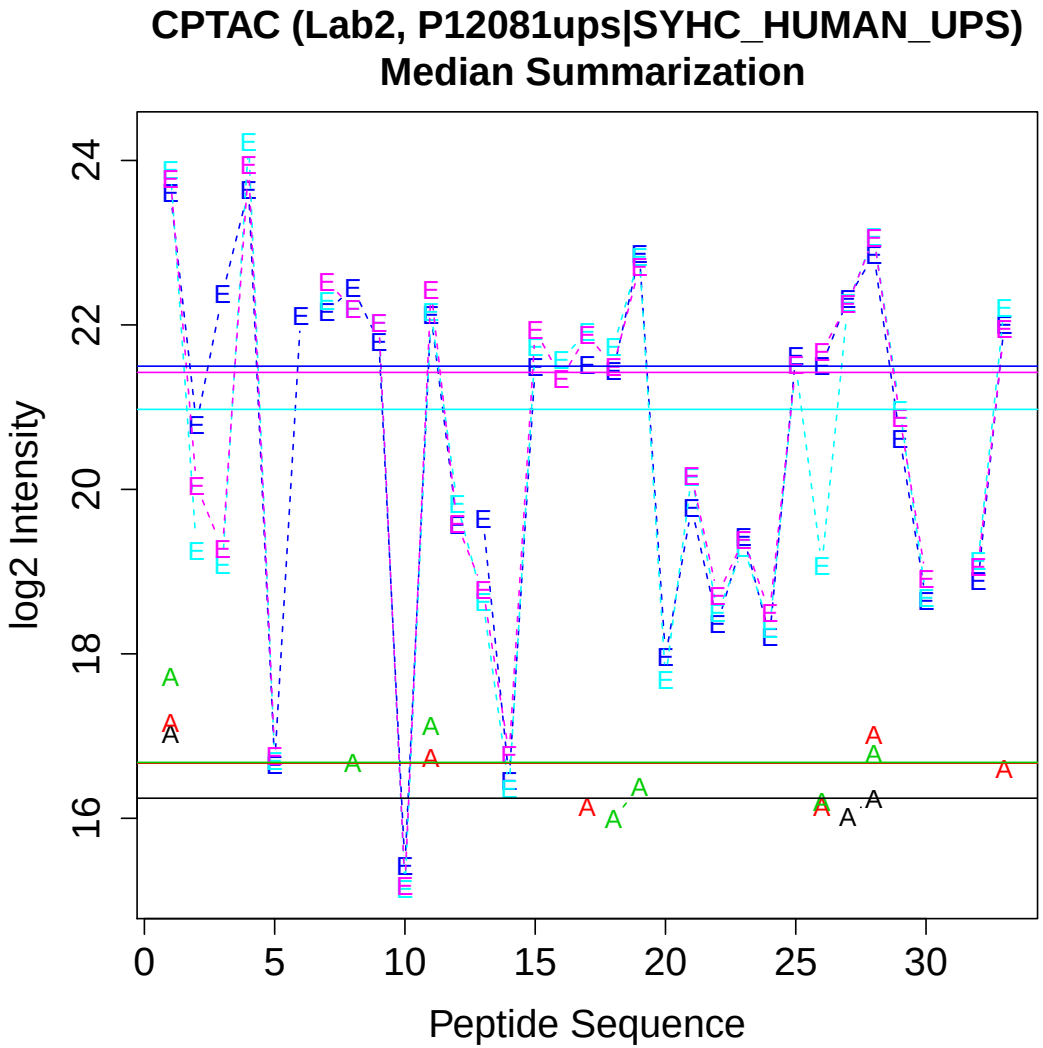
Summarization





Summarization

- Strong peptide effect
- Unbalanced peptide identification
- Summarization bias
- Different precision of protein level summaries



Impact of summarization?

Subsample of cptac study

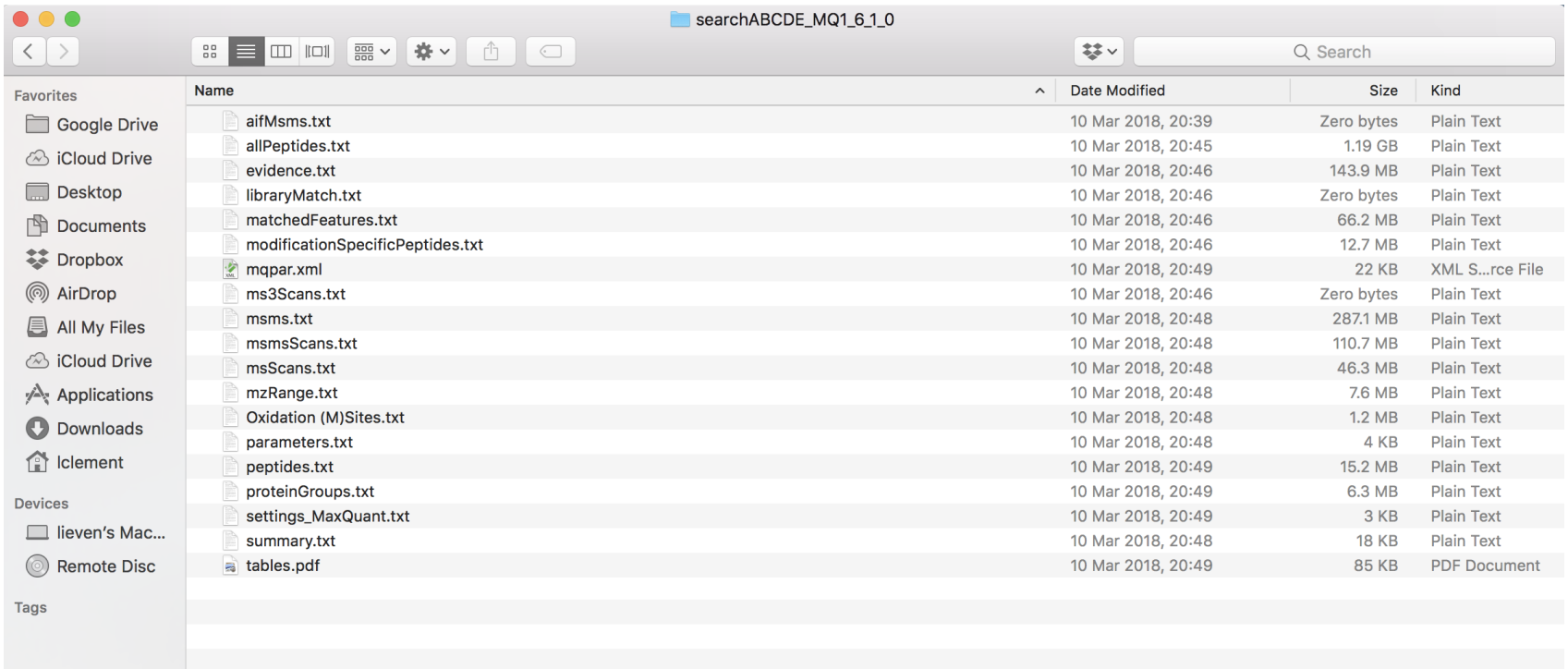
- Lab 3
- Spike in concentration A vs B

$\rightarrow$ Two group comparison

1. Median summarization
2. MaxLFQ (MaxQuant)
3. Robust summarization:

$$y_{sp} = \beta_p^{\text{pep}} + \beta_s^{\text{sample}} + \epsilon_{sp}$$

MaxQuant output



MaxLFQ summarization

a

>P63208
MPSIKLQSSDGEIFEVDVEIAKQSVTIKTMLEDLGMDDEGDD
DPVPLPNVNAAILKKVIQWCTHHKDDPPPPEDDENKEKRTDD
IPVWDQEFLKVDQGTLFELILAANYLDIKGLLDVTCKTVANM
IKGKTPEEIRKTFNIKNDFTEEEEAQVRKENQWCEEK

b

Peptide species	Sequence	Charge	Mod.
P_1	LQSSDGEIFEVDVEIAK	2	–
P_2	LQSSDGEIFEVDVEIAK	3	–
P_3	RTDDIPVWDQEFLK	2	–
P_4	TVANMIK	2	–
P_5	TVANMIK	2	Oxid.
P_6	TPEEIRK	3	–
P_7	NDFTEEEEAQVR	2	–

c

Sample	P_1	P_2	P_3	P_4	P_5	P_6	P_7
A		+				+	
B		+	+			+	
C	+	+	+	+		+	+
D	+	+		+		+	+
E		+		+			+
F		+			+		

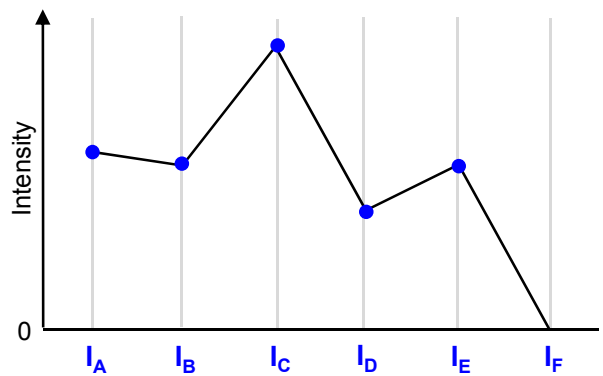
d

	A	B	C	D	E	F
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}	

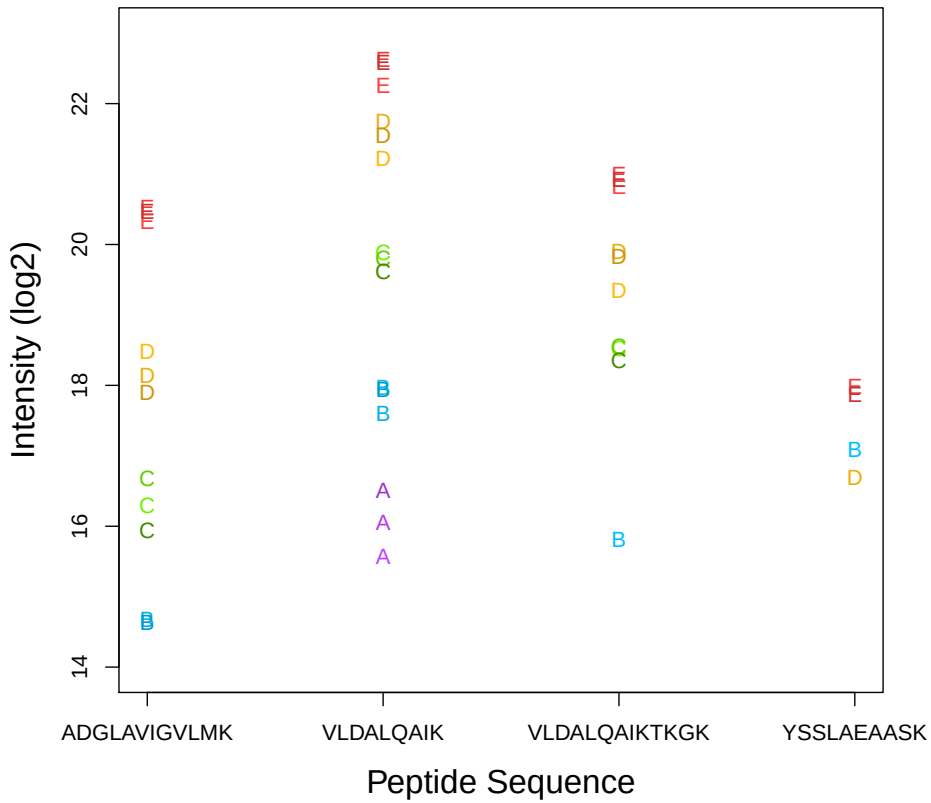
e

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

f



Summarisation with peptide based model



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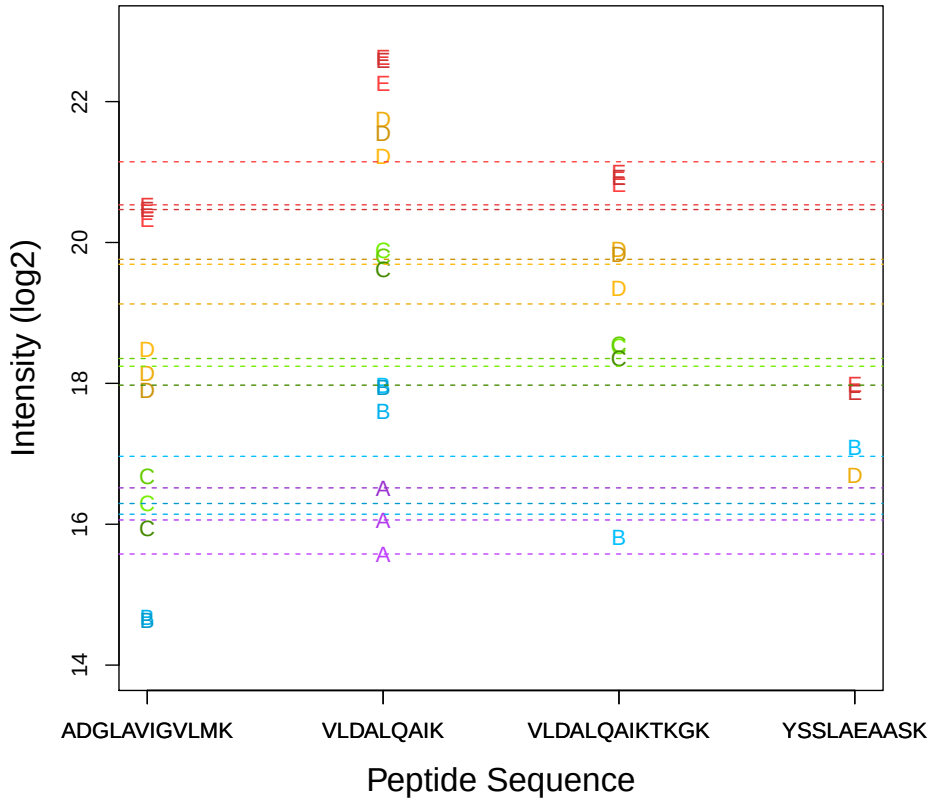
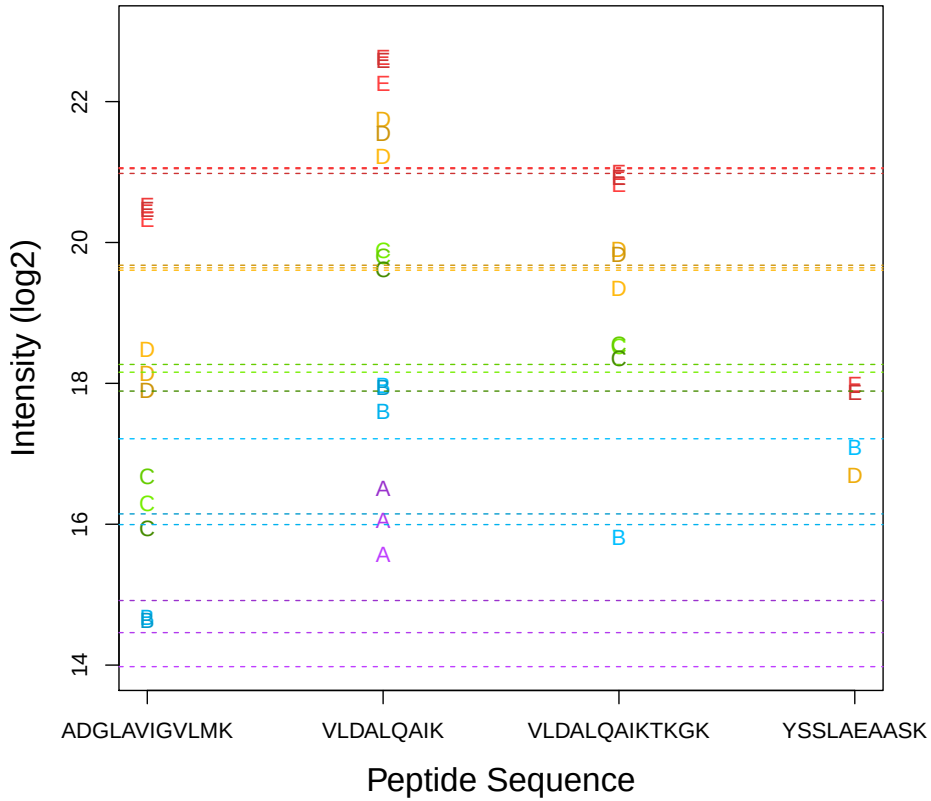


Protein by protein analysis of peptide data with linear model

peptide level protein level

$$y_{sp} = \epsilon_{sp} + \beta_s^{\text{sample}}$$

Summarisation with peptide based model



Protein by protein analysis of peptide data with linear model

peptide level + protein level

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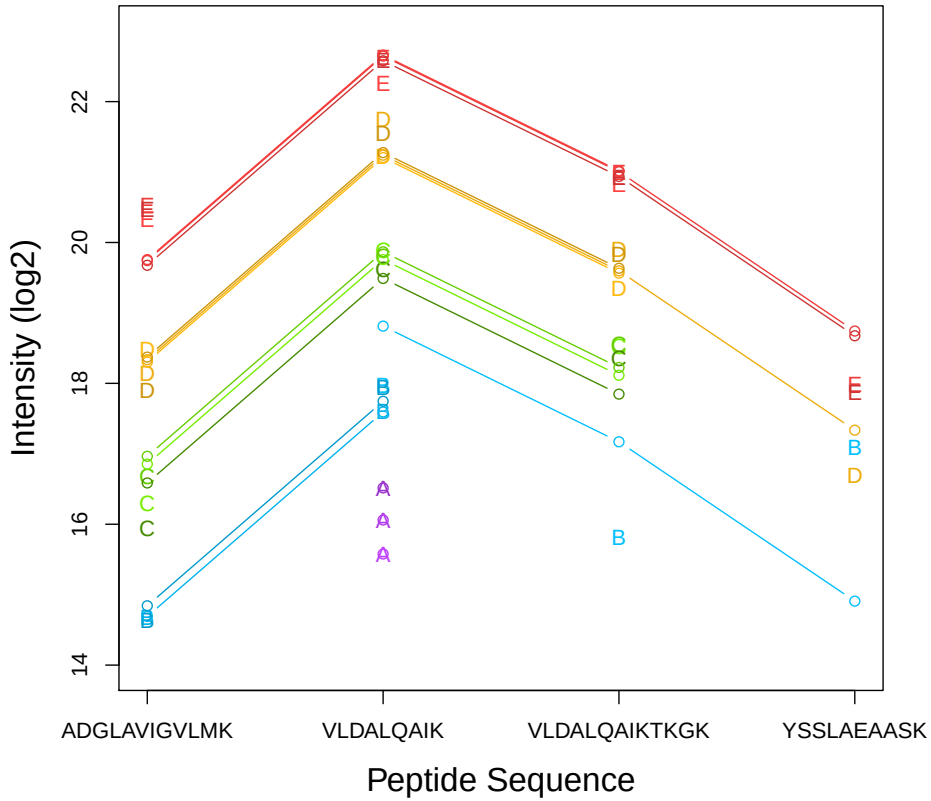

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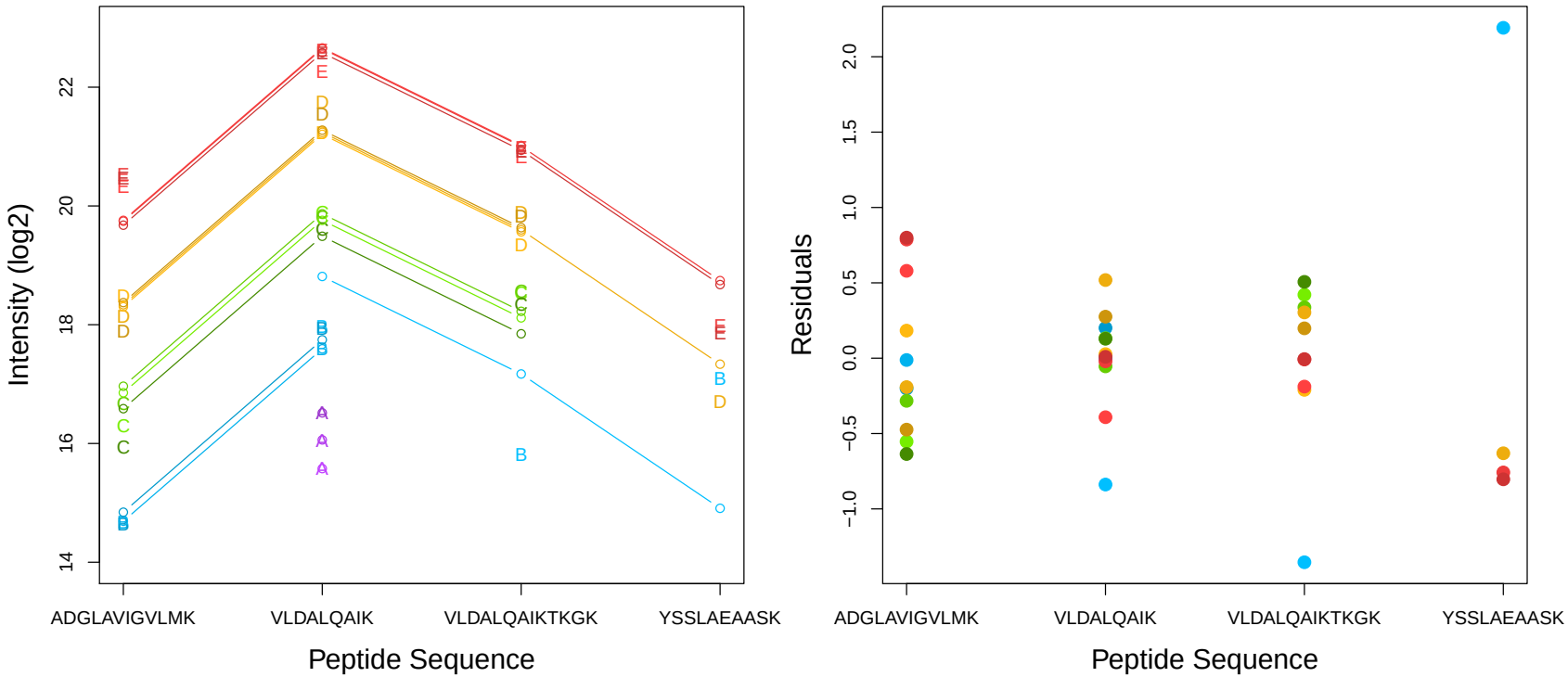
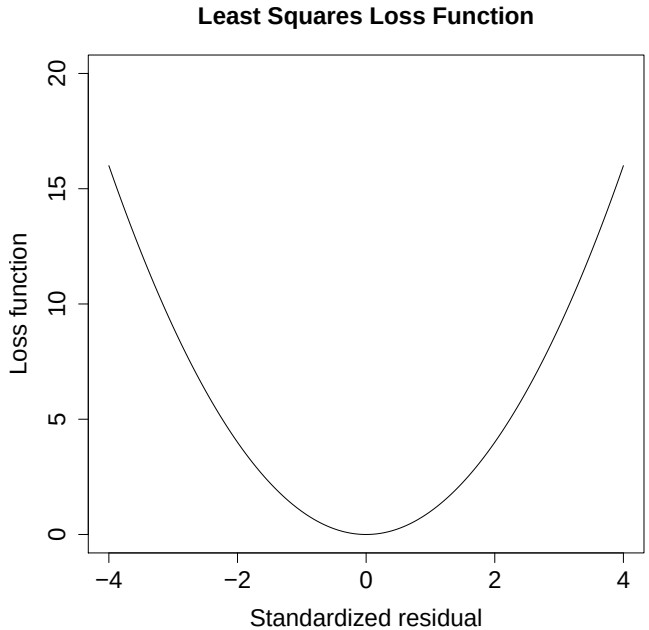


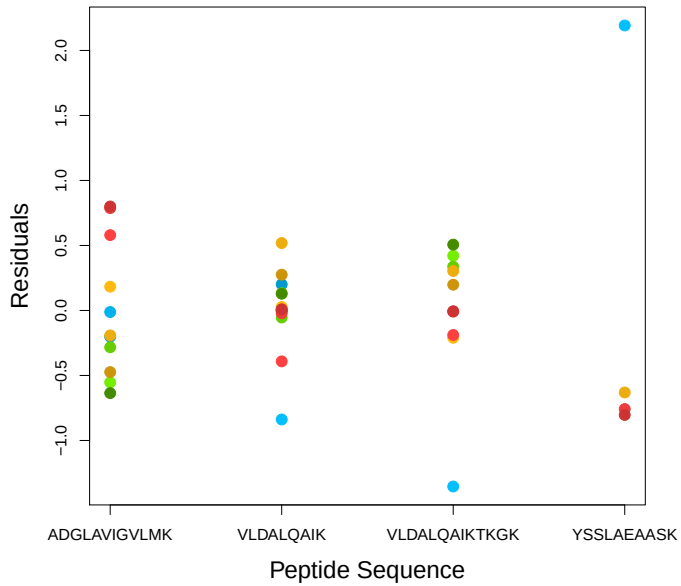
Protein by protein analysis of peptide data with linear model

$$\text{Estimation} \rightarrow \text{argmin}_{\beta^{\text{pep}}_{1\ldots P},\beta^{\text{samp}}_{1\ldots n}} \left[\sum_{i=1}^{n} \sum_{p}^{P} \left(y_{ip} - \beta^{\text{pep}}_{p} - \beta^{\text{samp}}_{i} \right)^2 \right]$$

Robust estimation using observation weights

- Outlying peptide intensities: incorrect peptide identification, post-translational modifications, ...





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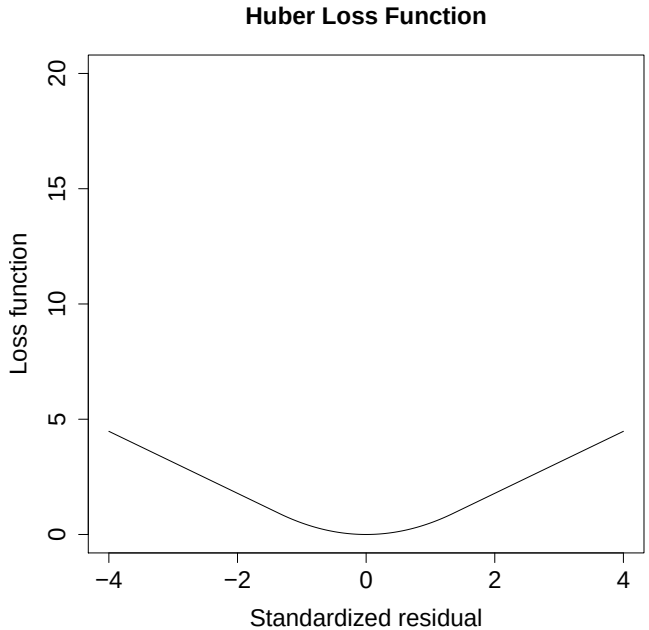

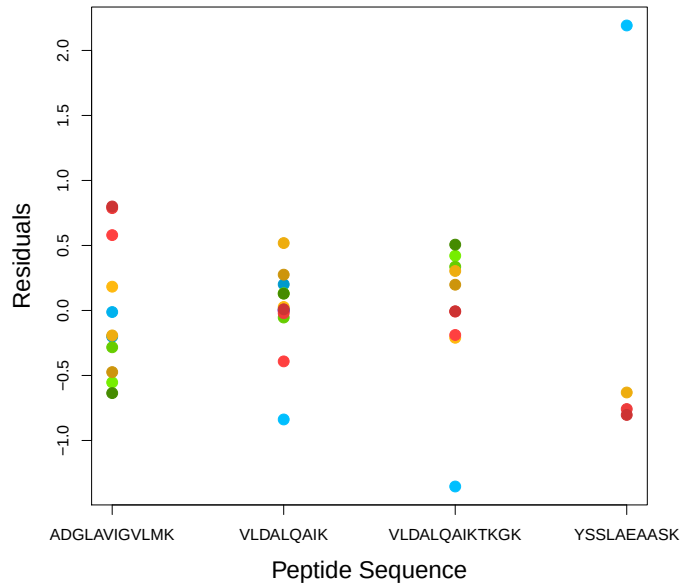

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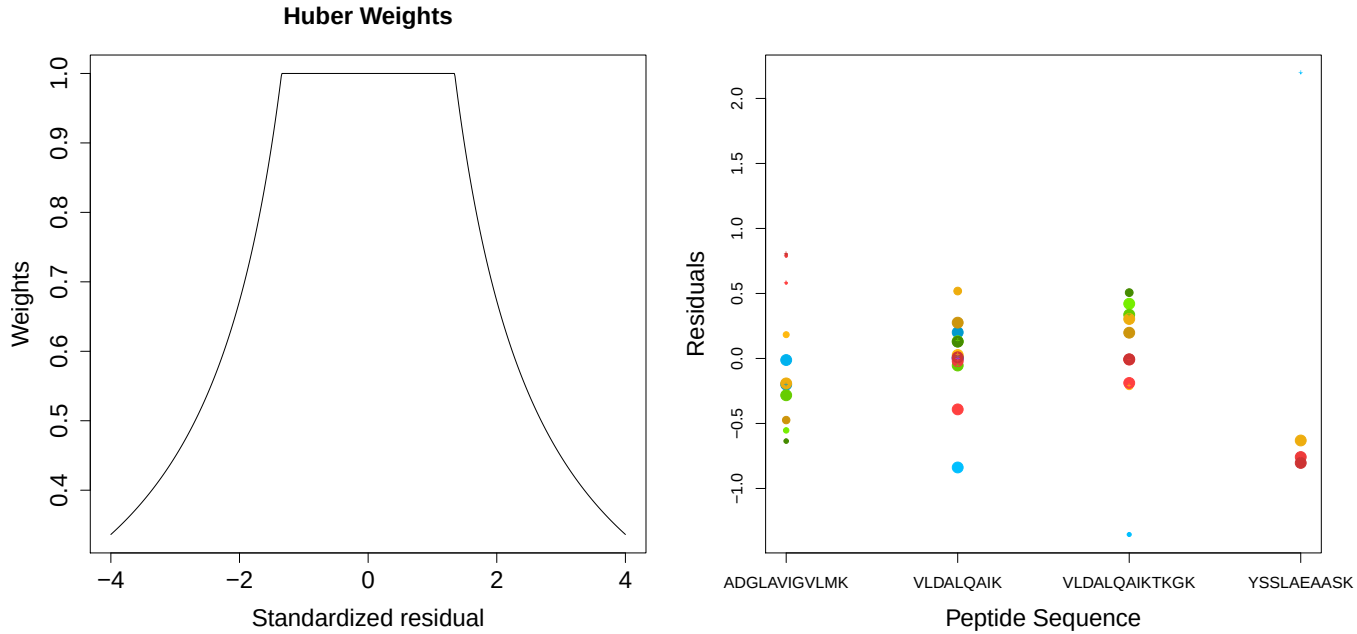

- Iteratively fit model with observation weights $w(\epsilon_{ip})$

$$\text{argmin}_{\beta^{\text{pep}}_{1\ldots P},\beta^{\text{samp}}_{1\ldots n}} \left[\sum_{i=1}^{n} \sum_{p}^{P} w(\epsilon_{ip}) \left(y_{ip} - \beta^{\text{pep}}_{p} - \beta^{\text{samp}}_{i} \right)^2 \right]$$

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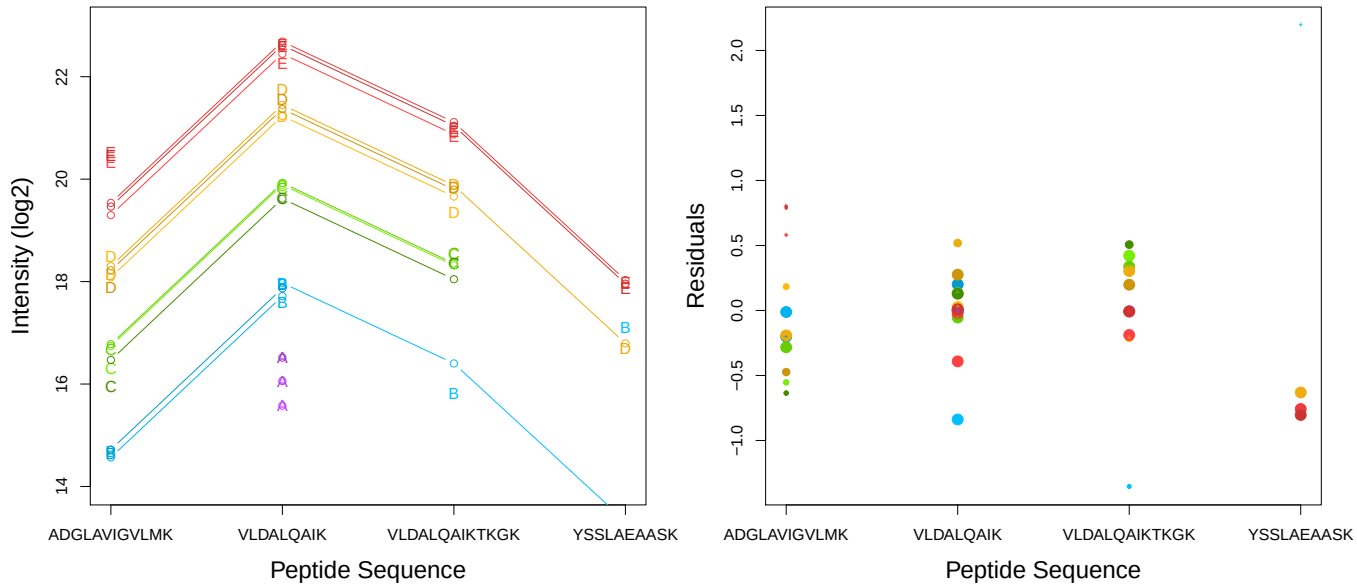

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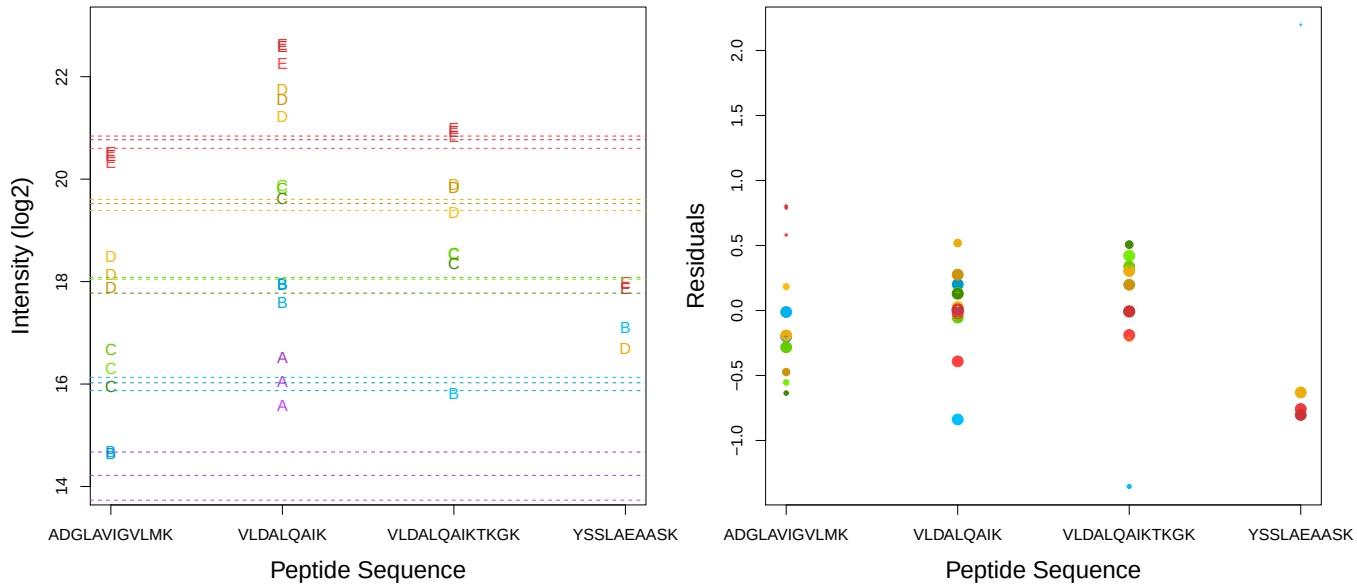

- Iteratively fit model with observation weights $w(\epsilon_{ip})$

$$\text{argmin}_{\beta_{1\ldots P}^{\text{pep}},\beta_{1\ldots n}^{\text{samp}}} \left[\sum_{i=1}^{n} \sum_{p}^{P} w(\epsilon_{ip}) \left(y_{ip} - \beta_{p}^{\text{pep}} - \beta_{i}^{\text{samp}} \right)^2 \right]$$