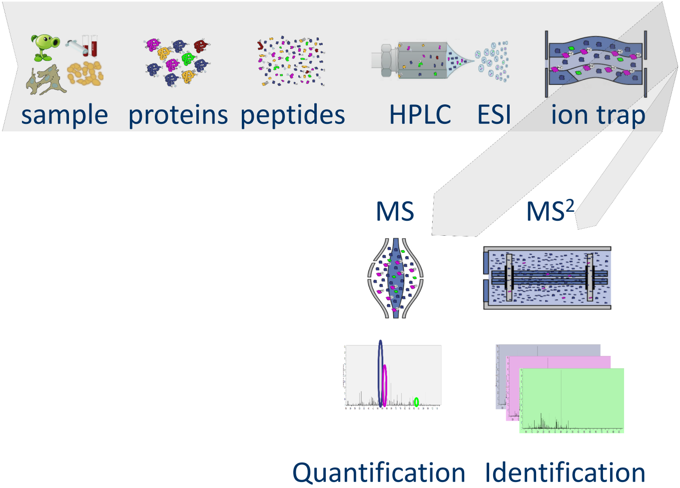
Part I: Normalization & Summarization

Lieven Clement

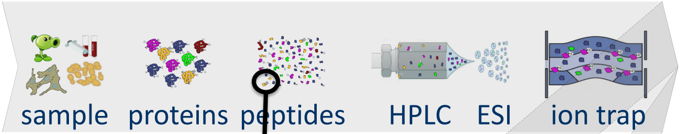
Proteomics Data Analysis Shortcourse

Outline

Challenges in Label Free Quantitative Proteomics

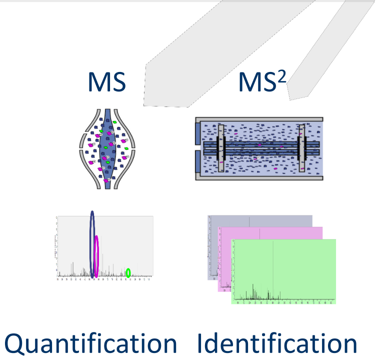


Challenges in Label Free Quantitative Proteomics

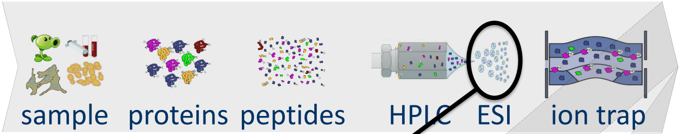


Peptide characteristics

- Modifications



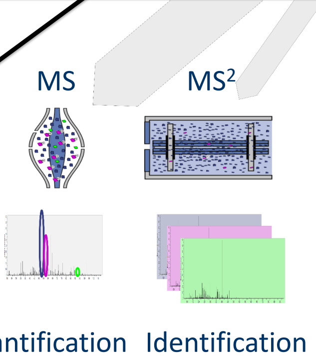
Challenges in Label Free Quantitative Proteomics



Peptide characteristics

- Modifications

- Ionisation efficiency
  - Outliers
  - Huge variability



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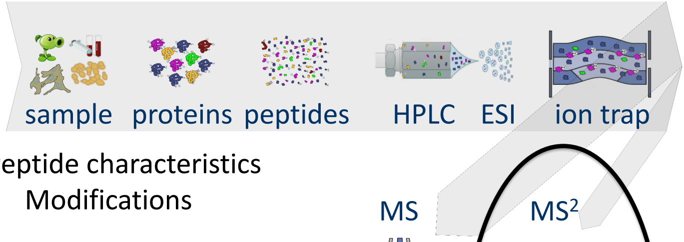
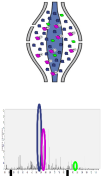
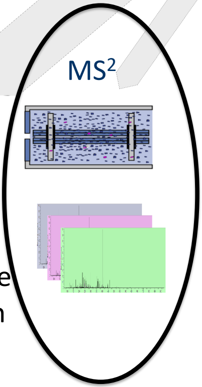


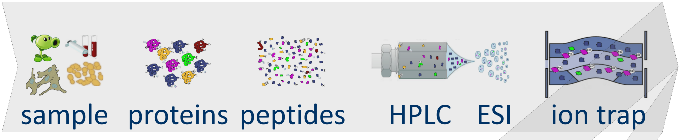




Peptide characteristics

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

Challenges in Label Free Quantitative Proteomics



Peptide characteristics

- Modifications

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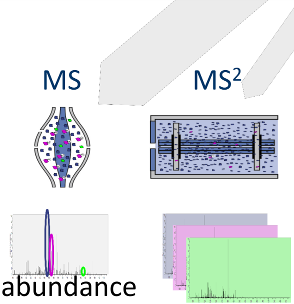
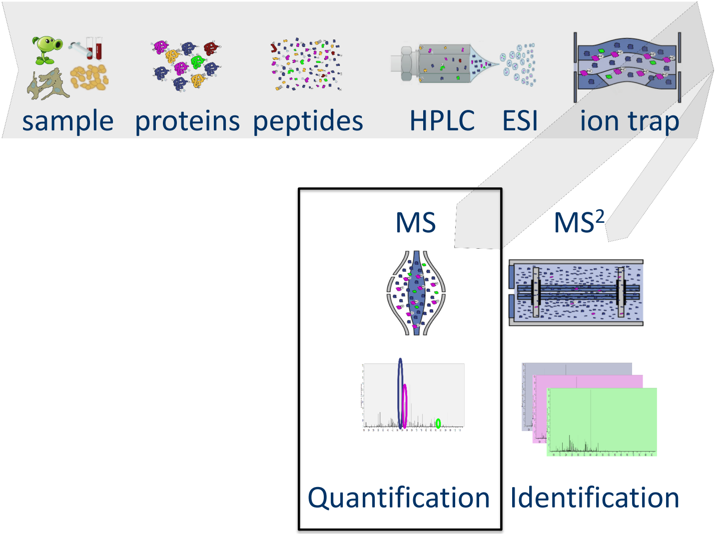


- MS^2 selection on peptide abundance
    - Context dependent Identification
    - Non-random missingness

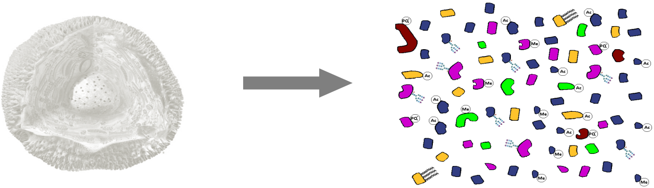
Unbalanced peptides identifications across samples and messy data

Challenges in Label Free MS-based Quatitative proteomics



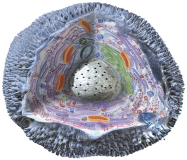
Challenges in Label Free MS-based Quatitative proteomics

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

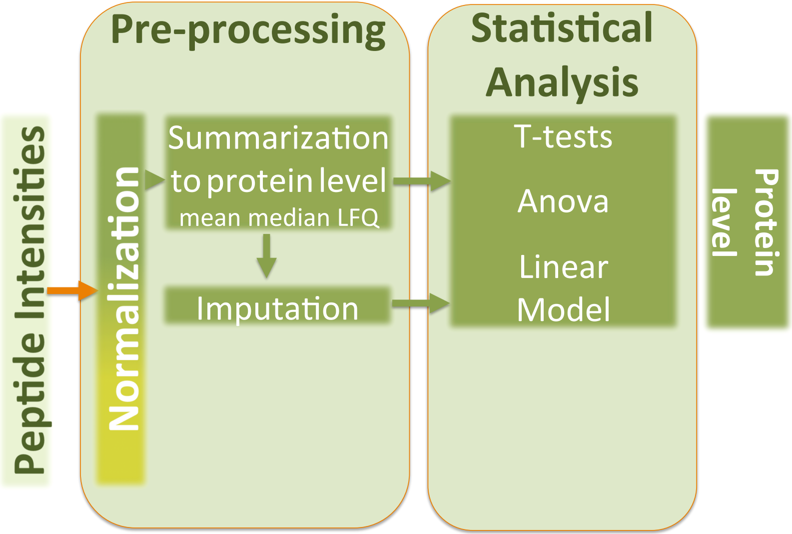


Challenges in Label Free MS-based Quatitative proteomics

We need information on protein level!



Label-free Quantitative Proteomics Data Analysis Pipelines



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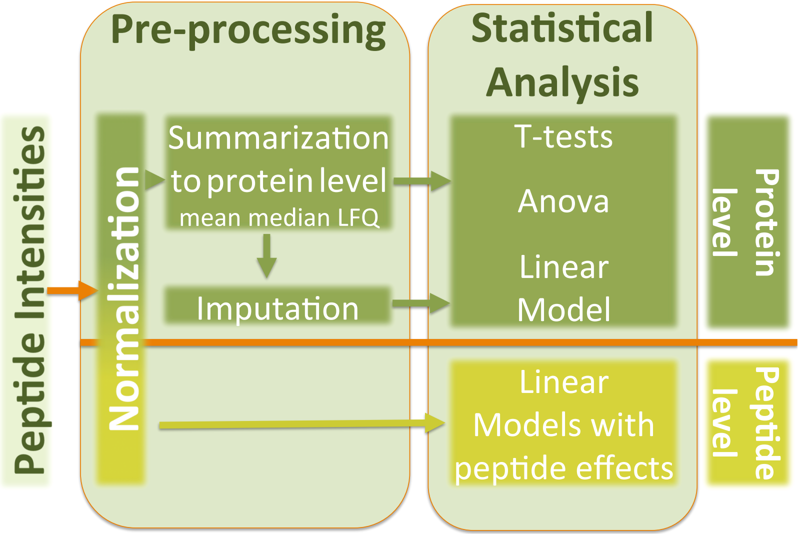
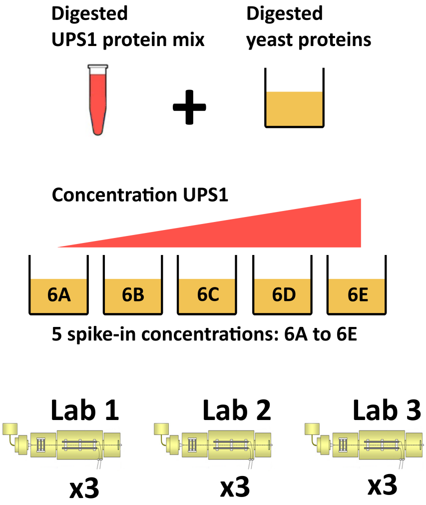


CPTAC Spike-in Study



- 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

→ **vast amount of missingness**

Preprocessing

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

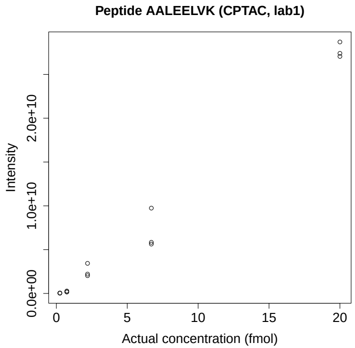
- Many methods exist

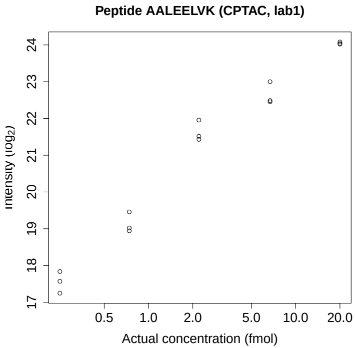
Filtering

- 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

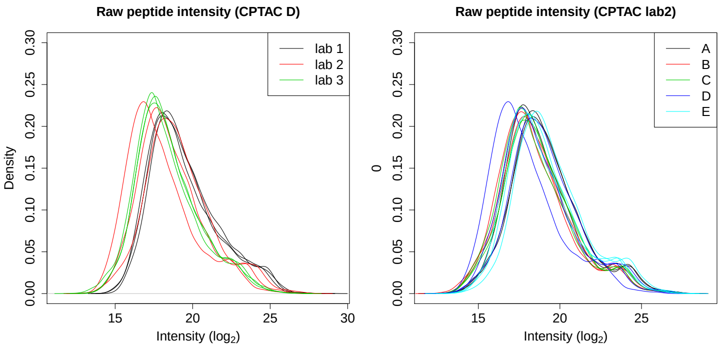
- Filtering does not induce bias if the criterion is independent from the downstream data analysis!

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?

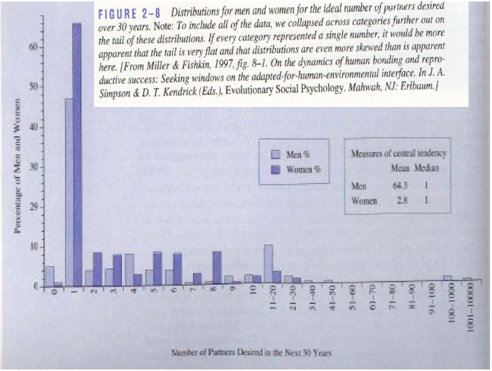
- Over a period of 30 years males desire to have on average 64.3 partners and females 2.8. (Miller and Fishkin, 1997)

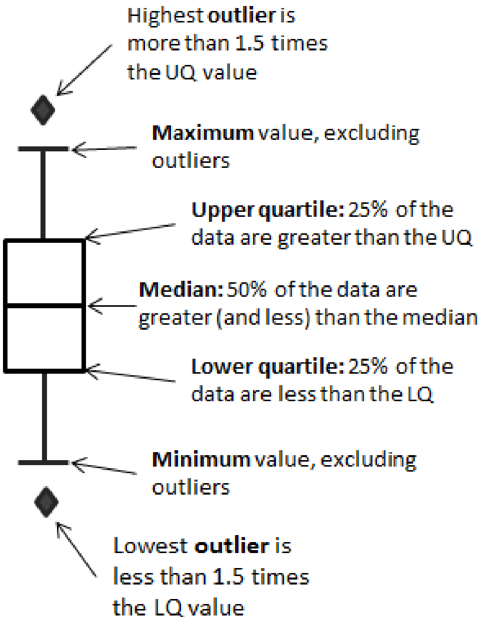
Mean or median?

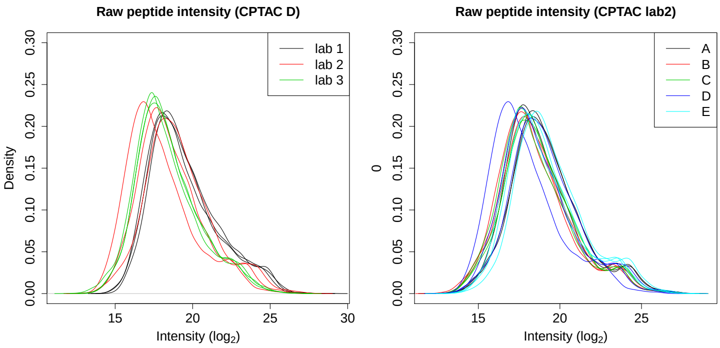
- Over a period of 30 years males desire to have on average 64.3 partners and females 2.8. (Miller and Fishkin, 1997)
- Over a period of 30 years males, is the median of the number of desired partners is 1 for both males and females. (Miller and Fishkin, 1997)

Mean or median?

Mean is very sensitive to outliers!




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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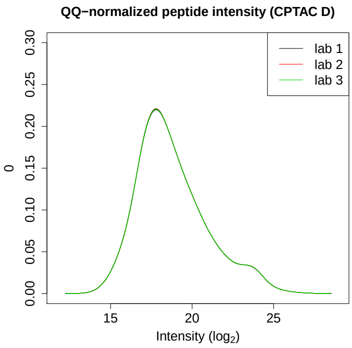

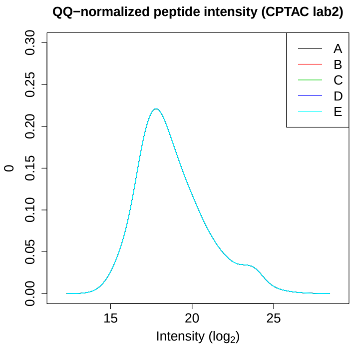

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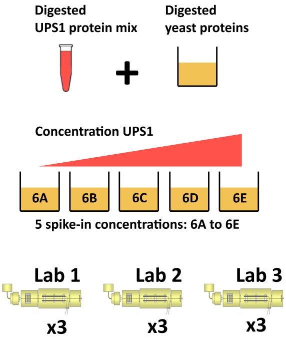
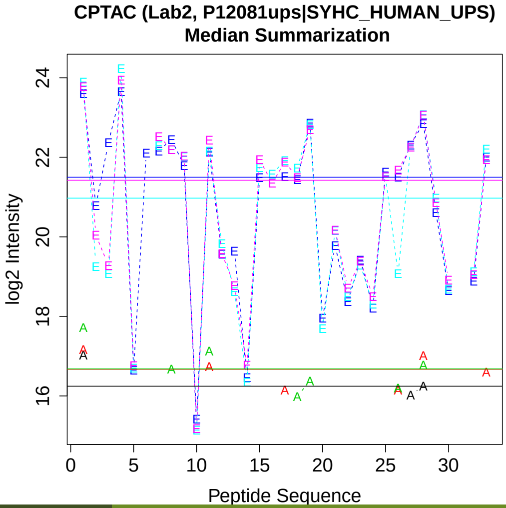
$\rightarrow$ Normalization is needed, e.g. **quantile normalization**

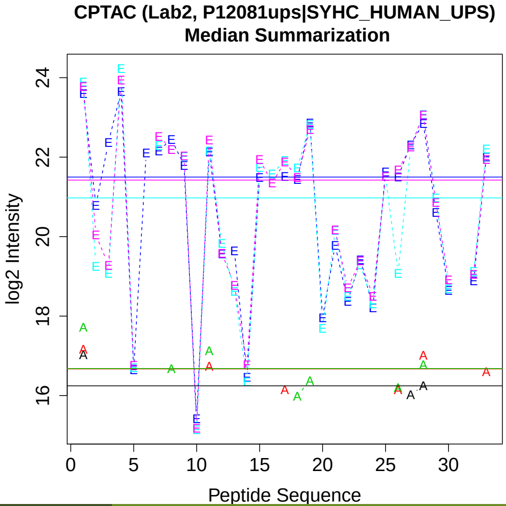
Summarization





Summarization

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



Impact of summarization?

Subsample of cptac study

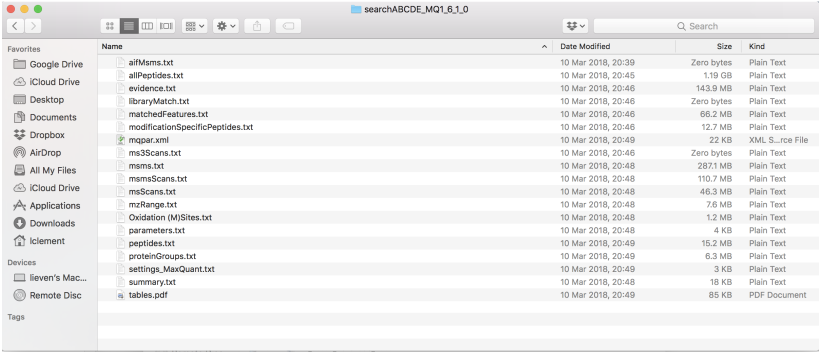
- Lab 3
- Spike in concentration A vs B

→ 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



searchABCDE_MQ1_6_1_0

Name	Date Modified	Size	Kind
aifMsms.txt	10 Mar 2018, 20:39	Zero bytes	Plain Text
allPeptides.txt	10 Mar 2018, 20:45	1.19 GB	Plain Text
evidence.txt	10 Mar 2018, 20:46	143.9 MB	Plain Text
libraryMatch.txt	10 Mar 2018, 20:46	Zero bytes	Plain Text
matchedFeatures.txt	10 Mar 2018, 20:46	66.2 MB	Plain Text
modificationSpecificPeptides.txt	10 Mar 2018, 20:46	12.7 MB	Plain Text
mqpar.xml	10 Mar 2018, 20:49	22 KB	XML S...rce File
ms3Scans.txt	10 Mar 2018, 20:46	Zero bytes	Plain Text
msms.txt	10 Mar 2018, 20:48	287.1 MB	Plain Text
msmsScans.txt	10 Mar 2018, 20:48	110.7 MB	Plain Text
msScans.txt	10 Mar 2018, 20:48	46.3 MB	Plain Text
mzRange.txt	10 Mar 2018, 20:48	7.6 MB	Plain Text
Oxidation (M)Sites.txt	10 Mar 2018, 20:48	1.2 MB	Plain Text
parameters.txt	10 Mar 2018, 20:48	4 KB	Plain Text
peptides.txt	10 Mar 2018, 20:49	15.2 MB	Plain Text
proteinGroups.txt	10 Mar 2018, 20:49	6.3 MB	Plain Text
settings_MaxQuant.txt	10 Mar 2018, 20:49	3 KB	Plain Text
summary.txt	10 Mar 2018, 20:48	18 KB	Plain Text
tables.pdf	10 Mar 2018, 20:49	85 KB	PDF Document

MaxLFQ summarization

a

>P63208

MPSIK**LQSSDGEIFEVDVEIAK**QSVTIKTMLEDLGMDDEGDD
DPVPLPNVNAAILKKVIQWCTHHKDDPPPPEDDENKEK**RTDD**
IPVWDQEFLKVDQGTLFELILAANYLDIKGLLDVTCK**TVANM**
IKGK**TPEEIRK**TFNIK**NDFTEEEEAQVR**KENQWCEEK

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		+			+		

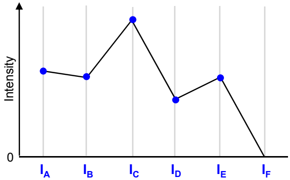
d

	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}	

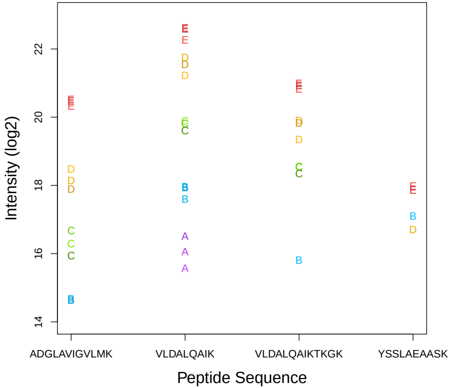
e

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

f



Summarisation with peptide based model



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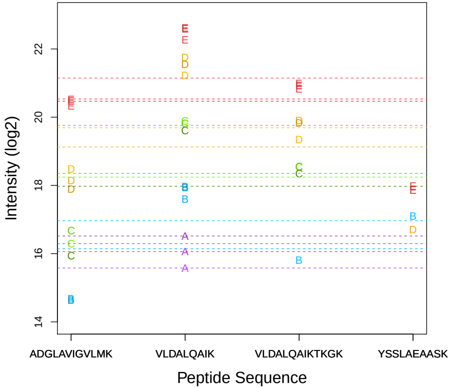
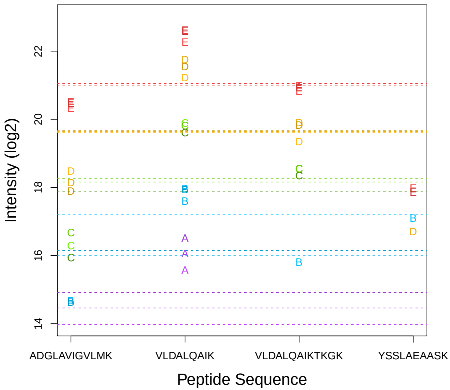


Protein by protein analysis of peptide data with linear model

peptide level protein level

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

Summarisation with peptide based model



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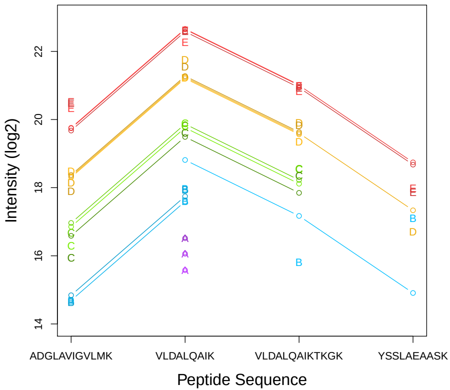

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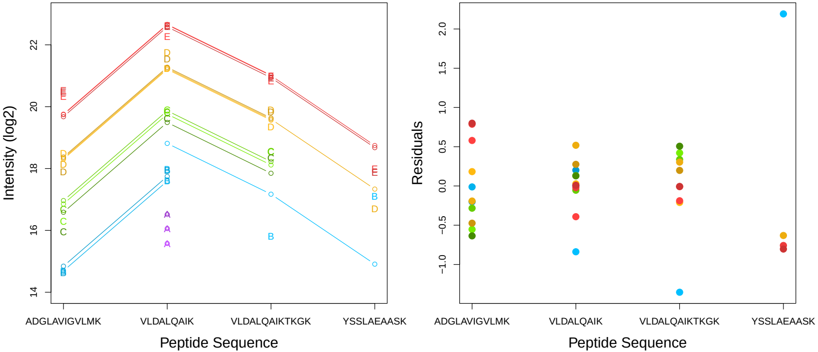
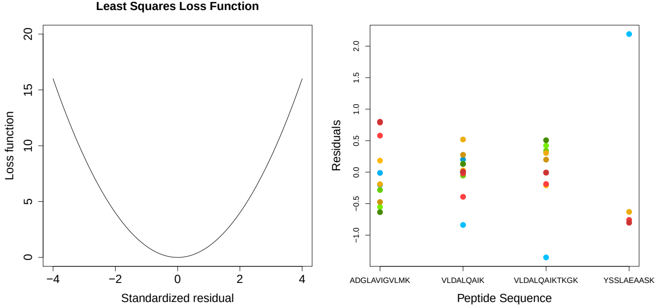


Protein by protein analysis of peptide data with linear model

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

Robust estimation using observation weights

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



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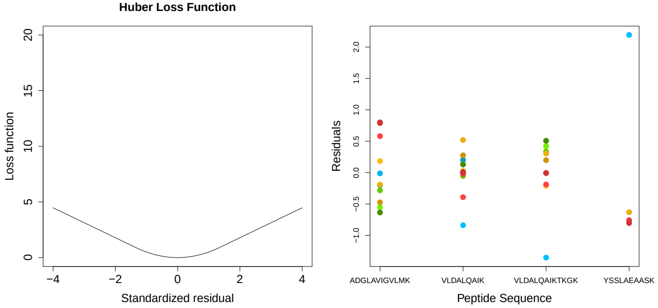

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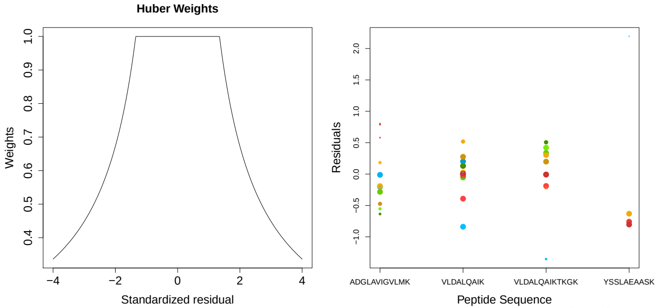

- Iteratively fit model with observation weights $w(\epsilon_{ip})$

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

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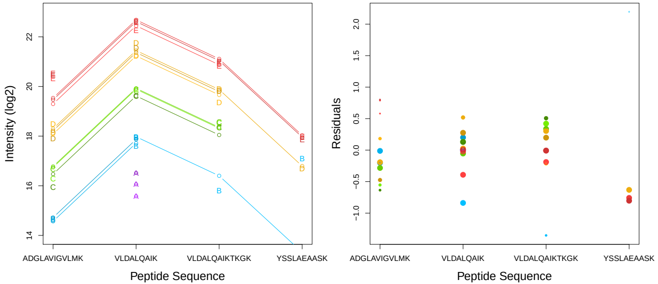

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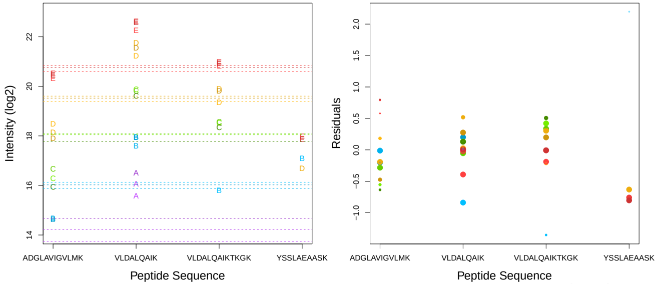

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