# Statistical Methods for Quantitative MS-based Proteomics: Peptide-level Models for Summarization and Inference

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This is part of the online course Proteomics Data Analysis (PDA)

```
library(tidyverse)
library(limma)
library(QFeatures)
library(msqrob2)
library(plotly)
library(gridExtra)
```

# 1 Subset of CPTAC study: A vs B comparison in lab 3

## 1.1 LFQ

Click to see background and code

1. Import data

```
proteinsFile <- "https://raw.githubusercontent.com/statOmics/PDA22GTPB/data/quantification/cptacAvsB_lal

ecols <- grep("LFQ\\.intensity\\.", names(read.delim(proteinsFile)))

peLFQ <- readQFeatures(
  table = proteinsFile, fnames = 1, ecol = ecols,
  name = "proteinRaw", sep = "\t"
)

cond <- which(
  strsplit(colnames(peLFQ)[[1]][1], split = "")[[1]] == "A") # find where condition is stored

colData(peLFQ)$condition <- substr(colnames(peLFQ), cond, cond) %>%
  unlist %>%
  as.factor
```

2. Preprocessing

```
rowData(peLFQ[["proteinRaw"]])$nNonZero <- rowSums(assay(peLFQ[["proteinRaw"]]) > 0)

peLFQ <- zeroIsNA(peLFQ, "proteinRaw") # convert 0 to NA

peLFQ <- logTransform(peLFQ, base = 2, i = "proteinRaw", name = "proteinLog")

peLFQ <- filterFeatures(peLFQ,~ Reverse != "+")
peLFQ <- filterFeatures(peLFQ,~ Potential.contaminant != "+")

peLFQ <- normalize(peLFQ,
                i = "proteinLog",
                name = "protein",
                method = "center.median")
```

3. Modeling and Inference

```
peLFQ <- msqrob(object = peLFQ, i = "protein", formula = ~condition)
```

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```

```
L <- makeContrast("conditionB=0", parameterNames = c("conditionB"))
peLFQ <- hypothesisTest(object = peLFQ, i = "protein", contrast = L)
```

```
volcanoLFQ <- ggplot(rowData(peLFQ[["protein"]])$conditionB,
                  aes(x = logFC, y = -log10(pval), color = adjPval < 0.05)) +
  geom_point(cex = 2.5) +
  scale_color_manual(values = alpha(c("black", "red"), 0.5)) +
  theme_minimal() +
  ggtitle(paste0("maxLFQ: TP = ",sum(rowData(peLFQ[["protein"]])$conditionB$adjPval<0.05&grepl(rownames
```

## 1.2 Median & robust summarization

Click to see background and code

1. Import Data

```
peptidesFile <- "https://raw.githubusercontent.com/statOmics/SGA2020/data/quantification/cptacAvsB_lab3/

ecols <- grep(
  "Intensity\\.",
  names(read.delim(peptidesFile))
  )

pe <- readQFeatures(
  table = peptidesFile,
  fnames = 1,
  ecol = ecols,
  name = "peptideRaw", sep="\t")

cond <- which(
  strsplit(colnames(pe)[[1]][1], split = "")[[1]] == "A") # find where condition is stored

colData(pe)$condition <- substr(colnames(pe), cond, cond) %>%
  unlist %>%
  as.factor
```

2. Preprocessing

```
rowData(pe[["peptideRaw"]])$nNonZero <- rowSums(assay(pe[["peptideRaw"]]) > 0)

pe <- zeroIsNA(pe, "peptideRaw") # convert 0 to NA

pe <- logTransform(pe, base = 2, i = "peptideRaw", name = "peptideLog")

pe <- filterFeatures(pe, ~ Proteins %in% smallestUniqueGroups(rowData(pe[["peptideLog"]])$Proteins))

pe <- filterFeatures(pe,~Reverse != "+")
pe <- filterFeatures(pe,~ Potential.contaminant != "+")

pe <- filterFeatures(pe,~ nNonZero >=2)
nrow(pe[["peptideLog"]])
```

```
## [1] 7011
```

```r
pe <- normalize(pe,
                i = "peptideLog",
                name = "peptideNorm",
                method = "center.median")

pe <- aggregateFeatures(pe,
  i = "peptideNorm",
  fcol = "Proteins",
  na.rm = TRUE,
  name = "proteinMedian",
  fun = matrixStats::colMedians)
```

```
## Your quantitative and row data contain missing values. Please read the
## relevant section(s) in the aggregateFeatures manual page regarding the
## effects of missing values on data aggregation.
```

```r
pe <- aggregateFeatures(pe,
  i = "peptideNorm",
  fcol = "Proteins",
  na.rm = TRUE,
  name = "proteinRobust")
```

```
## Your quantitative and row data contain missing values. Please read the
## relevant section(s) in the aggregateFeatures manual page regarding the
## effects of missing values on data aggregation.

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3. Modeling and inference

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pe <- msqrob(object = pe, i = "proteinMedian", formula = ~condition)
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```r
L <- makeContrast("conditionB=0", parameterNames = c("conditionB"))
pe <- hypothesisTest(object = pe, i = "proteinMedian", contrast = L)

pe <- msqrob(object = pe, i = "proteinRobust", formula = ~condition)
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## Warning in rlm.default(X, y, method = "M", maxit = maxitRob): 'rlm' failed to
## converge in 1 steps

## Warning in rlm.default(X, y, method = "M", maxit = maxitRob): 'rlm' failed to
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## Warning in rlm.default(X, y, method = "M", maxit = maxitRob): 'rlm' failed to
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## converge in 1 steps

## Warning in rlm.default(X, y, method = "M", maxit = maxitRob): 'rlm' failed to
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## Warning in rlm.default(X, y, method = "M", maxit = maxitRob): 'rlm' failed to
## converge in 1 steps

## Warning in rlm.default(X, y, method = "M", maxit = maxitRob): 'rlm' failed to
```

```
## converge in 1 steps

## Warning in rlm.default(X, y, method = "M", maxit = maxitRob): 'rlm' failed to
## converge in 1 steps

## Warning in rlm.default(X, y, method = "M", maxit = maxitRob): 'rlm' failed to
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## converge in 1 steps

## Warning in rlm.default(X, y, method = "M", maxit = maxitRob): 'rlm' failed to
## converge in 1 steps

## Warning in rlm.default(X, y, method = "M", maxit = maxitRob): 'rlm' failed to
## converge in 1 steps

## Warning in rlm.default(X, y, method = "M", maxit = maxitRob): 'rlm' failed to
## converge in 1 steps

## Warning in rlm.default(X, y, method = "M", maxit = maxitRob): 'rlm' failed to
## converge in 1 steps

## Warning in rlm.default(X, y, method = "M", maxit = maxitRob): 'rlm' failed to
## converge in 1 steps

## Warning in rlm.default(X, y, method = "M", maxit = maxitRob): 'rlm' failed to
## converge in 1 steps

## Warning in rlm.default(X, y, method = "M", maxit = maxitRob): 'rlm' failed to
## converge in 1 steps

## Warning in rlm.default(X, y, method = "M", maxit = maxitRob): 'rlm' failed to
## converge in 1 steps

## Warning in rlm.default(X, y, method = "M", maxit = maxitRob): 'rlm' failed to
## converge in 1 steps
```

```
pe <- hypothesisTest(object = pe, i = "proteinRobust", contrast = L)

volcanoMedian <- ggplot(rowData(pe[["proteinMedian"]])$conditionB,
                  aes(x = logFC, y = -log10(pval), color = adjPval < 0.05)) +
  geom_point(cex = 2.5) +
  scale_color_manual(values = alpha(c("black", "red"), 0.5)) +
  theme_minimal() +
  ggtitle(paste0("Median: TP = ",sum(rowData(pe[["proteinMedian"]])$conditionB$adjPval<0.05&grepl(rownar

volcanoRobust<- ggplot(rowData(pe[["proteinRobust"]])$conditionB,
                  aes(x = logFC, y = -log10(pval), color = adjPval < 0.05)) +
  geom_point(cex = 2.5) +
  scale_color_manual(values = alpha(c("black", "red"), 0.5)) +
```

```
  theme_minimal() +
  ggtitle(paste0("Robust: TP = ",sum(rowData(pe[["proteinRobust"]])$conditionB$adjPval<0.05&grepl(rowna
```

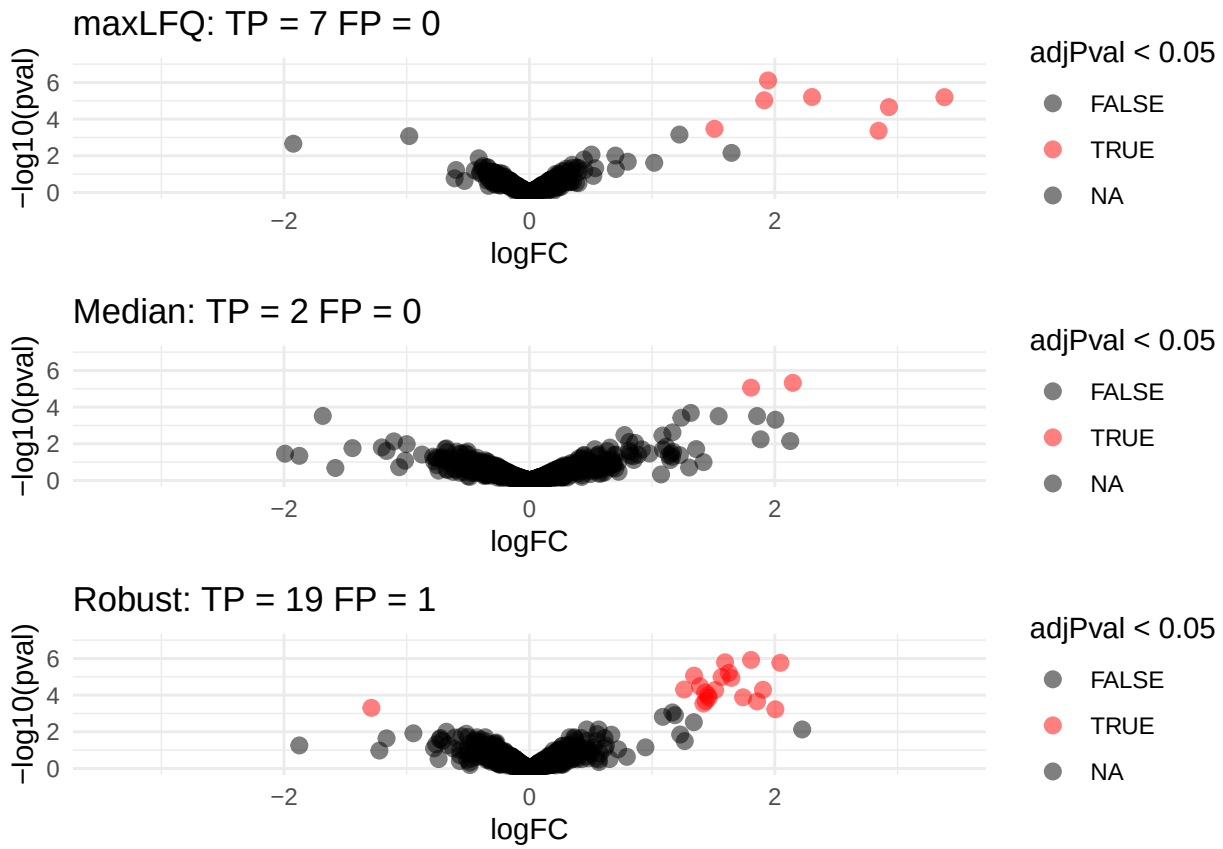
```
ylims <- c(0,
           ceiling(max(c(-log10(rowData(peLFQ[["protein"]])$conditionB$pval),
               -log10(rowData(pe[["proteinMedian"]])$conditionB$pval),
               -log10(rowData(pe[["proteinRobust"]])$conditionB$pval)),
               na.rm=TRUE))
)

xlims <- max(abs(c(rowData(peLFQ[["protein"]])$conditionB$logFC,
               rowData(pe[["proteinMedian"]])$conditionB$logFC,
               rowData(pe[["proteinRobust"]])$conditionB$logFC)),
               na.rm=TRUE) * c(-1,1)
```

```
compBoxPlot <- rbind(rowData(peLFQ[["protein"]])$conditionB %>% mutate(method="maxLFQ") %>% rownames_to
      rowData(pe[["proteinMedian"]])$conditionB %>% mutate(method="median")%>% rownames_to_column(var="
      rowData(pe[["proteinRobust"]])$conditionB%>% mutate(method="robust")%>% rownames_to_column(var="p
      mutate(ups= grepl(protein,pattern="UPS")) %>%
    ggplot(aes(x = method, y = logFC, fill = ups)) +
    geom_boxplot() +
    geom_hline(yintercept = log2(0.74 / .25), color = "#00BFC4") +
    geom_hline(yintercept = 0, color = "#F8766D")
```

## 1.3 Comparison summarization methods

```
grid.arrange(volcanoLFQ + xlim(xlims) + ylim(ylims),
             volcanoMedian + xlim(xlims) + ylim(ylims),
             volcanoRobust + xlim(xlims) + ylim(ylims),
             ncol=1)
```

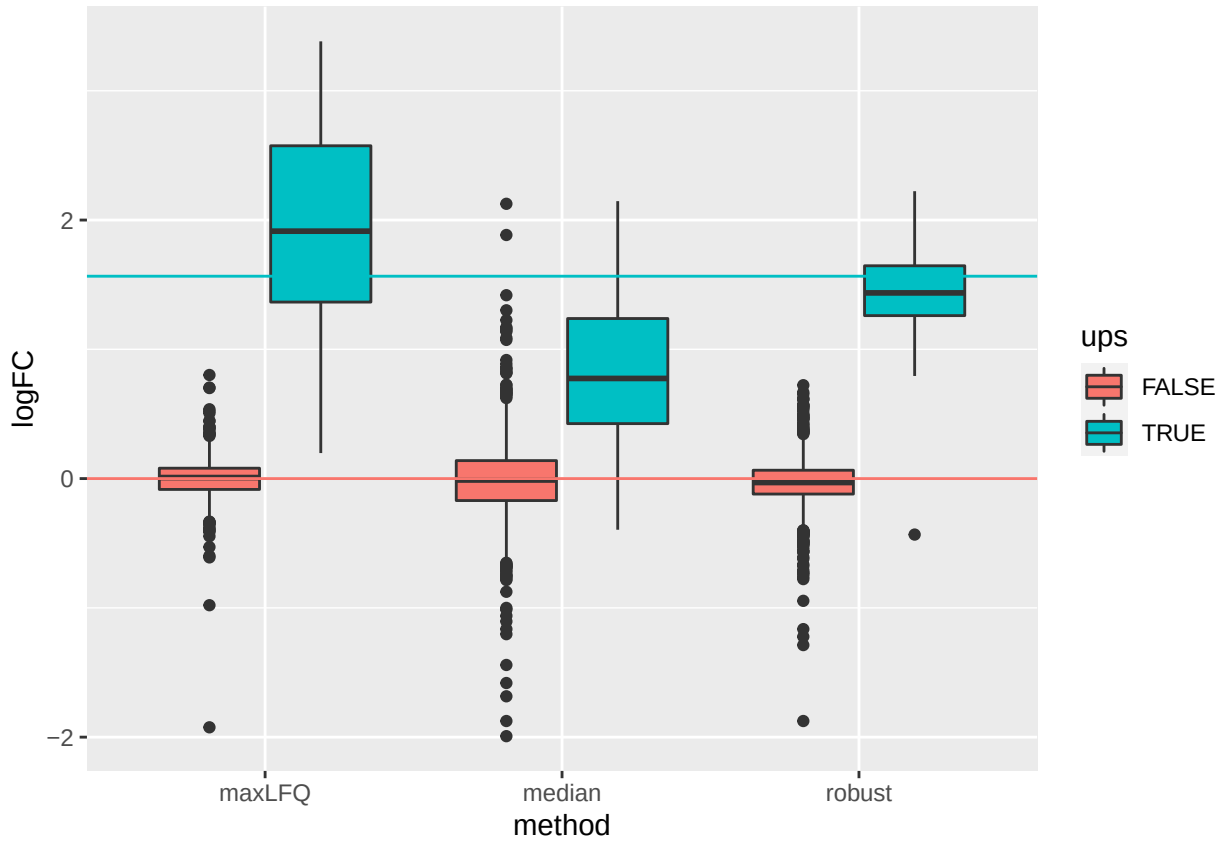
```
## Warning: Removed 746 rows containing missing values (geom_point).

## Warning: Removed 166 rows containing missing values (geom_point).

## Warning: Removed 167 rows containing missing values (geom_point).
```




- Robust summarization: highest power and still good FDR control: $FDP = \frac{1}{20} = 0.05$.

```
compBoxPlot
```

```
## Warning: Removed 1079 rows containing non-finite values (stat_boxplot).
```




- Median: biased logFC estimates for spike-in proteins
- maxLFQ: more variable logFC estiamtes for spike-in proteins

# 2 Full CPTAC study

## 2.1 Read data

Click to see background and code

1. We use a peptides.txt file from MS-data quantified with maxquant that contains MS1 intensities summarized at the peptide level.

```
peptidesFile <- "https://raw.githubusercontent.com/statOmics/PDA22GTPB/data/quantification/fullCptacData
```

2. Maxquant stores the intensity data for the different samples in columnns that start with Intensity. We can retreive the column names with the intensity data with the code below:

```
ecols <- grep("Intensity\\.", names(read.delim(peptidesFile)))
```

3. Read the data and store it in QFeatures object

```
pe <- readQFeatures(
  table = peptidesFile,
  fnames = 1,
  ecol = ecols,
  name = "peptideRaw", sep="\t")
```

## 2.2 Design

Click to see background and code

```
pe %>% colnames
```

```
## CharacterList of length 1
## [["peptideRaw"]] Intensity.6A_1 Intensity.6A_2 ... Intensity.6E_9
```

- Note, that the sample names include the spike-in condition.
- They also end on a number.
  - 1-3 is from lab 1,
  - 4-6 from lab 2 and
  - 7-9 from lab 3.
- We update the colData with information on the design

```
colData(pe)$lab <- rep(rep(paste0("lab",1:3),each=3),5) %>% as.factor
colData(pe)$condition <- pe[["peptideRaw"]] %>% colnames %>% substr(12,12) %>% as.factor
colData(pe)$spikeConcentration <- rep(c(A = 0.25, B = 0.74, C = 2.22, D = 6.67, E = 20),each = 9)
```

- We explore the colData

```
colData(pe)
```

```
## DataFrame with 45 rows and 3 columns
##                      lab condition spikeConcentration
##                 <factor>  <factor>          <numeric>
## Intensity.6A_1     lab1         A               0.25
## Intensity.6A_2     lab1         A               0.25
## Intensity.6A_3     lab1         A               0.25
## Intensity.6A_4     lab2         A               0.25
## Intensity.6A_5     lab2         A               0.25
## ...                 ...       ...                ...
## Intensity.6E_5     lab2         E                 20
## Intensity.6E_6     lab2         E                 20
## Intensity.6E_7     lab3         E                 20
## Intensity.6E_8     lab3         E                 20
## Intensity.6E_9     lab3         E                 20
```

## 2.3 Preprocessing

### 2.3.1 Log-transform

Click to see code to log-transfrom the data

- We calculate how many non zero intensities we have for each peptide and this can be useful for filtering.

```
rowData(pe[["peptideRaw"]])$nNonZero <- rowSums(assay(pe[["peptideRaw"]]) > 0)
```

- Peptides with zero intensities are missing peptides and should be represent with a `NA` value rather than `0`.

```
pe <- zeroIsNA(pe, "peptideRaw") # convert 0 to NA
```

- Logtransform data with base 2

```
pe <- logTransform(pe, base = 2, i = "peptideRaw", name = "peptideLog")
```

### 2.3.2 Filtering

Click to see code to filter the data

1. Handling overlapping protein groups

In our approach a peptide can map to multiple proteins, as long as there is none of these proteins present in a smaller subgroup.

```
pe <- filterFeatures(pe, ~ Proteins %in% smallestUniqueGroups(rowData(pe[["peptideLog"]])$Proteins))
```

2. Remove reverse sequences (decoys) and contaminants

We now remove the contaminants, peptides that map to decoy sequences, and proteins which were only identified by peptides with modifications.

```
pe <- filterFeatures(pe,~Reverse != "+")
pe <- filterFeatures(pe,~ Potential.contaminant != "+")
```

3. Drop peptides that were only identified in one sample

We keep peptides that were observed at last twice.

```
pe <- filterFeatures(pe,~ nNonZero >=2)
nrow(pe[["peptideLog"]])
```

```
## [1] 10478
```

We keep 10478 peptides upon filtering.

### 2.4 Normalization

Click to see R-code to normalize the data

```
pe <- normalize(pe,
                i = "peptideLog",
                name = "peptideNorm",
                method = "center.median")
```

---

# 3 Peptide-level models

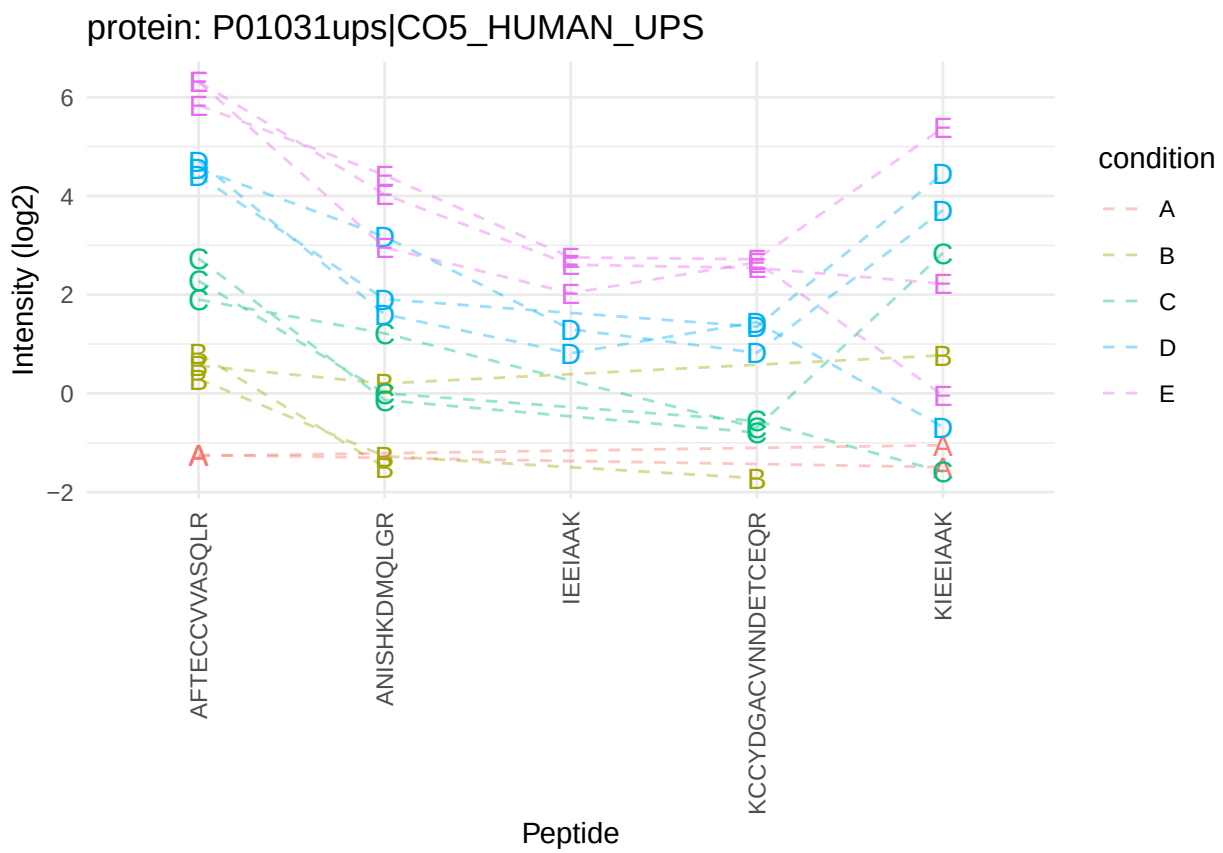
### 3.1 Summarization

Click to see code to make plot

```
prot <- "P01031ups|CO5_HUMAN_UPS"
data <- pe[["peptideNorm"]][
  rowData(pe[["peptideNorm"]])$Proteins == prot,
  colData(pe)$lab=="lab3"] %>%
  assay %>%
  as.data.frame %>%
  rownames_to_column(var = "peptide") %>%
  gather(sample, intensity, -peptide) %>%
  mutate(condition = colData(pe)[sample,"condition"]) %>%
  na.exclude
sumPlot <- data %>%
  ggplot(aes(x = peptide, y = intensity, color = condition, group = sample, label = condition), show.leg
  geom_text(show.legend = FALSE) +
  theme_minimal() +
  theme(axis.text.x = element_text(angle = 90, vjust = 0.5, hjust = 1)) +
  xlab("Peptide") +
  ylab("Intensity (log2)") +
  ggtitle(paste0("protein: ",prot))
```

Here, we will focus on the summarization of the intensities for protein P01031ups|CO5_HUMAN_UPS.

```
sumPlot +
  geom_line(linetype="dashed",alpha=.4)
```




### 3.1.1 Median summarization

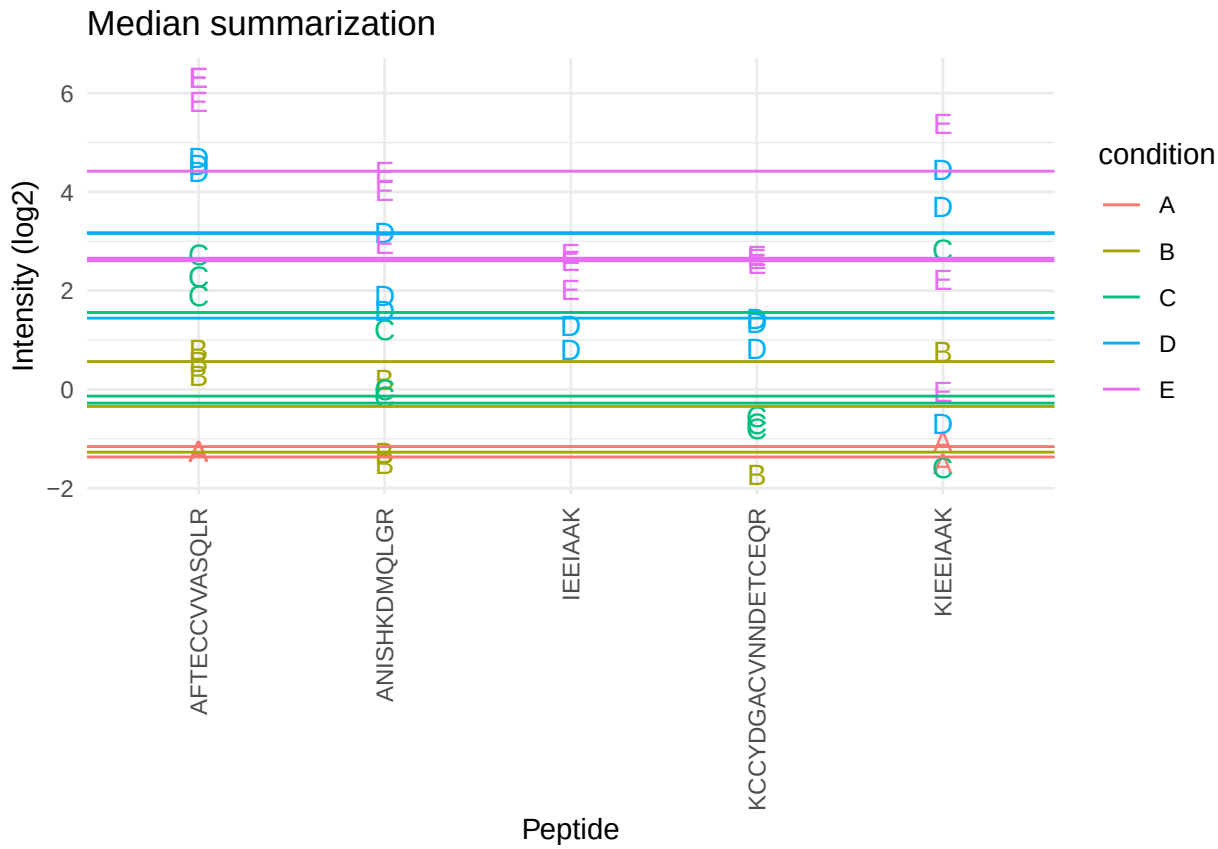
We first evaluate median summarization for protein P01031ups|CO5_HUMAN_UPS.

Click to see code to make plot

```
dataHlp <- pe[["peptideNorm"]][
    rowData(pe[["peptideNorm"]])$Proteins == prot,
    colData(pe)$lab=="lab3"] %>% assay

sumMedian <- data.frame(
  intensity= dataHlp
    %>% colMedians(na.rm=TRUE)
  ,
  condition= colnames(dataHlp) %>% substr(12,12) %>% as.factor )

sumMedianPlot <- sumPlot +
  geom_hline(
    data = sumMedian,
    mapping = aes(yintercept=intensity,color=condition)) +
  ggtitle("Median summarization")
```

```
sumMedianPlot
```

```
## Warning: Removed 1 rows containing missing values (geom_hline).
```




- The sample medians are not a good estimate for the protein expression value.
- Indeed, they do not account for differences in peptide effects
- Peptides that ionize poorly are also picked up in samples with high spike-in concencentration and not in samples with low spike-in concentration
- This introduces a bias.

### 3.1.2 Mean summarization

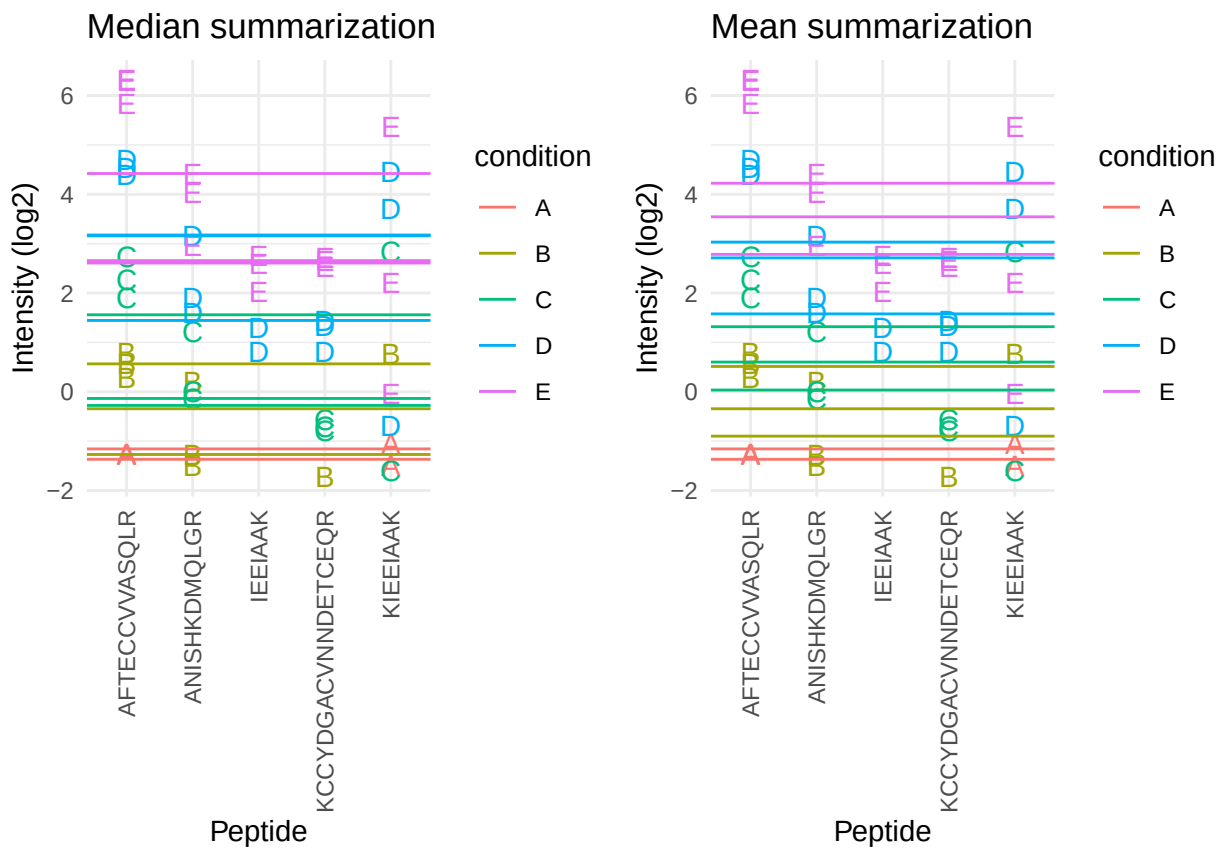
$$y_{ip} = \beta_i^{\text{sample}} + \epsilon_{ip}$$

Click to see code to make plot

```
sumMeanMod <- lm(intensity ~ -1 + sample,data)

sumMean <- data.frame(
  intensity=sumMeanMod$coef[grep("sample",names(sumMeanMod$coef))],
  condition= names(sumMeanMod$coef)[grep("sample",names(sumMeanMod$coef))] %>% substr(18,18) %>% as.fac



sumMeanPlot <- sumPlot + geom_hline(
    data = sumMean,
    mapping = aes(yintercept=intensity,color=condition)) +
    ggtitle("Mean summarization")
```

```
grid.arrange(sumMedianPlot, sumMeanPlot, ncol=2)
```

```
## Warning: Removed 1 rows containing missing values (geom_hline).
```




### 3.1.3 Model based summarization

We can use a linear peptide-level model to estimate the protein expression value while correcting for the peptide effect, i.e.

$$y_{ip} = \beta_i^{\text{sample}} + \beta_p^{peptide} + \epsilon_{ip}$$

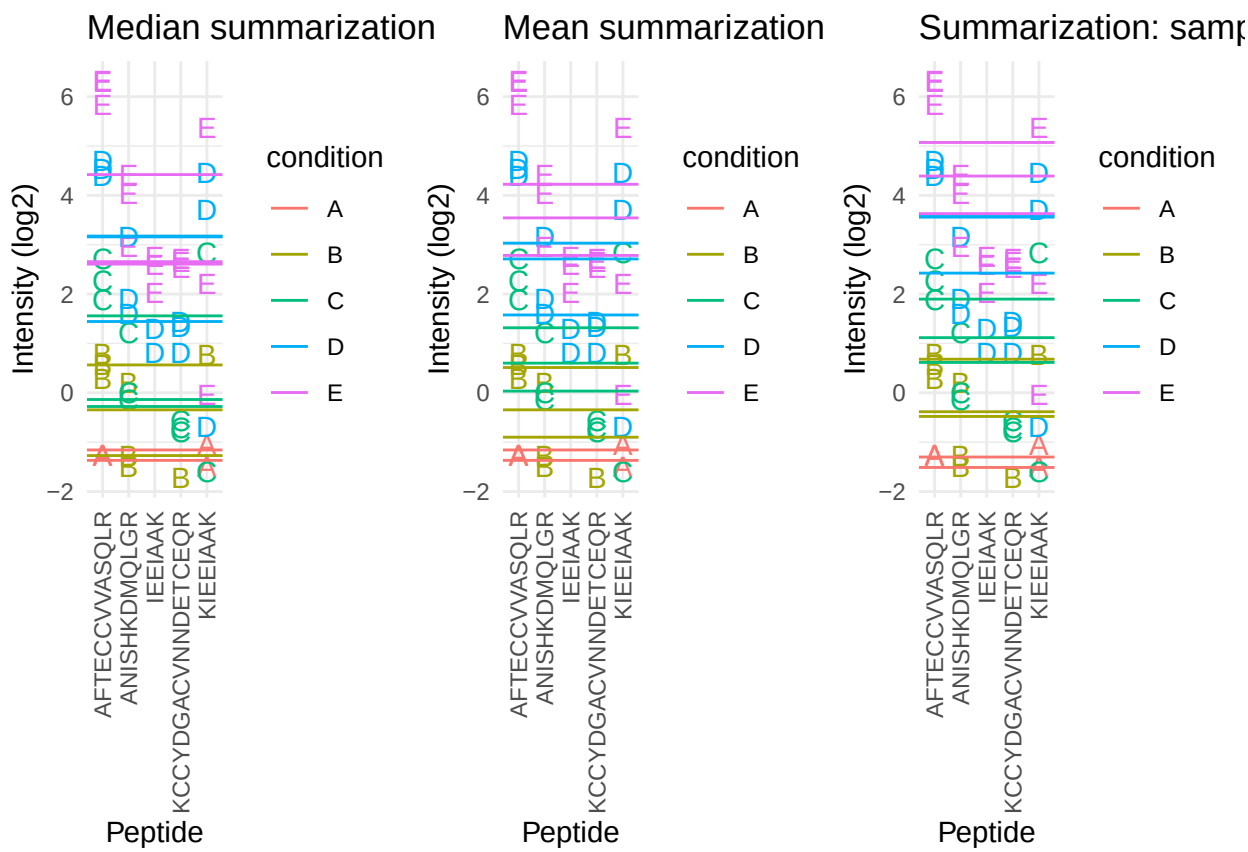
Click to see code to make plot

```
sumMeanPepMod <- lm(intensity ~ -1 + sample + peptide,data)

sumMeanPep <- data.frame(
  intensity=sumMeanPepMod$coef[grep("sample",names(sumMeanPepMod$coef))] + mean(data$intensity) - mean(s
  condition= names(sumMeanPepMod$coef)[grep("sample",names(sumMeanPepMod$coef))] %>% substr(18,18) %>% a


fitLmPlot <-  sumPlot + geom_line(
    data = data %>% mutate(fit=sumMeanPepMod$fitted.values),
    mapping = aes(x=peptide, y=fit,color=condition, group=sample)) +
```

```
    ggtitle("fit: ~ sample + peptide")
sumLmPlot <- sumPlot + geom_hline(
    data = sumMeanPep,
    mapping = aes(yintercept=intensity,color=condition)) +
    ggtitle("Summarization: sample effect")
```

```
grid.arrange(sumMedianPlot, sumMeanPlot, sumLmPlot, nrow=1)
```

```
## Warning: Removed 1 rows containing missing values (geom_hline).
```



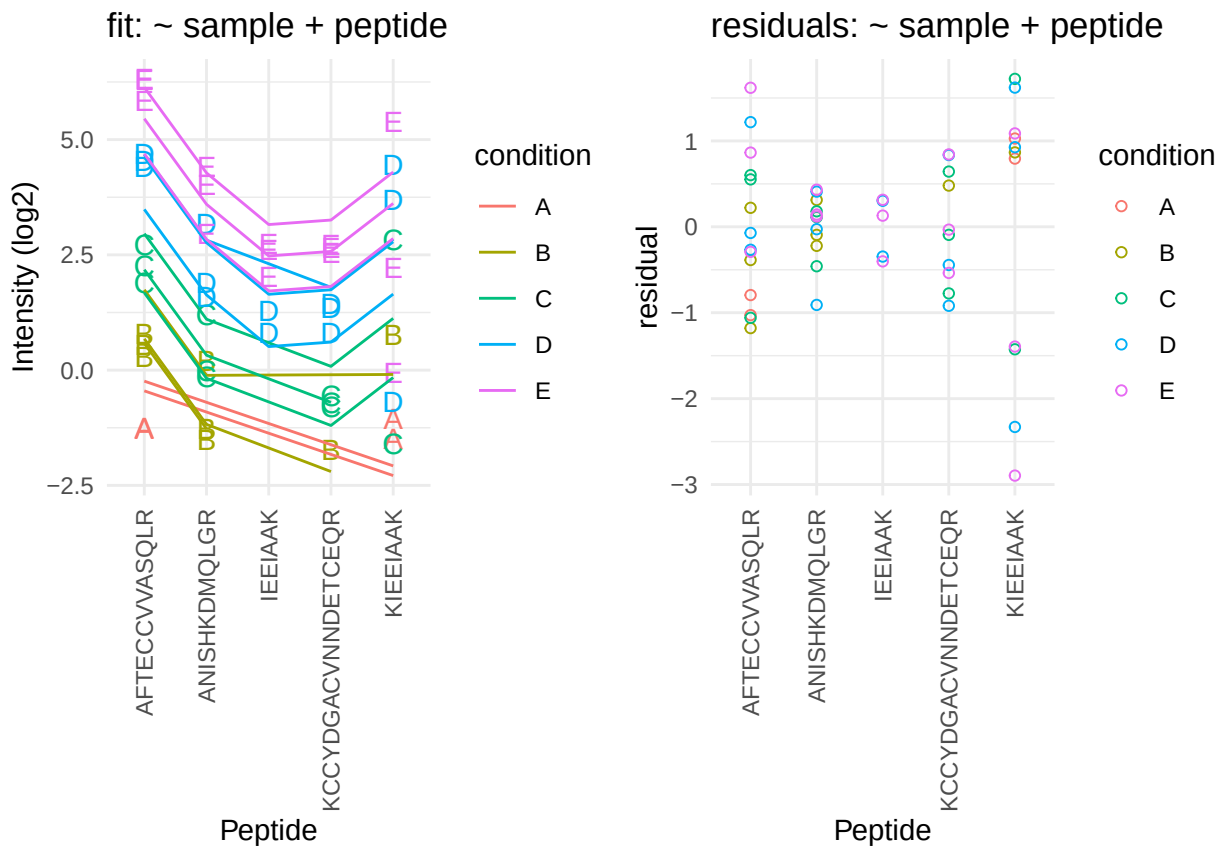

- By correcting for the peptide species the protein expression values are much better separated an better reflect differences in abundance induced by the spike-in condition.
- Indeed, it shows that median and mean summarization that do not account for the peptide effect indeed overestimate the protein expression value in the small spike-in conditions and underestimate that in the large spike-in conditions.
- Still there seem to be some issues with samples that for which the expression values are not well separated according to the spike-in condition.

A residual analysis clearly indicates potential issues:

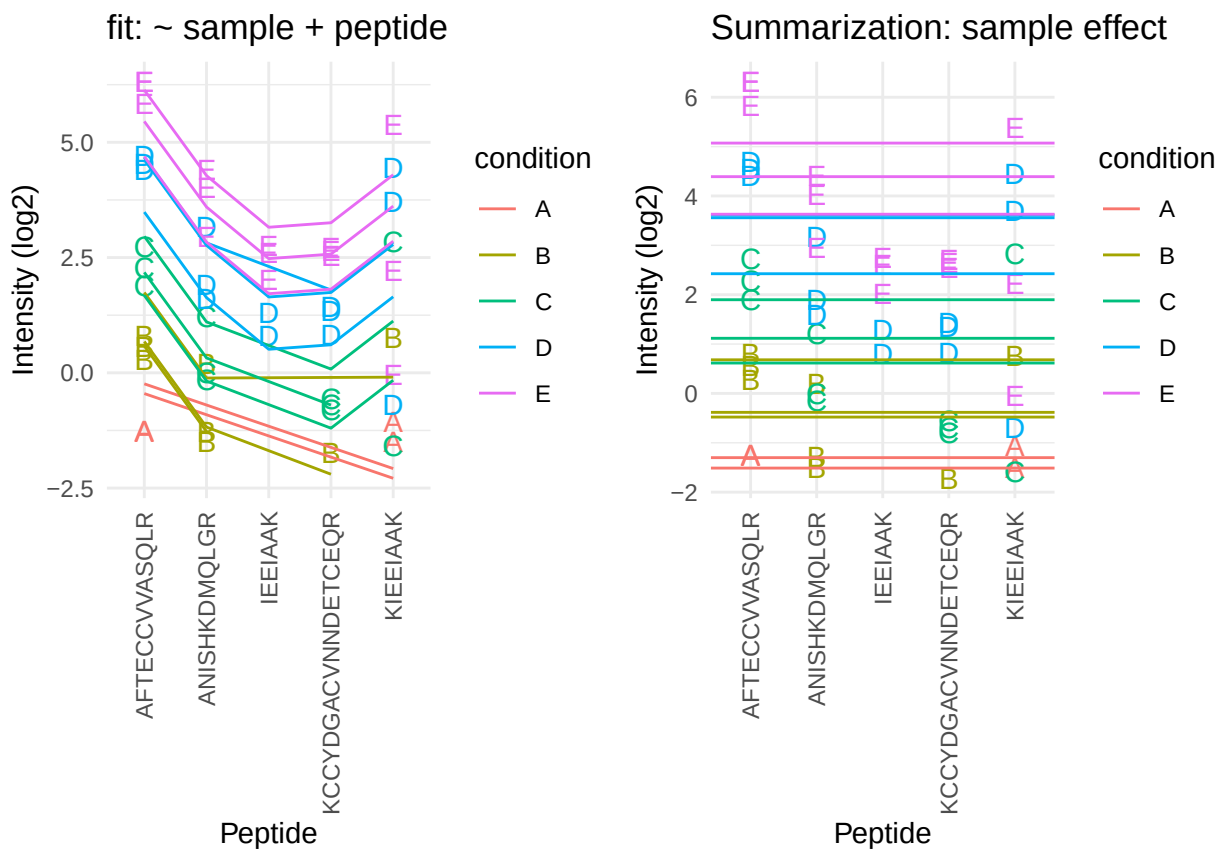
Click to see code to make plot

```
resPlot <- data %>%
  mutate(res=sumMeanPepMod$residuals) %>%
  ggplot(aes(x = peptide, y = res, color = condition, label = condition), show.legend = FALSE) +
  geom_point(shape=21) +
  theme_minimal() +
  theme(axis.text.x = element_text(angle = 90, vjust = 0.5, hjust = 1)) +
  xlab("Peptide") +
  ylab("residual") +
  ggtitle("residuals: ~ sample + peptide")
```

```
grid.arrange(fitLmPlot, resPlot, nrow = 1)
```




```
grid.arrange(fitLmPlot, sumLmPlot, nrow = 1)
```




- The residual plot shows some large outliers for peptide KIEEIAAK.
- Indeed, in the original plot the intensities for this peptide do not seem to line up very well with the concentration.
- This induces a bias in the summarization for some of the samples (e.g. for D and E)

### 3.1.4 Robust summarization using a peptide-level linear model

$$y_{ip} = \beta_i^{\text{sample}} + \beta_p^{peptide} + \epsilon_{ip}$$

- Ordinary least squares: estimate $\beta$ that minimizes

$$\text{OLS} : \sum_{i,p} \epsilon_{ip}^2 = \sum_{i,p} (y_{ip} - \beta_i^{\text{sample}} - \beta_p^{\text{peptide}})^2$$

We replace OLS by M-estimation with loss function

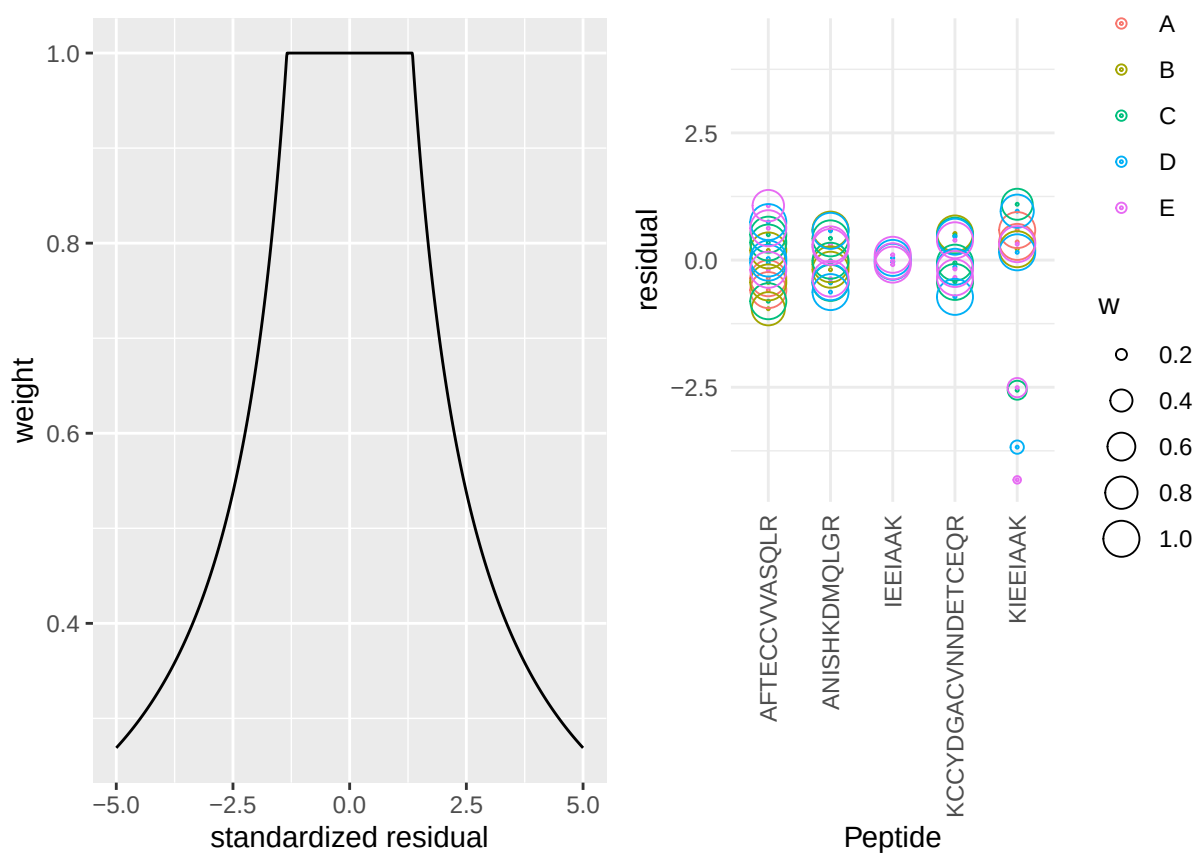
$$\sum_{i,p} w_{ip} \epsilon_{ip}^2 = \sum_{i,p} w_{ip} (y_{ip} - \beta_i^{\text{sample}} - \beta_p^{\text{peptide}})^2$$

- Iteratively fit model with observation weights $w_{ip}$ until convergence
- The weights are calculated based on standardized residuals

Click to see code to make plot

```
sumMeanPepRobMod <- MASS::rlm(intensity ~ -1 + sample + peptide,data)
resRobPlot <- data %>%
  mutate(res = sumMeanPepRobMod$residuals,
         w = sumMeanPepRobMod$w) %>%
  ggplot(aes(x = peptide, y = res, color = condition, label = condition,size=w), show.legend = FALSE) +
  geom_point(shape=21,size=.2) +
  geom_point(shape=21) +
  theme_minimal() +
  theme(axis.text.x = element_text(angle = 90, vjust = 0.5, hjust = 1)) +
  xlab("Peptide") +
  ylab("residual") +
  ylim(c(-1,1)*max(abs(sumMeanPepRobMod$residuals)))
weightPlot <- qplot(
  seq(-5,5,.01),
  MASS::psi.huber(seq(-5,5,.01)),
  geom="path") +
  xlab("standardized residual") +
  ylab("weight")
```

```
grid.arrange(weightPlot,resRobPlot,nrow=1)
```

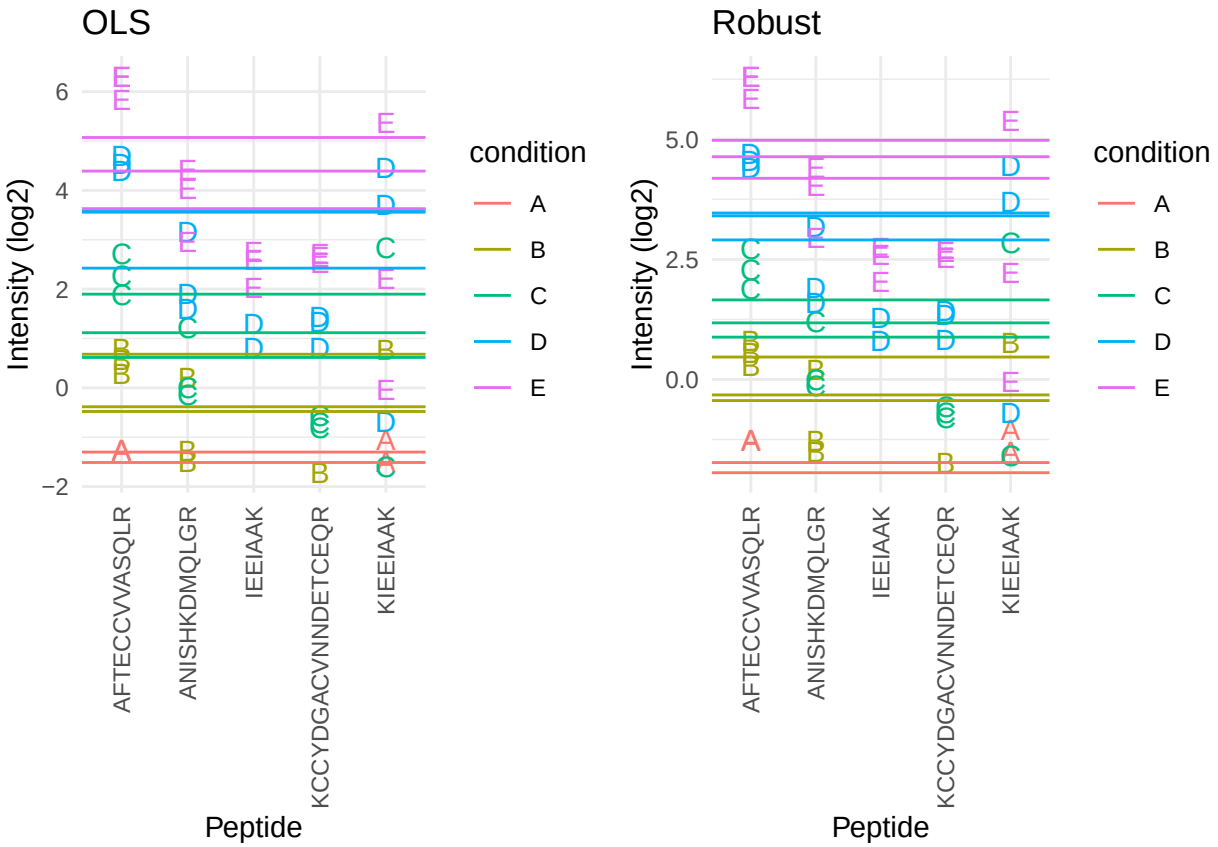



- We clearly see that the weights in the M-estimation procedure will down-weight errors associated with outliers for peptide KIEEIAAK.

Click to see code to make plot

```
sumMeanPepRob <- data.frame(
  intensity=sumMeanPepRobMod$coef[grep("sample",names(sumMeanPepRobMod$coef))] + mean(data$intensity) -
  condition= names(sumMeanPepRobMod$coef)[grep("sample",names(sumMeanPepRobMod$coef))] %>% substr(18,18

sumRlmPlot <- sumPlot + geom_hline(
    data=sumMeanPepRob,
    mapping=aes(yintercept=intensity,color=condition)) +
    ggtitle("Robust")
```

```
grid.arrange(sumLmPlot + ggtitle("OLS"), sumRlmPlot, nrow = 1)
```




- Robust regresion results in a better separation between the protein expression values for the different samples according to their spike-in concentration.

### 3.1.5 Comparison summarization methods

- maxLFQ

**a**

>P63208
MPSIK**LQSSDGEIFEVDVEIAK**QSVTIKTMLEDLGMDDEGDD
DPVPLPNVNAAILKKVIQWCTHHKDDPPPPEDDENKEK**RTDD**
**IPVWDQEFLK**VDQGTLFELILAANYLDIKGLLDVTCK**TVANM**
**IK**GK**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 | | + | | | + | | |

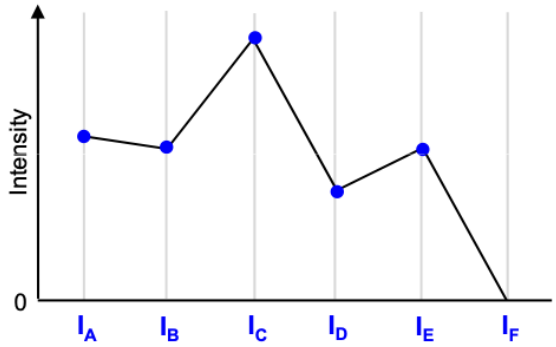
**d**

| | 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 | $r_{FA}$ | $r_{FB}$ | $r_{FC}$ | $r_{FD}$ | $r_{FE}$ | |

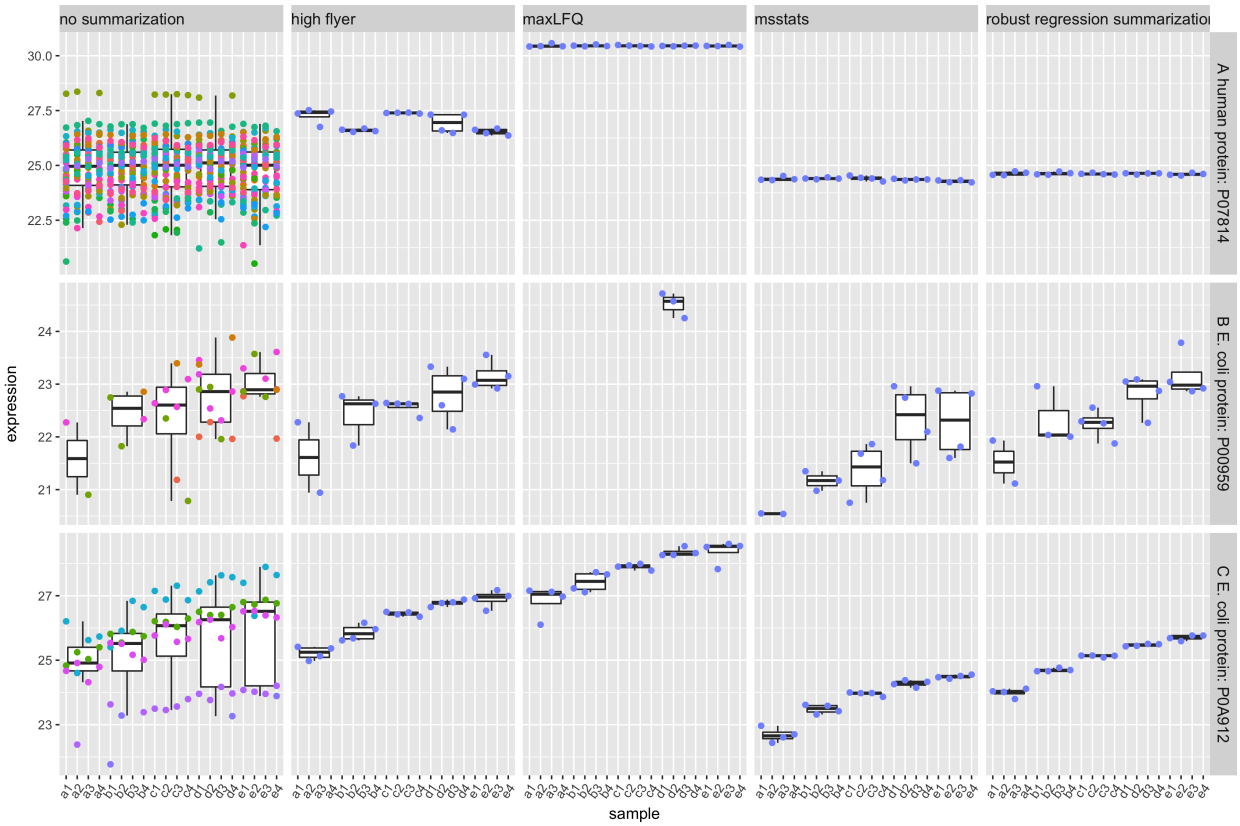
**e**

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

**f**




- MS-stats also uses a robust peptide level model to perform the summarization, however, they typically first impute missing values
- Proteus high-flyer method: mean of three peptides with highest intensity




- (Sticker et al. 2020)
- doi: https://doi.org/10.1074/mcp.RA119.001624

- pdf

## 3.2 Estimation of differential abundance using peptide level model

- Instead of summarising the data we can also directly model the data at the peptide-level.
- But, we will have to address the pseudo-replication.

$$y_{iclp} = \beta_0 + \beta_c^{\text{condition}} + \beta_l^{\text{lab}} + \beta_p^{\text{peptide}} + u_s^{\text{sample}} + \epsilon_{iclp}$$

- protein-level
  - $\beta_c^{\text{condition}}$: spike-in condition $c = b, \ldots, e$
  - $\beta_l^{\text{lab}}$: lab effect $l = l_2 \ldots l_3$
  - $u_r^{\text{run}} \sim N\left(0, \sigma_{\text{run}}^2\right) \rightarrow$ random effect addresses pseudo-replication
- peptide-level
  - $\beta_p^{\text{peptide}}$: peptide effect
  - $\epsilon_{rp} \sim N\left(0, \sigma_\epsilon^2\right)$ within sample (run) error
- DA estimates:

$$\log_2 FC_{B-A} = \beta_B^{\text{condition}}$$
$$\log_2 FC_{C-B} = \beta_C^{\text{condition}} - \beta_B^{\text{condition}}$$

- Mixed peptide-level models are implemented in msqrob2
- It has the advantages that
    1. it correctly addresses the difference levels of variability in the data
    2. it avoids summarization and therefore also accounts for the difference in the number of peptides that are observed in each sample
    3. more powerful analysis
- It has the disadvantage that
    1. protein summaries are no longer available for plotting
    2. it is difficult to correctly specify the degrees of freedom for the test-statistic leading to inference that is too liberal in experiments with small sample size
    3. sometimes sample level random effect variance are estimated to be zero, then the pseudo-replication is not addressed leading to inference that is too liberal for these specific proteins
    4. they are much more difficult to disseminate to users with limited background in statistics

Hence, for this course we opted to use peptide-level models for summarization, but not for directly inferring on the differential expression at the protein-level.

# References

Sticker, A., L. Goeminne, L. Martens, and L. Clement. 2020. "Robust Summarization and Inference in Proteome-wide Label-free Quantification." *Mol Cell Proteomics* 19 (7): 1209–19.