Using R and Bioconductor for proteomics data analysis.

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Abstract

This vignette shows and executes the code presented in the manuscript *Using R for proteomics data analysis*.

Keywords: bioinformatics, proteomics, mass spectrometry, tutorial

1 Introduction

1.1 General R ressources

The reader is expected to have basic R knowledge to find to document helpful. There are numerous R introductions freely available, some of which are listed below.

From the R project web-page:

- **An Introduction to R** is based on the former *Notes on R*, gives an introduction to the language and how to use R for doing statistical analysis and graphics. [browse HTML | download PDF]
- Several introductionary tutorials in the contributed documentation section.

1.2 Getting help

R has several mailing lists[1]. The most relevant here being the main R-help list, *for discussion about problem and solutions using R* . This one is for general R content and is not suitable for bioinformatics or proteomics questions.

Bioconductor also offers several mailing lists[2] dedicated to bioinformatics matters and Bioconductor packages. The main bioconductor list is the most relevant one. It is possible to post[3] questions without subscribing to the list.

It is important to read and comply to the posting guides (here and here) to maximise the chances to obtain good responses. It is important to specify the software versions using the sessionInfo() functions (see an example output at the end of this docuement, on page 29). It the question involves some code, make sure to isolate the relevant portion and filed it with your question and try to make your code/example reproducible[4].

All lists have browsable archives.

1.3 Installation

Since the package is not yet in Bioconductor, it is not yet possible to use its automatice installation and update framework. An ad hoc script is however available to facilitate installation of RforProteomics and all dependencies. Simply open R and type

```
source("http://proteome.sysbiol.cam.ac.uk/lgatto/RforProteomics/installR4P.R")
```

The script installs missing dependencies and then RforProteomics, which can then be loaded with

```
library("RforProteomics")

## This is the 'RforProteomics' version 0.2.2.
## Run 'RforProtemics()' in R or visit
## 'http://lgatto.github.com/RforProteomics/' to get started.
```

1.4 Obtaining the code

The code in this document describes all the examples presented in [1] and can be copy, pasted and executed. It is however more convenient to have it in a separate text file for better interaction with R (using ESS[5] for emacs or RStudio[6]) and to

[1] http://www.r-project.org/mail.html
[2] http://bioconductor.org/help/mailing-list/
[3] http://bioconductor.org/help/mailing-list/mailform/
[4] https://github.com/hadley/devtools/wiki/Reproducibility
[5] http://ess.r-project.org/
[6] http://rstudio.org/

easily modify and explore it. This can be achieved with the `Stangle` function. It only need the Sweave source of this document, extracts the code chunks and produces a clean `R` source file. If the package is installed, the following code chunk will create a `RforProteomics.R` file in your working directory containing all the annotated source code contained in this document.

```
## gets the vignette source
rnwfile <- dir(system.file(package = "RforProteomics", dir = "doc/vigsrc/"),
    full.name = TRUE, pattern = "RforProteomics.Rnw")
## produces an R file in the working directory
Stangle(rnwfile)

## Writing to file RforProteomics.R

dir(pattern = "RforProteomics.R$")

## [1] "RforProteomics.R"
```

1.5 Prepare the working environment

The packages that we will depend on to execture the examples will be loaded in the respective sections. Here, we pre-load packages that provide general functionality used throughout the document.

```
library("RColorBrewer")  ## Color palettes
library("ggplot2")  ## Convenient and nice plotting
library("reshape2")  ## Flexibly reshape data
```

2 Data standards and input/ouput

2.1 The mzR package

This code chunk, taken mainly from the `openMSfile` documentation illustrated how to open a connection to an raw data file. The example `mzML` data is taken from the `msdata` data package. The code below would be applicatble to an `mzXML` of `mzData` file.

```
## load the required packages
library("mzR")  ## the software package
library("msdata")  ## the data package
## below, we extract the releavant example file from the local 'msdata'
## installation
filepath <- system.file("microtofq", package = "msdata")
```

```
file <- list.files(filepath, pattern = "MM14.mzML", full.names = TRUE, recursive = T
## creates a commection to the mzML file
mz <- openMSfile(file)
## demonstraction of data access
fileName(mz)
```

```
## [1] "/home/lgatto/R/x86_64-unknown-linux-gnu-library/2.16/msdata/microtofq/MM14.m
```

```
isInitialized(mz)
```

```
## [1] TRUE
```

```
runInfo(mz)
```

```
## $scanCount
## [1] 112
##
## $lowMz
## [1] 0
##
## $highMz
## [1] 0
##
## $dStartTime
## [1] 270.3
##
## $dEndTime
## [1] 307.7
##
## $msLevels
## [1] 1
```

```
instrumentInfo(mz)
```

```
## $manufacturer
## [1] "Unknown"
##
## $model
## [1] "instrument model"
##
## $ionisation
## [1] "electrospray ionization"
##
## $analyzer
## [1] "mass analyzer type"
##
## $detector
## [1] "detector type"
```

```
## once finished, it is good to explicitely close the connection
close(mz)
```

3 Raw data abstraction with MSnExp objects

```
library("MSnbase")
mzXML <- dir(system.file(package = "MSnbase", dir = "extdata"), full.name = TRUE,
    pattern = "mzXML$")
raw <- readMSData(mzXML, verbose = FALSE)
raw

## Object of class "MSnExp"
##  Object size in memory: 0.2 Mb
## - - - Spectra data - - -
##  MS level(s): 2
##  Number of MS1 acquisitions: 1
##  Number of MSn scans: 5
##  Number of precursor ions: 5
##  4 unique MZs
##  Precursor MZ's: 437.8 - 716.34
##  MSn M/Z range: 100 2017
##  MSn retention times: 25:1 - 25:2 minutes
## - - - Processing information - - -
## Data loaded: Tue Oct 23 16:34:02 2012
##  MSnbase version: 1.7.2
## - - - Meta data  - - -
## phenoData
##   rowNames: 1
##   varLabels: sampleNames fileNumbers
##   varMetadata: labelDescription
## Loaded from:
##   dummyiTRAQ.mzXML
## protocolData: none
## featureData
##   featureNames: X1.1 X2.1 ... X5.1 (5 total)
##   fvarLabels: spectrum
##   fvarMetadata: labelDescription
## experimentData: use 'experimentData(object)'

## Extract a sigle spectrum
raw[[3]]
```

```
## Object of class "Spectrum2"
##  Precursor: 645.4
##  Retention time: 25:2
##  Charge: 2
##  MSn level: 2
##  Peaks count: 2125
##  Total ion count: 150838188
```

3.1 mgf read/write support

See `readMgfData` and `writeMgfData` in MSnbase.

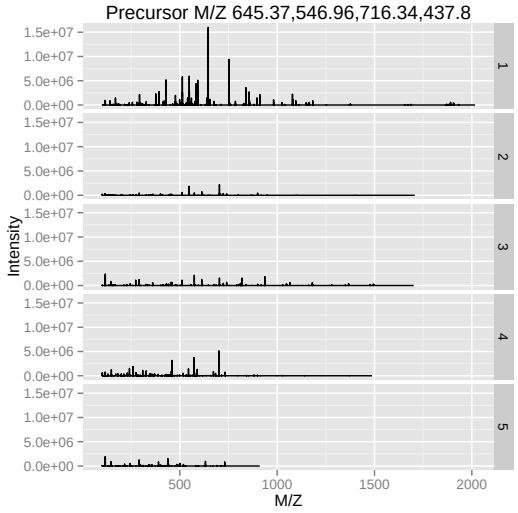
4 Quantitative proteomics

As an running example throughout this document, we will use the `PXD000001` data set. The code chunk below first downloads this data file from the ProteomeXchange server using the `getPXD000001mzXML` function from the RforProteomics package.

4.1 The mzTab format

```
mztab <- getPXD000001mzTab()
mztab  ## the mzTab file name

## [1] "./F063721.dat-mztab.txt"

## Load mzTabs's peptide data
qnt <- readMzTabData(mztab, what = "PEP")

## Detected a metadata section
## Detected a peptide section

sampleNames(qnt) <- reporterNames(TMT6)
head(exprs(qnt))

##   TMT6.126 TMT6.127 TMT6.128 TMT6.129 TMT6.130 TMT6.131
## 1 10630132 11238708 12424917 10997763  9928972 10398534
## 2 11105690 12403253 13160903 12229367 11061660 10131218
## 3  1183431  1322371  1599088  1243715  1306602  1159064
## 4  5384958  5508454  6883086  6136023  5626680  5213771
## 5 18033537 17926487 21052620 19810368 17381162 17268329
## 6  9873585 10299931 11142071 10258214  9664315  9518271
```

```
plot(raw, full = TRUE)
```




```
plot(raw[[3]], full = TRUE, reporters = iTRAQ4)
```

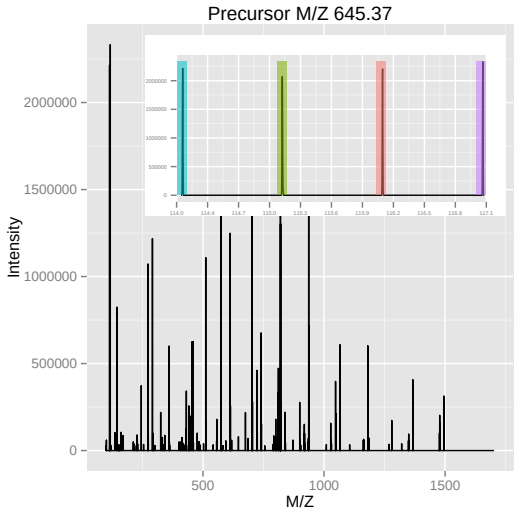


Figure 1: The `plot` method can be used on experiments, i.e. spectrum collections (left), or individual spectra (right).

```
## combine into proteins - usin the 'accession' feature meta data - sum
## the peptide intensities
protqnt <- combineFeatures(qnt, groupBy = fData(qnt)$accession, fun = sum)

## Combined 1528 features into 404 using user-defined function
```

```
cls <- brewer.pal(5, "Set1")
matplot(t(tail(exprs(protqnt), n = 5)), type = "b", lty = 1, col = cls, ylab = "Prot
    xlab = "TMT reporters")
legend("topright", tail(featureNames(protqnt), n = 5), lty = 1, bty = "n", cex = 0.8
    col = cls)
```

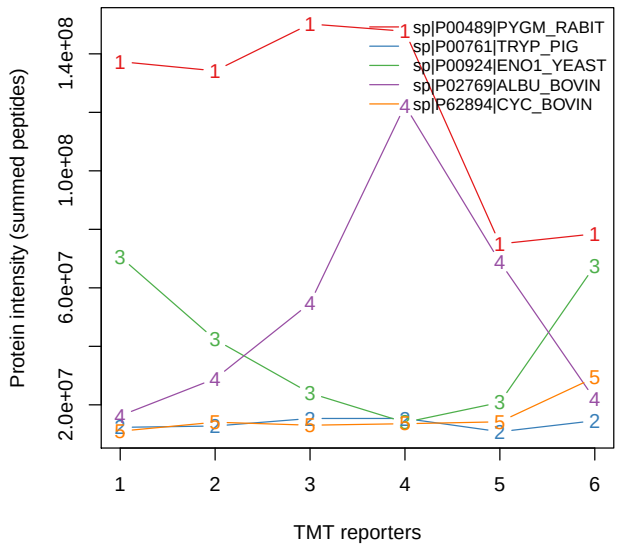


Figure 2: Protein quantitation data.

```
qntS <- normalise(qnt, "sum")
qntV <- normalise(qntS, "vsn")
qntV2 <- normalise(qnt, "vsn")

acc <- c("P00489", "P00924", "P02769", "P62894", "ECA")

idx <- sapply(acc, grep, fData(qnt)$accession)
idx2 <- sapply(idx, head, 3)
small <- qntS[unlist(idx2), ]

idx3 <- sapply(idx, head, 10)
medium <- qntV[unlist(idx3), ]

m <- exprs(medium)
colnames(m) <- c("126", "127", "128", "129", "130", "131")
rownames(m) <- fData(medium)$accession
rownames(m)[grep("CYC", rownames(m))] <- "CYT"
rownames(m)[grep("ENO", rownames(m))] <- "ENO"
```

```
rownames(m)[grep("ALB", rownames(m))] <- "BSA"
rownames(m)[grep("PYGM", rownames(m))] <- "PHO"
rownames(m)[grep("ECA", rownames(m))] <- "Background"

cls <- c(brewer.pal(length(unique(rownames(m))) - 1, "Set1"), "grey")
names(cls) <- unique(rownames(m))
wbcol <- colorRampPalette(c("white", "darkblue"))(256)
```

```
heatmap(m, col = wbcol, RowSideColors = cls[rownames(m)])
```

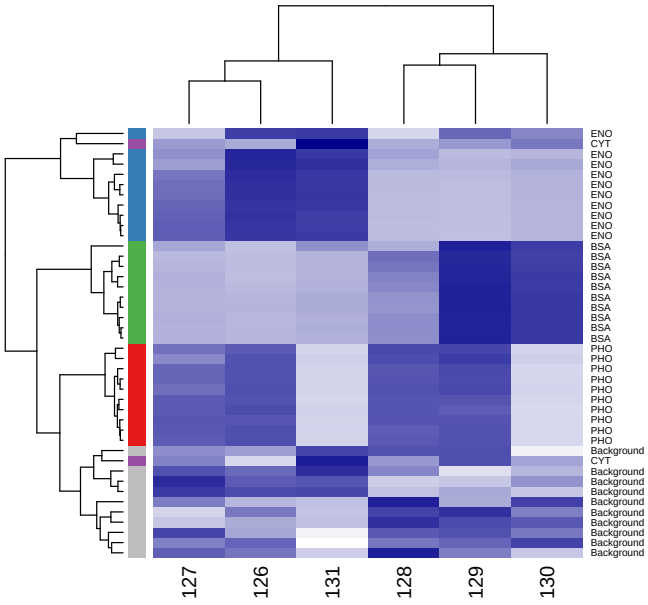


Figure 3: A heatmap.

```
dfr <- data.frame(exprs(small), Protein = as.character(fData(small)$accession),
    Feature = featureNames(small), stringsAsFactors = FALSE)
colnames(dfr) <- c("126", "127", "128", "129", "130", "131", "Protein", "Feature")
dfr$Protein[dfr$Protein == "sp|P00924|ENO1_YEAST"] <- "ENO"
dfr$Protein[dfr$Protein == "sp|P62894|CYC_BOVIN"] <- "CYT"
dfr$Protein[dfr$Protein == "sp|P02769|ALBU_BOVIN"] <- "BSA"
dfr$Protein[dfr$Protein == "sp|P00489|PYGM_RABIT"] <- "PHO"
dfr$Protein[grep("ECA", dfr$Protein)] <- "Background"
dfr2 <- melt(dfr)

## Using Protein, Feature as id variables

ggplot(aes(x = variable, y = value, colour = Protein), data = dfr2) + geom_point() +
    geom_line(aes(group = as.factor(Feature)), alpha = 0.5) + facet_grid(. ~
    Protein) + theme(legend.position = "none") + labs(x = "Reporters", y = "Normalis
```

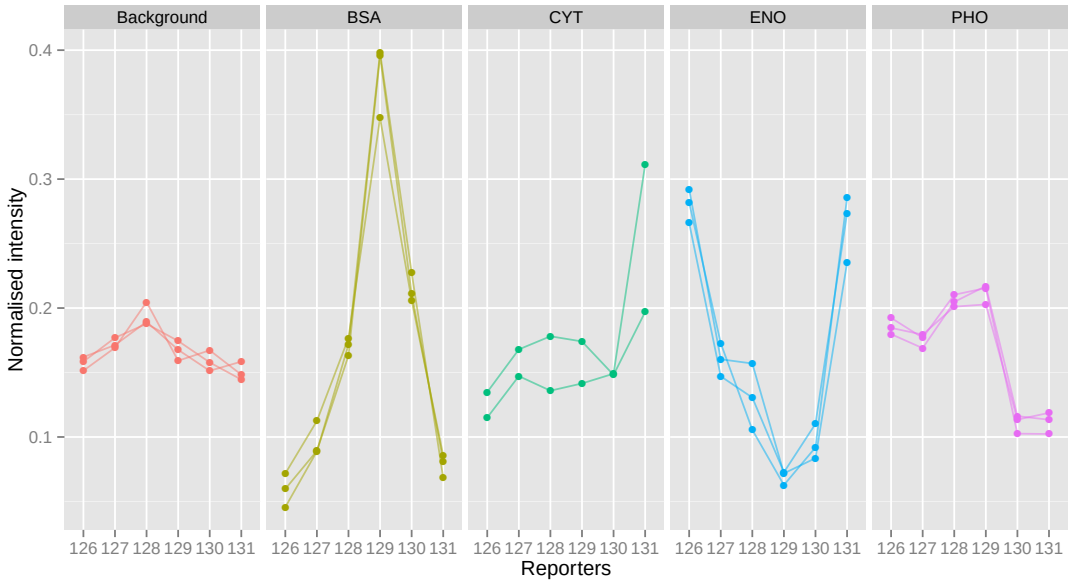


Figure 4: Spikes plot using ggplot2.

4.2 Working with raw data

```
mzxml <- getPXD000001mzXML()
rawms <- readMSData(mzxml, centroided = TRUE, verbose = FALSE)
qntms <- quantify(rawms, reporters = TMT7, method = "max", verbose = FALSE,
    parallel = TRUE)

## Loading required package:  foreach
## Loading required package:  doMC
## Loading required package:  iterators
## Loading required package:  multicore
##
## Attaching package:  'multicore'
## The following object is masked from 'package:lattice':
##
## parallel
## The following object is masked from 'package:parallel':
##
## mclapply, mcparallel, pvec


d <- data.frame(Signal = rowSums(exprs(qntms)[, 1:6]), Incomplete = exprs(qntms)[,
    7])
d <- log(d)
cls <- rep("#00000050", nrow(qnt))
pch <- rep(1, nrow(qnt))
cls[grep("P02769", fData(qnt)$accession)] <- "gold4"  ## BSA
cls[grep("P00924", fData(qnt)$accession)] <- "dodgerblue"  ## ENO
cls[grep("P62894", fData(qnt)$accession)] <- "springgreen4"  ## CYT
cls[grep("P00489", fData(qnt)$accession)] <- "darkorchid2"  ## PHO
pch[grep("P02769", fData(qnt)$accession)] <- 19
pch[grep("P00924", fData(qnt)$accession)] <- 19
pch[grep("P62894", fData(qnt)$accession)] <- 19
pch[grep("P00489", fData(qnt)$accession)] <- 19
```

```
mzp <- plotMzDelta(rawms, reporters = TMT6, verbose = FALSE) + ggtitle("")

## Scale for 'x' is already present.  Adding another scale for 'x', which
## will replace the existing scale.
## Warning:  Removed 2 rows containing missing values (geom_text).
```

```
mzp
```

```
## Warning:  Removed 2 rows containing missing values (geom_text).
```

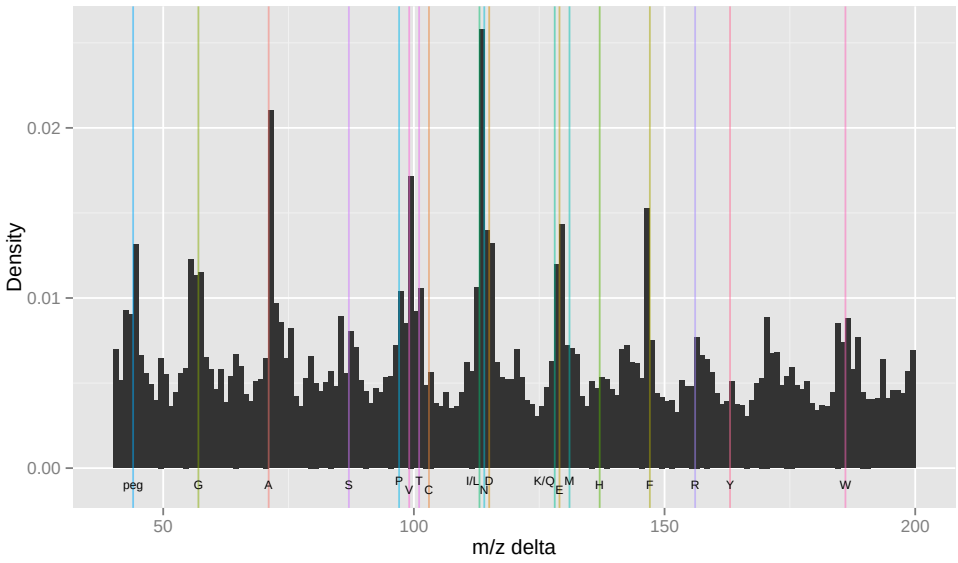


Figure 5: A m/z delta plot.

```
plot(Signal ~ Incomplete, data = d, xlab = expression(Incomplete ~ dissociation),
    ylab = expression(Sum ~ of ~ reporters ~ intensities), pch = 19, col = "#4582B38
grid()
abline(0, 1, lty = "dotted")
abline(lm(Signal ~ Incomplete, data = d), col = "darkblue")
```

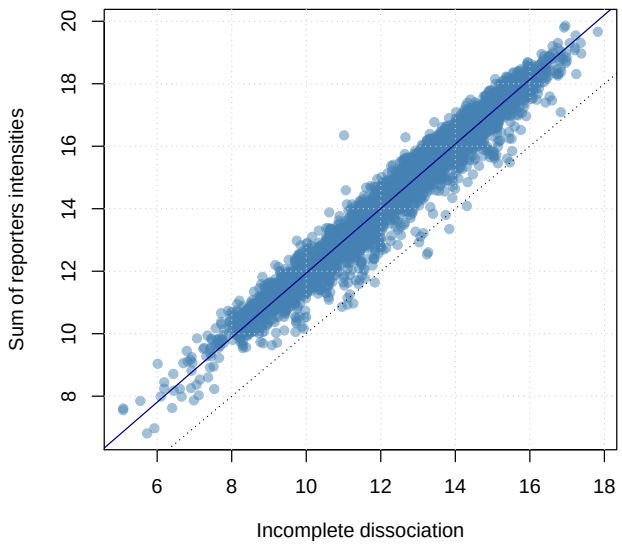


Figure 6: Incomplete dissociation.

```
MAplot(qnt[, c(4, 2)], cex = 0.9, col = cls, pch = pch, show.statistics = FALSE)
abline(lm(Signal ~ Incomplete, data = d), col = "darkblue")
```

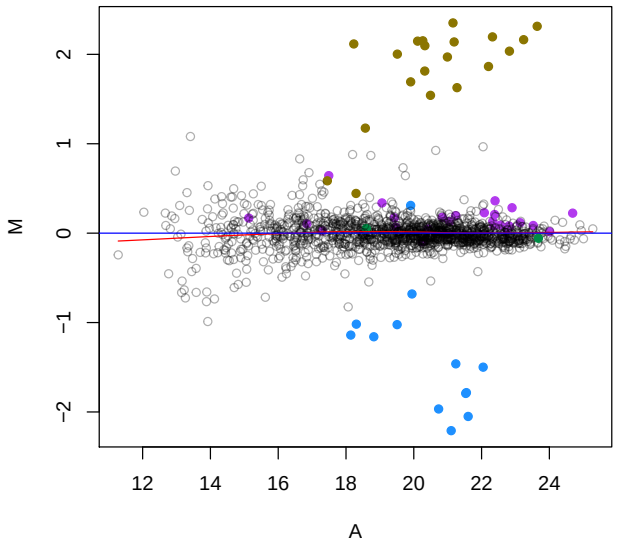


Figure 7: MAplot on an `MSnSet` instance.

4.3 The MALDIquant package

This section illustrates some of MALDIquant's data processing capabilities. The code is taken from the `processing-peaks.R` script downloaded from the package homepage[7].

Loading the data

```
## load packages
library("MALDIquant")
library("readBrukerFlexData")
datapath <- file.path(system.file("Examples", package = "readBrukerFlexData"),
    "2010_05_19_Gibb_C8_A1")
dir(datapath)

## [1] "0_A1" "0_A2"

sA1 <- mqReadBrukerFlex(datapath)
# in the following we use only the first spectrum
s <- sA1[[1]]

summary(mass(s))

##    Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
##    1000    2370    4330    4720    6870   10000

summary(intensity(s))

##    Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
##       4     180    1560    2840    4660   32600

head(as.matrix(s))

##        mass intensity
## [1,]  999.9     11278
## [2,] 1000.1     11350
## [3,] 1000.3     10879
## [4,] 1000.5     10684
## [5,] 1000.7     10740
## [6,] 1000.9     10947
```

Preprocessing

[7] http://strimmerlab.org/software/maldiquant/

```
plot(s)
```

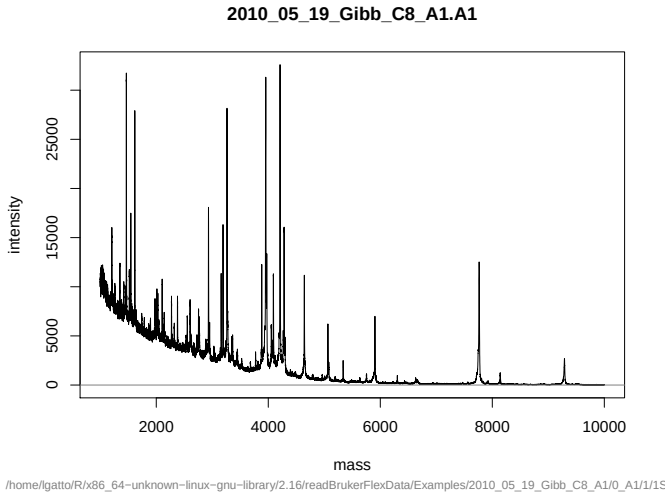


Figure 8: Spectrum plotting in MALDIquant.

```
## sqrt transform (for variance stabilization)
s2 <- transformIntensity(s, fun = sqrt)
s2

## S4 class type            : MassSpectrum
## Number of m/z values     : 22431
## Range of m/z values      : 999.939 - 10001.925
## Range of intensity values: 2e+00 - 1.805e+02
## Name                     : 2010_05_19_Gibb_C8_A1.A1
## File                     : /home/lgatto/R/x86_64-unknown-linux-gnu-library/2.16/


## smoothing
simpleSmooth <- function(y) {
    return(filter(y, rep(1, 5)/5, sides = 2))  # 5 point moving average
}

s3 <- transformIntensity(s2, simpleSmooth)
s3

## S4 class type            : MassSpectrum
## Number of m/z values     : 22427
## Range of m/z values      : 1000.324 - 10000.705
## Range of intensity values: 3.606e+00 - 1.792e+02
## Name                     : 2010_05_19_Gibb_C8_A1.A1
## File                     : /home/lgatto/R/x86_64-unknown-linux-gnu-library/2.16/
```

```
length(s2)  # 22431
```

```
## [1] 22431
```

```
length(s3)  # 22427 - at both ends data points have been removed
```

```
## [1] 22427
```

```
## baseline subtraction
s4 <- removeBaseline(s3, method = "SNIP")
s4
```

```
## S4 class type            : MassSpectrum
## Number of m/z values     : 22427
## Range of m/z values      : 1000.324 - 10000.705
## Range of intensity values: 0e+00 - 1.414e+02
## Name                     : 2010_05_19_Gibb_C8_A1.A1
## File                     : /home/lgatto/R/x86_64-unknown-linux-gnu-library/2.16/r
```

Peak picking

```
## peak picking
p <- detectPeaks(s4)
length(p)  # 181
```

```
## [1] 181
```

```
peak.data <- as.matrix(p)  # extract peak information
```

```
par(mfrow = c(2, 3))
xl <- range(mass(s))  # use same xlim on all plots for better comparison
plot(s, sub = "", main = "1: raw", xlim = xl)
plot(s2, sub = "", main = "2: variance stabilisation", xlim = xl)
plot(s3, sub = "", main = "3: smoothing", xlim = xl)
plot(s4, sub = "", main = "4: base line correction", xlim = xl)
plot(s4, sub = "", main = "5: peak detection", xlim = xl)
points(p)
top20 <- intensity(p) %in% sort(intensity(p), decreasing = TRUE)[1:20]
labelPeaks(p, index = top20, underline = TRUE)
plot(p, sub = "", main = "6: peak plot", xlim = xl)
labelPeaks(p, index = top20, underline = TRUE)
```

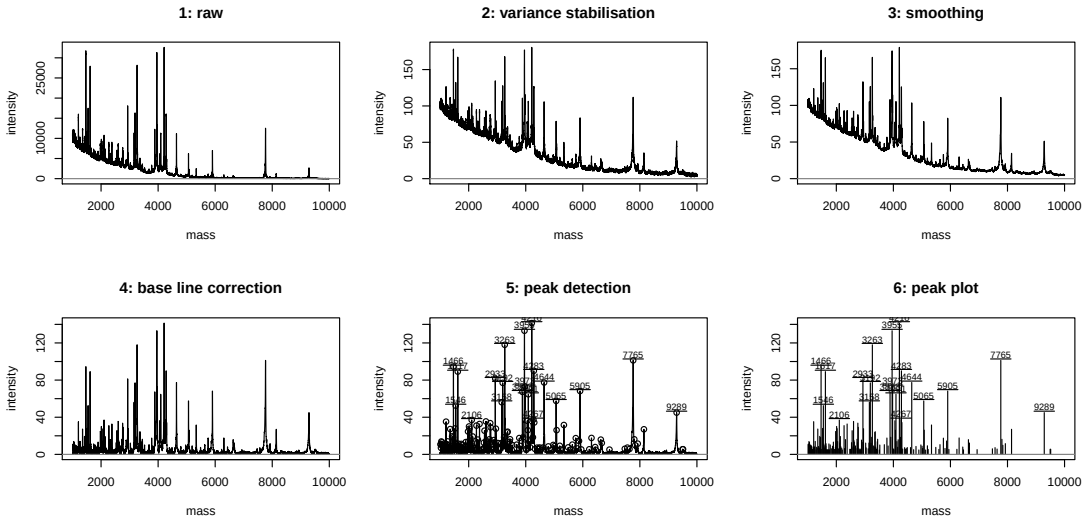


Figure 9: Spectrum plotting in MALDIquant.

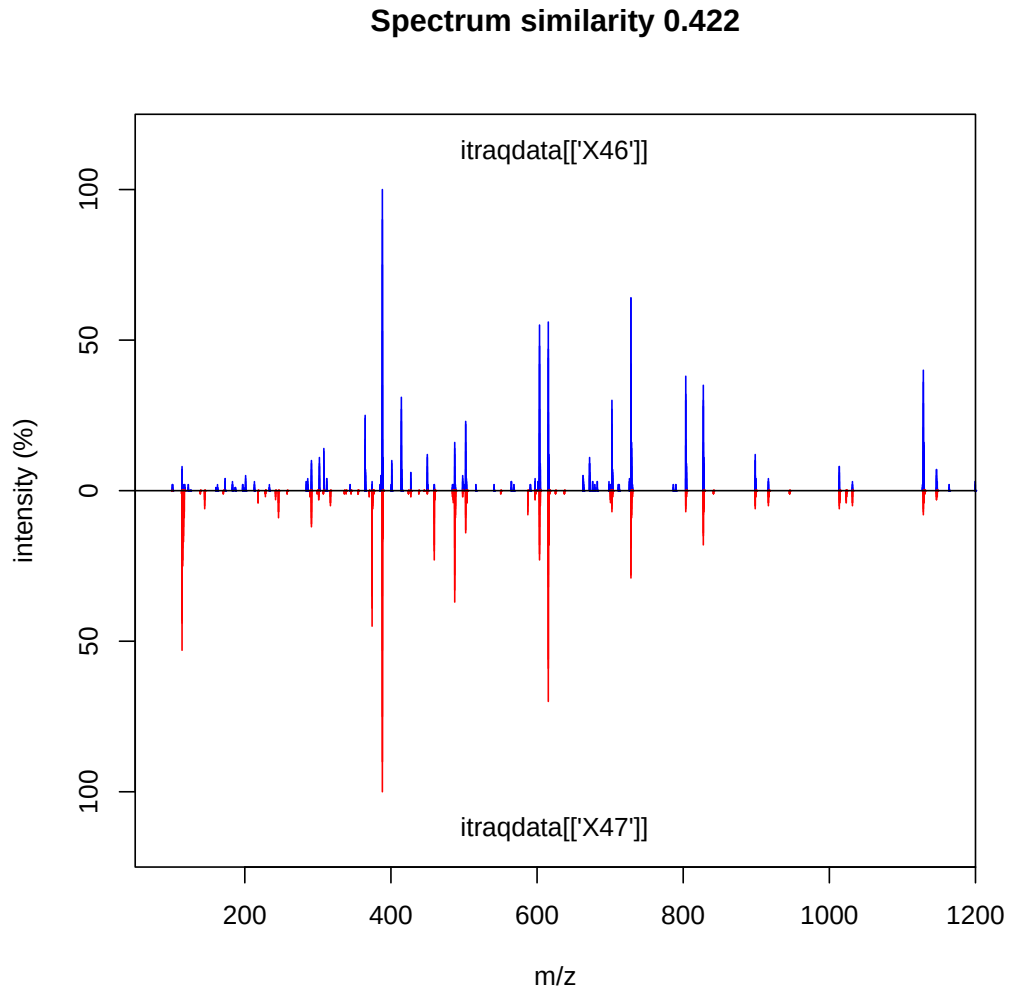
4.4 Working with peptide sequences

```
library(IPPD)
## x <- myo500[,'mz'] y <- myo500[,'intensities'] sel <- x <= 2500 y <-
## y[sel] x <- x[sel] see vignette

## add example

library(BRAIN)
atoms <- getAtomsFromSeq("SIVPSGASTGVHEALEMR")
unlist(atoms)

##   C   H   N   O   S
##  77 129  23  27   1


library(Rdisop)

## Loading required package:  RcppClassic

pepmol <- getMolecule(paste0(names(atoms), unlist(atoms), collapse = ""))
pepmol

## $formula
## [1] "C77H129N23O27S"
##
## $score
## [1] 1
##
## $exactmass
## [1] 1840
##
## $charge
## [1] 0
##
## $parity
## [1] "e"
##
## $valid
## [1] "Valid"
##
## $DBE
## [1] 25
##
## $isotopes
```

```
## $isotopes[[1]]
##           [,1]      [,2]      [,3]      [,4]      [,5]      [,6]      [,7]
## [1,] 1839.9149 1840.9177 1841.9197 1.843e+03 1.844e+03 1.845e+03 1.846e+03
## [2,]    0.3427    0.3353    0.1961 8.474e-02 2.953e-02 8.692e-03 2.226e-03
##           [,8]      [,9]     [,10]
## [1,] 1.847e+03 1.848e+03 1.849e+03
## [2,] 5.066e-04 1.040e-04 1.950e-05


##
library(OrgMassSpecR)
data(itraqdata)

simplottest <- itraqdata[featureNames(itraqdata) %in% paste0("X", 46:47)]
sim <- SpectrumSimilarity(as(simplottest[[1]], "data.frame"), as(simplottest[[2]],
    "data.frame"), top.lab = "itraqdata[['X46']]", bottom.lab = "itraqdata[['X47']]"
    b = 25)

## Warning:  the m/z tolerance is set too high

##        mz intensity.top intensity.bottom
## 1   114.1             0               44
## 2   114.1             0               53
## 3   114.1             0               43
## 4   115.1             0               25
## 5   364.7            25                0
## 6   374.2             0               39
## 7   374.2             0               45
## 8   374.2             0               35
## 9   388.2             0               35
## 10  388.3             0               75
## 11  388.3             0              100
## 12  388.3             0               90
## 13  388.3            35               53
## 14  388.3           100               53
## 15  388.3            90               53
## 16  388.3            53               53
## 17  388.3            75               53
## 18  414.3            31                0
## 19  414.3            27                0
## 20  487.3             0               33
## 21  487.3             0               37
## 22  487.3             0               28
## 23  603.3            42                0
## 24  603.4            55                0
```

```
## 25  603.4            48                 0
## 26  603.4            27                 0
## 27  615.3             0                28
## 28  615.3             0                56
## 29  615.4             0                70
## 30  615.4             0                59
## 31  615.4            26                32
## 32  615.4            44                32
## 33  615.4            56                32
## 34  615.4            47                32
## 35  702.4            27                 0
## 36  702.4            30                 0
## 37  728.4             0                28
## 38  728.5            64                29
## 39  728.5            64                29
## 40  728.5            42                29
## 41  728.5            42                29
## 42  803.4            30                 0
## 43  803.5            38                 0
## 44  803.5            32                 0
## 45  827.5            28                 0
## 46  827.5            35                 0
## 47  827.5            30                 0
## 48 1128.6            36                 0
## 49 1128.6            40                 0
## 50 1128.7            29                 0
```

```
title(main = paste("Spectrum similarity", round(sim, 3)))
```




```
MonoisotopicMass(formula = list(C = 2, O = 1, H = 6))

## [1] 46.04

molecule <- getMolecule("C2H5OH")
molecule$exactmass

## [1] 46.04

## x11() plot(t(.pepmol$isotopes[[1]]), type = 'h')

## x <- IsotopicDistribution(formula = list(C = 2, O = 1, H=6))
## t(molecule$isotopes[[1]]) par(mfrow = c(2,1))
## plot(t(molecule$isotopes[[1]]), type = 'h') plot(x[, c(1,3)], type =
## 'h')
```

```
## data(myo500) masses <- c(147.053, 148.056) intensities <- c(93, 5.8)
## molecules <- decomposeIsotopes(masses, intensities)

## experimental eno peptides
exppep <- as.character(fData(qnt[grep("ENO", fData(qnt)[, 2]), ])[, 1])  ## 13
minlength <- min(nchar(exppep))

eno <- download.file("http://www.uniprot.org/uniprot/P00924.fasta", destfile = "P009
eno <- paste(readLines("P00924.fasta")[-1], collapse = "")
enopep <- Digest(eno, missed = 1)
nrow(enopep)  ## 103

## [1] 103

sum(nchar(enopep$peptide) >= minlength)  ## 68

## [1] 68

pepcnt <- enopep[enopep[, 1] %in% exppep, ]
nrow(pepcnt)  ## 13

## [1] 13
```

```
## example code to generate an Texshade image to be included directly in a
## Latex document or R vignette
## seq1file <- 'seq1.tex' cat('\\begin{texshade}{Figures/P00924.fasta}
## \\setsize{numbering}{footnotesize} \\setsize{residues}{footnotesize}
## \\residuesperline*{70} \\shadingmode{functional} \\hideconsensus
## \\vsepspace{1mm} \\hidenames \\noblockskip\n', file = seq1file) tmp <-
## sapply(1:nrow(pepcnt), function(i) { col <- ifelse((i %% 2) == 0,
## 'Blue', 'RoyalBlue') cat('\\shaderegion{1}{', pepcnt$start[i], '..',
## pepcnt$stop[i], '}{White}{', col, '}\n', file = seq1file, append =
## TRUE) }) cat('\\end{texshade} \\caption{Visualising observed peptides
## for the Yeast enolase protein. Peptides are shaded in blue and black.
## The last peptide is a mis-cleavage and overlaps with
## \\texttt{IEEELGDNAVFAGENFHHGDK}.} \\label{fig:seq} \\end{center}
## \\end{figure}\n\n', file = seq1file, append = TRUE)
```

^{15}N incorporation

```
## 15N example
increate <- c(seq(0, 0.9, 0.1), 0.95, 1)
inc <- lapply(increate, function(inc) IsotopicDistributionN("YEVQGEVFTKPQLWP",
    inc))
par(mfrow = c(4, 3))
for (i in 1:length(inc)) plot(inc[[i]][, c(1, 3)], xlim = c(1823, 1848), type = "h",
    main = paste0("15N incorporation at ", increate[i], "%"))
```

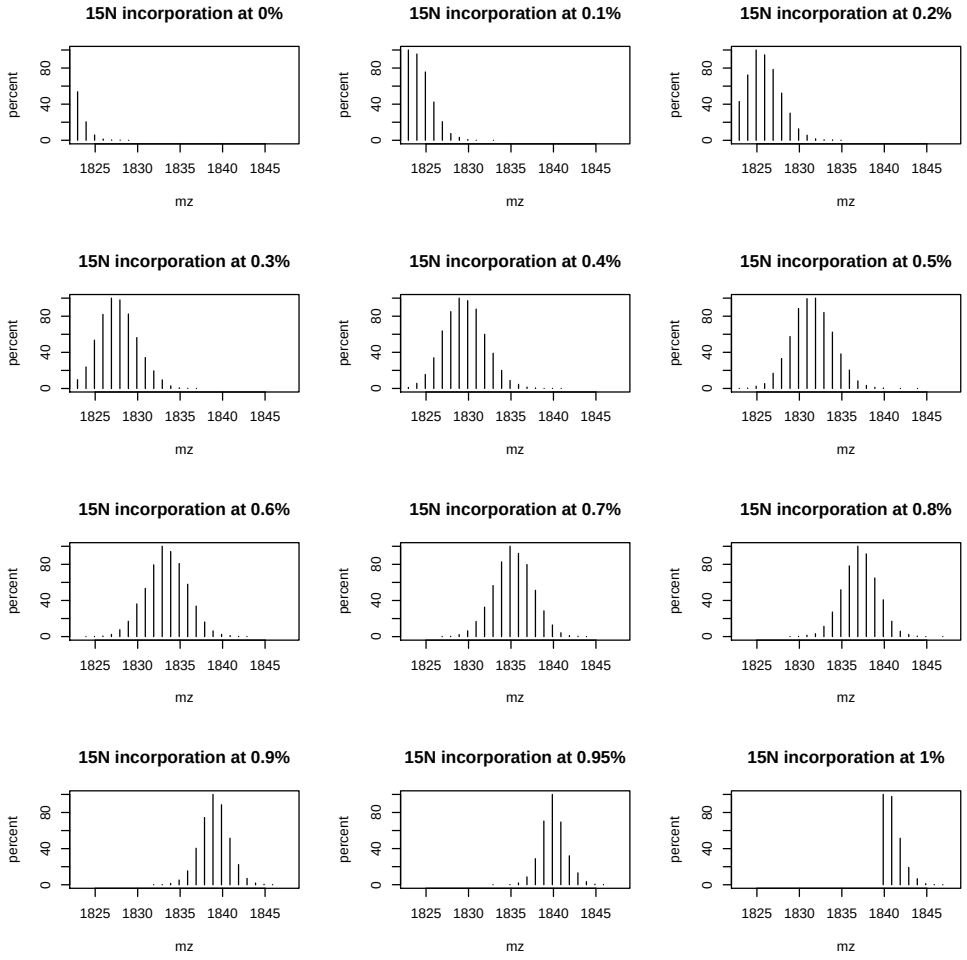


Figure 10: Isotopic envelope for the `YEVQGEVFTKPQLWP` peptide at different ^{15}N incorporation rates.

4.5 The isobar package

```
library(isobar)
.ions <- exprs(qnt)
.mass <- matrix(mz(TMT6), nrow(qnt), byrow = TRUE, ncol = 6)
colnames(.ions) <- colnames(.mass) <- reporterTagNames(new("TMT6plexSpectra"))
rownames(.ions) <- rownames(.mass) <- paste(fData(qnt)$accession, fData(qnt)$sequenc
    sep = ".")
pgtbl <- data.frame(spectrum = rownames(.ions), peptide = fData(qnt)$sequence,
    modif = ":", start.pos = 1, protein = fData(qnt)$accession, accession = fData(qn
x <- new("TMT6plexSpectra", pgtbl, .ions, .mass)

## merging identifications

featureData(x)$proteins <- as.character(fData(qnt)$accession)

x <- correctIsotopeImpurities(x)  ## useless, identity matrix

## LOG: isotopeImpurities.corrected:  TRUE

x <- normalize(x, per.file = FALSE)  ## little effect

## LOG: is.normalized:  TRUE
## LOG: normalization.multiplicative.factor channel 126:  1.1229
## LOG: normalization.multiplicative.factor channel 127:  1.0766
## LOG: normalization.multiplicative.factor channel 128:  1
## LOG: normalization.multiplicative.factor channel 129:  1.0537
## LOG: normalization.multiplicative.factor channel 130:  1.1524
## LOG: normalization.multiplicative.factor channel 131:  1.1154

spks <- c(protein.g(proteinGroup(x), "P00489"), protein.g(proteinGroup(x), "P00924")
    protein.g(proteinGroup(x), "P02769"), protein.g(proteinGroup(x), "P62894"))


cls2 <- rep("#00000040", nrow(x))
pch2 <- rep(1, nrow(x))
cls2[grep("P02769", featureNames(x))] <- "gold4"  ## BSA
cls2[grep("P00924", featureNames(x))] <- "dodgerblue"  ## ENO
cls2[grep("P62894", featureNames(x))] <- "springgreen4"  ## CYT
cls2[grep("P00489", featureNames(x))] <- "darkorchid2"  ## PHO
pch2[grep("P02769", featureNames(x))] <- 19
pch2[grep("P00924", featureNames(x))] <- 19
pch2[grep("P62894", featureNames(x))] <- 19
pch2[grep("P00489", featureNames(x))] <- 19

nm <- NoiseModel(x)
```

```
## [1]   0.07345 941.48624   2.82448

ib.background <- subsetIBSpectra(x, protein = spks, "exclude")
nm.background <- NoiseModel(ib.background)

## [1] 0.01346 2.85121 0.84631

ib.spks <- subsetIBSpectra(x, protein = spks, direction = "exclude others",
    specificity = "reporter-specific")
nm.spks <- NoiseModel(ib.spks, one.to.one = FALSE, pool = TRUE)

## 4 proteins with more than 10 spectra, taking top 50.
## [1] 1.000e-10 5.829e+00 6.610e-01


ratios <- 10^estimateRatio(x, nm, channel1 = "127", channel2 = "129", protein = spks
    combine = FALSE)[, "lratio"]

res <- estimateRatio(x, nm, channel1 = "127", channel2 = "129", protein = unique(fDa
    combine = FALSE, sign.level = 0.01)[, c(1, 2, 6, 8)]
res <- as.data.frame(res)
res$lratio <- -(res$lratio)

cls3 <- rep("#00000050", nrow(res))
pch3 <- rep(1, nrow(res))
cls3[grep("P02769", rownames(res))] <- "gold4"  ## BSA
cls3[grep("P00924", rownames(res))] <- "dodgerblue"  ## ENO
cls3[grep("P62894", rownames(res))] <- "springgreen4"  ## CYT
cls3[grep("P00489", rownames(res))] <- "darkorchid2"  ## PHO
## cls3[grep('P00761', rownames(res))] <- 'red' ## Trypsin
pch3[grep("P02769", rownames(res))] <- 19
pch3[grep("P00924", rownames(res))] <- 19
pch3[grep("P62894", rownames(res))] <- 19
pch3[grep("P00489", rownames(res))] <- 19
## pch3[grep('P00761', rownames(res))] <- 19

rat.exp <- c(PHO = 2/2, ENO = 5/1, BSA = 2.5/10, CYT = 1/1)
```

```
par(mfrow = c(1, 2))
maplot(x, noise.model = c(nm.background, nm.spks, nm), channel1 = "127", channel2 =
    pch = 19, col = cls2, main = "Spectra MA plot")
abline(h = 1, lty = "dashed", col = "grey")
legend("topright", c("BSA", "ENO", "CYT", "PHO"), pch = 19, col = c("gold4",
    "dodgerblue", "springgreen4", "darkorchid2"), bty = "n", cex = 0.7)
plot(res$lratio, -log10(res$p.value.rat), col = cls3, pch = pch3, xlab = expression(
    fold - change), ylab = expression(-log[10] ~ p - value), main = "Protein volcano
    xlim = c(-0.7, 0.7))
grid()
abline(h = -log10(0.01), lty = "dotted")
abline(v = log10(c(2, 0.5)), lty = "dotted")
abline(v = -0.003, col = "springgreen4", lty = "dashed", lwd = 2)
abline(v = 0.003, col = "darkorchid2", lty = "dashed", lwd = 2)
abline(v = log10(5), col = "dodgerblue", lty = "dashed", lwd = 2)
abline(v = log10(0.25), col = "gold4", lty = "dashed", lwd = 2)
points(res[spks, "lratio"], -log10(res[spks, "p.value.rat"]), col = c("darkorchid2",
    "dodgerblue", "gold4", "springgreen4"), pch = 19)
```

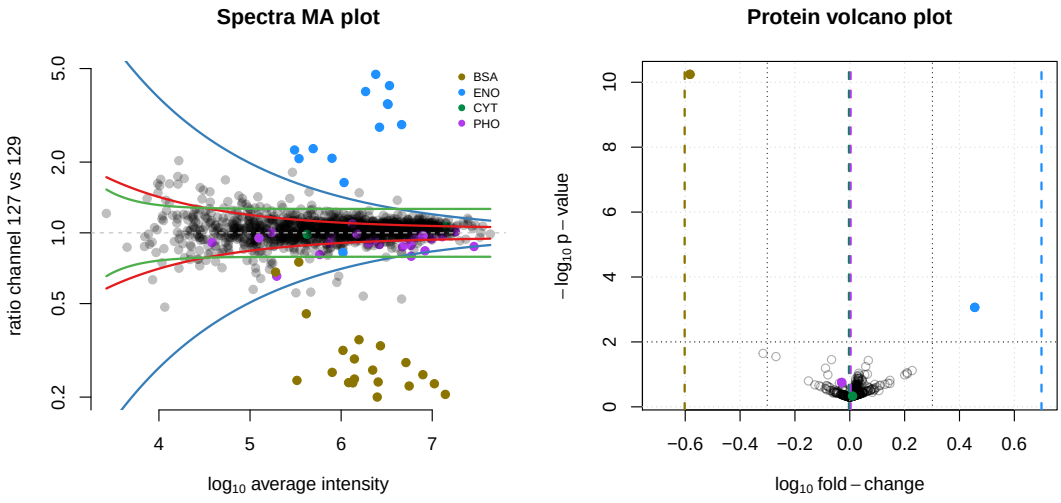


Figure 11: Result from the isobar pipeline.

4.6 The synapter package

The synapter package comes with a detailed vignette that describes how to prepare the MS^E data and then process it in R . Several interfaces are available provided the user with maximum control, easy batch processing capabilites or a graphical user interface. The conversion into `MSnSet` instances and filter and combination thereof as well as statistical analysis are also described.

```
## open the synapter vignette
library("synapter")
synapterGuide()
```

5 Annotation

See rols and hpar vignettes.

```
id <- "ENSG00000002746"
library("hpar")
getHpa(id, "SubcellularLoc")
```

```
##               Gene                       Main.location Other.location
## 24 ENSG00000002746 Nucleus but not nucleoli;Cytoplasm
##    Expression.type Reliability
## 24             APE        High
```

```
library(org.Hs.eg.db)
```

```
## Loading required package:  AnnotationDbi
##
## Attaching package:  'AnnotationDbi'
## The following object is masked from 'package:MASS':
##
## select
## Loading required package:  DBI
##
```

```
library(GO.db)
```

```
##
```

```
ans <- select(org.Hs.eg.db, keys = id, cols = c("ENSEMBL", "GO", "ONTOLOGY"),
    keytype = "ENSEMBL")
```

```
## Warning:  'select' resulted in 1:many mapping between keys and return
rows
```

```
ans <- ans[ans$ONTOLOGY == "CC", ]
ans

##           ENSEMBL         GO EVIDENCE ONTOLOGY
## 2 ENSG00000002746 GO:0005634      IDA       CC
## 3 ENSG00000002746 GO:0005737      IDA       CC

sapply(as.list(GOTERM[ans$GO]), slot, "Term")

##  GO:0005634  GO:0005737
##   "nucleus" "cytoplasm"


library("biomaRt")
ensembl <- useMart("ensembl", dataset = "hsapiens_gene_ensembl")
efilter <- "ensembl_gene_id"
eattr <- c("go_id", "name_1006", "namespace_1003")
bmres <- getBM(attributes = eattr, filters = efilter, values = id, mart = ensembl)
bmres[bmres$namespace_1003 == "cellular_component", "name_1006"]

## [1] "nucleus"       "cytoplasm"     "intracellular"



library("rols")
## see vignette
```

Session information

All software and version used in this document, as returned by `sessionInfo()` are detailed below.

- R Under development (unstable) (2012-09-27 r60832), `x86_64-unknown-linux-gnu`
- Locale: `LC_CTYPE=en_GB.UTF-8`, `LC_NUMERIC=C`, `LC_TIME=en_GB.UTF-8`, `LC_COLLATE=en_GB.UTF-8`, `LC_MONETARY=en_GB.UTF-8`, `LC_MESSAGES=en_GB.UTF-8`, `LC_PAPER=C`, `LC_NAME=C`, `LC_ADDRESS=C`, `LC_TELEPHONE=C`, `LC_MEASUREMENT=en_GB.UTF-8`, `LC_IDENTIFICATION=C`
- Base packages: base, datasets, graphics, grDevices, methods, parallel, stats, utils
- Other packages: AnnotationDbi 1.20.1, Biobase 2.18.0, BiocGenerics 0.4.0, biomaRt 2.14.0, Biostrings 2.26.2, bitops 1.0-4.1, BRAIN 1.2.0, DBI 0.2-5,

digest 0.5.2, doMC 1.2.5, foreach 1.4.0, ggplot2 0.9.2.1, GO.db 2.8.0, hpar 1.1.1, IPPD 1.6.0, IRanges 1.16.2, isobar 1.4.0, iterators 1.0.6, knitr 0.8, lattice 0.20-10, MALDIquant 1.3, MASS 7.3-22, Matrix 1.0-9, msdata 0.1.11, MSnbase 1.7.2, multicore 0.1-7, mzR 1.5.1, org.Hs.eg.db 2.8.0, OrgMassSpecR 0.3-12, plyr 1.7.1, PolynomF 0.94, RColorBrewer 1.0-5, Rcpp 0.9.15, RcppClassic 0.9.2, Rdisop 1.18.0, readBrukerFlexData 1.4, reshape2 1.2.1, RforProteomics 0.2.2, rols 1.1.1, RSQLite 0.11.2, XML 3.95-0.1

- Loaded via a namespace (and not attached): affy 1.36.0, affyio 1.26.0, BiocInstaller 1.8.2, codetools 0.2-8, colorspace 1.1-1, compiler 2.16.0, dichromat 1.2-4, distr 2.3.3, evaluate 0.4.2, formatR 0.6, grid 2.16.0, gtable 0.1.1, labeling 0.1, limma 3.14.1, memoise 0.1, munsell 0.4, preprocessCore 1.20.0, proto 0.3-9.2, RCurl 1.95-1.1, R.methodsS3 1.4.2, R.oo 1.9.9, R.utils 1.16.2, scales 0.2.2, sfsmisc 1.0-21, SSOAP 0.8-0, startupmsg 0.7.2, stats4 2.16.0, stringr 0.6.1, tools 2.16.0, vsn 3.26.0, XMLSchema 0.7-2, zlibbioc 1.4.0

References

[1] L. Gatto and A. Christoforou. Using R for proteomics data analysis. *BBA - Proteins and Proteomics*, 2012.