

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*. It also aims at being a general overview for users who wish to explore the *R* environment and programming language for the analysis of proteomics data.

Keywords: proteomics, mass spectrometry, tutorial.

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1 Introduction

This document illustrates some existing *R* infrastructure for the analysis of proteomics data. It presents the code for the use cases taken from [1, 2]. A pre-print of [1] available on arXiv¹ and [2] is open access².

There are however numerous additional *R* resources distributed by the *Bioconductor*³ and CRAN⁴ repositories, as well as packages hosted on personal websites. Section 8 on page 45 tries to provide a wider picture of available packages, without going into details.

1.1 General R resources

The reader is expected to have basic *R* knowledge to find the 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 introductory tutorials in the [contributed documentation](#) section.
- The TeachingMaterial repository⁵ contains several sets of slides and vignettes about *R* programming.

Relevant background on the *R* software and its application to computational biology in general and proteomics in particular can also be found in [1]. For details about the *Bioconductor* project, the reader is referred to [3].

1.2 Bioconductor resources

The Bioconductor offers many educational resources on its help page <http://bioconductor.org/help/>, in addition the package's vignettes (vignettes are a requirement for Bioconductor packages). We want to draw the attention to the Bioconductor work flows that offer a cross-package overview about a specific topic. In particular, there is now a *Mass spectrometry and proteomics data analysis*⁶ work flow.

1.3 Getting help

All *R* packages come with ample documentation. Every command (function, class or method) a user is susceptible to use is documented. The documentation can be accessed by preceding the command by a ? in the *R* console. For example, to obtain help about the `library` function, that will be used in the next section, one would type `?library`. In addition, all *Bioconductor* packages come with at least one vignette (this document is the vignette that comes with the *RforProteomics* package), a document that combines text and *R* code that is executed before the pdf is assembled. To look up all vignettes that come with a package, say *RforProteomics* and then open the vignette of interest, one uses the `vignette` function as illustrated below. More details can be found in `?vignette`.

```
## list all the vignettes in the RforProteomics package
vignette(package = "RforProteomics")
## Open the vignette called RforProteomics
vignette("RforProteomics", package = "RforProteomics")
## or just
vignette("RforProteomics")
```

¹<http://arxiv.org/abs/1305.6559>

²<http://onlinelibrary.wiley.com/doi/10.1002/pmic.201400392/full>

³<http://www.bioconductor.org>

⁴<http://cran.r-project.org/web/packages/>

⁵<https://github.com/lgatto/TeachingMaterial>

⁶<http://bioconductor.org/help/workflows/proteomics/>

R has several mailing lists⁷. The most relevant here being the main R-help list, *for discussion about problem and solutions using R*, ideal for general R content and is not suitable for bioinformatics or proteomics questions. Bioconductor also offers several resources dedicated to bioinformatics matters and Bioconductor packages, in particular the main Bioconductor support forum⁸ for Bioconductor-related queries. A dedicated *RforProteomics* Google group⁹ also welcomes questions/comments/announcements related to R and mass-spectrometry/proteomics, although the Bioconductor forum is the preferred channel.

It is advised 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 document, on page 50). If the question involves some code, make sure to isolate the relevant portion and report it with your question, trying to make your code/example reproducible¹⁰.

1.4 Installation

The package should be installed using as described below:

```
## only first time you install Bioconductor packages
source("http://www.bioconductor.org/biocLite.R")
## else
library("BiocInstaller")
biocLite("RforProteomics")
```

To install all dependencies and reproduce the code in the vignette, replace the last line in the code chunk above with:)

```
biocLite("RforProteomics", dependencies = TRUE)
```

Finally, the package can be loaded with

```
library("RforProteomics")

##
## This is the 'RforProteomics' version 1.12.0.
##
## To get started, visit
##   http://lgatto.github.com/RforProteomics/
##
## or, in R, open package vignettes by typing
##   RforProteomics() # R/Bioc for proteomics overview
##   RProtVis()      # R/Bioc for proteomics visualisation
##
## For a full list of available documents:
##   vignette(package='RforProteomics')
```

See also the *RforProteomics* web page¹¹ for more information on installation.

1.5 External dependencies

Some packages used in the document depend on external libraries that need to be installed prior to the R packages:

mzR depends on the Common Data Format¹² (CDF) to CDF-based raw mass-spectrometry data. On Linux, the `libcdf` library is required. On Debian-based systems, for instance, one needs to install the `libnetcdf-dev` package.

⁷<http://www.r-project.org/mail.html>

⁸<https://support.bioconductor.org/>

⁹<https://groups.google.com/forum/#!forum/rbioc-sig-proteomics>

¹⁰<https://github.com/hadley/devtools/wiki/Reproducibility>

¹¹<http://lgatto.github.io/RforProteomics/>

¹²<http://cdf.gsfc.nasa.gov/>

IPPD (and others) depend on the *XML* package which requires the `libxml2` infrastructure on Linux. On Debian-based systems, one needs to install `libxml2-dev`.

biomaRt performs on-line requests using the `curl`¹³ infrastructure. On Debian-based systems, you one needs to install `libcurl-dev` or `libcurl4-openssl-dev`.

MSGFplus Is based on the MS-GF+ java program and thus requires Java 1.7¹⁴ in order to work.

1.6 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 ESS¹⁵ for Emacs or RStudio¹⁶ for instance) to easily modify and explore it. This can be achieved with the `Stangle` function. One needs the Sweave source of this document (a document combining the narration and the R code) and the `Stangle` then specifically extracts the code chunks and produces a clean R source file. If the package is installed, the following code chunk will direct you to the `RforProteomics.R` file containing all the annotated source code contained in this document.

```
## gets the vignette source
rfile <- system.file("doc/RforProteomics.R",
                    package = "RforProteomics")
rfile
## [1] "/home/lg390/R/x86_64-pc-linux-gnu-library/3.3/RforProteomics/doc/RforProteomics.R"
```

Alternatively, you can obtain the `Rnw` file on the github page <https://github.com/lgatto/RforProteomics/blob/master/inst/doc/vigsrc/RforProteomics.Rnw>.

1.7 Prepare the working environment

The packages that we will depend on to execute 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/output

2.1 The mzR package

2.1.1 Raw MS data

The *mzR* package [4] provides a unified interface to various mass spectrometry open formats. This code chunk, taken 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 also be applicable to an `mzXML`, `mzData` or `netCDF` file.

```
## load the required packages
library("mzR") ## the software package
library("msdata") ## the data package
## below, we extract the relevant example file
```

¹³<http://curl.haxx.se/>

¹⁴<https://java.com>

¹⁵<http://ess.r-project.org/>

¹⁶<http://rstudio.org/>

```
## from the local 'msdata' installation
filepath <- system.file("microtofq", package = "msdata")
file <- list.files(filepath, pattern="MM14.mzML",
                  full.names=TRUE, recursive = TRUE)
## creates a connection to the mzML file
mz <- openMSfile(file)
## demonstration of data access
basename(fileName(mz))

## [1] "MM14.mzML"

isInitialized(mz)

## [1] TRUE

runInfo(mz)

## $scanCount
## [1] 112
##
## $lowMz
## [1] 0
##
## $highMz
## [1] 0
##
## $dStartTime
## [1] 270.334
##
## $dEndTime
## [1] 307.678
##
## $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 explicitly
## close the connection
close(mz)
```

mzR is used by other packages, like *MSnbase* [5], *TargetSearch* [6] and *xcms* [7, 8, 9], that provide a higher level abstraction to the data.

2.1.2 Identification data

The *mzR* package also provides very fast access to mzIdentML data by leveraging proteowizard's C++ parser.

```
file <- system.file("mzid", "Tandem.mzid.gz", package="msdata")
mzid <- openIDfile(file)
mzid

## Identification file handle.
## Filename: Tandem.mzid.gz
## Number of psms: 171
```

Once an mzIdent identification file handle has been established, various data and metadata can be extracted, as illustrated below.

```
softwareInfo(mzid)

## [1] "xtandem x! tandem CYCLONE (2010.06.01.5) "
## [2] "ProteoWizard MzIdentML 3.0.9490 ProteoWizard"

enzymes(mzid)

##      name nTermGain cTermGain minDistance missedCleavages
## 1 Trypsin      H      OH      0      1

names(psms(mzid))

## [1] "spectrumID"      "chargeState"
## [3] "rank"              "passThreshold"
## [5] "experimentalMassToCharge" "calculatedMassToCharge"
## [7] "sequence"         "modNum"
## [9] "isDecoy"          "post"
## [11] "pre"              "start"
## [13] "end"              "DatabaseAccess"
## [15] "DBseqLength"     "DatabaseSeq"
## [17] "DatabaseDescription" "acquisitionNum"

head(psms(mzid))[, 1:13]

##      spectrumID chargeState rank passThreshold
## 1 index=12      3 1 FALSE
## 2 index=285     3 1 FALSE
## 3 index=83      3 1 FALSE
## 4 index=21      3 1 FALSE
## 5 index=198     3 1 FALSE
## 6 index=13      2 1 FALSE
##      experimentalMassToCharge calculatedMassToCharge
## 1 903.7209 903.4032
## 2 792.3792 792.3899
## 3 792.5295 792.3899
## 4 850.0782 849.7635
## 5 527.2592 527.2849
## 6 724.8816 724.3771
##      sequence modNum isDecoy post pre start
## 1 LCYIALDFDEEMKAAEDSSDIEK 2 FALSE S K 217
## 2 KDLYGNVVLSSGGTTMYEGIGER 1 FALSE L R 292
## 3 KDLYGNVVLSSGGTTMYEGIGER 1 FALSE L R 292
## 4 VIDENFGLVEGLMTTVHAATGTQK 1 FALSE V K 842
## 5 GVGGAIVLVLYDEM 1 FALSE R R 297
```

```
## 6          HAVGGRYSSLCK      1  TRUE  D  K  392
## end
## 1 239
## 2 313
## 3 313
## 4 865
## 5 311
## 6 404
```

2.2 Handling MS² identification data with mzID

The *mzID* package allows to load and manipulate MS² data in the mzIdentML format. The main *mzID* function reads such a file and constructs an instance of class *mzID*.

```
library("mzID")
mzids <- list.files(system.file('extdata', package = 'mzID'),
                   pattern = '*.mzid', full.names = TRUE)

mzids

## [1] "/home/lg390/R/x86_64-pc-linux-gnu-library/3.3/mzID/extdata/55merge_omssa.mzid"
## [2] "/home/lg390/R/x86_64-pc-linux-gnu-library/3.3/mzID/extdata/55merge_tandem.mzid"
## [3] "/home/lg390/R/x86_64-pc-linux-gnu-library/3.3/mzID/extdata/Mascot_MSMS_example.mzid"
## [4] "/home/lg390/R/x86_64-pc-linux-gnu-library/3.3/mzID/extdata/Mascot_MSMS_example1.0.mzid"
## [5] "/home/lg390/R/x86_64-pc-linux-gnu-library/3.3/mzID/extdata/Mascot_NA_example.mzid"
## [6] "/home/lg390/R/x86_64-pc-linux-gnu-library/3.3/mzID/extdata/mascot_pmf_example.mzid"
## [7] "/home/lg390/R/x86_64-pc-linux-gnu-library/3.3/mzID/extdata/Mascot_top_down_example.mzid"
## [8] "/home/lg390/R/x86_64-pc-linux-gnu-library/3.3/mzID/extdata/MPC_example_Multiple_search_engines.mzid"
## [9] "/home/lg390/R/x86_64-pc-linux-gnu-library/3.3/mzID/extdata/Sequest_example_ver1.1.mzid"

id <- mzID(mzids[1])

## reading 55merge_omssa.mzid... DONE!

id

## An mzID object
##
## Software used:  OMSSA (version: NA)
##
## Rawfile:       D:/TestSpace/NeoTestMarch2011/55merge.mgf
##
## Database:     D:/Software/Databases/Neospora_3rndTryp/Neo_rndTryp_3times.fasta
##
## Number of scans: 39
## Number of PSM's: 99
```

Multiple files can be parsed in one go, possibly in parallel if the environment supports it. When this is done an *mzIDCollection* object is returned:

```
ids <- mzID(mzids[1:2])
ids

## An mzIDCollection object containing 2 samples
```

Peptides, scans, parameters, ... can be extracted with the respective *peptides*, *scans*, *parameters*, ... functions. The *mzID* object can also be converted into a *data.frame* using the *flatten* function.


```

fid <- flatten(id)
names(fid)

## [1] "spectrumid"           "spectrum title"
## [3] "acquisitionnum"       "passthreshold"
## [5] "rank"                 "calculatedmasstocharge"
## [7] "experimentalmasstocharge" "chargestate"
## [9] "omssa:evaluate"       "omssa:pvalue"
## [11] "isdecoy"              "post"
## [13] "pre"                  "end"
## [15] "start"                "accession"
## [17] "length"               "description"
## [19] "pepseq"               "modified"
## [21] "modification"         "idFile"
## [23] "spectrumFile"         "databaseFile"

dim(fid)

## [1] 101 24

```

3 Raw data abstraction with MSnExp objects

MSnbase [5] provides base functions and classes for MS-based proteomics that allow facile data and meta-data processing, manipulation and plotting (see for instance figure 1 on page 11).

```

library("MSnbase")
## uses a simple dummy test included in the package
mzXML <- dir(system.file(package="MSnbase",dir="extdata"),
             full.name=TRUE,
             pattern="mzXML$")
basename(mzXML)

## [1] "dummyiTRAQ.mzXML"

## reads the raw data into and MSnExp instance
raw <- readMSData(mzXML, verbose = FALSE, centroided = TRUE)
raw

## Object of class "MSnExp" (in memory)
## Object size in memory: 0.18 Mb
## - - - Spectra data - - -
## MS level(s): 2
## Number of spectra: 5
## MSn retention times: 25:1 - 25:2 minutes
## - - - Processing information - - -
## Data loaded: Tue Apr 18 21:47:51 2017
## MSnbase version: 2.0.2
## - - - Meta data - - -
## phenoData
##   rowNames: dummyiTRAQ.mzXML
##   varLabels: sampleNames
##   varMetadata: labelDescription
## Loaded from:
##   dummyiTRAQ.mzXML
## protocolData: none

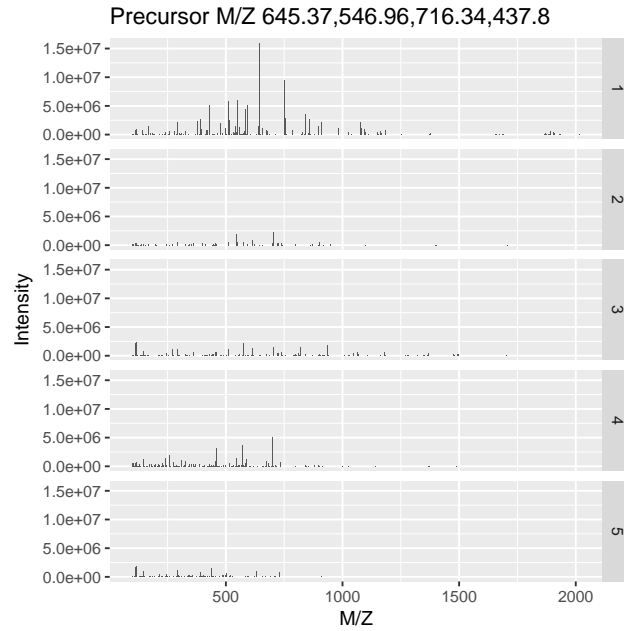
```

```
## featureData
##   featureNames: X1.1 X2.1 ... X5.1 (5 total)
##   fvarLabels: spectrum
##   fvarMetadata: labelDescription
## experimentData: use 'experimentData(object)'

## Extract a single spectrum
raw[[3]]

## Object of class "Spectrum2"
## Precursor: 645.3741
## Retention time: 25:2
## Charge: 2
## MSn level: 2
## Peaks count: 2125
## Total ion count: 150838188
```

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



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

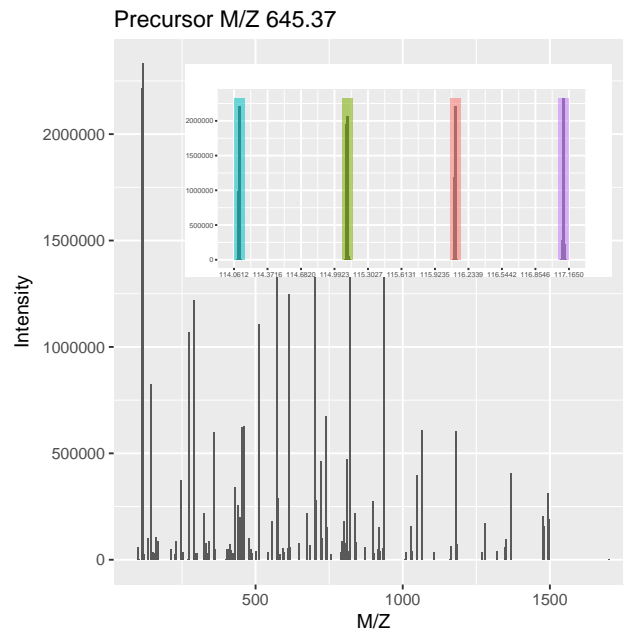


Figure 1: The `plot` method can be used on experiments, i.e. spectrum collections (top), or individual spectra (bottom).

3.1 mgf read/write support

Read and write support for data in the `mgf`¹⁷ and `mzTab`¹⁸ formats are available via the `readMgfData/writeMgfData` and `readMzTabData/writeMzTabData` functions, respectively. An example for the latter is shown in the next section.

4 Quantitative proteomics

As an running example throughout this document, we will use a TMT 6-plex data set, PXD000001 to illustrate quantitative data processing. The code chunk below first downloads this data file from the ProteomeXchange server using the `rpx` package.

4.1 The mzTab format

The first code chunk downloads the `mzTab` data from the ProteomeXchange repository [10].

```
## Experiment information
library("rpx")
px1 <- PXDataset("PXD000001")
px1

## Object of class "PXDataset"
## Id: PXD000001 with 12 files
## [1] 'F063721.dat' ... [12] 'generated'
## Use 'pxfiles(.)' to see all files.

pxfiles(px1)

## [1] "F063721.dat"
## [2] "F063721.dat-mztab.txt"
## [3] "PRIDE_Exp_Complete_Ac_22134.xml.gz"
## [4] "PRIDE_Exp_mzData_Ac_22134.xml.gz"
## [5] "PXD000001_mztab.txt"
## [6] "README.txt"
## [7] "TMT_Erwinia_1uLSike_Top10HCD_isol2_45stepped_60min_01-20141210.mzML"
## [8] "TMT_Erwinia_1uLSike_Top10HCD_isol2_45stepped_60min_01-20141210.mzXML"
## [9] "TMT_Erwinia_1uLSike_Top10HCD_isol2_45stepped_60min_01.mzXML"
## [10] "TMT_Erwinia_1uLSike_Top10HCD_isol2_45stepped_60min_01.raw"
## [11] "erwinia_carotovora.fasta"
## [12] "generated"

## Downloading the mzTab data
mztab <- pxget(px1, "PXD000001_mztab.txt")

## Downloading 1 file

mztab

## [1] "PXD000001_mztab.txt"
```

The code below loads the `mzTab` file into R and generates an `MSnSet` instance¹⁹, removes missing values and calculates protein intensities by summing the peptide quantitation data. Figure 2 illustrates the intensities for 5 proteins.

¹⁷<http://www.matrixscience.com/help/data.file.help.html#GEN>

¹⁸<https://code.google.com/p/mztab/>

¹⁹Here, we specify `mzTab` format version 0.9. Recent files have been generated according to the latest specifications, version 1.0, and the version does not need to be specified explicitly.

```

## Load mzTab peptide data
qnt <- readMzTabData(mztab, what = "PEP", version = "0.9")
## Warning: Version 0.9 is deprecated. Please see '?readMzTabData' and '?MzTab' for details.
## 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      NA      NA      NA      NA      NA      NA
## 2 10630132 11238708 12424917 10997763  9928972 10398534
## 3      NA      NA      NA      NA      NA      NA
## 4      NA      NA      NA      NA      NA      NA
## 5 11105690 12403253 13160903 12229367 11061660 10131218
## 6 1183431 1322371 1599088 1243715 1306602 1159064

## remove missing values
qnt <- filterNA(qnt)
processingData(qnt)

## - - - Processing information - - -
## mzTab read: Tue Apr 18 21:48:00 2017
## Subset [2351,6][1504,6] Tue Apr 18 21:48:00 2017
## Removed features with more than 0 NAs: Tue Apr 18 21:48:00 2017
## Dropped featureData's levels Tue Apr 18 21:48:00 2017
## MSnbase version: 2.0.2

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

## Combined 1504 features into 1504 using user-defined function

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

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")

```

```

cls <- brewer.pal(5, "Set1")
matplot(t(tail(exprs(protqnt), n = 5)), type = "b",
        lty = 1, col = cls,
        ylab = "Protein intensity (summed peptides)",
        xlab = "TMT reporters")
legend("topright", tail(featureNames(protqnt), n=5),
       lty = 1, bty = "n", cex = .8, col = cls)

```

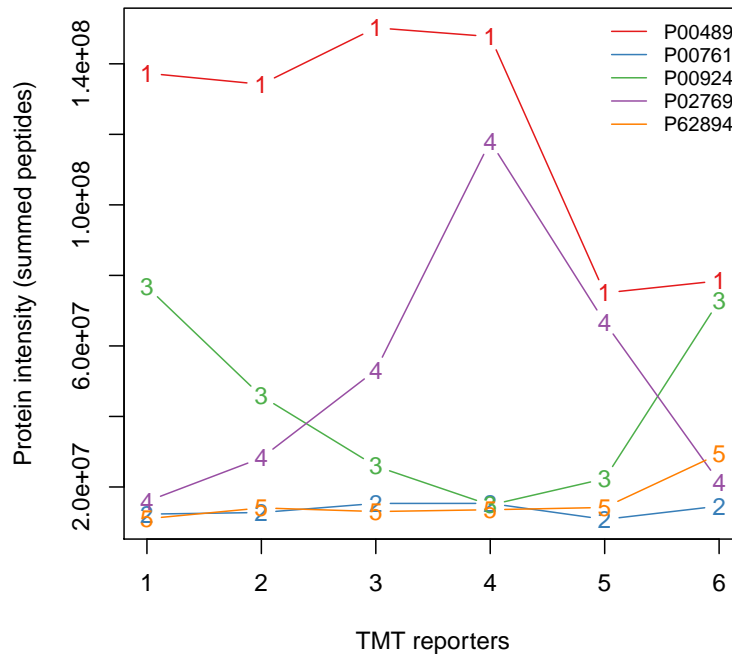


Figure 2: Protein quantitation data.

```

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))
wbccl <- colorRampPalette(c("white", "darkblue"))(256)

```

4.2 Third-party data

It is possible to import any arbitrary text-based spreadsheet as *MSnSet* object using either `readMSnSet` or `readMSnSet2`. The former takes three spreadsheets as input (for the expression data and the feature and sample meta-data). The latter

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

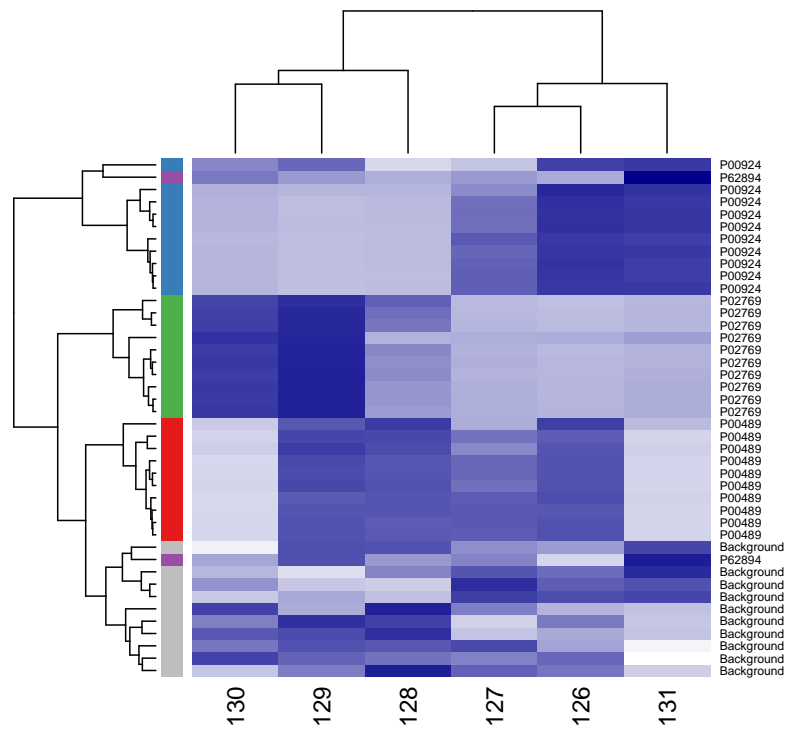
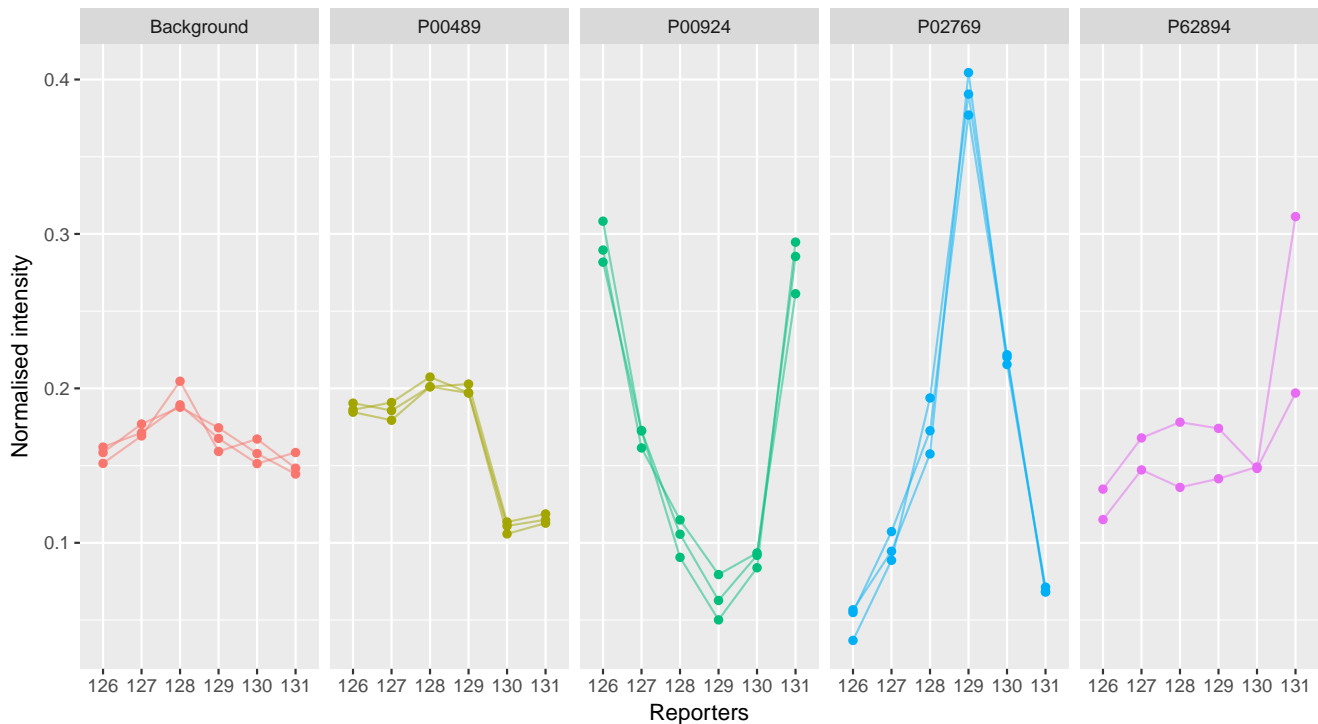


Figure 3: A heatmap.

uses a single spreadsheet and a vector of expression columns to populate the assay data and the feature meta-data. Detailed examples are provided in the MSnbase-io vignette, that can be consulted from R with `vignette("MSnbase-io")` or online²⁰.

²⁰<http://bioconductor.org/packages/release/bioc/vignettes/MSnbase/inst/doc/MSnbase-io.pdf>

```
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 = "Normalised intensity")
```

Figure 4: Spikes plot using *ggplot2*.

4.3 Working with raw data

We reuse our dedicated px1 ProteomeXchange data object to download the raw data (in mzXML format) and load it with the readMSData from the *MSnbase* package that produces a raw data experiment object of class MSnExp (a new readMSData2 function is now available to access the raw data on disk on demand, rather than loading it all in memory, enabling the management of more and larger files - see the benchmarking vignette in the *MSnbase* package for details). The raw data is then quantified using the quantify method specifying the TMT 6-plex isobaric tags and a 7th peak of interest corresponding to the un-dissociated reporter tag peaks (see the MSnbase-demo vignette in *MSnbase* for details).

```
mzxml <- pxget(px1, "TMT_Erwinia_1uLSike_Top10HCD_isol2_45stepped_60min_01.mzXML")
## Downloading 1 file

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

## Using default parallel backend: MulticoreParam
## Original MSnExp and new MSnSet have different number of samples in phenoData. Dropping original.
## Creating 'MSnSet' object

qntms
## MSnSet (storageMode: lockedEnvironment)
## assayData: 6103 features, 7 samples
## element names: exprs
## protocolData: none
## phenoData
## sampleNames: TMT7.126 TMT7.127 ... TMT7.230 (7
## total)
## varLabels: mz reporters
## varMetadata: labelDescription
## featureData
## featureNames: X0001.1 X0002.1 ... X6103.1 (6103
## total)
## fvarLabels: spectrum file ... collision.energy (12
## total)
## fvarMetadata: labelDescription
## experimentData: use 'experimentData(object)'
## Annotation: No annotation
## - - - Processing information - - -
## Data loaded: Tue Apr 18 21:50:09 2017
## TMT7 quantification by max: Tue Apr 18 21:55:40 2017
## MSnbase version: 2.0.2
```

Identification data in the mzIdentML format can be added to MSnExp or MSnSet instances with the addIdentificationData function. See the function documentation for examples.

```
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("")
```

```
mzp
```

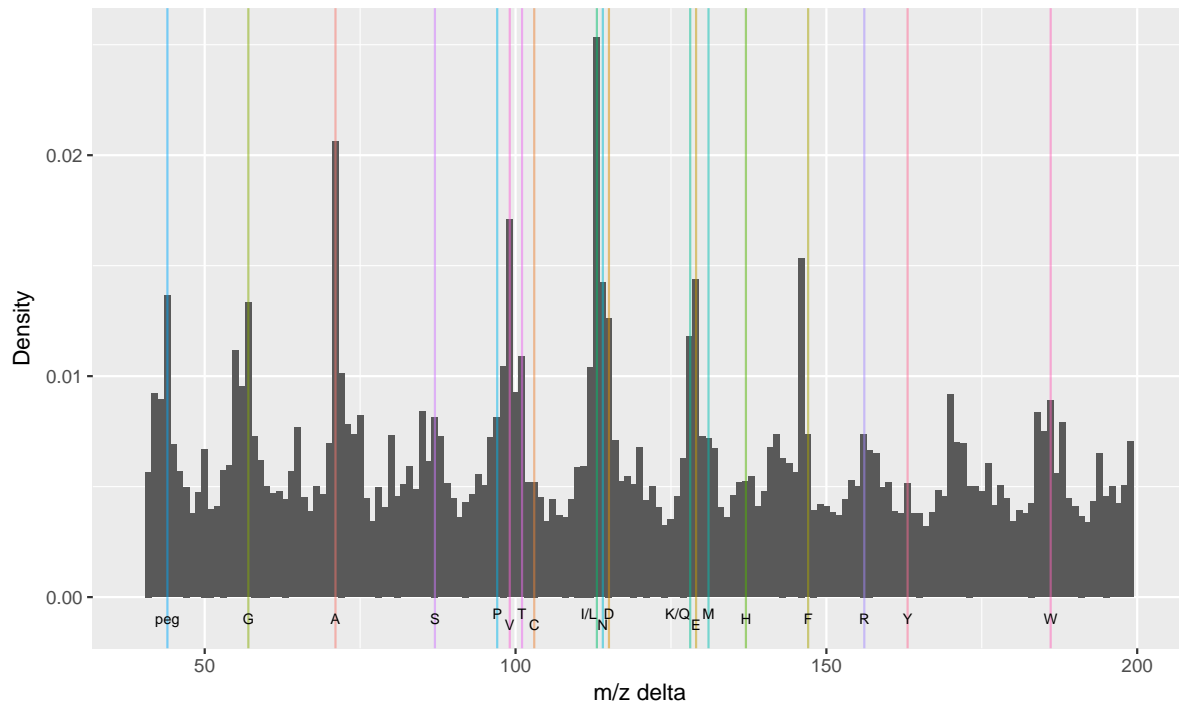


Figure 5: A m/z delta plot.

```
plot(Signal ~ Incomplete, data = d,  
     xlab = expression(Incomplete~dissociation),  
     ylab = expression(Sum~of~reporters~intensities),  
     pch = 19,  
     col = "#4582B380")  
grid()  
abline(0, 1, lty = "dotted")  
abline(lm(Signal ~ Incomplete, data = d), col = "darkblue")
```

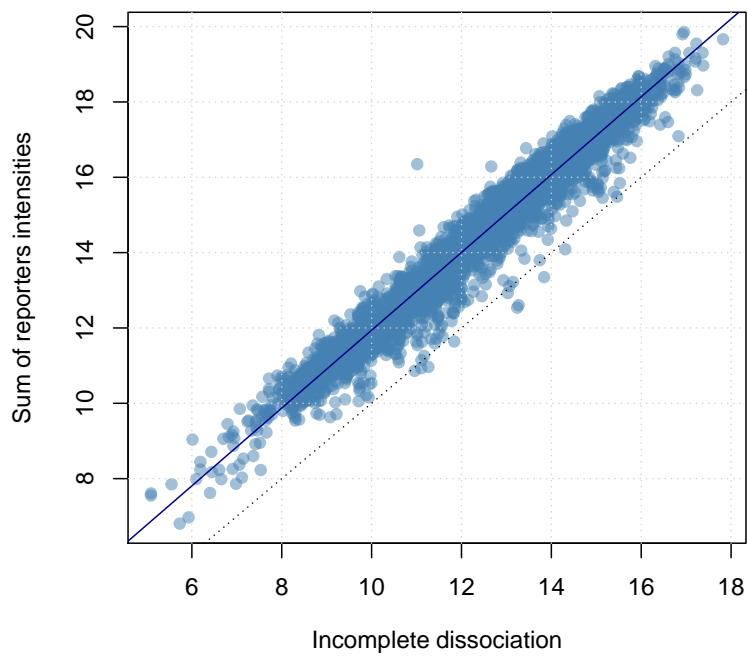


Figure 6: Incomplete dissociation.

```
Mplot(qnt[, c(4, 2)], cex = .9, col = cls, pch = pch, show.statistics = FALSE)
```

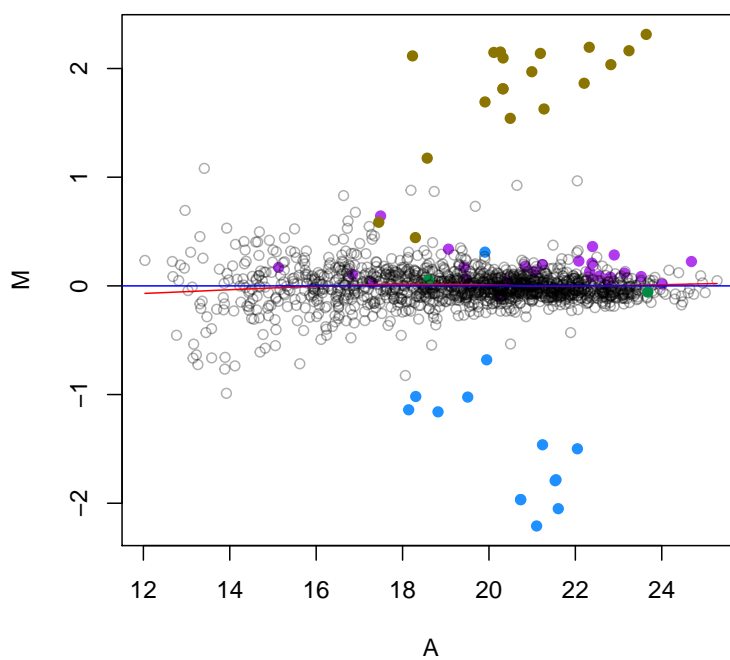


Figure 7: MAplot on an MSnSet instance.

4.4 The MALDIquant package

This section illustrates some of *MALDIquant*'s data processing capabilities [11]. The code is taken from the `processing-peaks.R` script downloaded from the package homepage²¹.

Loading the data

```
## load packages
library("MALDIquant")
library("MALDIquantForeign")
## getting test data
datapath <-
  file.path(system.file("Examples",
                        package = "readBrukerFlexData"),
            "2010_05_19_Gibb_C8_A1")
dir(datapath)
## [1] "O_A1" "O_A2"

sA1 <- importBrukerFlex(datapath, verbose=FALSE)
# in the following we use only the first spectrum
s <- sA1[[1]]

summary(mass(s))
##      Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
## 999.9  2373.0  4331.0  4721.0  6874.0 10000.0

summary(intensity(s))
##      Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
##      4      180     1562    2841    4656    32590

head(as.matrix(s))
##           mass intensity
## [1,] 999.9388     11278
## [2,] 1000.1316     11350
## [3,] 1000.3244     10879
## [4,] 1000.5173     10684
## [5,] 1000.7101     10740
## [6,] 1000.9030     10947
```

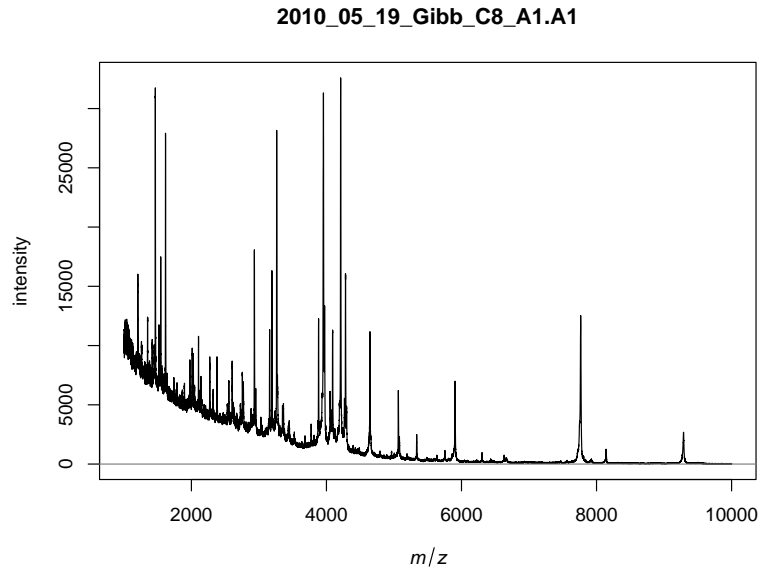
Preprocessing

```
## sqrt transform (for variance stabilization)
s2 <- transformIntensity(s, method="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
## Memory usage           : 359.875 KiB
## Name                   : 2010_05_19_Gibb_C8_A1.A1
```

²¹<http://strimmerlab.org/software/malDIquant/>

```
plot(s)
```



/home/lg390/R/x86_64-pc-linux-gnu-library/3.3/readBrukerFlexData/Examples/2010_05_19_Gibb_C8_A1/0_A1/1/1SLin

Figure 8: Spectrum plotting in *MALDIquant*.

```
## File : /home/lg390/R/x86_64-pc-linux-gnu-library/3.3/readBrukerFlexData/Examples/20
## smoothing - 5 point moving average
s3 <- smoothIntensity(s2, method="MovingAverage", halfWindowSize=2)
s3
## S4 class type : MassSpectrum
## Number of m/z values : 22431
## Range of m/z values : 999.939 - 10001.925
## Range of intensity values: 3.606e+00 - 1.792e+02
## Memory usage : 359.875 KiB
## Name : 2010_05_19_Gibb_C8_A1.A1
## File : /home/lg390/R/x86_64-pc-linux-gnu-library/3.3/readBrukerFlexData/Examples/20
## baseline subtraction
s4 <- removeBaseline(s3, method="SNIP")
s4
## S4 class type : MassSpectrum
## Number of m/z values : 22431
## Range of m/z values : 999.939 - 10001.925
## Range of intensity values: 0e+00 - 1.404e+02
## Memory usage : 359.875 KiB
## Name : 2010_05_19_Gibb_C8_A1.A1
## File : /home/lg390/R/x86_64-pc-linux-gnu-library/3.3/readBrukerFlexData/Examples/20
```

Peak picking

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

```

length(p) # 181
## [1] 186
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)

```

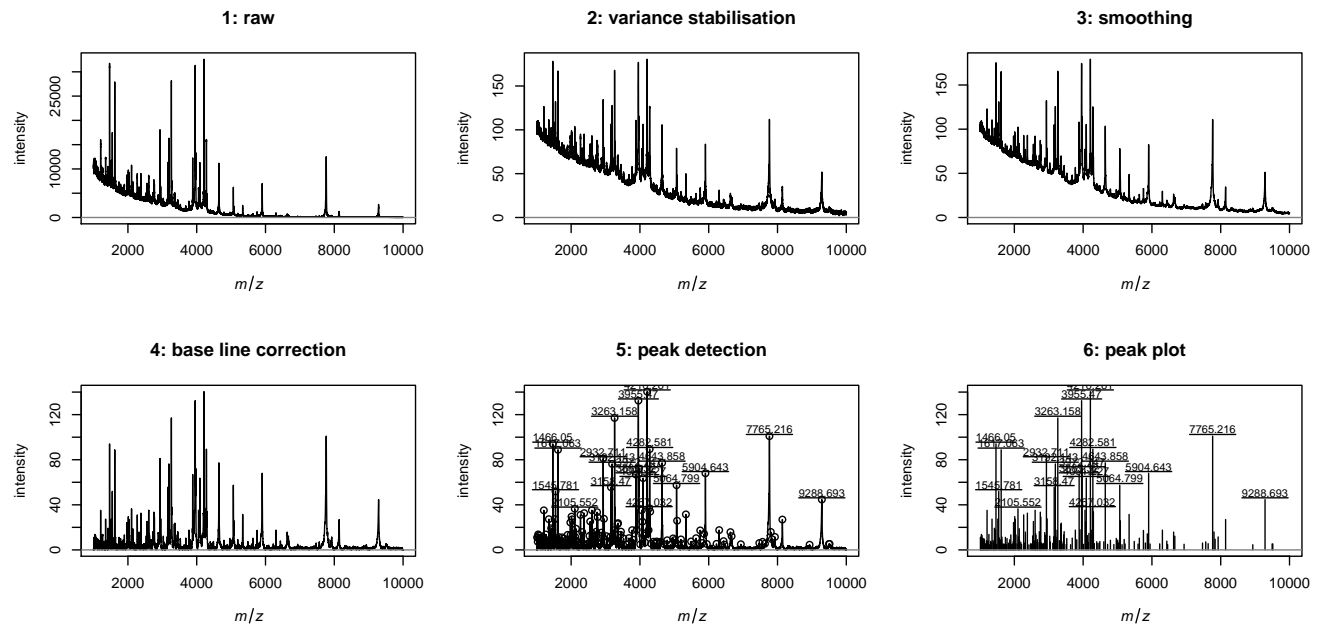


Figure 9: Spectrum plotting in *MALDIquant*.

4.5 Working with peptide sequences

```

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

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

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

pepmol

## $formula
## [1] "C77H129N23O27S"
##
## $score
## [1] 1
##
## $exactmass
## [1] 1839.915
##
## $charge
## [1] 0
##
## $parity
## [1] "e"
##
## $valid
## [1] "Valid"
##
## $DBE
## [1] 25
##
## $isotopes
## $isotopes[[1]]
##           [,1]      [,2]      [,3]      [,4]
## [1,] 1839.9148973 1840.9177412 1841.9196777 1.842921e+03
## [2,]  0.3427348   0.3353456   0.1960976 8.474135e-02
##           [,5]      [,6]      [,7]      [,8]
## [1,] 1.843923e+03 1.844925e+03 1.845927e+03 1.846928e+03
## [2,] 2.952833e-02 8.691735e-03 2.226358e-03 5.066488e-04
##           [,9]      [,10]
## [1,] 1.847930e+03 1.848932e+03
## [2,] 1.040196e-04 1.949686e-05

##
library(OrgMassSpecR)
data(itraqdata)

simplottest <-
  itraqdata[featureNames(itraqdata) %in% paste0("X", 46:47)]
sim <- SpectrumSimilarity(as(simplottest[[1]], "data.frame"),

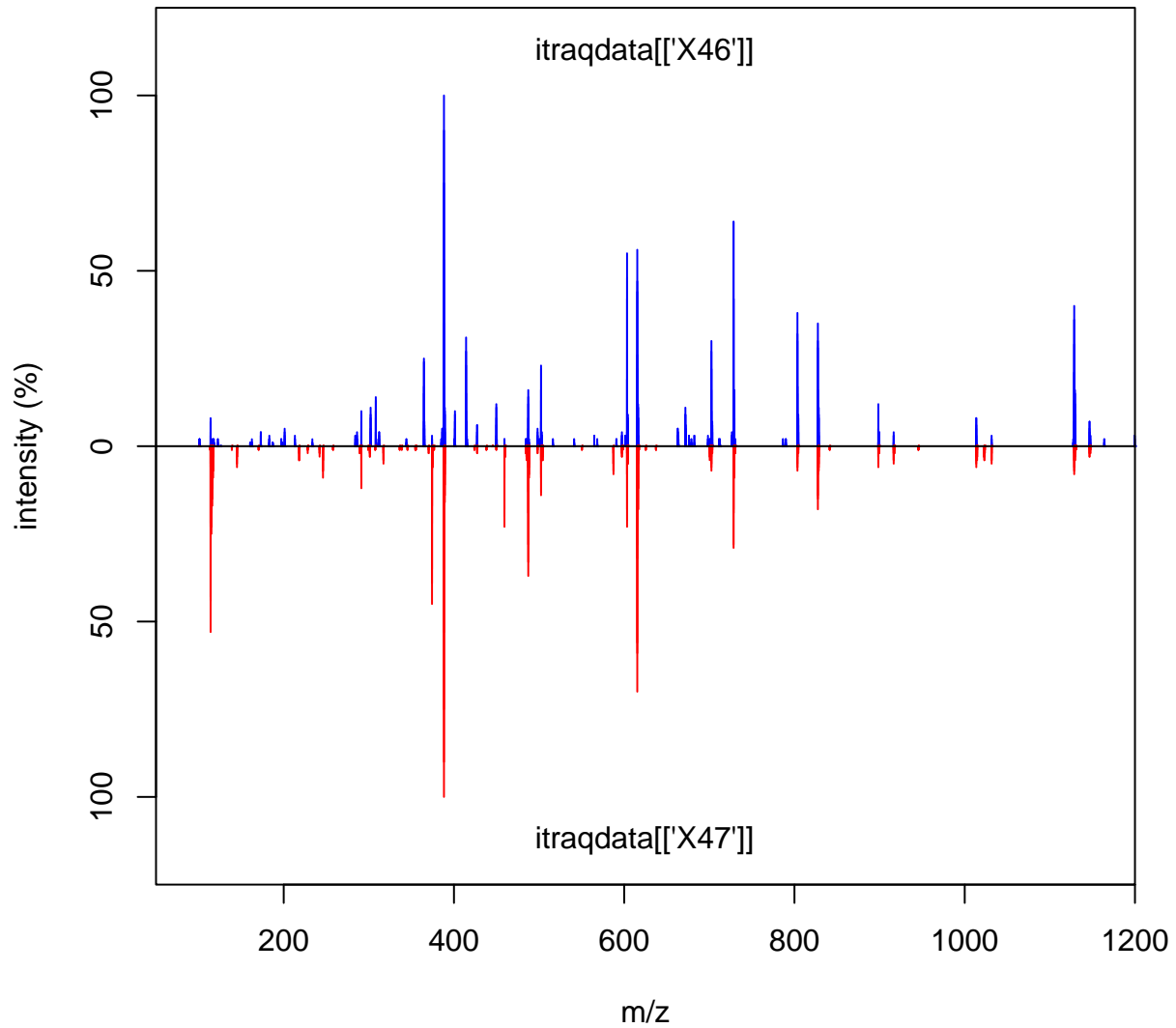
```



```
as(simplottest[[2]], "data.frame"),
top.lab = "itraqdata[['X46']]",
bottom.lab = "itraqdata[['X47']]",
b = 25)

##           mz intensity.top intensity.bottom
## 1  114.1091           0           44
## 2  114.1109           0           53
## 3  114.1127           0           43
## 4  115.1085           0           25
## 5  364.7215          25            0
## 6  374.2082           0           39
## 7  374.2191           0           45
## 8  374.2301           0           35
## 9  388.2442           0           35
## 10 388.2558           0           75
## 11 388.2673           0          100
## 12 388.2789           0           90
## 13 388.2904           35           53
## 14 388.2904          100           53
## 15 388.2904           90           53
## 16 388.2904           53           53
## 17 388.2904           75           53
## 18 414.2582           31            0
## 19 414.2709           27            0
## 20 487.2887           0           33
## 21 487.3050           0           37
## 22 487.3213           0           28
## 23 603.3339           42            0
## 24 603.3563           55            0
## 25 603.3787           48            0
## 26 603.4011           27            0
## 27 615.3124           0           28
## 28 615.3354           0           56
## 29 615.3585           0           70
## 30 615.3816           0           59
## 31 615.4047           26           32
## 32 615.4047           44           32
## 33 615.4047           56           32
## [ reached getOption("max.print") -- omitted 17 rows ]

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

Spectrum similarity 0.422

```
MonoisotopicMass(formula = list(C = 2, O = 1, H=6))  
## [1] 46.04186  
molecule <- getMolecule("C2H5OH")  
molecule$exactmass  
## [1] 46.04186  
## x11()  
## plot(t(.pepmol$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(moleculeIsotopes[[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))

if (!file.exists("P00924.fasta"))
  eno <- download.file("http://www.uniprot.org/uniprot/P00924.fasta",
                      destfile = "P00924.fasta")
eno <- paste(readLines("P00924.fasta")[-1], collapse = "")
eno pep <- Digest(eno, missed = 1)
nrow(eno pep) ## 103
## [1] 103
sum(nchar(eno pep$peptide) >= minlength) ## 68
## [1] 0
pepcnt <- eno pep[eno pep[, 1] %in% exppep, ]
nrow(pepcnt) ## 13
## [1] 0
```

The following code chunks demonstrate how to use the *cleaver* package for in-silico cleavage of polypeptides, e.g. cleaving of *Gastric juice peptide 1 (P01358)* using *Trypsin*:

```
library(cleaver)
cleave("LAAGKVEDSD", enzym = "trypsin")
## $LAAGKVEDSD
## [1] "LAAGK" "VEDSD"
```

Sometimes cleavage is not perfect and the enzyme miss some cleavage positions:

```
## miss one cleavage position
cleave("LAAGKVEDSD", enzym = "trypsin", missedCleavages = 1)
## $LAAGKVEDSD
## [1] "LAAGKVEDSD"

## miss zero or one cleavage positions
cleave("LAAGKVEDSD", enzym = "trypsin", missedCleavages = 0:1)
## $LAAGKVEDSD
## [1] "LAAGK" "VEDSD" "LAAGKVEDSD"
```

Example code to generate an *Texshade* image to be included directly in a *Latex* document or *R* vignette is presented below. The *R* code generates a *Texshade* environment and the annotated sequence display code that is written to a *TeX* file that can itself be included into a *LaTeX* of *Sweave* document.

```
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 = seqfile)
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 = seqfile, 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 = seqfile, append = TRUE)

```

¹⁵N incorporation

```
## 15N incorporation rates from 0, 0.1, ..., 0.9, 0.95, 1
incrate <- c(seq(0, 0.9, 0.1), 0.95, 1)
inc <- lapply(incrate, function(inc)
  IsotopicDistributionN("YEVQGEVFTKQLWP", 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 ", incrate[i]*100, "%"))
```

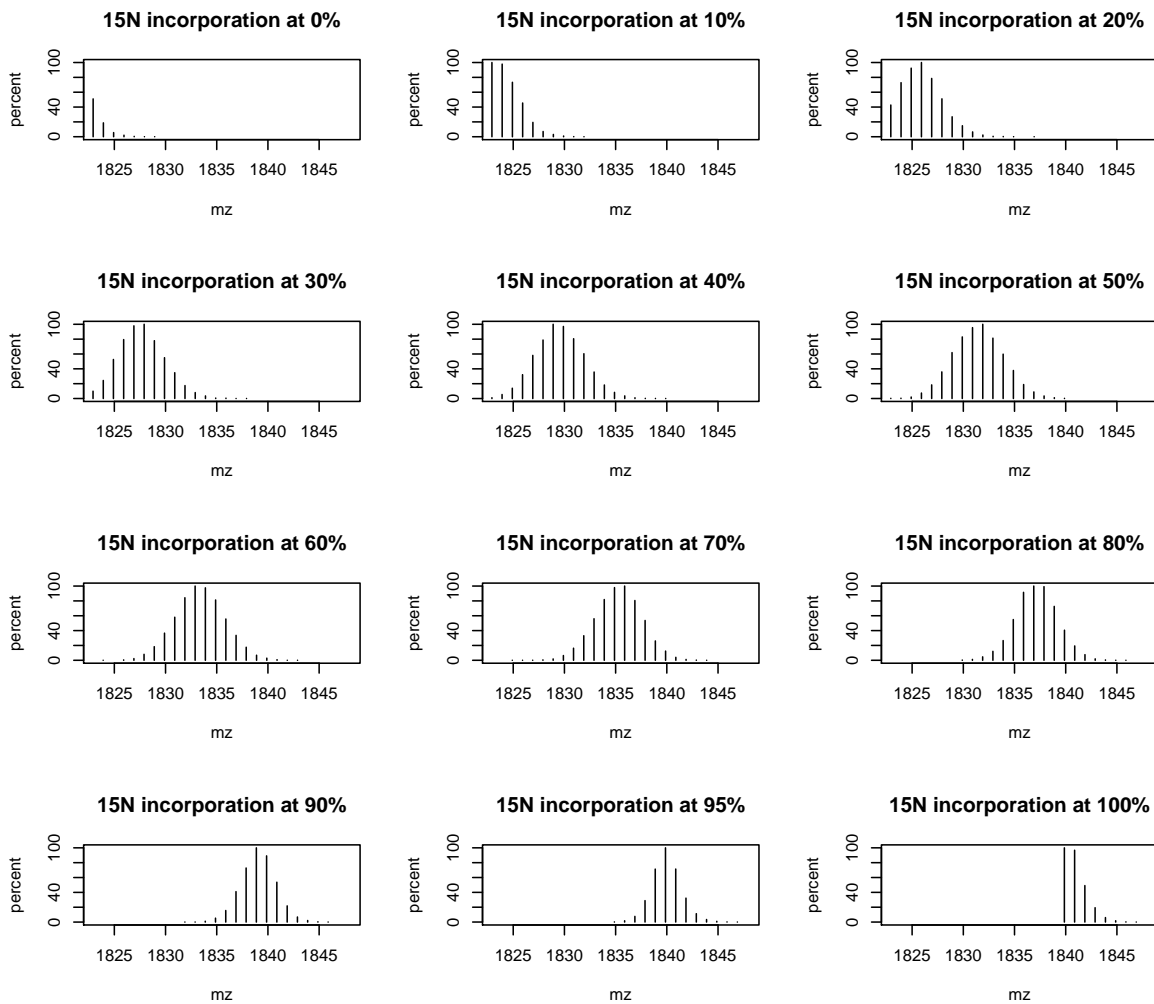


Figure 10: Isotopic envelope for the YEVQGEVFTKQLWP peptide at different ^{15}N incorporation rates.

4.6 The isobar package

The *isobar* package [12] provides methods for the statistical analysis of isobarically tagged MS² experiments.

```
library(isobar)

## Prepare the PXD000001 data for isobar analysis
.ions <- exprs(qnt)
.mass <- matrix(TMT6@mz, nrow(qnt), byrow=TRUE, ncol = 6)
colnames(.ions) <- colnames(.mass) <-
  reporterTagNames(new("TMT6plexSpectra"))
rownames(.ions) <- rownames(.mass) <-
  paste(fData(qnt)$accession, fData(qnt)$sequence, sep = ".")
pgtbl <- data.frame(spectrum = rownames(.ions),
                   peptide = fData(qnt)$sequence,
                   modif = ":",
                   start.pos = 1,
                   protein = fData(qnt)$accession,
                   accession = fData(qnt)$accession)
x <- new("TMT6plexSpectra", pgtbl, .ions, .mass)

## data.frame columns OK
## Creating ProteinGroup ...
## done

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

x <- correctIsotopeImpurities(x) ## using identity matrix here
## LOG: isotopeImpurities.corrected: TRUE

x <- normalize(x, per.file = FALSE)
## LOG: is.normalized: TRUE
## LOG: normalization.multiplicative.factor channel 126: 0.8846
## LOG: normalization.multiplicative.factor channel 127: 0.9244
## LOG: normalization.multiplicative.factor channel 128: 1
## LOG: normalization.multiplicative.factor channel 129: 0.9421
## LOG: normalization.multiplicative.factor channel 130: 0.8593
## LOG: normalization.multiplicative.factor channel 131: 0.889

## spikes
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] 7.306091e-02 1.140614e+04 3.489853e+00

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

## Creating ProteinGroup ...
## done

nm.background <- NoiseModel(ib.background)
## [1] 0.01425222 3.49812516 0.89685036

ib.spks <- subsetIBSpectra(x, protein = spks,
                           direction="include",
                           specificity="reporter-specific")

## Creating ProteinGroup ... done

nm.spks <- NoiseModel(ib.spks, one.to.one=FALSE, pool=TRUE)
## 4 proteins with more than 10 spectra, taking top 50.
## [1] 0.0000000001 6.1927071539 0.6721054619

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

res <- estimateRatio(x, nm,
                   channel1="127", channel2="129",
                   protein = unique(fData(x)$proteins),
                   combine = FALSE,
                   sign.level = 0.01)[, c(1, 2, 6, 8)]
res <- as.data.frame(res)
res$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
pch3[grep("P02769", rownames(res))] <- 19
pch3[grep("P00924", rownames(res))] <- 19
pch3[grep("P62894", rownames(res))] <- 19
pch3[grep("P00489", rownames(res))] <- 19

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

```

```
maplot(x,  
  noise.model = c(nm.background, nm.spks, nm),  
  channel1="127", channel2="129",  
  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 = .7)
```

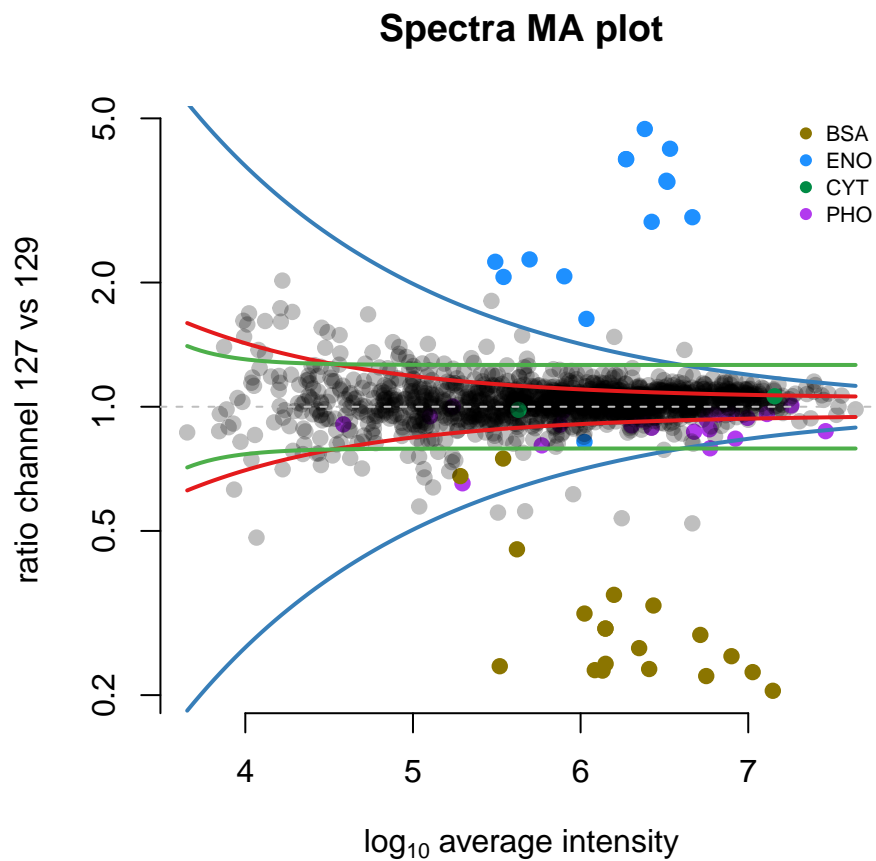


Figure 11: Result from the *isobar* pipeline.


```

                                package="rTANDEM"))
param <- setParamValue(param, 'output', 'path',
                        value = paste(getwd(),
                                      "output.xml", sep="/"))

```

5.1.2 Performing the search

The analysis is run using the `tandem` function (see also the `rtandem` function), which returns the results data file path (only the file name is displayed below).

```

resultPath <- tandem(param)

## Loading spectra
## (mgf). loaded.
## Spectra matching criteria = 242
## Starting threads . started.
## Computing models:
## testin
## sequences modelled = 5 ks
## Model refinement:
## partial cleavage ..... done.
## unanticipated cleavage ..... done.
## modified N-terminus ..... done.
## finishing refinement ... done.
## Creating report:
## initial calculations ..... done.
## sorting ..... done.
## finding repeats ..... done.
## evaluating results ..... done.
## calculating expectations ..... done.
## writing results ..... done.
##
## Valid models = 40
## Unique models = 41
## Estimated false positives = 1 +/- 1

basename(resultPath)

## [1] "output.2017_04_18_21_56_48.t.xml"

```

5.1.3 Import and analyse results

```

res <- GetResultsFromXML(resultPath)
## the inferred proteins
proteins <- GetProteins(res,
                        log.expect = -1.3,
                        min.peptides = 2)
proteins[, -(4:5), with = FALSE]

##      uid expect.value  label description num.peptides
## 1:  576      -27.2 YCR012W   YCR012W         5
## 2: 1811      -14.5 YFR053C   YFR053C         3
## 3: 2301      -12.8 YGR254W   YGR254W         3
## 4:   4       -12.0 YAL005C   YAL005C         3

```

```
## 5: 3517      -12.0 YLL024C      YLL024C      3
## 6: 3328      -10.3 YKL152C      YKL152C      2
## 7: 3386      -10.1 YKL216W      YKL216W      2
## 8: 2281      -7.9  YGR234W      YGR234W      2
## 9: 2568      -7.5  YHR174W      YHR174W      2
## 10: 2044     -7.1  YGL253W      YGL253W      2

## the identified peptides for YFR053C
peptides <- GetPeptides(protein.uid = 1811,
                        results = res,
                        expect = 0.05)
peptides[, c(1:4, 9, 10:16), with = FALSE]

##      pep.id prot.uid spectrum.id spectrum.mh expect.value
## 1: 102.1.1   1811         102    942.5147    0.00660
## 2: 250.1.1   1811         250   1212.5610    0.00043
## 3: 60.1.1    1811          60    863.4933    0.00870
##      tandem.score      mh      delta peak.count
## 1:          31.9  942.5370 -0.0220      NA
## 2:          35.0 1212.5531  0.0079      NA
## 3:          21.7  863.4985 -0.0052      NA
##      missed.cleavages start.position end.position
## 1:           0           166           173
## 2:           0           437           447
## 3:           0           309           315
```

More details are provided in the vignette available with (`vignette("rTANDEM")`), for instance the extraction of degenerated peptides, i.e. peptides found in multiple proteins.

The *shinyTANDEM* package offers a web-based graphical interface to *rTANDEM*.

5.2 MS-GF+

With the release of Bioconductor 3.0 the *MSGFplus* package has provided an interface to MS-GF+ [15, 16]. The package vignette describe in detail the different ways an MS-GF+ analysis can be initiated and only a simple example will be given here:

5.2.1 Preparation of the input data

```
library("MSGFplus")
## Create a parameter object with a set of parameters
param <- msgfPar(database = system.file('extdata',
                                       'milk-proteins.fasta',
                                       package='MSGFplus'),
                tolerance = '10 ppm',
                enzyme = 'Trypsin')

## Add parameters after creation
instrument(param) <- 'QEactive'
tda(param) <- TRUE
ntt(param) <- 2

## Add expected modifications
```

```

mods(param)[[1]] <- msgfParModification('Carbamidomethyl',
                                       composition = 'C2H3N1O1',
                                       residues = 'C',
                                       type = 'fix',
                                       position = 'any')

mods(param)[[2]] <- msgfParModification(name = 'Oxidation',
                                       mass = 15.994915,
                                       residues = 'M',
                                       type = 'opt',
                                       position = 'any')

nMod(param) <- 2 # Number of allowed modifications per peptide

## Get a summary of your parameters
show(param)

## An msgfPar object
##
## Database: /home/lg390/R/x86_64-pc-linux-gnu-library/3.3/MSGFplus/extdata/milk-protein
## Tolerance: 10 ppm
## TDA: TRUE
## Instrument: 3: QExactive
## Enzyme: 1: Trypsin
## No. tolerable termini: 2
##
## Modifications:
##
## Number of modifications per peptide: 2
##
## Carbamidomethyl: C2H3N1O1, C, fix, any
## Oxidation: 15.99492, M, opt, any

```

5.2.2 Performing the search

Initiating the search is done using the `runMSGF` method. As a minimum it takes a parameter object and a list of raw data files and performs the search for each data file in sequence. More specialised operations are also possible such as running it asynchronously, but interested readers should refer to the *MSGFplus* vignette for additional information.

The first time a search is initialised the MS-GF+ code is downloaded, so be sure to have an active internet connection (only applies to the first time a search is run).

```
result <- runMSGF(param, 'path/to/a/rawfile.mzML')
```

5.2.3 Import and analyse results

By default MSGFplus imports the results automatically using *mzID*. If only one file was analysed, the return value is an *mzID* object; if multiple files are analysed at once the return value is an *mzIDCollection* object.

If `import=FALSE` the results are not imported and can be accessed at a later time using the *mzID* package (see section 2.2 on page 8).

5.2.4 Running MS-GF+ through a GUI

MSGFplus comes with a sister package, *MSGFgui*, which provide a graphic interface to setting up and running MS-GF+ through R. Besides facilitating MS-GF+ analyses, which is arguably just as easy from the command line, it provides an intuitive way to investigate and evaluate the resulting identification data.

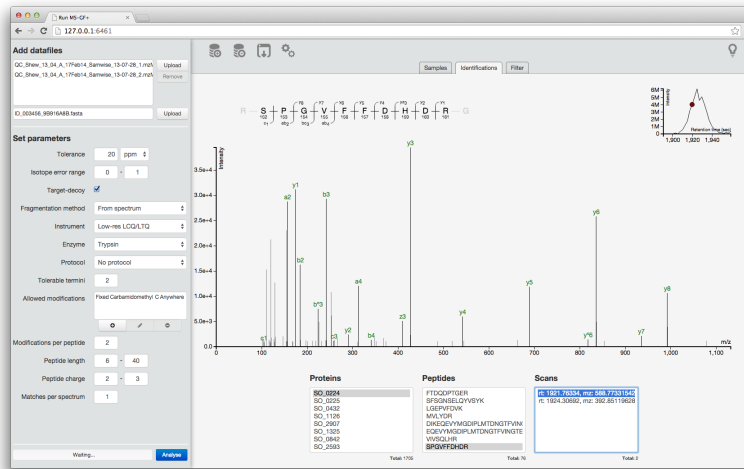


Figure 12: A screenshot of MSGFgui

Figure 12 shows an example of using *MSGFgui*. It is possible to gradually drill down in the results starting from the protein level and ending at the raw spectrum level. *mzIdentML* files already created with MS-GF+ (using *MSGFplus* or in other ways) can easily be imported into the gui to take advantage of the visualisation features, and results can be exported as either *rds* (for R), *xlsx* (for excel) or *txt* (for everything else) files.

5.3 Post-search Filtering of MS/MS IDs Using MSnID

The main purpose of *MSnID* package is to make sure that the peptide and protein identifications resulting from MS/MS searches are sufficiently confident for a given application. MS/MS peptide and protein identification is a process that prone to uncertainties. A typical and currently most reliable way to quantify uncertainty in the list of identify spectra, peptides or proteins relies on so-called decoy database. For bottom-up (i.e. involving protein digestion) approaches a common way to construct a decoy database is simple inversion of protein amino-acid sequences. If the spectrum matches to normal protein sequence it can be true or false match. Matches to decoy part of the database are false only (excluding the palindromes). Therefore the false discovery rate (FDR) of identifications can be estimated as ratio of hits to decoy over normal parts of the protein sequence database. There are multiple levels of identification that FDR can be estimated for. First, is at the level of peptide/protein- to-spectrum matches. Second is at the level of unique peptide sequences. Note, true peptides tend to be identified by more then one spectrum. False peptide tend to be sporadic. Therefore, after collapsing the redundant peptide identifications from multiple spectra to the level of unique peptide sequence, the FDR typically increases. The extend of FDR increase depends on the type and complexity of the sample. The same trend is true for estimating the identification FDR at the protein level. True proteins tend to be identified with multiple peptides, while false protein identifications are commonly covered only by one peptide. Therefore FDR estimate tend to be even higher for protein level compare to peptide level. The estimation of the FDR is also affected by the number of LC-MS (runs) datasets in the experiment. Again, true identifications tend to be more consistent from run to run, while false are sporadic. After collapsing the redundancy across the runs, the number of true identification reduces much stronger compare to false identifications. Therefore, the peptide and protein FDR estimates need to be re-evaluated. The main objective of the *MSnID* package is to provide convenience tools for handling tasks on estimation of FDR, defining and optimizing the filtering criteria and ensuring confidence in MS/MS identification data. The user can specify the criteria for filtering the data (e.g. goodness or p-value of matching of experimental and theoretical fragmentation mass spectrum,

deviation of theoretical from experimentally measured mass, presence of missed cleavages in the peptide sequence, etc), evaluate the performance of the filter judging by FDRs at spectrum, peptide and protein levels, and finally optimize the filter to achieve the maximum number of identifications while not exceeding maximally allowed FDR upper threshold.

5.3.1 Starting Project & Importing Data

To start a project one have to specify a directory. Currently the only use of the directory is for storing cached results.

```
library("MSnID")

##
## Attaching package: 'MSnID'
## The following object is masked from 'package:isobar':
##
## peptides
## The following object is masked from 'package:ProtGenerics':
##
## peptides

msnid <- MSnID(".")

## Note, the anticipated/suggested columns in the
## peptide-to-spectrum matching results are:
## -----
## accession
## calculatedMassToCharge
## chargeState
## experimentalMassToCharge
## isDecoy
## peptide
## spectrumFile
## spectrumID
```

Data can imported as data.frame or read from mzIdentML file.

```
PSMresults <- read.delim(system.file("extdata", "human_brain.txt",
                                   package="MSnID"),
                        stringsAsFactors=FALSE)

psms(msnid) <- PSMresults
show(msnid)

## MSnID object
## Working directory: "."
## #Spectrum Files: 1
## #PSMs: 767 at 49 % FDR
## #peptides: 687 at 57 % FDR
## #accessions: 665 at 65 % FDR

mzids <- system.file("extdata", "c_elegans.mzid.gz", package="MSnID")
msnid <- read_mzIDs(msnid, mzids)

## Reading from mzIdentMLs ...
## reading c_elegans.mzid.gz... DONE!
show(msnid)

## MSnID object
```

```
## Working directory: "."
## #Spectrum Files: 1
## #PSMs: 12263 at 36 % FDR
## #peptides: 9489 at 44 % FDR
## #accessions: 7414 at 76 % FDR
```

5.3.2 Analysis of Peptide Sequences

A particular properties of peptide sequences we are interested in are

1. irregular cleavages at the termini of the peptides and
2. missing cleavage site within the peptide sequences.

A particular properties of peptide sequences we are interested in are (1) irregular cleavages at the termini of the peptides and (2) missing cleavage site within the peptide sequences:

- Counting the number of irregular cleavage termimi (0, 1 or 2) in peptides sequence creates a new column `numIrregCleavages`.
- Counting the number of missed cleavages in peptides sequence correspondingly creates a `numMissCleavages` column.

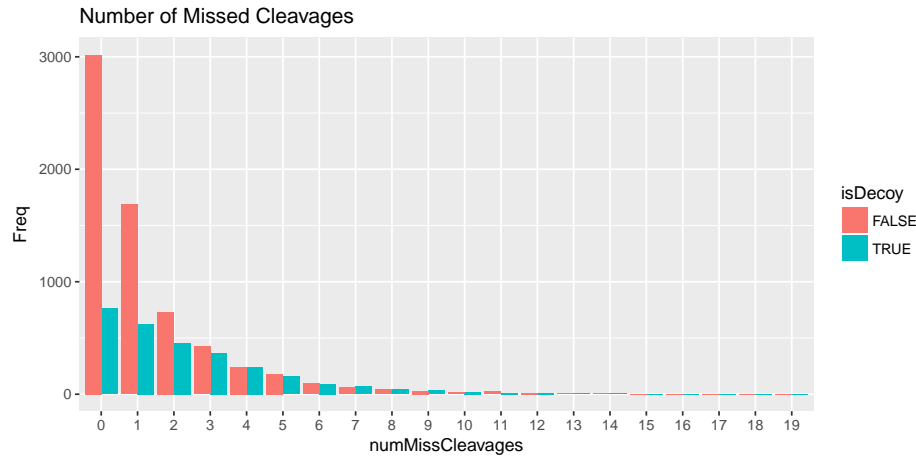
The default regular expressions for the `validCleavagePattern` and `missedCleavagePattern` correspond to trypsin specificity.

```
msnid <- assess_termini(msnid, validCleavagePattern="[KR]\\. [^P]")
msnid <- assess_missed_cleavages(msnid, missedCleavagePattern="[KR] (?=[^P$])")
prop.table(table(msnid$numIrregCleavages))

##
##          0          1          2
## 0.801574390 0.189294149 0.009131462
```

Now the object has two more columns, `numIrregCleavages` and `numMissCleavages`, evidently corresponding to the number of termini with irregular cleavages and number of missed cleavages within the peptide sequence. The figure below shows that peptides with 2 or more missed cleavages are likely to be false identifications.

```
pepClev <- unique(psms(msnid)[,c("numMissCleavages", "isDecoy", "peptide")])
pepClev <- as.data.frame(table(pepClev[,c("numMissCleavages", "isDecoy")]))
library("ggplot2")
ggplot(pepClev, aes(x=numMissCleavages, y=Freq, fill=isDecoy)) +
  geom_bar(stat='identity', position='dodge') +
  ggtitle("Number of Missed Cleavages")
```



5.3.3 Defining the Filter

The criteria that will be used for filtering the MS/MS data has to be present in the `MSnID` object. We will use $-\log_{10}$ transformed MS-GF+ Spectrum E-value, reflecting the goodness of match experimental and theoretical fragmentation patterns as one the filtering criteria. Let's store it under the "msmsScore" name. The score density distribution shows that it is a good discriminant between non-decoy (red) and decoy hits (green).

For alternative MS/MS search engines refer to the engine-specific manual for the names of parameters reflecting the quality of MS/MS spectra matching. Examples of such parameters are E-Value for X!Tandem and XCorr and $\Delta Cn2$ for SEQUEST.

As a second criterion we will be using the absolute mass measurement error (in ppm units) of the parent ion. The mass measurement errors tend to be small for non-decoy (enriched with real identification) hits (red line) and is effectively uniformly distributed for decoy hits.

```
msnid$msmsScore <- -log10(msnid$`MS-GF:SpecEValue`)
msnid$absParentMassErrorPPM <- abs(mass_measurement_error(msnid))
```

MS/MS filters are handled by a special `MSnIDFilter` class objects. Individual filtering criteria can be set by name (that is present in `names(msnid)`), comparison operator (`<`, `>`, `=`, ...) defining if we should retain hits with higher or lower given the threshold and finally the threshold value itself. The filter below set in such a way that retains only those matches that has less than 5 ppm of parent ion mass measurement error and more than 10^7 MS-GF:SpecEValue.

```
filtObj <- MSnIDFilter(msnid)
filtObj$absParentMassErrorPPM <- list(comparison="<", threshold=5.0)
filtObj$msmsScore <- list(comparison=">", threshold=8.0)
show(filtObj)

## MSnIDFilter object
## (absParentMassErrorPPM < 5) & (msmsScore > 8)
```

The stringency of the filter can be evaluated at different levels.

```
evaluate_filter(msnid, filtObj, level="PSM")

##           fdr      n
## PSM 0.00272745 5147

evaluate_filter(msnid, filtObj, level="peptide")

##           fdr      n
## peptide 0.00424371 3313
```



```
evaluate_filter(msnid, filtObj, level="accession")
##           fdr    n
## accession 0.01770658 1207
```

5.3.4 Optimizing the Filter

The threshold values in the example above are not necessarily optimal and set just be in the range of probable values. Filters can be optimized to ensure maximum number of identifications (peptide-to-spectrum matches, unique peptide sequences or proteins) within a given FDR upper limit.

First, the filter can be optimized simply by stepping through individual parameters and their combinations. The idea has been described in [17]. The resulting MSnIDFilter object can be used for final data filtering or can be used as a good starting parameters for follow-up refining optimizations with more advanced algorithms.

```
filtObj.grid <- optimize_filter(filtObj, msnid, fdr.max=0.01,
                               method="Grid", level="peptide",
                               n.iter=500)
show(filtObj.grid)
## MSnIDFilter object
## (absParentMassErrorPPM < 10) & (msmsScore > 7.8)
```

The resulting `filtObj.grid` can be further fine tuned with such optimization routines as simulated annealing or Nelder-Mead optimization.

```
filtObj.nm <- optimize_filter(filtObj.grid, msnid, fdr.max=0.01,
                              method="Nelder-Mead", level="peptide",
                              n.iter=500)
show(filtObj.nm)
## MSnIDFilter object
## (absParentMassErrorPPM < 10) & (msmsScore > 7.8)
```

Evaluate non-optimized and optimized filters.

```
evaluate_filter(msnid, filtObj, level="peptide")
##           fdr    n
## peptide 0.00424371 3313
evaluate_filter(msnid, filtObj.grid, level="peptide")
##           fdr    n
## peptide 0.009220702 3393
evaluate_filter(msnid, filtObj.nm, level="peptide")
##           fdr    n
## peptide 0.009777778 3408
```

Finally applying filter to remove predominantly false identifications.

```
msnid <- apply_filter(msnid, filtObj.nm)
show(msnid)
## MSnID object
## Working directory: "."
## #Spectrum Files: 1
## #PSMs: 5288 at 0.63 % FDR
## #peptides: 3408 at 0.98 % FDR
```

```
## #accessions: 1253 at 3.8 % FDR
```

Removing hits to decoy and contaminant sequences using the same `apply_filter` method.

```
msnid <- apply_filter(msnid, "isDecoy == FALSE")
show(msnid)

## MSnID object
## Working directory: "."
## #Spectrum Files: 1
## #PSMs: 5255 at 0 % FDR
## #peptides: 3375 at 0 % FDR
## #accessions: 1207 at 0 % FDR

msnid <- apply_filter(msnid, "!grepl('Contaminant',accession)")
show(msnid)

## MSnID object
## Working directory: "."
## #Spectrum Files: 1
## #PSMs: 5246 at 0 % FDR
## #peptides: 3368 at 0 % FDR
## #accessions: 1205 at 0 % FDR
```

5.3.5 Interface with Other Bioconductor Packages

One can extract the entire PSMs tables as `data.frame` or `data.table`

```
psm.df <- psms(msnid)
psm.dt <- as(msnid, "data.table")
```

If only interested in the non-redundant list of confidently identified peptides or proteins

```
peps <- peptides(msnid)
head(peps)

## [1] "K.AISQIQEYVDYYGGSGVQHIALNTSDIITAIEALR.A"
## [2] "K.SAGSGYLVGDSLTFVDLLVAQHTADLLAANAALLDEFQFK.A"
## [3] "K.NSIFTNVAETANGEYFWEGLEDEIADKNVDITTWLGEK.W"
## [4] "R.VFCLLDGESAEGSVWEAAAFASIYKLDNLVAIVDVNR.L"
## [5] "R.TTDSGNNTGLDLYTVDQVEHSNYVEQNFLDFIFVFR.K"
## [6] "R.KFDADGSGKLEFDEFKALVYTVANTVDKETLEKELR.E"

prots <- accessions(msnid)
head(prots)

## [1] "CE02347" "CE07055" "CE12728" "CE36358" "CE36359"
## [6] "CE36360"

prots <- proteins(msnid) # may be more intuitive than accessions
head(prots)

## [1] "CE02347" "CE07055" "CE12728" "CE36358" "CE36359"
## [6] "CE36360"
```

The *MSnID* package is aimed at providing convenience functionality to handle MS/MS identifications. Quantification *per se* is outside of the scope of the package. The only type of quantitation that can be seamlessly tied with MS/MS identification analysis is so-called *spectral counting* approach. In such an approach a peptide abundance is considered to be directly proportional to the number of matched MS/MS spectra. In its turn protein abundance is proportional to

the sum of the number of spectra of the matching peptides. The *MSnID* object can be converted to an *MSnSet* object defined in *MSnbase* that extends generic Bioconductor *eSet* class to quantitative proteomics data. The spectral count data can be analyzed with *msmsEDA*, *msmsTests* or *DESeq* packages.

```
msnset <- as(msnid, "MSnSet")
library("MSnbase")
head(fData(msnset))

##                                     peptide
## -.APPSQDVLKEIFNLYDEELD GK.I          -.APPSQDVLKEIFNLYDEELD GK.I
## -.APPSQDVLKEIFNLYDEELD GKIDGTQVGDVAR.A -.APPSQDVLKEIFNLYDEELD GKIDGTQVGDVAR.A
## -.APPTFADLGK.S                       -.APPTFADLGK.S
## -.GFQNLWFSHPR.K                      -.GFQNLWFSHPR.K
## -.GIDINHKHDR.V                       -.GIDINHKHDR.V
## -.MFSNLFIFL.V                        -.MFSNLFIFL.V
##                                     accession
## -.APPSQDVLKEIFNLYDEELD GK.I          CE01236, CE30652
## -.APPSQDVLKEIFNLYDEELD GKIDGTQVGDVAR.A CE01236, CE30652
## -.APPTFADLGK.S                       CE29443
## -.GFQNLWFSHPR.K                      CE26849
## -.GIDINHKHDR.V                       CE16650
## -.MFSNLFIFL.V                        CE21589

head(exprs(msnset))

##                                     c_elegans_A_3_1_21Apr10_Draco_10-03-04_dta.txt
## -.APPSQDVLKEIFNLYDEELD GK.I          1
## -.APPSQDVLKEIFNLYDEELD GKIDGTQVGDVAR.A 4
## -.APPTFADLGK.S                       2
## -.GFQNLWFSHPR.K                      1
## -.GIDINHKHDR.V                       2
## -.MFSNLFIFL.V                        1
```

Note, the conversion from *MSnID* to *MSnSet* uses peptides as features. The number of redundant peptide observations represent so-called spectral count that can be used for rough quantitative analysis. Summing of all of the peptide counts to a proteins level can be done with `combineFeatures` function from *MSnbase* package.

```
msnset <- combineFeatures(msnset,
                          fData(msnset)$accession,
                          redundancy.handler="unique",
                          fun="sum",
                          cv=FALSE)

## Combined 2082 features into 2082 using sum

head(fData(msnset))

##                                     peptide accession
## CE00078                             K.RLPVAPR.G   CE00078
## CE00103 K.LPNDDIGVQVSYLGEPHTFTPEQVLAALLTK.L   CE00103
## CE00134                             I.PAEVAEHLK.A   CE00134
## CE00209                             K.ALEGP GGEDAAHSENNPPR.N   CE00209
## CE00302                             K.LTYFDIHGLAEPIR.L   CE00302
## CE00318                             K.ALNALCAQLMTELADALEVLDTK.S   CE00318

head(exprs(msnset))

##                                     c_elegans_A_3_1_21Apr10_Draco_10-03-04_dta.txt
## CE00078                             4
```

```
## CE00103 3
## CE00134 4
## CE00209 8
## CE00302 2
## CE00318 11
```

6 Quality control

Quality control (QC) is an essential part of any high throughput data driven approach. Bioconductor has a rich history of QC for various genomics data and currently two packages support proteomics QC.

proteoQC provides a dedicated pipeline that will produce a dynamic and extensive html report. It uses the *rTANDEM* package to automate the generation of identification data and uses information about the experimental/replication design.

The *qcmetrics* package is a general framework to define QC metrics and bundle them together to generate html or pdf reports. It provides some ready made metrics for MS data and ¹⁵N labelled data.

7 Annotation

In this section, we briefly present some *Bioconductor* annotation infrastructure.

We start with the *hpar* package, an interface to the *Human Protein Atlas* [18, 19], to retrieve subcellular localisation information for the ENSG00000002746 ensemble gene.

```
id <- "ENSG00000105323"
library("hpar")
getHpa(id, "hpaSubcellularLoc")

##           Gene Gene.name           Main.location
## 2000 ENSG00000105323 HNRNPUL1 Nucleus but not nucleoli
##           Other.location Expression.type Reliability
## 2000                               APE Supportive
##           Main.location.GO.id Other.location.GO.id
## 2000                               GO:0005654
```

Below, we make use of the human annotation package *org.Hs.eg.db* and the Gene Ontology annotation package *GO.db* to retrieve compatible information with above.

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

## 'select()' returned 1:many mapping between keys and
## columns

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

##           ENSEMBL           GO EVIDENCE ONTOLOGY
## 4 ENSG00000105323 GO:0005634 IDA CC
## 5 ENSG00000105323 GO:0005654 IDA CC
```

```
## 6 ENSG00000105323 GO:0005654 TAS CC
## 12 ENSG00000105323 GO:0030529 IEA CC

sapply(as.list(GOTERM[ans$GO]), slot, "Term")
##          GO:0005634
##          "nucleus"
##          GO:0005654
##          "nucleoplasm"
##          GO:0005654
##          "nucleoplasm"
##          GO:0030529
## "intracellular ribonucleoprotein complex"
```

Finally, this information can also be retrieved from on-line databases using the *biomaRt* package [20].

```
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] "nucleoplasm"
## [2] "intracellular ribonucleoprotein complex"
## [3] "viral nucleocapsid"
## [4] "nucleus"
```

8 Other packages

8.1 Bioconductor packages

This section provides a complete list of packages available in the relevant *Bioconductor* version 3.4 (as of April 18, 2017) *biocView*²² categories. Tables 1, 2 and 3 represent the packages for the Proteomics (88 packages), MassSpectrometry (61 packages) and MassSpectrometryData (17 experiment packages) categories.

Package	Title	Version
ASEB	Predict Acetylated Lysine Sites	1.18.0
bioassayR	Cross-target analysis of small molecule bioactivity	1.12.1
biobroom	Turn Bioconductor objects into tidy data frames	1.6.0
biosigner	Signature discovery from omics data	1.2.4
BRAIN	Baffling Recursive Algorithm for Isotope distributioN calculations	1.20.0
Cardinal	A mass spectrometry imaging toolbox for statistical analysis	1.6.0
CellNOptR	Training of boolean logic models of signalling networks using prior knowledge networks and perturbation data.	1.20.0
ChemmineOB	R interface to a subset of OpenBabel functionalities	1.12.0
ChemmineR	Cheminformatics Toolkit for R	2.26.1
cisPath	Visualization and management of the protein-protein interaction networks.	1.14.0
cleaver	Cleavage of Polypeptide Sequences	1.12.0
clippda	A package for the clinical proteomic profiling data analysis	1.24.0
CNORdt	Add-on to CellNOptR: Discretized time treatments	1.16.0
CNORfeeder	Integration of CellNOptR to add missing links	1.14.0
CNORode	ODE add-on to CellNOptR	1.16.0
customProDB	Generate customized protein database from NGS data, with a focus on RNA-Seq data, for proteomics search.	1.14.1
DAPAR	Tools for the Differential Analysis of Proteins Abundance with R	1.6.0
deltaGseg	deltaGseg	1.14.0
EGSEA	Ensemble of Gene Set Enrichment Analyses	1.2.0
eiR	Accelerated similarity searching of small molecules	1.14.1
EWCE	Expression Weighted Celltype Enrichment	1.2.0

²²<http://www.bioconductor.org/packages/devel/BiocViews.html>

fCI	f-divergence Cutoff Index for Differential Expression Analysis in Transcriptomics and Proteomics	1.4.0
fmcsR	Mismatch Tolerant Maximum Common Substructure Searching	1.16.0
GraphPAC	Identification of Mutational Clusters in Proteins via a Graph Theoretical Approach.	1.16.0
hpar	Human Protein Atlas in R	1.16.0
iPAC	Identification of Protein Amino acid Clustering	1.18.0
IPPD	Isotopic peak pattern deconvolution for Protein Mass Spectrometry by template matching	1.22.0
isobar	Analysis and quantitation of isobarically tagged MSMS proteomics data	1.20.0
kimod	A k-tables approach to integrate multiple Omics-Data	1.2.0
LPEadj	A correction of the local pooled error (LPE) method to replace the asymptotic variance adjustment with an unbiased adjustment based on sample size.	1.34.0
MassSpecWavelet	Mass spectrum processing by wavelet-based algorithms	1.40.0
MSGFgui	A shiny GUI for MSGFplus	1.8.0
MSGFplus	An interface between R and MS-GF+	1.8.0
msmsEDA	Exploratory Data Analysis of LC-MS/MS data by spectral counts	1.12.0
msmsTests	LC-MS/MS Differential Expression Tests	1.12.0
MSnbase	Base Functions and Classes for MS-based Proteomics	2.0.2
MSnID	Utilities for Exploration and Assessment of Confidence of LC-MSn Proteomics Identifications	1.8.0
mzID	An mzIdentML parser for R	1.12.0
mzR	parser for netCDF, mzXML, mzData and mzML and mzIdentML files (mass spectrometry data)	2.8.1
PAA	PAA (Protein Array Analyzer)	1.8.0
PAnnBuilder	Protein annotation data package builder	1.38.0
Path2PPI	Prediction of pathway-related protein-protein interaction networks	1.4.0
pathview	a tool set for pathway based data integration and visualization	1.14.0
Pbase	Manipulating and exploring protein and proteomics data	0.14.0
PCpheno	Phenotypes and cellular organizational units	1.36.0
PECA	Probe-level Expression Change Averaging	1.10.0
pepXMLTab	Parsing pepXML files and filter based on peptide FDR.	1.8.0
PGA	An package for identification of novel peptides by customized database derived from RNA-Seq	1.4.0
plgem	Detect differential expression in microarray and proteomics datasets with the Power Law Global Error Model (PLGEM)	1.46.0
PLPE	Local Pooled Error Test for Differential Expression with Paired High-throughput Data	1.34.0
ppiStats	Protein-Protein Interaction Statistical Package	1.40.0
proBAMr	Generating SAM file for PSMs in shotgun proteomics data	1.8.0
PROcess	Ciphergen SELDI-TOF Processing	1.50.0
procoil	Prediction of Oligomerization of Coiled Coil Proteins	2.2.0
ProCoNA	Protein co-expression network analysis (ProCoNA).	1.12.0
proFIA	Preprocessing of FIA-HRMS data	1.0.10
pRoloc	A unifying bioinformatics framework for spatial proteomics	1.14.6
pRolocGUI	Interactive visualisation of spatial proteomics data	1.8.2
Prostar	Provides a GUI for DAPAR	1.6.1
prot2D	Statistical Tools for volume data from 2D Gel Electrophoresis	1.12.0
ProteomicsAnnotationHubData	Transform public proteomics data resources into Bioconductor Data Structures	1.4.0
proteoQC	An R package for proteomics data quality control	1.10.0
ProtGenerics	S4 generic functions for Bioconductor proteomics infrastructure	1.6.0
Pviz	Peptide Annotation and Data Visualization using Gviz	1.8.0
qcmetrics	A Framework for Quality Control	1.12.0
QuartPAC	Identification of mutational clusters in protein quaternary structures.	1.6.0
rain	Rhythmicity Analysis Incorporating Non-parametric Methods	1.8.0
RankProd	Rank Product method for identifying differentially expressed genes with application in meta-analysis	3.0.0
RCASPAR	A package for survival time prediction based on a piecewise baseline hazard Cox regression model.	1.20.0
Rchemcpp	Similarity measures for chemical compounds	2.12.0
Rcpi	Molecular Informatics Toolkit for Compound-Protein Interaction in Drug Discovery	1.10.6
readat	Functionality to Read and Manipulate SomaLogic ADAT files	1.0.0
ropls	PCA, PLS(-DA) and OPLS(-DA) for multivariate analysis and feature selection of omics data	1.6.2
ROTS	Reproducibility-Optimized Test Statistic	1.2.0
RpsiXML	R interface to PSI-MI 2.5 files	2.16.0
rpx	R Interface to the ProteomeXchange Repository	1.10.2
rTANDEM	Interfaces the tandem protein identification algorithm in R	1.14.2
sapFinder	A package for variant peptides detection and visualization in shotgun proteomics.	1.12.0
ScISI	In Silico Interactome	1.46.0
shinyTANDEM	Provides a GUI for rTANDEM	1.12.0
SLGI	Synthetic Lethal Genetic Interaction	1.34.0
SpacePAC	Identification of Mutational Clusters in 3D Protein Space via Simulation.	1.12.0
specl	specl - Prepare Peptide Spectrum Matches for Use in Targeted Proteomics	1.8.0
spliceSites	A bioconductor package for exploration of alignment gap positions from RNA-seq data	1.22.1
SWATH2stats	Transform and Filter SWATH Data for Statistical Packages	1.4.1
synapter	Label-free data analysis pipeline for optimal identification and quantitation	1.16.0
tofsims	Import, process and analysis of Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS) imaging data	1.2.0
TPP	Analyze thermal proteome profiling (TPP) experiments	3.0.3

Table 1: Packages available under the Proteomics *biocViews* category.

Package	Title	Version
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apComplex	Estimate protein complex membership using AP-MS protein data	2.40.0
BRAIN	Baffling Recursive Algorithm for Isotope distribution calculations	1.20.0
CAMERA	Collection of annotation related methods for mass spectrometry data	1.30.0
Cardinal	A mass spectrometry imaging toolbox for statistical analysis	1.6.0
cosmiq	cosmiq - COmbining Single Masses Into Quantities	1.8.0
customProDB	Generate customized protein database from NGS data, with a focus on RNA-Seq data, for proteomics search.	1.14.1
DAPAR	Tools for the Differential Analysis of Proteins Abundance with R	1.6.0
flagme	Analysis of Metabolomics GC/MS Data	1.30.0
gaga	GaGa hierarchical model for high-throughput data analysis	2.20.0
iontree	Data management and analysis of ion trees from ion-trap mass spectrometry	1.20.0
IPO	Automated Optimization of XCMS Data Processing parameters	1.0.0
isobar	Analysis and quantitation of isobarically tagged MSMS proteomics data	1.20.0
LOBSTAHS	Lipid and Oxylipin Biomarker Screening through Adduct Hierarchy Sequences	1.0.0
MAIT	Statistical Analysis of Metabolomic Data	1.8.0
MassArray	Analytical Tools for MassArray Data	1.26.0
MassSpecWavelet	Mass spectrum processing by wavelet-based algorithms	1.40.0
Metab	Metab: An R Package for a High-Throughput Analysis of Metabolomics Data Generated by GC-MS.	1.8.0
metabomxtr	A package to run mixture models for truncated metabolomics data with normal or lognormal distributions	1.8.0
metaMS	MS-based metabolomics annotation pipeline	1.10.0
MetCirc	A workflow to analyse and visualise metabolomics data	1.0.1
MSGFgui	A shiny GUI for MSGFplus	1.8.0
MSGFplus	An interface between R and MS-GF+	1.8.0
msmsEDA	Exploratory Data Analysis of LC-MS/MS data by spectral counts	1.12.0
msmsTests	LC-MS/MS Differential Expression Tests	1.12.0
MSnbase	Base Functions and Classes for MS-based Proteomics	2.0.2
MSnID	Utilities for Exploration and Assessment of Confidence of LC-MSn Proteomics Identifications	1.8.0
msPurity	Automated Evaluation of Precursor Ion Purity for Mass Spectrometry Based Fragmentation in Metabolomics	1.0.0
mzID	An mzIdentML parser for R	1.12.0
mzR	parser for netCDF, mzXML, mzData and mzML and mzIdentML files (mass spectrometry data)	2.8.1
PAPi	Predict metabolic pathway activity based on metabolomics data	1.14.0
Pbase	Manipulating and exploring protein and proteomics data	0.14.0
pepXMLTab	Parsing pepXML files and filter based on peptide FDR.	1.8.0
PGA	An package for identification of novel peptides by customized database derived from RNA-Seq	1.4.0
plgem	Detect differential expression in microarray and proteomics datasets with the Power Law Global Error Model (PLGEM)	1.46.0
proBAMr	Generating SAM file for PSMs in shotgun proteomics data	1.8.0
PROcess	Ciphergen SELDI-TOF Processing	1.50.0
proFIA	Preprocessing of FIA-HRMS data	1.0.10
pRoloc	A unifying bioinformatics framework for spatial proteomics	1.14.6
Prostar	Provides a GUI for DAPAR	1.6.1
proteoQC	An R package for proteomics data quality control	1.10.0
ProtGenerics	S4 generic functions for Bioconductor proteomics infrastructure	1.6.0
qcmetrics	A Framework for Quality Control	1.12.0
Rdisop	Decomposition of Isotopic Patterns	1.34.0
Risa	Converting experimental metadata from ISA-tab into Bioconductor data structures	1.16.0
RMassBank	Workflow to process tandem MS files and build MassBank records	2.2.1
rols	An R interface to the Ontology Lookup Service	2.2.5
ropls	PCA, PLS(-DA) and OPLS(-DA) for multivariate analysis and feature selection of omics data	1.6.2
rpx	R Interface to the ProteomeXchange Repository	1.10.2
rTANDEM	Interfaces the tandem protein identification algorithm in R	1.14.2
sapFinder	A package for variant peptides detection and visualization in shotgun proteomics.	1.12.0
shinyTANDEM	Provides a GUI for rTANDEM	1.12.0
SIMAT	GC-SIM-MS data processing and alaysis tool	1.6.1
specL	specL - Prepare Peptide Spectrum Matches for Use in Targeted Proteomics	1.8.0
statTarget	Statistical Analysis of Metabolite Profile	1.4.12
SWATH2stats	Transform and Filter SWATH Data for Statistical Packages	1.4.1
synapter	Label-free data analysis pipeline for optimal identification and quantitation	1.16.0
TargetSearch	A package for the analysis of GC-MS metabolite profiling data	1.30.0
tofsims	Import, process and analysis of Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS) imaging data	1.2.0
TPP	Analyze thermal proteome profiling (TPP) experiments	3.0.3
xcms	LC/MS and GC/MS Data Analysis	1.50.1
yamss	Tools for high-throughput metabolomics	1.0.4

Table 2: Packages available under the MassSpectrometry *biocViews* category.

Package	Title	Version
CardinalWorkflows	Datasets and workflows for the Cardinal mass spectrometry imaging package	1.6.0
DAPARdata	Data accompanying the DAPAR and Prostar packages	1.2.0
faahKO	Saghatelian et al. (2004) FAAH knockout LC/MS data	1.14.0

gcspikelite	Spike-in data for GC/MS data and methods within flagme	1.12.0
iontreeData	Data provided to show the usage of functions in iontree package	1.10.0
metaMSdata	Example CDF data for the metaMS package	1.10.0
msdata	Various Mass Spectrometry raw data example files	0.14.0
msPurityData	Data to test the msPurity package	1.0.0
msqc1	Sigma mix MSQC1 data	1.2.0
mtbls2	MetaboLights MTBLS2: Comparative LC/MS-based profiling of silver nitrate-treated Arabidopsis thaliana leaves of wild-type and cyp79B2 cyp79B3 double knockout plants. Bttcher et al. (2004)	1.4.0
plasFIA	FIA-HRMS plasma dataset	1.0.0
ProData	SELDI-TOF data of Breast cancer samples	1.12.0
pRolocdata	Data accompanying the pRoloc package	1.12.0
PtH2O2lipids	P. tricornutum HPLC-ESI-MS Lipid Data from van Creveld et al. (2015)	1.0.0
RforProteomics	Companion package to the 'Using R and Bioconductor for proteomics data analysis' publication	1.12.0
RMassBankData	Test dataset for RMassBank	1.12.0
synapterdata	Data accompanying the synapter package	1.12.0

Table 3: Experimental Packages available under the MassSpectrometryData *biocViews* category.

The tables can easily be generated with the `proteomicsPackages`, `massSpectrometryPackages` and `massSpectrometryDataPackage` functions. The respective package tables can then be interactively explored using the `display` function.

```
pp <- proteomicsPackages()
display(pp)
```

8.2 Other CRAN packages

The CRAN task view on Chemometrics and Computational p Physics²³ is another useful resource listing 86 packages, including a set of packages for mass spectrometry and proteomics, some of which are illustrated in this document.

MALDIquant provides tools for quantitative analysis of MALDI-TOF mass spectrometry data, with support for baseline correction, peak detection and plotting of mass spectra (<http://cran.r-project.org/web/packages/MALDIquant/index.html>).

OrgMassSpecR is for organic/biological mass spectrometry, with a focus on graphical display, quantification using stable isotope dilution, and protein hydrogen/deuterium exchange experiments (<http://cran.r-project.org/web/packages/OrgMassSpecR/index.html>).

FTICRMS provides functions for Analyzing Fourier Transform-Ion Cyclotron Resonance Mass Spectrometry Data (<http://cran.r-project.org/web/packages/FTICRMS/index.html>).

titan provides a GUI to analyze mass spectrometric data on the relative abundance of two substances from a titration series (<http://cran.r-project.org/web/packages/titan/index.html>).

digeR provides a GUI interface for analysing 2D DIGE data. It allows to perform correlation analysis, score plot, classification, feature selection and power analysis for 2D DIGE experiment data. (<http://cran.r-project.org/web/packages/digeR/index.html>)

protViz helps with quality checks, visualizations and analysis of mass spectrometry data, coming from proteomics experiments. The package is developed, tested and used at the Functional Genomics Center Zurich. (<http://cran.r-project.org/web/packages/protViz/index.html>)

²³<http://cran.r-project.org/web/views/ChemPhys.html>

Suggestions for additional *R* packages are welcome and will be added to the vignette. Please send suggestions and possibly a short description and/or an example utilisation with code to lg390@cam.ac.uk. The only requirement is that the package must be available on an official package channel (CRAN, *Bioconductor*, R-forge, Omegahat), i.e. not only available through a personal web page.

9 Session information

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

- R version 3.3.2 Patched (2016-11-01 r71616), x86_64-pc-linux-gnu
- Base packages: base, datasets, graphics, grDevices, methods, parallel, stats, stats4, utils
- Other packages: AnnotationDbi 1.36.2, Biobase 2.34.0, BiocGenerics 0.20.0, BiocInstaller 1.24.0, BiocParallel 1.8.1, biomaRt 2.30.0, Biostrings 2.42.1, bitops 1.0-6, BRAIN 1.20.0, cleaver 1.12.0, data.table 1.10.4, digest 0.6.12, ggplot2 2.2.1, GO.db 3.4.0, hpar 1.16.0, IPPD 1.22.0, IRanges 2.8.2, isobar 1.20.0, knitr 1.15.1, lattice 0.20-35, MALDIquant 1.16.2, MALDIquantForeign 0.10, MASS 7.3-45, Matrix 1.2-8, msdata 0.14.0, MSGFgui 1.8.0, MSGFplus 1.8.0, MSnbase 2.0.2, MSnID 1.8.0, mzID 1.12.0, mzR 2.8.1, org.Hs.eg.db 3.4.0, OrgMassSpecR 0.4-6, PolynomF 0.94, ProtGenerics 1.6.0, RColorBrewer 1.1-2, Rcpp 0.12.10, RcppClassic 0.9.6, Rdisop 1.34.0, reshape2 1.4.2, RforProteomics 1.12.0, rJava 0.9-8, rols 2.2.5, rpx 1.10.2, rTANDEM 1.14.0, S4Vectors 0.12.2, xlsx 0.5.7, xlsxjars 0.6.1, XML 3.98-1.6, xtable 1.8-2, XVector 0.14.1
- Loaded via a namespace (and not attached): affy 1.52.0, affyio 1.44.0, annotate 1.52.1, assertthat 0.1, base64enc 0.1-3, BiocStyle 2.2.1, biocViews 1.42.0, Category 2.40.0, codetools 0.2-15, colorspace 1.3-2, compiler 3.3.2, DBI 0.6-1, distr 2.6, doParallel 1.0.10, dplyr 0.5.0, evaluate 0.10, foreach 1.4.3, genefilter 1.56.0, graph 1.52.0, grid 3.3.2, gridSVG 1.5-0, GSEABase 1.36.0, gtable 0.2.0, highr 0.6, htmltools 0.3.5, httpuv 1.3.3, httr 1.2.1, impute 1.48.0, interactiveDisplay 1.12.0, interactiveDisplayBase 1.12.0, iterators 1.0.8, jsonlite 1.3, labeling 0.3, lazyeval 0.2.0, limma 3.30.13, magrittr 1.5, memoise 1.0.0, mime 0.5, munsell 0.4.3, pcaMethods 1.66.0, plyr 1.8.4, preprocessCore 1.36.0, prettyunits 1.0.2, progress 1.1.2, R.cache 0.12.0, R.methodsS3 1.7.1, R.oo 1.21.0, R.utils 2.5.0, R6 2.2.0, RBGL 1.50.0, RCurl 1.95-4.8, readBrukerFlexData 1.8.3, readMzXmlData 2.8.1, RJSONIO 1.3-0, RSQLite 1.1-2, RUnit 0.4.31, scales 0.4.1, sfsmisc 1.1-0, shiny 1.0.0, shinyFiles 0.6.2, splines 3.3.2, startupmsg 0.9.3, stringi 1.1.3, stringr 1.2.0, survival 2.41-3, SweaveListingUtils 0.7.5, tibble 1.3.0, tools 3.3.2, vsn 3.42.3, zlibbioc 1.20.0

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