protViz: Visualizing and Analyzing Mass Spectrometry Related Data in Proteomics

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Abstract

protViz is an R package to do 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. We use this package mainly for prototyping, teaching, and having *fun* with proteomics data. But it can also be used to do data analysis for small scale data sets. Nevertheless, if one is patient, it also handles large data sets.

Keywords: proteomics, mass spectrometry, fragment-ion.

Contents

1. Related Work

The method of choice in proteomics is mass spectrometry. There are already packages in R which deal with mass spec related data. Some of them are listed here:

- **OrgMassSpec**: Organic Mass Spectrometry
- **MSnbase** package (basic functions for mass spec data including quant aspect with iTRAQ data)
  http://bioconductor.org/packages/MSnbase/
- **plgem** – spectral counting quantification, applicable to MudPIT experiments
  http://www.bioconductor.org/packages/plgem/
- **synapter** – MSe (Hi3 = Top3 Quantification) for Waters Q-tof data aquired in MSe mode
  http://bioconductor.org/packages/synapter/
- **mzR**
  http://bioconductor.org/packages/mzR/
- isobar iTRAQ/TMT quantification package
  http://bioconductor.org/packages/isobar/
- **readMzXmlData**
  https://CRAN.R-project.org/package=readMzXmlData
- **rawDiag** - an R package supporting rational LC-MS method optimization for bottom-up proteomics on multiple OS platforms (Trachsel, Panse, Kockmann, Wolski, Grossmann, and Schlapbach 2018)

2. Get Data In – Preprocessing

The most time consuming and challenging part of data analysis and visualization is shaping the data the way that they can easily further process. In this package, we intentionally left this part away because it is very infrastructure dependent. Moreover, we use also commercial tools to analyze data and export the data into R accessible formats. We provide a different kind of importers if these formats are available, but with little effort, one can bring other exports in a similar format which will make it easy to use our package for a variety of tools.

2.1. Identification - In-silico from Proteins to Peptides

For demonstration, we use a sequence of peptides derived from a tryptic digest using the Swiss-Prot `FETUA_BOVIN Alpha-2-HS-glycoprotein` protein (P12763).

`fcat` and `tryptic-digest` are commandline programs which are included in the package. `fcat` removes the lines starting with `>` and all 'new line' character within the protein sequence while `tryptic-digest` is doing the triptic digest of a protein sequence applying the rule: cleave after arginine (R) and lysine (K) except followed by proline(P).

Both programs can be used through the `Fasta` Rcpp module.

```
R> library(protViz)
R> fname <- system.file("extdata", name='P12763.fasta', package = "protViz")
R> F <- Fasta$new(fname)
```

print the first 60 characters of P12763.

```
R> substr(F$getSequences(), 1, 60)

[1] "MKSFVLLFCLAQLWGCHSIPLDPVAGYKEPACDDPDTEQAALAAVDYINKHLPRGYKHTL"

R> (fetuin <- F$getTrypticPeptides())

 [1] "MK"
 [2] "SFVLLFCLAQLWGCHSIPLDPVAGYK"
 [3] "EPACDDPDTEQAALAAVDYINK"
 [4] "HLPR"
 [5] "GYK"
 [6] "HTLNQIDSVK"
 [7] "VWPR"
 [8] "RPTGEVYDIEIDTLETTCHVLDPTPLANCSVR"
 [9] "QQTQHAVEGDCDIHVLK"
[10] "QDGQFSVLFTK"
[11] "CDSSPDSAEDVR"
[12] "K"
[13] "LCPDCPLLAPLNDSR"
[14] "VVHAVEVALATFNAESNGSYLQLVEISR"
[15] "AQFVPLPVSVSVEFAVAATDCIAK"
[16] "EVVDPTK"
[17] "CNLLAEK"
[18] "QYGFCK"
[19] "GSVIQK"
[20] "ALGGEDVR"
[21] "VTCTLFQTQPVIPQPQPDGAEAEAPSAVPDAAGPTPSAAGPPVASVVVGPSVVAVPLPLHR"
[22] "AHYDLR"
[23] "HTFSGVASVESSSGEAFHVGK"
[24] "TPIVGQPSIPGGPVR"
[25] "LCPGR"
[26] "IR"
[27] "YFK"
[28] "I"
```

3. Peptide Identification

The currency in proteomics are the peptides. In proteomics, proteins are digested to so-called peptides since peptides are much easier to handle biochemically than proteins. Proteins are very different in nature some are very sticky while others are soluble in aqueous solutions

while again are only sitting in membranes. Therefore, proteins are chopped up into peptides because it is fair to assume, that for each protein, there will be many peptides behaving well so that they can be measured with the mass spectrometer. This step introduces another problem, the so-called protein inference problem. In this package here, we do not touch at all upon the protein inference.

3.1. Computing Mass and Hydrophobicity of a Peptide Sequence

`parentIonMass` computes the mass of an amino acid sequence.

```
R> mass <- protViz::parentIonMass(fetuin)
```

The `ssrc` function derives a measure for the hydrophobicity based on the method described in (Krokhin, Craig, Spicer, Ens, Standing, Beavis, and Wilkins 2004).

```
R> hydrophobicity <- protViz::ssrc(fetuin)
```

The content of `mass` and `hydrophobicity` can be seen in the Table 1.

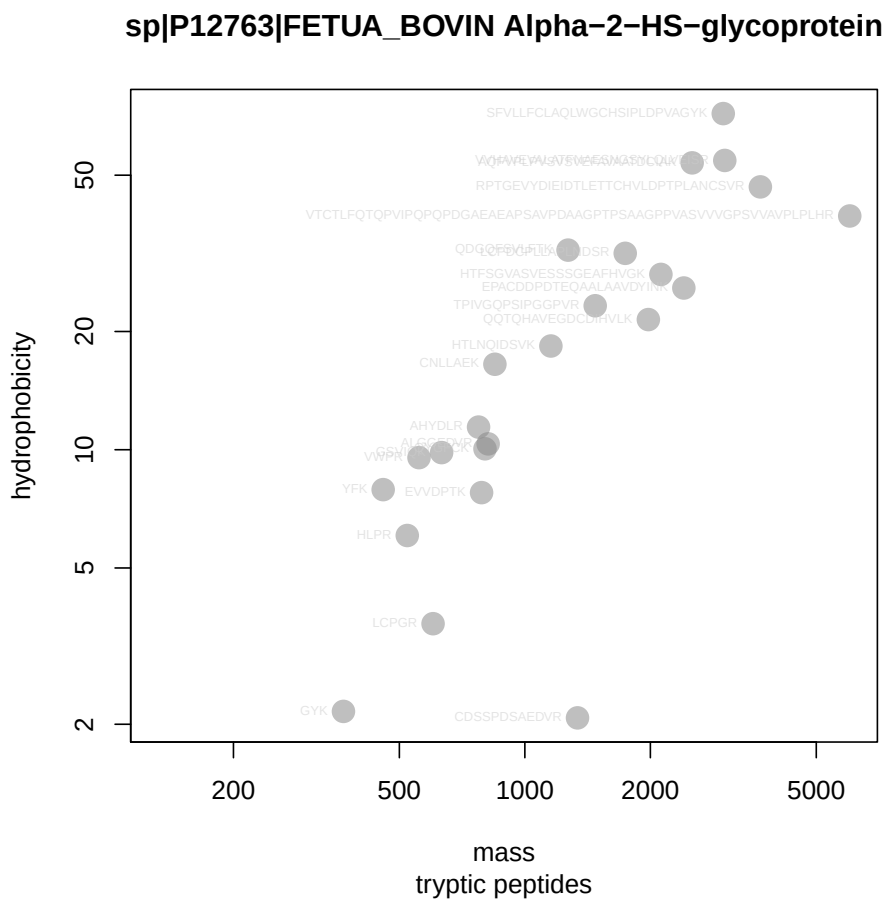
peptide	mass	hydrophobicity
MK	278.15	
SFVLLFCLAQLWGCHSIPLDPVAGYK	2991.53	71.74
EPACDDPDTEQAALAAVDYINK	2406.08	25.81
HLPR	522.31	6.05
GYK	367.20	2.16
HTLNQIDSVK	1154.62	18.37
VWPR	557.32	9.55
RPTGEVYDIEIDTLETTCHVLDPTPLANCSVR	3671.77	46.69
QQTQHAVEGDCDIHVLK	1977.94	21.45
QDGQFSVLFTK	1269.65	32.22
CDSSPDSAEDVR	1337.53	2.08
K	147.11	
LCPDCPLLAPLNDSR	1740.84	31.62
VVHAVEVALATFNAESNGSYLQLVEISR	3016.57	54.51
AQFVPLPVSVSVEFAVAATDCIAK	2519.32	53.75
EVVDPTK	787.42	7.78
CNLLAEK	847.43	16.51
QYGFCK	802.36	10.05
GSVIQK	631.38	9.83
ALGGEDVR	816.42	10.35
VTCTLFQTQPVIPQPQPDGAEAEAPSAVPDAAGPTPSAAGPPVASVVVGPSVVAVPLPLHR	6015.13	39.37
AHYDLR	774.39	11.42
HTFSGVASVESSSGEAFHVGK	2120.00	27.95
TPIVGQPSIPGGPVR	1474.84	23.26
LCPGR	602.31	3.61
IR	288.20	
YFK	457.24	7.91
I	132.10	

Table 1: parent ion mass and hydrophobicity values of the tryptic digested protein extttP12763.

A figure below shows a scatter plot graphing the parent ion mass versus the hydrophobicity value of each in-silico tryptic digested peptide of the `FETUA BOVIN` (P12763) protein.

```
R> op <- par(mfrow = c(1, 1))
R> plot(hydrophobicity ~ mass,
```

```
+    log = 'xy', pch = 16, col = '#88888888', cex = 2,
+    main = "sp|P12763|FETUA_BOVIN Alpha-2-HS-glycoprotein",
+    sub = 'tryptic peptides')
R> text(mass, hydrophobicity, fetuin, pos=2, cex=0.5, col = '#CCCCCC88')
```



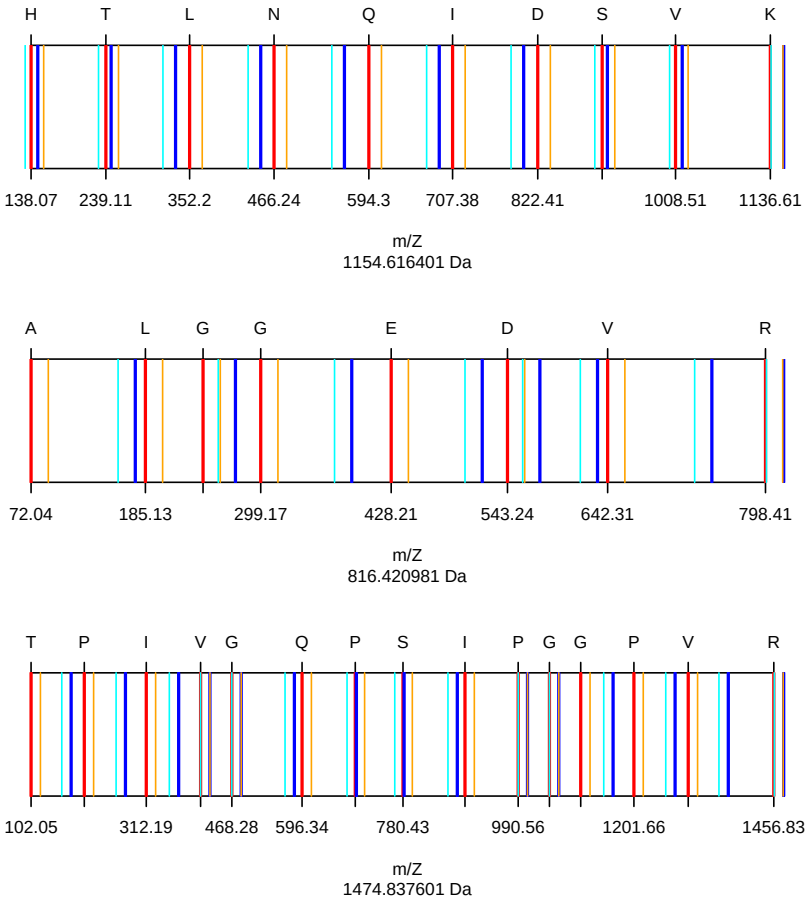
3.2. In-silico Peptide Fragmentation

The fragment ions computation of a peptide follows the rules proposed in (Roepstorff and Fohlman 1984). Beside the `b` and `y` ions the `FUN` argument of `fragmentIon` defines which ions are computed. the default ions beeing computed are defined in the function `defaultIon`. The are no limits for defining other forms of fragment ions for ETD (c and z ions) CID (b and y ions).

```
R> defaultIon

function (b, y)
{
    Hydrogen <- 1.007825
    Oxygen <- 15.994915
    Nitrogen <- 14.003074
```

```
    c <- b + (Nitrogen + (3 * Hydrogen))
    z <- y - (Nitrogen + (3 * Hydrogen))
    return(cbind(b, y, c, z))
}
<bytecode: 0x5652514fdfa0>
<environment: namespace:protViz>
```

```
R> ## plot in-silico fragment ions of
R> peptides <- c('HTLNQIDSVK', 'ALGGEDVR', 'TPIVGQPSIPGGPVR')
R> pims <- peptides |> protViz::parentIonMass()
R> fis <- peptides |> protViz::fragmentIon()
R> par(mfrow = c(3, 1));
R> rv <- mapply(FUN = function(fi, pim, peptide){
+      plot(0,0,
+          xlab='m/Z', ylab='',
+          xlim = range(c(fi$b, fi$y)),
+          ylim = c(0,1),
+          type = 'n', axes = FALSE,
+          sub=paste(pim, "Da"));
+
+      axis(1, fi$b,round(fi$b, 2))
+
+      pepSeq <- strsplit(peptide, "")
+      axis(3, fi$b, pepSeq[[1]])
+
+      abline(v = fi$b, col='red', lwd=2)
+      abline(v = fi$y, col='blue',lwd=2)
+      abline(v = fi$c, col='orange')
+      abline(v = fi$z, col='cyan')
+    }, fis, pims, peptides)
```



The next lines compute the singly and doubly charged fragment ions of the `HTLNQIDSVK` peptide. Which are usually the ones that can be used to make an identification.

```
R> Hydrogen<-1.007825
R> (fi.HTLNQIDSVK.1 <- fragmentIon('HTLNQIDSVK'))[[1]]
```

```
           b         y         c         z
1   138.0662  147.1128  155.0927  130.0863
2   239.1139  246.1812  256.1404  229.1547
3   352.1979  333.2132  369.2245  316.1867
4   466.2409  448.2402  483.2674  431.2136
5   594.2994  561.3242  611.3260  544.2977
6   707.3835  689.3828  724.4100  672.3563
7   822.4104  803.4258  839.4370  786.3992
8   909.4425  916.5098  926.4690  899.4833
9  1008.5109 1017.5575 1025.5374 1000.5309
10 1136.6058 1154.6164 1153.6324 1137.5899
```

```
R> (fi.HTLNQIDSVK.2 <-(fi.HTLNQIDSVK.1[[1]] + Hydrogen) / 2)
```

```
           b         y         c         z
1   69.53701  74.06031  78.05028  65.54704
```

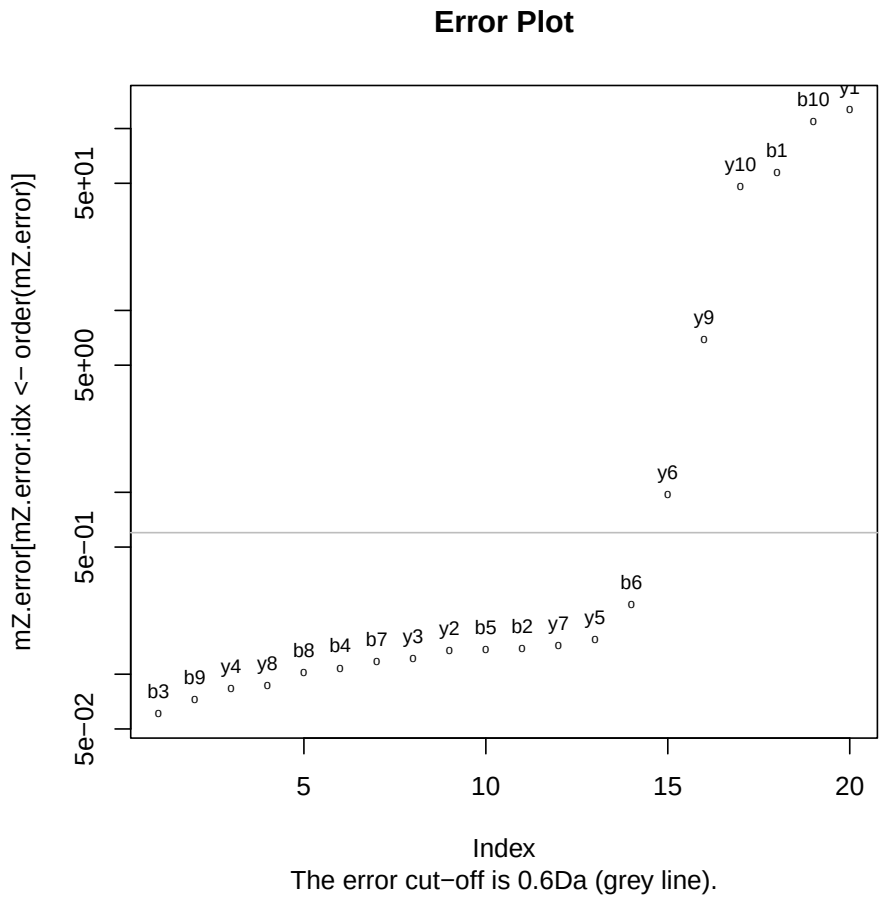
```
2  120.06085 123.59452 128.57412 115.08124
3  176.60288 167.11053 185.11615 158.59726
4  233.62434 224.62400 242.13761 216.11073
5  297.65363 281.16603 306.16691 272.65276
6  354.19566 345.19532 362.70894 336.68205
7  411.70913 402.21679 420.22241 393.70351
8  455.22515 458.75882 463.73842 450.24554
9  504.75935 509.28266 513.27262 500.76938
10 568.80683 577.81211 577.32010 569.29884
```

3.3. Peptide Sequence – Fragment Ion Matching

Given a peptide sequence and a tandem mass spectrum. For the assignment of a candidate peptide an in-silico fragment ion spectra `fi` is computed. The function `findNN` determines for each fragment ion the closed peak in the MS2. If the difference between the in-silico mass and the measured mass is inside the 'accuracy' mass window of the mass spec device the in-silico fragment ion is considered as a potential hit.

```
R>     peptideSequence <- 'HTLNQIDSVK'
R>     spec <- list(scans=1138,
+          title="178: (rt=22.3807) [20080816_23_fetuin_160.RAW]",
+          rtinseconds=1342.8402,
+          charge=2,
+          mZ=c(195.139940, 221.211970, 239.251780, 290.221750,
+      316.300770, 333.300050, 352.258420, 448.384360, 466.348830,
+      496.207570, 509.565910, 538.458310, 547.253380, 556.173940,
+      560.358050, 569.122080, 594.435500, 689.536940, 707.624790,
+      803.509240, 804.528220, 822.528020, 891.631250, 909.544400,
+      916.631600, 973.702160, 990.594520, 999.430580, 1008.583600,
+      1017.692500, 1027.605900),
+          intensity=c(931.8, 322.5, 5045, 733.9, 588.8, 9186, 604.6,
+      1593, 531.8, 520.4, 976.4, 410.5, 2756, 2279, 5819, 2.679e+05,
+      1267, 1542, 979.2, 9577, 3283, 9441, 1520, 1310, 1.8e+04,
+      587.5, 2685, 671.7, 3734, 8266, 3309))
R>     fi <- protViz::fragmentIon(peptideSequence)
R>     n <- nchar(peptideSequence)
R>     by.mZ <- c(fi[[1]]$b, fi[[1]]$y)
R>     by.label <- c(paste("b",1:n,sep=''), paste("y",n:1,sep=''))
R>     # should be a R-core function as findInterval!
R>     idx <- protViz::findNN(by.mZ, spec$mZ)
R>     mZ.error <- abs(spec$mZ[idx]-by.mZ)
R>     plot(mZ.error[mZ.error.idx<-order(mZ.error)],
+          main="Error Plot",
+          pch='o',
+          cex=0.5,
+          sub='The error cut-off is 0.6Da (grey line).',
```

```
+          log='y')
R>     abline(h=0.6,col='grey')
R>     text(1:length(by.label),
+          mZ.error[mZ.error.idx],
+          by.label[mZ.error.idx],
+          cex=0.75,pos=3)
```



The error cut−off is 0.6Da (grey line).

The graphic above is showing the mass error of the assignment between the MS2 `spec` and the singly charged fragment ions of `HTLNQIDSVK`. The function `psm` is doing the peptide sequence matching. Of course, the more theoretical ions match (up to a small error tolerance, given by the system) the measured ion chain, the more likely it is, that the measured spectrum indeed is from the inferred peptide (and therefore the protein is identified)

3.4. Modifications

```
R> library(protViz)
R> ptm.0 <- cbind(AA="-",
+      mono=0.0, avg=0.0, desc="unmodified", unimodAccID=NA)
R> ptm.616 <- cbind(AA='S',
+      mono=-27.010899, avg=NA, desc="Substituition",
```

```
+      unimodAccID=616)
R> ptm.651 <- cbind(AA='N',
+      mono=27.010899, avg=NA, desc="Substituition",
+      unimodAccID=651)
R> m <- as.data.frame(rbind(ptm.0, ptm.616, ptm.651))
R> genMod(c('TAFDEAIAELDTLNEESYK','TAFDEAIAELDTLSEESYK'), m$AA)

[[1]]
[1] "0000000000000000000" "0000000000000200000" "0000000000000000100"
[4] "0000000000000100200"

[[2]]
[1] "0000000000000000000" "0000000000000100000" "0000000000000000100"
[4] "0000000000000100100"

R> fi <- protViz::fragmentIon(c('TAFDEAIAELDTLSEESYK',
+      'TAFDEAIAELDTLNEESYK', 'TAFDEAIAELDTLSEESYK',
+      'TAFDEAIAELDTLNEESYK'),
+          modified=c('0000000000000200000',
+          '0000000000000100000', '0000000000000000000',
+          '0000000000000000000'),
+      modification=m$mono)
```

3.5. Labeling Peaklists

The `peakplot` Panse, Gerrits, and Schlapbach (2009) function performs the labeling of the tandem mass spectra.

```
R> data(msms)
R> op <- par(mfrow = c(2, 1))
R> protViz::peakplot("TAFDEAIAELDTLNEESYK", msms[[1]])

$mZ.Da.error
 [1]  232.331344  161.294234   14.225824   -0.032616   -0.143306
 [6]    0.032244    0.054604   -0.004076   -0.071746   -0.084536
[11]   -0.097076   -0.038856   -0.061816    0.004554   -0.122336
[16]   -0.139626   -1.071256  -18.783686 -146.878646  187.273499
[21]   24.210169    0.048669    0.177779    0.027939    0.049579
[26]    0.052379    0.044579    0.036749    0.043189   -0.035101
[31]   -0.061011    0.000729   -0.092081    2.011029   -8.412111
[36]    7.195579  -63.841531 -164.889211  215.304795  144.267685
[41]   -2.800725  -17.059165    2.034875    2.264105    4.008125
[46]    1.292875   -0.003965  -13.612585   -0.060925  -17.065405
[51]    3.897535    3.000405  -17.148885  -17.166175  -18.097805
[56]  -35.810235 -163.905195  204.300048   41.236718   17.075218
[61]   -0.843372   -1.091812    0.129908   17.078928   -0.372162
```

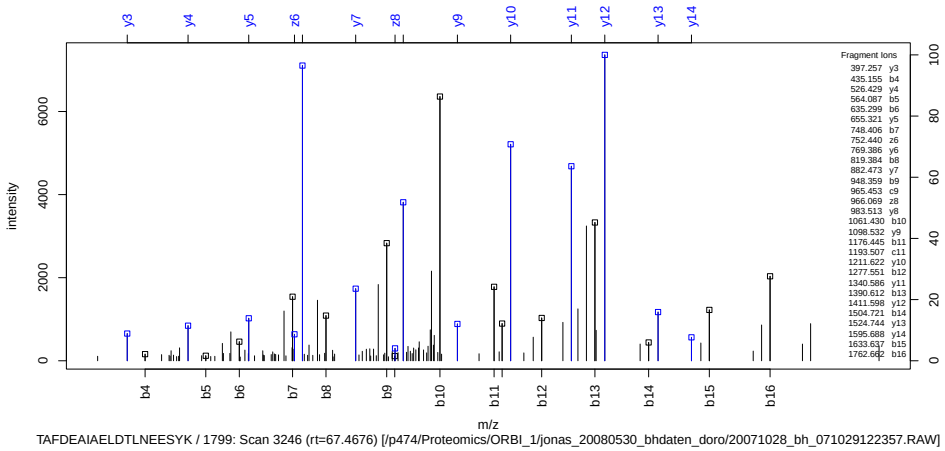
```
[66]  -16.539502   -1.044962   -1.000952   -1.409062   -2.995122
[71]   16.934468   19.037578    8.614438   24.222128  -46.814982
[76] -147.862662

$mZ.ppm.error
 [1]  2.276532e+06  9.318407e+05  4.443342e+04 -7.494702e+01
 [5] -2.539851e+02  5.075660e+01  7.296574e+01 -4.974443e+00
 [9] -7.564705e+01 -7.963713e+01 -8.250960e+01 -3.041352e+01
[13] -4.445040e+01  3.026484e+00 -7.488007e+01 -7.920687e+01
[17] -5.791093e+02 -9.331667e+03 -6.860308e+04  1.272993e+06
[21]  7.805297e+04  1.225277e+02  3.378218e+02  4.263587e+01
[25]  6.444386e+01  5.935833e+01  4.532837e+01  3.345395e+01
[29]  3.564687e+01 -2.618263e+01 -4.321937e+01  4.781134e-01
[33] -5.770282e+01  1.165934e+03 -4.572174e+03  3.621478e+03
[37] -3.102183e+04 -7.637286e+04  1.808046e+06  7.588299e+05
[41] -8.306147e+03 -3.772366e+04  3.500821e+03  3.470990e+03
[45]  5.236793e+03  1.545734e+03 -4.106862e+00 -1.262129e+04
[49] -5.104441e+01 -1.318183e+04  2.768725e+03  1.971690e+03
[53] -1.038832e+04 -9.644849e+03 -9.694247e+03 -1.764117e+04
[57] -7.595171e+04  1.570497e+06  1.406678e+05  4.491332e+04
[61] -1.656190e+03 -1.710589e+03  1.726789e+02  1.973544e+04
[65] -3.850849e+02 -1.529356e+04 -8.747728e+02 -7.562373e+02
[69] -1.010347e+03 -1.986529e+03  1.072648e+04  1.114745e+04
[73]  4.725878e+03  1.229618e+04 -2.293808e+04 -6.903096e+04

$idx
 [1]   1   1   1   3  14  21  38  49  64  87  91  97 102 106 110 113
[17] 115 116 116   1   1   2  12  25  41  53  70  89  94  99 104 107
[33] 108 111 114 116 116 116   1   1   1   3  16  24  41  52  67  88
[49]  93  97 104 107 110 113 115 116 116   1   1   2  11  22  40  53
[65]  68  88  93  98 103 106 108 111 114 116 116 116

$label
 [1] "b1"  "b2"  "b3"  "b4"  "b5"  "b6"  "b7"  "b8"  "b9"  "b10" "b11"
[12] "b12" "b13" "b14" "b15" "b16" "b17" "b18" "b19" "y1"  "y2"  "y3"
[23] "y4"  "y5"  "y6"  "y7"  "y8"  "y9"  "y10" "y11" "y12" "y13" "y14"
[34] "y15" "y16" "y17" "y18" "y19" "c1"  "c2"  "c3"  "c4"  "c5"  "c6"
[45] "c7"  "c8"  "c9"  "c10" "c11" "c12" "c13" "c14" "c15" "c16" "c17"
[56] "c18" "c19" "z1"  "z2"  "z3"  "z4"  "z5"  "z6"  "z7"  "z8"  "z9"
[67] "z10" "z11" "z12" "z13" "z14" "z15" "z16" "z17" "z18" "z19"

$score
[1] -1

$sequence
[1] "TAFDEAIAELDTLNEESYK"
```

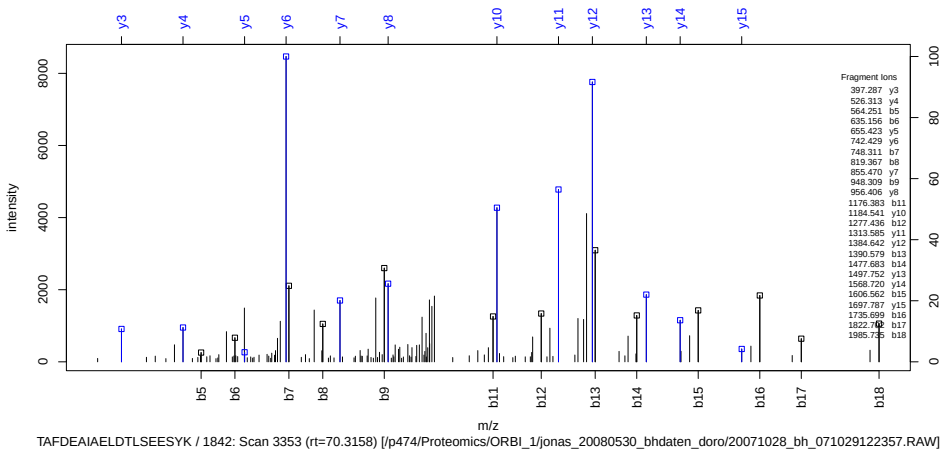
```
$fragmentIon
           b         y         c         z
1   102.0550  147.1128  119.0815  130.0863
2   173.0921  310.1761  190.1186  293.1496
3   320.1605  397.2082  337.1870  380.1816
4   435.1874  526.2508  452.2140  509.2242
5   564.2300  655.2933  581.2566  638.2668
6   635.2671  769.3363  652.2937  752.3097
7   748.3512  882.4203  765.3777  865.3938
8   819.3883  983.4680  836.4148  966.4415
9   948.4309 1098.4950  965.4574 1081.4684
10 1061.5149 1211.5790 1078.5415 1194.5525
11 1176.5419 1340.6216 1193.5684 1323.5951
12 1277.5896 1411.6587 1294.6161 1394.6322
13 1390.6736 1524.7428 1407.7002 1507.7162
14 1504.7165 1595.7799 1521.7431 1578.7533
15 1633.7591 1724.8225 1650.7857 1707.7959
16 1762.8017 1839.8494 1779.8283 1822.8229
17 1849.8338 1986.9178 1866.8603 1969.8913
18 2012.8971 2057.9549 2029.9236 2040.9284
19 2140.9920 2159.0026 2158.0186 2141.9761

R> protViz::peakplot("TAFDEAIAELDTLSEESYK", msms[[2]])

$mZ.Da.error
 [1]  245.264254  174.227144   27.158734   14.444434    0.021404
 [6]   -0.111266   -0.039926   -0.021626   -0.121916   -8.079236
[11]   -0.158376   -0.153156   -0.094316   -0.022946   -0.186736
[16]   -0.092226   -0.120456   -0.151686 -128.246646  200.206409
[21]   37.143079    0.078909    0.062269    0.129769    0.103729
[26]    0.060869   -0.051451  -18.048351   -0.027511   -0.025601
[31]   -0.006211    0.020529   -0.048781   -0.024771   -9.166311
[36]    6.953579  -45.209531 -146.257211  228.237705  157.200595
[41]   10.132185   -2.582115    1.626855    2.722405    9.009025
[46]   -1.130895    1.216385   13.347315   -3.671525    0.960295
[51]  -17.120865    3.020205  -17.213285  -17.118775  -17.147005
[56]  -17.178235 -145.273195  217.232958   54.169628   17.105458
[61]   -0.833452   -1.260332   -0.899352   -3.098942   -1.173512
[66]   -1.021802   -0.939162   -1.007752   -1.377062   -3.022622
[71]   16.977768   17.001778    7.860238   23.980128  -28.182982
[76] -129.230662

$mZ.ppm.error
 [1]  2.403257e+06  1.006558e+06  8.482850e+04  3.319130e+04
 [5]  3.793488e+01 -1.751484e+02 -5.335196e+01 -2.639286e+01
 [9] -1.285450e+02 -7.611043e+03 -1.346114e+02 -1.198789e+02
[13] -6.782037e+01 -1.552813e+01 -1.162198e+02 -5.313198e+01
```

```
[17] -6.608212e+01 -7.638202e+01 -6.066594e+04  1.360904e+06
[21]  1.197483e+05  1.986591e+02  1.183257e+02  1.980319e+02
[25]  1.397352e+02  7.115774e+01 -5.379332e+01 -1.684426e+04
[29] -2.322450e+01 -1.948903e+01 -4.485617e+00  1.370673e+01
[33] -3.109508e+01 -1.458996e+01 -5.056331e+03  3.547913e+03
[37] -2.226035e+04 -6.860121e+04  1.916651e+06  8.268554e+05
[41]  3.004915e+04 -5.709941e+03  2.798859e+03  4.173588e+03
[45]  1.177069e+04 -1.352074e+03  1.259905e+03  1.237534e+04
[49] -3.076091e+03  7.417604e+02 -1.216230e+04  2.020566e+03
[53] -1.060078e+04 -9.766434e+03 -9.319787e+03 -8.576627e+03
[57] -6.817113e+04  1.669915e+06  1.847849e+05  4.499286e+04
[61] -1.636709e+03 -1.974616e+03 -1.239974e+03 -3.696333e+03
[65] -1.249174e+03 -9.690310e+02 -8.043928e+02 -7.772361e+02
[69] -1.006903e+03 -2.041339e+03  1.094110e+04  1.011538e+04
[73]  4.376983e+03  1.234257e+04 -1.399411e+04 -6.110297e+04

$idx
 [1]   1   1   1   3  11  20  39  45  64  90  96 106 116 121 126 129
[17] 131 133 133   1   1   2   7  24  38  49  65  90  97 110 115 122
[33] 123 127 130 132 133 133   1   1   1   3  13  23  40  47  67  91
[49]  98 108 116 122 126 129 131 133 133   1   1   2   6  21  36  47
[65]  62  90  95 108 113 121 123 127 130 132 133 133

$label
 [1] "b1"  "b2"  "b3"  "b4"  "b5"  "b6"  "b7"  "b8"  "b9"  "b10" "b11"
[12] "b12" "b13" "b14" "b15" "b16" "b17" "b18" "b19" "y1"  "y2"  "y3"
[23] "y4"  "y5"  "y6"  "y7"  "y8"  "y9"  "y10" "y11" "y12" "y13" "y14"
[34] "y15" "y16" "y17" "y18" "y19" "c1"  "c2"  "c3"  "c4"  "c5"  "c6"
[45] "c7"  "c8"  "c9"  "c10" "c11" "c12" "c13" "c14" "c15" "c16" "c17"
[56] "c18" "c19" "z1"  "z2"  "z3"  "z4"  "z5"  "z6"  "z7"  "z8"  "z9"
[67] "z10" "z11" "z12" "z13" "z14" "z15" "z16" "z17" "z18" "z19"

$score
[1] -1

$sequence
[1] "TAFDEAIAELDTLSEESYK"

$fragmentIon
          b         y         c         z
1  102.0550  147.1128  119.0815  130.0863
2  173.0921  310.1761  190.1186  293.1496
3  320.1605  397.2082  337.1870  380.1816
4  435.1874  526.2508  452.2140  509.2242
5  564.2300  655.2933  581.2566  638.2668
6  635.2671  742.3254  652.2937  725.2988
7  748.3512  855.4094  765.3777  838.3829
```

```
8   819.3883  956.4571  836.4148  939.4306
9   948.4309 1071.4841  965.4574 1054.4575
10 1061.5149 1184.5681 1078.5415 1167.5416
11 1176.5419 1313.6107 1193.5684 1296.5842
12 1277.5896 1384.6478 1294.6161 1367.6213
13 1390.6736 1497.7319 1407.7002 1480.7053
14 1477.7056 1568.7690 1494.7322 1551.7424
15 1606.7482 1697.8116 1623.7748 1680.7850
16 1735.7908 1812.8385 1752.8174 1795.8120
17 1822.8229 1959.9069 1839.8494 1942.8804
18 1985.8862 2030.9440 2002.9127 2013.9175
19 2113.9811 2131.9917 2131.0077 2114.9652
```

```
R> par(op)
```





The following code snippet combines all the functions to implement a simple peptide search engine. As default arguments, the mass spectrum `x`, mZ and intensity arrays list, and a character vector of peptide sequences are given.

```
R> .peptideFragmentIonSpectrumMatch <- function (x,
+                                    peptideSet,
```

```
+                             framentIonMassToleranceDa = 0.1)
+ {
+   ## Here we ignore the peptide mass
+   # peptideMassTolerancePPM = 5
+   # query.mass <- ((x$pepmass[1] * x$charge)) - (1.007825 * (x$charge - 1))
+   # eps <- query.mass * peptideMassTolerancePPM * 1e-06
+   # pimIdx <- protViz::parentIonMass(peptideSequence)
+   # lower <- protViz::findNN(query.mass - eps, pimIdx)
+   # upper <- protViz::findNN(query.mass + eps, pimIdx)
+
+
+   rv <- lapply(peptideSet, FUN = protViz::psm, spec = x, plot = FALSE) |>
+     lapply(FUN = function(p) {
+       ## determine peaks considered as hits
+       idx <- abs(p$mZ.Da.error) < framentIonMassToleranceDa
+       intensityRatio <- sum(x$intensity[idx]) / sum(x$intensity)
+
+       ## derive objectives for a good match
+       data.frame(nHits=sum(idx), intensityRatio = intensityRatio)
+     }) |>
+     Reduce(f=rbind)
+
+
+   idx.tophit <- which(rv$nHits == max(rv$nHits))[1]
+
+   data.frame(peptideMatch = peptideSet[idx.tophit],
+              nHits = max(rv$nHits),
+              nPeaks = length(x$mZ),
+              intensityRatio = rv$intensityRatio[idx.tophit]
+   )
+ }
```

define a set of peptide sequences

```
R> peptideSet <- c("ELIVSK", 'TAFDEAIAELDTLNEESYK','TAFDEAIAELDTLSEESYK')
```

generate a in-silico tandem mass spectrum

```
R> mZ <- protViz::fragmentIon("TAFDEAIAELDTLNEESYK")[[1]] |>
+   unlist() |> sort()
R> intensity <- mZ |> length() |> sample()
R> msms.insilico <- list(mZ = mZ, intensity = intensity)
```

generate reverse peptide sequences

```
R> peptideSet.rev <- peptideSet |>
+   sapply(FUN = function(x){
+     strsplit(x, "")[[1]] |> rev() |> paste0(collapse = "")
+   })
```

The output is an assignment of the best matching peptide.

```
R> lapply(list(msms[[1]], msms[[2]], msms.insilico),
+         FUN = .peptideFragmentIonSpectrumMatch,
+         peptideSet = c(peptideSet, peptideSet.rev),
+         framentIonMassToleranceDa = 0.05) |>
+    Reduce(f=rbind)

         peptideMatch nHits nPeaks intensityRatio
1 TAFDEAIAELDTLNEESYK    14    116      0.2195071
2 TAFDEAIAELDTLSEESYK    10    133      0.1513915
3 TAFDEAIAELDTLNEESYK    76     76      1.0000000
```

4. Quantification

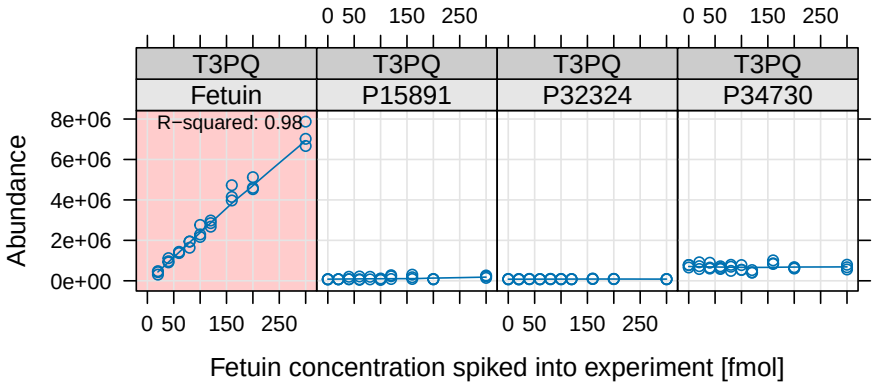
For an overview on Quantitative Proteomics read Bantscheff, Lemeer, Savitski, and Kuster (2012); Cappadona, Baker, Cutillas, Heck, and van Breukelen (2012). The authors are aware that meaningful statistics usually require a much higher number of biological replicates. In almost all cases there are not more than three to six repetitions. For the moment there are limited options due to the availability of machine time and the limits of the technologies.

4.1. Label-free methods on protein level

The data set `fetuinLFQ` contains a subset of our results descriped in Grossmann, Roschitzki, Panse, Fortes, Barkow-Oesterreicher, Rutishauser, and Schlapbach (2010). The example below shows a visualization using trellis plots. It graphs the abundance of four protein independency from the fetuin concentration spiked into the sample.

```
R> library(lattice)
R> data(fetuinLFQ)
R> cv<-1-1:7/10
R> t<-trellis.par.get("strip.background")
R> t$col<-(rgb(cv,cv,cv))
R> trellis.par.set("strip.background",t)
R> print(xyplot(abundance~conc|prot*method,
+      groups=prot,
+      xlab="Fetuin concentration spiked into experiment [fmol]",
+      ylab="Abundance",
+      aspect=1,
+      data=fetuinLFQ$t3pq[fetuinLFQ$t3pq$prot
+          %in% c('Fetuin', 'P15891', 'P32324', 'P34730'),],
+      panel = function(x, y, subscripts, groups) {
+          if (groups[subscripts][1] == "Fetuin")  {
+              panel.fill(col="#ffcccc")
+          }
+          panel.grid(h=-1,v=-1)
```

```
+           panel.xyplot(x, y)
+           panel.loess(x,y, span=1)
+           if (groups[subscripts][1] == "Fetuin")  {
+               panel.text(min(fetuinLFQ$t3pq$conc),
+                   max(fetuinLFQ$t3pq$abundance),
+                   paste("R-squared:",
+                   round(summary(lm(x~y))$r.squared,2)),
+                   cex=0.75,
+                   pos=4)
+           }
+       }
+   ))
```



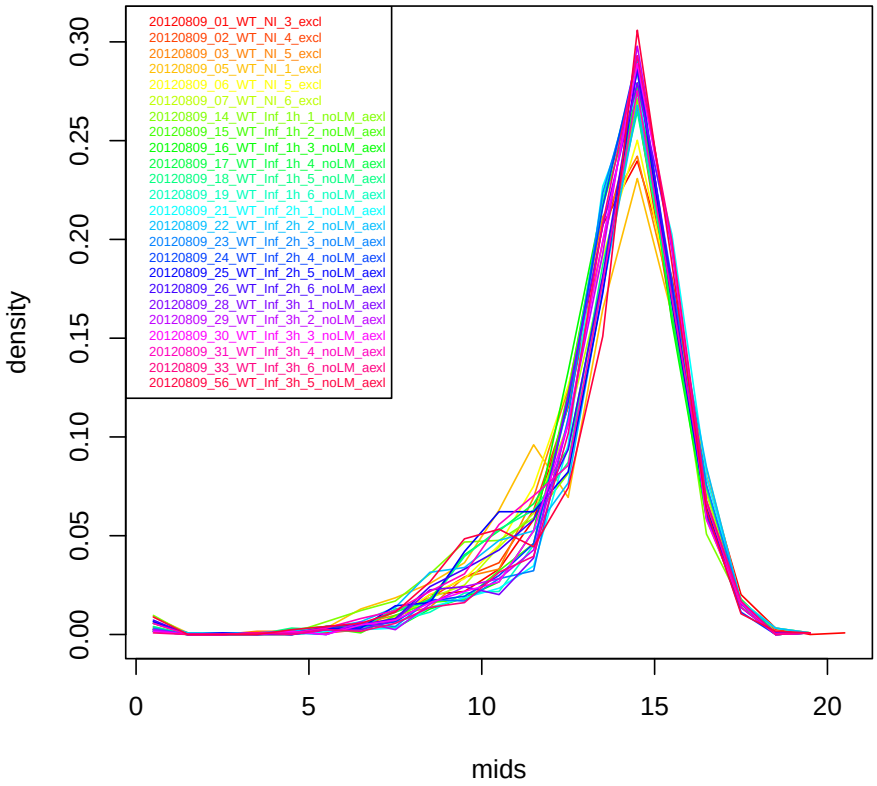
The plot shows the estimated concentration of the four proteins using the top three most intense peptides. The Fetuin peptides are spiked in with increasing concentration while the three other yeast proteins are kept stable in the background.

4.2. pgLFQ – LCMS based label-free quantification

LC-MS based label-free quantification (LFQ) is a very popular method to extract relative quantitative information from mass spectrometry experiments. At the FGCZ we use the soft-

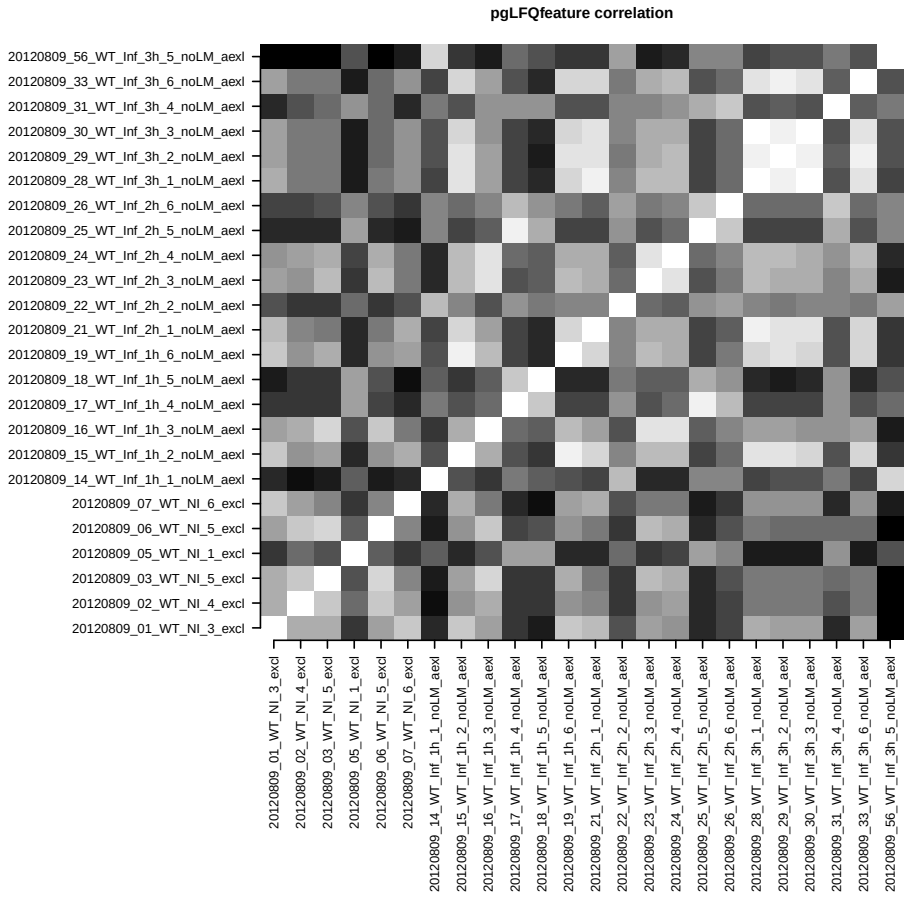
ware ProgenesisLCMS for this workflow http://www.nonlinear.com/products/progenesis/lc-ms/overview/. Progenesis is a graphical software which does the aligning between several LCMS experiments, extracts signal intensities from LCMS maps and annotates the master map with peptide and protein labels.

```
R> data(pgLFQfeature)
R> data(pgLFQprot)
R> featureDensityPlot<-function(data, n=ncol(data), nbins=30){
+      my.col<-rainbow(n);
+      mids<-numeric()
+      density<-numeric()
+      for (i in 1:n) {
+          h<-hist(data[,i],nbins, plot=FALSE)
+          mids<-c(mids, h$mids)
+          density<-c(density, h$density)
+      }
+      plot(mids,density, type='n')
+      for (i in 1:n) {
+          h<-hist(data[,i],nbins, plot=FALSE)
+          lines(h$mids,h$density, col=my.col[i])
+      }
+      legend("topleft", names(data), cex=0.5,
+          text.col=my.col
+      )
+  }
R> par(mfrow=c(1,1));
R> featureDensityPlot(asinh(pgLFQfeature$"Normalized abundance"),
+      nbins=25)
```



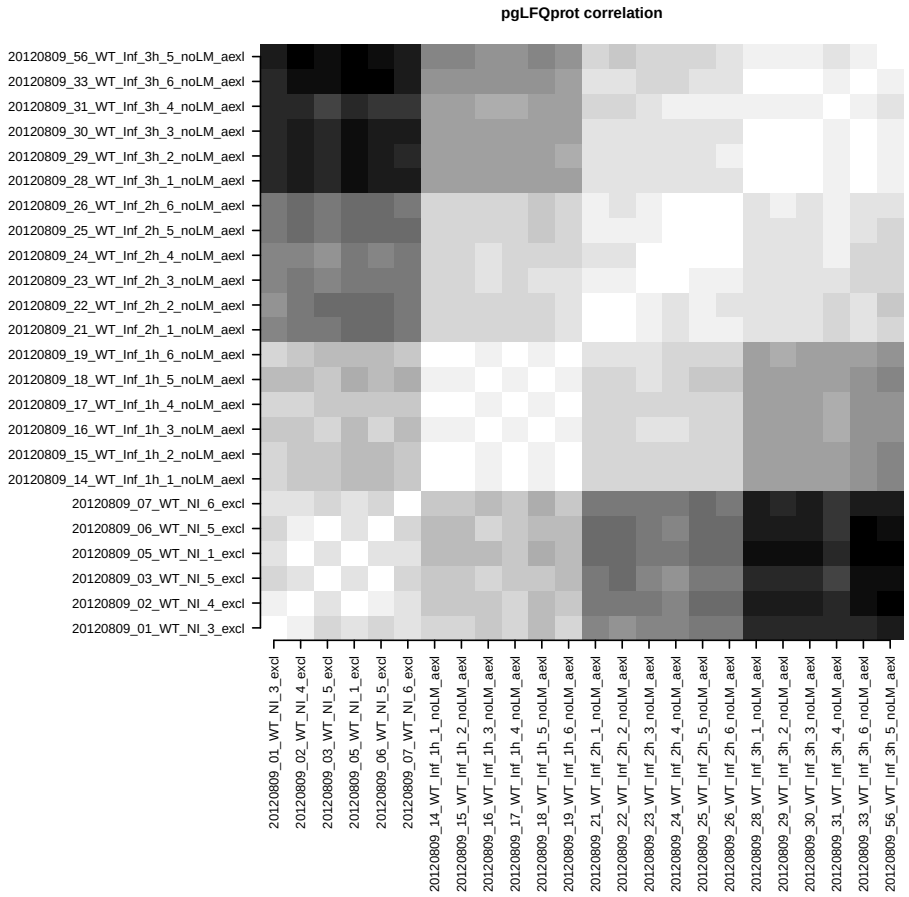
The `featureDensityPlot` shows the normalized signal intensity distribution (asinh transformed) over 24 LCMS runs which are aligned in this experiment.

```
R> op<-par(mfrow=c(1,1),mar=c(18,18,4,1),cex=0.5)
R> samples<-names(pgLFQfeature$"Normalized abundance")
R> image(cor(asinh(pgLFQfeature$"Normalized abundance")),
+      col=gray(seq(0,1,length=20)),
+      main='pgLFQfeature correlation',
+      axes=FALSE)
R> axis(1,at=seq(from=0, to=1,
+      length.out=length(samples)),
+      labels=samples, las=2)
R> axis(2,at=seq(from=0, to=1,
+      length.out=length(samples)), labels=samples, las=2)
R> par(op)
```



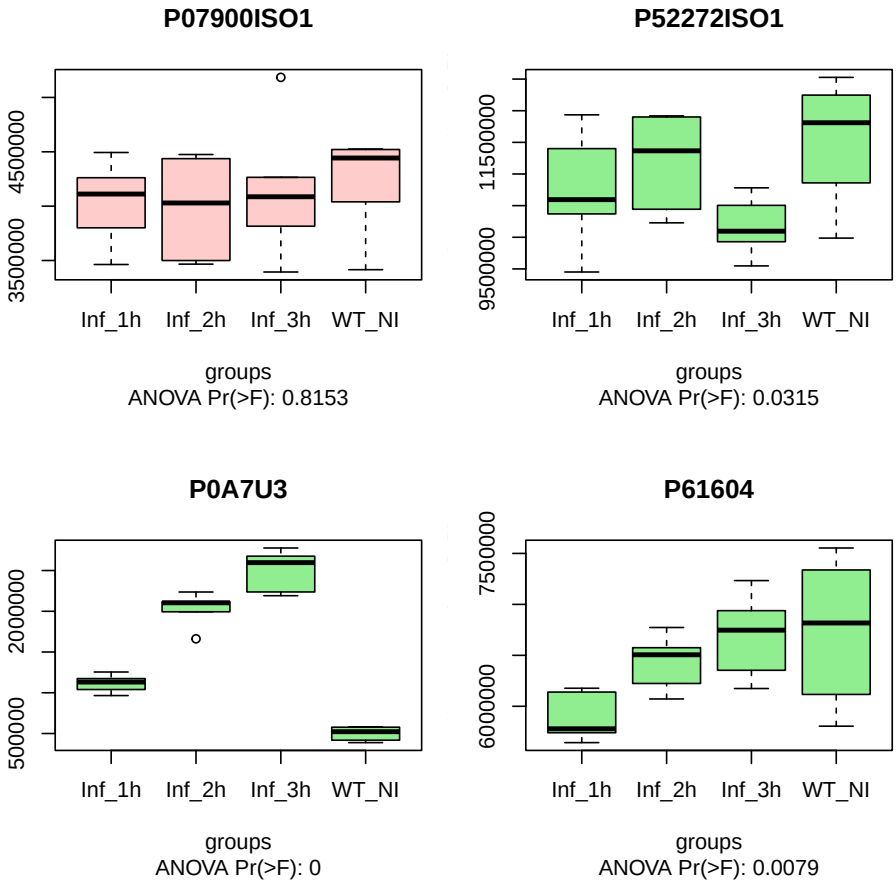
This image plot shows the correlation between runs on feature level (values are `asinh` transformed). White is perfect correlation while black indicates a poor correlation.

```
R> op<-par(mfrow=c(1,1),mar=c(18,18,4,1),cex=0.5)
R> image(cor(asinh(pgLFQprot$"Normalized abundance")),
+      main='pgLFQprot correlation',
+      axes=FALSE,
+      col=gray(seq(0,1,length=20)))
R> axis(1,at=seq(from=0, to=1,
+      length.out=length(samples)), labels=samples, las=2)
R> axis(2,at=seq(from=0, to=1,
+      length.out=length(samples)), labels=samples, las=2)
R> par(op)
```



This figure shows the correlation between runs on protein level (values are `asinh` transformed). White is perfect correlation while black indicates a poor correlation. Striking is the fact that the six biological replicates for each condition cluster very well.

```
R> par(mfrow=c(2,2),mar=c(6,3,4,1))
R> ANOVA<-pgLFQaov(pgLFQprot$"Normalized abundance",
+       groups=as.factor(pgLFQprot$grouping),
+       names=pgLFQprot$output$Accession,
+       idx=c(15,16,196,107),
+       plot=TRUE)
```



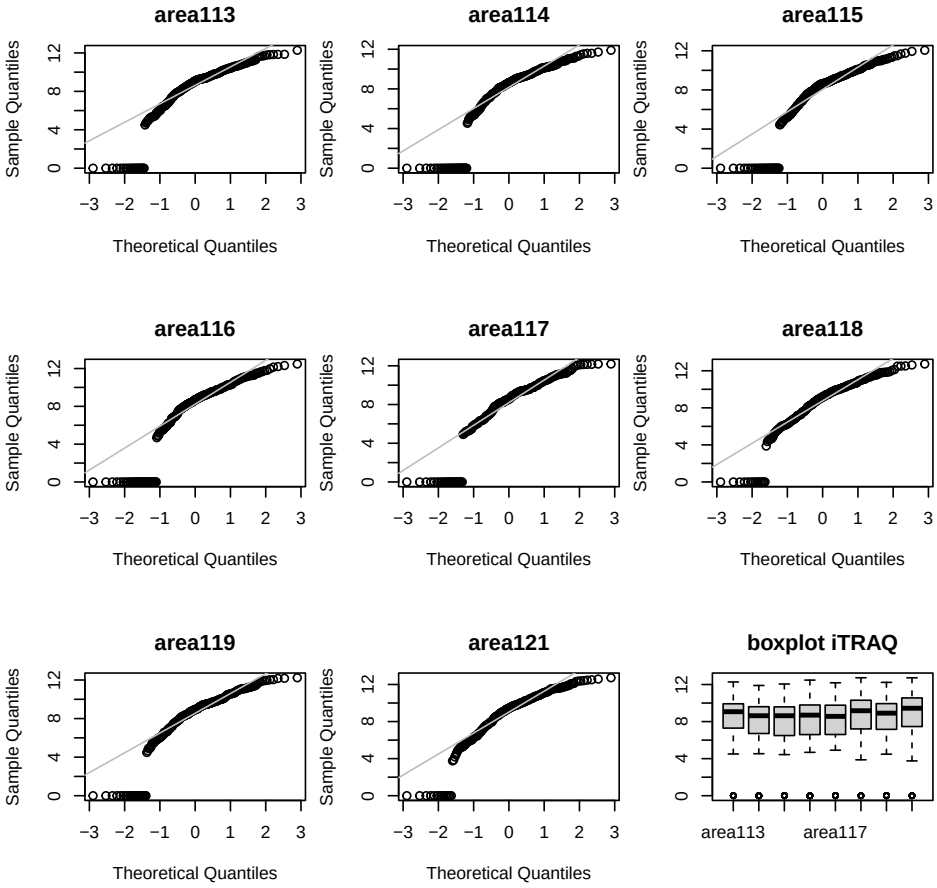
This figure shows the result for four proteins which either differ significantly in expression across conditions (green boxplots) using an analysis of variance test, or non-differing protein expression (red boxplot).

4.3. iTRAQ – Two Group Analysis

The data for the next section is an iTRAQ-8-plex experiment where two conditions are compared (each condition has four biological replicates)

Sanity Check

```
R> data(iTRAQ)
R> x<-rnorm(100)
R> par(mfrow=c(3,3),mar=c(6,4,3,0.5));
R> for (i in 3:10){
+       qqnorm(asinh(iTRAQ[,i]),
+           main=names(iTRAQ)[i])
+       qqline(asinh(iTRAQ[,i]), col='grey')
+  }
R> b<-boxplot(asinh(iTRAQ[,c(3:10)]), main='boxplot iTRAQ')
```

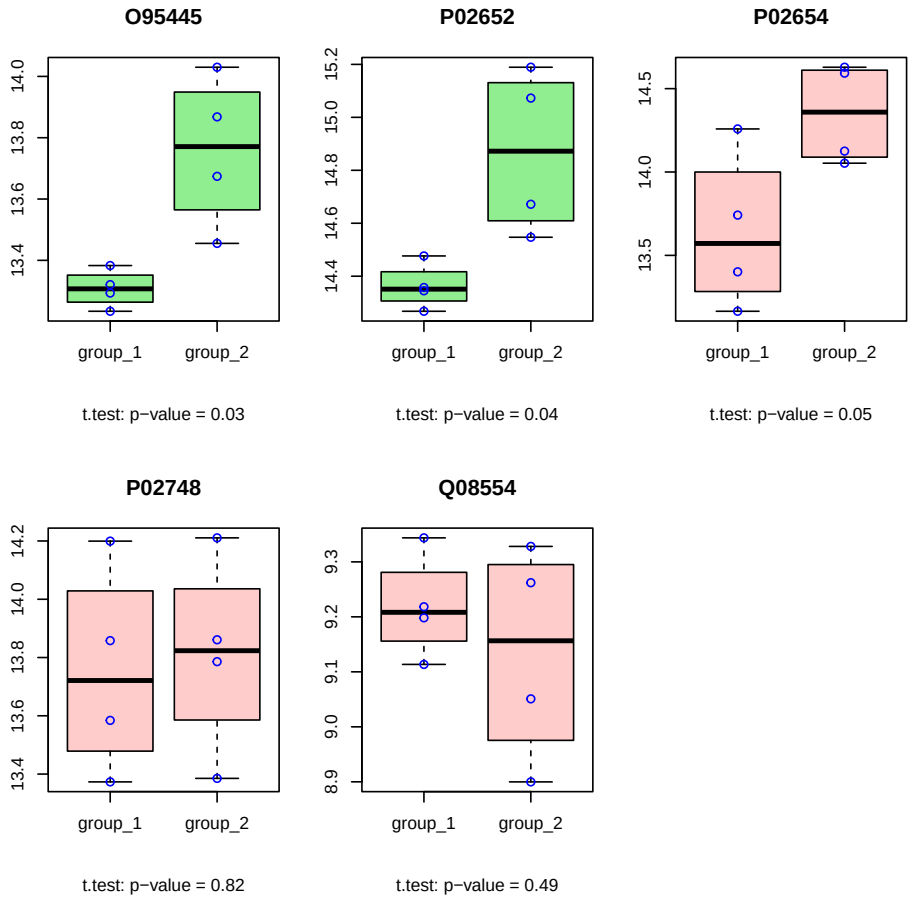


A first quality check to see if all reporter ion channels are having the same distributions. Shown in the figure are Q-Q plots of the individual reporter channels against a normal distribution. The last is a boxplot for all individual channels.

On Protein Level

```
R> data(iTRAQ)
R> group1Protein<-numeric()
R> group2Protein<-numeric()
R> for (i in c(3,4,5,6))
+      group1Protein<-cbind(group1Protein,
+          asinh(tapply(iTRAQ[,i], paste(iTRAQ$prot), sum, na.rm=TRUE)))
R> for (i in 7:10)
+      group2Protein<-cbind(group2Protein,
+          asinh(tapply(iTRAQ[,i], paste(iTRAQ$prot), sum, na.rm=TRUE)))
R> par(mfrow=c(2,3),mar=c(6,3,4,1))
R> for (i in 1:nrow(group1Protein)){
+      boxplot.color="#ffcccc"
+      tt.p_value <- t.test(as.numeric(group1Protein[i,]),
+          as.numeric(group2Protein[i,]))$p.value
```

```
+
+      if (tt.p_value < 0.05)
+          boxplot.color='lightgreen'
+
+      b <- boxplot(as.numeric(group1Protein[i,]),
+          as.numeric(group2Protein[i,]),
+          main=row.names(group1Protein)[i],
+          sub=paste("t.test: p-value =", round(tt.p_value,2)),
+          col=boxplot.color,
+          axes=FALSE)
+      axis(1, 1:2, c('group_1','group_2')); axis(2); box()
+
+      points(rep(1,b$n[1]), as.numeric(group1Protein[i,]), col='blue')
+      points(rep(2,b$n[2]), as.numeric(group2Protein[i,]), col='blue')
+  }
```



This figure shows five proteins which are tested if they differ across conditions using the four biological replicates with a t statistic.

On Peptide Level

The same can be done on peptide level using the `protViz` function `iTRAQ2GroupAnalysis`.

```
R> data(iTRAQ)
R> q <- iTRAQ2GroupAnalysis(data=iTRAQ,
+      group1=c(3,4,5,6),
+      group2=7:10,
+      INDEX=paste(iTRAQ$prot,iTRAQ$peptide),
+      plot=FALSE)
R> q[1:10,]
```

```
                          name p_value Group1.area113
1                 O95445 AFLLTPR   0.056        1705.43
2                 O95445 DGLCVPR   0.161        2730.41
3               O95445 MKDGLCVPR   0.039       28726.38
4              O95445 NQEACELSNN   0.277        4221.31
5               O95445 SLTSCLDSK   0.036       20209.66
6    P02652 AGTELVNFLSYFVELGTQPA   0.640        4504.97
7   P02652 AGTELVNFLSYFVELGTQPAT   0.941       67308.30
8  P02652 AGTELVNFLSYFVELGTQPATQ   0.338        4661.54
9    P02652 EPCVESLVSQYFQTVTDYGK   0.115        4544.56
10              P02652 EQLTPLIK   0.053       24596.42
   Group1.area114 Group1.area115 Group1.area116 Group2.area117
1         1459.10         770.65        3636.40        3063.48
2         1852.90        1467.65        2266.88        2269.57
3        15409.81       19050.13       58185.02       51416.05
4         4444.28        2559.23        6859.71        5545.12
5        14979.02       12164.94       37572.56       30687.57
6         4871.88        2760.53        9213.41        6728.62
7        46518.21       33027.14      111629.30       94531.76
8         3971.82        2564.39        8269.73        6045.30
9         4356.51        2950.48        6357.90        6819.99
10       22015.94       18424.56       49811.91       33197.47
   Group2.area118 Group2.area119 Group2.area121
1         4046.73        2924.49        5767.87
2         3572.32        2064.82        2208.92
3        70721.05       38976.42       60359.72
4        11925.66        6371.50       15656.92
5        39176.99       34417.66       54439.22
6        14761.96        7796.29       18681.60
7       168775.00       83526.72      168032.50
8        13724.92        7426.84       17214.87
9        10265.84        7012.92       14279.22
10       67213.62       40030.86       87343.38
```

5. Pressure Profiles QC

A common problem with mass spec setup is the pure reliability of the high-pressure pump. The following graphics provide visualizations for quality control.

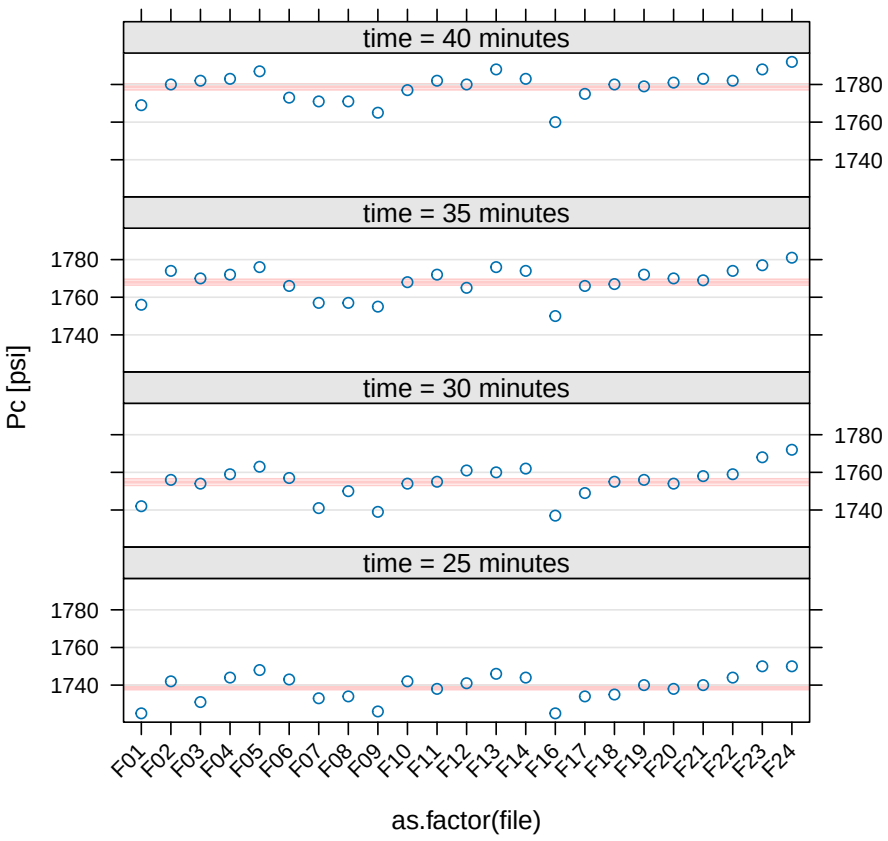
An overview of the pressure profile data can be seen by using the `ppp` function.

```
R> data(pressureProfile)
R> ppp(pressureProfile)
```

The lines plots the pressure profiles data on a scatter plot "Pc" versus "time" grouped by time range (no figure because of too many data items).

The Trellis `xyplot` shows the Pc development over each instrument run to a specified relative runtime $(25, 30, \ldots)$.

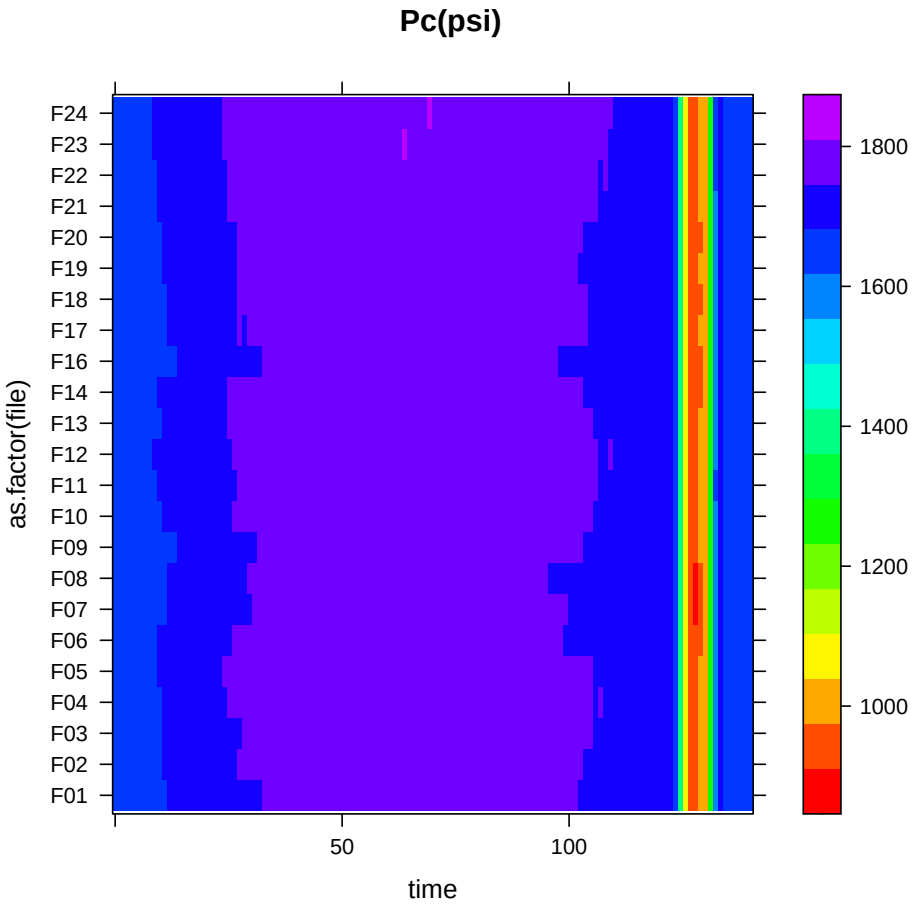
```
R> pp.data<-pps(pressureProfile, time=seq(25,40,by=5))
R> print(xyplot(Pc ~ as.factor(file) | paste("time =",
+      as.character(time), "minutes"),
+      panel = function(x, y){
+          m<-sum(y)/length(y)
+          m5<-(max(y)-min(y))*0.05
+          panel.abline(h=c(m-m5,m,m+m5),
+              col=rep("#ffcccc",3),lwd=c(1,2,1))
+          panel.grid(h=-1, v=0)
+          panel.xyplot(x, y)
+      },
+      ylab='Pc [psi]',
+      layout=c(1,4),
+      sub='The three red lines indicate the average plus min 5%.',
+      scales = list(x = list(rot = 45)),
+      data=pp.data))
```



The three red lines indicate the average plus min 5%.

While each panel in the `xyplot` above shows the data to a given point in time, we try to use the levelplot to get an overview of the whole pressure profile data.

```
R> pp.data<-pps(pressureProfile, time=seq(0,140,length=128))
R> print(levelplot(Pc ~ time * as.factor(file),
+       main='Pc(psi)',
+       data=pp.data,
+       col.regions=rainbow(100)[1:80]))
```



The **protViz** package has also been used in Grossmann *et al.* 2010; Nanni, Panse, Gehrig, Mueller, Grossmann, and Schlapbach 2013; Panse, Trachsel, Grossmann, and Schlapbach 2015; Kockmann, Trachsel, Panse, Wahlander, Selevsek, Grossmann, Wolski, and Schlapbach 2016; Bilan, Leutert, Nanni, Panse, and Hottiger 2017; Egloff, Zimmermann, Arnold, Hutter, Morger, Opitz, Poveda, Keserue, Panse, Roschitzki, and Seeger 2018; Gehrig, Nowak, Panse, Leutert, Grossmann, Schlapbach, and Hottiger 2020; Kockmann and Panse 2021.

References

Bantscheff M, Lemeer S, Savitski MM, Kuster B (2012). "Quantitative mass spectrometry in proteomics: critical review update from 2007 to the present." *Anal Bioanal Chem*, **404**(4), 939–965. doi:10.1007/s00216-012-6203-4.

Bilan V, Leutert M, Nanni P, Panse C, Hottiger MO (2017). "Combining Higher-Energy Collision Dissociation and Electron-Transfer/Higher-Energy Collision Dissociation Fragmentation in a Product-Dependent Manner Confidently Assigns Proteomewide ADP-Ribose Acceptor Sites." *Anal. Chem.*, **89**(3), 1523–1530. doi:10.1021/acs.analchem.6b03365.

Cappadona S, Baker PR, Cutillas PR, Heck AJ, van Breukelen B (2012). "Current challenges

in software solutions for mass spectrometry-based quantitative proteomics." *Amino Acids*, **43**(3), 1087–1108. doi:10.1007/s00726-012-1289-8.

Egloff P, Zimmermann I, Arnold FM, Hutter CA, Morger D, Opitz L, Poveda L, Keserue HA, Panse C, Roschitzki B, Seeger M (2018). "Engineered Peptide Barcodes for In-Depth Analyses of Binding Protein Ensembles." doi:10.1101/287813. URL https://doi.org/10.1101/287813.

Gehrig PM, Nowak K, Panse C, Leutert M, Grossmann J, Schlapbach R, Hottiger MO (2020). "Gas-Phase Fragmentation of ADP-Ribosylated Peptides: Arginine-Specific Side-Chain Losses and Their Implication in Database Searches." *Journal of the American Society for Mass Spectrometry*, **32**(1), 157–168. doi:10.1021/jasms.0c00040. URL https://doi.org/10.1021/jasms.0c00040.

Grossmann J, Roschitzki B, Panse C, Fortes C, Barkow-Oesterreicher S, Rutishauser D, Schlapbach R (2010). "Implementation and evaluation of relative and absolute quantification in shotgun proteomics with label-free methods." *J Proteomics*, **73**(9), 1740–1746. doi:10.1016/j.jprot.2010.05.011.

Kockmann T, Panse C (2021). "The rawrr R Package: Direct Access to Orbitrap Data and Beyond." *Journal of Proteome Research*. doi:10.1021/acs.jproteome.0c00866. URL https://doi.org/10.1021/acs.jproteome.0c00866.

Kockmann T, Trachsel C, Panse C, Wahlander A, Selevsek N, Grossmann J, Wolski WE, Schlapbach R (2016). "Targeted proteomics coming of age - SRM, PRM and DIA performance evaluated from a core facility perspective." *Proteomics*, **16**(15-16), 2183–2192. doi:10.1002/pmic.201500502.

Krokhin OV, Craig R, Spicer V, Ens W, Standing KG, Beavis RC, Wilkins JA (2004). "An improved model for prediction of retention times of tryptic peptides in ion pair reversed-phase HPLC: its application to protein peptide mapping by off-line HPLC-MALDI MS." *Mol. Cell Proteomics*, **3**(9), 908–919. doi:10.1074/mcp.M400031-MCP200.

Nanni P, Panse C, Gehrig P, Mueller S, Grossmann J, Schlapbach R (2013). "PTM MarkerFinder, a software tool to detect and validate spectra from peptides carrying post-translational modifications." *Proteomics*, **13**(15), 2251–2255. doi:10.1002/pmic.201300036.

Panse C, Gerrits B, Schlapbach R (2009). "PEAKPLOT: Visualizing Fragmented Peptide Mass Spectra in Proteomics." UseR!2009 conference, Rennes, F, URL https://www.r-project.org/conferences/useR-2009/abstracts/pdf/Panse+Gerrits+Schlapbach.pdf.

Panse C, Trachsel C, Grossmann J, Schlapbach R (2015). "specL–an R/Bioconductor package to prepare peptide spectrum matches for use in targeted proteomics." *Bioinformatics*, **31**(13), 2228–2231. doi:10.1093/bioinformatics/btv105.

Roepstorff P, Fohlman J (1984). "Proposal for a common nomenclature for sequence ions in mass spectra of peptides." *Biomed. Mass Spectrom.*, **11**(11), 601. doi:10.1002/bms.1200111109.

Trachsel C, Panse C, Kockmann T, Wolski WE, Grossmann J, Schlapbach R (2018). "rawDiag - an R package supporting rational LC-MS method optimization for bottom-up proteomics." doi:10.1101/304485. URL https://doi.org/10.1101/304485.

A. Session information

An overview of the package versions used to produce this document are shown below.

- R version 4.3.2 (2023-10-31), `x86_64-pc-linux-gnu`
- Locale: `LC_CTYPE=C`, `LC_NUMERIC=C`, `LC_TIME=en_US.UTF-8`, `LC_COLLATE=C`, `LC_MONETARY=en_US.UTF-8`, `LC_MESSAGES=en_US.UTF-8`, `LC_PAPER=en_US.UTF-8`, `LC_NAME=C`, `LC_ADDRESS=C`, `LC_TELEPHONE=C`, `LC_MEASUREMENT=en_US.UTF-8`, `LC_IDENTIFICATION=C`
- Time zone: `Europe/Zurich`
- TZcode source: `system (glibc)`
- Running under: `Debian GNU/Linux trixie/sid`
- Matrix products: default
- BLAS: `/usr/lib/x86_64-linux-gnu/blas/libblas.so.3.11.0`
- LAPACK: `/usr/lib/x86_64-linux-gnu/lapack/liblapack.so.3.11.0`
- Base packages: base, datasets, grDevices, graphics, methods, stats, utils
- Other packages: lattice 0.22-5, protViz 0.7.9, xtable 1.8-4
- Loaded via a namespace (and not attached): Rcpp 1.0.11, codetools 0.2-19, compiler 4.3.2, grid 4.3.2, tools 4.3.2

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