protViz: Visualizing and Analyzing Mass Spectrometry Related Data in Proteomics

Christian Panse
Functional Genomics Center Zurich

Jonas Grossmann
Functional Genomics Center Zurich

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.

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
• **protViz** - 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
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
R> substr(F$getSequences(), 1, 60)
[1] "MKSFVLLFCALQLWGCHSIPLDVAGYKEPACDDPTEQAALAAVDYINKHLPRGYKHTL"
```

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

[1] "MK"
[2] "SFVLLFCALQLWGCHSIPLDVAGYK"
[3] "EPACDDPTEQAALAAVDYINK"
[4] "HLPR"
[5] "GYK"
[6] "HTLNQIDSVK"
[7] "VWPR"
[8] "RPTGEVYIDETLCHVLDPTLANCSVR"
[9] "QQTQHAVEGCDIHLVK"
[10] "QDGQFSVLF" 
```
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

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

\[ R> \text{mass} \leftarrow \text{parentIonMass(fetuin)} \]

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

\[ R> \text{hydrophobicity} \leftarrow \text{ssrc(fetuin)} \]

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

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.

\[ R> \text{op} \leftarrow \text{par(mfrow = c(1, 1))} \]
\[ R> \text{plot(hydrophobicity} \sim \text{mass,} \]

\[ [11] "CDSSPDSAEDVR"
[12] "K"
[13] "LCPDCPLLAPLNDSR"
[14] "VVHAVEVALTFNAESNGYQLVEISR"
[15] "AQFVLPVSVSEVFAAATDCIAK"
[16] "EVVDPTK"
[17] "CNLLAEK"
[18] "QYGFC"
[19] "GSVIQK"
[20] "ALGGEDVR"
[21] "VTCTLFQTQVIPQPQPDGAEEAEPSAVPDAAGPTPSAAGPPVASVVGPSVVAVPLPLHR"
[22] "AHYDLR"
[23] "HTFSGVASVESSGEAFHVGK"
[24] "TPIVQPSIPGPPVR"
[25] "LCGGR"
[26] "IR"
[27] "YFK"
[28] "I" \]
Table 1: parent ion mass and hydrophobicity values of the tryptic digested protein extttP12763.

<table>
<thead>
<tr>
<th>peptide</th>
<th>mass</th>
<th>hydrophobicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>MK</td>
<td>278.15</td>
<td></td>
</tr>
<tr>
<td>SFVLLFCLAQLWGCHSIPLDPVAGYK</td>
<td>2991.53</td>
<td>71.74</td>
</tr>
<tr>
<td>EPACDDPDTEQAALAANVDYINK</td>
<td>2406.08</td>
<td>25.81</td>
</tr>
<tr>
<td>HLPR</td>
<td>522.31</td>
<td>6.05</td>
</tr>
<tr>
<td>GYK</td>
<td>367.20</td>
<td>2.16</td>
</tr>
<tr>
<td>HTHLNQIDSVK</td>
<td>1154.62</td>
<td>18.37</td>
</tr>
<tr>
<td>VWPR</td>
<td>557.32</td>
<td>9.55</td>
</tr>
<tr>
<td>RPTGEVYDIEIDTLETTCHVLDPTPLANCVR</td>
<td>3671.77</td>
<td>46.69</td>
</tr>
<tr>
<td>QTQHAVEGDCDIIHVLK</td>
<td>1977.94</td>
<td>21.45</td>
</tr>
<tr>
<td>QDQGFSYLFTK</td>
<td>1269.65</td>
<td>32.22</td>
</tr>
<tr>
<td>CDSPPDSEADVR</td>
<td>1337.53</td>
<td>2.98</td>
</tr>
<tr>
<td>K</td>
<td>147.11</td>
<td></td>
</tr>
<tr>
<td>LCPDCPLLAPLNSR</td>
<td>1740.84</td>
<td>31.62</td>
</tr>
<tr>
<td>VHHAVEVALATFNAESNGSYLQLEISR</td>
<td>3016.57</td>
<td>54.51</td>
</tr>
<tr>
<td>AQFVPLPVSVSVEFAATDCIAK</td>
<td>2519.32</td>
<td>53.75</td>
</tr>
<tr>
<td>EVVDPTK</td>
<td>787.42</td>
<td>7.78</td>
</tr>
<tr>
<td>CNLLAEK</td>
<td>847.43</td>
<td>16.51</td>
</tr>
<tr>
<td>QYGFCQ</td>
<td>802.36</td>
<td>10.05</td>
</tr>
<tr>
<td>GSVIPQK</td>
<td>631.38</td>
<td>9.83</td>
</tr>
<tr>
<td>AGLGDVR</td>
<td>816.42</td>
<td>10.35</td>
</tr>
<tr>
<td>VICTLFQTOPVQPDQASAEASVPDAAGPTSAAGPPVAVGSPVAVPLPLHR</td>
<td>6015.13</td>
<td>39.37</td>
</tr>
<tr>
<td>AHYDRL</td>
<td>774.39</td>
<td>11.42</td>
</tr>
<tr>
<td>HTFSGVASVESSGEAFHVVK</td>
<td>2120.00</td>
<td>27.95</td>
</tr>
<tr>
<td>TPIVGQPSIPGGPYR</td>
<td>1474.84</td>
<td>23.26</td>
</tr>
<tr>
<td>LCPR</td>
<td>602.31</td>
<td>3.61</td>
</tr>
<tr>
<td>IR</td>
<td>288.20</td>
<td></td>
</tr>
<tr>
<td>YFK</td>
<td>457.24</td>
<td>7.91</td>
</tr>
<tr>
<td>I</td>
<td>132.10</td>
<td></td>
</tr>
</tbody>
</table>

+ 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 being computed are defined in the function defaultIon. There 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: 0x558dbb9bbd40>
protViz

<environment: namespace:protViz>

```r
R> peptides<-c('HTLNQIDSVK', 'ALGGEDVR', 'TPIVGQPSIPGGPVR')
R> pim<-parentIonMass(peptides)
R> fi<-fragmentIon(peptides)
R> par(mfrow=c(3,1));
R> for (i in 1:length(peptides)){
+   plot(0,0,
+       xlab='m/Z',
+       ylab=''
+       xlim=range(c(fi[i][[1]]$b,fi[i][[1]]$y)),
+       ylim=c(0,1),
+       type='n',
+       axes=FALSE,
+       sub=paste( pim[i], "Da"));
+   box()
+   axis(1,fi[i][[1]]$b,round(fi[i][[1]]$b,2))
+   pepSeq<-strsplit(peptides[i],"")
+   axis(3,fi[i][[1]]$b,pepSeq[[1]])
+   abline(v=fi[i][[1]]$b, col='red',lwd=2)
+   abline(v=fi[i][[1]]$c, col='orange')
+   abline(v=fi[i][[1]]$y, col='blue',lwd=2)
+   abline(v=fi[i][[1]]$z, col='cyan')
+ }
```
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
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
```
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
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 <- 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 <- findNN(by.mZ, spec$mZ)
R> mZ.error <- abs(spec$mZ[idx]-by.mZ)
R> plot(mZ.error[idx<-order(mZ.error)],
+ main="Error Plot",
+ pch='o',
+ cex=0.5,
+ sub='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
R> library(proteinViz)
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="Substitution",
```
protViz

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

[[1]]
[1] "0000000000000000000" "0000000000000200000" "0000000000000001000"
[4] "000000000000000010200"

[[2]]
[1] "0000000000000000000" "0000000000000100000" "0000000000000000100"
[4] "0000000000000000100100"

R> fi <- fragmentIon(c('TAFDEIAEELDTLNEESYK',
+ 'TAFDEIAEELDTLSEESYK', 'TAFDEIAEELDTLNEESYK',
+ 'TAFDEIAEELDTLSEESYK'),
+ modified=c('0000000000000200000',
+ '0000000000000100000', '0000000000000000000',
+ '0000000000000000000'),
+ modification=m$mono)
R> #bh<-c('TAFDEIAEELDTLNEESYK', 'TAFDEIAEELDTLSEESYK')
R> #fi<-fragmentIon(rep('HTLNQIDSVK',2),
R> # modified=c('00000000100', '0000000000'),
R> # modification=m[,2])

3.5. Labeling Peaklists

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

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

$mZ.Da.error

<table>
<thead>
<tr>
<th></th>
<th>232.331344</th>
<th>161.294234</th>
<th>14.225824</th>
<th>-0.032616</th>
<th>-0.143306</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>0.032244</td>
<td>0.054604</td>
<td>-0.004076</td>
<td>-0.071746</td>
<td>-0.084536</td>
</tr>
<tr>
<td>11</td>
<td>-0.097076</td>
<td>-0.038856</td>
<td>-0.061816</td>
<td>0.004554</td>
<td>-0.122336</td>
</tr>
<tr>
<td>16</td>
<td>-0.139626</td>
<td>-1.071256</td>
<td>-18.783686</td>
<td>-146.878646</td>
<td>187.273499</td>
</tr>
<tr>
<td>21</td>
<td>24.210169</td>
<td>0.048669</td>
<td>0.177779</td>
<td>0.027939</td>
<td>0.049579</td>
</tr>
<tr>
<td>26</td>
<td>0.052379</td>
<td>0.044579</td>
<td>0.036749</td>
<td>0.043189</td>
<td>-0.035101</td>
</tr>
<tr>
<td>31</td>
<td>-0.061011</td>
<td>0.000729</td>
<td>-0.092081</td>
<td>2.011029</td>
<td>-8.412111</td>
</tr>
<tr>
<td>36</td>
<td>7.195579</td>
<td>-63.841531</td>
<td>-164.889211</td>
<td>215.304795</td>
<td>144.267685</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td>[41]</td>
<td>-2.800725</td>
<td>-17.059165</td>
<td>2.034875</td>
<td>2.264105</td>
<td>4.008125</td>
</tr>
<tr>
<td>[46]</td>
<td>1.292875</td>
<td>-0.003965</td>
<td>-13.612585</td>
<td>-0.060925</td>
<td>-17.065405</td>
</tr>
<tr>
<td>[51]</td>
<td>3.897535</td>
<td>3.000405</td>
<td>-17.148885</td>
<td>-17.166175</td>
<td>-18.097805</td>
</tr>
<tr>
<td>[56]</td>
<td>-35.810235</td>
<td>-163.905195</td>
<td>204.300048</td>
<td>41.236718</td>
<td>17.075218</td>
</tr>
<tr>
<td>[61]</td>
<td>-0.843372</td>
<td>-1.091812</td>
<td>0.129908</td>
<td>17.078928</td>
<td>-0.372162</td>
</tr>
<tr>
<td>[66]</td>
<td>-16.539502</td>
<td>-1.044962</td>
<td>-1.000952</td>
<td>-1.409062</td>
<td>-2.995122</td>
</tr>
<tr>
<td>[76]</td>
<td>-147.862662</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$mZ.ppm.error$

<p>| | | | | | | | | | | | | | | | | | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>[1]</td>
<td>2.276532e+06</td>
<td>9.318407e+05</td>
<td>4.443342e+04</td>
<td>-7.494702e+01</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[5]</td>
<td>-2.539851e+02</td>
<td>5.075660e+01</td>
<td>7.296574e+01</td>
<td>-4.974443e+00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[9]</td>
<td>7.564705e+01</td>
<td>-7.963713e+01</td>
<td>-8.250960e+01</td>
<td>-3.041352e+01</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[13]</td>
<td>-4.445040e+01</td>
<td>3.026848e+00</td>
<td>-7.488007e+01</td>
<td>-7.920687e+01</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[17]</td>
<td>-5.791093e+02</td>
<td>-9.331667e+03</td>
<td>-6.860308e+04</td>
<td>1.272993e+06</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[21]</td>
<td>7.805297e+04</td>
<td>1.225277e+02</td>
<td>3.378218e+02</td>
<td>4.263587e+01</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[25]</td>
<td>6.444386e+01</td>
<td>5.935833e+01</td>
<td>4.532837e+01</td>
<td>3.345395e+01</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[29]</td>
<td>3.564687e+01</td>
<td>-2.618263e+01</td>
<td>-4.321937e+01</td>
<td>4.781134e-01</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[33]</td>
<td>-5.770282e+01</td>
<td>1.165934e+03</td>
<td>-4.572174e+03</td>
<td>3.621478e+03</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[37]</td>
<td>-3.102183e+04</td>
<td>-7.637286e+04</td>
<td>1.808046e+06</td>
<td>7.588299e+05</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[41]</td>
<td>-8.306147e+03</td>
<td>-3.772366e+04</td>
<td>3.500821e+03</td>
<td>3.470990e+03</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[45]</td>
<td>5.236793e+03</td>
<td>1.545734e+03</td>
<td>-4.106862e+00</td>
<td>-1.262129e+04</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[49]</td>
<td>-5.104441e+01</td>
<td>-1.318183e+04</td>
<td>2.768725e+03</td>
<td>1.971690e+03</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[53]</td>
<td>-1.038832e+04</td>
<td>-9.648489e+03</td>
<td>-9.694247e+03</td>
<td>-1.764117e+04</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[57]</td>
<td>-7.595171e+04</td>
<td>1.570497e+06</td>
<td>1.406678e+05</td>
<td>4.491332e+04</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[61]</td>
<td>-1.656190e+03</td>
<td>-1.710589e+03</td>
<td>1.726789e+02</td>
<td>1.973544e+04</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[65]</td>
<td>-3.850849e+02</td>
<td>-1.529356e+04</td>
<td>-8.747728e+02</td>
<td>-7.562373e+02</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[69]</td>
<td>-1.010347e+03</td>
<td>-1.986529e+03</td>
<td>1.072648e+04</td>
<td>1.114745e+04</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[73]</td>
<td>4.725878e+03</td>
<td>1.229618e+04</td>
<td>-2.293808e+04</td>
<td>-6.903096e+04</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$idx$

<p>| | | | | | | | | | | | | | | | | | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>[1]</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>12</td>
<td>41</td>
<td>53</td>
<td>70</td>
<td>89</td>
<td>94</td>
<td>99</td>
<td>104</td>
<td>107</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[17]</td>
<td>115</td>
<td>116</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>12</td>
<td>41</td>
<td>53</td>
<td>70</td>
<td>89</td>
<td>94</td>
<td>99</td>
<td>104</td>
<td>107</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[33]</td>
<td>108</td>
<td>111</td>
<td>116</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>16</td>
<td>24</td>
<td>41</td>
<td>52</td>
<td>67</td>
<td>88</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[49]</td>
<td>93</td>
<td>97</td>
<td>104</td>
<td>107</td>
<td>110</td>
<td>113</td>
<td>115</td>
<td>116</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>11</td>
<td>22</td>
<td>40</td>
<td>53</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[65]</td>
<td>68</td>
<td>88</td>
<td>93</td>
<td>98</td>
<td>103</td>
<td>106</td>
<td>108</td>
<td>111</td>
<td>114</td>
<td>116</td>
<td>116</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$label$

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

$score$
protViz

[1] "TAFDEIAELDLNEESYK"

$sequence

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 1524.7428 1294.6161 1394.6322
13 1390.6736 1595.7799 1323.5951 1465.6322
14 1504.7165 1595.7799 1521.7431 1578.7533
15 1633.7591 1724.8225 1650.7857 1707.7959
16 1762.8017 1839.8494 1779.8263 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> peakplot("TAFDEIAELDLNEESYK", msms[[2]])

$mZ.Da.error

1 245.264254 174.227144 27.158734 14.444434 0.021404
2 -0.11266 -0.039926 -0.21626 -0.121916 -8.079236
3 -0.158376 -0.153156 -0.34316 -0.022946 -0.186736
4 -0.092226 -0.120456 -0.21686 -0.246664 200.206409
5 37.143079 0.078909 0.62269 0.129769 0.103729
6 0.060869 -0.051451 -18.048351 -0.027511 -0.025601
7 -0.006211 0.20529 -0.048781 -0.024771 -9.166311
8 6.953579 -45.209531 -146.257211 228.237705 157.200595
9 10.132185 -2.582115 1.626855 2.722405 9.009025
10 -1.30895 1.216385 13.347315 -3.671525 0.960295
11 -17.120865 3.020205 -17.213285 -17.118775 -17.147005
12 -17.178235 -145.273195 217.232985 54.169628 17.105458
13 -0.833452 -1.260332 -0.899352 -3.098942 -1.173512
14 -1.021802 -0.939162 -1.007752 -1.377062 -3.022622
15 16.977768 17.001778 7.860238 23.980128 23.980128
16 -129.230662
$mZ.ppm.error$

<p>| | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>[1]</td>
<td>2.403257e+06</td>
<td>1.006558e+06</td>
<td>8.482850e+04</td>
<td>3.319130e+04</td>
<td></td>
</tr>
<tr>
<td>[5]</td>
<td>3.793488e+01</td>
<td>-1.751484e+02</td>
<td>-5.335196e+01</td>
<td>-2.639286e+01</td>
<td></td>
</tr>
<tr>
<td>[9]</td>
<td>-1.285450e+02</td>
<td>-7.611043e+04</td>
<td>-1.346114e+02</td>
<td>-1.198789e+02</td>
<td></td>
</tr>
<tr>
<td>[13]</td>
<td>-6.782037e+01</td>
<td>-1.552813e+01</td>
<td>-1.162198e+02</td>
<td>-5.313198e+01</td>
<td></td>
</tr>
<tr>
<td>[17]</td>
<td>-6.608212e+01</td>
<td>-7.638202e+01</td>
<td>-6.066594e+04</td>
<td>1.360904e+06</td>
<td></td>
</tr>
<tr>
<td>[21]</td>
<td>1.197483e+05</td>
<td>1.986591e+02</td>
<td>1.183257e+02</td>
<td>1.980319e+02</td>
<td></td>
</tr>
<tr>
<td>[25]</td>
<td>1.397352e+02</td>
<td>7.115774e+01</td>
<td>-1.552813e+01</td>
<td>-4.85617e+01</td>
<td></td>
</tr>
<tr>
<td>[29]</td>
<td>-2.322450e+01</td>
<td>-1.948903e+01</td>
<td>-4.485617e+00</td>
<td>1.370673e+01</td>
<td></td>
</tr>
<tr>
<td>[33]</td>
<td>-3.109508e+01</td>
<td>-1.458996e+01</td>
<td>-5.056331e+03</td>
<td>3.547913e+03</td>
<td></td>
</tr>
<tr>
<td>[37]</td>
<td>-2.226035e+04</td>
<td>-6.860121e+04</td>
<td>1.916651e+06</td>
<td>8.268554e+05</td>
<td></td>
</tr>
<tr>
<td>[41]</td>
<td>3.004915e+04</td>
<td>5.709941e+03</td>
<td>2.798859e+03</td>
<td>4.173588e+03</td>
<td></td>
</tr>
<tr>
<td>[45]</td>
<td>1.177069e+04</td>
<td>1.352074e+03</td>
<td>1.259905e+03</td>
<td>1.237534e+04</td>
<td></td>
</tr>
<tr>
<td>[49]</td>
<td>-3.076091e+03</td>
<td>7.417604e+02</td>
<td>-1.216230e+04</td>
<td>2.020566e+03</td>
<td></td>
</tr>
<tr>
<td>[53]</td>
<td>-1.60078e+04</td>
<td>-9.766434e+03</td>
<td>-9.319787e+03</td>
<td>-8.576627e+03</td>
<td></td>
</tr>
<tr>
<td>[57]</td>
<td>-6.817113e+04</td>
<td>1.669915e+06</td>
<td>1.847849e+05</td>
<td>4.499286e+04</td>
<td></td>
</tr>
<tr>
<td>[61]</td>
<td>-1.636709e+03</td>
<td>-1.974616e+03</td>
<td>-1.239974e+03</td>
<td>-3.696333e+03</td>
<td></td>
</tr>
<tr>
<td>[65]</td>
<td>-1.249174e+03</td>
<td>-9.690310e+02</td>
<td>-8.043928e+02</td>
<td>-7.772361e+02</td>
<td></td>
</tr>
<tr>
<td>[69]</td>
<td>-1.006903e+03</td>
<td>-2.041339e+03</td>
<td>1.094110e+04</td>
<td>1.011538e+04</td>
<td></td>
</tr>
<tr>
<td>[73]</td>
<td>4.376983e+03</td>
<td>1.234257e+04</td>
<td>-1.399411e+04</td>
<td>-6.110297e+04</td>
<td></td>
</tr>
</tbody>
</table>

$idx$

<p>| | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>[1]</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>11</td>
</tr>
<tr>
<td>[17]</td>
<td>131</td>
<td>133</td>
<td>133</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>[33]</td>
<td>123</td>
<td>130</td>
<td>130</td>
<td>130</td>
<td>133</td>
</tr>
<tr>
<td>[49]</td>
<td>98</td>
<td>108</td>
<td>116</td>
<td>122</td>
<td>126</td>
</tr>
<tr>
<td>[65]</td>
<td>62</td>
<td>90</td>
<td>95</td>
<td>108</td>
<td>113</td>
</tr>
</tbody>
</table>

$label$

<p>| | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>[1]</td>
<td>&quot;b1&quot;</td>
<td>&quot;b2&quot;</td>
<td>&quot;b3&quot;</td>
<td>&quot;b4&quot;</td>
<td>&quot;b5&quot;</td>
</tr>
<tr>
<td>[12]</td>
<td>&quot;b12&quot;</td>
<td>&quot;b13&quot;</td>
<td>&quot;b14&quot;</td>
<td>&quot;b15&quot;</td>
<td>&quot;b16&quot;</td>
</tr>
<tr>
<td>[23]</td>
<td>&quot;y4&quot;</td>
<td>&quot;y5&quot;</td>
<td>&quot;y6&quot;</td>
<td>&quot;y7&quot;</td>
<td>&quot;y8&quot;</td>
</tr>
<tr>
<td>[34]</td>
<td>&quot;y15&quot;</td>
<td>&quot;y16&quot;</td>
<td>&quot;y17&quot;</td>
<td>&quot;y18&quot;</td>
<td>&quot;y19&quot;</td>
</tr>
<tr>
<td>[45]</td>
<td>&quot;c7&quot;</td>
<td>&quot;c8&quot;</td>
<td>&quot;c9&quot;</td>
<td>&quot;c10&quot;</td>
<td>&quot;c11&quot;</td>
</tr>
<tr>
<td>[56]</td>
<td>&quot;c18&quot;</td>
<td>&quot;c19&quot;</td>
<td>&quot;z1&quot;</td>
<td>&quot;z2&quot;</td>
<td>&quot;z3&quot;</td>
</tr>
<tr>
<td>[67]</td>
<td>&quot;z10&quot;</td>
<td>&quot;z11&quot;</td>
<td>&quot;z12&quot;</td>
<td>&quot;z13&quot;</td>
<td>&quot;z14&quot;</td>
</tr>
</tbody>
</table>

$score$

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>[1]</td>
<td>-1</td>
</tr>
</tbody>
</table>

$sequence$

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>[1]</td>
<td>&quot;TAFDEIAIAELDTSEESYK&quot;</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$fragmentIon$

<table>
<thead>
<tr>
<th></th>
<th>b</th>
<th>y</th>
<th>c</th>
<th>z</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>102.0550</td>
<td>147.1128</td>
<td>119.0815</td>
<td>130.0863</td>
</tr>
<tr>
<td>2</td>
<td>173.0921</td>
<td>310.1761</td>
<td>190.1186</td>
<td>293.1496</td>
</tr>
<tr>
<td>m/z</td>
<td>Intensity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------</td>
<td>-----------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>397.2082</td>
<td>337.1870</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>330.1605</td>
<td>380.1816</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>526.2508</td>
<td>452.2140</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>655.2933</td>
<td>638.2668</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>742.3254</td>
<td>725.2988</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>855.4094</td>
<td>838.3829</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>956.4571</td>
<td>939.4306</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1071.4841</td>
<td>1167.5416</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1184.5681</td>
<td>1296.5842</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1313.6107</td>
<td>1367.6213</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1384.6478</td>
<td>1480.7053</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1477.7056</td>
<td>1551.7424</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1623.7748</td>
<td>1680.7850</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1752.8174</td>
<td>1795.8120</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1839.8494</td>
<td>1942.8804</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2002.9127</td>
<td>2013.9175</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2131.0077</td>
<td>2114.9652</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

R> par(op)

---

**Fragment Ions**

<table>
<thead>
<tr>
<th>m/z</th>
<th>Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>397.257</td>
<td>y3</td>
</tr>
<tr>
<td>435.155</td>
<td>b4</td>
</tr>
<tr>
<td>526.429</td>
<td>y4</td>
</tr>
<tr>
<td>564.087</td>
<td>b5</td>
</tr>
<tr>
<td>635.299</td>
<td>b6</td>
</tr>
<tr>
<td>655.321</td>
<td>y5</td>
</tr>
<tr>
<td>748.406</td>
<td>b7</td>
</tr>
<tr>
<td>752.440</td>
<td>z6</td>
</tr>
<tr>
<td>769.386</td>
<td>y6</td>
</tr>
<tr>
<td>819.384</td>
<td>b8</td>
</tr>
<tr>
<td>882.473</td>
<td>y7</td>
</tr>
<tr>
<td>948.359</td>
<td>b9</td>
</tr>
<tr>
<td>965.453</td>
<td>c9</td>
</tr>
<tr>
<td>966.069</td>
<td>z8</td>
</tr>
<tr>
<td>983.513</td>
<td>y8</td>
</tr>
<tr>
<td>1061.430</td>
<td>b10</td>
</tr>
<tr>
<td>1098.532</td>
<td>y9</td>
</tr>
<tr>
<td>1176.445</td>
<td>b11</td>
</tr>
<tr>
<td>1193.507</td>
<td>c11</td>
</tr>
<tr>
<td>1211.622</td>
<td>y10</td>
</tr>
<tr>
<td>1277.551</td>
<td>b12</td>
</tr>
<tr>
<td>1313.585</td>
<td>y11</td>
</tr>
<tr>
<td>1340.586</td>
<td>y12</td>
</tr>
<tr>
<td>1390.612</td>
<td>b13</td>
</tr>
<tr>
<td>1411.598</td>
<td>y12</td>
</tr>
<tr>
<td>1504.721</td>
<td>b14</td>
</tr>
<tr>
<td>1524.744</td>
<td>y13</td>
</tr>
<tr>
<td>1595.688</td>
<td>y14</td>
</tr>
<tr>
<td>1633.637</td>
<td>b15</td>
</tr>
<tr>
<td>1762.662</td>
<td>b16</td>
</tr>
</tbody>
</table>

---

**Fragment Ions**

<table>
<thead>
<tr>
<th>m/z</th>
<th>Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>397.287</td>
<td>y3</td>
</tr>
<tr>
<td>526.313</td>
<td>y4</td>
</tr>
<tr>
<td>564.251</td>
<td>b5</td>
</tr>
<tr>
<td>635.156</td>
<td>b6</td>
</tr>
<tr>
<td>655.423</td>
<td>y5</td>
</tr>
<tr>
<td>742.429</td>
<td>y6</td>
</tr>
<tr>
<td>748.311</td>
<td>b7</td>
</tr>
<tr>
<td>819.367</td>
<td>b8</td>
</tr>
<tr>
<td>855.470</td>
<td>y7</td>
</tr>
<tr>
<td>948.309</td>
<td>b9</td>
</tr>
<tr>
<td>956.406</td>
<td>y8</td>
</tr>
<tr>
<td>1176.383</td>
<td>b11</td>
</tr>
<tr>
<td>1184.541</td>
<td>y10</td>
</tr>
<tr>
<td>1277.436</td>
<td>b12</td>
</tr>
<tr>
<td>1313.585</td>
<td>y11</td>
</tr>
<tr>
<td>1384.642</td>
<td>y12</td>
</tr>
<tr>
<td>1390.579</td>
<td>b13</td>
</tr>
<tr>
<td>1477.683</td>
<td>b14</td>
</tr>
<tr>
<td>1497.752</td>
<td>y13</td>
</tr>
<tr>
<td>1568.720</td>
<td>y14</td>
</tr>
<tr>
<td>1606.562</td>
<td>b15</td>
</tr>
<tr>
<td>1697.787</td>
<td>y15</td>
</tr>
<tr>
<td>1735.699</td>
<td>b16</td>
</tr>
<tr>
<td>1822.702</td>
<td>b17</td>
</tr>
<tr>
<td>1985.735</td>
<td>b18</td>
</tr>
</tbody>
</table>

---

**R> par(op)**
The following code snippet combine all the function to a simple peptide search engine. As default arguments the mass spec measurement, a list of mZ and intensity arrays, and a character vector of peptide sequences is given.

```r
R> peptideSearch <- function (x,
+   peptideSequence,
+   pimIdx = parentIonMass(peptideSequence),
+   peptideMassTolerancePPM = 5,
+   fragmentIonMassToleranceDa = 0.01,
+   FUN = .byIon)
+ {
+   query.mass <- ((x$pepmass * x$charge)) - (1.007825 * (x$charge -
+     1))
+   eps <- query.mass * peptideMassTolerancePPM * 1e-06
+   lower <- findNN(query.mass - eps, pimIdx)
+   upper <- findNN(query.mass + eps, pimIdx)
+   rv <- lapply(peptideSequence[lower:upper], function(p) {
+     psm(p, x, plot = FALSE, FUN = FUN)
+   })
+   rv.error <- sapply(rv, function(p) {
+     sum(abs(p$mZ.Da.error) < fragmentIonMassToleranceDa)
+   })
+   idx.tophit <- which(rv.error == max(rv.error))[1]
+   data.frame(mass_error = eps,
+     idxDiff = upper - lower,
+     charge = x$charge,
+     pepmass = query.mass,
+     peptideSequence = rv[[idx.tophit]]$sequence,
+     groundTrue.peptideSequence = x$peptideSequence,
+     ms2hit = (rv[[idx.tophit]]$sequence ==
+     x$peptideSequence), hit = (x$peptideSequence %in%
+     peptideSequence[lower:upper]))
+ }
```

## 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 fetuinLFO contains a subset of our results described in Grossmann, Roschitzki, Panse, Fortes, Barkow-Oesterreicher, Rutishauser, and Schlapbach (2010). The example be-
low shows a visualization using trellis plots. It graphs the abundance of four protein independence from the fetuin concentration spiked into the sample.

```r
R> library(lattice)
R> data(fetuinLFQ)
R> cv<-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

LCMS based label-free quantification is a very popular method to extract relative quantitative information from mass spectrometry experiments. At the FGCZ we use the software ProgenesisLCMS for this workflow [http://www.nonlinear.com/products/progenesis/lc-ms/overview/](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
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 \texttt{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,
This figure shows the correlation between runs on protein level (values are \texttt{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
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
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:row(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[,]),
  as.numeric(group2Protein[,]),
  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$n[1]), as.numeric(group1Protein[,]), col='blue')
points(rep(2,b$n[2]), as.numeric(group2Protein[,]), col='blue')

This figure shows five proteins which are tested if they differ across conditions using the four biological replicates with a t-test.
On Peptide Level

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

```r
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.area1 Group1.area2  Group1.area3
1  O95445  0.0560000     1705.43      3636.40      51416.05
2  O95445  0.1610000      2730.41      2266.88      2269.57
3  O95445  0.0390000     28726.38     15409.81     111629.30
4  O95445  0.2770000      4221.31       9859.71      6859.71
5  O95445  0.0360000     20209.66     14979.02     12164.94
6 P02652  0.6400000     4504.97      3636.40      51416.05
7 P02652  0.9410000     67308.30     15409.81     111629.30
8 P02652  0.3380000     4661.54       9859.71      6859.71
9 P02652  0.1150000      4544.56       9859.71      6859.71
10 P02652  0.0530000     24596.42
```
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
R> data(pressureProfile)
R> ppp(pressureProfile)
```

The lines plot 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, ...).

```r
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))
```
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).

References


A. Session information

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

- R version 3.6.3 (2020-02-29), x86_64-pc-linux-gnu
• Locale: LC_CTYPE=en_US.UTF-8, LC_NUMERIC=C, LC_TIME=en_US.UTF-8, LC_COLLATE=en_US.UTF-8, LC_MONETARY=en_US.UTF-8, LC_MESSAGES=en_US.UTF-8, LC_PAPER=en_US.UTF-8, LC_ADDRESS=C, LC_TELEPHONE=C, LC_MEASUREMENT=en_US.UTF-8, LC_IDENTIFICATION=C

• Running under: Debian GNU/Linux 10 (buster)

• Matrix products: default

• BLAS: /usr/lib/x86_64-linux-gnu/atlas/libblas.so.3.10.3

• LAPACK: /usr/lib/x86_64-linux-gnu/atlas/liblapack.so.3.10.3

• Base packages: base, datasets, graphics, grDevices, methods, stats, utils

• Other packages: lattice 0.20-40, protViz 0.6.8, xtable 1.8-4

• Loaded via a namespace (and not attached): codetools 0.2-16, compiler 3.6.3, grid 3.6.3, Rcpp 1.0.3, tools 3.6.3

Affiliation:

Jonas Grossmann and Christian Panse
Functional Genomics Center Zurich, UZH|ETHZ
Winterthurerstr. 190
CH-8057, Zürich, Switzerland
Telephone: +41-44-63-53912
E-mail: cp@fgcz.ethz.ch
URL: http://www.fgcz.ch