

# 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.

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## 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).

`fcut` and `tryptic-digest` are commandline programs which are included in the package. `fcut` 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] "LCPDCPLLAPLNSR"
[14] "VVHAVEVALATFNAESNGSYLQLVEISR"
[15] "AQFVPLPVSVSVEFAVAATDCIAK"
[16] "EVVDPTK"
[17] "CNLLAEK"
[18] "QYGFCK"
[19] "GSVIQK"
[20] "ALGGEDVR"
[21] "VTCTLFQTQPVIPQPQPDGAEAEAPSAVPDAAGPTPSAAGPPVASVVVGPVAVPLPLHR"
[22] "AHYDLR"
[23] "HTFSGVASVESSSGEAFHV GK"
[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
QDGQFSVLF TK	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
VTCTLFQTQPVIPQPQPDGAEAEAPSAPVDAAGPTPSAAGPPVASVVVGPSVVAVPLPLHR	6015.13	39.37
AHYDLR	774.39	11.42
HTFSGVASVESSSGEAFHV GK	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.

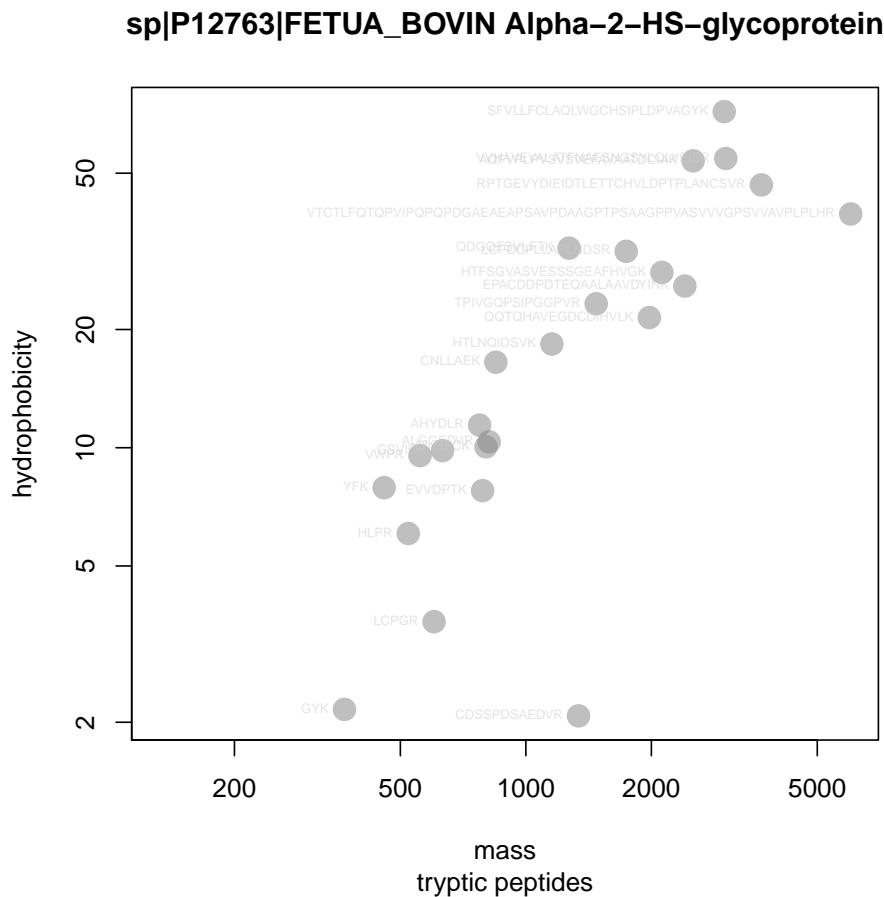
A figure below shows a scatter plot graphing the parent ion mass versus the hydrophobicity value of each in-silico tryptic digested peptide of the FETUA BOVIN (P12763) protein.

```
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 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
}

```

```

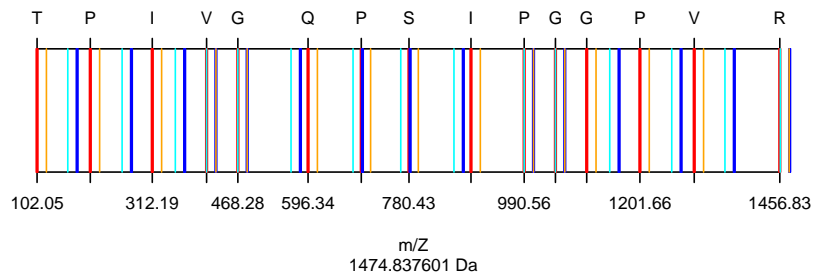
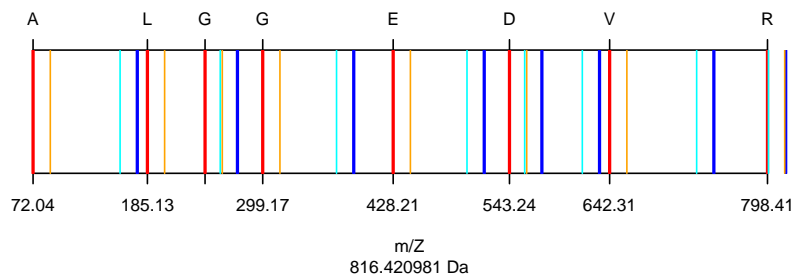
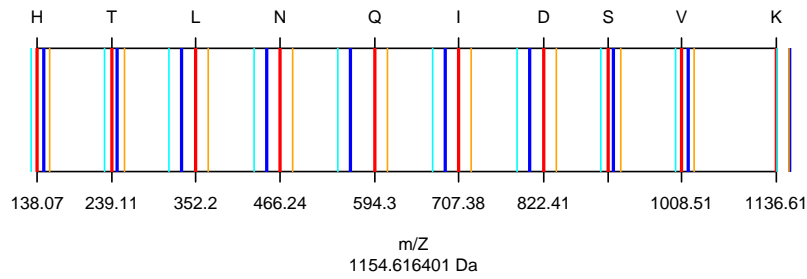
    c <- b + (Nitrogen + (3 * Hydrogen))
    z <- y - (Nitrogen + (3 * Hydrogen))
    return(cbind(b, y, c, z))
}
<bytecode: 0x55874d0e78c8>
<environment: namespace:protViz>

```

```

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).',

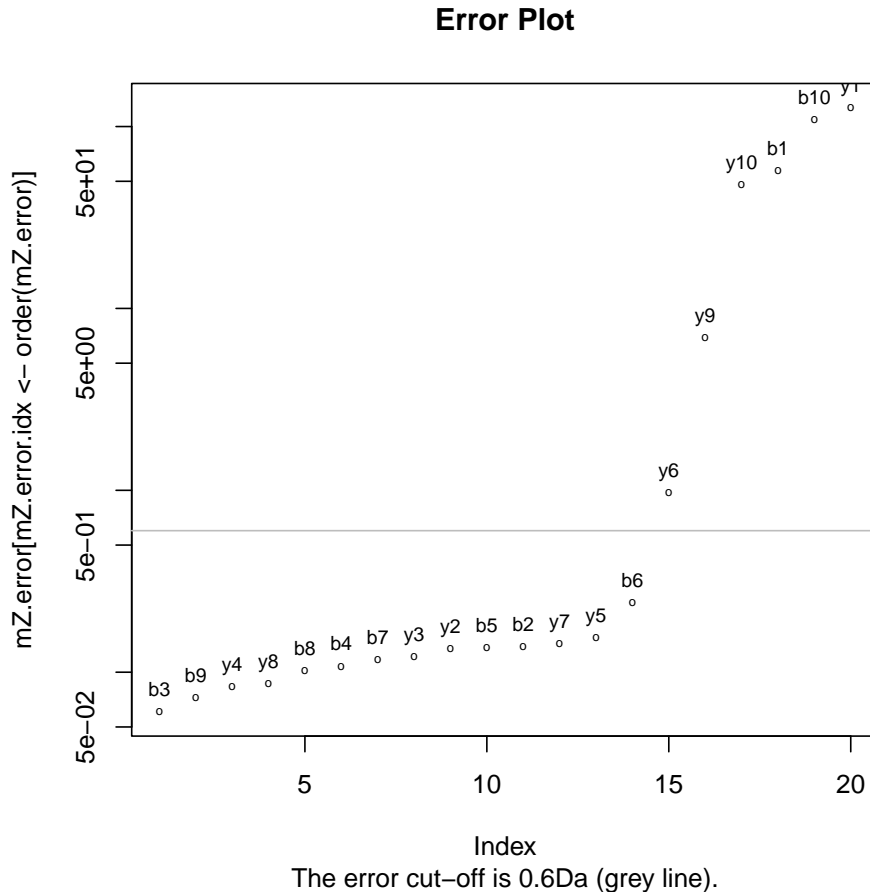
```



```

+       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 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="Substitution",

```

```

+       unimodAccID=616)
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('TAFDEAIAELDTLNEESYK', 'TAFDEAIAELDTLSEESYK'), m$AA)

[[1]]
[1] "00000000000000000000" "0000000000000200000" "0000000000000000100"
[4] "00000000000000100200"

[[2]]
[1] "00000000000000000000" "0000000000000100000" "0000000000000000100"
[4] "00000000000000100100"

R> fi <- protViz::fragmentIon(c('TAFDEAIAELDTLSEESYK',
+ 'TAFDEAIAELDTLNEESYK', 'TAFDEAIAELDTLSEESYK',
+ 'TAFDEAIAELDTLNEESYK'),
+       modified=c('0000000000000200000',
+       '0000000000000100000', '0000000000000000000',
+       '00000000000000000000'),
+       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

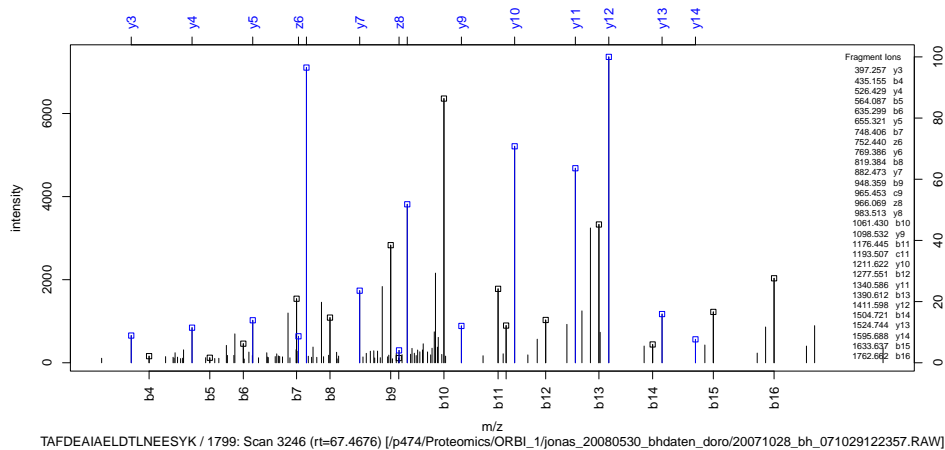
	b	y	c	z
1	102.0550	147.1128	119.0815	130.0863
2	173.0921	310.1761	190.1186	293.1496
3	320.1605	397.2082	337.1870	380.1816
4	435.1874	526.2508	452.2140	509.2242
5	564.2300	655.2933	581.2566	638.2668
6	635.2671	742.3254	652.2937	725.2988
7	748.3512	855.4094	765.3777	838.3829

```

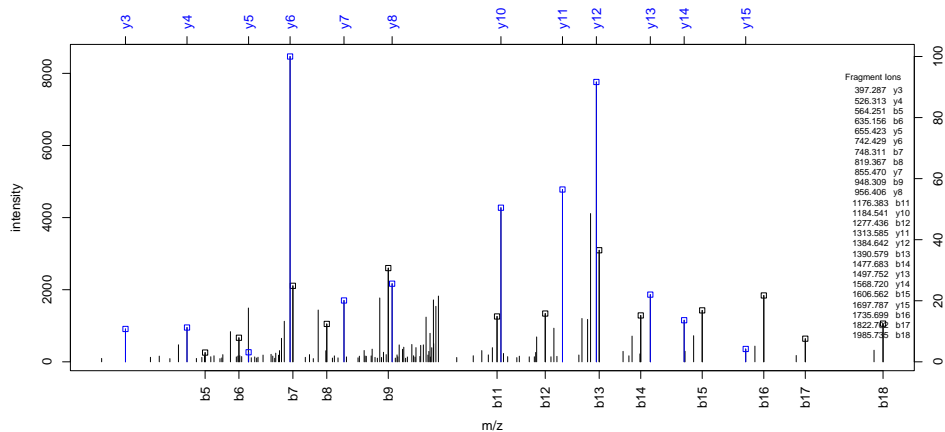
8 819.3883 956.4571 836.4148 939.4306
9 948.4309 1071.4841 965.4574 1054.4575
10 1061.5149 1184.5681 1078.5415 1167.5416
11 1176.5419 1313.6107 1193.5684 1296.5842
12 1277.5896 1384.6478 1294.6161 1367.6213
13 1390.6736 1497.7319 1407.7002 1480.7053
14 1477.7056 1568.7690 1494.7322 1551.7424
15 1606.7482 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)
```



TAFDEAIAELDTLNEESYK / 1799: Scan 3246 (rt=67.4676) [/p474/Proteomics/ORBI\_1/jonas\_20080530\_bhdaten\_doro/20071028\_bh\_071029122357.RAW]



TAFDEAIAELDTLSEESYK / 1842: Scan 3353 (rt=70.3158) [/p474/Proteomics/ORBI\_1/jonas\_20080530\_bhdaten\_doro/20071028\_bh\_071029122357.RAW]

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,
```

```

+                                     fragmentIonMassToleranceDa = 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) < fragmentIonMassToleranceDa
+       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),
+         fragmentIonMassToleranceDa = 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 described 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.

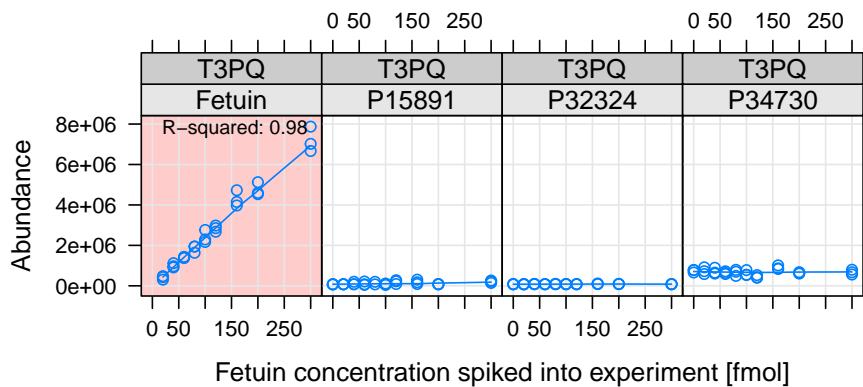
```
R> library(lattice)
R> data(fetuinLFQ)
R> cv<-1-1:7/10
R> t<-trellis.par.get("strip.background")
R> t$col<-(rgb(cv,cv,cv))
R> trellis.par.set("strip.background",t)
R> print(xyplot(abundance~conc|prot*method,
+             groups=prot,
+             xlab="Fetuin concentration spiked into experiment [fmol]",
+             ylab="Abundance",
+             aspect=1,
+             data=fetuinLFQ$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)
+     }
+ }
+ ))

```



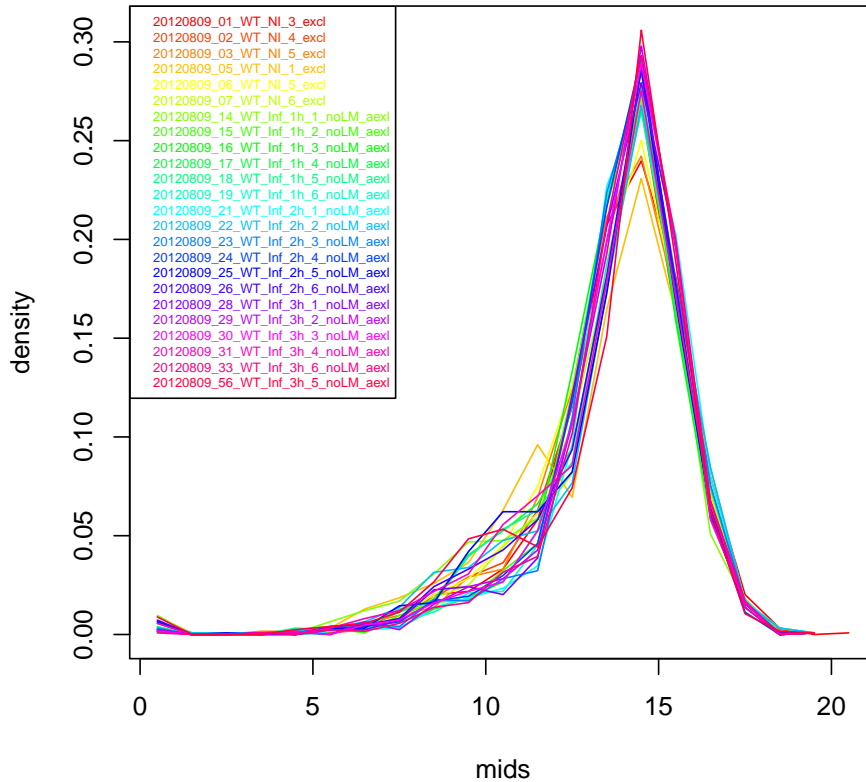
The plot shows the estimated concentration of the four proteins using the top three most intense peptides. The Fetuin peptides are spiked in with increasing concentration while the three other yeast proteins are kept stable in the background.

#### 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-

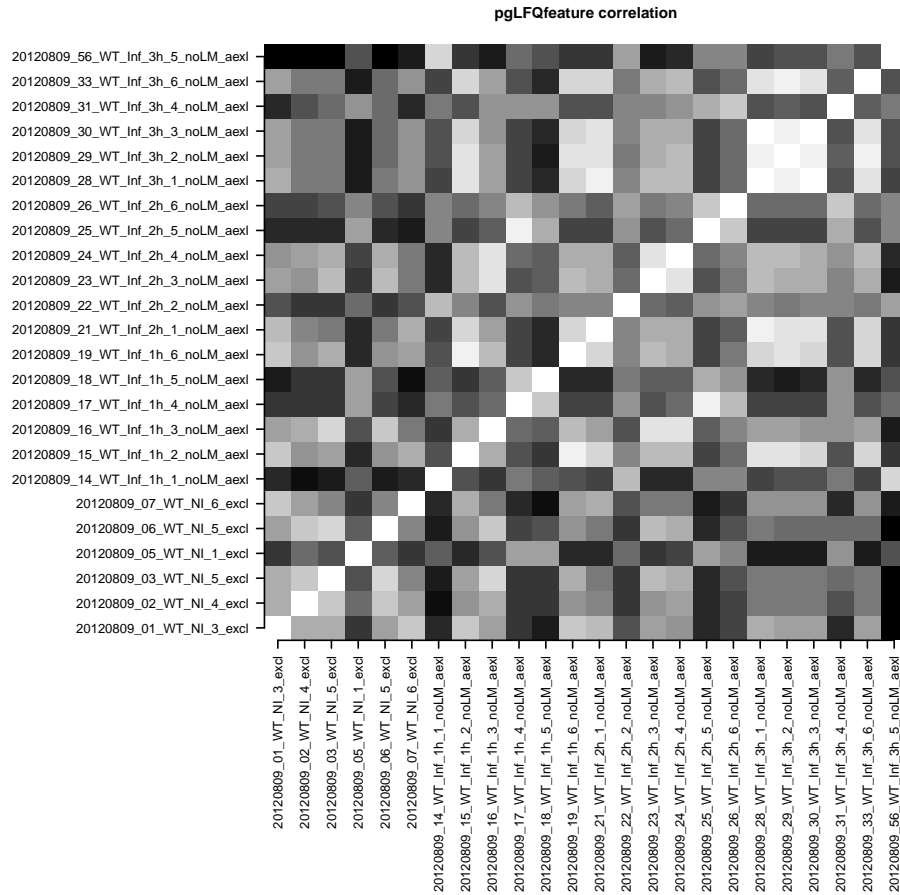
ware ProgenesisLCMS for this workflow <http://www.nonlinear.com/products/progenesis/lc-ms/overview/>. Progenesis is a graphical software which does the aligning between several LCMS experiments, extracts signal intensities from LCMS maps and annotates the master map with peptide and protein labels.

```
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)
```



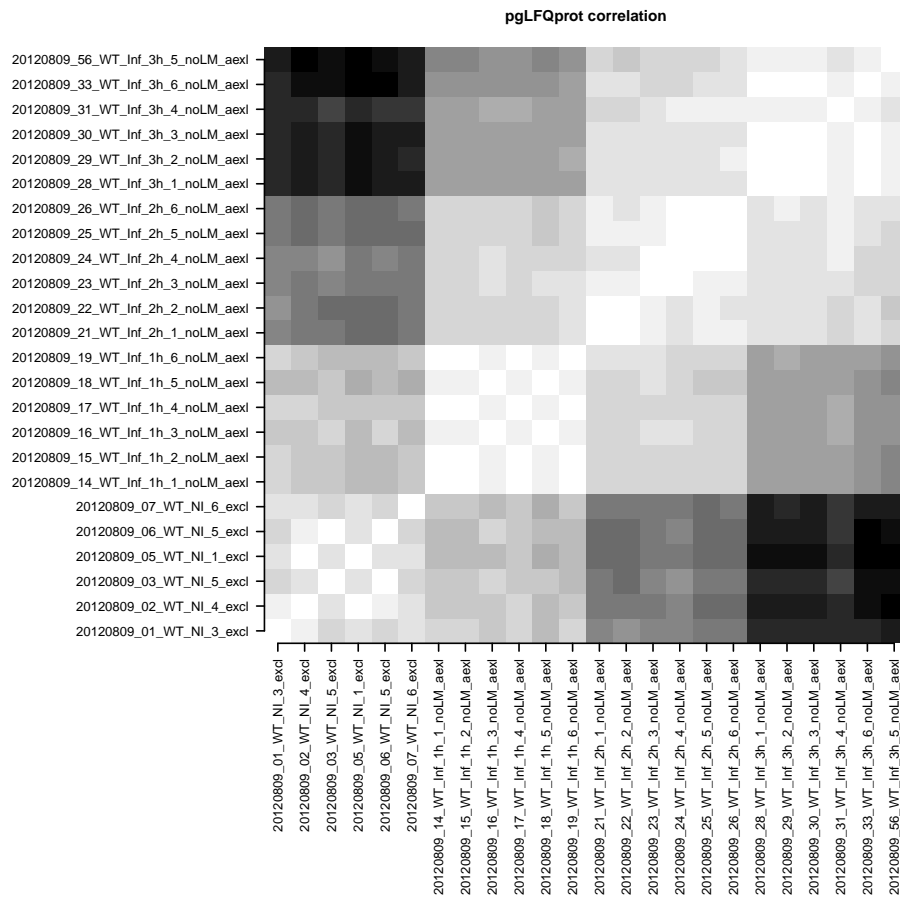
The `featureDensityPlot` shows the normalized signal intensity distribution (asinh transformed) over 24 LCMS runs which are aligned in this experiment.

```
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)
```



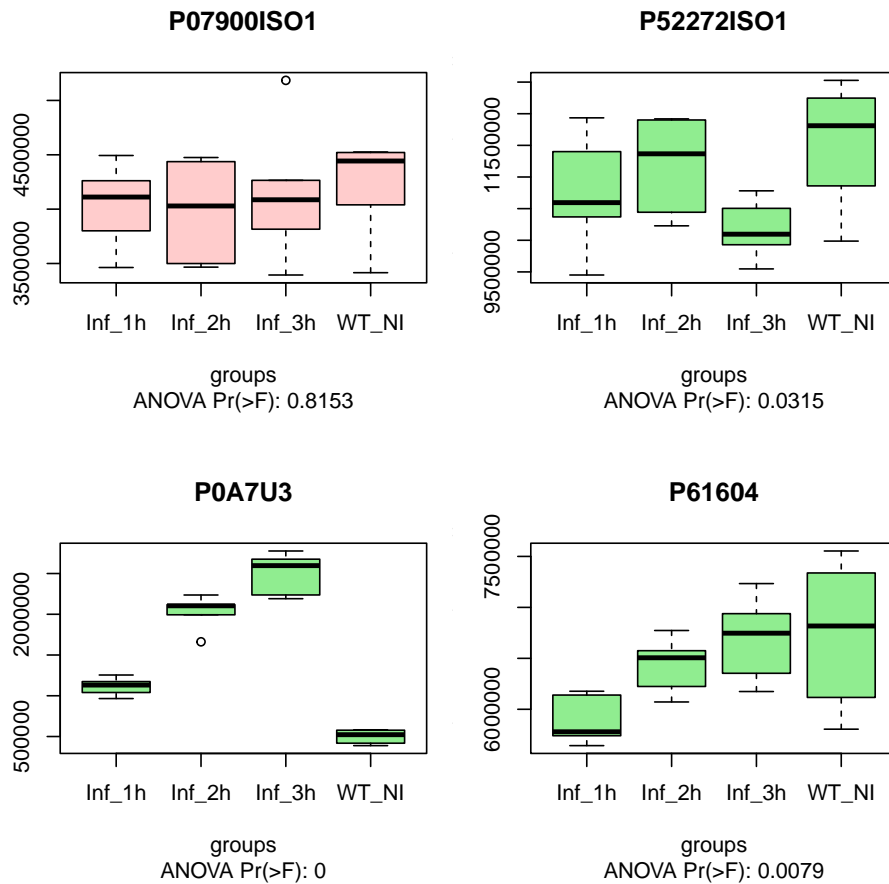
This image plot shows the correlation between runs on feature level (values are `asinh` transformed). White is perfect correlation while black indicates a poor correlation.

```
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)
```



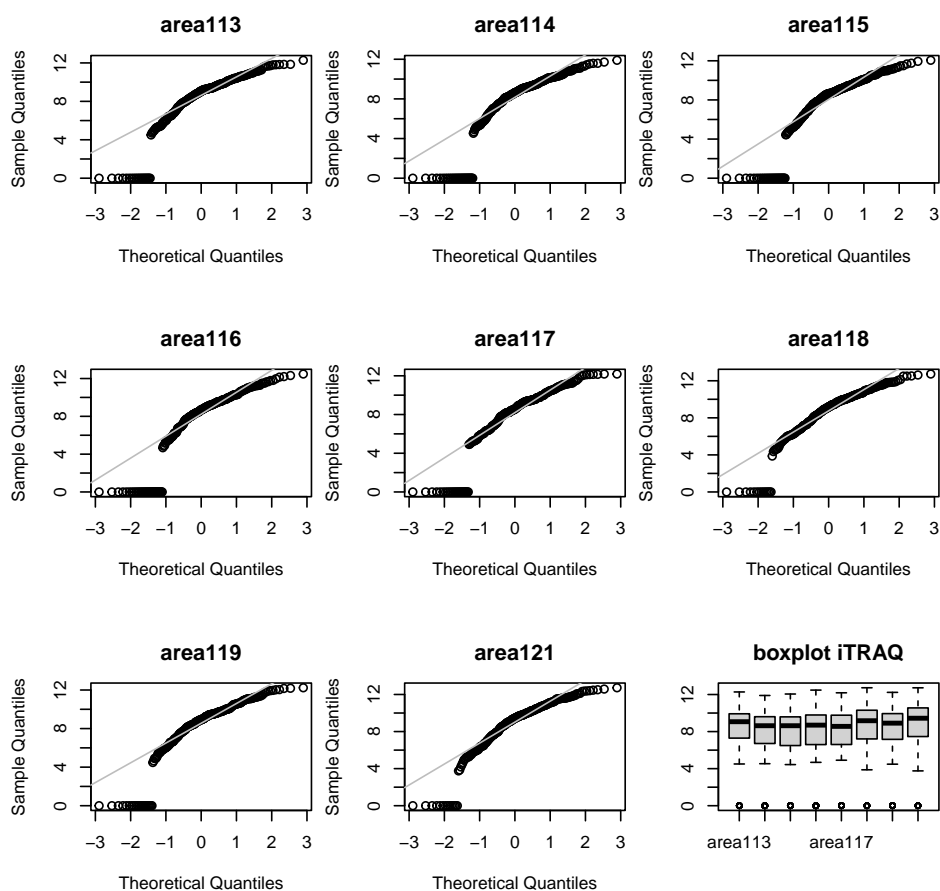
This figure shows the result for four proteins which either differ significantly in expression across conditions (green boxplots) using an analysis of variance test, or non-differing protein expression (red boxplot).

### 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.

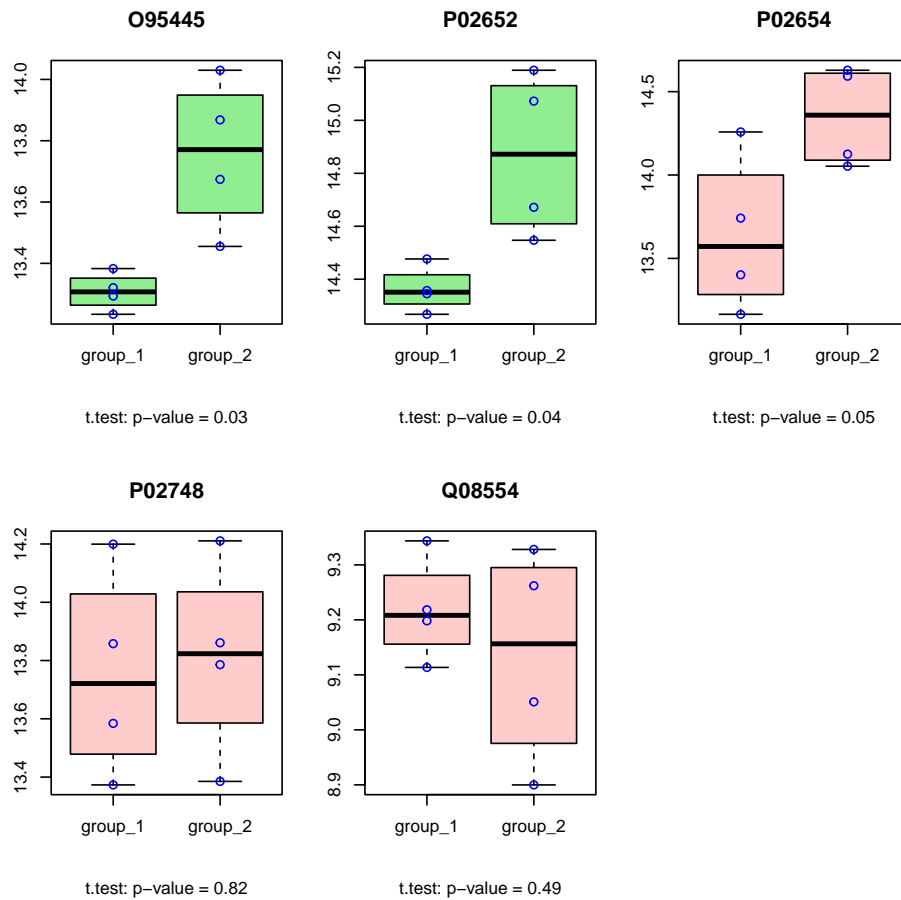
### *On Protein Level*

```
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	095445 AFLLTPR	0.056	1705.43	
2	095445 DGLCVPR	0.161	2730.41	
3	095445 MKDGLCVPR	0.039	28726.38	
4	095445 NQEACELSNN	0.277	4221.31	
5	095445 SLTSCCLDSK	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.

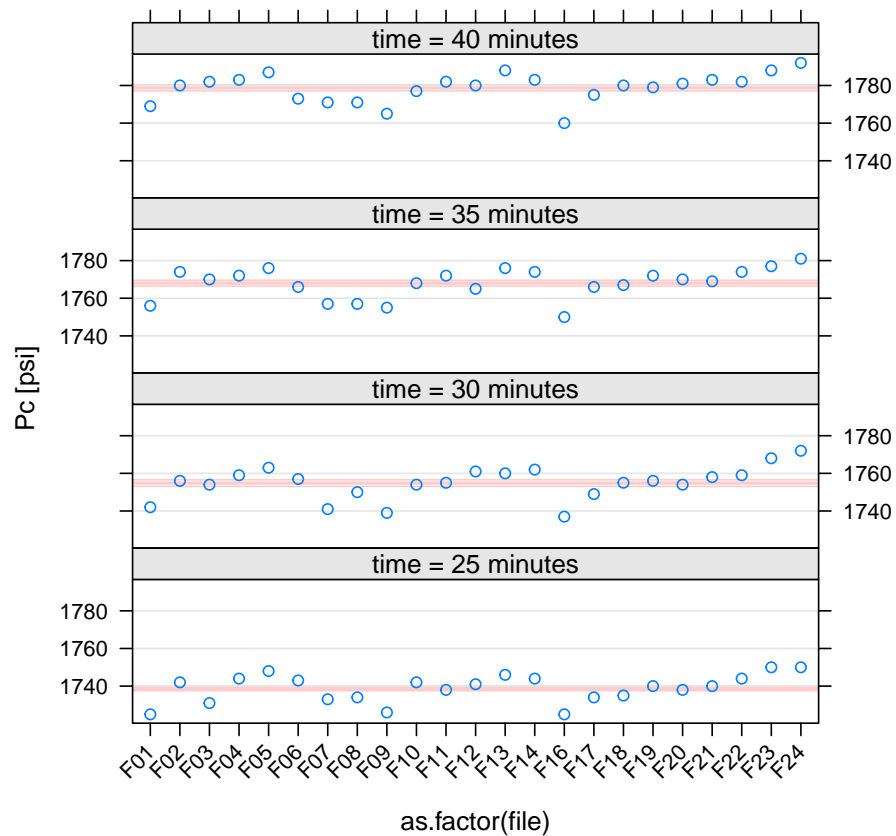
An overview of the pressure profile data can be seen by using the `ppp` function.

```
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, ...).

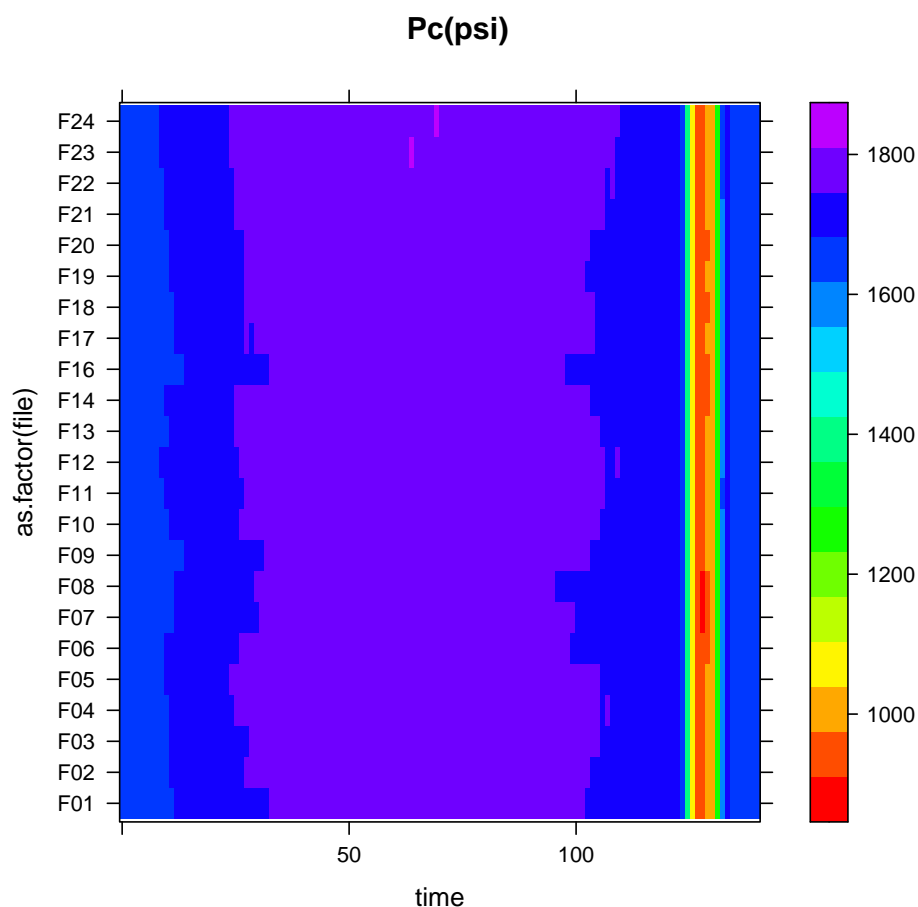
```
R> pp.data<-pps(pressureProfile, time=seq(25,40,by=5))
R> print(xyplot(Pc ~ as.factor(file) | paste("time =",
+       as.character(time), "minutes"),
+       panel = function(x, y){
+         m<-sum(y)/length(y)
+         m5<-(max(y)-min(y))*0.05
+         panel.abline(h=c(m-m5,m,m+m5),
+           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.

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## A. Session information

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

- R version 4.2.2 Patched (2022-11-10 r83330), x86\_64-pc-linux-gnu
- Locale: LC\_CTYPE=en\_US.UTF-8, 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
- Running under: Debian GNU/Linux bookworm/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 0.20-45, protViz 0.7.7, xtable 1.8-4
- Loaded via a namespace (and not attached): Rcpp 1.0.9, codetools 0.2-18, compiler 4.2.2, grid 4.2.2, tools 4.2.2

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