

Analysis of MS Data in R

Investigating Mass Spectra and Neutral Loss Trigger

Sebastian Gibb

2017-11-27

<https://github.com/sgibb/talk-odense-20171127/>

github: @sgibb / twitter: @sebastiangibb

Loading Data with MSnbase (Gatto and Lilley 2011)

```
library("MSnbase")

msfile <- file.path(
  "data",
  "neutral-loss",
  "LUM2_01470_KS_L1-5-2_EC17-123-150In2_55oC_NLtrig.mzML.gz"
)

ms <- readMSData(msfile, mode="onDisk")
```

The MSnExp object

```
ms
# MSn experiment data ("OnDiskMSnExp")
# Object size in memory: 50.52 Mb
# - - - Spectra data - - -
# MS level(s): 1 2 3
# Number of spectra: 108844
# MSn retention times: 0:0 - 120:0 minutes
# - - - Processing information - - -
# Data loaded [Fri Nov 24 14:09:00 2017]
# MSnbase version: 2.5.0
# - - - Meta data - - -
# phenoData
# rowNames:
#   LUM2_01470_KS_L1-5-2_EC17-123-150In2_55oC_NLtrig.mzML.gz
# varLabels: sampleNames
# varMetadata: labelDescription
# Loaded from:
#   LUM2_01470_KS_L1-5-2_EC17-123-150In2_55oC_NLtrig.mzML.gz
# protocolData: none
# featureData
#   featureNames: F1.S000001 F1.S000002 ... F1.S108844 (108844
#     total)
#   fvarLabels: fileIdx spIdx ... spectrum (29 total)
#   fvarMetadata: labelDescription
# experimentData: use 'experimentData(object)'
```

The MSnExp object 2

```
head(featureNames(ms))
```

```
# [1] "F1.S000001" "F1.S000002" "F1.S000003" "F1.S000004" "F1.S000005"  
# [6] "F1.S000006"
```

```
fData(ms)[1:2, 1:6]
```

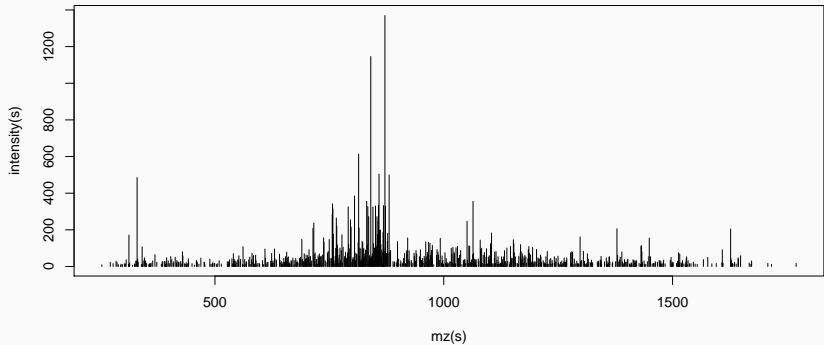
```
#           fileIdx spIdx centroided smoothed seqNum acquisitionNum  
# F1.S000001      1     1         NA        NA          1           1  
# F1.S000002      1     2         NA        NA          2           2
```

```
ms[[1]]
```

```
# Object of class "Spectrum1"  
# Retention time: 0:0  
# MSn level: 1  
# Total ion count: 32197  
# Polarity: 1
```

Plot a Spectrum of an MSnExp object

```
s <- ms[[95]]  
plot(mz(s), intensity(s), type="h")
```



Filter by MS level

```
ms1 <- ms[fData(ms)$msLevel == 1]
```

```
ms1 <- filterMsLevel(ms, 1)
```

```
ms2 <- filterMsLevel(ms, 2)
```

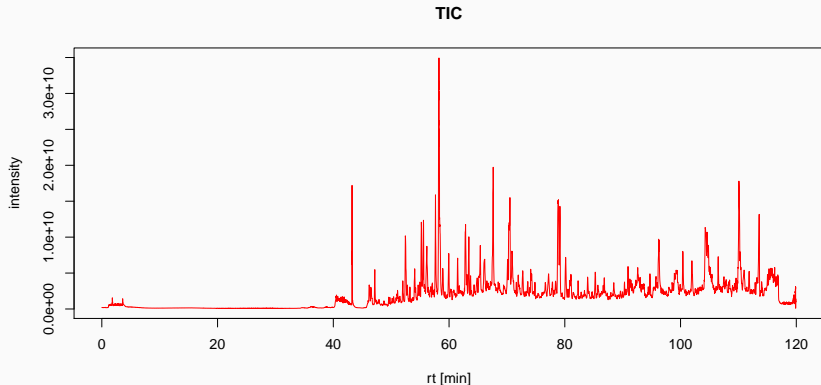
```
ms3 <- filterMsLevel(ms, 3)
```

The MSnExp object 3

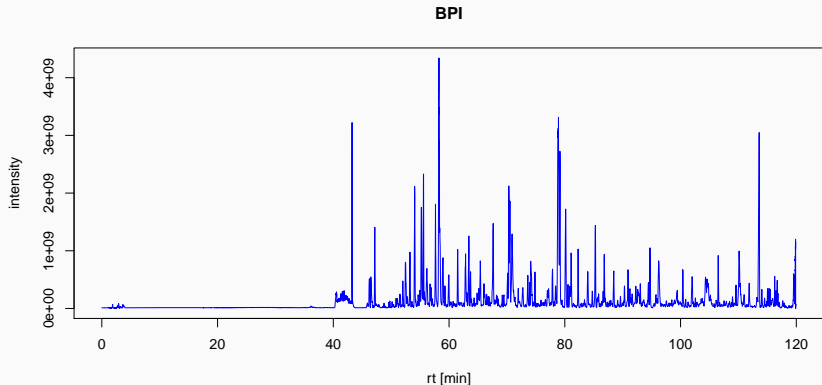
```
ms1
# MSn experiment data ("OnDiskMSnExp")
# Object size in memory: 1.81 Mb
# - - - Spectra data - - -
# MS level(s): 1
# Number of spectra: 5565
# MSn retention times: 0:0 - 119:58 minutes
# - - - Processing information - - -
# Data loaded [Fri Nov 24 14:09:00 2017]
# Filter: select MS level(s) 1 [Sat Nov 25 17:29:30 2017]
# MSnbase version: 2.5.0
# - - - Meta data - - -
# phenoData
#   rowNames:
#     LUM2_01470_KS_L1-5-2_EC17-123-150In2_55oC_NLtrig.mzML.gz
#   varLabels: sampleNames
#   varMetadata: labelDescription
# Loaded from:
#   LUM2_01470_KS_L1-5-2_EC17-123-150In2_55oC_NLtrig.mzML.gz
# protocolData: none
# featureData
#   featureNames: F1.S000001 F1.S000002 ... F1.S108817 (5565 total)
#   fvarLabels: fileIdx spIdx ... spectrum (29 total)
#   fvarMetadata: labelDescription
# experimentData: use 'experimentData(object)'
```



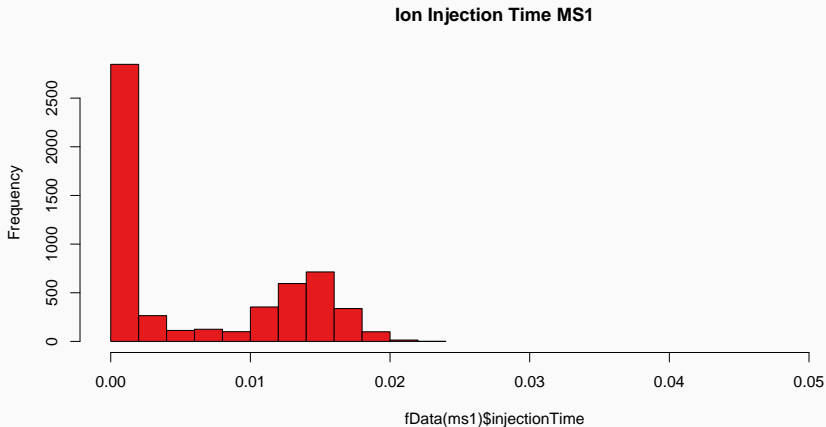
```
plot(rtime(ms1) / 60, tic(ms1),  
     type="l", col="red",  
     main="TIC", xlab="rt [min]", ylab="intensity")
```



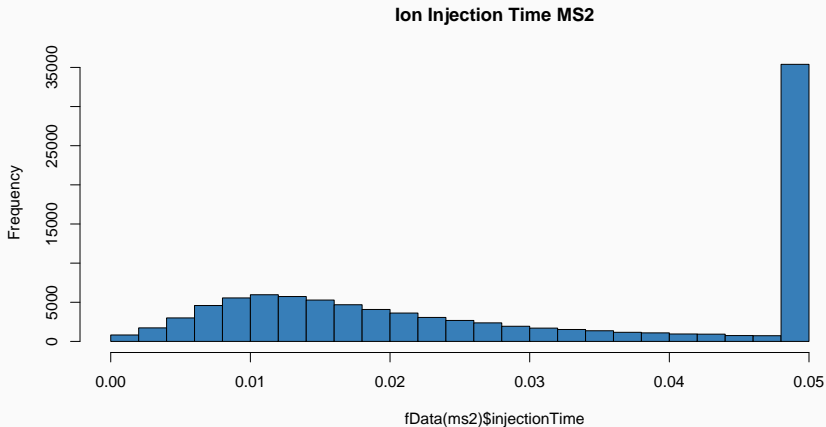
```
plot(rtime(ms1) / 60, fData(ms1)$basePeakIntensity,  
     type="l", col="blue",  
     main="BPI", xlab="rt [min]", ylab="intensity")
```



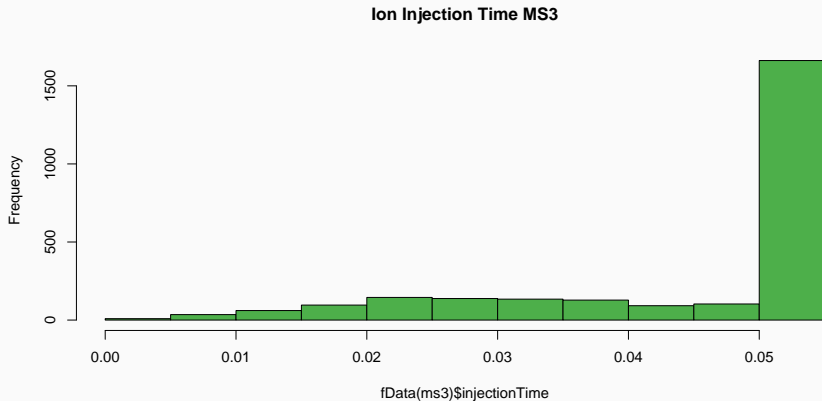
```
hist(fData(ms1)$injectionTime,  
     col=col["MS1"],  
     main="Ion Injection Time MS1", xlim=c(0, 0.055))
```



```
hist(fData(ms2)$injectionTime,  
     col=col["MS2"],  
     main="Ion Injection Time MS2", xlim=c(0, 0.055))
```

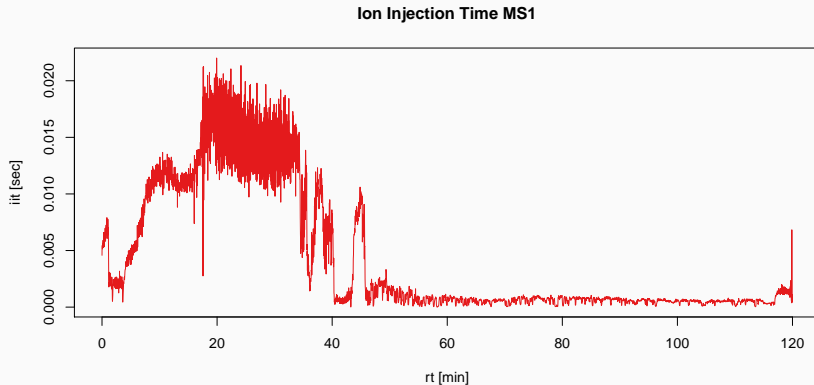


```
hist(fData(ms3)$injectionTime,  
     col=col["MS3"],  
     main="Ion Injection Time MS3", xlim=c(0, 0.055))
```



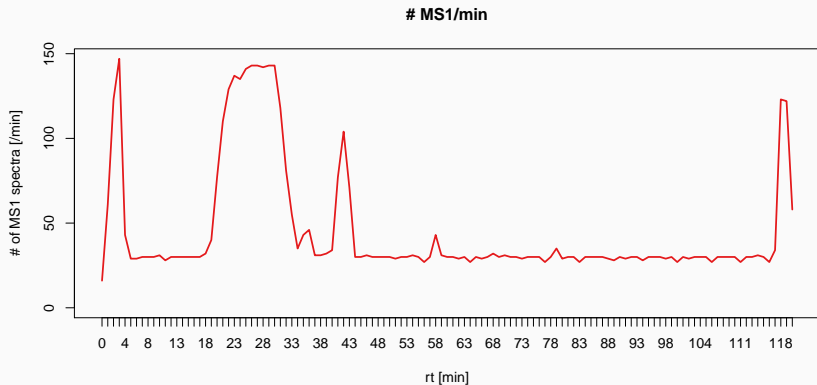
IIT vs RT MS1

```
plot(rtime(ms1) / 60, fData(ms1)$injectionTime,  
     type="l", col=col["MS1"],  
     main="Ion Injection Time MS1",  
     xlab="rt [min]", ylab="iit [sec]")
```



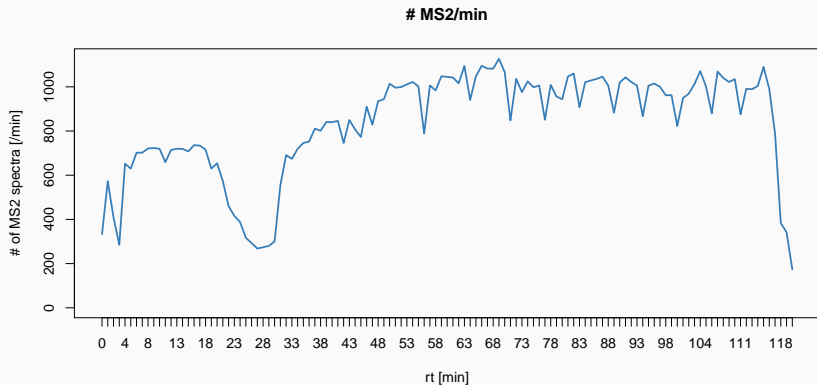
MS1 per minute

```
plot(table(round(rtime(ms1) / 60)),  
      type="l", col=col["MS1"], main="# MS1/min",  
      xlab="rt [min]", ylab="# of MS1 spectra [/min])")
```



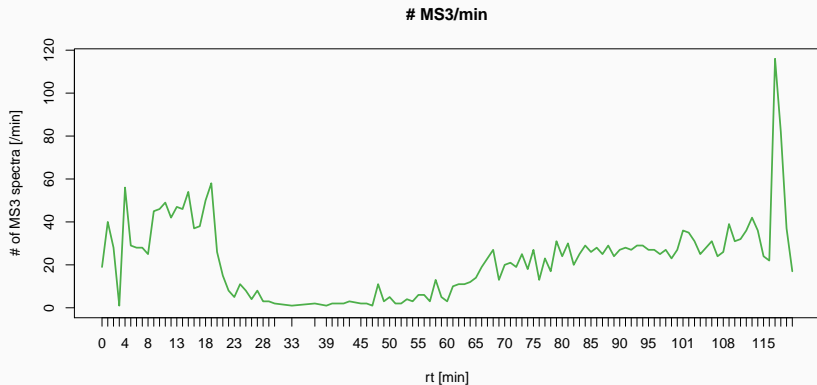
MS2 per minute

```
plot(table(round(rtime(ms2) / 60)),  
      type="l", col=col["MS2"], main="# MS2/min",  
      xlab="rt [min]", ylab="# of MS2 spectra [/min])
```



MS3 per minute

```
plot(table(round(rtime(ms3) / 60)),  
      type="l", col=col["MS3"], main="# MS3/min",  
      xlab="rt [min]", ylab="# of MS3 spectra [/min]")
```



NL trigger (jiang et al. 2017)

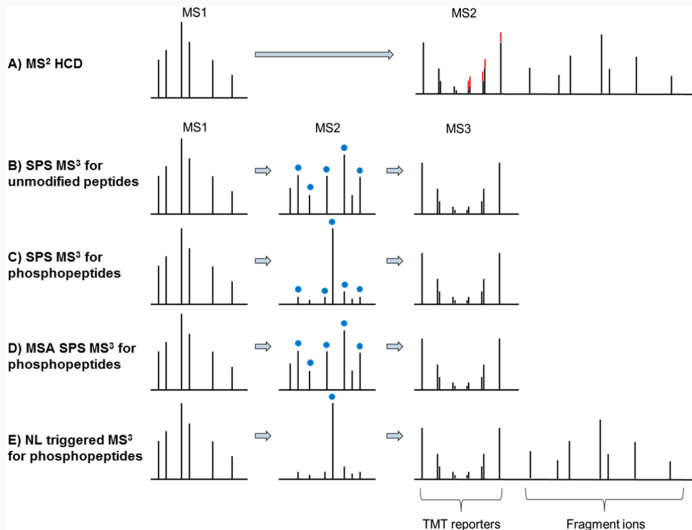


Figure: (Fig. 1 in Jiang et al. 2017)

NL trigger/Thermo Orbitrap Lumos

The screenshot displays the 'Method Editor' window for a Thermo Xcalibur instrument. The window is divided into several sections:

- Method Timeline:** Shows a duration of 28 minutes. The timeline is marked with time points: 4.7, 9.3, 14, 18.7, 23.3, and 28.
- Global Parameters:** Includes 'Experiment 1' and 'Time Range' set to '0-28 min'.
- System Templates:** A list of templates including Glycans, Metabolites, Peptides-ID, Peptides-PTM, Peptides-Quan, Peptides-Xlink, and Proteins-ID.
- Method Flowchart:** A central flowchart showing the sequence of MS steps:
 - MS OT
 - MIPS
 - Intensity
 - Charge State
 - Dynamic Exclusion
 - ddMS² IT CID
 - Targeted Loss Trigger (highlighted in green)
 - ddMS² OT HCD
- Targeted Loss Trigger Properties:** A panel on the right with the following settings:
 - Compound Table:**

Compound	m/z
1 phosphoL	97.9763
2 phosphoL	80
 - Mass Tolerance:** Set to 'm/z'.
 - Low:** 0.5
 - High:** 0.5
 - Trigger Only with Detection of at Least N Ions from the List:** ☒
 - N:** 1
 - Only Ion(s) Within Top N Most Intense:** ☒
 - N:** 5
 - Only Ion(s) Above the Threshold (Relative Intensity, %):** ☐
 - Trigger Only with Detection of Correct Charge State of the Product Ion:** ☐
 - Ignore Charge State Requirement for Unassigned Ions:** ☐

Figure

Own NL trigger

MS2: m/z of top 5 peaks - precursor m/z $\approx 80/97$ [Da]

Thermo NL trigger

MS2 \leftrightarrow MS3

Verification

Comparison of MS2 scan numbers

NL trigger/How to find our own trigger? 1

```
findTop5mz <- function(x) {  
  mz(x)[order(intensity(x), decreasing=TRUE)[1:5]]  
}  
top5mz <- spectrapply(ms2, findTop5mz)  
top5mz <- do.call(rbind, top5mz)  
top5mz[1:2, ]  
#           [,1]      [,2]      [,3]      [,4]      [,5]  
# F1.S000003 507.9660 619.1350 427.9571 699.134 601.090  
# F1.S000004 676.1671 427.9181 756.1360 508.006 409.902  
  
deltamz <- precursorMz(ms2) - top5mz  
deltamz[1:2, ]  
#           [,1]      [,2]      [,3]      [,4]      [,5]  
# F1.S000003 55.58902 -55.57996 135.5980 -135.57898 -37.53497  
# F1.S000004 -84.10120 164.14780 -164.0702 84.05984 182.16382
```

NL trigger/How to find our own trigger? 2

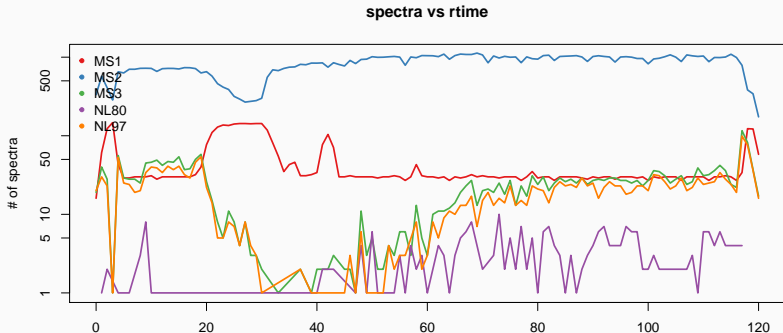
```
nl <- c(phosphate=80, phosphoNL=97.9763)
tolerance <- 0.5 # [Da]

# How many phosphates?
nl80 <- as.logical(rowSums(
  abs(deltamz - nl["phosphate"]) <= tolerance)
)
sum(nl80)
# [1] 276

# How many phosphoNL?
nl97 <- as.logical(rowSums(
  abs(deltamz - nl["phosphoNL"]) <= tolerance)
)
sum(nl97)
# [1] 2124
```

NL trigger/Spectra + NL vs RT log

```
plot(NA, ylim=c(1, 1100), xlim=c(0, 120), log="y",  
     main="spectra vs rtime", xlab="rt [min]", ylab="# of spectra")  
l <- list(ms1, ms2, ms3, ms2[nl80], ms2[nl97])  
for (i in seq(along=l)) {  
  lines(table(round(rtime(l[[i]]) / 60)), col=col[i], type="l")  
}  
legend("topleft", legend=names(col), col=col, pch=20, bty="n")
```



NL trigger/Find device trigger; the tricky part 1

```
fData(ms2)$filterString[1:2]
# [1] "ITMS + c NSI t d Full ms2 563.5551@cid35.00 [150.0000-1138.0000]"
# [2] "ITMS + c NSI t d Full ms2 592.0658@cid35.00 [158.0000-1195.0000]"
fData(ms3)$filterString[1:2]
# [1] "FTMS + p NSI d Full ms3 933.4402@cid35.00 915.4110@hcd30.00 [100.0000-2000.0000]"
# [2] "FTMS + p NSI d Full ms3 850.0895@cid35.00 752.1000@hcd30.00 [100.0000-2000.0000]"

ms2df <- data.frame(scanId=scanIndex(ms2),
                    pcCid=gsub(".*ms2 *([0-9.]+"@cid[0-9.]+" .*", "\\1",
                               fData(ms2)$filterString),
                    stringsAsFactors=FALSE)
```

	scanId	pcCid
F1S000003	3	563.5551@cid35.00
F1S000004	4	592.0658@cid35.00

```
ms3df <- data.frame(scanId=scanIndex(ms3),
                    pcCid=gsub(".*ms3 *([0-9.]+"@cid[0-9.]+" .*", "\\1",
                               fData(ms3)$filterString),
                    stringsAsFactors=FALSE)
```

	scanId	pcCid
F1S000040	40	933.4402@cid35.00
F1S000054	54	850.0895@cid35.00

NL trigger/Find device trigger; the tricky part 2

```
nldf <- merge(ms3df, ms2df, by="pcCid",  
              all.x=TRUE, all.y=FALSE,  
              sort=FALSE, suffixes=c(".ms3", ".ms2"))  
nldf$delta <- nldf$scanId.ms3 - nldf$scanId.ms2
```

pcCid	scanId.ms3	scanId.ms2	delta
933.4402@cid35.00	40	38	2
850.0895@cid35.00	54	52	2
644.5992@cid35.00	67	11506	-11439
644.5992@cid35.00	67	11081	-11014
644.5992@cid35.00	67	4763	-4696
644.5992@cid35.00	67	4364	-4297

NL trigger/Find device trigger; the tricky part 3

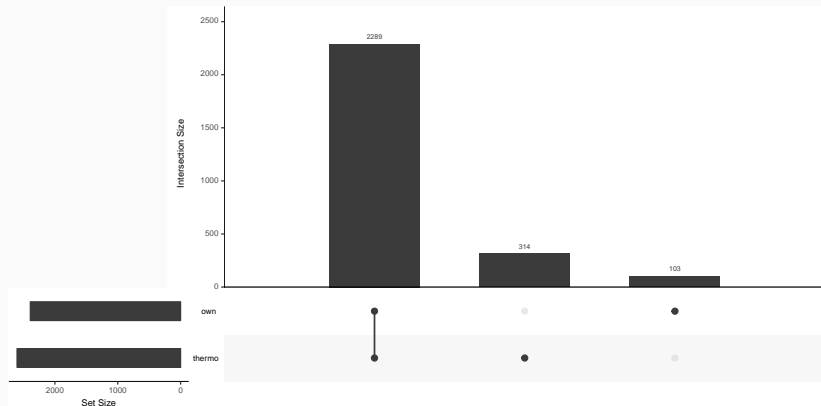
```
nldf <- nldf[nldf$delta > 0,]  
  
nldf <- nldf[order(nldf$scanId.ms3, nldf$delta),]  
  
nldf <- nldf[!duplicated(nldf$scanId.ms3),]
```

	pcCid	scanId.ms3	scanId.ms2	delta
1	933.4402@cid35.00	40	38	2
2	850.0895@cid35.00	54	52	2
10	644.5992@cid35.00	67	65	2
12	699.0558@cid35.00	82	80	2
15	636.8463@cid35.00	83	81	2
16	757.3283@cid35.00	108	106	2

NL trigger/Find common trigger/differences 1

```
ms2idNL <- list(own=scanIndex(ms2)[nl80 | nl97],  
               thermo=nldf$scanId.ms2)
```

```
library("UpSetR")  
upset(fromList(ms2idNL), order.by="freq")
```

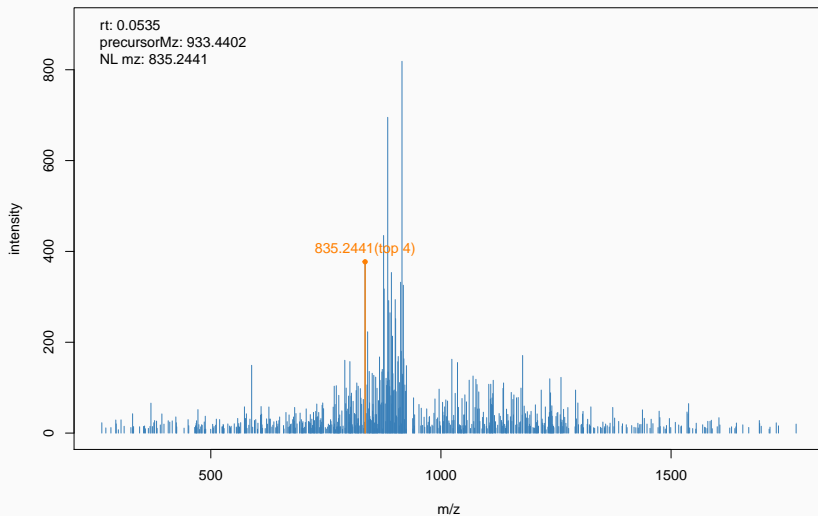


NL trigger/Find common trigger/differences 2

```
common <- intersect(ms2idNL$own, ms2idNL$thermo)
uniqueOwn <- setdiff(ms2idNL$own, ms2idNL$thermo)
uniqueThermo <- setdiff(ms2idNL$thermo, ms2idNL$own)
```

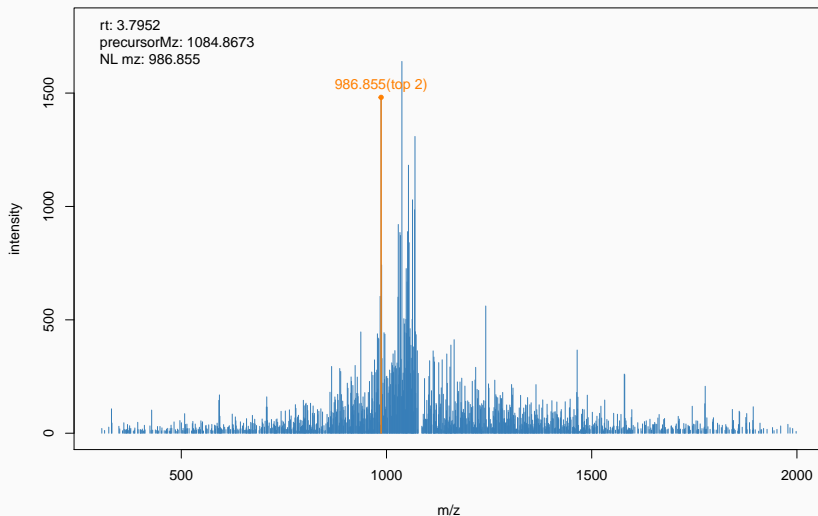
NL trigger/Common trigger

```
plotMs2(ms[[common[1]]])
```



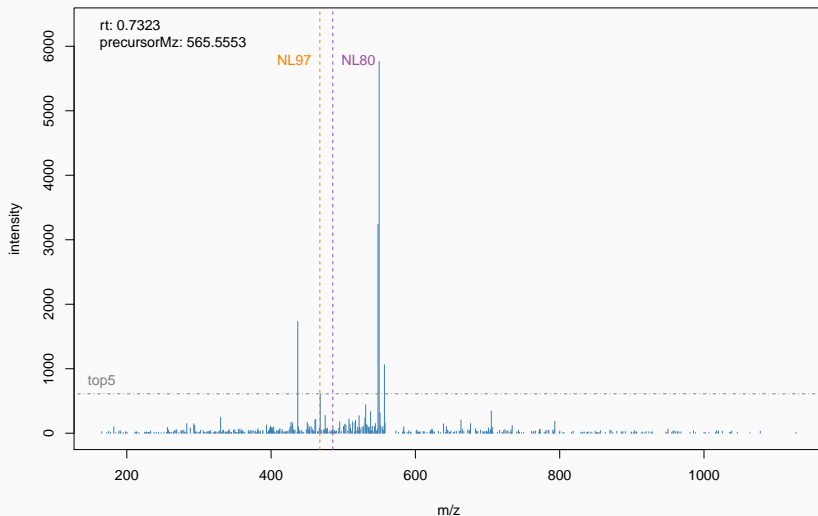
NL trigger/Should have been triggered

```
plotMs2(ms[[uniqueOwn[1]]])
```



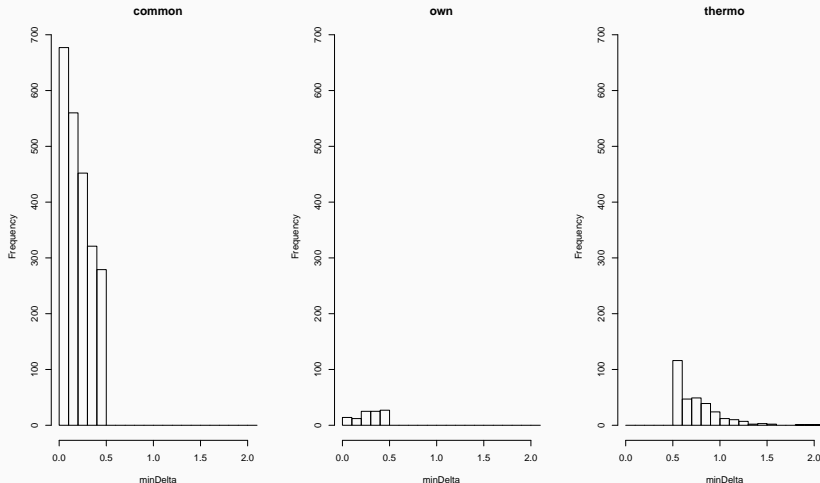
NL trigger/Was triggered but shouldn't

```
plotMs2(ms[[uniqueThermo[1]]])
```



NL trigger/Trigger tolerance

```
l <- list(common=common, own=uniqueOwn, thermo=uniqueThermo)
i <- lapply(1, match, scanIndex(ms2))
for (j in seq(along=l)) {
  minDelta <- apply(deltamz[i[[j]], ], 1, function(r)min(abs(r - rep(nl, each=length(r)))))
  hist(minDelta, breaks=seq(0, 2.1, by=0.1), ylim=c(0, 675), main=names(l)[j])
}
```



- MSnbase: @lgatto, @jotsetzung
- mzR: @sneumann, @lgatto, @jotsetzung

<https://github.com/sgibb/talk-odense-20171127/>

github: @sgibb / twitter: @sebastiangibb

Gatto, Laurent, and Kathryn S. Lilley. 2011. “MSnbase—an R/Bioconductor Package for Isobaric Tagged Mass Spectrometry Data Visualization, Processing and Quantitation.” *Bioinformatics* 28 (2). Oxford University Press (OUP): 288–89. doi:[10.1093/bioinformatics/btr645](https://doi.org/10.1093/bioinformatics/btr645).

Jiang, Xiaoyue, Ryan Bomgarden, Joseph Brown, Devin L. Drew, Aaron M. Robitaille, Rosa Viner, and Andreas R. Huhmer. 2017. “Sensitive and Accurate Quantitation of Phosphopeptides Using TMT Isobaric Labeling Technique.” *Journal of Proteome Research* 16 (11). American Chemical Society (ACS): 4244–52. doi:[10.1021/acs.jproteome.7b00610](https://doi.org/10.1021/acs.jproteome.7b00610).