

# XIC using rawDiag

*CP & CT*

*6/29/2018*

## Requirements

If not already installed please install the packages: - rawDiag download and install from [http://fgcz-ms.uzh.ch/~cpanse/rawDiag\\_0.0.6.tar.gz](http://fgcz-ms.uzh.ch/~cpanse/rawDiag_0.0.6.tar.gz) - protViz from <https://CRAN.R-project.org/package=protViz> - get the example data from [http://fgcz-ms.uzh.ch/~cpanse/20180220\\_14\\_autoQC01.raw](http://fgcz-ms.uzh.ch/~cpanse/20180220_14_autoQC01.raw)

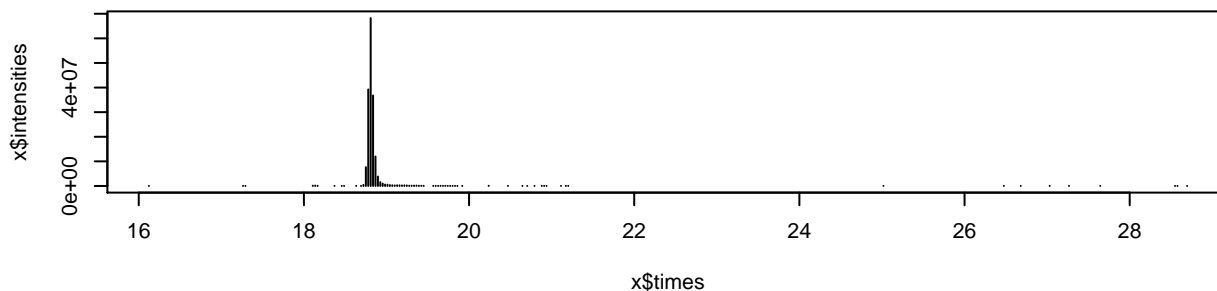
Define raw file we work on and we calculate the doubly charged mass of three iRT peptides.

```
#adapt to location where the example data file is located  
rawfile <- "C:\\Users\\christian\\Documents\\RawFiles\\20180220_14_autoQC01.raw"  
pepSeq <- c("AGGSSEPVTGLADK", "GDLDAASYAPVR", "FLLQFGAQGSPLFK")  
mass2Hplus <- (parentIonMass(pepSeq) + 1.008) / 2
```

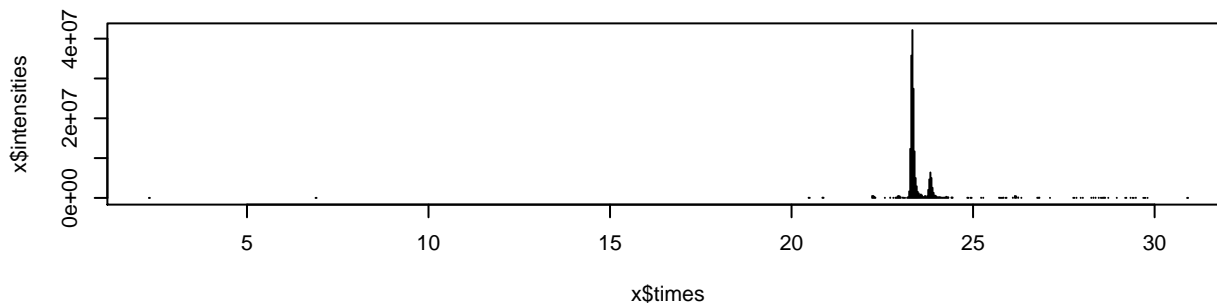
Extract the XIC of the three defined iRT peptides from the raw file.

```
op <- par(mfrow = c(3, 1), mar=c(4,4,4,1))  
X <- readXICs(rawfile = rawfile, masses = mass2Hplus)  
op <- par(mfrow = c(3, 1))  
rv <- lapply(1:3, function(i){plot(X[[i]], main = pepSeq[i])})
```

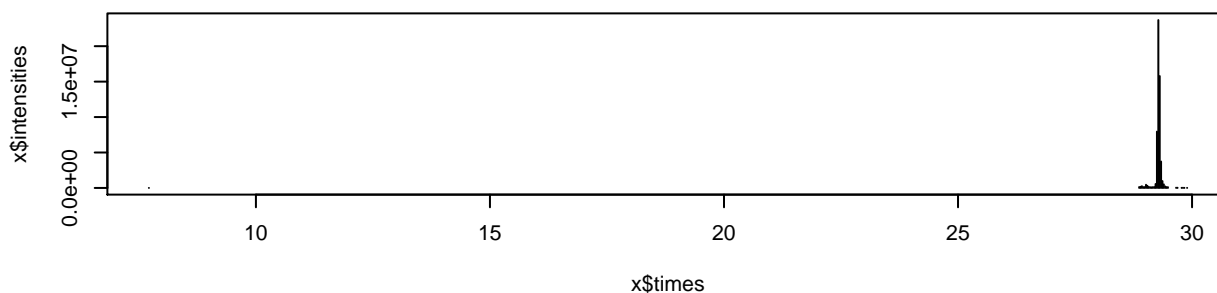
### AGGSSEPVTGLADK



### GDLDAASYYPVR



### FLLQFGAQGSPLFK



load the meta data of the specified raw file

```
metadata <- read.raw(rawfile)
```

```
## system2 is writing to tempfile C:\Users\CHRIST-1\AppData\Local\Temp\RtmpktyRn8\file333c7c4ad25tsv
```

```
## unlinking C:\Users\CHRIST-1\AppData\Local\Temp\RtmpktyRn8\file333c7c4ad25tsv ...
```

```
## Warning in is.rawDiag(object): missing column name(s): MasterScanNumber,
```

```
## LMCORrection, ElapsedScanTimesec, transient, AGCMode, PrescanMode
```

```
## MasterScanNumber calculated
```

```
## Warning: package 'bindrcpp' was built under R version 3.4.4
```

```
## renamed LmZCorrectionppm to LMCORrection
```

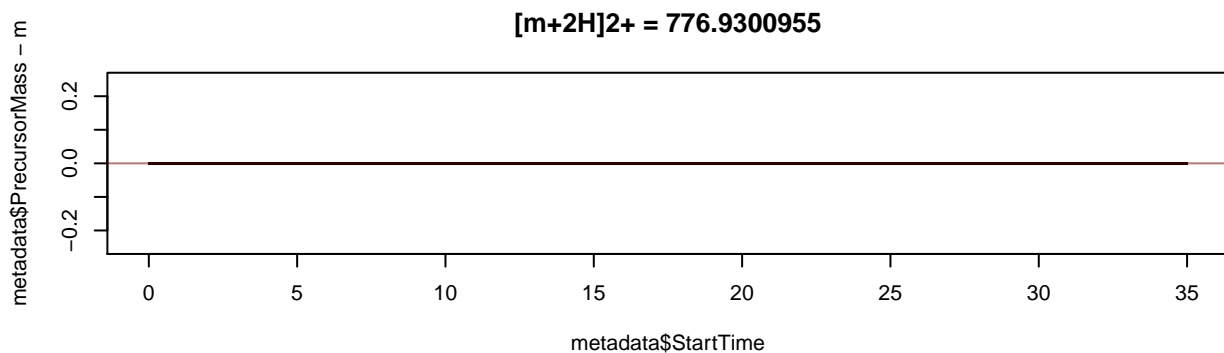
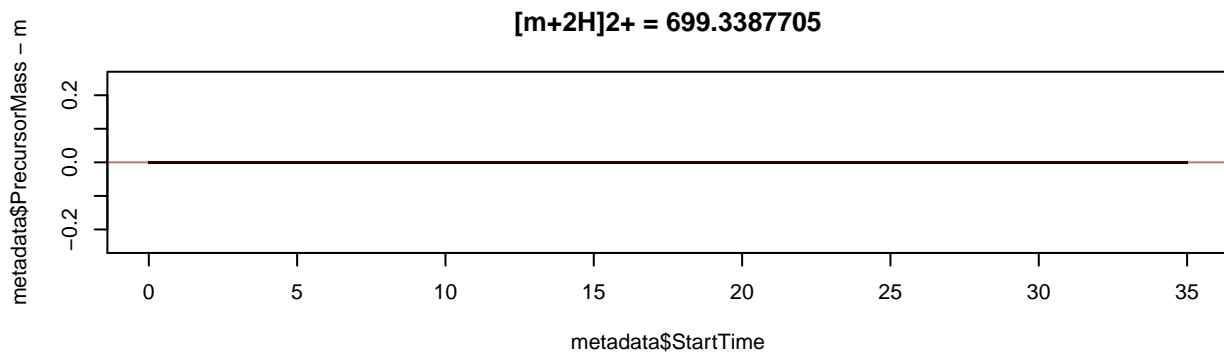
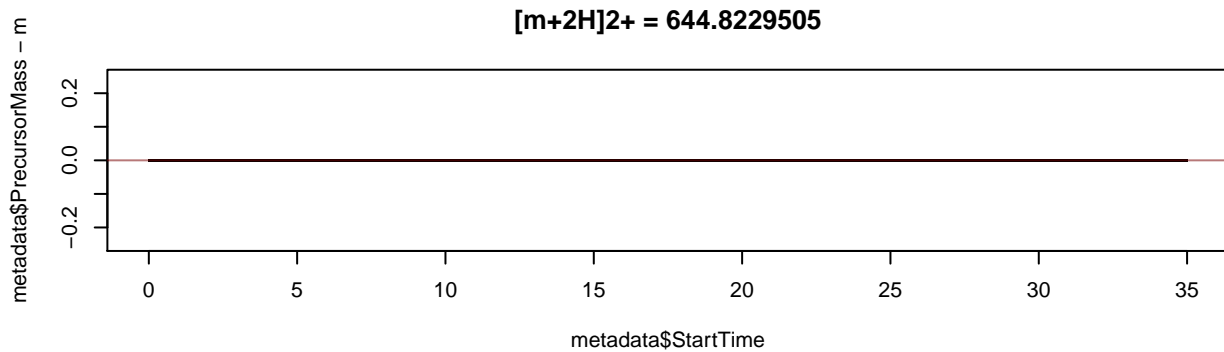
```
## renamed AGCPSMode to PrescanMode
```

Now we check in which scan the delta of the isolated precursor mass and the calculated precursor mass is smaller as 0.1 Da and return a plot for visual inspection.

```

op <- par(mfrow = c(3, 1), mar=c(4,4,4,1))
rv <- lapply(mass2Hplus, function(m){
  plot(metadata$StartTime, metadata$PrecursorMass - m, pch='.', ylim=c(-0.25,0.25), main = paste("[m+2H]2+",
  abline(h=0, col="#77000088"));
})

```



uff...so many scans...looks like we have PRM data. How do we verify the peak produced in the XIC plot with the peptide ID?

let's see what else we have.

we extract all scan numbers where our precursor filter is true.

we define a set of fragment ions for labeling our spectra (from package protviz)

```

HCDIons <- 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))
return(cbind(b, y))
}

```

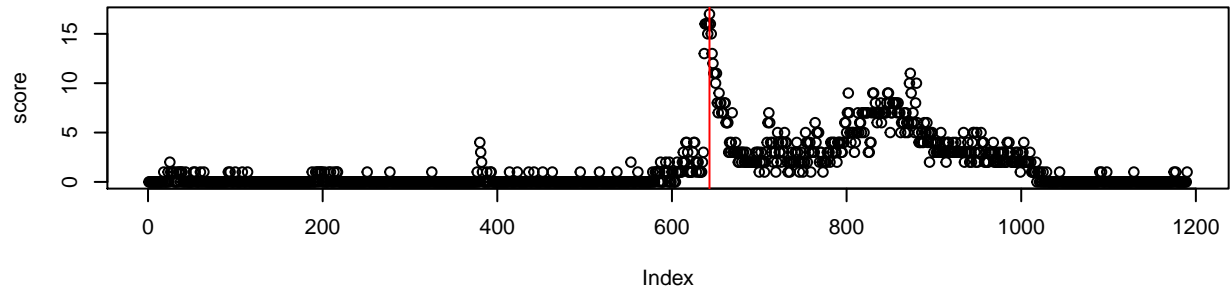
Now we extract from the raw file the peak list of all scans which match our precursor filter, pass these scans to peakplot for labeling the b- and y-ions and use a simple score (largest number of assigned ions) to select the best matching spectrum for each peptide. The score distribution is plotted for visual inspection and the best matching scan is highlighted by a red line.

```

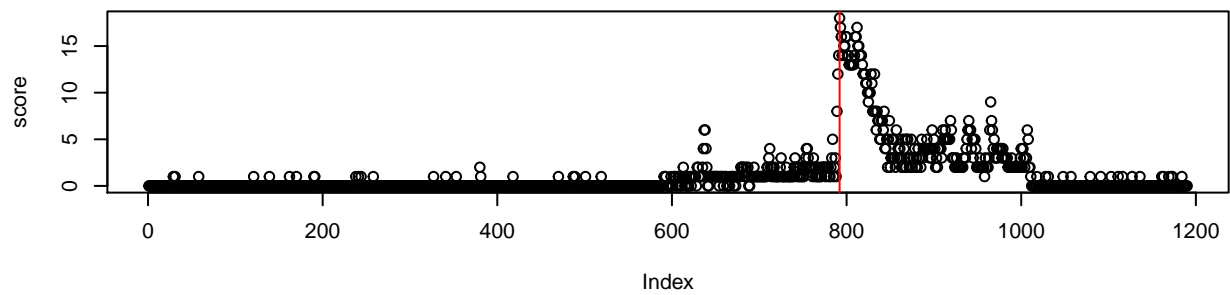
op <- par(mfrow = c(3, 1), mar=c(4,4,4,1))
bestMatchingMS2Scan <- sapply(1:3, function(i){
  PL <- readScans(rawfile, scans = scanNumbers[[i]])
  pp <- lapply(PL, function(x){psm(pepSeq[i], x, FUN = HCDIons, plot = FALSE)})
  score <- sapply(pp, function(x){sum(abs(x$mZ.Da.error) < 0.1)}) #find best scoring spectra
  plot(score, main = pepSeq[i])
  bestFirstMatch <- which(max(score) == score)[1]
  abline(v = bestFirstMatch, col = "red")
  scanNumbers[[i]][bestFirstMatch]
})

```

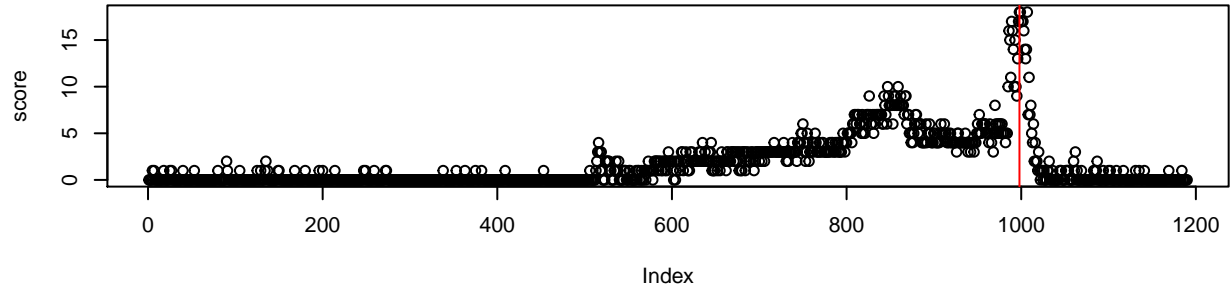
### AGGSSEPVTGLADK



### GDLDAASYYPVR

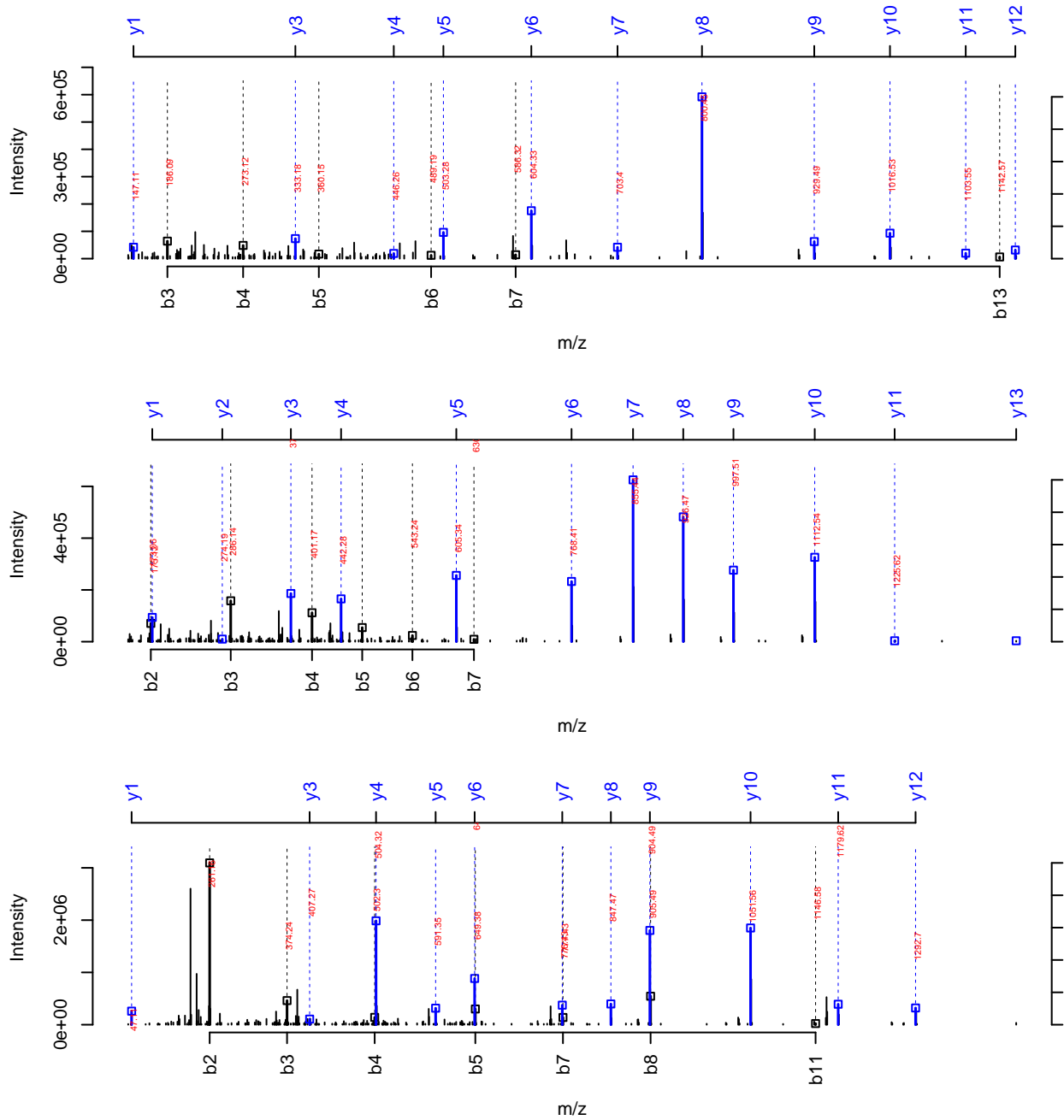


### FLLQFGAQGSPLFK



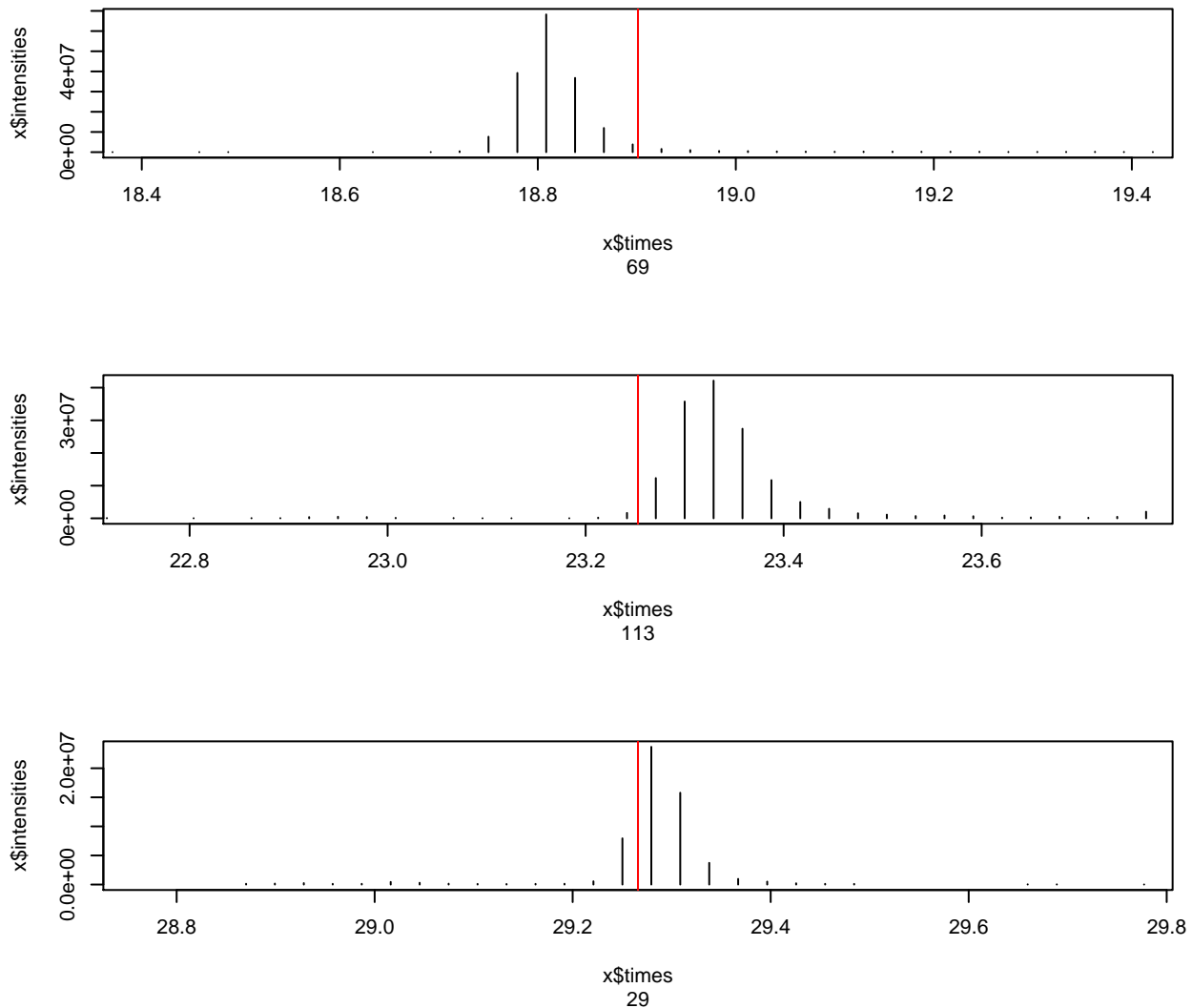
To check the actual fragment spectra, we plot for each peptide the best matching spectrum and label the corresponding b- and y-Ions.

```
op <- par(mfrow = c(3, 1), mar=c(4,4,4,1))
PL <- readScans(rawfile, scans = bestMatchingMS2Scan)
pp <- lapply(1:3, function(i){peakplot(pepSeq[i], PL[[i]], FUN = HCDIons)})
```



Now we can plot the XIC again, but zoom with a range 1 min around the best matching scan and we are sure we look at the proper peak.

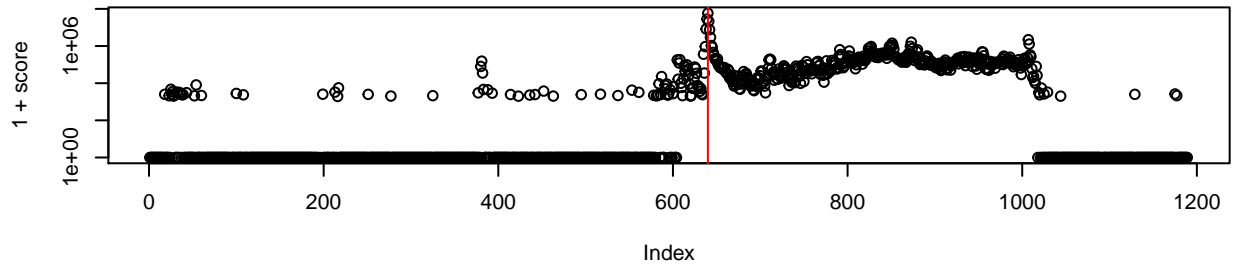
```
op <- par(mfrow = c(3, 1), mar=c(5,4,4,1))
p <- lapply(1:3, function(i){t <- metadata$StartTime[bestMatchingMS2Scan[i]];
plot(X[[i]], xlim = c(t - 0.5, t + 0.5)); abline(v=t,col='red')}})
```



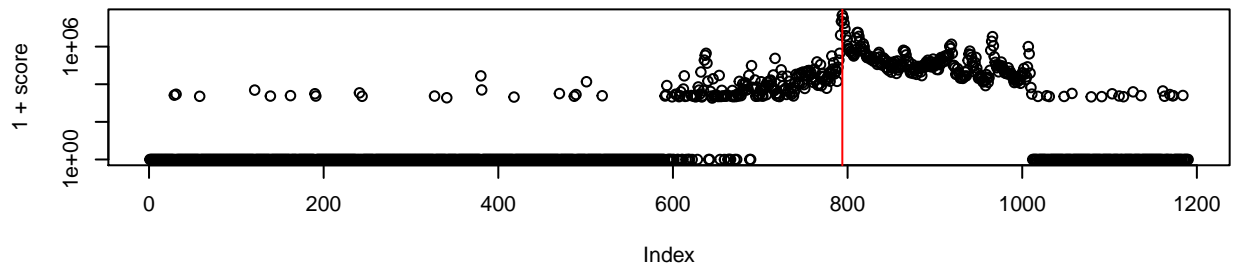
Now we could start to refine the scoring function by additional parameters...e.g. we take the intensity into consideration.

```
op <- par(mfrow = c(3, 1), mar = c(5,4,4,1))
bestMatchingMS2Scan <- sapply(1:3, function(i){
  PL <- readScans(rawfile, scans = scanNumbers[[i]])
  pp <- lapply(PL, function(x){psm(pepSeq[i], x, FUN = HCDIons, plot =
FALSE)})
  score <- sapply(1:length(pp),
function(j){sum(PL[[j]]$intensity[abs(pp[[j]]$mZ.Da.error) < 0.1]})}
  plot(1+score, main = paste(pepSeq[i], max(score, na.rm = TRUE)), log='y')
  bestFirstMatch <- which(max(score, na.rm = TRUE) == score)[1]
  abline(v = bestFirstMatch, col = "red")
  scanNumbers[[i]][bestFirstMatch]
})
```

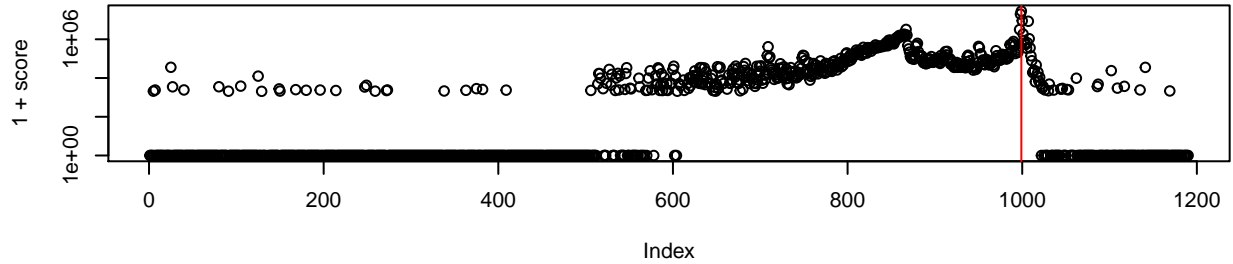
**AGGSSEPVTGLADK 58907595.8671875**



**GDLDAASYYPVR 46927761.953125**



**FLLQFGAQGSPLFK 28366938.4609375**



And plot again the XIC with the zoom around the best matching scan.

```
op <- par(mfrow = c(3, 1), mar=c(5,4,4,1))
p <- lapply(1:3, function(i){t <- metadata$StartTime[bestMatchingMS2Scan[i]];
plot(X[[i]], xlim = c(t - 0.5, t + 0.5)); abline(v=t,col='red')}})
```



