# On Finding putative PTM (pPTM) Marker Ion in HCD scans using `PTM_MarkerFinder`

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**Abstract**

Glycopeptides as well as acetylated, methylated and other modified peptides release specific fragment ions during CID (collision-induced dissociation) and HCD (higher energy collisional dissociation) fragmentation. These fragment ions can be used to validate the presence of the PTM (post translational modifications) on the peptides. `PTM_MarkerFinder`, an R function of the **protViz** package that takes advantage of such marker ions. `PTM_MarkerFinder` scans the MS/MS spectra in the output of a peptide spectrum match search, e.g., Mascot, for marker ions specific for selected PTMs.

While the software tool has been described by Nanni, Panse, Gehrig, Mueller, Grossmann, and Schlapbach (2013) here we provide a step-by-step guide on how the software can be used.

*Keywords*: MarkerFinder, putative post translational modifications, R.

## 1. Howto get the software and data

The method for finding the marker ions is contained in the R package **protViz** available through CRAN using https://cran.r-project.org/package=protViz. The package requires R (R Development Core Team 2008) installed.

The minimal data structure requirement for the `PTM_MarkerFinder` function looks as follow.

```
R> library(protViz)
R> data(HexNAc)
R> str(HexNAc[[1]], nchar.max = 30)

List of 12
 $ peptideSequence   : chr "STMQELNSR"
 $ mascotScore       : num 49.5
 $ modification      : chr "00000000000"
 $ MonoisotopicAAmass: num [1:9] 0 0 0 0 0 0 0 0 0
 $ proteinInformation: chr "zz|ZZ_FGCZCont0219|"
 $ title             : chr "NGlycoFASP_NH"| __truncated__
```

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```
$ pepmass           : num 533
$ charge            : num 2
$ scans             : num 2659
$ rtinseconds       : num 1846
$ mZ                : num [1:150] 101 104 105 110 112 ...
$ intensity         : num [1:150] 369.3 2860 37.3 103.8 190.7 ...
```

Here we have listed the `HexNAc` data which is included in **protViz**.

**protViz** also provides and perl script `protViz_mascotDat2RData.pl`[1] taking mascot server dat files as input and producing RData output.

```
$ /usr/local/lib/R/site-library/protViz/exec/protViz_mascotDat2RData.pl \
  -d=/usr/local/mascot/data/20130116/F178287.dat \
  -m=$HOME/mod_file
```

`mascotDat2RData.pl` requires the mascot server `mod_file` keeping all the configured modification of the mascot server.

In theory `PTM_MarkerFinder` can process the output of any search engine for peptide identification. It is up to the R user writing a wrapper script converting the output of any particular peptide identification search engine to the data structure listed above.

# 2. Finding the Marker Ions

## 2.1. HexNAc – Example

`PTM_MarkerFinder` can search for any Marker ion series. The next lines define the `HexNAc_MarkerIons`.

```
R> HexNAc_MarkerIons <- c(126.05495, 138.05495, 144.06552,
+     168.06552, 186.07608, 204.08665)
```

The lines below configure the modification information used by the search engine. The HexNAc modification below is described on unimod http://www.unimod.org/modifications_view.php?editid1=43.

```
R> ptm.0 <- cbind(AA = "-",
+          mono = 0.0, avg = 0.0, desc = "unmodified", unimodAccID = NA)
R> ptm.1 <- cbind(AA='N',
+          mono = 317.122300, avg = NA, desc = "HexNAc",
+          unimodAccID=2)
R> ptm.2 <- cbind(AA='M',
+          mono = 147.035400, avg = NA, desc = "Oxidation",
+          unimodAccID=1)
R> m <- as.data.frame(rbind(ptm.0, ptm.1, ptm.2))
```

[1] The prefix `protViz_` is used to benefit from the `bash` tab completion.

`PTM_MarkerFinder` is called.

```
R> S <- PTM_MarkerFinder(data = HexNAc,
+          modification = m$mono,
+          modificationName = m$desc,
+          minMarkerIntensityRatio = 3,
+          itol_ppm = 20,
+          mZmarkerIons = HexNAc_MarkerIons)
```

The content of `S` can be seen in the Table below.

| scans | mZ | markerIonMZ | markerIonIntensity | markerIonMzError | markerIonPpmError | query | pepmass | peptideSequence | modification |
|---|---|---|---|---|---|---|---|---|---|
| 3687 | 126.06 | 126.05 | 9945.00 | -0.00 | -0.642576494978 98 | 4 | 713.36 | IMNVTTDSLTK | 0001000000000 |
| 3687 | 138.06 | 138.05 | 1933.00 | -0.00 | -2.49175522390729 | 4 | 713.36 | IMNVTTDSLTK | 0001000000000 |
| 3687 | 144.07 | 144.07 | 412.30 | -0.00 | -1.59649326794302 | 4 | 713.36 | IMNVTTDSLTK | 0001000000000 |
| 3687 | 168.07 | 168.07 | 810.20 | -0.00 | -2.36811844277867 | 4 | 713.36 | IMNVTTDSLTK | 0001000000000 |
| 3687 | 204.09 | 204.09 | 3273.00 | -0.00 | -1.74435407225623 | 4 | 713.36 | IMNVTTDSLTK | 0001000000000 |
| 2540 | 126.06 | 126.05 | 2945.00 | -0.00 | -0.825036336847078 | 6 | 490.56 | HSFNGNQSTFK | 0000001000000 |
| 2540 | 138.06 | 138.05 | 759.20 | -0.00 | -10.3725737215287 | 6 | 490.56 | HSFNGNQSTFK | 0000001000000 |
| 2540 | 144.07 | 144.07 | 195.40 | -0.00 | -0.118001850879316 | 6 | 490.56 | HSFNGNQSTFK | 0000001000000 |
| 2540 | 168.07 | 168.07 | 262.90 | -0.00 | -0.916308466469431 | 6 | 490.56 | HSFNGNQSTFK | 0000001000000 |
| 2540 | 186.08 | 186.08 | 188.50 | -0.00 | -2.95577150125756 | 6 | 490.56 | HSFNGNQSTFK | 0000001000000 |
| 2540 | 204.09 | 204.09 | 998.40 | -0.00 | -1.5189603491234 | 6 | 490.56 | HSFNGNQSTFK | 0000001000000 |
| 4393 | 126.06 | 126.05 | 13620.00 | -0.00 | -1.03922824020165 | 9 | 891.41 | EASGLSDNETEWLK | 0000000010000000 |
| 4393 | 138.06 | 138.05 | 3798.00 | -0.00 | -0.420122390602973 | 9 | 891.41 | EASGLSDNETEWLK | 0000000010000000 |
| 4393 | 168.07 | 168.07 | 1526.00 | -0.00 | -0.642606113437682 | 9 | 891.41 | EASGLSDNETEWLK | 0000000010000000 |
| 4393 | 186.08 | 186.08 | 1014.00 | -0.00 | -0.983467730223809 | 9 | 891.41 | EASGLSDNETEWLK | 0000000010000000 |
| 4393 | 204.09 | 204.09 | 5041.00 | -0.00 | -1.06817259804309 | 9 | 891.41 | EASGLSDNETEWLK | 0000000010000000 |
| 2739 | 126.06 | 126.05 | 7327.00 | -0.00 | -0.690174721011021 | 10 | 665.59 | NA | NA |
| 2739 | 138.05 | 138.05 | 1963.00 | -0.00 | -0.311470082107949 | 10 | 665.59 | NA | NA |
| 2739 | 144.07 | 144.07 | 468.60 | -0.00 | -0.5344787486255 | 10 | 665.59 | NA | NA |
| 2739 | 168.07 | 168.07 | 624.30 | -0.00 | -0.642606113437682 | 10 | 665.59 | NA | NA |
| 2739 | 204.09 | 204.09 | 2496.00 | -0.00 | -0.622284313992652 | 10 | 665.59 | NA | NA |

Table 1: Result

```
R> summary(S)
```

```
 scans          mZ          markerIonMZ     markerIonIntensity
2540:6   Min.   :126.1   Min.   :126.1   Min.   :  188.5
2739:5   1st Qu.:138.1   1st Qu.:138.1   1st Qu.:  624.3
3687:5   Median :144.1   Median :144.1   Median : 1526.0
4393:5   Mean   :159.5   Mean   :159.5   Mean   : 2838.1
         3rd Qu.:186.1   3rd Qu.:186.1   3rd Qu.: 3273.0
         Max.   :204.1   Max.   :204.1   Max.   :13620.0

markerIonMzError             markerIonPpmError query
Min.   :-0.0014320   -0.642606113437682: 2      10:5
1st Qu.:-0.0003100   -0.118001850879316: 1      4 :5
Median :-0.0001310   -0.311470082107949: 1      6 :6
Mean   :-0.0002436   -0.420122390602973: 1      9 :5
3rd Qu.:-0.0000870   -0.5344787486255  : 1
Max.   :-0.0000170   -0.622284313992652: 1
                     (Other)           :14
   pepmass             peptideSequence           modification
Min.   :490.6   EASGLSDNETEWLK:5      0000000010000000:5
1st Qu.:490.6   HSFNGNQSTFK   :6      0000001000000   :6
Median :665.6   IMNVTTDSLTK   :5      0001000000000   :5
```

```
 Mean   :680.7   NA            :5      NA              :5
 3rd Qu.:713.4
 Max.   :891.4
```

**Some overview graphics** just an overview of the sample data set `HexNAc`.

```
R> op <- par(mfrow = c(2, 2), mar=c(4, 4, 4, 1))
R> dump <- lapply(split(S, S$query),
+      function(x){
+        plot(x$mZ, x$markerIonIntensity,
+          type = 'h',
+          col = 'lightblue',
+          cex = 2,
+          ylab = 'intensity', xlab='m/z',
+          xlim = range(c(HexNAc_MarkerIons,
+              max(HexNAc_MarkerIons)
+                  + 0.1 * (max(HexNAc_MarkerIons) - min(HexNAc_MarkerIons)),
+              min(HexNAc_MarkerIons)
+                  - 0.1 * (max(HexNAc_MarkerIons) - min(HexNAc_MarkerIons)))),
+          ylim = range(S$markerIonIntensity),
+              log = 'y',
+              main = paste("scan=", unique(x$scans),
+                  "/query=", unique(x$query), sep=''));
+              text(x$mZ, x$markerIonIntensity,
+                  round(x$mZ,2),col='red',cex=0.7)
+          }
+      )
R> par(op)
```

Figure 1 dislays the output of `PTM_MarkerFinder`.

## 2.2. Reshaping the output and export

The R method `reshape` transforms the data frame `S` from a long format to a wide format.

```
R> names(S)[4] <- "mII"
R> S.wide <- reshape(S[,c(1,7,3,4)],
+                    direction = 'wide',
+                    timevar = "markerIonMZ",
+                    idvar = c('scans','query'))
R>
```

export as comma separeted file

```
R> write.table(S.wide,
+          file = file.path(tempdir(), "HexNAc_PTM_markerFinder.csv"),
```

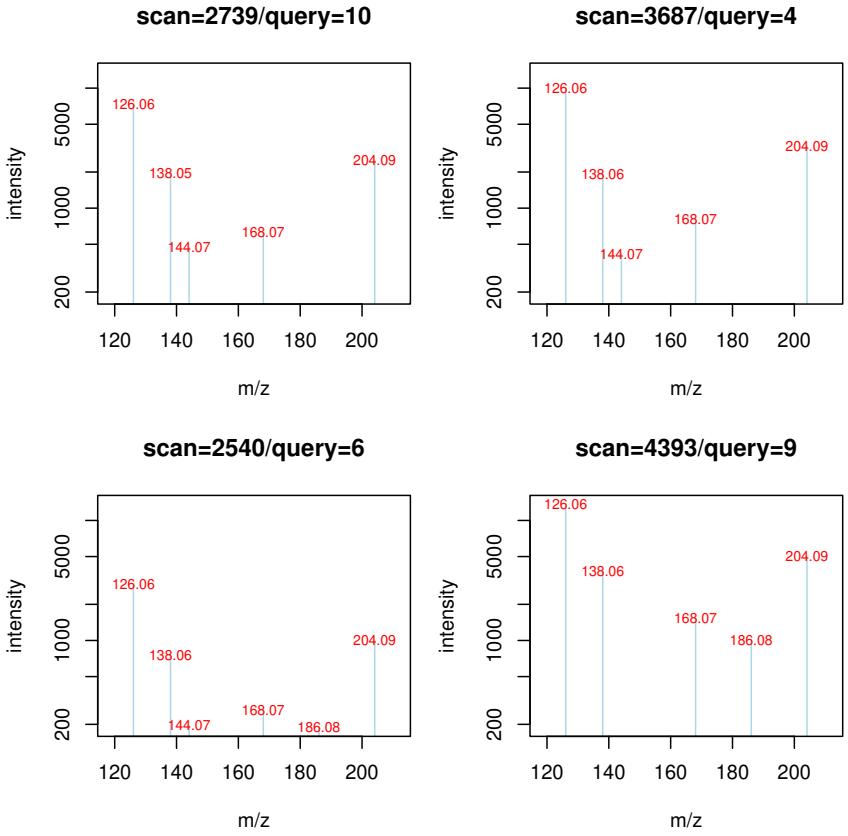


Figure 1: Overview of the marker ions.

| scans | query | mII.126.05495 | mII.138.05495 | mII.144.06552 | mII.168.06552 | mII.204.08665 | mII.186.07608 |
|---|---|---|---|---|---|---|---|
| 3687 | 4 | 9945.00 | 1933.00 | 412.30 | 810.20 | 3273.00 | |
| 2540 | 6 | 2945.00 | 759.20 | 195.40 | 262.90 | 998.40 | 188.50 |
| 4393 | 9 | 13620.00 | 3798.00 | | 1526.00 | 5041.00 | 1014.00 |
| 2739 | 10 | 7327.00 | 1963.00 | 468.60 | 624.30 | 2496.00 | |

Table 2: Result

```
+             sep = ',',
+         row.names = FALSE,
+         col.names = TRUE,
+         quote = FALSE)
```

## 2.3. Visualization of the Result

```
R> # prepare the input
R> d <- list(); d[[1]] <- HexNAc[[3]]; d[[2]] <- HexNAc[[4]]; d[[3]] <- HexNAc[[5]]
R> S <- PTM_MarkerFinder(data = d, modification = m$mono,
+         modificationName = m$desc,
+         minMarkerIntensityRatio = 3,
```

```
+          itol_ppm = 20,
+          mZmarkerIons = HexNAc_MarkerIons)
```

The graphics can be seen in Figure 2.

# 3. Demonstartion

The user can call the demonstration with

```
R> demo(PTM_MarkerFinder)
```

## 3.1. Other examples

The following ADP-Ribose marker ions configuration was described by Bilan, Leutert, Nanni, Panse, and Hottiger (2017).

```
R> ADP_Ribose <- c(136.0618, 250.0935, 348.0704, 428.0367)
```

# 4. Session information

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

- R version 4.1.3 (2022-03-10), `x86_64-pc-linux-gnu`
- Locale: `C`
- Running under: `Debian GNU/Linux 10 (buster)`
- Matrix products: default
- BLAS: `/usr/lib/x86_64-linux-gnu/blas/libblas.so.3.8.0`
- LAPACK: `/usr/lib/x86_64-linux-gnu/lapack/liblapack.so.3.8.0`
- Base packages: base, datasets, grDevices, graphics, methods, stats, utils
- Other packages: protViz 0.7.2, xtable 1.8-4
- Loaded via a namespace (and not attached): Rcpp 1.0.7, codetools 0.2-18, compiler 4.1.3, tools 4.1.3

# References

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

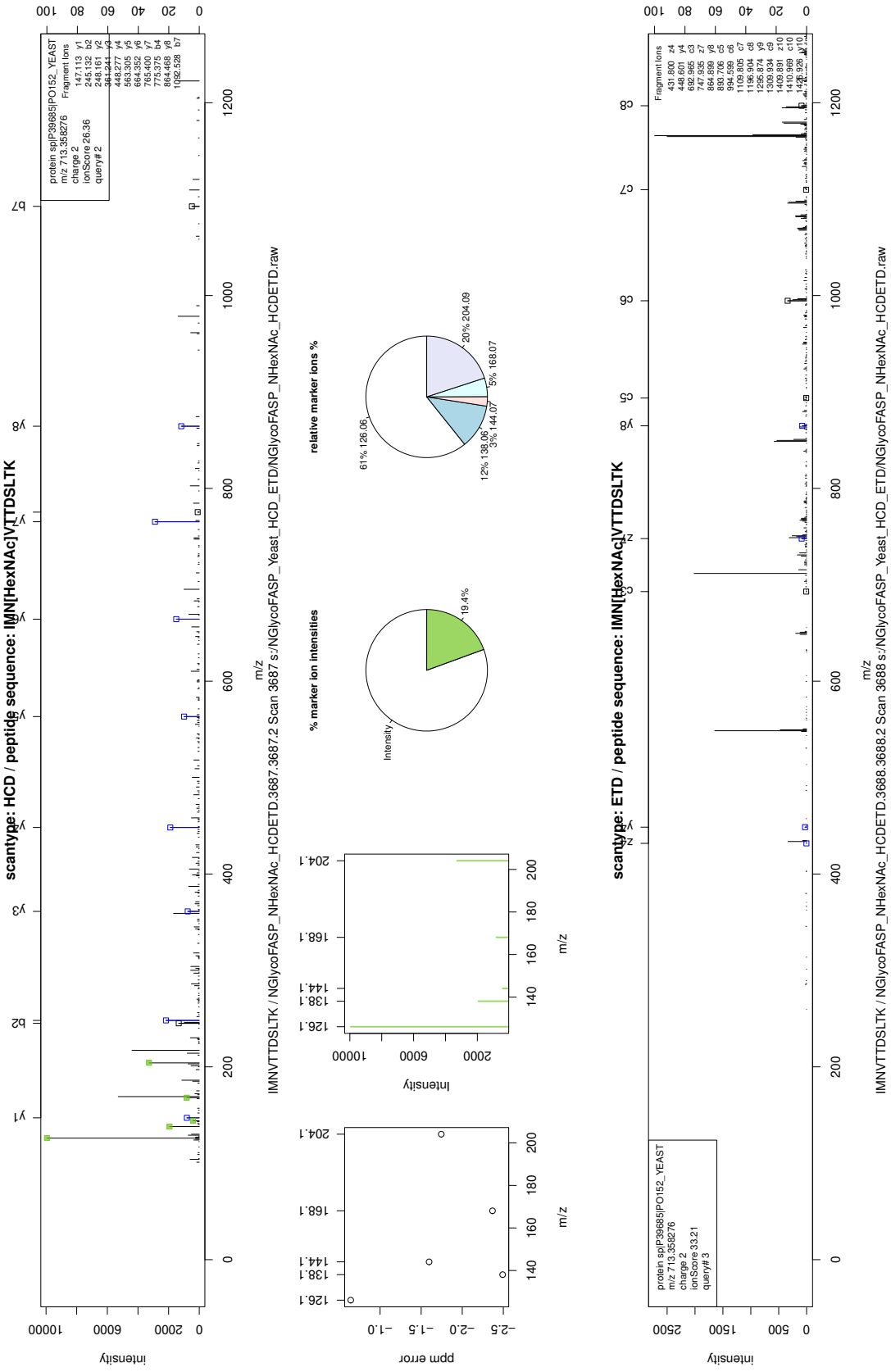


Figure 2: Graphical output of the method.

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

R Development Core Team (2008). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

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