

Finding putative PTM (pPTM) Marker Ion in HCD scans

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1 Get Data In – Preprocessing

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

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

List of 12

```
$ peptideSequence      : chr "STMQELNSR"
$ mascotScore          : num 49.5
$ modification         : chr "000000000000"
$ MonoisotopicAAMass   : num [1:9] 0 0 0 0 0 0 0 0 0
$ proteinInformation: chr "zz|ZZ_FGCZCont0219|"
$ title                : chr "NGlycoFASP_NHexNAc_HCDETD.265"| __truncated__
$ 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 a perl script protViz_mascotDat2RData.pl¹ 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

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

```
> 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.

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

```

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

```

PTM_MarkerFinder is called.

```

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

```

	scans	mZ	markerIonMZ	markerIonIntensity	markerIonMzError
1	3687	126.0550	126.0550	9945.0	-0.000081
2	3687	138.0553	138.0549	1933.0	-0.000344
3	3687	144.0658	144.0655	412.3	-0.000230
4	3687	168.0659	168.0655	810.2	-0.000398
5	3687	204.0870	204.0866	3273.0	-0.000356
6	2540	126.0551	126.0550	2945.0	-0.000104
7	2540	138.0564	138.0549	759.2	-0.001432
8	2540	144.0655	144.0655	195.4	-0.000017
9	2540	168.0657	168.0655	262.9	-0.000154
10	2540	186.0766	186.0761	188.5	-0.000550
11	2540	204.0870	204.0866	998.4	-0.000310
12	4393	126.0551	126.0550	13620.0	-0.000131
13	4393	138.0550	138.0549	3798.0	-0.000058
14	4393	168.0656	168.0655	1526.0	-0.000108
15	4393	186.0763	186.0761	1014.0	-0.000183
16	4393	204.0869	204.0866	5041.0	-0.000218
17	2739	126.0550	126.0550	7327.0	-0.000087
18	2739	138.0550	138.0549	1963.0	-0.000043
19	2739	144.0656	144.0655	468.6	-0.000077
20	2739	168.0656	168.0655	624.3	-0.000108
21	2739	204.0868	204.0866	2496.0	-0.000127

	markerIonPpmError	query	pepmass
1	-0.6425765	4	713.3583
2	-2.4917552	4	713.3583
3	-1.5964933	4	713.3583
4	-2.3681184	4	713.3583
5	-1.7443541	4	713.3583
6	-0.8250363	6	490.5612

7	-10.3725737	6 490.5612
8	-0.1180019	6 490.5612
9	-0.9163085	6 490.5612
10	-2.9557715	6 490.5612
11	-1.5189603	6 490.5612
12	-1.0392282	9 891.4088
13	-0.4201224	9 891.4088
14	-0.6426061	9 891.4088
15	-0.9834677	9 891.4088
16	-1.0681726	9 891.4088
17	-0.6901747	10 665.5916
18	-0.3114701	10 665.5916
19	-0.5344787	10 665.5916
20	-0.6426061	10 665.5916
21	-0.6222843	10 665.5916

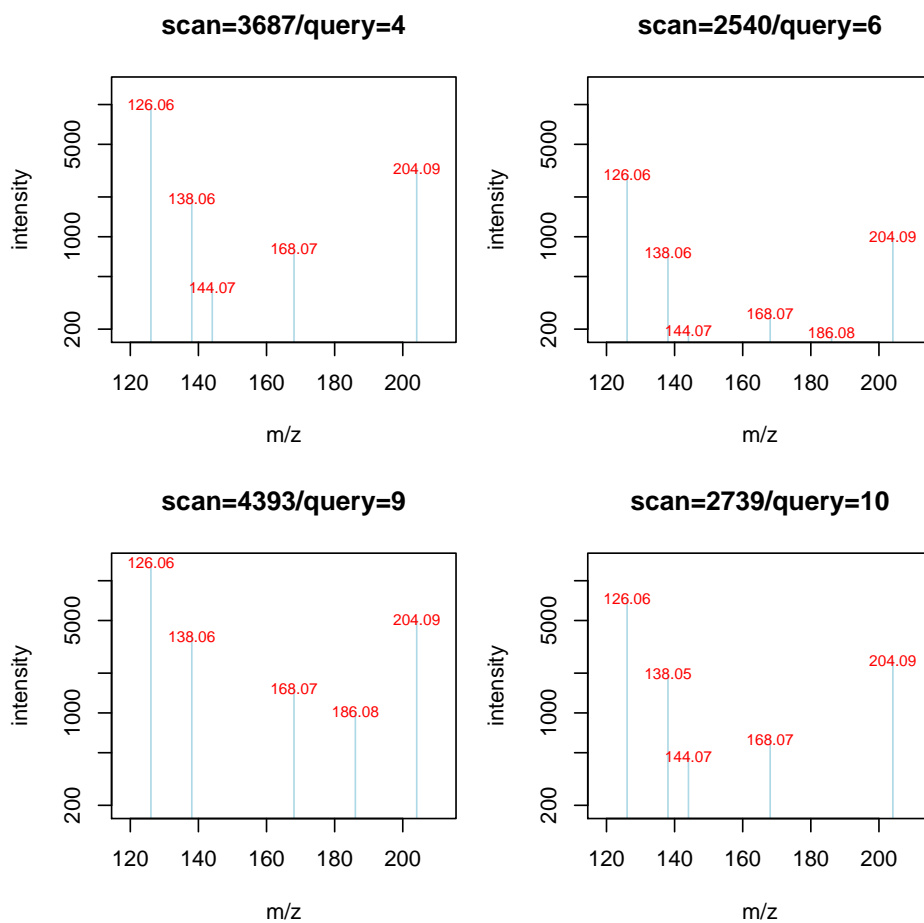
The user can call the demonstration with

```
> demo(PTM_MarkerFinder)
```

3 Some overview graphics

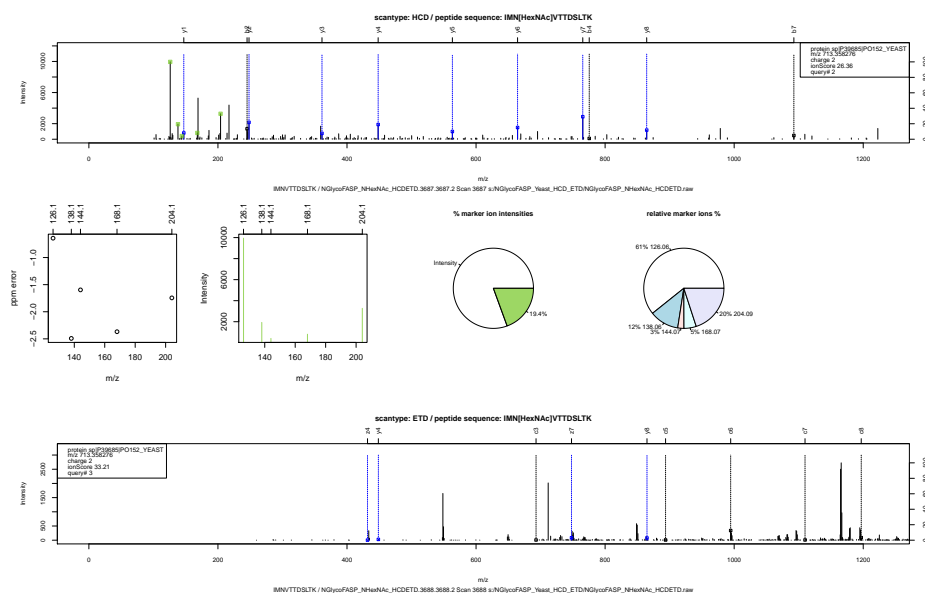
just an overview of the sample data set HexNAC.

```
> op<-par(mfrow=c(2,2), mar=c(4,4,4,1));
> 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)
+   })
+ )
> par(op)
```



The next graphics show the output of PTM_MarkerFinder.

```
> d<-list(); d[[1]]<-HexNAc[[3]]; d[[2]]<-HexNAc[[4]];
> d[[3]]<-HexNAc[[5]]
> ss<-PTM_MarkerFinder(data=d, modification=m$mono,
+      modificationName=m$desc,
+      minMarkerIntensityRatio=3,
+      itol_ppm=20,
+      mZmarkerIons=HexNAc_MarkerIons)
>
```



4 Reshaping the output and export

reshape the table:

```
> w<-reshape(s[,c(1,7,3,4)], direction='wide',
+           timevar="markerIonMZ", idvar=c('scans','query'))
> w
```

	scans	query	markerIonIntensity.126.05495	markerIonIntensity.138.05495
1	3687	4	9945	1933.0
6	2540	6	2945	759.2
12	4393	9	13620	3798.0
17	2739	10	7327	1963.0
			markerIonIntensity.144.06552	markerIonIntensity.168.06552
1			412.3	810.2
6			195.4	262.9
12			NA	1526.0
17			468.6	624.3
			markerIonIntensity.204.08665	markerIonIntensity.186.07608
1			3273.0	NA
6			998.4	188.5
12			5041.0	1014.0
17			2496.0	NA

export as comma separated file

```
> write.table(w, file="HexNAc_PTM_markerFinder.csv",
+           sep=',', row.names=FALSE,col.names=TRUE, quote=FALSE)
>
```

References

- [1] P. Nanni, C. Panse, P. Gehrig, S. Mueller, J. Grossmann, and R. Schlapbach. PTM MarkerFinder, a software tool to detect and validate spectra from peptides carrying post-translational modifications. *Proteomics*, 13(15):2251–2255, Aug 2013. [DOI:10.1002/pmic.201300036] [PubMed:23713006].