USC Department Of Translational Genomics
Keck Medicine of USC

TRGN 527: Applied Data Science and Bioinformatics

UNIT I. Introduction and Basic Data Science

Week 4 – Lecture 1 – Case Study Part 1

Enrique I. Velazquez Villarreal, M.D., Ph.D., M.P.H., M.S. | **Assistant Professor**

Dept. of Translational Genomics

USC | Keck School of Medicine | Norris Comprehensive Cancer Center

Leader of the USC Bioinformatics Core – *USC CaRE2 Health Equity Center*

David W. Craig, Ph.D. | **Professor and Vice Chair**

Dept. of Translational Genomics

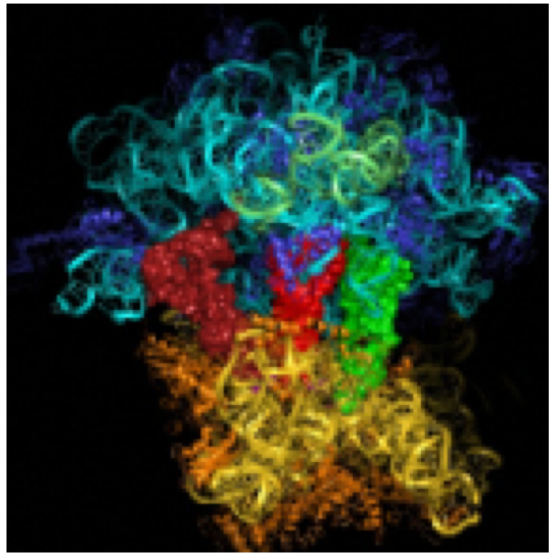
USC | Keck School of Medicine | Norris Comprehensive Cancer Center

Co-Director, Institute of Translational Genomics

Case Study – Proteomics Analysis

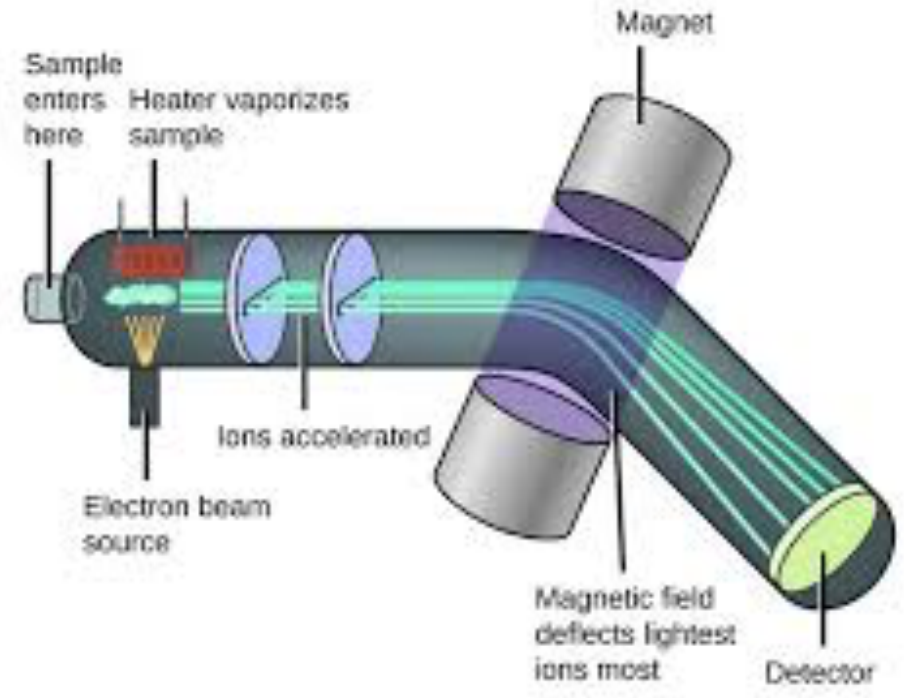
Case Study – Proteomics Analysis

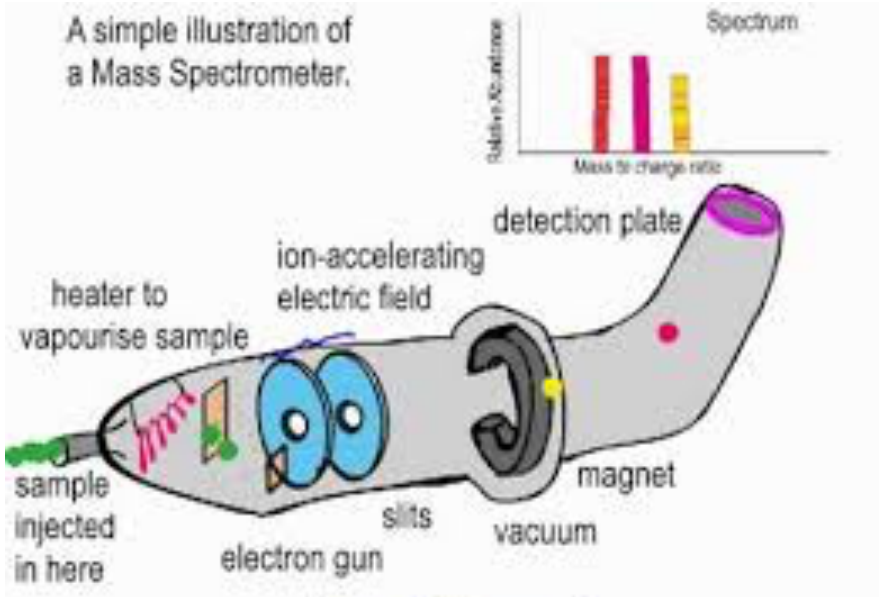
- Introduction
  - It is crucial to study cellular processes also at the level of proteome and to measure the abundance of the proteins in biological samples.
  - The segment of molecular biology that investigates the behavior of proteins is called “proteomics”.
  - There are several methods in this field that aim to measure the abundance of proteins in parallel in different cellular systems.
  - Mass spectrometry (MS) is one of the essential approaches that is capable of the identification of proteins, sometimes even with quantitative results.



Case Study – Proteomics Analysis

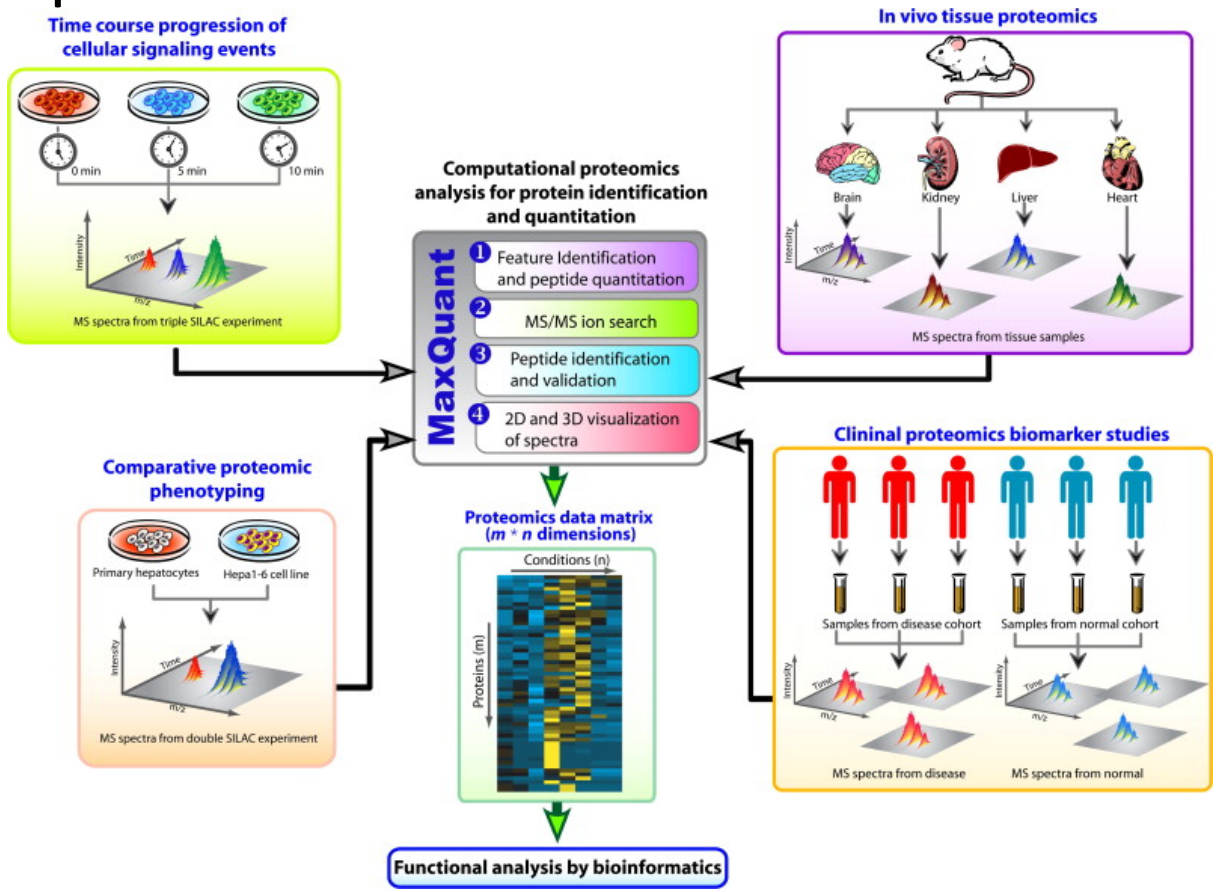
- Proteomics: Mass spectrometry (MS)
- MS is an analytic technique that produces the mass-to-charge ratio spectra of peptide fragment in samples.
- Typically samples contain mainly purified proteins or mixtures representing biological specimens.





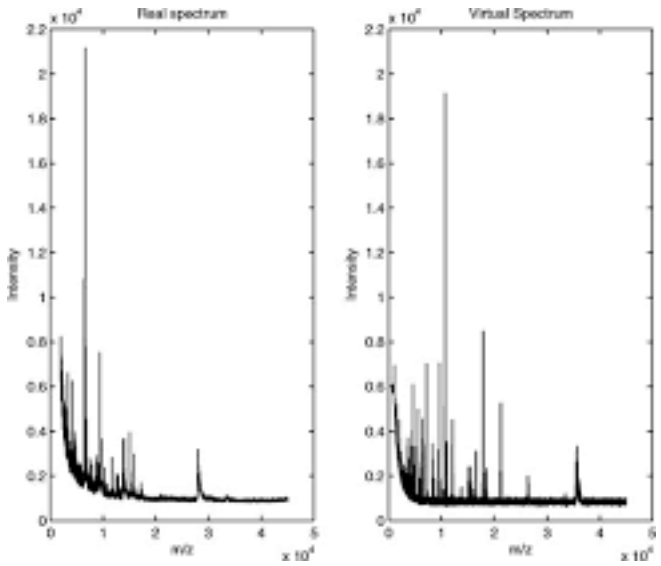
Case Study – Proteomics Analysis

- The GOAL of the analysis is to identify (one or more) peptides from the original protein or proteins, because these peptides are used for the qualitative or quantitative determination of the proteins in the specimens, or sometimes, for the identification of the fine modifications (i.e., phosphorylation, glycosylation, etc…) of the original protein.



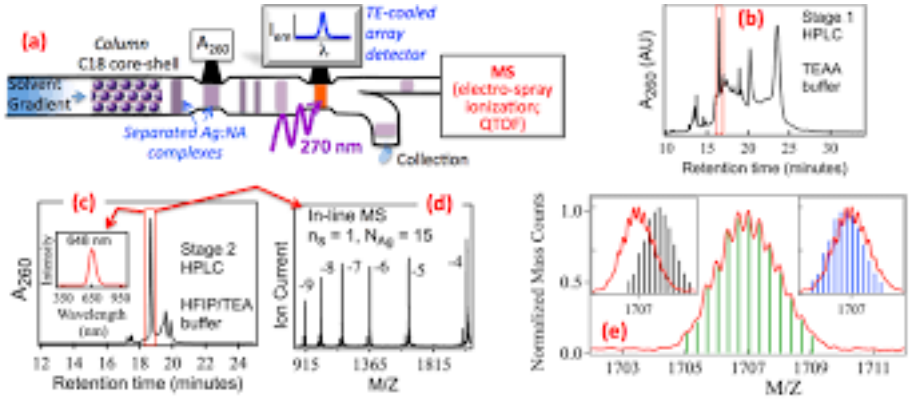
Case Study – Proteomics Analysis

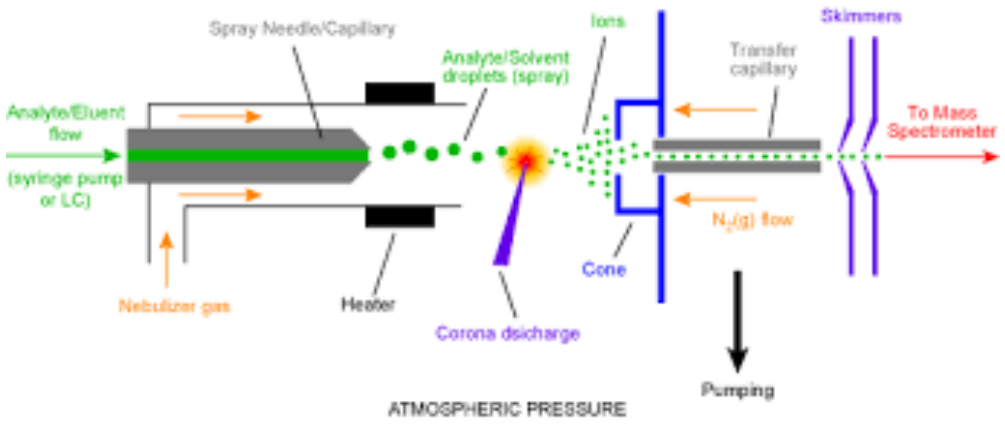
- It is very important to consider the origin of the samples.
  - 1) Proteins are isolated from tissue samples or cell cultures according to the nature of the experiment.
  - 2) As these samples typically contain 5-40 thousand kinds of proteins, it would be hopeless to find differences without further separation.
  - 3) The most often used separation method is the two-dimensional polyacrylamide gel electrophoresis that distributes the proteins along two dimensions based on their isoelectric point and molecular mass.
  - 4) The spots on the gel contain a mixture of proteins that are cut and submitted to the procedure of MS.



Case Study – Proteomics Analysis

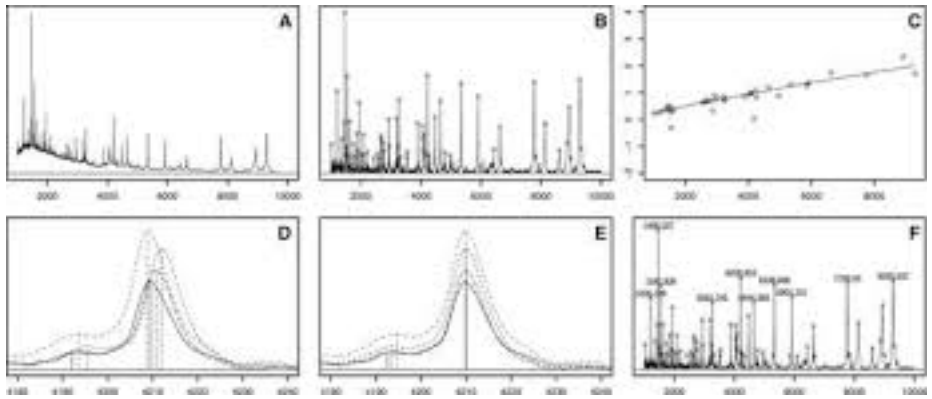
- The proteins isolated from the 2D-gel or the samples loaded to the HPLCD columns are digested with a protease enzyme
- MS always detects peptides—the fragments of the original proteins.
- The actual MS follows the preparation of separated peptide mixture.
- The spectrometer itself is built from three parts:
  - Ion source
  - Mass analyzer
  - Detector





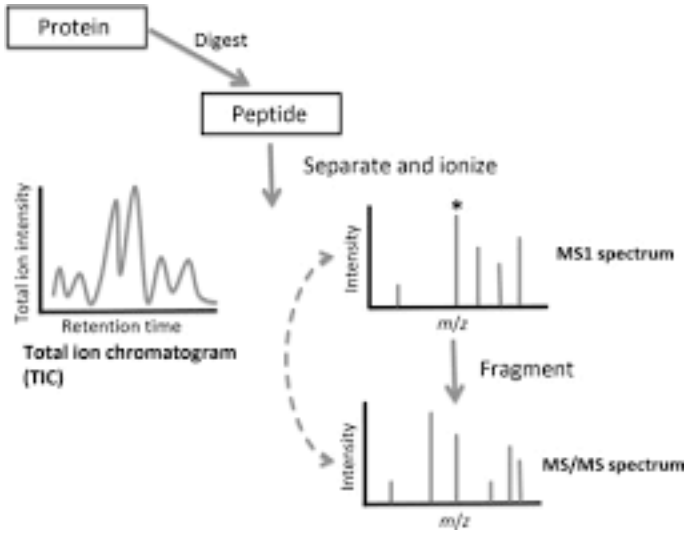
Case Study – Proteomics Analysis

- The Role of Data science/Bioinformatics in this kind of experiment is:
  - To Identify the peaks originating from peptides in the spectrum
  - To identify the source proteins of the peptides
  - Optionally to detect most translational modifications in the proteins
  - To find qualitative and quantitative differences between the biological samples



Case Study – Proteomics Analysis

- Dedicated software is available to analyze MS spectra, usually from the producers of the measurement equipment.
- Independent data analysis tools are available from Bioconductor packages.



Case Study – Proteomics Analysis

- File formats for MS data
  - The diversity formats known from the sequence segment of bioinformatics can also be seen in MS data.
  - The used file formats can be divided into two categories:
    - Files containing raw measurement data.
    - Files carrying processed data.
  - Standard formats for data exchange:
    - ANDI-MS
    - XML-based formats
  - mzR package from Bioconductor contains parsers for the following file formats:
    - netCDF
    - mzXML
    - mzData
    - mzML

Case Study – Proteomics Analysis

- Installing Packages:

```
---
title: "Rmarkdown_TRGN_599_Week_4_Proteomics_Analysis"
author: "Enrique I. Velazquez Villarreal, MD, PhD"
date: "1/25/2019"
output: html_document
---

# Checking R working directory
```{R}
# Check your current working directory
getwd()

# Set your working directory
setwd("/Users/enriquevelazquez/Documents/R_working_directory")
```

Install mzR

To install this package, start R (version "3.5") and enter:
if (!requireNamespace("BiocManager", quietly = TRUE))
install.packages("BiocManager")
BiocManager::install("mzR", version = "3.8")

Install msdata

#if (!requireNamespace("BiocManager", quietly = TRUE))
install.packages("BiocManager")
#BiocManager::install("msdata", version = "3.8")

Install magrittr
install.packages("magrittr")

Accessing the raw data of published studies
```

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- Let us use the data:

```
35 ```{R}
36 library(mzR)
37 library(msdata)
38 library(magrittr)
39
40 msfile <- system.file("threonine/threonine_i2_e35_pH_tree.mzXML",
41                       package = "msdata")
42
43 my.data <- openMSfile(msfile)
44
45 ```
```

- openMSfile() function can deter the file format, and interprets the details in the my.data variable.

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- It is important that after we have finished working on the data package, it would be closed:

````
49 ```{R}
50 close(my.data)
51 ```
````

Case Study – Proteomics Analysis

- Accessing the metadata itself:

````
56
57 # Accessing the measurement - metadata:
58
59 ```{r}
60
61 runInfo(my.data)
62
63 ```
64
65 ```{r}
66 instrumentInfo(my.data)
67
68 ```
69
70 ```{r}
71 header(my.data)
72 ```
````

Case Study – Proteomics Analysis

```
59 ```{r}
60
61 runInfo(my.data)
62
63 ```
```

```
$scanCount
[1] 55

$lowMz
[1] 50.0036

$highMz
[1] 298.673

$dStartTime
[1] 0.3485

$dEndTime
[1] 390.027

$msLevels
[1] 1 2 3 4

$startTimeStamp
[1] NA
```

Case Study – Proteomics Analysis

```
65 ```{r}
66 instrumentInfo(my.data)
67
68 ```
```

```
$manufacturer
[1] "Thermo Scientific"

$model
[1] "LTQ Orbitrap"

$ionisation
[1] "electrospray ionization"

$analyzer
[1] "fourier transform ion cyclotron resonance mass spectrometer"

$detector
[1] "unknown"

$software
[1] "Xcalibur software 2.2 SP1"

$sample
[1] ""

$source
[1] ""
```

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```r
header(my.data)
```

	seqNum	acquisitionNum	msLevel	polarity	peaksCount	totIonCurrent	retentionTime	basePeakMZ	basePeakIntensity
1	1	1	1	1	684	341427000	0.3485	120.0660	211860000.0
2	2	2	2	1	432	160473000	5.8561	120.0660	139169000.0
3	3	3	2	1	340	58862000	12.5000	102.0560	27036600.0
4	4	4	3	1	273	30770400	19.7568	102.0550	26736000.0
5	5	5	3	1	238	5291800	26.6056	56.0497	2188950.0
6	6	6	3	1	140	14294000	34.0324	74.0605	12518700.0
7	7	7	3	1	186	1413070	40.8786	56.0498	11830.4
8	8	8	4	1	78	1492820	48.3417	56.0497	886536.0
9	9	9	4	1	278	2354340	55.3885	110.0830	79565.8
10	10	10	4	1	317	2875910	62.7088	84.0448	213506.0
11	11	11	4	1	300	2368190	70.0325	110.0850	72235.1
12	12	12	1	1	798	313900000	77.2653	120.0660	201865000.0
13	13	13	2	1	378	113438000	84.3054	120.0660	97457600.0
14	14	14	2	1	370	63132600	90.9602	102.0560	28619300.0
15	15	15	3	1	273	28086600	98.2067	102.0550	24283500.0
16	16	16	3	1	227	4741460	105.0660	56.0497	1933080.0
17	17	17	3	1	167	15533800	112.4830	74.0604	13427500.0
18	18	18	3	1	148	1159100	119.3430	53.7828	13460.9
19	19	19	4	1	88	1455980	126.7930	56.0497	816687.0
20	20	20	4	1	220	1717640	133.8520	110.0850	94071.9
21	21	21	4	1	337	2904410	141.1620	84.0448	173552.0
22	22	22	4	1	301	2473480	148.4970	110.0850	40811.0
23	23	23	1	1	760	298444000	155.7190	120.0660	189928000.0
24	24	24	2	1	448	156749000	162.7680	120.0660	134812000.0
25	25	25	2	1	307	54785800	169.4140	102.0560	25553800.0
26	26	26	3	1	259	28587300	176.6710	102.0560	24676000.0
27	27	27	3	1	193	4729110	183.5190	56.0497	2071830.0
28	28	28	3	1	176	15149600	190.9490	74.0605	13006800.0
29	29	29	3	1	163	1281970	197.7980	64.5802	23298.9
30	30	30	4	1	87	1544430	205.2600	56.0497	849991.0
31	31	31	4	1	257	2099510	212.3040	110.0860	74823.3
32	32	32	4	1	287	2449720	219.6240	84.0449	222710.0
33	33	33	4	1	253	2073550	226.9490	110.0860	82527.0
34	34	34	1	1	762	324080000	234.1820	120.0660	210152000.0
35	35	35	2	1	458	150504000	241.2210	120.0660	133003000.0
36	36	36	2	1	316	56265300	247.8760	102.0560	25598100.0
37	37	37	3	1	217	28866800	255.1210	102.0550	25688200.0
38	38	38	3	1	220	5248920	261.9830	56.0497	2218720.0

	collisionEnergy	ionisationEnergy	lowMZ	highMZ	precursorScanNum	precursorMZ	precursorCharge
1	0	0	50.3254	298.6730	0	0.0000	0

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37	37	37	3	1	217	28866800	255.1210	102.0550	25688200.0
38	38	38	3	1	220	5248920	261.9830	56.0497	2218720.0

	collisionEnergy	ionisationEnergy	lowMZ	highMZ	precursorScanNum	precursorMZ	precursorCharge
1	0	0	50.3254	298.6730	0	0.0000	0
2	0	0	50.4459	134.1380	1	120.0661	1
3	35	0	50.0658	134.1430	1	120.0661	1
4	0	0	50.2843	114.6510	3	102.0600	0
5	35	0	50.1967	114.9470	3	102.0600	0
6	0	0	50.2254	84.8802	3	74.0600	0
7	35	0	50.1161	84.3040	3	74.0600	0
8	0	0	50.0186	69.7022	7	56.0500	0
9	35	0	50.0940	124.1700	7	56.0500	0
10	0	0	50.0818	179.1560	7	84.0400	0
11	35	0	50.2006	179.5180	7	84.0400	0
12	0	0	50.0608	297.5910	0	0.0000	0
13	0	0	50.1201	133.7830	12	120.0661	1
14	35	0	50.0570	134.6390	12	120.0661	1
15	0	0	50.4447	113.9020	14	102.0600	0
16	35	0	50.0036	114.8890	14	102.0600	0
17	0	0	50.0271	84.8065	14	74.0600	0
18	35	0	50.0058	84.7754	14	74.0600	0
19	0	0	50.0887	69.0518	18	56.0500	0
20	35	0	50.2412	124.7270	18	56.0500	0
21	0	0	50.4238	179.9800	18	84.0400	0
22	35	0	50.2533	178.8750	18	84.0400	0
23	0	0	50.0057	296.6160	0	0.0000	0
24	0	0	50.0101	134.8170	23	120.0661	1
25	35	0	50.0852	134.8220	23	120.0661	1
26	0	0	50.0392	114.6410	25	102.0600	0
27	35	0	50.2574	114.1250	25	102.0600	0
28	0	0	50.1784	84.5998	25	74.0600	0
29	35	0	50.0834	84.9637	25	74.0600	0
30	0	0	50.1508	69.7567	29	56.0500	0
31	35	0	50.0291	124.4360	29	56.0500	0
32	0	0	50.4089	178.1340	29	84.0400	0
33	35	0	50.0503	179.8680	29	84.0400	0
34	0	0	50.1337	297.2720	0	0.0000	0
35	0	0	50.0615	134.5250	34	120.0661	1
36	35	0	50.0294	134.7460	34	120.0661	1
37	0	0	50.9147	112.8510	36	102.0600	0
38	35	0	50.6186	114.6040	36	102.0600	0

	precursorIntensity	mergedScan	mergedResultScanNum	mergedResultStartScanNum	mergedResultEndScanNum
1	0.00000e+00	0	0	0	0
2	2.10140e+08	0	0	0	0

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```
37              0              0 50.9147 112.8510              36    102.0600              0
38             35              0 50.6186 114.6040              36    102.0600              0
   precursorIntensity mergedScan mergedResultScanNum mergedResultStartScanNum mergedResultEndScanNum
1        0.00000e+00          0                   0                         0                       0
2        2.10140e+08          0                   0                         0                       0
3        2.10140e+08          0                   0                         0                       0
4        1.44188e+06          0                   0                         0                       0
5        1.44188e+06          0                   0                         0                       0
6        1.55609e+06          0                   0                         0                       0
7        1.55609e+06          0                   0                         0                       0
8        2.02325e+04          0                   0                         0                       0
9        2.02325e+04          0                   0                         0                       0
10       1.28766e+04          0                   0                         0                       0
11       1.28766e+04          0                   0                         0                       0
12       0.00000e+00          0                   0                         0                       0
13       2.00932e+08          0                   0                         0                       0
14       2.00932e+08          0                   0                         0                       0
15       1.62085e+06          0                   0                         0                       0
16       1.62085e+06          0                   0                         0                       0
17       1.66728e+06          0                   0                         0                       0
18       1.66728e+06          0                   0                         0                       0
19       1.75168e+04          0                   0                         0                       0
20       1.75168e+04          0                   0                         0                       0
21       8.48349e+03          0                   0                         0                       0
22       8.48349e+03          0                   0                         0                       0
23       0.00000e+00          0                   0                         0                       0
24       1.89890e+08          0                   0                         0                       0
25       1.89890e+08          0                   0                         0                       0
26       1.47298e+06          0                   0                         0                       0
27       1.47298e+06          0                   0                         0                       0
28       1.63855e+06          0                   0                         0                       0
29       1.63855e+06          0                   0                         0                       0
30       9.33749e+03          0                   0                         0                       0
31       9.33749e+03          0                   0                         0                       0
32       1.09943e+04          0                   0                         0                       0
33       1.09943e+04          0                   0                         0                       0
34       0.00000e+00          0                   0                         0                       0
35       2.06837e+08          0                   0                         0                       0
36       2.06837e+08          0                   0                         0                       0
37       1.52646e+06          0                   0                         0                       0
38       1.52646e+06          0                   0                         0                       0
```

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```
38       1.52646e+06          0                  0                        0                     0
   injectionTime filterString                                spectrumId centroided ionMobilityDriftTime
1              0         <NA>  controllerType=0 controllerNumber=1 scan=1       TRUE                   NA
2              0         <NA>  controllerType=0 controllerNumber=1 scan=2       TRUE                   NA
3              0         <NA>  controllerType=0 controllerNumber=1 scan=3       TRUE                   NA
4              0         <NA>  controllerType=0 controllerNumber=1 scan=4       TRUE                   NA
5              0         <NA>  controllerType=0 controllerNumber=1 scan=5       TRUE                   NA
6              0         <NA>  controllerType=0 controllerNumber=1 scan=6       TRUE                   NA
7              0         <NA>  controllerType=0 controllerNumber=1 scan=7       TRUE                   NA
8              0         <NA>  controllerType=0 controllerNumber=1 scan=8       TRUE                   NA
9              0         <NA>  controllerType=0 controllerNumber=1 scan=9       TRUE                   NA
10             0         <NA> controllerType=0 controllerNumber=1 scan=10       TRUE                   NA
11             0         <NA> controllerType=0 controllerNumber=1 scan=11       TRUE                   NA
12             0         <NA> controllerType=0 controllerNumber=1 scan=12       TRUE                   NA
13             0         <NA> controllerType=0 controllerNumber=1 scan=13       TRUE                   NA
14             0         <NA> controllerType=0 controllerNumber=1 scan=14       TRUE                   NA
15             0         <NA> controllerType=0 controllerNumber=1 scan=15       TRUE                   NA
16             0         <NA> controllerType=0 controllerNumber=1 scan=16       TRUE                   NA
17             0         <NA> controllerType=0 controllerNumber=1 scan=17       TRUE                   NA
18             0         <NA> controllerType=0 controllerNumber=1 scan=18       TRUE                   NA
19             0         <NA> controllerType=0 controllerNumber=1 scan=19       TRUE                   NA
20             0         <NA> controllerType=0 controllerNumber=1 scan=20       TRUE                   NA
21             0         <NA> controllerType=0 controllerNumber=1 scan=21       TRUE                   NA
22             0         <NA> controllerType=0 controllerNumber=1 scan=22       TRUE                   NA
23             0         <NA> controllerType=0 controllerNumber=1 scan=23       TRUE                   NA
24             0         <NA> controllerType=0 controllerNumber=1 scan=24       TRUE                   NA
25             0         <NA> controllerType=0 controllerNumber=1 scan=25       TRUE                   NA
26             0         <NA> controllerType=0 controllerNumber=1 scan=26       TRUE                   NA
27             0         <NA> controllerType=0 controllerNumber=1 scan=27       TRUE                   NA
28             0         <NA> controllerType=0 controllerNumber=1 scan=28       TRUE                   NA
29             0         <NA> controllerType=0 controllerNumber=1 scan=29       TRUE                   NA
30             0         <NA> controllerType=0 controllerNumber=1 scan=30       TRUE                   NA
31             0         <NA> controllerType=0 controllerNumber=1 scan=31       TRUE                   NA
32             0         <NA> controllerType=0 controllerNumber=1 scan=32       TRUE                   NA
33             0         <NA> controllerType=0 controllerNumber=1 scan=33       TRUE                   NA
34             0         <NA> controllerType=0 controllerNumber=1 scan=34       TRUE                   NA
35             0         <NA> controllerType=0 controllerNumber=1 scan=35       TRUE                   NA
36             0         <NA> controllerType=0 controllerNumber=1 scan=36       TRUE                   NA
37             0         <NA> controllerType=0 controllerNumber=1 scan=37       TRUE                   NA
38             0         <NA> controllerType=0 controllerNumber=1 scan=38       TRUE                   NA
 [ reached 'max' / getOption("max.print") -- omitted 17 rows ]
```

Case Study – Proteomics Analysis

- Investigating the number of actual spectra in the set:

````
75 # Investigating the number of actual spectra in the set:
76 ```{r}
77 runInfo(my.data)$scanCount
78 ```
````

```
[1] 55
```

Case Study – Proteomics Analysis

- Investigating the ion source for the experiment:

````
80 # Investigating the ion source for the experiment
81 ```{r}
82 instrumentInfo(my.data)$ionisation
83 ```
````

```
[1] "electrospray ionization"
```

Case Study – Proteomics Analysis

- The data itself can be accessed by using the peaks() function:

```
# Accessing the data itself using the peaks() function.
```{r}
peaks(my.data,1)
```
```

```
          [,1]        [,2]
  [1,]  50.32539 1.185034e+04
  [2,]  50.65150 1.201500e+04
  [3,]  50.91941 1.068692e+04
  [4,]  51.02643 1.053706e+04
  [5,]  51.14503 1.140725e+04
  [6,]  51.16061 1.060811e+04
  [7,]  51.23557 1.214280e+04
  [8,]  51.28455 1.084886e+04
  [9,]  51.32047 9.873077e+03
 [10,]  51.66521 1.217426e+04
 [11,]  51.81630 1.007943e+04
 [12,]  52.61617 1.240245e+04
 [13,]  52.61874 1.033738e+04
 [14,]  52.61882 9.897575e+03
 [15,]  52.65551 1.015991e+04
 [16,]  52.82106 1.264292e+04
 [17,]  53.28766 1.157443e+04
 [18,]  53.46381 1.192924e+04
 [19,]  54.06857 1.218992e+04
 [20,]  54.64257 1.271298e+04
 [21,]  55.02473 1.083895e+04
 [22,]  55.16191 1.197379e+04
 [23,]  55.39608 1.318504e+04
 [24,]  55.69463 1.107968e+04
 [25,]  55.69698 1.182305e+04
 [26,]  55.73802 1.141378e+04
 [27,]  55.99680 1.449282e+04
 [28,]  56.01848 1.053534e+04
 [29,]  56.04968 2.106181e+04
 [30,]  56.07721 1.168999e+04
 [31,]  56.37001 1.271762e+04
 [32,]  56.49357 1.116523e+04
 [33,]  56.59671 1.161248e+04
 [34,]  57.03291 1.253886e+04
 [35,]  57.44817 1.237266e+04
 [36,]  57.56149 1.232441e+04
 [37,]  57.57074 1.251690e+04
 [38,]  57.63020 1.122068e+04
 [39,]  57.80737 1.249781e+04
 [40,]  57.87675 1.230323e+04
```

Case Study – Proteomics Analysis

```
90 ```{r}
91 peaks(my.data)
92 ```
```

```
[[1]]
           [,1]        [,2]
  [1,]  50.32539 1.185034e+04
  [2,]  50.65150 1.201500e+04
  [3,]  50.91941 1.068692e+04
  [4,]  51.02643 1.053706e+04
  [5,]  51.14503 1.140725e+04
  [6,]  51.16061 1.060811e+04
  [7,]  51.23557 1.214280e+04
  [8,]  51.28455 1.084886e+04
  [9,]  51.32047 9.873077e+03
 [10,]  51.66521 1.217426e+04
 [11,]  51.81630 1.007943e+04
 [12,]  52.61617 1.240245e+04
 [13,]  52.61874 1.033738e+04
 [14,]  52.61882 9.897575e+03
 [15,]  52.65551 1.015991e+04
 [16,]  52.82106 1.264292e+04
 [17,]  53.28766 1.157443e+04
 [18,]  53.46381 1.192924e+04
 [19,]  54.06857 1.218992e+04
 [20,]  54.64257 1.271298e+04
 [21,]  55.02473 1.083895e+04
 [22,]  55.16191 1.197379e+04
 [23,]  55.39608 1.318504e+04
 [24,]  55.69463 1.107968e+04
 [25,]  55.69698 1.182305e+04
 [26,]  55.73802 1.141378e+04
 [27,]  55.99680 1.449282e+04
 [28,]  56.01848 1.053534e+04
 [29,]  56.04968 2.106181e+04
 [30,]  56.07721 1.168999e+04
 [31,]  56.37001 1.271762e+04
 [32,]  56.49357 1.116523e+04
 [33,]  56.59671 1.161248e+04
 [34,]  57.03291 1.253886e+04
 [35,]  57.44817 1.237266e+04
 [36,]  57.56149 1.232441e+04
 [37,]  57.57074 1.251690e+04
 [38,]  57.63020 1.122068e+04
 [39,]  57.80737 1.249781e+04
 [498,] 153.57797 1.261890e+04
 [499,] 155.22195 1.483236e+04
 [500,] 156.39238 1.324974e+04
  [ reached getOption("max.print") -

[[2]]
           [,1]        [,2]
  [1,]  50.44595 7.130229e+03
  [2,]  50.63268 6.751621e+03
  [3,]  51.19802 7.744562e+03
  [4,]  51.30608 7.552050e+03
  [5,]  52.19186 7.821688e+03
  [6,]  52.27118 8.311925e+03
  [7,]  52.30317 7.661416e+03
  [8,]  52.33964 7.897834e+03
  [9,]  52.54125 8.598500e+03
 [10,]  52.55791 7.412984e+03
 [11,]  52.71907 7.608189e+03
 [12,]  52.84742 8.373299e+03
 [13,]  52.90979 8.570965e+03
 [14,]  53.12151 7.852208e+03
 [15,]  53.89261 7.181609e+03
 [16,]  54.12863 7.886996e+03
 [17,]  54.25117 8.021970e+03
 [18,]  54.25359 6.982733e+03
 [19,]  54.31281 8.117685e+03
 [20,]  54.57345 6.943318e+03
 [21,]  54.81168 7.215416e+03
 [22,]  54.92728 7.199123e+03
 [23,]  56.04983 1.157029e+04
 [24,]  56.19535 7.446009e+03
 [25,]  56.46938 7.716136e+03
 [26,]  56.74446 9.424041e+03
 [27,]  56.87177 7.570626e+03
 [28,]  57.27736 7.771037e+03
 [29,]  57.48447 8.067558e+03
 [30,]  57.54039 7.521494e+03
 [31,]  57.84854 8.147912e+03
 [32,]  57.90528 7.368232e+03
 [33,]  58.21653 9.569272e+03
 [34,]  58.56902 8.423535e+03
 [35,]  58.58501 8.095901e+03
 [36,]  58.66974 8.602247e+03
 [37,]  58.86484 7.274165e+03
 [38,]  59.64755 7.135571e+03
 [39,]  59.82327 8.698951e+03
 [431,] 134.07140 7.364540e+03
 [432,] 134.13841 7.647712e+03

[[3]]
           [,1]        [,2]
  [1,]  50.06576   6794.749
  [2,]  50.59673   7079.568
  [3,]  51.19412   7668.185
  [4,]  51.27377   8077.836
  [5,]  51.46772   7213.749
  [6,]  51.65720   8776.008
  [7,]  51.85004   7182.117
  [8,]  52.56889   6912.155
  [9,]  52.67318   7243.696
 [10,]  52.82322   7927.134
 [11,]  52.97269   7437.494
 [12,]  53.31343   9220.775
 [13,]  53.49500   6592.752
 [14,]  53.60342   7440.715
 [15,]  53.77227   7104.609
 [16,]  54.30957   6826.388
 [17,]  54.54459   7425.452
 [18,]  54.79221   8127.149
 [19,]  54.85666   8938.672
 [20,]  54.89362   7470.742
 [21,]  55.12350   6944.706
 [22,]  55.59793   9447.896
 [23,]  56.01242   7472.028
 [24,]  56.04912  10787.321
 [25,]  56.04974 821494.125
 [26,]  56.05036  18899.715
 [27,]  56.31257   7571.338
 [28,]  56.99893   8132.565
 [29,]  57.25984   7353.479
 [30,]  57.90335   8372.167
 [31,]  58.13220   7369.997
 [32,]  58.13994   7681.747
 [33,]  58.24904   7391.480
 [34,]  58.85498   7444.690
 [35,]  58.96836   7671.243
 [36,]  59.00388   8874.579
 [37,]  59.02112   7104.393
 [38,]  59.08067   9936.309
 [39,]  60.35535   8014.486
 [40,]  61.06535   6873.027
 [41,]  61.14135   7170.367
```