# Introduction to XCMS in R

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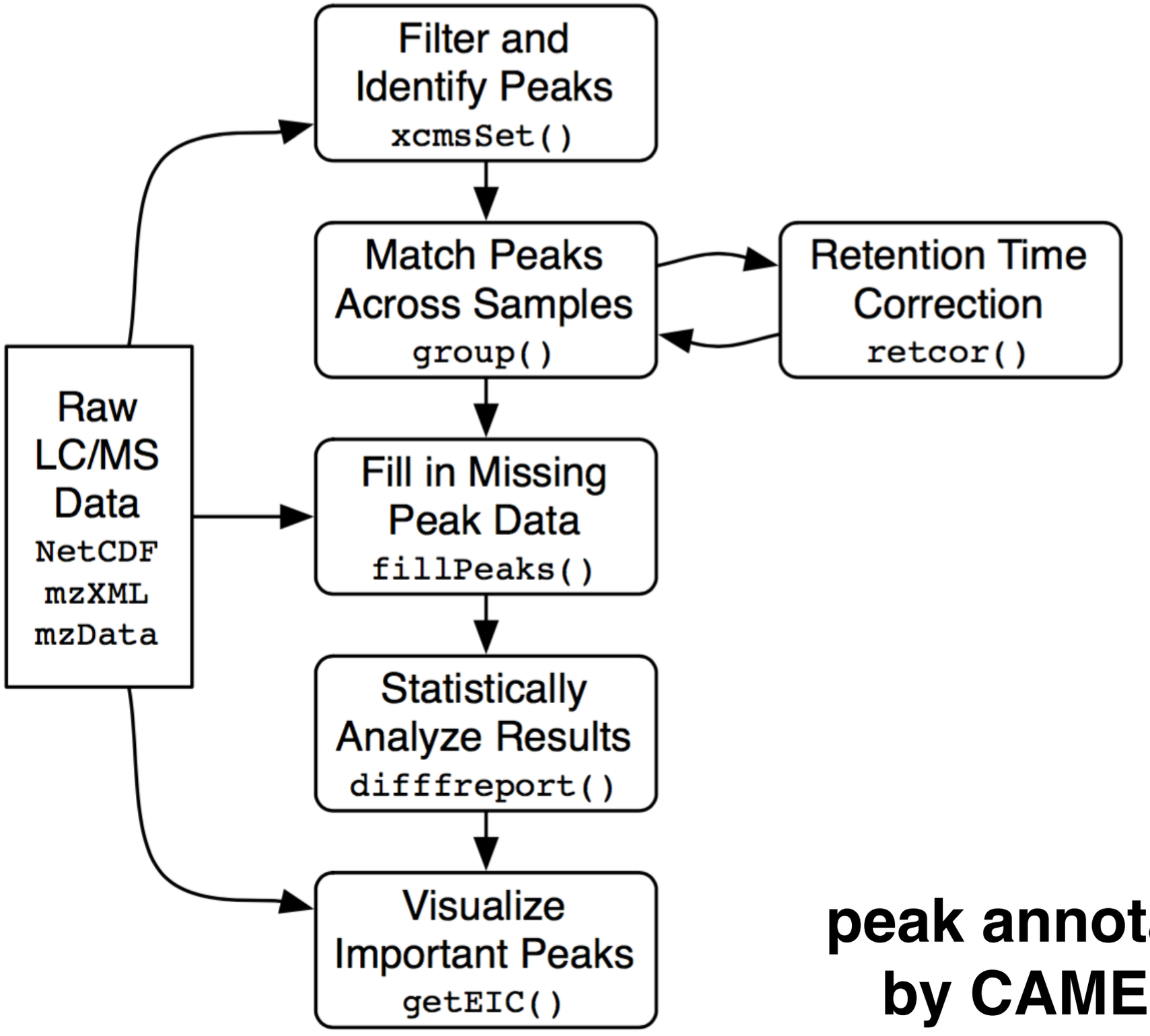
# XCMS in R

- Primarily used for preprocessing untargeted LC/MS metabolomics data in centroid mode
- Primary related publications:
  - Smith, C.A., Want, E.J., O'Maille, G., Abagyan,R., Siuzdak and G. (2006). "XCMS: Processing mass spectrometry data for metabolite profiling using nonlinear peak alignment, matching and identification." Analytical Chemistry, 78, pp. 779–787.
  - Tautenhahn R, Boettcher C and Neumann S (2008). "Highly sensitive feature detection for high resolution LC/MS." BMC Bioinformatics, 9, pp. 504.
- Information about XCMS R package can be found at

  `https://bioconductor.org/packages/release/bioc/html/xcms.html`

# Preprocessing steps



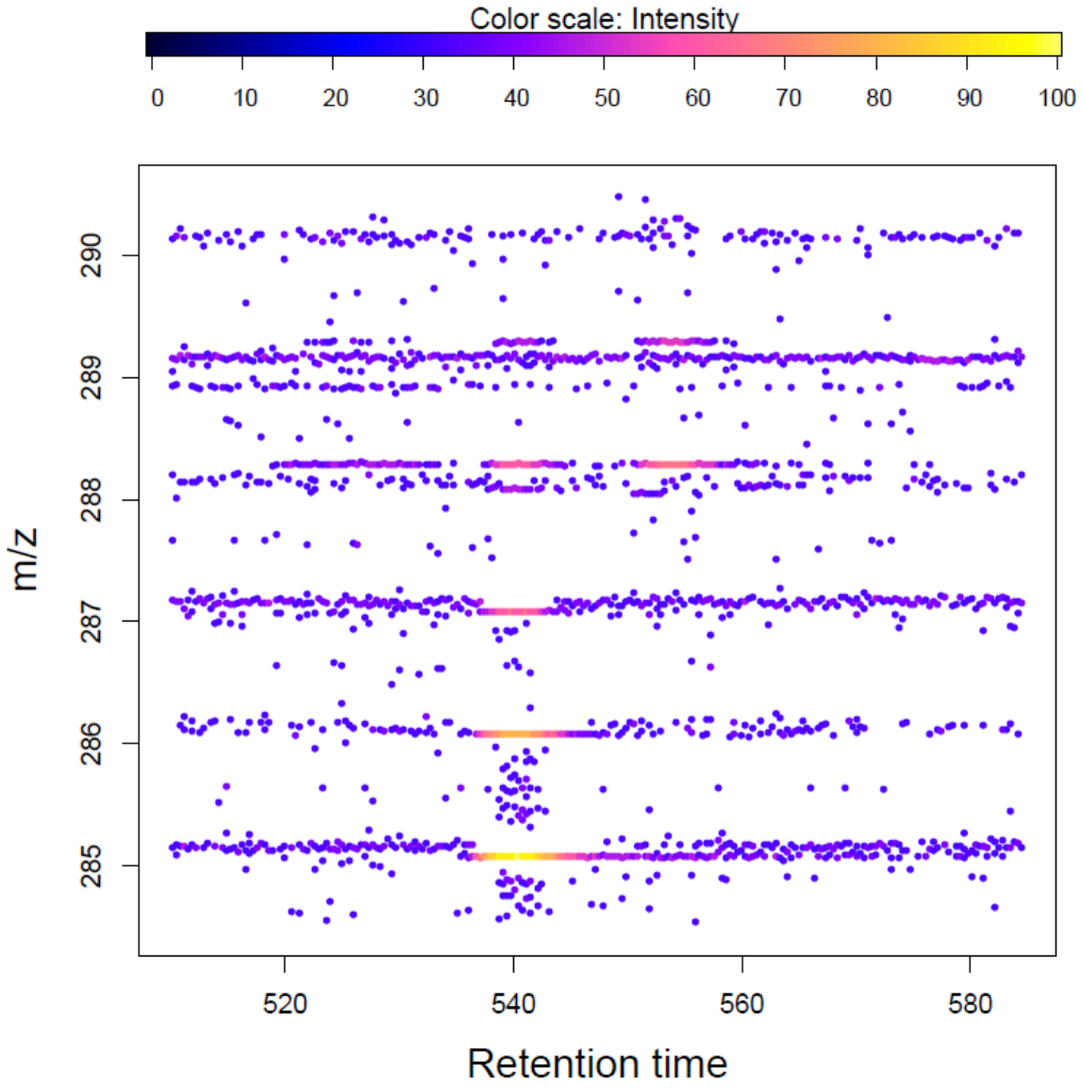

**peak annotation by CAMERA**

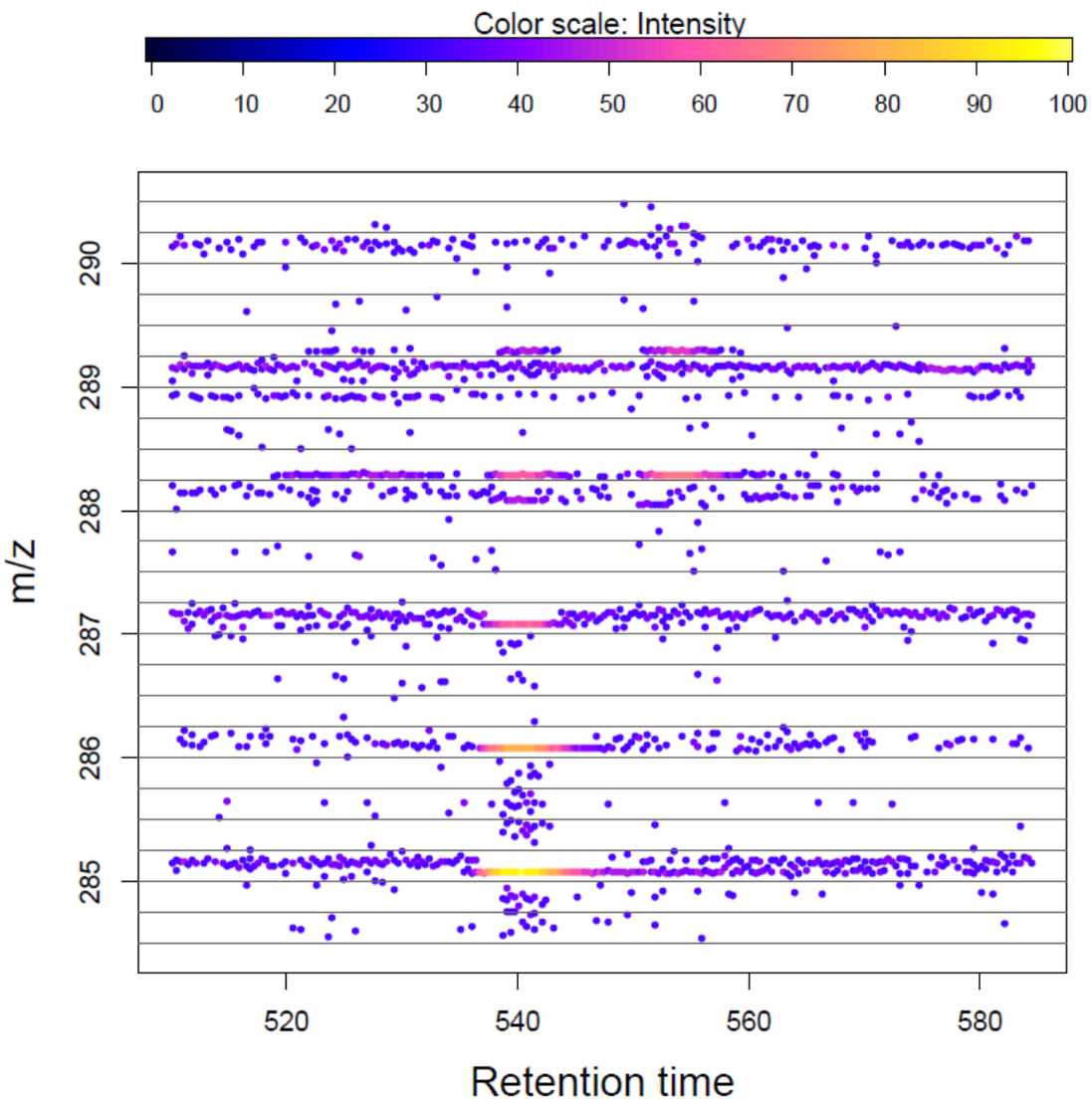
# EIC construction and peak picking

- Achieved by the function *xcmsSet()*

```
xset <- xcmsSet(all_raw_files,
                method="centWave",
                ppm=500,
                peakwidth=c(20, 120),
                snthresh=10,
                prefilter=c(3,400),
                mzCenterFun="wMean",
                integrate=1,
                mzdiff=-0.001,
                fitgauss=F,
                noise=100,
                sleep=0)
```

# Construct EICs

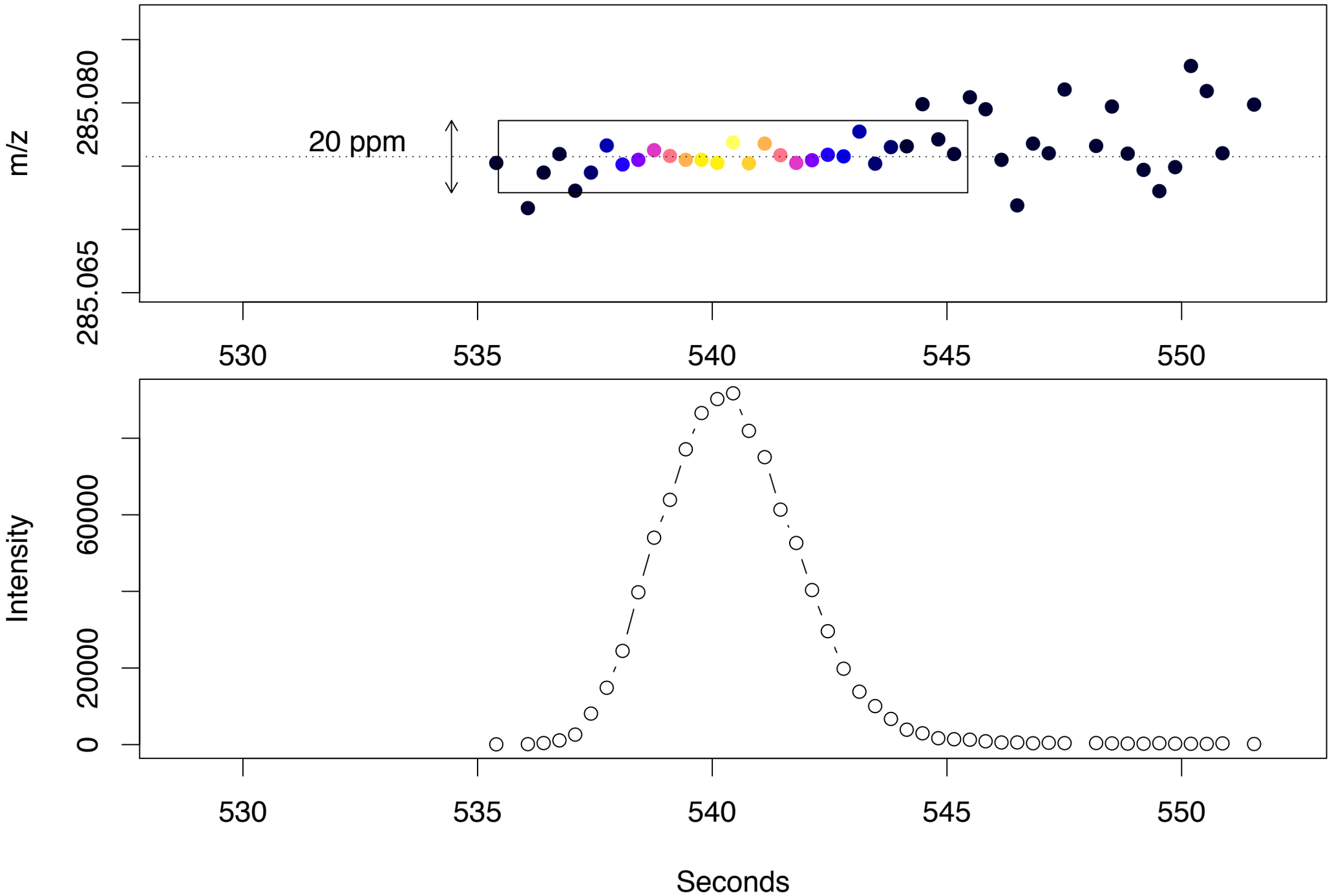





Tautenhahn, R.; Bottcher, C.; Neumann, S., Highly sensitive feature detection for high resolution LC/MS. *BMC bioinformatics* **2008,** *9*, 504.

# Construct EICs

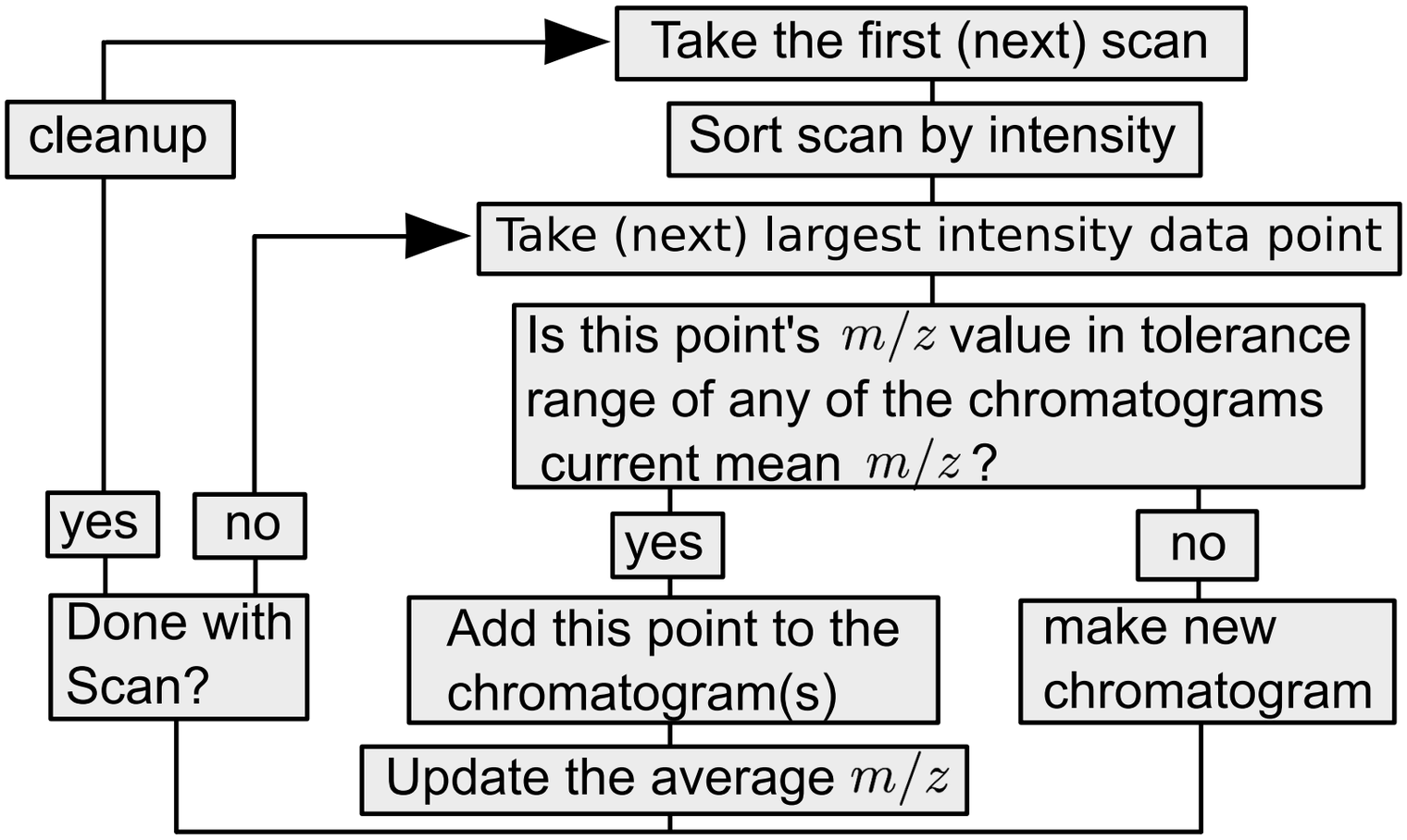





Tautenhahn, R.; Bottcher, C.; Neumann, S., Highly sensitive feature detection for high resolution LC/MS. *BMC bioinformatics* **2008,** *9*, 504.

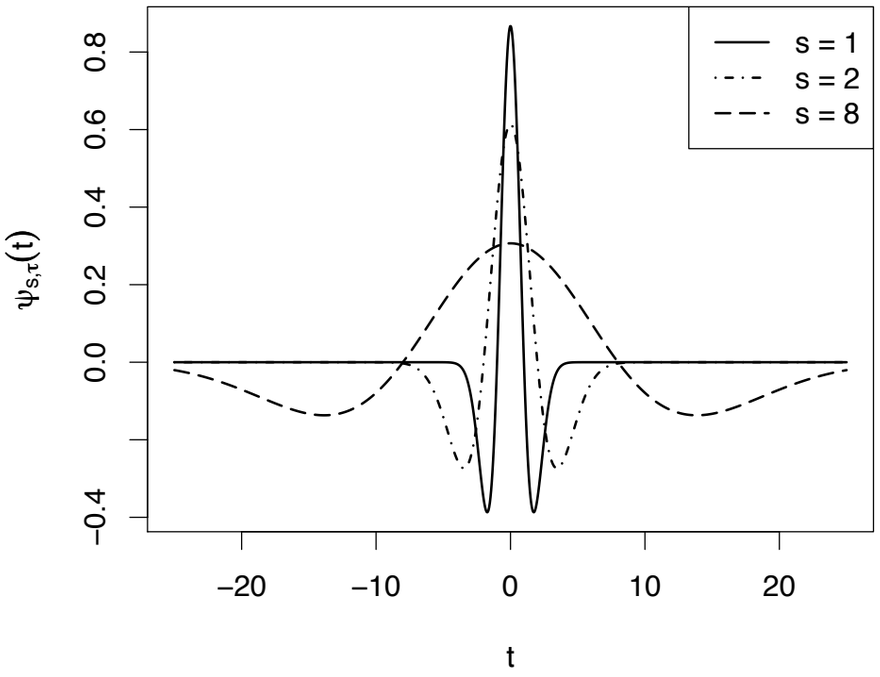
# Construct EICs

- Workflow

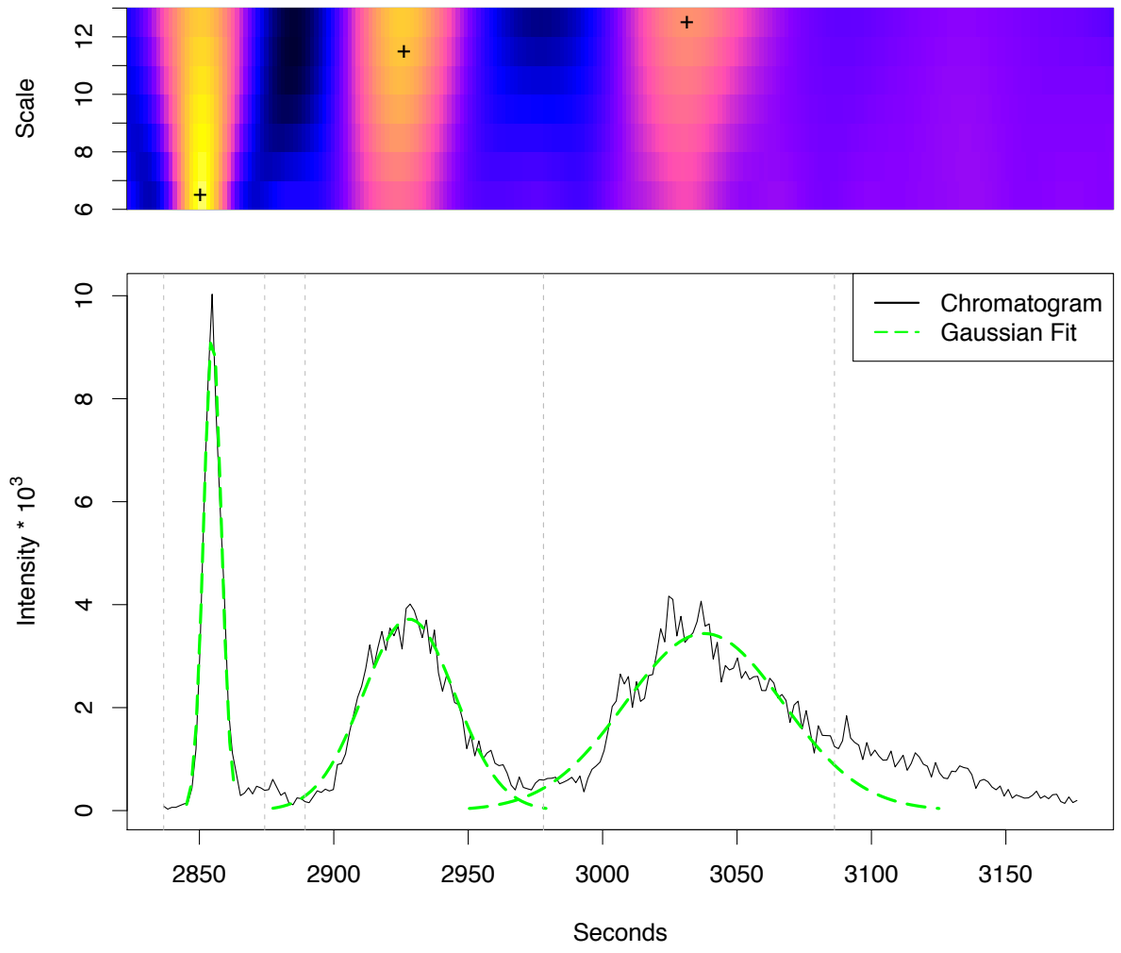


# Detect EIC peaks

- Use wavelet transform



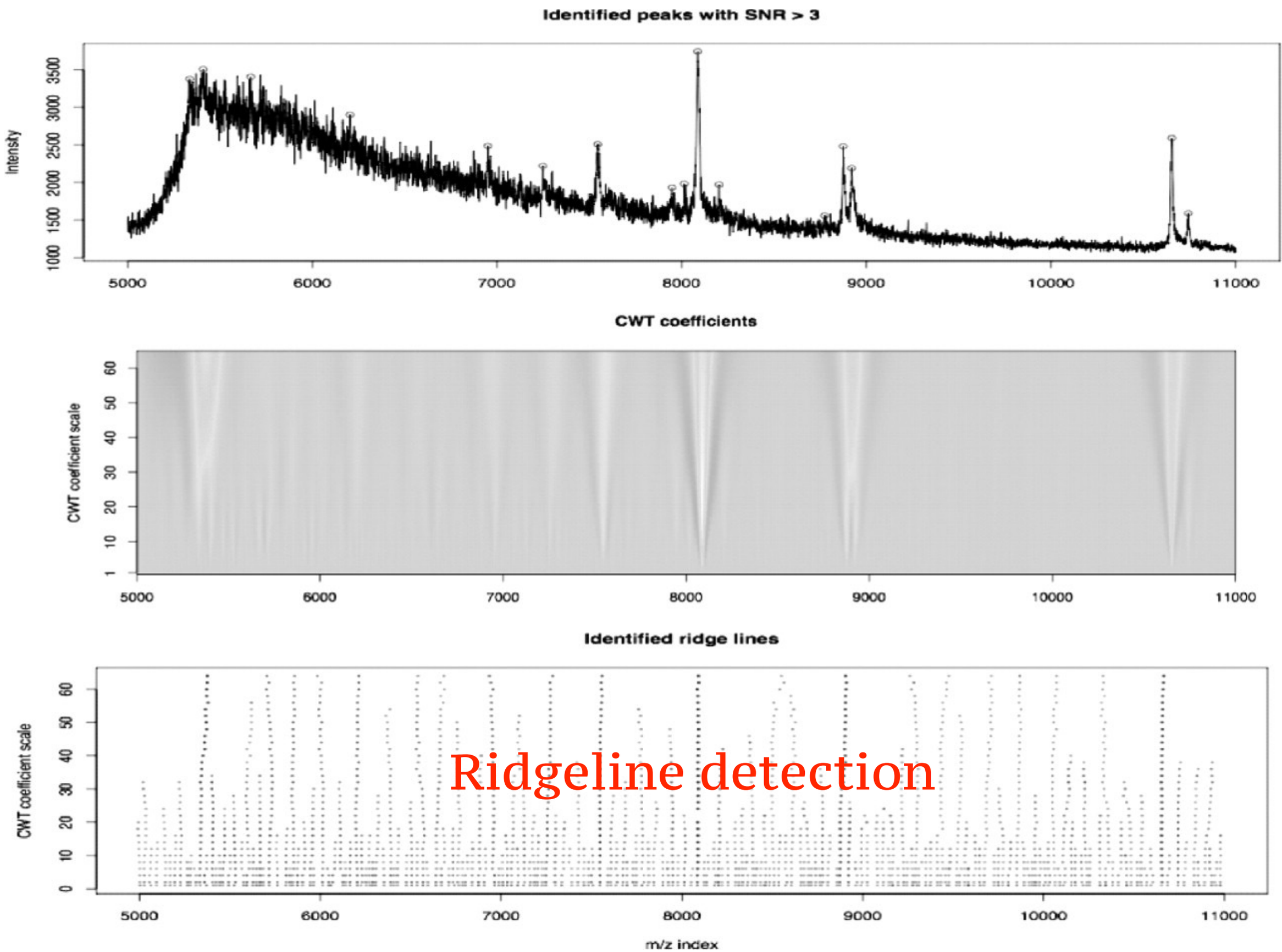

mexican hat wavelet




- Implemented as the *`findPeaks.centWave()`* method



Tautenhahn, R.; Bottcher, C.; Neumann, S., Highly sensitive feature detection for high resolution LC/MS. *BMC bioinformatics* **2008,** *9*, 504.






Du P, Kibbe WA, Lin SM: Improved peak detection in mass spectrum by incorporating continuous wavelet transform-based pattern matching. *Bioinformatics* 2006, 22(17):2059-2065.

# Detect EIC peaks

- Results of peak detection

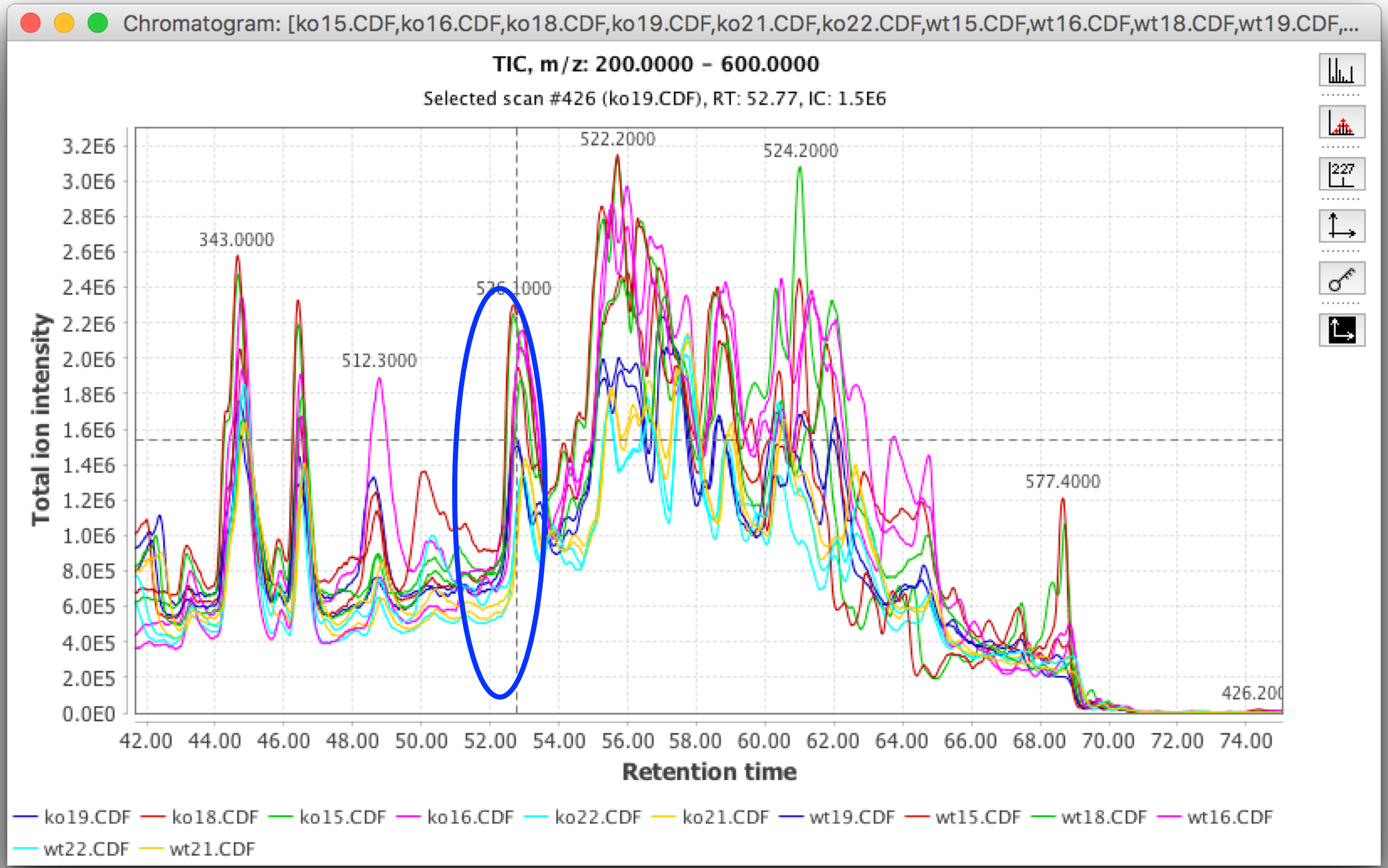
| | mz | mzmin | mzmax | rt | rtmin | rtmax | into | intb | maxo | sn | sample |
|---|---|---|---|---|---|---|---|---|---|---|---|
| [1,] | 412.5000 | 412.5 | 412.5 | 2506.073 | 2501.378 | 2509.203 | 4089.345 | 4083.085 | 693 | 692 | 1 |
| [2,] | 548.4161 | 548.4 | 548.5 | 2506.073 | 2501.378 | 2510.768 | 4482.160 | 4474.335 | 646 | 645 | 1 |
| [3,] | 511.6397 | 511.6 | 511.7 | 2513.898 | 2509.203 | 2518.593 | 3266.155 | 3258.330 | 461 | 460 | 1 |
| [4,] | 214.9000 | 214.9 | 214.9 | 2517.028 | 2512.333 | 2520.158 | 8339.885 | 8333.625 | 1467 | 1466 | 1 |
| [5,] | 564.9000 | 564.9 | 564.9 | 2515.463 | 2510.768 | 2520.158 | 3868.680 | 3860.855 | 587 | 586 | 1 |
| [6,] | 571.5853 | 571.5 | 571.6 | 2518.593 | 2512.333 | 2523.287 | 4065.499 | 3938.182 | 526 | 11 | 1 |

# Peak information

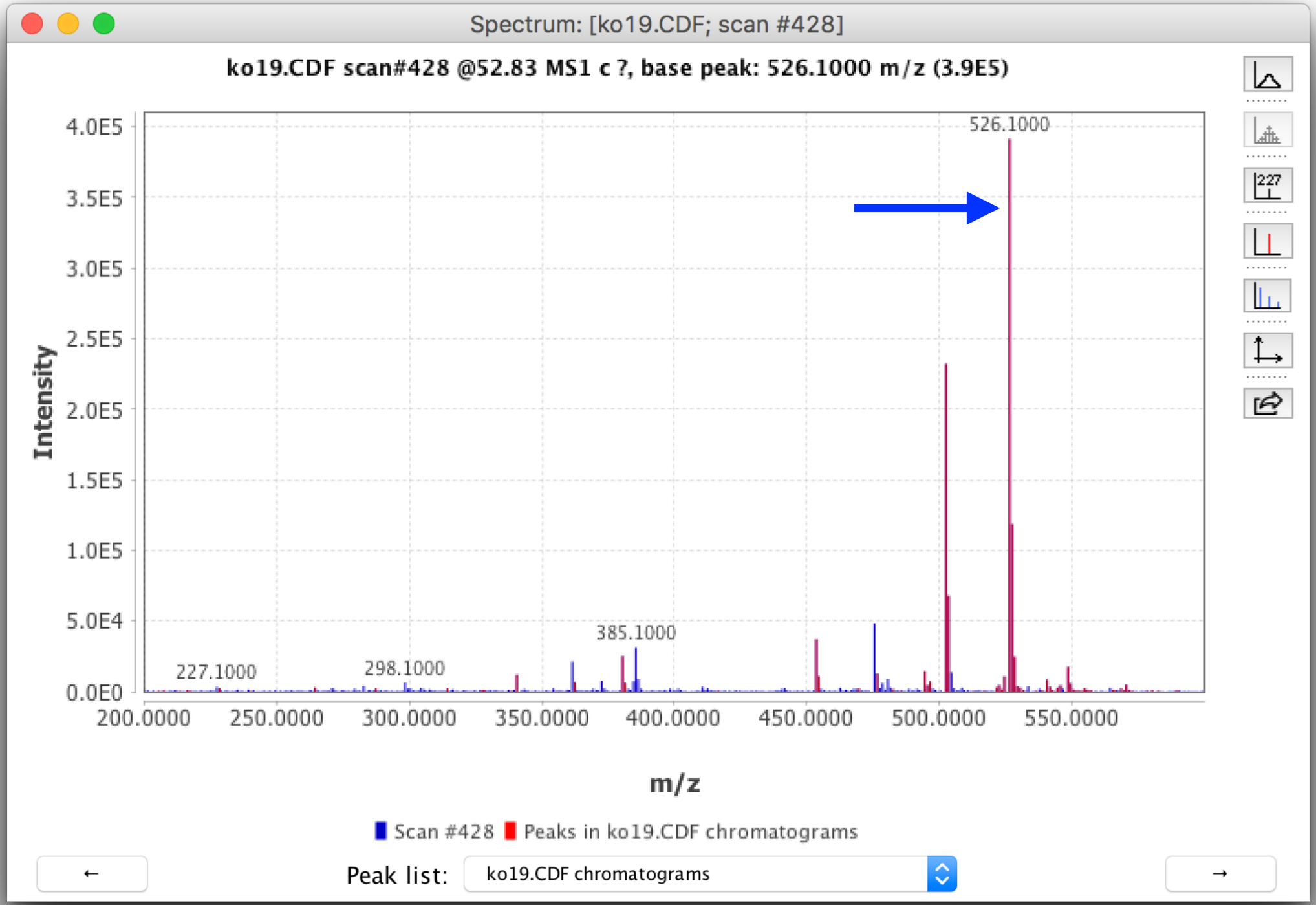
| | |
|---|---|
| `mz` | weighted (by intensity) mean of peak m/z across scans |
| `mzmin` | m/z peak minimum |
| `mzmax` | m/z peak maximum |
| `rt` | retention time of peak midpoint |
| `rtmin` | leading edge of peak retention time |
| `rtmax` | trailing edge of peak retention time |
| `into` | integrated peak intensity |
| `intb` | baseline corrected integrated peak intensity |
| `maxo` | maximum peak intensity |
| `sn` | Signal/Noise ratio, defined as `(maxo - baseline)/sd`, where `maxo` is the maximum peak intensity, `baseline` the estimated baseline value and `sd` the standard deviation of local chromatographic noise. |

# Why alignment?

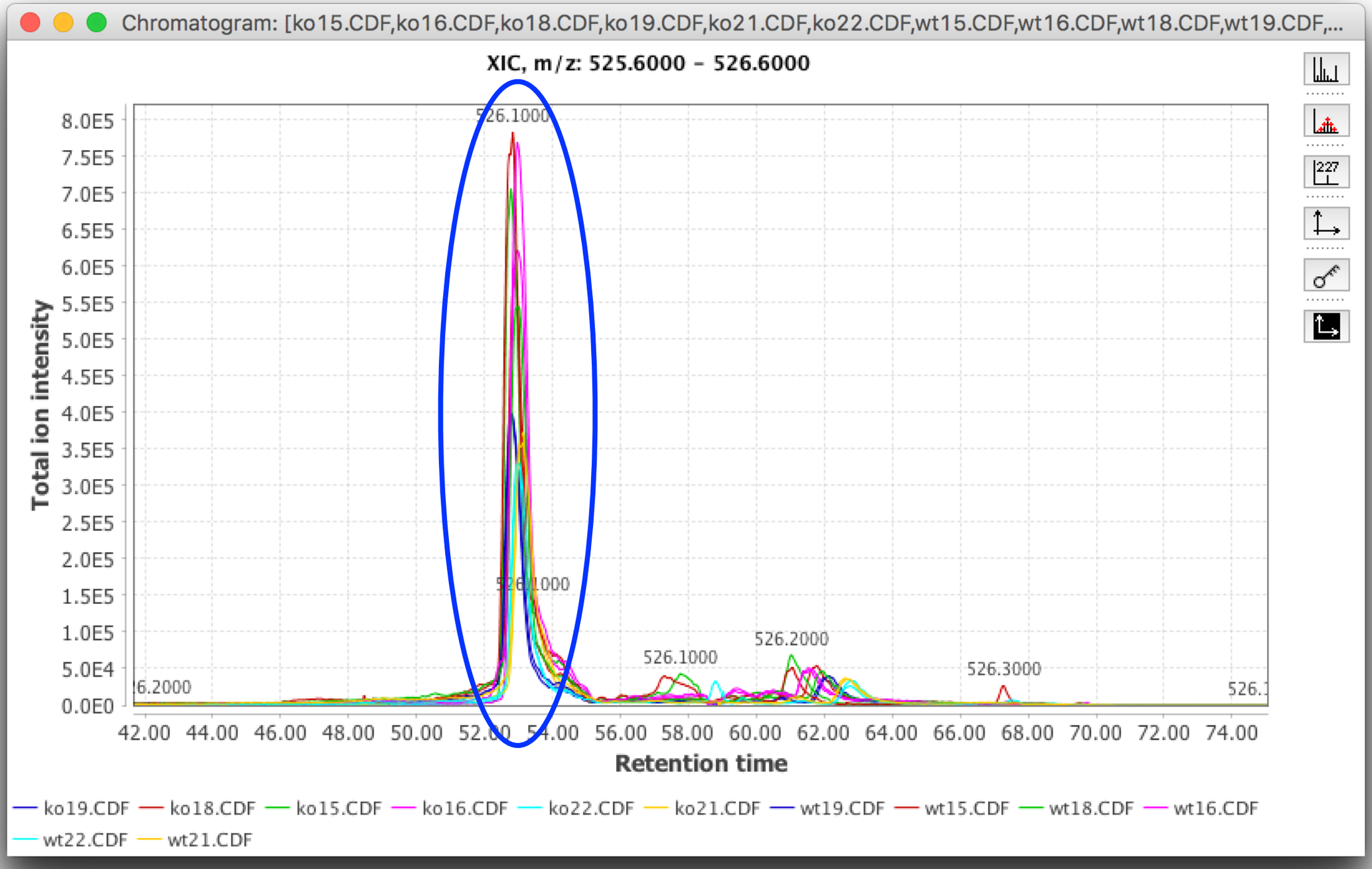
- faahKO



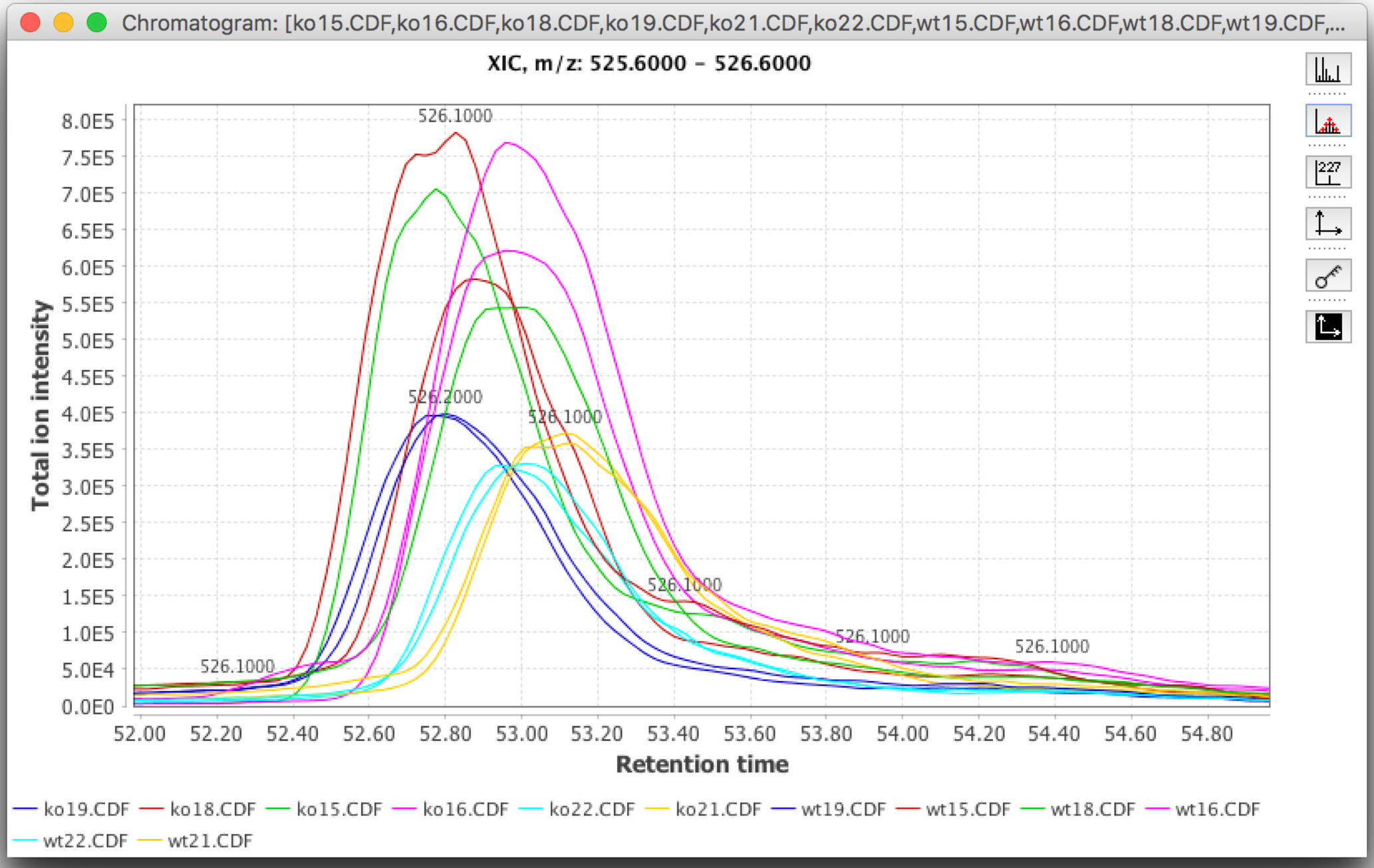
# Why alignment?



# Why alignment?



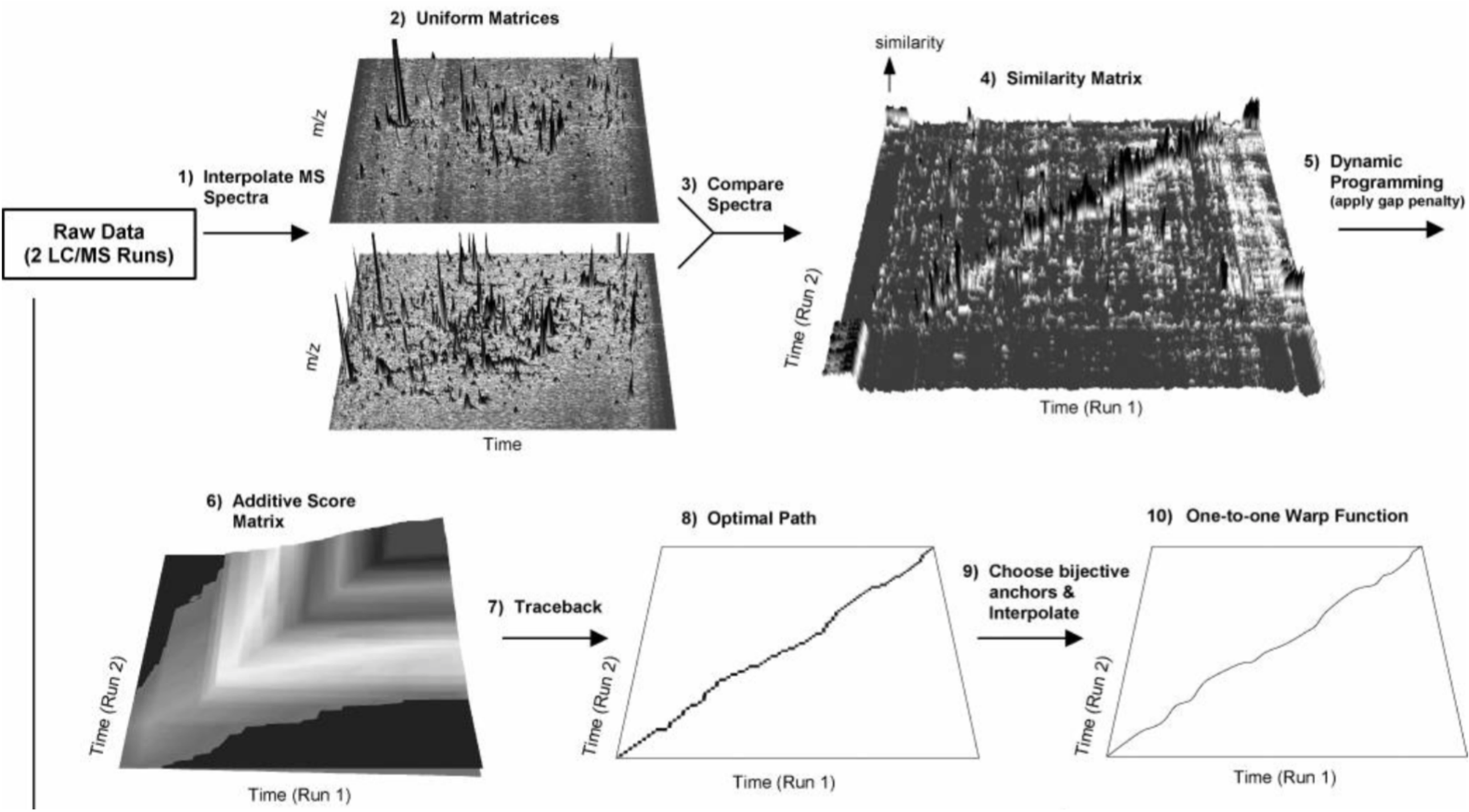
# Why alignment?



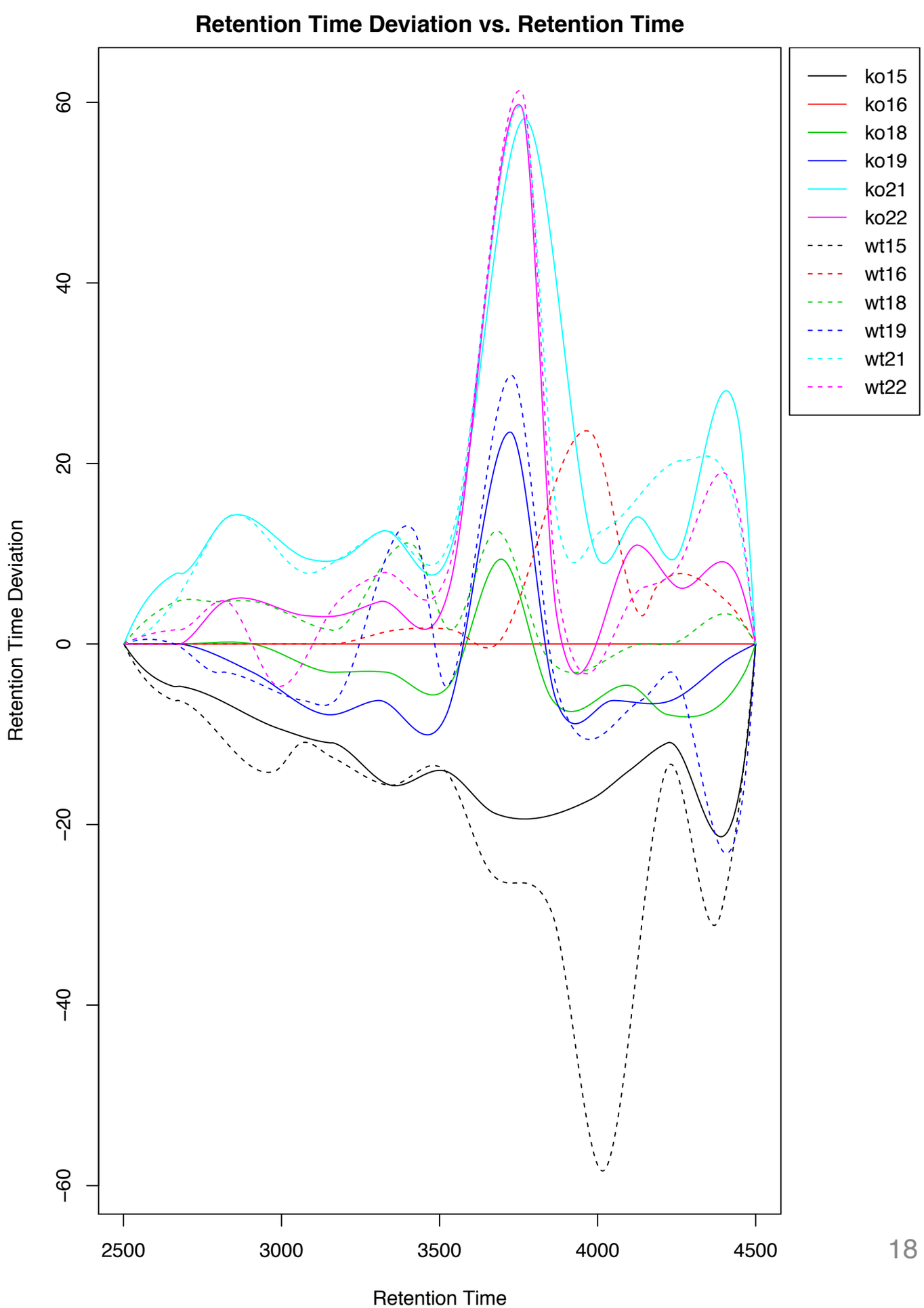
# Alignment

- Achieved by the `retcor()` function
- Principle: Obiwarp algorithm
- Related publication
  - Chromatographic Alignment of ESI-LC-MS Proteomics Data Sets by Ordered Bijective Interpolated Warping, John T. Prince and, Edward M. Marcotte Analytical Chemistry 2006 78 (17), 6140-6152

# Alignment


Raw Data
(2 LC/MS Runs)
1) Interpolate MS Spectra
2) Uniform Matrices
m/z
m/z
Time
3) Compare Spectra
similarity
4) Similarity Matrix
Time (Run 2)
Time (Run 1)
5) Dynamic Programming
(apply gap penalty)
6) Additive Score Matrix
Time (Run 2)
Time (Run 1)
7) Traceback
8) Optimal Path
Time (Run 2)
Time (Run 1)
9) Choose bijective anchors & Interpolate
10) One-to-one Warp Function
Time (Run 2)
Time (Run 1)

# Alignment

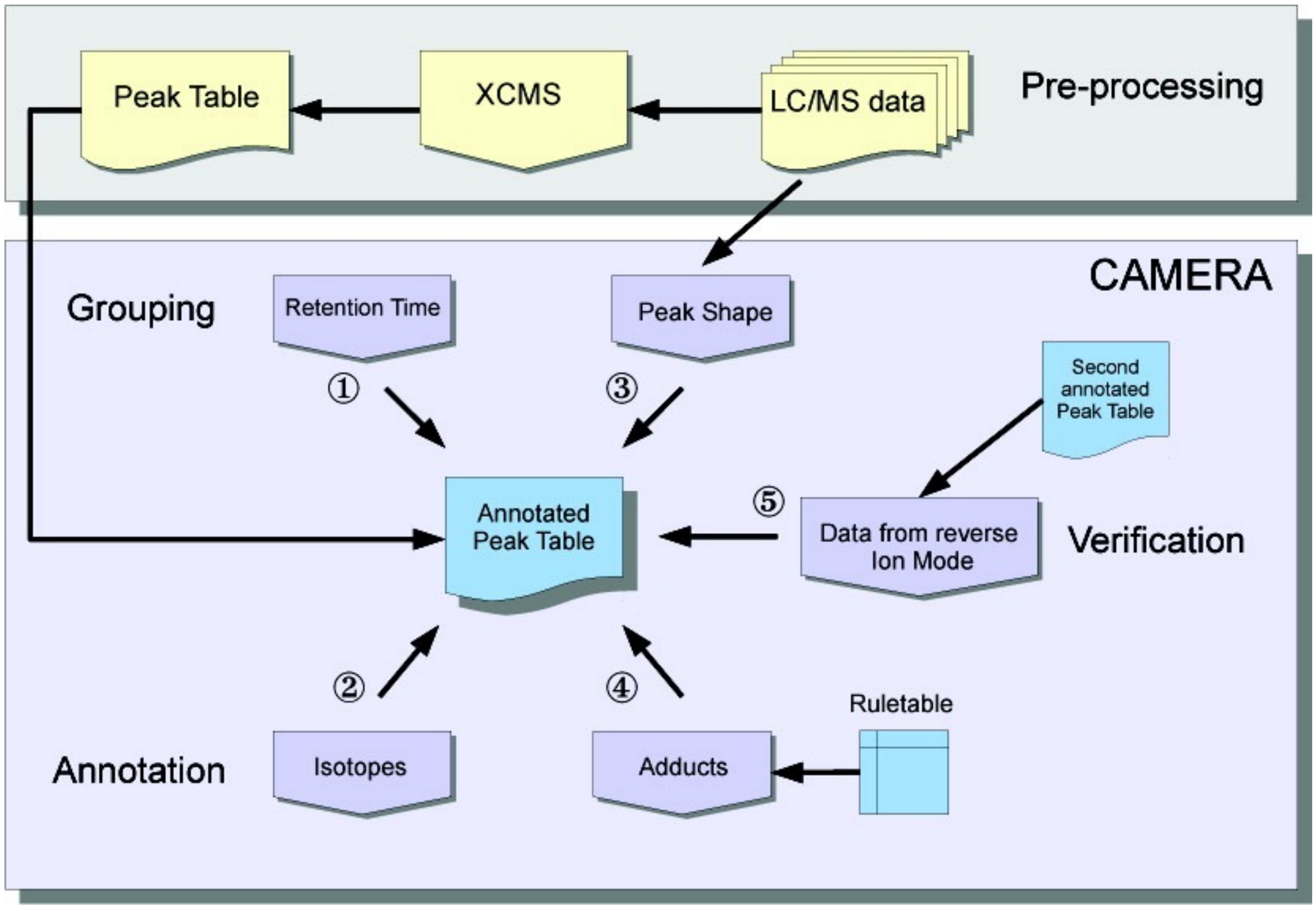

Retention Time Deviation vs. Retention Time
Retention Time Deviation
60
40
20
0
−20
−40
−60
2500
3000
3500
4000
4500
Retention Time
ko15
ko16
ko18
ko19
ko21
ko22
wt15
wt16
wt18
wt19
wt21
wt22

# Peak annotation

- Achieved by the *CAMERA* package

- Related publication
  - Kuhl C, Tautenhahn R, Boettcher C, Larson TR and Neumann S (2012). "CAMERA: an integrated strategy for compound spectra extraction and annotation of liquid chromatography/mass spectrometry data sets." Analytical Chemistry, 84, pp. 283–289. http://pubs.acs.org/doi/abs/10.1021/ac202450g.

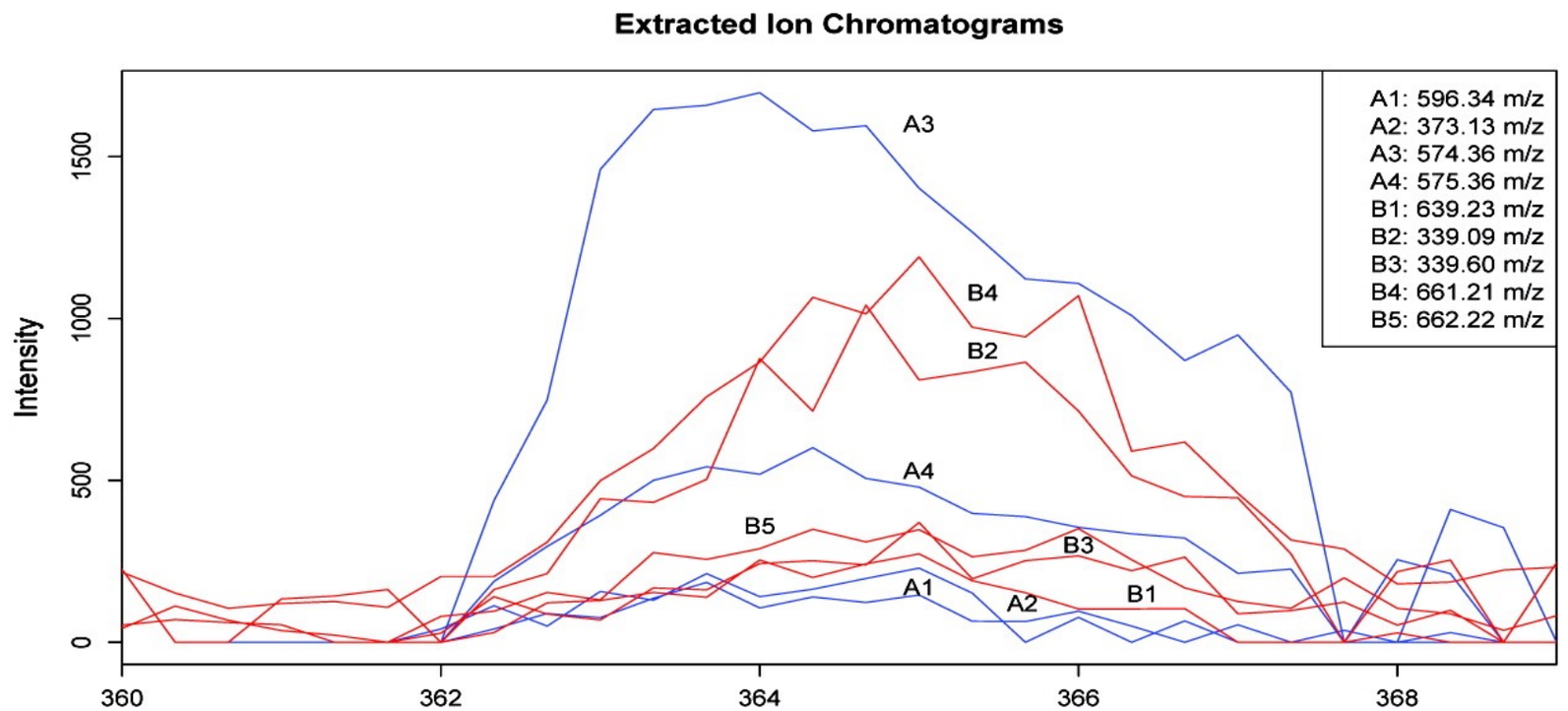
# Peak annotation



# Peak annotation

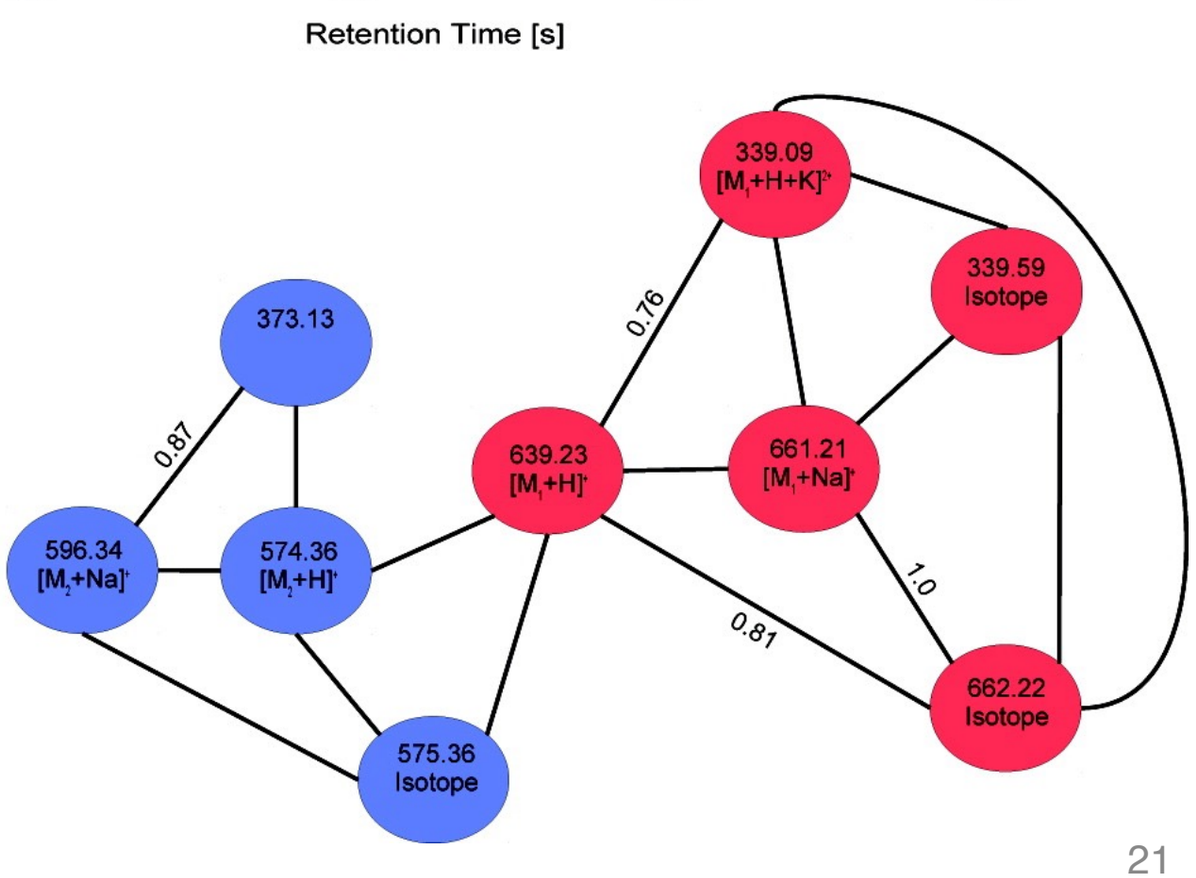
| id | m/z | rt [s] |
|---|---|---|
| 1 | 339.09 | 369.1 |
| 2 | 339.59 | 369.1 |
| 3 | 373.13 | 368.6 |
| 4 | 574.36 | 368.7 |
| 5 | 575.36 | 368.7 |
| 6 | 596.34 | 368.4 |
| 7 | 639.23 | 368.7 |
| 8 | 661.21 | 369.1 |
| 9 | 662.22 | 369.1 |

Initial compound spectrum




| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| 2 | 1.0 | | | | | | | |
| 3 | 0.34 | 0.36 | | | | | | |
| 4 | 0.72 | 0.67 | 0.90 | | | | | |
| 5 | 0.68 | 0.47 | 0.68 | 1 | | | | |
| 6 | 0.38 | 0.38 | 0.87 | 0.86 | 0.75 | | | |
| 7 | 0.76 | 0.72 | 0.73 | 0.82 | 0.88 | 0.70 | | |
| 8 | 0.94 | 0.79 | 0.35 | 0.70 | 0.67 | 0.51 | 0.79 | |
| 9 | 0.88 | 0.84 | 0.46 | 0.62 | 0.70 | 0.62 | 0.81 | 1.0 |

Relationship scoring table



# Peak annotation

| id | mz | rt | isotopes | adduct | pc |
|---|---|---|---|---|---|
| 65 | 176.04 | 280.09 | | | 4 |
| 76 | 136.05 | 280.43 | [14][M+1]1+ | | 5 |
| 77 | 135.05 | 280.43 | [14][M]1+ | | 5 |
| 74 | 153.06 | 280.43 | | [M+H]+ 152.05437 | 5 |
| 75 | 175.04 | 280.43 | | [M+Na]+ 152.05437 | 5 |
| 73 | 197.02 | 280.76 | | [M+2Na-H]+ 152.05437 | 5 |
| 78 | 377.74 | 286.15 | | | 6 |
| 79 | 732.5 | 286.49 | | | 6 |
| 83 | 488.32 | 286.82 | | [M+Na]+ 465.33205 | 7 |
| 82 | 466.34 | 286.82 | | [M+H]+ 465.33205 | 7 |
| ... | | | | | |

# R functions

- Peak grouping based on retention time
  - *`groupFWHM()`*
- Peak group refinement based on peak shape
  - *`groupCorr()`*
- Discovery of isotopes
  - *`findIsotopes()`*
- Annotation of adducts and determination of molecular mass
  - *`findAdducts()`*

# Annotation rules

- Primary adduct rules

| name | nmol | charge | massdiff | oidscore | quasi | ips |
|---|---|---|---|---|---|---|
| [M+H]+ | 1 | 1 | 1.007276 | 1 | 1 | 1 |
| [M+Na]+ | 1 | 1 | 22.989218 | 8 | 1 | 1 |
| [M+K]+ | 1 | 1 | 38.963158 | 10 | 1 | 1 |
| [M+NH4]+ | 1 | 1 | 18.033823 | 16 | 1 | 1 |

# Annotation rules

Extended adduct rules

| name | nmol | charge | massdiff | oidscore | quasi | ips |
|---|---|---|---|---|---|---|
| [M+H]+ | 1 | 1 | 1.007276 | 1 | 1 | 1 |
| [M+2H]2+ | 1 | 2 | 2.014552 | 2 | 0 | 0.75 |
| [M+3H]3+ | 1 | 3 | 3.021828 | 3 | 0 | 0.75 |
| [M+H+Na]2+ | 1 | 2 | 23.996494 | 4 | 0 | 0.5 |
| [M+H+K]2+ | 1 | 2 | 39.970434 | 6 | 0 | 0.5 |
| [M+H+NH4]2+ | 1 | 2 | 19.041099 | 7 | 0 | 0.5 |
| [M+Na]+ | 1 | 1 | 22.989218 | 8 | 1 | 1 |
| [M+2Na]2+ | 1 | 2 | 45.978436 | 9 | 0 | 0.5 |
| [M+K]+ | 1 | 1 | 38.963158 | 10 | 1 | 1 |
| [M+Na+K]2+ | 1 | 2 | 61.952376 | 11 | 0 | 0.5 |
| [M+2K]2+ | 1 | 2 | 77.926316 | 13 | 0 | 0.5 |
| [M+NH4]+ | 1 | 1 | 18.033823 | 16 | 1 | 1 |
| [M+2Na-H]+ | 1 | 1 | 44.97116 | 34 | 0 | 0.5 |
| [M+2K-H]+ | 1 | 1 | 76.91904 | 60 | 0 | 0.5 |
| [2M+H]+ | 2 | 1 | 1.007276 | 1 | 0 | 0.5 |
| [2M+2H]2+ | 2 | 2 | 2.014552 | 2 | 0 | 0.5 |
| [2M+3H]3+ | 2 | 3 | 3.021828 | 3 | 0 | 0.5 |
| [2M+H+Na]2+ | 2 | 2 | 23.996494 | 4 | 0 | 0.5 |
| [2M+H+K]2+ | 2 | 2 | 39.970434 | 6 | 0 | 0.5 |
| [2M+H+NH4]2+ | 2 | 2 | 19.041099 | 7 | 0 | 0.5 |
| [2M+Na]+ | 2 | 1 | 22.989218 | 8 | 0 | 0.5 |
| [2M+2Na]2+ | 2 | 2 | 45.978436 | 9 | 0 | 0.5 |
| [2M+K]+ | 2 | 1 | 38.963158 | 10 | 0 | 0.5 |
| [2M+Na+K]2+ | 2 | 2 | 61.952376 | 11 | 0 | 0.5 |
| [2M+2K]2+ | 2 | 2 | 77.926316 | 13 | 0 | 0.5 |
| [2M+NH4]+ | 2 | 1 | 18.033823 | 16 | 0 | 0.5 |
| [2M+2Na-H]+ | 2 | 1 | 44.97116 | 34 | 0 | 0.25 |

# Thank you!