Metabolomics

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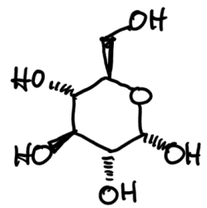
- Preprocessing of LC-MS data in Bioconductor
- Normalization
- Annotation/identification

Metabolite? Metabolism?

- Glycolysis

glucose 

Metabolite? Metabolism?

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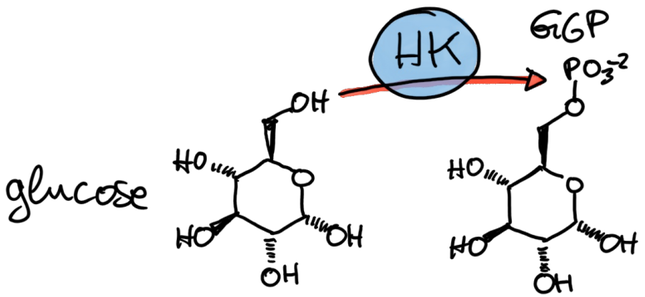

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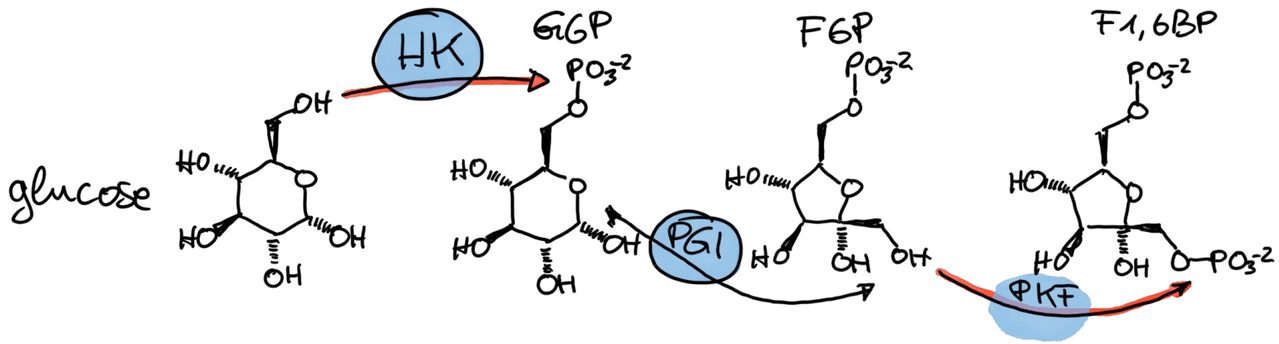

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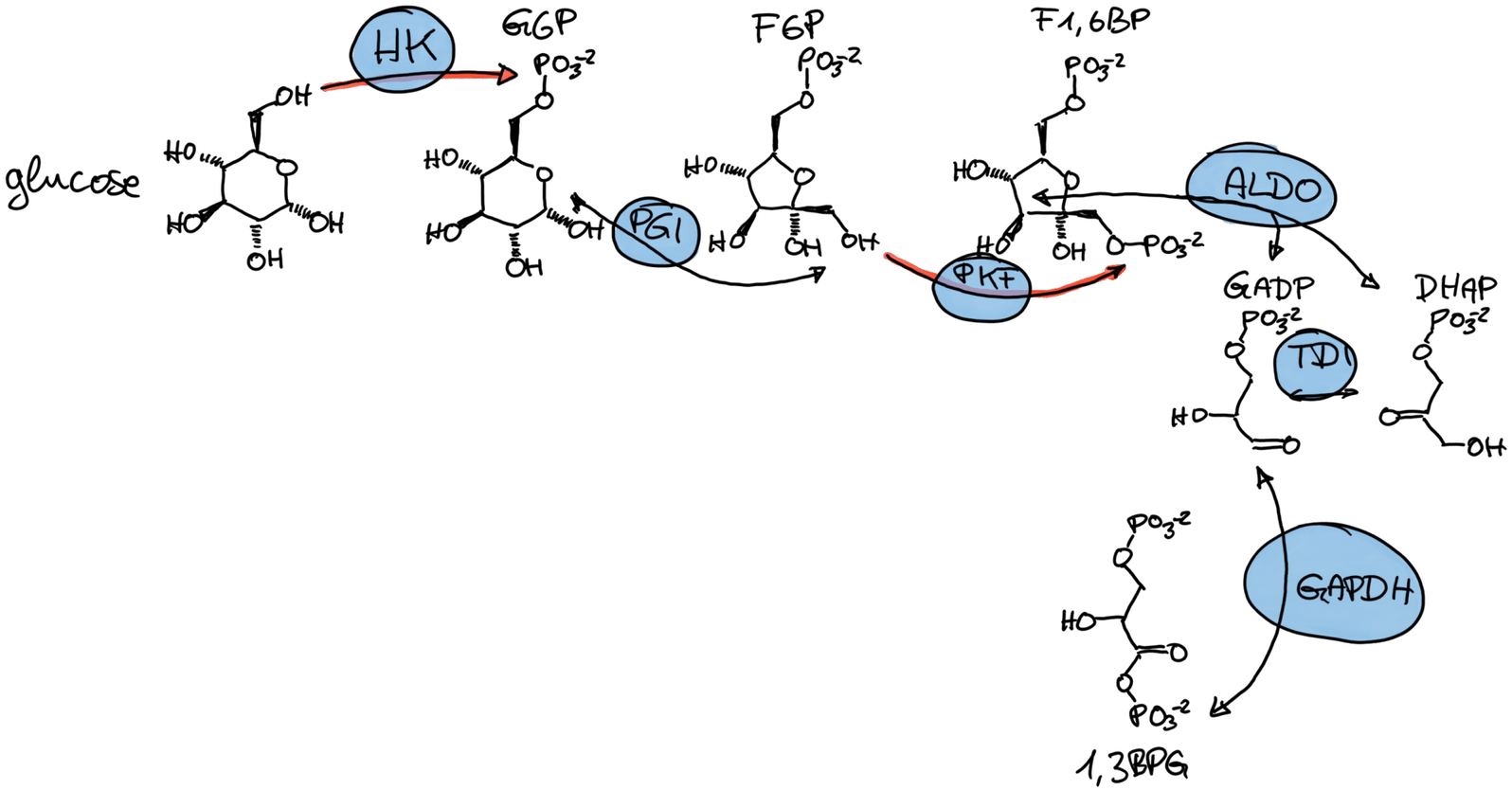

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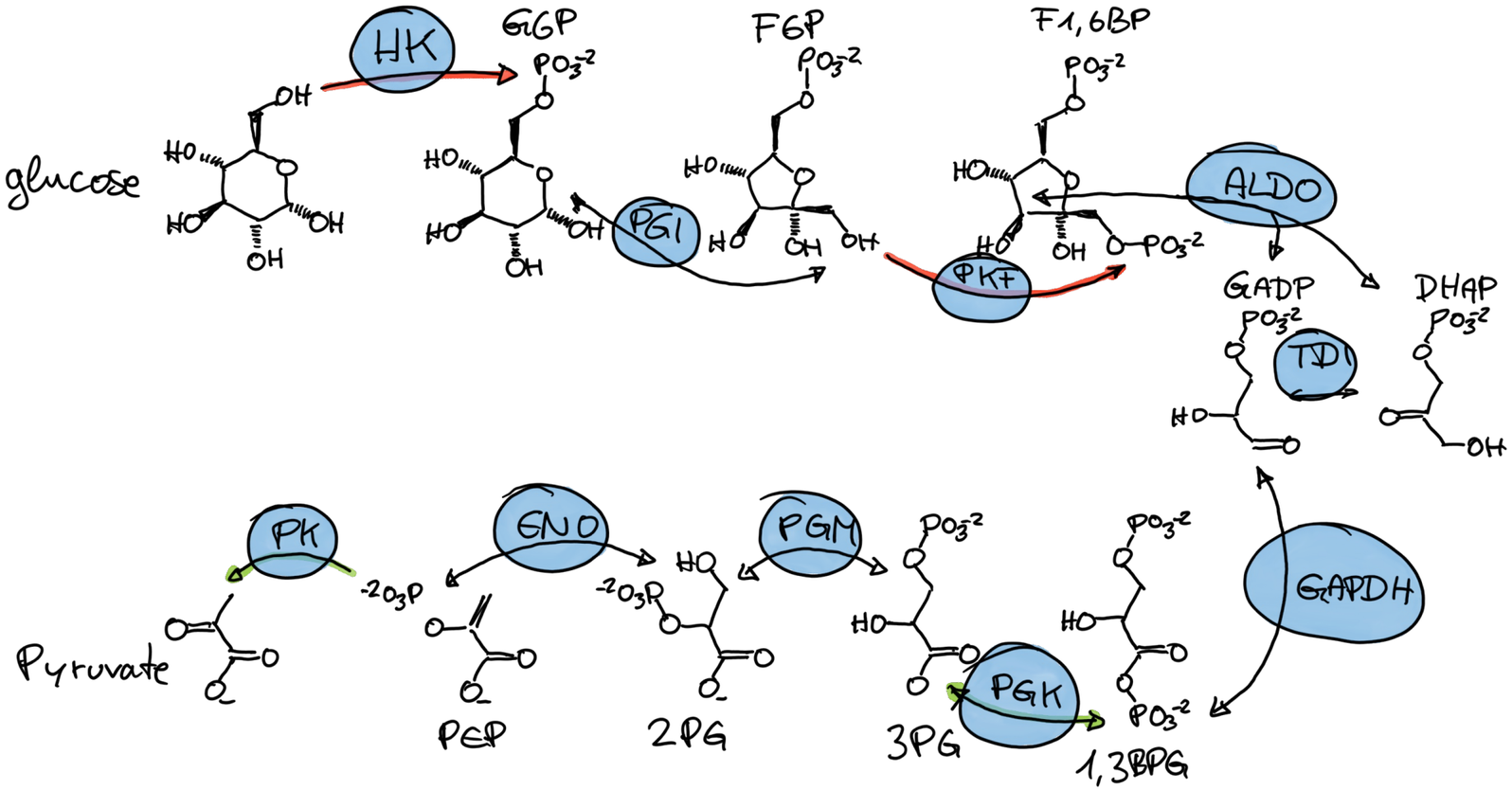

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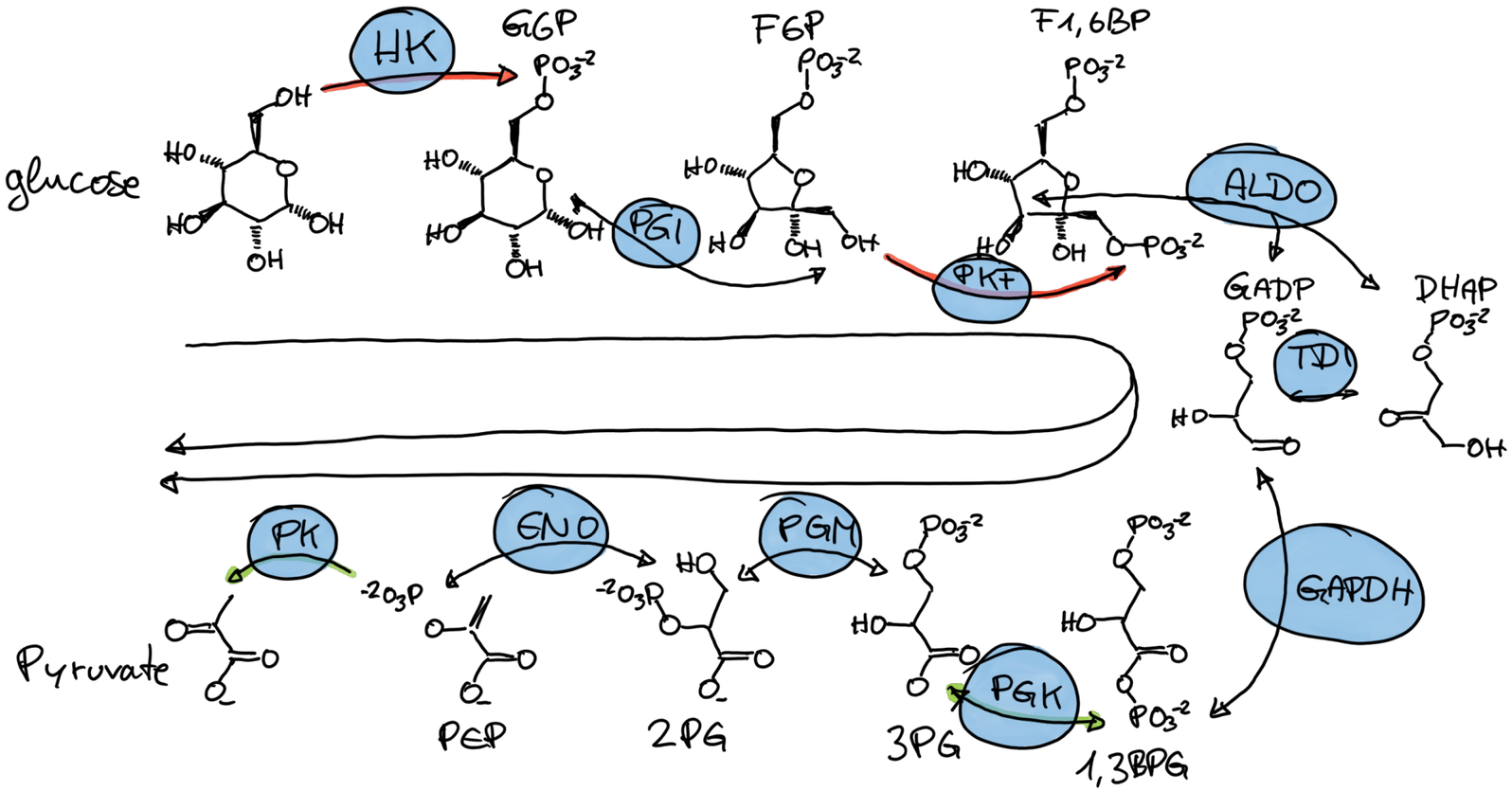

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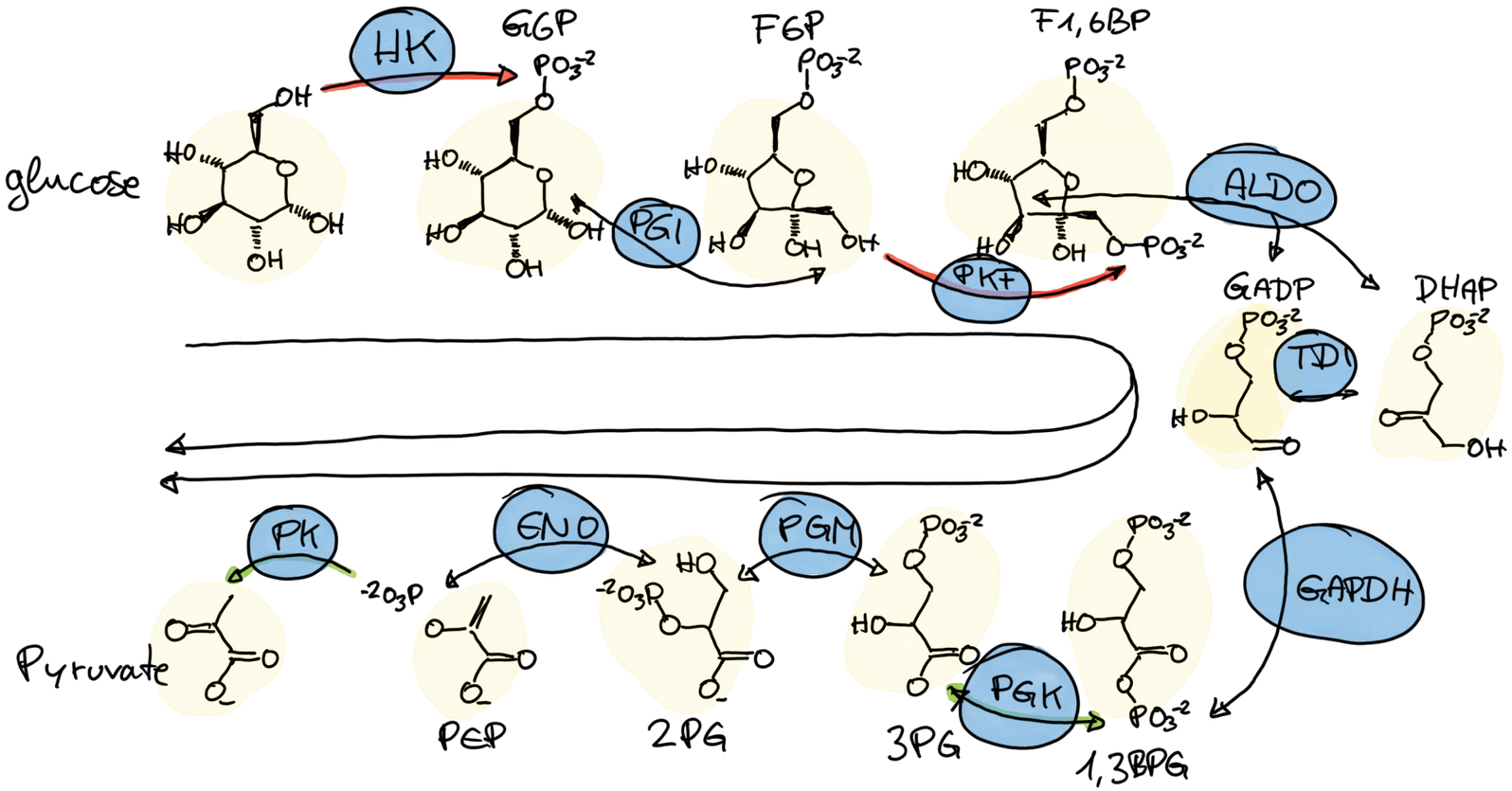


- Metabolites: intermediates and products of cellular processes.

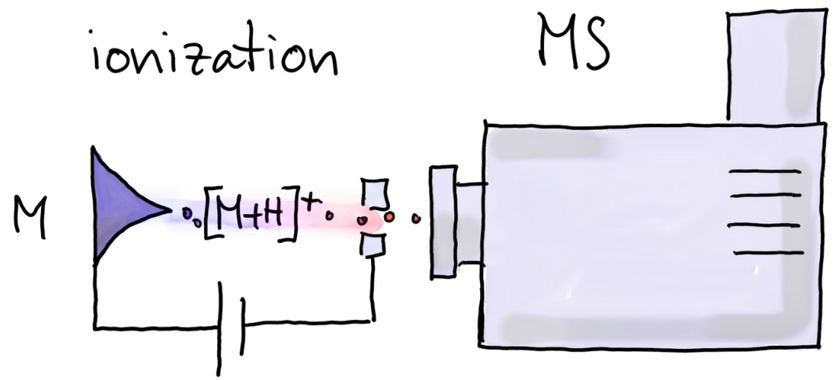
Metabolomics?

- Large-scale study of small molecules (metabolites) in a system (cell, tissue, organism).
- Comparison of the different -omes:
- **Genome**: what can happen.
- **Transcriptome**: what appears to be happening.
- **Proteome**: what makes it happen.
- **Metabolome**: what actually happened.
- Metabolome influenced by genetic **and** environmental factors.

How can we measure metabolites?

- Nuclear Magnetic Resonance (NMR) - not covered here.
- Mass spectrometry (MS)-based metabolomics.
- Targeted/untargeted metabolomics:
- **targeted**: quantitative measurement of selected metabolites.
- **untargeted**: semi-quantitative measurement of all metabolites (detectable with the setup) in a sample.

Mass Spectrometry (MS)



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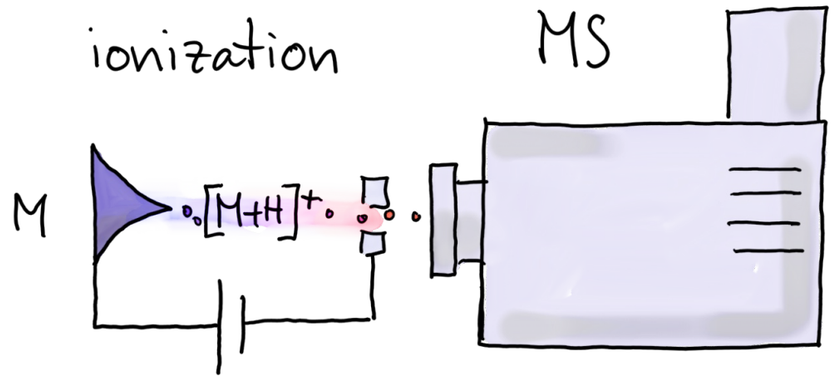
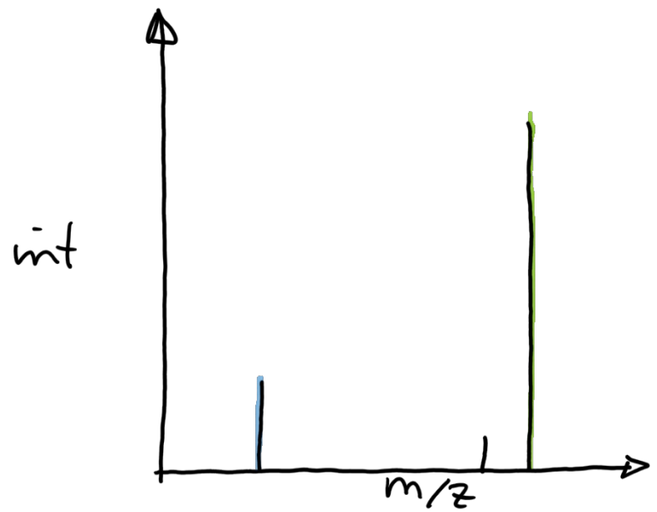
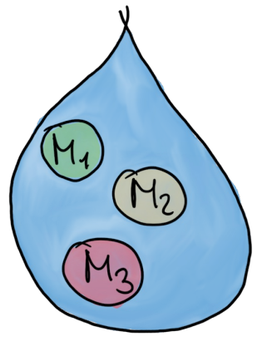




- **Problem:** unable to distinguish between metabolites with the same mass-to-charge ratio (m/z).
- **Solution:** separate metabolites prior to MS by another property.

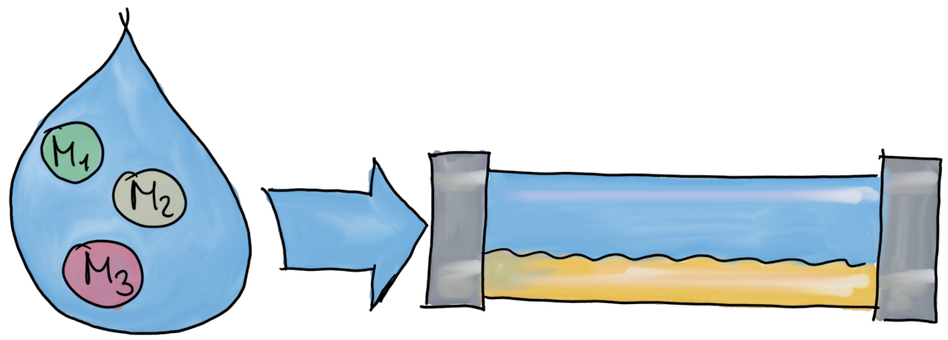
Liquid chromatography

- Sample is dissolved in a fluid (mobile phase).



Liquid chromatography

- Sample is dissolved in a fluid (mobile phase).
- Mobile phase carries analytes through a column with a stationary phase.



Liquid chromatography

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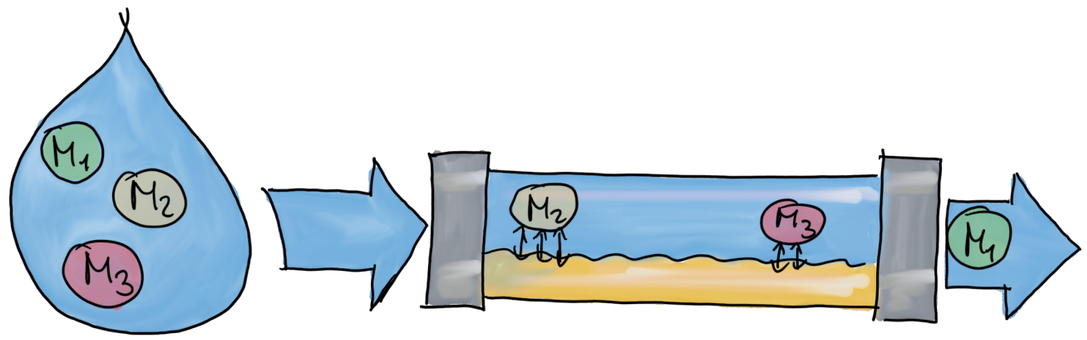
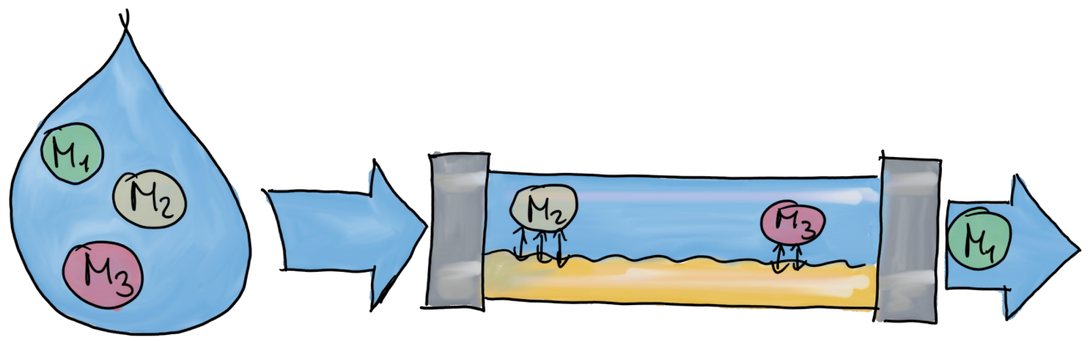
- Separation based on affinity for the column’s stationary phase.

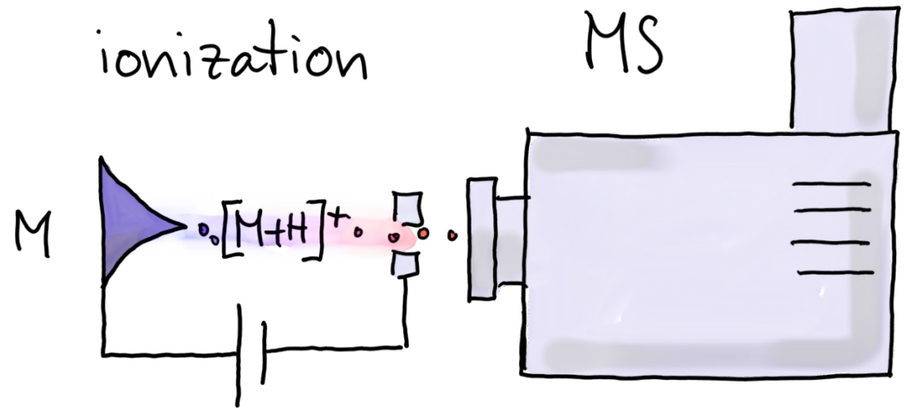


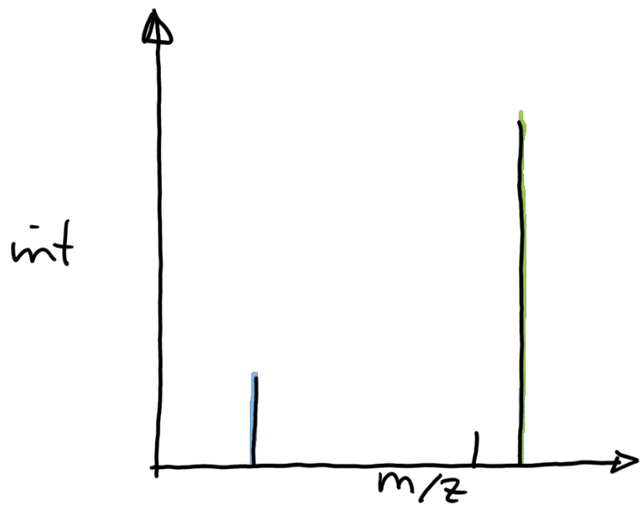
Liquid chromatography

- Sample is dissolved in a fluid (mobile phase).
- Mobile phase carries analytes through a column with a stationary phase.
- Separation based on affinity for the column's stationary phase.
- HILIC (hyrophilic liquid interaction chromatography):
  - Hydrophilic, polar stationary phase.
  - Analytes solved in mobile phase.
  - Analytes separated by polarity: low polarity elute first, high polarity later.



Liquid Chromatography Mass Spectrometry (LC-MS)





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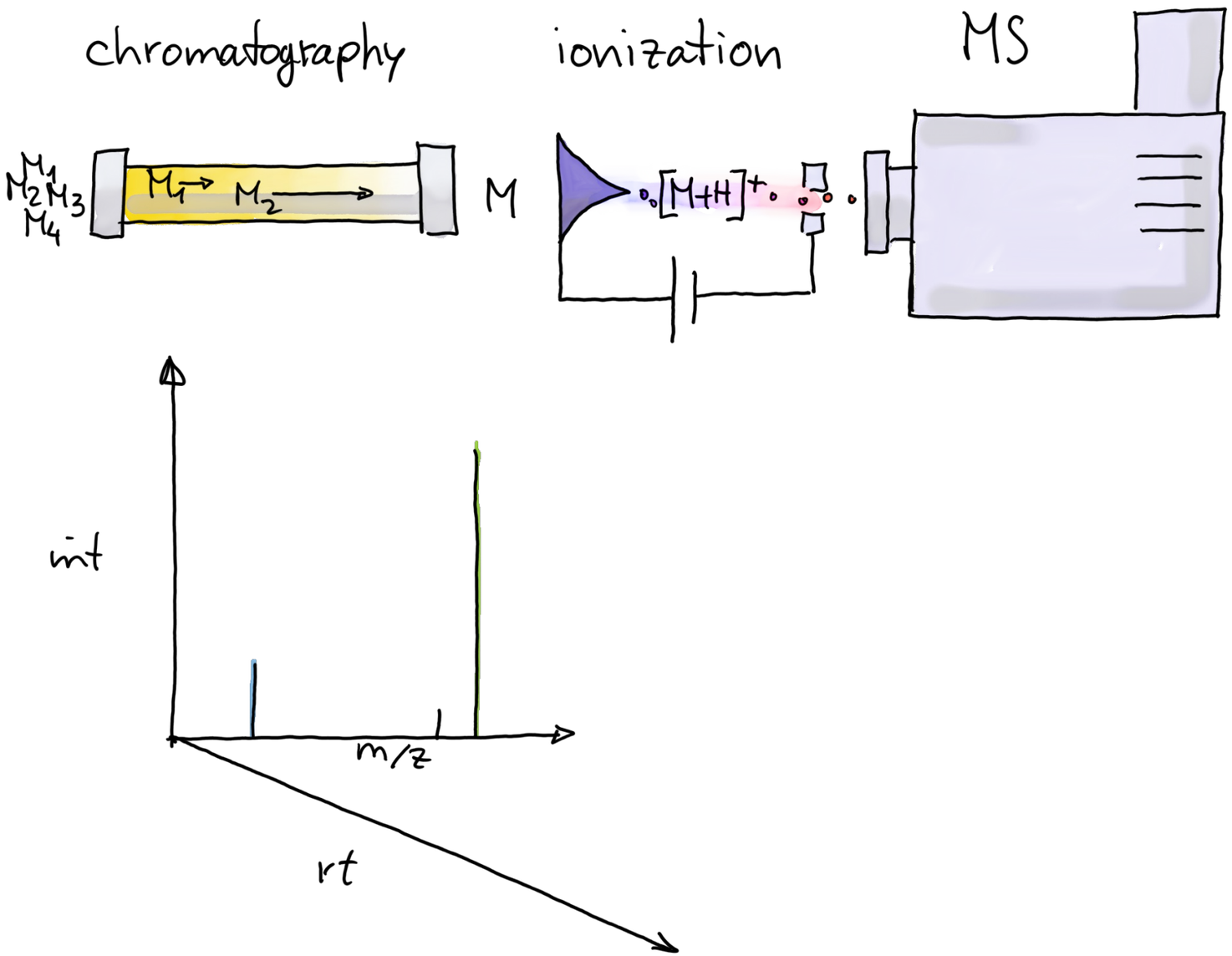

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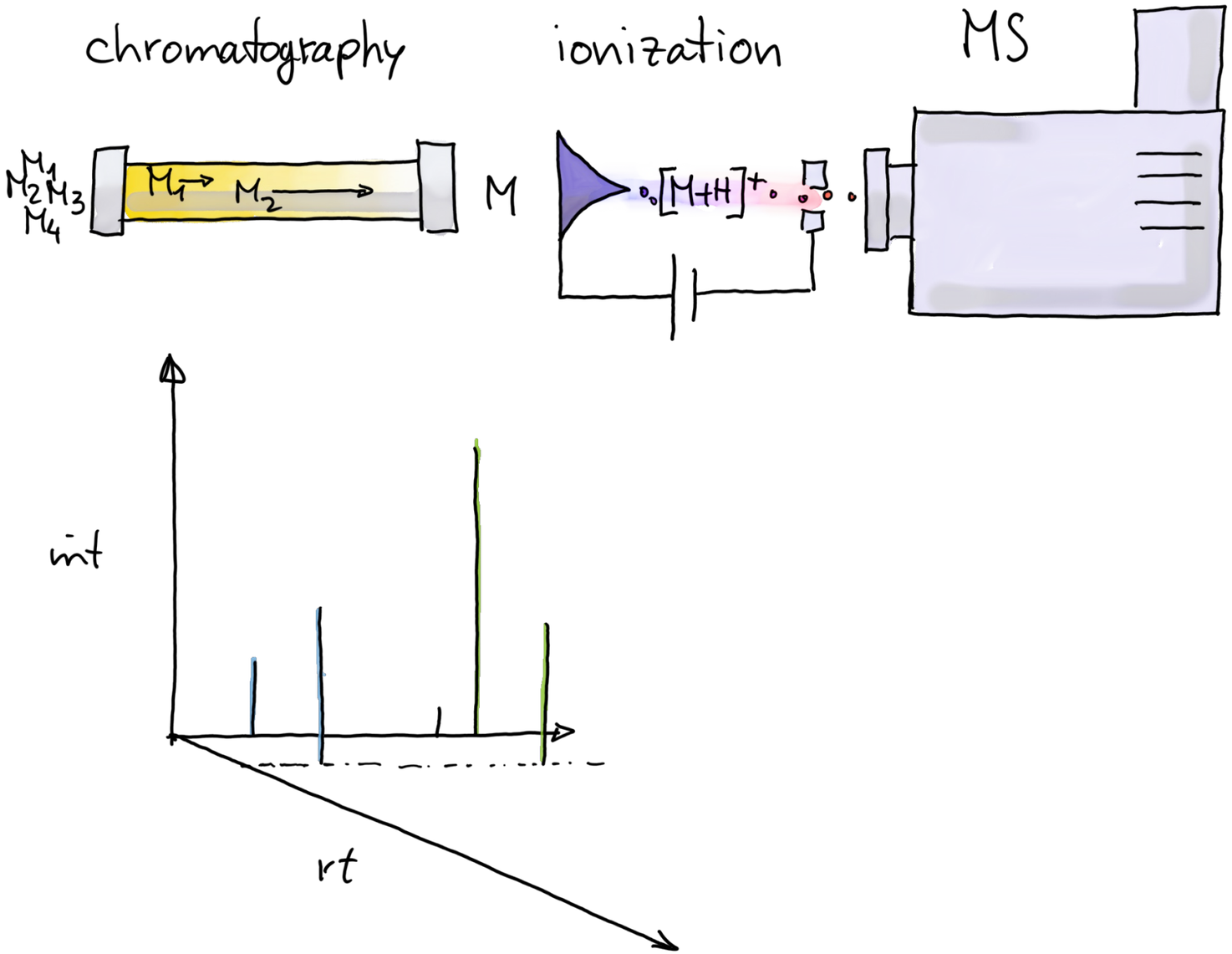

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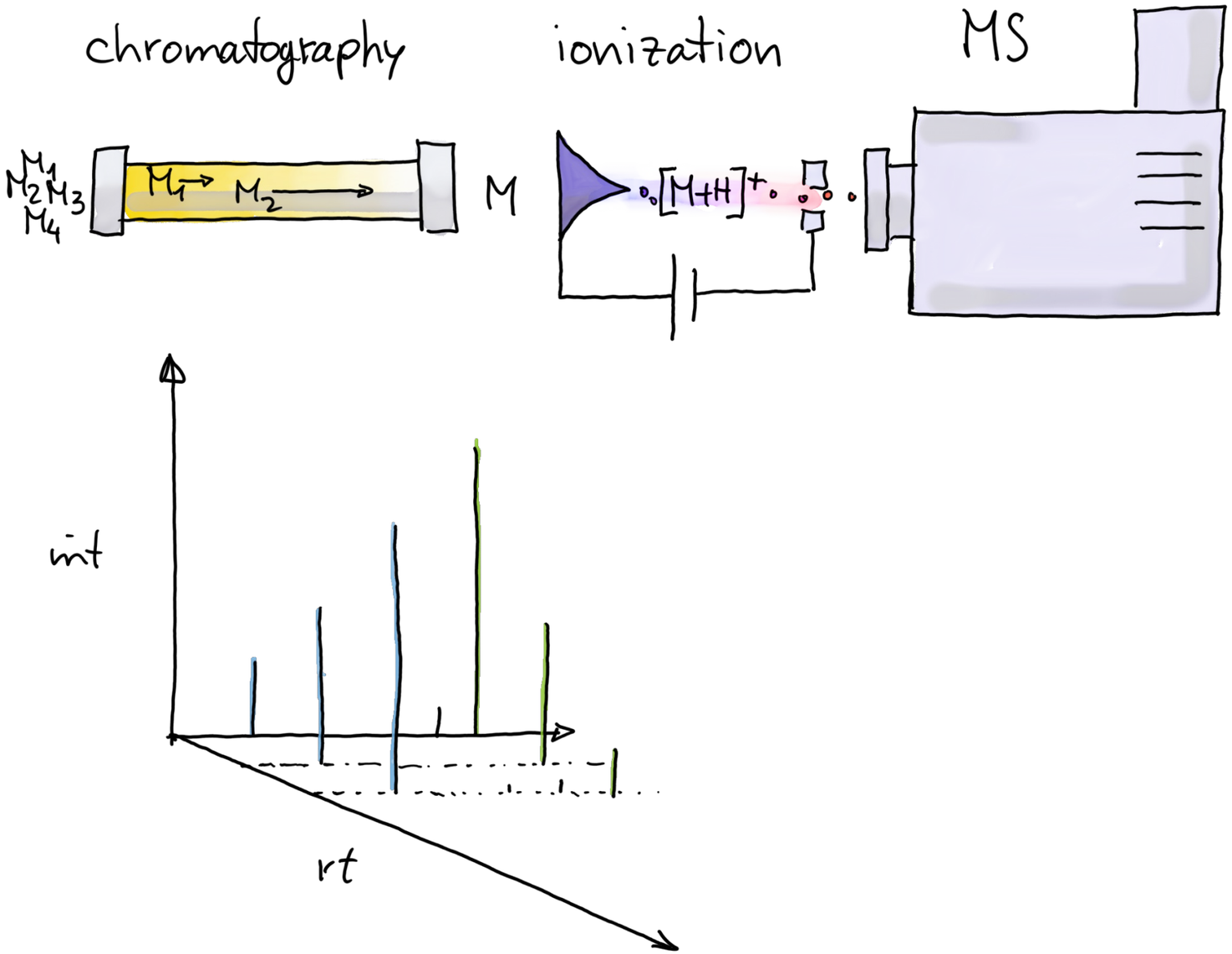

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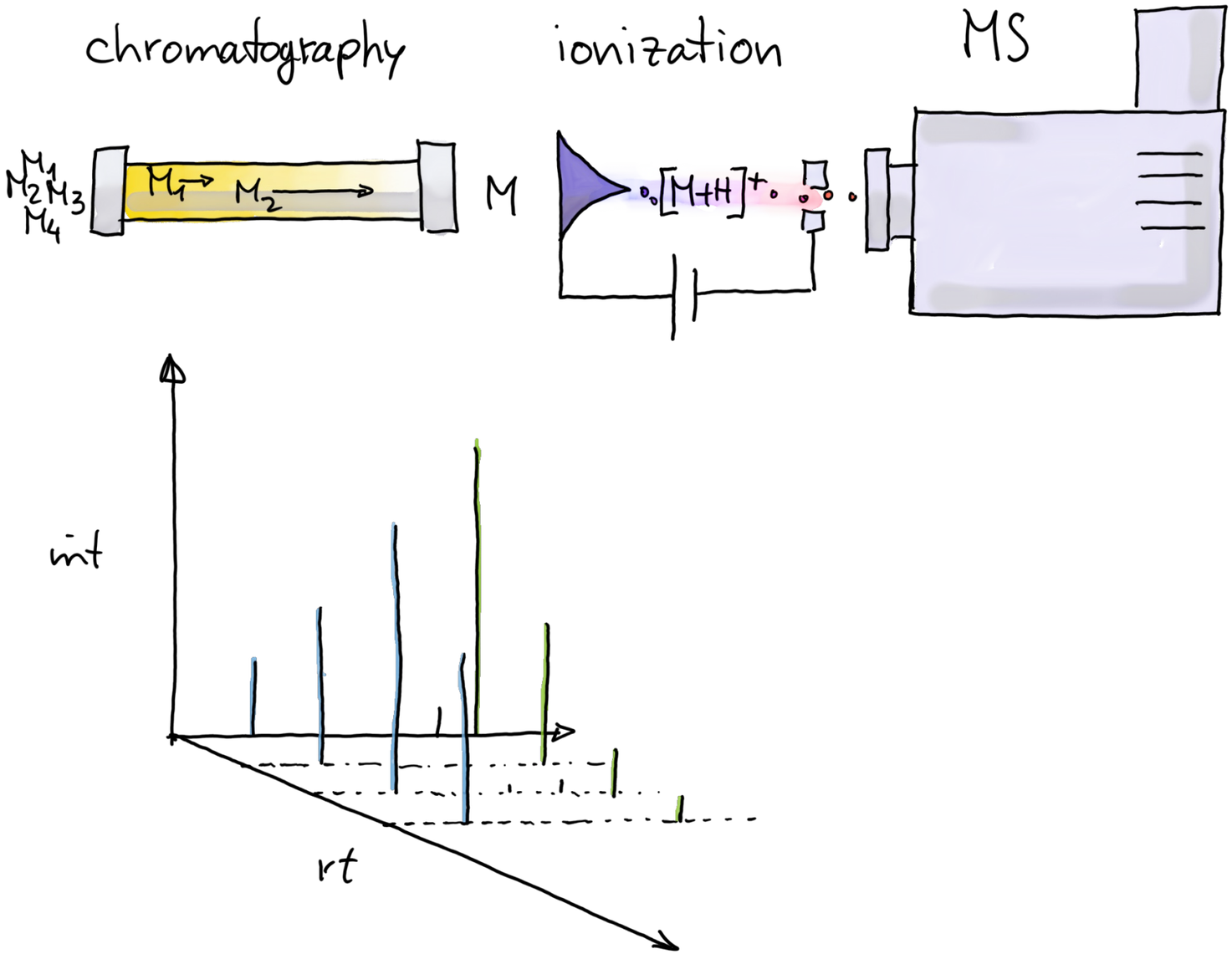

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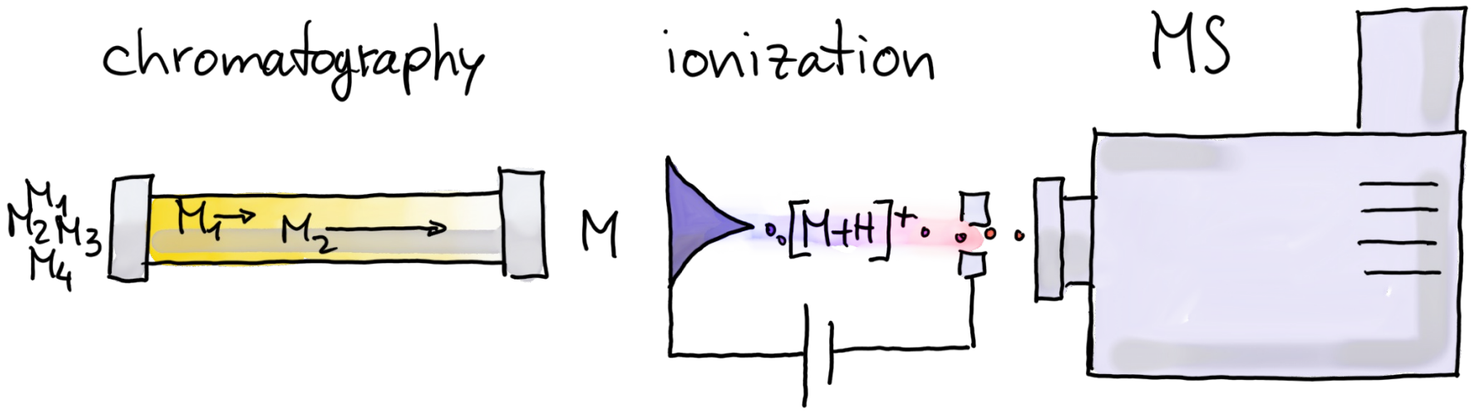

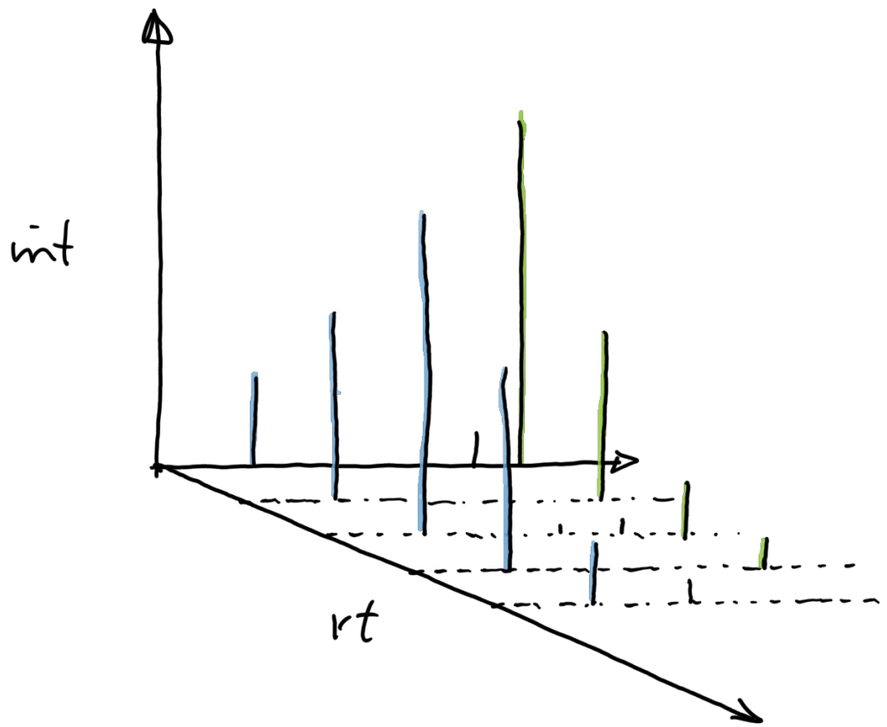

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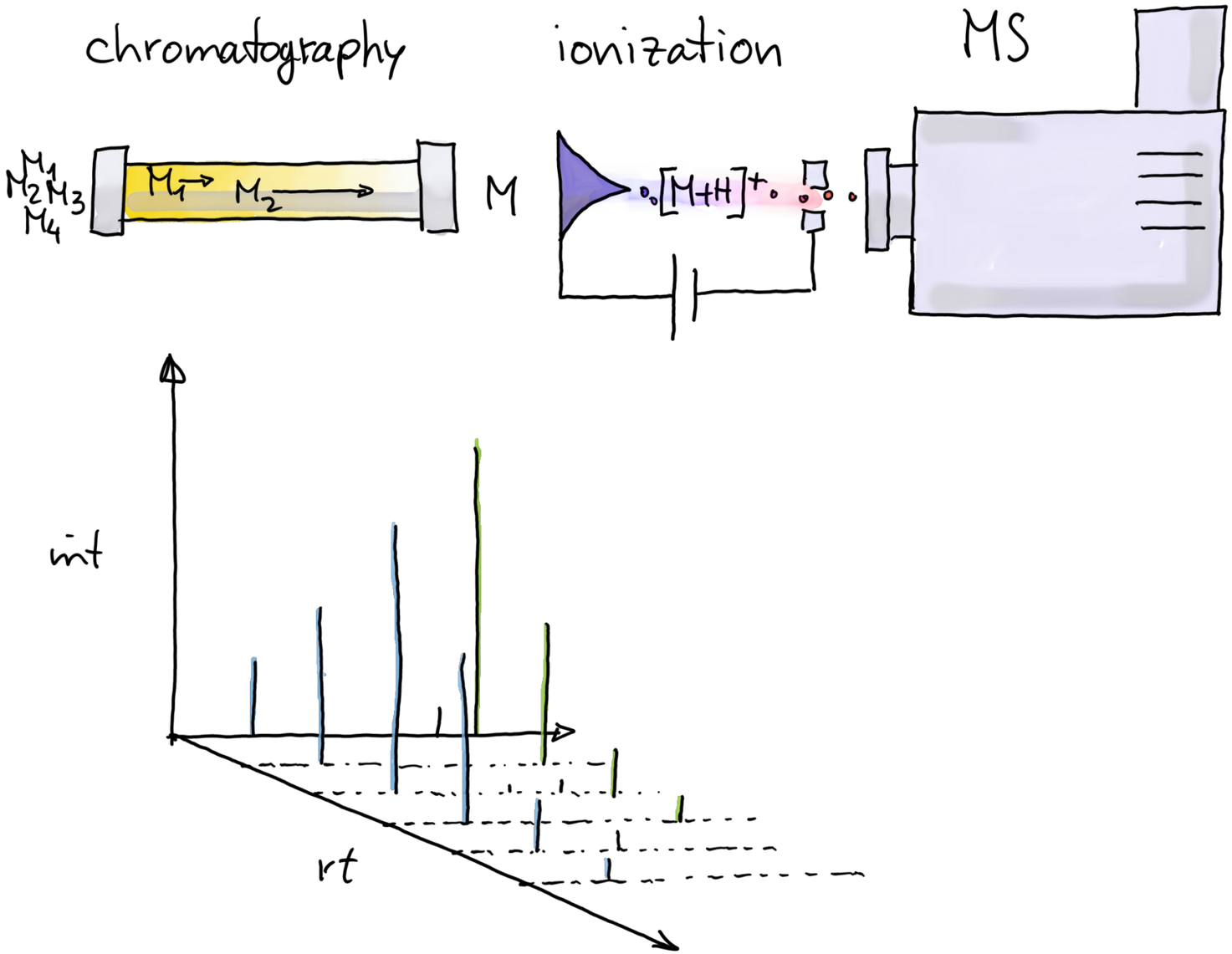

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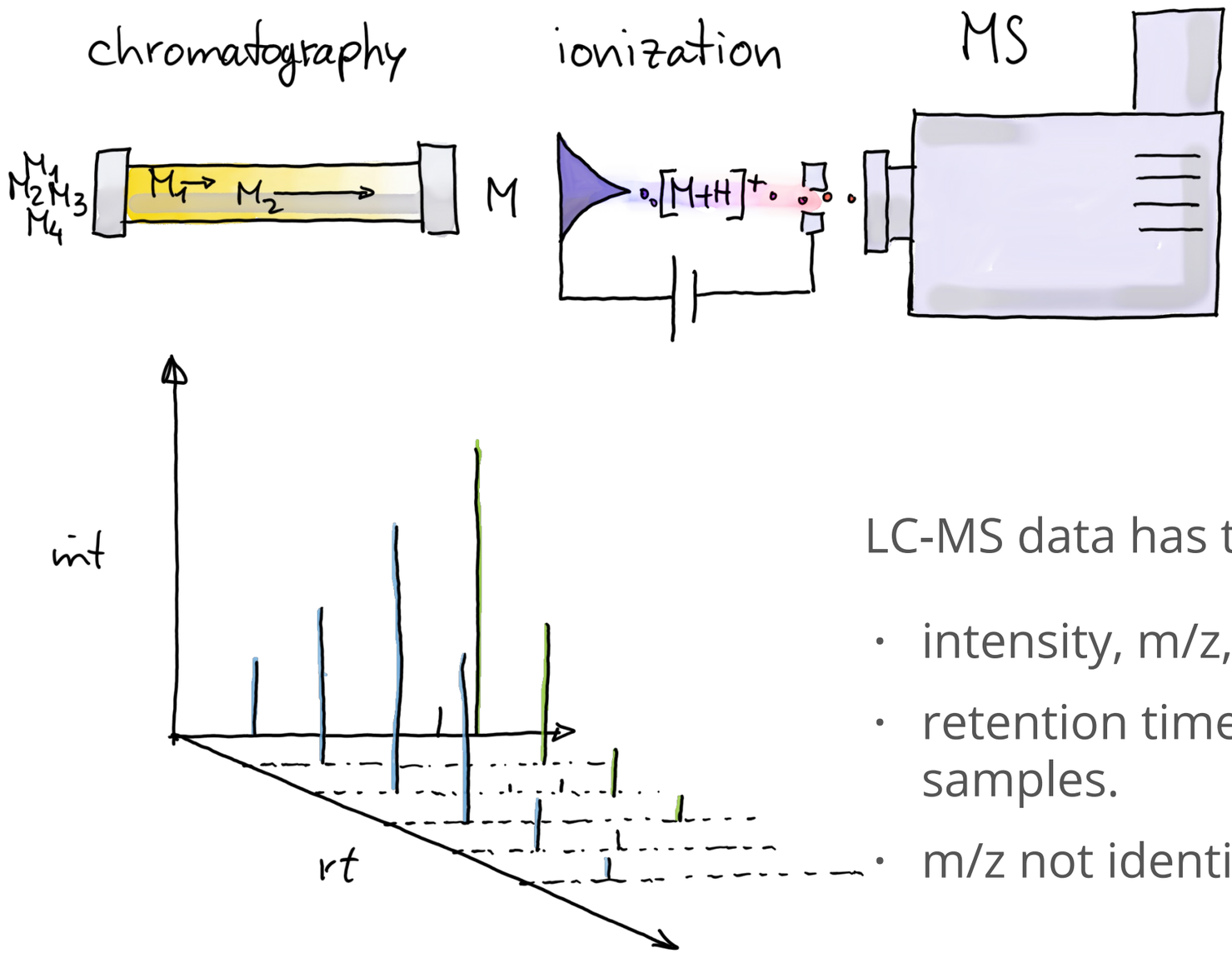


LC-MS data has thus 3 dimensions:

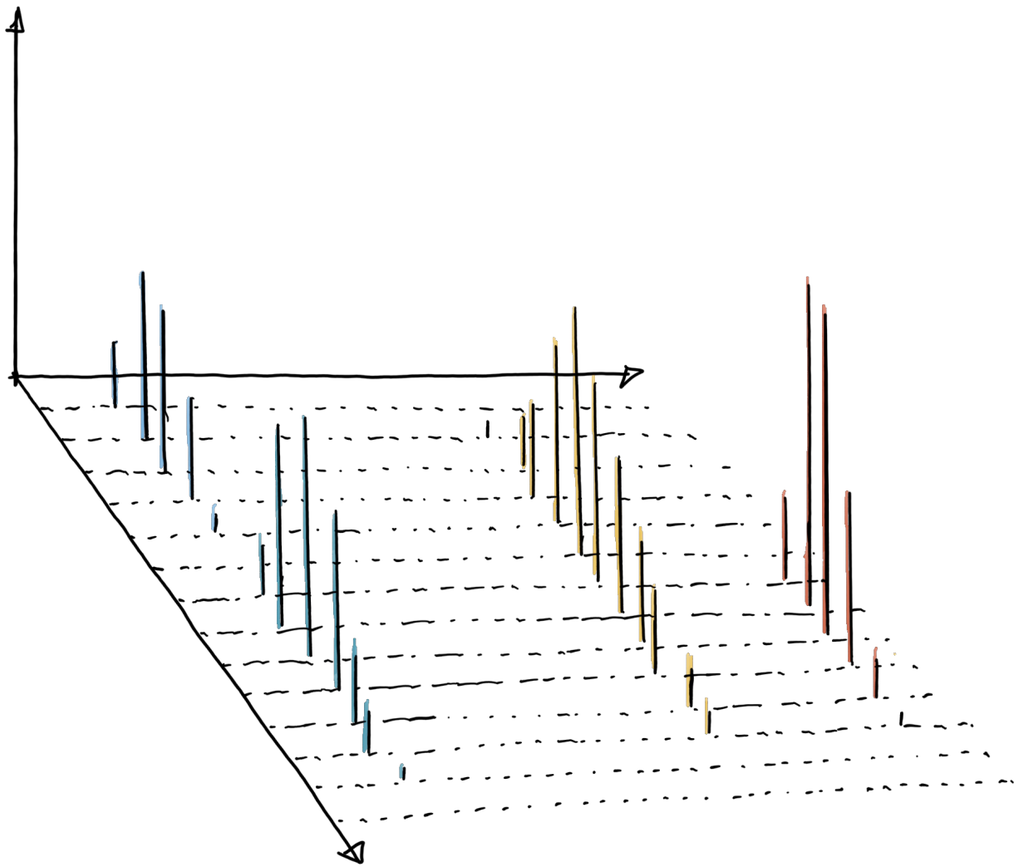
- intensity, m/z, retention time tuples.
- retention time not constant between samples.
- m/z not identical between samples.

LC-MS data preprocessing

- Chromatographic peak detection
- Alignment
- Correspondence

Chromatographic peak detection

- Aim: identify chromatographic peaks in the data.



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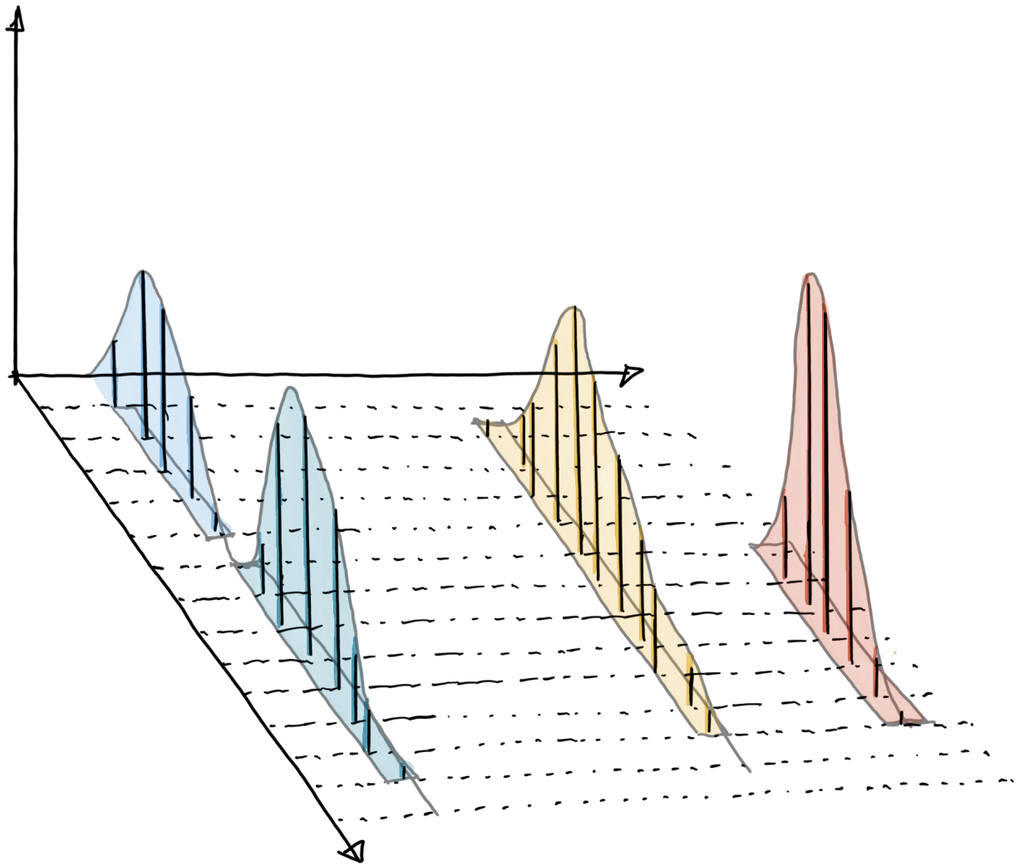

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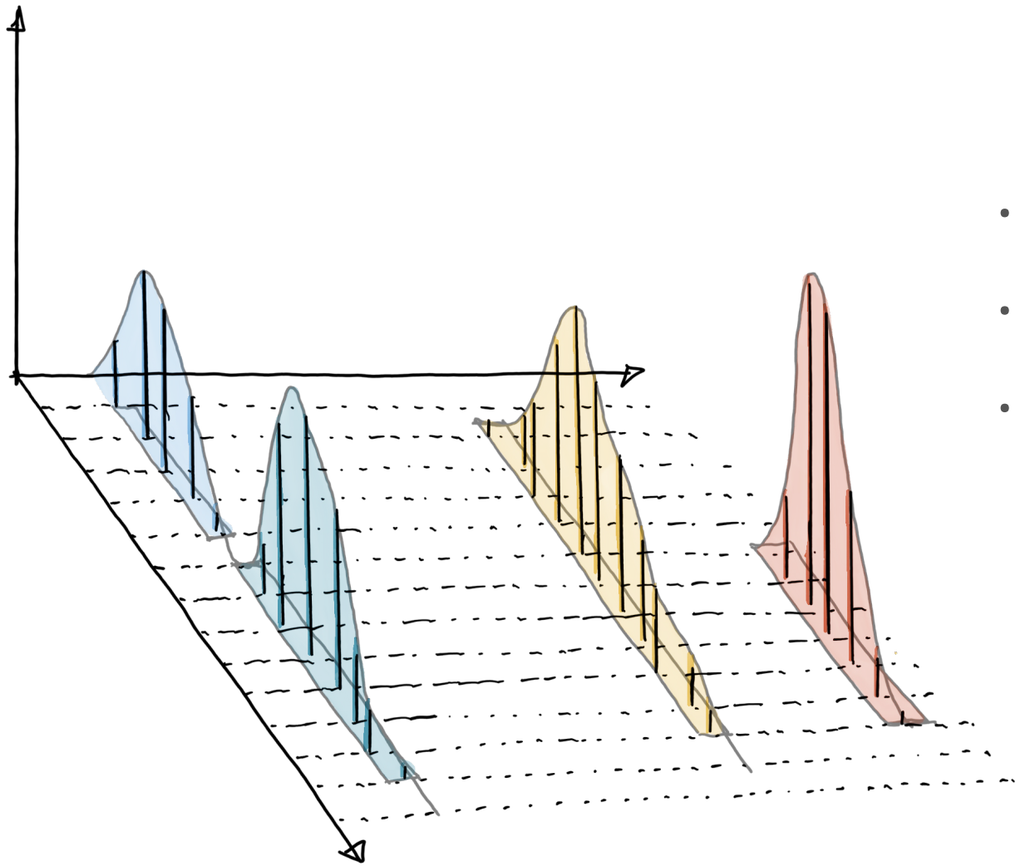
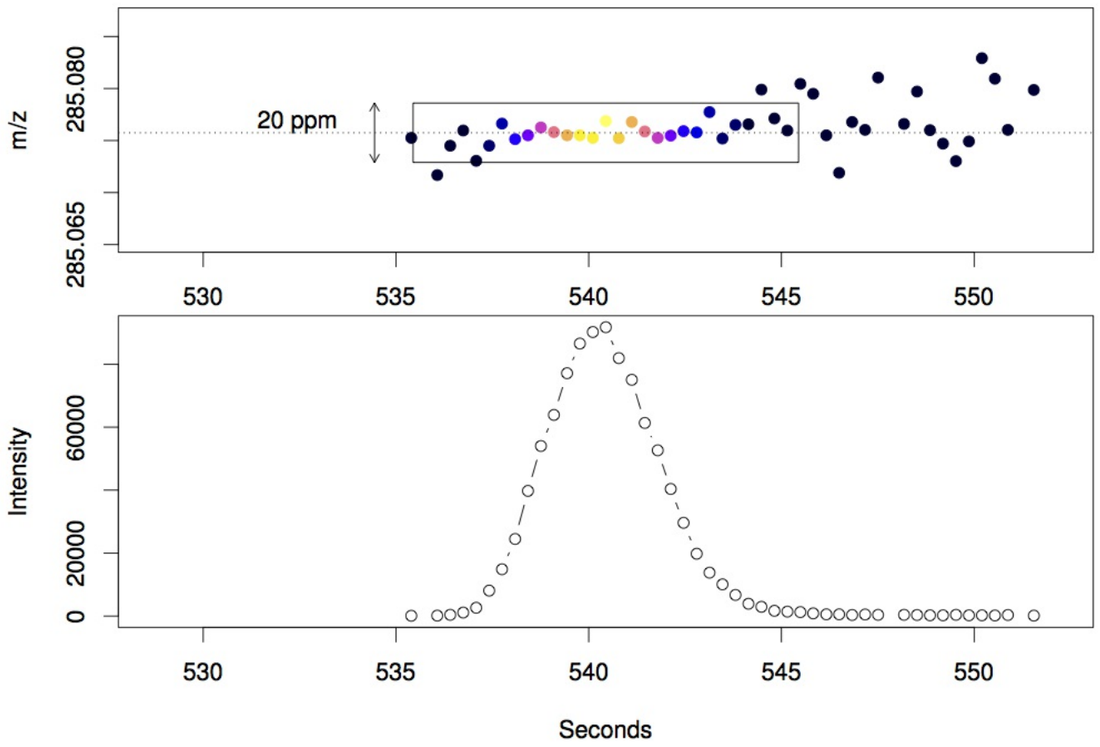


- allow different rt-widths
- allow some scattering on m/z
- identify peak boundaries

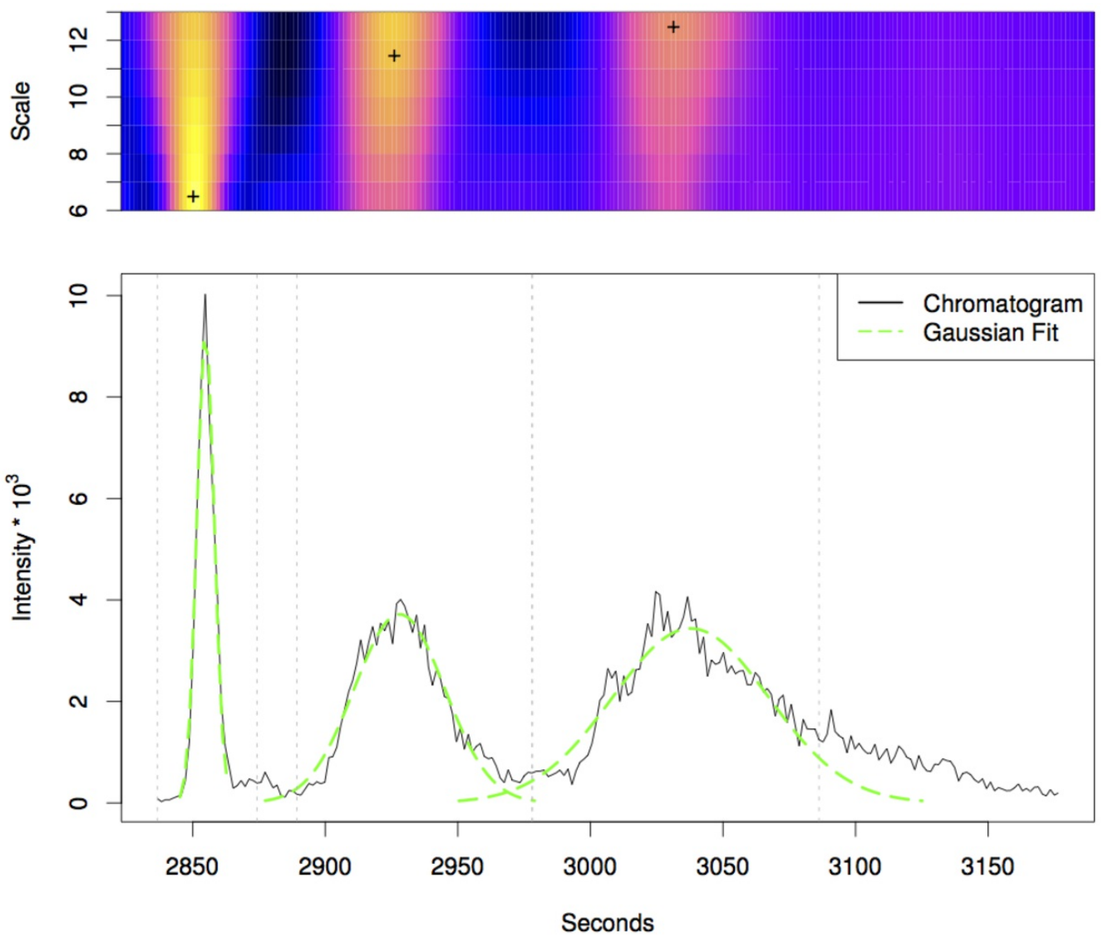
Chromatographic peak detection

- **centWave** [Tautenhahn et al. BMC Bioinformatics, 2008]:
- Step 1: identify regions of interest.



Chromatographic peak detection

- Step 2: peak detection using continuous wavelet transform.
- Allows detection of peaks with different widths.



Chromatographic peak detection

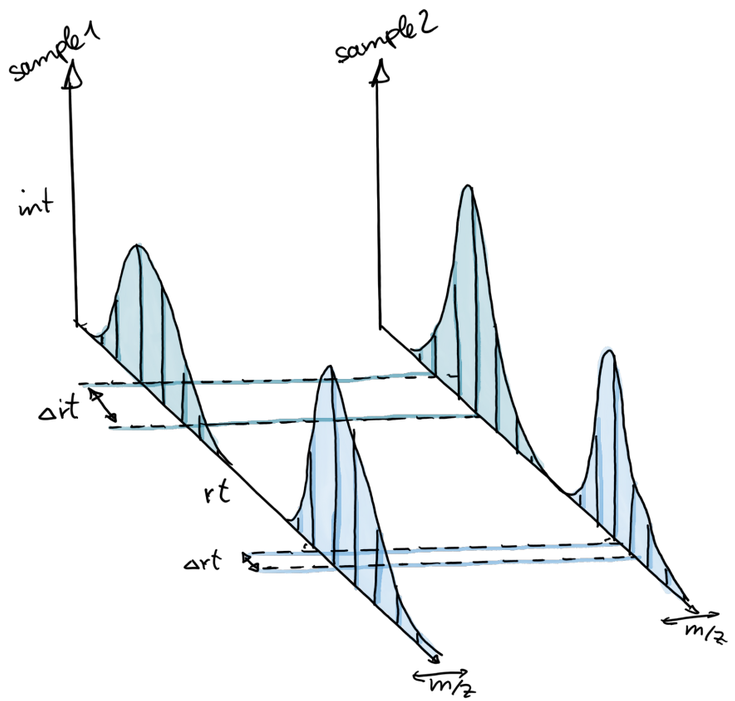
- After reading the data with `readMSData` (`MSnbase` package):
- `xcms`: `findChromPeaks` function, passing settings along with an algorithm-specific parameter object.

```
cwp <- CentWaveParam(peakwidth = c(2, 10), snthresh = 5)
data <- findChromPeaks(data, param = cwp)
head(chromPeaks(data), n = 3)
```

```
##              mz    mzmin    mzmax     rt rtmin  rtmax     into     intb
## [1,] 114.0907 114.0899 114.0929  1.954 0.280  3.907 1559.829 1555.923
## [2,] 114.0913 114.0884 114.0929  5.860 4.465  8.650 1890.221 1885.757
## [3,] 114.0914 114.0899 114.0929 10.882 8.650 13.114 1950.953 1946.210
##          maxo  sn sample is_filled
## [1,] 584.9510 584      1         0
## [2,] 601.8881 601      1         0
## [3,] 691.9580 691      1         0
```

Alignment

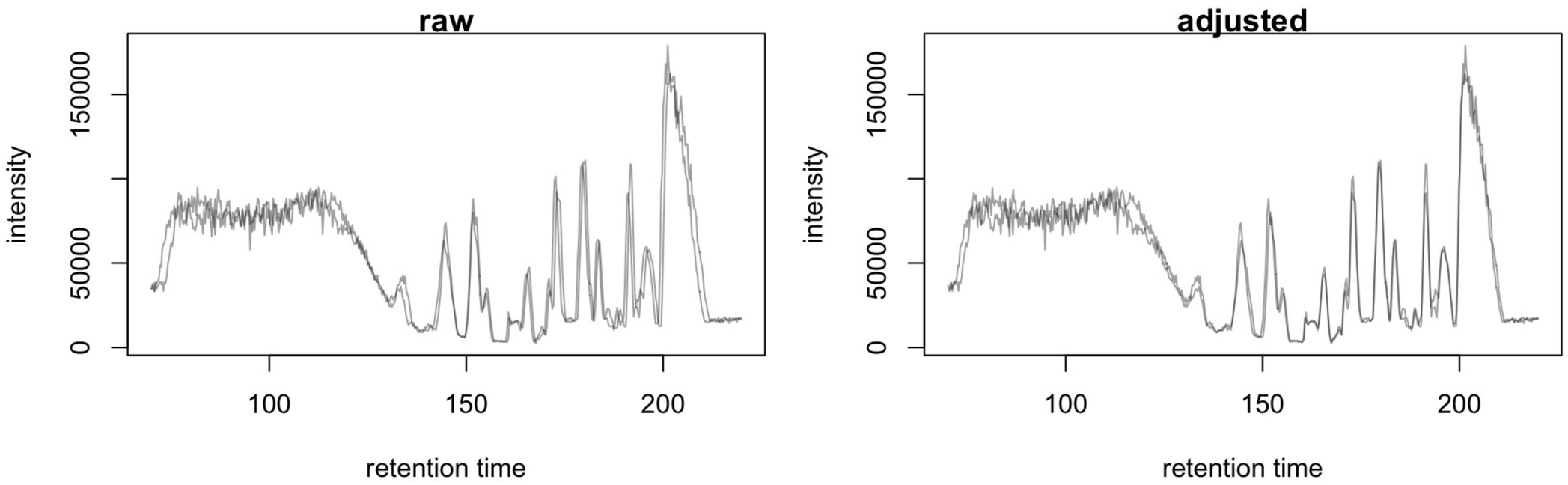
- Chromatography subject to (random and systematic) noise.
- Same analyte may elute at different time in different runs.



- Shifts are LC-setup dependent, seem to be also analyte dependent.

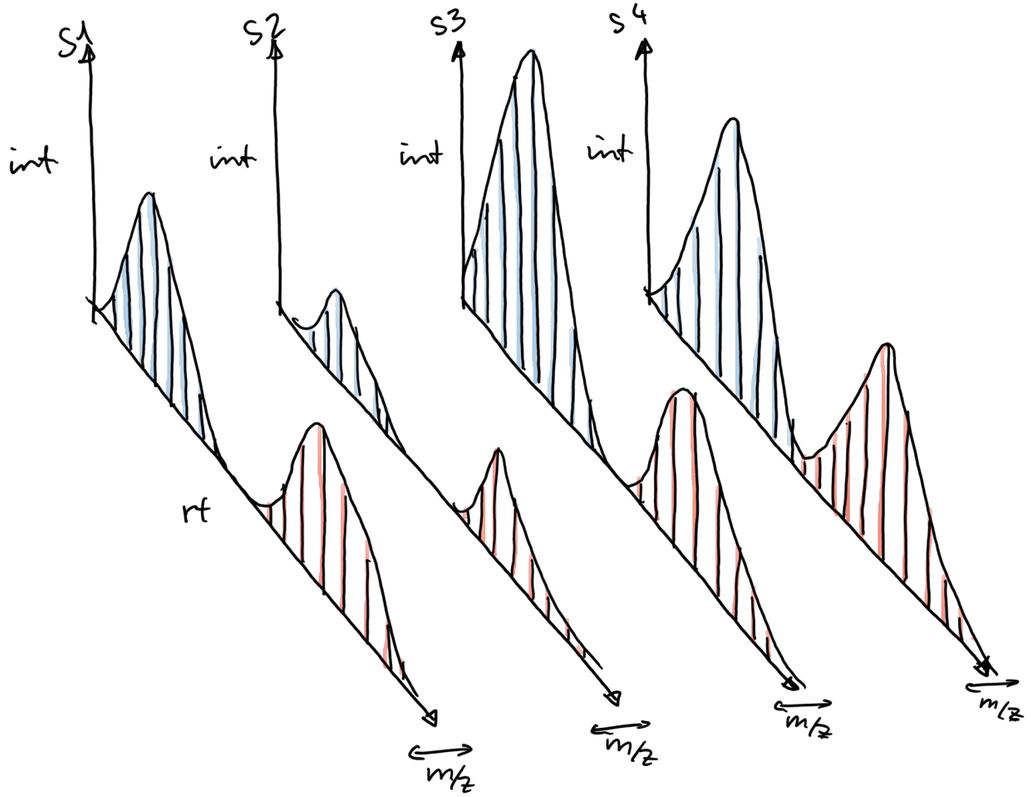
Alignment

- How strong the shifts are depends on the LC-setup.
- Many algorithms available [Smith et al. Brief Bioinformatics 2013]
- Main assumption: analytes elute in the same order.
- `xcms`: `adjustRtime` function with `PeakDensityParam` [Smith et al. Anal. chem. 2006] or `ObiwarpParam` [Prince et al. Anal. chem. 2006].



Correspondence

- Aim: group peaks across samples, assuming they represent the same ion.
- Depends on proper alignment.



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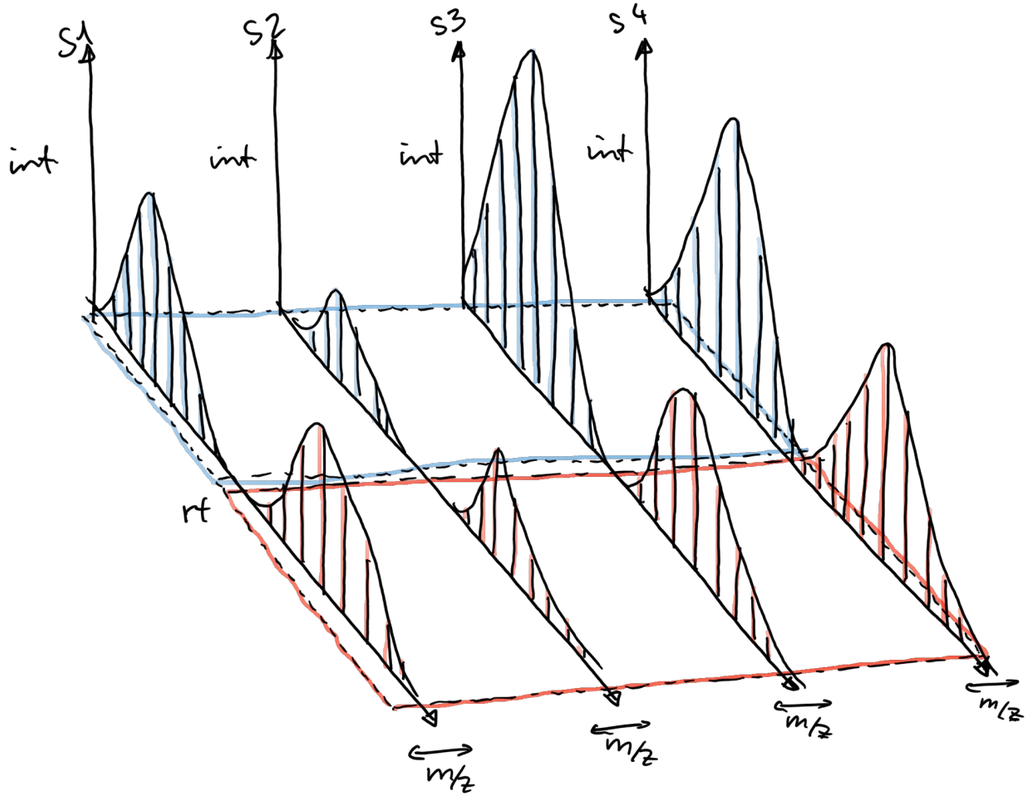

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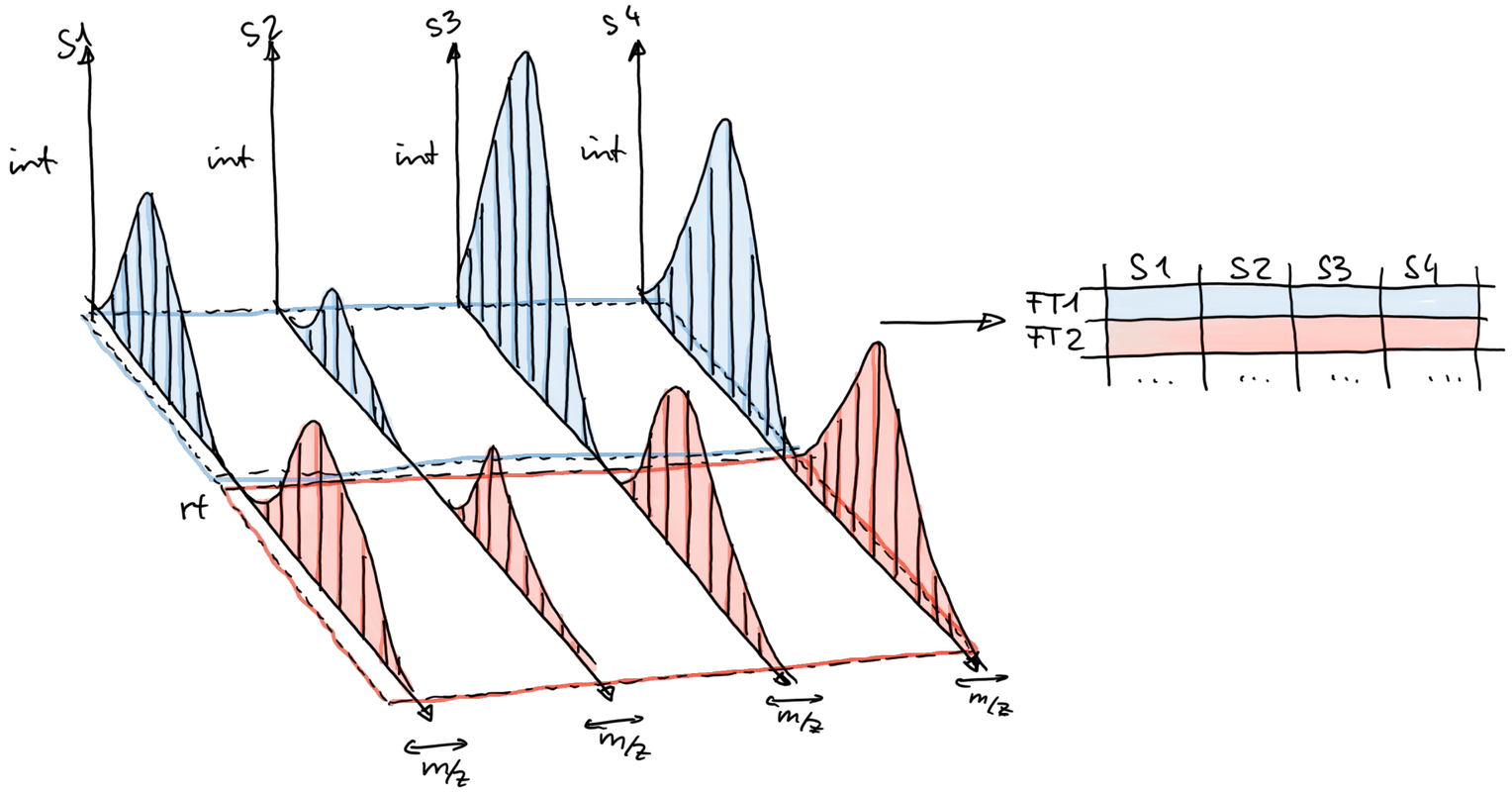
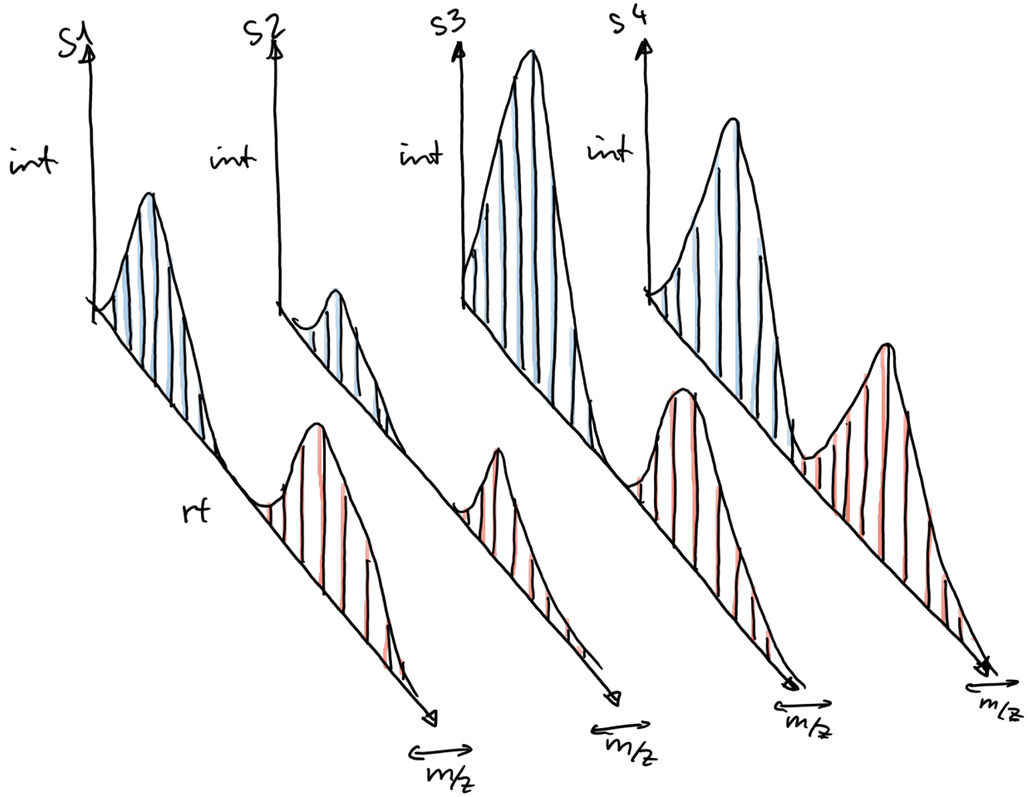


Correspondence

- `xcms`: `groupChromPeaks` with `NearestPeaksParam` [Katajamaa et al. Bioinformatics 2006] and `PeakDensityParam` [Smith et al. Anal. chem. 2006].

- peak density approach (for a given m/z slice):



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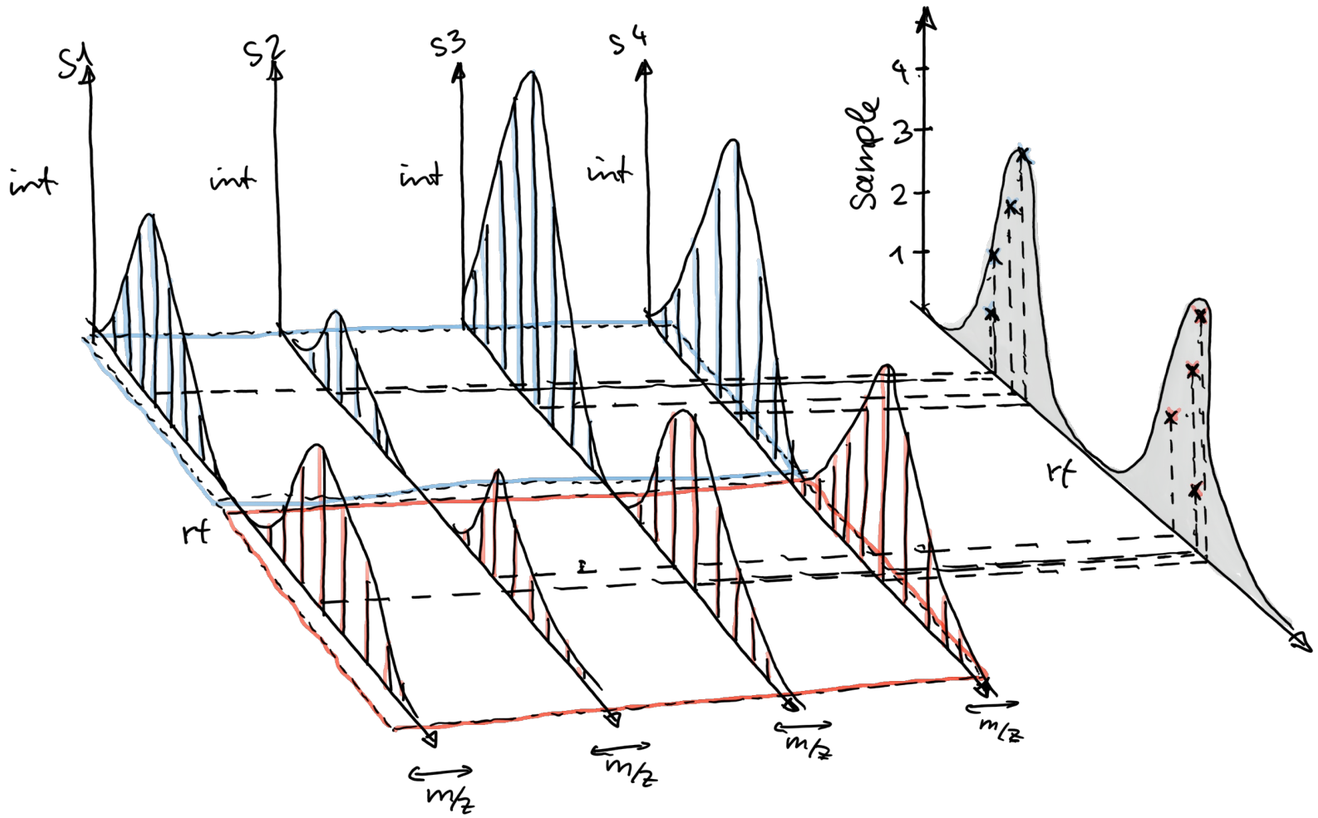
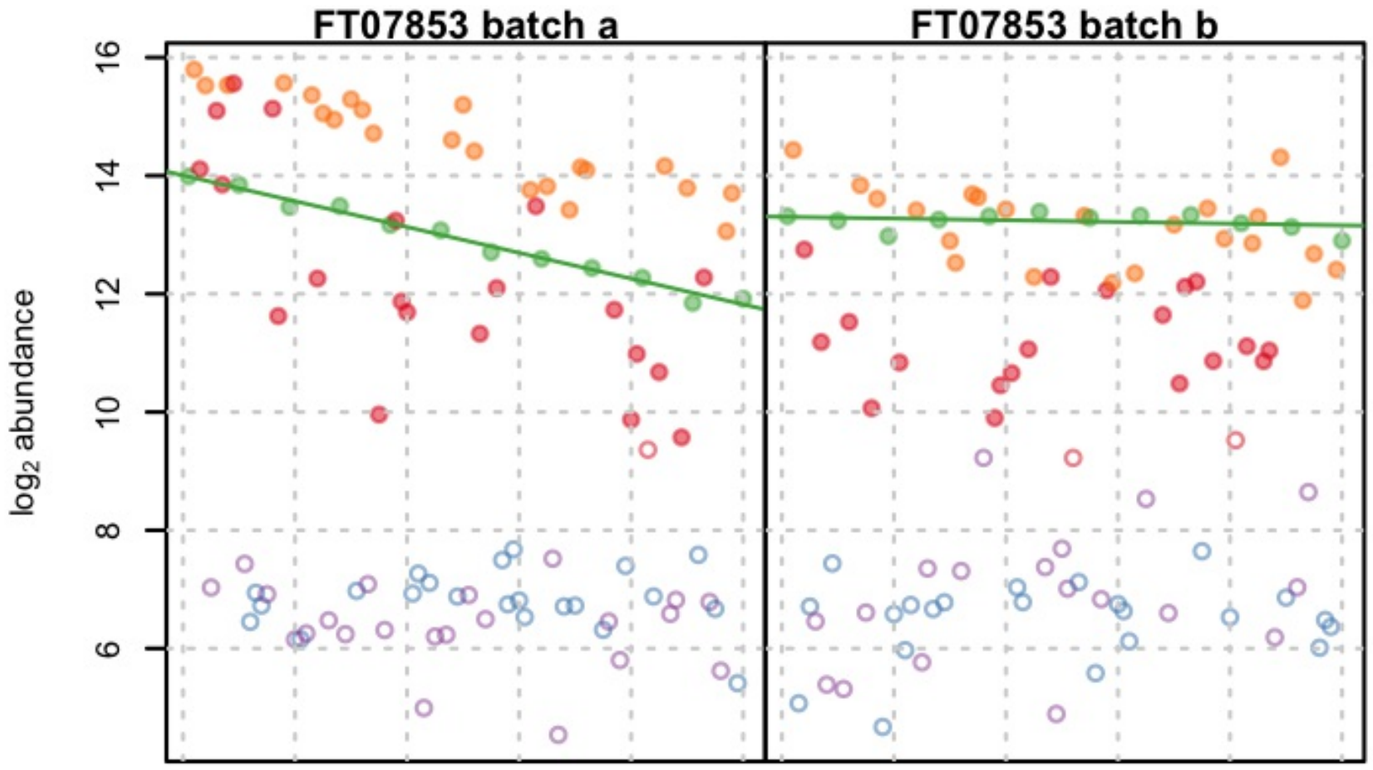


Normalization

- Batch effects.
- LC-dependent effects: seem to affect each metabolite in a different way.



Normalization

- QC controls required for proper noise estimation and adjustment.
- Commonly used: pooled samples measured repeatedly.
- MS runs usually not very expensive, running replicates, QC controls etc not a problem.
- Popular methods:
  - RUV [De Livera et al. Anal. Chem. 2015]
  - linear models [Wehrens et al. Metabolomics 2016]
  - linear and higher order models [Brunius et al. Metabolomics 2016].

Identification

- Features have to be annotated to metabolites.

```
## DataFrame with 4 rows and 4 columns
##                    mzmed             rtmed            POOL_1            POOL_2
##                <numeric>         <numeric>         <numeric>         <numeric>
## FT001 105.041814839707 167.961095453642 229.490739260736 3093.75184315684
## FT002 105.041653033614 157.083057856508 4762.39872227772 6601.45091358641
## FT003 105.069636149683 31.8108067962868 699.723986763235 1033.23232267732
## FT004  105.11027064078 63.7513630255991 20211.2633706294 15839.5504368189
```

- Match compounds based on features' m/z.
- Will result in an 1:n mapping.

Identification based on m/z matching

- Databases containing masses of (synthetic and biological) compounds.
- m/z is **not** the mass.
- The mass of the charged molecule has to be considered too: [M+H]+ adduct: mass of metabolite M + mass of hydrogen.
- We can have different adducts from the same compound in a sample: [M+H]+, [M+Na]+, …
- Main databases providing compound masses (and spectra):
  - The Human Metabolome Database (HMDB): https://hmdb.ca
  - Chemical Entities of Biological Interest: https://www.ebi.ac.uk/chebi
  - PubChem (human and other animals) https://pubchem.ncbi.nlm.nih.gov
  - …

Improved identification

Identify compounds based on m/z **and**:

- **retention time**: requires lab-internal database with approximate retention times for specific compounds (manually defined).
- **MS2 spectrum**:
  - Needs MS2 data of the compounds (e.g. SWATH data).
  - Only works if reference spectrum available in database.
- Up and coming: `CompoundDb` package (similar to `ensembldb` and alike).

Afternoon metabolomics lab

- LC-MS data handling (`MSnbase`).
- LC-MS data preprocessing using `xcms`.