Ion mobility enhanced matching between LC-MS runs improves identification and quantification in MaxQuant

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Introduction

Trapped ion mobility spectrometry (TIMS) provides an additional dimension of separation to LC-MS which potentially can boost proteome coverage, quantification accuracy and dynamic range in shotgun proteomics experiments. Required for this is suitable software that extracts the information contained in the 4D data space spanned by m/z, retention time, ion mobility and signal intensity.

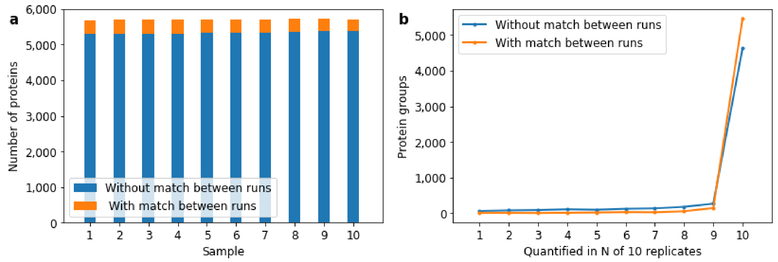
The MaxQuant workflow

We adapted the complete MaxQuant shotgun proteomics workflow to process data with the added ion mobility dimension. Most adaptations were done in the feature detection workflow which now produces 4D peaks.

Workflow component	Changes for IMS
Feature detection	+++
MS/MS preparation	+++
Initial search	-
Recalibration	++
Main search	-
PSM FDR	-
Alignment	++
Match between runs	++
Protein groups / FDR	-
Label-free quantification	-
Writing of results	+

Matching between runs

Matching MS1 features between runs, the transfer of identifications without MS/MS identifications, is making use of the ion mobility coordinates to become more specific.



Feature detection

Deisotoping and assembling of MS1 labeling multiplets utilize intensity profile correlations also over the ion mobility direction.

4D feature space

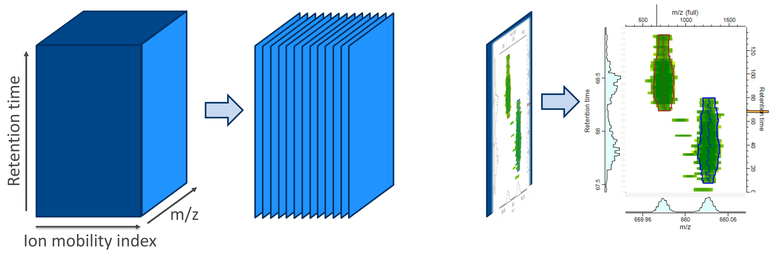
LC-MS

- 2D input space: mass, retention time
- Peaks/Features are 3D objects
- Features can be visualized as heat maps or 3D surface plots

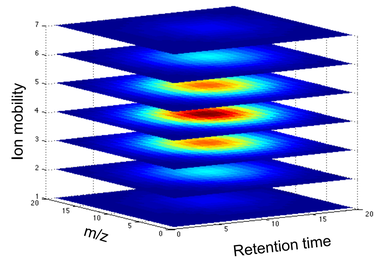
LC-IMS-MS

- 3D input space: mass, retention time, ion mobility index
- Peaks/Features are 4D objects
- Full feature is difficult to visualize.
  - Projections
  - Contour surface

Slicing

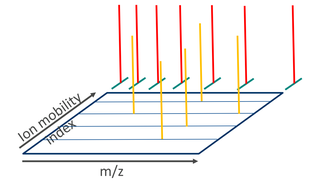


Assembling features across slices

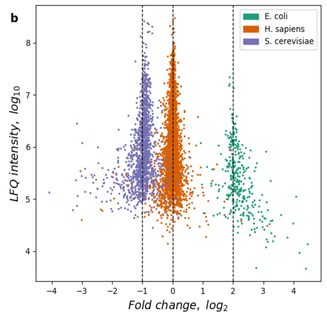


MS/MS spectra

MS/MS spectra corresponding to one precursor are projected down to conventional spectra over ion mobility axis to m/z grid



Benchmark measurements



We performed benchmark measurements on a Bruker timsTOF Pro instrument with Parallel Accumulation Serial Fragmentation (PASEF) functionality for the acquisition of MS/MS spectra. We generated benchmark datasets in which HeLa proteins were spiked with E. coli and yeast proteomes at 1:4 and 2:1 ratios and compared the replicates with the MaxLFQ algorithm for label-free quantification.