dia-PASEF: Bottom-up proteomics with near optimal ion usage

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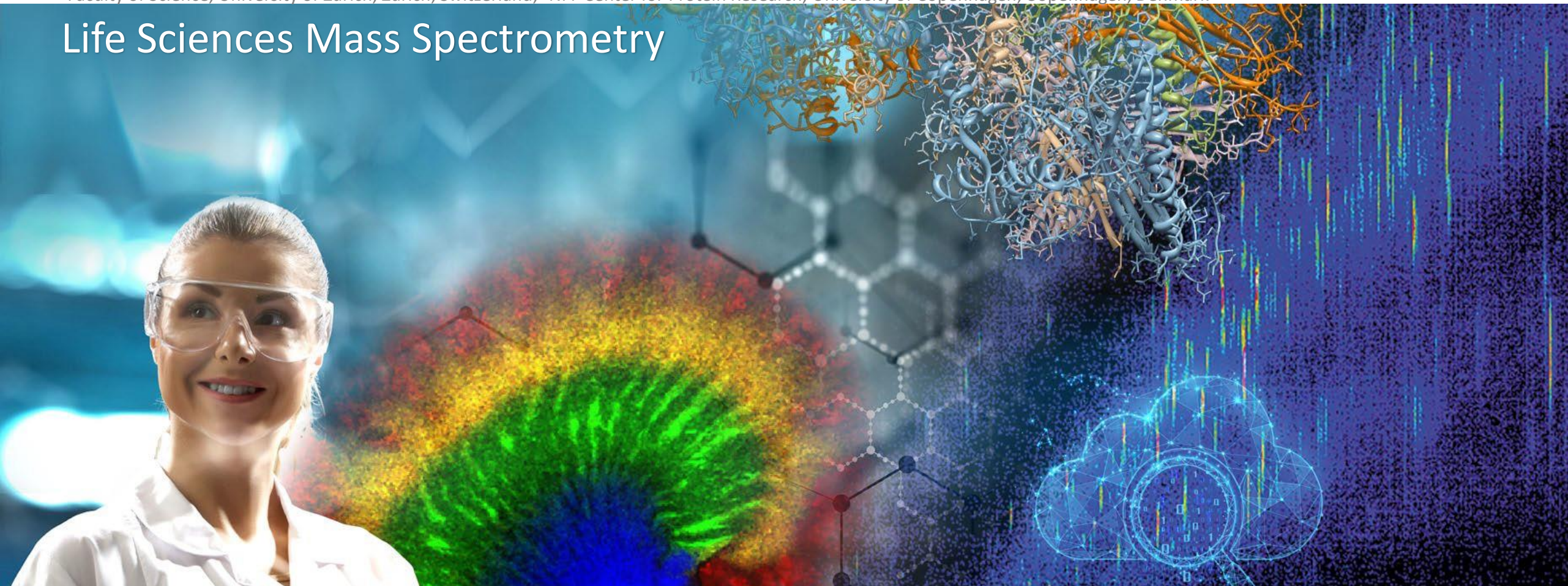
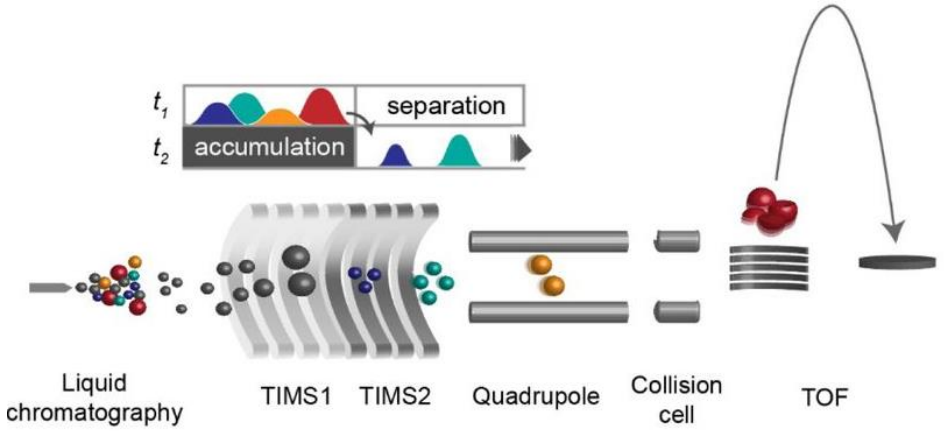
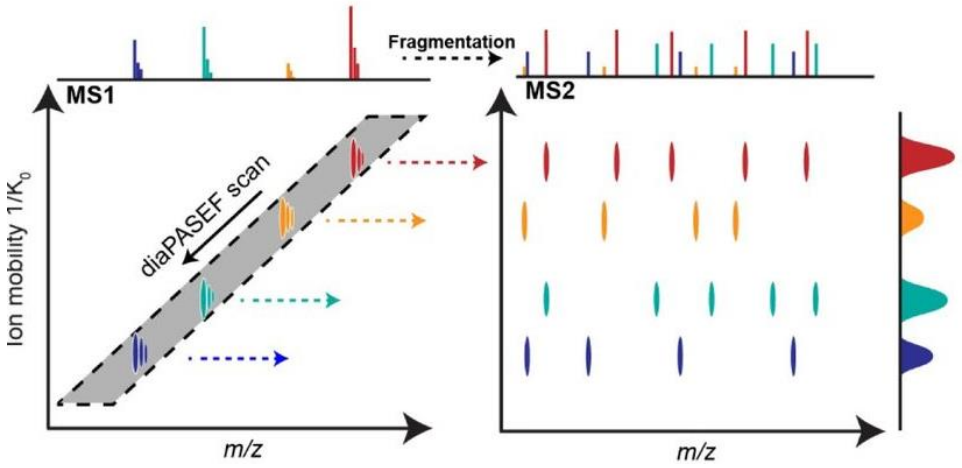


Introduction: dia-PASEF acquisition mode

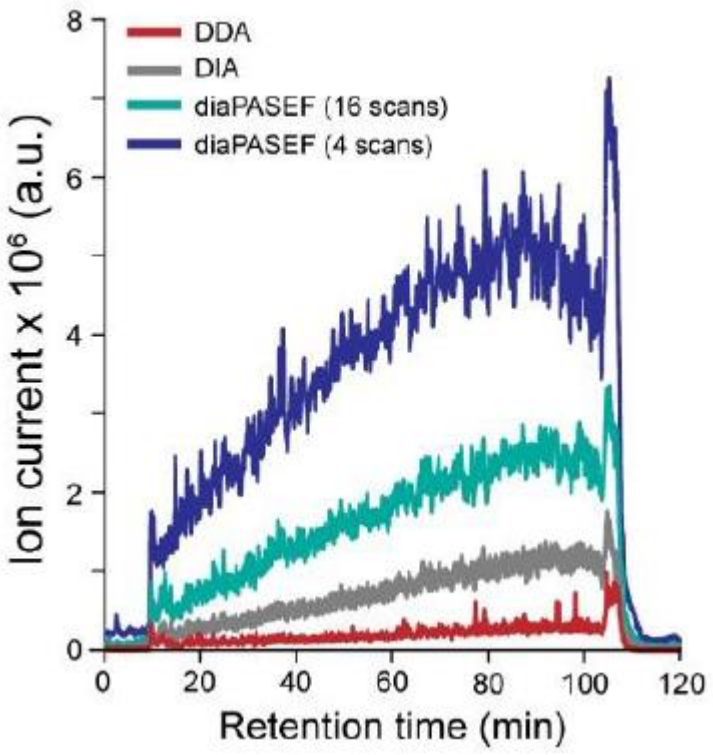




- Synchronizing trapped ion mobility spectrometry (TIMS) and precursor ion selection in a quadrupole time-of-flight mass spectrometer increases MS/MS sequencing rates more than ten-fold in a novel acquisition mode termed PASEF.
- Here, we applied the PASEF principle to DIA and investigate its performance.

Meier et al., 2019: https://www.biorxiv.org/content/10.1101/656207v2

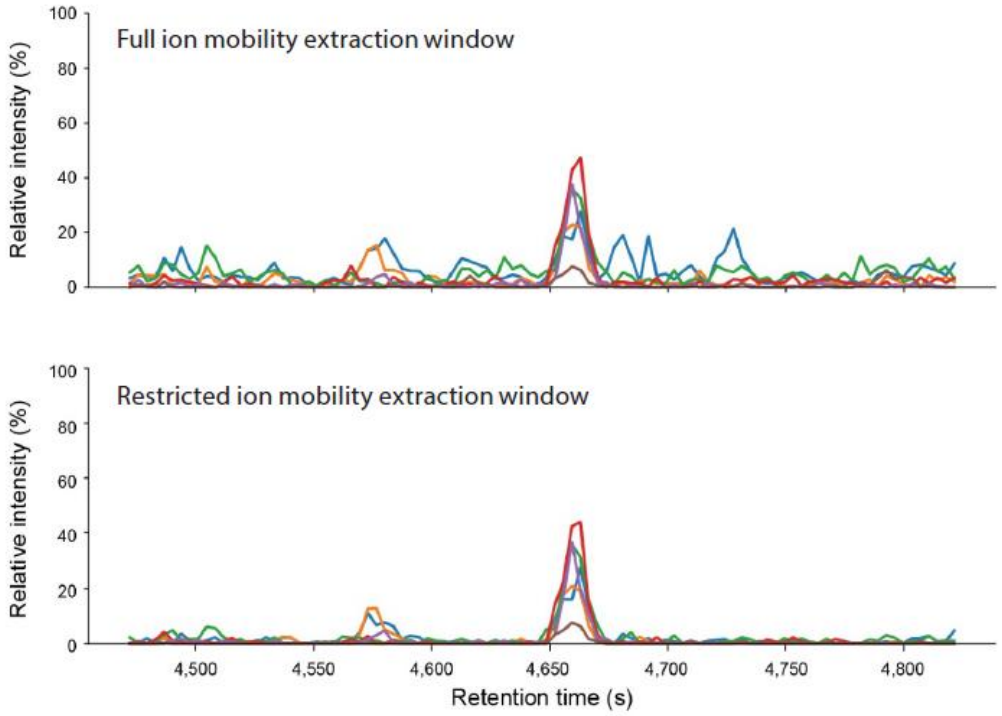
Results: Increase in ion sampling efficiency



- Ion sampling efficiency for a tryptic HeLa digest increased by five-fold by applying dia-PASEF and a 4 scan window placement when comparing to common DIA window placement.

Meier et al., 2019: https://www.biorxiv.org/content/10.1101/656207v2

Results: Ion Mobility-Aware Targeted Data Extraction

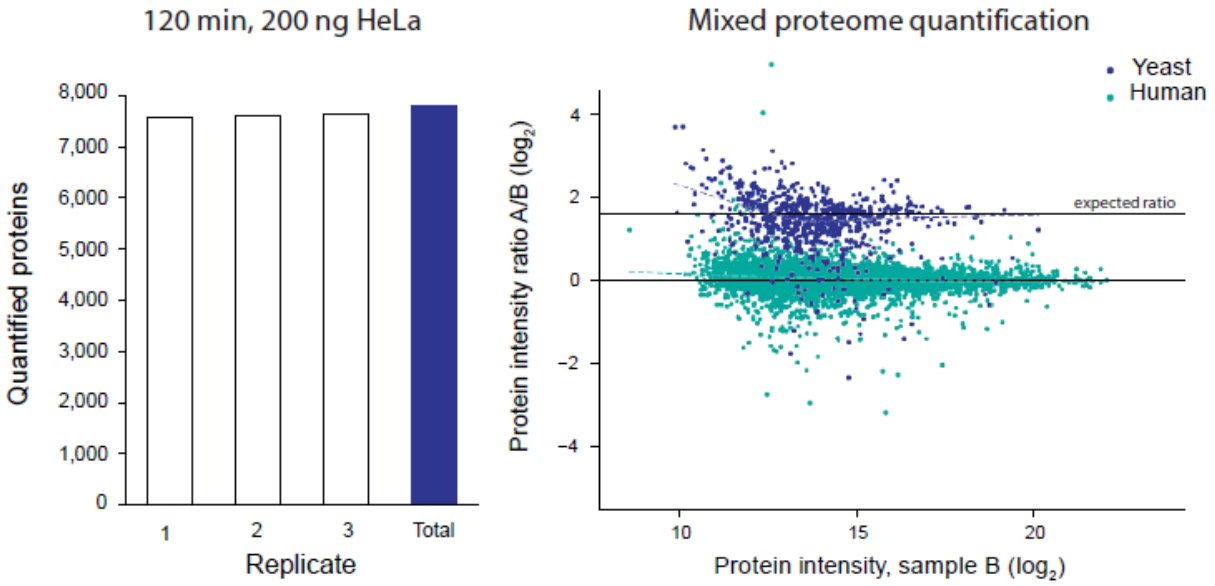


- To handle the novel 4D data structure Mobi-DIK (Ion Mobility DIA Analysis Kit) was developed.
- Restricting the extraction data to a user-defined width in the ion mobility dimension improves the signal-to-noise ratio by 4-fold.

Fragment ion chromatograms of DGLLIIGVHSAK extracted with and without restriction in the ion mobility dimension

Meier et al., 2019: https://www.biorxiv.org/content/10.1101/656207v2

Results: Single Run Proteome Quantification

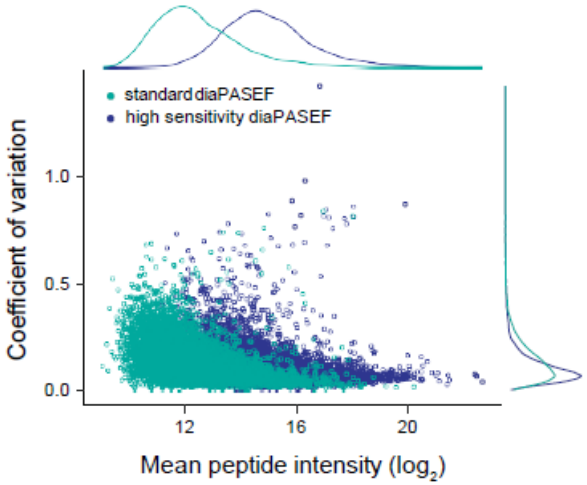


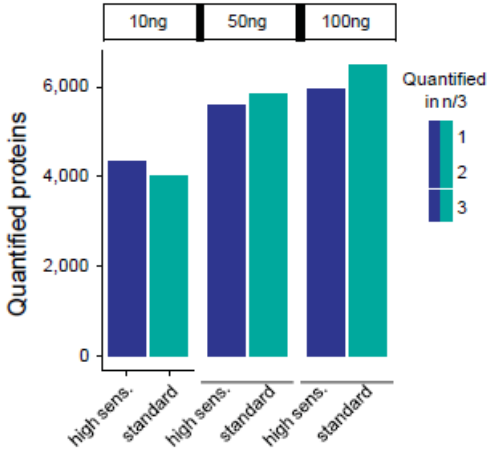
- With a library-based analysis in total 7,800 proteins were identified at a global protein FDR of 1%. Remarkably 85% of all proteins in the library were covered.

- Using a combined human and yeast library 82,808 (7,943)human and 7,483 (2,250) yeast unique peptides (proteins) could be quantified. Their protein abundance ratios split according to the mixed ratios.

Meier et al., 2019: https://www.biorxiv.org/content/10.1101/656207v2

High-sensitivity Proteome Analysis





- Lowering the number of dia-PASEF scans and increasing the quadrupole isolation width (high sensitivity dia-PASEF method) increased the detected fragment ion signal on average about 4-fold as compared to the standard method. The higher ion signal translated into a more precise quantification.

- With the high sensitivity dia-PASEF method 4,310 proteins could be quantified in triplicates of 10 ng injection.

Meier et al., 2019: https://www.biorxiv.org/content/10.1101/656207v2

Conclusion

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- Standard DIA acquisition schemes utilize only a few percent of the ion current by cycling through segments of the precursor m/z range
- dia-PASEF makes use of the correlation of molecular weight and ion mobility in a trapped ion mobility mass spectrometer (timsTOF Pro)
- Synchronizing ion mobility separation and precursor selection allows to sample up to 100% of the peptide precursor ion current
- CCS-Aware targeted data extraction increases the specificity for precursor identification
- Single run analysis of whole proteome digests and mixed organism samples demonstrates deep proteome coverage and exceptional sensitivity

Questions and Answers