dia-PASEF: Bottom-up proteomics with near optimal ion usage
Florian Meier¹, Stephanie Kaspar-Schoenefeld², Andreas-David Brunner¹, Max Frank³, Annie Ha³, Isabell Bludau¹, Eugenia Voytik¹, Scarlet Koch², Markus Lubeck², Oliver Raether², Ruedi Aebersold⁴⁵, Ben C. Collins ⁴, Hannes L. Röst³, Matthias Mann¹⁶

¹Proteomics and Signal Transduction, Max Planck Institute of Biochemistry, Martinsried, Germany; ²Bruker Daltonik GmbH, Bremen, Germany; ³Donnelly Centre for Cellular and Biomolecular Research, Toronto, ON; ⁴Department of Biology, Institute of Molecular Systems Biology, ETH Zürich, Zurich, Switzerland; ⁵Faculty of Science, University of Zürich, Zurich, Switzerland; ⁶NFF Center for Protein Research, University of Copenhagen, Copenhagen, Denmark
• 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
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.

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

• 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