diaPASEF: toward the ideal mass analyzer with data-independent acquisition and parallel accumulation – serial fragmentation

HUPO 2019

Florian Meier[1], Andreas-David Brunner[1], Annie Ha[2], Max Frank[2], Eugenia Voytik[1], Stephanie Kaspar-Schoenefeld[3], Markus Lubeck[3], Heiner Koch[3], Scarlet Koch[3], Oliver Raether[3], Ruedi Aebersold[4,5], Ben Collins[4], Hannes Rost[2], Matthias Mann[1,6]

[1] Max Planck Institute of Biochemistry, Martinsried, Germany
[2] University of Toronto, Toronto, Canada
[3] Bruker Daltonik GmbH, Bremen, Germany
[4] ETH Zurich, Zurich, Switzerland
[5] University of Zurich, Zurich, Switzerland
[6] NNF Center for Protein Research, University of Copenhagen, Copenhagen, Denmark

Introduction

Data-independent acquisition (DIA) workflows have gained in popularity as they overcome the issue of stochastic selection of peptide precursors encountered in typical data-dependent approaches (DDA). The success of DIA relies on key instrumental capabilities, namely: resolution, sensitivity, accuracy and dynamic range uncompromised by a fast-spectral acquisition rate (>40 Hz) demanded by DIA. Trapped ion mobility spectrometry adds an additional dimension for separation of complex proteomics samples. In addition, the collisional cross section (CCS) term allows for unbiased alignment of precursor and fragment information. Here we combine Parallel Accumulation Serial Fragmentation (PASEF, [1]) with a DIA approach, called diaPASEF [2], to investigate its potential for the in-depth analysis of complex proteomics samples.

Methods

Whole-cell proteomes were extracted from a human cancer cell line (HeLa S3, ATCC, USA) and digested with LysC and trypsin (1:100 enzyme:protein (wt/wt) for both. A nanoLC system was coupled online to a timsTOF Pro (Bruker Daltonics). Peptides were separated on a reversed-phase column (50 cm x 75 µm i.d.) with a pulled emitter tip, packed with 1.9 µm C18-coated porous silica beads (Dr. Maisch, Germany) using a linear gradient from 5 to 30% B (80/20/0.1% ACN/water/formic acid) within 95 minutes followed by an increase to 60% B within 5 minutes. We adapted the instrument firmware to perform data-independent isolation of multiple precursor windows within a single TIMS separation (100 ms). This new scan mode is termed diaPASEF (**Fig. 1**)
Analysis of the four-dimensional data space was performed using Mobi-DIK (Ion Mobility DIA Analysis Kit [3]).

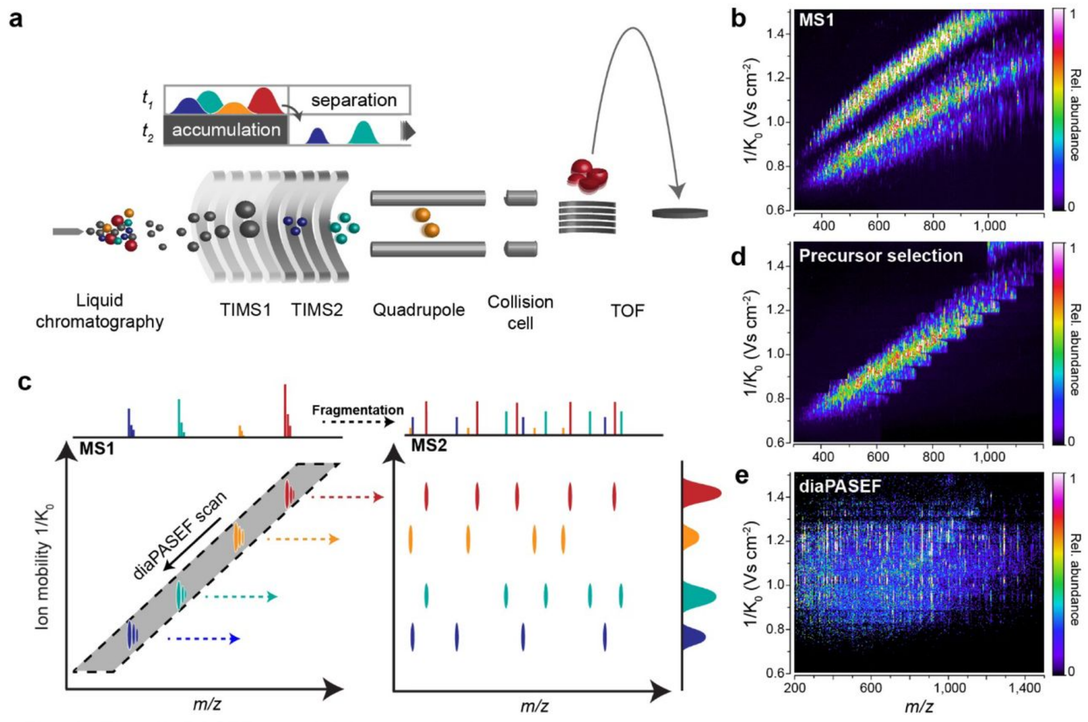

Fig. 1: The diaPASEF acquisition method.
a) Schematic ion path of the timsTOF Pro. b) Correlation of peptide ion mobility and m/z in a tryptic digest of HeLa cell lysate. c) In diaPASEF, the quadrupole isolation window (grey) is dynamically positioned as a function of ion mobility (arrow). In a single TIMS scan, ions from the selected mass ranges are fragmented to record ion mobility-resolved MS2 spectra of all precursors. d) Implementation of diaPASEF precursor selection with a stepped quadrupole isolation scheme. e) Representative example of a single diaPASEF scan with the precursor selection scheme from d.

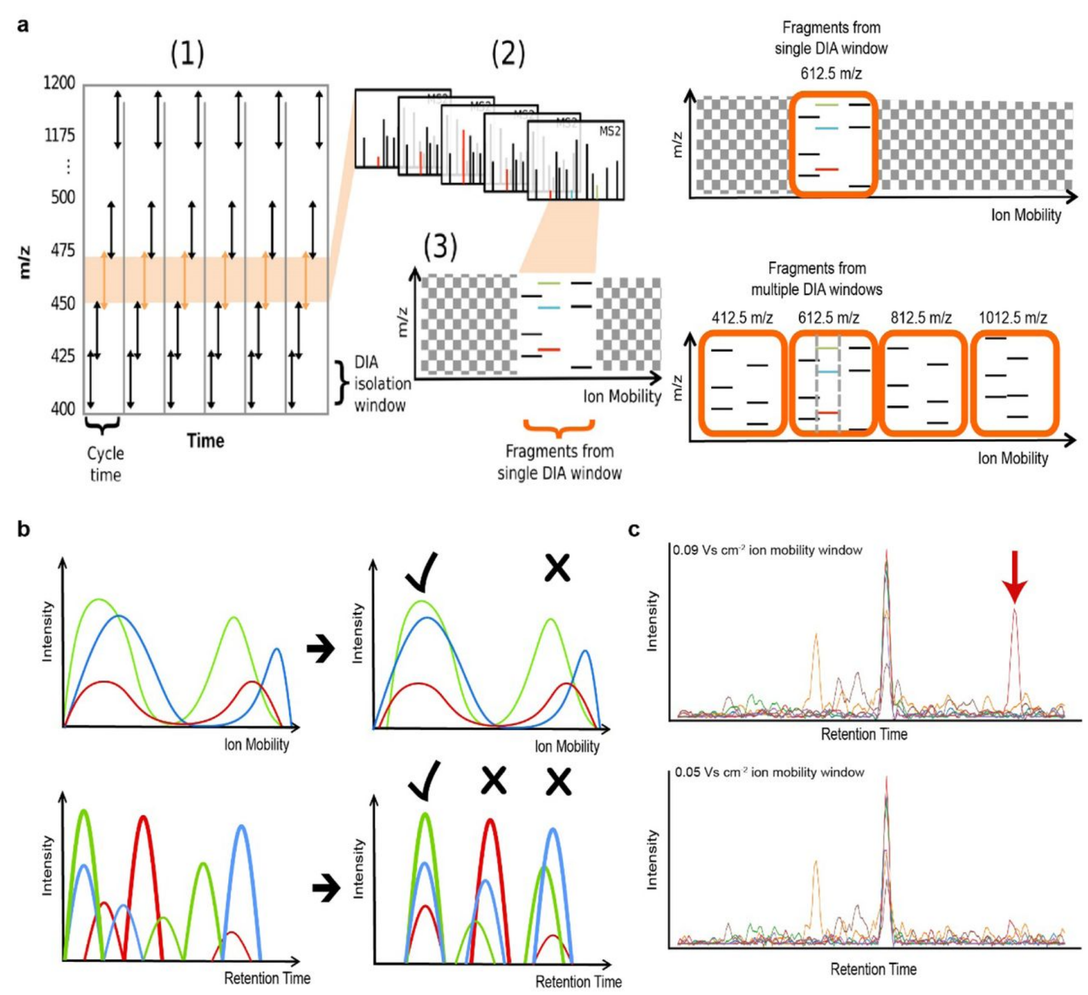

Fig. 2: Ion mobility-aware targeted data extraction.
a) Steps in the Mobi-DIK workflow to extract fragment ion chromatograms from multiple DIA windows in a single diaPASEF scan. b) Illustration of the data extraction from the four-dimensional data cuboid for a precursor with three transitions: red, blue and green. c) Extracted fragment ion chromatograms from a HeLa experiment with and without ion mobility-enhanced extraction to remove interfering signals from co-eluting precursors in the same DIA window.

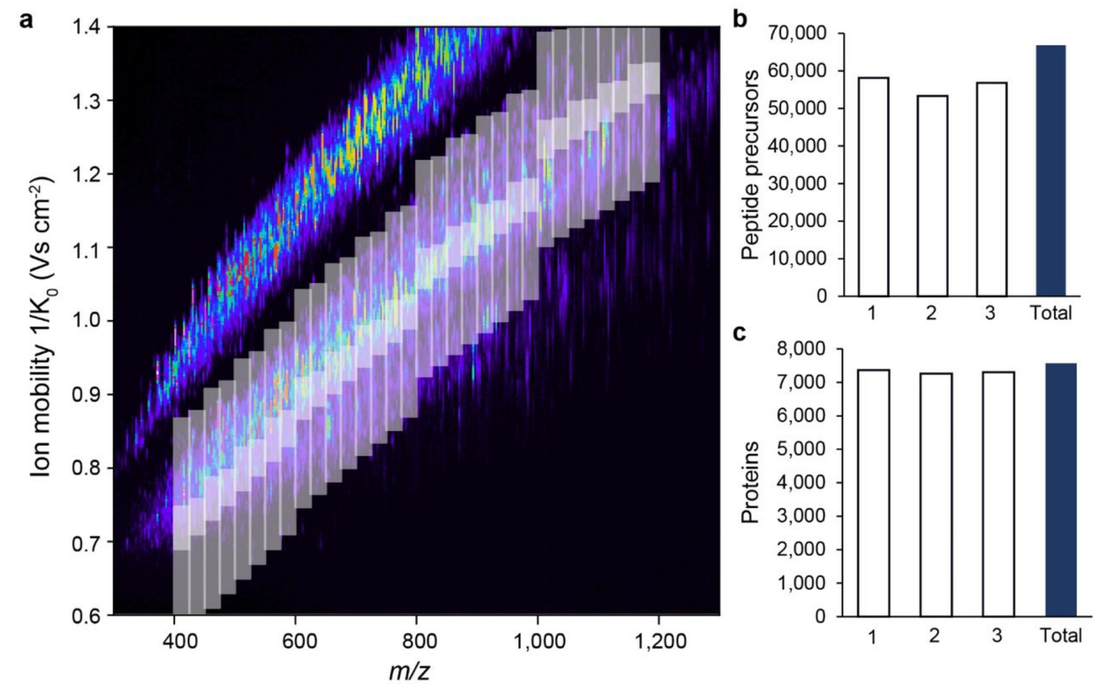

Fig. 3: HeLa proteome analysis with diaPASEF.
a) Position of the precursor isolation windows in the diaPASEF acquisition scheme overlaid on the average precursor ion intensity in an 120 min LC-MS experiment. b), c) Number of b, peptide precursor ions and c, proteins identified in triplicate injections of 200 ng HeLa digest.

Results

To identify and quantify peptides from the diaPASEF data, we used Mobi-DIK, a novel software based on the OpenMS package capable of analyzing the four-dimensional diaPASEF data (**Fig. 2**). The workflow generates ion mobility-enabled spectral libraries directly from highly fractionated DDA PASEF runs (using MaxQuant output files), which are used for targeted data extraction. The software automatically calibrates mass (non-linear), retention time (non-linear) and mobility (linear) between the generated assay library and experimental diaPASEF runs.

We investigated the performance of diaPASEF in single-run proteome analysis of a human HeLa cancer cell line. First, we built a project-specific library from 24 high-pH reversed-phase peptide fractions with DDA PASEF comprising 135,671 target precursors and 9,140 target proteins. For sample amounts on column of at least 200 ng and 120 min LC-MS runs, we reasoned that a diaPASEF method with a somewhat lower duty cycle, but higher precursor selectivity should be beneficial. We devised a method with four windows in each 100 ms diaPASEF scan. Eight of these scans covered the diagonal scan line for doubly charged peptides in the m/z-ion mobility plane and added a second, parallel scan line to ensure coverage of triply charged species with narrow 25 m/z precursor isolation windows.

Overall, we identified a total of 56,071 unique peptide sequences at 1% FDR, from which we inferred 7,565 proteins at a global protein FDR of 1% (**Fig. 3**). Out of these, 6,974 proteins were quantified in all three replicates, 406 in two and only 106 proteins in a single replicate, which translates into a data completeness of 96%.

Conclusions

- More than 7,000 proteins were reproducibly identified and quantified in single runs of 200 ng HeLa digest.
- With these excellent identification rates diaPASEF helps to go one step further towards the ideal mass analyzer.

timsTOF Pro

[1] Meier F. et al. (2018) Mol Cell Proteomics, doi:10.1074/mcp.TIR118.000900
[2] Meier F. et al. (2019) doi: https://doi.org/10.1101/656207
[3] http://openswath.org/en/latest/docs/mobi-dik.html