bioRxiv preprint doi: https://doi.org/10.1101/2022.10.31.514544; this version posted October 31, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

Slice-PASEF: fragmenting all ions for maximum sensitivity in proteomics

Lukasz Szyrwiel[1,*], Ludwig Sinn[1,*], Markus Ralser[1,2,✉] and Vadim Demichev[1,✉]

[1]Department of Biochemistry, Charité – Universitätsmedizin Berlin, Berlin, Germany
[2]The Wellcome Centre for Human Genetics, Nuffield Department of Medicine, University of Oxford, UK

*These authors contributed equally
✉ Correspondence to: markus.ralser@charite.de and vadim.demichev@charite.de

Abstract

We present Slice-PASEF, a novel mass spectrometry technology based on trapped ion mobility separation of ions. Slice-PASEF allows to achieve the theoretical maximum of MS/MS sensitivity and boosts proteomics of low sample amounts. Leveraging Slice-PASEF, we show, for the first time, that comprehensive profiling of single cell-level peptide amounts is possible using ultra-fast microflow chromatography and a general-purpose mass spectrometer, allowing quantification of 1417 proteins from 200 picograms of a HeLa cell peptide standard on an Evosep One LC system coupled to a timsTOF Pro 2, at a 200 samples per day throughput. We implemented a Slice-PASEF module in our DIA-NN data processing software, to make it readily available for the proteomics community.

bioRxiv preprint doi: https://doi.org/10.1101/2022.10.31.514544; this version posted October 31, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

Introduction

Mass spectrometry-based proteomics has seen a number of technological innovations in recent years, both at the level of instrumentation and data processing algorithms. These have enabled the emergence of novel applications with improved proteomic depth, robustness, quantitative accuracy, acquisition throughput and sensitivity[1,2]. Large-scale proteomic screens for systems biology experiments and biomarker discovery in patient cohorts are now streamlined[3–12]. Furthermore, recent high-sensitivity applications, such as single-cell proteomics and spatial tissue proteomics, have been rapidly gaining traction in the field[13–18]. High sensitivity applications in proteomics are currently hampered by two key limitations. First, the proteomic depth and quantification precision achieved from profiling low sample amounts is fold-change lower than that obtained from analysing bulk samples. Further gains here would thus provide a substantial boost to the ability to derive valuable biological conclusions or clinical implications. Second, low-sample-amount LC-MS methods benefit greatly from nanoflow liquid chromatography which facilitates the high sensitivity but comes at the cost of throughput and higher batch-variability.

Separation of analytes by ion mobility before they enter the quadrupole 1 (Q1) on a trapped ion mobility time-of-flight (TIMS-TOF) mass spectrometer[19] offers both enhanced selectivity and sensitivity of MS/MS acquisition, in a technology termed parallel accumulation and serial fragmentation (PASEF), and is one of the approaches that have improved throughput or sensitivity for a range of novel proteomic applications. The data-dependent acquisition (DDA) method termed PASEF[19,20], as well as a data-independent acquisition (DIA) method termed dia-PASEF[21], have been established for TIMS-capable mass spectrometers. We further increased sensitivity of dia-PASEF by developing a neural network-enabled data processing workflow for TIMS based on FragPipe and DIA-NN[2], yielding one of the most sensitive discovery proteomics methods to date. Soon after, this workflow was also demonstrated to enable comprehensive label-free single-cell proteomics using the timsTOF SCP mass spectrometer[22].

In this work, we introduce a novel family of data-independent TIMS-TOF methods, termed Slice-PASEF. Slice-PASEF is able to maximise the sensitivity through exploiting MS/MS duty cycles up 100%. To achieve this, Slice-PASEF employs continuous slicing of the precursor ion space with fragmentation of all ions in each slice. We show that the new method significantly increases the proteomic depth when analysing low sample amounts, compared to dia-PASEF. For instance, in the analysis of 10ng of a K562 peptide standard, Slice-PASEF increases precursor ion identification by 85% and increases the number of precisely quantified proteins 3.4-fold. Benchmarking the new method using a microflow LC system (Evosep One[23]) operated at 200 SPD (samples per day) throughput, we identify and quantify 1417 proteins from 200 picograms of a HeLa peptide preparation, using a timsTOF Pro 2 mass spectrometer, paving the way for robust and accessible large-scale single-cell proteomics. Slice-PASEF can be readily deployed using the standard instrument control software. We also integrated a Slice-PASEF module in our easy to use automated DIA-NN software suite[24] for streamlined application in any proteomics laboratory.

bioRxiv preprint doi: https://doi.org/10.1101/2022.10.31.514544; this version posted October 31, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

Results

Slice-PASEF: sliced fragmentation of precursor ions

A trapped ion mobility spectrometry (TIMS) system accumulates incoming ions and subsequently releases them - gradually and depending on their mobility in a gas (represented by the '1/K0' value). The released ions can be analysed directly (MS1 acquisition) or first be subjected to fragmentation. In the latter case, the acquisition is referred to as PASEF[19] (Parallel Accumulation – SErial Fragmentation). Peptide ions with z = 2 or 3 and a similar ion mobility have a limited spread in the m/z dimension, with an interquartile m/z range of approximately 50 m/z. This allows tuning the Q1 quadrupole to match with the release of precursor ions from the TIMS device, such that the Q1 isolation window, used to select precursor ions for fragmentation, changes depending on their ion mobility. The set of Q1 isolation windows corresponding to the release of all accumulated precursors from the TIMS device is referred to as 'frame', and different frames can feature different Q1 windows, constituting an acquisition scheme which can either be data-dependent or data-independent. Previously, the dia-PASEF technology has been introduced, wherein each frame features a low number (typically 2 to 5) of predefined, non-overlapping Q1 windows[21].

Conversely, in Slice-PASEF, which consists of a family of related methods (Figure 1), the precursor ions are sampled by splitting 'diagonally' the m/z * 1/K0 space into in general continuous slices and 'scanning' each of these slices using a high number of Q1 windows (10 - 15), independent of each other in the m/z dimension, with each window corresponding to a narrow 1/K0 range (0.03 - 0.045). The method is different in nature from the Scanning SWATH technology we introduced previously[6] as well as from the recently proposed implementation of the scanning quadrupole concept on TIMS-TOF mass spectrometers[25], since in the case of Slice-PASEF each fragment ion signal is captured with a single Q1 isolation window.

In this work, we describe and benchmark three types of Slice-PASEF schemes (Figure 1). First, a '1-frame' (1F) scheme, wherein a single frame is used to fragment all individual peptides with charge state 2 or 3, achieving 100% MS/MS duty cycle and theoretically maximal MS/MS sensitivity. Second, a '2-frame' (2F) family of methods, wherein each cycle is formed by a pair of frames, with the m/z boundary between these being varied across cycles. 2F methods achieve a 50% duty cycle. Third, a multi-frame (MF) family of methods, wherein the precursor ion space is sliced into multiple frames, with m/z boundaries between these being varied across cycles. Herein we benchmark a '4-frame' (4F) MF method, which has a 25% duty cycle.

bioRxiv preprint doi: https://doi.org/10.1101/2022.10.31.514544; this version posted October 31, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

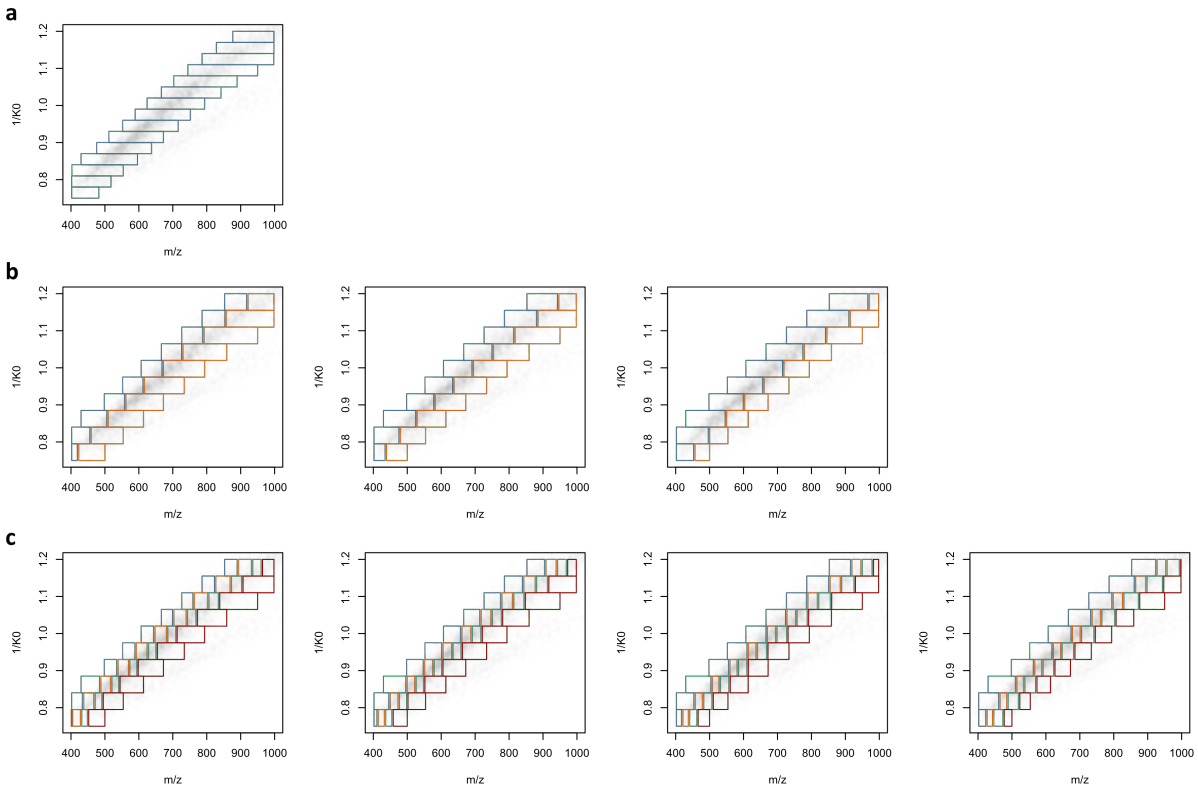

Fig. 1. Visualisation of Slice-PASEF methods. a. 1-frame (1F). **b.** 2-frame (2F). **c.** 4-frame (4F). Each individual plot indicates a method cycle. Each cycle is preceded by an MS1 scan. Within each cycle, the precursor ion space is fragmented in 'diagonal' slices, with each slice corresponding to a single PASEF frame and consisting of a number of isolation windows, which can overlap in the m/z dimension. Different slices are highlighted with different colours.

Slice-PASEF maximises sensitivity for the analysis of low sample amounts

To comprehensively benchmark Slice-PASEF against dia-PASEF, we assessed its performance on a dilution series of a commercial tryptic digest standard produced from the K562 human myelogenous leukaemia cell line, acquired in triplicates. We chose an analytical flow (500 µl/min) platform (1290 Infinity II LC, Agilent) coupled to a first-generation timsTOF Pro instrument (Bruker) and operated with a 5-minute chromatographic gradient. While analytical flow rate chromatography is not the system of choice for sensitive proteomics due to the high sample dilution, it is a convenient choice for conducting comparative benchmarks of acquisition methods and has the advantage of highly reproducible chromatography and high ion spray stability[4]. We compared Slice-PASEF to an 8-frame dia-PASEF scheme featuring 25 Da isolation windows and 12.5% MS/MS duty cycle, which we have optimised for this analytical flow platform. We note that this scheme is similar in its characteristics to the scheme (8-frame, 25 Da, 12.5% duty cycle) proposed by Brunner et al for single-cell dia-PASEF[22].

We observed that Slice-PASEF yields a substantial increase in protein identification numbers specifically with low injection amounts, particularly when using the high duty cycle schemes (Figure 2). For example, analysing 10ng K562 digest on the analytical flow setup, precursor numbers were increased by 85% and protein numbers by 52% using the 1F Slice-PASEF scheme, in comparison to dia-PASEF. As expected for a high-sensitivity method, the better coverage was achieved via the

bioRxiv preprint doi: https://doi.org/10.1101/2022.10.31.514544; this version posted October 31, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

identification of low-abundant peptides, missed by dia-PASEF (Figure 2c, left). For the jointly detected peptides, 1F recorded a higher signal (Figure 2c, right), with an average 5.7-times signal boost.

Further, all the Slice-PASEF methods demonstrated more precise quantification, at all injection amounts. For example, the 1F method was able to precisely quantify (coefficient of variation (CV) < 10%) 3.4-times more proteins than dia-PASEF from 10ng acquisitions.

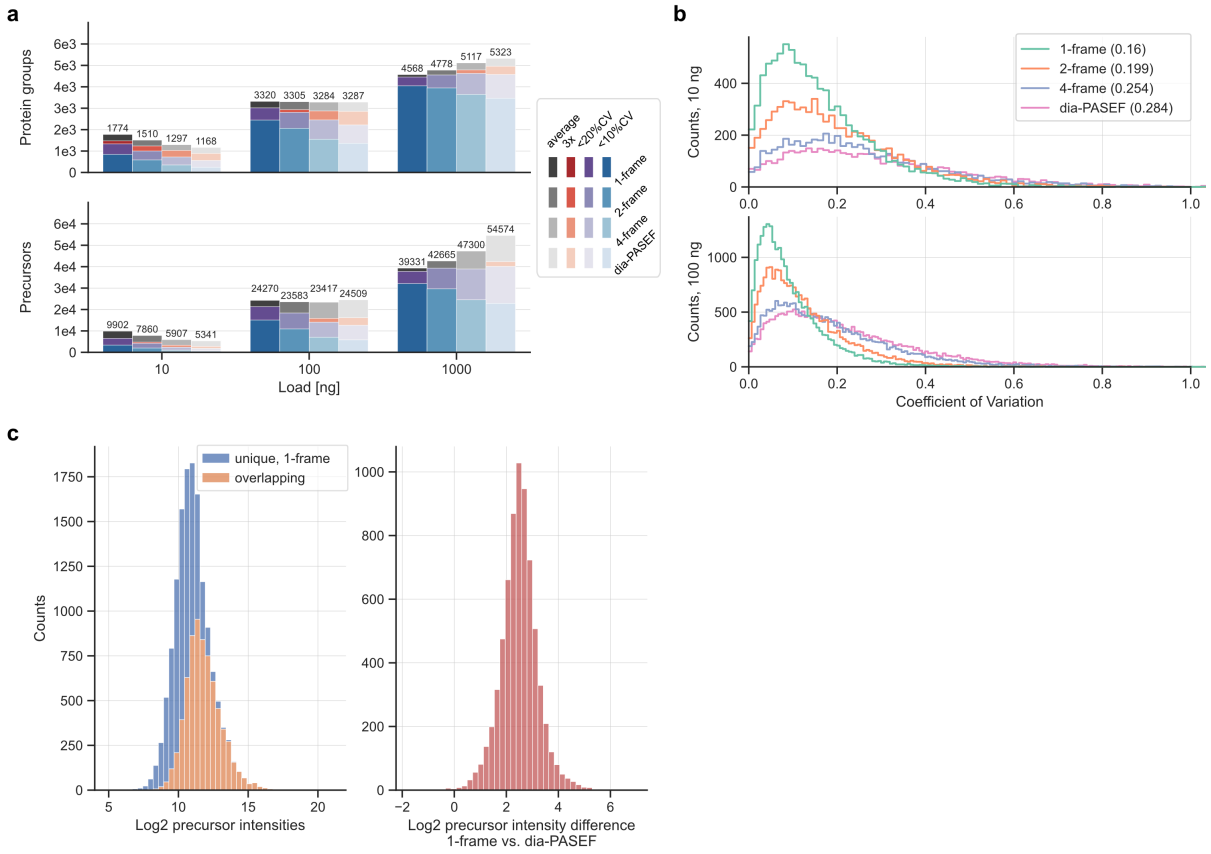

Fig. 2. Slice-PASEF increases sensitivity and quantitative precision in proteomic experiments with low sample amounts. a. Numbers of protein groups (upper panel) and precursors (lower panel) identified and quantified from different injection amounts of a K562 tryptic digest analysed in triplicates with a 5-minute 500 µl/min analytical flow gradient on Agilent 1290 II coupled to Bruker timsTOF Pro. **b.** Quantification precision, expressed as coefficient of variation (CV) distributions for precursor quantities in the 10ng and 100ng acquisitions. Median values for the 10ng acquisitions are indicated. **c.** Left: the distribution of log2-transformed precursor intensities in 1F 10ng acquisitions, with identifications unique to 1F and shared with dia-PASEF highlighted. Right: the distribution of log2-transformed intensity ratios for the shared precursors.

Finally, to validate the precursor quantities obtained for ultra low injection amounts of the peptide preparations (10ng), we plotted the respective log2-transformed quantities against the quantities of the same precursors obtained from the 100ng acquisitions used as a reference (Figure 3). In addition to a significantly higher number of precursors detected from 10ng of the standard, the 1F method also showed higher correlation between 10ng and 100ng sample quantities, indicative of better accuracy. In fact, even the correlation between 1F 10ng and dia-PASEF 100ng was higher than between 10ng and 100ng both acquired in dia-PASEF mode.

bioRxiv preprint doi: https://doi.org/10.1101/2022.10.31.514544; this version posted October 31, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

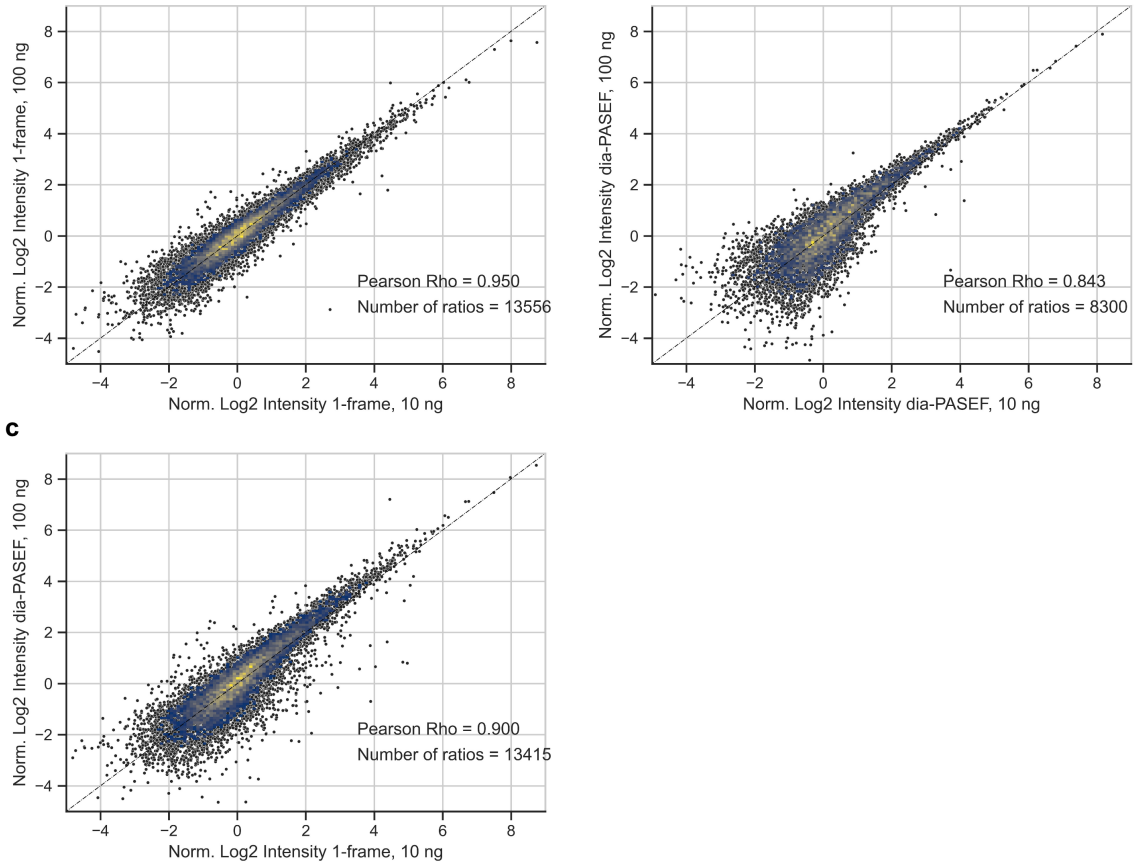

Fig. 3. Quantitative similarity between methods and injection amounts. Normalised log2-transformed intensities were plotted for precursor identifications shared between method and injection amount combinations, recorded for a K562 tryptic digest with a 5-minute 500 µl/min analytical flow gradient on Agilent 1290 II coupled to Bruker timsTOF Pro. In each case, a median was taken across three technical replicates. **a.** Comparison of 1F 100 ng and 1F 10ng acquisitions. **b.** Comparison of dia-PASEF 100ng and dia-PASEF 10ng acquisitions. **c.** Comparison of dia-PASEF 100ng and 1F 10ng acquisitions.

Slice-PASEF facilitates high-throughput microflow analysis of single cell-level amounts

Recent progress in mass spectrometry instrumentation and data processing methods has led to a rapidly increasing interest in novel technologies and applications that involve proteomic profiling of ultra-low peptide amounts. These facilitate, for example, single-cell proteomics or spatial proteomic profiling of tissues, which can offer unique biological and biomedical insights[13–18,26–29]. Still, so far these methods have inevitably required peptide separation using nanoflow gradients to achieve the required sensitivity, including, in some cases, flow rates below 100 nl/min. While highly sensitive, such setups have a number of limitations. First, they tend to have limited throughput. Second, in comparison to microflow setups, nanoflow setups are more prone to column clogging and emitter damage, and in general are more difficult to achieve reproducible chromatography on while requiring sophisticated operation and maintenance. Aiming at scaling up the throughput of sensitive proteomics applications, we speculated that the sensitivity of Slice-PASEF can allow to benefit from the greater

bioRxiv preprint doi: https://doi.org/10.1101/2022.10.31.514544; this version posted October 31, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

robustness and throughput of microflow chromatography[30,31], while still achieving comprehensive identification and quantification performance when analysing ultra-low peptide amounts.

Previously, acquisition on timsTOF series mass spectrometers coupled to data processing with DIA-NN has been shown to be suitable for both label-free[22] and multiplexed[27] single-cell proteomics. Brunner et al[22] demonstrated the capability of the Evosep One preformed gradient system, operated in a low-nanoflow (100 nl/min) mode, for deep proteomic profiling of single cells on a timsTOF SCP mass spectrometer, which is specifically optimised for the measurement of low sample amounts and has significantly higher sensitivity in this setting than timsTOF Pro. The same Evosep One system can also run microflow gradients at the throughput of hundreds of samples per day, and here we aimed to explore this capability for the measurement of ultra-low peptide amounts using Slice-PASEF. Specifically, we tested the 200 SPD (200 samples per day) Evosep method (2 µl/min flow) using the general-purpose timsTOF Pro 2 mass spectrometer.

To benchmark this setup, we analysed 0.2ng and 1ng of a HeLa cell line tryptic digest (Figure 4). With the 0.2ng injections, which roughly correspond to a peptide amount per single HeLa cell[32], we quantified, on average, 4840 precursors and 1417 proteins. The respective median CV was 13.8% on the protein level, from four replicate injections. Further, the quantities obtained from 0.2ng were similar to those obtained from 1ng, for jointly identified precursors, with the Pearson correlation between the log2-transformed quantities being 0.95.

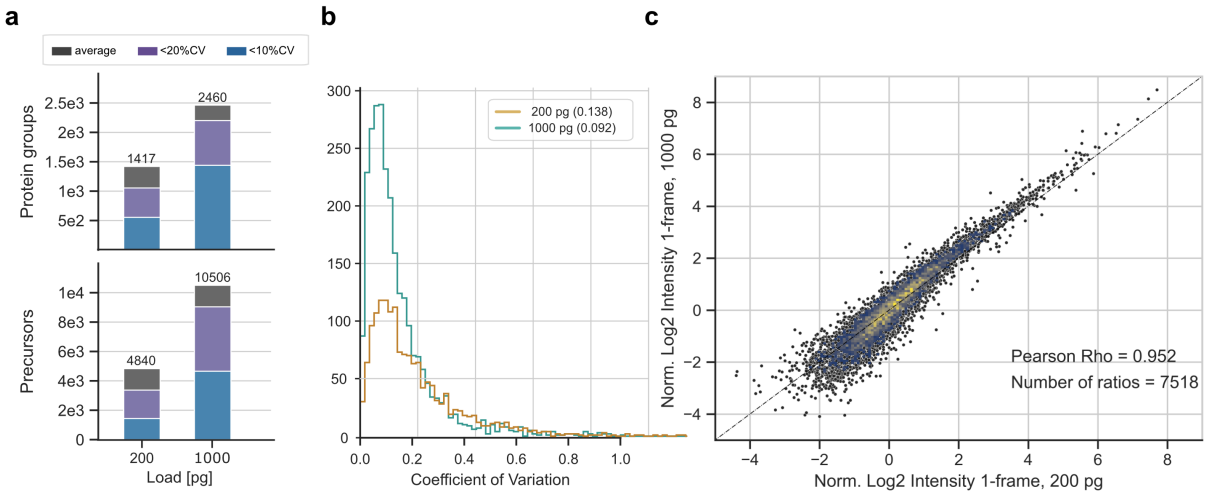

Fig. 4. Slice-PASEF combined with fast microflow chromatography. a. Numbers of protein groups (upper panel) and precursors (lower panel) identified and quantified from 200pg of a Hela tryptic standard analysed in 4 replicates using a 200 samples per day 2 µl/min method on Evosep One coupled to Bruker timsTOF Pro 2. **b.** CV distributions for protein quantities, median values are indicated. **c.** Normalised log2-transformed intensities plotted for precursor identifications shared between 200pg and 1ng acquisitions. In each case, a median was taken across four technical replicates.

bioRxiv preprint doi: https://doi.org/10.1101/2022.10.31.514544; this version posted October 31, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

Discussion

We describe Slice-PASEF, a family of data-independent proteomic methods utilising trapped ion mobility separation. In Slice-PASEF, precursor ion space is split into slices for fragmentation. Slice-PASEF allows for high flexibility in method design. First, Slice-PASEF allows for arbitrarily high or low MS/MS duty cycles, thus balancing sensitivity and selectivity. This includes enabling 100% MS/MS duty cycle by fragmenting almost all individual charge 2 or 3 peptide ions released by the TIMS device, i.e. achieving the theoretical maximum of MS/MS sensitivity. Second, Slice-PASEF allows to tailor the Q1 isolation window boundaries to achieve optimal separation of peptides in each 1/K0 range bin. Third, Slice-PASEF allows to vary the isolation window boundaries in different DIA cycles in an arbitrary way, benefiting mass deconvolution of MS/MS signals in the DIA-NN software, improving selectivity. Fourth, any of the Slice-PASEF frames can be repeated a number of times (up to 5 times, in the methods described herein), with the signals from these repeats being merged by the DIA-NN software. This can be done to either boost sensitivity or to fit MS cycle time to a specific LC gradient.

We report that Slice-PASEF significantly outperforms dia-PASEF for proteome profiling of low sample amounts, both in terms of identification and quantification performance. To our knowledge, Slice-PASEF is hence currently the most sensitive approach for discovery proteomics. For instance, Slice-PASEF facilitated proteomics of single cell-level peptide amounts using high-throughput microflow chromatography, paving the way for large-scale single-cell experiments and other high-sensitivity applications, such as spatial profiling of tissues. Slice-PASEF works using the production acquisition software. Importantly, we have incorporated a Slice-PASEF module into DIA-NN[24], a universal DIA data processing software suite, to make this technology broadly accessible to the proteomics community.

In this work, we tested Slice-PASEF on a readily available LC-MS platform - the Evosep One 200 SPD setup coupled to a timsTOF Pro 2. Naturally, we would expect a further gain in sensitivity if using dedicated instruments, such as timsTOF SCP[22]. Further, the Evosep One system also features even faster methods, such as 300 SPD, which can likewise be coupled to Slice-PASEF. In addition, the Slice-PASEF module in DIA-NN is compatible with plexDIA, and hence throughput can be further tripled by using multiplexing with mTRAQ labels, as we have described previously for regular DIA[27]. So far, we have benchmarked only some of the potential Slice-PASEF methods. The concept of Slice-PASEF is highly flexible, i.e. in each DIA cycle a different slicing approach can be used to fragment precursor ions. For example, one can devise a scheme which combines 1-frame fragmentation for even cycles and multi-frame fragmentation for odd cycles, thus achieving both, a >50% overall MS/MS duty cycle, as well as the selectivity in the m/z dimension afforded by the multi-frame methods.

bioRxiv preprint doi: https://doi.org/10.1101/2022.10.31.514544; this version posted October 31, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

Methods

LC-MS

Both analysed samples, a HeLa tryptic digest (prepared as described previously[2]) and a K562 tryptic digest (V6951, Promega) were diluted in 0.1% FA.

For analytical flow proteomics we used the Agilent 1290 Infinity II liquid chromatography system coupled to the Bruker timsTOF Pro mass spectrometer equipped with the VIP-HESI source (3000 V of Capillary voltage, 10.0 l/min of Dry gas and temperature 280 °C, probe gas flow 4.8 l/min and temperature 450 °C). The peptide separation was performed on a Luna Omega 1.6 µm C18 100 Å 30 x 2.1 mm column at 60°C using a linear gradient ramping from 3% B to 36% B in 5 minutes (Buffer A: 0.1% FA; Buffer B: ACN/0.1% FA) with a flow rate of 500 µl/min. The column was washed using an increase to 80% B in 0.5 min and a flow rate of 850 µl/min, maintained for another 0.2 min. In the next 0.1 min, the B proportion was changed to 3% and flow was reduced to 600 µl/min after 1.2 min and 500 µl/min after 0.3 min. For the dia-PASEF method, the MS/MS precursor mass range was m/z 401 to 1226 and 1/*K*0 0.72 to 1.29, with 33 x 25 Th windows with ramp and accumulation time 72 ms and cell cycle estimate 0.7 s. For the 1F, 2F and 4F Slice-PASEF methods the ramp and accumulation time were 100 ms and the windows setup was chosen as presented on Figure 1 (method definition files are available at https://osf.io/t2ymc/?view_only=7462fffb20e648fc83afc75d8c67e9f8). The m/z range was 400 to 1000 and the 1/*K*0 range 0.75 to 1.2. All methods were used in the high sensitivity mode of the mass spectrometer.

For the 200 SPD method, the Evosep One system was coupled with the Bruker timsTOF Pro 2 mass spectrometer equipped with the Bruker Captive Spray source. The Endurance Column 4 cm x 150 µm ID, 1.9 µm beads (EV1107, Evosep) was connected to a Captive Spray emitter (ZDV) with a diameter 20 µm (1865710, Bruker). The source parameters were kept as in standard methods offered by Bruker (Capillary voltage 1400 V, Dry Gas 3.0 l/min and Dry Temp 180 °C). The Evotips were loaded and maintained following the protocol by the manufacturer.

The Slice-PASEF and dia-PASEF methods were set up in the Bruker timsControl software (v3.0.0, analytical flow setup, and v1.1.19 68, Evosep One), by importing a text table method definition file containing the isolation window specification.

Raw data processing

The data were processed using DIA-NN 1.8.2 beta 11, which is available, along with the DIA-NN pipeline that specifies all the settings used to process the data sets described in this manuscript, at https://osf.io/t2ymc/?view_only=7462fffb20e648fc83afc75d8c67e9f8. Briefly, the mass accuracies were fixed to 15ppm (both MS1 and MS2), and the scan window was set to 6 (analytical flow) or 7 (Evosep) analyses. Protein inference was disabled, to use the protein grouping already present in the spectral library. The spectral library[2] that was used to analyse the HeLa 0.2ng and 1ng acquisitions on the Evosep One system was first refined using an analysis of 5ng HeLa acquisitions, with the Library generation strategy set to IDs, RT & IM profiling. The --tims-scan option was supplied to DIA-NN for the analysis of Slice-PASEF acquisitions. Acquisitions corresponding to each combination of the method and the injection amount were analysed separately, and for protein-level benchmarks, the

bioRxiv preprint doi: https://doi.org/10.1101/2022.10.31.514544; this version posted October 31, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

output was filtered using 1% global protein q-value. For direct comparison of precursor quantities between different methods and injection amounts, joint analyses of the respective acquisitions were performed. In the case of the analytical flow acquisitions, DIA-NN was then supplied with the --restrict-fr and --no-fr-selection commands, which ensured that it used the same fragments to quantify precursor ions in different runs.

Data availability

Slice-PASEF and dia-PASEF mass spectrometry acquisitions have been deposited to the OSF repository https://osf.io/t2ymc/?view_only=7462fffb20e648fc83afc75d8c67e9f8. The Slice-PASEF methods, the DIA-NN 1.8.2 beta 11 setup file, the DIA-NN output reports, the DIA-NN pipeline file and the spectral libraries have likewise been deposited by the same link.

Acknowledgements

This work is funded by the German Ministry of Education and Research (BMBF), as part of the National Research Node “Mass spectrometry in Systems Medicine” (MSCoreSys), under grant agreements 161L0221 (to V.D.) and 031L0220 (to M.R.).

References

1. Ludwig, C. *et al.* Data-independent acquisition-based SWATH-MS for quantitative proteomics: a tutorial. *Mol. Syst. Biol.* **14**, e8126 (2018).
2. Demichev, V. *et al.* dia-PASEF data analysis using FragPipe and DIA-NN for deep proteomics of low sample amounts. *Nat. Commun.* **13**, 3944 (2022).
3. Bruderer, R. *et al.* Analysis of 1508 Plasma Samples by Capillary-Flow Data-Independent Acquisition Profiles Proteomics of Weight Loss and Maintenance. *Mol. Cell. Proteomics* **18**, 1242–1254 (2019).
4. Messner, C. B. *et al.* Ultra-High-Throughput Clinical Proteomics Reveals Classifiers of COVID-19 Infection. *Cell Syst* **11**, 11–24.e4 (2020).
5. Demichev, V. *et al.* A time-resolved proteomic and prognostic map of COVID-19. *Cell Syst* **12**, 780–794.e7 (2021).
6. Messner, C. B. *et al.* Ultra-fast proteomics with Scanning SWATH. *Nat. Biotechnol.* 1–9 (2021).

bioRxiv preprint doi: https://doi.org/10.1101/2022.10.31.514544; this version posted October 31, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

7. Slavov, N. Increasing proteomics throughput. *Nature biotechnology* vol. 39 809–810 (2021).

8. Geyer, P. E. *et al.* High-resolution serum proteome trajectories in COVID-19 reveal patient-specific seroconversion. *EMBO Mol. Med.* **13**, e14167 (2021).

9. Niu, L., Thiele, M., Geyer, P. & Rasmussen, D. N. A derivation and validation study of paired liver and plasma proteomics in 659 individuals reveals circulating biomarkers for alcohol-related liver disease. *J. At. Mol. Phys.*

10. Messner, C. B. *et al.* The Proteomic Landscape of Genome-Wide Genetic Perturbations. *bioRxiv* 2022.05.17.492318 (2022) doi:10.1101/2022.05.17.492318.

11. Muenzner, J. *et al.* The natural diversity of the yeast proteome reveals chromosome-wide dosage compensation in aneuploids. *bioRxiv* 2022.04.06.487392 (2022) doi:10.1101/2022.04.06.487392.

12. Gonçalves, E. *et al.* Pan-cancer proteomic map of 949 human cell lines. *Cancer Cell* **40**, 835–849.e8 (2022).

13. Slavov, N. Driving Single Cell Proteomics Forward with Innovation. *J. Proteome Res.* **20**, 4915–4918 (2021).

14. Mund, A., Brunner, A.-D. & Mann, M. Unbiased spatial proteomics with single-cell resolution in tissues. *Mol. Cell* **82**, 2335–2349 (2022).

15. Mund, A. *et al.* Deep Visual Proteomics defines single-cell identity and heterogeneity. *Nat. Biotechnol.* **40**, 1231–1240 (2022).

16. Slavov, N. Scaling Up Single-Cell Proteomics. *Mol. Cell. Proteomics* **21**, 100179 (2022).

17. Burnum-Johnson, K. E. *et al.* New Views of Old Proteins: Clarifying the Enigmatic Proteome. *Mol. Cell. Proteomics* **21**, 100254 (2022).

18. Nwosu, A. J. *et al.* In-Depth Mass Spectrometry-Based Proteomics of Formalin-Fixed, Paraffin-Embedded Tissues with a Spatial Resolution of 50-200 μm. *J. Proteome Res.* **21**, 2237–2245 (2022).

19. Meier, F. *et al.* Parallel Accumulation–Serial Fragmentation (PASEF): Multiplying Sequencing Speed and Sensitivity by Synchronized Scans in a Trapped Ion Mobility Device. *J. Proteome Res.* **14**, 5378–5387 (2015).

20. Meier, F. *et al.* Online Parallel Accumulation-Serial Fragmentation (PASEF) with a Novel

bioRxiv preprint doi: https://doi.org/10.1101/2022.10.31.514544; this version posted October 31, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

Trapped Ion Mobility Mass Spectrometer. *Mol. Cell. Proteomics* **17**, 2534–2545 (2018).

21. Meier, F. *et al.* diaPASEF: parallel accumulation–serial fragmentation combined with data-independent acquisition. *Nat. Methods* **17**, 1229–1236 (2020).
22. Brunner, A.-D. *et al.* Ultra-high sensitivity mass spectrometry quantifies single-cell proteome changes upon perturbation. *Mol. Syst. Biol.* **18**, e10798 (2022).
23. Bache, N. *et al.* A Novel LC System Embeds Analytes in Pre-formed Gradients for Rapid, Ultra-robust Proteomics. *Mol. Cell. Proteomics* **17**, 2284–2296 (2018).
24. Demichev, V., Messner, C. B., Vernardis, S. I., Lilley, K. S. & Ralser, M. DIA-NN: neural networks and interference correction enable deep proteome coverage in high throughput. *Nat. Methods* **17**, 41–44 (2020).
25. Tenzer, S. Method and apparatus for data independent combined ion mobility and mass spectroscopy analysis. *US Patent* (2022).
26. Specht, H. *et al.* Single-cell proteomic and transcriptomic analysis of macrophage heterogeneity using SCoPE2. *Genome Biol.* **22**, 50 (2021).
27. Derks, J. *et al.* Increasing the throughput of sensitive proteomics by plexDIA. *Nat. Biotechnol.* (2022) doi:10.1038/s41587-022-01389-w.
28. Huffman, R. G. *et al.* Prioritized single-cell proteomics reveals molecular and functional polarization across primary macrophages. Preprint at https://doi.org/10.1101/2022.03.16.484655.
29. Webber, K. G. I. *et al.* Label-Free Profiling of up to 200 Single-Cell Proteomes per Day Using a Dual-Column Nanoflow Liquid Chromatography Platform. *Anal. Chem.* **94**, 6017–6025 (2022).
30. Vowinckel, J. *et al.* Cost-effective generation of precise label-free quantitative proteomes in high-throughput by microLC and data-independent acquisition. *Sci. Rep.* **8**, 4346 (2018).
31. Bian, Y. *et al.* Robust, reproducible and quantitative analysis of thousands of proteomes by micro-flow LC–MS/MS. *Nat. Commun.* **11**, 1–12 (2020).
32. Kulak, N. A., Pichler, G., Paron, I., Nagaraj, N. & Mann, M. Minimal, encapsulated proteomic-sample processing applied to copy-number estimation in eukaryotic cells. *Nat. Methods* **11**, 319–324 (2014).