Significantly faster library-free dia-PASEF analysis with a Spectronaut integrated workflow in ProteoScape

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Introduction:

Bruker ProteoScape (BPS), formerly PaSER, has been transforming into a comprehensive proteomics data analysis platform that can integrate third-party tools while utilizing the concept of data streaming to realize fully customizable real-time processing workflows including on-the fly decision making based on the data generated. Recent advances in technology have decidedly allowed the field to migrate towards fast LC gradients, allowing for increasingly larger sample cohorts in studies in both data-dependent acquisition (DDA) and data-independent acquisition (DIA) modes. "Library-free" analysis of DIA data has also grown to be an increasingly alternative to generating project specific spectral library from DDA acquisitions. A significant downside of these "library-free" workflows is longer processing times due to the increased search space, especially when PTMs are considered.

Here we've integrated a workflow including a Spectronaut (SN) module in Bruker ProteoScape (BPS) to take advantage of the synergistic capabilities of the two software. We show that BPS is a natural platform for faster library free analysis of dia-PASEF data using Spectronaut's directDIA+ workflow by:

1. Allowing data to be processed at a single injection level, before combining at the project level.
2. Providing same results, including FDR control, for both single injection and project level analysis.
3. Providing an intuitive user interface for the entire workflow.

We utilize this new workflow on three datasets, a multi-species dataset with know ratios to help evaluate precision and accuracy of the workflow and can show more than 40% savings in time-to-results vs analysis fully after the acquisition and two large datasets. BPS can provide quick visualization of both injection level data and project level data, while detailed peptide\spectrum level analysis can be explored in a standalone Spectronaut version.

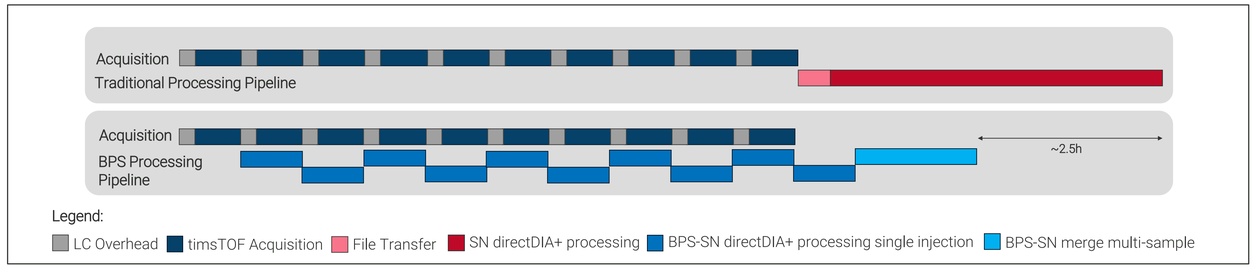

Fig. 1: BPS allows for automated dia-PASEF data processing. In this example with 2 samples and 5 replicates, with a 45min LC gradient and 15min LC overhead, users can get fully processed data 2.5h faster or ~ 40% faster. With traditional processing pipelines, users need to wait until all files have been acquired before transfer and subsequent processing. With BPS processing, run-by-run analysis is performed during the acquisition queue, so users are able to evaluate the data quality for each injection. At the end of the acquisition queue, users can trigger a multi-sample combine workflow, providing a project-wide view for further analysis.

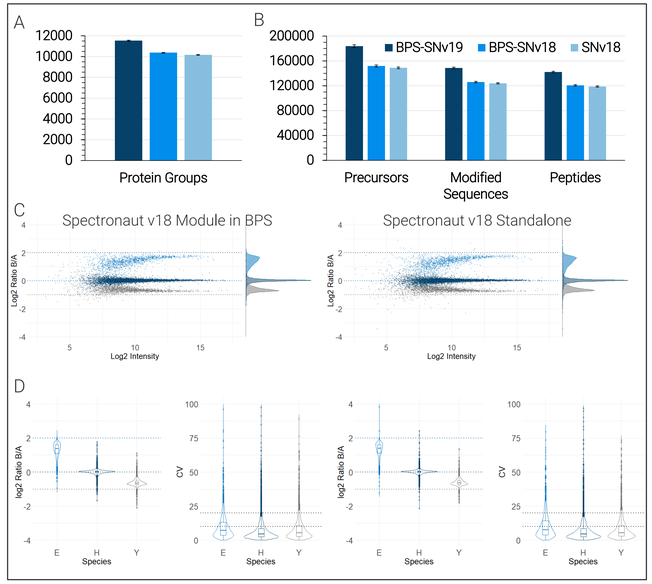

Fig. 2: The Spectronaut module in BPS and standalone version produce comparable results with regards to identification as well as quantitation accuracy and precision. Spectronaut 19 shows marked increase in the average number of identifications across all 10 replicates by Protein Groups (A) and by Precursor, Modified Sequences and Peptides are similar. (C) Shows intensity vs ratio distribution by species for a dataset with 5 replicates. (D) The expected and measure ratios for all protein groups and the CV by species are all comparable.

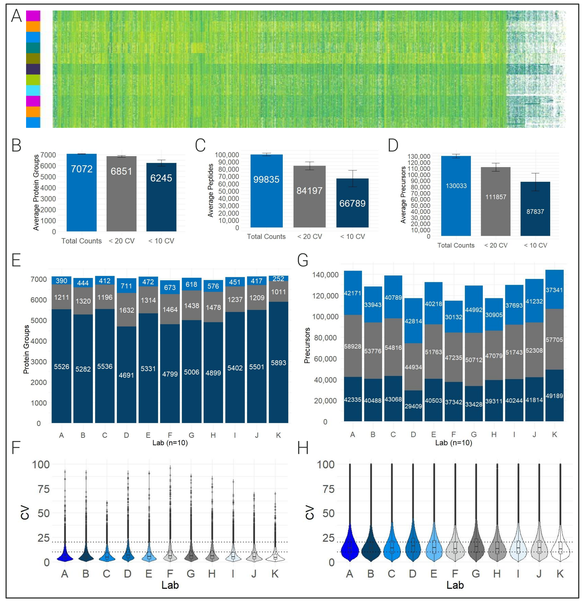

Fig. 3: An interlaboratory study, involving 11 sites, where each site injected 10 replicates of 200ng K562 (Promega) lysate using nanoElute with 5min active LC gradient (7min total acquisition) and analyzed by dia-PASEF on timsTOF HT instruments, was re-analyzed using the directDIA workflow in BPS. All 110 samples were processed as ID runs and then quantified together. (A) An heatmap view generated by BPS for all quantified protein groups. The average number of (B) protein group, (C) peptides and (D) precursors identified, quantified with CV<20% and CV<10%. Similarly, a site-based breakdown for identified, quantified with CV<20% and CV<10% at the (E-F) protein group level and (G-H) precursors level, as well as their respective CV violin plots (dashed lines indicating 10 and 20%).
In summary, the timsTOF platform enables high throughput proteomics with deep proteome coverage and highly reproducible quantitation in short gradients of 5 minutes. More than 7000 protein groups can be identified on average in 5-minute gradient time across 11 different labs.

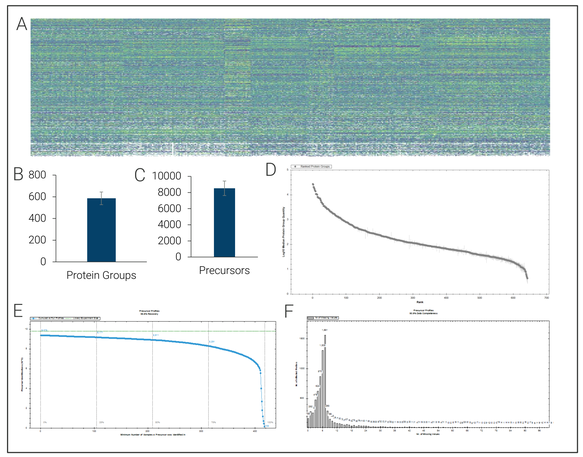

Fig. 4: The Spectronaut module in BPS is capable of scaling to process larger studies that are becoming typical in proteomics. 418 neat Plasma samples were acquired with 5min active gradient on timsTOF HT in dia-PASEF mode using PepSep Max columns with nanoElute2. The data were analyzed using the SNv19 directDIA workflow in BPS. (A) A heatmap of the 418 samples, with vertical white streaks showing poor quality samples. Average protein groups (B) and precursors (C) identified across all samples. The data is readily exported from BPS and available in Spectronaut standalone for further exploration such as (D) ranked protein groups, (E) data completeness and (F) data missingness among other more through visualizations.

Acknowledgements
Bruker thanks the creation of the Mestrelab Research Center (CIM) subsidized by the Galician Innovation Agency. Operation funded by the Xunta de Galicia, through the business aid program for the creation and integration of new business research centers 001_IN853D_2022.

Conclusion

- ProteoScape has workflows integrating academic algorithms, such as DIA-NN, or 3rd party algorithms, such as Novor, and now Spectronaut greatly reducing time-to-results for large projects while simultaneously providing detailed results at the single injection level.
- ProteoScape Spectronaut projects can be readily exported and examined in Spectronaut standalone, or they can be exported for 3rd party post-analysis, such as Mass Dynamics, R or Python.

Technology