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dia-PASEF Visualization Tool: a shiny App for data visualization and exploration of dia-PASEF data

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Introduction

- dia-PASEF has matured into a powerful and widely used analytical technique in the biological and clinical field[1].
- The throughput of sample acquisition and data analysis has increased drastically due to the improvements in TIMS technology and data analysis software tools.
- To quickly inspect the results of dia-PASEF, we developed a dia-PASEF Tools Shiny App. This app is a user-friendly tool designed to streamline the process of exploring and visualizing dia-PASEF results.
- The app uses the reports from common software tools, DIA-NN, Spectronaut and tims DIA-NN, and offers a user-friendly interface and a variety of interactive charts and graphs to help users better understand their data. We illustrate its use with a dilution series experiment acquired using dia-PASEF.

Methods

- BSA peptides were spiked into a commercially available human digest (Promega K562) to generate a dilution series sample set with peptide concentrations ranging from 380 amol to 12.5 fmol.
- The samples were analyzed using dia-PASEF on a Bruker TimsTOF HT coupled to an EvoSep system (30SPD).
- dia-PASEF covered the range of 350-1200 m/z and 0.7-1.3 1/k0 with a cycle time of 1.5 seconds.
- The data analysis was done using Spectronaut (v17.6 Biognosys).

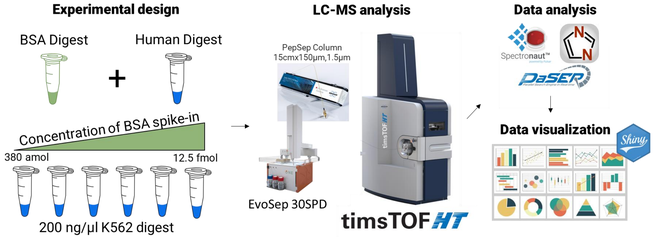

Fig. 1. Experimental design for the test dataset. BSA peptides were spiked in a human digest (Promega K562) to create a dilution series sample set, ranging in peptide concentrations from 380 amol to 12.5 fmol. The data was acquired on a Bruker timsTOF HT coupled to an EvoSep (30SPD). The shiny App takes the input from Spectronaut, TIMS DIA-NN or DIA-NN and facilitates the data visualization

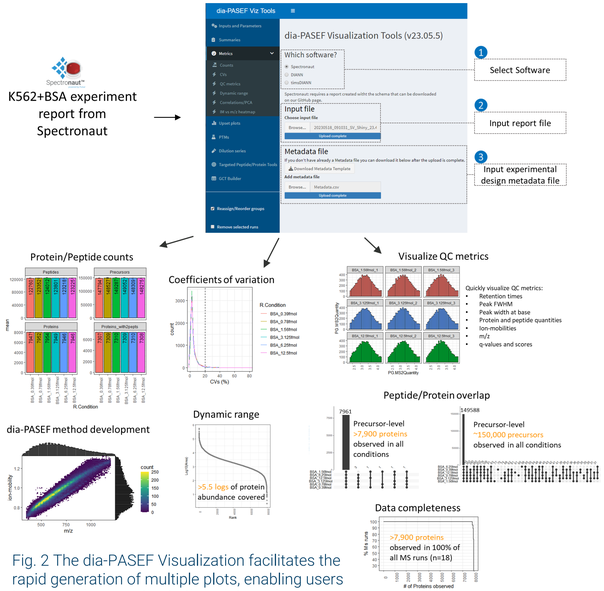

Fig. 2 The dia-PASEF Visualization facilitates the rapid generation of multiple plots, enabling users to thoroughly investigate their data and make prompt decisions.

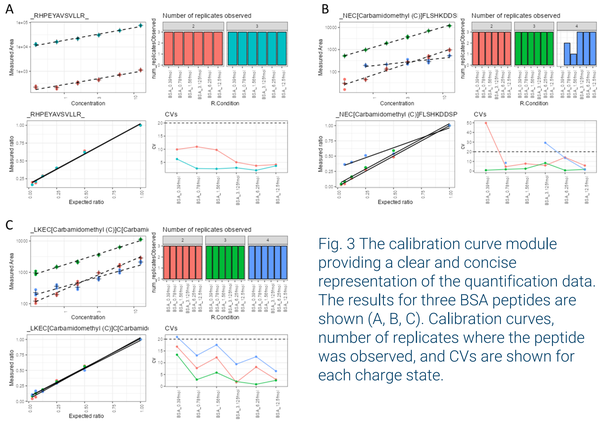

Fig. 3 The calibration curve module providing a clear and concise representation of the quantification data. The results for three BSA peptides are shown (A, B, C). Calibration curves, number of replicates where the peptide was observed, and CVs are shown for each charge state.

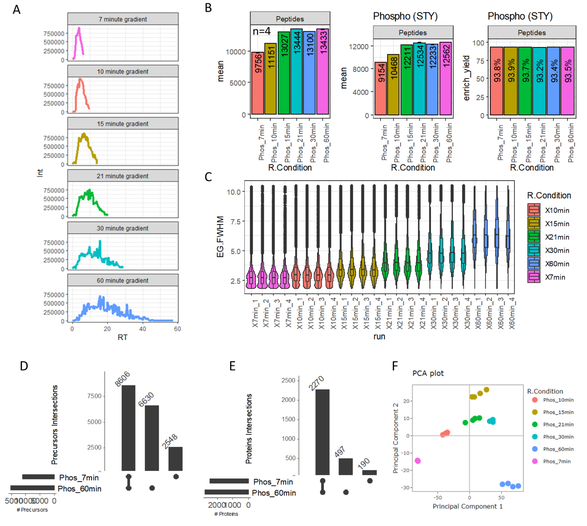

Fig. 4 The PTM module enables the exploration of PTM data. The data was taken form the work of *Oliinyk et al. Proteomics, 2023* (ProteomeXchange Consortium ID: PXD033904). The phosphoproteomics data was acquired on a timsTOF HT using multiple gradient lengths (7 to 60 minutes)[2]. The dia-PASEF Visualization Tool, allows for a swift exploration of the dataset and gain insights in a timely manner. Total ion chromatograms (A), peptide/phosphopeptides counts, phospho enrichment yield (B), chromatographic FWHM (C) are shown as an example. We compared the results from 7-min and 60-min gradient. The peptide (D) and protein (E) identification overlaps, a PCA plot (F) and correlations of quantified areas (G) are shown.

Code availability

Sebastian-Vaca/diaPASEF_Viz_Tool

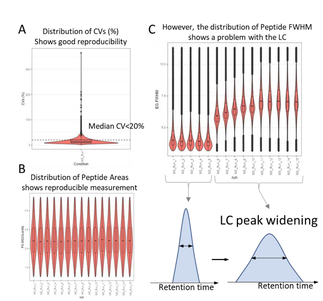

Fig. 5 Evaluation of instrument performance can rapidly be assessed by the App. In this example, 15 MS runs where acquired with excellent reproducibility having a median CV<20% (A) and showing consistent peak areas (B). However, the FHWM increased over time (C). A leak on the LC system could be identified and repaired before the quantification performance was hindered.

Results

- The dia-PASEF Visualization Tool provides a streamlined and efficient solution for the visualization and analysis of complex quantitative proteomic results.
- The App is compatible with native reports from DIA-NN, TIMS DIA-NN or Spectronaut. The experimental design can be entered with a metadata file.
- The user can input a list of target proteins or peptides, giving them the ability to extract information for a subpopulation of peptides of interest (PTMs, quantification targets)
- For our test dataset:
  - We identified ~9K proteins and ~140K peptide precursors ions in each 40-min MS run
  - Data completeness was higher than 90% covering 5-logs in protein abundance.
  - The mean and median coefficient of variation was below 10%.
  - The dilution series analysis shows excellent linearity (r^2>0.95) for most of the targets across the entire concentration range.

Conclusion

- The dia-PASEF Visualization Tool, is a user-friendly app that provides a streamlined and efficient solution for visualization and analysis of dia-PASEF results from several DIA software tools.
- Better data visualization can make it easier to identify patterns, trends, and relationships that may not be immediately apparent from raw data.
- Clear and concise data representation accelerates decision-making and help QC instrument performance.
- The dia-PASEF Visualization Tool can save time and resources by allowing users to quickly identify areas of interest and make informed decisions without having to manually sift through large amounts of raw data.

Technology

[1] Meier, F., Brunner, AD., Frank, M. et al. Nat Methods 17, 1229–1236 (2020). https://doi.org/10.1038/s41592-020-00998-0

[2] Oliinyk, D., & Meier, F. (2023) Proteomics, 23, e2200032. https://doi.org/10.1002/pmic.202200032