timsplot: A Python Shiny App for Visualizing timsTOF Proteomics Results

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Introduction

The timsTOF platform has garnered a strong foothold in the field of proteomics, and propagation of high-throughput methods in industry and academia necessitates having tools for fast and straightforward data inspection.

We developed timsplot as a tool for visualizing timsTOF data. The app uses reports from common search software (Spectronaut, FragPipe, DIA-NN, and Bruker ProteoScape) as well as raw data files generated by timsTOF instruments to generate figures for data exploration and presentation in a user-friendly interface.

We demonstrate its use with a dilution series acquired using DIA-PASEF and immunopeptidomics data acquired using DDA-PASEF.

Methods

A series of dilutions were made from a Promega K562 human digest to generate a dilution series sample set with injection loads ranging from 15 pg to 16 ng. These samples were separated on an IonOpticks 25 cm C18 column using a 30-minute gradient and were analyzed using DIA-PASEF mode on a Bruker timsTOF Ultra with a NanoElute 2 LC system. Post-acquisition analysis was done using Spectronaut 19.4 (Biognosys).

The second group of samples were class I and class II immunopeptides. These were separated on an IonOpticks 25 cm C18 column using a 60-minute gradient and were analyzed using DDA-PASEF mode on a Bruker timsTOF Ultra 2 with a NanoElute 2 LC system. Post-acquisition analysis was done using FragPipe.

Code Availability

- The timsplot code can be freely downloaded and installed from GitHub.
  github.com/zack-kirsch/timsplot
- DISCLAIMER: the timsplot app is not a Bruker product and is not supported by Bruker. It is provided “as-is”. Any inquiries, bug reports, or feature requests should not be directed to Bruker.

COI Statement:
Z.K., M.K.P., D.A., R.S., and M.W. are employees of Bruker Daltonics.

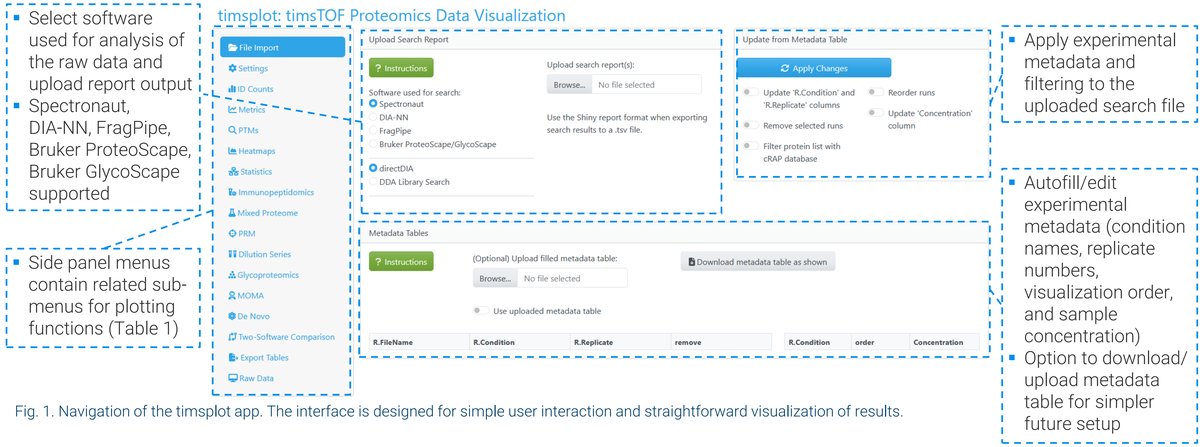

Fig. 1. Navigation of the timsplot app. The interface is designed for simple user interaction and straightforward visualization of results.

Results

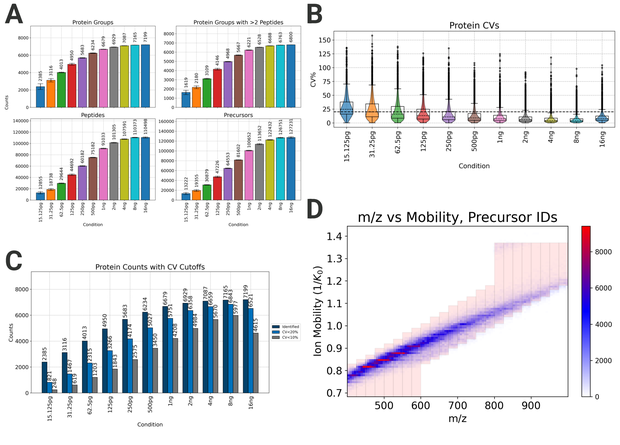

Fig. 2. Dilution series results from ID Counts and Heatmaps modules. Average identifications (A), CV violin plots (B), and protein ID counts above CV cutoffs (C) per sample condition. IM vs m/z heatmap of identified precursors with overlaid DIA windows (D).

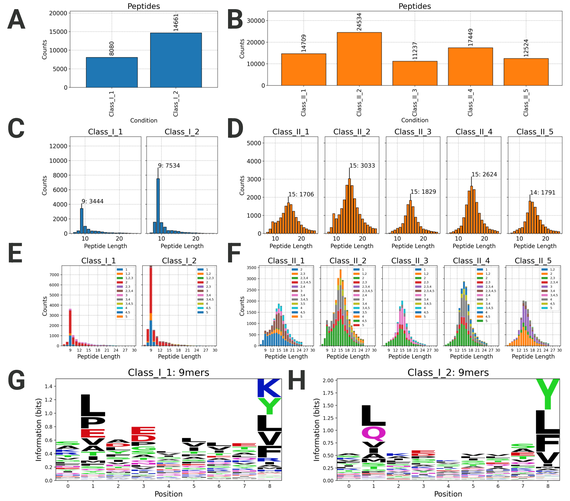

Fig. 3. Immunopeptide results from ID Counts, Metrics, and Immunopeptidomics modules. Counts for the number of unique modified peptide sequences per run (A,B). Distributions of stripped peptide lengths per run (C,D). Distributions of charge states per stripped peptide length (including precursors detected in multiple charge states) per run (E,F). 9-mer sequence motifs from class I immunopeptide samples (G,H).

Table 1. Modules available in the timsplot app.

Sidebar Module	Submodules/Description
ID Counts	Counts per Condition, CV Plots, IDs with CV Cutoffs, UpSet Plots, Protein Signal Tracker
Metrics	Charge States, Peptide Lengths, Peptides per Protein, Dynamic Range, Mass Accuracy, Data Completeness, Peak Width, Missed Cleavages
PTMs	Detected PTMs, Counts per Condition, CV Plots, Mass Accuracy, PTMs per Precursor
Heatmaps	Heatmaps of RT, m/z, and IM, Precursor Charge Heatmap, # of IDs per RT
Statistics	Volcano Plot, Feature Plot, Up/Down Regulation, Dendrograms, PCA
Immunopeptidomics	Sequence Motifs, Charge States per Peptide Length
Mixed Proteome	Counts per Organism, Intensities per Organism, Quantitative Ratios
PRM	Peptide Tracker, Peptide Intensity Across Runs, PRM Table Setup
Dilution Series	Dilution Ratios
Glycoproteomics	Glyco ID Counts, Glycan/Peptide Tracker
MOMA	Extract mobility offset-mass aligned (MOMA) events from search file and from raw data
De Novo	IDs Found in FASTA, Position Confidence Comparison between BPS Novor with other software
Two-Software Comparison	Compare IDs across two search software
Export Tables	Export tables of ID information
Raw Data[1]	TIC, BPC, EIC, EIM, tims Accumulation Time

Conclusion

- The timsplot app provides a comprehensive and user-friendly platform for interfacing with and visualizing timsTOF results, including multiple modules for different plotting functions depending on the experiment that was performed
- In the dilution series, we identified up to 7,000 protein groups and 110,000 peptides. >2,000 proteins were identified with as little as 15 pg of sample injected
- For the immunopeptides, we identified up to 5,000 proteins and 30,000 precursors

Acknowledgements

- Thanks to Lynn Spruce from CHOP for permission to use these immunopeptidomics samples to highlight the features in timsplot

1. Willems, S. et al. Mol. Cell Proteomics, 2020, 20, 100149. https://doi.org/10.1016/j.mcpro.2021.100149