A Deep Dive into Positional Isomers Leveraging CCS Information and Novel Software Tools

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Introduction
MOMA and TIMS Viz

The PASEF acquisition mode on the timsTOF platform has the power to separate and isolate co-eluting quasi-isobaric peptides for fragmentation, based on differences in their ion mobility. Such events are called Mobility Offset Mass Aligned (MOMA) and result in non-chimeric spectra, despite the quadrupoles fidelity not being sufficient to separate MOMA peptides by their *m/z*. This can especially be valuable in PTM analysis, e.g. to resolve positional isomers in phosphoproteomics.

PaSER is a GPU-driven search platform that delivers search results in real-time during acquisition on the timsTOF Pro, flex and SCP platforms. TIMS Viz, introduced in PaSER 2022, provides the ability to explore 4D-Proteomics data directly after the run is done, including visualizing and searching of MOMA events in the data.

Methods

200 µg starting material were enriched for phosphorylated peptides. Commercially available HeLa digest (Pierce) was used as representative lysate sample. Digests were separated on a nanoElute (Bruker Daltonics) coupled to a timsTOF Pro2 (Bruker Daltonics), operating in PASEF acquisition mode. An Aurora Series UHPLC column (25 cm x 75 µm, 1.6 µm C18, IonOpticks) was used for chromatographic separation. Cell lysates were measured on 100 min, 45 min and 21 min gradients. Data analysis was performed using the real-time database search engine in PaSER 2022 (Bruker Daltonics), operating at 1% FDR level. The real-time searches were carried out against human swissprot database with min. 1 peptide per protein and max. 1 missed cleavages. The novel TIMS Viz tool was used for data visualization in form of a precursor heatmap in *m/z* and ion mobility dimensions and for identification of MOMA features.

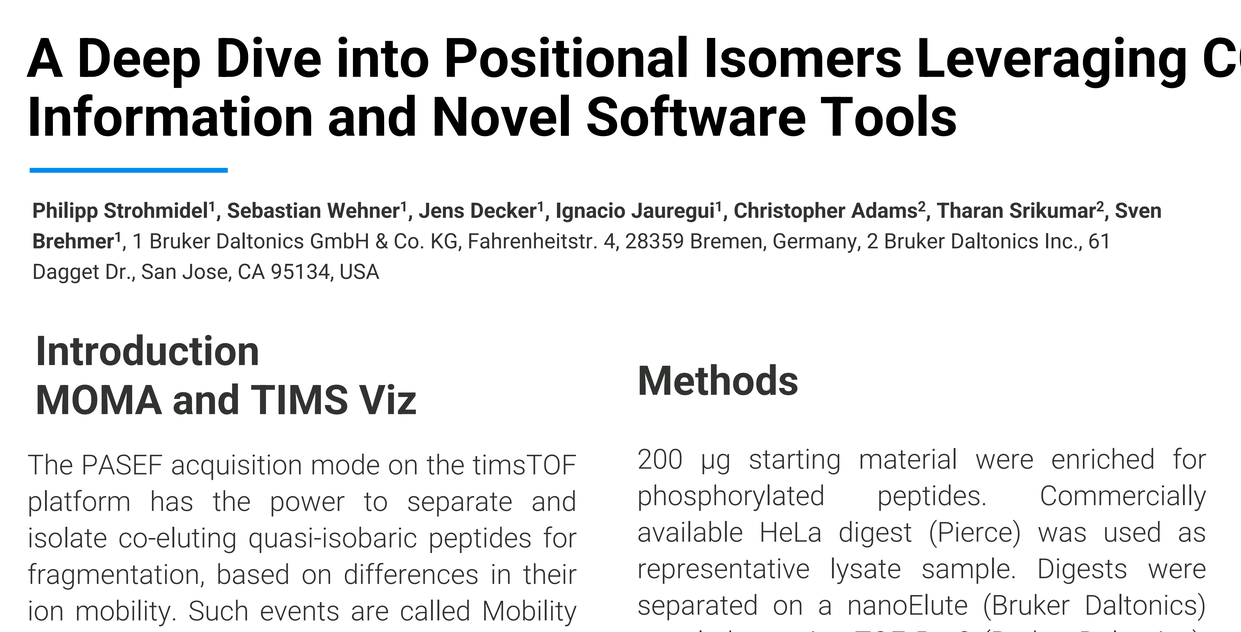

Fig. 1 Graphical user interface of TIMS Viz in PaSER 2022. (A) Mobility and m/z distribution over the entire course of a LCMS run, where yellow dots represent a "MOMA" event. (B) 10 sec. capture of co-eluting peptide isomers resolved in ion mobility and m/z space.

Sample type	Conditions	0.5 Da MOMA Groups	0.5 Da MOMA Spectra	0.1 Da MOMA Groups	0.1 Da MOMA Spectra	0.025 Da MOMA Groups	0.025 Da MOMA Spectra
Cell lysate	200 ng, 100 min	43908	105270	23666	53526	16472	37022
	200 ng, 45 min	37839	95196	17059	37521	9918	21262
	100 ng, 21 min	23836	62245	10807	23834	5786	12295
Phospho	120 min	56802	146577	33295	78090	27729	65007

Table. 1 Number of mapped MOMA groups and corresponding MOMA spectra for cell lysate and phosphopeptide-enriched analyses.

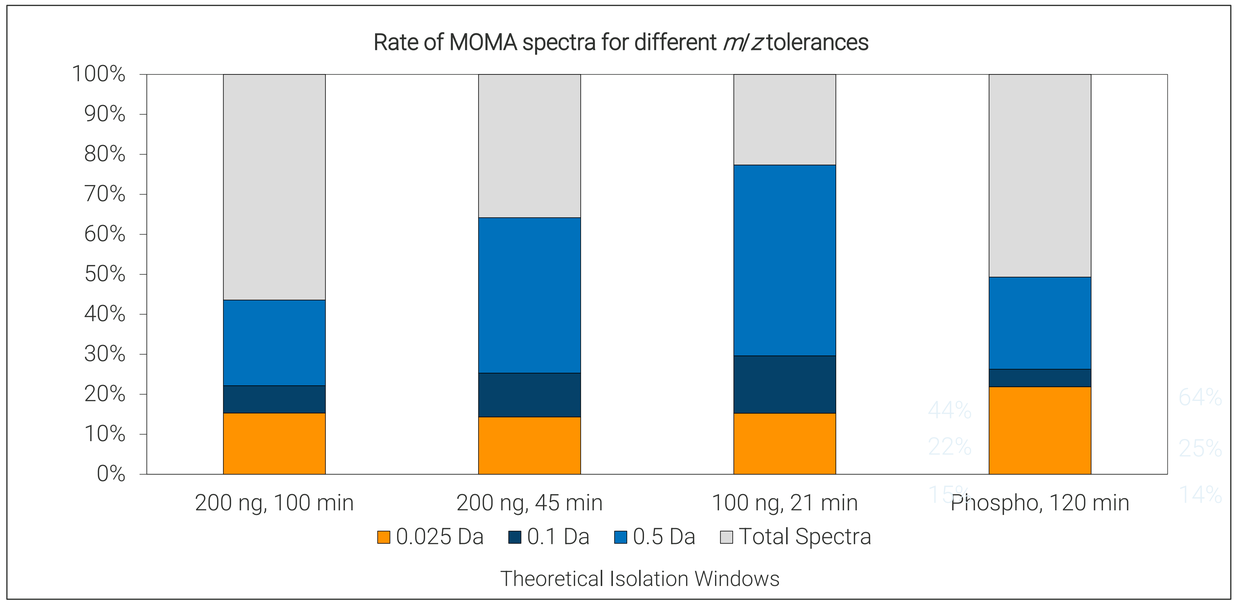

Fig. 2 Amount of MOMA spectra for varying *m/z* tolerances relative to the total acquired spectra.

Results

TIMS Viz can be accessed in PaSER 2022 as soon as the run is finished. An interactive heatmap plot of mobility against *m/z* allows to visualize and explore 4D-Proteomics data sets in a fast and convenient way (Fig. 1A). Features, e.g. MOMA groups or PSMs, can be highlighted in the heatmap with overlays and be exported for further data analysis.

MOMA groups are sets of at least two individually picked precursors that fit the MOMA criteria and the resulting fragment spectra are called MOMA spectra. The lower compartment in Fig. 1A shows the TIC and the selected retention time range for the heatmap visualization is marked in blue. Fig. 1B depicts a section of a heatmap plot of a 50 s retention time slice and MOMA groups are highlighted in orange. When selecting a MOMA group on the interactive heatmap, TIMS Viz displays the features of the precursors assigned to that MOMA group and the precursors are highlighted in green.

The criteria for MOMA group assignment are:

- Retention time difference of max. 10 s
- Ion mobility difference of min. 0.03 Vs/cm^2
- *m/z* difference was set to max. 0.5, 0.1 and 0.025 Da

Tab. 1 contains the number of mapped MOMA groups and corresponding MOMA spectra for cell lysate and phospopeptide-enriched analyses, each with the results for varying max. *m/z* differences as MOMA criterion. The typical lower limit of quadrupole isolation can be considered as 0.5 Da, hence all in Table 1 reported MOMA spectra would likely be chimeric without ion mobility separation.

The amount of MOMA spectra for varying *m/z* tolerances relative to the total acquired spectra is shown for different gradient lengths in Fig. 2. It can be seen, that with 0.5 Da set as max. *m/z* difference, the amount of MOMA spectra strongly increases with shorter gradient times. This is expected, due to the reduced chromatographic separation of peptides and demonstrates the power of ion mobility separation to reduce the chimeric nature of selected precursors, especially on shorter gradients.

When setting the tolerance to 0.025 Da, >14% of all spectra are based on quasi-isobaric precursors that can be resolved by ion mobility. With 22%, the phosphopeptide-enriched analyses have the highest amount of nearly-isobaric precursors to be individually fragmented due to ion mobility.

Conclusion

- TIMS Viz was introduced to the real-time search engine PaSER 2022
- Users can explore their 4D-Proteomics data in a fast and convenient way
- Relative amount of MOMA spectra increases with shorter gradient lengths
- Ion mobility has the power to improve spectral quality, especially on short gradients for high-throughput applications

Bioinformatics