BRUKER AT ASMS 2021 - ADVANCES IN 4D-PROTEOMICS™

TIMS Viz for Mobility Offset Mass Aligned interrogation of complex samples

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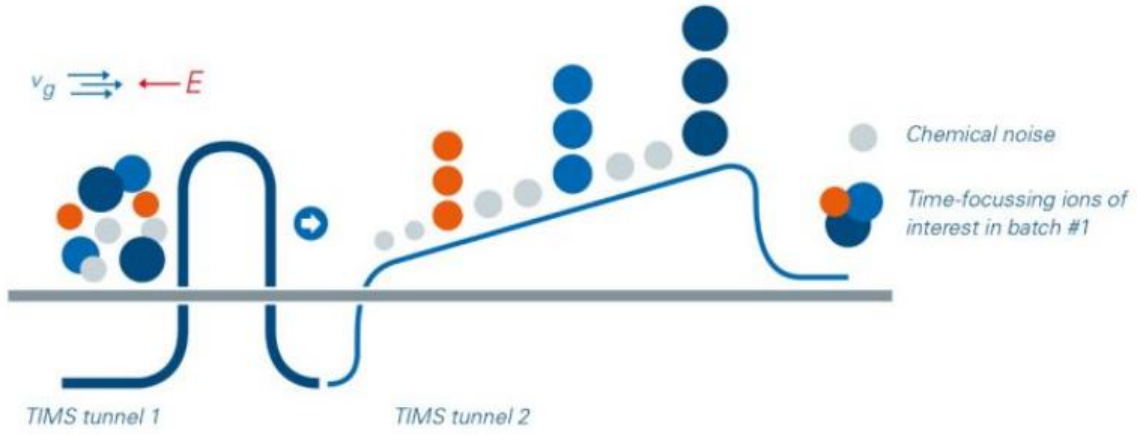
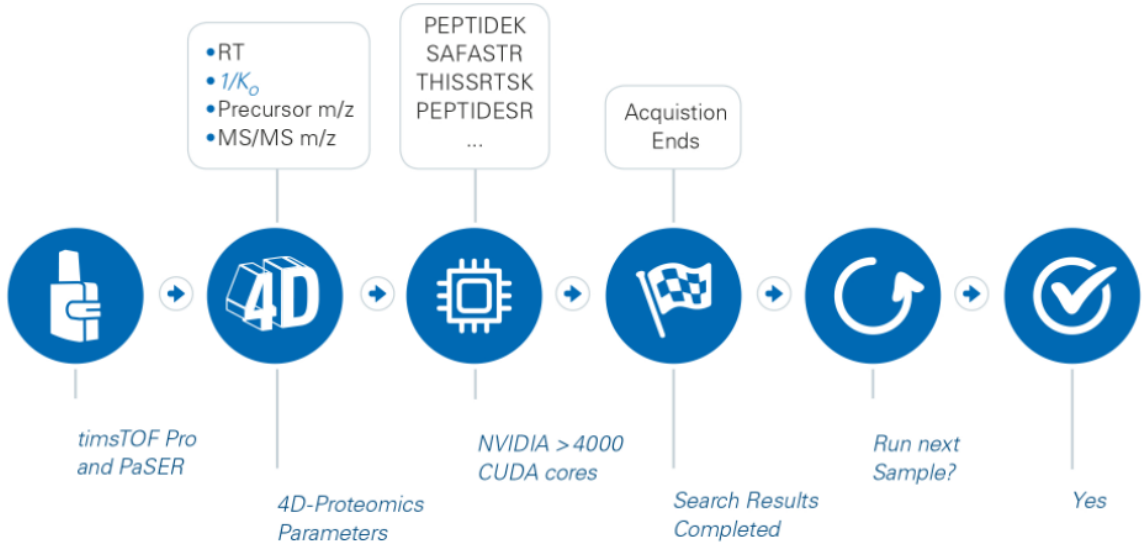
Introduction – MOMA in 4D-Proteomics™

- The PASEF acquisition mode of the timsTOF Pro 2 has the power to separate and isolate co-eluting, quasi-isobaric peptides for fragmentation
- The separation of quasi-isobaric peptides is based on differences in their ion mobility
- Such events are called Mobility Offset Mass Aligned (MOMA)
- MOMA events 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



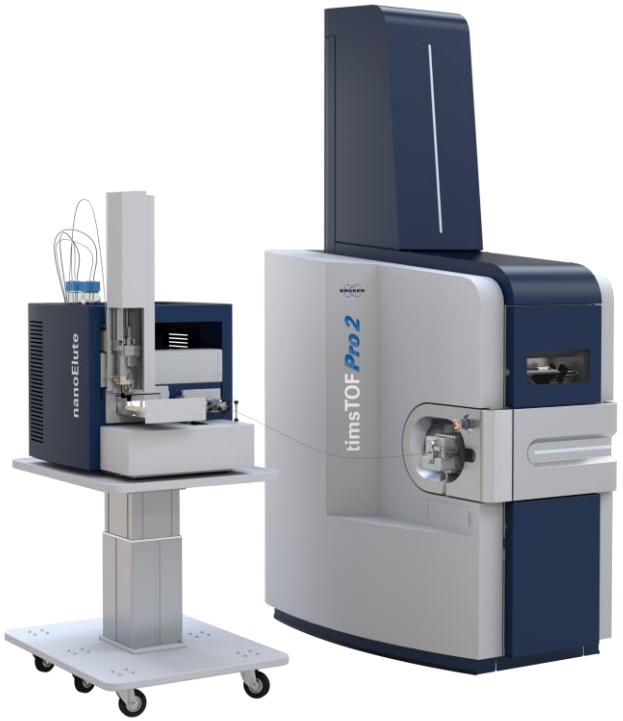
Introduction – TIMS Viz in PaSER 2022

- PaSER is a GPU-driven search platform that delivers search results in real-time during the acquisition on the timsTOF Pro, fleX and SCP platforms
- The GPU-powered processing facilitates thousands of processing cores for massive parallelization, which allows for a real-time database search without compromising the search space
- The PaSER data viewer can be accessed from the instrument pc or other computers in the same network to dive deep into the data
- TIMS Viz provides the ability to visualize 4D-Proteomics data directly after the acquisition ends
- MOMA viewer powered by TIMS Viz allows one to visualize, search and sort MOMA events



Method

- 200 µg starting material were enriched for phosphorylated peptides
- Commercially available HeLa digest (Pierce) was used as representative cell lysate sample
- Digests were separated on a nanoElute (Bruker Daltonics) coupled to a timsTOF Pro 2 (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
- Data analysis was performed using the real-time database search engine PaSER 2022 (Bruker Daltonics).
- 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



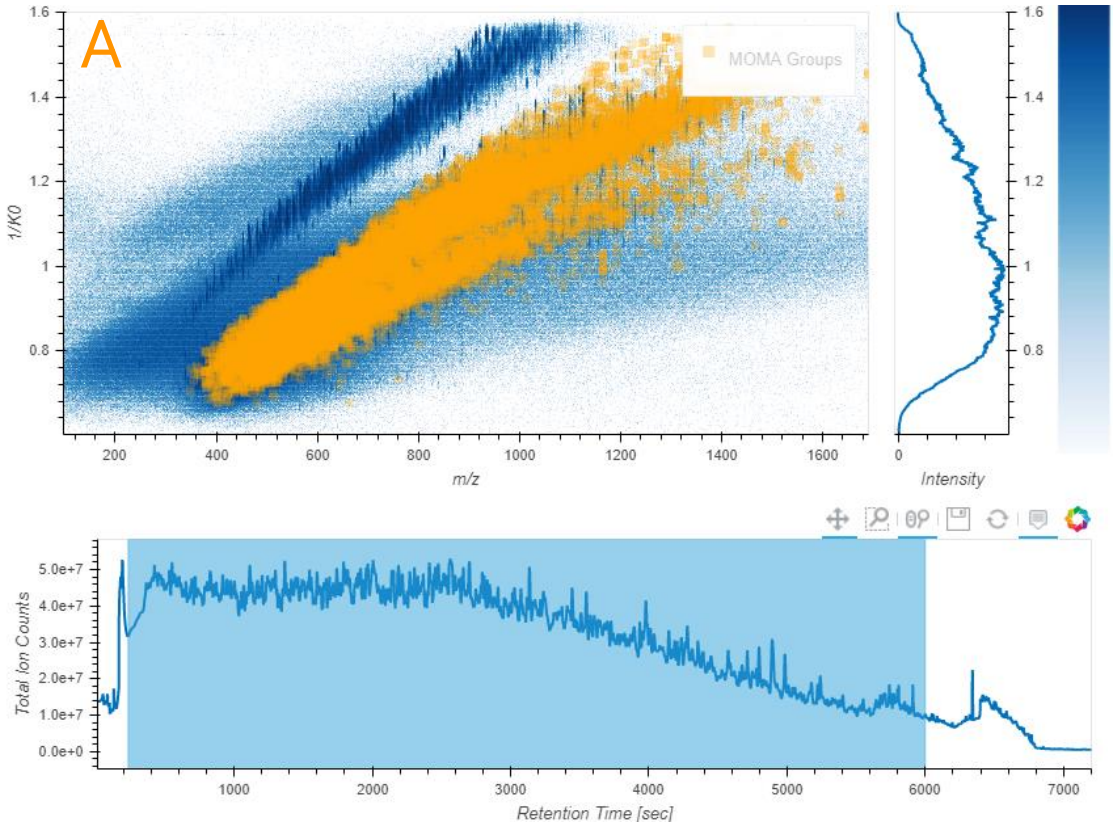
Results – PaSER database search results

- Cell lysates were measured on 100 min, 45 min and 21 min gradient lengths
- All runs were acquired as triplicates
- Search settings:
  - Database: Human Swissprot
  - Fixed PTM: Carbimidomethylation (C)
  - Variable PTMs: Oxidation (M), Deamidation (NQ), Acetylation (N-term), Phosphorylation (STY, only Phospho-enriched sample)
  - FDR: 1% at protein level
  - Min. peptides per protein: 1
  - Max. missed cleavages: 1

Sample Type	Condition	Protein Groups	Peptides	Phospho Peptides
Cell lysate	200 ng, 100 min	5598	47145	
		5507	46347	
		5480	46135	
	200 ng, 45 min	4674	30144	
		4711	30115	
		4675	30023	
	100 ng, 21 min	3603	19423	
		3576	19146	
		3542	19090	
Phospho	140 min	4724	34942	27210
		5210	27750	18085
		5028	25919	18647

Results – Interactive 4D data visualization in TIMS Viz

- TIMS Viz can be accessed in PaSER 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
- Features, e.g. MOMA groups or PSMs, can be highlighted with overlays
- Highlighted features can easily be exported for further data analysis



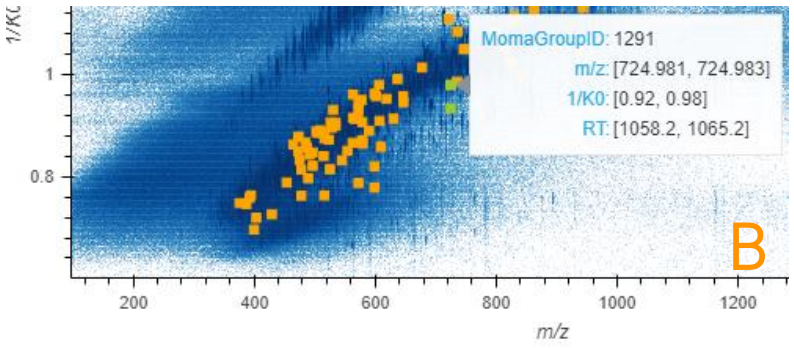


Fig A: Heatmap plot of the inverse reduced mobility $1/K_0$ depended on *m/z* with MOMA groups marked in orange. The lower compartment shows the TIC and the selected retention time range for the heatmap visualization is marked in blue. Fig B: Section of the heatmap plot for a retention time range of 50 s. MOMA groups are marked in orange, while both MOMA precursors of one selected group is shown in green. When selecting a MOMA group on the interactive heatmap, TIMS Viz displays the features of the precursors assigned to that MOMA group.

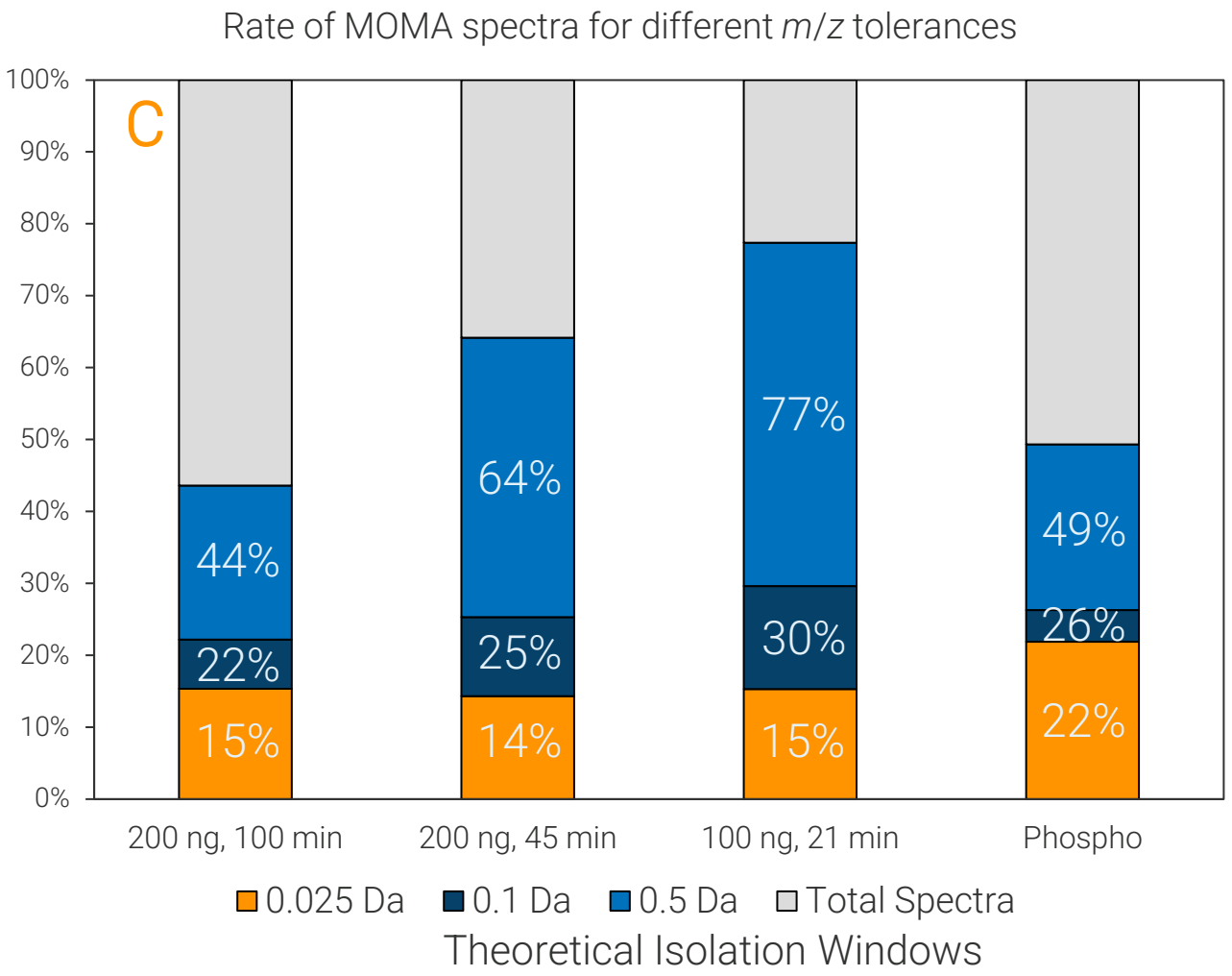
Results – Comparison of MOMA groups and spectra

Sample type	Conditions	0.5 Da		0.1 Da		0.025 Da	
		MOMA Groups	MOMA Spectra	MOMA Groups	MOMA Spectra	MOMA Groups	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		56802	146577	33295	78090	27729	65007

- MOMA groups, which are sets of at least two MOMA features, were mapped by TIMS Viz
- Criteria for MOMA group assignment: retention time difference of max. 10 s, ion mobility difference of min. 0.03 Vs/cm^2, *m/z* difference of max. 0.5, 0.1 and 0.025 Da
- 0.5 Da can be considered as typical lower limit of quadrupole isolation, so all herein reported MOMA spectra would likely be chimeric without ion mobility separation

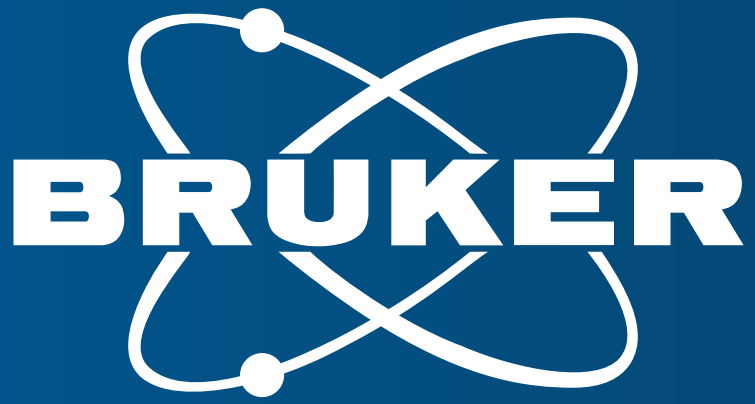
Results – Comparison of MOMA groups and spectra

- Fig. C shows the amount of MOMA spectra for different *m/z* tolerances relative to the total acquired spectra
- With 0.5 Da the amount of MOMA spectra strongly increases with shorter gradient times
- Setting the tolerance to 0.025 Da shows that >14% of all spectra are based on quasi-isobaric precursors that can be resolved by ion mobility separation
- This demonstrates the power of ion mobility to improve spectral quality, especially on shorter gradients



Conclusion

- TIMS Viz was introduced to the real-time search engine PaSER
- The interactive heatmap in ion mobility and *m/z* space enables users to explore 4D-Proteomics data in a fast and convenient way
- MOMA groups and features can be mapped on the heatmap and can be exported for further analysis
- Different MOMA m/z tolerances were compared for different gradient length of a cell lysate sample and for a phosphopeptide-enriched sample
- It could be shown that the relative amount of MOMA spectra increases with shorter gradient lengths
- While those features can be resolved by ion mobility, they would likely be chimeric without this additional separation
- This demonstrates the power of ion mobility separation to improve spectral quality, especially on short gradients for high-throughput applications



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