Ion-mobility-based Gas-Phase Fractionation dramatically improves Ubiquitylated and Acetylated peptide identifications and chimeric resolution.

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Introduction

Ion mobility is a powerful extension to mass spectrometry that delivers information about the three-dimensional structure of a peptide.

Trapped ion-mobility spectrometry (TIMS) increases selectivity and peak capacity, allowing for confident identifications and CCS measurement for each analyte.

Here, we used of ion-mobility-based Gas-phase Fractionation (GPF)[1] combined with parallel accumulation-serial fragmentation (PASEF) on a Bruker timsTOF SCP to evaluate advantages in K-GG and K-Ac peptide characterization.

Methods

- GPF optimization was performed with K562 digest evaluating the use of a single narrow (0.7-1.3 V s/cm2) and large ion-mobility ranges (0.6-1.6 V s/cm2) as compared to ion-mobility fractions (>2 gas-phase fractions).
- The peptides were separated using a Bruker NanoElute system with an IonOpticks Aurora C18 column (25cmx75um, 1.7um) couple to a TimsTOF SCP system.
- The data was acquired in DDA-PASEF mode. The cycle time was 1.9 seconds, the TIMS ramp time and accumulation time was set at 100ms and 10 PASEF frames were acquired per cycle.
- The data was processed with PASER 2022c and MsFragger v18[2]

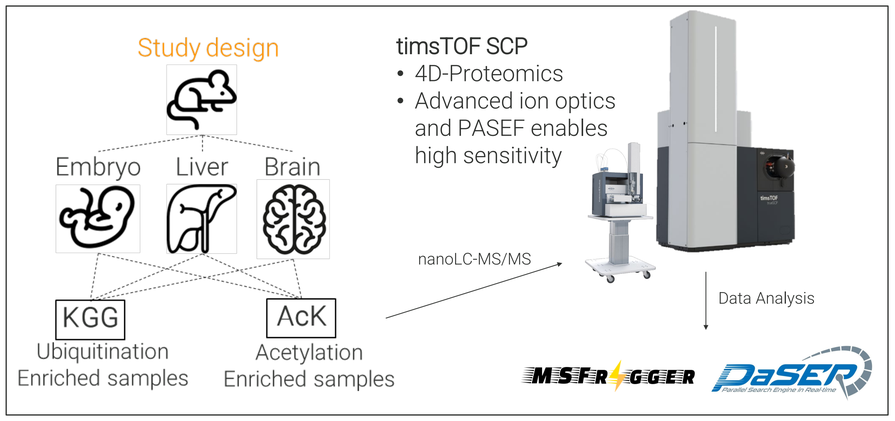

Fig.1 Experimental design of the analysis of enriched samples of ubiquitylated and acetylated peptides from three different tissues (brain, embryo and liver).

Results

- The ion-mobility based GPF allowed identification of a combined >28,000 ubiquitylated peptides and >26,000 acetylated peptides.
- This represents an increase of ~20% more peptide identifications, respectively, as compared to the unfractionated runs.
- Additionally, GPF using TIMS for K-GG and K-Ac peptides resolves thousands of co-eluting chimeric species by mobility offset mass alignment (MOMA)
- These CCS values can be used in quantification and to train deep learning algorithms.

[1] Guergues et al. JPR, 2022, 21 (8), 2036-2044
[2] Yu, et al. Molecular & Cellular Proteomics,2020, 19(9), 1575-1585.

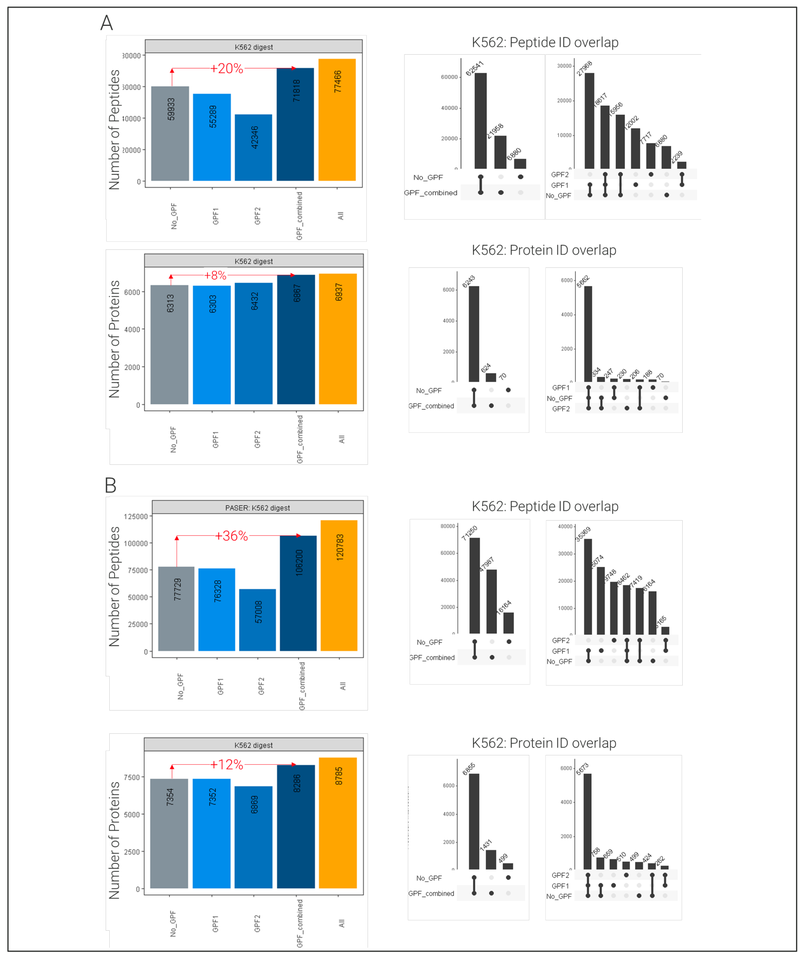

Fig. 2 Evaluation of Gas-Phase Fractionation on K562 digest. Number of peptide and protein identifications using MsFragger (A) and PASER (B). In an 85 min gradient, injecting 50ng on column, we observed GPF methods produced more than 8,000 proteins groups and more than 100,000 precursors. This represented more than 36% more identifications than an unfractionated run analyzed with the same LC-MS conditions.

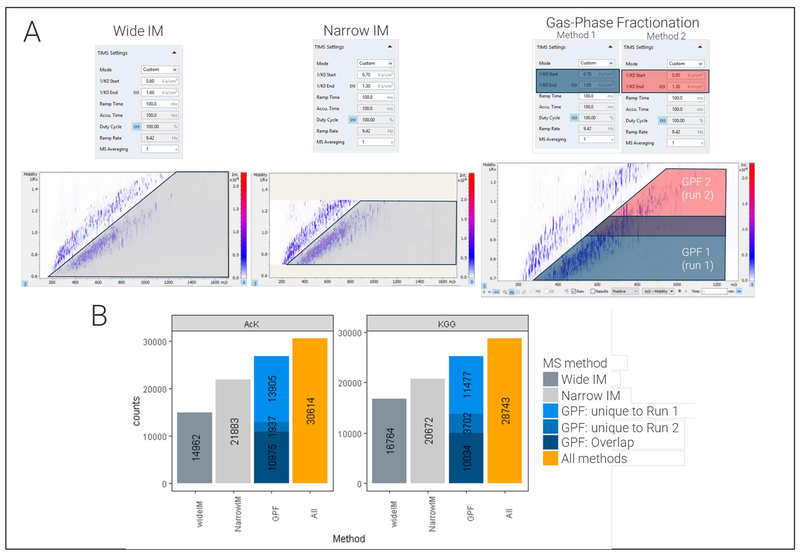

Fig. 3 Evaluation of Gas-Phase fractionation to the analysis of ubiquitylated and acetylated peptides. (A) Three methods were evaluated: single narrow (0.7-1.3 V s/cm^2) and large ion-mobility ranges (0.6-1.6 V s/cm^2) as compared to ion-mobility fractions (2 GPF). (B) The ion-mobility based GPF allowed identification of a combined >26,000 acetylated peptides and >28,000 ubiquitylated peptides.

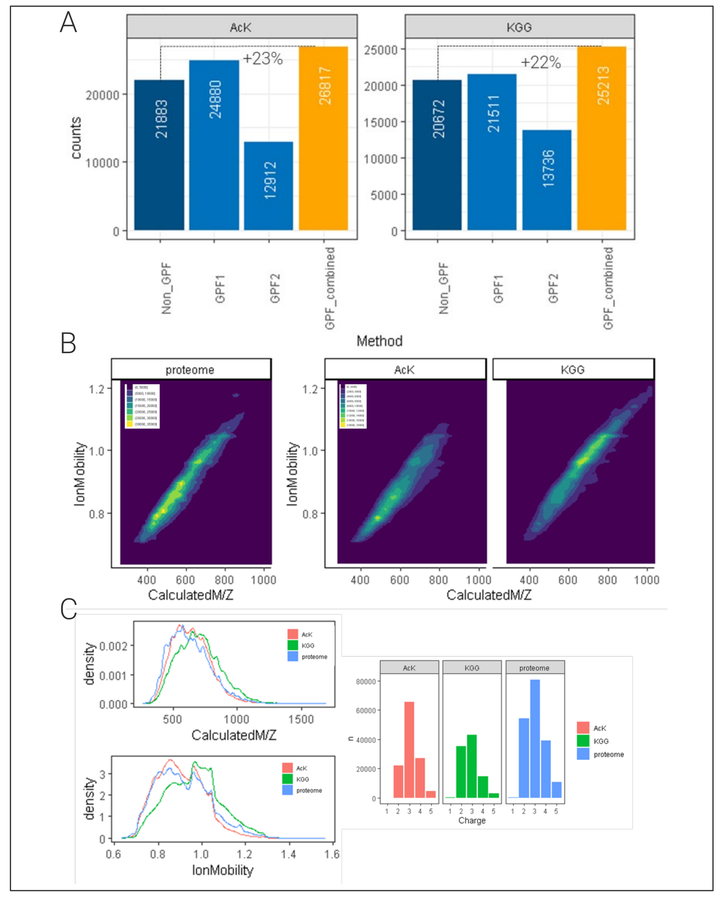

Fig. 4 (A) Number of ubiquitylated and acetylated peptide identifications using MsFragger. (B) Peptide distribution of proteome, K-Ac and K-GG peptides in the m/z and ion-mobility dimension. (C) m/z, ion-mobility distribution and charge distribution of K-Ac and K-GG peptides.

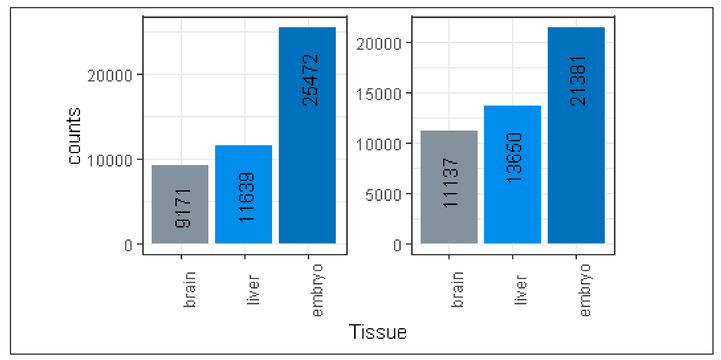

Fig. 5 Number of K-Ac and K-GG peptides per tissue.

Conclusion

- Gas-phase fractionation is a powerful approach to increase proteome coverage
- Gas-phase fractionation is ideal for sample-limited projects where off-line fractionation is not an option
- Gas-phase fractionation can be used to generate comprehensive spectral libraries for dia-PASEF

Technology