Analysis of the global histone modification landscape in mouse tissue using nano C18-monolithic column and timsTOF HT

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Introduction

The histone tail regions' PTMs play a role in key processes such as DNA replication, repair, and transcription. A single peptide may undergo multiple modifications and thus exist in numerous peptidoforms. This complexity and the overlap of many histone PTMs with tryptic cleavage sites make the proteomics characterization of the histone PTM landscape challenging. In this study, we conduct an unprecedented concurrent analysis of PTMs) to unveil the global histone modification landscape in mouse tissue, using for the first time a combination of C18 monolithic columns and timsTOF detection for fast separation and identification of histone modifications.

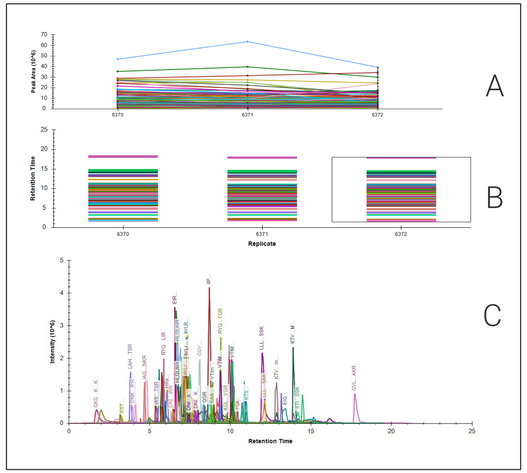

Fig. 1 A) Retention time reproducibility, B) The peak area of all quantified histone peptides, and C) Extracted ion chromatograms of identified histone peptides.

Methods

- The C18 silica-based monolithic separation column was operated on a nanoElute2 nanoLC system with a 5mm trap column. The separation gradient was 5-30% B in 17.8 min. MS acquisitions were performed DDA and DIA modes. Histones were extracted in 0.2 M H_2SO_4 and precipitated in 33% trichloroacetic acid (TCA). The protein digest was performed using ArgC and following the protocol provided by Promega.
- A spectral library was generated by searching the DDA runs using FragPipe and a UniProtKB Mus Musculus whole proteome database. A second search was performed using the filtered database, with KR mono-, di-, and tri-methylation, K acetylation, STY phosphorylation, and K acetylation as variables.
- In the third step, the DIA runs were searched using DIA-NN 2.1, with the library result generated from the FragPipe PTM search. All results were manually validated in Skyline
- Over 100 modified histone sites were quantified from pooled mouse histone samples. The average sequence coverage for the H1 family was 30%, 60% for the H2A family 60% for the H2B family, 80% for the H3 family, and 95% for the H4 family.

Results

Due to the increased physicochemical complexity, the post-translational modifications of proteins and the resulting digested peptides often result in broader peaks. However, the separation of peptides originating from the enzymatic digestion of histones using the C18 silica monolithic column resulted in peak widths of 3-5 seconds, depending on the amount and nature of the post-translational modifications (PTMs).

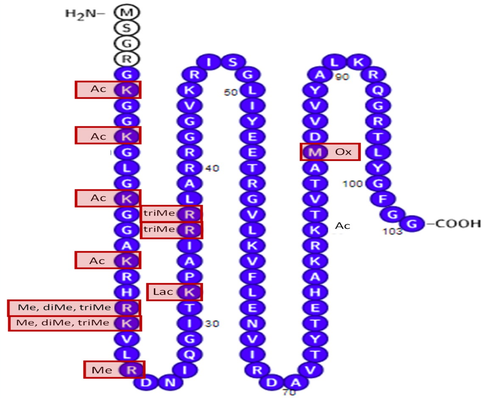

Fig. 3 Quantified (blue) and identified (red) PTMs for the Histone H4.
Excellent LC separation of the H4 peptides enabled almost the complete identification of the H4 sequence and a number of PTMs.

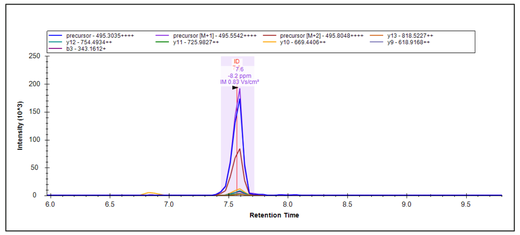

Fig. 4 Transition pattern for the H4 peptide DNIQGITK(Lac)PAIR(triMe)R(triMe)LAR4+

- Additionally, it was possible to separate peptides bearing identical modifications on different amino acids in the peptide backbone, i.e., to separate positional isomers. The ability to separate those peptides increases the number of identified sites by decluttering the ion clouds and increasing ionization efficiency. The median CV from the triplicate injections was 13.54%

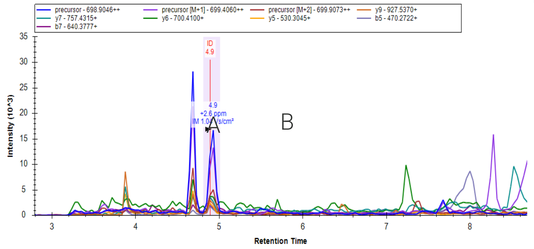

Fig. 4 Chromatographic separation of posiitonal isomers for the H4 peptide A) GK(Ac)GGK(Ac)GLGKGGAK(Ac)R and B) GKGGK(Ac)GLGK(Ac)GGAK(Ac)R .

Summary

The monolithic column for peptide separation, coupled with the timsTOF HT, enabled fast sample throughput, reproducible separation, and high sensitivity detection. Positional isomers of several post-translationally modified histones were detected and identified. The

- Literature

1. Fioriniello S, Marano D, Fiorillo F, D'Esposito M, Della Ragione F. Epigenetic Factors that Control Pericentric Heterochromatin Organization in Mammals. Genes. 2020;11(6):595.

2. Marano D, Fioriniello S, D'Esposito MLandscape, Della Ragione F. Transcriptomic and Epigenomic in Rett Syndrome. Biomolecules. 2021;11(7):967.

Conflict of interest

Authors declare no conflict of interests.

Conclusion

- High throughput for profiling of large sample cohorts
- The use of a monolithic separation column enabled the separation and detection of positional isomers.

Technology