Analysis of Histones from HEK293T Cells using a QTOF with Trapped Ion Mobility and PASEF Workflows

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Introduction

Histones form the fundamental unit of the eukaryotic nucleosome consisting of 146 bp of DNA wrapped around protein octamer units. Histones contain diverse post translational modifications that dictate all critical chromatin derived functionalities. Bottom-up approaches to studying histones result in a mixture of isobaric species arising from multiple modifications on the same molecule, making them challenging to analyze via routine LC-MS/MS. Here, we describe the utility of trapped ion mobility on a QTOF, to separate isobaric species leading to the detection of modforms that might otherwise go unidentified. Utilizing a combination of low sample load and PASEF workflows, we detect over 2000 peptide sequences and achieve increased depth and coverage of histone modforms without specialized chromatography and data interpretation.

References

Kelleher et al.; Curr Opin Chem Biol 2016, 33:142-150
Garcia et al.; Methods Enzymol 2017, 586:359-378

Methods

Histones were acid-extracted from Human embryonic kidney 293T cells and derivatized with propionic anhydride/ACN (1:3) pre- and post-digestion with trypsin. Peptides were separated on a 1.6µm C18 25cm x 75µm column (Ionoptiks) using a nanoElute nano LC (Bruker Daltonics) coupled to a trapped ion mobility equipped Q-TOF mass spectrometer (timsTOF Pro). 270ng of sample was injected and resolved over a 90-minute gradient ranging from 2% to 25% Acetonitrile (0.1% FA) at 400nl/min. Data derived from PASEF scans were recorded using otofControl and HyStar (Bruker Daltonics) software. Acquired data were analyzed via Data Analysis (Bruker Daltonics) and PEAKS (Bioinformatics Solutions, Inc) software platforms.

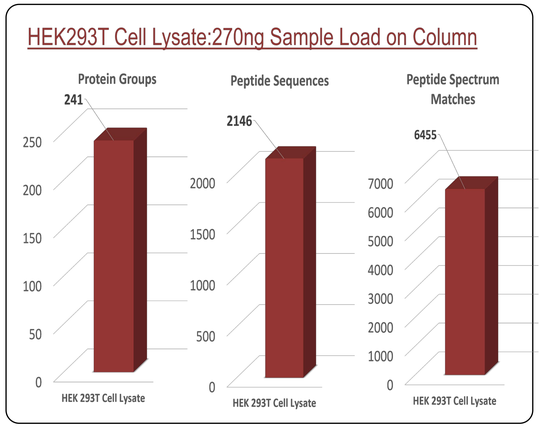

Fig. 2. Protein Groups, unique peptide sequences and PSM identified from 270ng of HEK293T cell digests 90 min gradient.

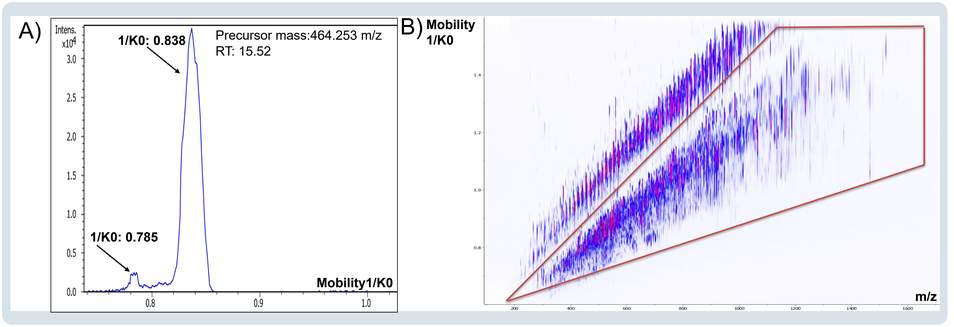

Fig.1 A). Ion mobility separation of isobaric species that differ only by their PTM localization site B) MS heatmap of peptides separated by charge state from the nano LC separation over the 90 min gradient in the 1/K0 vs m/z space. Area inside polygon consisting of only higher charged species selected for MS/MS.

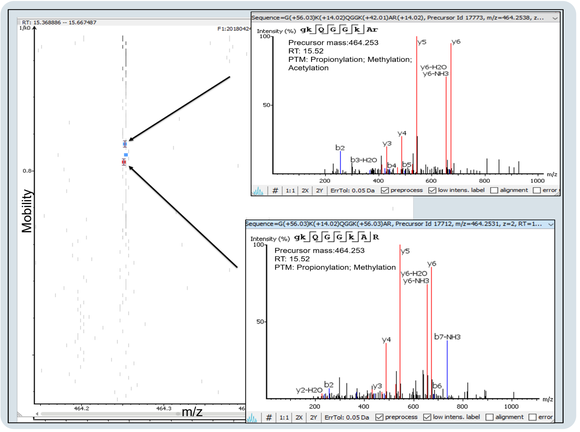

Fig. 3. Co-eluting peptides that differ only by the PTM localization site are separated by TIMS, resulting in non-chimeric MS/MS spectra that enable localization of the different sites of modifications

Results

Histone ID with PASEF

The increased peak capacity from the extra dimension of separation provided by the TIMS and increased sequencing speed of the PASEF method enables very large numbers of Histone identifications from very low sample amounts.

More than 200 protein groups, 2000 peptide sequences and 6000 peptide spectrum matches (PSM) (at 1% FDR as estimated by PEAKS using decoy fusion) were identified from 270ng of protein (Figure 2).

Resolution of Co-eluting Isobaric Peptides

Co-eluting peptides that are isobaric or have overlapping precursor ion isotope envelopes can be resolved using Trapped Ion Mobility Spectrometry resulting in clean MS/MS spectra.

Figure 3 shows an example where two co-eluting peptides with the same sequence but different modification sites were separated based on their collisional cross sections enabling discrete MS/MS spectra which were confidently assigned to different peptides by PEAKS database search.

This would not be possible on a conventional non-TIMS mass spectrometer

Conclusions

- PASEF on the timsTOF Pro identified more than 200 protein groups from a 90 min gradient.
- Almost 2000 peptides were confidently identified in a 90 min gradient.
- Extra dimension of separation provided by TIMS allowed resolution of co-eluting, isobaric peptides

timsTOF Pro