Top-Down Mapping of Protein Modifications by Multimodal MS^n on the New timsOmni Platform

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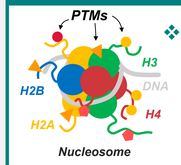
Aims

- Evaluate multimodal **Omnitrap™** (MS²/MS³) **workflows for rapid top-down proteoform identification** and high-coverage amino acid sequencing of **intact proteins**.
- **Precisely localize Post-translational modification (PTM)** sites on intact histone proteins, capturing **co-occurring modifications and resolving positional isomers**.

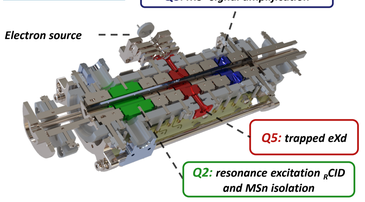
Introduction



- **Biological context:** Histone proteins carry dense array of **PTMs** that orchestrate chromatin structure and gene expression. **Their spatial patterns, not merely their presence, drive functional outcomes** and are frequently disrupted in disease.
- **Analytical challenge: determining** the presence and **exact location of PTMs on intact proteins** remains challenging, especially when **isomers (same m/z)** are involved[1].
- **Complementary fragmentation: Combining electron- and collision-based dissociation** in one device broadens sequence coverage and enables residue-level discrimination of PTM positional isomers[2].
- **This study:** We deploy eXd MS²/MS³ on the new timsOmni platform to **localise multiple and co-occurring lysine acetylations on histones H3/H4.**

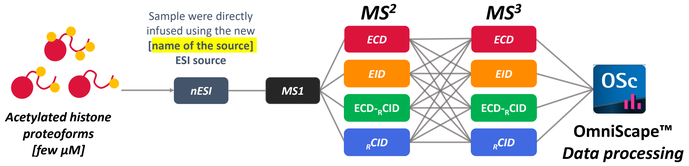
timsOmni Platform

timsOmni™ integrates TIMS-Q-TOF with a Omnitrap™ platform for advanced top-down proteomics[3].

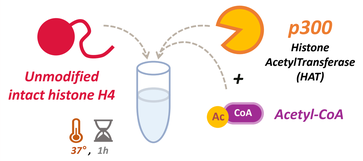


- **Omnitrap™ eXd:** The Omnitrap™ segmented linear ion trap integrate **multimodal fragmentation** workflow delivering the rich spectral detail required for **precise PTM mapping on intact proteins**.

Multimodal MS^n eXd worklows:



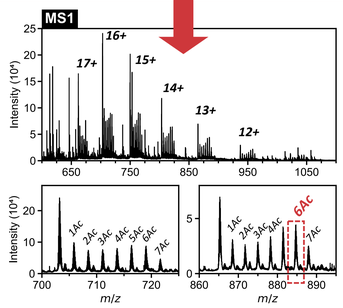
Proteoform Mapping of Multiply Acetylated Histone H4 (11.2 kDa)



Ratio histone:AcCoA:p300: 20:100:0.1
Incubation 1h in 50 mM HEPES, 0.1 mM EDTA and 1 mM DTT; Reaction quenched with 10% (v/v) of FA.

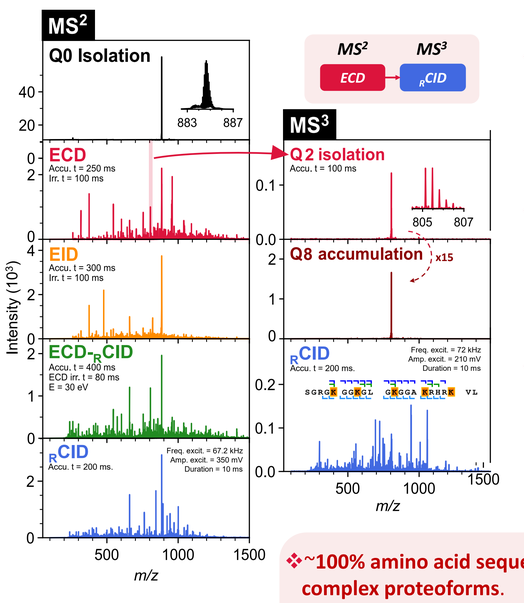
p300 is known to targets several lysine residues on the H4 N-term. tail (primarily K5, K8, K12, and K16)[4].

In vitro acetylation reaction with p300

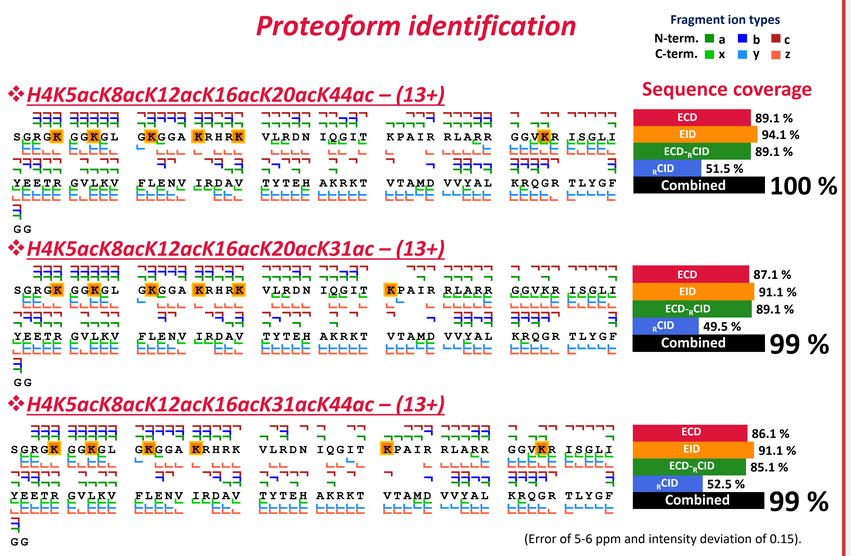


Mass spectrum reveals H4 proteoforms bearing 1 to 7 acetylations following p300 treatment (1h)

eXd MS² and MS³ of H4(Kac)6 (13+)



Proteoform identification

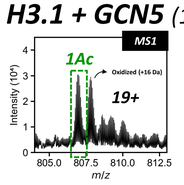


(Error of 5-6 ppm and intensity deviation of 0.15).

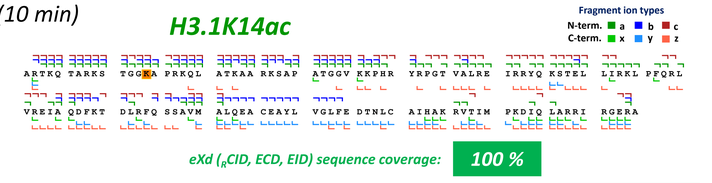
- **~100% amino acid sequence coverage achieved** using eXd workflow, enabling residue-level **PTM mapping of complex proteoforms**.
- eXd MS2/3 of H4(Kac)$_6$–(13$^+$) localized **7 acetylation sites: K5–K20, K31, and K44**. Results were consistent at z = 16$^+$.
- For lower acetylation states (Kac1–Kac4), **acetylation preferentially targeted N-tail lysine residues** (K5–K16).

Site-Specific Monoacetylation of H3.1 at K14 by GCN5 and PCAF

H3.1 + GCN5 (10 min)

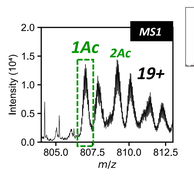


H3.1K14ac

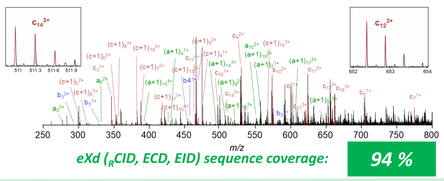


eXd ($_R$CID, ECD, EID) sequence coverage: **100 %**

H3.1 + PCAF (30 min)

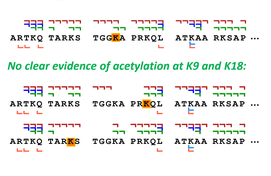


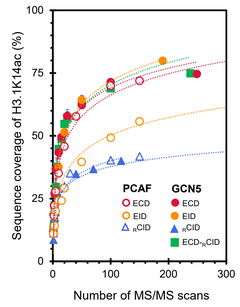
H3.1K14ac



eXd ($_R$CID, ECD, EID) sequence coverage: **94 %**

ECD Fragmentation map (residues 1–29):





- H3.1K14ac is confidently identified as the dominant monoacetylated proteoform, even with minimal MS/MS averaging.
- Sequence coverage increase logarithmically with the number of MS/MS spectra.

Enzymes GCN5 and PCAF are highly specific and K14 is identified as acetylation site for H3.1(Kac)$_1$

Conclusion

- **Multimodal MS^n (eXd) on the timsOmni™ platform enables residue-level mapping of co-occurring lysine acetylations on intact histones H3.1 and H4.**
- **Nearly ~100 % sequence coverage is achieved.**
- **For heavily acetylated H4, we confidently localized up to 7 acetylation sites using combined MS²/MS³ strategies, with consistent results across charge states.**
- **Monoacetylated H3 proteoforms from GCN5 and PCAF treatments were also characterized, confirming enzyme specificity (e.g. H3K14ac).**

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+ Jasmin funding

References: [1] F. Berthias et al. Proteomics (2024), 24, e2200471. [2] P. V. Shliaha et al. *Anal. Chem.* 2018 90 (21), 12519-12526
[3] D. Papanastasiou et al. *JASMS* 2022 33 (10), 1990-2007, [4] R. A. Henry, Y.-. Kuo, and A. J. Andrews. *Biochemistry* 2013 52 (34), 5746-5759

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Conflict of Interest Disclosure

C. A., A. S., M. K., D. P. are employees of Bruker Daltonics, which develops and manufactures timsOmni.