Probing histone tail interactions by HX-ETD-Orbitrap-MS

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Introduction

- Hydrogen/deuterium-exchange mass spectrometry (HX-MS) probes protein dynamics and protein interactions (with ligands, DNA etc).
- Part of histone N-terminal tails are absent in nucleosome structures reported to date.
- HX-MS using pepsin to analyze histone dimers, tetramers, nucleosomes and chaperones provided poor coverage of histone tails (i.e. [1,2]).
- Cathepsin-L improved resolution in this region, however cannot be leveraged for the analysis of large complexes [3].
- Protease XIII [4] shows superior resolution for histone tails and can be leveraged for deuterium measurements at the MS1 and MS2 level.

Optimization of digestion conditions using H4

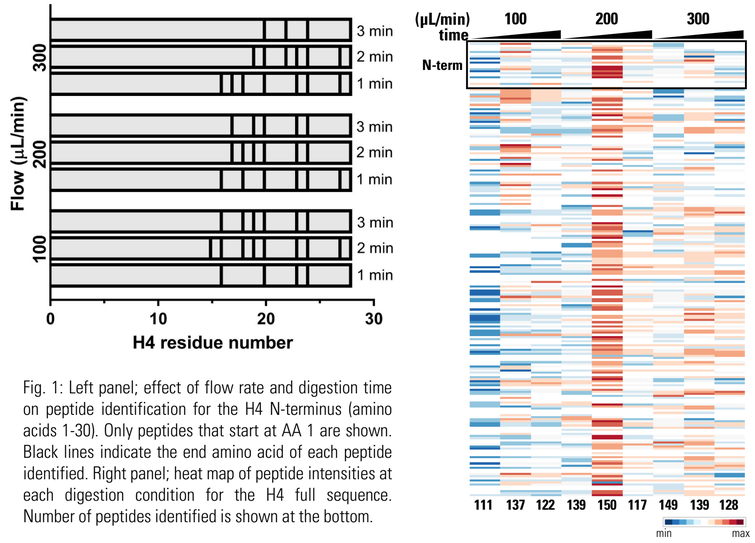
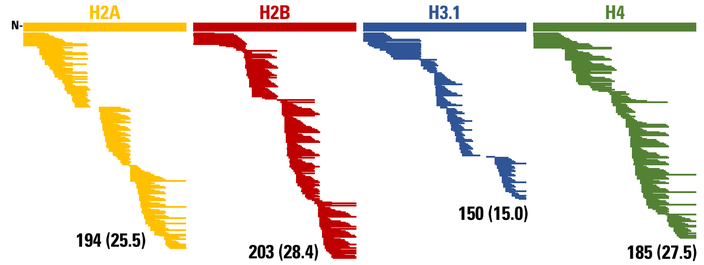


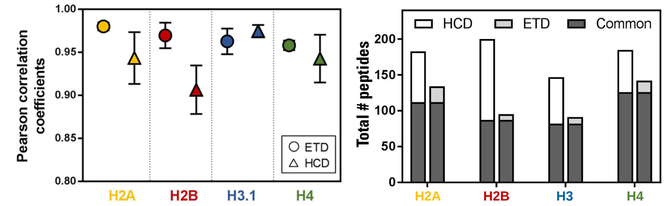
Fig. 1: Left panel; effect of flow rate and digestion time on peptide identification for the H4 N-terminus (amino acids 1-30). Only peptides that start at AA 1 are shown. Black lines indicate the end amino acid of each peptide identified. Right panel; heat map of peptide intensities at each digestion condition for the H4 full sequence. Number of peptides identified is shown at the bottom.

Peptide identification of histone monomers using HCD or ETD

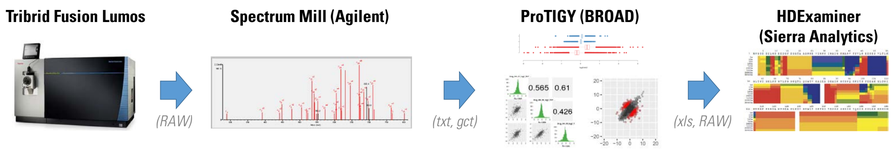


MS1 Resolution	60 K
MS1 AGC	2e5
m/z range	270-1300
Source T (°C)	50
Capillary T (°C)	220
Isolation width	3
MS2 Resolution	30 K
MS2 AGC	1e5
Microscan	2
Reagent Target	4e5

Fig. 3: Top panel; peptide maps of histone monomers using identifications from HCD and ETD fragmentation. MS conditions are shown to the right. Bottom left panel; reproducibility of digestions in triplicate. Pearson correlation coefficients were calculated using normalized peptide intensities. Bottom right panel; unique peptides identified by HCD or ETD and peptide overlap between the two methods.



The HX-MS workflow



- Purified histone monomers from New England Biolabs and mononucleosomes from Epicypher.
- Samples were run using a DDA Top 15 method with either HCD or ETD (Thermo Fisher).
- Immobilized protease XIII/pepsin (1:1) from NovaBioAssays; digestions occurred at 8 °C.
- Samples were introduced with a HDx-PAL™ system (LEAP).
- Trapping with PepMap300 (5 µM); separation with Hypersil C18 (1.9 µM).

References

[1] Black *et al*, Nature, 2004
[2] D'arcy *et al*, Mol Cell, 2013
[3] Papanastasiou *et al*, in revision
[4] Zhang et al, Anal Chem, 2008

ETD spectra of H4 (1-20) peptide

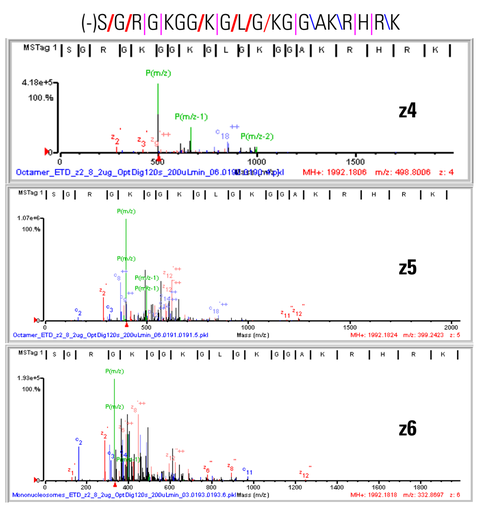


Fig. 2: ETD spectra of a H4 N-terminal peptide. Rich fragmentation spectra are obtained for higher charge states. ETD reaction times were 25 msec for z4 and 20 msec for z5 and z6.

Tail peptides (1-30) and redundancy at the residue level using ETD

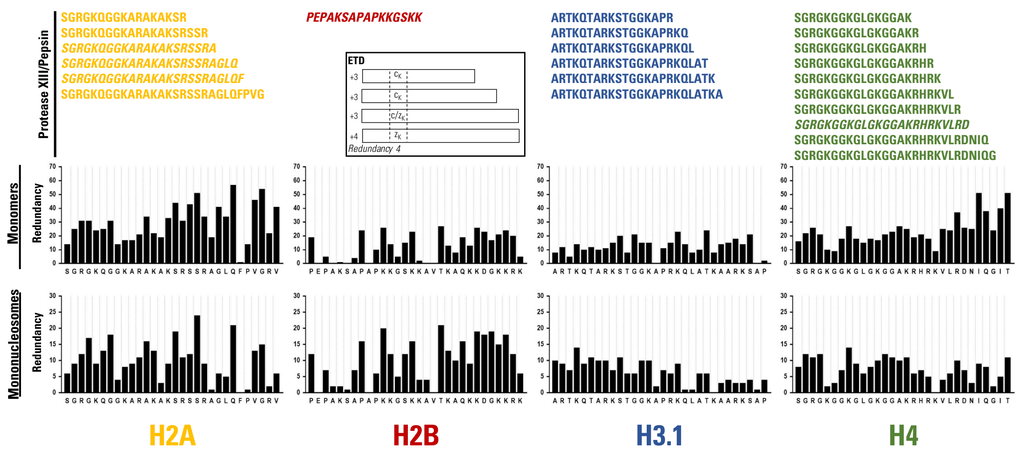


Fig. 4: Top panel; Sequences of peptides identified for each histone using the dual protease column; digestion occurred for 2 min at 200 µL/min. Pepsin generated peptides [3] are featured in italic fonts. Protease XIII is shown superior resolution for histone tails compared to other proteases available to date. Bottom panel; redundancy values calculated (as shown in the inset) at the residue level using ETD. All peptides identified were taken into account, including the ones that extend beyond residue 30 and that are not shown here. Results obtained from histone monomers and mononucleosomes are shown.