Comparison of CID, ETD, and HCD for Top-down Characterization of Histones

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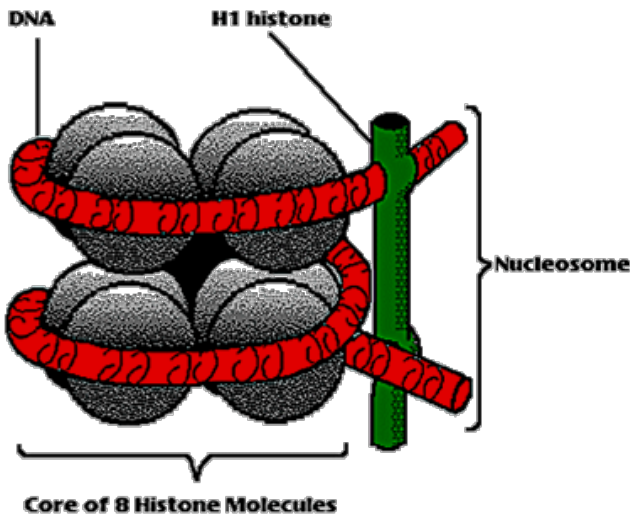
Histones and post-translational modifications (PTMs)

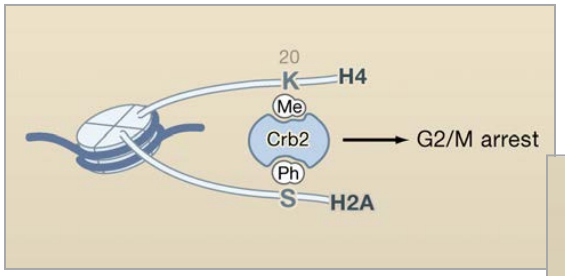


Nucleosome

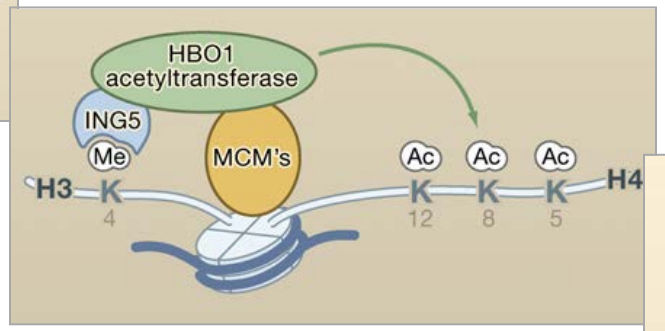
http://www.accessexcellence.com/AB/GG/nucleosome.gif

Core histones: H4, H2B, H2A, H3

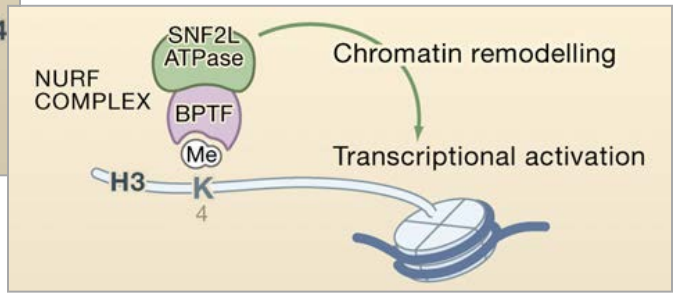
DNA Repair[1]



DNA Transcription[3]

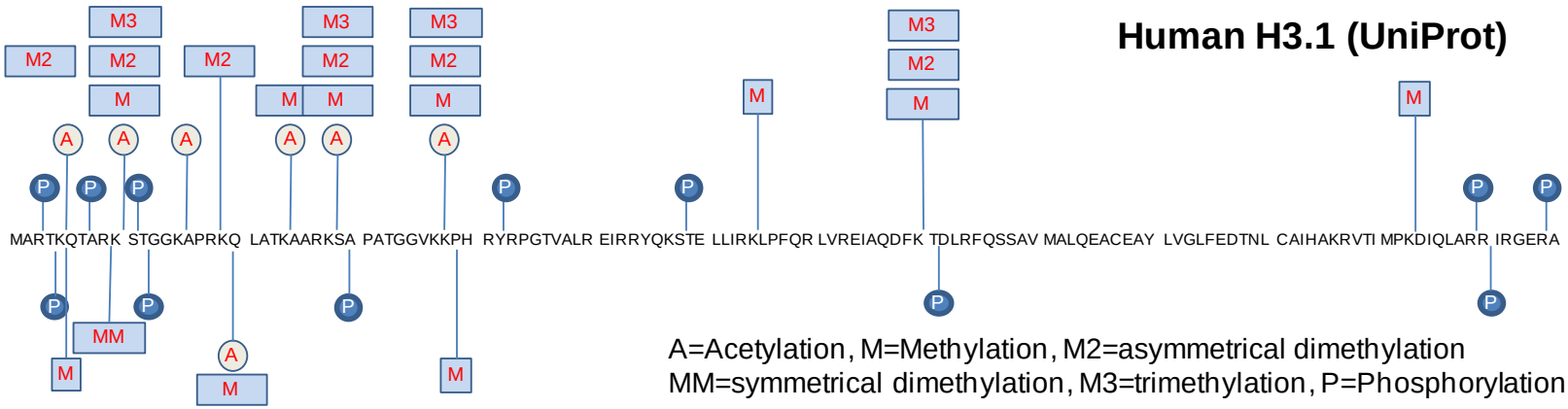


DNA Replication[2]

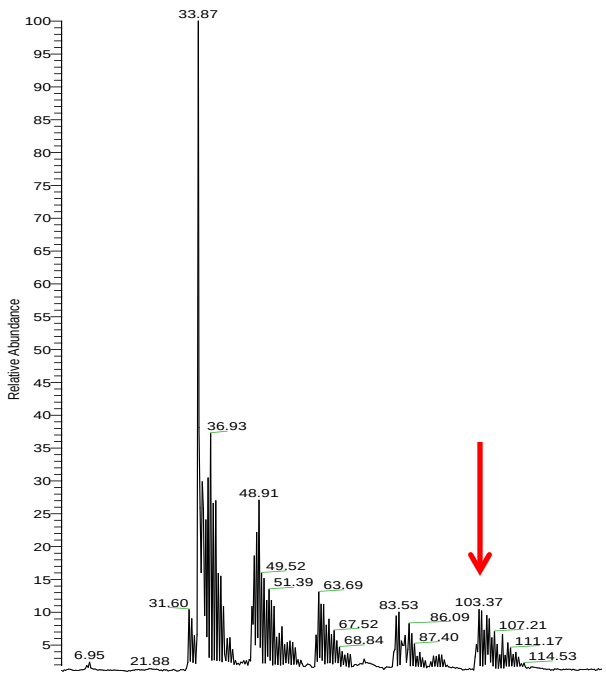


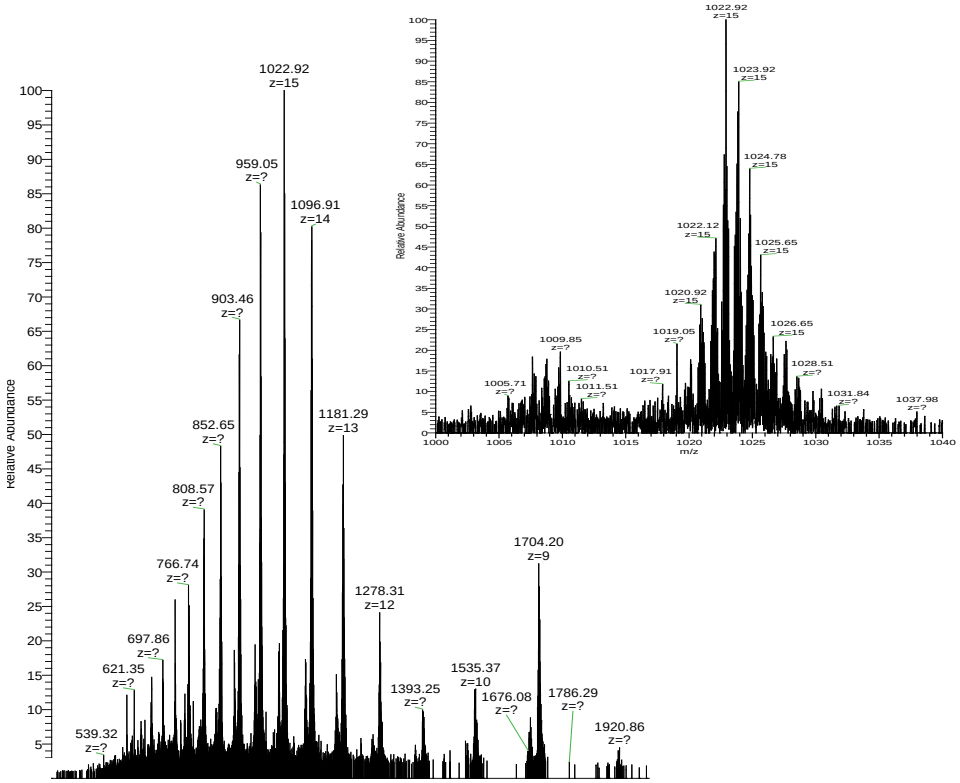
1. Nature Reviews Mol. Cell Bio. 6, 757 (2005); 2. Molecules and Cells 28, 149 (2009); 3. Science 272, 371 (1996)

Histones are heavily modified and very complex



RPLC-MS/MS of HeLa core histones

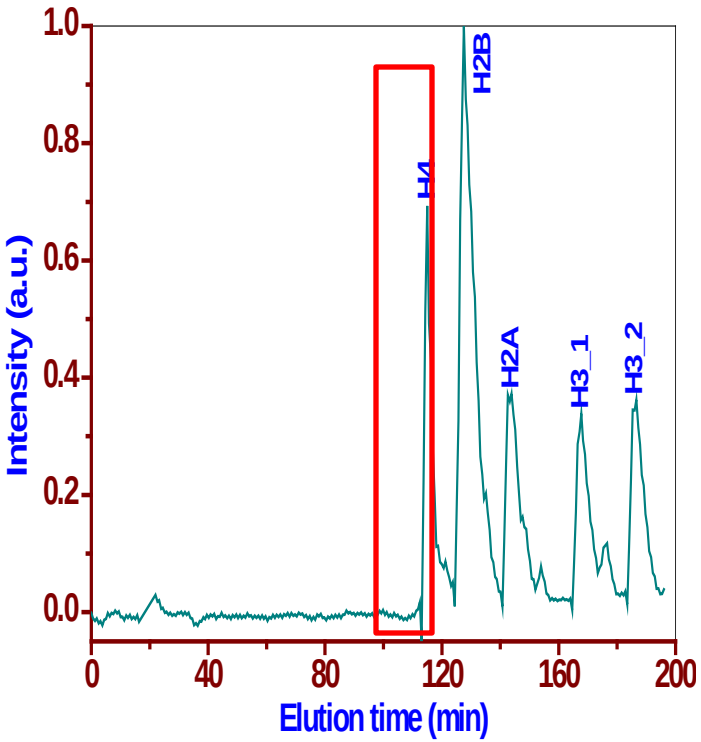




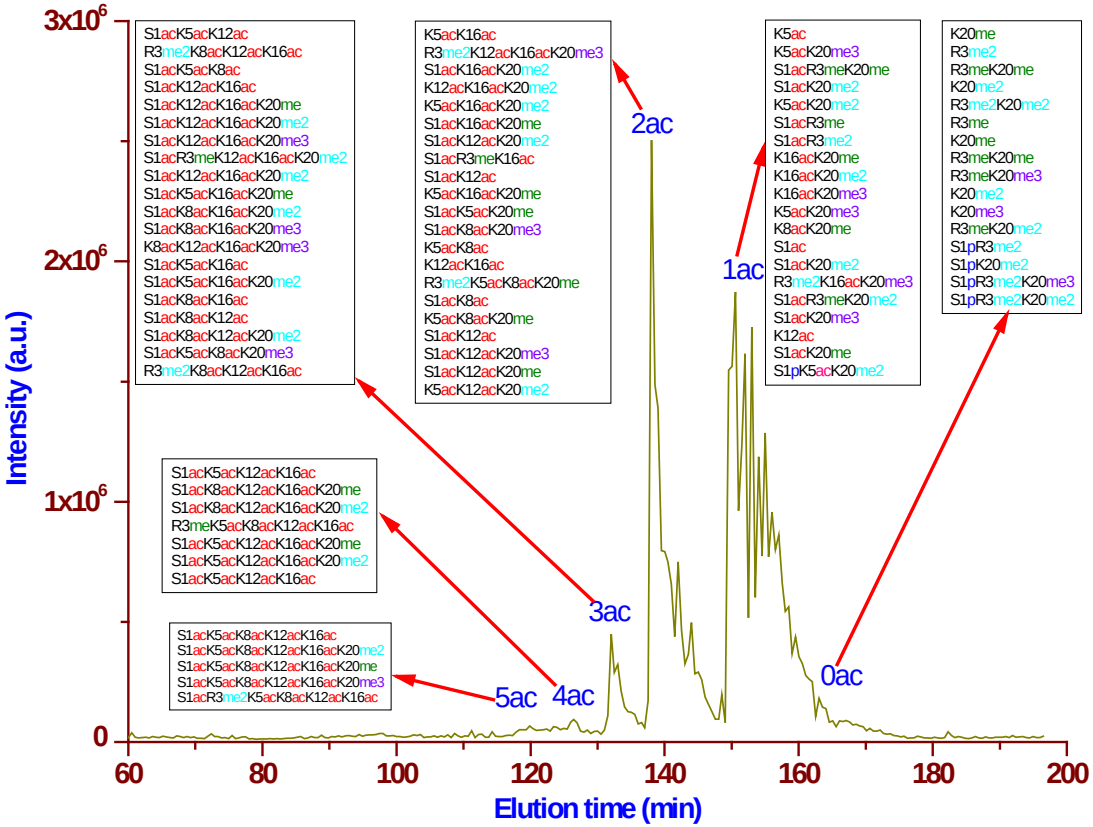
Online RPLC/WCX-MS/MS HeLa core histones

Methods	IDs
RPLC-MS/MS	127
WCX-MS/MS	135
RPLC-WCX-MS/MS	708

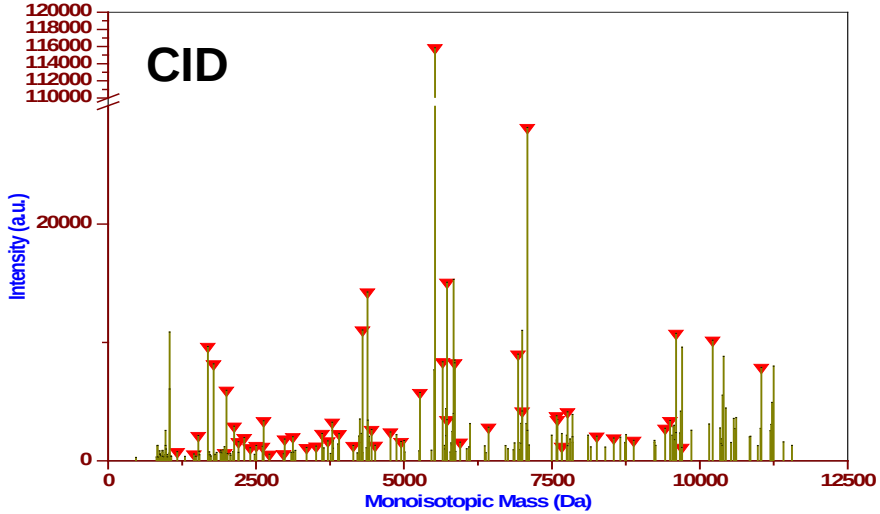
RPLC separation of histones

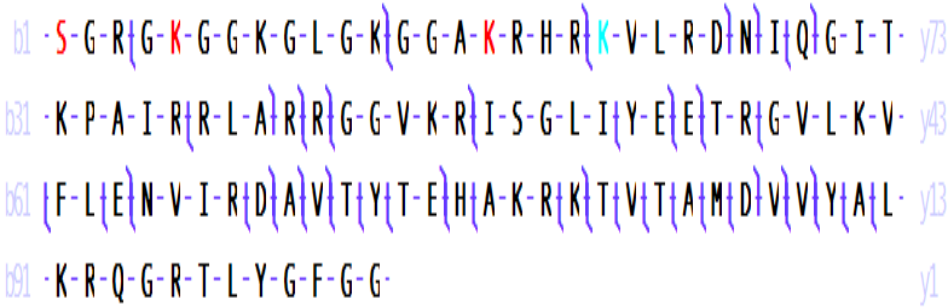


WCX-MS/MS of H4

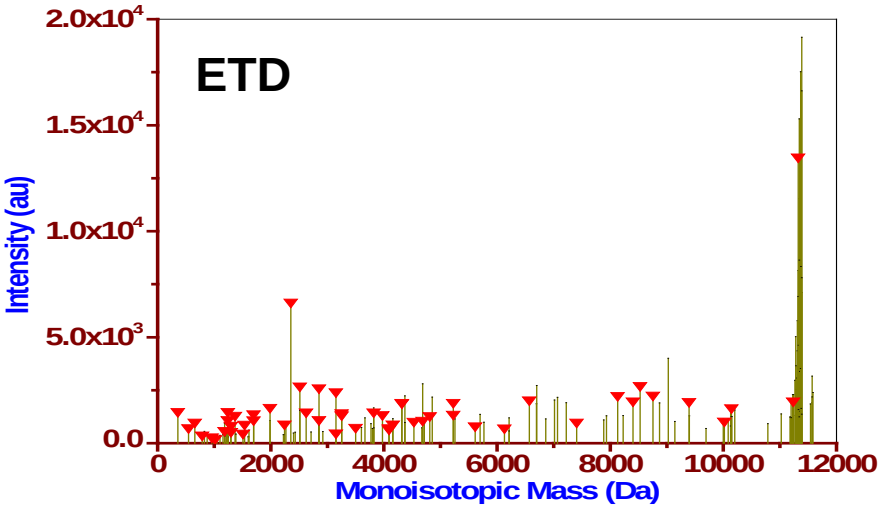


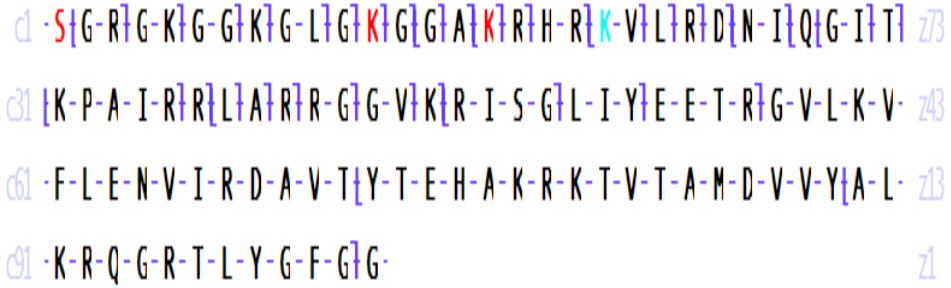
Unambiguous localization of K12ac in histone H4





Ambiguous identifications:
H4_S1ac**K{5, 8, 12}ac**K16acK20me2



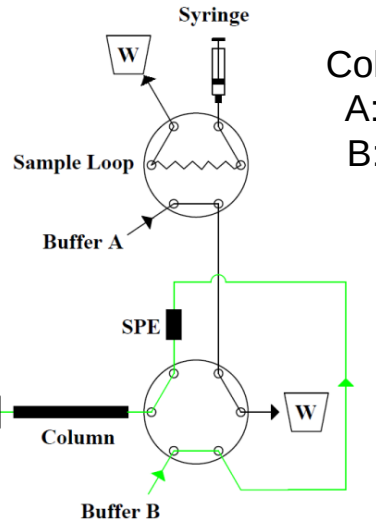


Unique identification:
H4_S1ac**K12ac**K16acK20me2

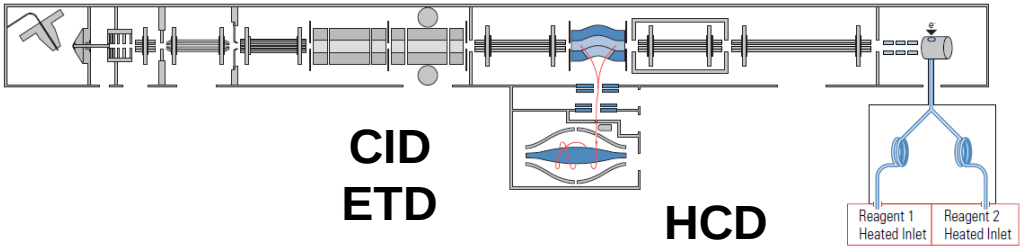
Methylation (mono) Acetylation Phosphorylation Dimethylation Trimethylation

Optimizing fragmentation for histone characterization

RPLC



Column (200 µid*70cm, C5(J), 5µ, 300Å)
A: 20%ACN, 5%IPA, 0.6%FA
B: 45%ACN, 45%IPA, 0.6%FA



LTQ Orbitrap Velos

HeLa core histones (3.75 µg)

	NCE (%)	AT (ms)
HCD_45_30	45	30
HCD_45_60	45	60
HCD_60_60	60	60
CID_35_30	35	30
CID_45_60	45	60
CID_60_60	60	60
ETD_10	/	10
ETD_15	/	15
ETD_20	/	20

.RAW

ProSightPC 2.0

IDs

Different fragmentation conditions

	NCE (%)	RT (ms)	IDs	Avg [–log(P_Score)]
HCD_45_30	45	30	123	16.9
HCD_45_60	45	60	136	15.3
HCD_60_60	60	60	144 **±8**	20.4 **±0.3**
CID_35_30	35	30	62	10.3
CID_45_60	45	60	56	16.5
CID_60_60	60	60	65	21.2
ETD_10	/	10	83	23.3
ETD_15	/	15	160	34.5
ETD_20	/	20	138	28.9

HCD conditions identification of H4_S1acK16ack20me2

HCD_45_30: Matching fragments=**27**, P_Score=**6.5E-31**

```
b1  - S - G - R - G - K - G - G - K - G - L - G - K - G - G - A - K - R - H - R - K - V - L - R - D - N - I - Q - G - I - T -  y73
b31 - K - P - A - I - R - R - L - A - R - R - G - G - V - K - R - I - S - G - L - I - Y - E - E - T - R - G - V - L - K - V -  y43
b61 - F - L - E - N - V - I - R - D - A - V - T - Y - T - E - H - A - K - R - K - T - V - T - A - M - D - V - V - Y - A - L -  y13
b91 - K - R - Q - G - R - T - L - Y - G - F - G - G -                                                                          y1
```

HCD_45_60: Matching fragments=**38**, P_Score=**5.9E-39**

```
b1  - S - G - R - G - K - G - G - K - G - L - G - K - G - G - A - K - R - H - R - K - V - L - R - D - N - I - Q - G - I - T -  y73
b31 - K - P - A - I - R - R - L - A - R - R - G - G - V - K - R - I - S - G - L - I - Y - E - E - T - R - G - V - L - K - V -  y43
b61 - F - L - E - N - V - I - R - D - A - V - T - Y - T - E - H - A - K - R - K - T - V - T - A - M - D - V - V - Y - A - L -  y13
b91 - K - R - Q - G - R - T - L - Y - G - F - G - G -                                                                          y1
```

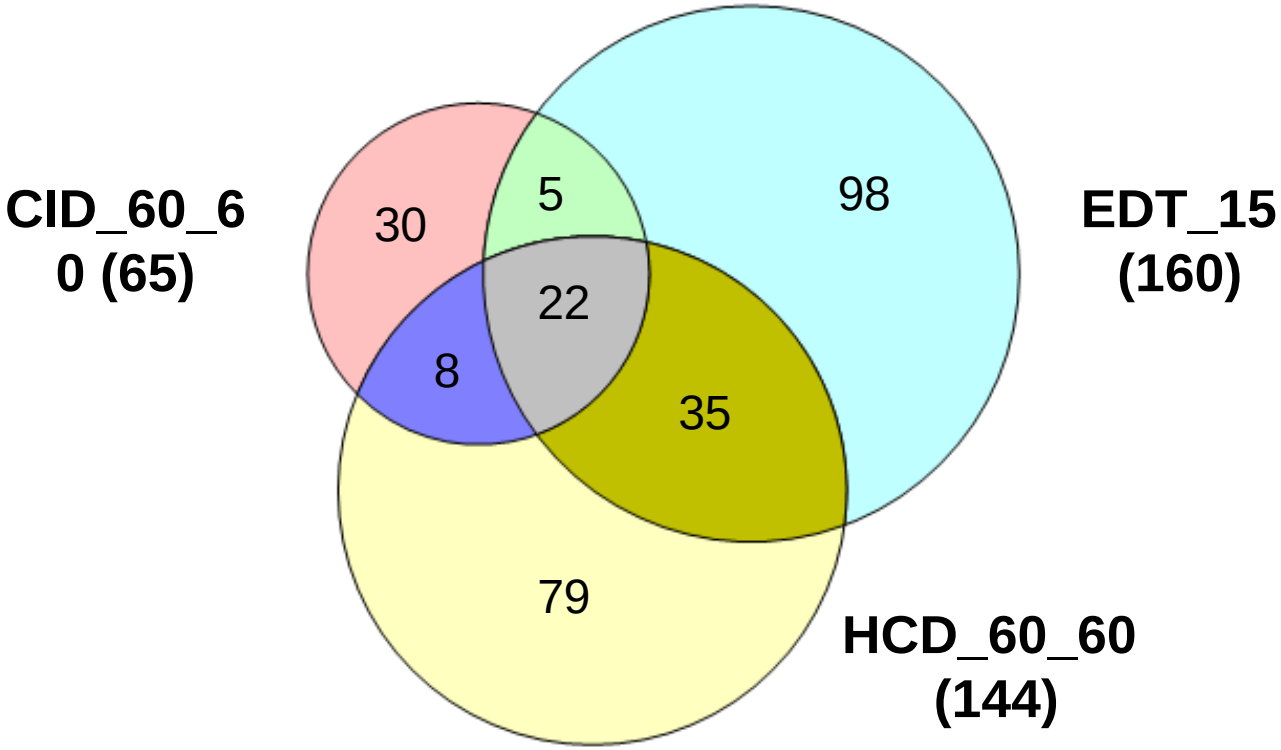
HCD_60_60: **Matching fragments=42, P_Score=1.3E-50**

```
b1  - S - G - R - G - K - G - G - K - G - L - G - K - G - G - A - K - R - H - R - K - V - L - R - D - N - I - Q - G - I - T -  y73
b31 - K - P - A - I - R - R - L - A - R - R - G - G - V - K - R - I - S - G - L - I - Y - E - E - T - R - G - V - L - K - V -  y43
b61 - F - L - E - N - V - I - R - D - A - V - T - Y - T - E - H - A - K - R - K - T - V - T - A - M - D - V - V - Y - A - L -  y13
b91 - K - R - Q - G - R - T - L - Y - G - F - G - G -                                                                          y1
```

Methylation (mono) Acetylation Phosphorylation Dimethylation Trimethylation

Optimal CID, ETD, and HCD

	NCE (%)	RT (ms)	IDs	Avg [–log(P_Score)]
HCD_60_60	60	60	144	20.4
CID_60_60	60	60	65	21.2
ETD_15	/	15	160	34.5



ETD, HCD, and CID: H4_S1acK16ack20me2

ETD: Matching fragments=**56**, P_Score=**1.1E-76**

```
c1  -S-G-R-G-K-G-G-K-G-L-G-K-G-G-A-K-R-H-R-K-V-L-R-D-N-I-Q-G-I-T-  z73
c31 -K-P-A-I-R-R-L-A-R-R-G-G-V-K-R-I-S-G-L-I-Y-E-E-T-R-G-V-L-K-V-  z43
c61 -F-L-E-N-V-I-R-D-A-V-T-Y-T-E-H-A-K-R-K-T-V-T-A-M-D-V-V-Y-A-L-  z13
c91 -K-R-Q-G-R-T-L-Y-G-F-G-G-                                      z1
```

HCD: Matching fragments=**46**, P_Score=**3.3E-56**

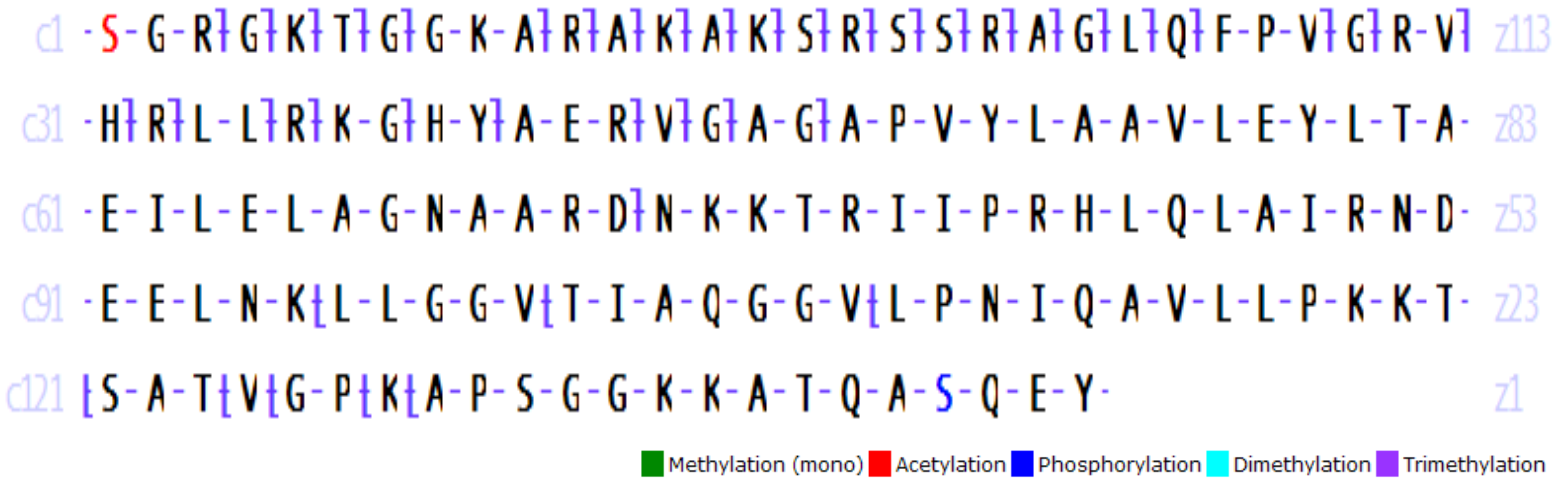
```
b1  -S-G-R-G-K-G-G-K-G-L-G-K-G-G-A-K-R-H-R-K-V-L-R-D-N-I-Q-G-I-T-  y73
b31 -K-P-A-I-R-R-L-A-R-R-G-G-V-K-R-I-S-G-L-I-Y-E-E-T-R-G-V-L-K-V-  y43
b61 -F-L-E-N-V-I-R-D-A-V-T-Y-T-E-H-A-K-R-K-T-V-T-A-M-D-V-V-Y-A-L-  y13
b91 -K-R-Q-G-R-T-L-Y-G-F-G-G-                                      y1
```

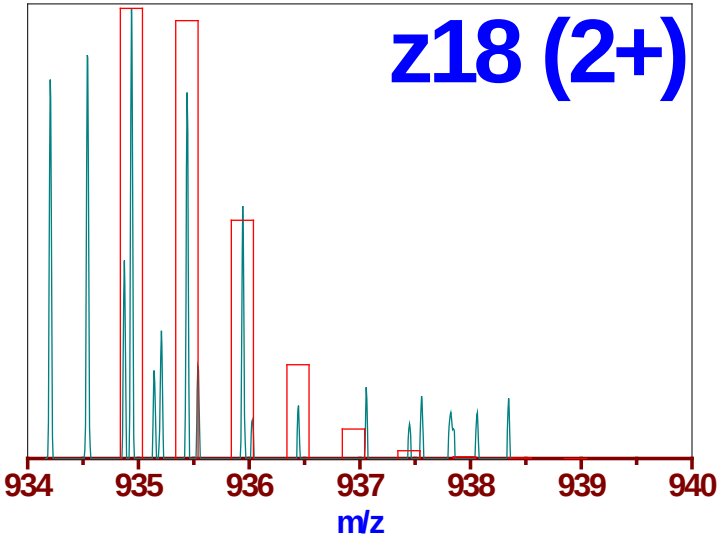
CID: Matching fragments=**31**, P_Score=**1.1E-40**

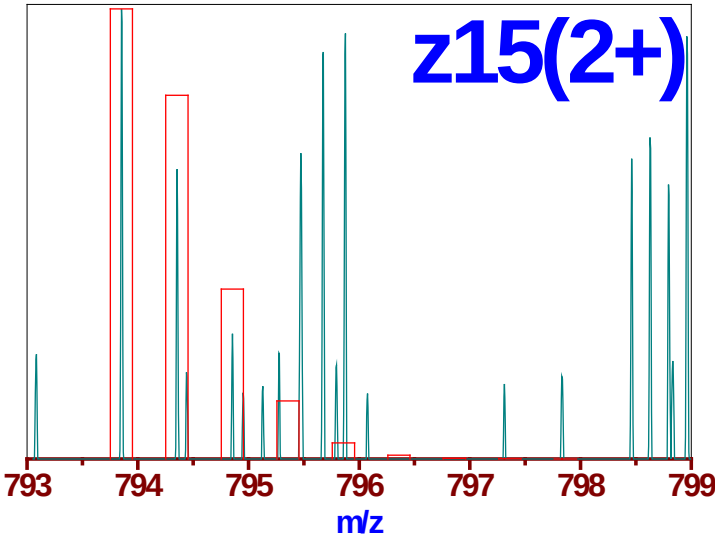
```
b1  -S-G-R-G-K-G-G-K-G-L-G-K-G-G-A-K-R-H-R-K-V-L-R-D-N-I-Q-G-I-T-  y73
b31 -K-P-A-I-R-R-L-A-R-R-G-G-V-K-R-I-S-G-L-I-Y-E-E-T-R-G-V-L-K-V-  y43
b61 -F-L-E-N-V-I-R-D-A-V-T-Y-T-E-H-A-K-R-K-T-V-T-A-M-D-V-V-Y-A-L-  y13
b91 -K-R-Q-G-R-T-L-Y-G-F-G-G-                                      y1
```

Methylation (mono) Acetylation Phosphorylation Dimethylation Trimethylation

H2AX_S1acS139p uniquely identified only by ETD







Top-down only! Not with bottom-up or middle-down!

Advantage of top-down fragmentation

HCD_60_60: H31_K9me2K27me2K36meK79me

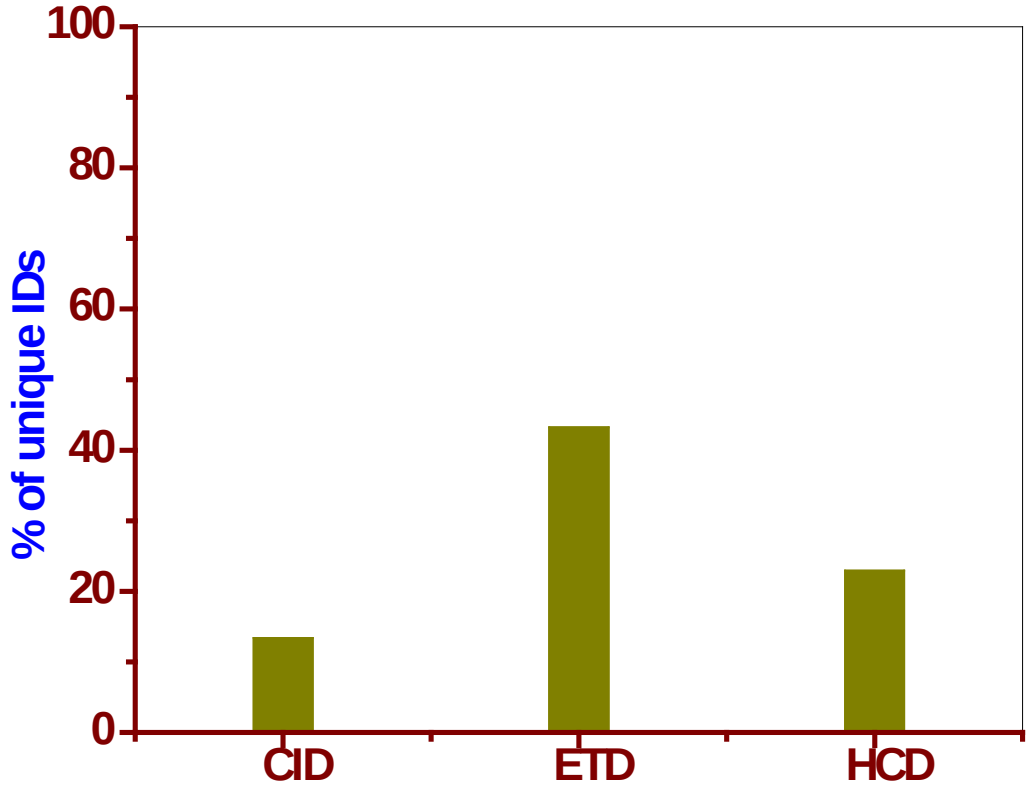
```
b1   - A - R - T - K - Q - T - A - R - K - S - T - G - G - K - A - P - R - K - Q - L - A - T - K - A - A - R - K - S - A - P -   y106
b31  - A - T - G - G - V - K - K - P - H - R - Y - R - P - G - T - V - A - L - R - E - I - R - R - Y - Q - K - S - T - E - L -   y76
b61  - L - I - R - K - L - P - F - Q - R - L - V - R - E - I - A - Q - D - F - K - T - D - L - R - F - Q - S - S - A - V - M -   y46
b91  - A - L - Q - E - A - C - E - A - Y - L - V - G - L - F - E - D - T - N - L - C - A - I - H - A - K - R - V - T - I - M -   y16
b121 - P - K - D - I - Q - L - A - R - R - I - R - G - E - R - A -   y1
```

CID_60_60: H2B1O_K46meK57me2

```
b1   - P - D - P - A - K - S - A - P - A - P - K - K - G - S - K - K - A - V - T - K - A - Q - K - K - D - G - K - E - R - K -   y96
b31  - R - S - R - K - E - S - Y - S - I - Y - V - Y - K - V - L - K - Q - V - H - P - D - T - G - I - S - S - K - A - M - G -   y66
b61  - I - M - N - S - F - V - N - D - I - F - E - R - I - A - G - E - A - S - R - L - A - H - Y - N - K - R - S - T - I - T -   y36
b91  - S - R - E - I - Q - T - A - V - R - L - L - L - P - G - E - L - A - K - H - A - V - S - E - G - T - K - A - V - T - K -   y6
b121 - Y - T - S - S - K -   y1
```

Methylation (mono) Acetylation Phosphorylation Dimethylation Trimethylation

Fragmentation efficiency of CID, ETD, HCD



$$\%_of_unique_IDs = \frac{unique_IDs}{unique_IDs + ambiguous_IDs} \times 100$$

Conclusions

- An online 2D LC-FTMS platform dramatically improved throughput and sensitivity compared to more traditional platforms, and resulted in identification of hundreds of histone isoforms from microgram levels of protein, including phosphorylation.
- For a single fragmentation method, ETD provides the most confident identiifcations on average.
- Total number of identifications more than doubled when CID was switched to ETD or HCD.
- Combinatorial modifications across the entire protein sequence could only be identified using top-down proteomics.

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