Electron-induced and electron-capture dissociation in data-dependent acquisition mode performed on the Omnitrap platform coupled to an Orbitrap MS

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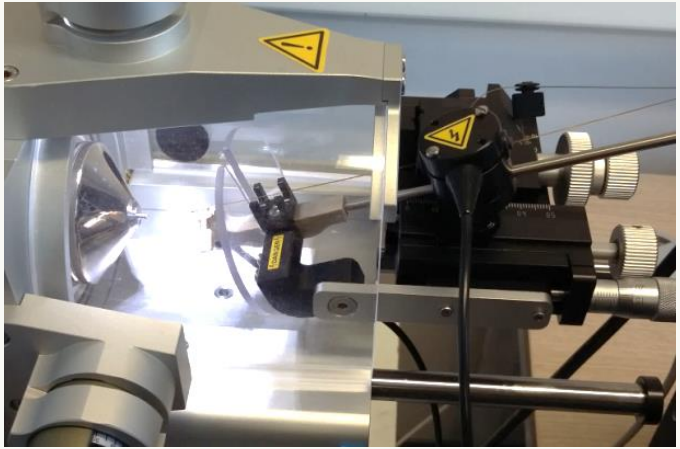
FasmaTECH science and technology

Karolinska Institutet

The Omnitrap platform coupled to Q Exactive HF and EASY-nLC 1000

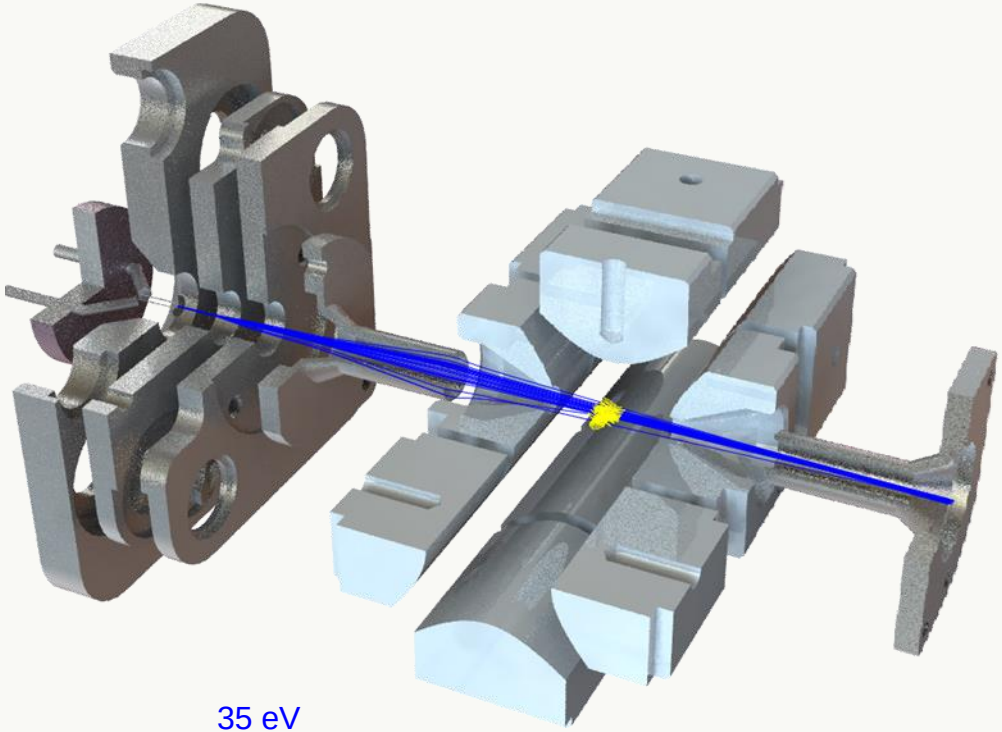
Fasmatech's OmniLab



NanoSpray Flex™ Ion Source
(Thermo Fisher Scientific)

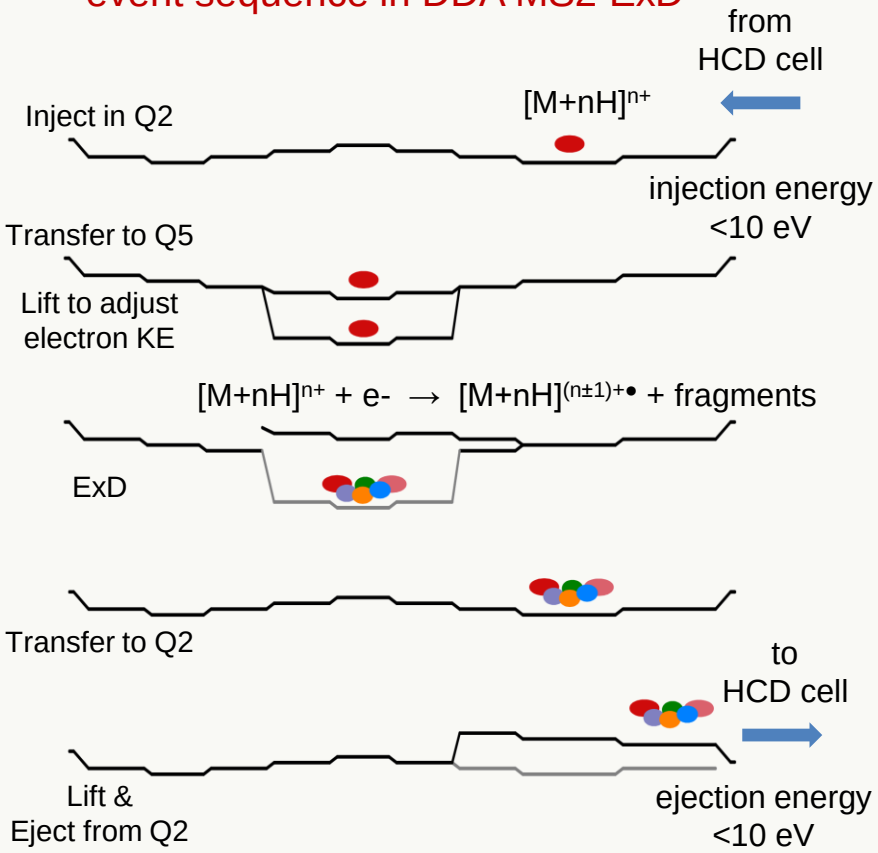
C18 PepSep nLC column
(25cm×75μm, 3μm)

Electron-based Activation in Segment Q5 of the Omnitrap Platform



35 eV
electron energy
for optimum EID

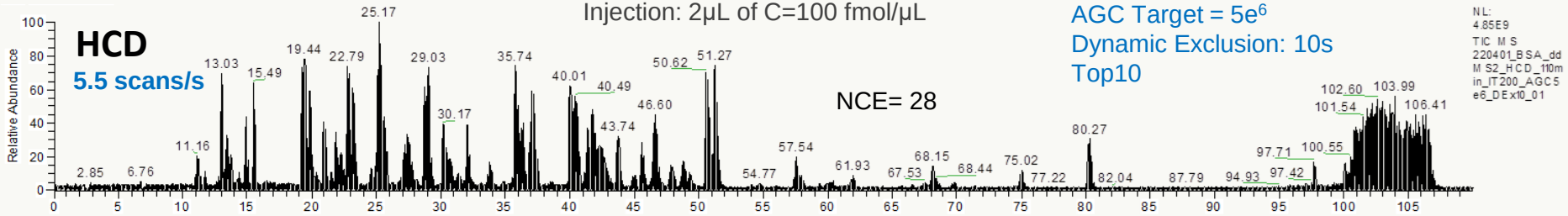
event sequence in DDA MS2 ExD

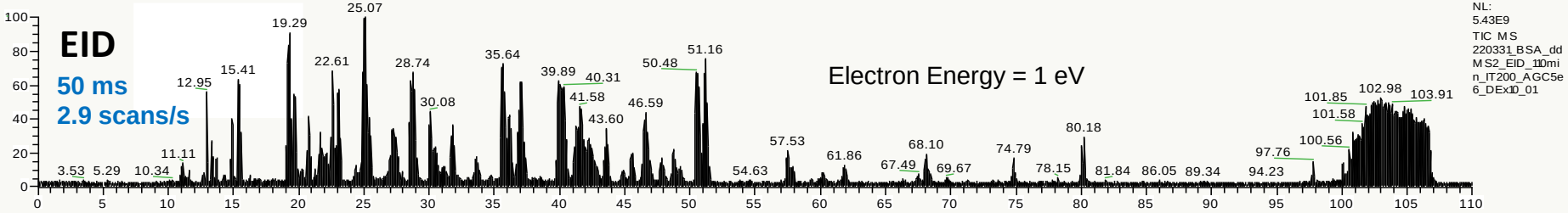


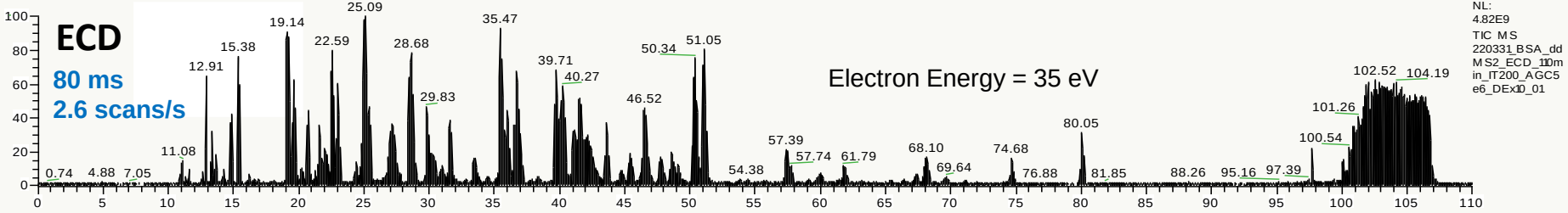
Data-Depended Acquisition in the Omnitrap Q Exactive HF Platform

BSA Digest H2O:FA:ACN 92:5:3 v/v
Injection: 2µL of C=100 fmol/µL

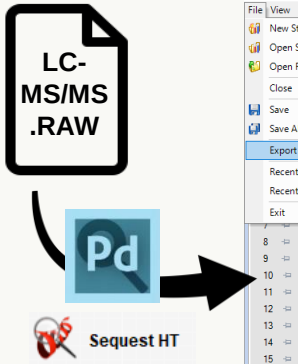
Max Injection Time = 200ms
AGC Target = $5e^6$
Dynamic Exclusion: 10s
Top10

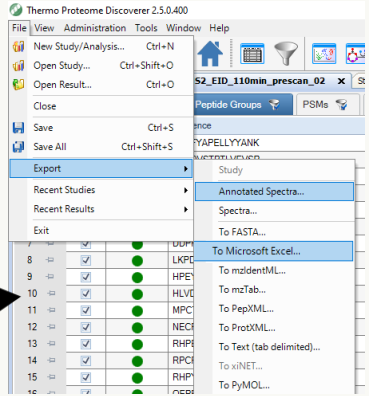


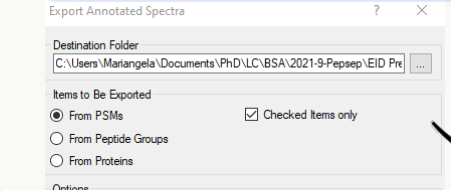


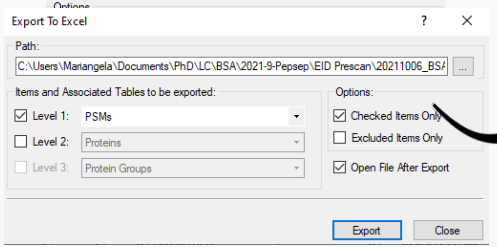


Data Analysis

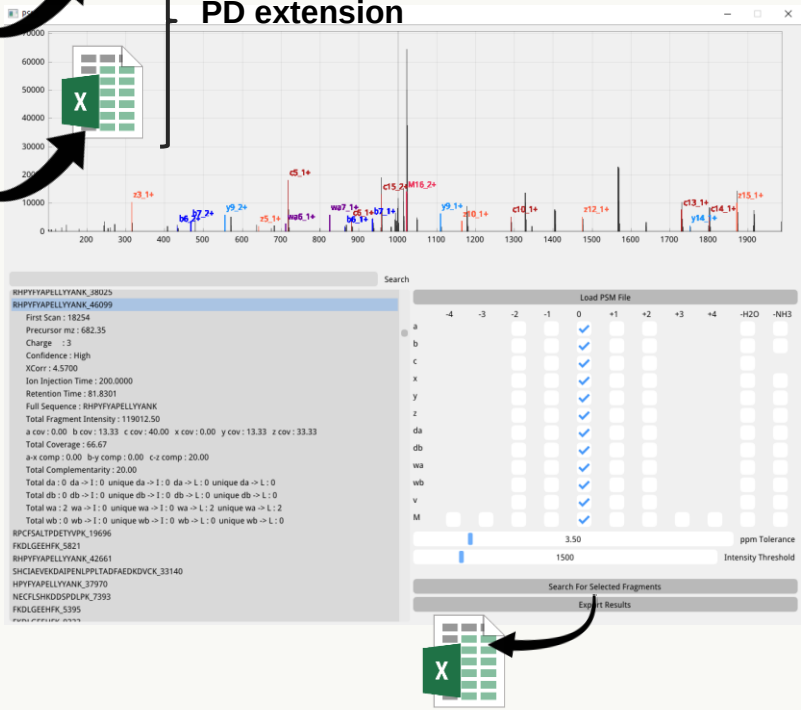




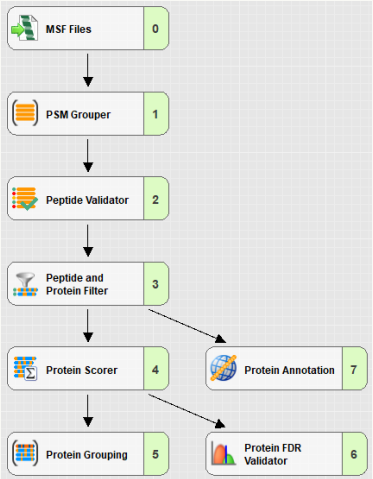




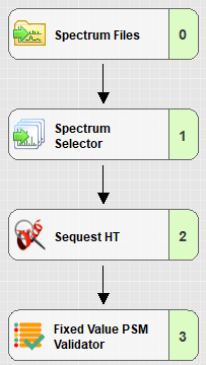
PD extension



Consensus step:
CWF_Basic_Annotation



Processing step:
PWF_OT_Basic_SequestHT

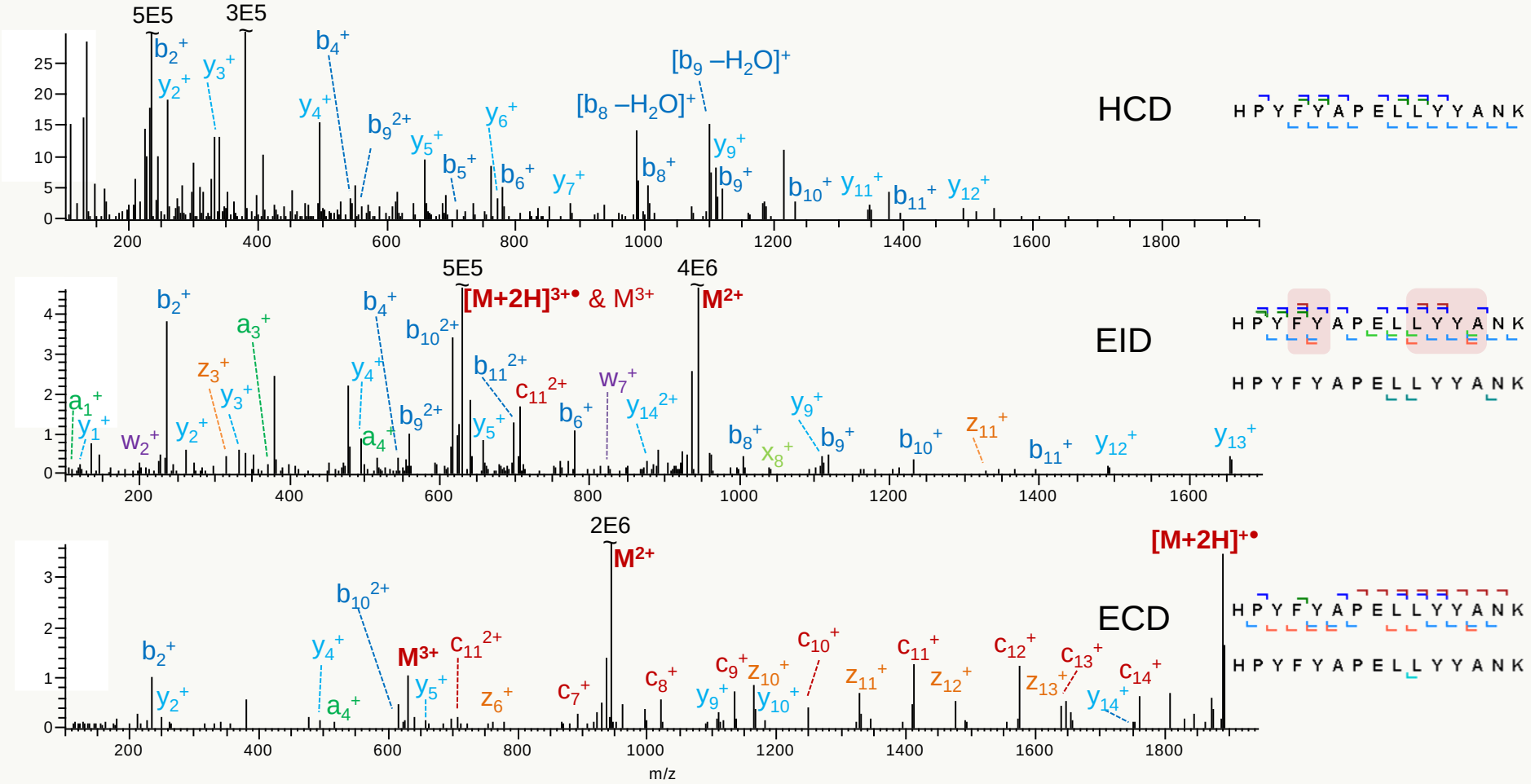


All primary fragments (a, b, c, x, y, z) are included in the ExD (x=I, C) search, while the HCD search is limited to a, b and y.

Fasmatech's PD extension software recalculates and reassigns fragments to all MS2 peptide mass spectra identified in PD.

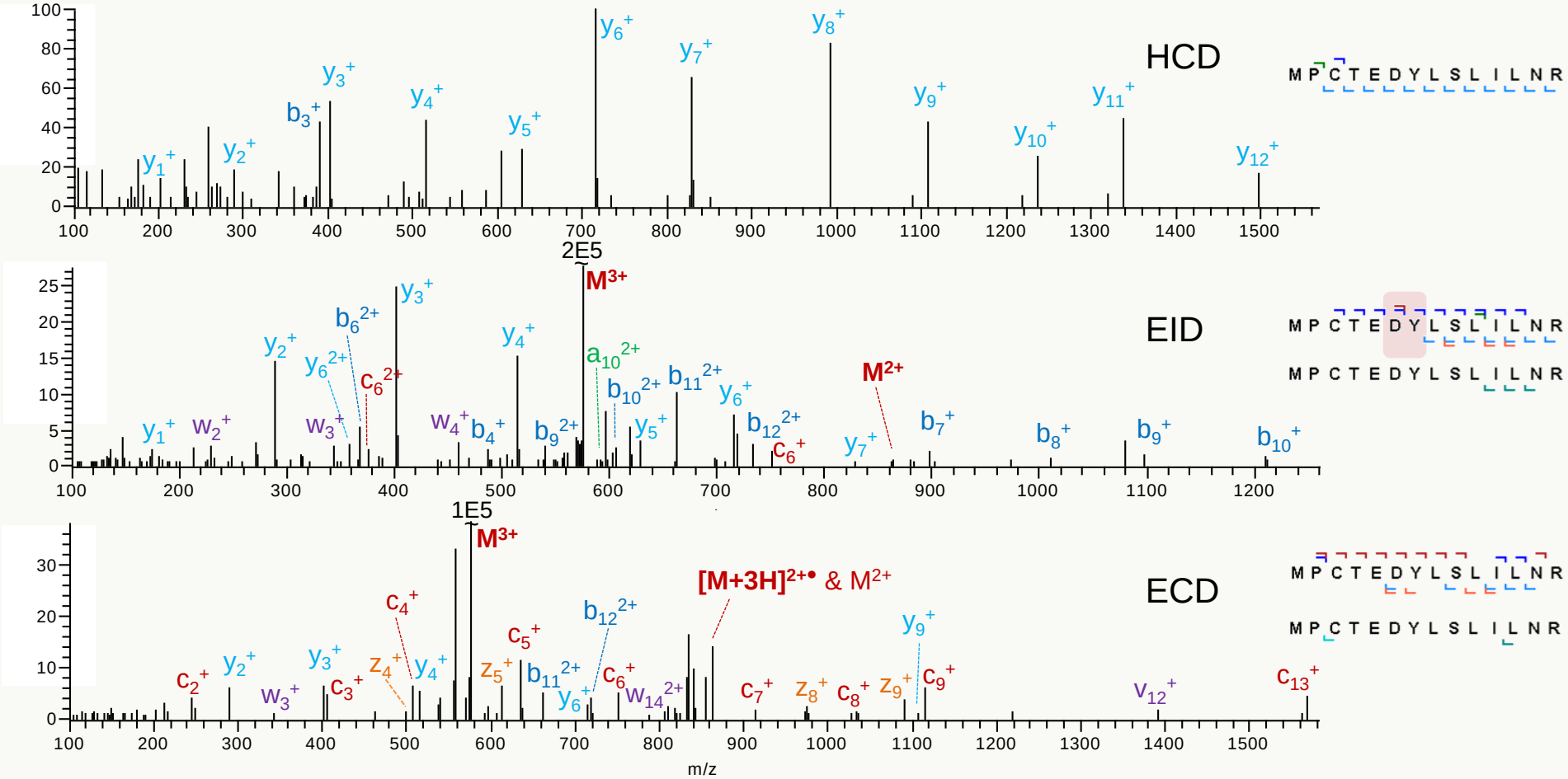
2+

Enhancing Sequence Coverage, Complementarity and side chain information



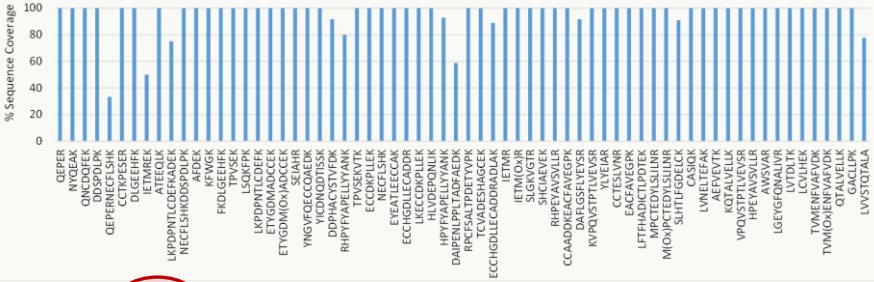
3+

Enhancing Sequence Coverage, Complementarity and side chain information

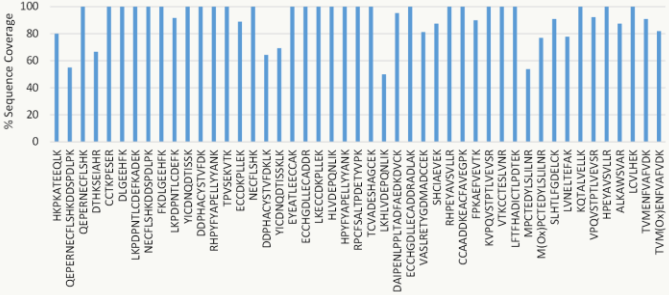


Sequence coverage *vs* Peptide Charge State

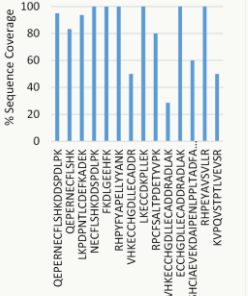
HCD 2+ 96%



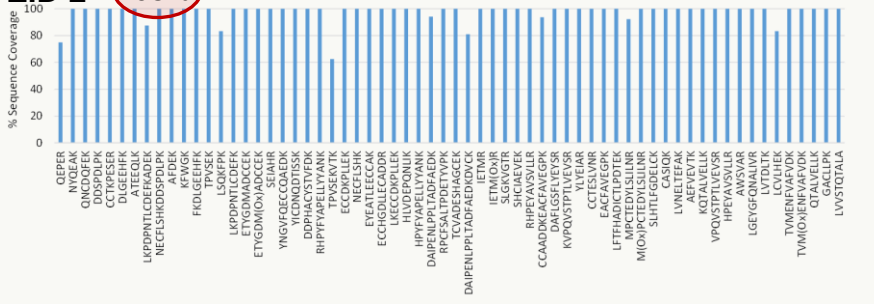
HCD 3+ 91%



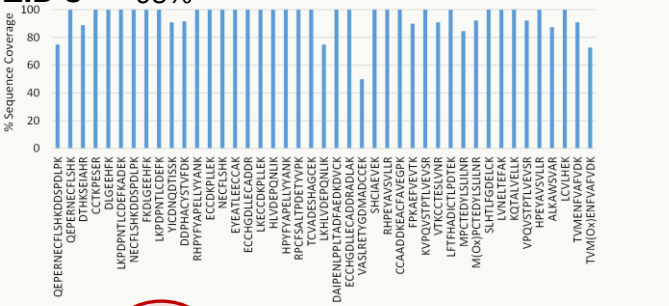
HCD 4+ 81%



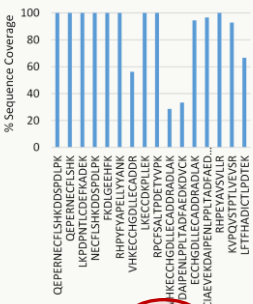
EID 2+ 98%



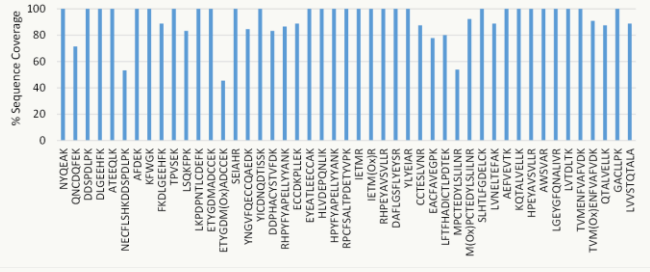
EID 3+ 95%



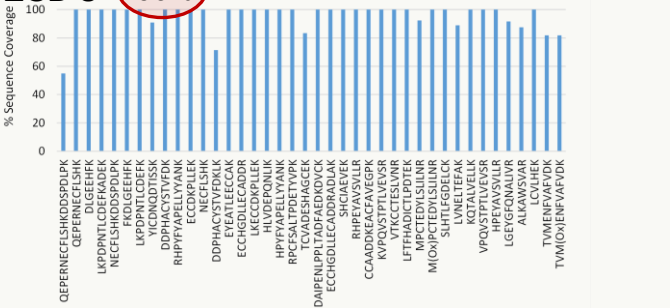
EID 4+ 86%



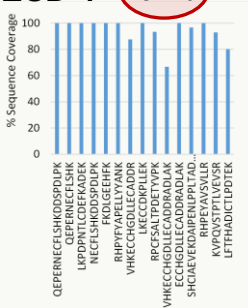
ECD 2+ 92%



ECD 3+ 96%

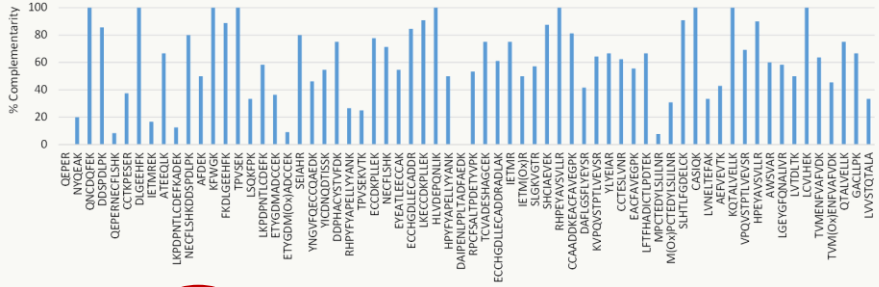


ECD 4+ 94%

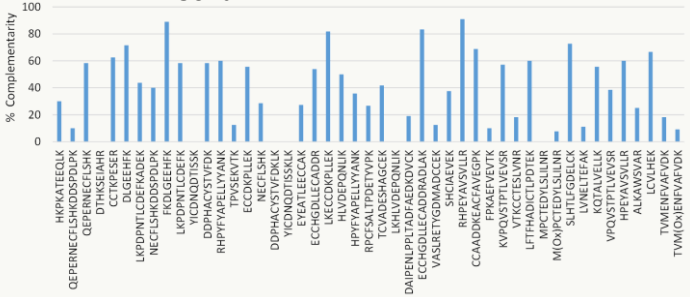


Complementarity *vs* Peptide Charge State

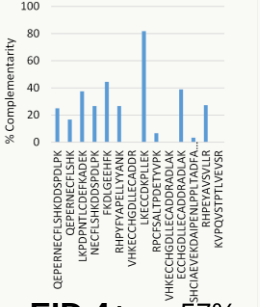
HCD 2+ 60%



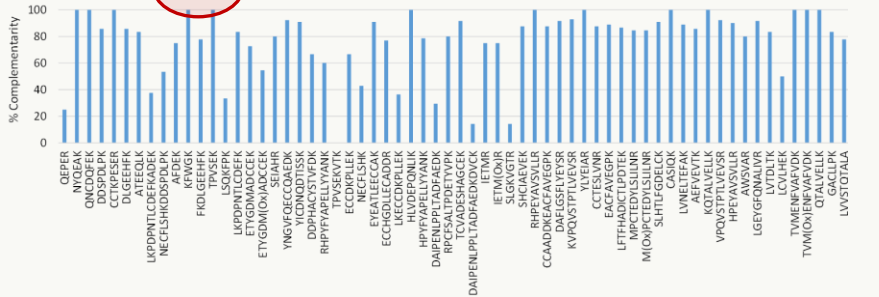
HCD 3+ 39%



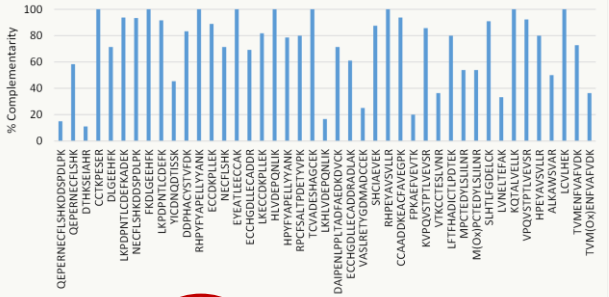
HCD 4+ 24%



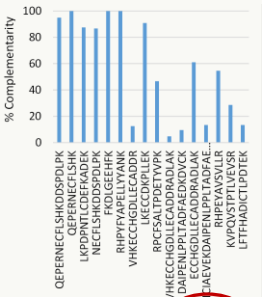
EID 2+ 77%



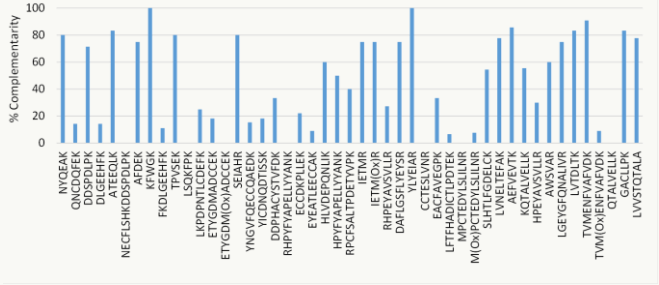
EID 3+ 71%



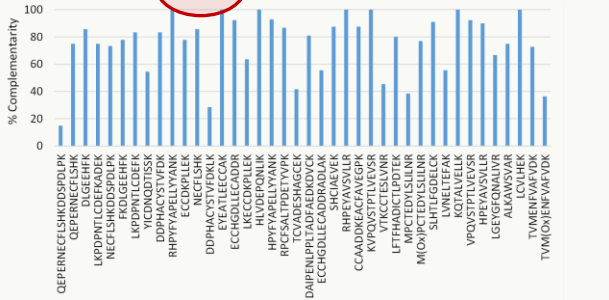
EID 4+ 57%



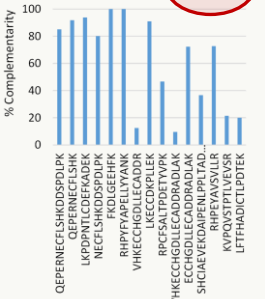
ECD 2+ 44%



ECD 3+ 76%



ECD 4+ 62%

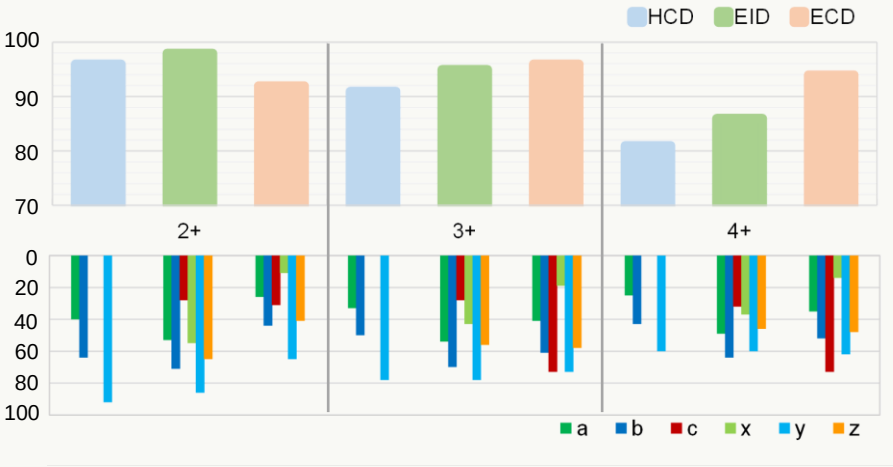


Summary Table & Results

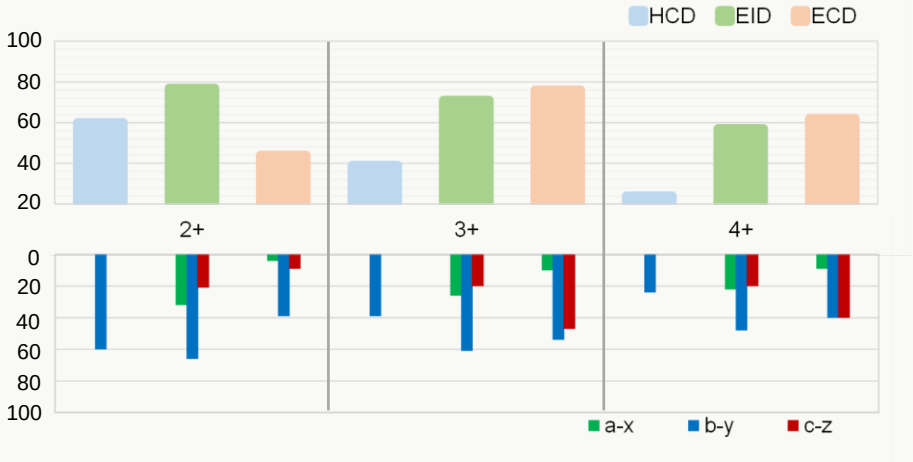
Exp	BSA PSMs	Total PSMs	MS/MS	Peptides	Sequence Coverage (%)
HCD	2044	3241	18907	76	89
EID	1600	3094	14608	72	88
ECD	1214	2598	13073	63	82

Max Injection time = 200 ms
AGC Target=$5e^6$
Dynamic Exclusion=10s
Top10

Sequence Coverage

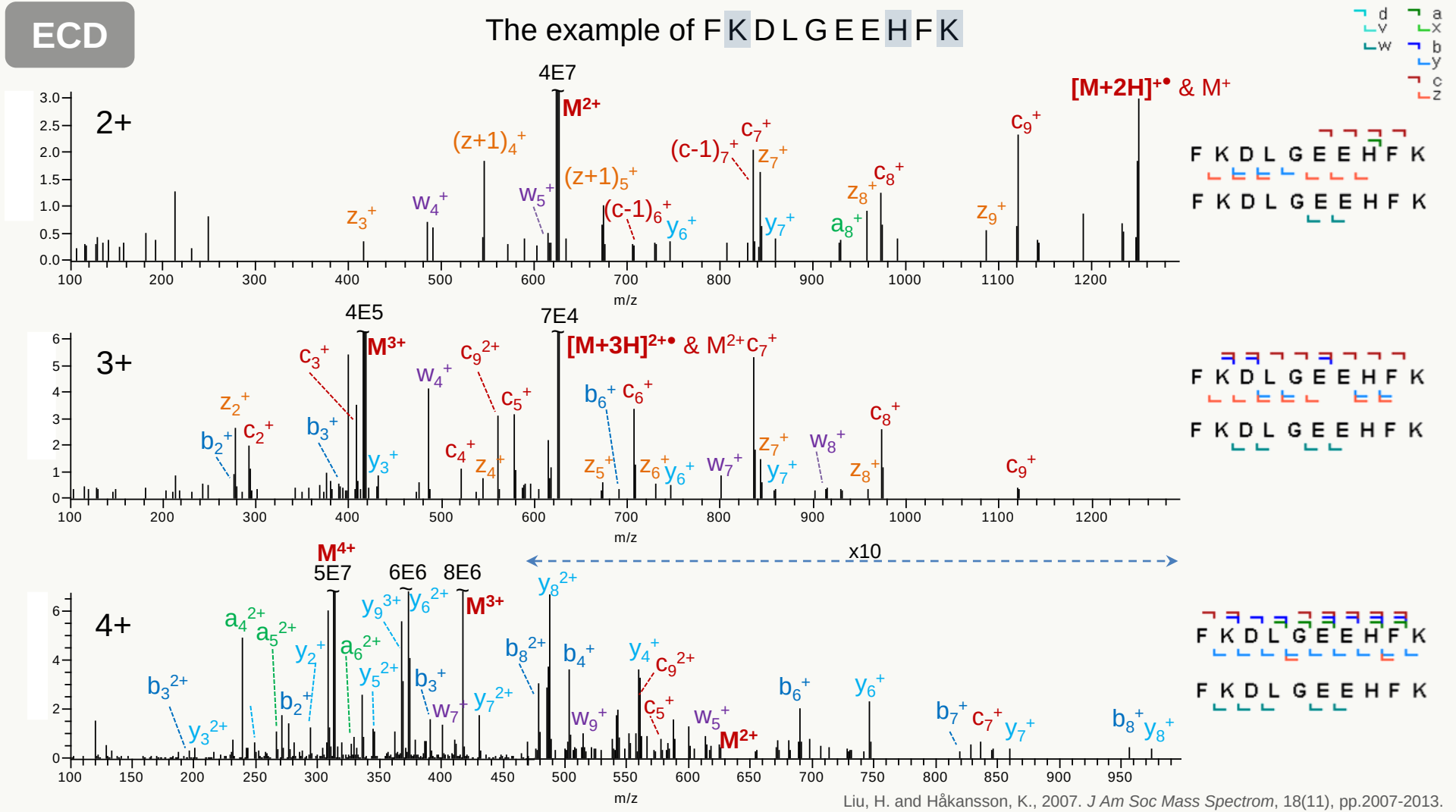


Complementarity

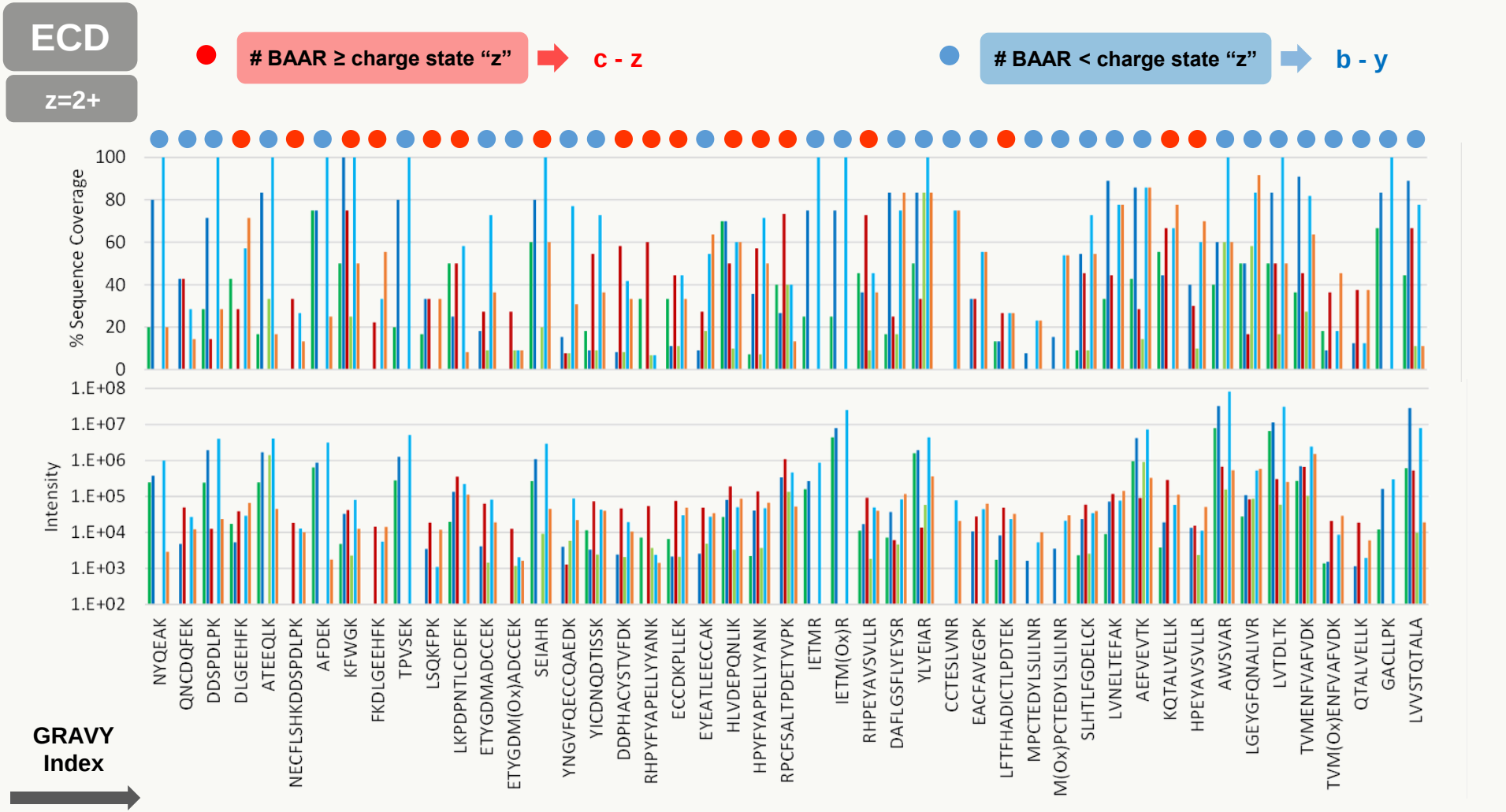


ECD

The example of F K D L G E E H F K



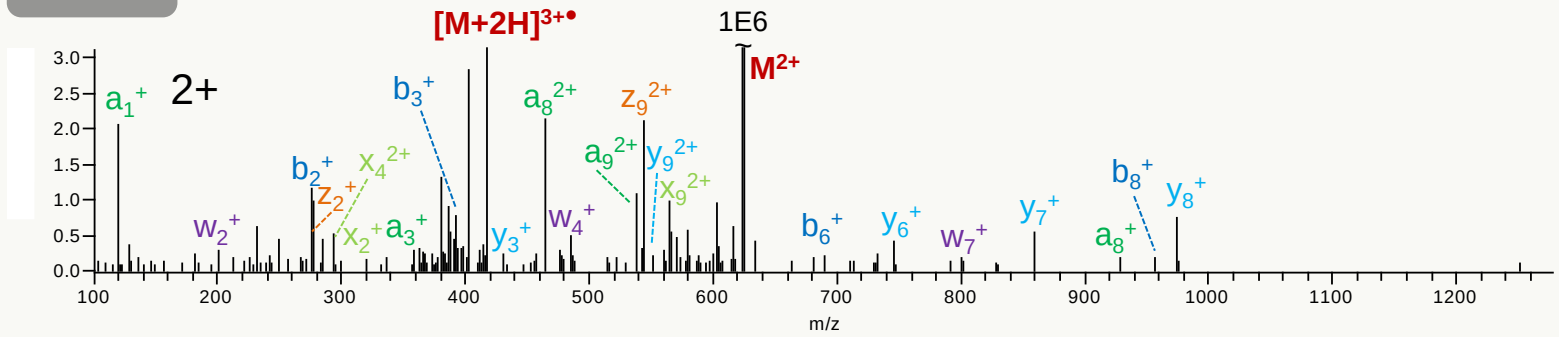
Liu, H. and Håkansson, K., 2007. *J Am Soc Mass Spectrom*, 18(11), pp.2007-2013.

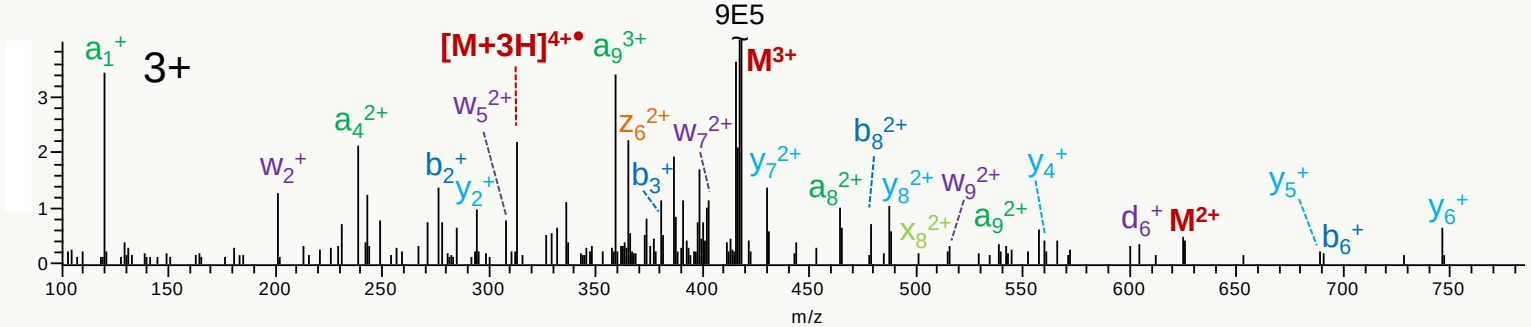


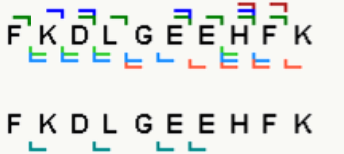
Bakken, V., Helgaker, T. and Uggerud, E., 2004. *European J Mass Spectrom*, 10(5), pp.625-638.

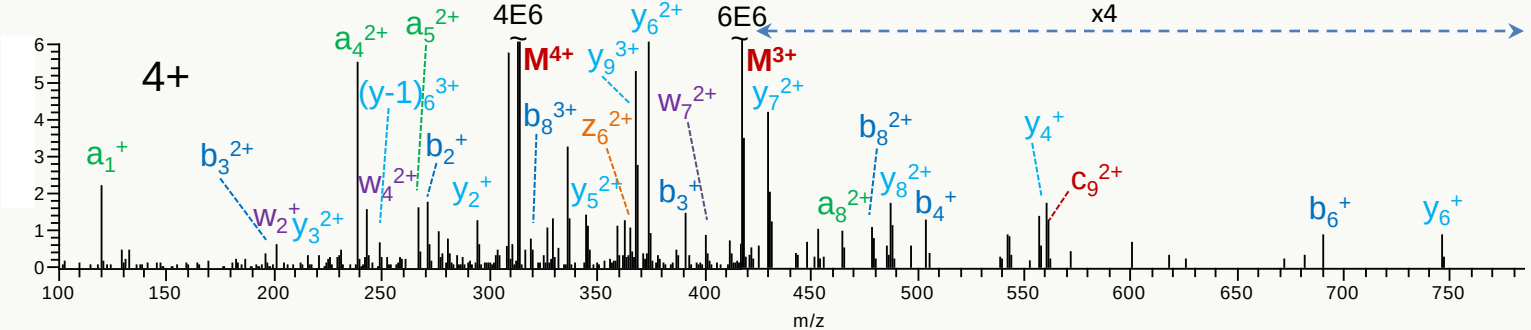
EID

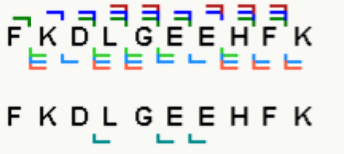
The example of F K D L G E E H F K







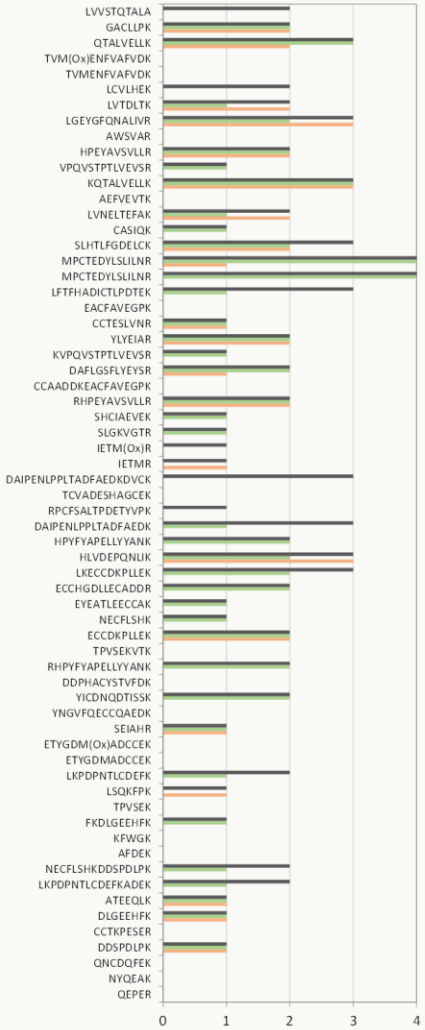




Leucine & Isoleucine w diagnostic fragments

Leu + Ile | EID w | ECD w

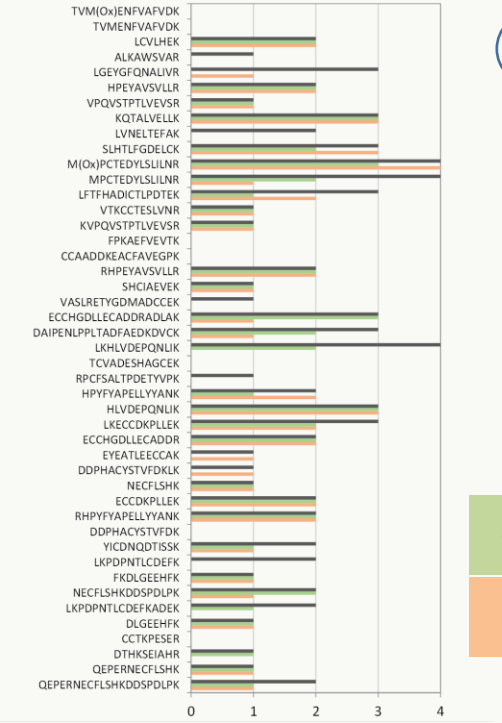
2+



72%

55%

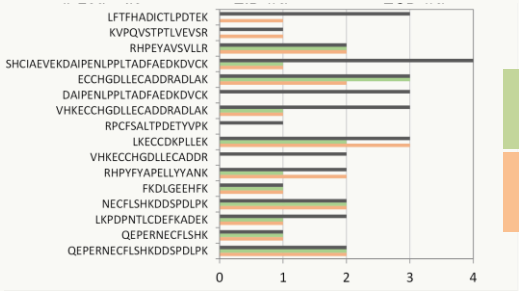
3+



69%

69%

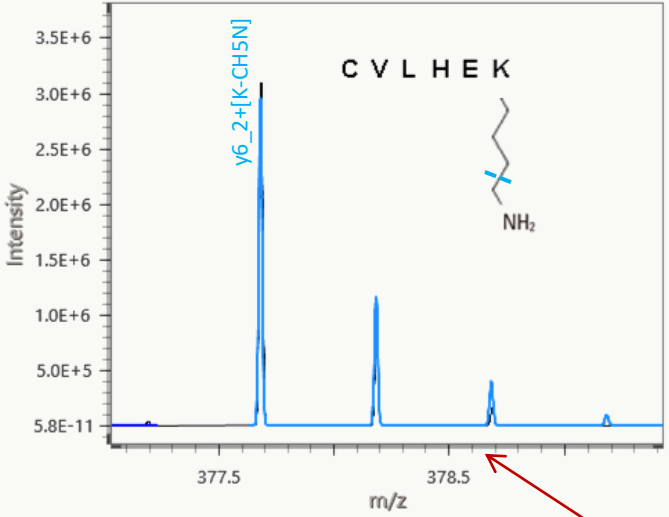
4+



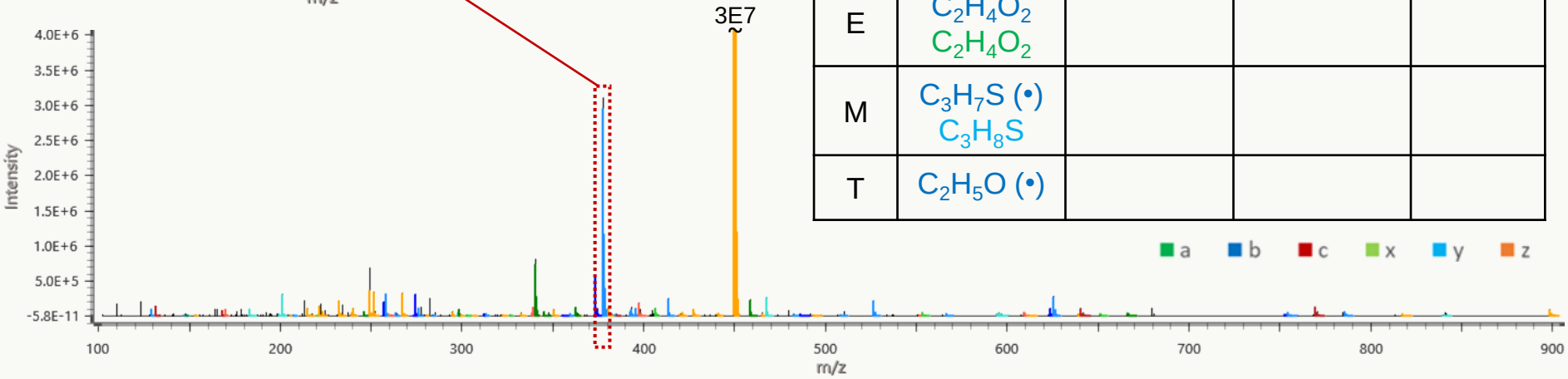
49%

63%

EID fragments with neutral side chain losses

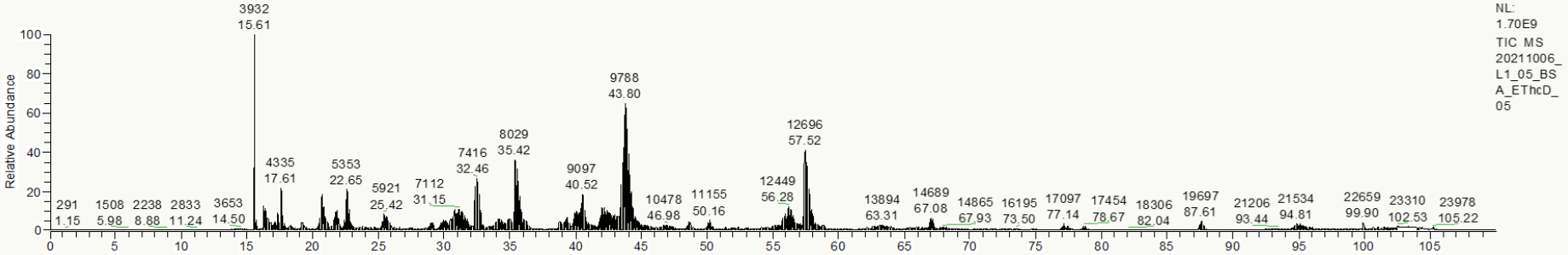


LCVLHEK 2+
*carbamidomethyl



AA	Side chain cleavage position			
	C_α / C_β	C_β / C_γ	C_γ / C_δ	C_δ / C_ε
K		C_3H_9N		CH_5N
R			$C_2H_6N_3$ (•)	CH_3N_3
Y		C_6H_6O C_6H_5O (•) C_6H_5O (•)		
E	$C_2H_4O_2$ $C_2H_4O_2$			
M	C_3H_7S (•) C_3H_8S			
T	C_2H_5O (•)			

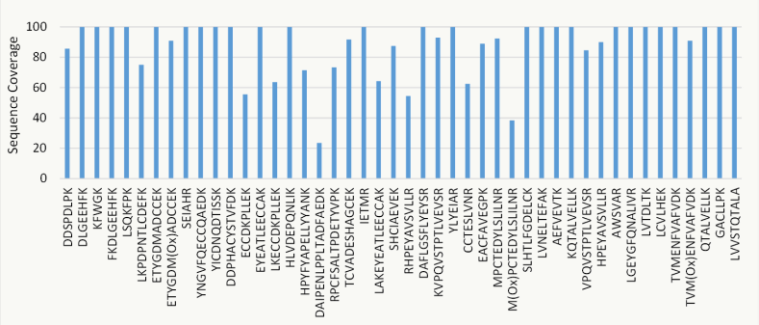
Comparing ExD on the Omnitrap with EThcD on the Fusion Lumos



EID 2+ 98%

ECD 2+ 92%

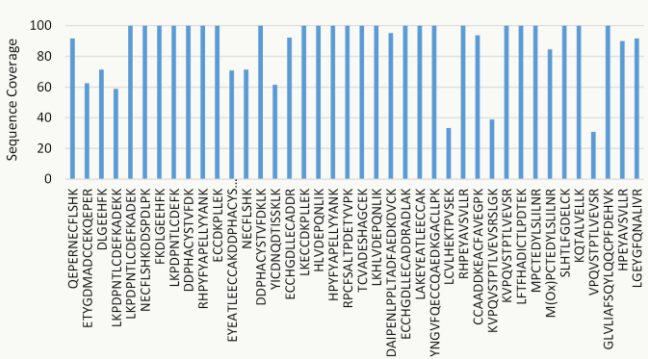
EThcD 2+ 89%



EID 3+ 95%

ECD 3+ 96%

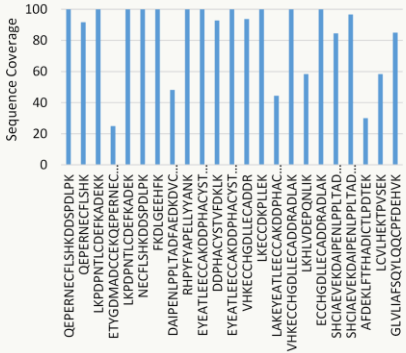
EThcD 3+ 88%

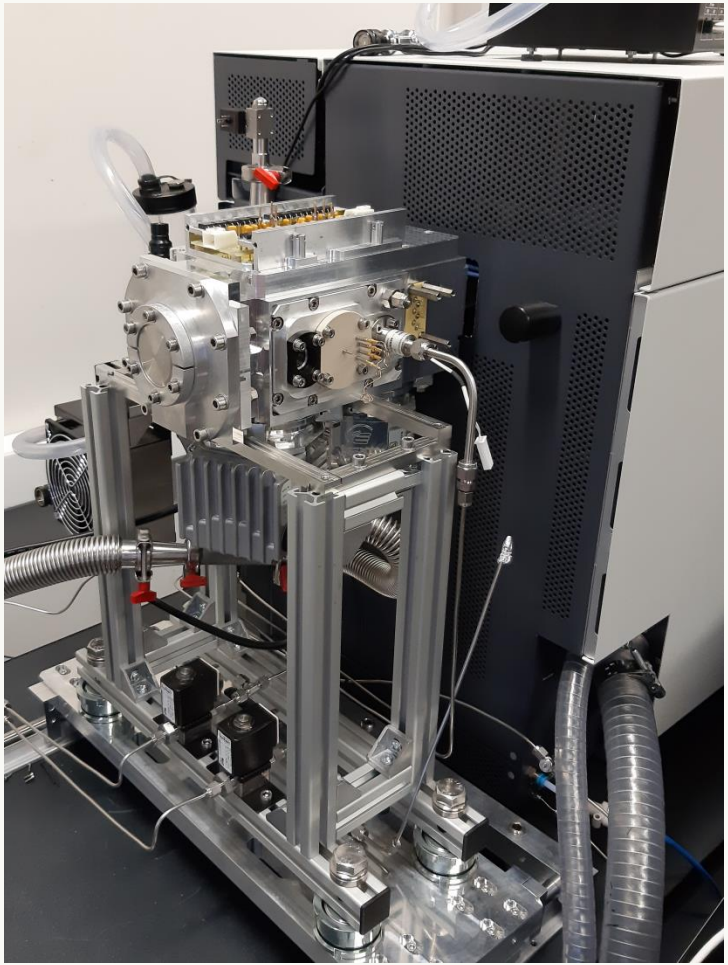


EID 4+ 86%

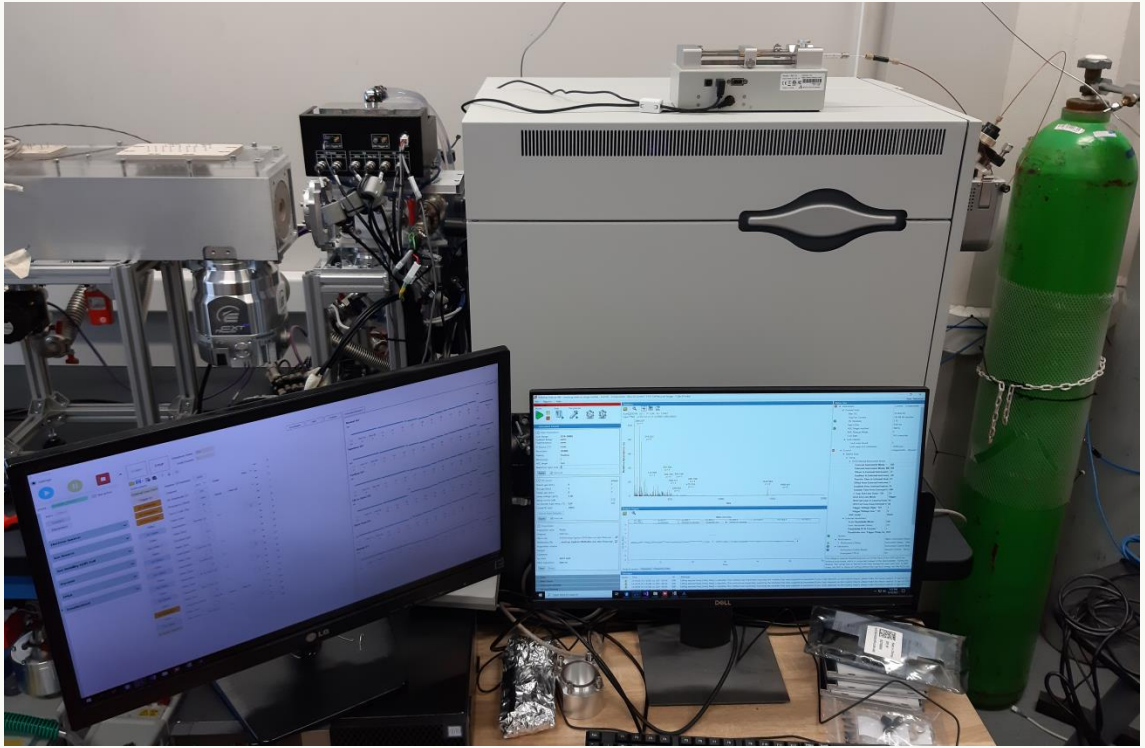
ECD 4+ 94%

EThcD 4+ 83%



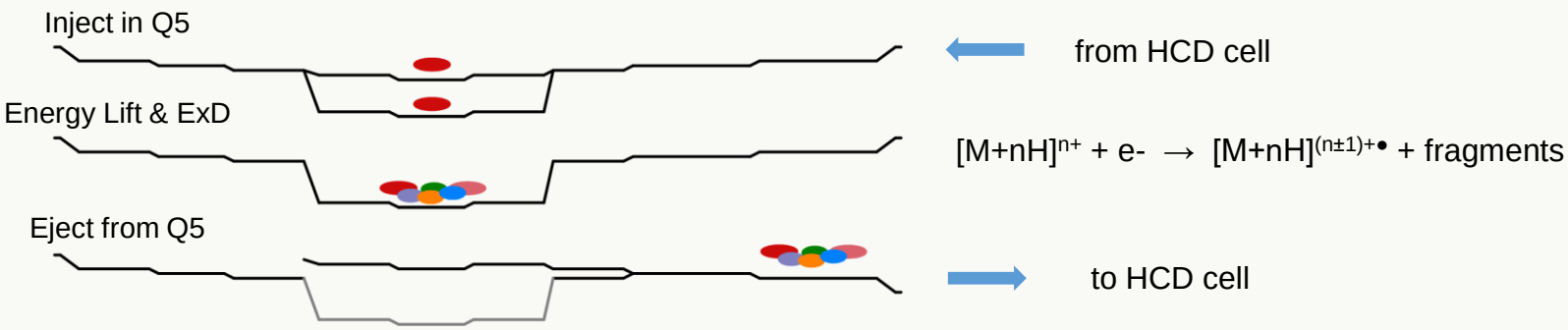


Omnitrap platform coupled to Exploris 480 Mass Spectrometer



Electron-based Activation in Segment Q5 of the Omnitrap Platform

Optimized event sequence for maximum speed in DDA MS2 ExD



EID reaction time: 35 ms
Transfer time from and to HCD cell: 35 ms
OMNITRAP Processing cycle: ~**70 ms**

processing in HCD – c-trap: 10 ms
max Injection time: 35 ms
ORBITRAP Processing cycle: ~**45 ms**

~9 Hz

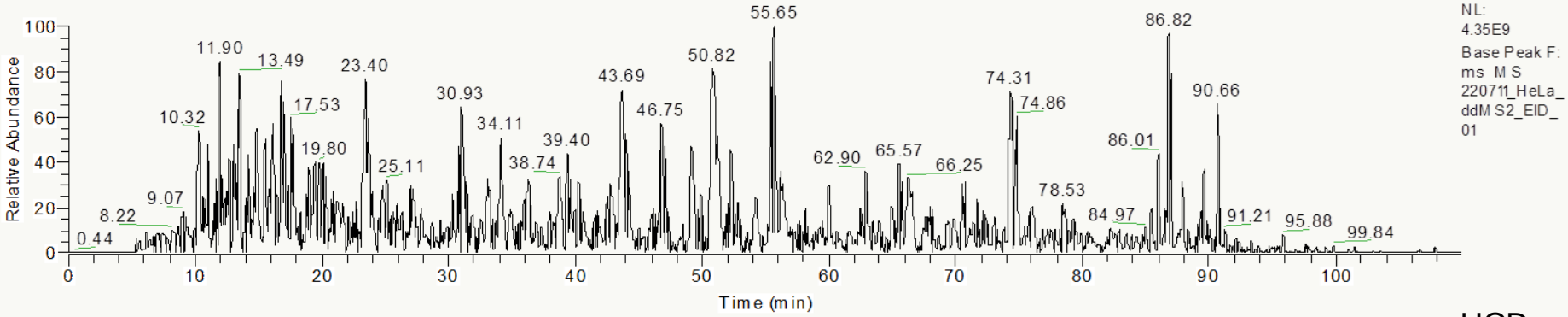
ECD reaction time: 100 ms
Transfer time from and to HCD cell: 35 ms
OMNITRAP Processing cycle: ~**135 ms**

processing in HCD – c-trap: 10 ms
max Injection time: 35 ms
ORBITRAP Processing cycle: ~**45 ms**

~6 Hz

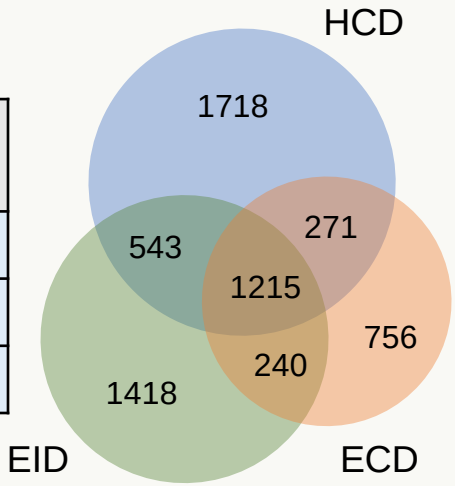
Orbitrap Mass Resolving Power:15k, 32 ms

Extending the analysis to complex protein samples – HeLa digest



Fragm. Method	ddMS2 AGC target	ddMS2 max IT (ms)	electron reaction time (ms)	scan rate (scans/s)	Proteins *High confidence*	Peptides* (±10 ppm)	MS/MS*	PSMs*
HCD	1e6	35	-	~ 16.4	**3747**	31438 / 70465	42903 / 89739	41783 / 88432
EID	1e6	35	35	~ 8.5	**3416**	21342 / 41767	23255 / 48909	22879 / 46683
ECD	1e6	35	100	~ 5.9	**2482**	13676 / 23786	15242 / 35397	15169 / 26302

* Peptides, MS/MS and PSMs refer to “high-confidence protein identifications” using both medium and high confidence peptides



High confidence protein Venn diagram

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