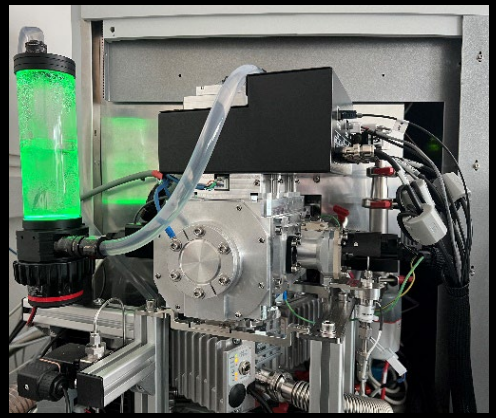
The 71st ASMS Conference, Houston, TX

Omnitrap-Orbitrap performance enhancement via unreduced data processing



Anton Kozhinov, Konstantin Nagornov, & Yury Tsybin

Spectroswiss, Lausanne, Switzerland

Conflict of interest: these co-authors are employees of Spectroswiss, which develops MS hardware & software

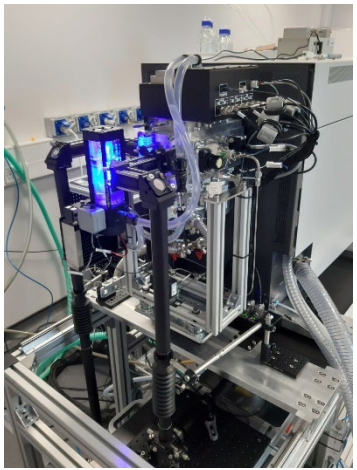
Camille Garcia, Martial Rey, Tingting Fu, & Julia Chamot-Rooke

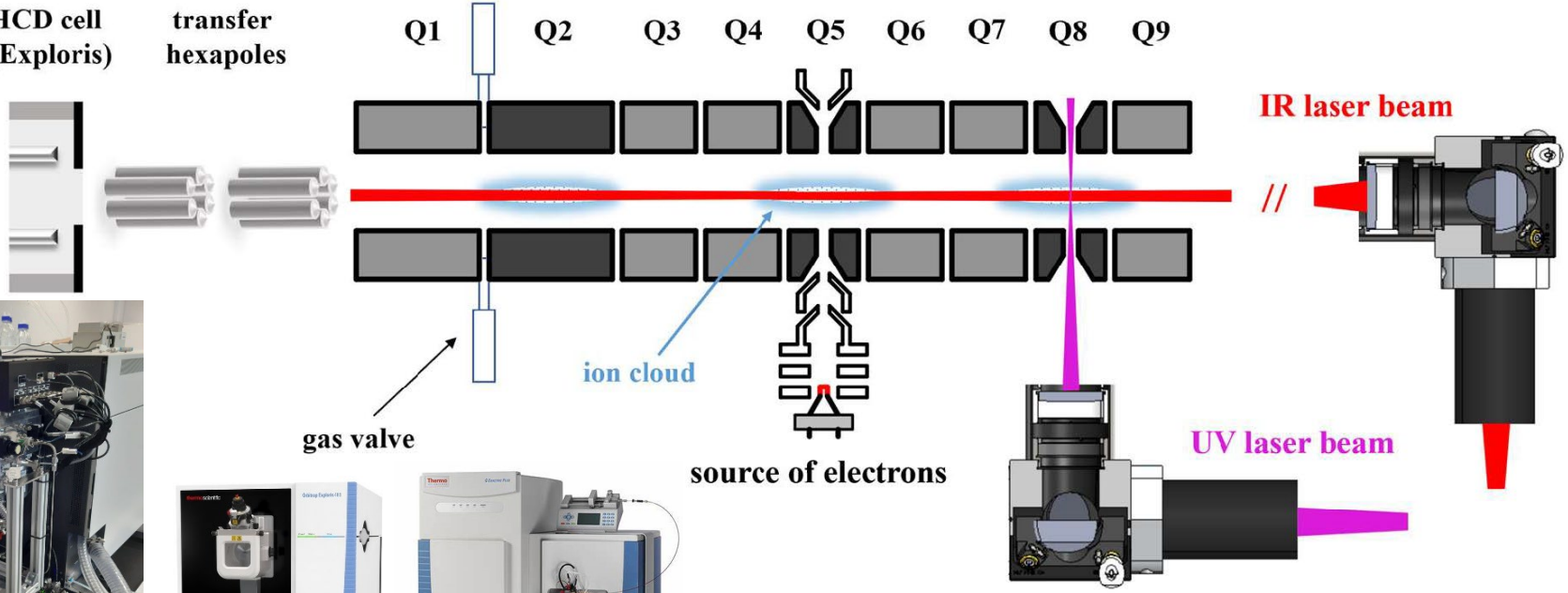
Mass Spectrometry for Biology Unit, Institut Pasteur, Paris, France

Wednesday, June 7, 2023: WOA am Instrumentation: New Hybrid and Multimodal Approaches

Omnitrap-Orbitrap: A Multimodal MS/MS Platform

- **Omnitrap**: an add-on linear ion trap for the multimodal & multiple-stage tandem MS (MS/MS)
- Transforms the **HCD** (*b/y*-ions) Q Exactive & Exploris Orbitraps into versatile MS/MS platforms
- **ECD** (*c/z*-ions), electron ionization dissociation (**EID**), photo dissociation (**IR**, **UV**), **CID**, & more

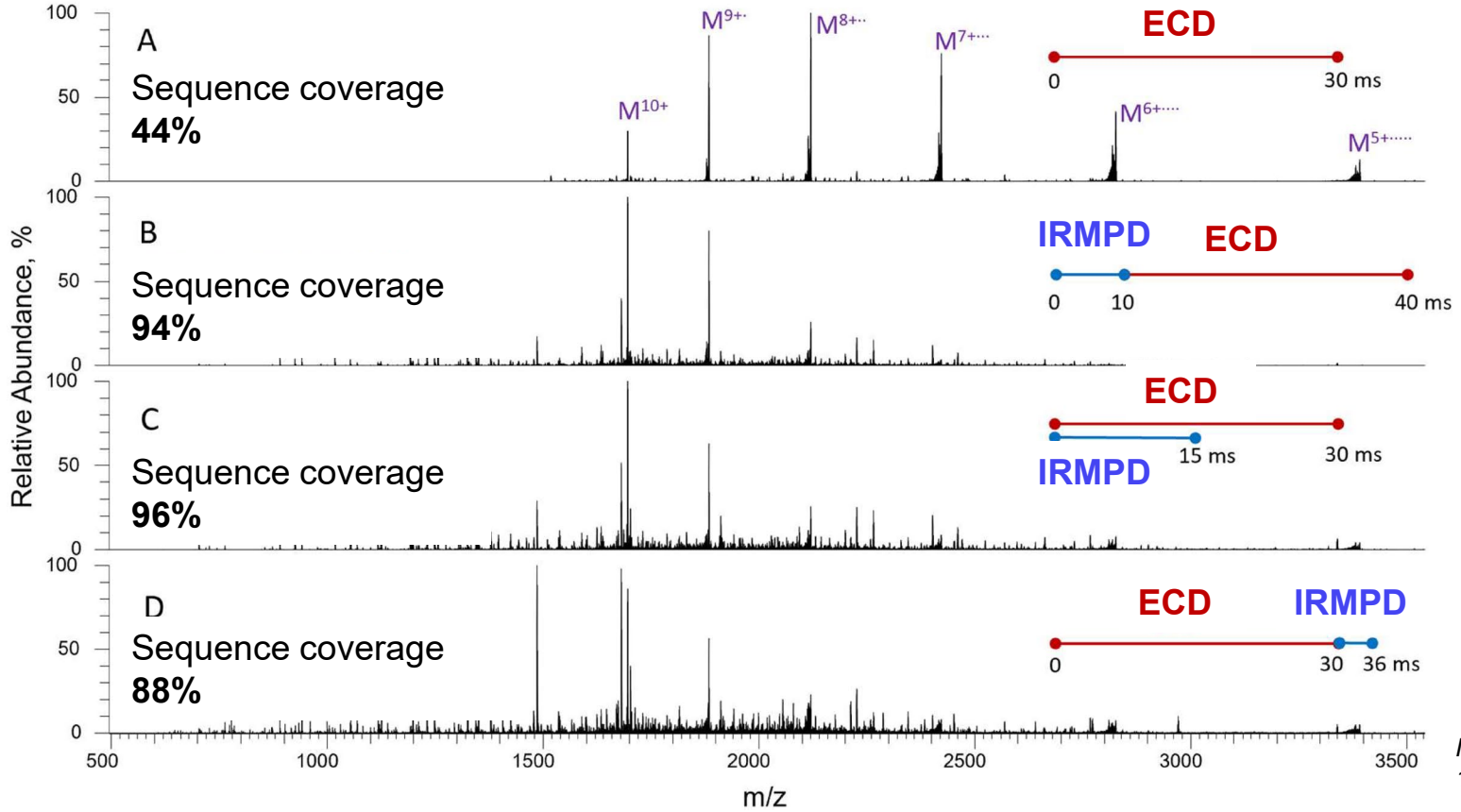




Papanastasiou et al., JASMS, 2022, 33, 10, 1990–2007

Mohammed et al., DOI 10.1101/2023.05.15.540788

ECD and Activated-Ion ECD of Myoglobin (16.7 kDa)



Direct infusion

60 scans (1 us)

AGC 1e6

480k @ *m/z* 200

Mohammed et al., DOI 10.1101/2023.05.15.540788

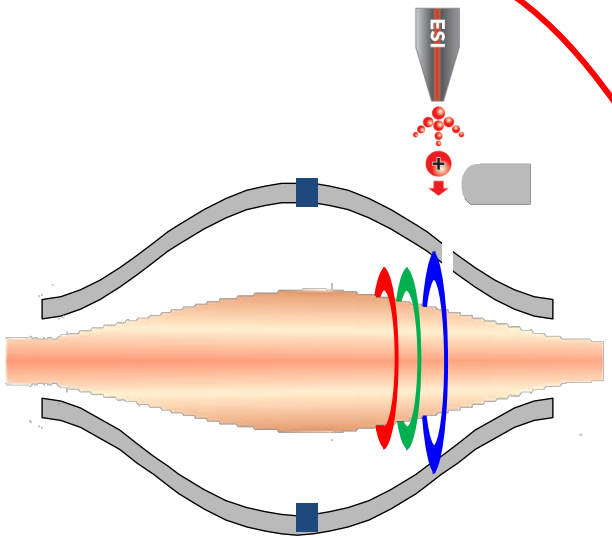
How to increase sequence coverage & confidence? The unreduced data!

Ion Signals in Orbitrap FTMS: **Transients**

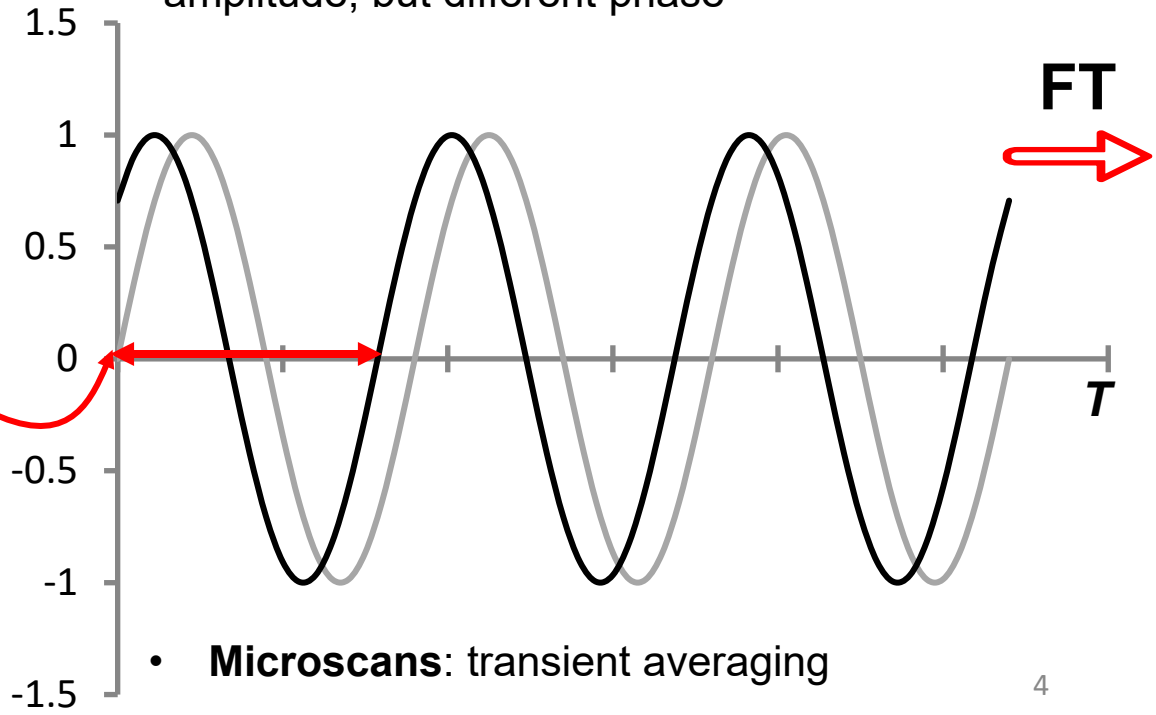
- Ion identity (*m/z*) is encoded as a frequency of ion oscillations in an orbitrap
- Frequencies of ion oscillations are measured as time-domain signals (**transients**)

Three components to define a wave:

- Frequency
- Magnitude (measure of amplitude)
- Phase (angle)



- Example: two waves of the same frequency and amplitude, but different phase



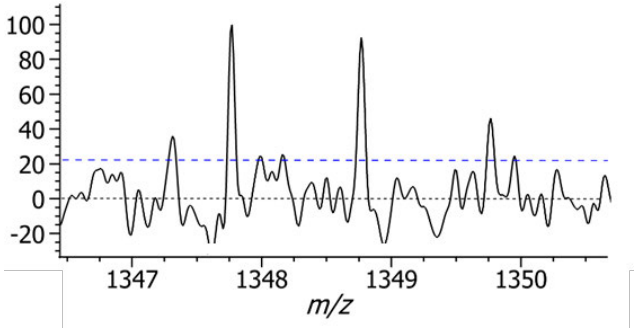
- **Microscans**: transient averaging

Orbitrap Mass Spectra: aFT vs. eFT

- **Absorption FT (aFT)** preserves all information (*f, A,* phase), equal to transients = **unreduced**
- **Enhanced FT (eFT)** mass spectra in full or reduced profile (.RAW) = **reduced data**

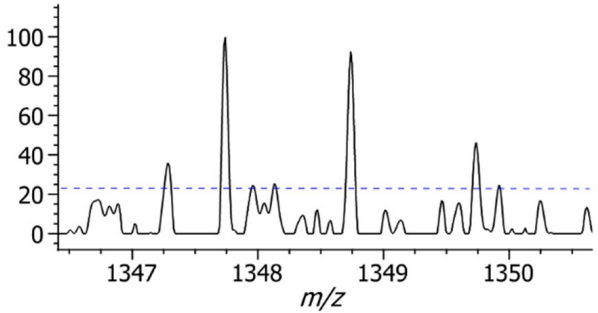
unreduced data | **reduced data**

Desired: full profile aFT



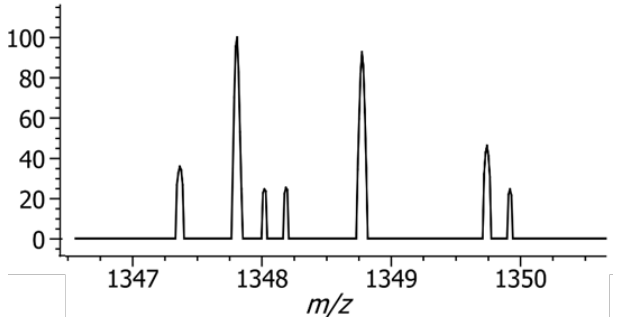
$$\frac{S}{N} \sim \sqrt{n}$$

Full profile eFT



$$\frac{S}{N} < \sqrt{n}$$

Reduced profile eFT

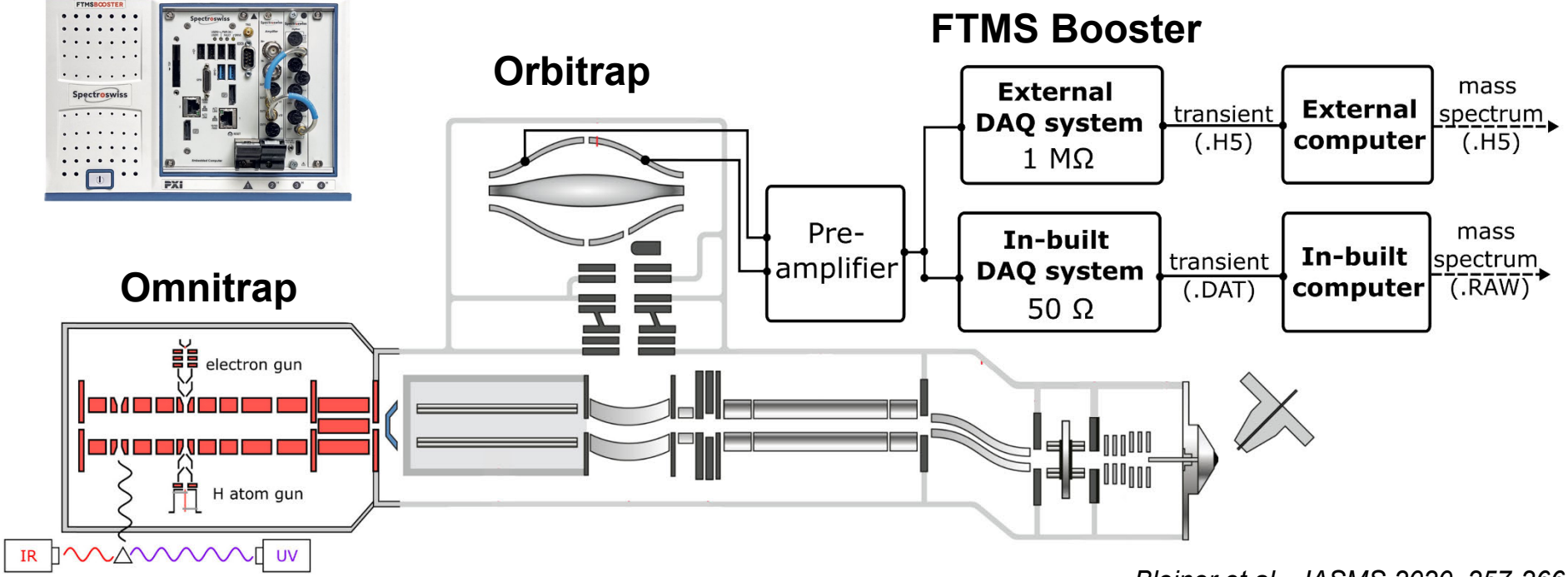


$$\frac{S}{N} < \sqrt{n}$$

How to access the unreduced data?

Accessing the Unreduced Data from an Omnitrap-Orbitrap Platform

- Parallel transient acquisition via a high-performance external data acquisition (DAQ) system



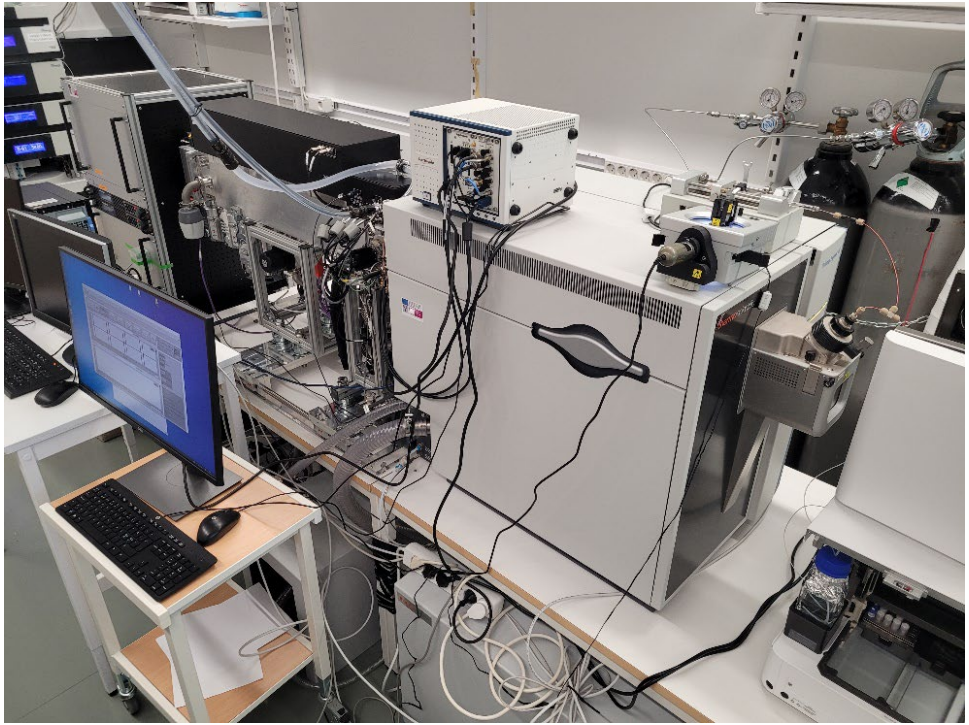
Exploris™ schematics: https://planetorbitrap.com/

Bleiner et al., JASMS 2020, 257-266

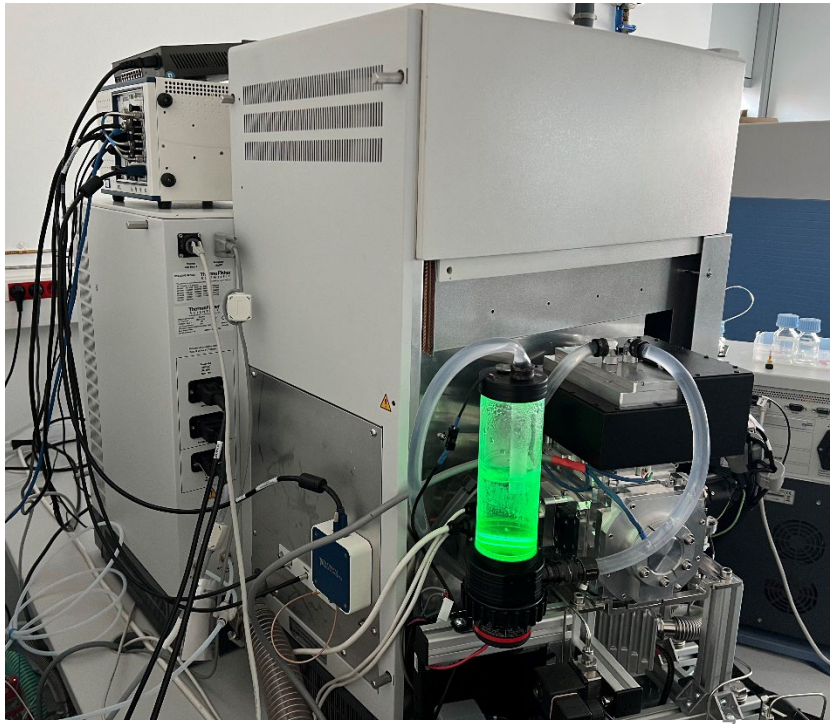
Marcus et al., JASMS 2021, 1224−1236

Orbitrap - Omnitrap - FTMS Booster: A Powerful MS/MS Platform

Exploris 480 Set-Up
Karolinska Institute, Sweden
Roman Zubarev's Lab



Q Exactive HF Set-Up
Pasteur Institute, France
Julia Chamot-Rooke's Lab



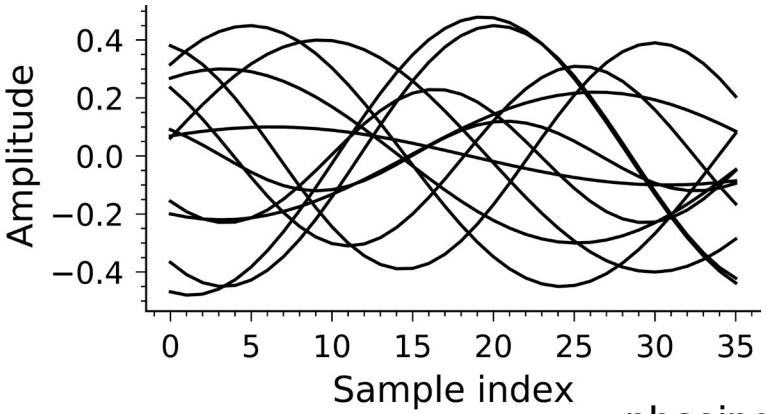
FTMS Booster Provides **aFT Mass Spectra** via Phased Transients

transients

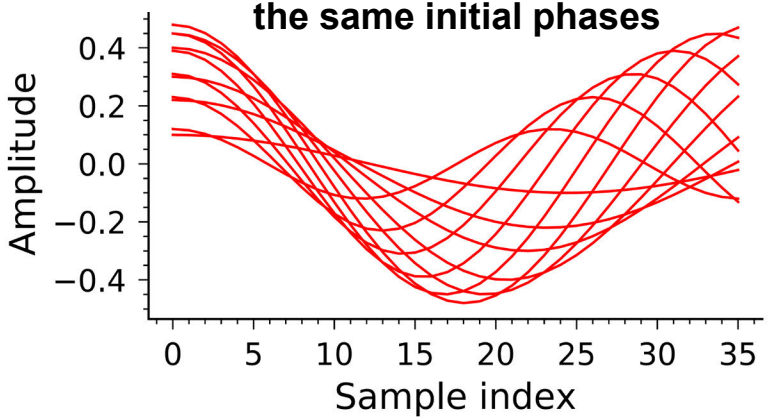
FT

aFT mass spectra

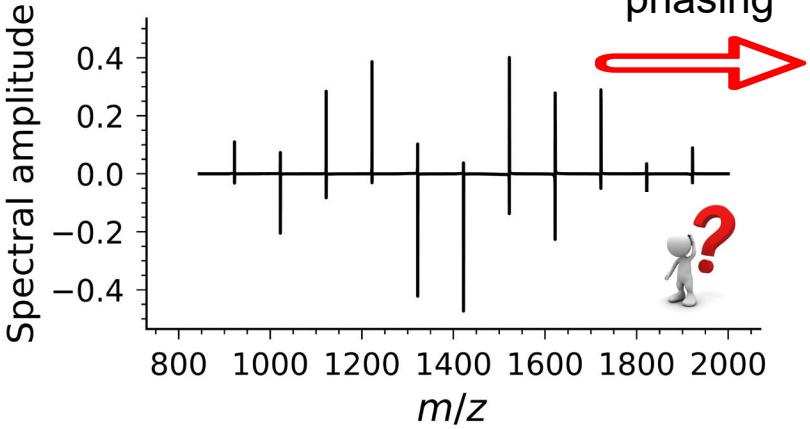
Regular

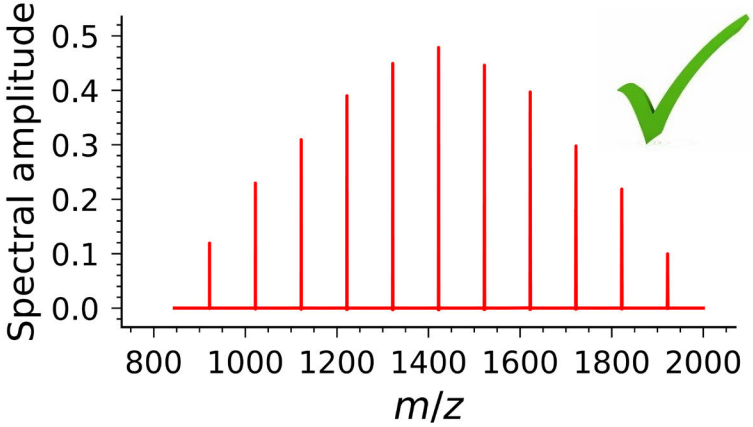


Phased



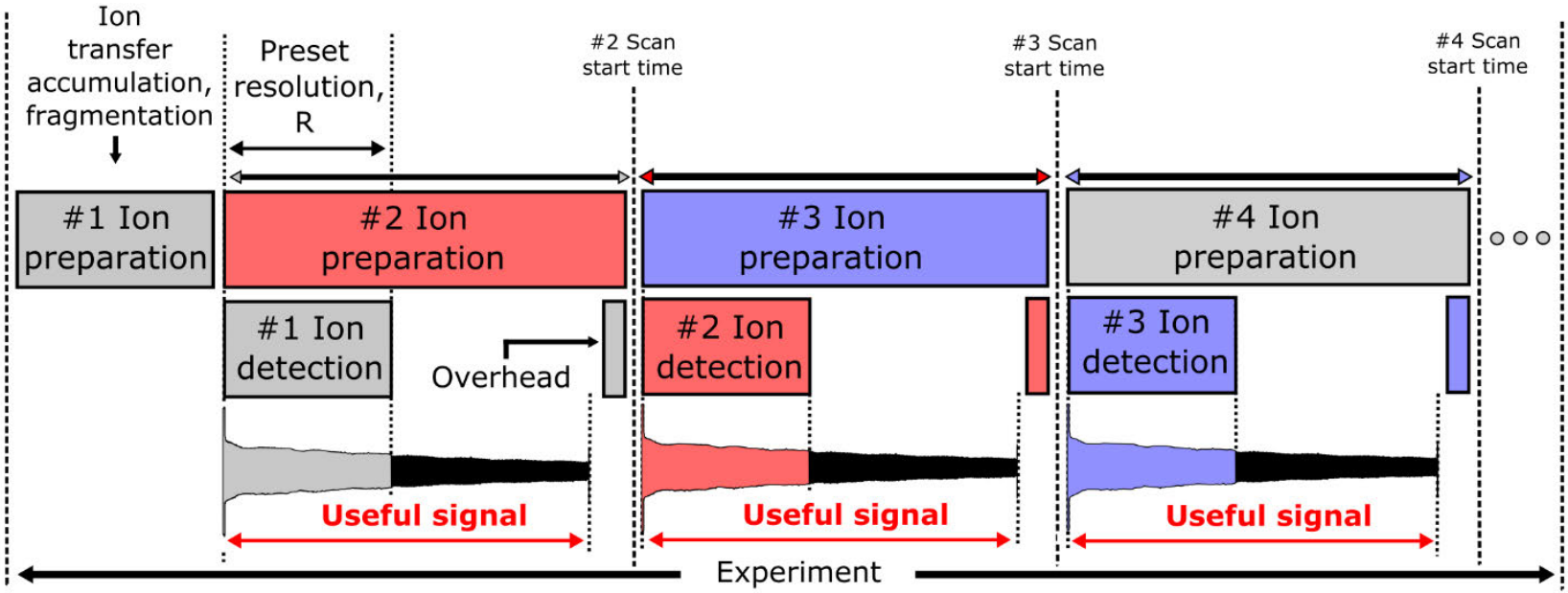
phasing





FTMS Booster Provides the Unreduced (Full) Transients

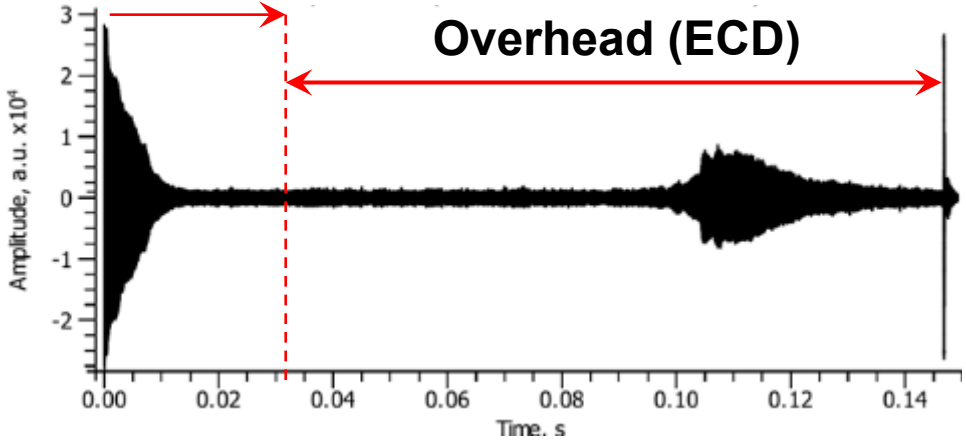
- Records ion signals from all ions, all the time they oscillate (decodes both start & **stop** triggers)
- Overheads are included into the transients: full and extended length transients (ultra-high res.)
- Ion accumulation + fragmentation times for scan ***N*** may exceed OT transient length for scan ***N-1***

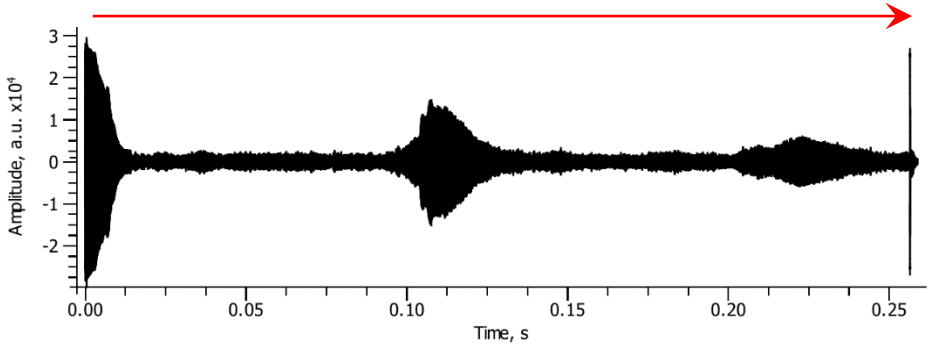


What benefits can we harvest by using the unreduced data?

Full Transients Benefits: Overheads

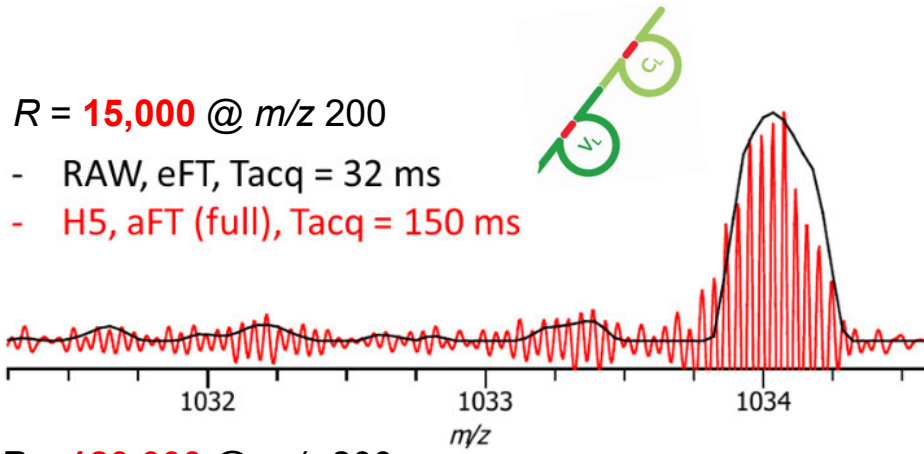
- Optimization of a 25 kDa mAb light chain ECD analysis on a Q Exactive HF - Omnitrap





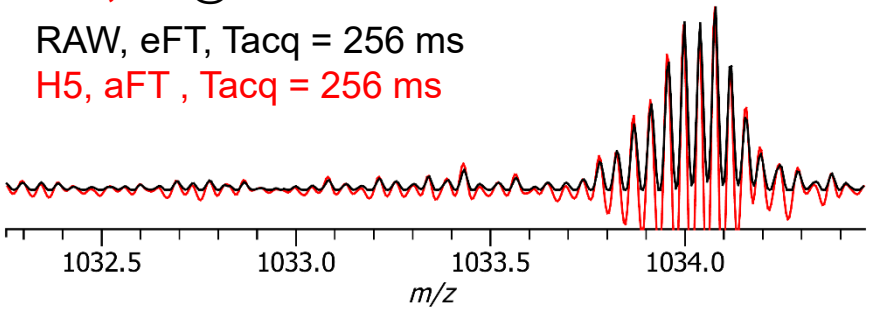
R = **15,000** @ *m/z* 200

- RAW, eFT, Tacq = 32 ms
- H5, aFT (full), Tacq = 150 ms



R = **120,000** @ *m/z* 200

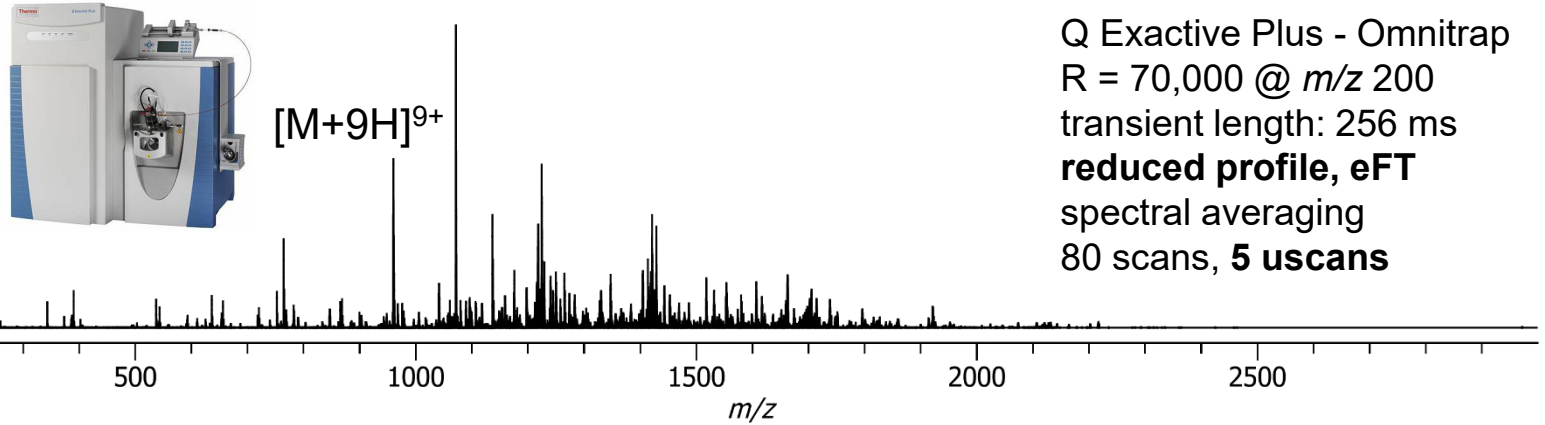
- RAW, eFT, Tacq = 256 ms
- H5, aFT , Tacq = 256 ms



Ubiquitin (8.6 kDa) Top-Down Analysis (**ECD**)

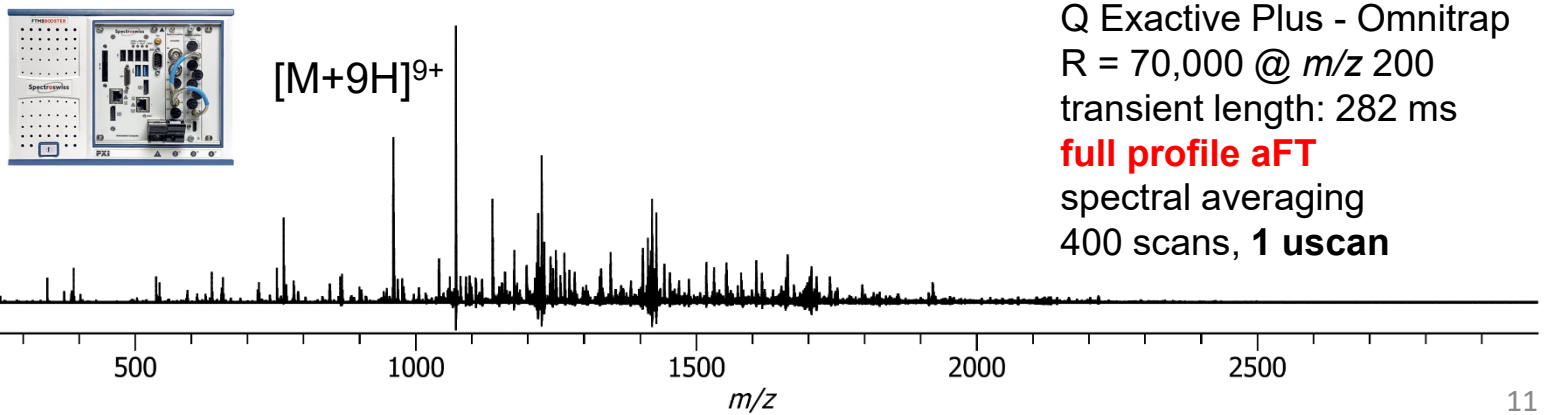
- Direct infusion ESI MS/MS for a total of 400 transients per experiment: similar outlook

1



Q Exactive Plus - Omnitrap
R = 70,000 @ *m/z* 200
transient length: 256 ms
reduced profile, eFT
spectral averaging
80 scans, **5 uscans**

2



Q Exactive Plus - Omnitrap
R = 70,000 @ *m/z* 200
transient length: 282 ms
full profile aFT
spectral averaging
400 scans, **1 uscan**

Ubiquitin Top-Down Analysis: 100% Sequence Coverage

1 **eFT: 94.7 %,** 243 fragments

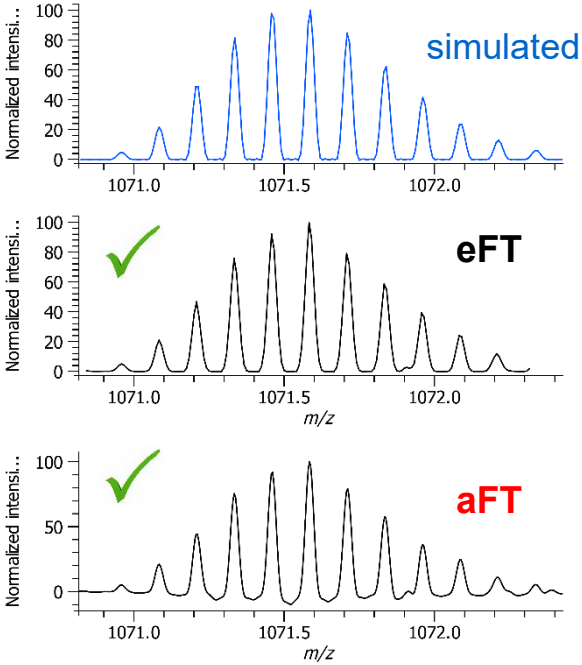
N M Q I F V K T L T G 10
11 K T I T L E V E P S 20
21 D T I E N V K A K I 30
31 Q D K E G I P P D Q 40
41 Q R L I F A G K Q L 50
51 E D G R T L S D Y N 60
61 I Q K E S T L H L V 70
71 L R L R G G C

2 **aFT: 100.0 %,** 317 fragments

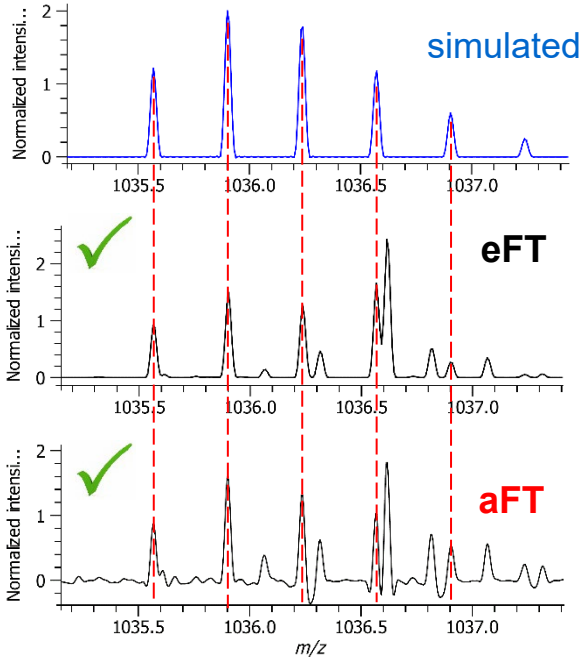
N M Q I F V K T L T G 10
11 K T I T L E V E P S 20
21 D T I E N V K A K I 30
31 Q D K E G I P P D Q 40
41 Q R L I F A G K Q L 50
51 E D G R T L S D Y N 60
61 I Q K E S T L H L V 70
71 L R L R G G C

- Abundant peaks lead to product ion detection in eFT & aFT

y_{76}^{8+}, 8559.617 Da (base peak)



c_{28}^{3+}, 3103.684 Da



FTMS data simulations: *Nagornov et al., JASMS 2020, 31, 9, 1927*

Ubiquitin Top-Down Analysis: 100% Sequence Coverage

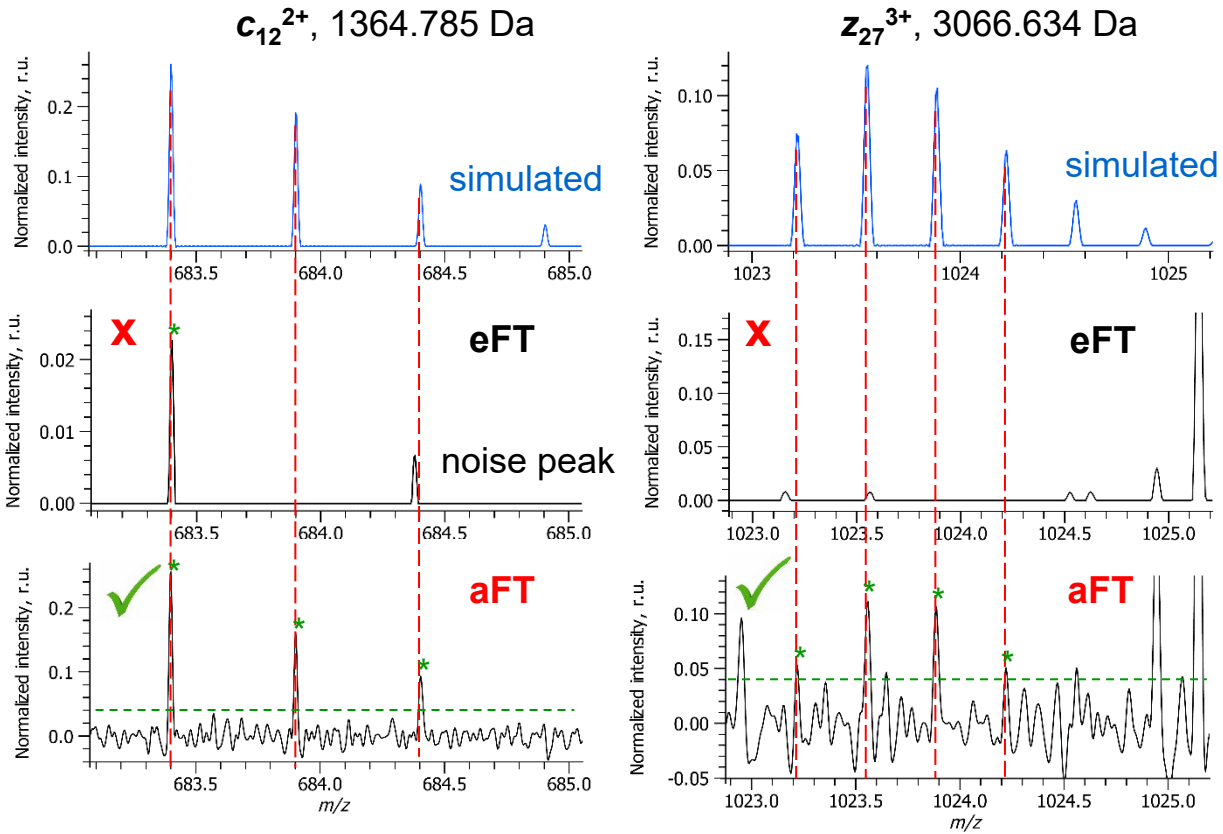
1 **eFT: 94.7 %,** 243 fragments

```
N M Q I F V K T L T G 10
11 K T I T L E V E P S 20
21 D T I E N V K A K I 30
31 Q D K E G I P P D Q 40
41 Q R L I F A G K Q L 50
51 E D G R T L S D Y N 60
61 I Q K E S T L H L V 70
71 L R L R G G C
```

2 **aFT: 100.0 %,** 317 fragments

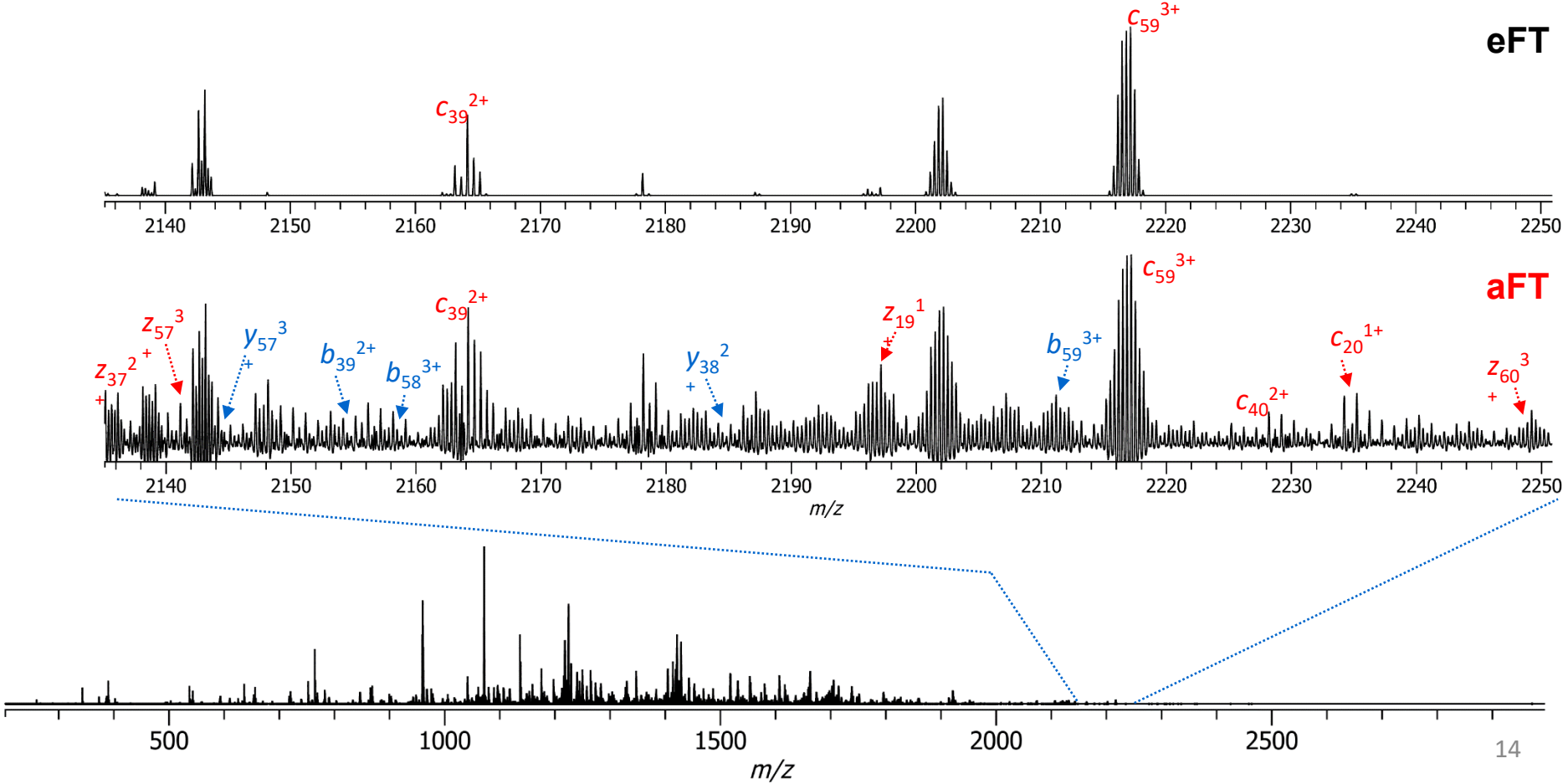
```
N M Q I F V K T L T G 10
11 K T I T L E V E P S 20
21 D T I E N V K A K I 30
31 Q D K E G I P P D Q 40
41 Q R L I F A G K Q L 50
51 E D G R T L S D Y N 60
61 I Q K E S T L H L V 70
71 L R L R G G C
```

- The aFT data helps to detect the missing product ions



Ubiquitin Top-Down Analysis: Detecting More Product Ions

- aFT data shows enhanced sensitivity and dynamic range, particularly in the high mass range



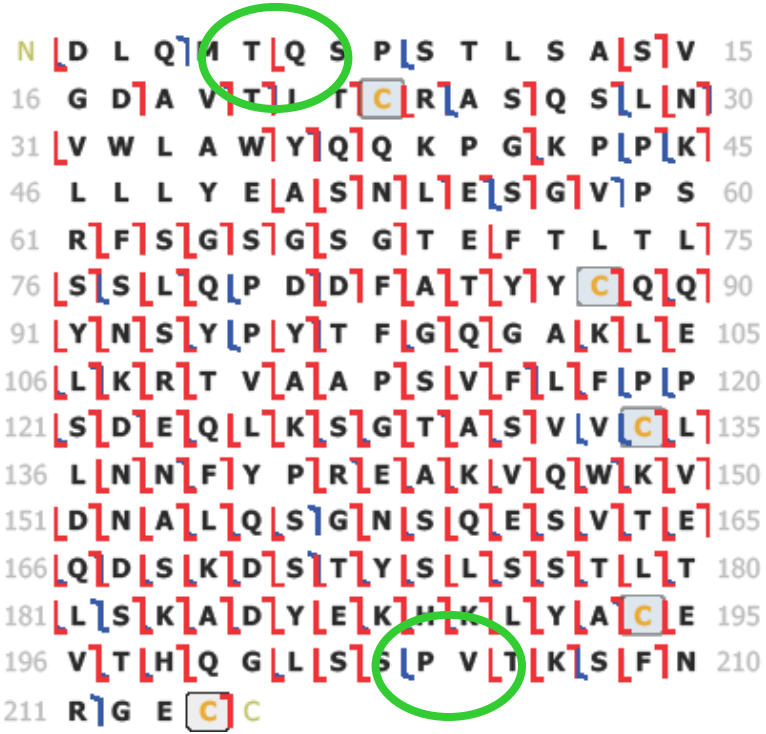
LC-MS/MS Analysis: ECD of a mAb's Light Chain (25 kDa, 23+)

- Light chain extracted from the urine of patient with multiple myeloma
- Reduced light chain, QE HF, 1 LC-MS/MS run, **500** scans (1 uscan), 50 ms ECD, **10 ppm**

eFT

Sequence coverage: **78.0 %**

Total: 489; *c* - 163, *z* - 208, *b* - 27, *y* – 95

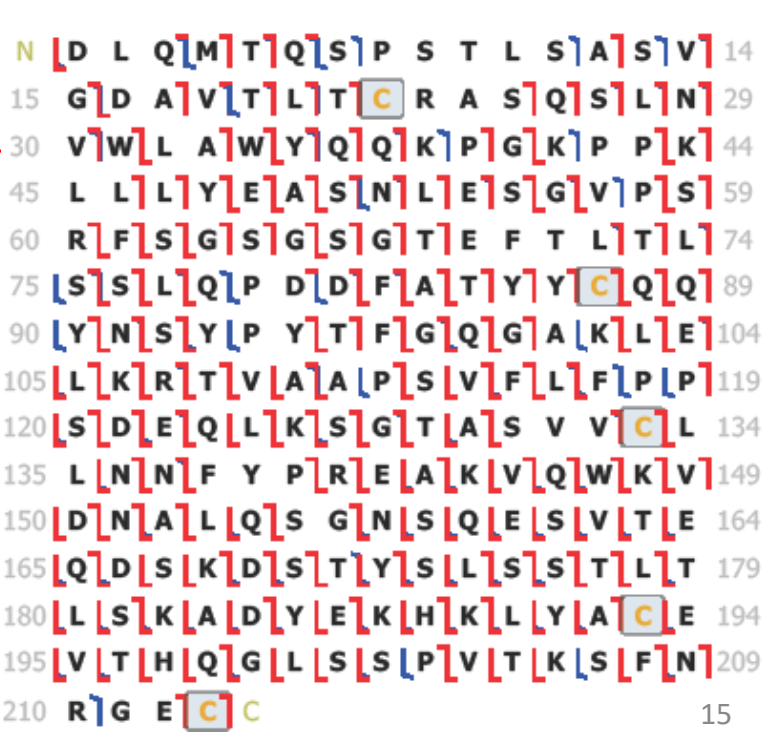


+10 %

aFT

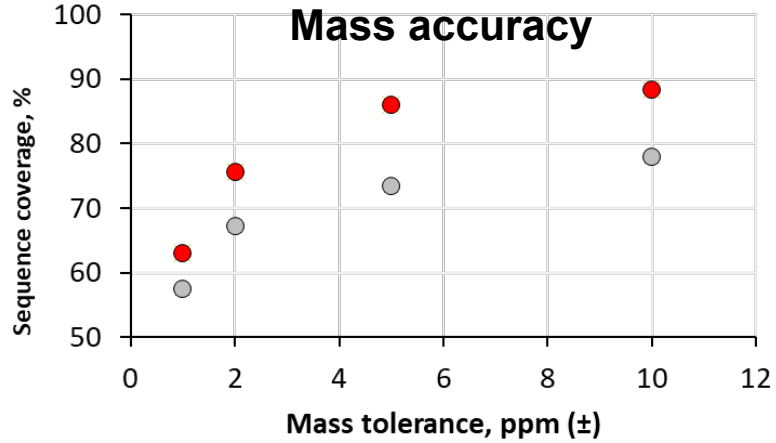
Sequence coverage: **88.8%**

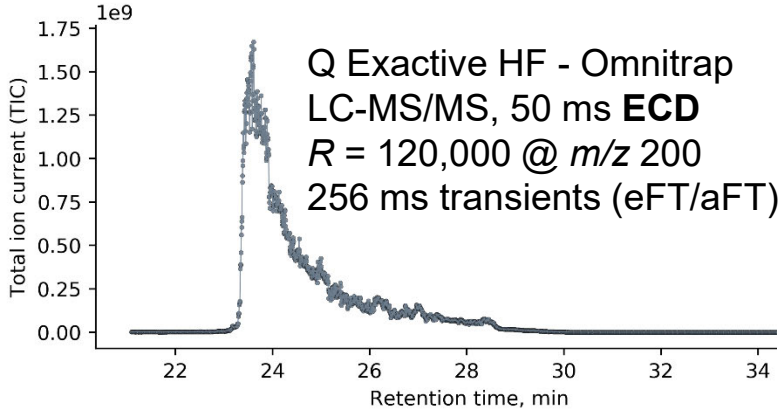
Total: 552; *c* - 201, *z* - 213, *b* - 48, *y* – 90



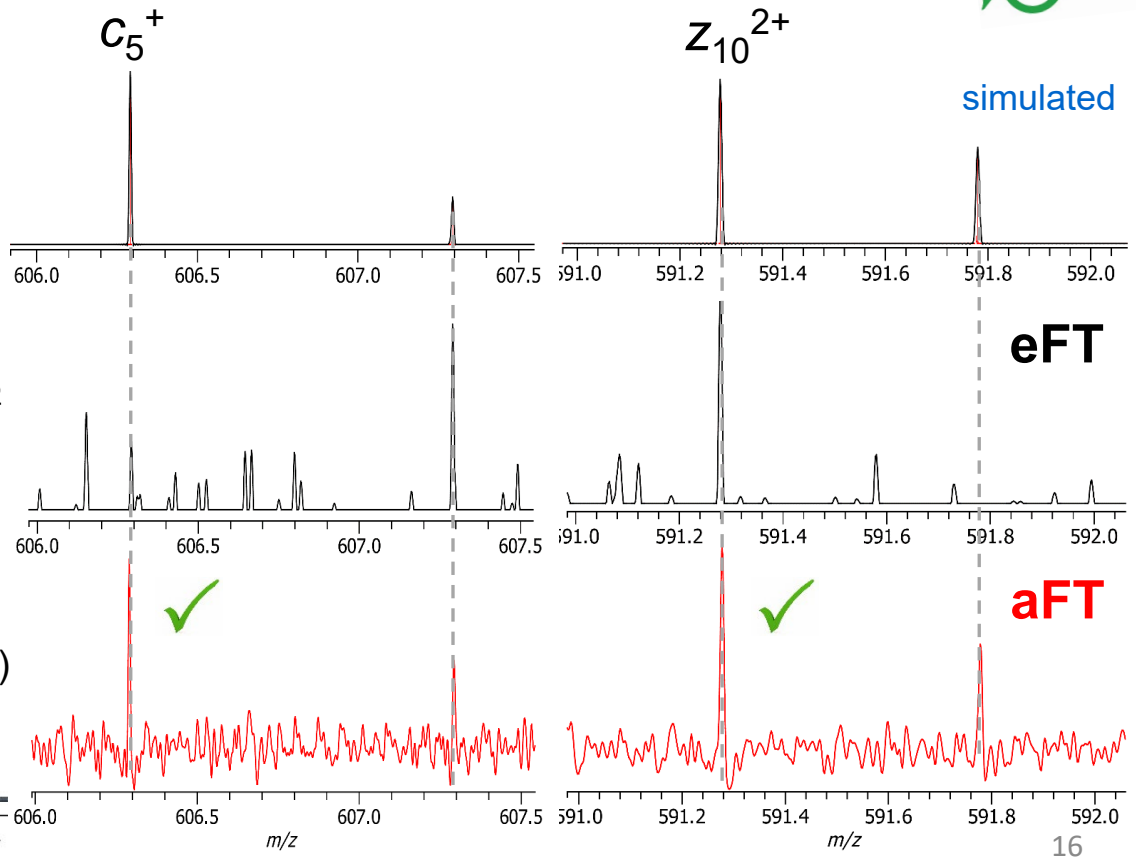
LC-MS/MS Analysis: ECD of a mAb's Light Chain (25 kDa, 23+)

- Reduced light chain, single LC-MS/MS run, 1 uscan per scan (500 averaged scans)



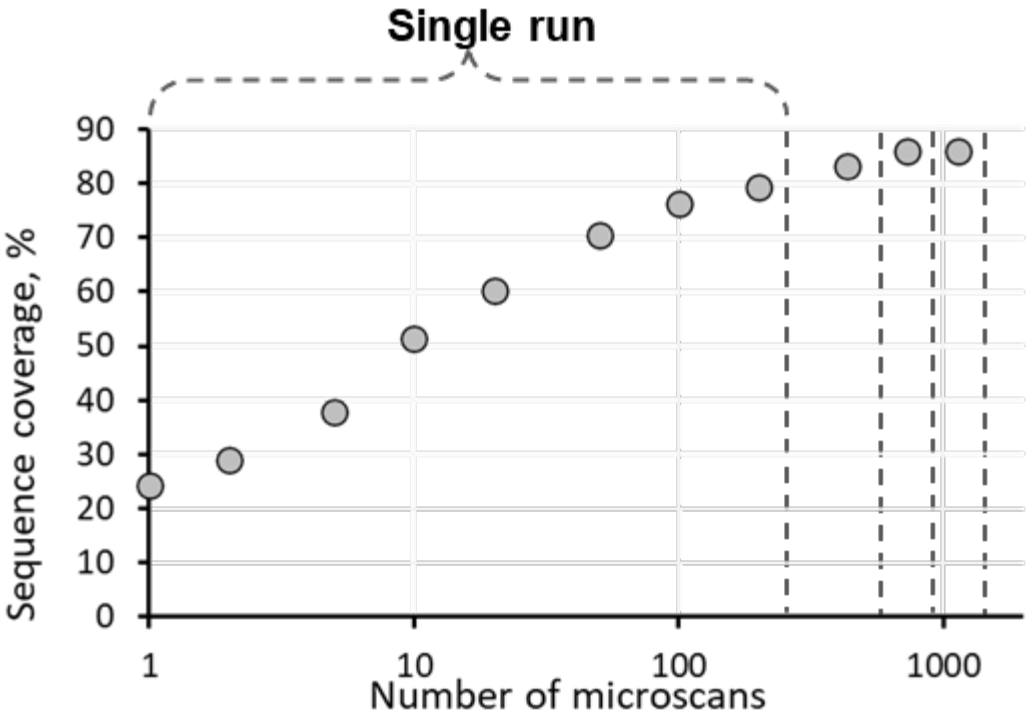


Q Exactive HF - Omnitrap
LC-MS/MS, 50 ms **ECD**
R = 120,000 @ *m/z* 200
256 ms transients (eFT/aFT)



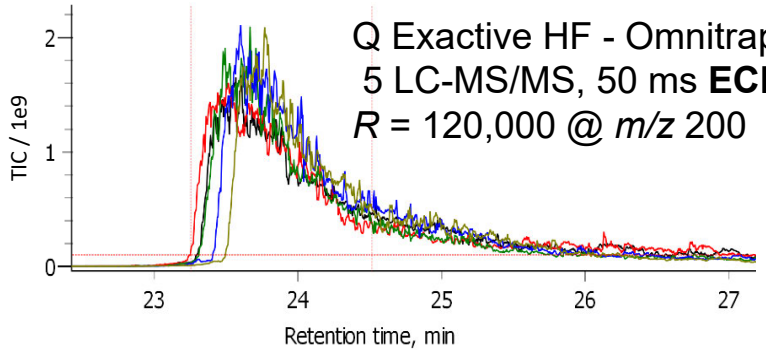
LC-MS/MS Analysis: ECD of a mAb's Light Chain (25 kDa, 23+)

- **5** LC-MS/MS runs (technical replicates), **2300** microscans total, **aFT** mass spectral averaging



1 replicate: Sequence coverage: **88.8%**
Total: 552; *c* - 201, *z* - 213, *b* - 48, *y* – 90

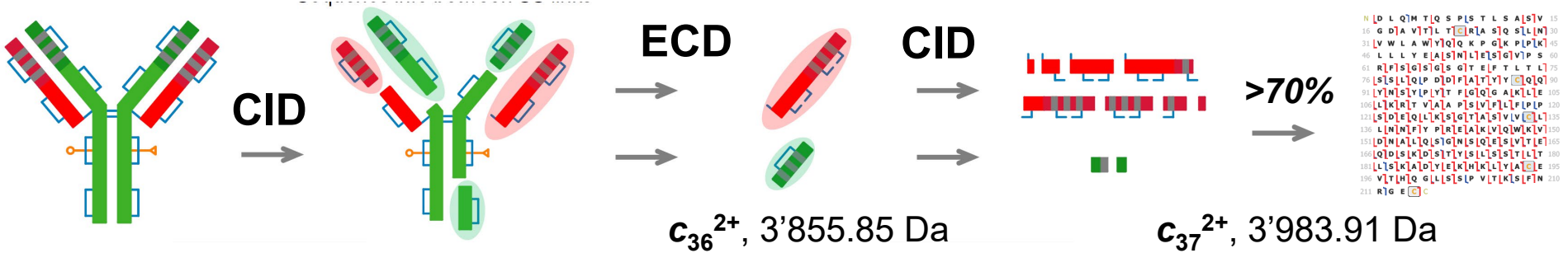
5 replicates: Sequence coverage: **90.7 %**
Total: 596; *c* - 220, *z* - 225, *b* – 48, *y* – 103



Sequence coverage is maximized for a given charge state – fragment another one

MS3/MS4 Workflows To Tackle S-S Bonds in Top-Down MS

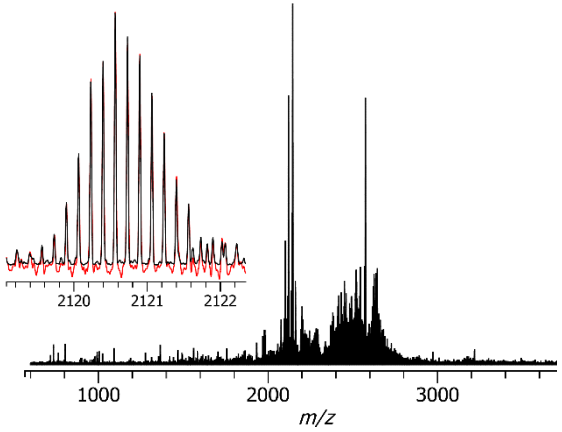
- CID of an intact trastuzumab (49+), followed by ECD of its light chain (11+),& suppl. CID (9+··)

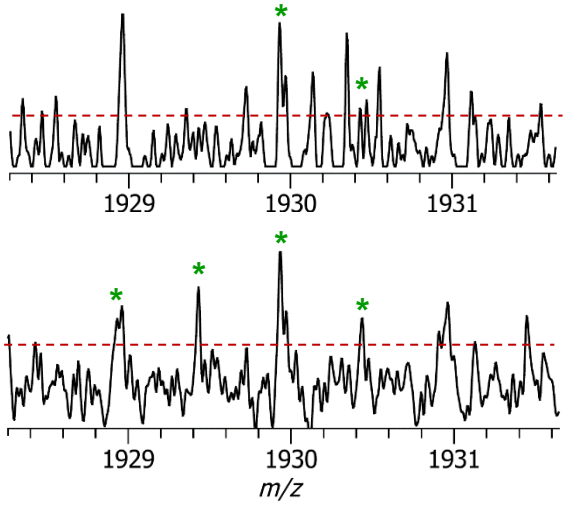


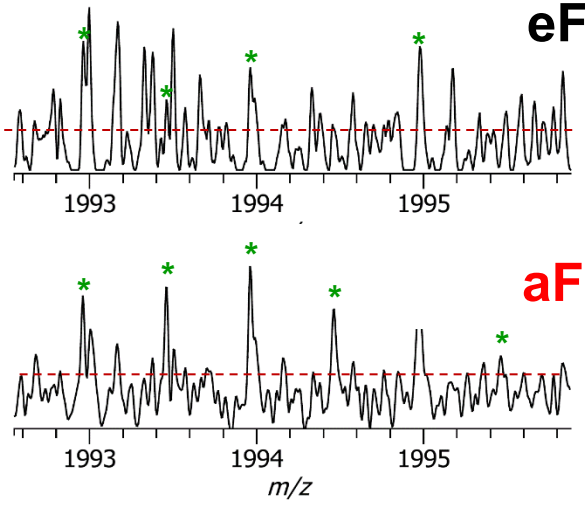
c_{36}^{2+}, 3'855.85 Da

c_{37}^{2+}, 3'983.91 Da

ECD of 11+ light chain (QE Plus)

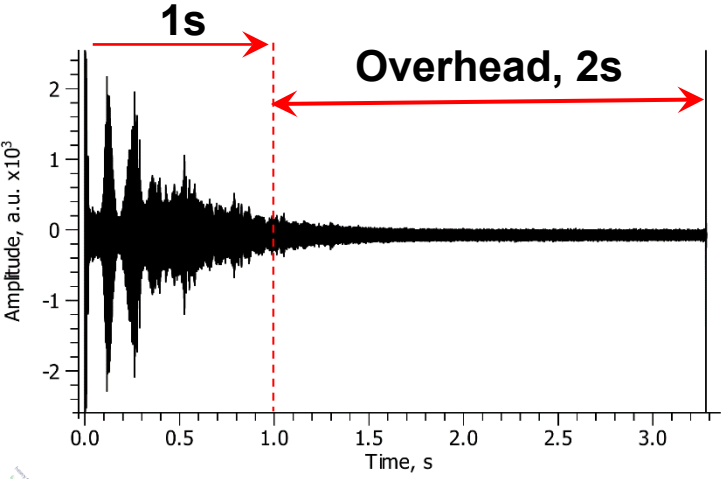


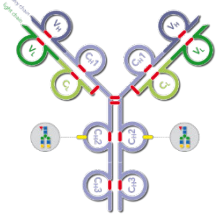




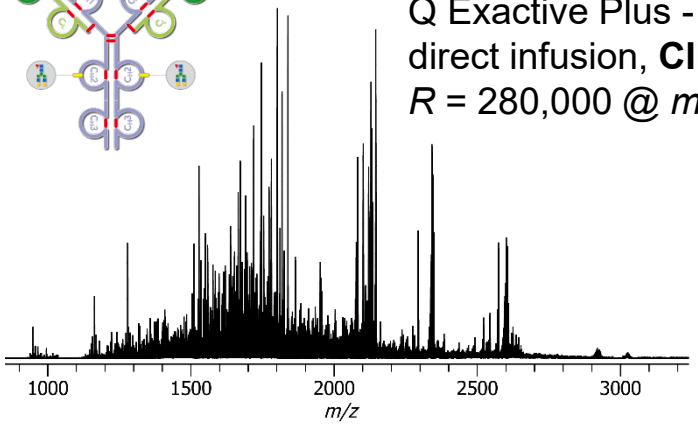
Toward maximum sequence coverage in top-down MS

Ultra-High Resolution (Long Transients): Top-Down MS of a mAb

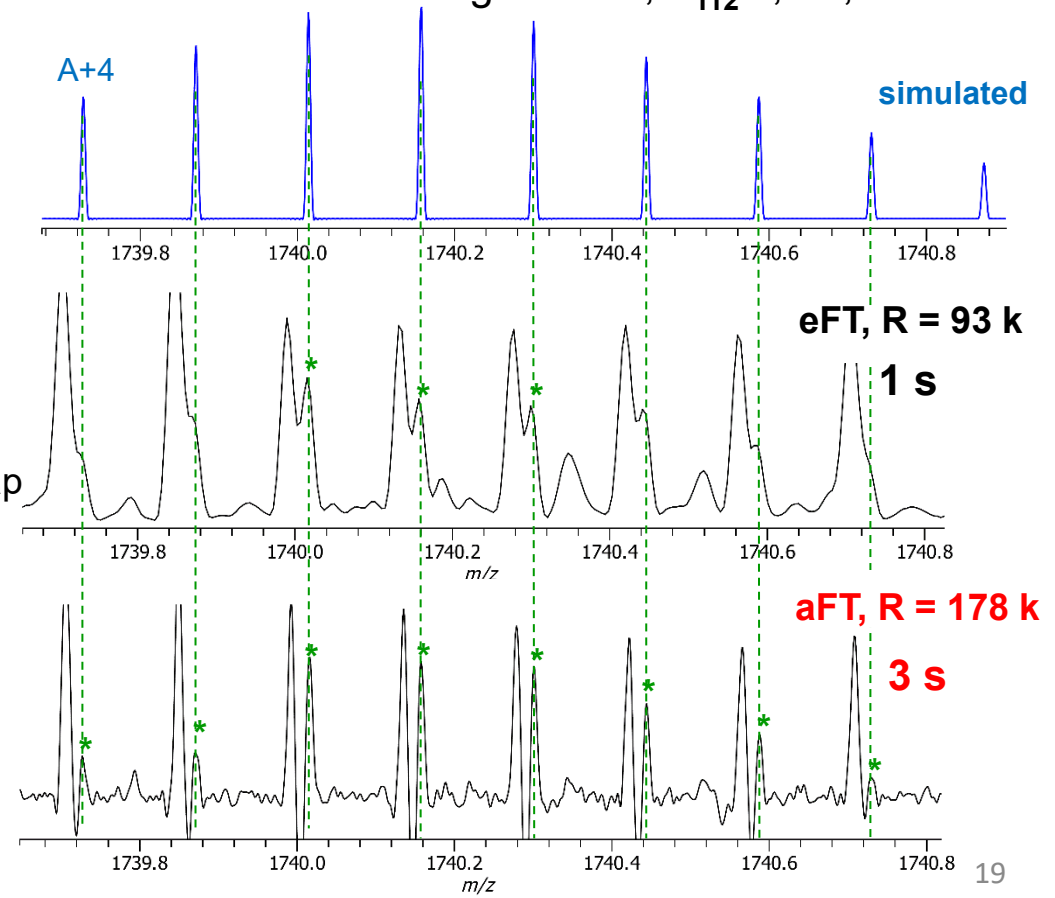




Trastuzumab, 150 kDa
Q Exactive Plus - Omnitrap
direct infusion, **CID**
R = 280,000 @ *m/z* 200



Light chain, b_{112}^{7+}, 12,167.03 Da

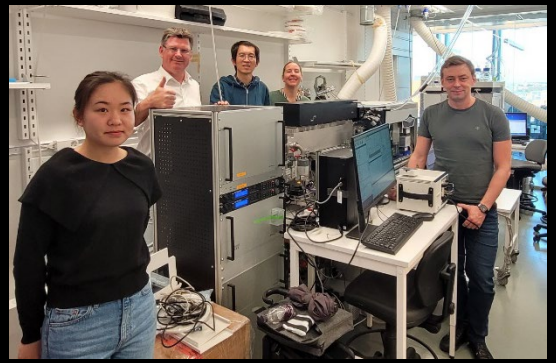


Conclusions

- Novel MS/MS platforms, based on the **Omnitrap-Orbitrap-Booster** combination, have been developed on the basis of a Q Exactive Plus, a Q Exactive HF, and an Exploris 480 Orbitraps
- FTMS Boosters help to obtain the Orbitraps' **unreduced data**: transients and aFT spectra
- The **eFT** and aFT mass spectra are comparable for the same # & length transients (uscans)
- Benefits of the **unreduced data** on the employed Omnitrap-Orbitrap platforms:
  - reveal the Omnitrap's overheads: increased duty cycle and experiment optimization
  - offer parallel ion detection and fragmentation: interaction times (50-500 ms) in Omnitrap
  - main beneficial factors: longer transients and/or full profile (pos/neg) mass spectra
  - averaging the **aFT spectra** increases sensitivity and enhances protein **sequence coverage**
  - ultra-high resolution improves analysis of complex product ion distributions in top-down MS
- The **unreduced data** benefits other multimodal and hybrid MS/MS platforms, e.g., tribrids
- Outlook: use of a single ion counting (CDMS) to improve Omnitrap-Orbitrap workflows. CDMS benefits from **ultra-long transients** (25 s!) on a Q Exactive UHMR (WOA 2:50 PM, Heck *et al.*)

Maximizing MS/MS information output from the multimodal MS/MS platforms

Thank you!



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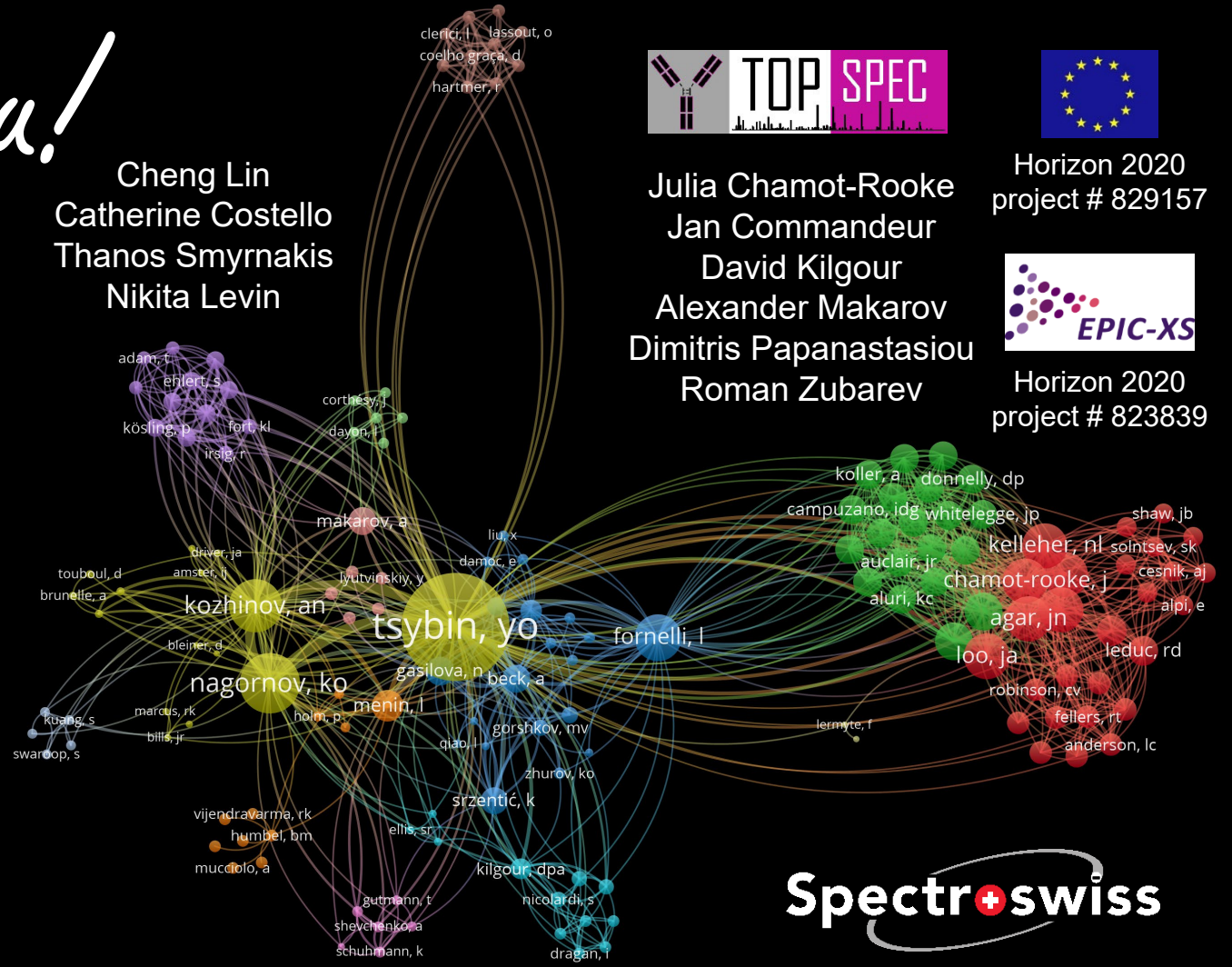

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Horizon 2020
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