Update from Institut Pasteur

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24/10/2022

Contents

1. Analysis of the P15 light chain with the omnitrap
2. First data with the booster
3. Analysis of the NIST mAb
4. Conclusions/Future direction

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P15: Light chain from urine of patients (multiple myeloma)

analytical chemistry

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Article

De Novo Sequencing of Antibody Light Chain Proteoforms from Patients with Multiple Myeloma

Mathieu Dupré,[§] Magalie Duchateau,[§] Rebecca Sternke-Hoffmann, Amelie Boquoi, Christian Malosse, Roland Fenk, Rainer Haas, Alexander K. Buell, Martial Rey, and Julia Chamot-Rooke*

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Data acquisition status on the HF-Omnitrap-Booster platform

1. Top-down LC-MSn analysis of Light Chains (HF-Omnitrap)
   Data acquired from April to July 2022, processed with PeakFinder

2. Top-down LC-MSn analysis of Light Chains (HF-Omnitrap)
   After filament replacement, data acquired from Sept to Oct 2022, to be processed with Cronus

3. Top-down LC-MSn analysis of Light Chains (Lumos)
   Data acquired in August 2022, to be processed with Cronus

4. HF-Omnitrap-Booster Characterization (HF-Omnitrap-Booster)

5. Top-down LC-MSn analysis of NISTmAb (HF-Omnitrap)

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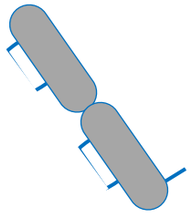
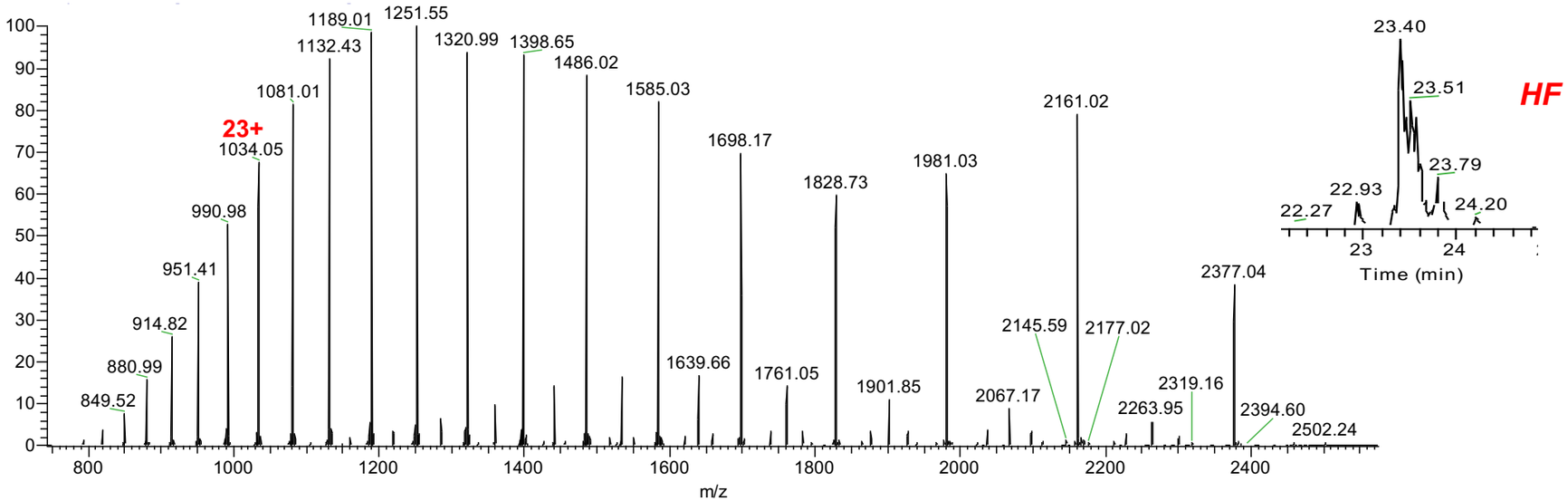
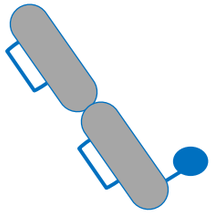
Reduced, alkylated and intact Light chain: MS1

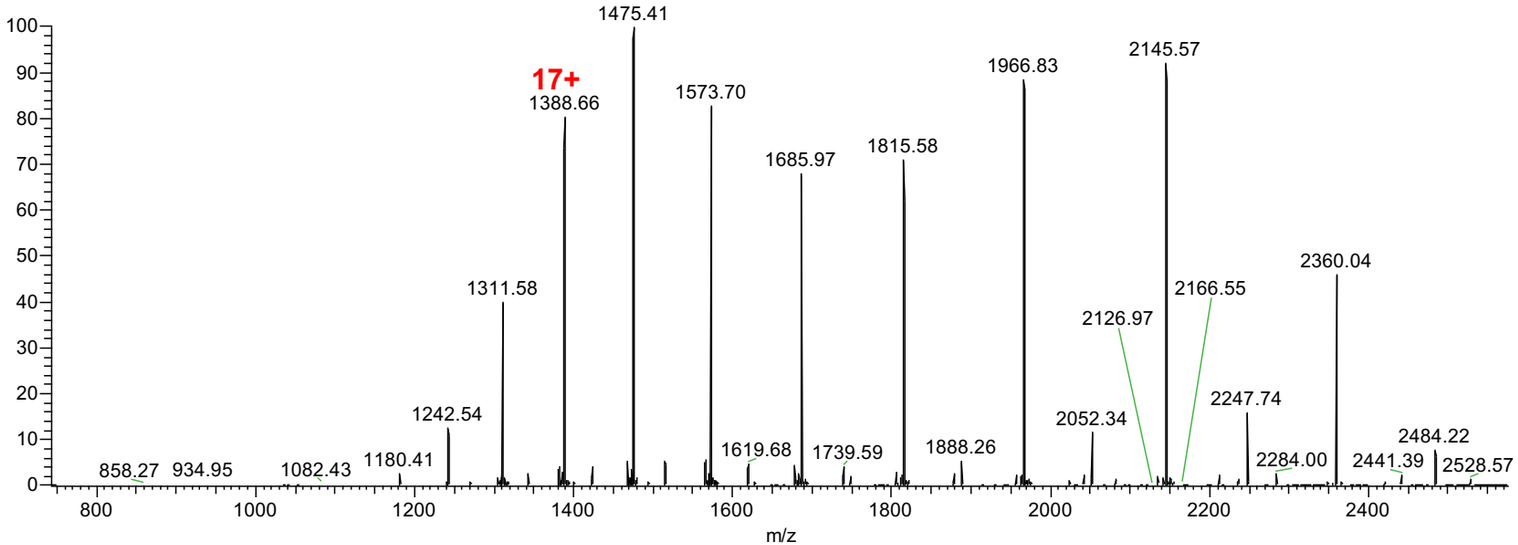


Reduced, alkylated





Intact



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Data acquired from April to July 2022 (nLC + PeakFinder)

Reduced, alkylated LC (*m/z* 1034, 23+)

Exp.		Seq. coverage	Parameters
	HCD NCE20	36% (deconv.)	AGC5E5,10 µscan, 240k
MS2	ET10HCD10 (Lumos)	77.9%	AGC1E6,20 µscan, 120k
	ECD 70ms	78.9%	AGC5E5,10 µscan, 120k
	EID 50ms	85.0%	AGC5E5,10 µscan, 120k
	ECD 50ms	83.1%	AGC5E5,10 µscan, 120k
	ECD 50ms+suppl. CID activation	91.5%	AGC5E5,10 µscan, 120k 2 datasets combined

Intact LC (*m/z* 1388, 17+)

Exp.		Seq. coverage	Parameters
MS2	HCD NCE20	20% (deconv.)	AGC5E5,10 µscan, 240k
	ECD 70ms	37%	AGC5E5,10 µscan, 120k
	ECD 70ms+suppl. CID activation	44% (deconv.)	AGC5E5,10 µscan, 120k 2 datasets combined
	EID 50ms		AGC5E5,10 µscan, 120k
	EID 150ms	39%	AGC5E6,3 µscan, 240k
	ECD 250ms		AGC5E6,3 µscan, 240k
	ECD 500ms		AGC5E6,3 µscan, 240k
	ECD 750ms		AGC5E6,3 µscan, 240k
MS3	ECD 250ms+MS3CID of 16+•	39.0%	AGC5E6,3 µscan, 240k
	ECD 250ms+MS3CID of 15+••	25.4%	AGC5E6,3 µscan, 240k
	ECD 500ms+MS3CID of 16+•	41.3%	AGC5E6,3 µscan, 240k
	ECD 750ms+MS3CID of 16+•	29.6%	AGC5E6,3 µscan, 240k
	EID 150ms+MS3CID of 18+•	28.2%	AGC5E6,3 µscan, 240k
	EID 150ms+MS3CID of 19+••	23.0%	AGC5E6,3 µscan, 240k
MS4 y96	HCD20-ECD50ms-MS4CID	33.7% (y96)	AGC5E6,3 µscan, 240k
	HCD20-ECD500ms-MS4CID	26.3% (y96)	AGC5E6,3 µscan, 240k

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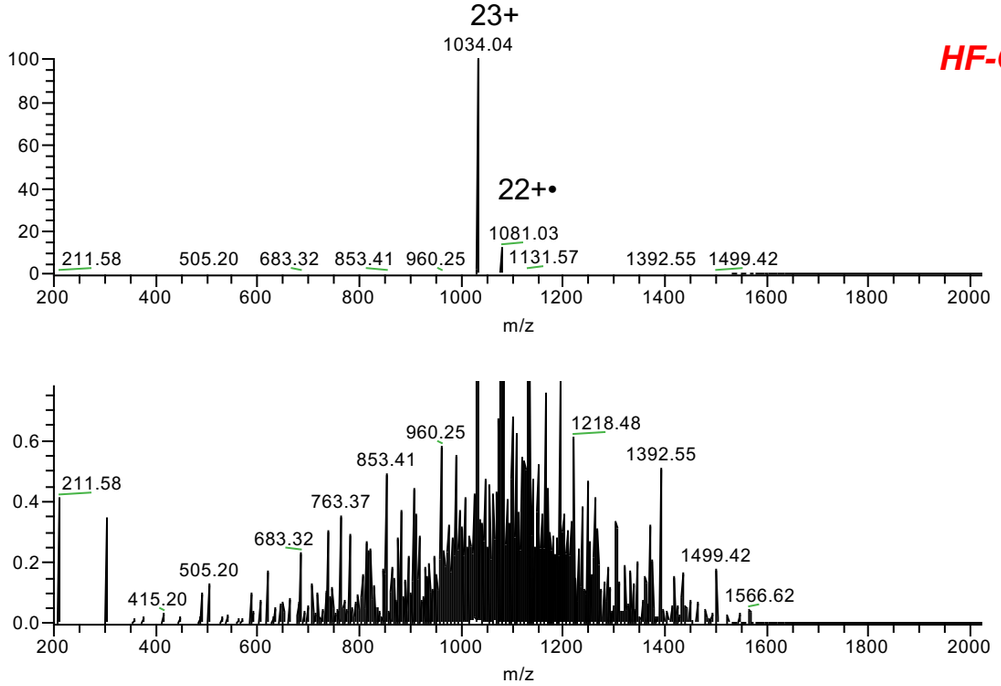
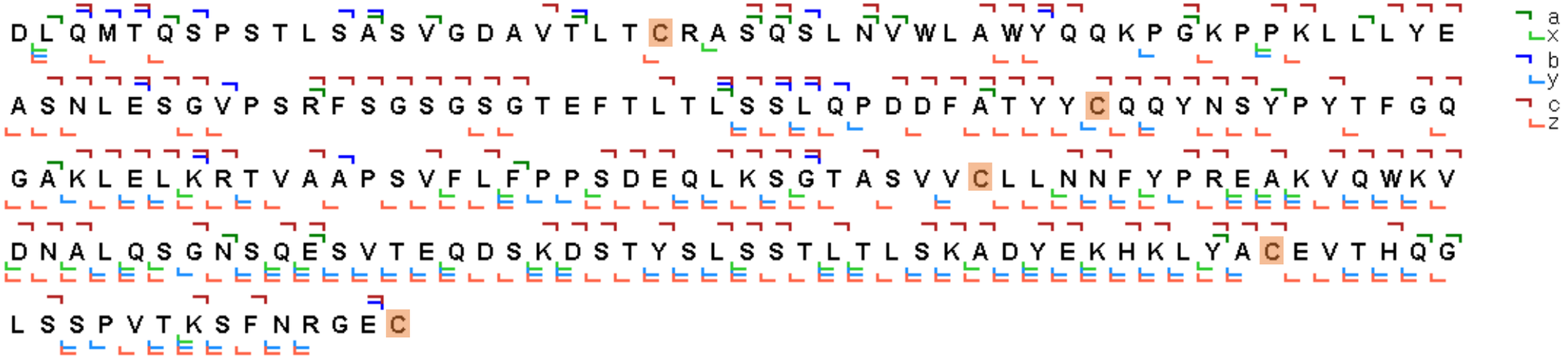
Reduced, alkylated Light chain: ECD (m/z 1034, 23+)

ECD 50 ms

a : 10% x : 14%
b : 9% y : 31%
c : 50% z : 60%

Sequence coverage : 83.1%





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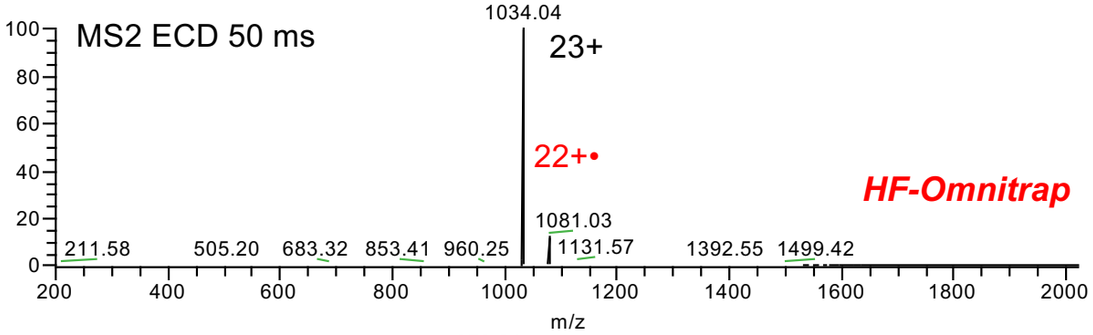
Reduced, alkylated Light chain: ECD (m/z 1034, 23+) + CID

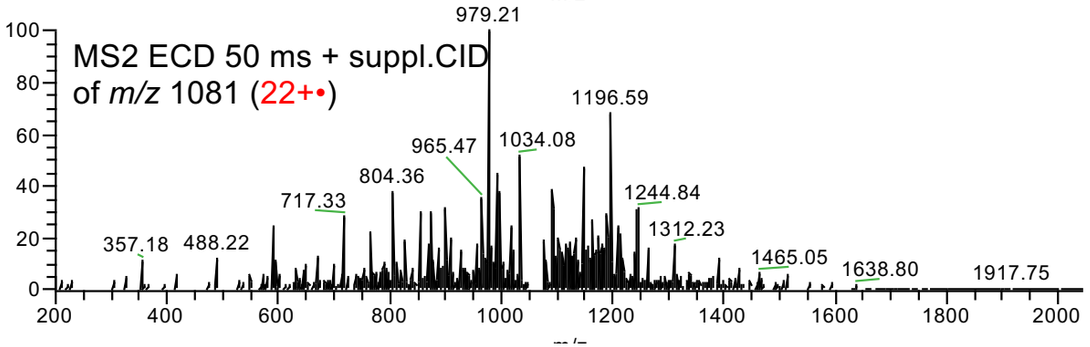
ECD 50 ms + CID supp activation

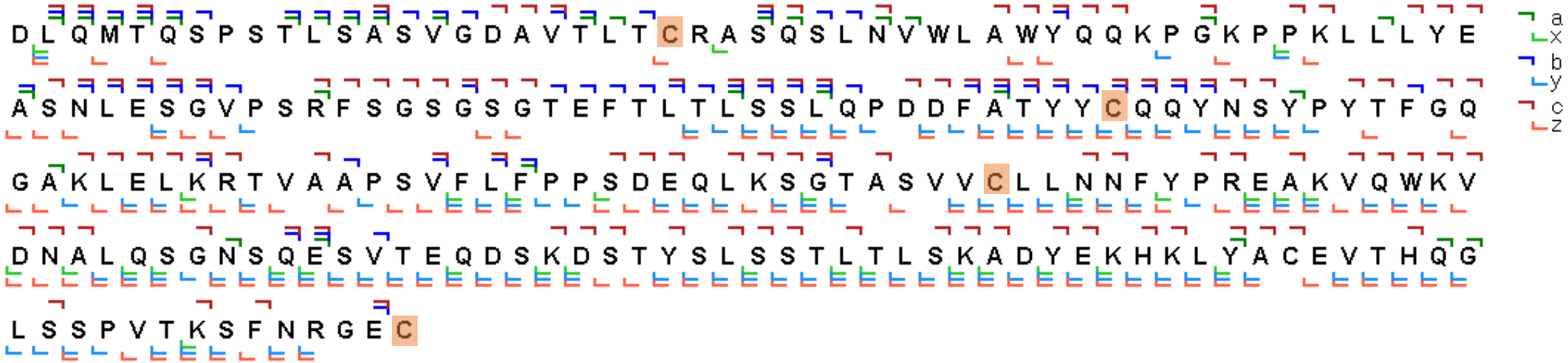
a : 15% x : 15%
b : 28% y : 51%
c : 53% z : 62%

2 datasets combined

Sequence coverage : 91.5%





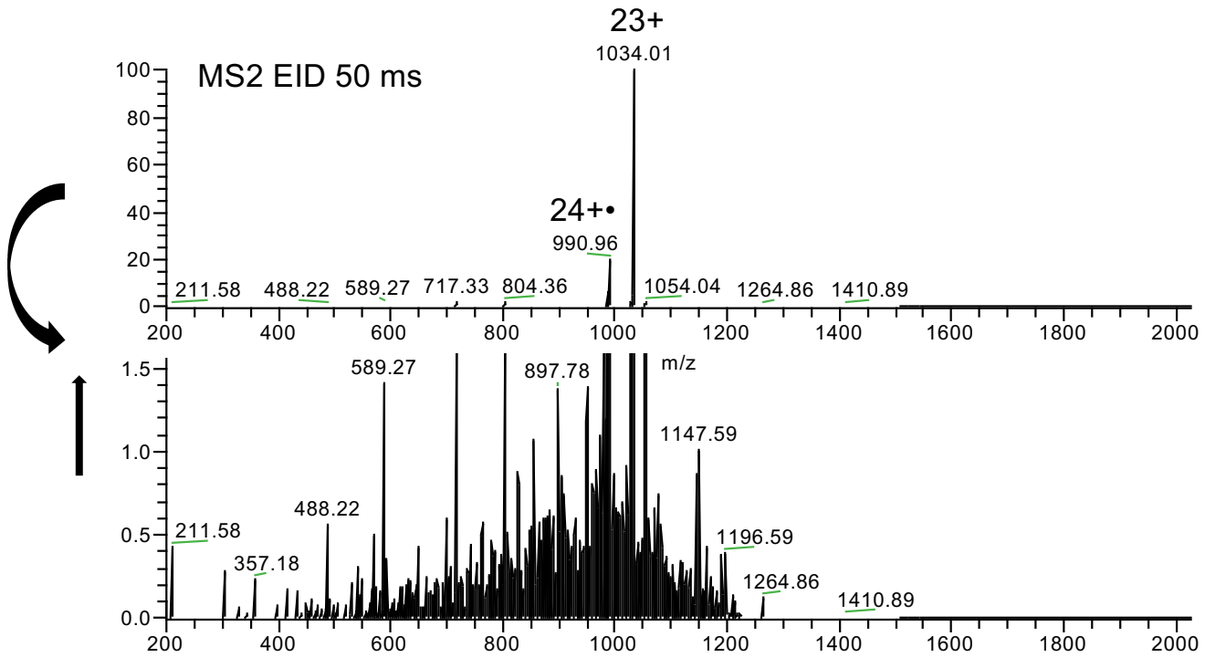


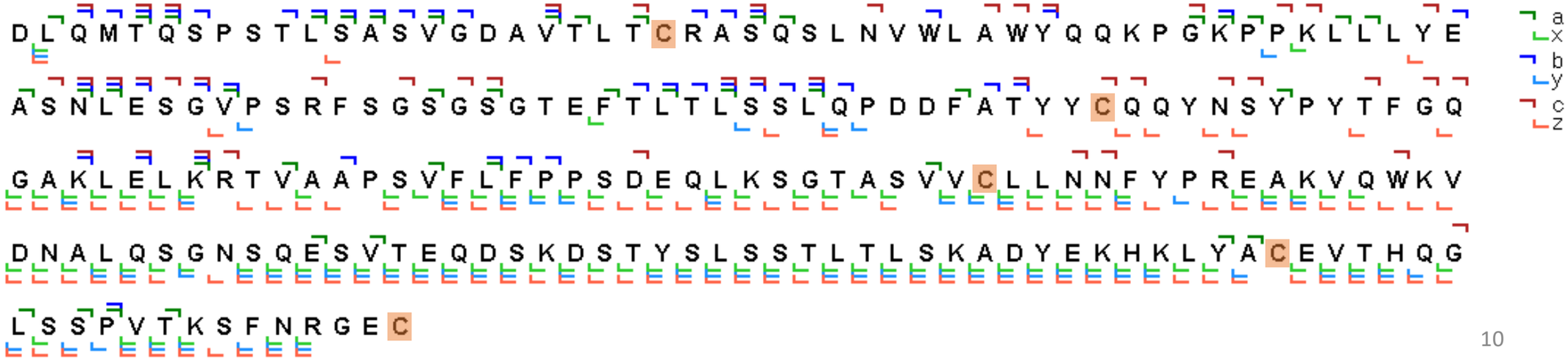
Reduced, alkylated Light chain: EID (m/z 1034, 23+)

EID 50 ms

a : 20% x : 42%
b : 20% y : 31%
c : 21% z : 52%

Sequence coverage : 85%





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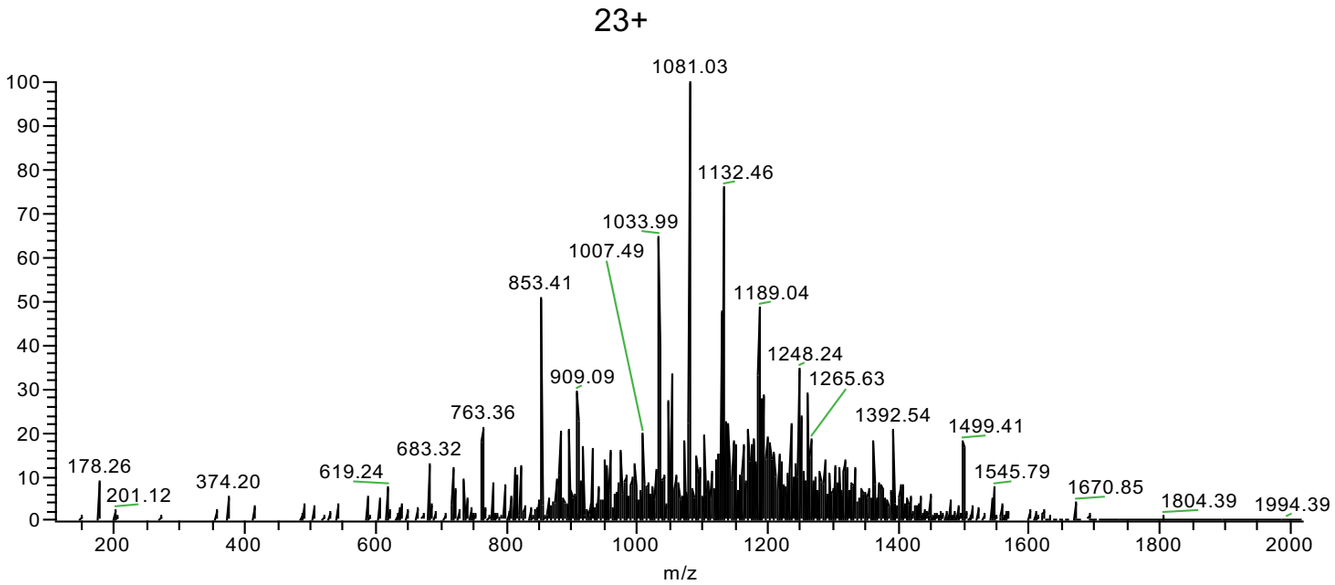
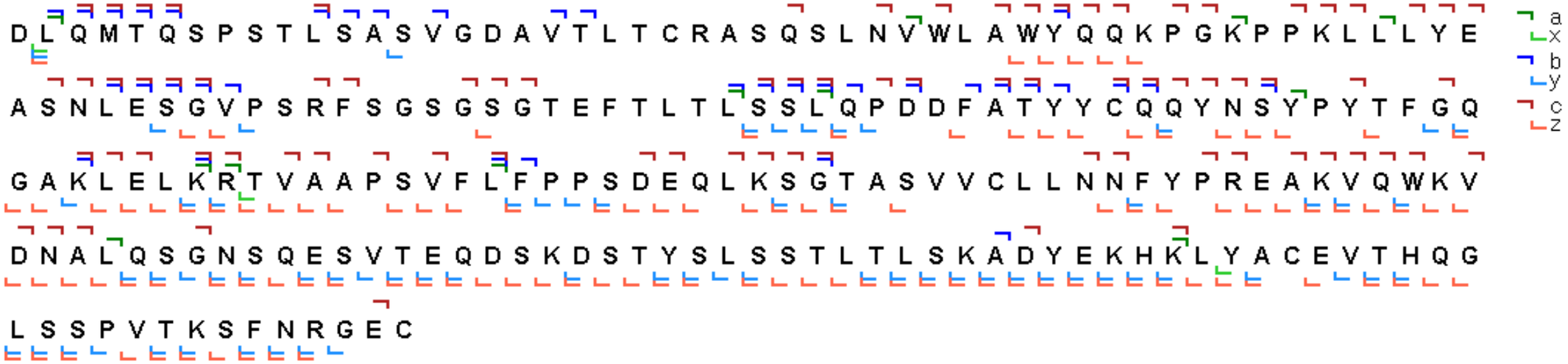
Reduced, alkylated Light chain: EThCD Lumos

ET10 HCD10 on Lumos

a : 6% x : 1%
b : 17% y : 31%
c : 38% z : 53%

Sequence coverage : 77.9%





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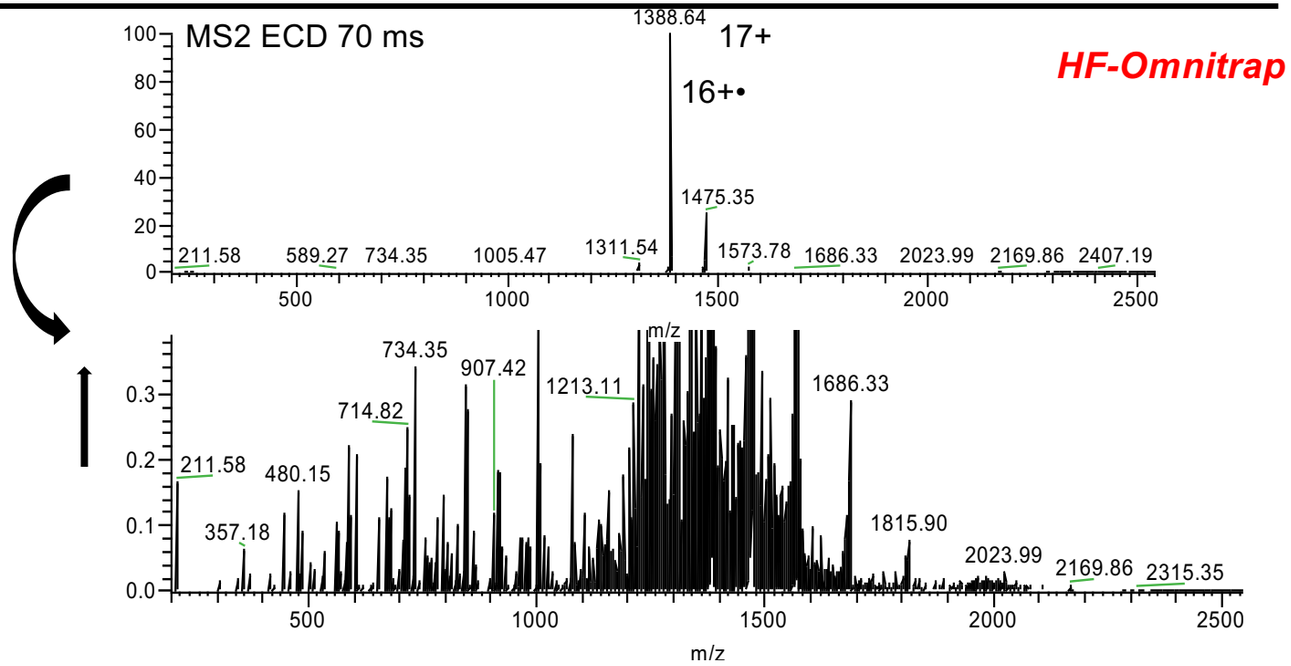
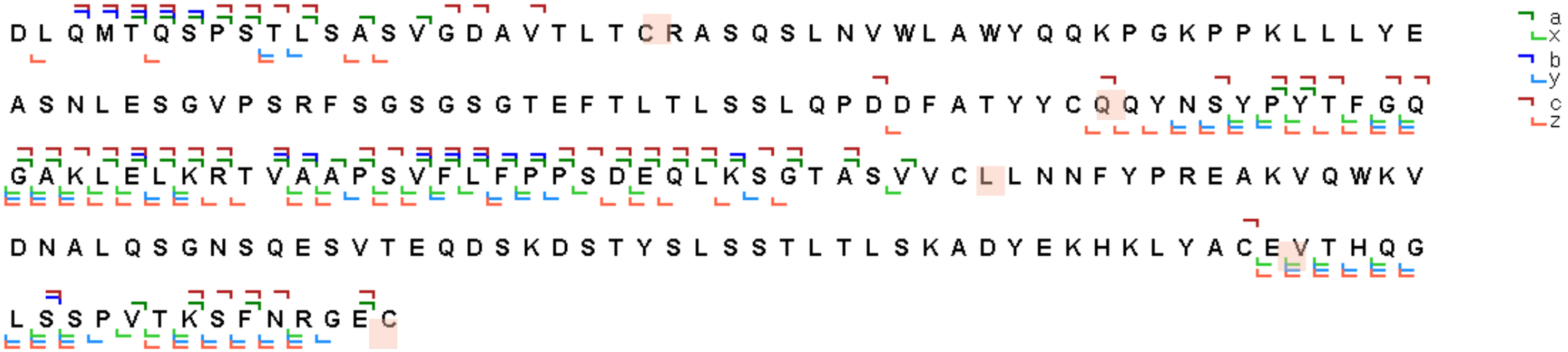
Intact Light chain: ECD (m/z 1388, 17+)

ECD 70 ms

a : 18% x : 15%
b : 7% y : 16%
c : 23% z : 24%

Sequence coverage : 37.1%





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Intact Light chain: ECD (m/z 1388, 17+) + CID

ECD 70 ms + suppl. CID activation

2 datasets combined

Sequence coverage : 44%

```
N  D L Q M T Q S P S T L S A S V G D A V T L T C R A  25
26 S Q S L N V W L A W Y Q Q K P G K P P K L L L Y E  50
51 A S N L E S G V P S R F S G S G S G T E F T L T L  75
76 S S L Q P D D F A T Y Y C Q Q Y N S Y P Y T F G Q  100
101 G A K L E L K R T V A A P S V F L F P P S D E Q L  125
126 K S G T A S V V C L L N N F Y P R E A K V Q W K V  150
151 D N A L Q S G N S Q E S V T E Q D S K D S T Y S L  175
176 S S T L T L S K A D Y E K H K L Y A C E V T H Q G  200
201 L S S P V T K S F N R G E C C
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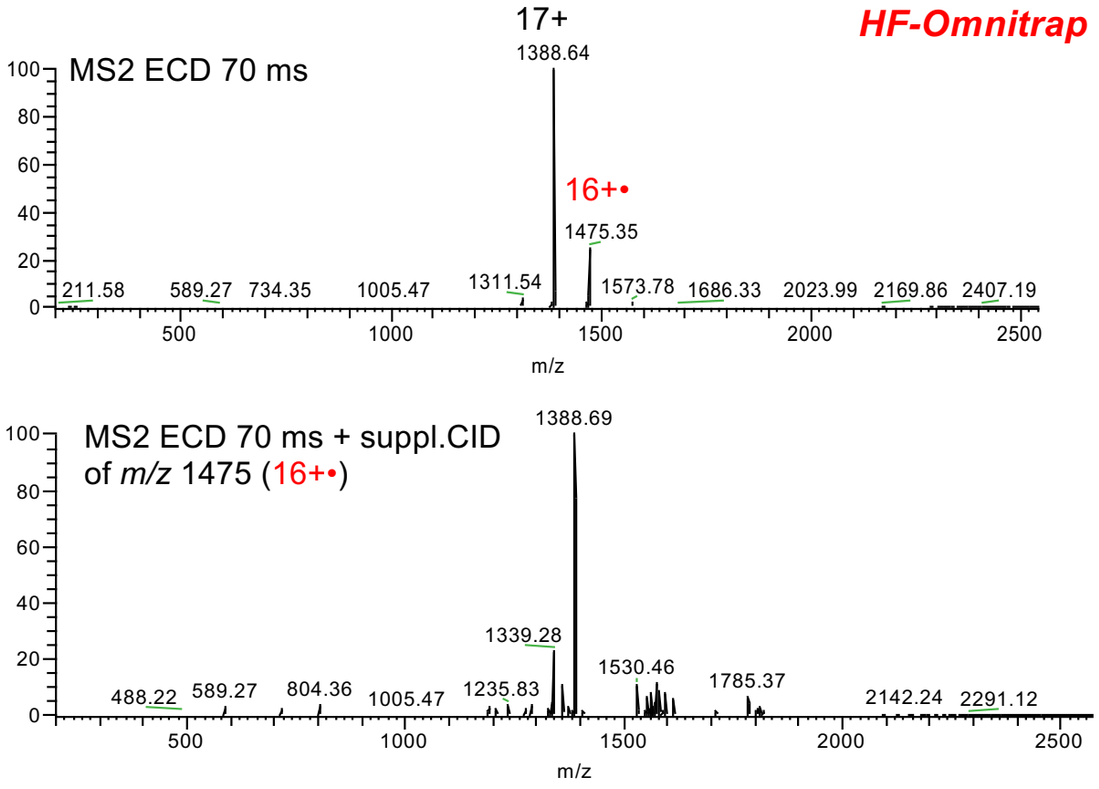
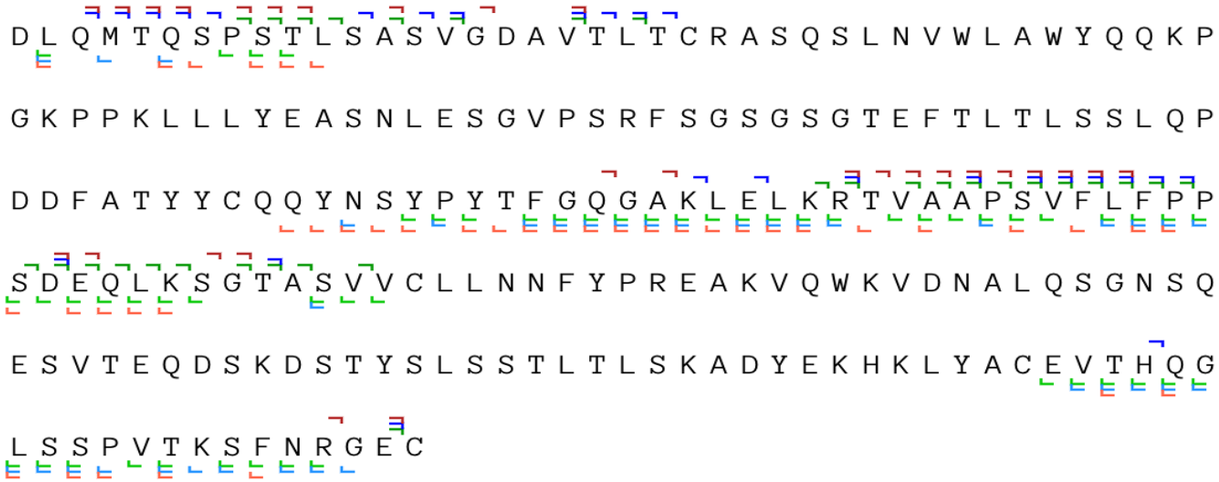


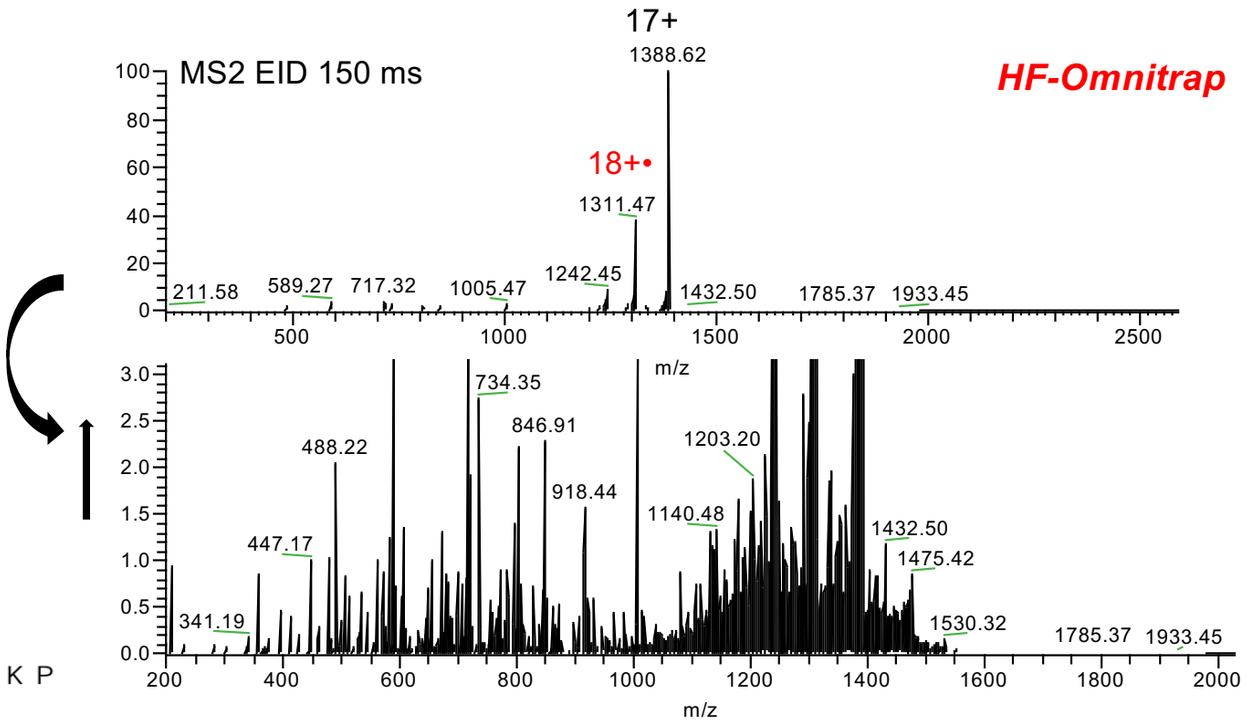
Intact Light chain: EID (m/z 1388, 17+)

EID 150 ms

a : 15% x : 25%
b : 12% y : 17%
c : 13% z : 19%

Sequence coverage : 39%



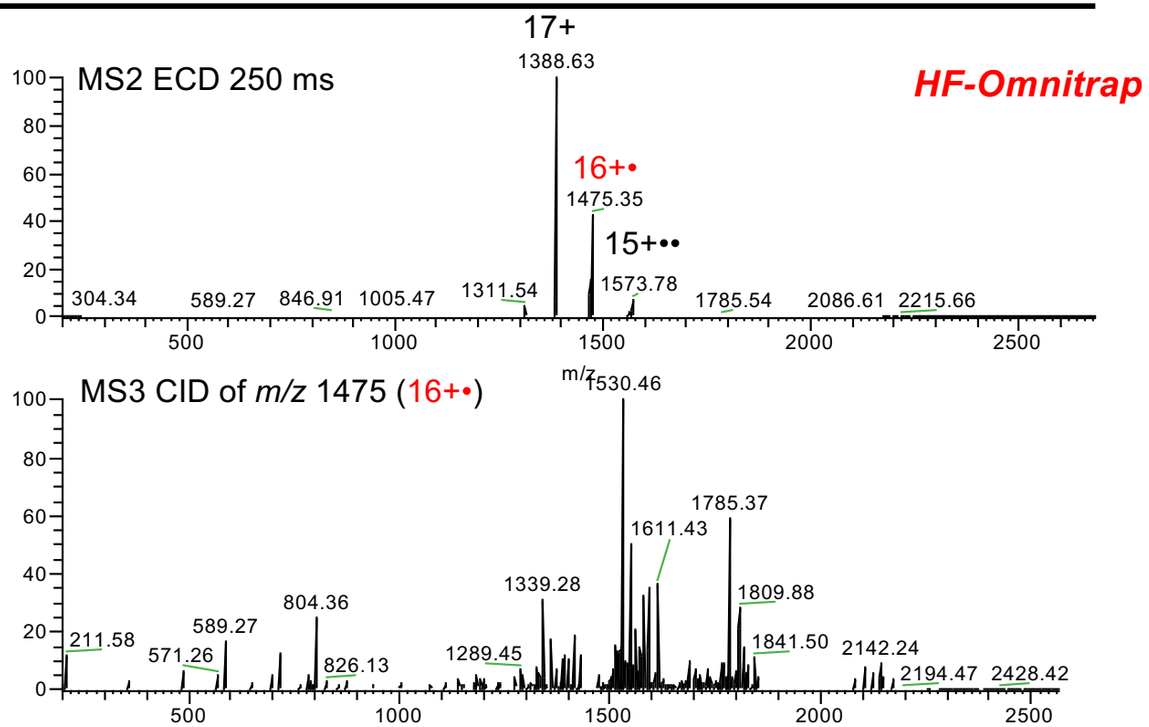


Intact Light chain: MS3

ECD 250 ms, MS3 CID of *m/z* 1475 (16+•)

a : 10% x : 7%
b : 22% y : 19%
c : 14% z : 14%

Sequence coverage : 39%



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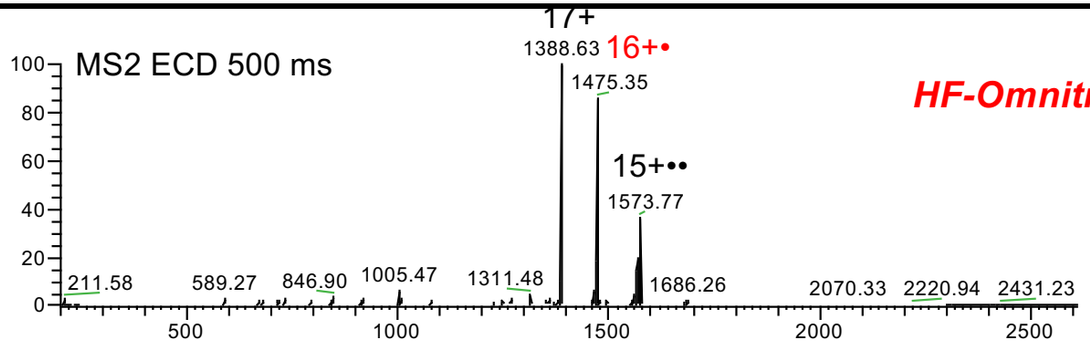
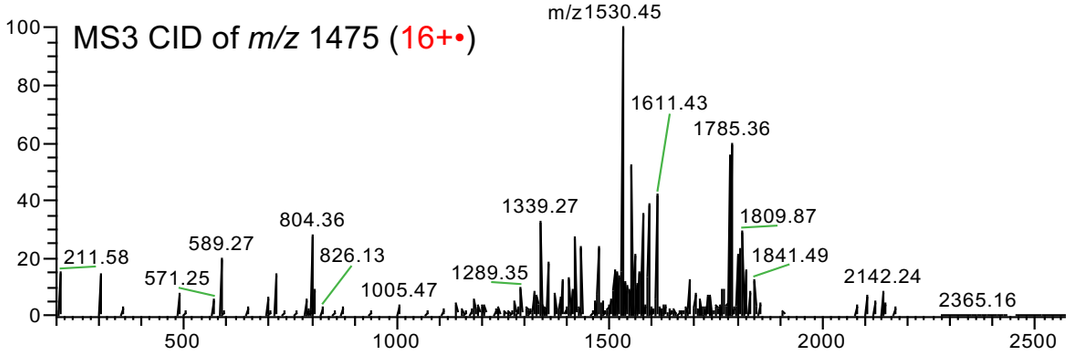
Intact Light chain: MS3

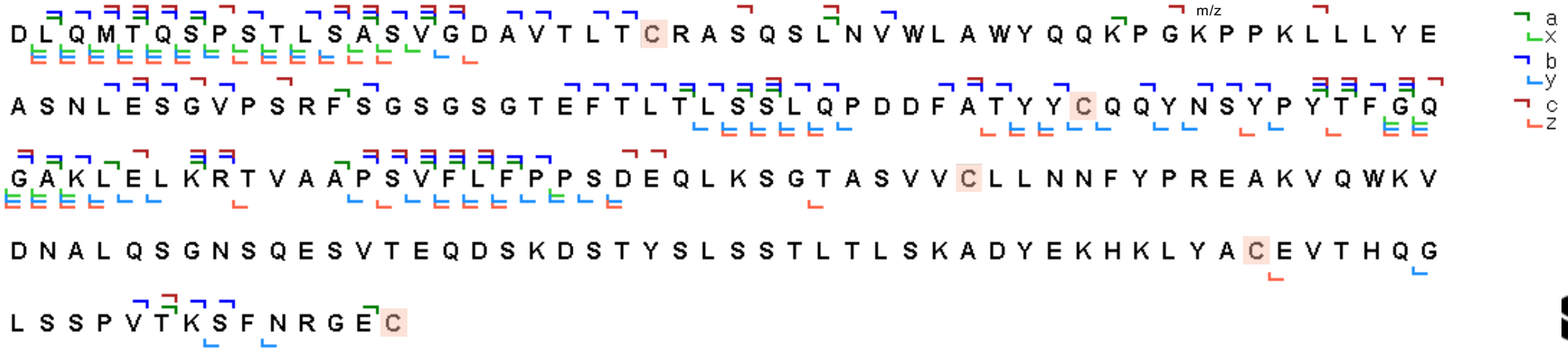
ECD 500 ms, MS3 CID of *m/z* 1475 (16+•)

a : 11% x : 9%
b : 28% y : 21%
c : 15% z : 17%

Sequence coverage : 41.3%







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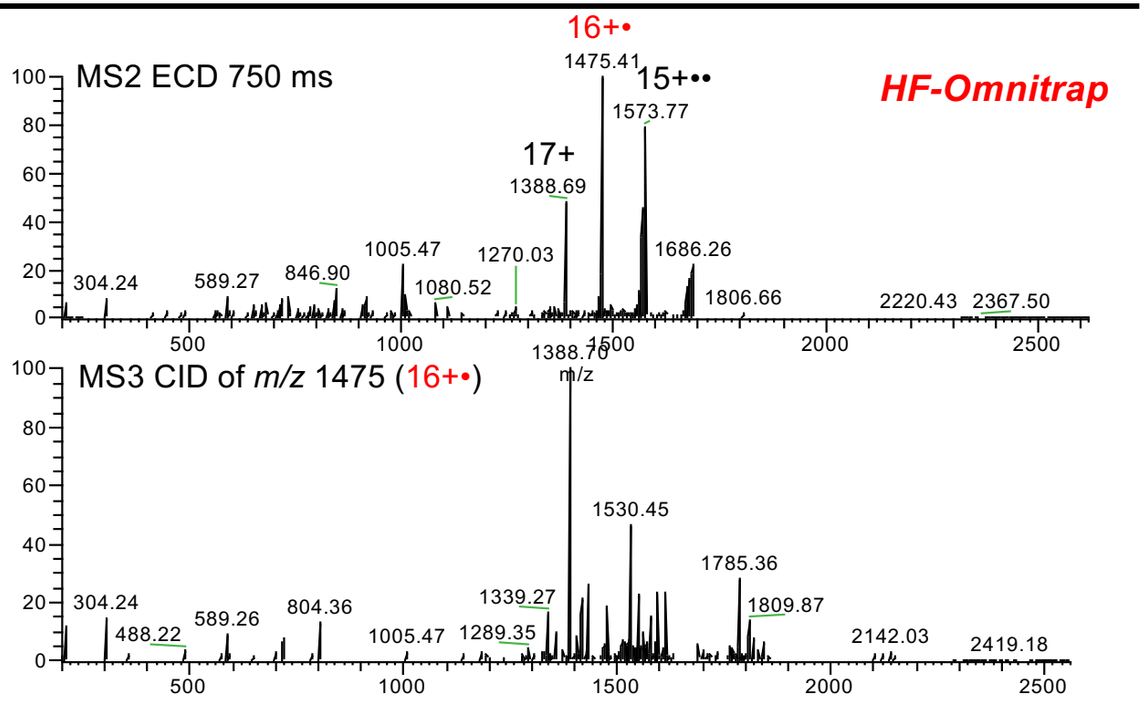
Intact Light chain: MS3

ECD 750 ms, MS3 CID of *m/z* 1475 (16+•)

a : 9% x : 6%
b : 15% y : 16%
c : 13% z : 11%

Sequence coverage : 29.6%



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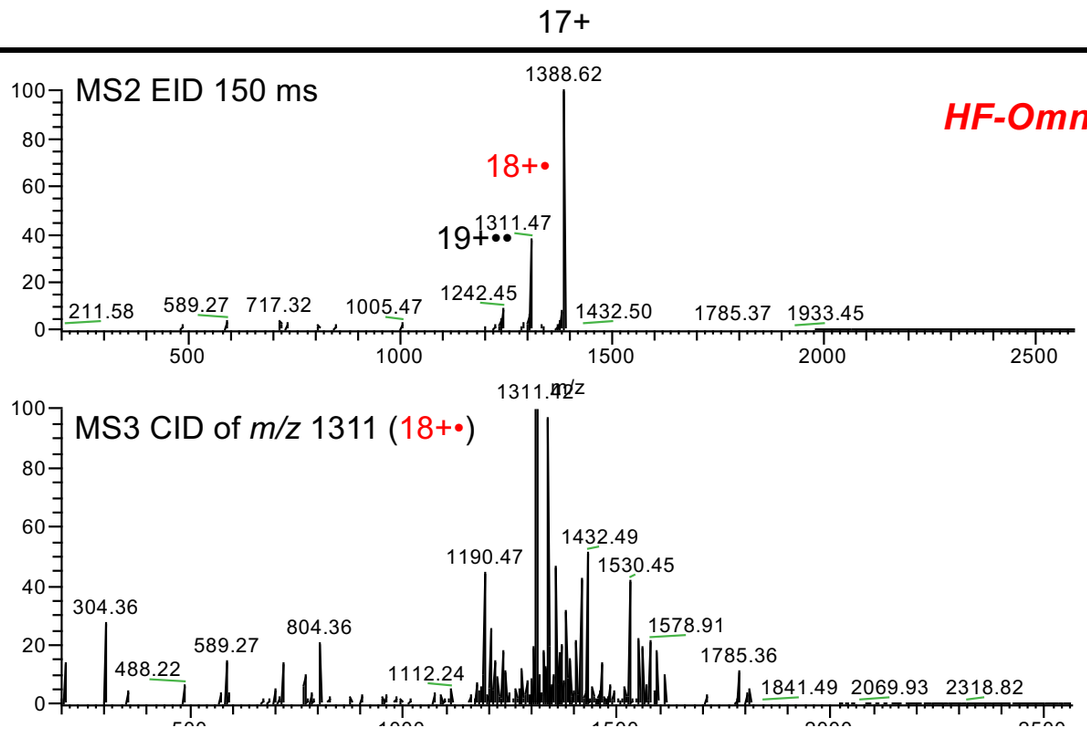
Intact Light chain: MS3

EID 150 ms, MS3 CID of *m/z* 1311 (18+•)

a : 8% x : 8%
b : 19% y : 13%
c : 8% z : 9%

Sequence coverage : 28.2%



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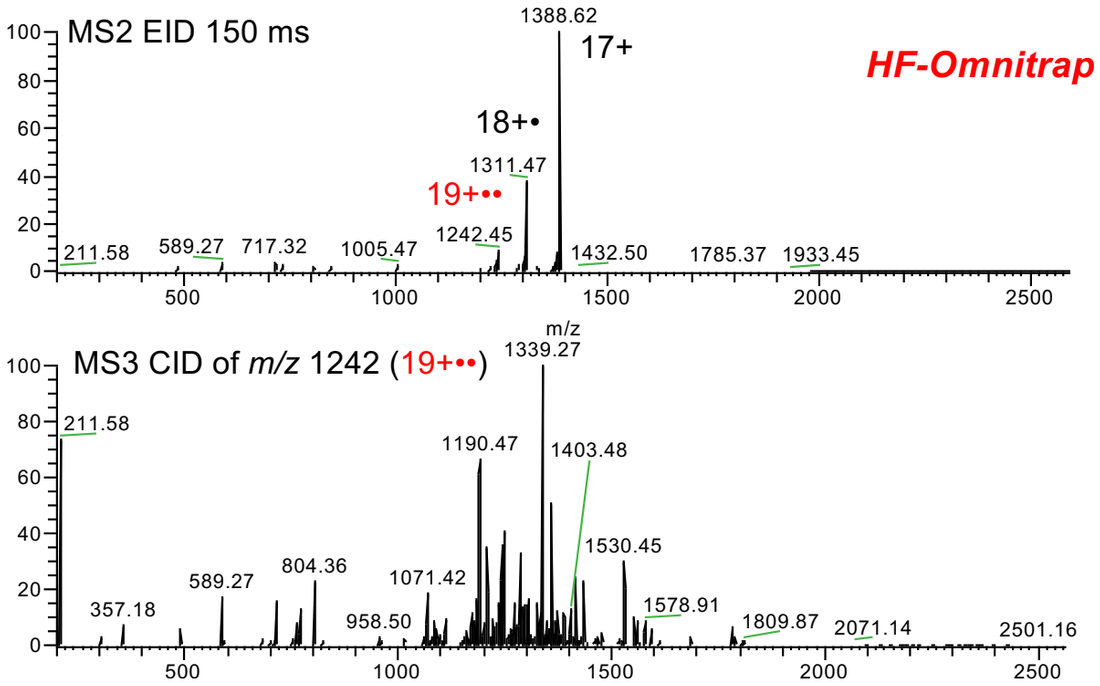
Intact Light chain: MS3

EID 150 ms, MS3 CID of *m/z* 1242 (19+••)

a : 6% x : 7%
b : 13% y : 13%
c : 6% z : 10%

Sequence coverage : 23%



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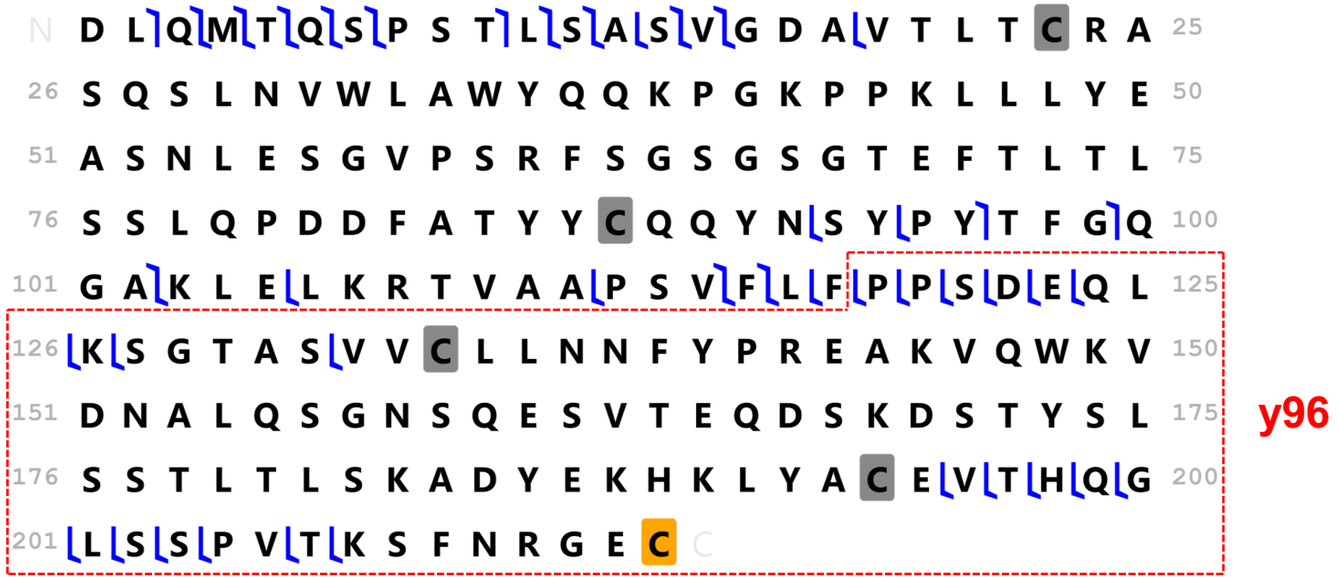
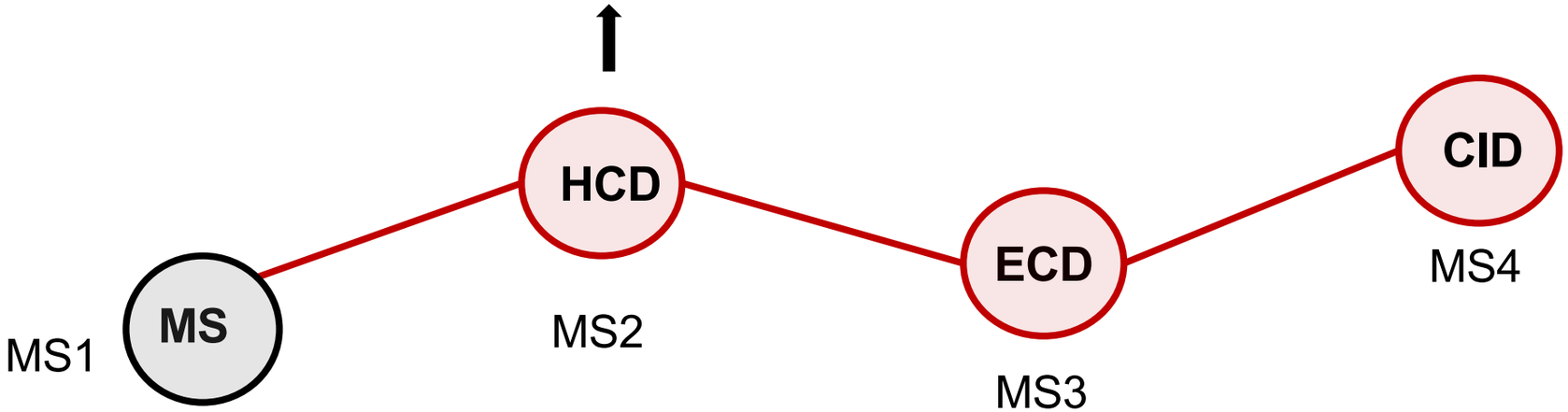
Intact Light chain: MS4

HCD NCE20

HF-Omnitrap



y96



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Intact Light chain: MS4

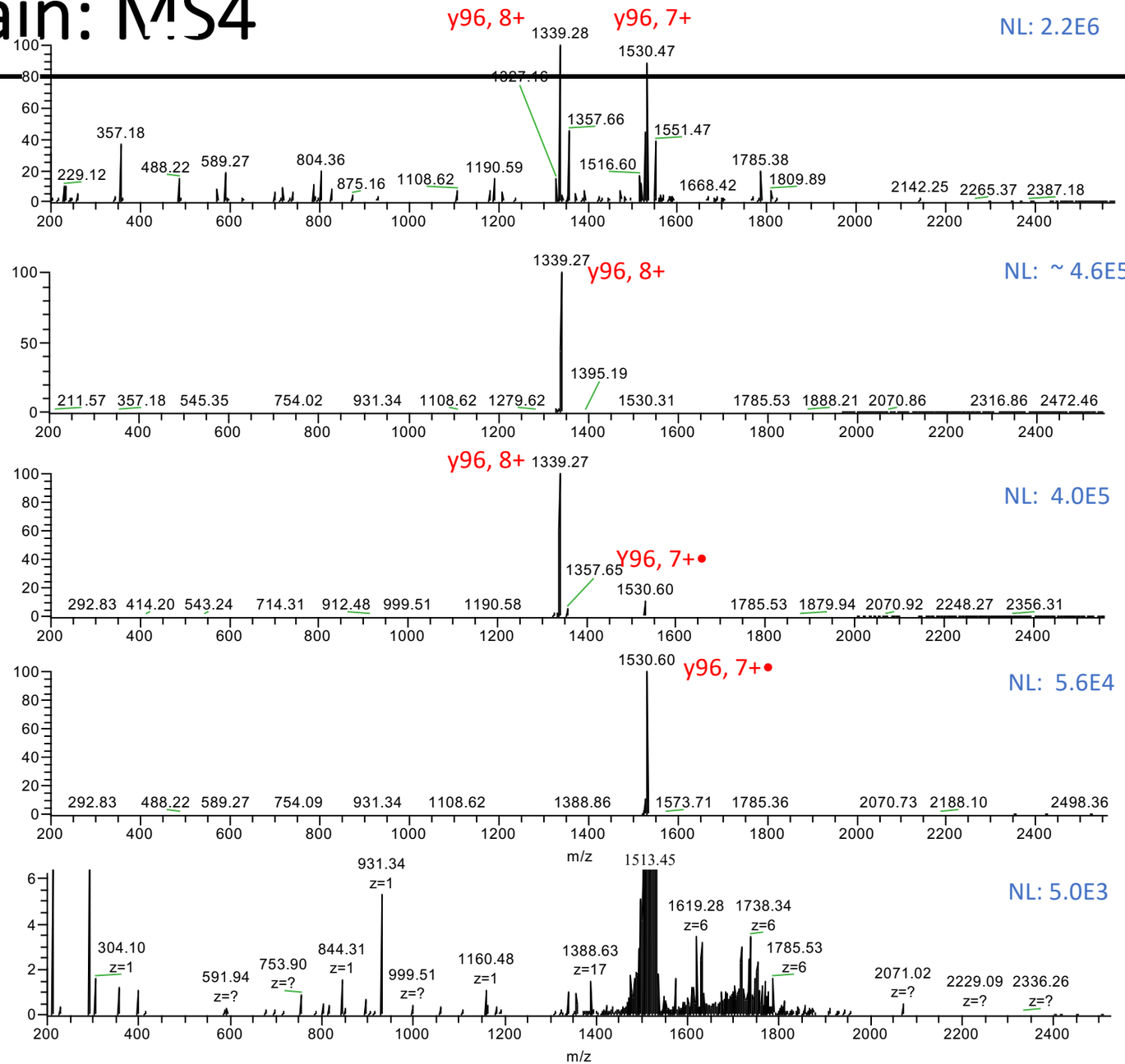
MS2 **HCD** NCE 20

Res DC isolation

MS3 **ECD** 50 ms

Res DC isolation

MS4 **CID**



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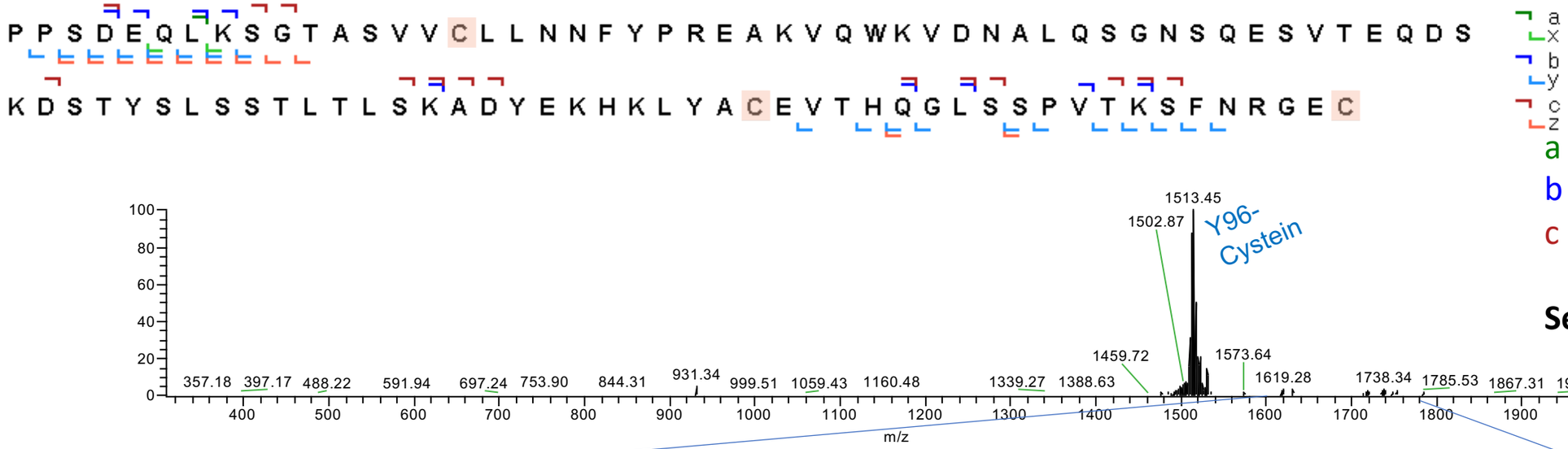
Intact Light chain: MS4

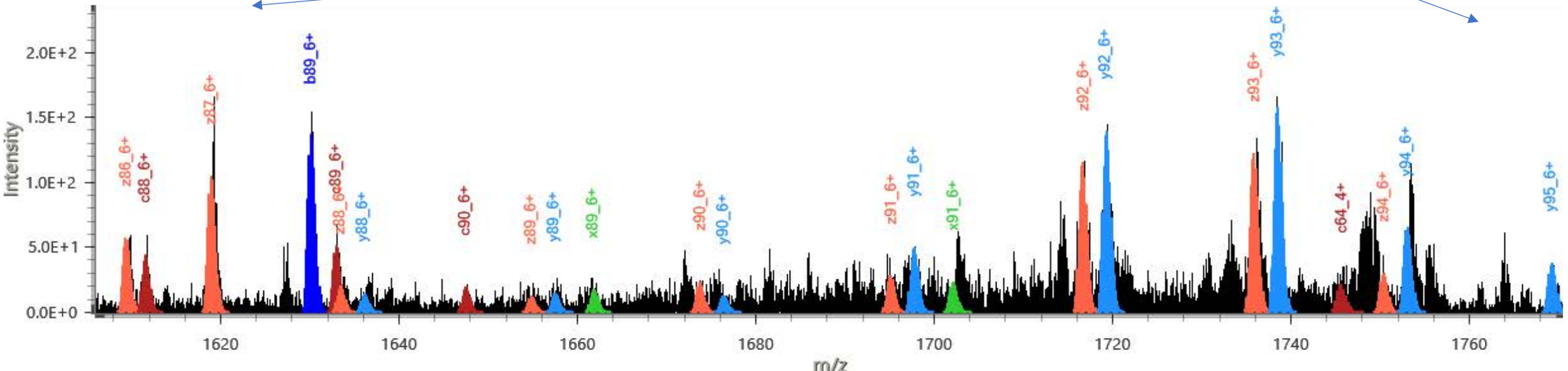
MS2 HCD20-MS3 ECD 50ms-MS4 CID

HF-Omnitrap



a : 1% x : 5%
b : 11% y : 24%
c : 15% z : 13%

Sequence coverage : 33.7%



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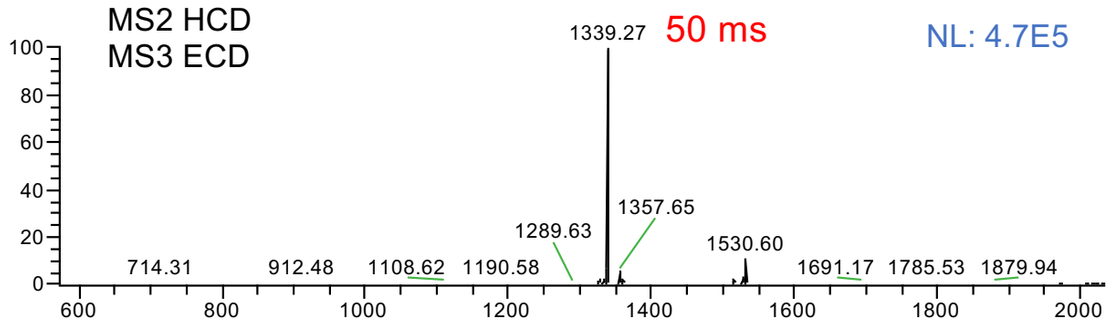
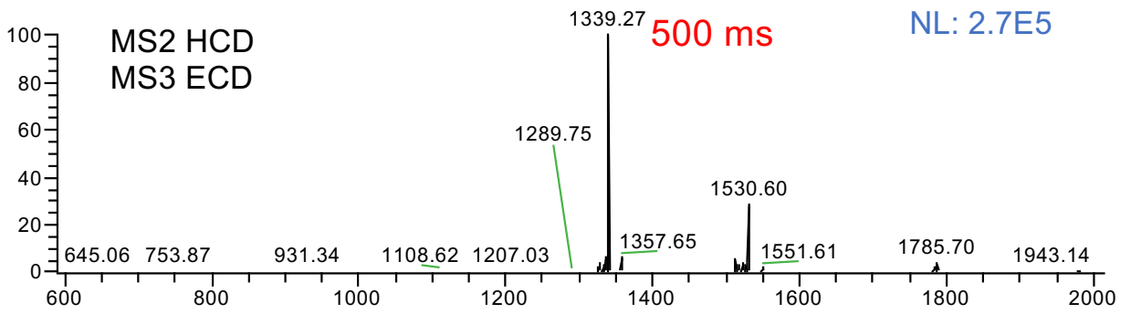
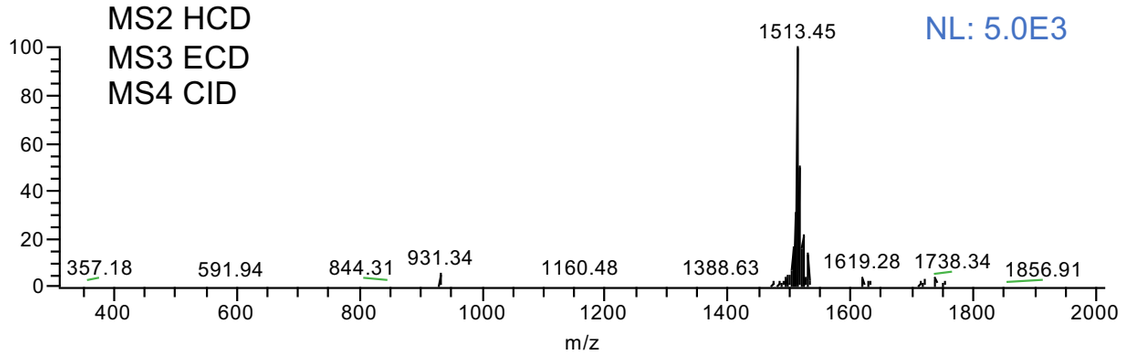
Intact Light chain: MS4 (extending MS3 ECD activation time)

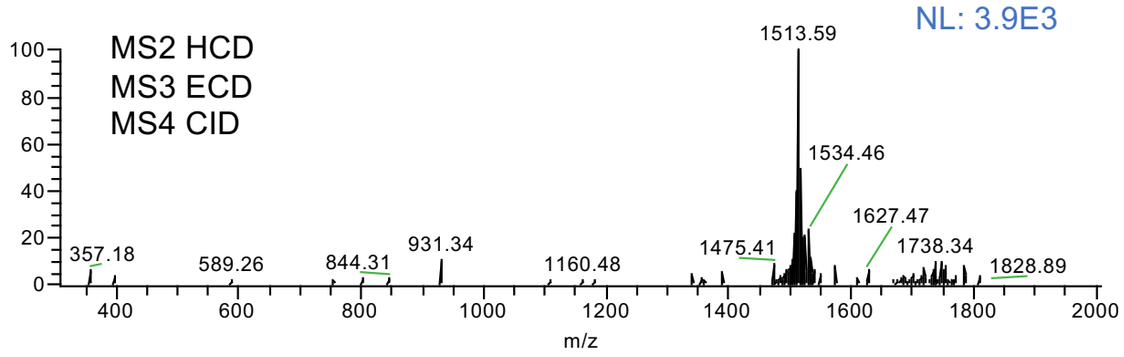
MS3





MS4





Sequence coverage : 33.7%

Sequence coverage : 26.3%

Data acquired in Sept./Oct. 2022, to be processed w. Cronus

Reduced, alkylated LC (*m/z* 1034, 23+)

Exp.		Seq. coverage	Parameters
MS2	ECD 25ms		AGC5E6,3 µscan, 240k
	ECD 50ms		AGC5E6,3µscan, 240k
	ECD 100ms		AGC5E6,3µscan, 240k
	ECD 50ms[a]		AGC5E5,3µscan, 240k
	ECD 50ms+suppl. CID activation (*m/z* 1081)[a]		AGC5E5,3µscan, 240k
	EID 25 ms		AGC5E6,3µscan, 240k
	EID 50ms		AGC5E6,3µscan, 240k

[a] AGC target was lowered to 1E6 to obtain charge reduced species

Intact LC (*m/z* 1388, 17+)

Exp.		Seq. coverage	Parameters
MS2	ECD 50 ms		AGC5E6,3 µscan, 240k
	ECD 100ms		AGC5E6,3 µscan, 240k
	ECD 100ms+suppl. CID activation		AGC5E6,3 µscan, 240k
	ECD 200ms		AGC5E5,3 µscan, 240k
	ECD 250ms		AGC5E5,3 µscan, 240k
	EID 50ms		AGC5E6,3 µscan, 240k
	EID 100ms		AGC5E6,3 µscan, 240k
MS3	ECD 100ms+MS3CID of 16+•		AGC5E6,3 µscan, 240k
	ECD 200ms+MS3CID of 16+•		AGC5E6,3 µscan, 240k
	EID 50ms+MS3CID of 18+•		AGC5E6,3 µscan, 240k
MS3 (y96)	HCD20-EID 50ms		AGC5E6,3 µscan, 240k
	HCD20-EID 200ms		AGC5E6,3 µscan, 240k
	HCD20-ECD 50ms		AGC5E6,3 µscan, 240k
	HCD20-ECD 500ms		AGC5E6,3 µscan, 240k
MS4 (y96)	HCD20-ECD50ms-MS4CID		AGC5E6,3 µscan, 240k
	HCD20-ECD500ms-MS4CID		AGC5E6,3 µscan, 240k
	In source CID-m/z1339-ECD50ms-MS4CID		AGC5E6,3 µscan, 240k
	In source CID-m/z1339-ECD500ms-MS4CID		AGC5E6,3 µscan, 240k
MS3 (b118)	In source CID-m/z1432-ECD50ms		AGC5E6,3 µscan, 240k
MS4 (b118)	In source CID-m/z1432-ECD500ms		AGC5E6,3 µscan, 240k
	In source CID-m/z1432-ECD50ms-MS4CID		AGC5E6,3 µscan, 240k

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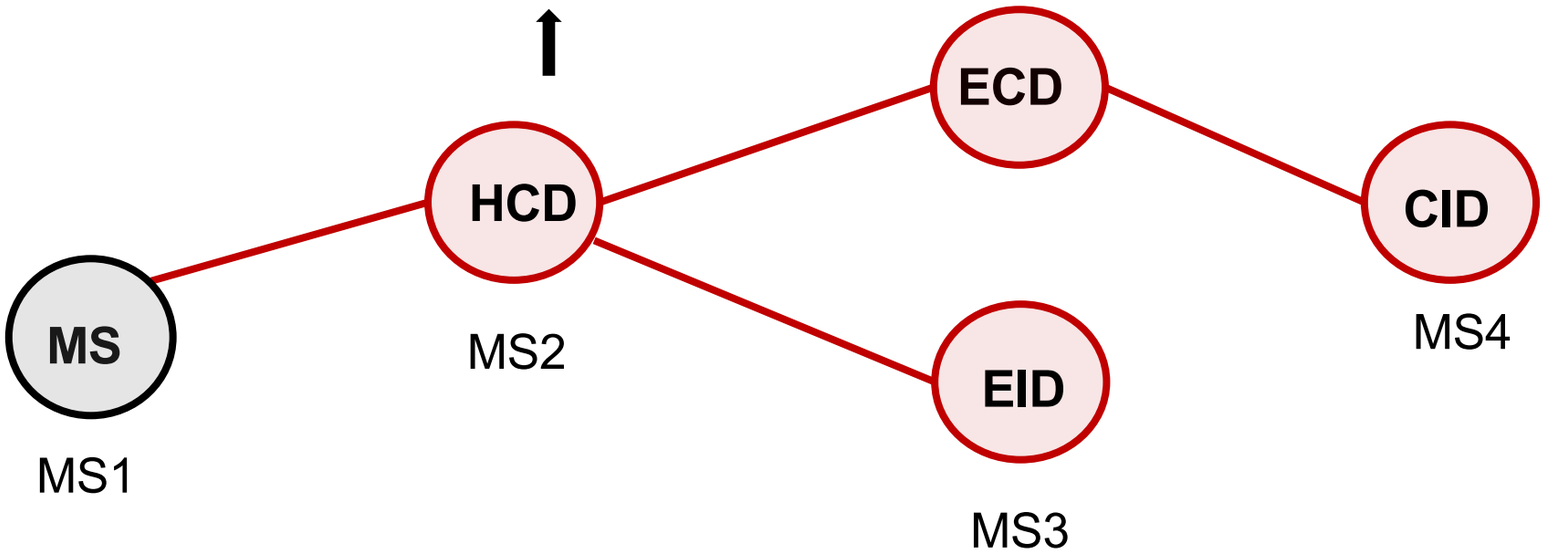
Intact Light chain: MS4 (*m/z* 1388, 17+)

Targeting C terminal fragment *y96 ion*

HCD NCE20

```
N   D L Q M T Q S P S T L S A S V G D A V T L T C R A   25
26  S Q S L N V W L A W Y Q Q K P G K P P K L L L Y E   50
51  A S N L E S G V P S R F S G S G S G T E F T L T L   75
76  S S L Q P D D F A T Y Y C Q Q Y N S Y P Y T F G Q   100
101 G A K L E L K R T V A A P S V F L F P P S D E Q L   125
126 K S G T A S V V C L L N N F Y P R E A K V Q W K V   150
151 D N A L Q S G N S Q E S V T E Q D S K D S T Y S L   175
176 S S T L T L S K A D Y E K H K L Y A C E V T H Q G   200
201 L S S P V T K S F N R G E C C
```

y96



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Intact Light chain: MS4 (*m/z* 1388, 17+)

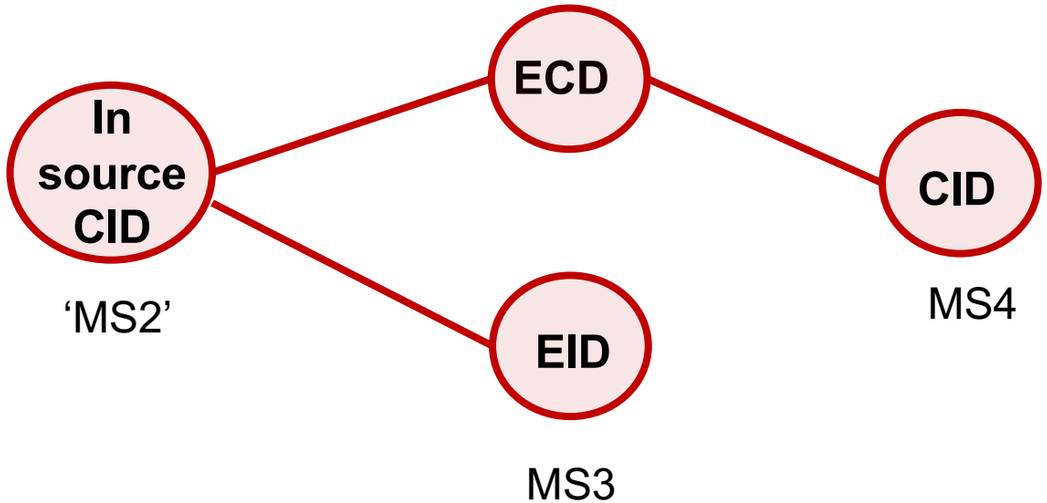
Targeting N terminal fragment *b118 ion*

HCD NCE20

```
N   D L Q M T Q S P S T L S A S V G D A V T L T C R A   25
26  S Q S L N V W L A W Y Q Q K P G K P P K L L L Y E   50
51  A S N L E S G V P S R F S G S G S G T E F T L T L   75
76  S S L Q P D D F A T Y Y C Q Q Y N S Y P Y T F G Q   100
101 G A K L E L K R T V A A P S V F L F P P S D E Q L   125
126 K S G T A S V V C L L N N F Y P R E A K V Q W K V   150
151 D N A L Q S G N S Q E S V T E Q D S K D S T Y S L   175
176 S S T L T L S K A D Y E K H K L Y A C E V T H Q G   200
201 L S S P V T K S F N R G E C C
```

b118

y96



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Data acquired on Lumos (Aug. 2022) to be processed with Cronus

Lumos

Reduced, alkylated LC (*m/z* 1034, 23+)

Exp.		Seq. coverage	Parameters
MS2[d]	ETD10		AGC5E6,3 µscan, 240k
	ET5HCD5		AGC5E6,3µscan, 240k
	ET10HCD10		AGC5E6,3µscan, 240k
	ET15HCD15		AGC5E5,3µscan, 240k

[d] Low AGC target data (1E6, 3 and 10 µscan) were also acquired

Intact LC (*m/z* 1388, 17+)

Exp.		Seq.coverage	Parameters
MS2[a]	ETD15		AGC5E6,3 µscan, 240k
	ETD20		AGC5E6,3 µscan, 240k
	ETD25		AGC5E6,3 µscan, 240k
	ET10HCD10		AGC5E6,3 µscan, 240k
	ET15HCD15		AGC5E6,3 µscan, 240k
	ET10HCD10		AGC5E6,3 µscan, 240k
	ET25HCD25		AGC5E6,3 µscan, 240k
MS3[b] (*m/z* 1475, 16+•)	ETD-MS3HCD15		AGC5E6,3 µscan, 240k
	ETD-MS3HCD20		AGC5E6,3 µscan, 240k
	ETD-MS3HCD25		AGC5E6,3 µscan, 240k
MS3[c] (*m/z* 1339, 8+, y96)	HCD-MS3ETD15		AGC1E6,10 µscan, 240k
	HCD-MS3ETD20		AGC1E6,10 µscan, 240k
	HCD-MS3ET15HCD15		AGC1E6,10 µscan, 240k
	HCD-MS3ET20HCD20		AGC1E6,10 µscan, 240k

[a] Low AGC target data (1E6, 3 and 10 µscan) were also acquired
[b] Low AGC target data (1E6, 10 µscan) were also acquired
[c] High AGC target data (5E6, 3µscan) were not usable

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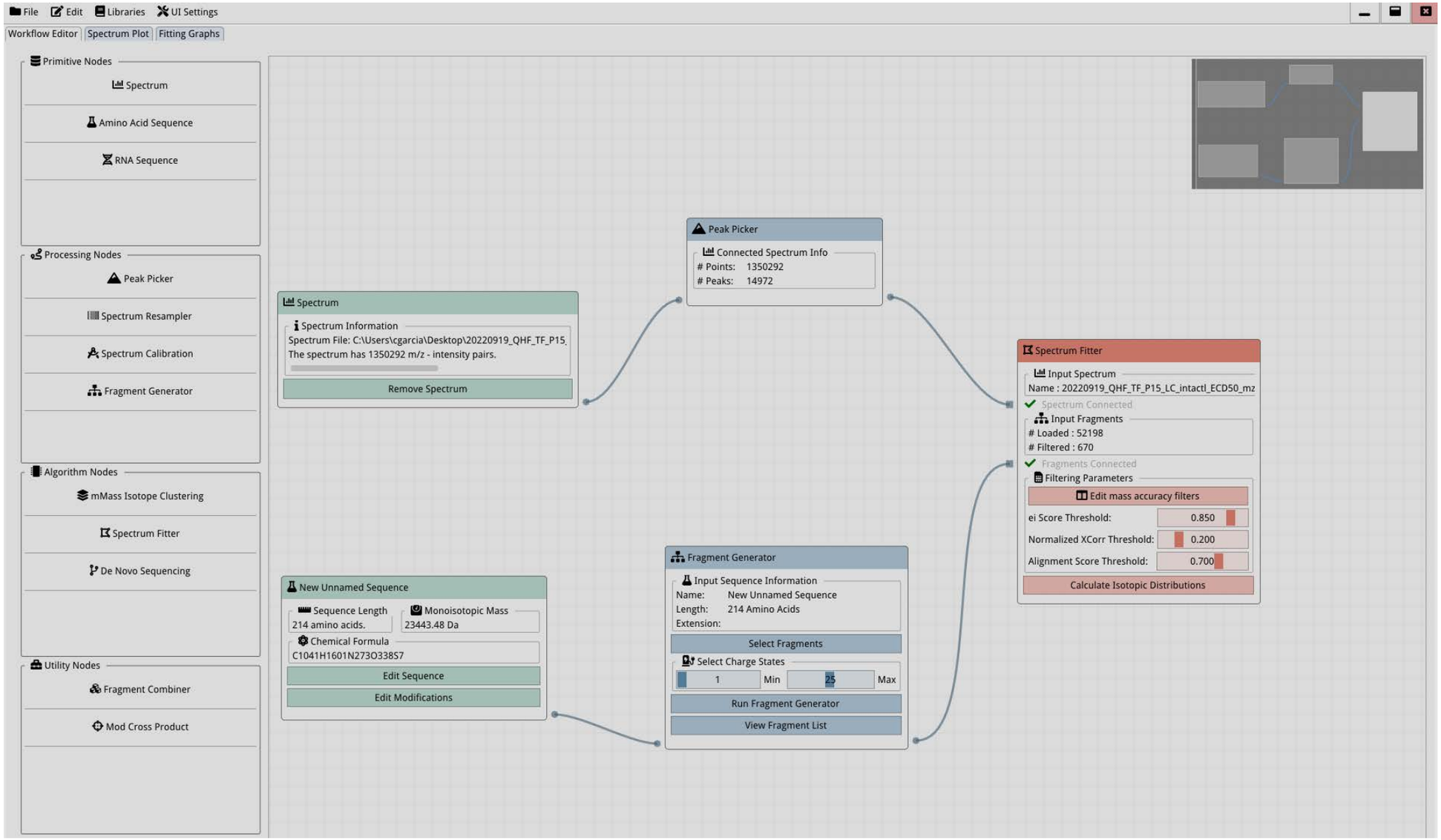
Cronus analysis



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1. Analysis of the P15 light chain with the omnitrap
2. First data with the booster
3. Analysis of the NIST mAb
4. Conclusion/Future direction

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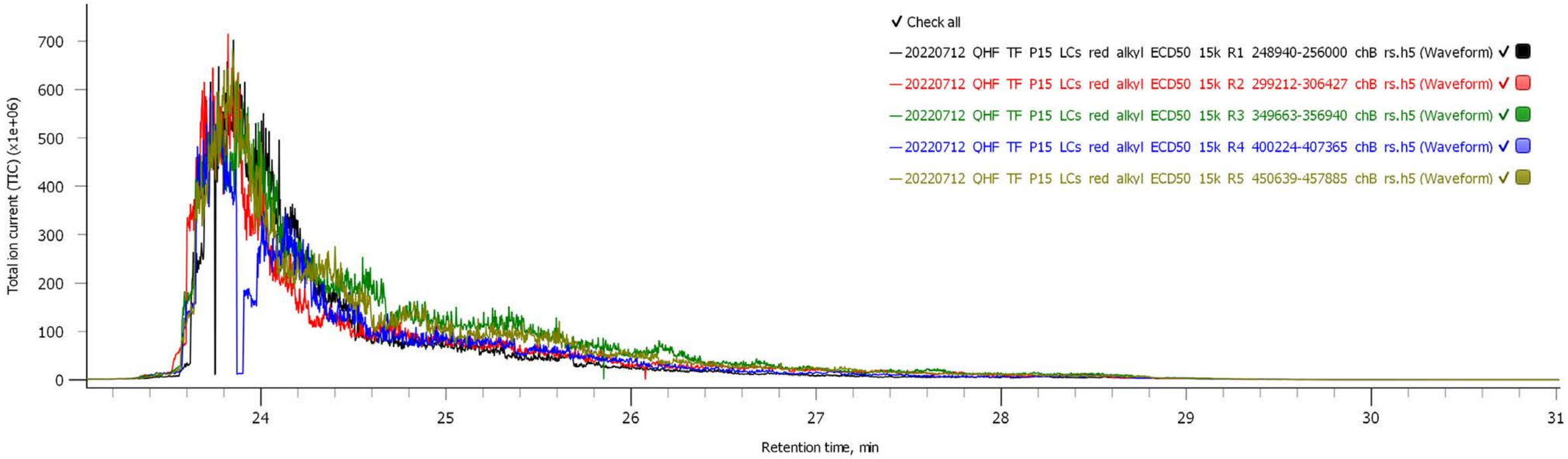
HF-Omnitrap-Booster Characterization

LC-MSn analysis of red/alkyl Light chain

ECD 50 ms, 15k resolution, 5 replicates



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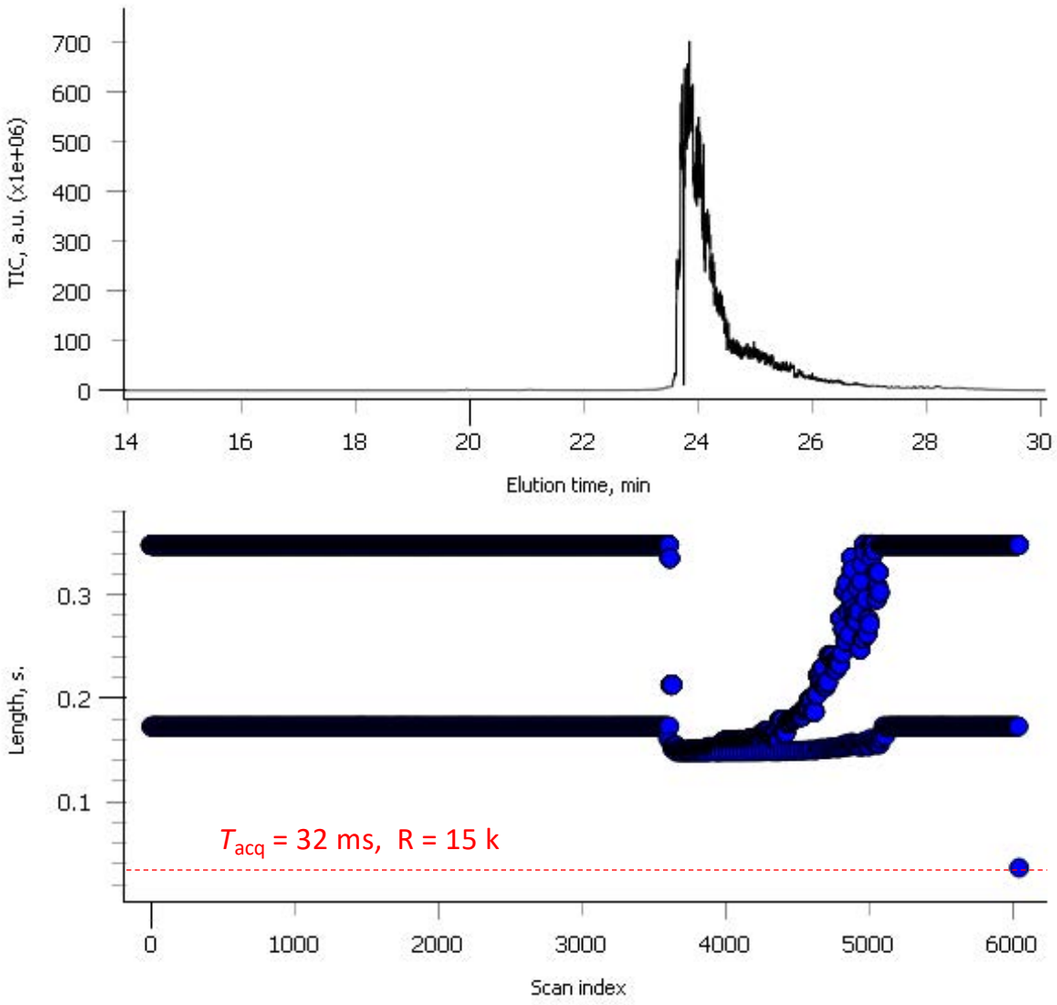
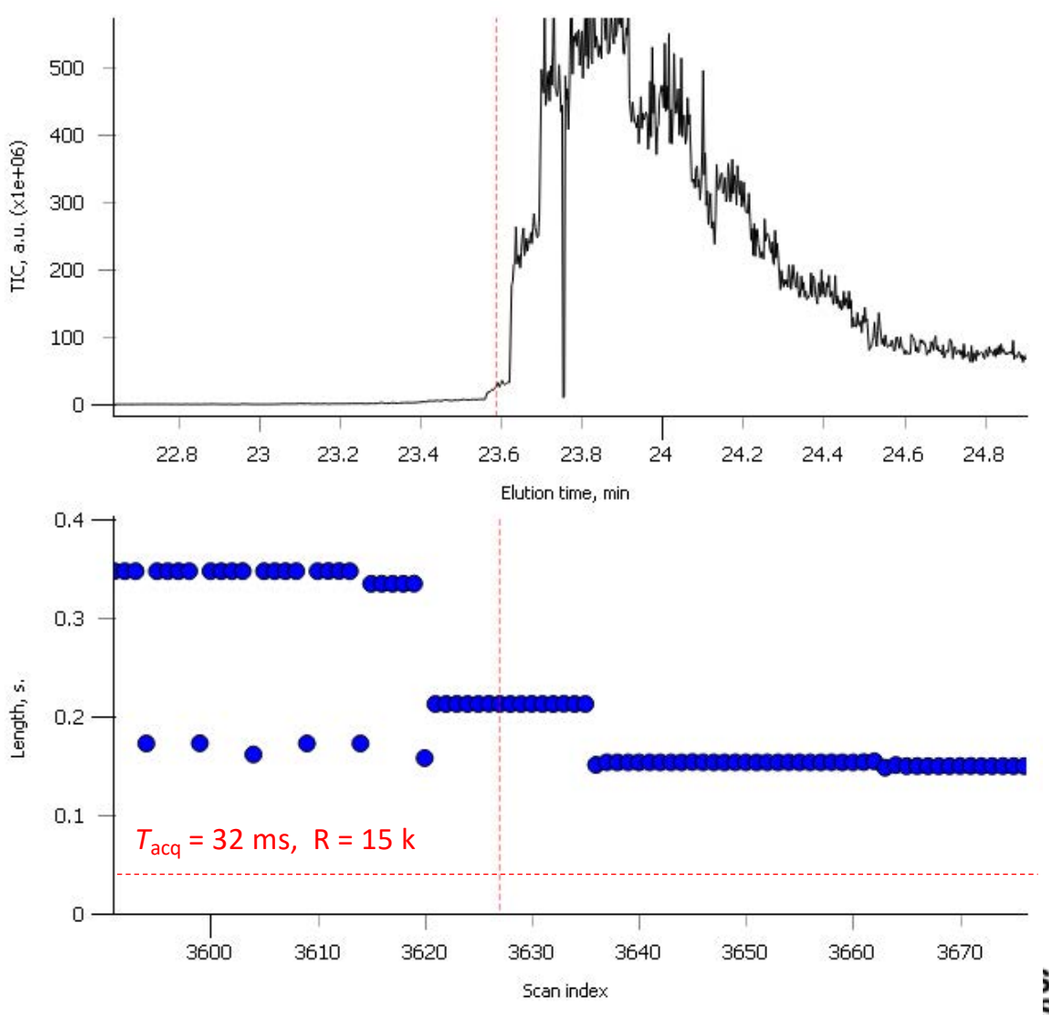
QExHF, TF_P15_LCs_red_alkyl_ECD50, R = 15 K , AGC = 5e5, ITmax = 200 ms

20220712_QHF_TF_P15_LCs_red_alkyl_ECD50_15k_R1

HF-Omnitrap-Booster





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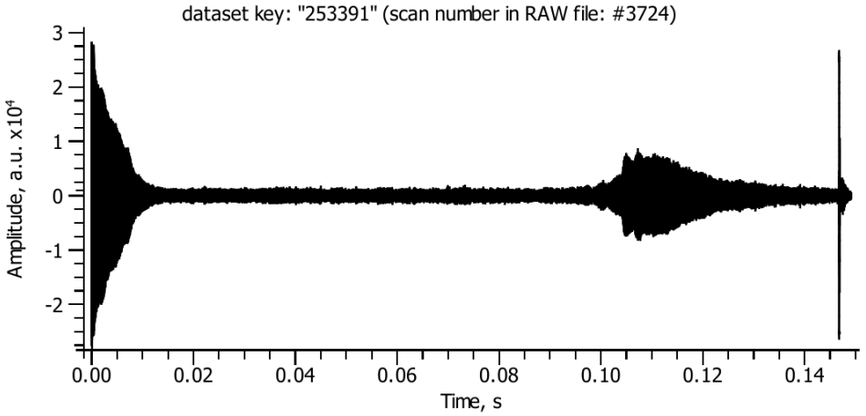
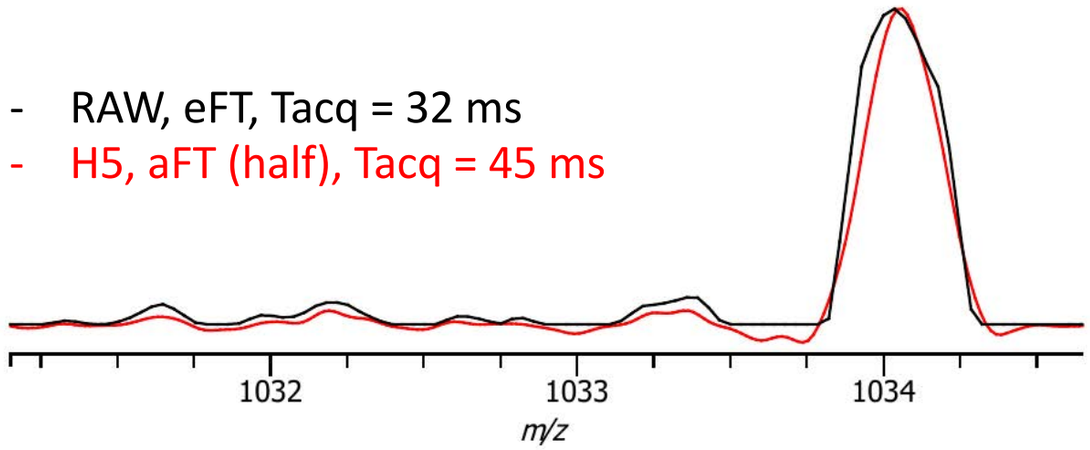
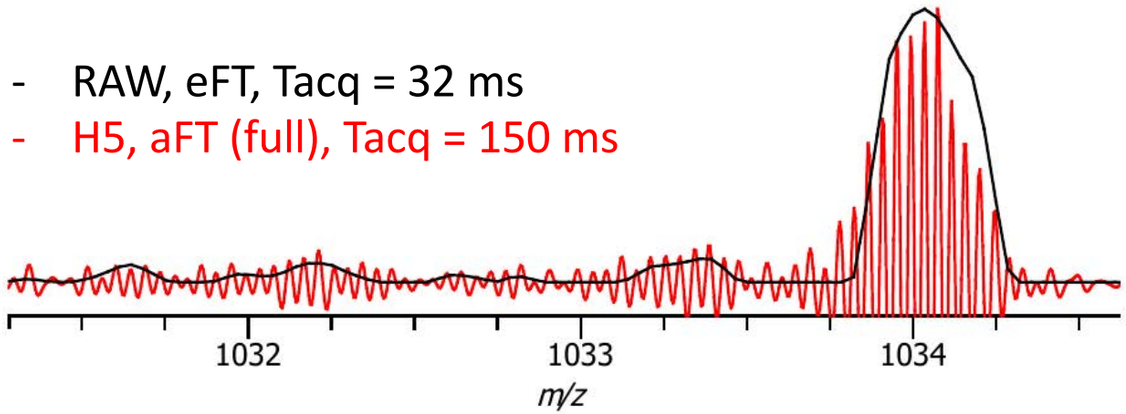
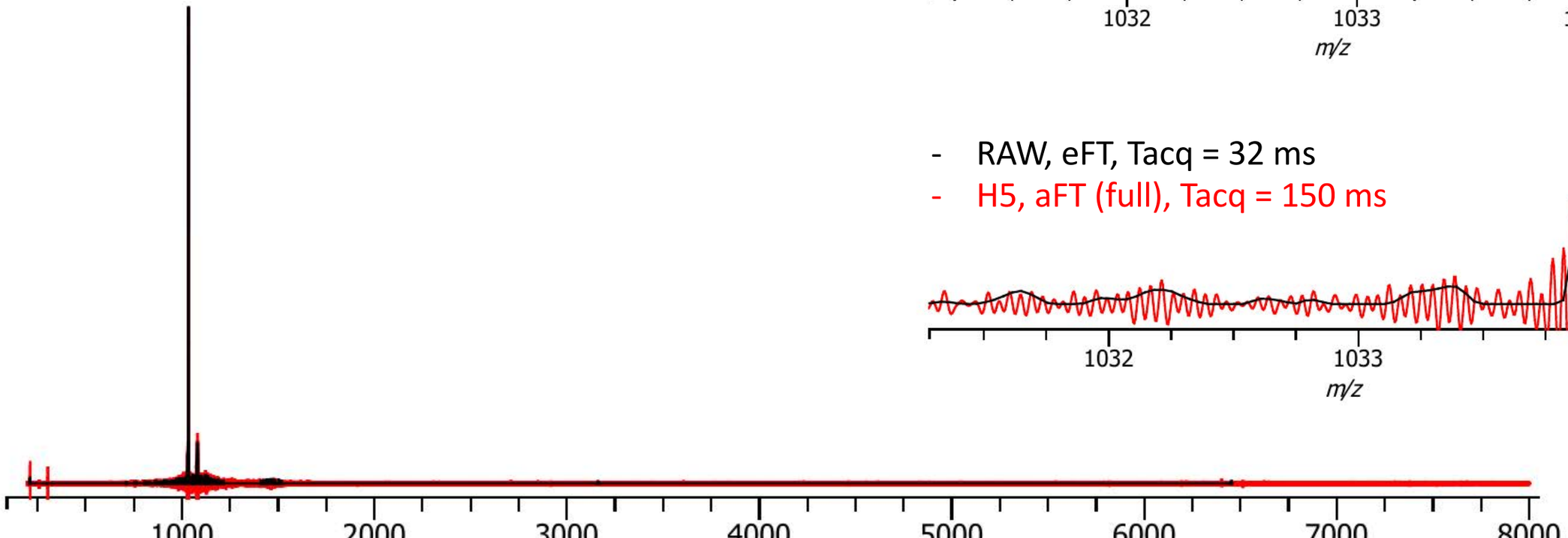
QExHF, TF_P15_LCs_red_alkyl_ECD50, R1

HF-Omnitrap-Booster









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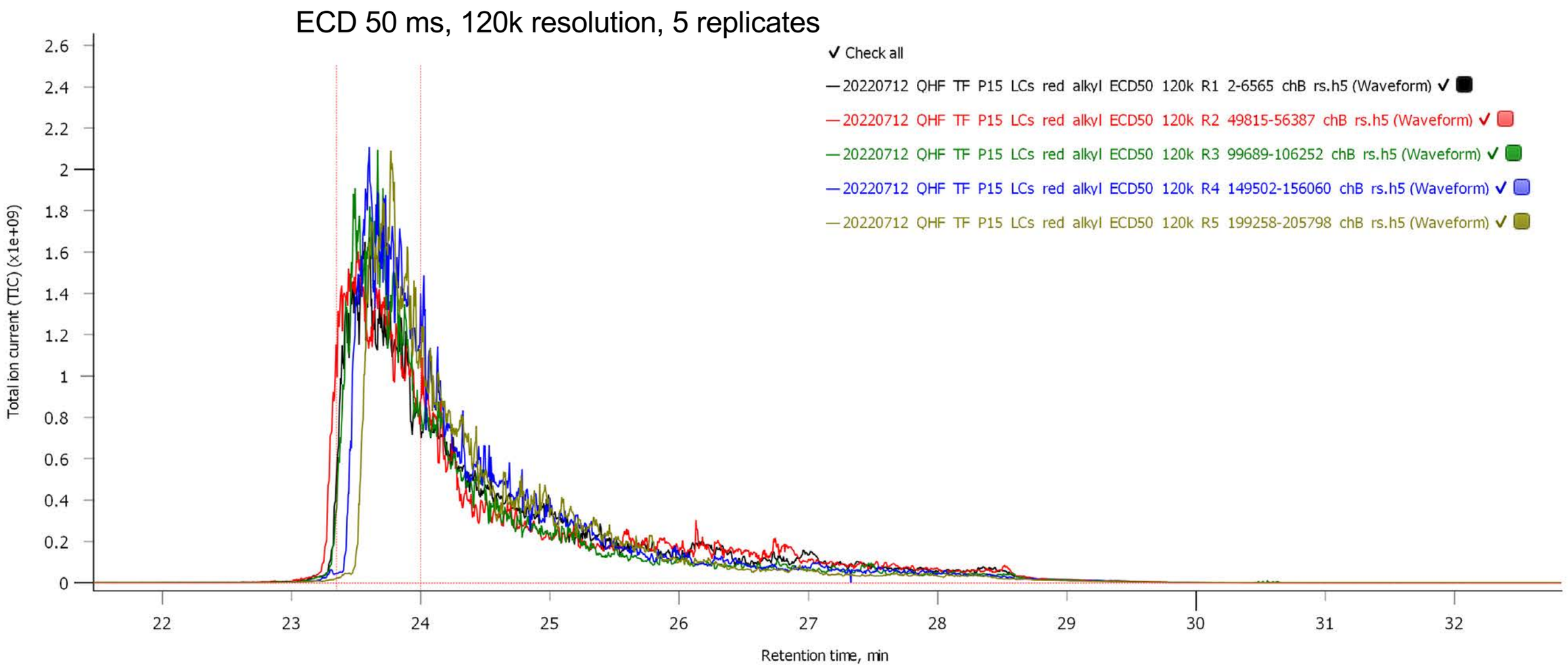
QExHF, TF_P15_LCs_red_alkyl_ECD50, R = 120 K
AGC = 5e5, ITmax = 200 ms

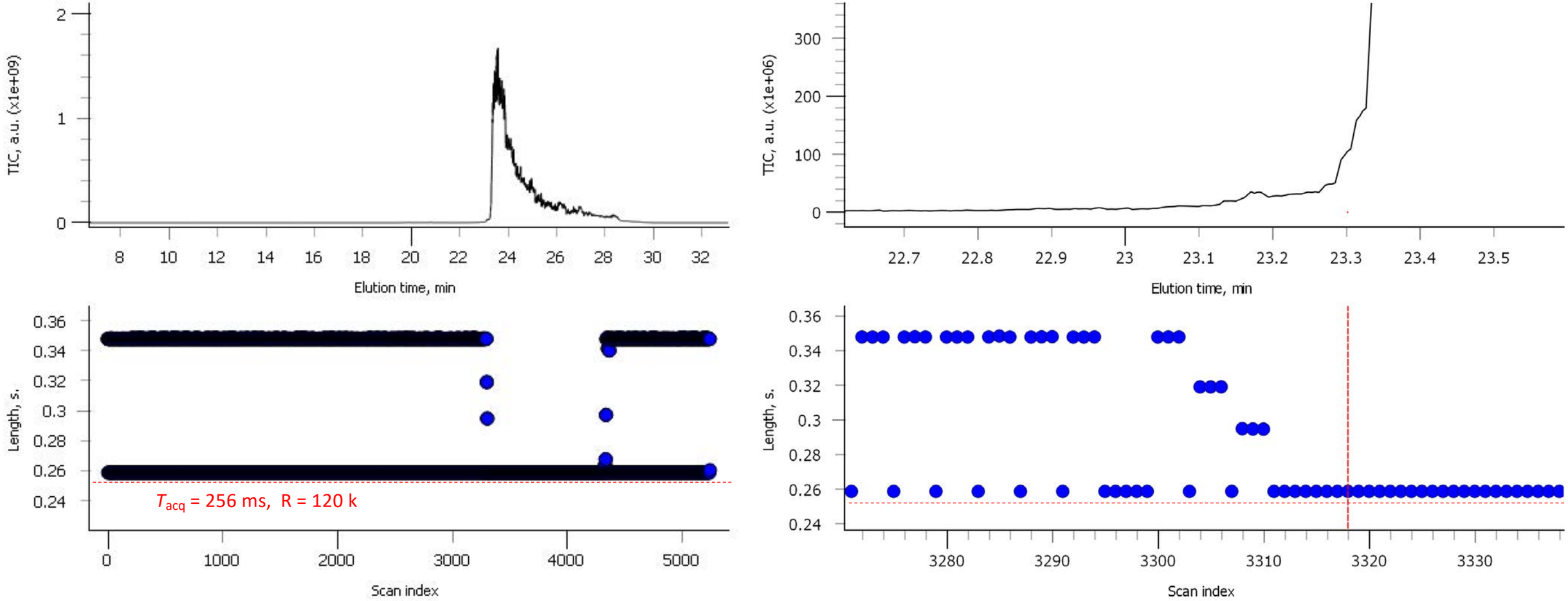


INSTITUT PASTEUR

QExHF, TF_P15_LCs_red_alkyl_ECD50, R = 120 K
AGC = 5e5, ITmax = 200 ms

20220712_QHF_TF_P15_LCs_red_alkyl_ECD50_120k_R1

HF-Omnitrap-Booster



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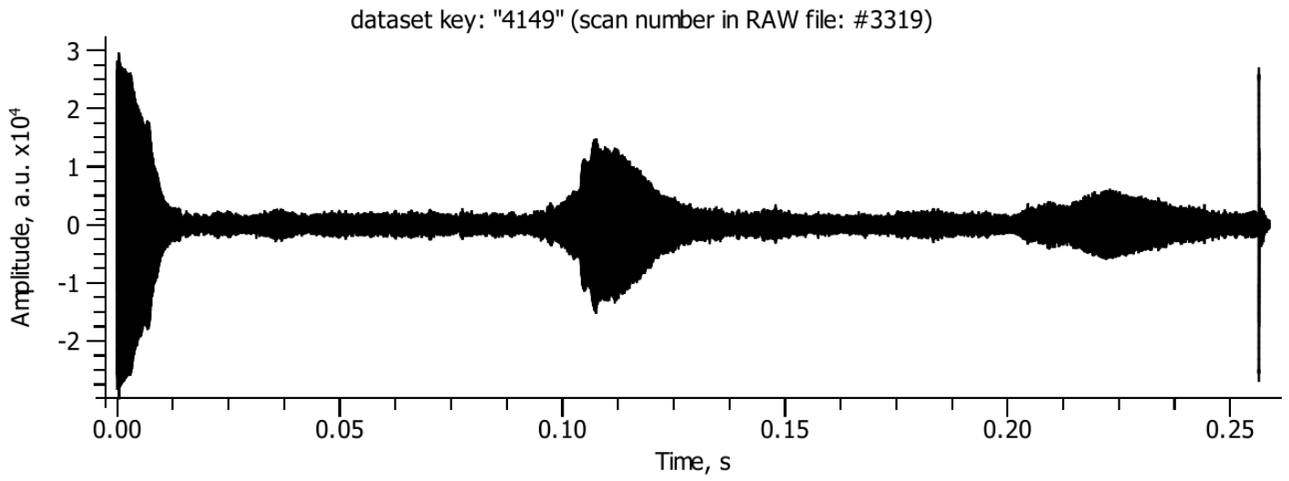
QExHF, TF_P15_LCs_red_alkyl_ECD50, R = 120 K
AGC = 5e5, ITmax = 200 ms

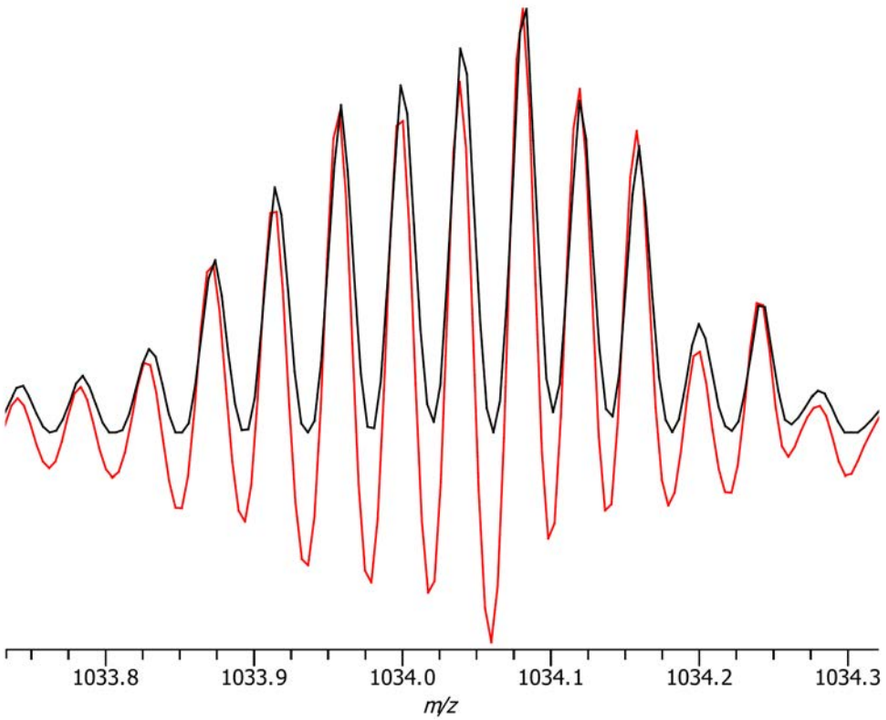
QExHF, TF_P15_LCs_red_alkyl_ECD50, R = 120 K, AGC = 5e5, ITmax = 200 ms

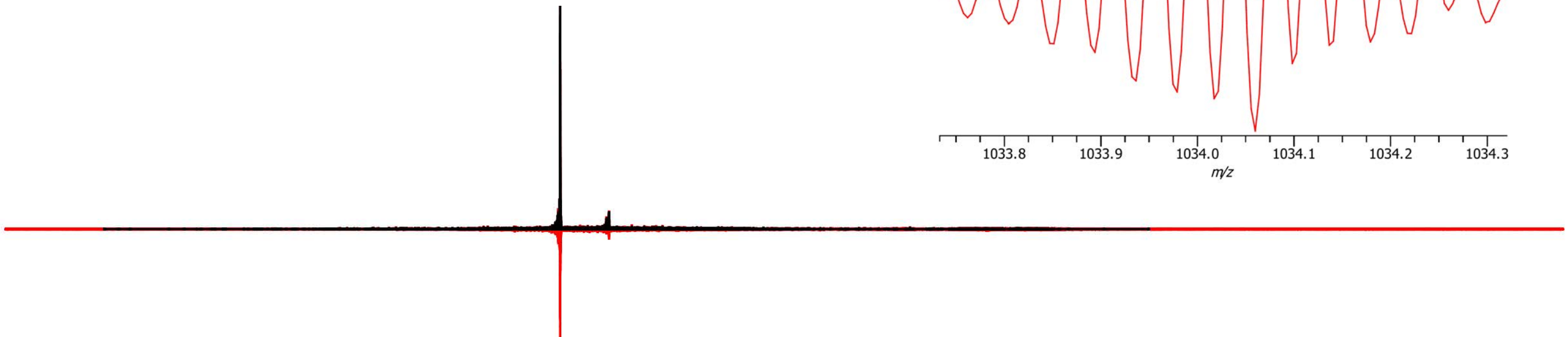
HF-Omnitrap-Booster

Single scan

- RAW, eFT, Tacq = 256 ms
- H5, aFT (half), Tacq = 256 ms

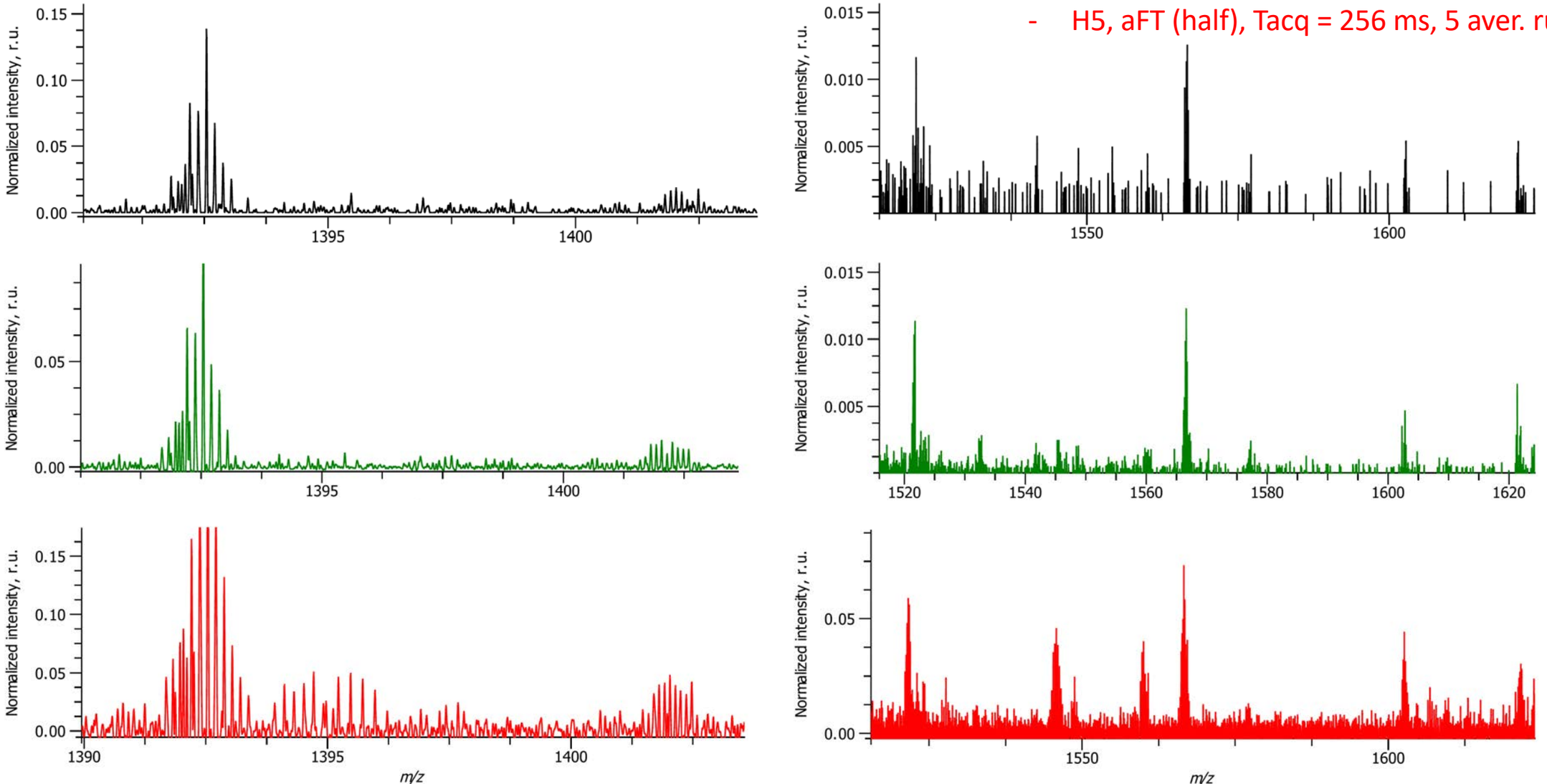






QExHF, TF_P15_LCs_red_alkyl_ECD50, R = 120 K
AGC = 5e5, ITmax = 200 ms

- RAW, eFT, Tacq = 256 ms, single run
- RAW, eFT, Tacq = 256 ms, 5 aver. runs
- H5, aFT (half), Tacq = 256 ms, 5 aver. runs



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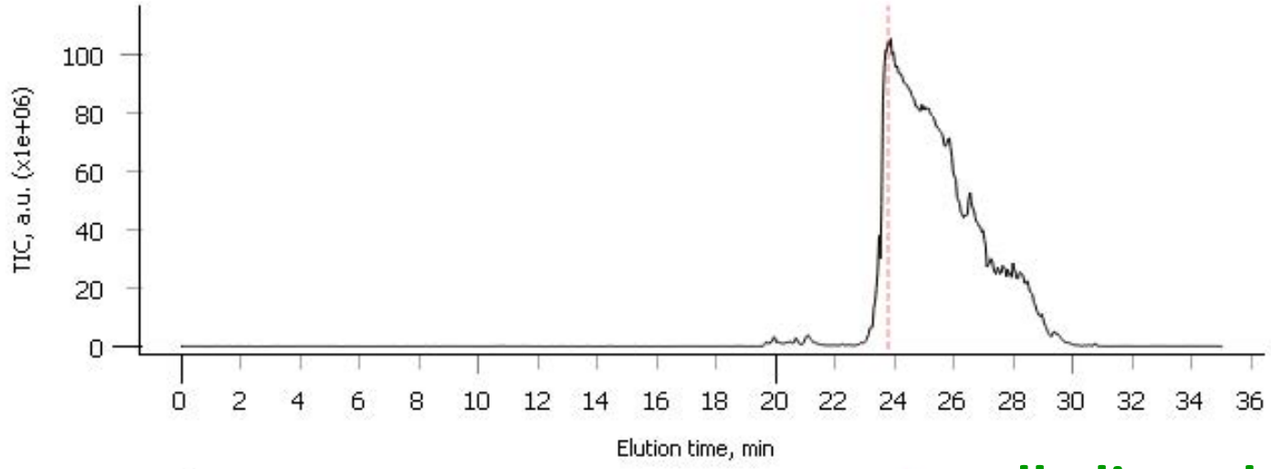
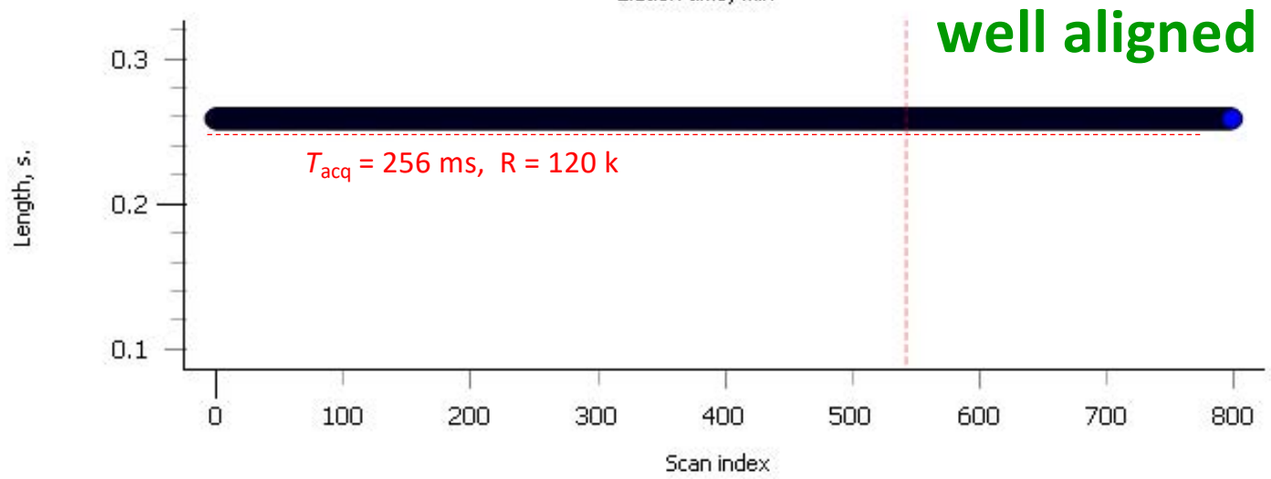
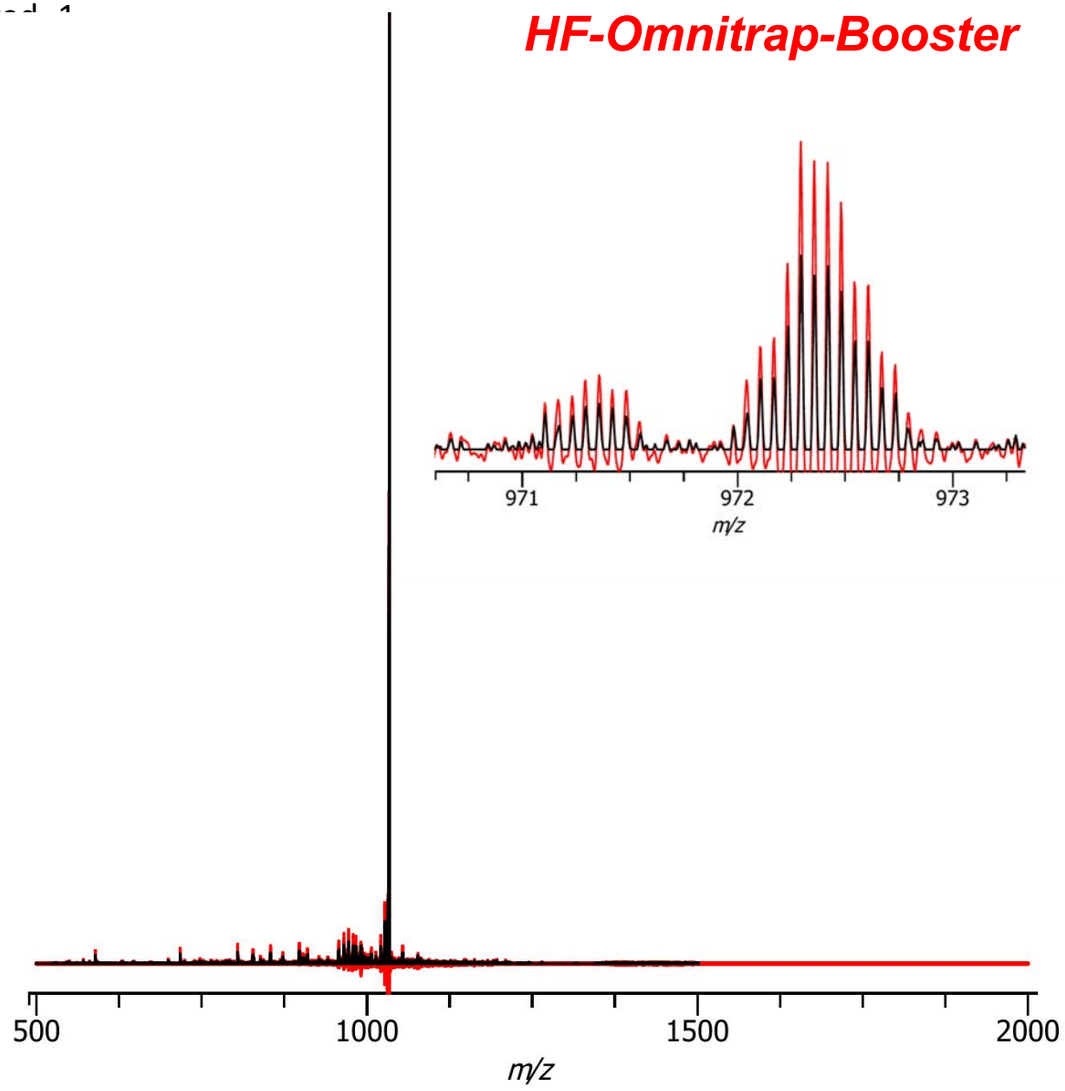
QExHF, TF_P15_LCs_red_alkyl_ECD50, R = 120 K , **AGC = fixed**, ITmax = 50 ms, **10 us**

20220712_QHF_TF_P15_LCs_red_alkyl_ECD50_120k_10µscan_AGC_f







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HF-Omnitrap: TDMS analysis of Intact antibody

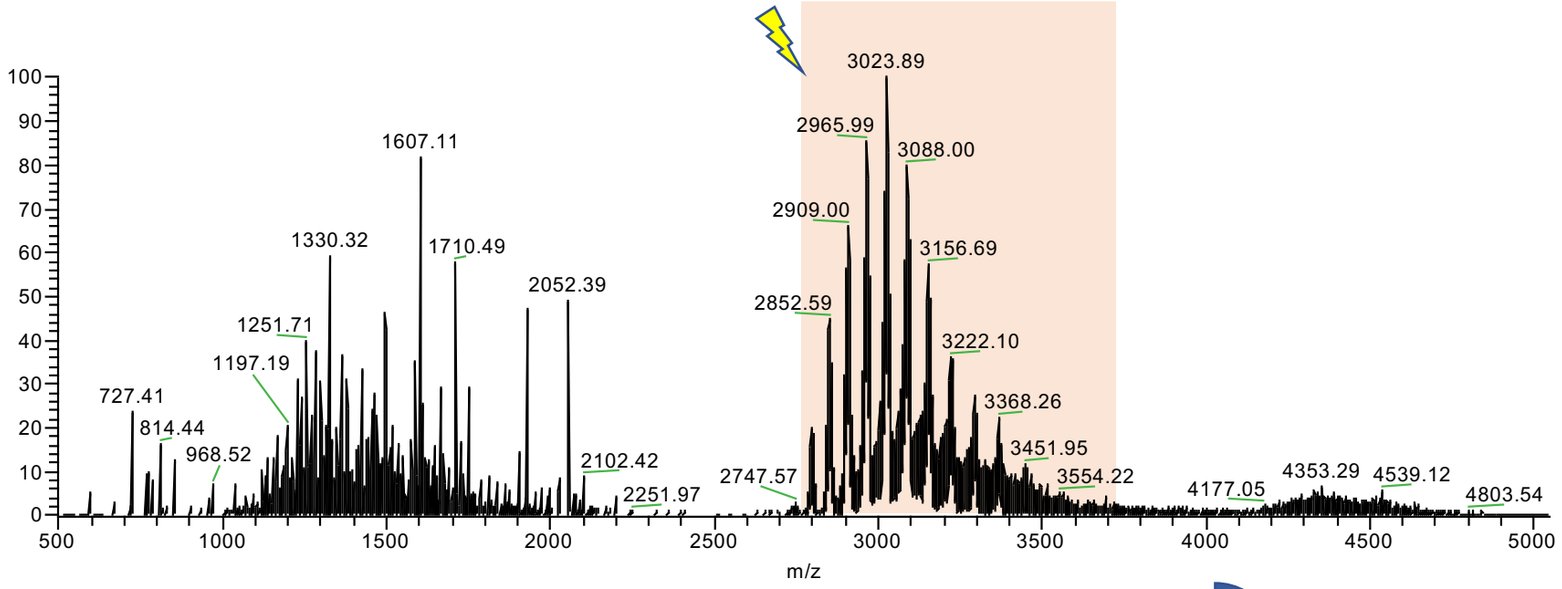
HF-Omnitrap

Intact NISTmAb, *m/z* 2797, 53+

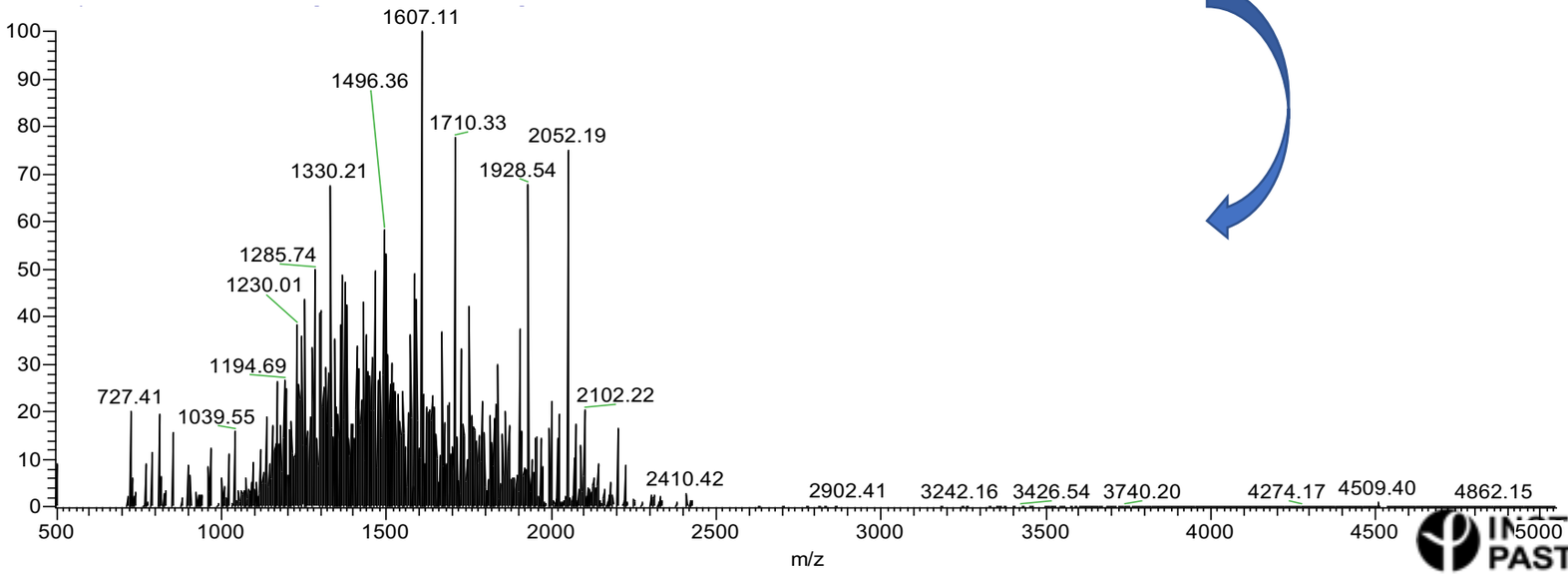
Isolation window: 30 *m/z*

Broadband excitation

MS2 ECD 90 ms



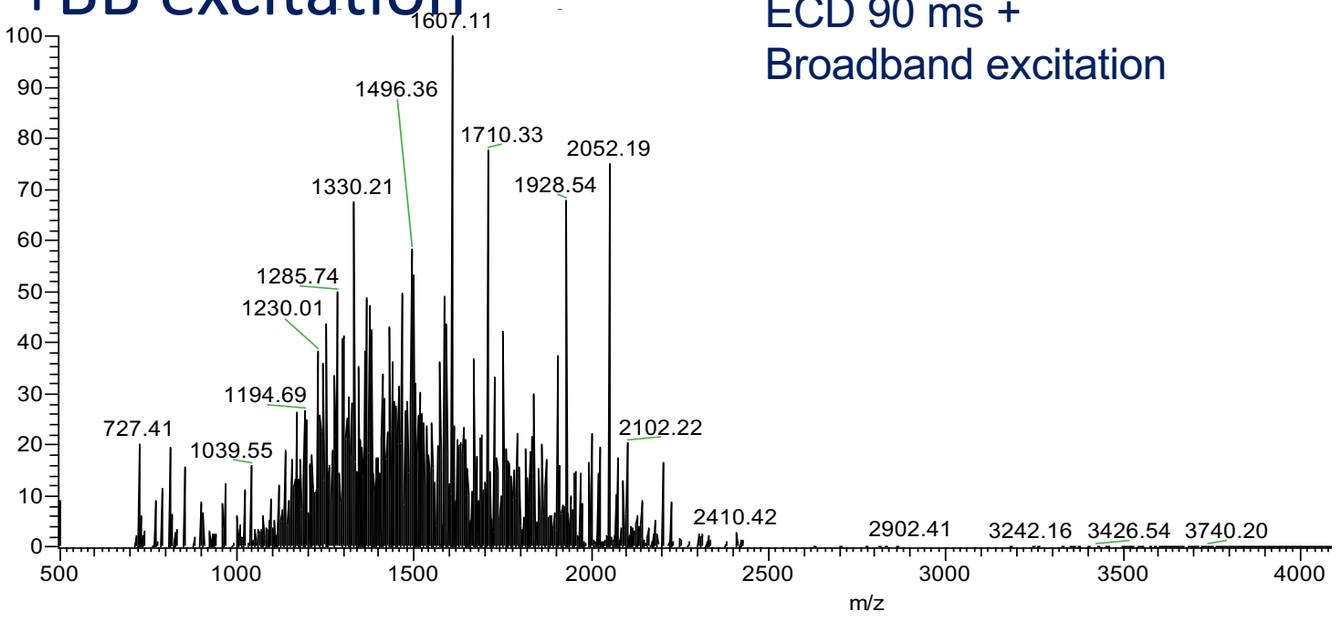
MS2 ECD 90 ms +
Broadband excitation



Sequence coverage obtained from MS2 ECD +BB excitation

FreeStyle ProSight Lite

ECD 90 ms +
Broadband excitation



Residue cleavage: 16%

```
N  D I Q M T Q S P S T L S A S V G D R V T I T C S A  25
26 S S R V G Y M H W Y Q Q K P G K A P K L L I Y D T  50
51 S K L A S G V P S R F S G S G S G T E F T L T I S  75
76 S L Q P D D F A T Y Y C F Q G S G Y P F T F G G G  100
101 T K V E I K R T V A A P S V F I F P P S D E Q L K  125
126 S G T A S V V C L L N N F Y P R E A K V Q W K V D  150
151 N A L Q S G N S Q E S V T E Q D S K D S T Y S L S  175
176 S T L T L S K A D Y E K H K V Y A C E V T H Q G L  200
201 S S P V T K S F N R G E C C
```

Residue cleavage: 21%

```
N  Q V T L R E S G P A L V K P T Q T L T L T C T F S  25
26 G F S L S T A G M S V G W I R Q P P G K A L E W L  50
51 A D I W W D D K K H Y N P S L K D R L T I S K D T  75
76 S K N Q V V L K V T N M D P A D T A T Y Y C A R D  100
101 M I F N F Y F D V W G Q G T T V T V S S A S T K G  125
126 P S V F P L A P S S K S T S G G T A A L G C L V K  150
151 D Y F P E P V T V S W N S G A L T S G V H T F P A  175
176 V L Q S S G L Y S L S S V V T V P S S S L G T Q T  200
201 Y I C N V N H K P S N T K V D K R V E P K S C D K  225
226 T H T C P P C P A P E L L G G P S V F L F P P K P  250
251 K D T L M I S R T P E V T C V V V D V S H E D P E  275
276 V K F N W Y V D G V E V H N A K T K P R E E Q Y N  300
301 S T Y R V V S V L T V L H Q D W L N G K E Y K C K  325
326 V S N K A L L P A P I E K T I S K A K G Q P R E P Q  350
351 V Y T L P P S R E E M T K N Q V S L T C L V K G F  375
376 Y P S D I A V E W E S N G Q P E N N Y K T T P P V  400
401 L D S D G S F F L Y S K L T V D K S R W Q Q G N V  425
426 F S C S V M H E A L H N H Y T Q K S L S L S P G C
```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MSn experiments

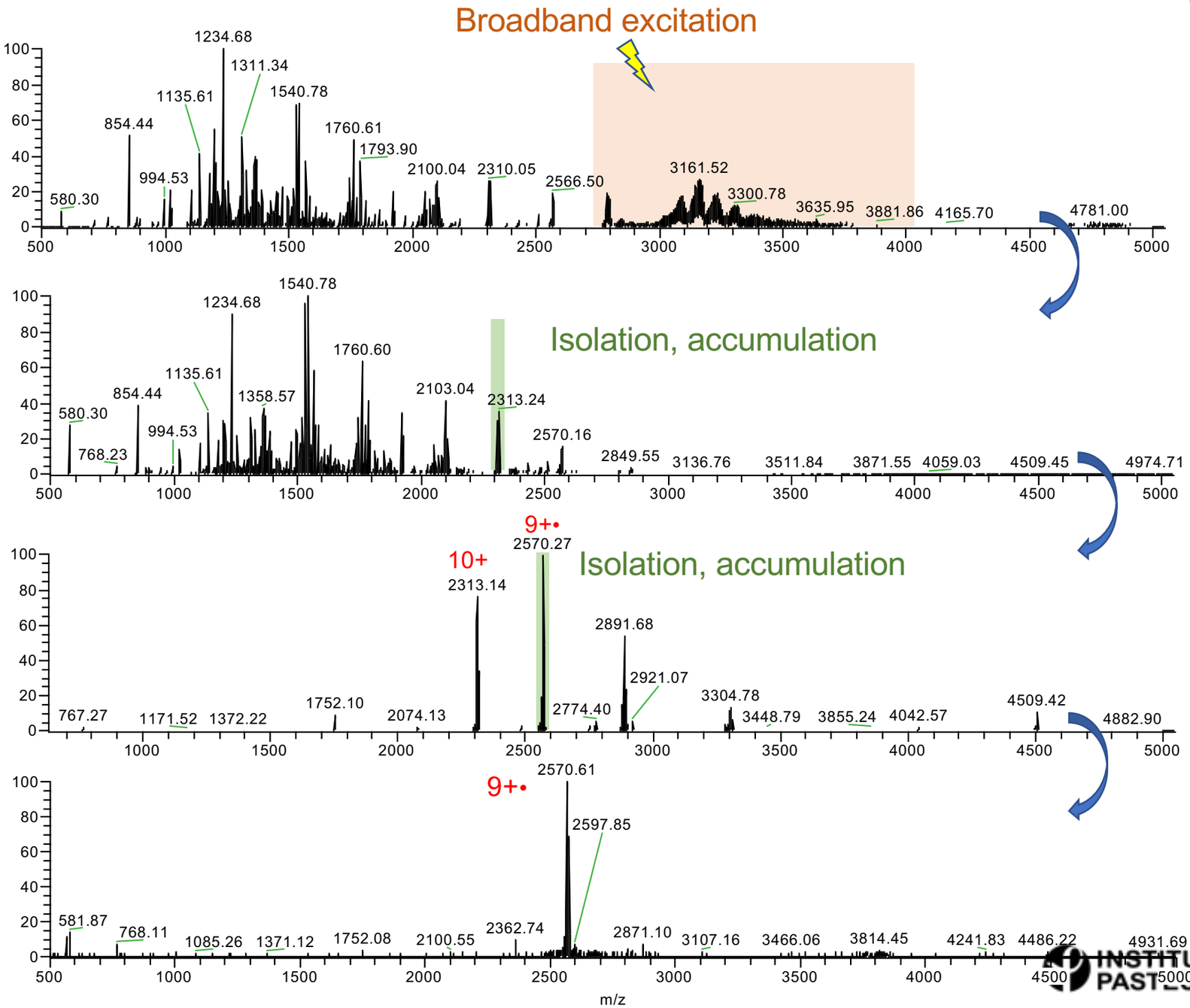
HF-Omnitrap

MS2: CID of *m/z* 2797 (53+)

MS2: CID + broadband excitation

MS3: ECD of light chain 10+

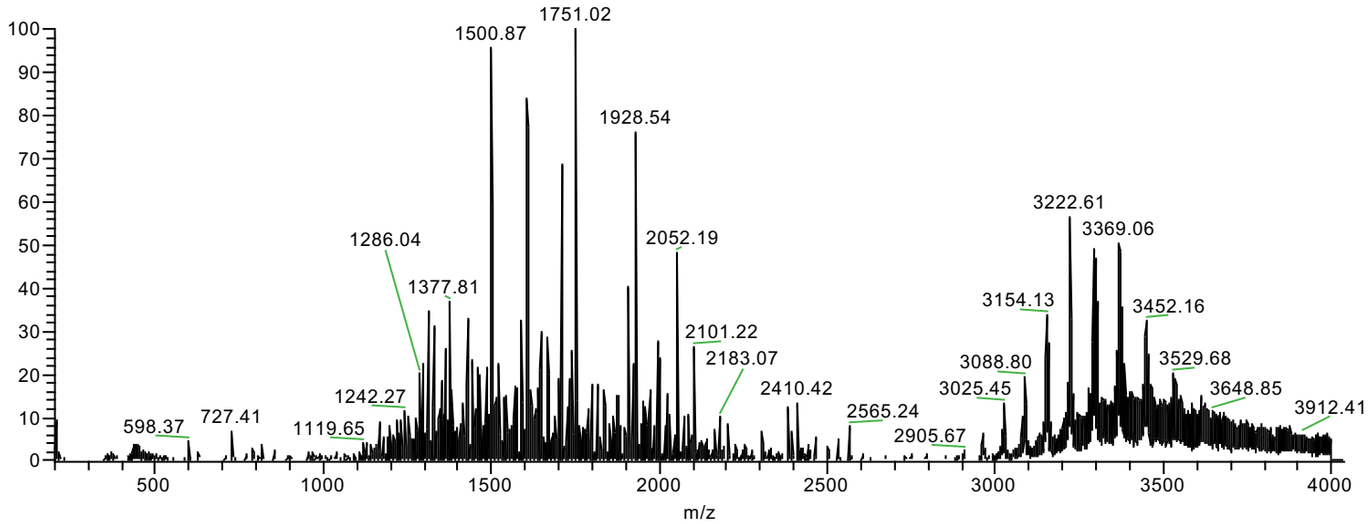
MS4: …



Lumos ETD 15 ms

HF-Omnitrap

m/z 2797, 53+, isolation window: 2 *m/z*



Residue cleavage: 9%

```
N  D I Q M T Q S P S T L S A S V G D R V T I T C S A  25
26 S S R V G Y M H W Y Q Q K P G K A P K L L I Y D T  50
51 S K L A S G V P S R F S G S G S G T E F T L T I S  75
76 S L Q P D D F A T Y Y C F Q G S G Y P F T F G G G  100
101 T K V E I K R T V A A P S V F I F P P S D E Q L K  125
126 S G T A S V V C L L N N F Y P R E A K V Q W K V D  150
151 N A L Q S G N S Q E S V T E Q D S K D S T Y S L S  175
176 S T L T L S K A D Y E K H K V Y A C E V T H Q G L  200
201 S S P V T K S F N R G E C C
```

Residue cleavage: 10%

```
N  Q V T L R E S G P A L V K P T Q T L T L T C T F S  25
26 G F S L S T A G M S V G W I R Q P P G K A L E W L  50
51 A D I W W D D K K H Y N P S L K D R L T I S K D T  75
76 S K N Q V V L K V T N M D P A D T A T Y Y C A R D  100
101 M I F N F Y F D V W G Q G T T V T V S S A S T K G  125
126 P S V F P L A P S S K S T S G G T A A L G C L V K  150
151 D Y F P E P V T V S W N S G A L T S G V H T F P A  175
176 V L Q S S G L Y S L S S V V T V P S S S L G T Q T  200
201 Y I C N V N H K P S N T K V D K R V E P K S C D K  225
226 T H T C P P C P A P E L L G G P S V F L F P P K P  250
251 K D T L M I S R T P E V T C V V V D V S H E D P E  275
276 V K F N W Y V D G V E V H N A K T K P R E E Q Y N  300
301 S T Y R V V S V L T V L H Q D W L N G K E Y K C K  325
326 V S N K A L L P A P I E K T I S K A K G Q P R E P Q  350
351 V Y T L P P S R E E M T K N Q V S L T C L V K G F  375
376 Y P S D I A V E W E S N G Q P E N N Y K T T P P V  400
401 L D S D G S F F L Y S K L T V D K S R W Q Q G N V  425
426 F S C S V M H E A L H N H Y T Q K S L S L S P G C
```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Conclusions/future directions

- Easy and smooth installation (3 days), very stable system (filament changed only once, no other major cleaning), but manual operation (4-5 LC-MS/MS experiments /day)
- Very promising data both on light chain (LC time scale) and mAb analysis
- Added value of booster for data acquisition
- Data analysis very long and difficult (improved with Cronus but still requires more automation)

- **To be done (by Dec 2022?)**
  - Analyse all acquired data and **acquire new ones on intact mAbs (infusion) with the booster (C. Garcia)**
  - Install and test the PI trap
  - Install VUV on the omnitrap
  - Write the paper on the P15 light chain (> ASMS abstracts)

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