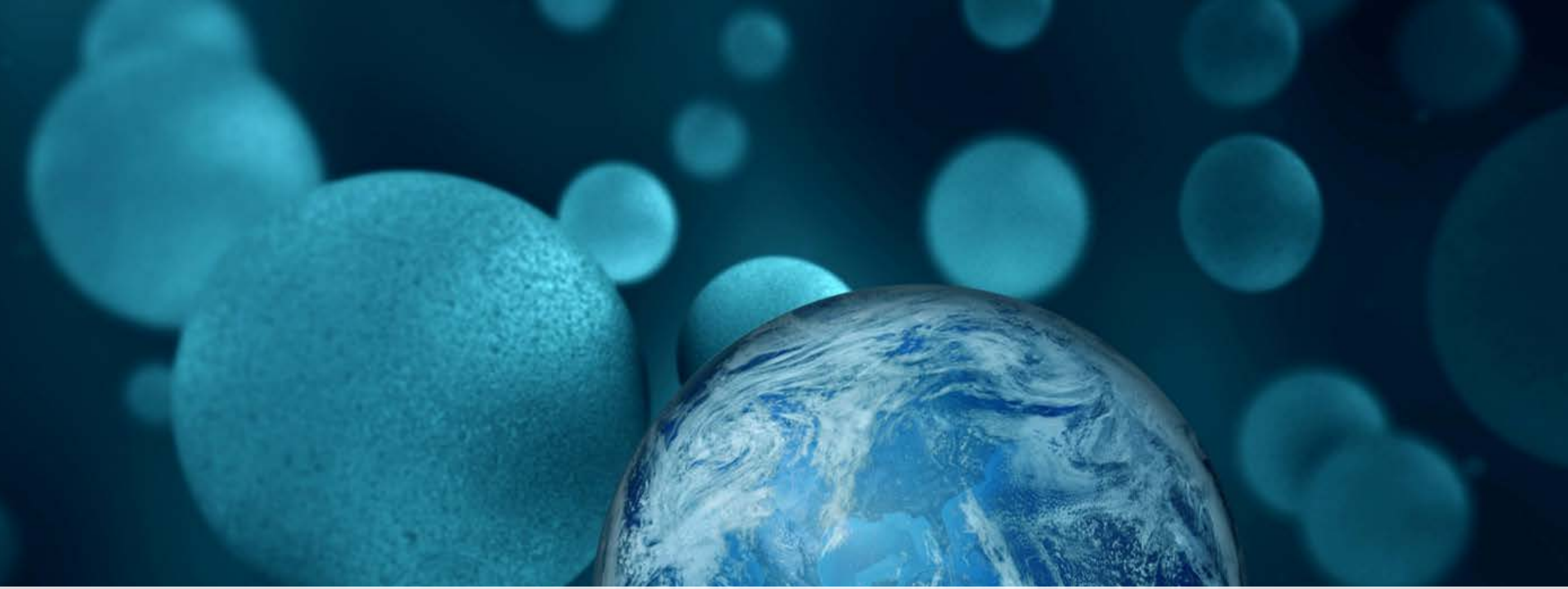
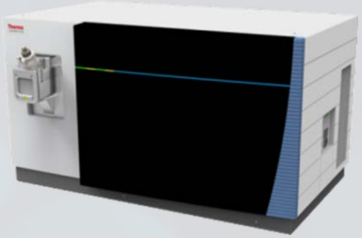


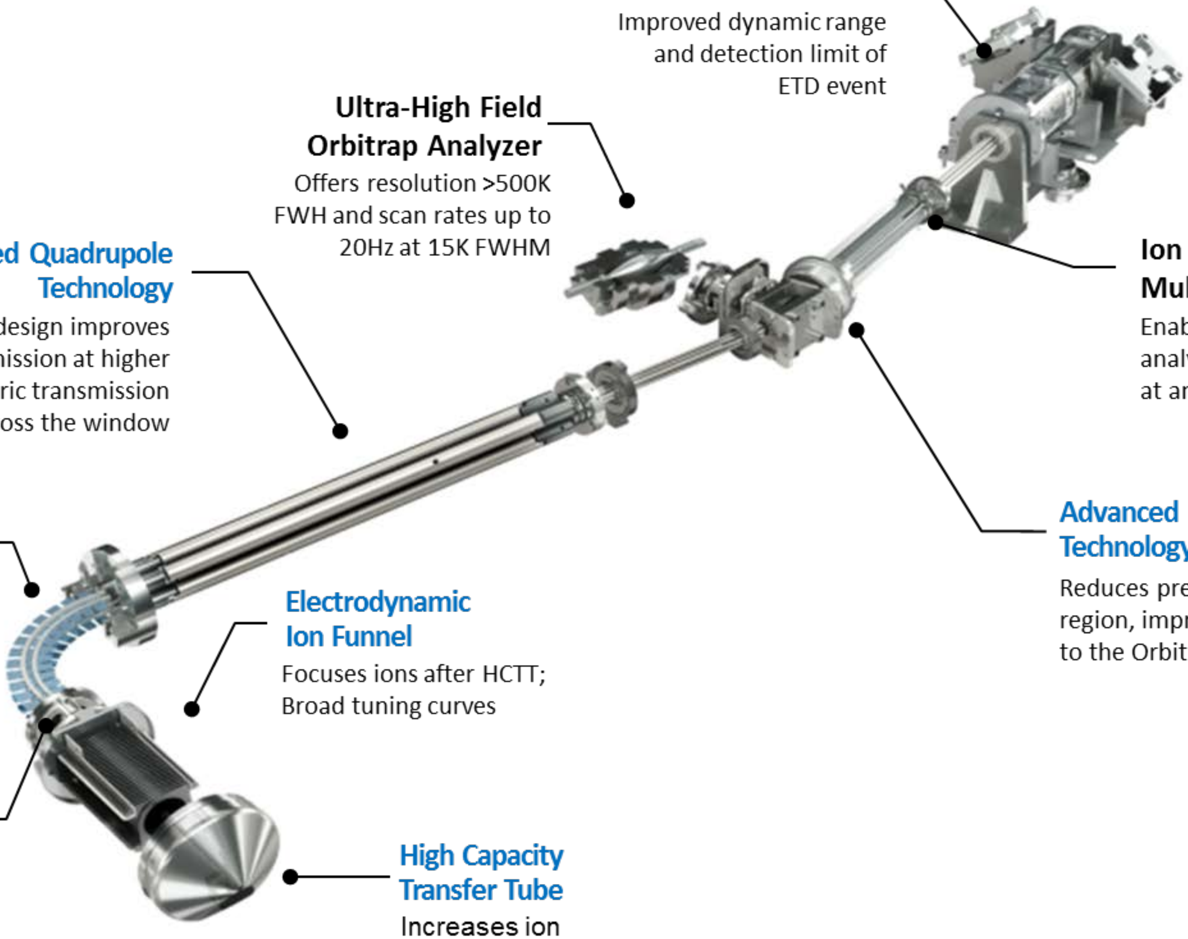
ThermoFisher
SCIENTIFIC

Intact and Top-Down Protein Analysis Guide: Orbitrap Fusion Lumos



Seema Sharma
October 2016

Orbitrap Fusion Lumos



ETD HD
Improved dynamic range and detection limit of ETD event

Dual-Pressure Linear Ion Trap
MS^n and sensitive mass analysis of four fragmentation types: CID, HCD, ETD and EThcD

Ultra-High Field Orbitrap Analyzer
Offers resolution >500K FWH and scan rates up to 20Hz at 15K FWHM

Advanced Quadrupole Technology
Segmented design improves transmission at higher resolution; symmetric transmission across the window

Ion Routing Multipole
Enables parallel analysis; performs HCD at any MS^n stage

Advanced Vacuum Technology
Reduces pressure in UHV region, improving transmission to the Orbitrap analyzer

Advanced Active Ion Beam Guide
Prevents neutrals and high velocity clusters from entering mass resolving quadrupole

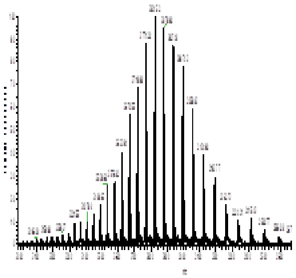
Electrodynamic Ion Funnel
Focuses ions after HCTT; Broad tuning curves

EASY ETD Source
Based on Townsend discharge; reliable and easy to use

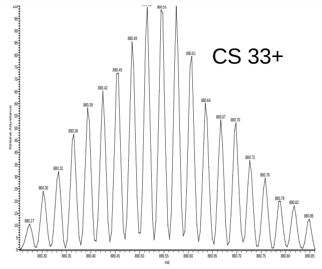
High Capacity Transfer Tube
Increases ion flux into the mass spectrometer

Application Dependency: Pressure & Resolution

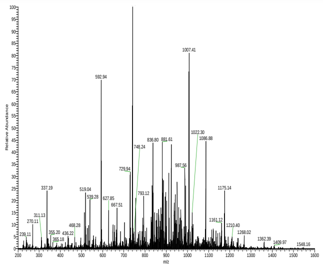
MS Protein >50 kDa	MS Protein <40kDa	MS/MS	MS Protein 40-50 kDa
Standard Pressure Mode	**Intact Protein Mode** **IRM @ 2mtorr**	**Intact Protein Mode** **IRM @ 2mtorr**	**Intact Protein Mode** **IRM @ 1mtorr**
R @ 15000	**R @ 120000**	**R @ 120000**	**R @ 240000**



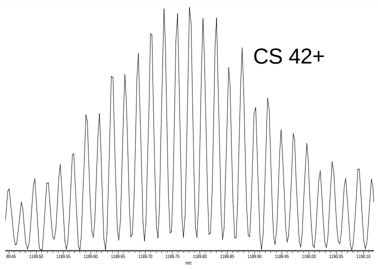
Intact mAb Charge States



Carbonic Anhydrase
Isotopic Resolution

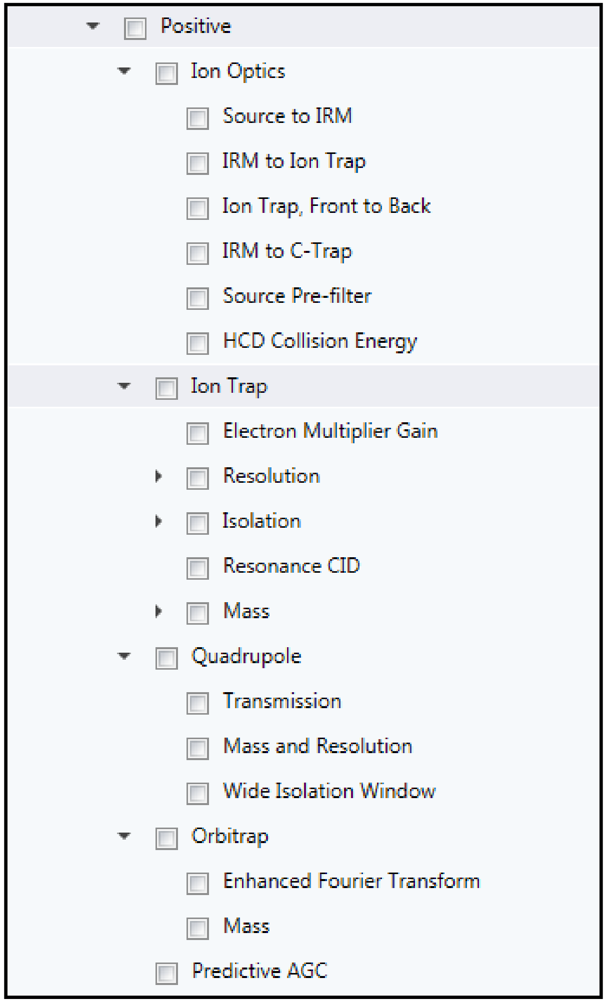


Carbonic Anhydrase
HCD MS/MS



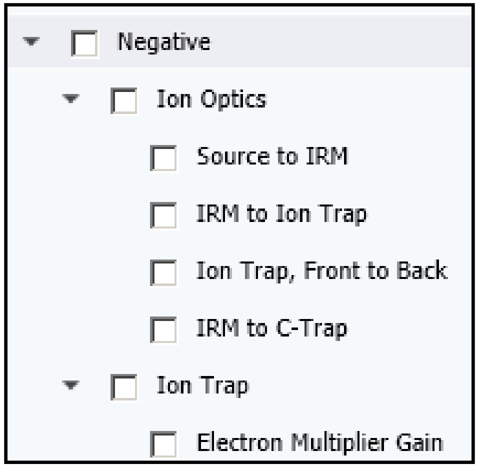
Reduced mAb Heavy Chain
Isotopic Resolution

General Calibrations

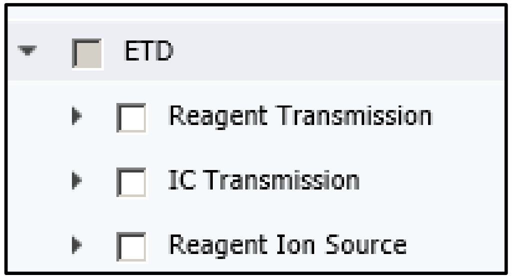


- ☐ Positive
  - ☐ Ion Optics
    - ☐ Source to IRM
    - ☐ IRM to Ion Trap
    - ☐ Ion Trap, Front to Back
    - ☐ IRM to C-Trap
    - ☐ Source Pre-filter
    - ☐ HCD Collision Energy
  - ☐ Ion Trap
    - ☐ Electron Multiplier Gain
    - ☐ Resolution
    - ☐ Isolation
    - ☐ Resonance CID
    - ☐ Mass
  - ☐ Quadrupole
    - ☐ Transmission
    - ☐ Mass and Resolution
    - ☐ Wide Isolation Window
  - ☐ Orbitrap
    - ☐ Enhanced Fourier Transform
    - ☐ Mass
  - ☐ Predictive AGC

- Check all positive calibrations before proceeding to extended positive calibrations.
- Check Negative mode Ion optics & Ion Trap electron multiplier gain calibrations prior to ETD calibration.



- ☐ Negative
  - ☐ Ion Optics
    - ☐ Source to IRM
    - ☐ IRM to Ion Trap
    - ☐ Ion Trap, Front to Back
    - ☐ IRM to C-Trap
  - ☐ Ion Trap
    - ☐ Electron Multiplier Gain



- ☐ ETD
  - ☐ Reagent Transmission
  - ☐ IC Transmission
  - ☐ Reagent Ion Source

Extended Calibrations

MS
Protein
>50 kDa

<u>Normal Mass Range</u>
No Extended
Calibrations Required

<u>High Mass Range</u>
Positive Extended:
High Mass Range
CalMix & Enfuvirtide

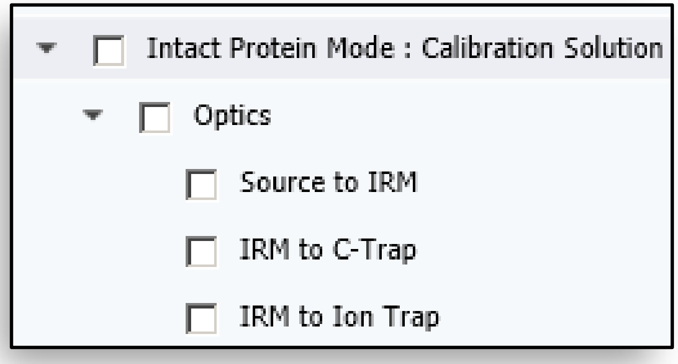
☐ High Mass Range : Calibration Solution

☐ High Mass Range : Enfuvirtide

Extended Calibrations Contd.

MS
Protein
<40 kDa

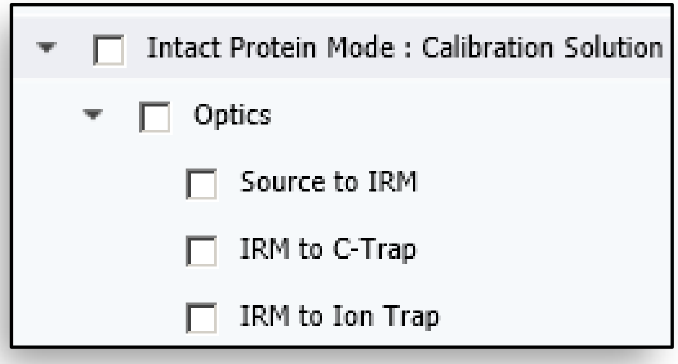
Set IRM Pressure @ 2mtorr
Positive Extended: Intact Protein Mode



☐ Intact Protein Mode : Calibration Solution
- ☐ Optics
  - ☐ Source to IRM
  - ☐ IRM to C-Trap
  - ☐ IRM to Ion Trap

MS
Protein
40-50 kDa

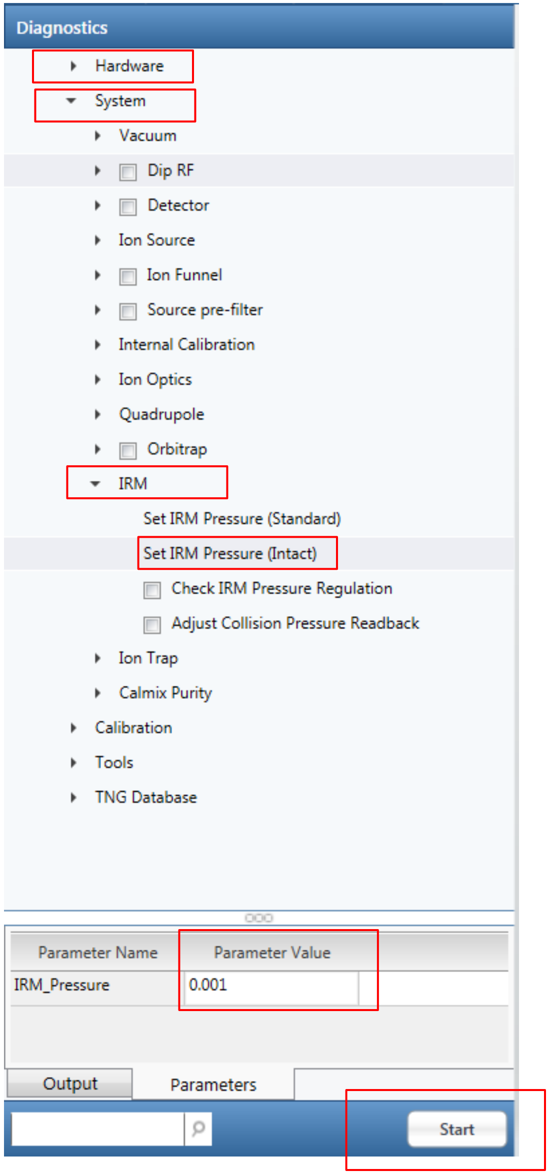
Set IRM Pressure @ 1mtorr
Positive Extended: Intact Protein Mode



☐ Intact Protein Mode : Calibration Solution
- ☐ Optics
  - ☐ Source to IRM
  - ☐ IRM to C-Trap
  - ☐ IRM to Ion Trap

IPM Pressure Setting

- IPM pressure can be changed in diagnostics, by changing the parameter value under ‘Set IRM pressure (Intact)’ and then click on Start.
- Hardware→System→IRM→ Set IRM Pressure (Intact)→ ‘IRM_Pressure’ Parameter Value
- Note: The instrument can only be calibrated for one ‘IRM Pressure (Intact)’ at a given time.
- The intact protein mode calibration (optics) needs to be run at the desired IRM pressure (Intact).



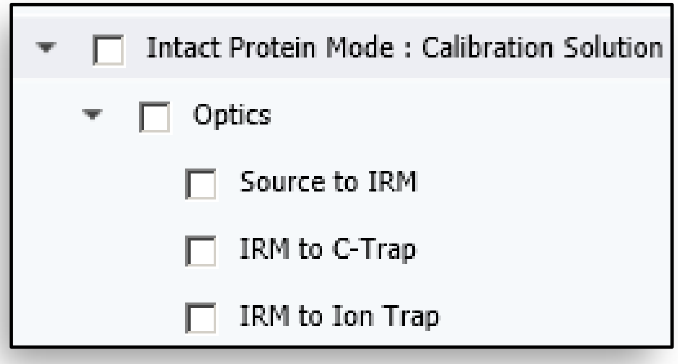
Parameter Name	Parameter Value
IRM_Pressure	0.001

Extended Calibrations Contd.

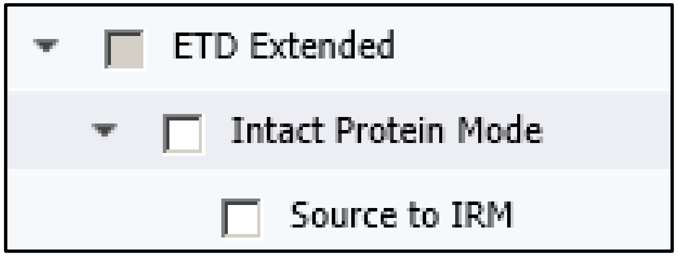
MS/MS

Set IRM Pressure @ 2mtorr

Positive Extended:
Intact Protein Mode
&
ETD Extended:
Intact Protein Mode



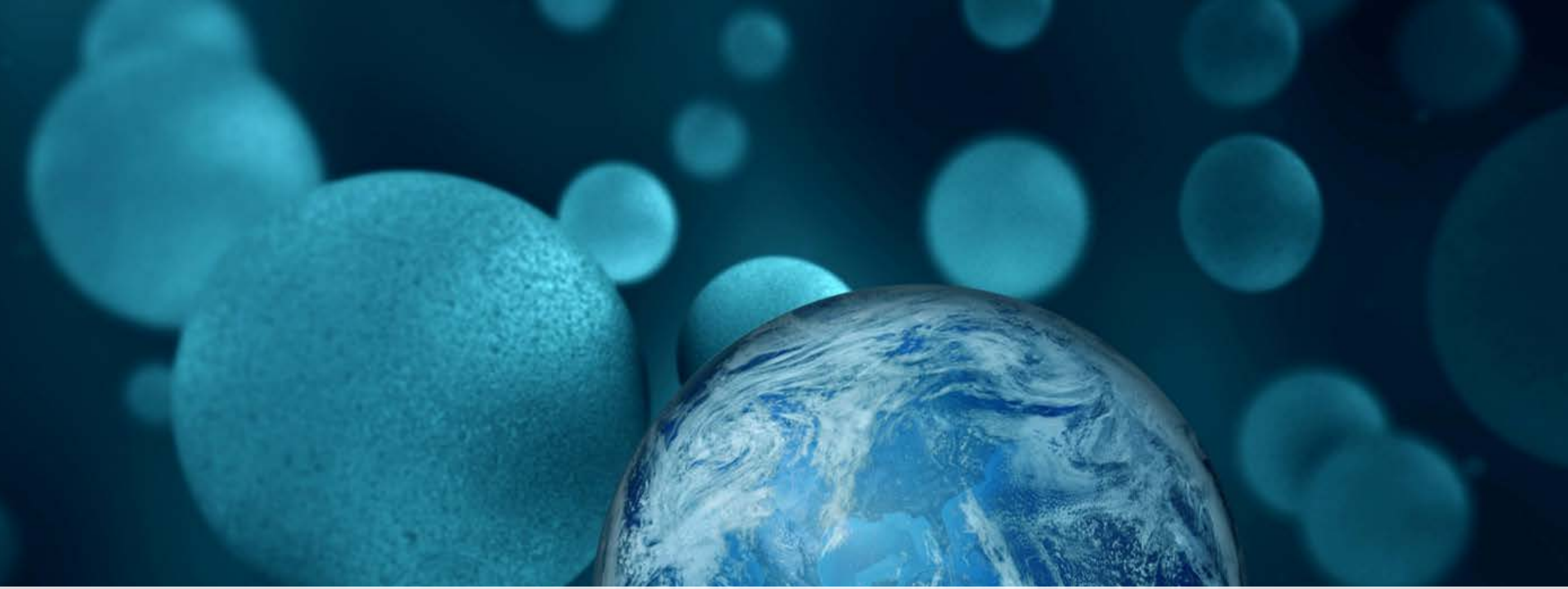
- ☐ Intact Protein Mode : Calibration Solution
  - ☐ Optics
    - ☐ Source to IRM
    - ☐ IRM to C-Trap
    - ☐ IRM to Ion Trap



- ☐ ETD Extended
  - ☐ Intact Protein Mode
    - ☐ Source to IRM

FT Signal: Averaged Transients vs. Averaged Scans

- To improve spectra S/N use either microscans (each is a single transient) or Averager in Tune. S/N improves as square root of number of transients averaged.
- When spectra are averaged in Qual browser, both signal and noise are averaged, the gain in S/N is somewhat lower.
- Whenever possible try to average as many transients as possible to improve spectral S/N.
- Averager updates the scan with every transient added.
- Microscans update the scan only when all transients are added.
- The end result is exactly the same whether you use *n* microscans or average *n* transients through Averager. The only difference is in the scan update frequency.
- Averager uses rolling average routine.
- When using microscans (*n*) the scan appear after averaging the last *n* transients.



Thermo Fisher
SCIENTIFIC

Direct Infusion: Carbonic Anhydrase

The world leader in serving science

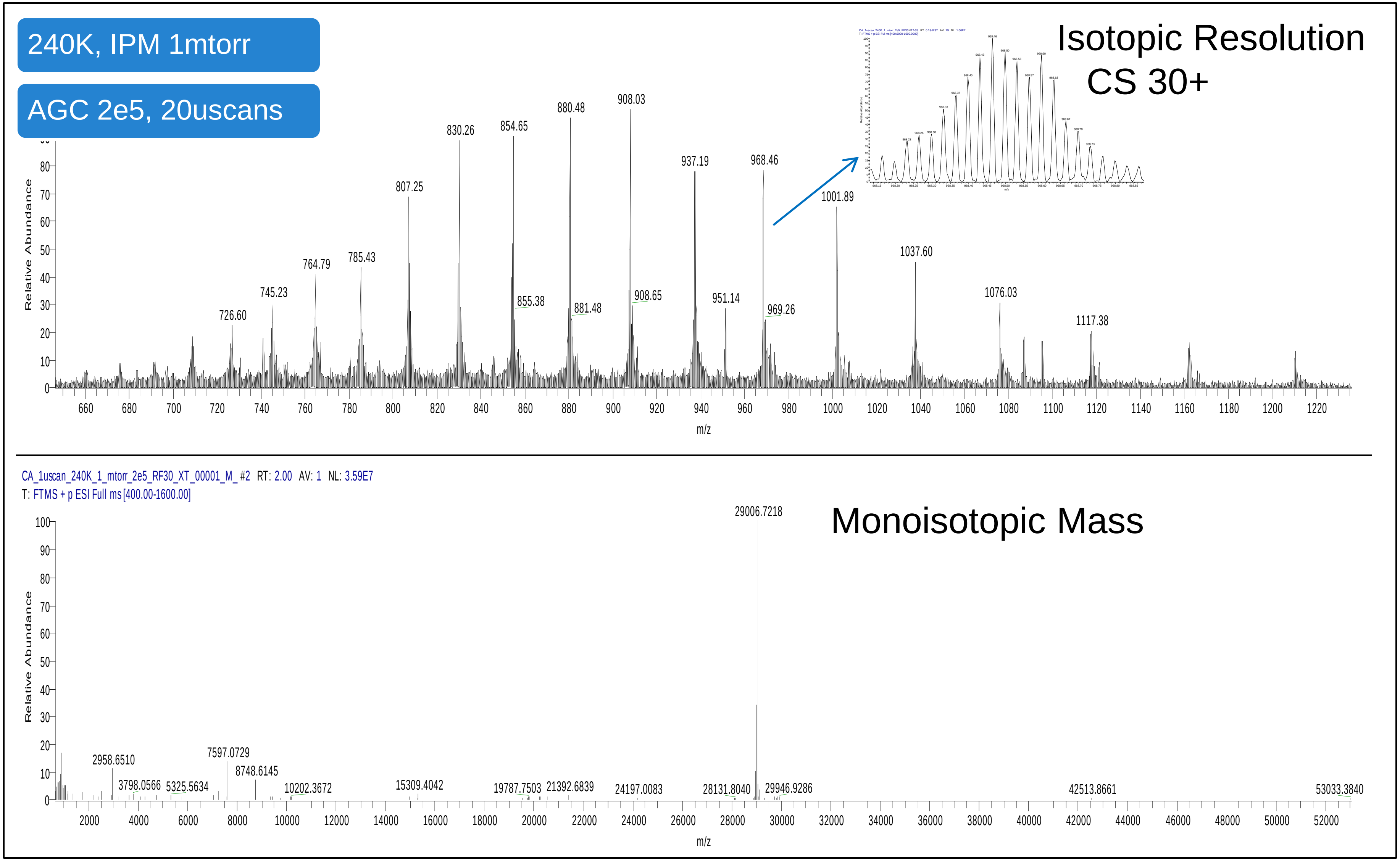
Carbonic Anhydrase (29 kDa)

- P/N C2522, Sigma
- Direct Infusion: Flow rate 3ul/min
- 1-5 pmol/ul in 50:50 AcN: H_2O, 0.1% FA
- HESI probe (same source conditions as for calmix)

N-terminal acetylation

(42.0106) SHHWGYGKH NGPEHWHKDF PIANGERQSP
VDIDTKAVVQ DPALKPLALV YGEATSRRMV NNGHSFNVEY
DDSQDKAVLK DGPLTGTYRL VQFHFHWGSS DDQGSEHTVD
RKKYAAELHLVHWNTKYGDF GTAAQQPDGL AVVGVFLKVG
DANPALQKVL DALDSIKTKG KSTDFPNFDPGSLLPNVLDY
WTYPGSLTTP PLLESVTWIV LKEPISVSSQ QMLKFRTLNF
NAEGEPELLM LANWRPAQPL KNRQVRGFPK

Carbonic Anhydrase Full MS



240K, IPM 1mtorr

AGC 2e5, 20uscans

Isotopic Resolution
CS 30+

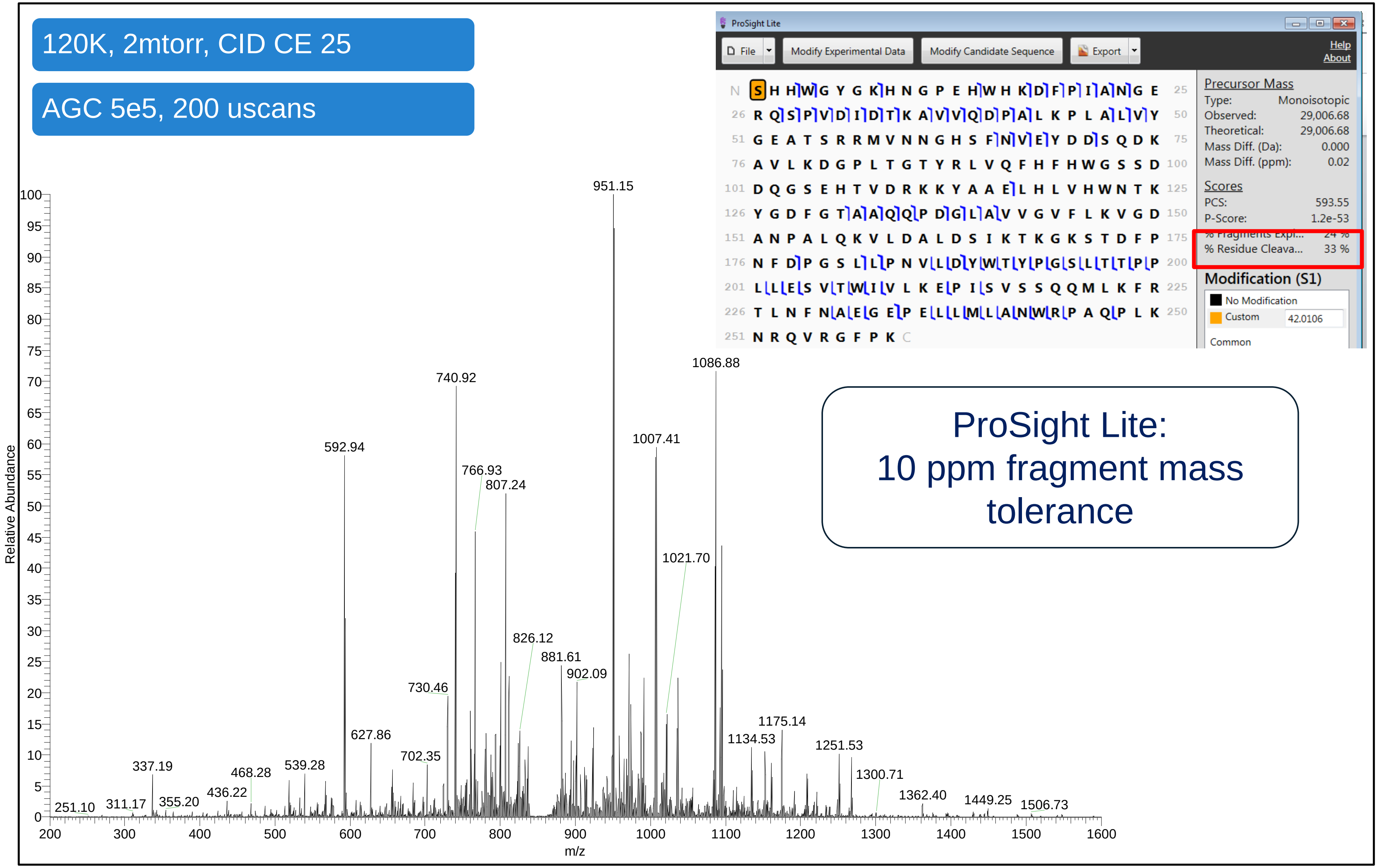
Monoisotopic Mass

CA_1uscan_240K_1_mtorr_2e5_RF30_XT_00001_M_ #2 RT: 2.00 AV: 1 NL: 3.59E7
T: FTMS + p ESI Full ms [400.00-1600.00]

Carbonic Anhydrase MS/MS CID

120K, 2mtorr, CID CE 25

AGC 5e5, 200 uscans



N SHHWGYGKHNGPEHWHKDFPIANGE 25
26 RQSPVDIDTKAVVQDPALKPLALVY 50
51 GEATSRRMVNNGHSFNVEYDDSQDK 75
76 AVLKDGPLTGTYRLVQFHFHWGSSD 100
101 DQGSEHTVDRKKYAAELHLVHWNTK 125
126 YGDFGTAAQQPDGLAVVGVFLKVGD 150
151 ANPALQKVLDALDSIKTKGKSTDFP 175
176 NFDPGSLLPNVLDYWTYPGSLTTPP 200
201 LLESVTWIVLKEPISVSSQQMLKFR 225
226 TLNFNAEGEPELLMLANWRPAQPLK 250
251 NRQVRGFPK C

Precursor Mass

Type:	Monoisotopic
Observed:	29,006.68
Theoretical:	29,006.68
Mass Diff. (Da):	0.000
Mass Diff. (ppm):	0.02

Scores

PCS:	593.55
P-Score:	1.2e-53
% Fragments Expl...	24 %
% Residue Cleava...	33 %

Modification (S1)

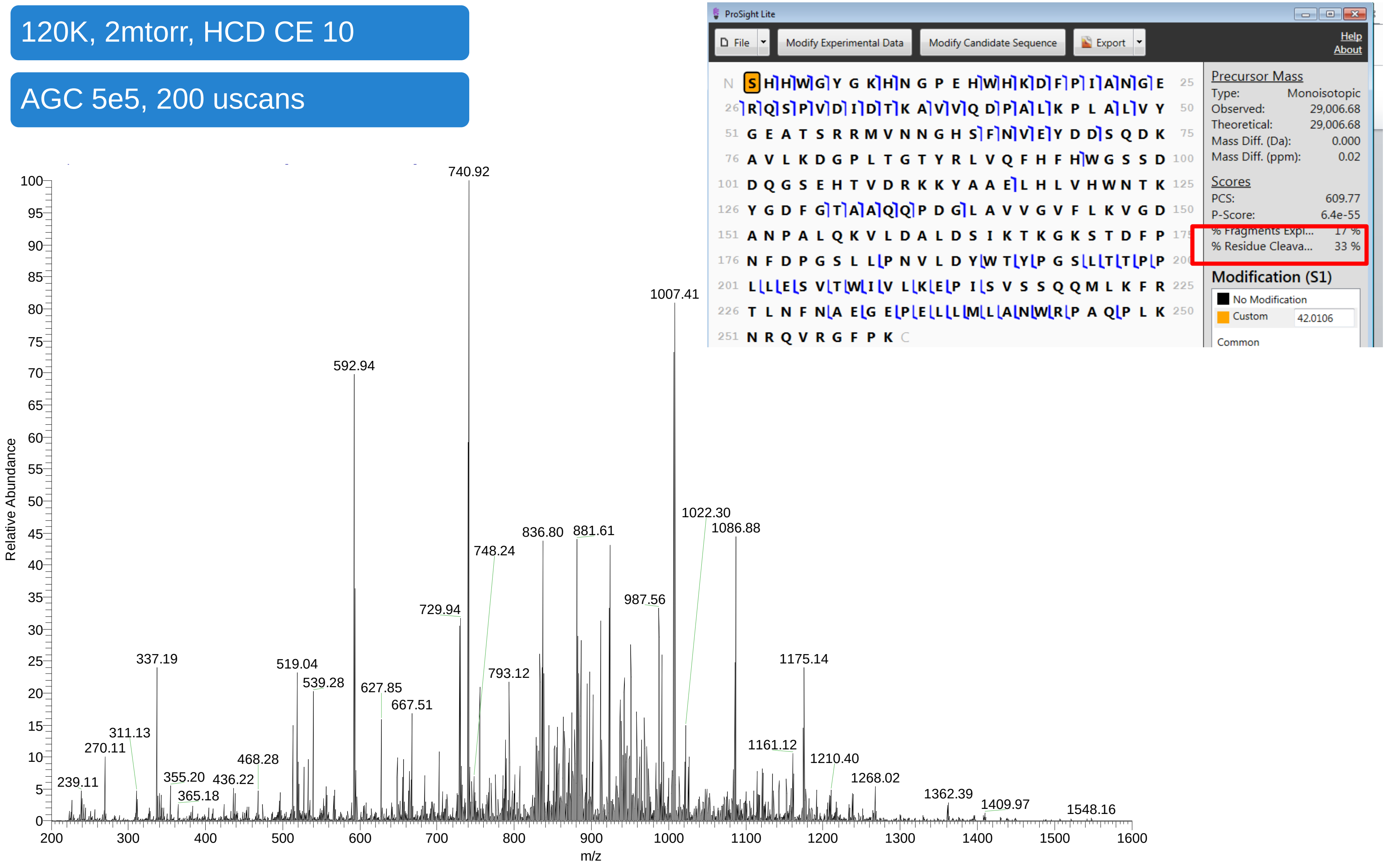
■ No Modification
🟧 Custom 42.0106
Common

ProSight Lite:
10 ppm fragment mass tolerance

Carbonic Anhydrase MS/MS HCD

120K, 2mtorr, HCD CE 10

AGC 5e5, 200 uscans



```
N   S H H W G Y G K H N G P E H W H K D F P I A N G E    25
26  R Q S P V D I D T K A V V Q D P A L K P L A L V Y    50
51  G E A T S R R M V N N G H S F N V E Y D D S Q D K    75
76  A V L K D G P L T G T Y R L V Q F H F H W G S S D   100
101 D Q G S E H T V D R K K Y A A E L H L V H W N T K   125
126 Y G D F G T A A Q Q P D G L A V V G V F L K V G D   150
151 A N P A L Q K V L D A L D S I K T K G K S T D F P   175
176 N F D P G S L L P N V L D Y W T Y P G S L T T P P   200
201 L L E S V T W I V L K E P I S V S S Q Q M L K F R   225
226 T L N F N A E G E P E L L M L A N W R P A Q P L K   250
251 N R Q V R G F P K C
```

Precursor Mass

Type: Monoisotopic
Observed: 29,006.68
Theoretical: 29,006.68
Mass Diff. (Da): 0.000
Mass Diff. (ppm): 0.02

Scores

PCS: 609.77
P-Score: 6.4e-55
% Fragments Expl... 17 %
% Residue Cleava... 33 %

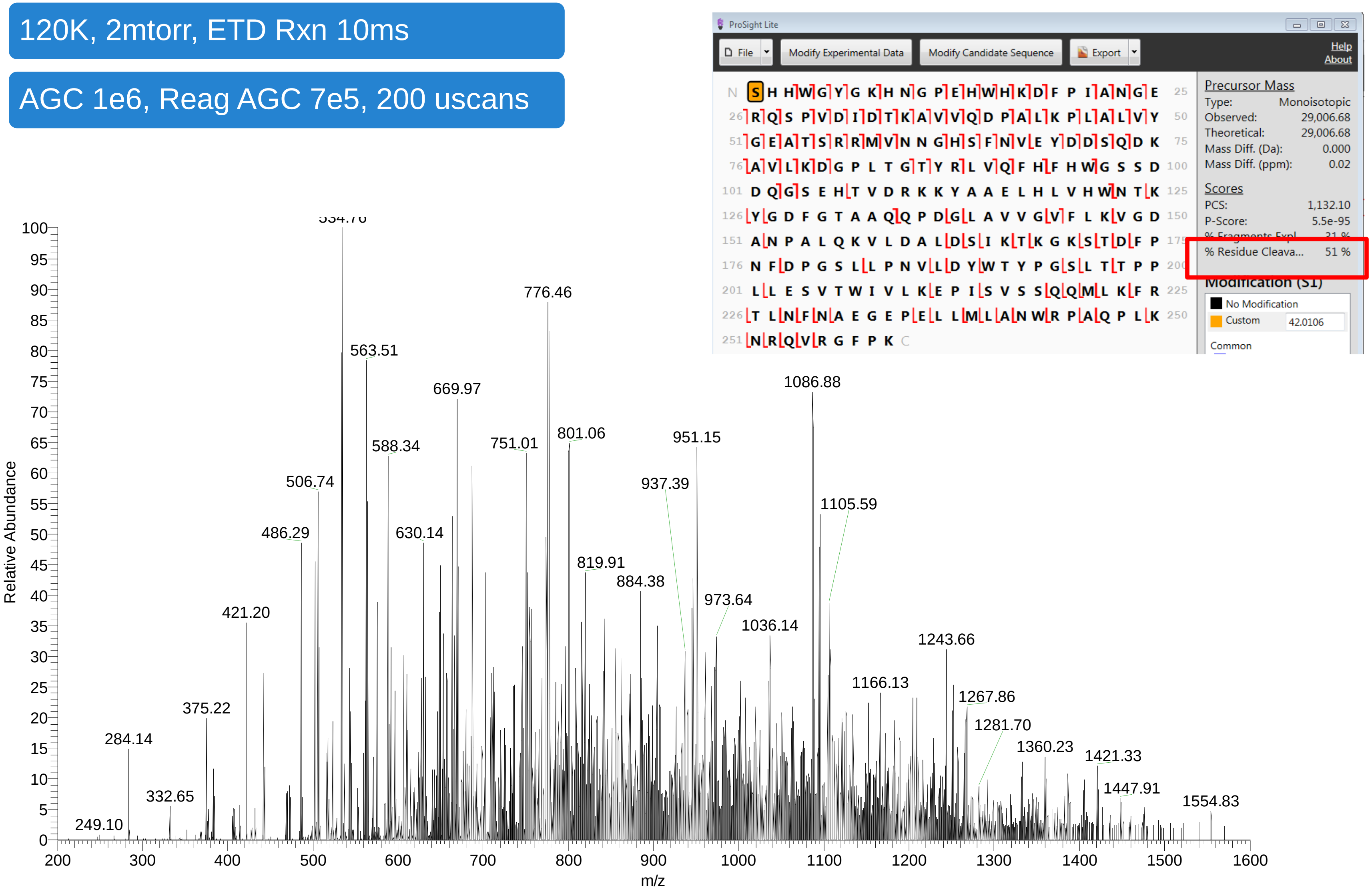
Modification (S1)

■ No Modification
🟧 Custom 42.0106
Common

Carbonic Anhydrase ETD HD

120K, 2mtorr, ETD Rxn 10ms

AGC 1e6, Reag AGC 7e5, 200 uscans



N S H H W G Y G K H N G P E H W H K D F P I A N G E 25
26 R Q S P V D I D T K A V V Q D P A L K P L A L V Y 50
51 G E A T S R R M V N N G H S F N V E Y D D S Q D K 75
76 A V L K D G P L T G T Y R L V Q F H F H W G S S D 100
101 D Q G S E H T V D R K K Y A A E L H L V H W N T K 125
126 Y G D F G T A A Q Q P D G L A V V G V F L K V G D 150
151 A N P A L Q K V L D A L D S I K T K G K S T D F P 175
176 N F D P G S L L P N V L D Y W T Y P G S L T T P P 200
201 L L E S V T W I V L K E P I S V S S Q Q M L K F R 225
226 T L N F N A E G E P E L L M L A N W R P A Q P L K 250
251 N R Q V R G F P K C

Precursor Mass

Type:	Monoisotopic
Observed:	29,006.68
Theoretical:	29,006.68
Mass Diff. (Da):	0.000
Mass Diff. (ppm):	0.02

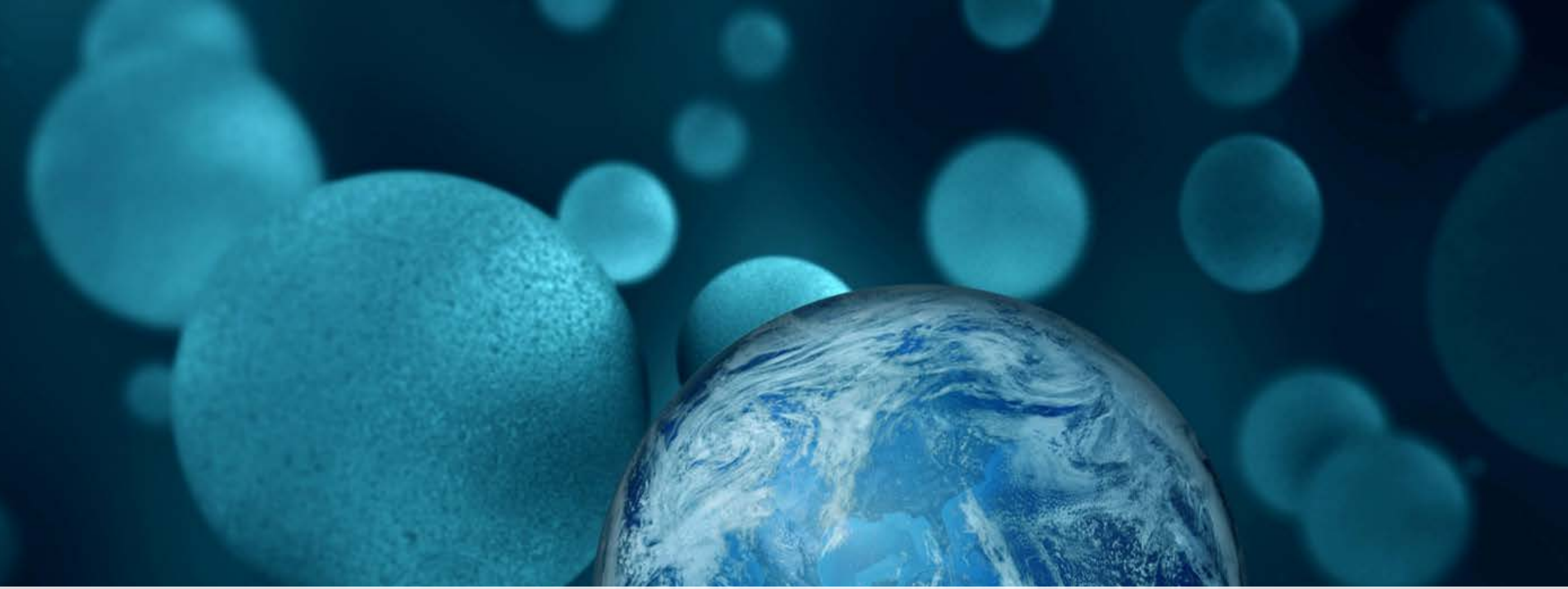
Scores

PCS:	1,132.10
P-Score:	5.5e-95
% Fragments Expl...	31 %
% Residue Cleava...	51 %

Modification (S1)

■ No Modification
🟧 Custom 42.0106

Common



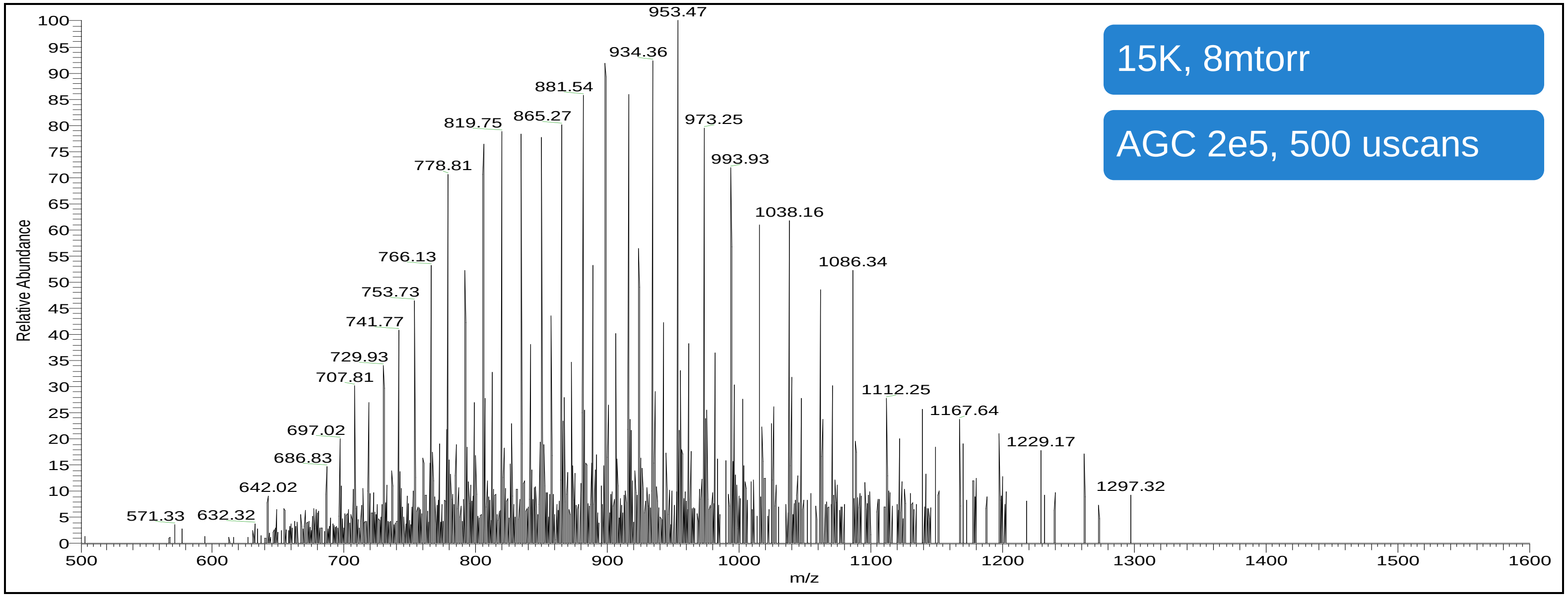
Direct Infusion: Enolase

Enolase (47 kDa)

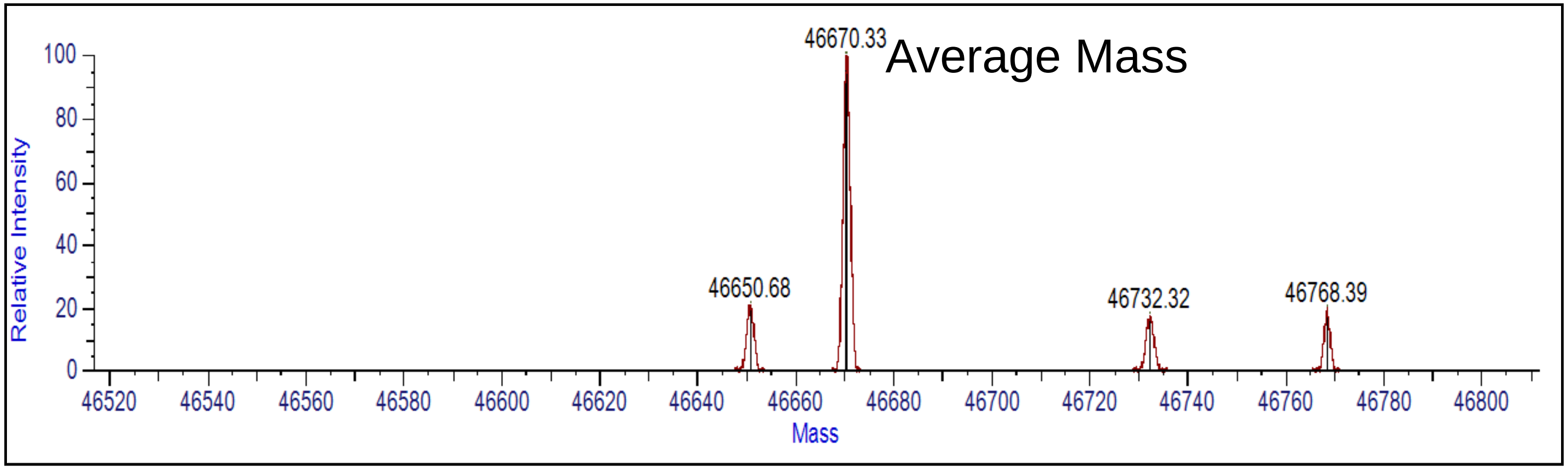
- P/N E6126, Sigma
- Direct Infusion: Flow rate 3ul/min
- Clean-up using Bio-Rad Micro spin columns (follow manufacturer’s instructions)
- 10 -20 pmol/ul in 50:50 AcN: H_2O, 0.1% FA

AVSKVYARSVYDSRGNPTVEVELTTEKGVFRSIVPSGASTGVHEALEMRDGDKSKWMGKG
VLHAVKNVNDVIAPAFVKANIDVKDQKAVDDFLISLDGTANKSKLGANAILGVSLAASRAAAAE
KNVPLYKHLADLSKSKTSPYVLPVPFLNVLNGGSHAGGALALQEFMIAPTGAKTFAEALRIGS
EVYHNLKSLTKKRYGASAGNVGDEGGVAPNIQTAEEALDLIVDAIKAAGHDGKVKIGLDCASS
EFFKDGKYDLDFKNPNSDKSKWLTGPQLADLYHSLMKRYPIVSIEDPFAEDDWEAWSHFFKT
AGIQIVADDLTVTNPKRIATAIEKKAADALLLKVNQIGTLSESIKAAQDSFAAGWGVMVSHRSG
ETEDTFIADLVVGLRTGQIKTGAPARSERLAKLNQLLRIEEELGDNAVFAGENFHHGDKL

Enolase Full MS


15K, 8mtorr

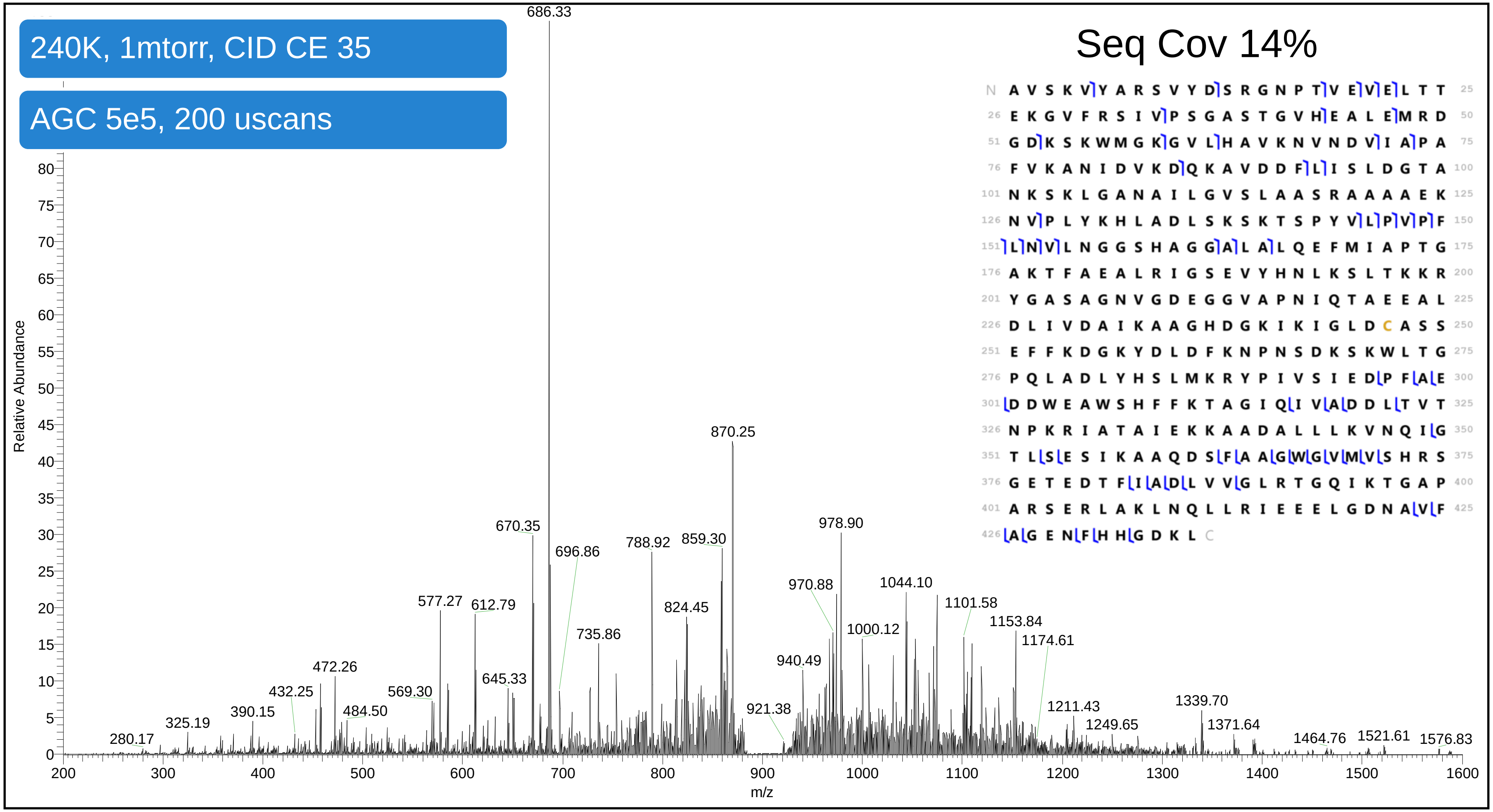
AGC 2e5, 500 uscans


Average Mass

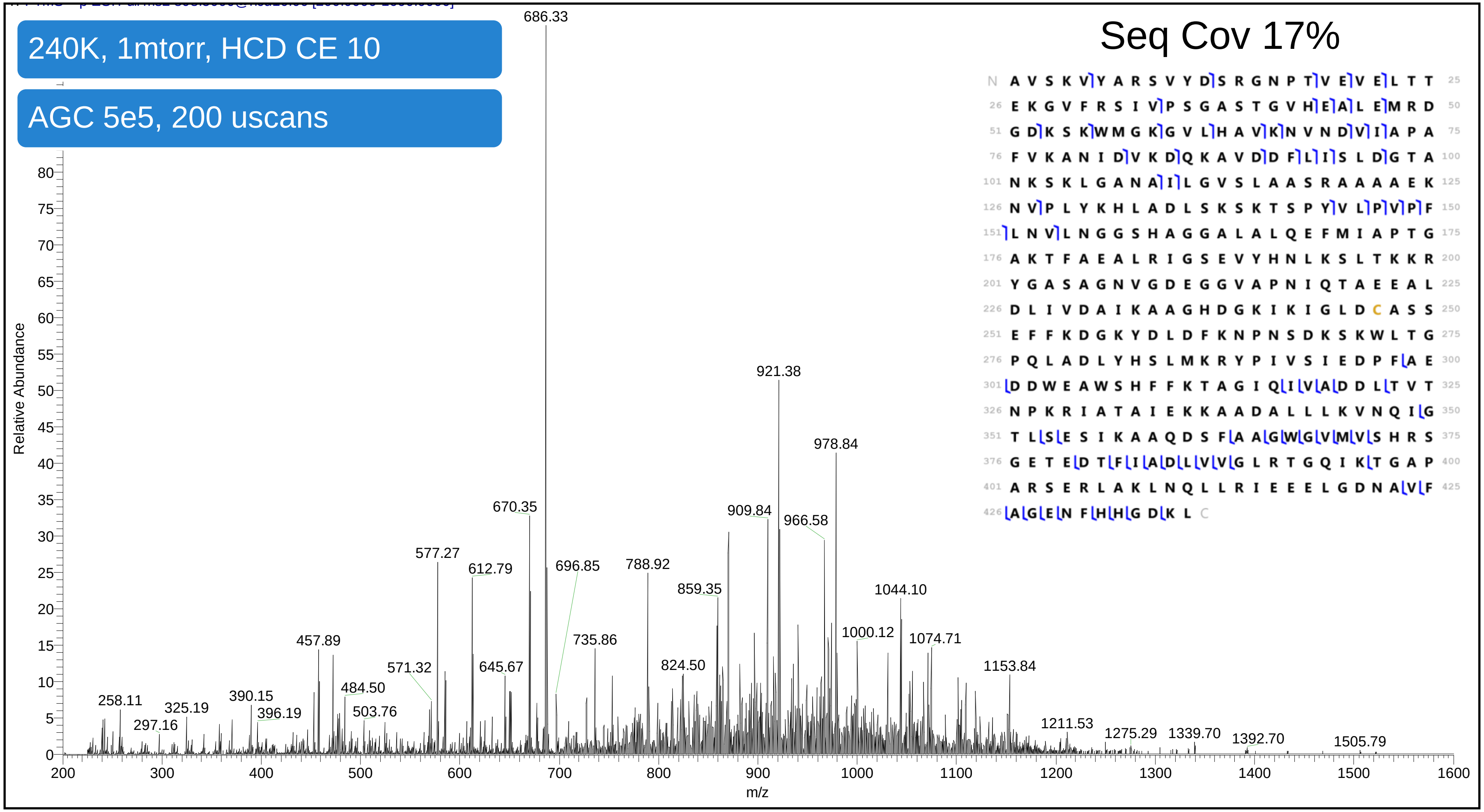
Enolase 47 kDa, MS/MS CID

240K, 1mtorr, CID CE 35

AGC 5e5, 200 uscans



Enolase MS/MS HCD

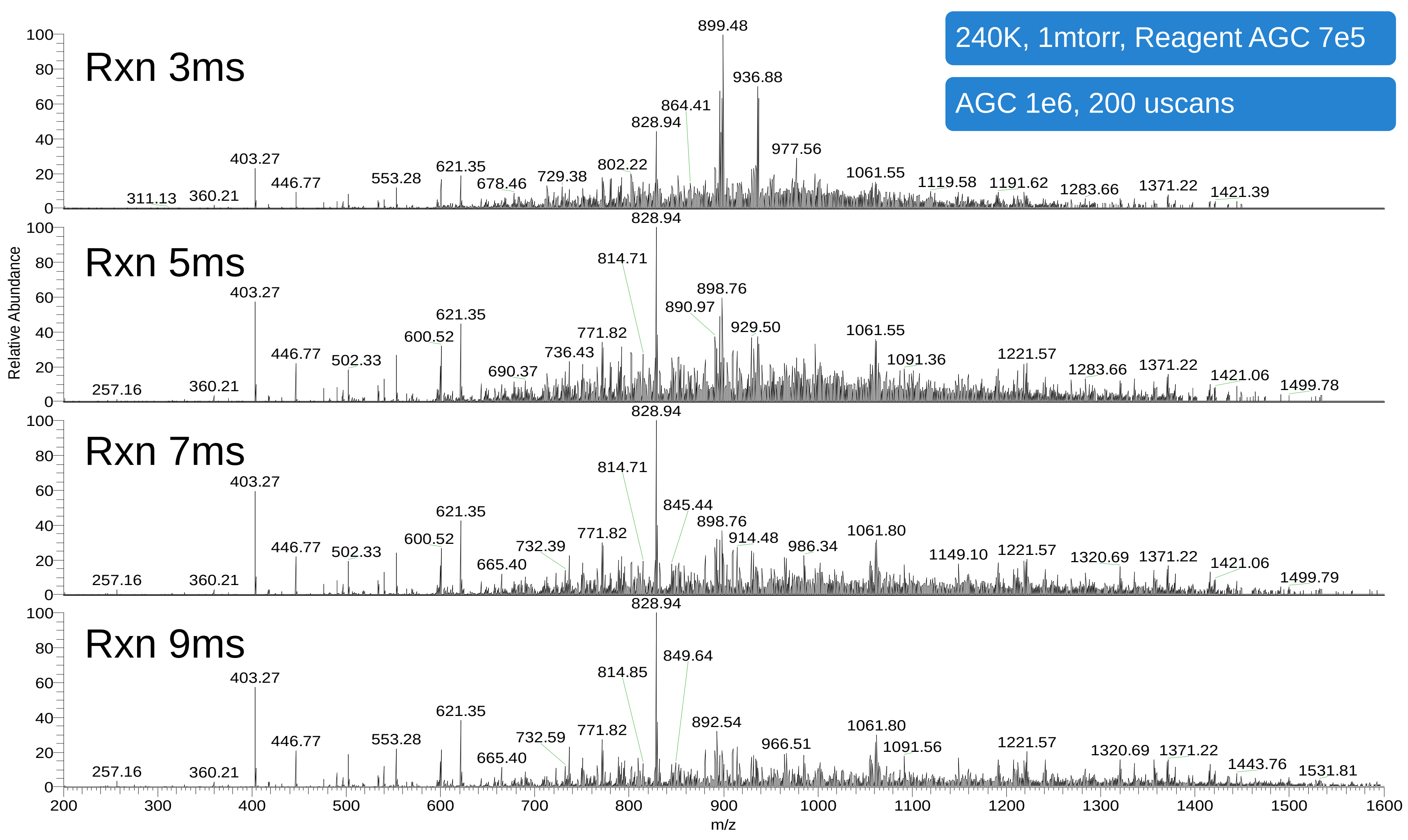

240K, 1mtorr, HCD CE 10

AGC 5e5, 200 uscans

Seq Cov 17%

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

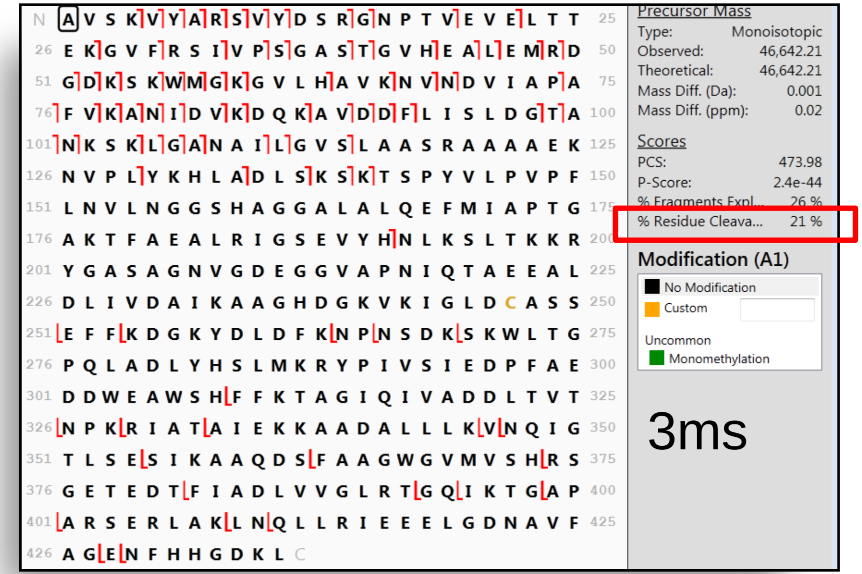
Enolase ETD HD CS 52+



240K, 1mtorr, Reagent AGC 7e5

AGC 1e6, 200 uscans

Enolase CS 52+, ETD HD Sequence Coverage



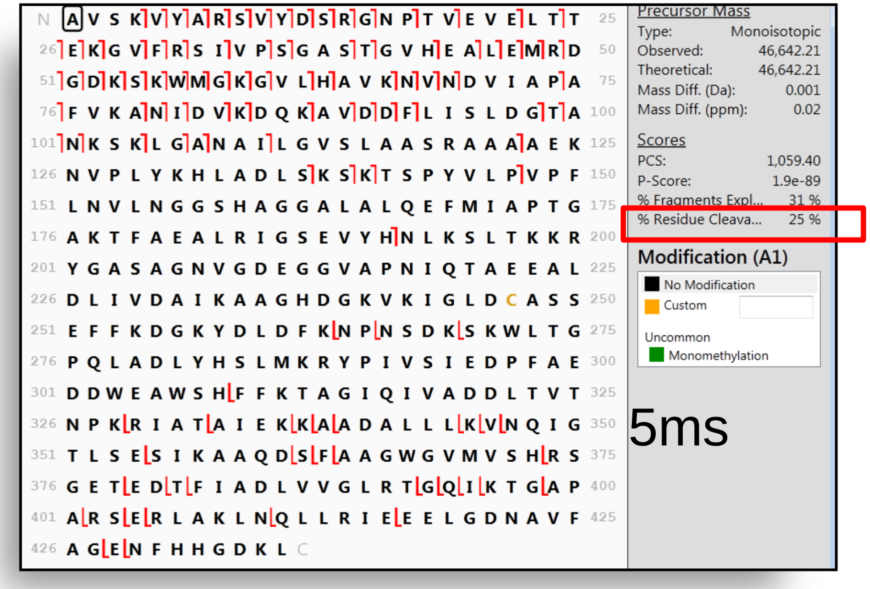
N A V S K V Y A R S V Y D S R G N P T V E V E L T T 25
26 E K G V F R S I V P S G A S T G V H E A L E M R D 50
51 G D K S K W M G K G V L H A V K N V N D V I A P A 75
76 F V K A N I D V K D Q K A V D D F L I S L D G T A 100
101 N K S K L G A N A I L G V S L A A S R A A A A E K 125
126 N V P L Y K H L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

Precursor Mass
Type: Monoisotopic
Observed: 46,642.21
Theoretical: 46,642.21
Mass Diff. (Da): 0.001
Mass Diff. (ppm): 0.02

Scores
PCS: 473.98
P-Score: 2.4e-44
% Fragments Expl... 26 %
% Residue Cleava... 21 %

Modification (A1)

3ms



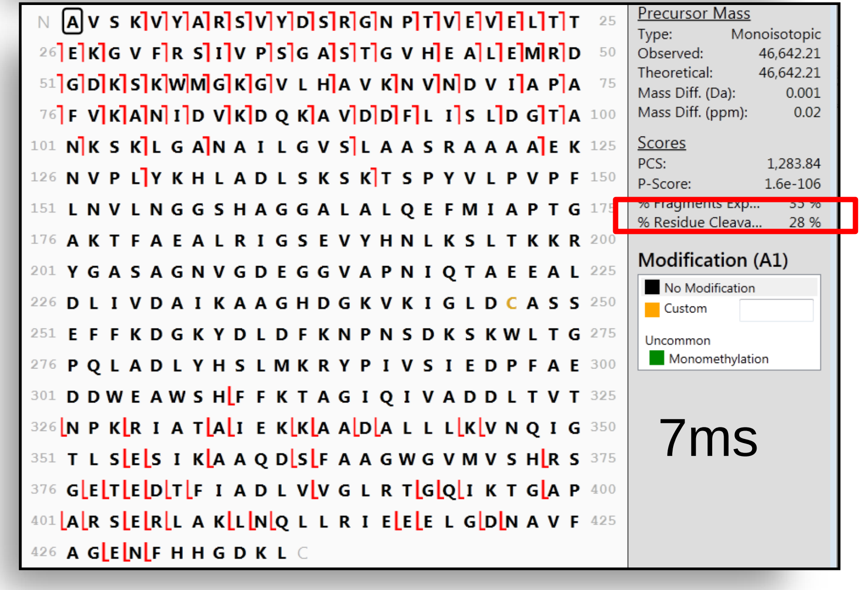
N A V S K V Y A R S V Y D S R G N P T V E V E L T T 25
26 E K G V F R S I V P S G A S T G V H E A L E M R D 50
51 G D K S K W M G K G V L H A V K N V N D V I A P A 75
76 F V K A N I D V K D Q K A V D D F L I S L D G T A 100
101 N K S K L G A N A I L G V S L A A S R A A A A E K 125
126 N V P L Y K H L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

Precursor Mass
Type: Monoisotopic
Observed: 46,642.21
Theoretical: 46,642.21
Mass Diff. (Da): 0.001
Mass Diff. (ppm): 0.02

Scores
PCS: 1,059.40
P-Score: 1.9e-89
% Fragments Expl... 31 %
% Residue Cleava... 25 %

Modification (A1)

5ms



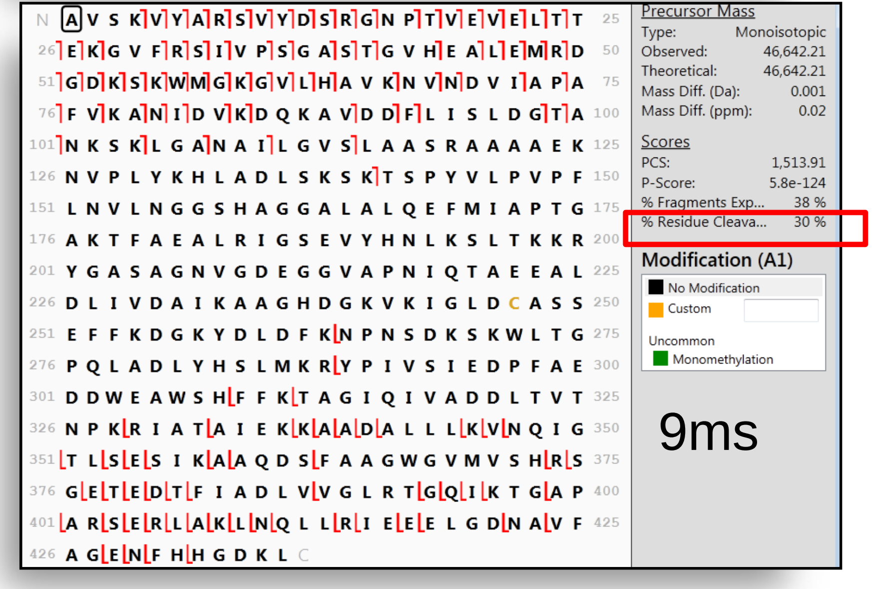
N A V S K V Y A R S V Y D S R G N P T V E V E L T T 25
26 E K G V F R S I V P S G A S T G V H E A L E M R D 50
51 G D K S K W M G K G V L H A V K N V N D V I A P A 75
76 F V K A N I D V K D Q K A V D D F L I S L D G T A 100
101 N K S K L G A N A I L G V S L A A S R A A A A E K 125
126 N V P L Y K H L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

Precursor Mass
Type: Monoisotopic
Observed: 46,642.21
Theoretical: 46,642.21
Mass Diff. (Da): 0.001
Mass Diff. (ppm): 0.02

Scores
PCS: 1,283.84
P-Score: 1.6e-106
% Fragments Exp... 33 %
% Residue Cleava... 28 %

Modification (A1)

7ms



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

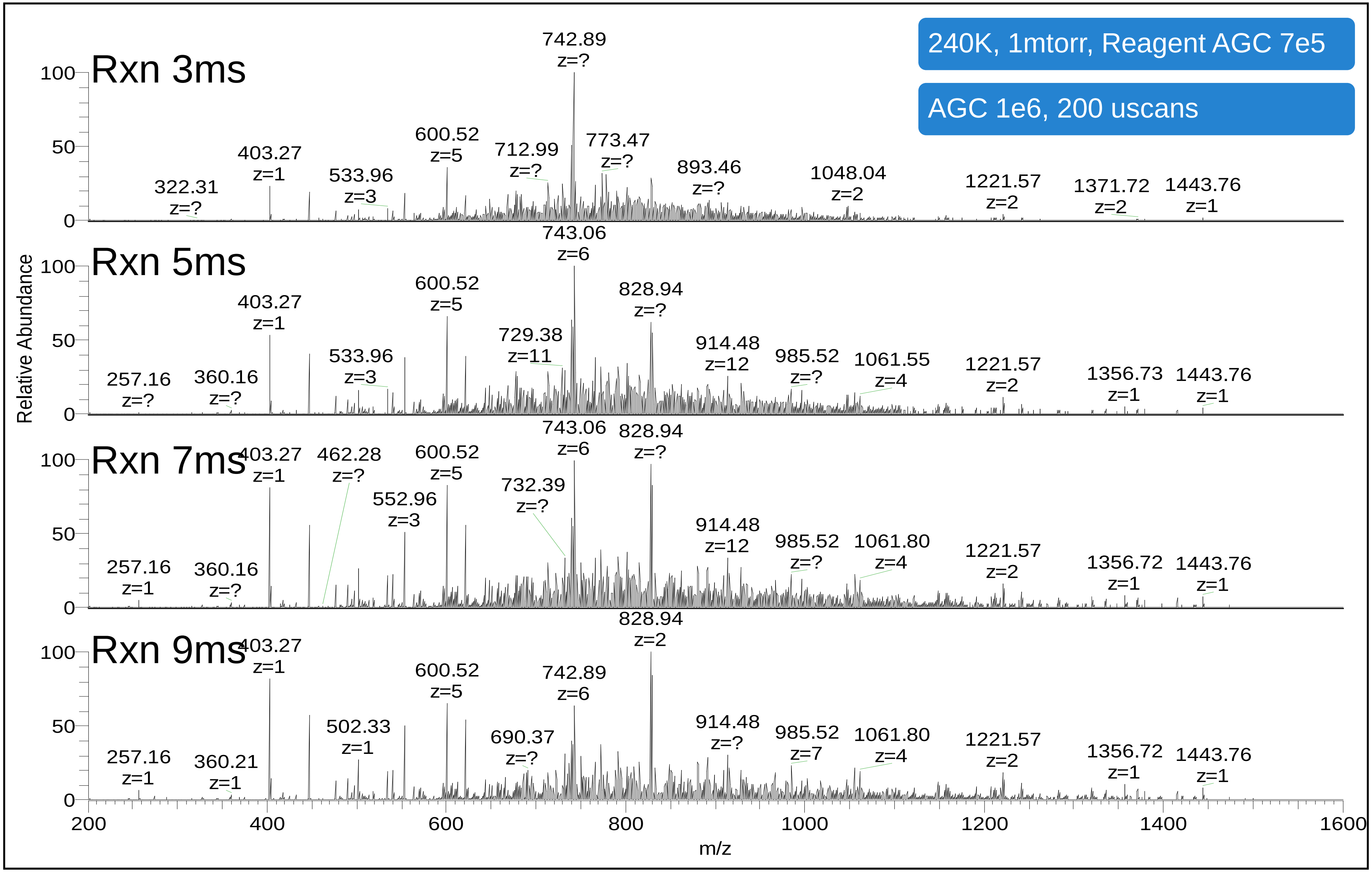
Precursor Mass
Type: Monoisotopic
Observed: 46,642.21
Theoretical: 46,642.21
Mass Diff. (Da): 0.001
Mass Diff. (ppm): 0.02

Scores
PCS: 1,513.91
P-Score: 5.8e-124
% Fragments Exp... 38 %
% Residue Cleava... 30 %

Modification (A1)

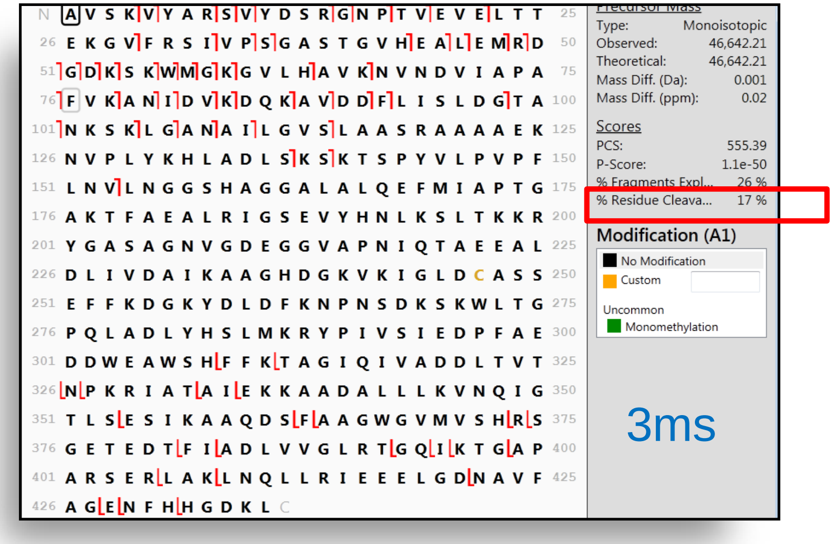
9ms

Enolase ETD HD CS 63+


240K, 1mtorr, Reagent AGC 7e5

AGC 1e6, 200 uscans

Enolase CS 63+, ETD HD Seq Coverage



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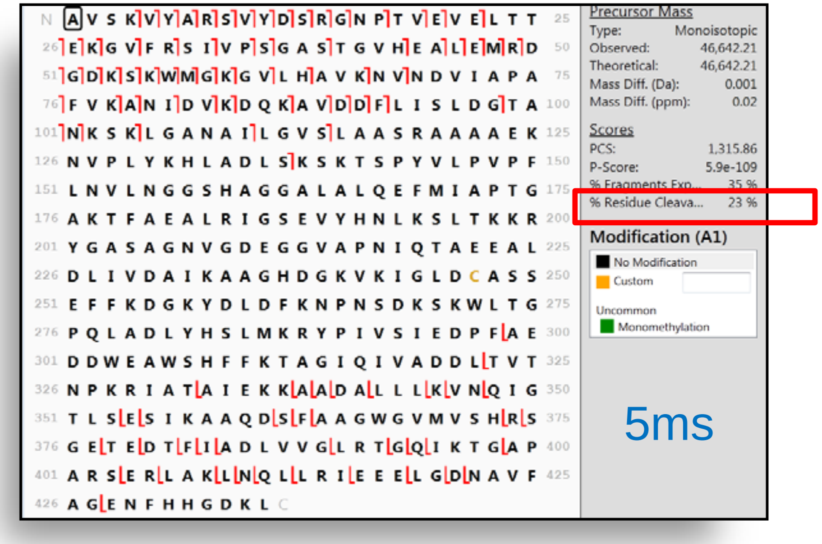
N   A V S K V Y A R S V Y D S R G N P T V E V E L T T  25
26  E K G V F R S I V P S G A S T G V H E A L E M R D  50
51  G D K S K W M G K G V L H A V K N V N D V I A P A  75
76  F V K A N I D V K D Q K A V D D F L I S L D G T A  100
101 N K S K L G A N A I L G V S L A A S R A A A A E K  125
126 N V P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

Precursor Mass
Type: Monoisotopic
Observed: 46,642.21
Theoretical: 46,642.21
Mass Diff. (Da): 0.001
Mass Diff. (ppm): 0.02

Scores
PCS: 555.39
P-Score: 1.1e-50
% Fragments Expl... 26 %
% Residue Cleava... 17 %

Modification (A1)
■ No Modification
■ Custom
Uncommon
🟩 Monomethylation

3ms



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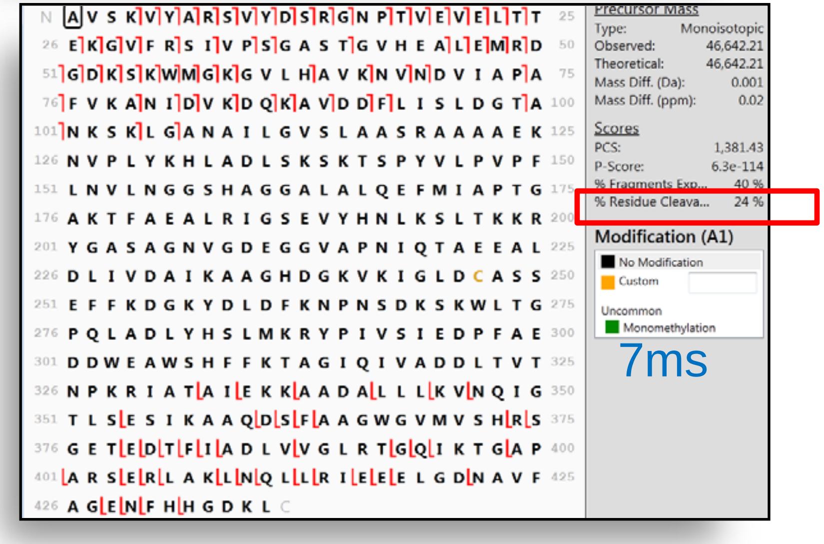

Precursor Mass
Type: Monoisotopic
Observed: 46,642.21
Theoretical: 46,642.21
Mass Diff. (Da): 0.001
Mass Diff. (ppm): 0.02

Scores
PCS: 1,315.86
P-Score: 5.9e-109
% Fragments Exp... 35 %
% Residue Cleava... 23 %

Modification (A1)
■ No Modification
■ Custom
Uncommon
🟩 Monomethylation

5ms



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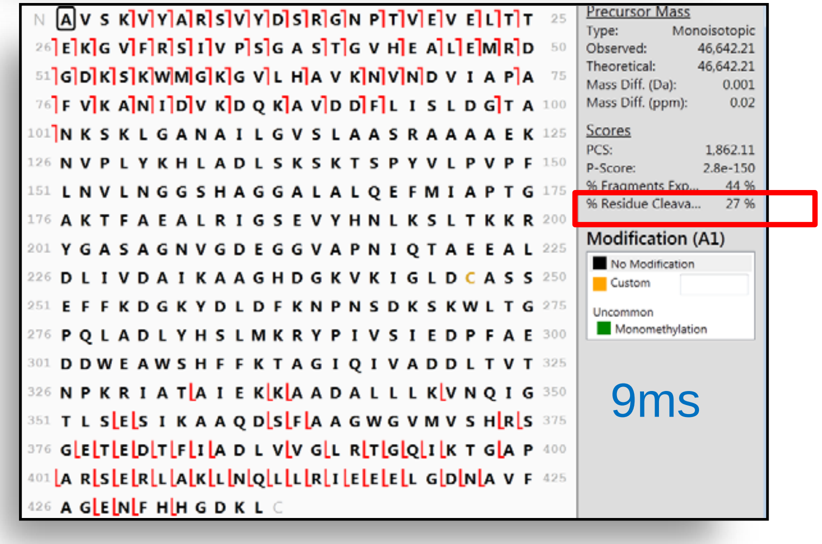
N   A V S K V Y A R S V Y D S R G N P T V E V E L T T  25
26  E K G V F R S I V P S G A S T G V H E A L E M R D  50
51  G D K S K W M G K G V L H A V K N V N D V I A P A  75
76  F V K A N I D V K D Q K A V D D F L I S L D G T A  100
101 N K S K L G A N A I L G V S L A A S R A A A A E K  125
126 N V P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

Precursor Mass
Type: Monoisotopic
Observed: 46,642.21
Theoretical: 46,642.21
Mass Diff. (Da): 0.001
Mass Diff. (ppm): 0.02

Scores
PCS: 1,381.43
P-Score: 6.3e-114
% Fragments Exp... 40 %
% Residue Cleava... 24 %

Modification (A1)
■ No Modification
■ Custom
Uncommon
🟩 Monomethylation

7ms



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

Precursor Mass
Type: Monoisotopic
Observed: 46,642.21
Theoretical: 46,642.21
Mass Diff. (Da): 0.001
Mass Diff. (ppm): 0.02

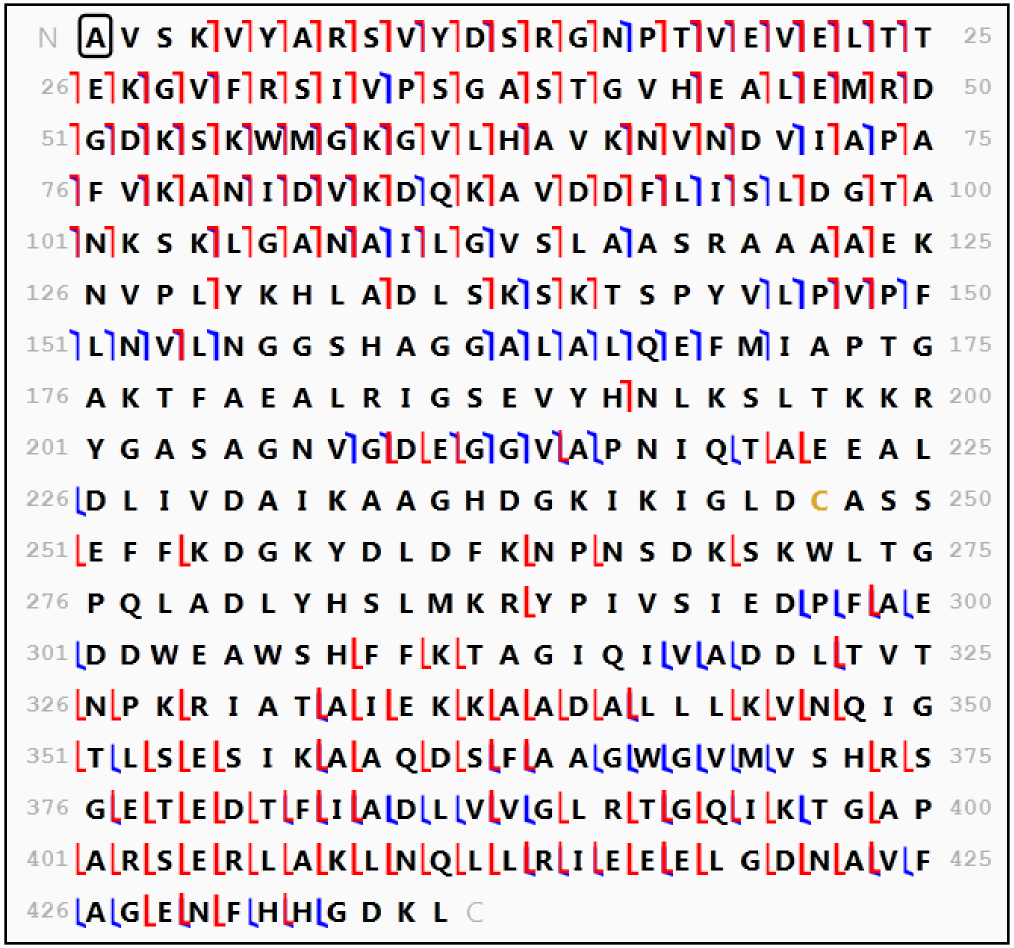
Scores
PCS: 1,862.11
P-Score: 2.8e-150
% Fragments Exp... 44 %
% Residue Cleava... 27 %

Modification (A1)
■ No Modification
■ Custom
Uncommon
🟩 Monomethylation

9ms

Enolase Combined Sequence Coverage

56% Seq Coverage



N A V S K V Y A R S V Y D S R G N P T V E V E L T T 25

26 E K G V F R S I V P S G A S T G V H E A L E M R D 50

51 G D K S K W M G K G V L H A V K N V N D V I A P A 75

76 F V K A N I D V K D Q K A V D D F L I S L D G T A 100

101 N K S K L G A N A I L G V S L A A S R A A A A E K 125

126 N V P L Y K H L A D L S K S K T S P Y V L P V P F 150

151 L N V L N G G S H A G G A L A L Q E F M I A P T G 175

176 A K T F A E A L R I G S E V Y H N L K S L T K K R 200

201 Y G A S A G N V G D E G G V A P N I Q T A E E A L 225

226 D L I V D A I K A A G H D G K I K I G L D C A S S 250

251 E F F K D G K Y D L D F K N P N S D K S K W L T G 275

276 P Q L A D L Y H S L M K R Y P I V S I E D P F A E 300

301 D D W E A W S H F F K T A G I Q I V A D D L T V T 325

326 N P K R I A T A I E K K A A D A L L L K V N Q I G 350

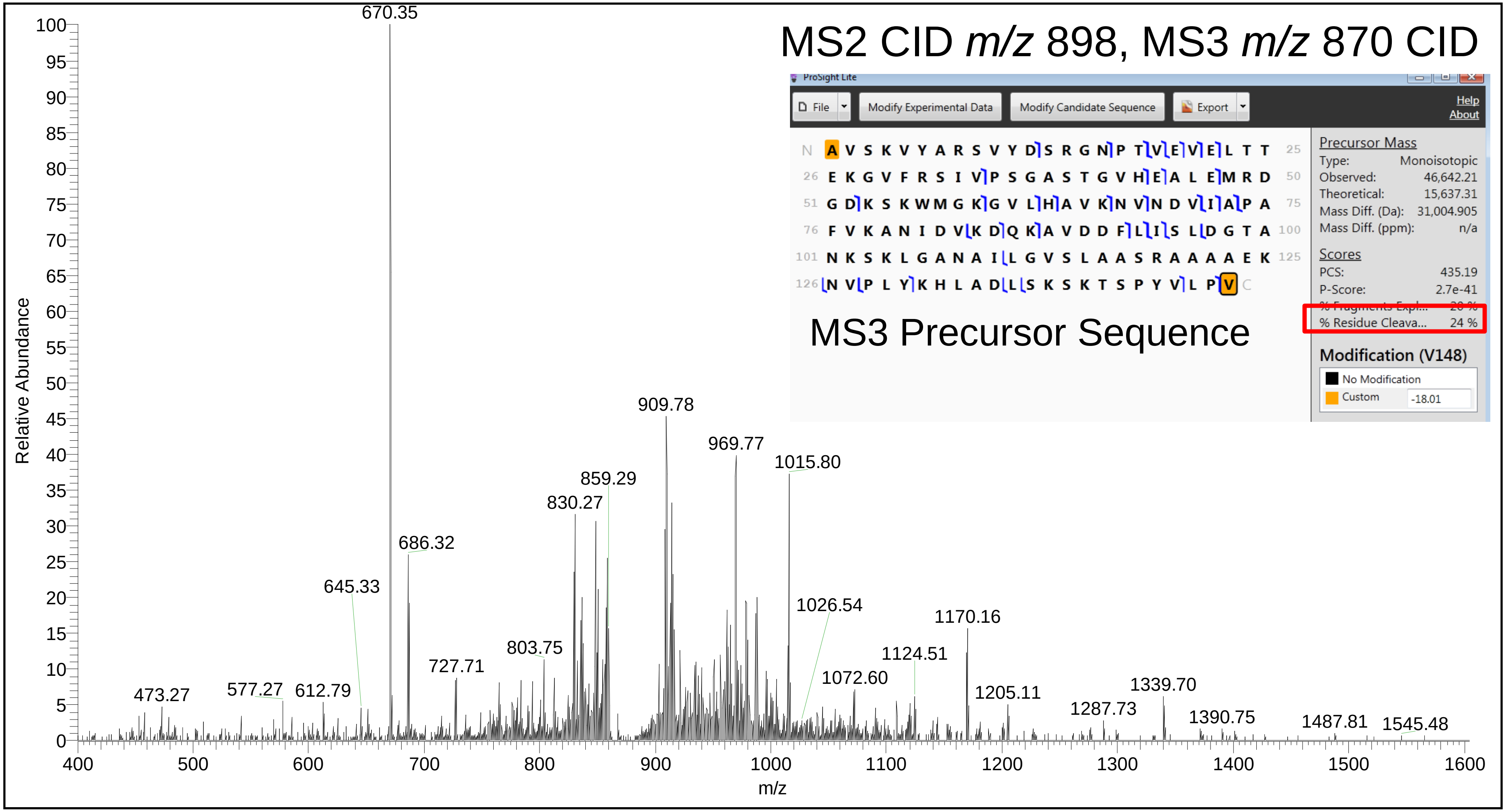
351 T L S E S I K A A Q D S F A A G W G V M V S H R S 375

376 G E T E D T F I A D L V V G L R T G Q I K T G A P 400

401 A R S E R L A K L N Q L L R I E E E L G D N A V F 425

426 A G E N F H H G D K L C

Enolase MS2 CID MS3 CID



MS2 CID *m/z* 898, MS3 *m/z* 870 CID

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

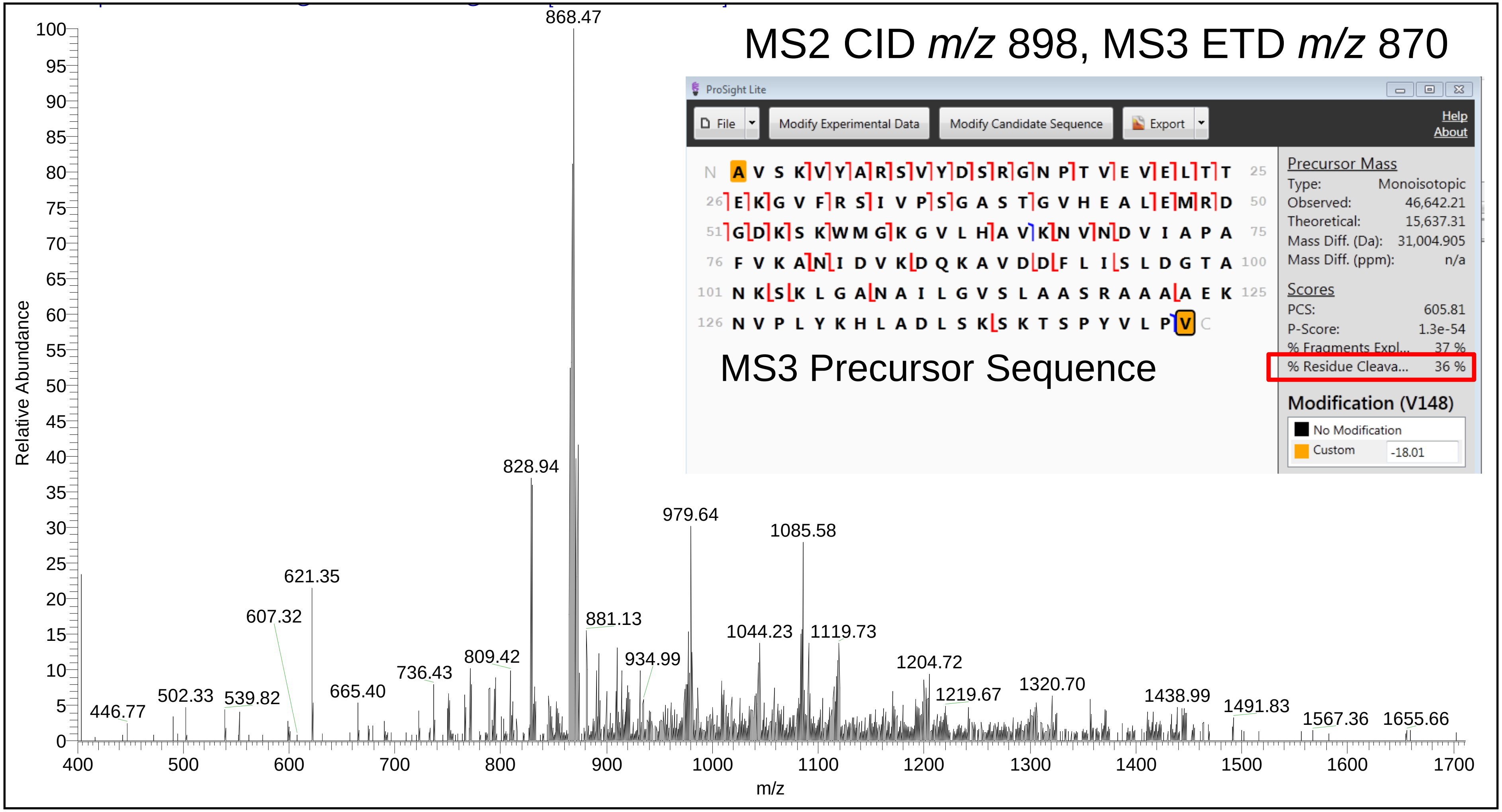
Precursor Mass
Type: Monoisotopic
Observed: 46,642.21
Theoretical: 15,637.31
Mass Diff. (Da): 31,004.905
Mass Diff. (ppm): n/a

Scores
PCS: 435.19
P-Score: 2.7e-41
% Fragments Expl... 20 %
% Residue Cleava... 24 %

Modification (V148)
■ No Modification
🟧 Custom -18.01

MS3 Precursor Sequence

Enolase MS2 CID MS3 ETD



MS2 CID *m/z* 898, MS3 ETD *m/z* 870

ProSight Lite

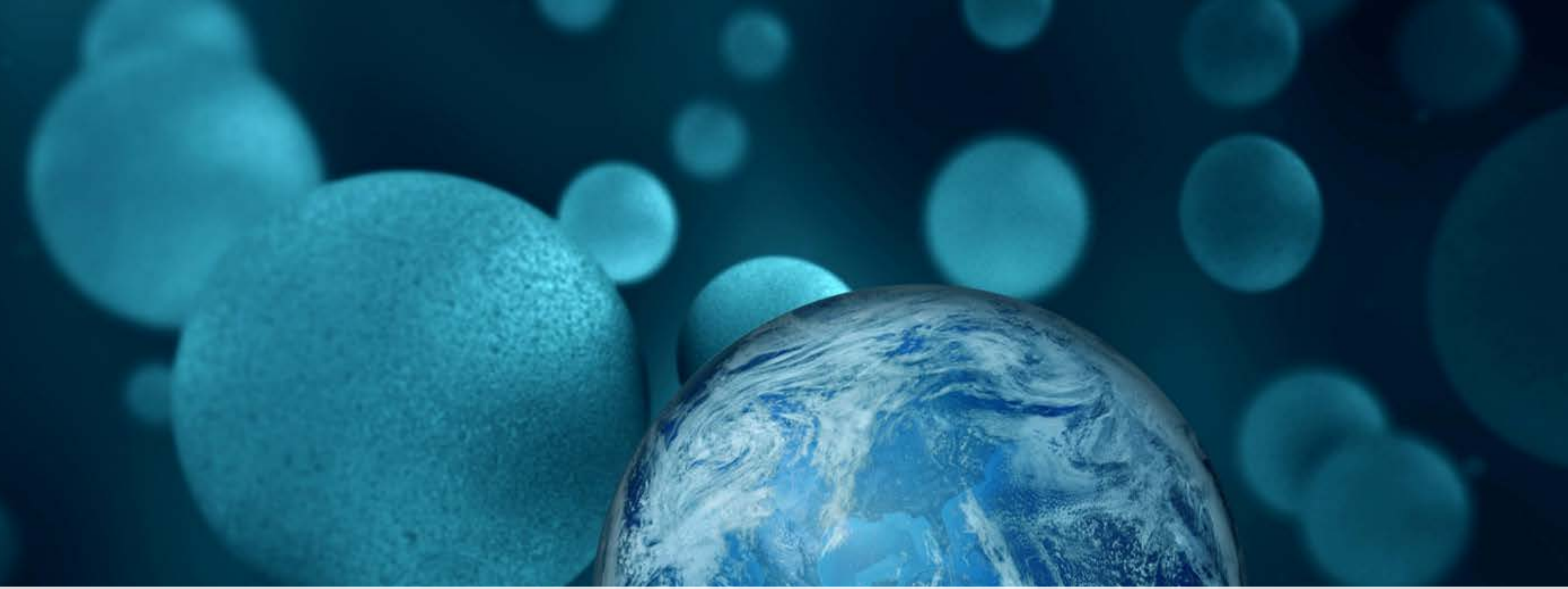
N A V S K V Y A R S V Y D S R G N P T V E V E L T T 25
26 E K G V F R S I V P S G A S T G V H E A L E M R D 50
51 G D K S K W M G K G V L H A V K N V N D V I A P A 75
76 F V K A N I D V K D Q K A V D D F L I S L D G T A 100
101 N K S K L G A N A I L G V S L A A S R A A A A E K 125
126 N V P L Y K H L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

MS3 Precursor Sequence

Precursor Mass
Type: Monoisotopic
Observed: 46,642.21
Theoretical: 15,637.31
Mass Diff. (Da): 31,004.905
Mass Diff. (ppm): n/a

Scores
PCS: 605.81
P-Score: 1.3e-54
% Fragments Expl... 37 %
% Residue Cleava... 36 %

Modification (V148)
■ No Modification
🟧 Custom -18.01



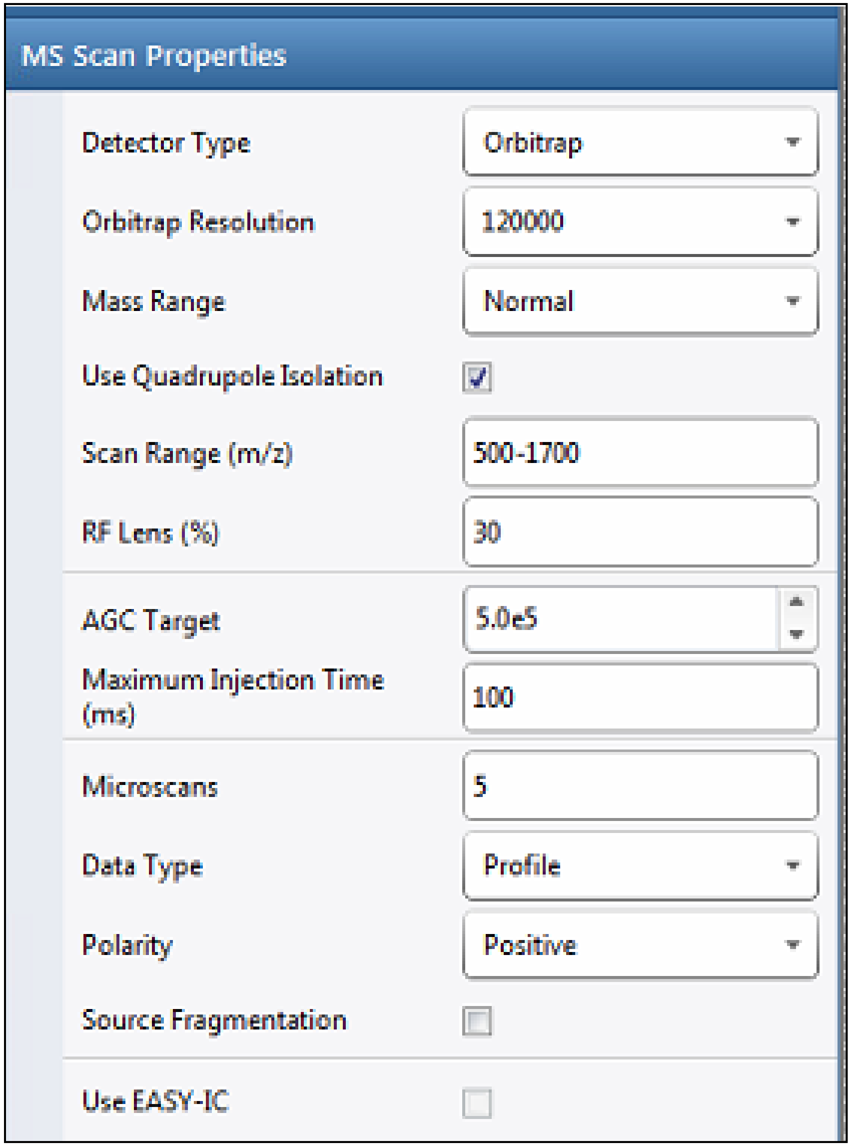
DDA LC-MS Analysis: Bacterial Lysates

The world leader in serving science

Chromatography

- LC: Dionex Ultimate 3000RSLCnano
- Flow rate: 12 ul/min
- Column: ProSwift Monolithic RP-4H 200 µm x 25cm
- Trap Column (optional): Prototype C4, 200 µm x 5mm
- Solvents: A: H_2O, 0.1% FA
  B: AcN, 0.1% FA
- Gradient: 20% B to 60% B in 90min

Instrument Method Full MS

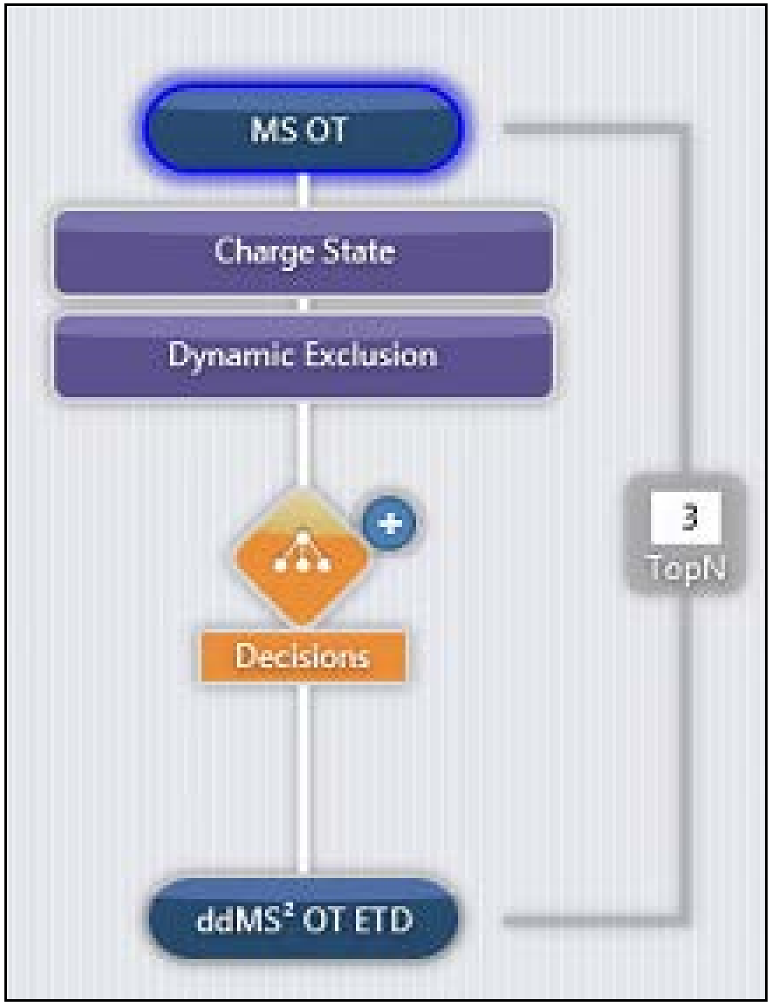


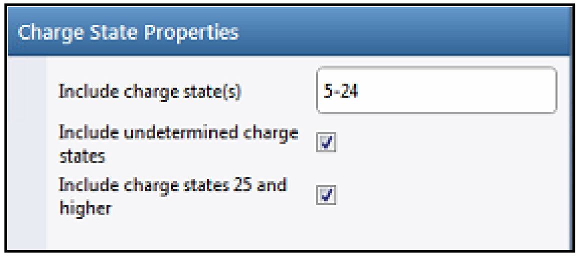
MS Scan Properties

Detector Type	Orbitrap
Orbitrap Resolution	120000
Mass Range	Normal
Use Quadrupole Isolation	☑
Scan Range (m/z)	500-1700
RF Lens (%)	30
AGC Target	5.0e5
Maximum Injection Time (ms)	100
Microscans	5
Data Type	Profile
Polarity	Positive
Source Fragmentation	☐
Use EASY-IC	☐

- High / High Method
  - Full MS @ 120000 resolution
  - MS2 @ 120000 resolution
- Medium / High Method
  - Full MS @ 15000 resolution
  - MS2 @ 120000 resolution

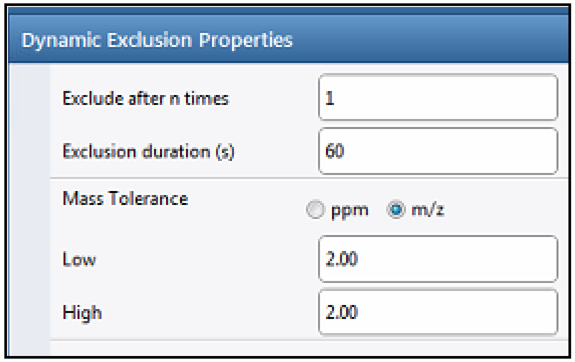
Instrument Method DDA





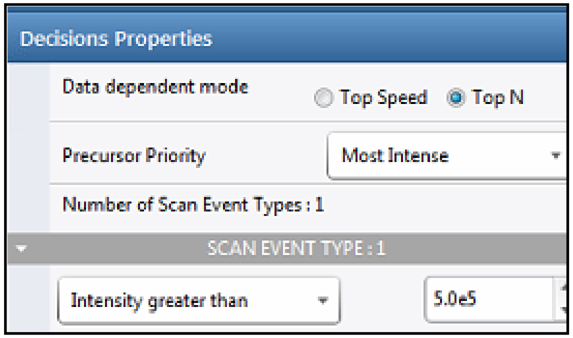
Charge State Properties

Include charge state(s)	5-24
Include undetermined charge states	☑
Include charge states 25 and higher	☑



Dynamic Exclusion Properties

Exclude after n times	1
Exclusion duration (s)	60
Mass Tolerance	○ ppm ● m/z
Low	2.00
High	2.00



Decisions Properties

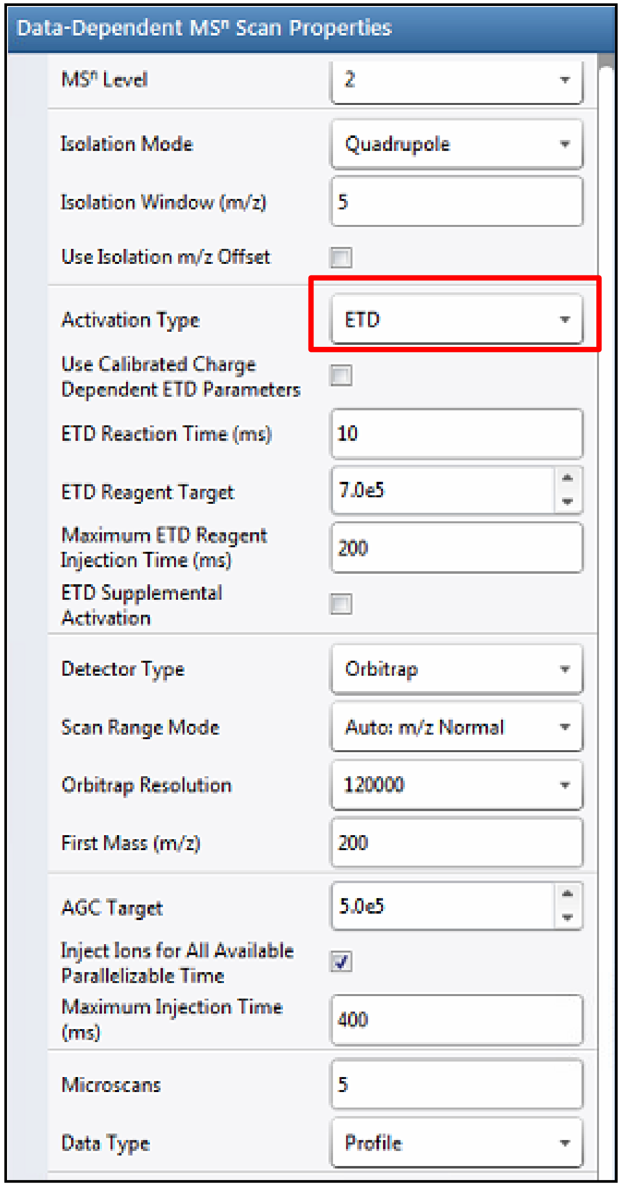
Data dependent mode	○ Top Speed ● Top N
Precursor Priority	Most Intense

Number of Scan Event Types : 1

SCAN EVENT TYPE : 1

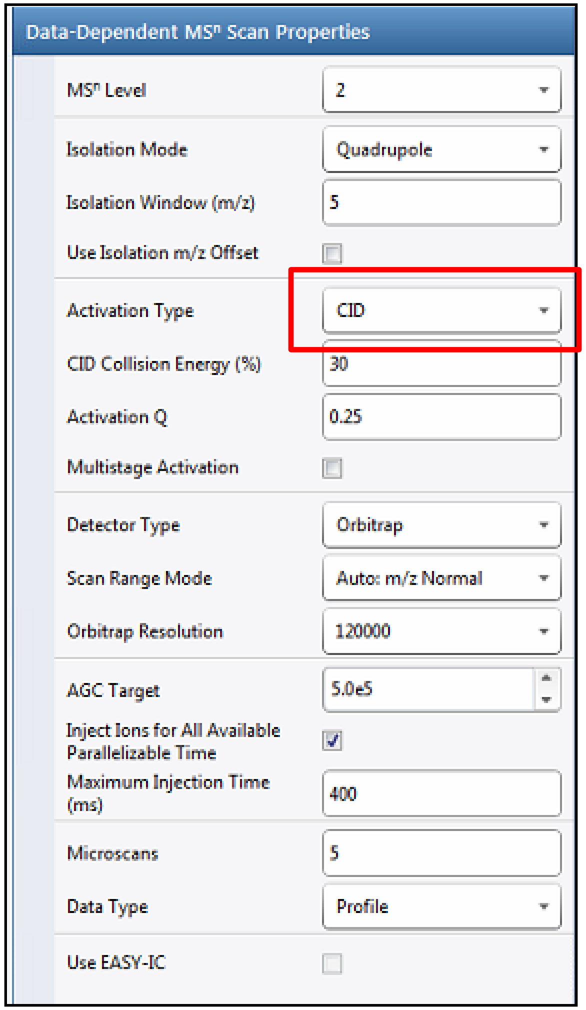
Intensity greater than	5.0e5

DDA Parameters



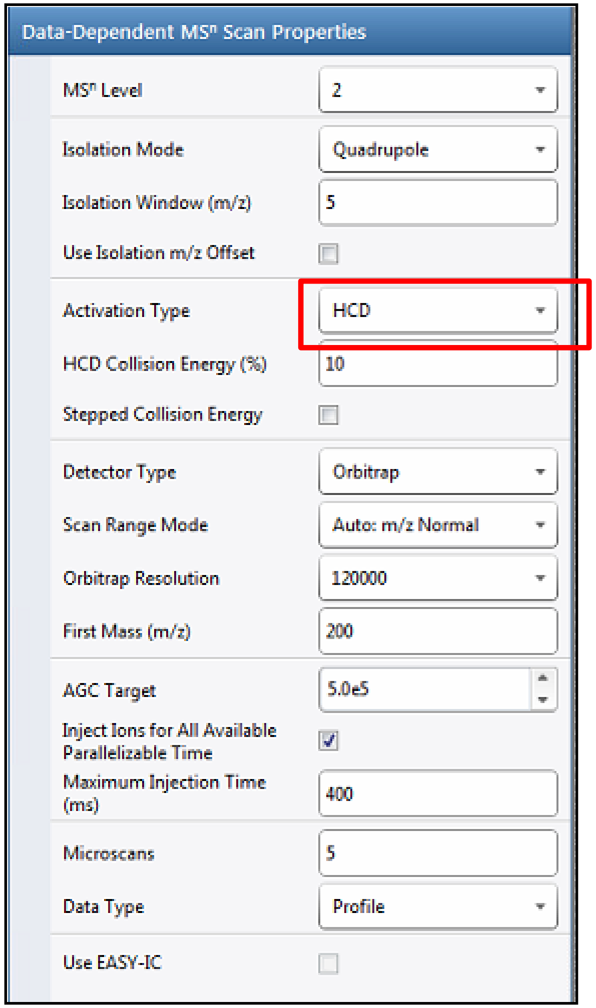
Data-Dependent MSⁿ Scan Properties

MSⁿ Level	2
Isolation Mode	Quadrupole
Isolation Window (m/z)	5
Use Isolation m/z Offset	☐
Activation Type	ETD
Use Calibrated Charge Dependent ETD Parameters	☐
ETD Reaction Time (ms)	10
ETD Reagent Target	7.0e5
Maximum ETD Reagent Injection Time (ms)	200
ETD Supplemental Activation	☐
Detector Type	Orbitrap
Scan Range Mode	Auto: m/z Normal
Orbitrap Resolution	120000
First Mass (m/z)	200
AGC Target	5.0e5
Inject Ions for All Available Parallelizable Time	☑
Maximum Injection Time (ms)	400
Microscans	5
Data Type	Profile



Data-Dependent MSⁿ Scan Properties

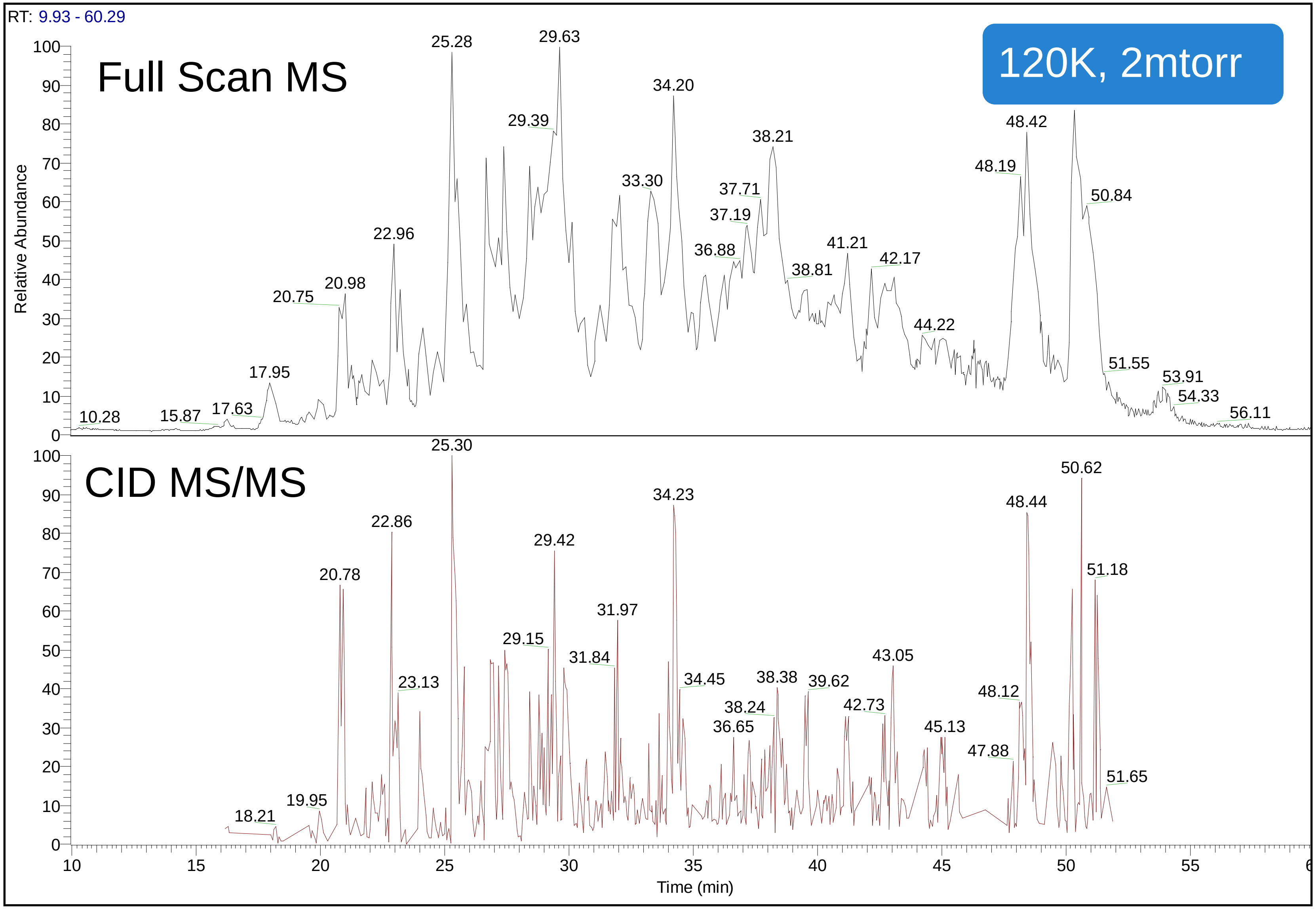
MSⁿ Level	2
Isolation Mode	Quadrupole
Isolation Window (m/z)	5
Use Isolation m/z Offset	☐
Activation Type	CID
CID Collision Energy (%)	30
Activation Q	0.25
Multistage Activation	☐
Detector Type	Orbitrap
Scan Range Mode	Auto: m/z Normal
Orbitrap Resolution	120000
AGC Target	5.0e5
Inject Ions for All Available Parallelizable Time	☑
Maximum Injection Time (ms)	400
Microscans	5
Data Type	Profile
Use EASY-IC	☐



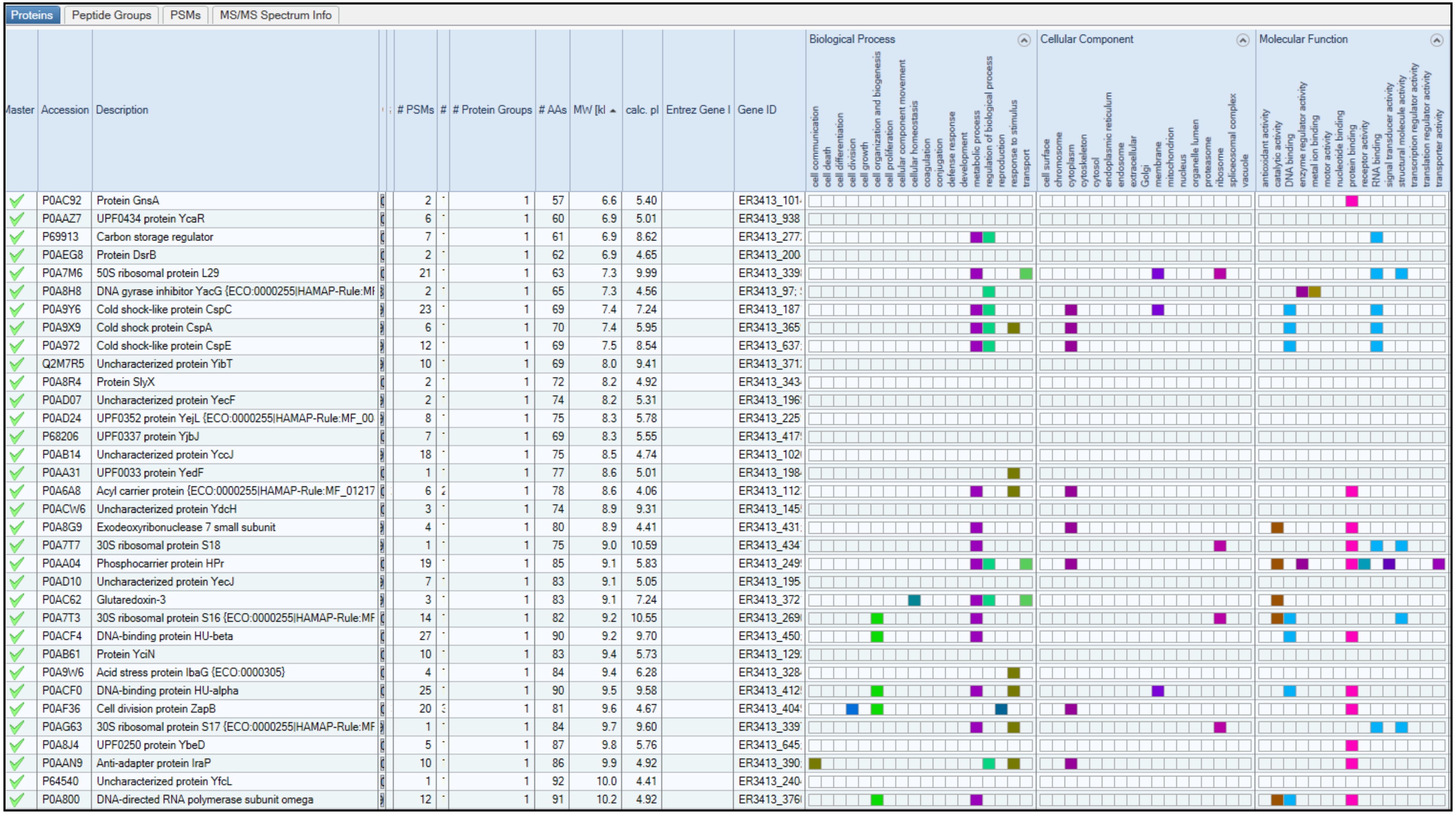
Data-Dependent MSⁿ Scan Properties

MSⁿ Level	2
Isolation Mode	Quadrupole
Isolation Window (m/z)	5
Use Isolation m/z Offset	☐
Activation Type	HCD
HCD Collision Energy (%)	10
Stepped Collision Energy	☐
Detector Type	Orbitrap
Scan Range Mode	Auto: m/z Normal
Orbitrap Resolution	120000
First Mass (m/z)	200
AGC Target	5.0e5
Inject Ions for All Available Parallelizable Time	☑
Maximum Injection Time (ms)	400
Microscans	5
Data Type	Profile
Use EASY-IC	☐

E. coli DDA Total Ion Chromatogram



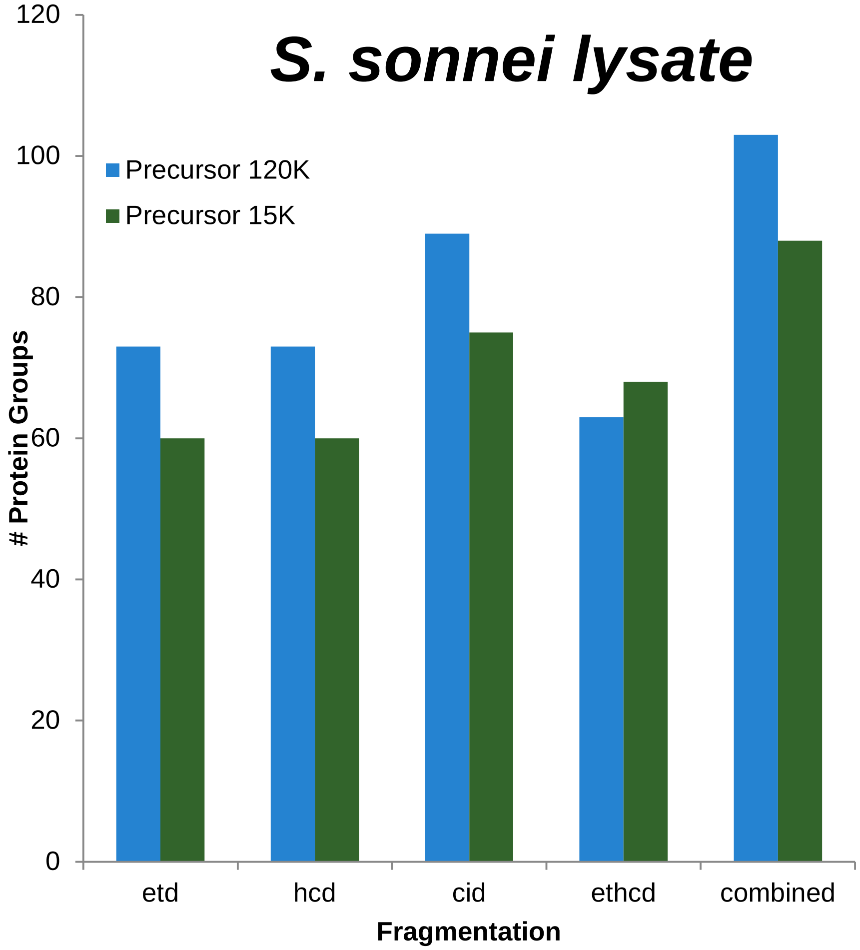
Data Processing: ProSight PD

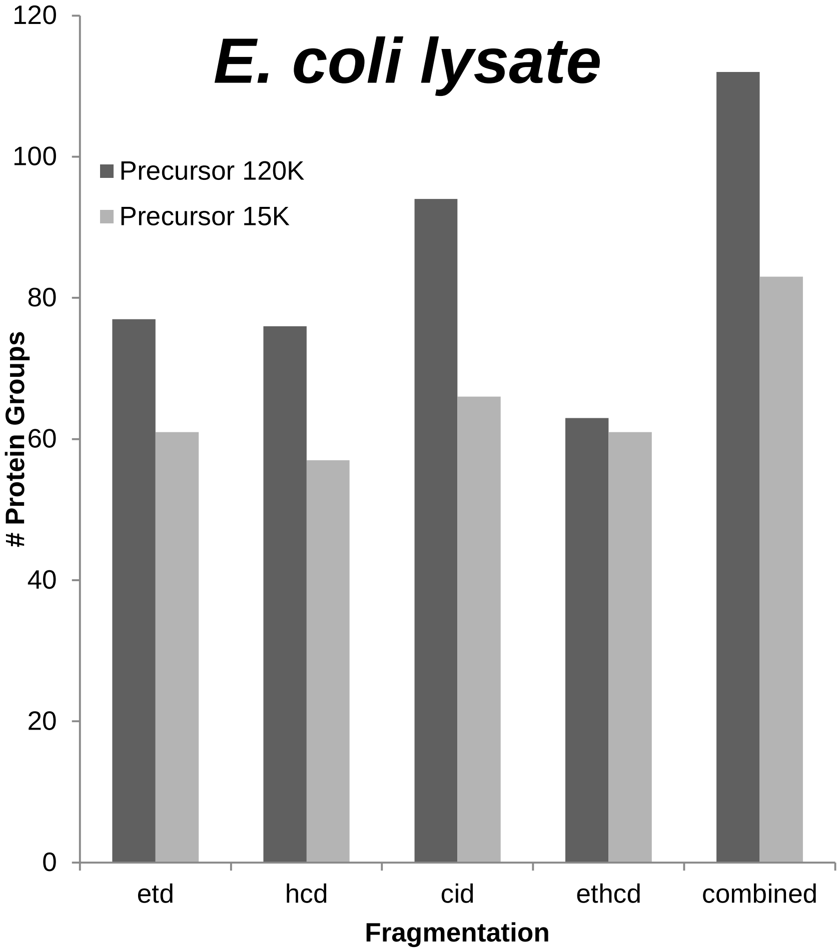


Master	Accession	Description	# PSMs	# Protein Groups	# AAs	MW [kl ▲	calc. pI	Entrez Gene I	Gene ID
✓	P0AC92	Protein GnsA	2	1	57	6.6	5.40		ER3413_101
✓	P0AAZ7	UPF0434 protein YcaR	6	1	60	6.9	5.01		ER3413_938
✓	P69913	Carbon storage regulator	7	1	61	6.9	8.62		ER3413_277
✓	P0AEG8	Protein DsrB	2	1	62	6.9	4.65		ER3413_200
✓	P0A7M6	50S ribosomal protein L29	21	1	63	7.3	9.99		ER3413_339
✓	P0A8H8	DNA gyrase inhibitor YacG {ECO:0000255|HAMAP-Rule:MI	2	1	65	7.3	4.56		ER3413_97;
✓	P0A9Y6	Cold shock-like protein CspC	23	1	69	7.4	7.24		ER3413_187
✓	P0A9X9	Cold shock protein CspA	6	1	70	7.4	5.95		ER3413_365
✓	P0A972	Cold shock-like protein CspE	12	1	69	7.5	8.54		ER3413_637
✓	Q2M7R5	Uncharacterized protein YibT	10	1	69	8.0	9.41		ER3413_371
✓	P0A8R4	Protein SlyX	2	1	72	8.2	4.92		ER3413_343
✓	P0AD07	Uncharacterized protein YecF	2	1	74	8.2	5.31		ER3413_196
✓	P0AD24	UPF0352 protein YejL {ECO:0000255|HAMAP-Rule:MF_00	8	1	75	8.3	5.78		ER3413_225
✓	P68206	UPF0337 protein YjbJ	7	1	69	8.3	5.55		ER3413_417
✓	P0AB14	Uncharacterized protein YccJ	18	1	75	8.5	4.74		ER3413_102
✓	P0AA31	UPF0033 protein YedF	1	1	77	8.6	5.01		ER3413_198
✓	P0A6A8	Acyl carrier protein {ECO:0000255|HAMAP-Rule:MF_01217	6	1	78	8.6	4.06		ER3413_112
✓	P0ACW6	Uncharacterized protein YdcH	3	1	74	8.9	9.31		ER3413_145
✓	P0A8G9	Exodeoxyribonuclease 7 small subunit	4	1	80	8.9	4.41		ER3413_431
✓	P0A7T7	30S ribosomal protein S18	1	1	75	9.0	10.59		ER3413_434
✓	P0AA04	Phosphocarrier protein HPr	19	1	85	9.1	5.83		ER3413_249
✓	P0AD10	Uncharacterized protein YecJ	7	1	83	9.1	5.05		ER3413_195
✓	P0AC62	Glutaredoxin-3	3	1	83	9.1	7.24		ER3413_372
✓	P0A7T3	30S ribosomal protein S16 {ECO:0000255|HAMAP-Rule:MF	14	1	82	9.2	10.55		ER3413_269
✓	P0ACF4	DNA-binding protein HU-beta	27	1	90	9.2	9.70		ER3413_450
✓	P0AB61	Protein YciN	10	1	83	9.4	5.73		ER3413_129
✓	P0A9W6	Acid stress protein IbaG {ECO:0000305}	4	1	84	9.4	6.28		ER3413_328
✓	P0ACF0	DNA-binding protein HU-alpha	25	1	90	9.5	9.58		ER3413_412
✓	P0AF36	Cell division protein ZapB	20	1	81	9.6	4.67		ER3413_404
✓	P0AG63	30S ribosomal protein S17 {ECO:0000255|HAMAP-Rule:MF	1	1	84	9.7	9.60		ER3413_339
✓	P0A8J4	UPF0250 protein YbeD	5	1	87	9.8	5.76		ER3413_645
✓	P0AAN9	Anti-adapter protein IraP	10	1	86	9.9	4.92		ER3413_390
✓	P64540	Uncharacterized protein YfcL	1	1	92	10.0	4.41		ER3413_240
✓	P0A800	DNA-directed RNA polymerase subunit omega	12	1	91	10.2	4.92		ER3413_376

Protein Identifications

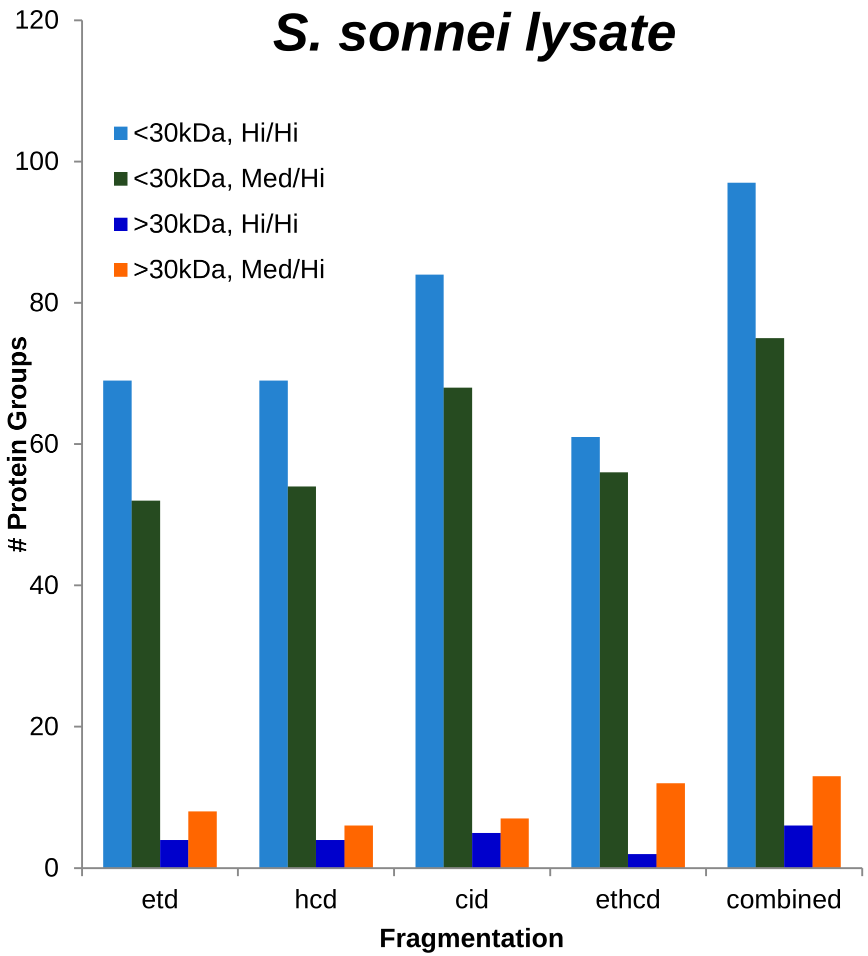
Higher number of proteins identified using High/High method

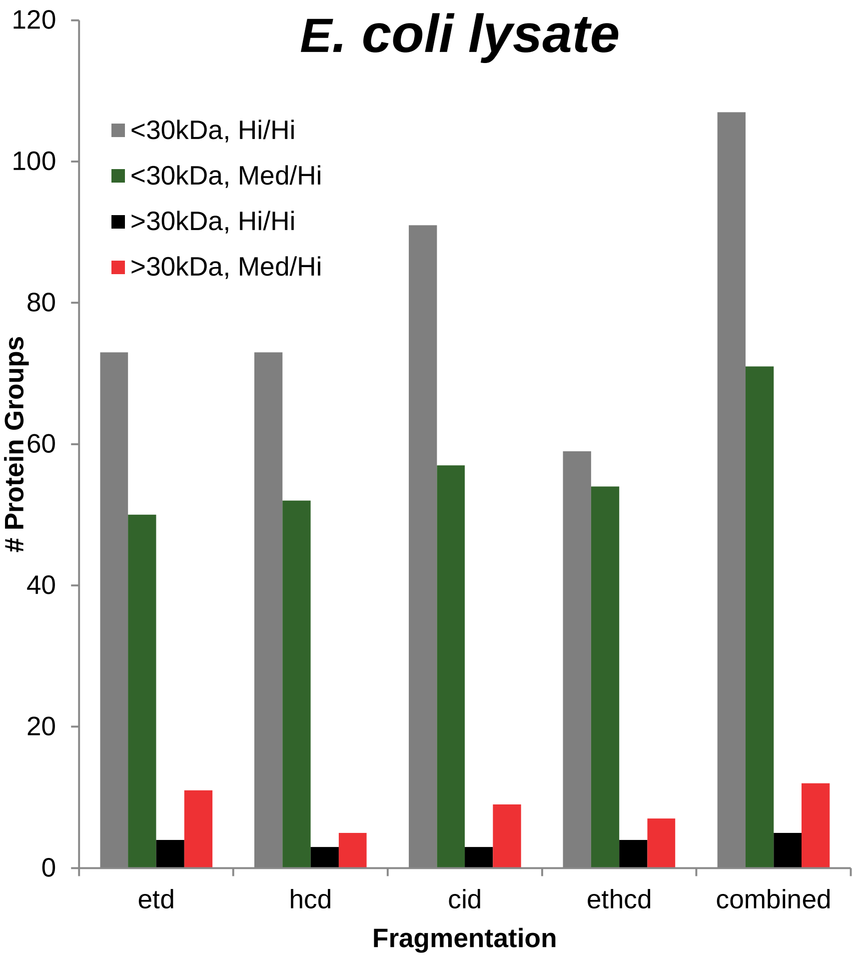


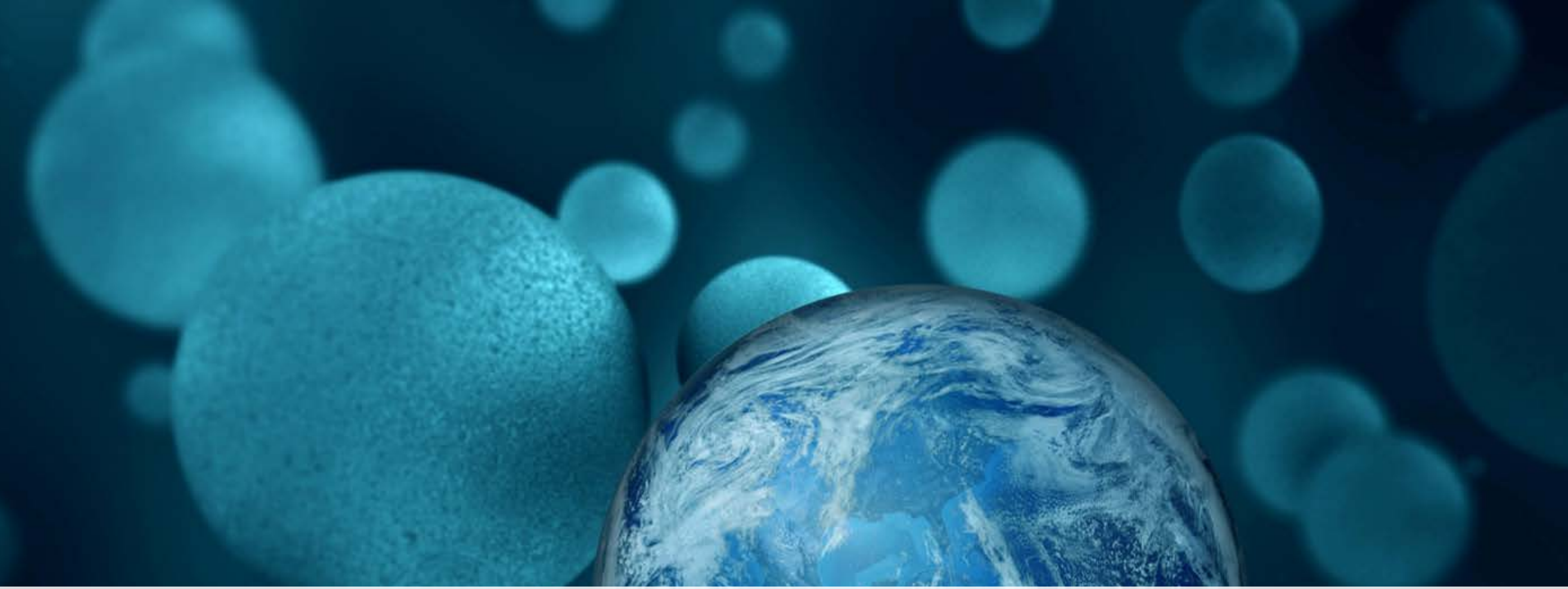


Identification of Large Proteins

Greater number of large proteins (>30kDa) identified using Med/High

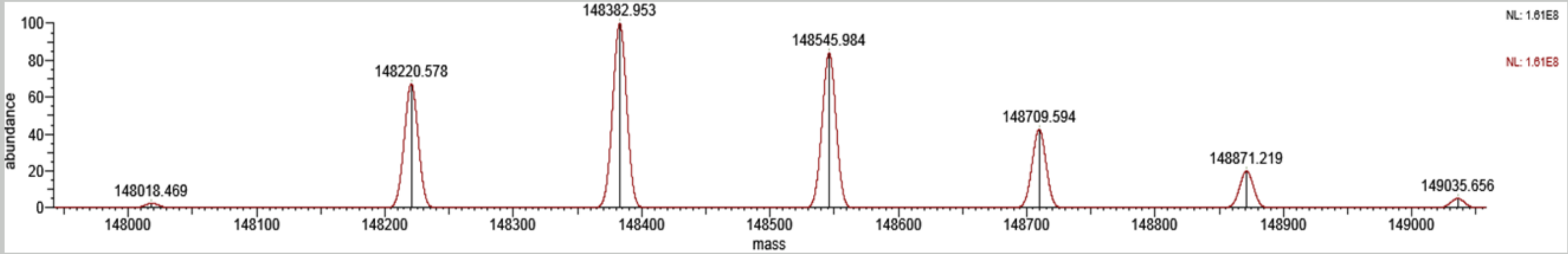




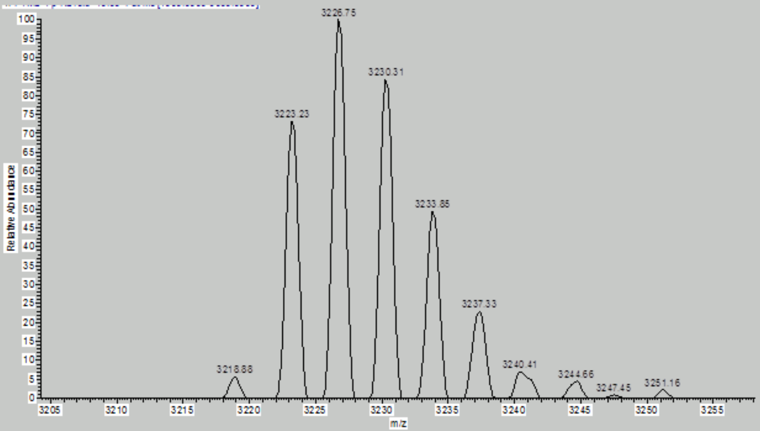


High Flow LC-MS Analysis: Intact mAb

Intact IgG: Seven Major Glycosylated Forms

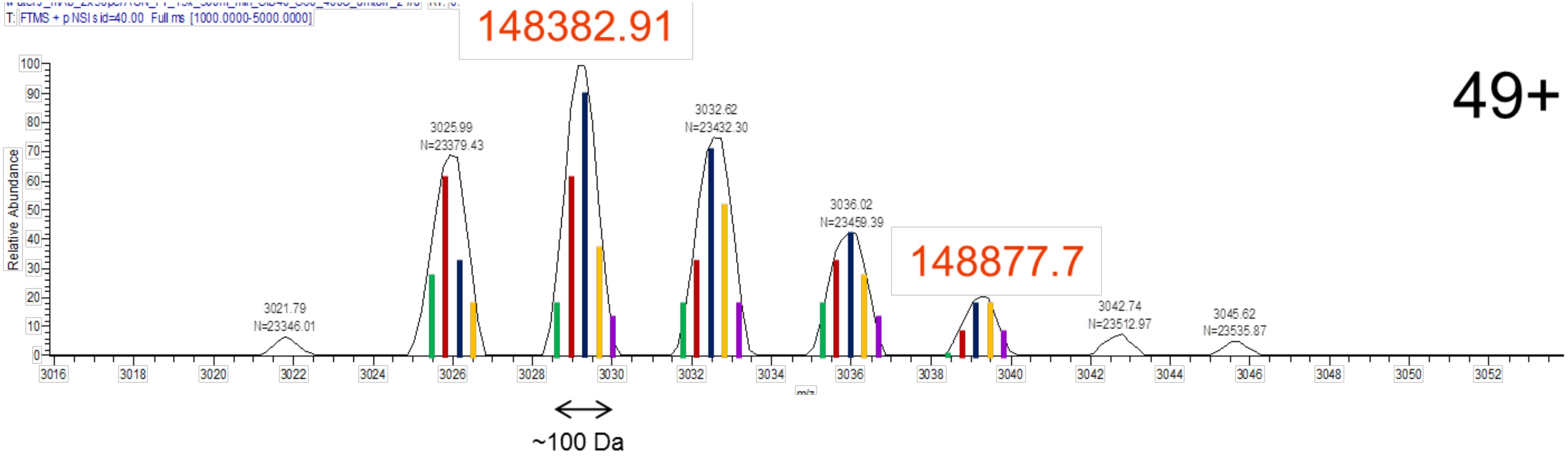


Deconvoluted Mass, Da	Reported Mass, Da
148018.5	
148220.6	148220.4
148383.0	148382.5
148546.0	148544.6
148709.6	148706.7
148871.2	148868.8
149035.7	



Why mass shifts?

How Many Components Are In Each Peak?



148382.91

148877.7

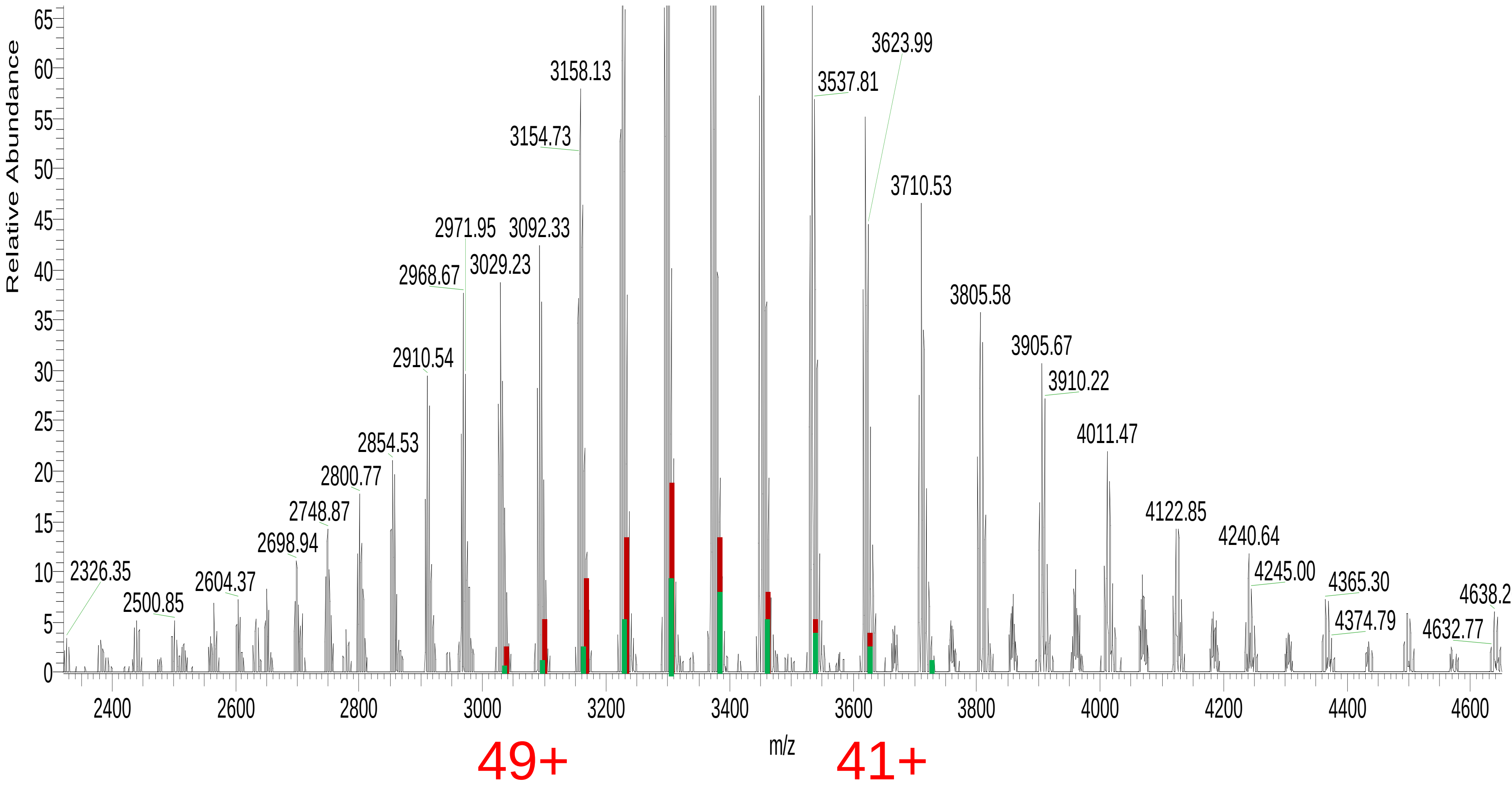
49+

~100 Da

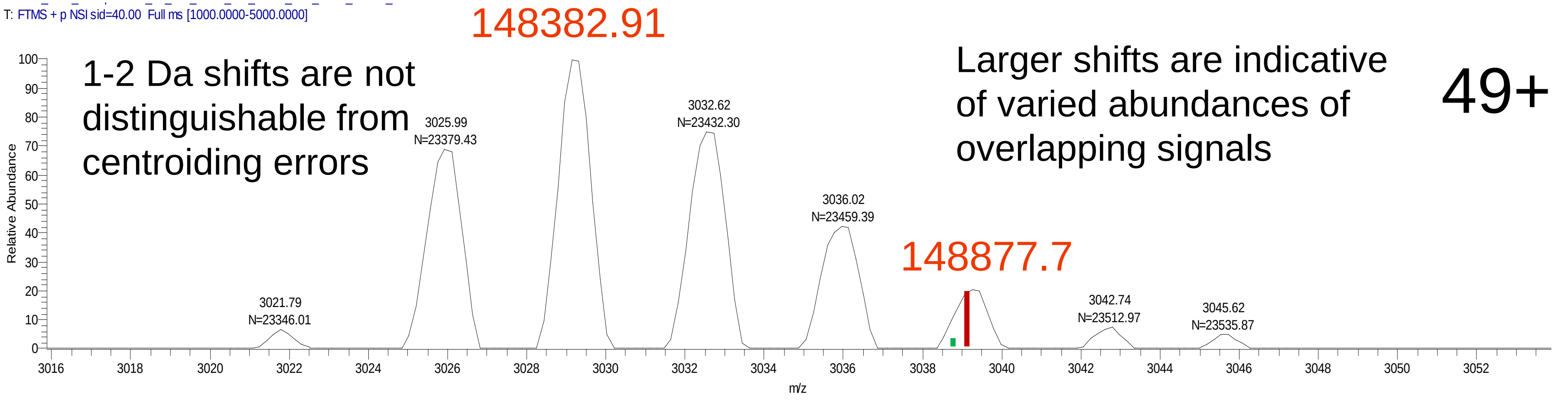
- Covalent mods can include: SS red/ox, oxidation, deamidation, aa substitutions, Lys variants, sialated forms

- Non-covalent mods can include: metal adducts, phosphate adducts

Commonly Observed CS Distribution…….s????



Evidence For Multiple Overlapping Distributions



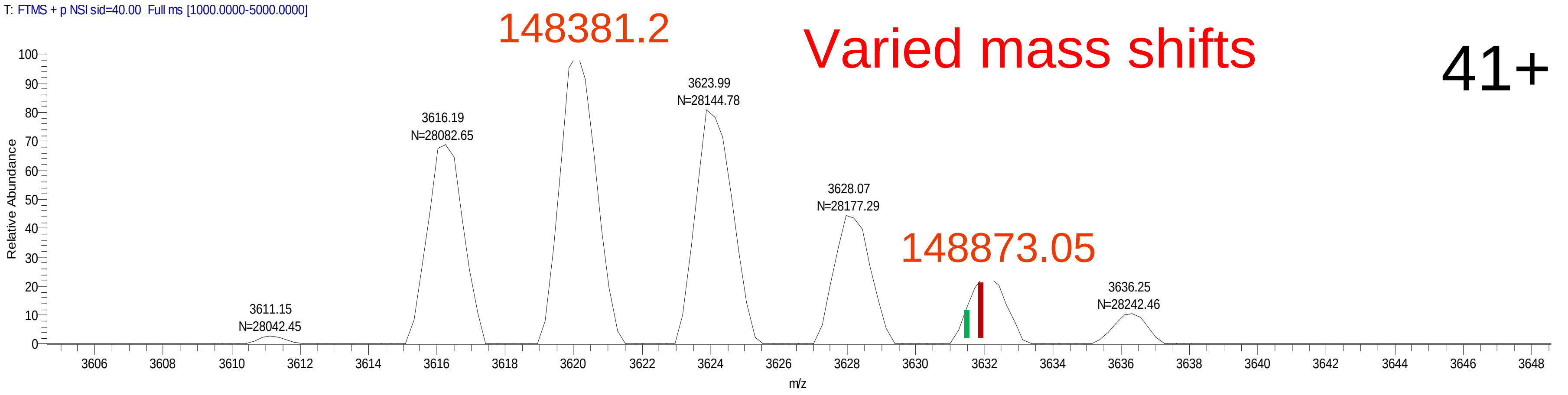
148382.91

1-2 Da shifts are not distinguishable from centroiding errors

Larger shifts are indicative of varied abundances of overlapping signals

49+

148877.7



148381.2

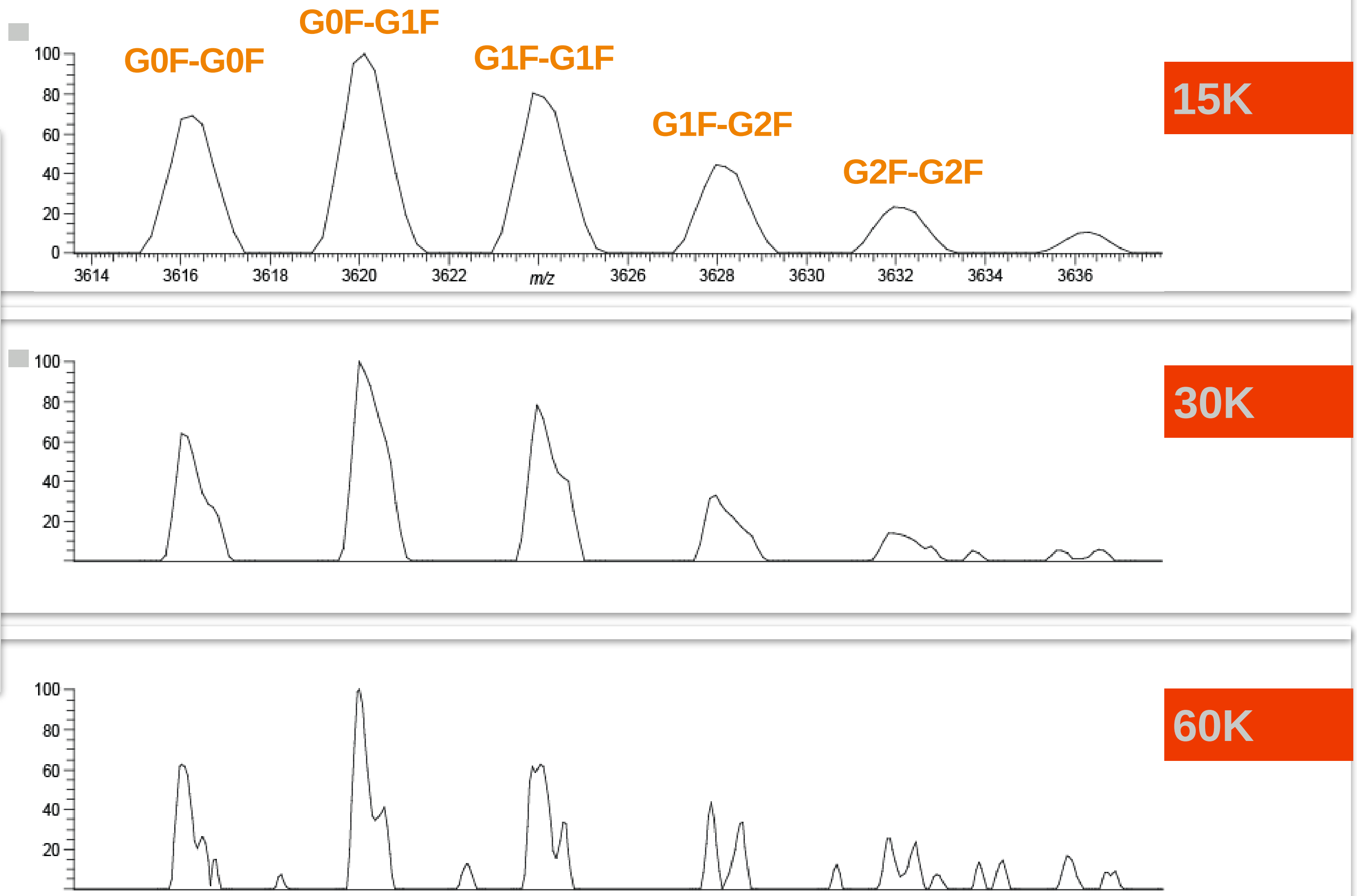
Varied mass shifts

41+

148873.05

41+: Higher Resolution Reveals Multiple Isoforms

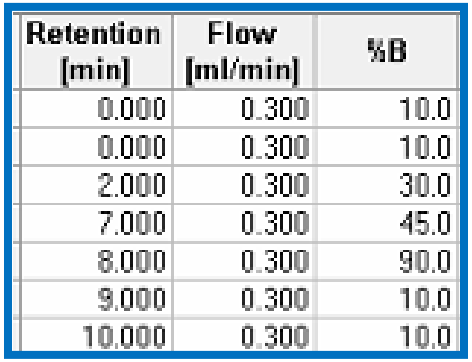
Unique to Orbitrap Analyzer



Chromatography

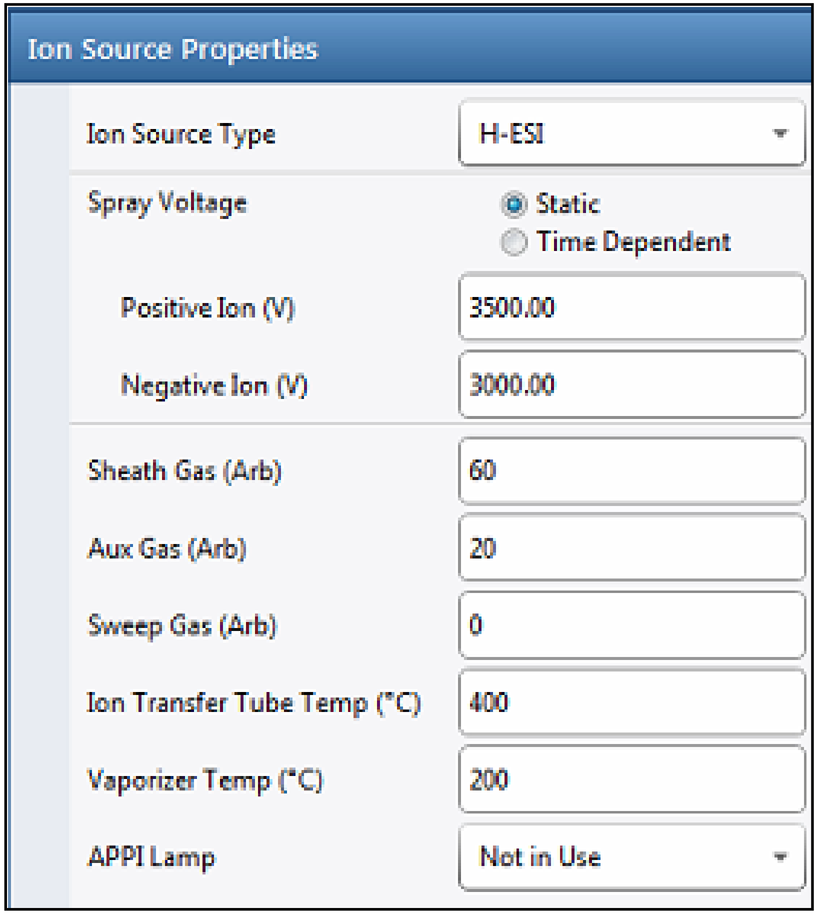
- Sigma mAb (p/n MSQC4)
- Sample Load: 1ug
- LC : Dionex Ultimate 3000
- Column: MabPac RP 2.1 x 10 cm

LC Gradient



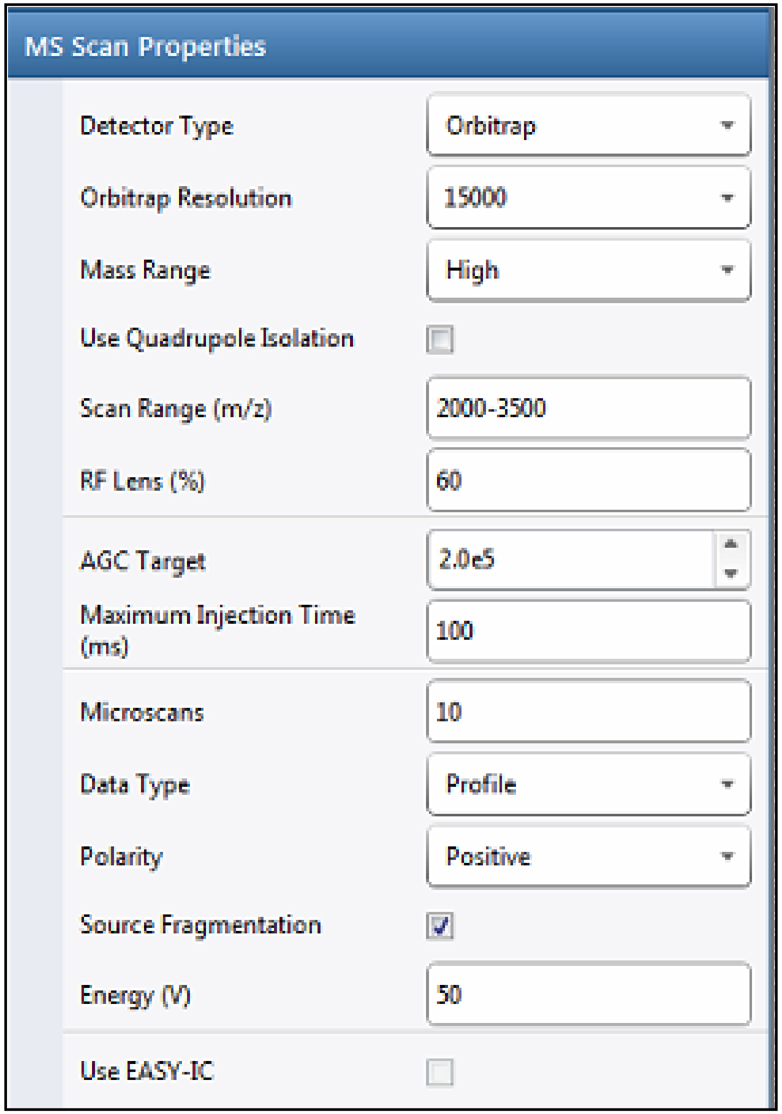
Retention [min]	Flow [ml/min]	%B
0.000	0.300	10.0
0.000	0.300	10.0
2.000	0.300	30.0
7.000	0.300	45.0
8.000	0.300	90.0
9.000	0.300	10.0
10.000	0.300	10.0

Instrument Method Full MS



Ion Source Properties

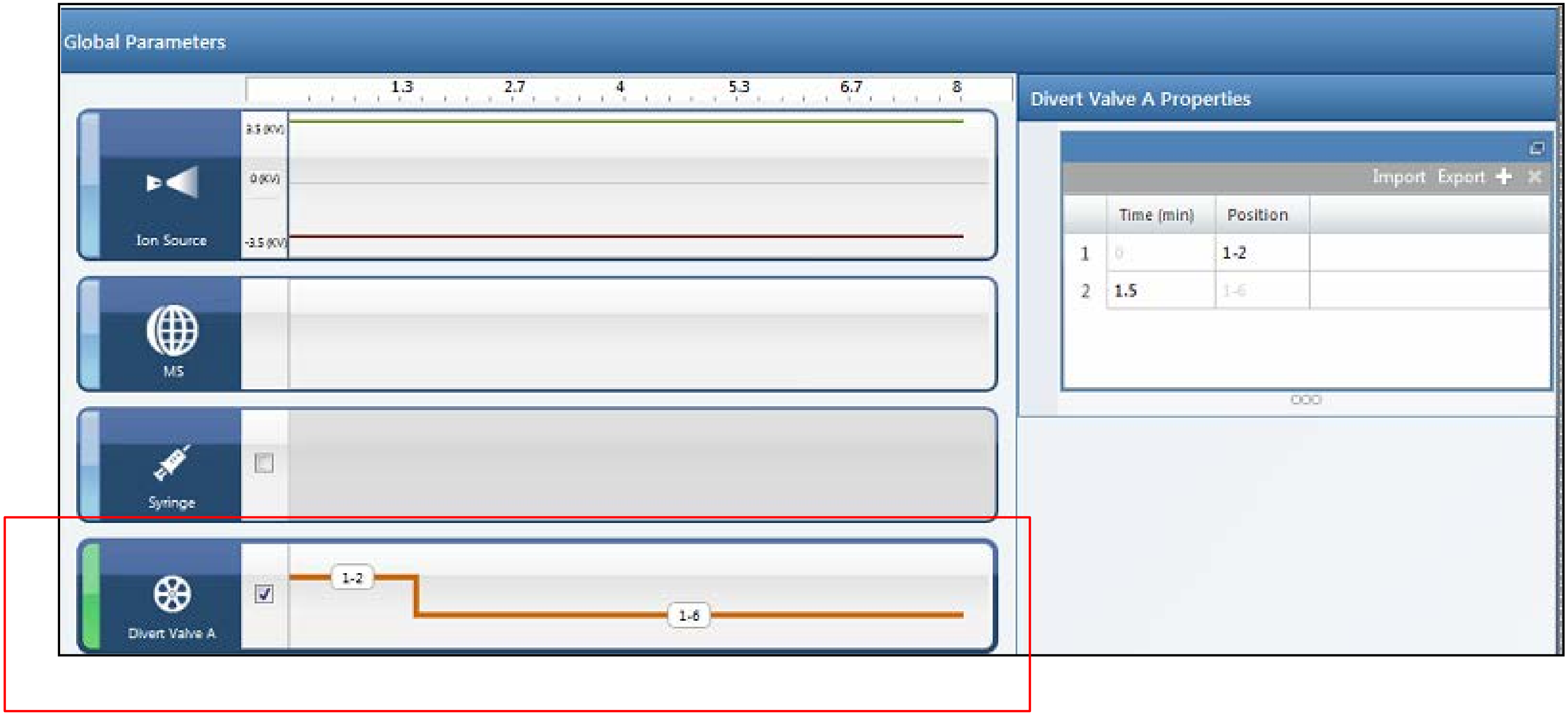
Ion Source Type	H-ESI
Spray Voltage	● Static ○ Time Dependent
Positive Ion (V)	3500.00
Negative Ion (V)	3000.00
Sheath Gas (Arb)	60
Aux Gas (Arb)	20
Sweep Gas (Arb)	0
Ion Transfer Tube Temp (°C)	400
Vaporizer Temp (°C)	200
APPI Lamp	Not in Use



MS Scan Properties

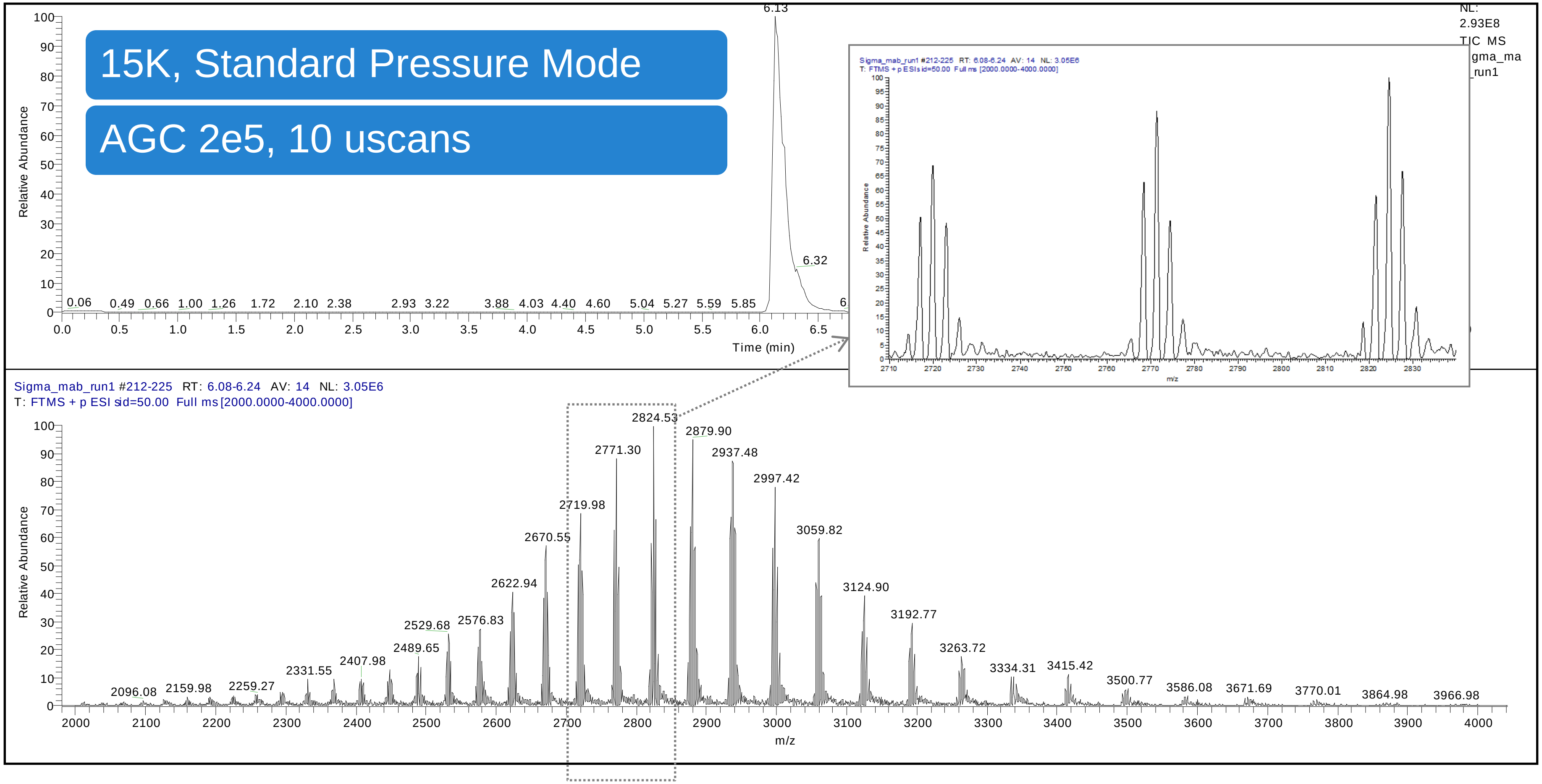
Detector Type	Orbitrap
Orbitrap Resolution	15000
Mass Range	High
Use Quadrupole Isolation	☐
Scan Range (m/z)	2000-3500
RF Lens (%)	60
AGC Target	2.0e5
Maximum Injection Time (ms)	100
Microscans	10
Data Type	Profile
Polarity	Positive
Source Fragmentation	☑
Energy (V)	50
Use EASY-IC	☐

Divert Valve Set-up



	Time (min)	Position
1	0	1-2
2	1.5	1-6

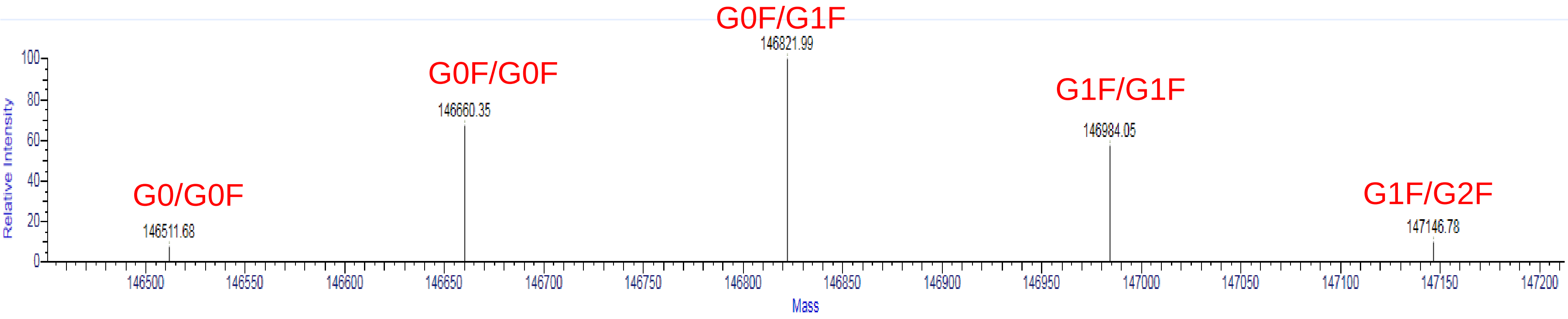
Sigma mAb LCMS: 15K Full MS

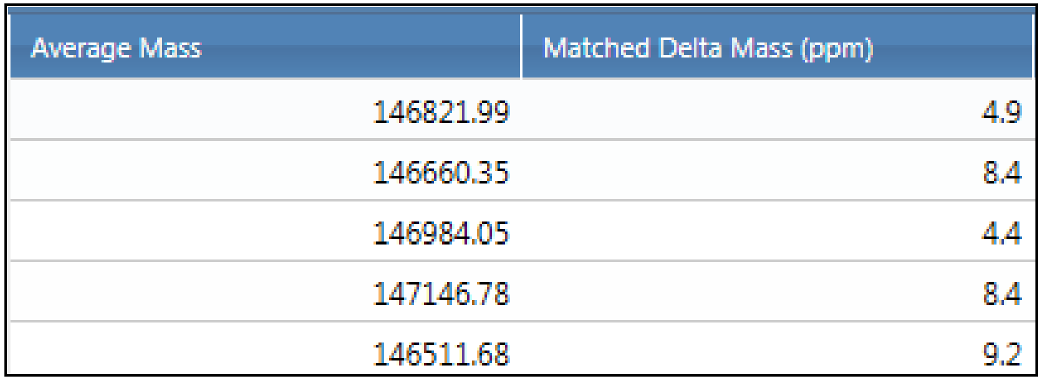


15K, Standard Pressure Mode

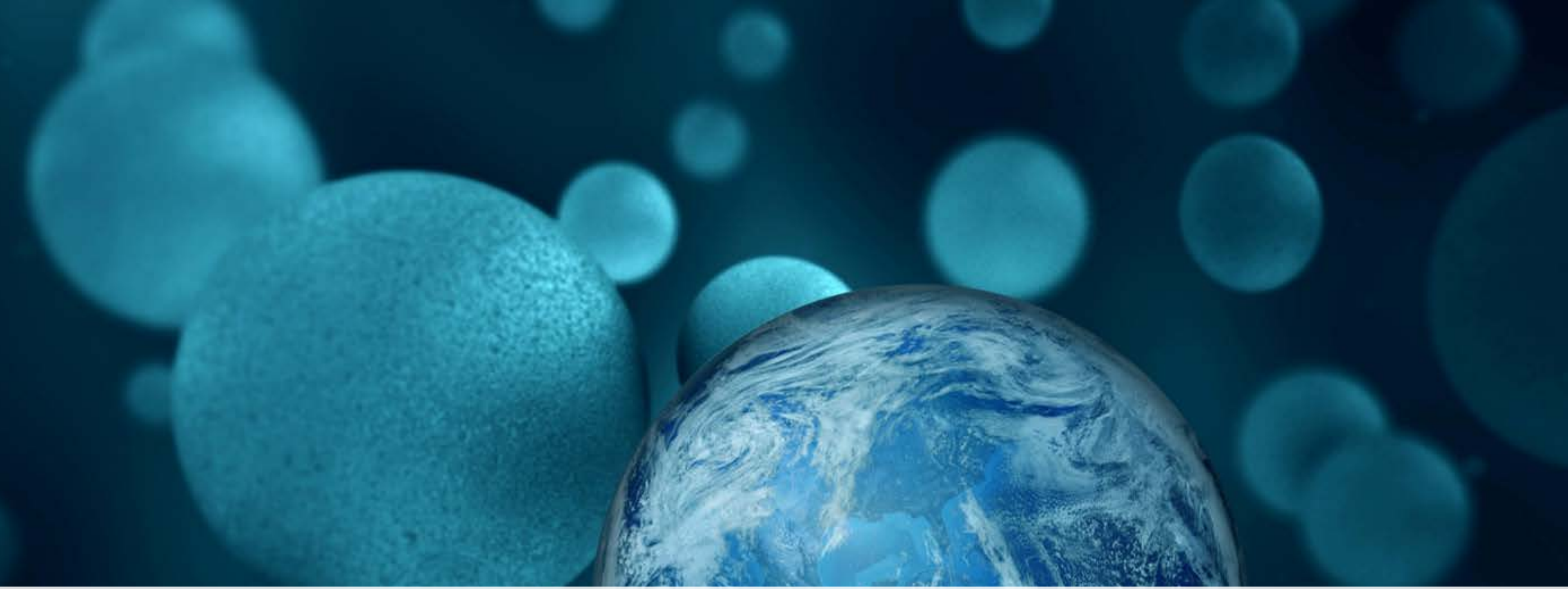
AGC 2e5, 10 uscans

BioPharma Finder Deconvolution Results





Average Mass	Matched Delta Mass (ppm)
146821.99	4.9
146660.35	8.4
146984.05	4.4
147146.78	8.4
146511.68	9.2



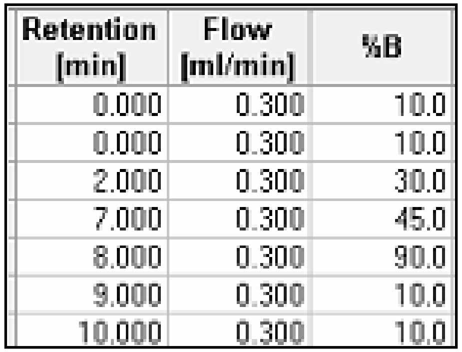
ThermoFisher
SCIENTIFIC

Targeted LC-MS Analysis: Reduced mAb

Chromatography:Flow Rate 300 ul/min

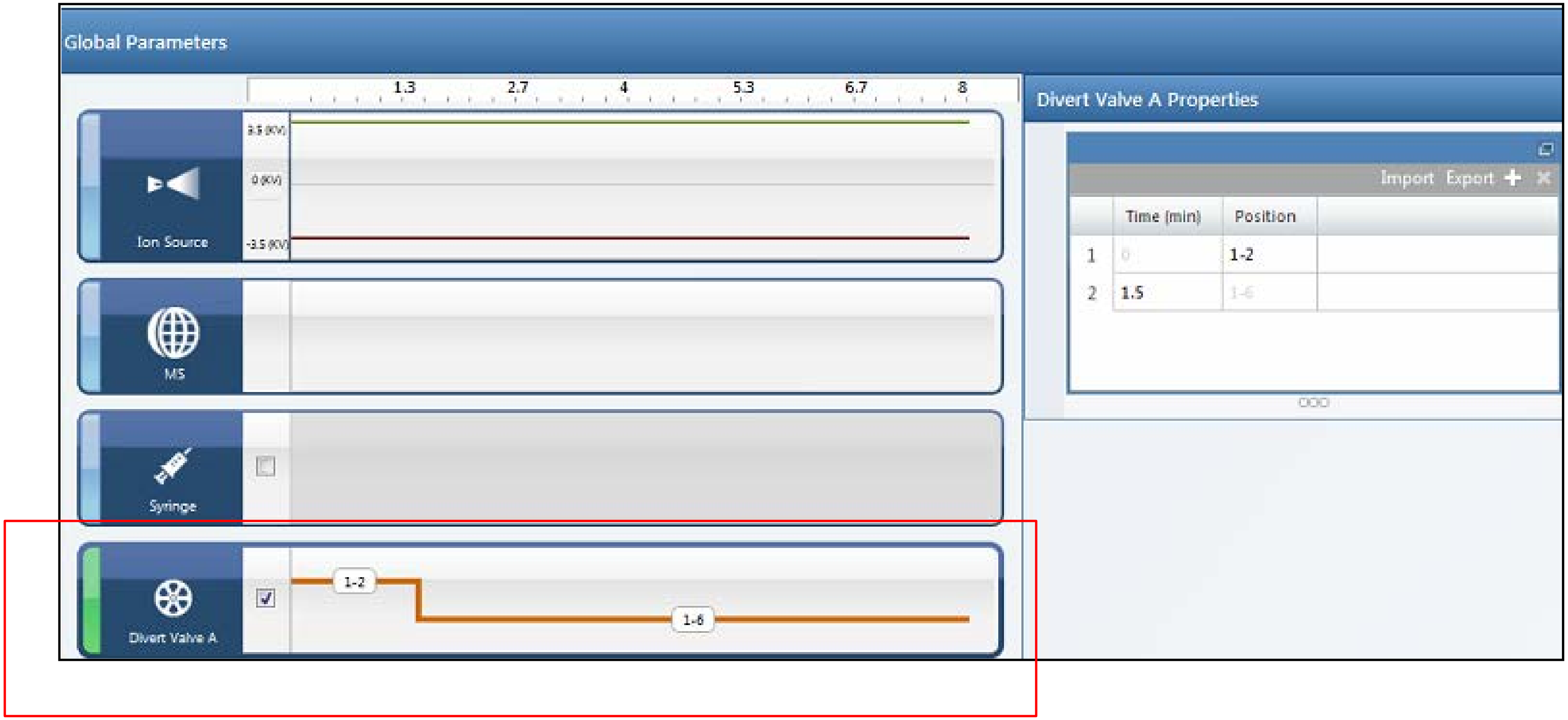
- Sample Load: 3ug
- Sigma mAb (p/n MSQC4) Reduced deglycosylated
- LC : Dionex Ultimate 3000 (High Flow)
- Column: MabPac RP 2.1 x 10 cm

LC Gradient



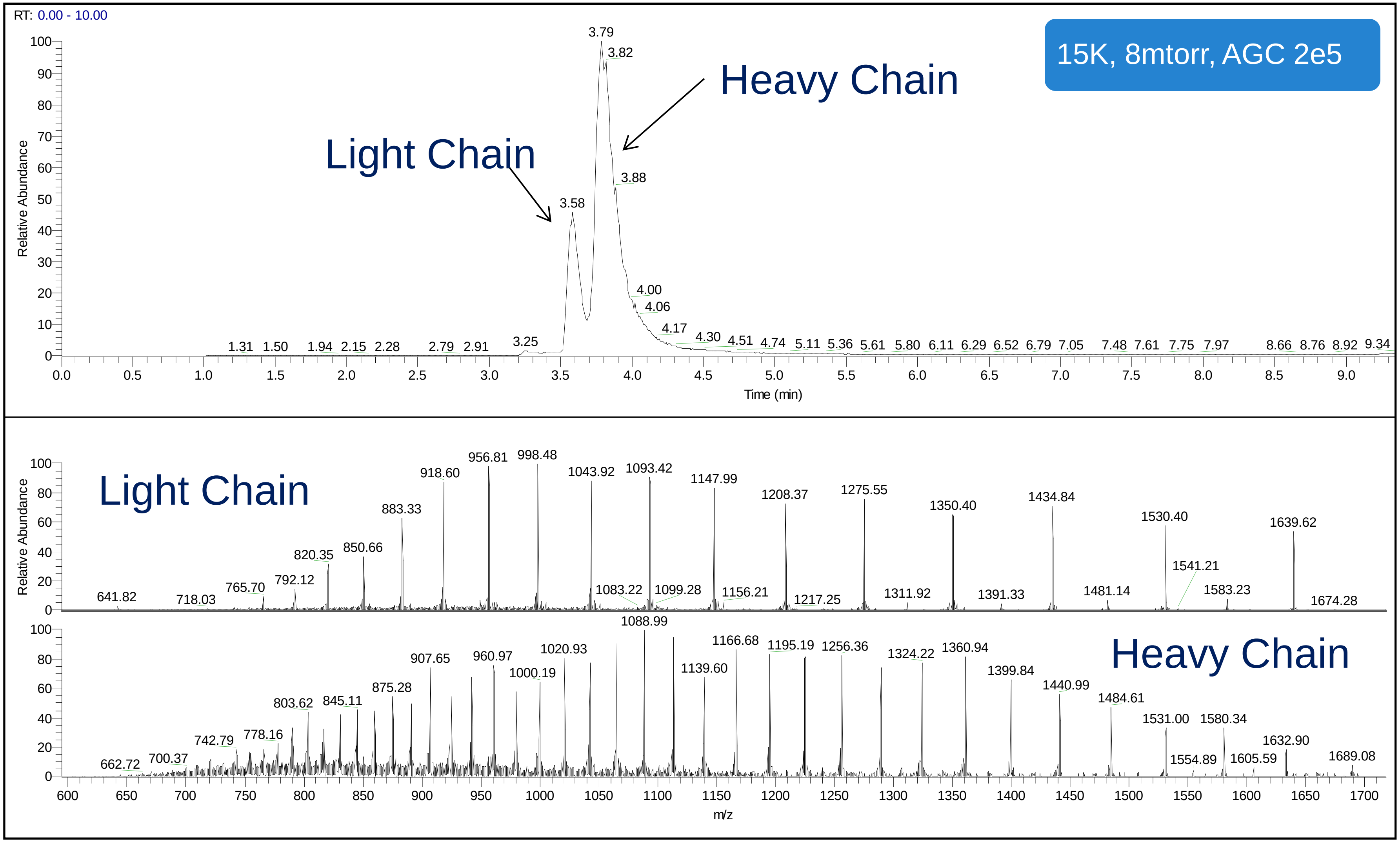
Retention [min]	Flow [ml/min]	%B
0.000	0.300	10.0
0.000	0.300	10.0
2.000	0.300	30.0
7.000	0.300	45.0
8.000	0.300	90.0
9.000	0.300	10.0
10.000	0.300	10.0

Divert Valve Set-up



	Time (min)	Position
1	0	1-2
2	1.5	1-6

Deglycosylated Reduced mAb

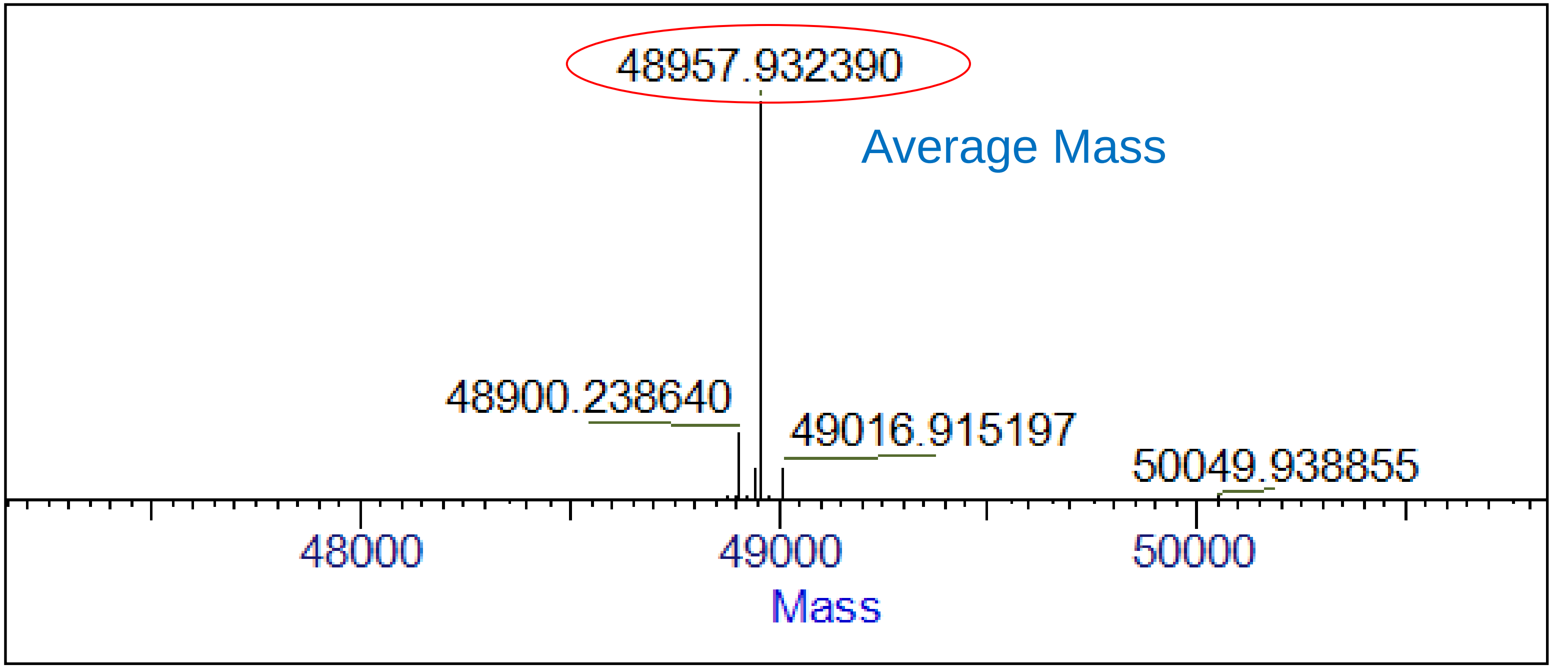


15K, 8mtorr, AGC 2e5

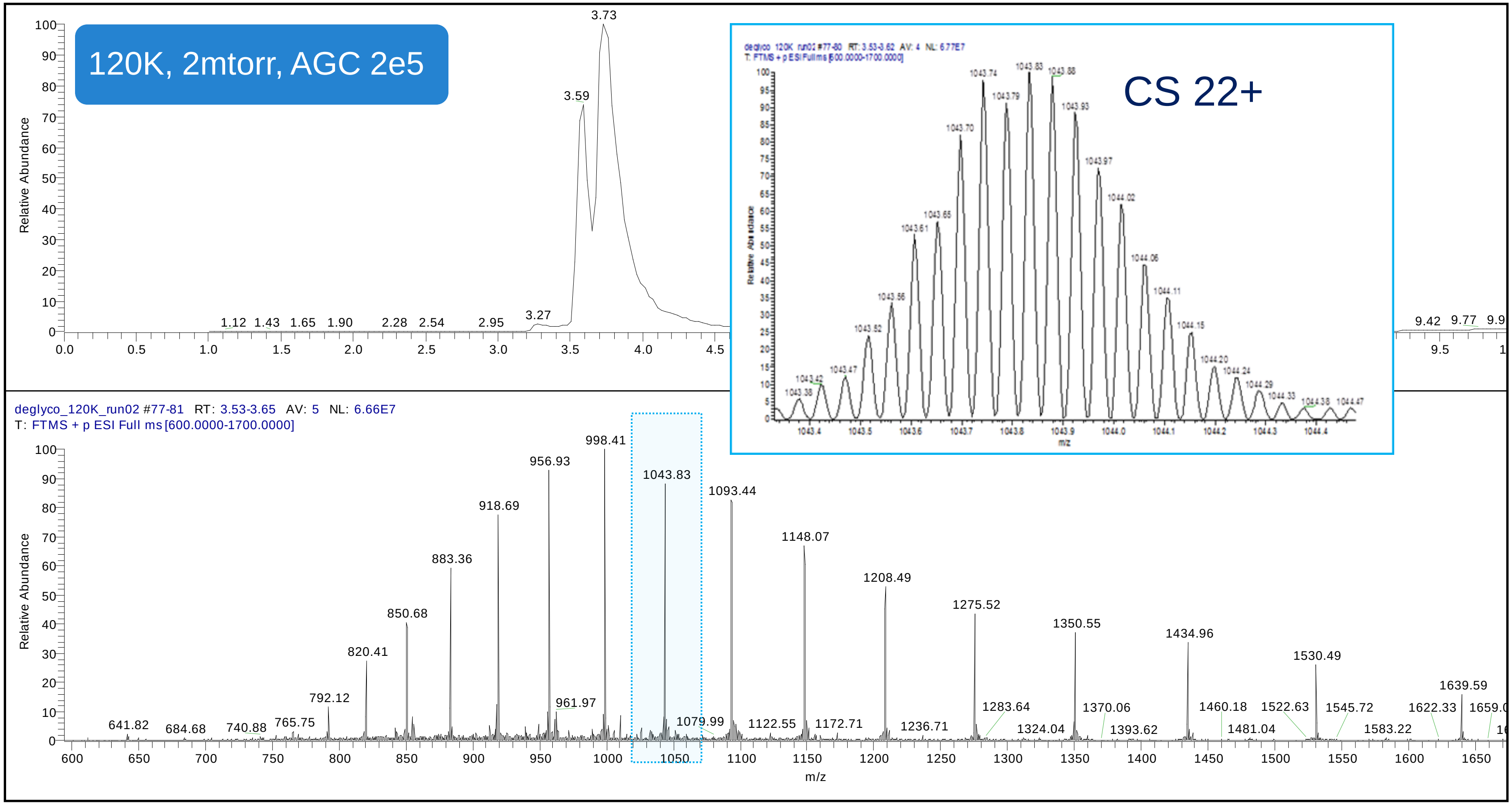
Light Chain

Heavy Chain

Heavy Chain: ReSpect Sliding Window Deconvolution



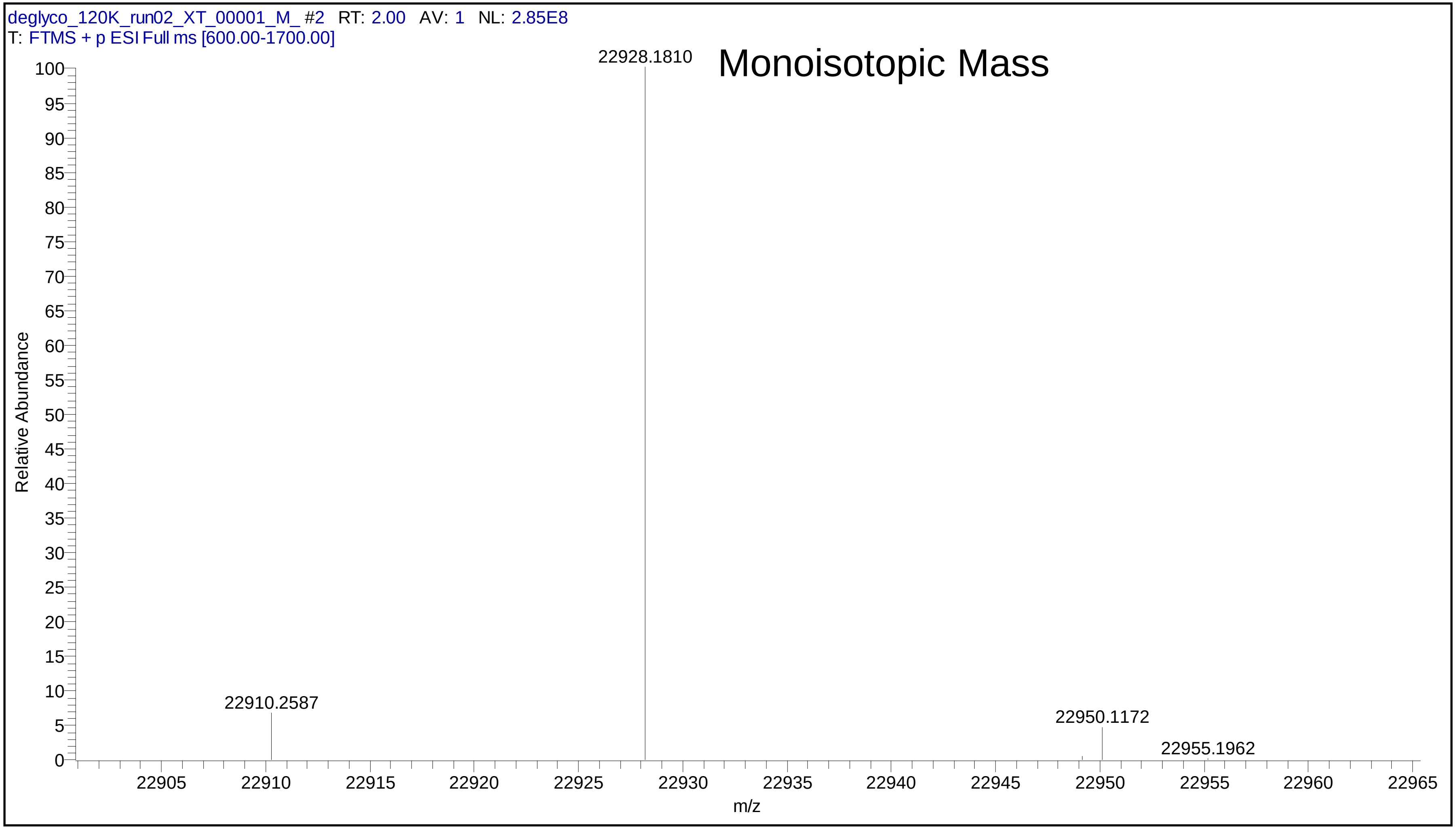
Light Chain Isotopic Resolution



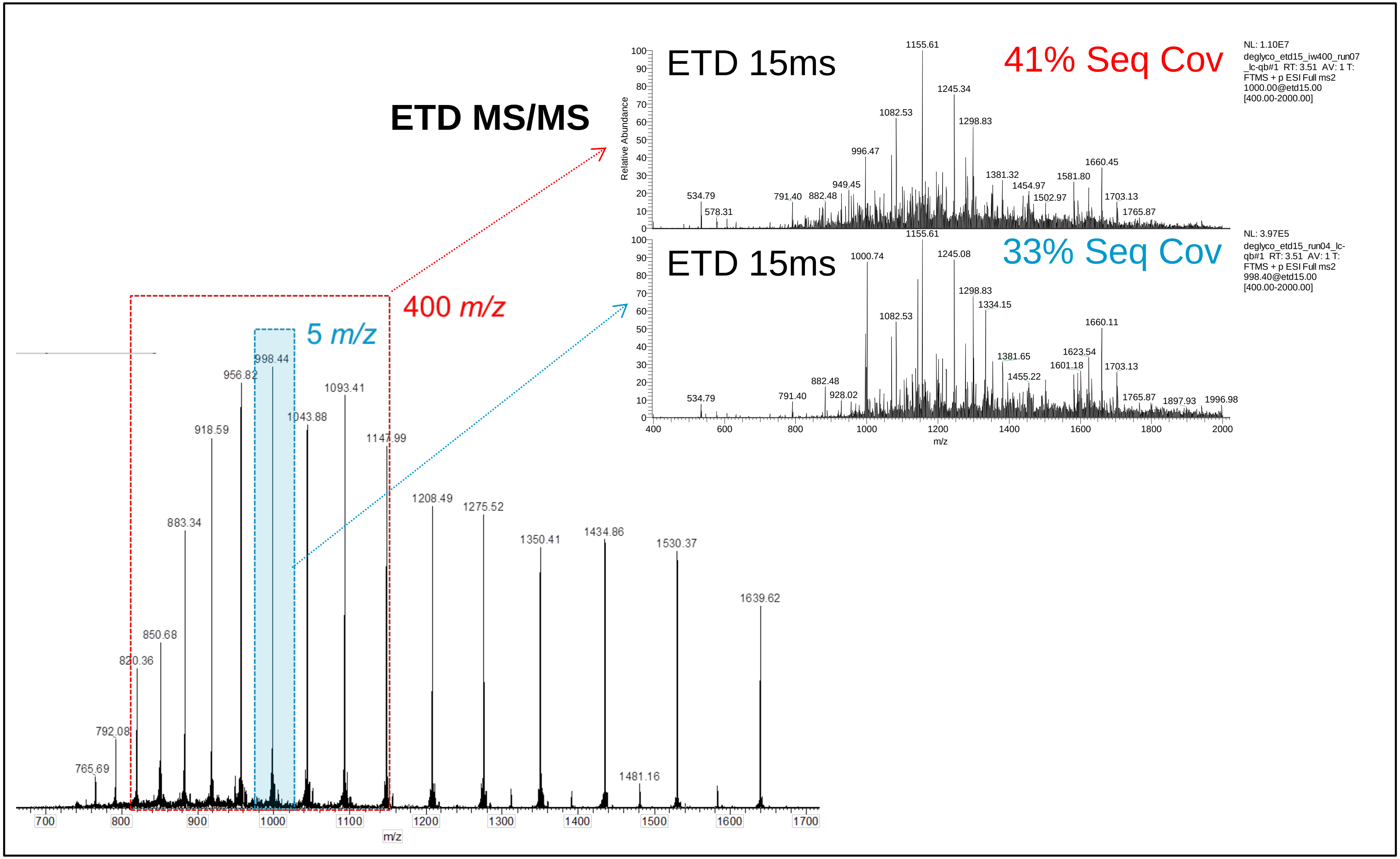
120K, 2mtorr, AGC 2e5

CS 22+

Light Chain: Xtract Deconvolution



Light Chain: Wide Isolation Window

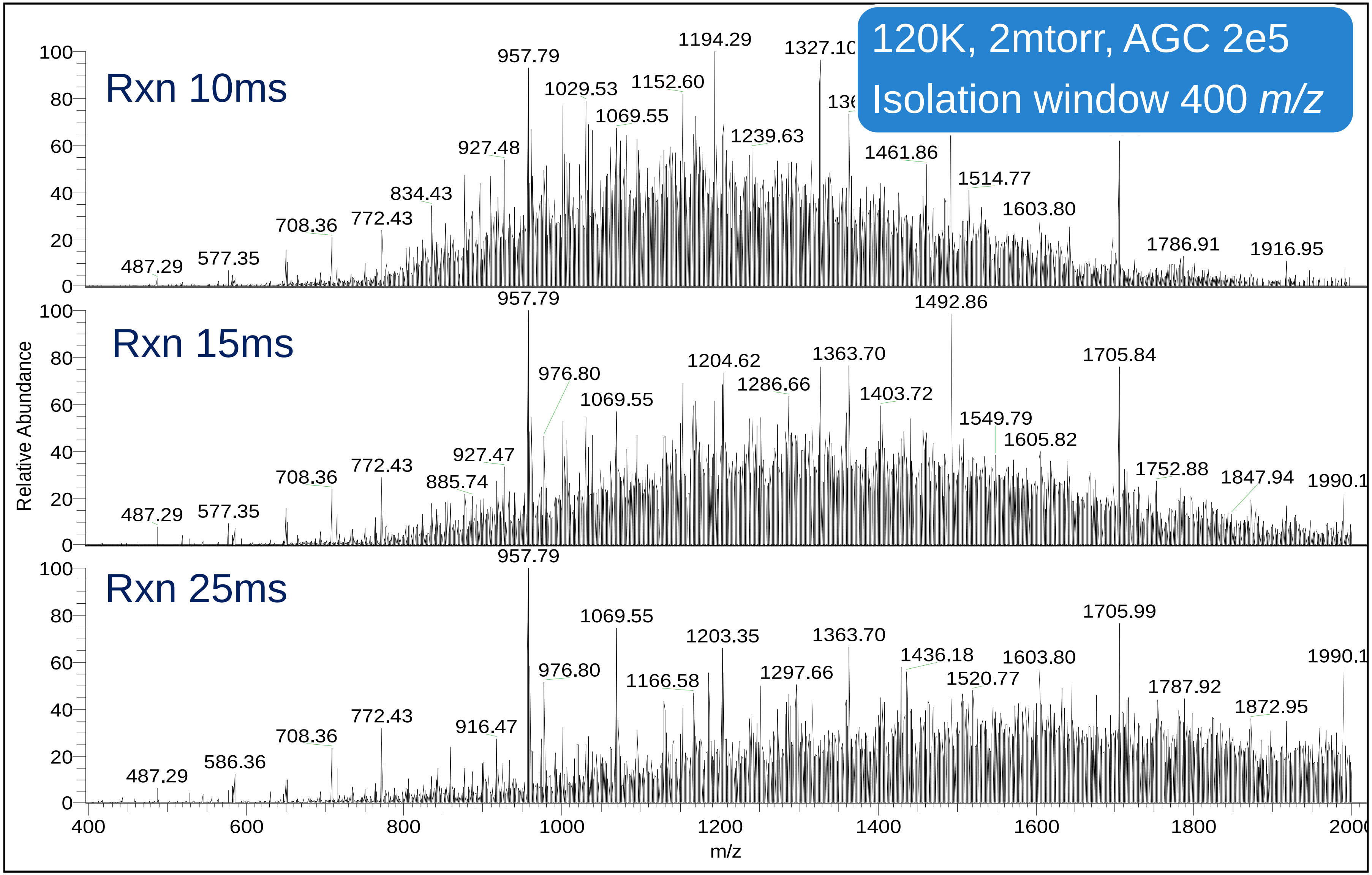


Light Chain: ETD & EThcD

65% Sequence Coverage

- EThcD: 15ms, 25ms
- ETD iw 400: 7ms, 15ms

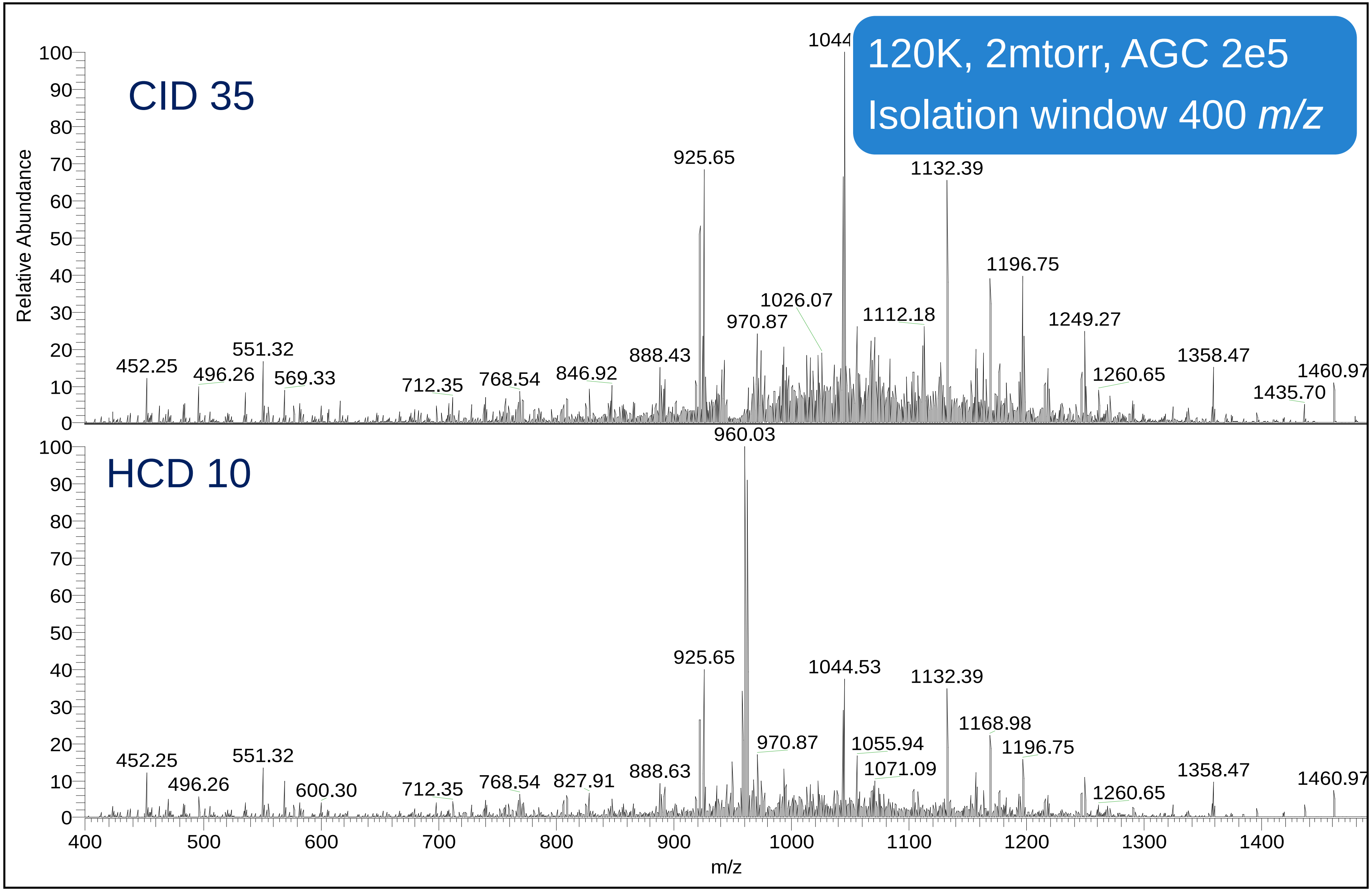
Heavy Chain: ETD Fragmentation



120K, 2mtorr, AGC 2e5

Isolation window 400 *m/z*

Heavy Chain:CID, HCD Fragmentation



120K, 2mtorr, AGC 2e5

Isolation window 400 *m/z*

Combined Seq Cov: ETD, HCD, CID

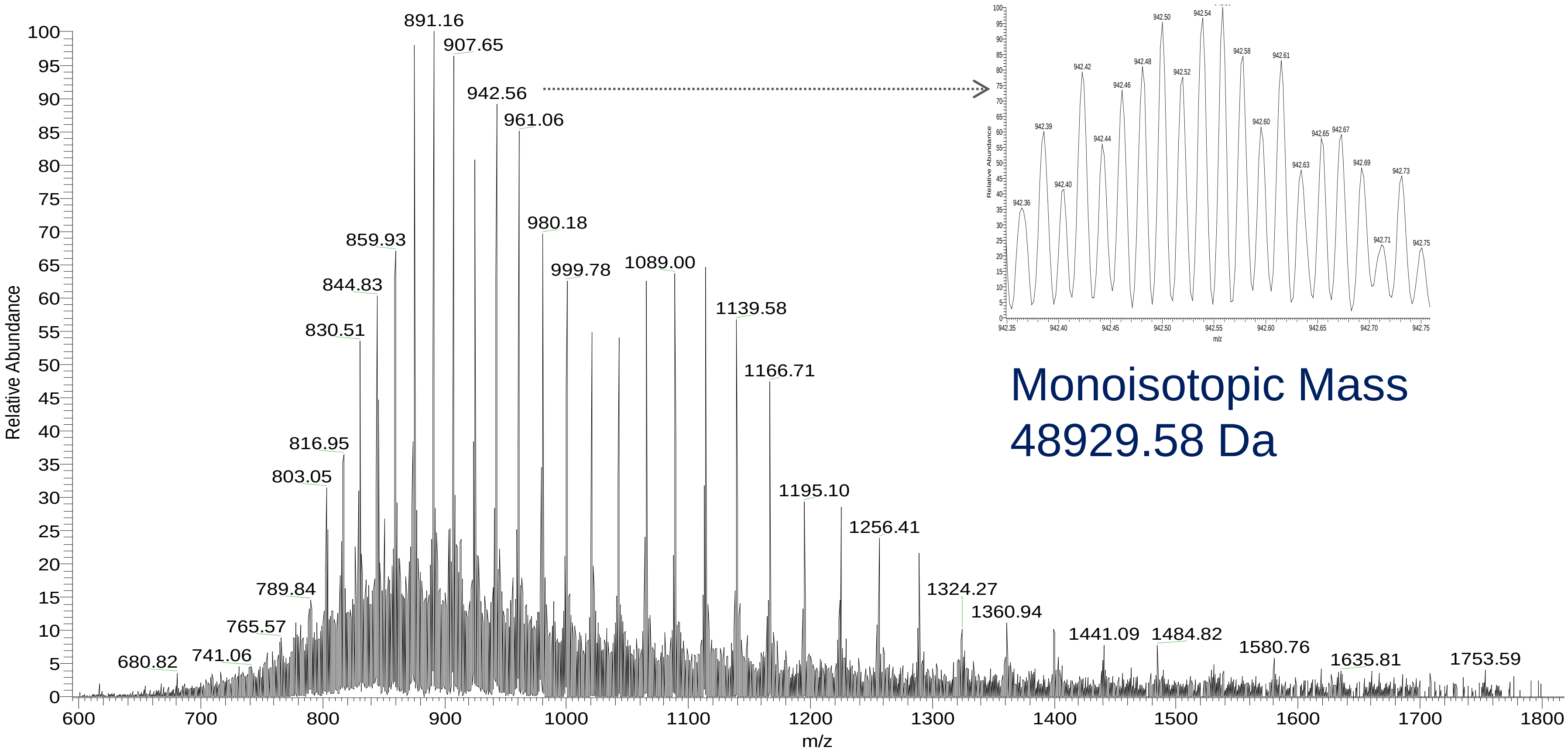
- ETD 10ms, 15ms, 25ms
- CID 35%, HCD 10%

25% Sequence Coverage

Heavy Chain Isotopic Resolution: LC-MS

240K, 1mtorr, AGC 2e5

8ug sample on column, 5uscans, spectrum averaged over LC-MS peak

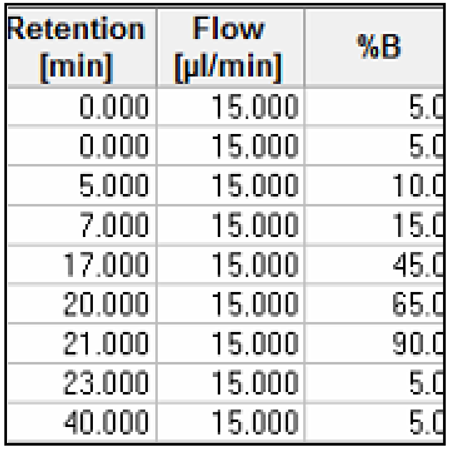


Monoisotopic Mass
48929.58 Da

Chromatography: Flow Rate 15ul/min

- Sample load: 500ng
- Sigma mAb (p/n MSQC4)
  Reduced deglycosylated
- LC : Dionex Ultimate 3000
  (Microflow)
- Column: ProSwift Monolithic RP-4H
  200um x 25cm

LC Gradient



Retention [min]	Flow [µl/min]	%B
0.000	15.000	5.0
0.000	15.000	5.0
5.000	15.000	10.0
7.000	15.000	15.0
17.000	15.000	45.0
20.000	15.000	65.0
21.000	15.000	90.0
23.000	15.000	5.0
40.000	15.000	5.0

Light Chain: Top-down Fragmentation

HCD 10: 22% Seq Cov

N QSALTQPRSVSGSPGQSVTISCTGT 25
26 SSDIGGYNFVSWYQQHPGKAPKLMI 50
51 YDATKRPSGVPDRFSGSKSGNTASL 75
76 TISGLQAEDEADYYCCSYAGDYTPG 100
101 VVFGGGTKLTVLGQPKAAPSVTLFP 125
126 PSSEELQANKATLVCLISDFYPGAV 150
151 TVAWKADSSPVKAGVETTTPSKQSN 175
176 NKYAASSYLSLTPEQWKSHRSYSCQ 200
201 VTHEGSTVEKTVAPTECS C

CID 30: 22% Seq Cov

N QSALTQPRSVSGSPGQSVTISCTGT 25
26 SSDIGGYNFVSWYQQHPGKAPKLMI 50
51 YDATKRPSGVPDRFSGSKSGNTASL 75
76 TISGLQAEDEADYYCCSYAGDYTPG 100
101 VVFGGGTKLTVLGQPKAAPSVTLFP 125
126 PSSEELQANKATLVCLISDFYPGAV 150
151 TVAWKADSSPVKAGVETTTPSKQSN 175
176 NKYAASSYLSLTPEQWKSHRSYSCQ 200
201 VTHEGSTVEKTVAPTECS C

ETD 15ms: 46% Seq Cov

N QSALTQPRSVSGSPGQSVTISCTGT 25
26 SSDIGGYNFVSWYQQHPGKAPKLMI 50
51 YDATKRPSGVPDRFSGSKSGNTASL 75
76 TISGLQAEDEADYYCCSYAGDYTPG 100
101 VVFGGGTKLTVLGQPKAAPSVTLFP 125
126 PSSEELQANKATLVCLISDFYPGAV 150
151 TVAWKADSSPVKAGVETTTPSKQSN 175
176 NKYAASSYLSLTPEQWKSHRSYSCQ 200
201 VTHEGSTVEKTVAPTECS C

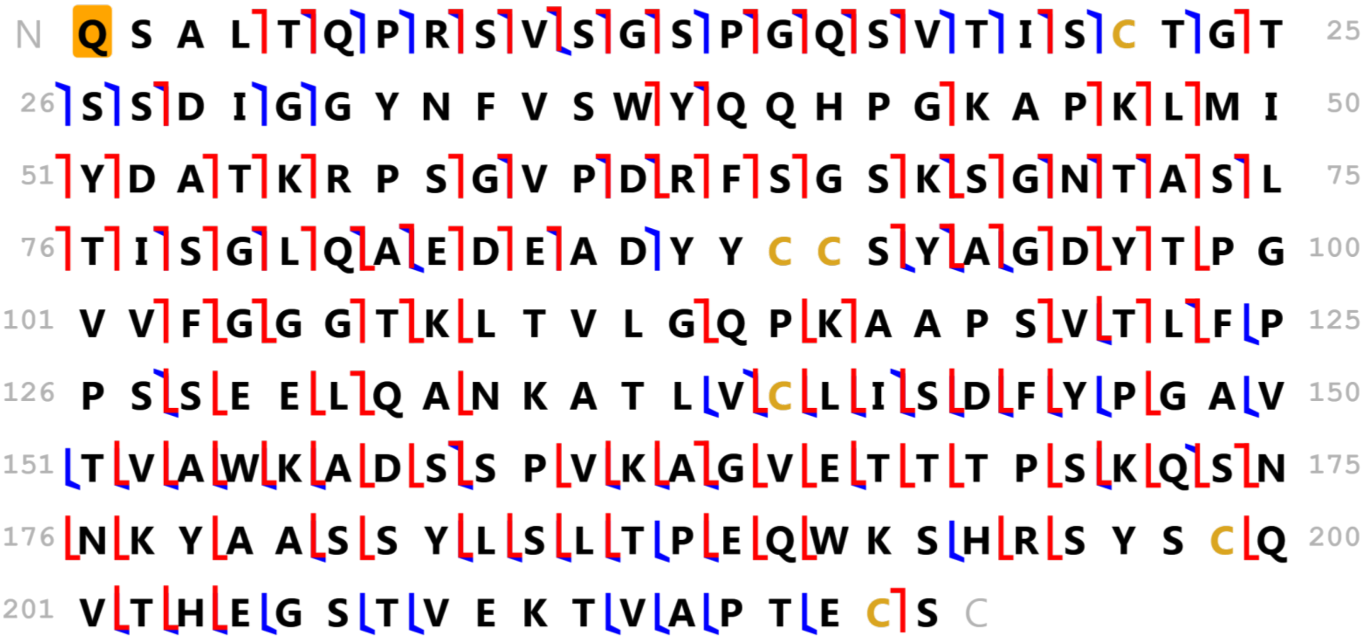
ETD 25ms: 44% Seq Cov

N QSALTQPRSVSGSPGQSVTISCTGT 25
26 SSDIGGYNFVSWYQQHPGKAPKLMI 50
51 YDATKRPSGVPDRFSGSKSGNTASL 75
76 TISGLQAEDEADYYCCSYAGDYTPG 100
101 VVFGGGTKLTVLGQPKAAPSVTLFP 125
126 PSSEELQANKATLVCLISDFYPGAV 150
151 TVAWKADSSPVKAGVETTTPSKQSN 175
176 NKYAASSYLSLTPEQWKSHRSYSCQ 200
201 VTHEGSTVEKTVAPTECS C

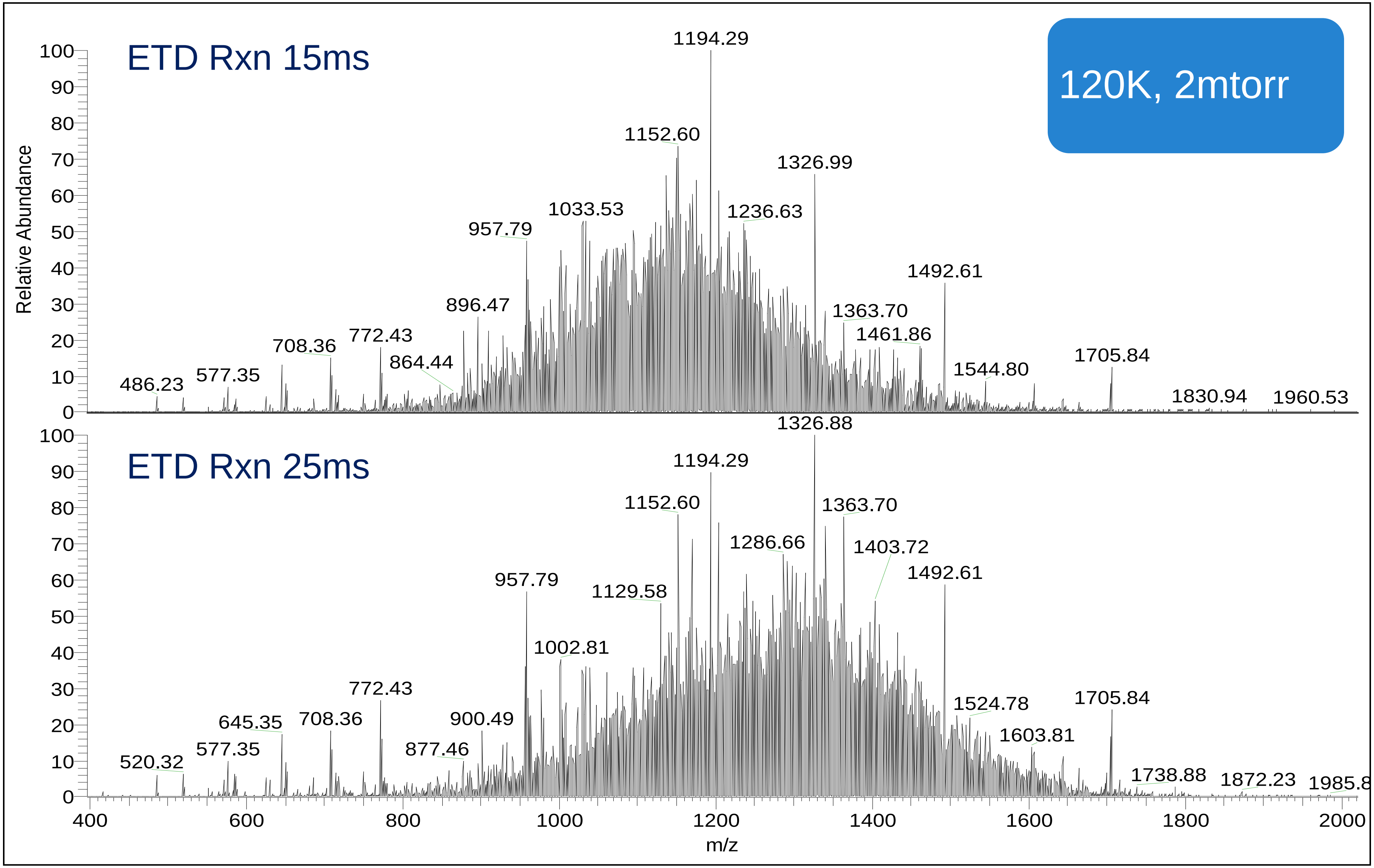
Light Chain Combined Coverage

70% Sequence Coverage

- ETD 15ms, 25ms
- HCD 10
- CID 30



Heavy Chain: ETD HD Fragmentation (CS 52+)

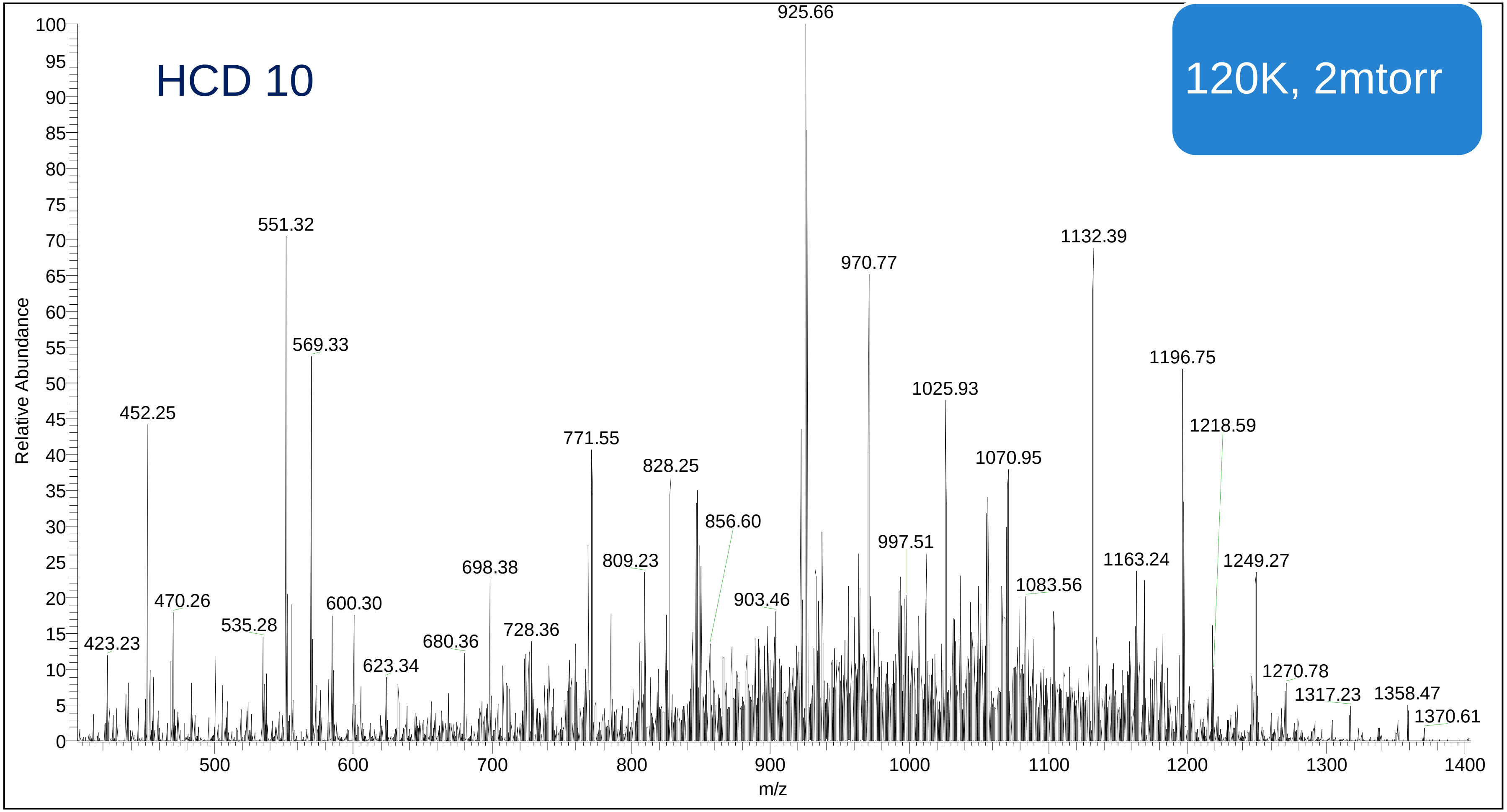


120K, 2mtorr

ETD Rxn 15ms

ETD Rxn 25ms

Heavy Chain HCD Fragmentation (CS 52+)



HCD 10

120K, 2mtorr

Combined Sequence Coverage

31% Sequence Coverage

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

- ETD 15ms, 25ms
- HCD 10%