# The exclusive ion activation arsenal of the Omnitrap platform illustrated Applications in top down and bottom up proteomics

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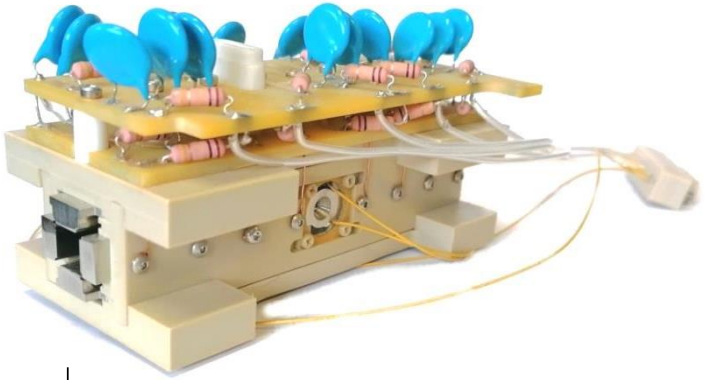
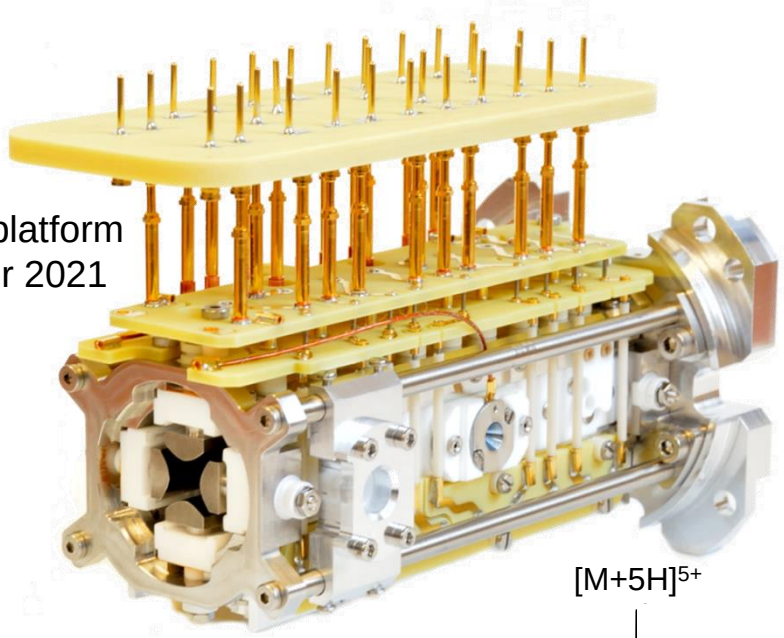
# The Omnitrap™ platform – Past & Present

Linear Ion Trap
June 2015



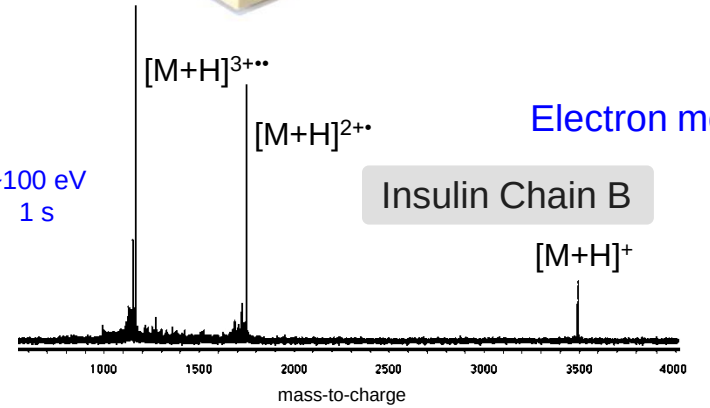
Omnitrap platform
November 2021



Electron meta-Ionization

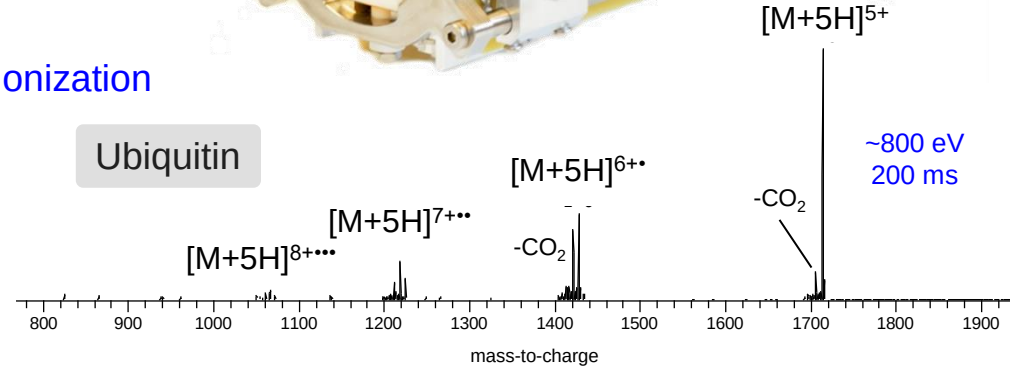
~100 eV
1 s

Insulin Chain B

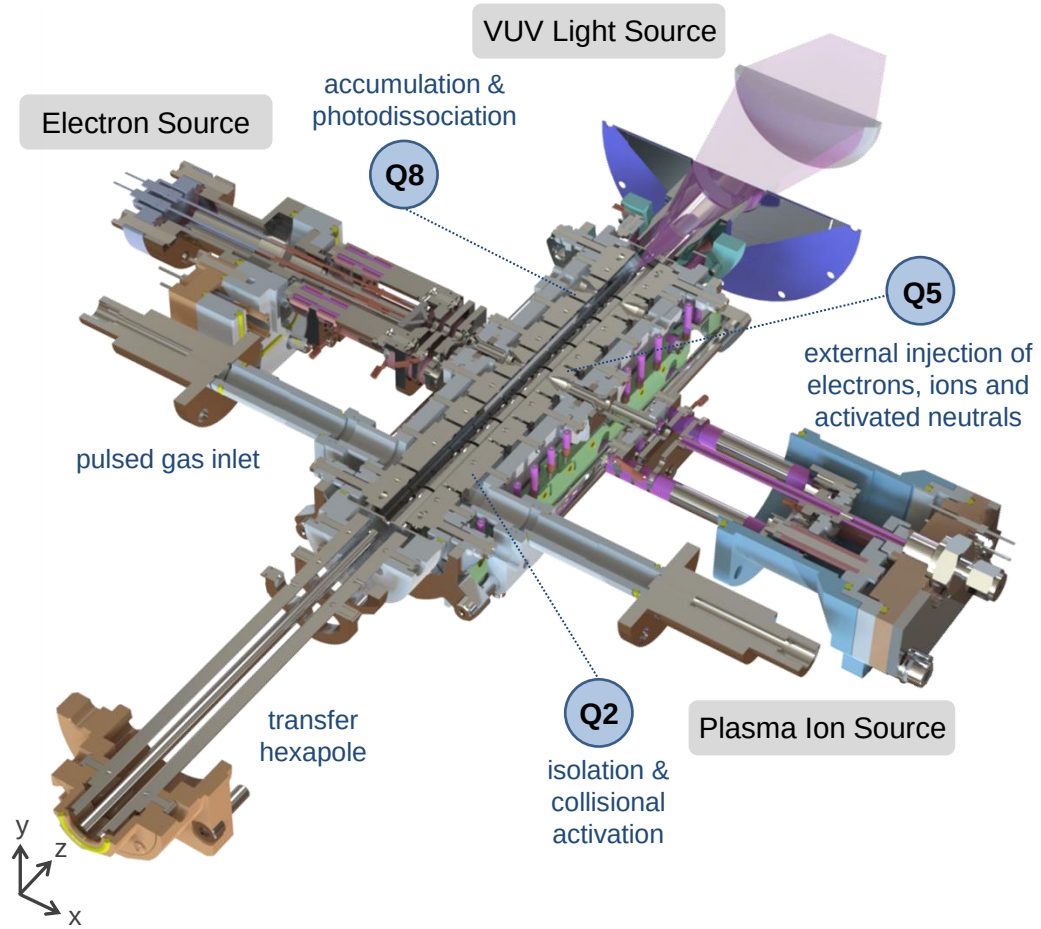


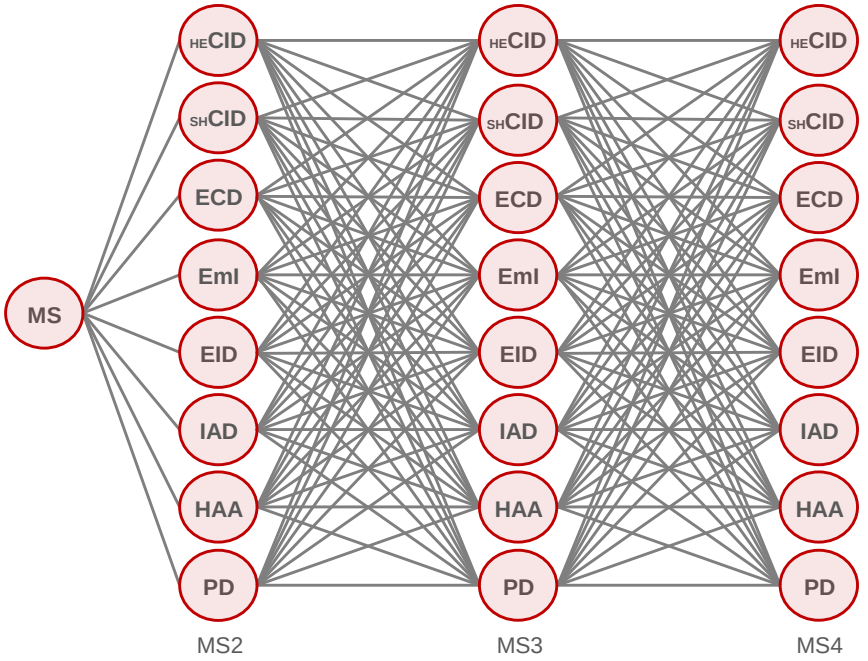

Ubiquitin

~800 eV
200 ms

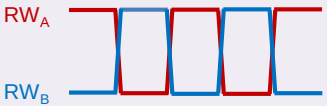


# The Omnitrap™ platform – Design aspects & Activation network

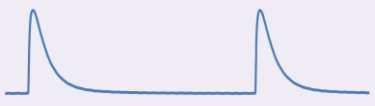


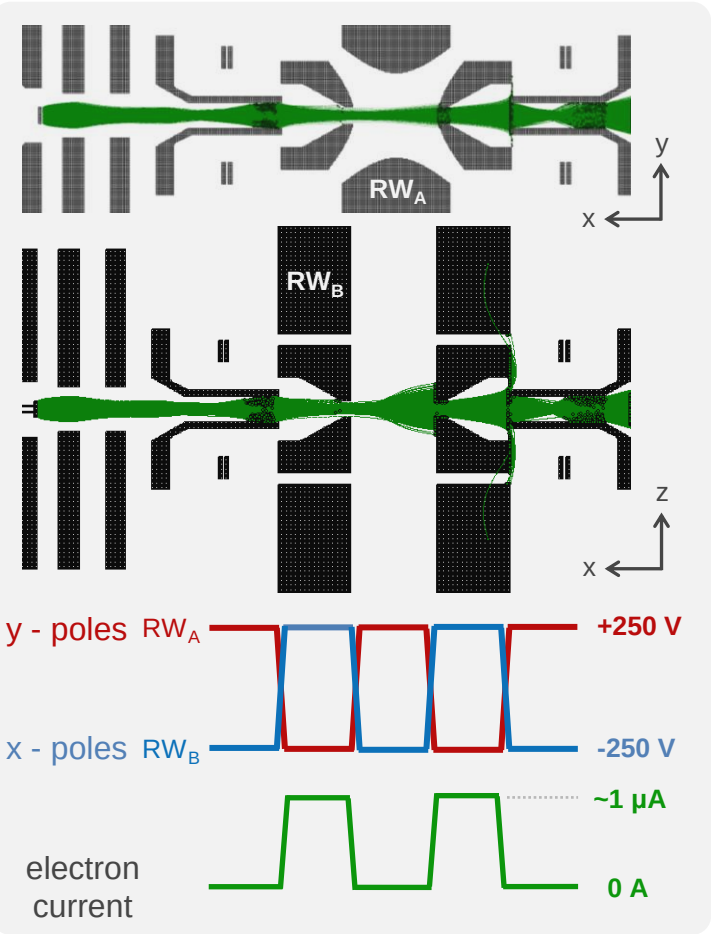

## Rectangular Waveforms

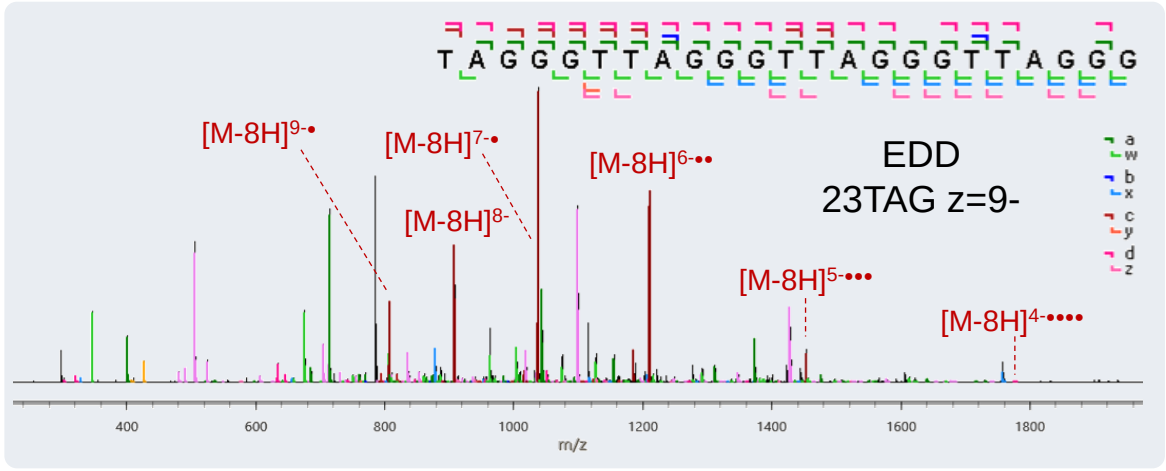


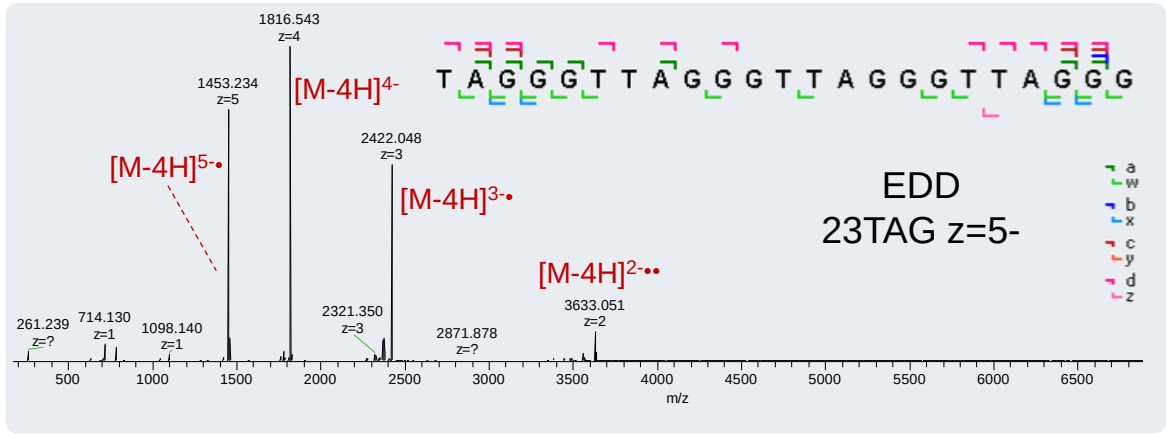

## Dynamic Pressure Control



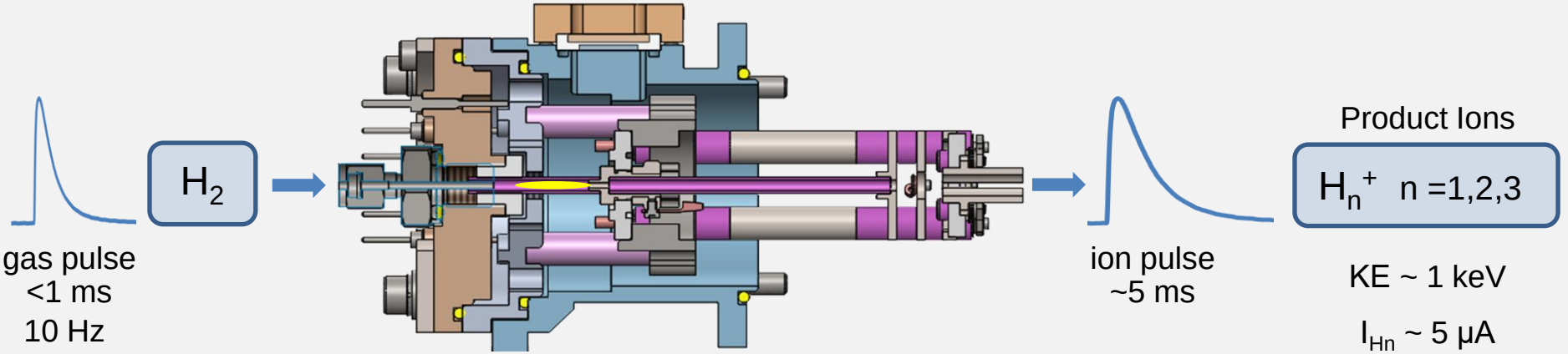
# Electron Source

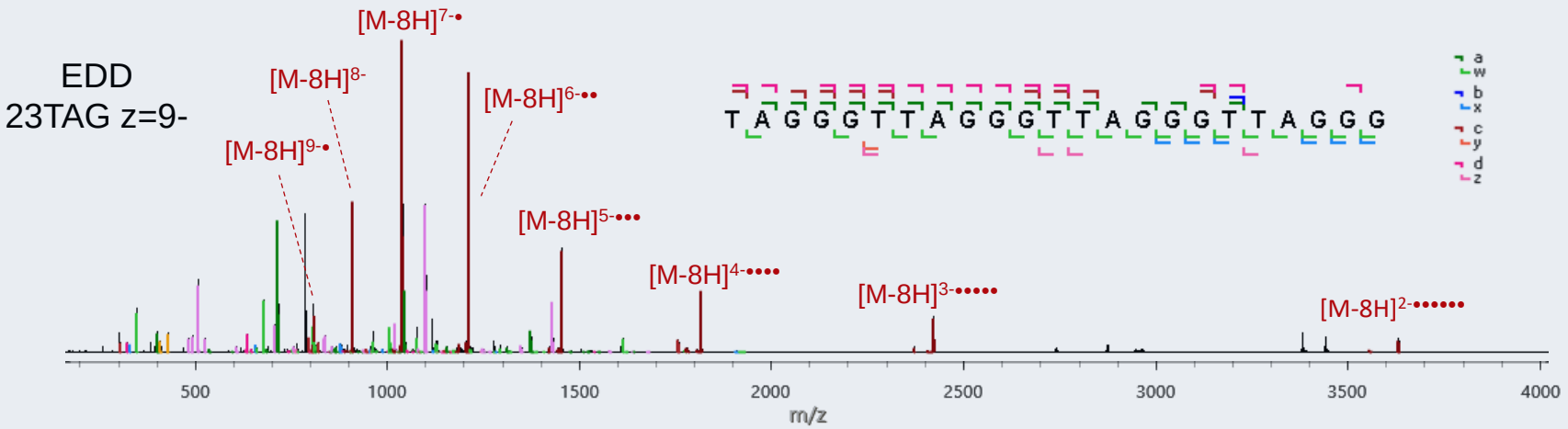


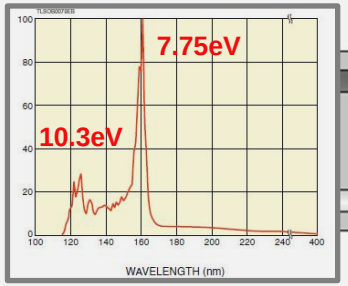



# Plasma Ion Source

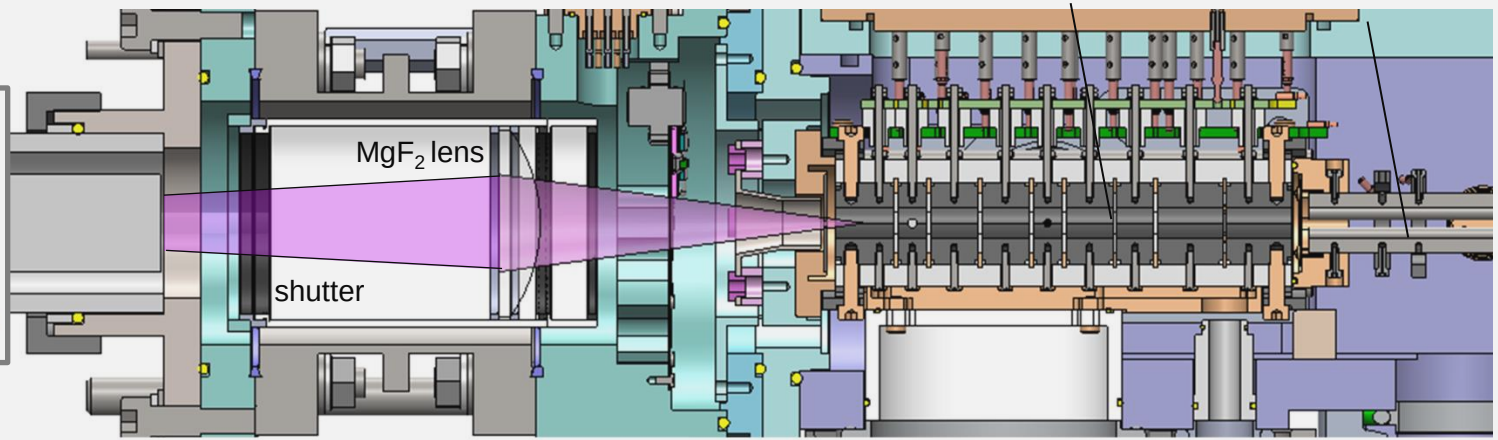


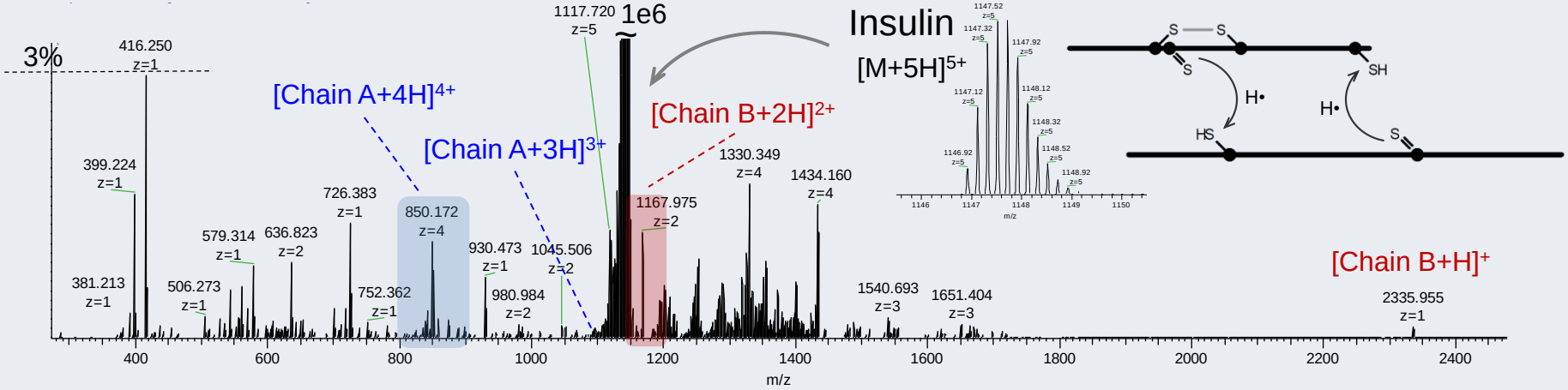



# VUV Light Source



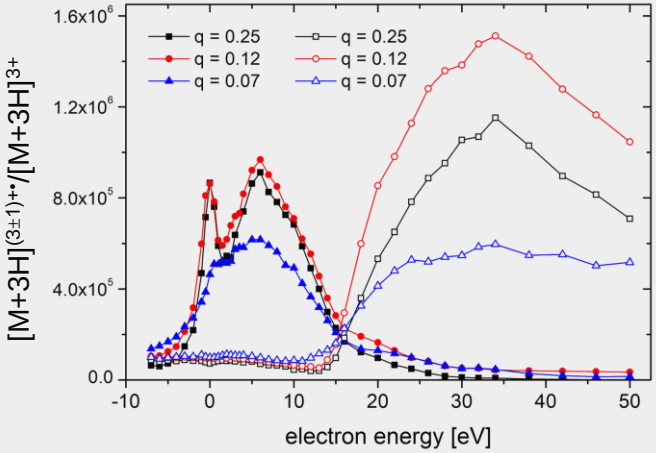

Hamamatsu $D_2$ lamp



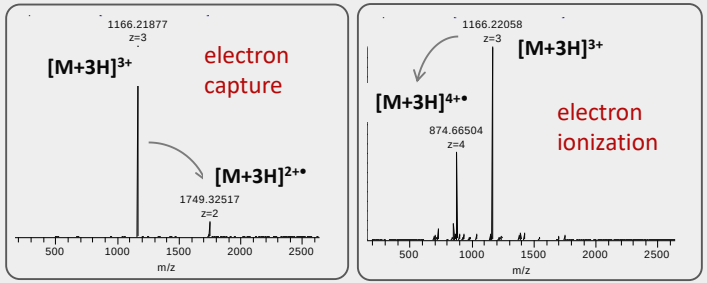
**submitted to JASMS**

# The Omnitrap Platform: a Versatile Segmented Linear Ion Trap for Multidimensional Multiple-Stage Tandem MS

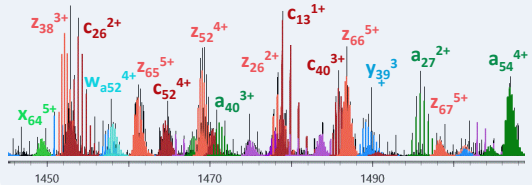


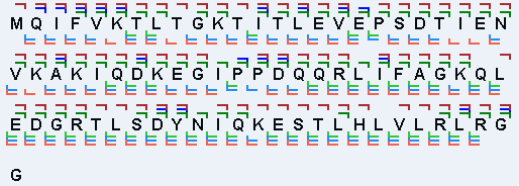

**Electron Capture:** $[M+3H]^{3+} + e_s^- \longrightarrow [M+3H]^{2+\bullet}$

**Electron Ionization:** $[M+3H]^{3+} + e_f^- \longrightarrow [M+3H]^{4+\bullet} + e_s^- + e_f^-$

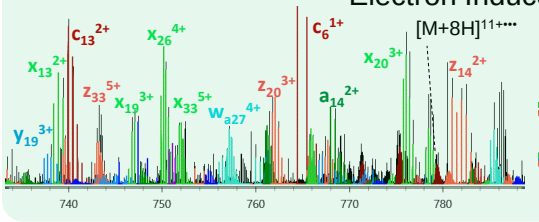


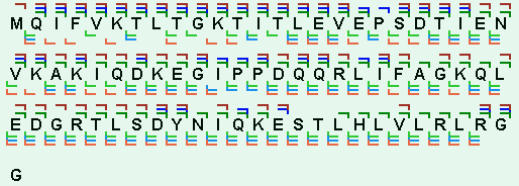

## Electron Capture Dissociation



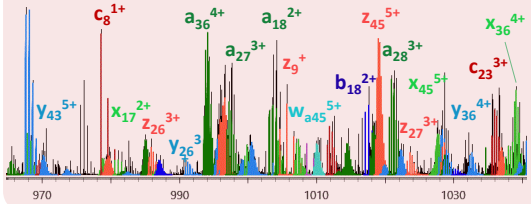



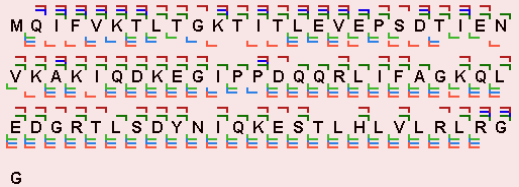

## Electron Induced Dissociation



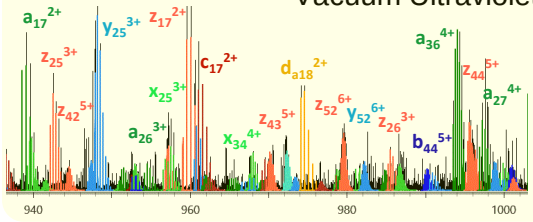



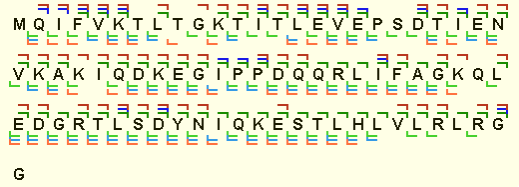

## Hydrogen Ion Activated Dissociation




## Vacuum Ultraviolet Photodissociation

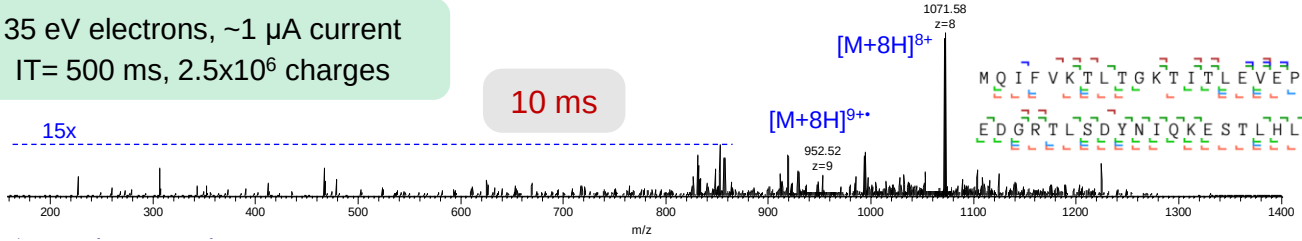


# Electron Induced Dissociation of ubiquitin $[M+8H]^{8+}$

35 eV electrons, ~1 μA current
IT= 500 ms, 2.5x$10^6$ charges

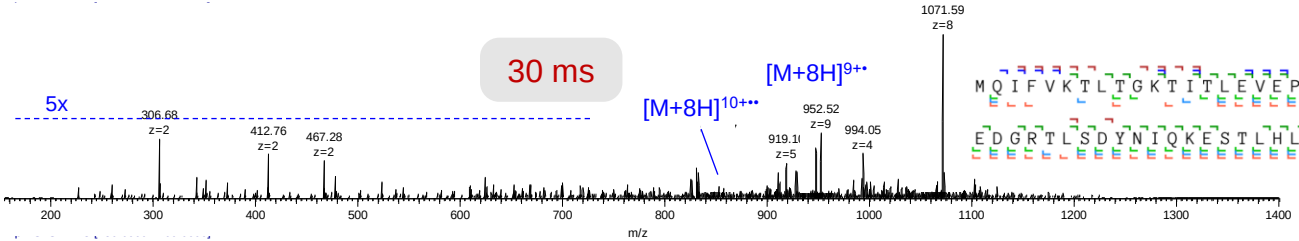
## 10 ms





93.3%

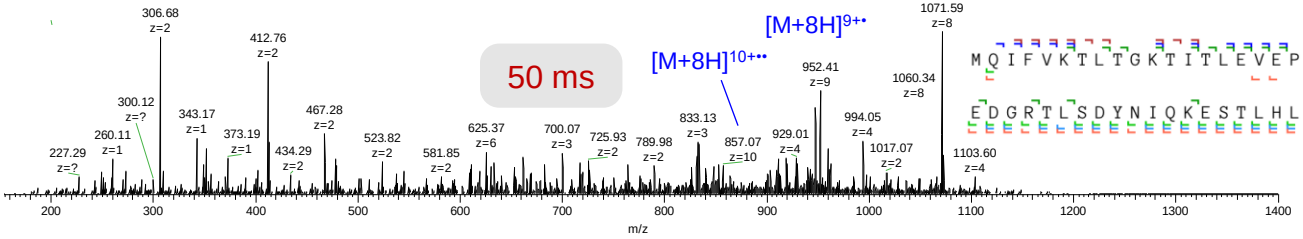
## 30 ms





97.3%

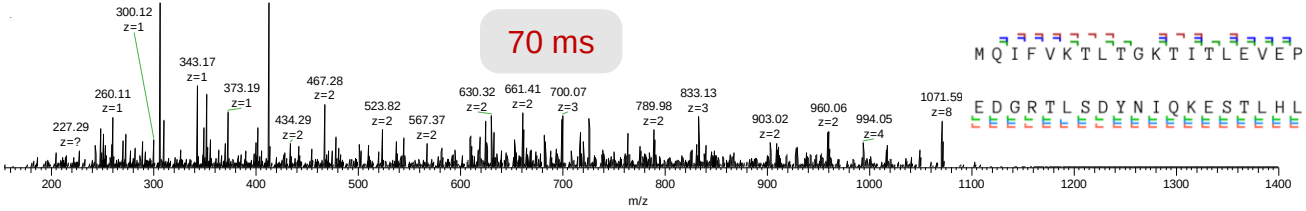
## 50 ms





93.3%

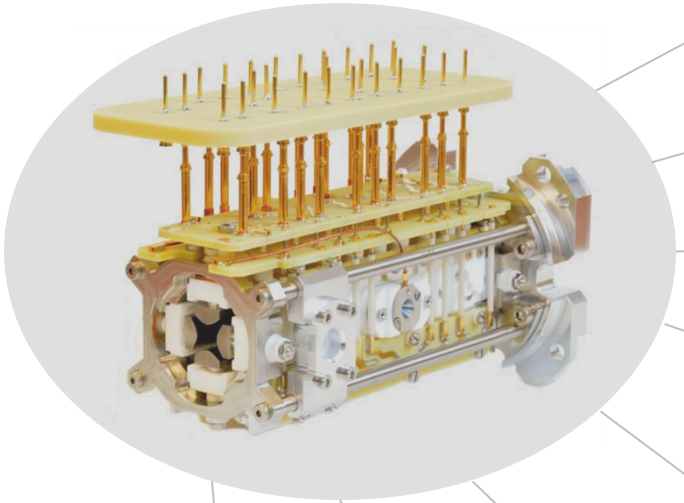
## 70 ms





89.3%

# New Applications based on the Omnitrap platform



- **Top Down (MSn)** — Antibody sequencing — INSTITUT PASTEUR
- **Bottom-up (ExD)** — Peptide sequencing — Karolinska Institutet
- **Bottom-up (IR/UV)** — Speed / PTMs — The Rosalind Franklin Institute
- **Glycans (EID)** — Structural characterization — BU Boston University School of Medicine
- **SS bond dissociation (VUV)** — Protein structure & sequencing
- **Oligonucleotides (EDD)** — université de BORDEAUX
- **Lipids (EID)** — Identification & structural characterization
- **Radical Ions (EmI – MS3)** — Radical migration network / fundamental studies

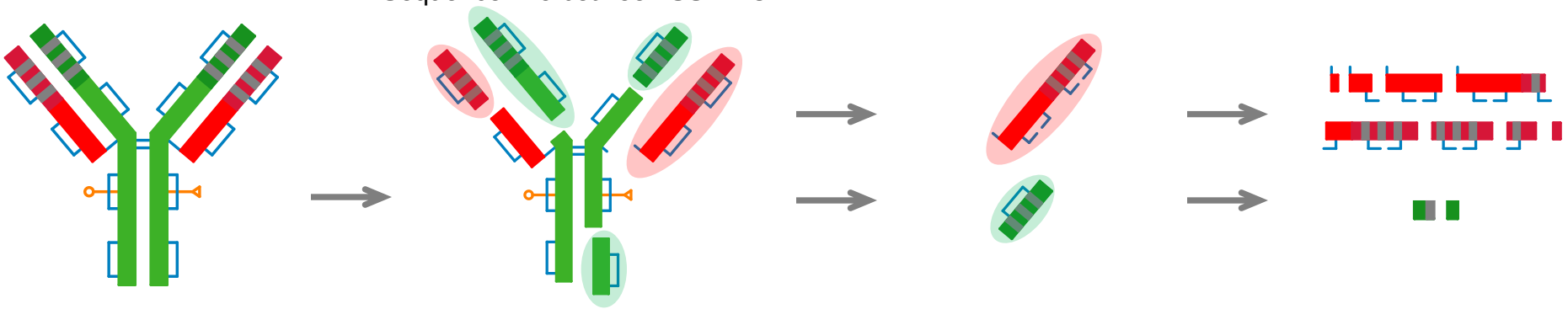
# MS4 Analysis of Intact mAbs in the Omnitrap platform coupled to a QE Plus

Intact non-reduced denatured mAb

Light Chain
LC & HC Variable Regions
Sequence info between SS links

Charge-reduced LC ions
Sequence info between SS links

Sequence info across entire Light Chain



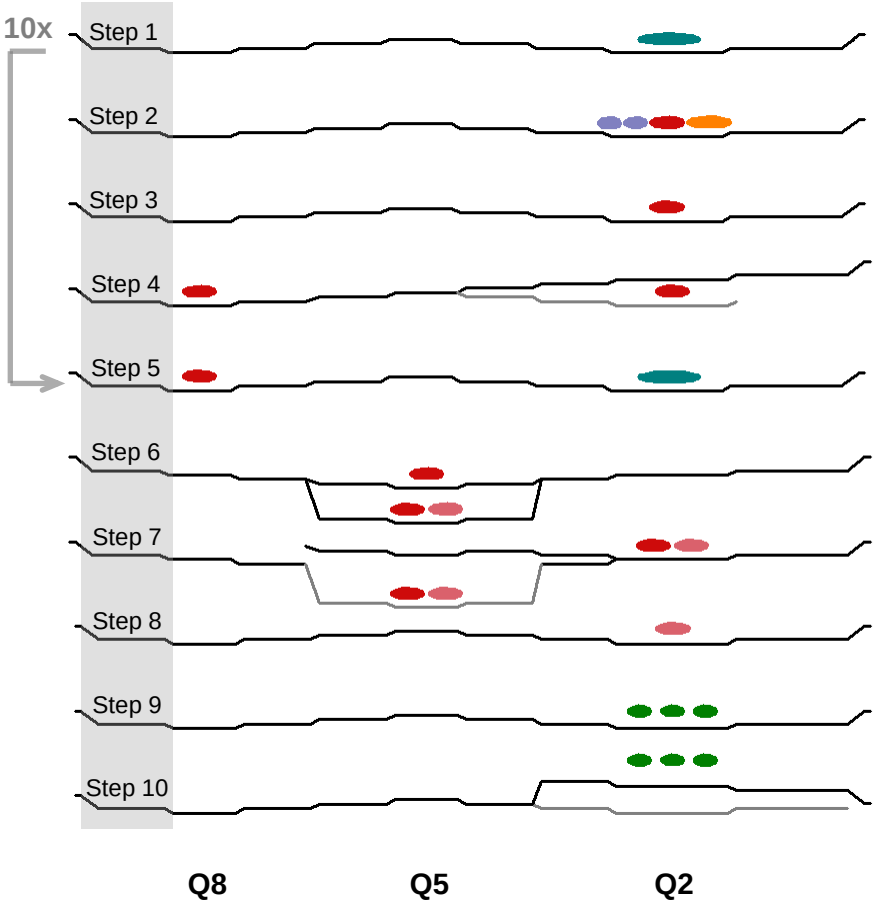
MS | slow heating CID | MS2 | ECD | MS3 | Slow heating CID | MS4

Two-step CID process involving collisional re-activation of high m/z complementary fragments by broadband excitation. Dissociation of the intermolecular bond connecting the light and heavy chains produces an heterogeneous population of fragment ions with variations on the cysteine side chain.

Electron capture leads to charge reduced intact light chain ions and low intensity primary fragments with cleavage positions external to the SS linked regions. Electron capture initiates homolytic cleavage of the SS bonds.

Collisional activation of charge reduced ions produces sequence information throughout the light chain. Spectral complexity due to the heterogeneity of the intermolecular SS bond cleavage is further enhanced by the heterogeneity of the intramolecular SS bond cleavage.

# MS4 Experimental Workflow in the Omnitrap QE platform




**Q2 Injection / denatured Herceptin 49+ (intact, non-reduced)**

**Q2 Slow heating CID in Ar (MS2) / Heavy Chain, Light Chain & Fragments**

**Q2 Resolving DC Isolation / Light Chain**

**Q8 Transfer for Accumulation / Light Chain**

**Q8 Parking / Enriched Light Chain Population, Q2 Injection / Herceptin 49+**

**Q5 Electron Capture Dissociation (MS3) / Charge Reduced Light Chain**

**Q2 Transfer / Charge Reduced Light Chain**

**Q2 Resolving DC Isolation / Charge Reduced Light Chain**

**Q2 Slow Heating CID (MS4) / Charge Reduced Light Chain Fragments**

**Q2 Transfer to Orbitrap / Charge Reduced Light Chain Fragments**

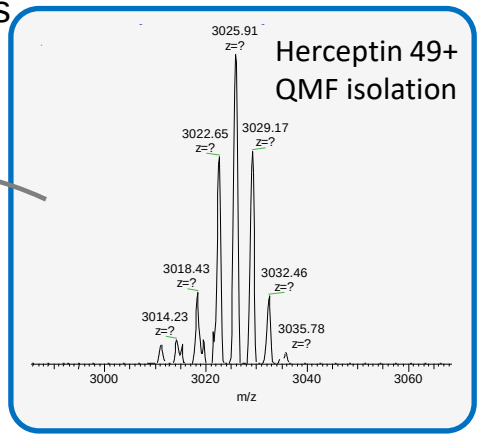
Step 2

# MS2 Slow-heating CID of Herceptin® 49+ & Broadband-excitation CID of high-mass fragment ions

Slow-heating CID produces a remarkably non-congested spectrum that allows to further select fragment ions for MSn interrogation. ECD or UVPD appear less favorable as a starting step for MSn workflows.

**Slow-heating CID settings**:

$f_{RF}$ = 710.6 kHz (q=0.1)
$\omega_{exc}$ = 30 kHz
$V_{ampl}$ = 3300 mV
t = 10 ms





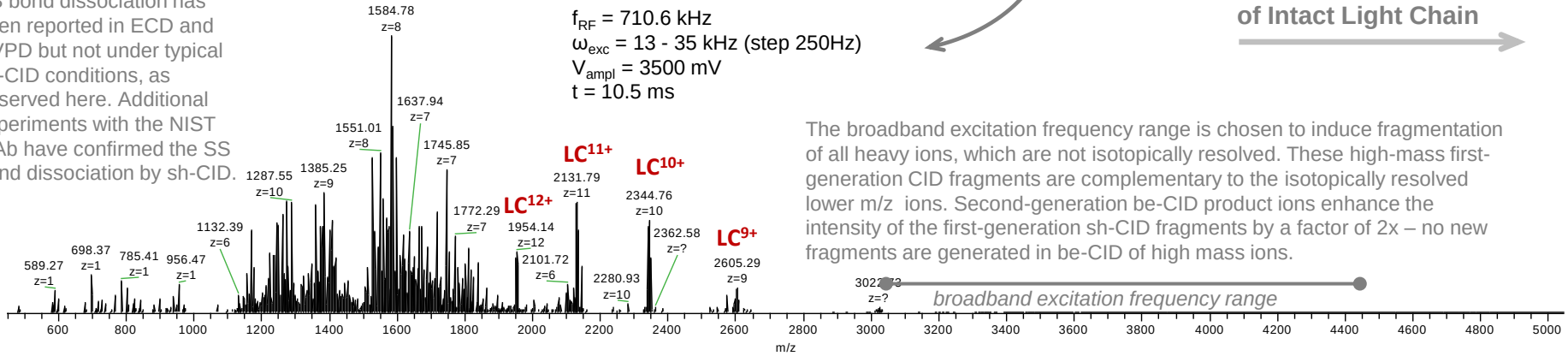


SS bond dissociation has been reported in ECD and UVPD but not under typical sh-CID conditions, as observed here. Additional experiments with the NIST mAb have confirmed the SS bond dissociation by sh-CID.

**Broadband-excitation CID settings**:

$f_{RF}$ = 710.6 kHz
$\omega_{exc}$ = 13 - 35 kHz (step 250Hz)
$V_{ampl}$ = 3500 mV
t = 10.5 ms

**Isolation & Enrichment of Intact Light Chain**




The broadband excitation frequency range is chosen to induce fragmentation of all heavy ions, which are not isotopically resolved. These high-mass first-generation CID fragments are complementary to the isotopically resolved lower m/z ions. Second-generation be-CID product ions enhance the intensity of the first-generation sh-CID fragments by a factor of 2x – no new fragments are generated in be-CID of high mass ions.

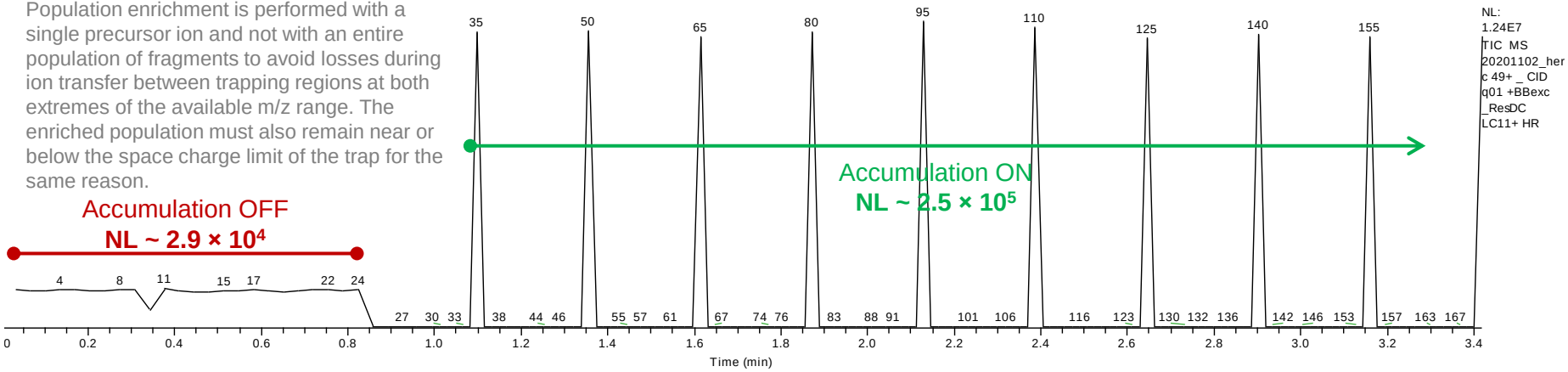
Steps 3 – 5

# Resolving DC isolation of Light Chain z=11+ in Accumulation Mode

Population enrichment is performed with a single precursor ion and not with an entire population of fragments to avoid losses during ion transfer between trapping regions at both extremes of the available m/z range. The enriched population must also remain near or below the space charge limit of the trap for the same reason.

Accumulation OFF
**NL ~ 2.9 × $10^4$**

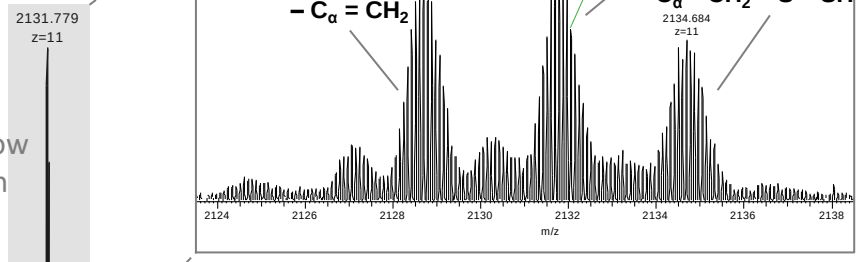
Accumulation ON
**NL ~ 2.5 × $10^5$**




**Resolving DC settings:**

$f_{RF}$ = 355.3KHz
$V_{DC}$ = 53.3V
t = 6ms

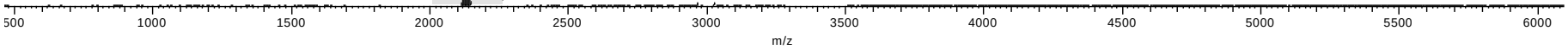
isolation window selected for ion enrichment




The isolation window contains three main precursor ions produced by dissociation of the intermolecular disulfide bond linking the two chains at three consecutive positions.


Three consecutive cleavages across the intermolecular SS bond linking the heavy and light chains together are identified with high mass accuracy (<±5ppm).

**ECD of Enriched Light Chain**



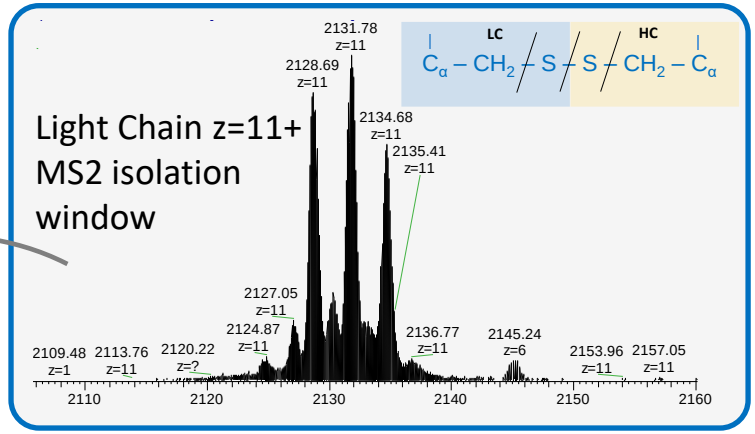
Step 6

# MS3 ECD of intact light chain produced by a two-step MS2 CID process

Gas phase reactions performed in the omnitrap platform involve a two-step CID process producing intact light chain ions in high abundance. The population of the light chain is enriched prior to reactions with low energy electrons. The ECD step produces a series of charge-reduced light chain ions and low abundance ECD fragments.

$[M+49H]^{49+} \xrightarrow{\text{sh-CID + be-CID}} \textit{fragments} + [LC+nH]^{n+}$ (n=9, 10, 11, 12)

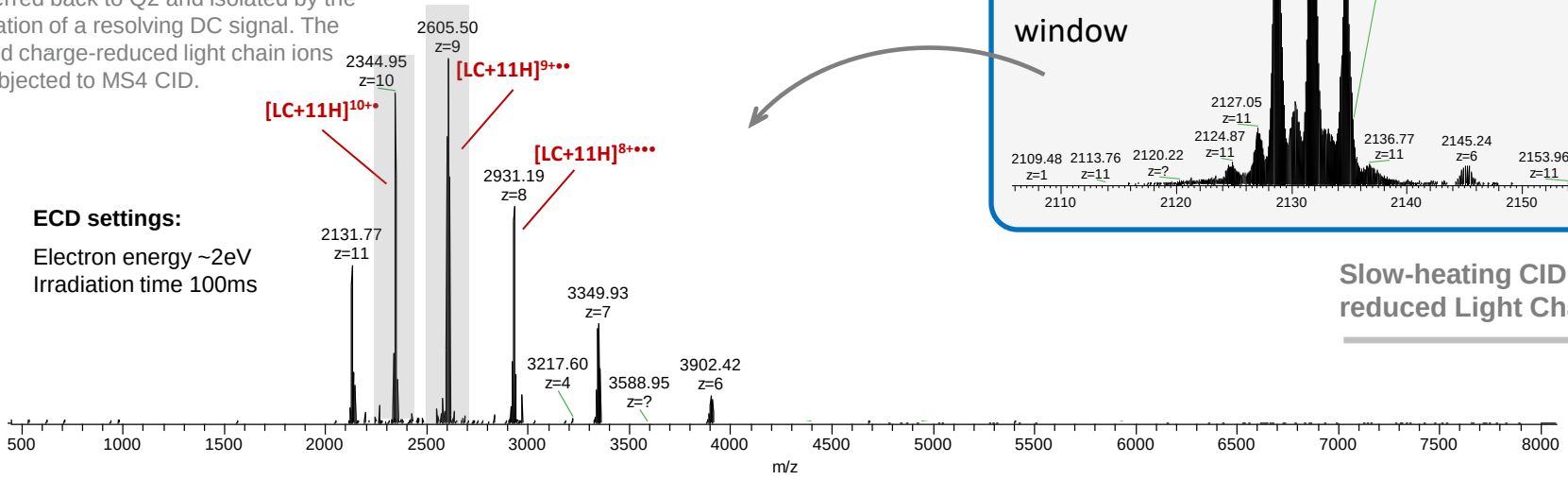
$[LC+11H]^{11+} \xrightarrow{\text{ECD}} [LC+11H]^{10+\bullet} + [LC+11H]^{9+\bullet\bullet} + \ldots + \text{fragments}$




Charge-reduced light chain ions are transferred back to Q2 and isolated by the application of a resolving DC signal. The isolated charge-reduced light chain ions are subjected to MS4 CID.

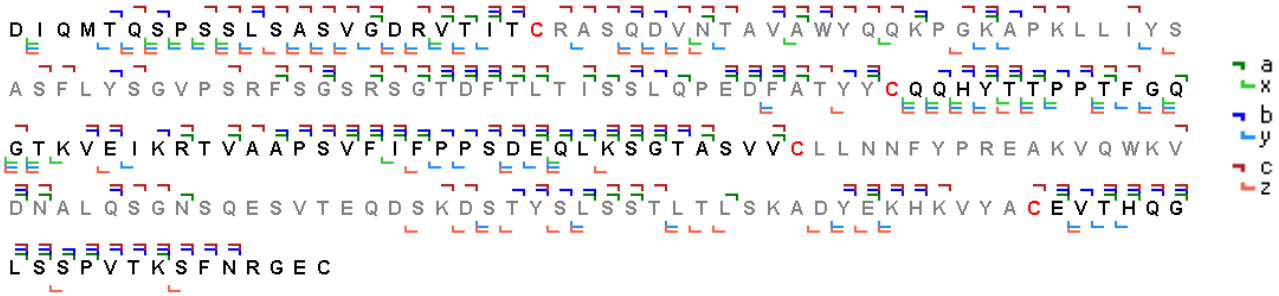
**ECD settings:**

Electron energy ~2eV
Irradiation time 100ms




Slow-heating CID of charge-reduced Light Chain ions

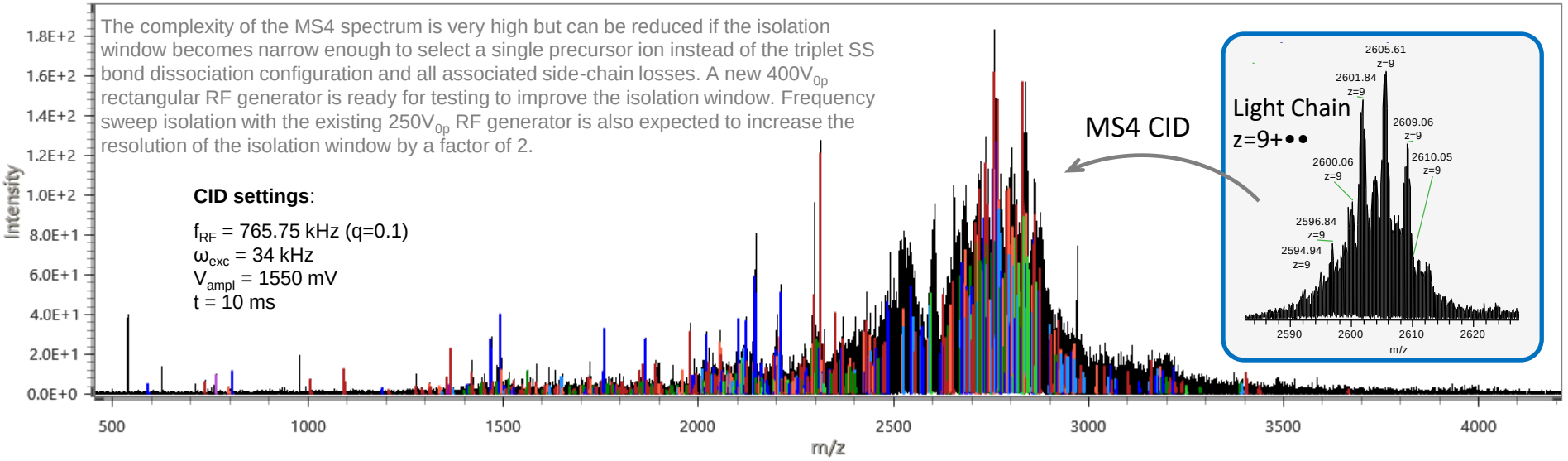
# Step 9 Data Processing of the MS4 Collisionally Activated EC(no)D of Light Chain 9+•• Ions




a : 29% x : 10%
b : 37% y : 26%
c : 53% z : 24%

**Sequence coverage : 73.7%**

*Data processing is performed in PeakFinder*



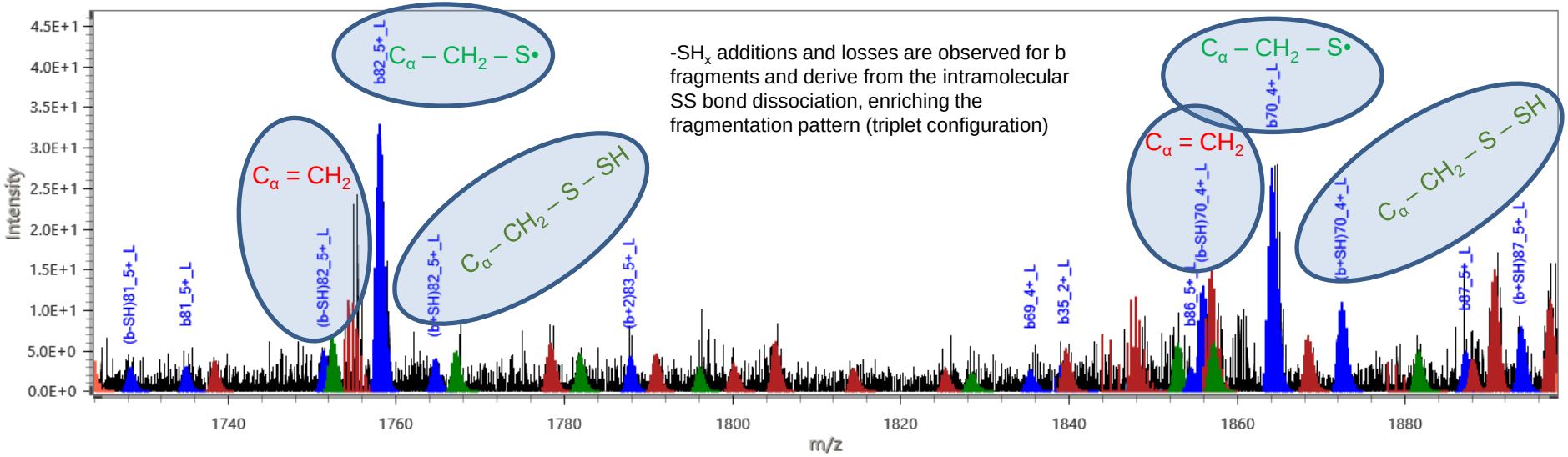
# Step 9 Data Processing of the MS4 Collisionally Activated EC(no)D of Light Chain 9+•• Ions


a : 29% x : 10%
b : 37% y : 26%
c : 53% z : 24%

**Sequence coverage : 73.7%**
*(including modifications)*

Modifications to the side chain of cysteines participating in intramolecular SS bonds were taken into consideration and a high number of fragments with modified side-chains on cysteine residues were identified.

These modifications add further to the complexity of the fragmentation pattern originating from the dissociation pattern of the intermolecular SS bond dissociation connecting heavy and light chains.




-$SH_x$ additions and losses are observed for b fragments and derive from the intramolecular SS bond dissociation, enriching the fragmentation pattern (triplet configuration)

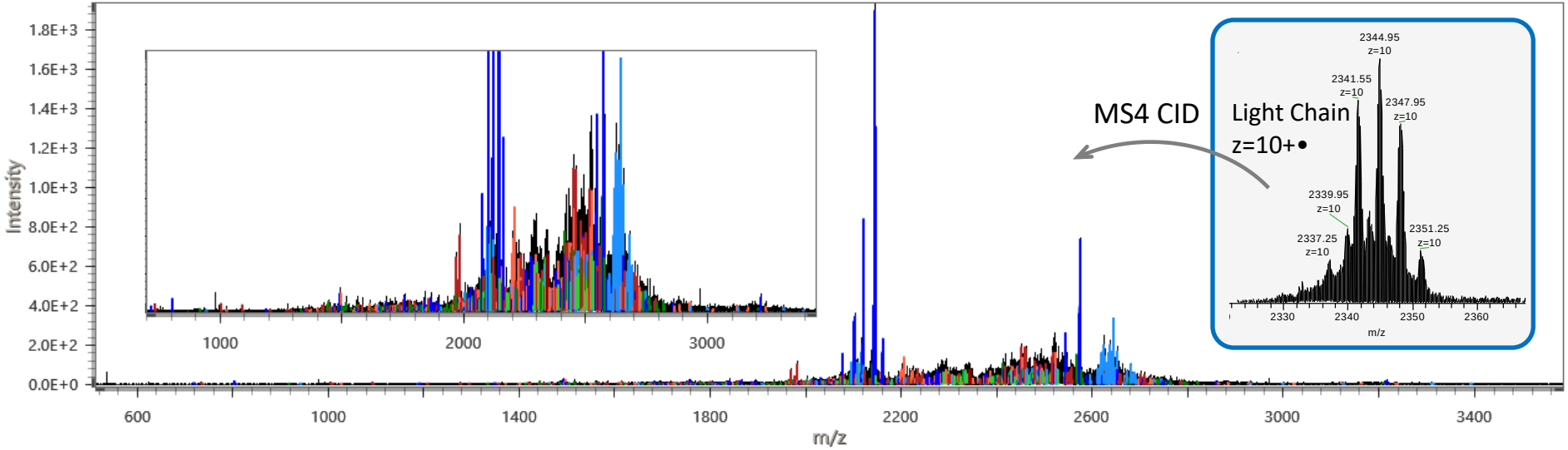
Step 9

# Data Processing of the MS4 Collisionally Activated EC(no)D of Light Chain 10+• Ions

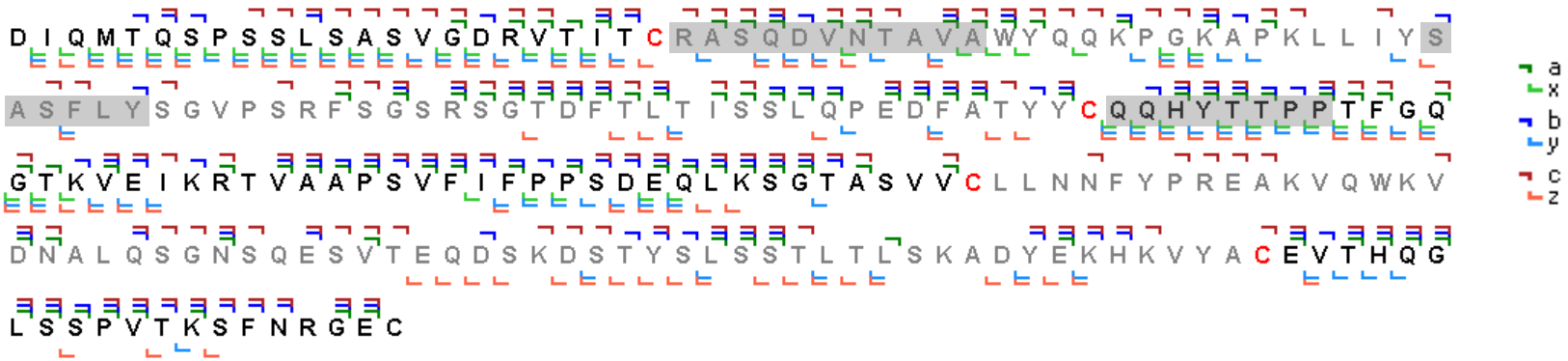

a : 28% x : 14%
b : 30% y : 23%
c : 40% z : 29%

**Sequence coverage : 70.9%**

*Data processing is performed in PeakFinder*



Combined sequence information obtained from MS4 CID of $LC^{10+\bullet}$ and MS4 CID of $LC^{9+\bullet\bullet}$




a : 40.8%
b : 45.1%
c : 61.5%

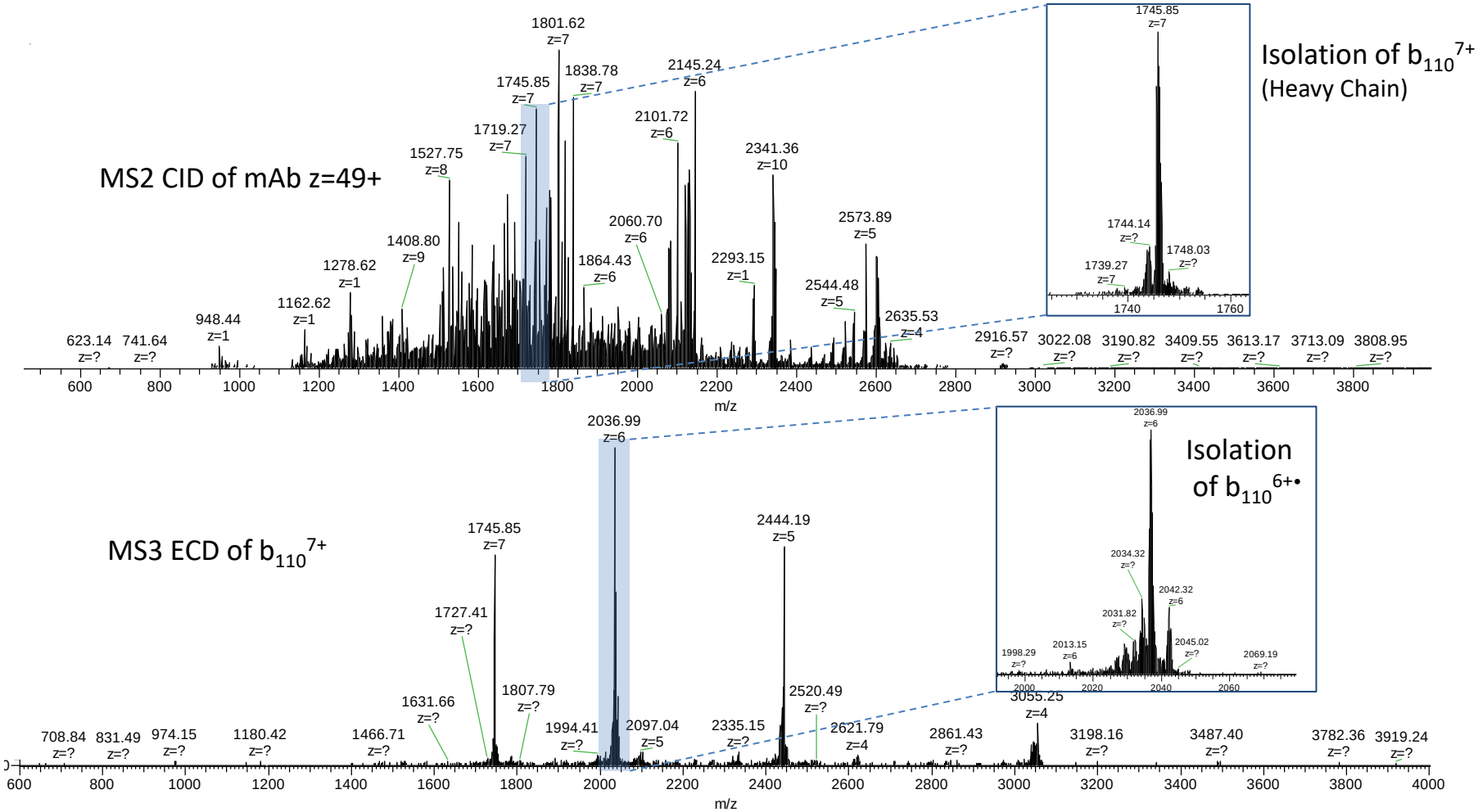
x : 20.2%
y : 33.8%
z : 38.5%

CDR regions

**Sequence coverage : 83.1%**

Steps 1 – 8

# MS2 Slow-heating CID of Herceptin® 49+ & MS3 EC(no)D of Heavy Chain b-type fragment



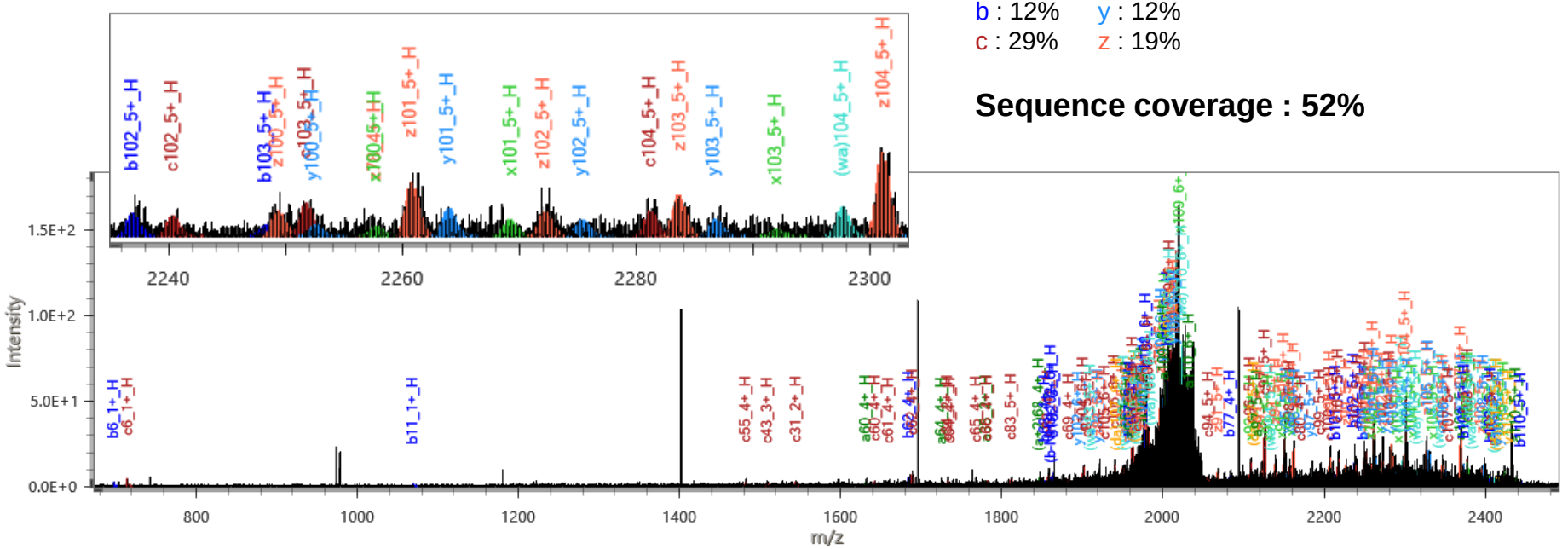
Step 9

# MS4 Collisional Activation of the Charge-Reduced $b_{110}^{6+\bullet}$ Ions & Data Processing

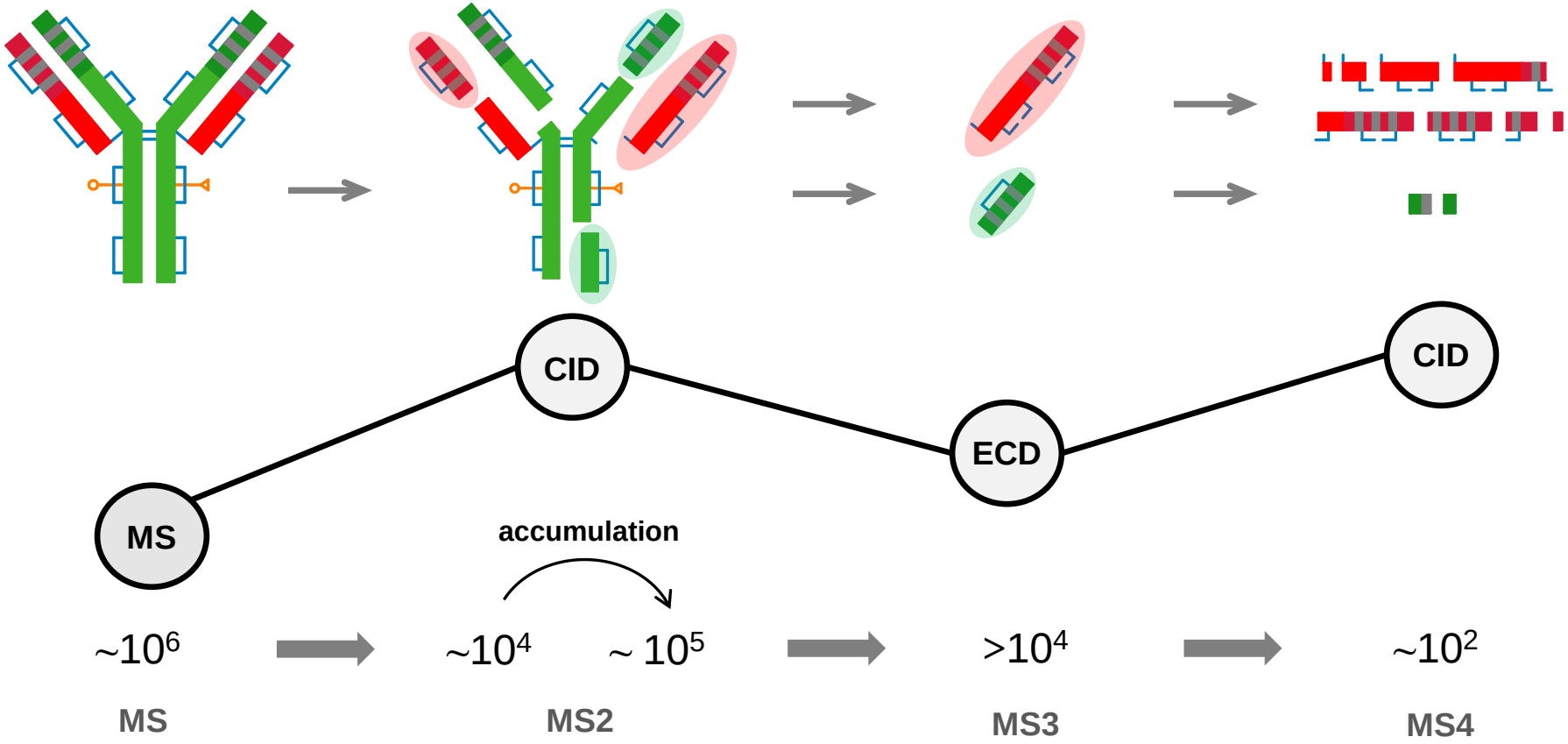

CDR regions

a : 9%  x : 11%
b : 12%  y : 12%
c : 29%  z : 19%

**Sequence coverage : 52%**



# Dynamic range of ion concentration in MS4 ion accumulation mode



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