10.15 - 10.45 WP1. Theory, instrument overview and status
10.45 – 11.15 WP1. IMS Electronics & IMS Mechanical design

11.15 – 11.45 Coffee break

11.45 – 12.15 WP2. Implementation of CAD, ECD, HECD, UV, IR, PD, and EID MS/MS techniques in Omnitrap

12.15-12.45 WP3. Development and application of H-atom bombardment techniques

12.45-13.45 Lunch

13.45-14.15 WP4. Development and application of Coulomb explosion MS/MS technique

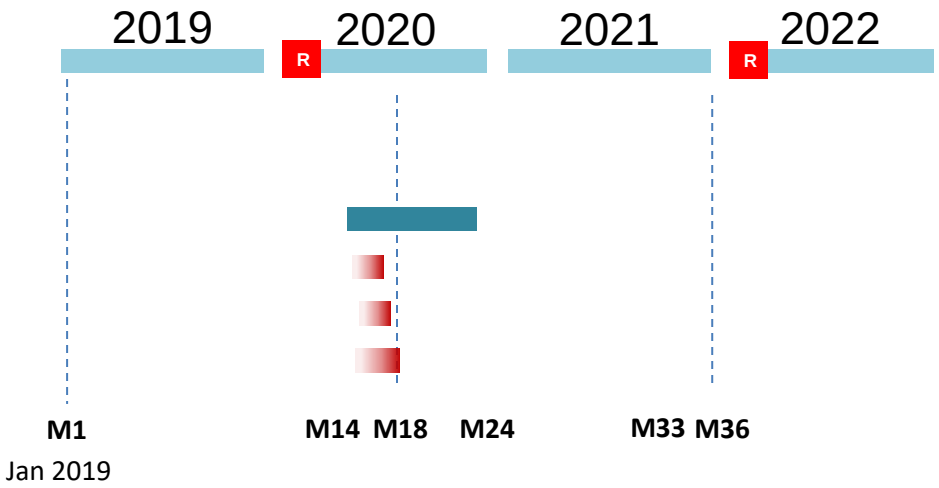
TopSpec

WP2 – Implementation of Activation Techniques

D2.1 – Ion Isolation

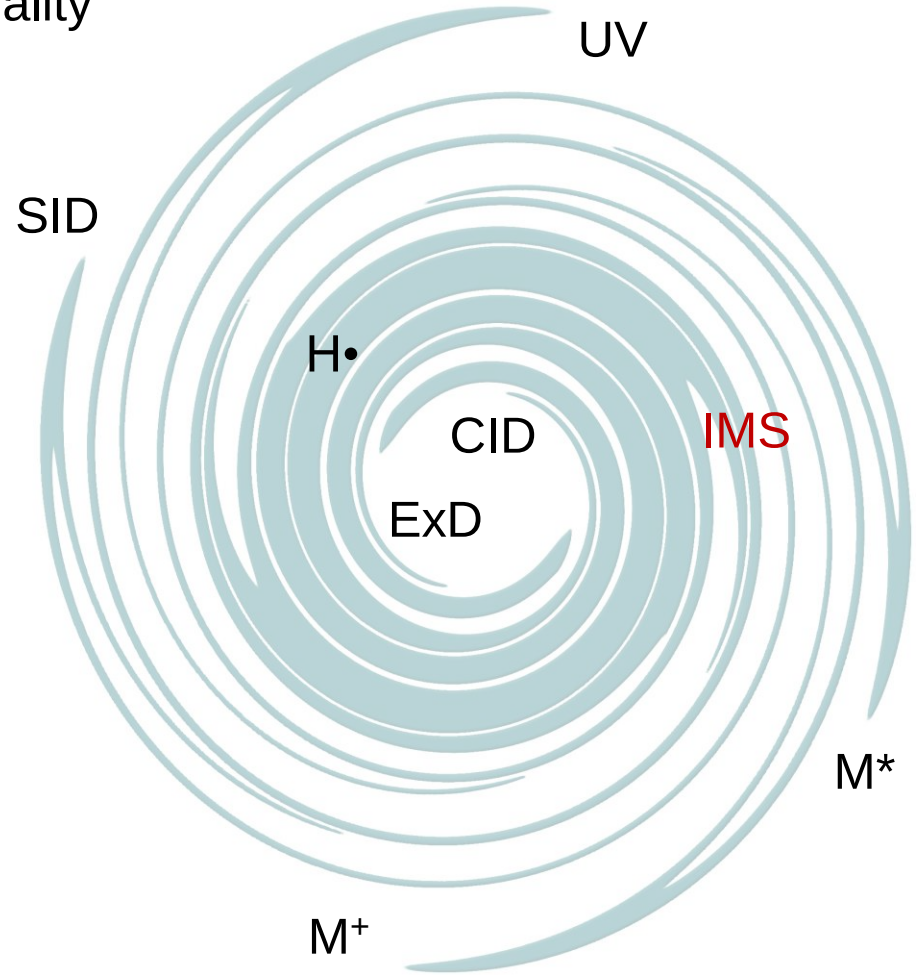
D2.2 – CID

D2.3 – ECD, HAB & CED

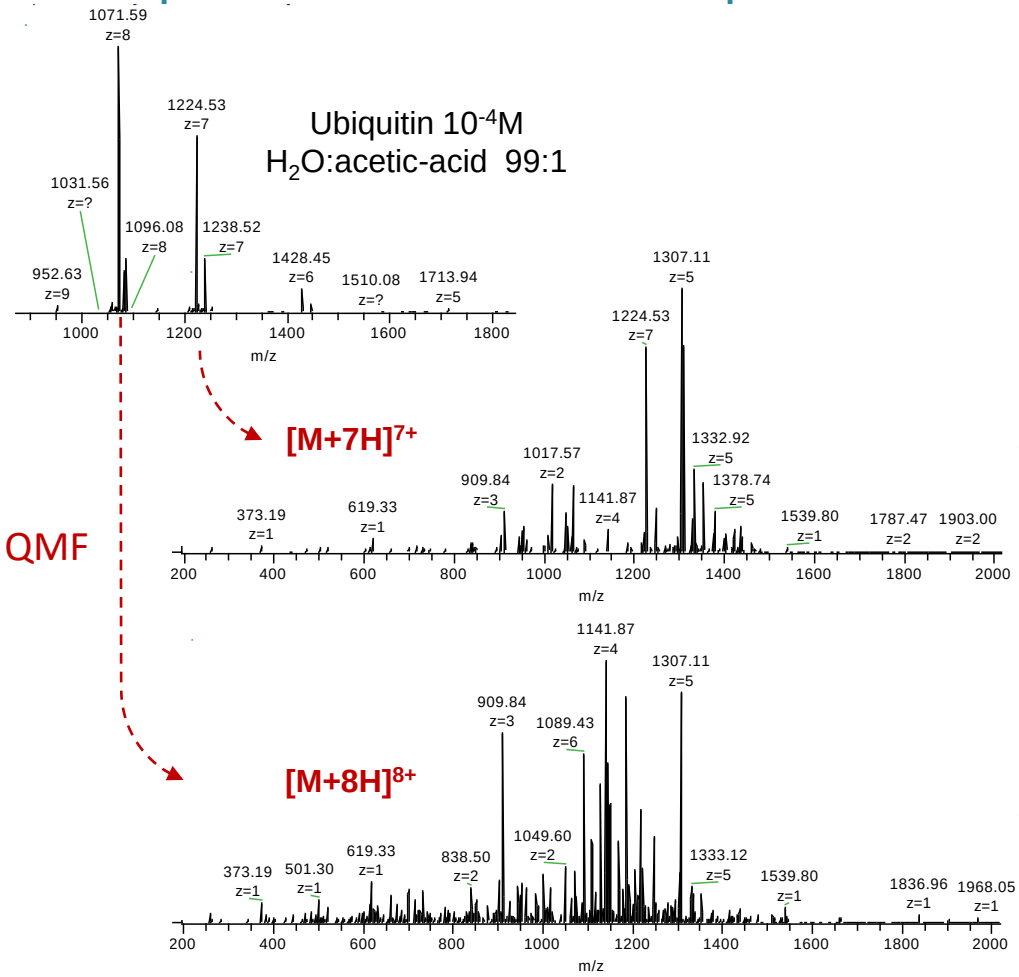


FasmaTECH
science and technology

Omnitrap Functionality



WP2 – Implementation of Activation Techniques



Collision Induced Dissociation

Slow heating CID parameters:

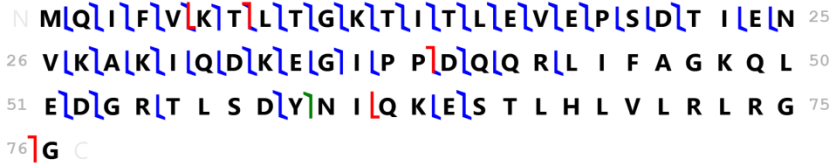
RF: $100V_{0p}$ - 600KHz

ω_{exc}=39.75KHz - $125mV_{0p}$ (q~0.15)

20ms excitation in Ar gas pulse

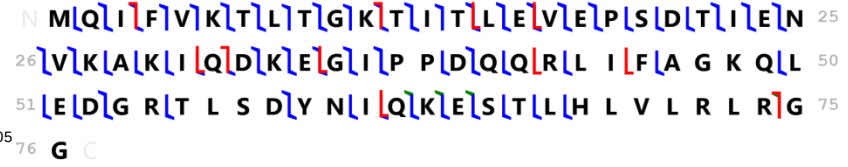
63% residue cleavage

18% fragments explained



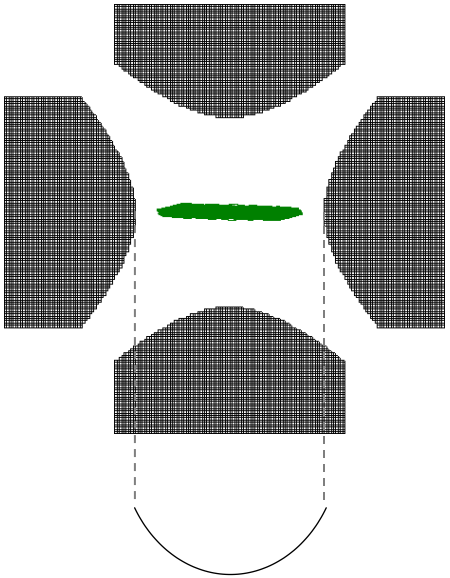
77% residue cleavage

11% fragments explained



Collision Induced Dissociation

RF: 600KHz - 100V_{0p}



Effective potential

$$D_{rec} = 0.411\ q_z\ V_{0p}$$

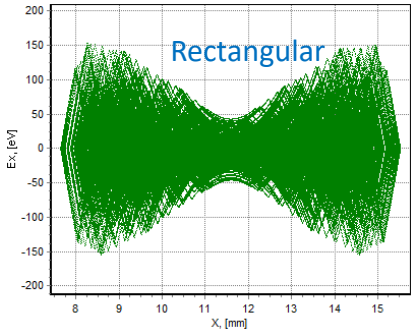
$$D_{sin} = 0.250\ q_z\ V_{0p}$$

Ubiquitin

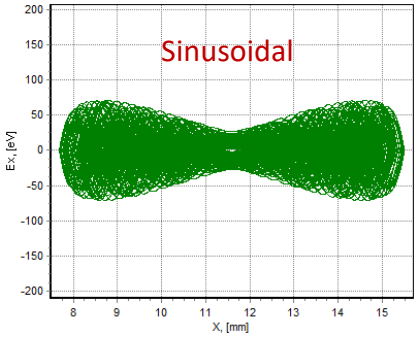
$[M+8H]^{8+}$

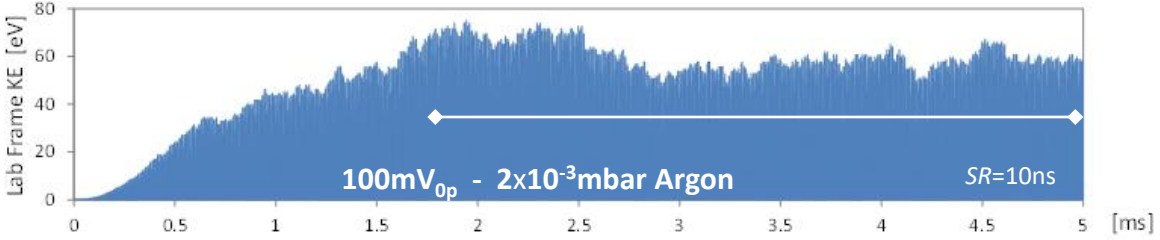
m/z=1071.61

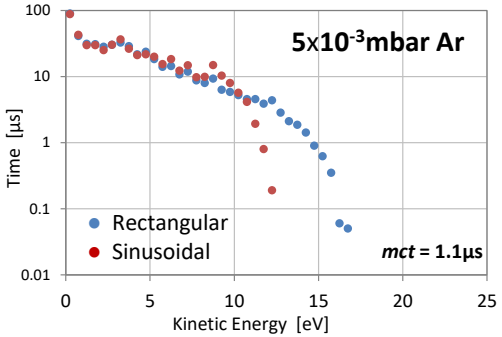
q=0.15: **ω=41.5KHz - 50mV_{0p}**

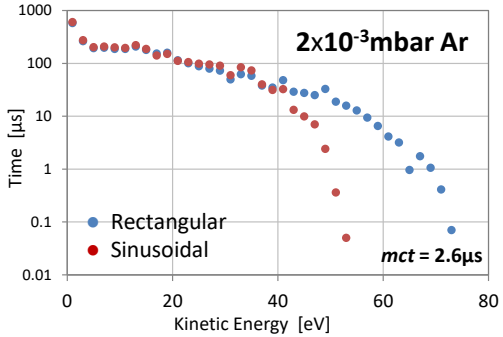


ω=32.3KHz - 50mV_{0p}



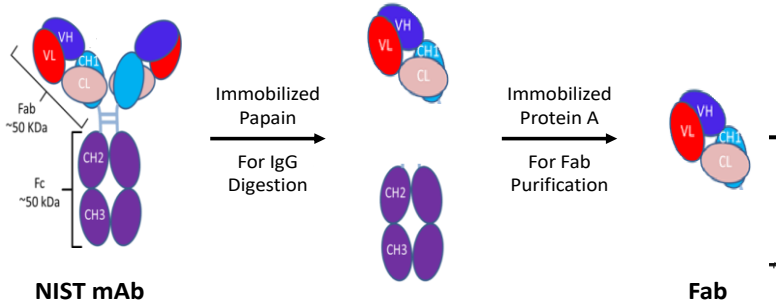


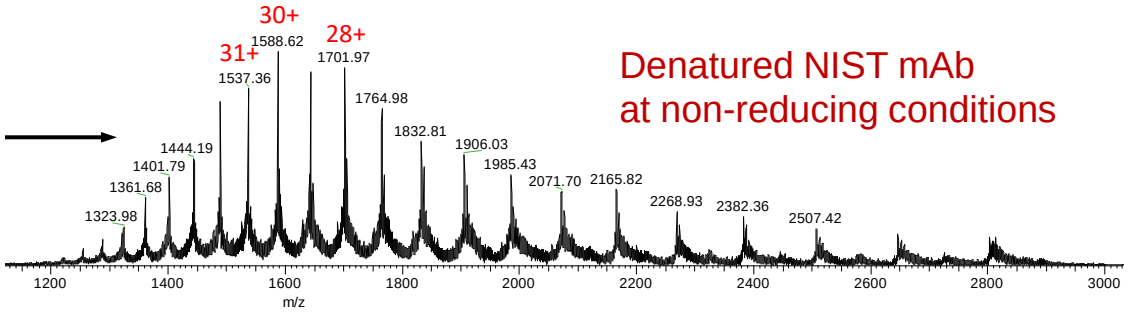




WP2 – Implementation of Activation Techniques

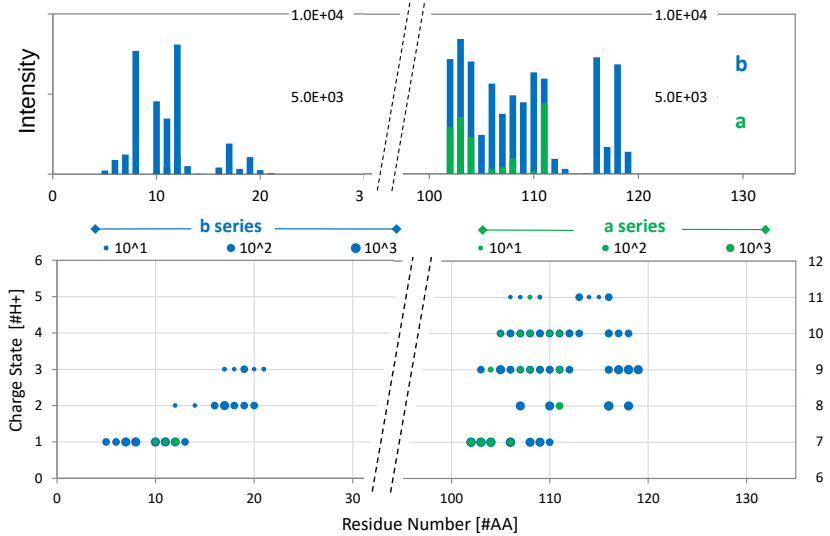
Collision Induced Dissociation

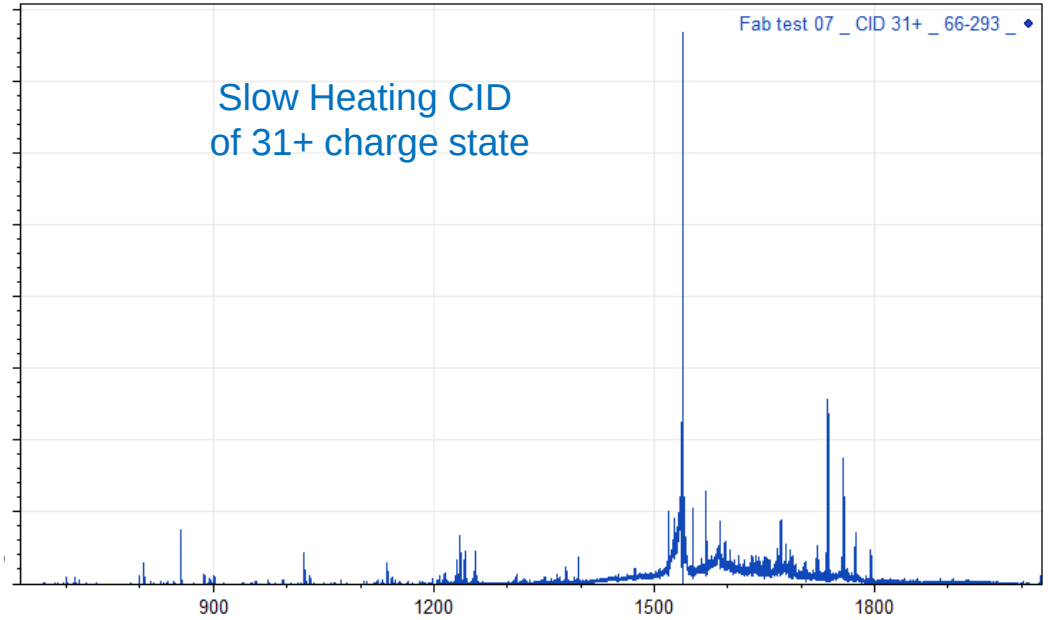




Denatured NIST mAb
at non-reducing conditions

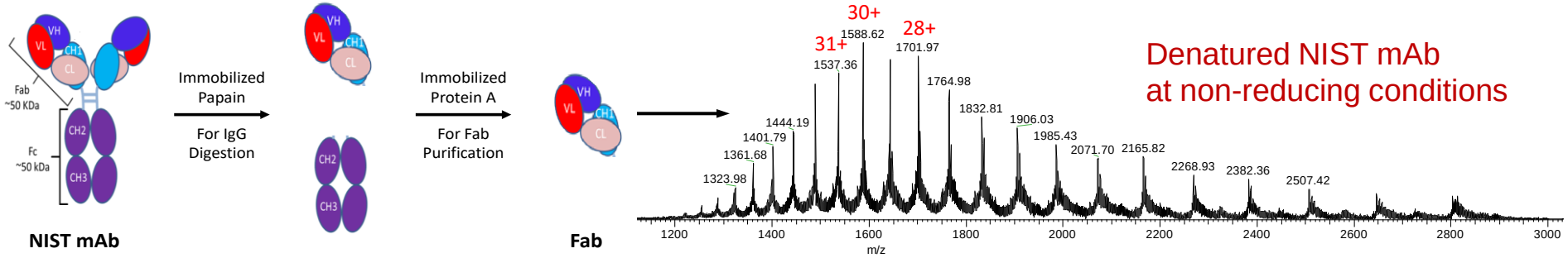
CID (31+) Heavy Chain





WP2 – Implementation of Activation Techniques

Collision Induced Dissociation



Denatured NIST mAb
at non-reducing conditions

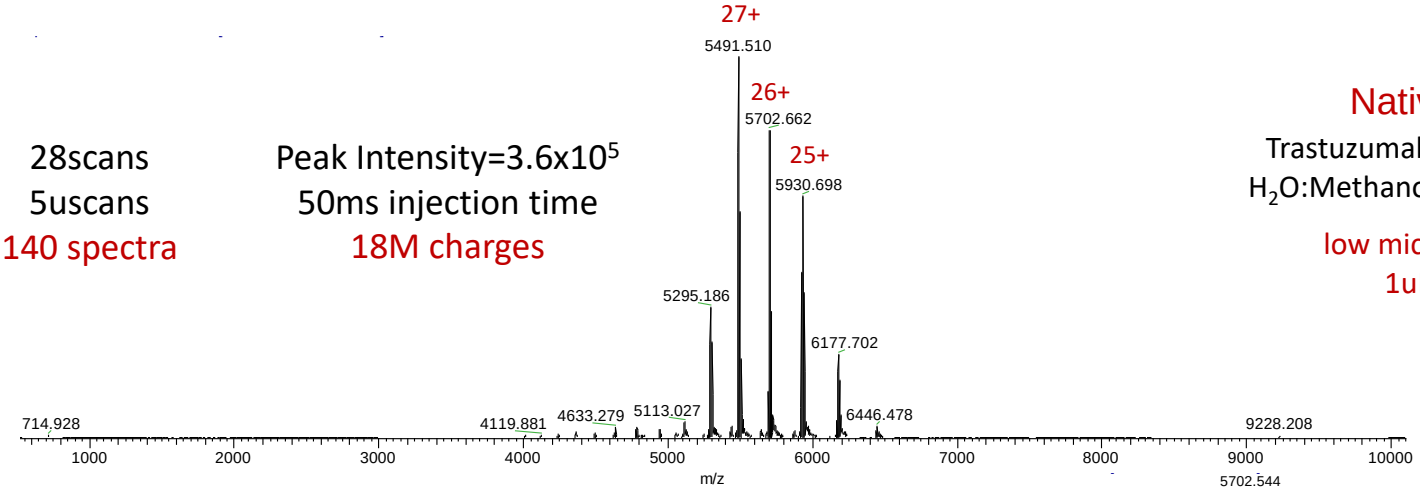
HCD – Light Chain

WP2 – Implementation of Activation Techniques

Collision Induced Dissociation

28scans
5uscans
140 spectra

Peak Intensity=3.6x10^5
50ms injection time
18M charges



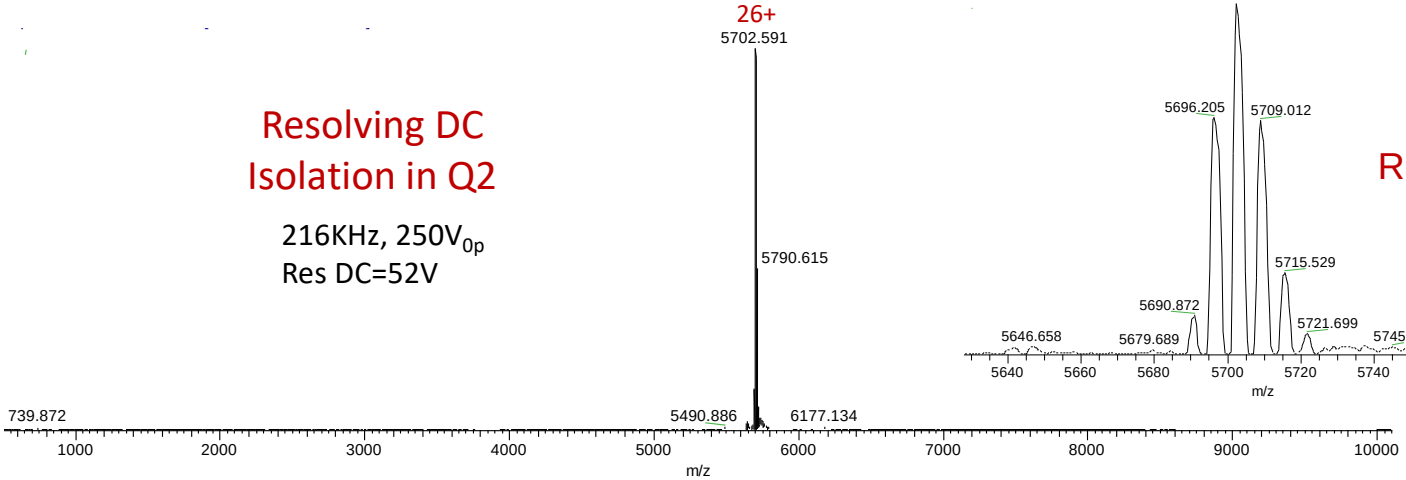
Native ESI

Trastuzumab (HERCEPTIN)
H_2O:Methanol (50:50), 10^{-5}M

low microflow ESI
1uL/min

Resolving DC
Isolation in Q2

216KHz, $250V_{0p}$
Res DC=52V



R=17,500

Collision Induced Dissociation

25scans
5uscans
125 spectra

Peak Intensity=6x10^3

slow heating CID of 26+ in Q2

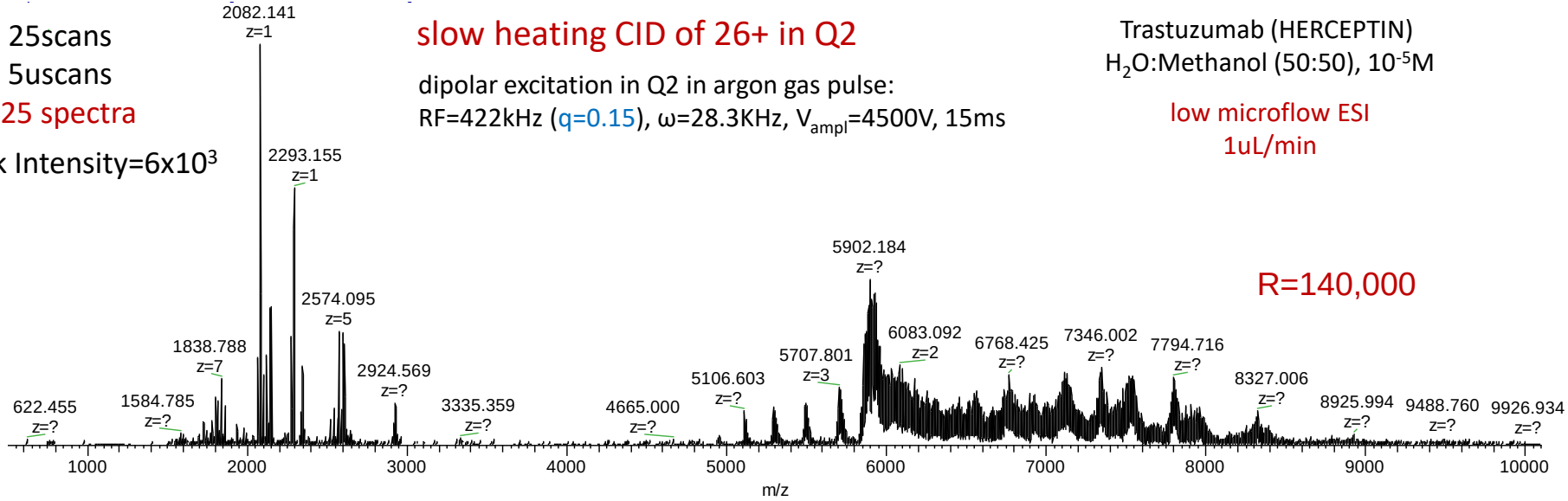
dipolar excitation in Q2 in argon gas pulse:
RF=422kHz (q=0.15), ω=28.3KHz, V_{ampl}=4500V, 15ms

Native ESI

Trastuzumab (HERCEPTIN)
H_2O:Methanol (50:50), 10^{-5}M

low microflow ESI
1uL/min

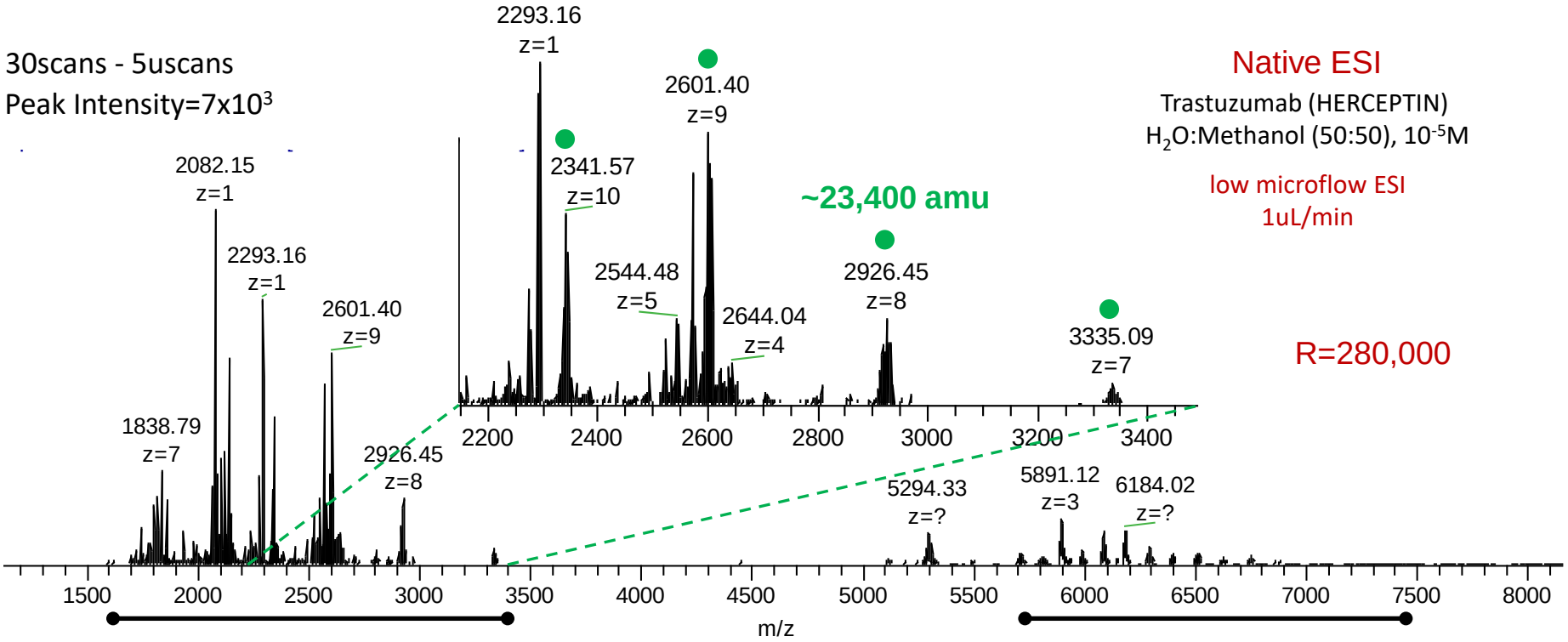
R=140,000



slow heating CID fragments

high mass fragment ions &
charged reduced precursor species

Collision Induced Dissociation



slow heating CID fragments

dipolar excitation in Q2 in argon gas pulse:
RF=366kHz (q=0.2), ω=33.0KHz, V_{ampl}=4500V, 7ms

Broadband Excitation Window

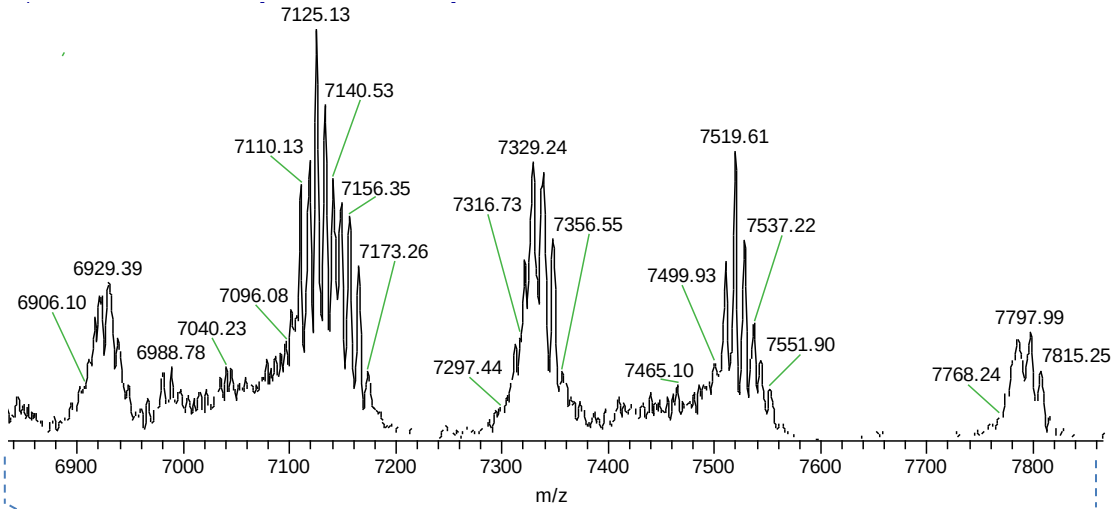
broadband excitation in Q2 in argon gas pulse:
RF=366kHz, ω=15-33KHz, δω=500Hz, V_{ampl}=1300V, 10ms

WP2 – Implementation of Activation Techniques

Collision Induced Dissociation

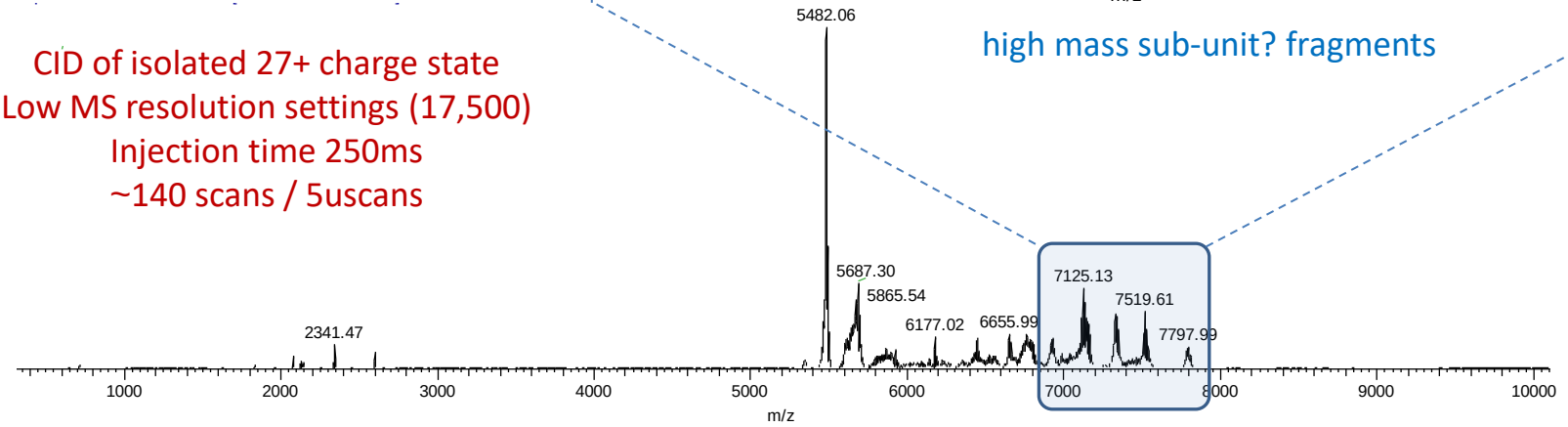
Native ESI

Trastuzumab (HERCEPTIN)
5uM in 100mM Am Ac
Static nESI



high mass sub-unit? fragments

CID of isolated 27+ charge state
Low MS resolution settings (17,500)
Injection time 250ms
~140 scans / 5uscans



WP2 – Implementation of Activation Techniques

Collision Induced Dissociation

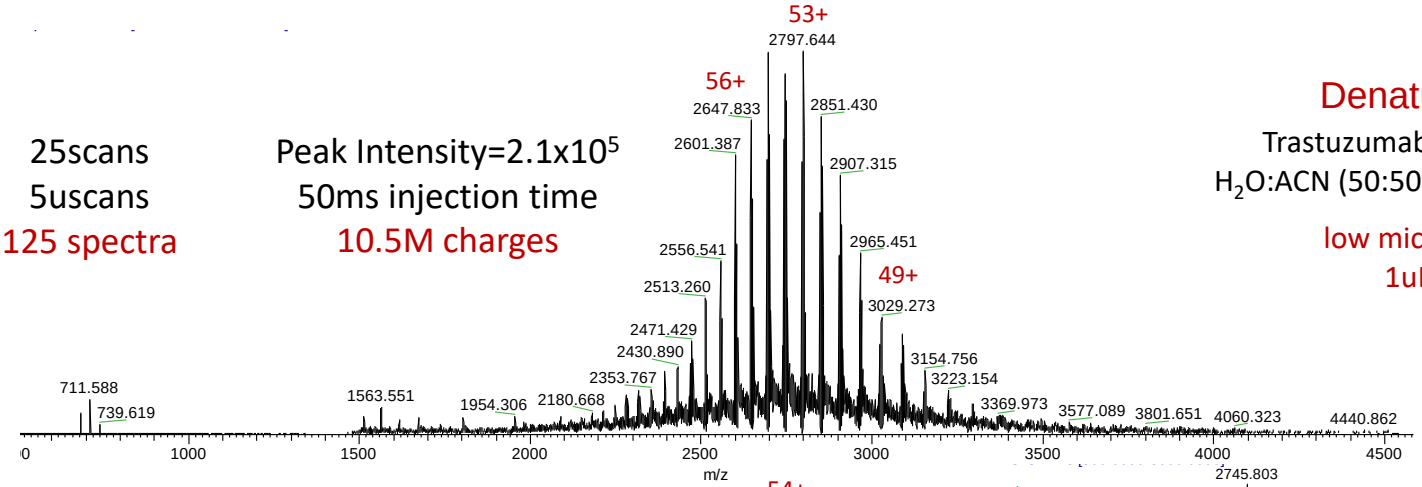
25scans
5uscans
125 spectra

Peak Intensity=2.1x10^5
50ms injection time
10.5M charges

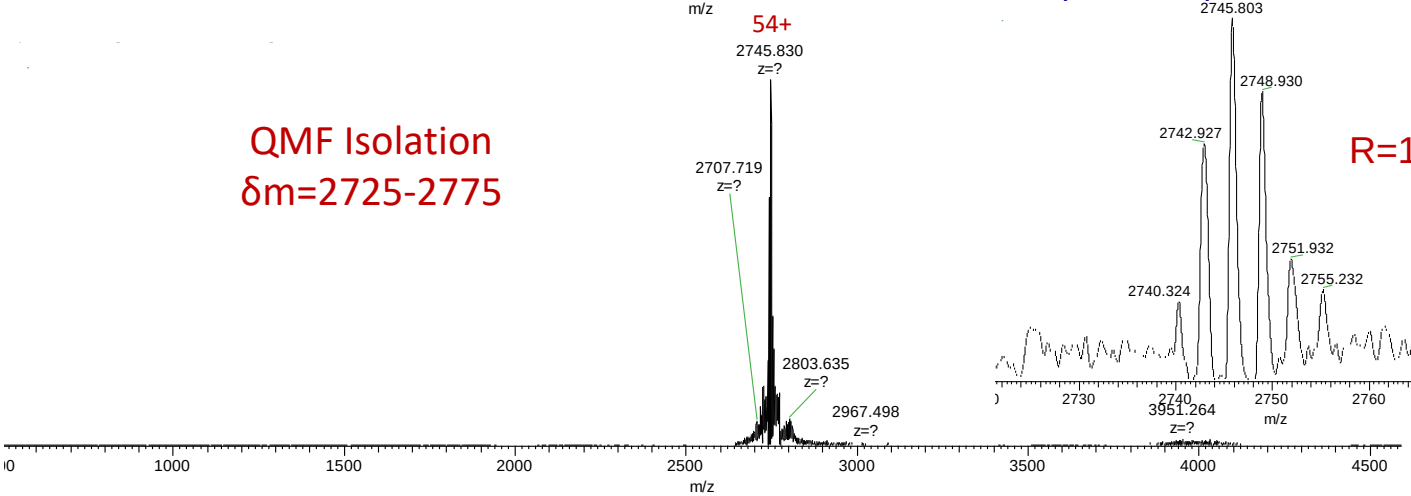
Denatured ESI

Trastuzumab (HERCEPTIN)
H_2O:ACN (50:50) & 0.1% FA, 10^{-5}M

low microflow ESI
1uL/min



QMF Isolation
δm=2725-2775



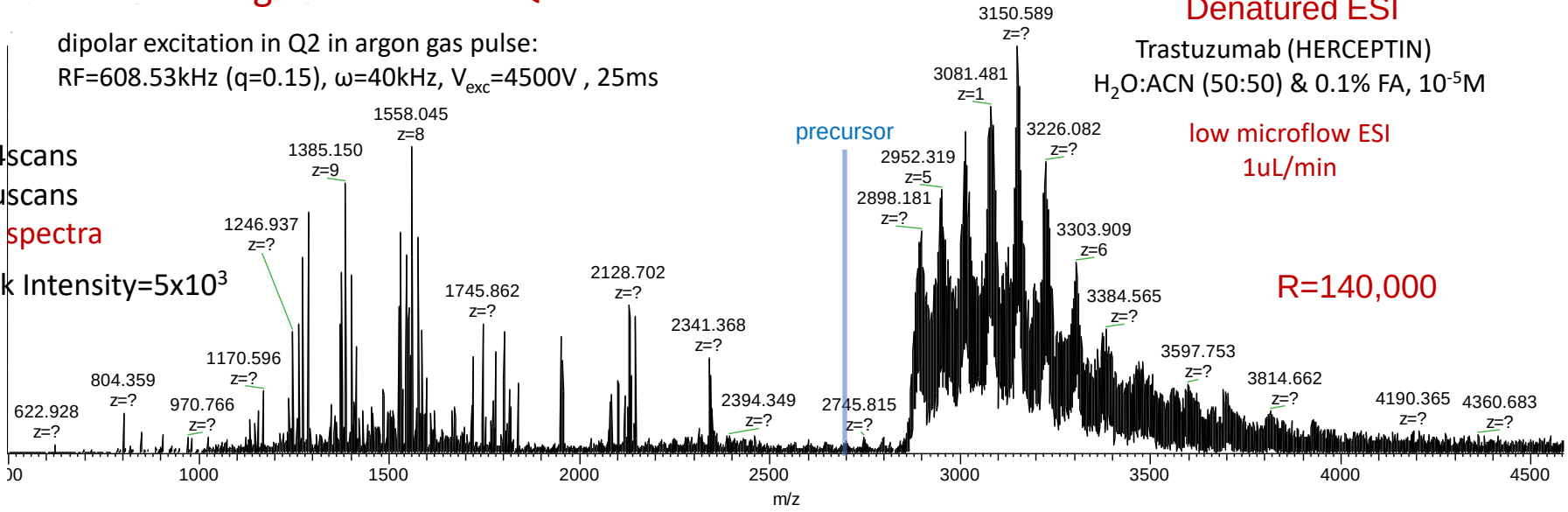
R=17,500

Collision Induced Dissociation

slow heating CID of 54+ in Q2

dipolar excitation in Q2 in argon gas pulse:
RF=608.53kHz (q=0.15), ω=40kHz, V_{exc}=4500V , 25ms

34scans
5uscans
170 spectra
Peak Intensity=$5x10^3$



Denatured ESI

Trastuzumab (HERCEPTIN)
H_2O:ACN (50:50) & 0.1% FA, 10^{-5}M

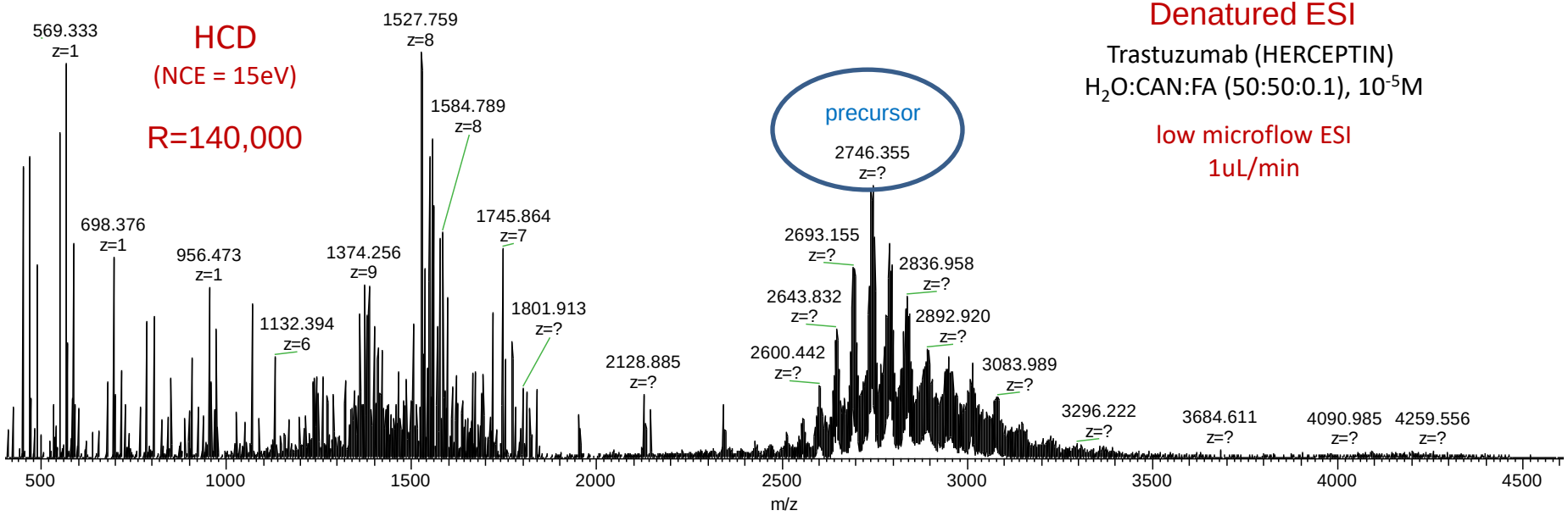
low microflow ESI
1uL/min

R=140,000

slow heating CID fragments

charged reduced precursor ions (proton loss)
& high mass fragments

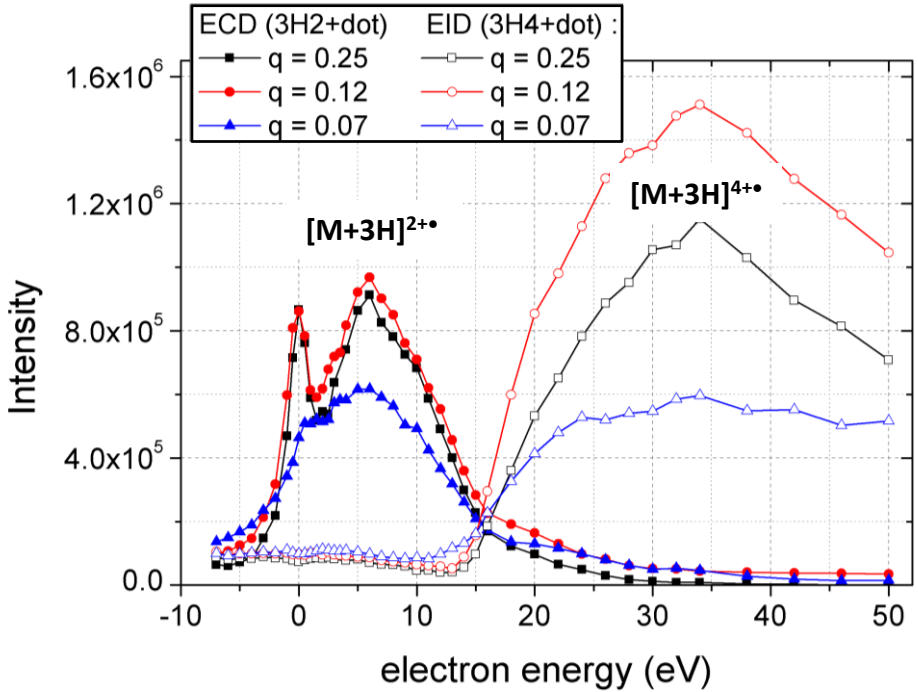
Higher-Energy CID



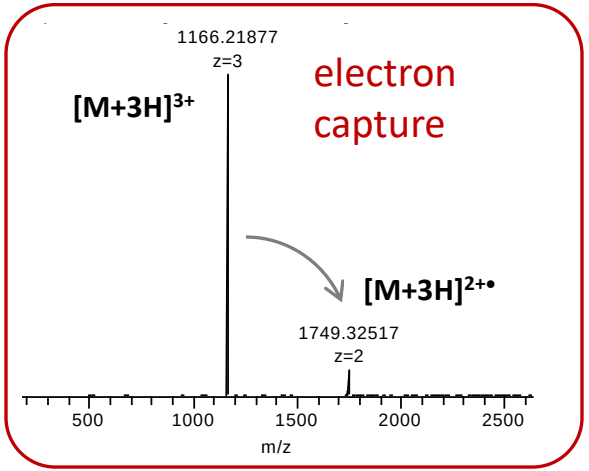
Insulin Chain B

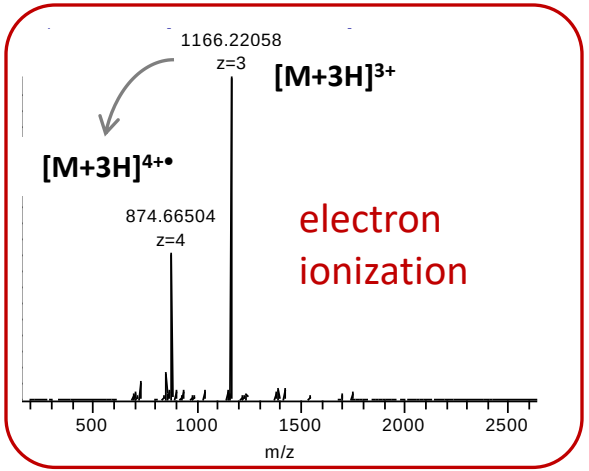
Electron Capture : $[M+3H]^{3+} + e_s^- \longrightarrow \mathbf{[M+3H]^{2+\bullet}}$

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



Electron-based Dissociation

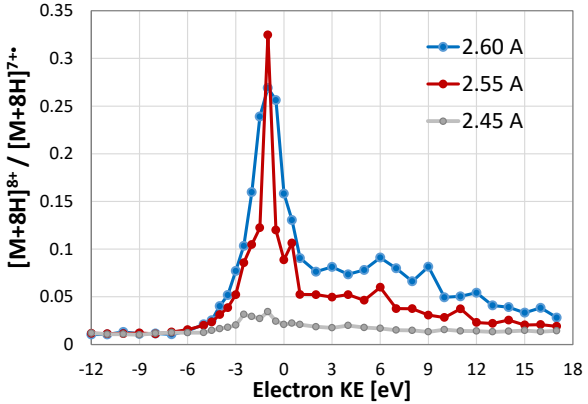


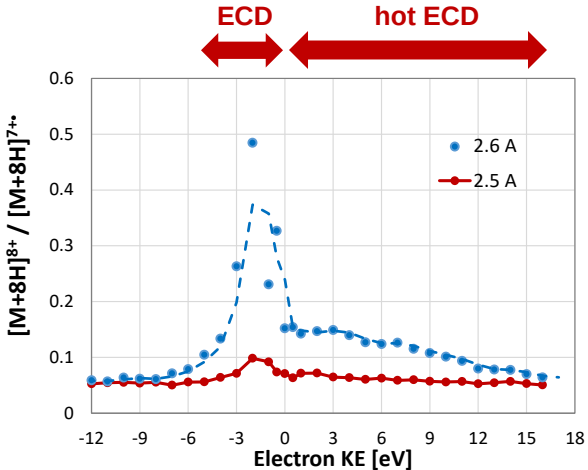


WP2 – Implementation of Activation Techniques

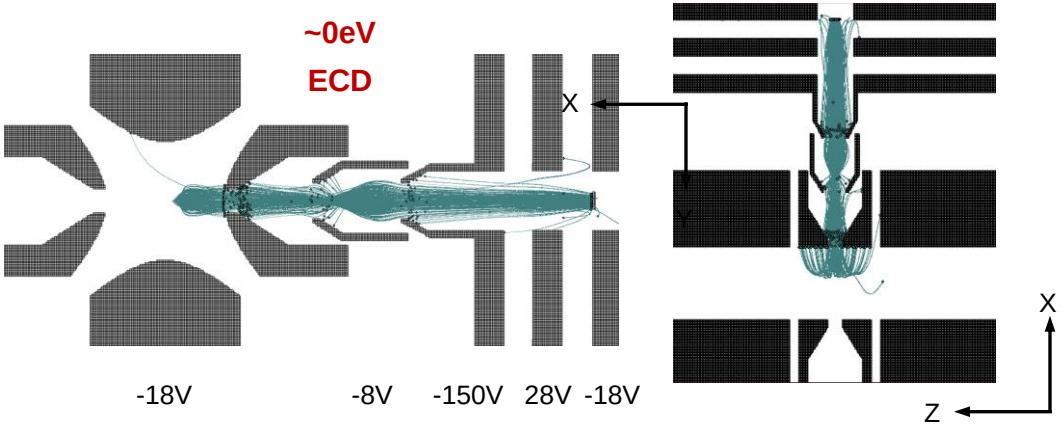
ECD of ubiquitin

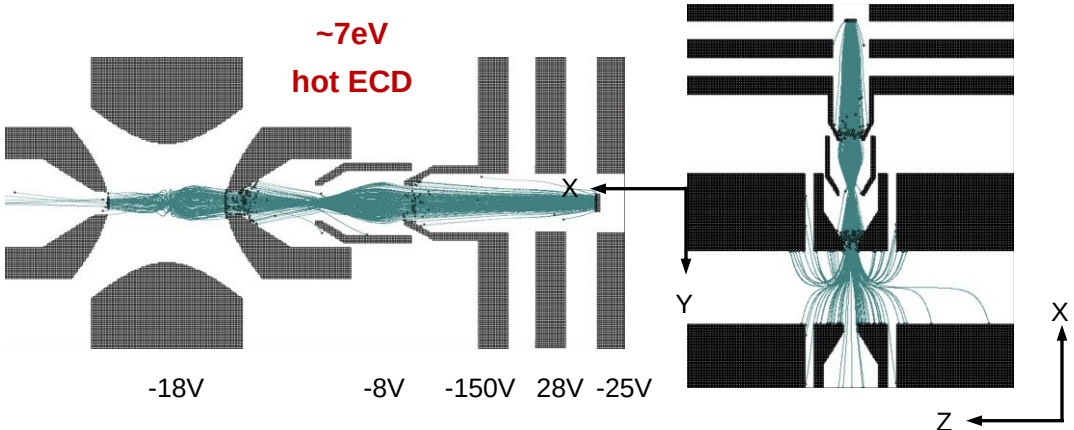
charge reduction: $[M+8H]^{8+} + e^- \rightarrow [M+8H]^{7+\bullet}$



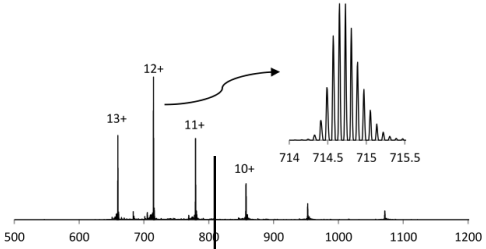


Electron Capture & Hot Electron Capture Dissociation





Electron Capture/Induced Dissociation

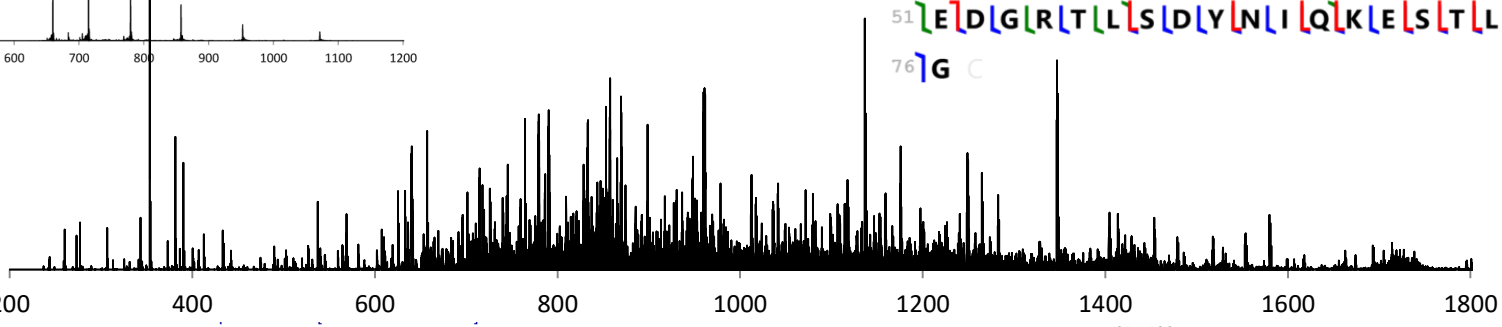


ECD of all charge states

8+ → 13+

Ubiquitin

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

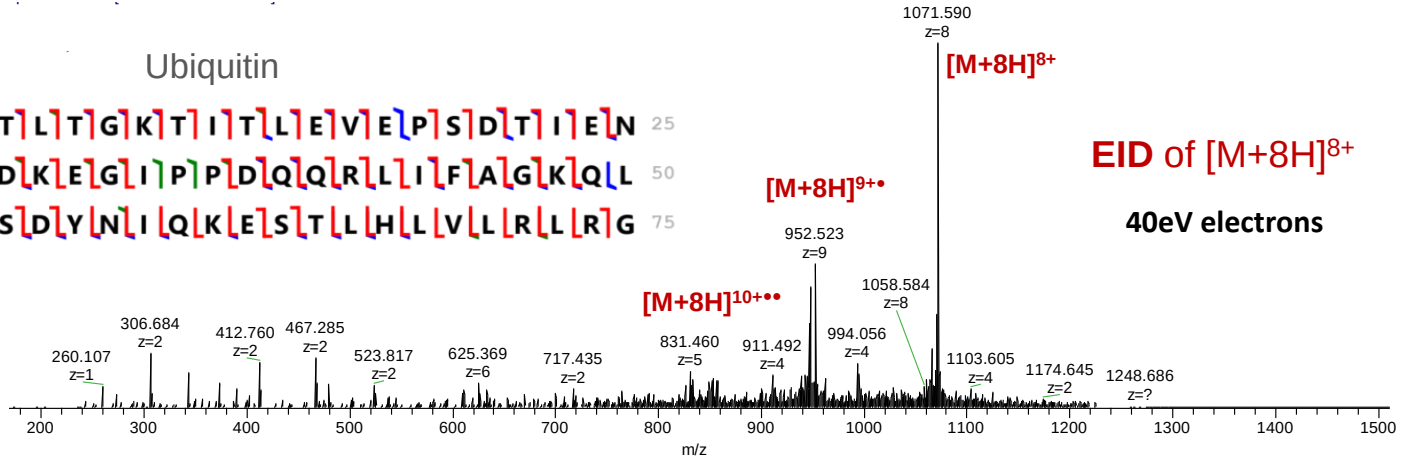


Ubiquitin

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

EID of $[M+8H]^{8+}$

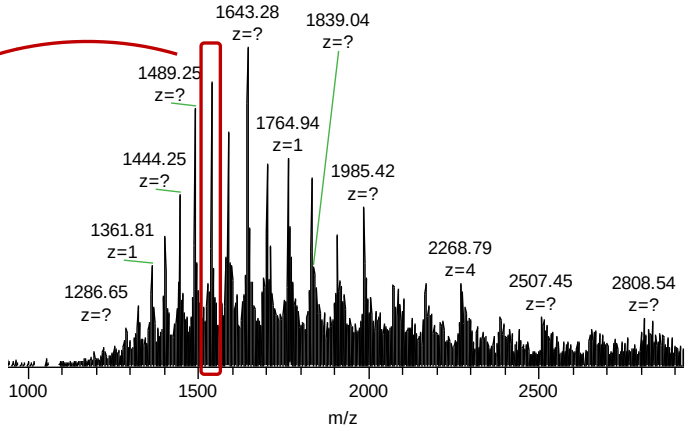
40eV electrons

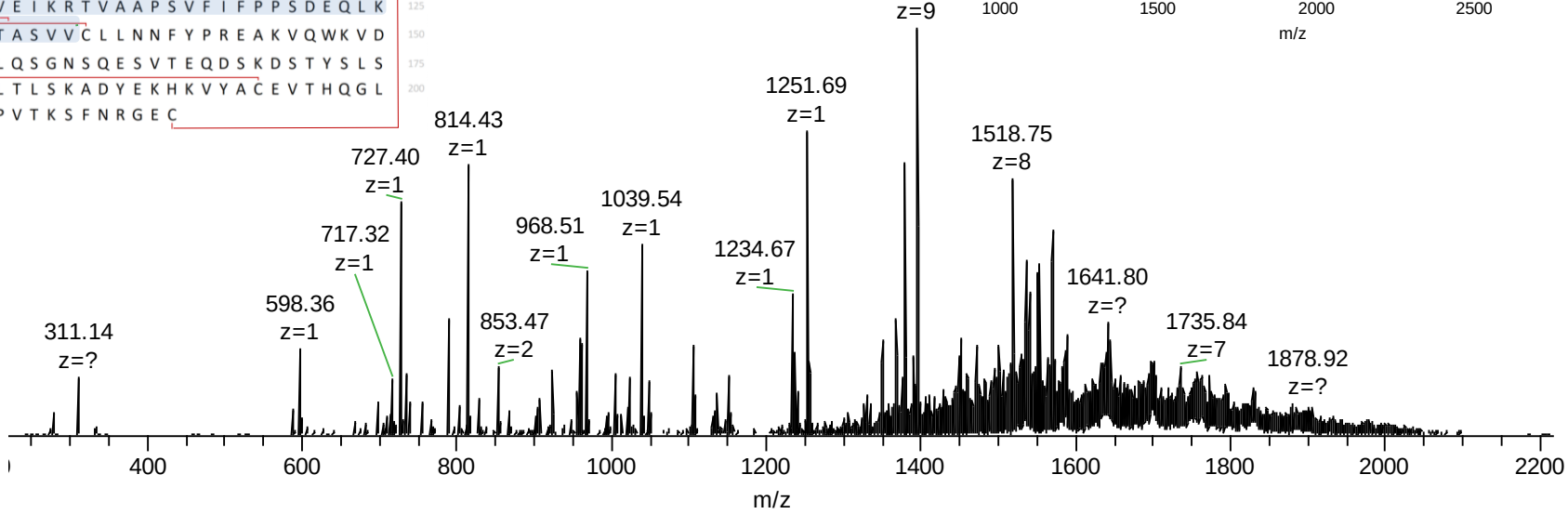


Electron Capture Dissociation

ECD of NIST mAb Fab

Isolation of charge state 31+

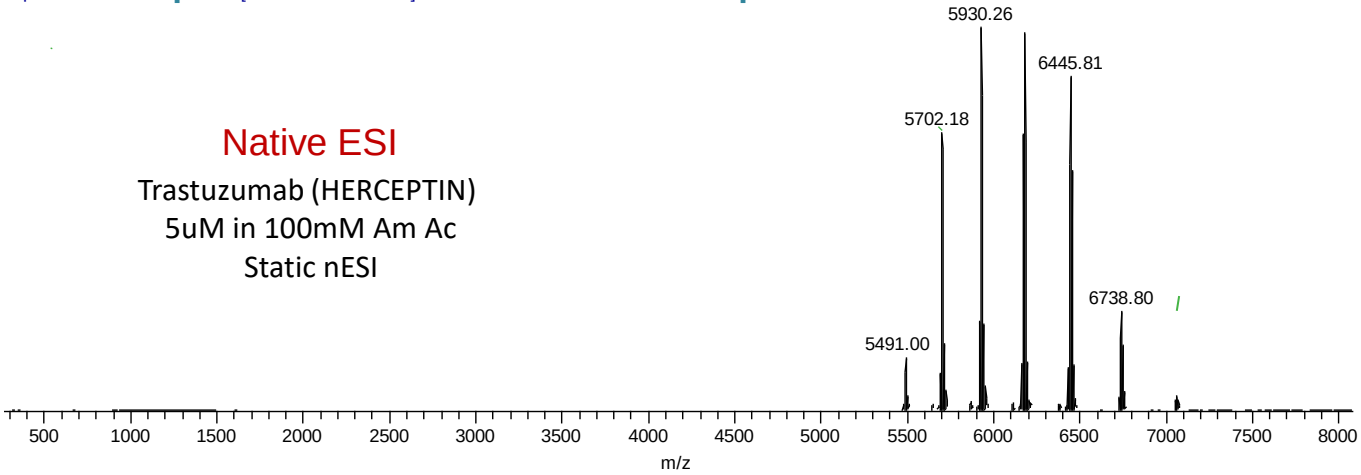




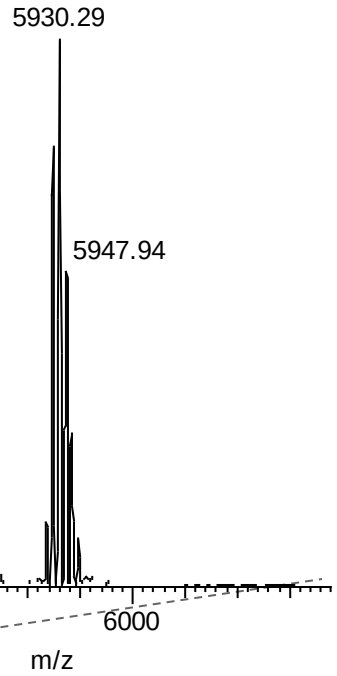
Isolation of Native Species

Native ESI

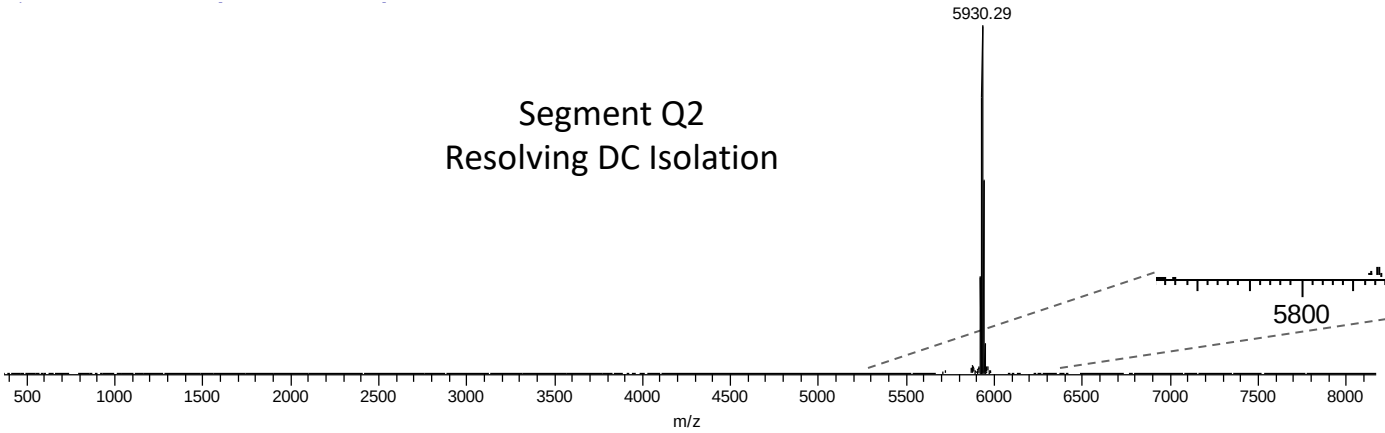
Trastuzumab (HERCEPTIN)
5uM in 100mM Am Ac
Static nESI



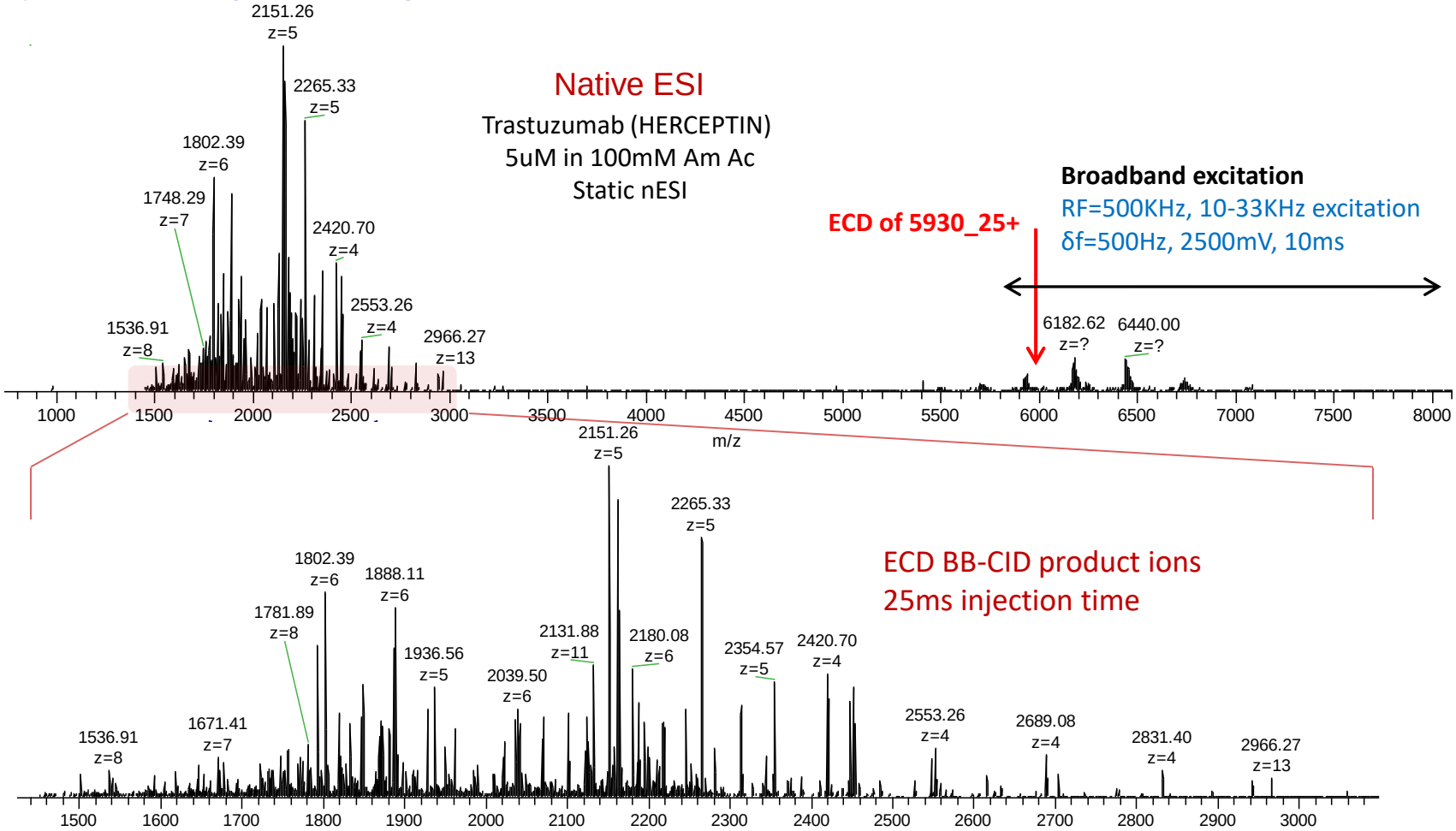
25+



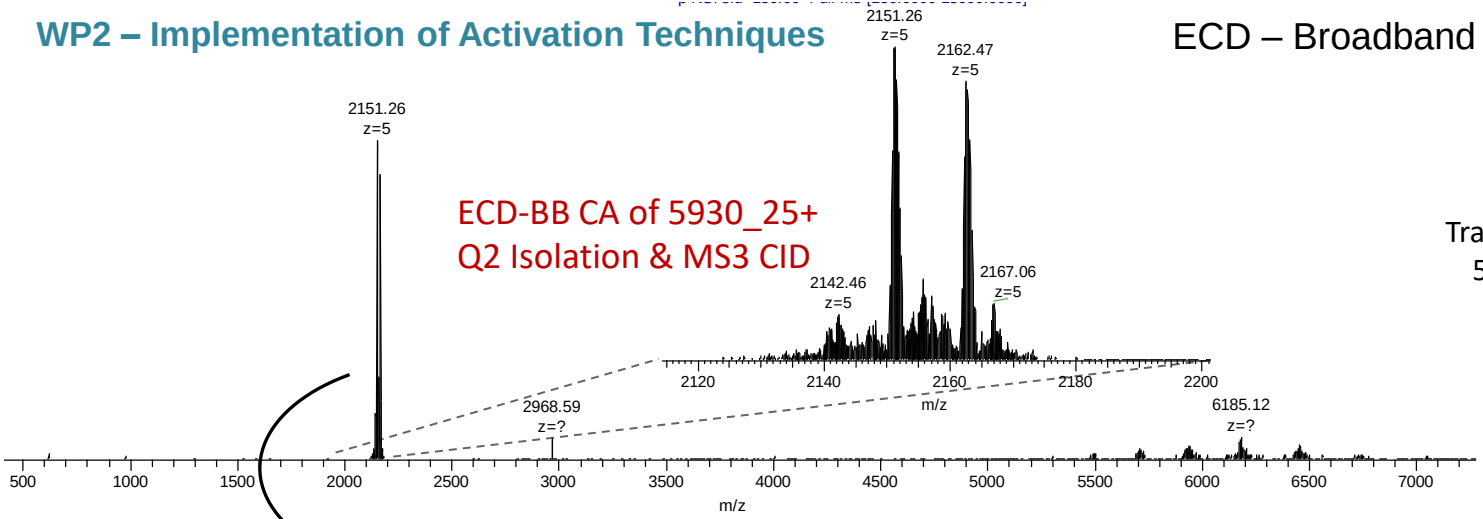
Segment Q2
Resolving DC Isolation



ECD & Broadband CID



ECD – Broadband CID – MS3 CID

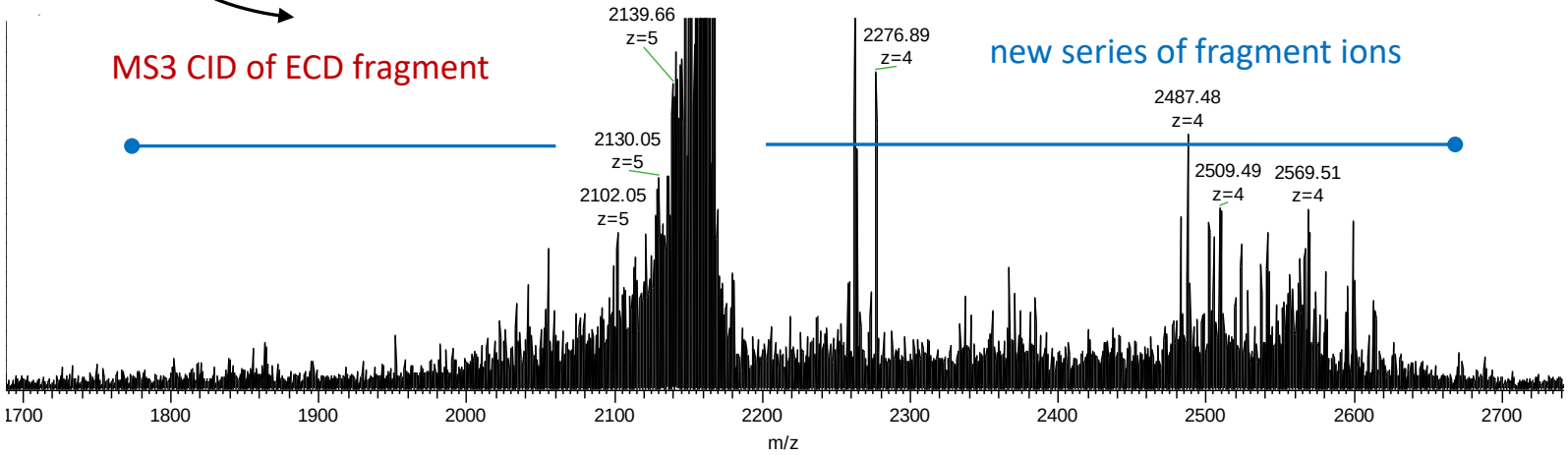


ECD-BB CA of 5930_25+
Q2 Isolation & MS3 CID

Native ESI

Trastuzumab (HERCEPTIN)
5uM in 100mM Am Ac
Static nESI

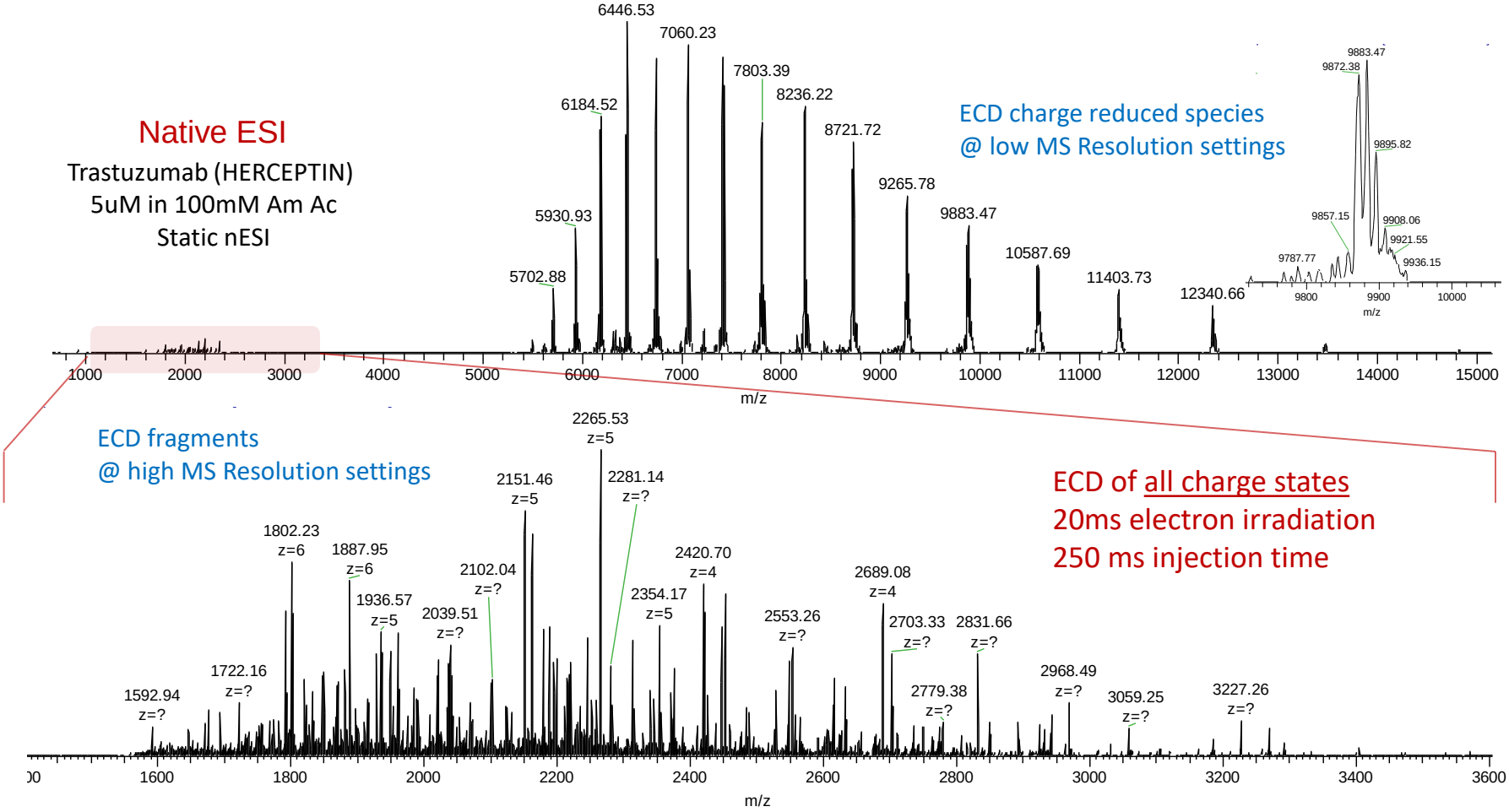
MS3 CID of ECD fragment



new series of fragment ions

WP2 – Implementation of Activation Techniques

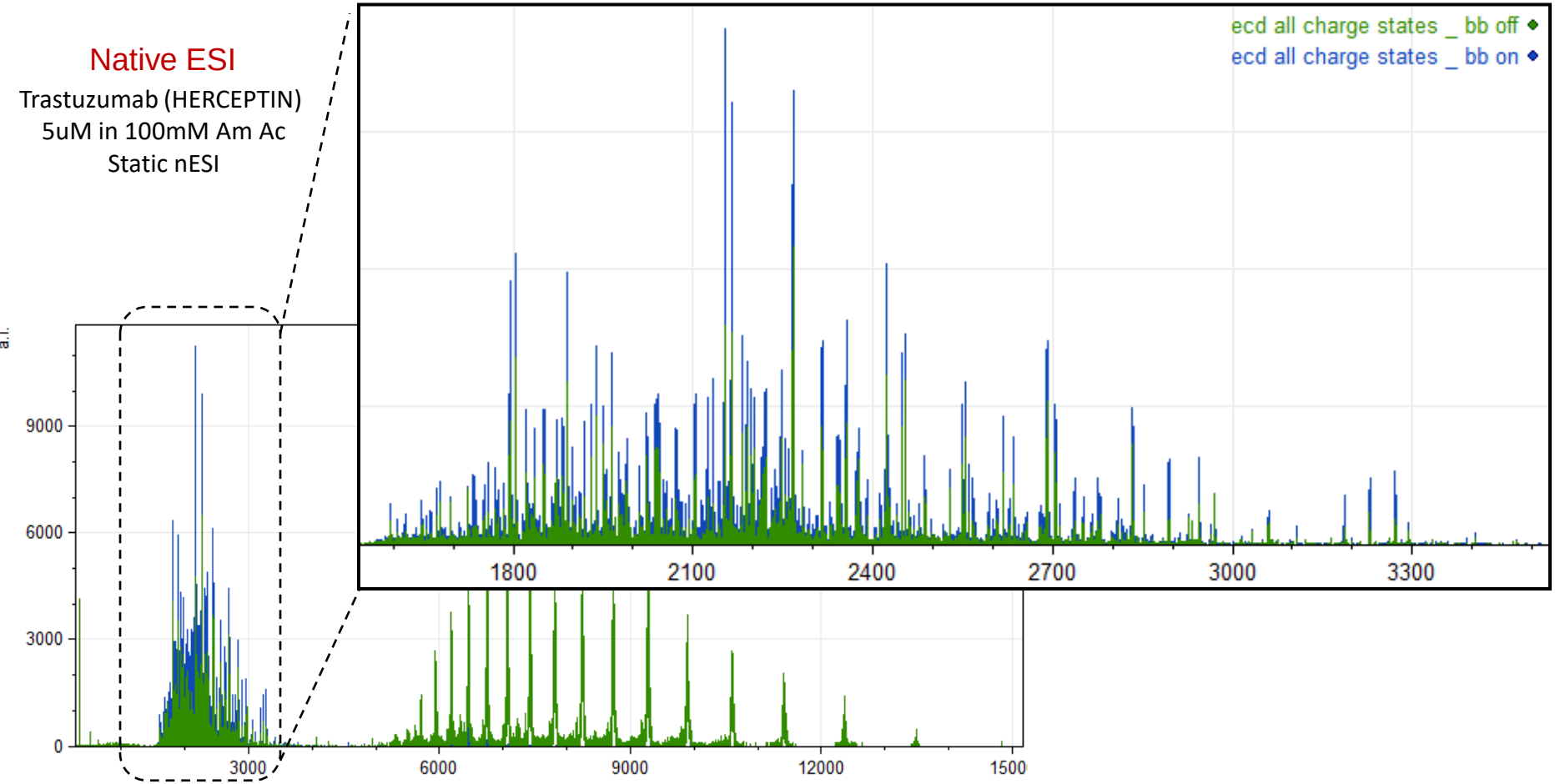
Electron Capture Dissociation



ECD & Broadband Excitation CID

Native ESI

Trastuzumab (HERCEPTIN)
5uM in 100mM Am Ac
Static nESI

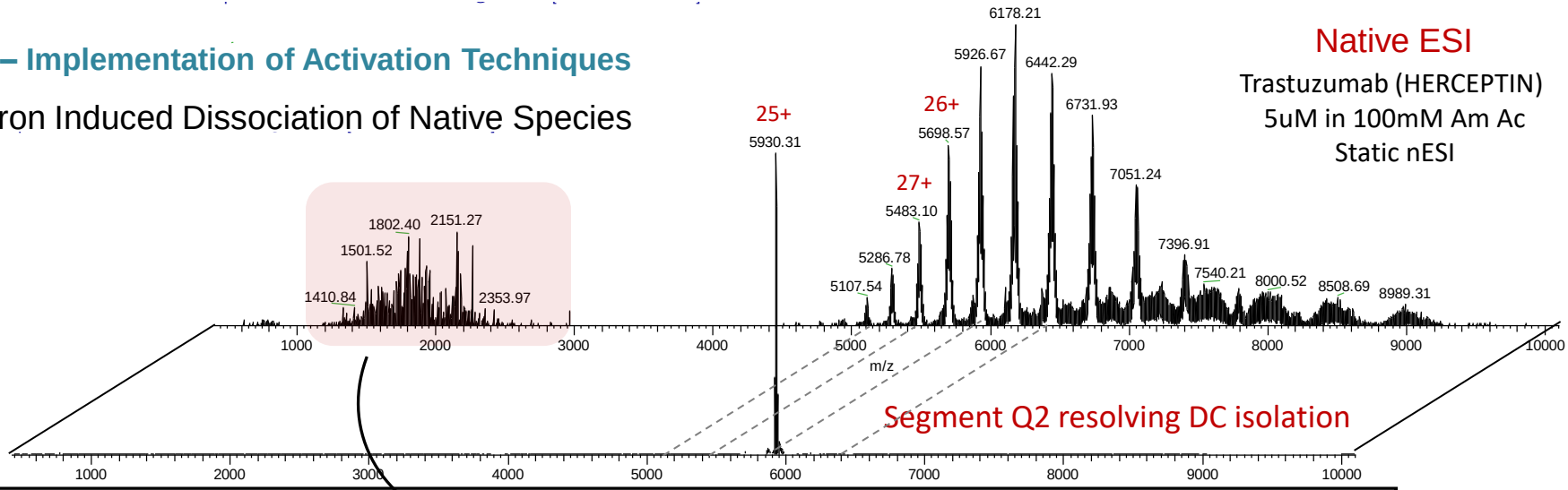


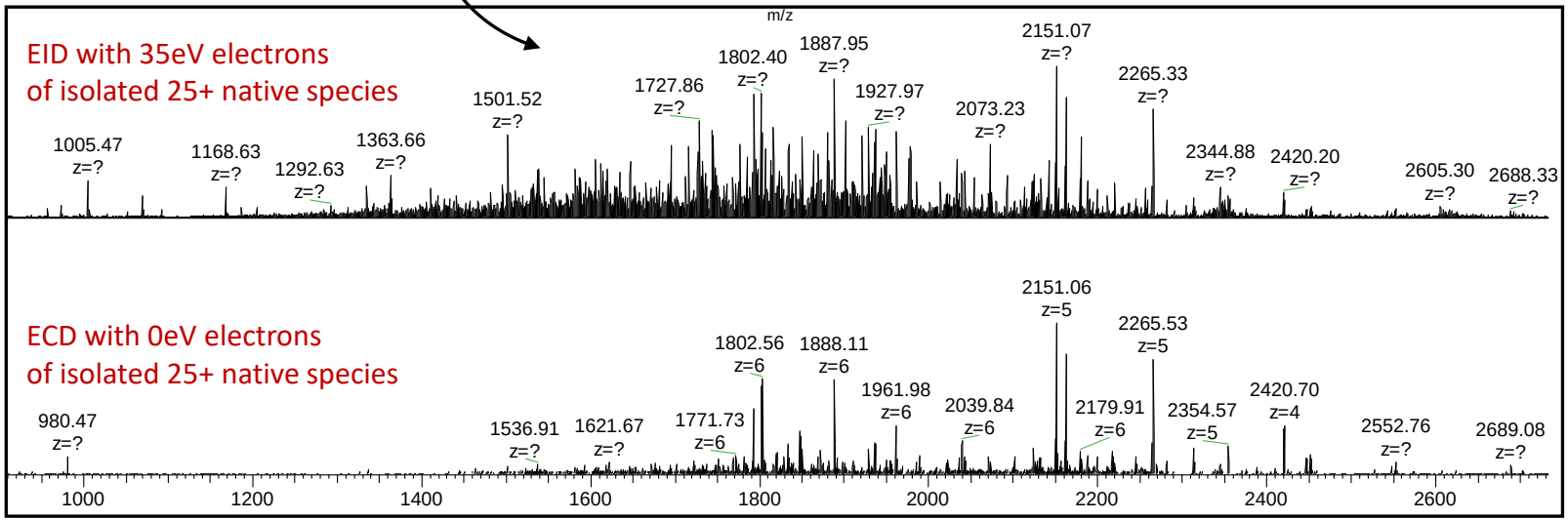
WP2 – Implementation of Activation Techniques

Electron Induced Dissociation of Native Species

Native ESI

Trastuzumab (HERCEPTIN)
5uM in 100mM Am Ac
Static nESI

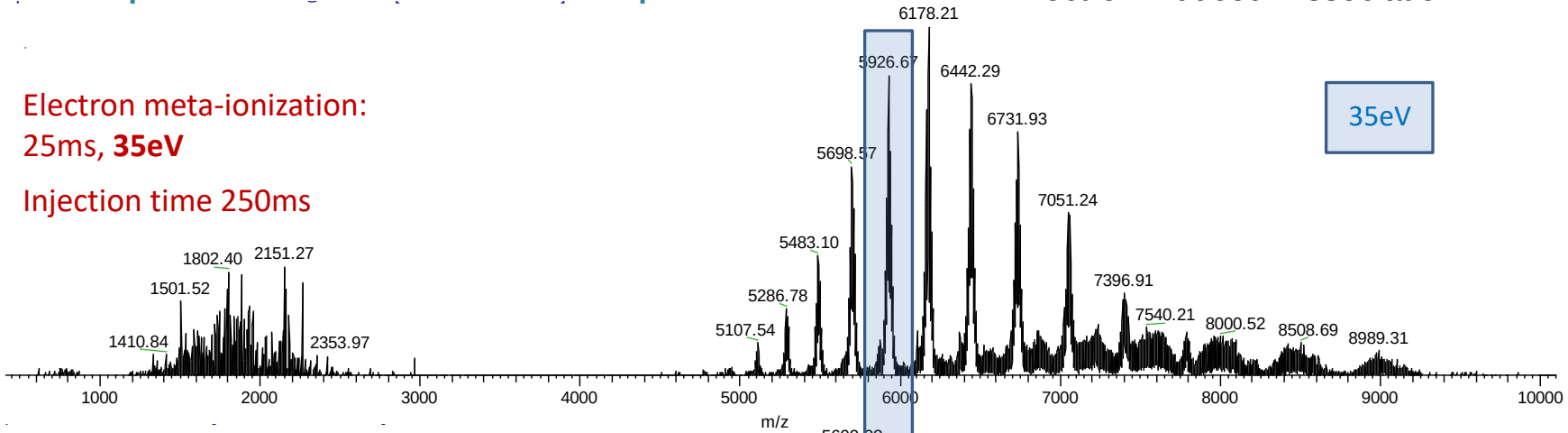




Electron Induced Dissociation

Electron meta-ionization:
25ms, **35eV**

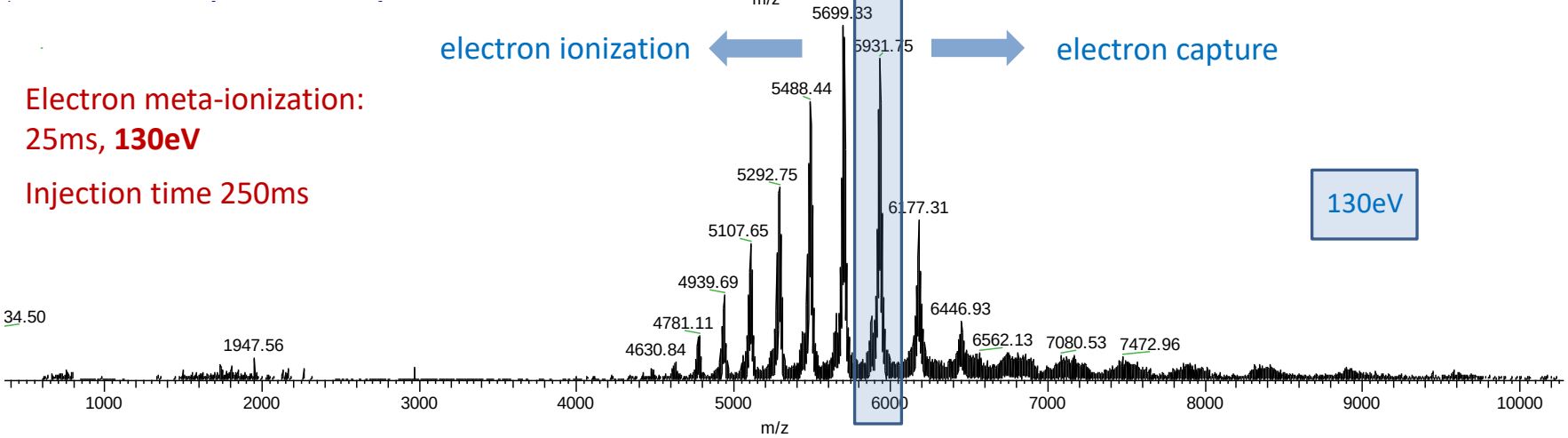
Injection time 250ms



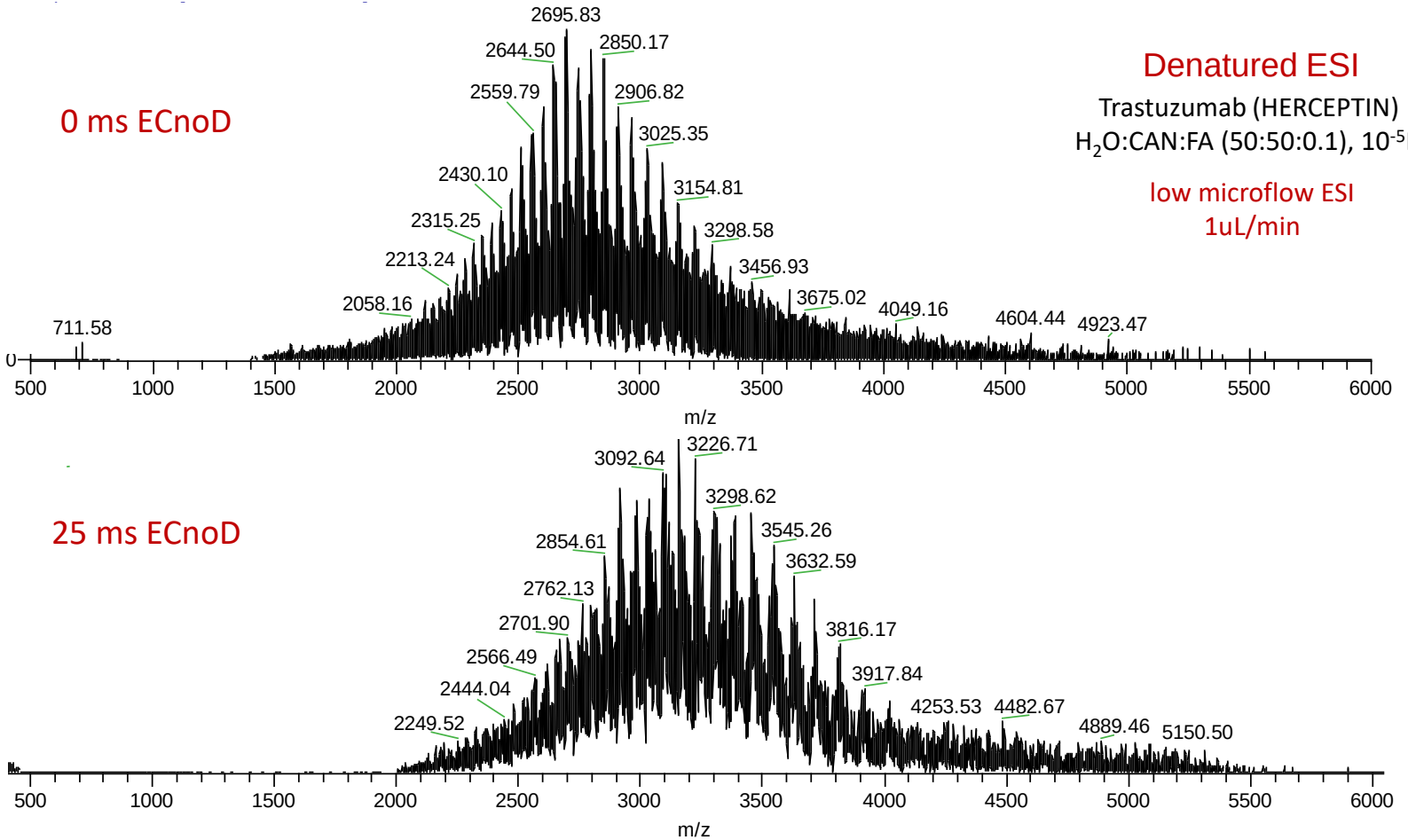
electron ionization ⟵ ⟶ electron capture

Electron meta-ionization:
25ms, **130eV**

Injection time 250ms



ECnoD



Denatured ESI

Trastuzumab (HERCEPTIN)

H_2O:CAN:FA (50:50:0.1), 10^{-5}M

low microflow ESI

1uL/min

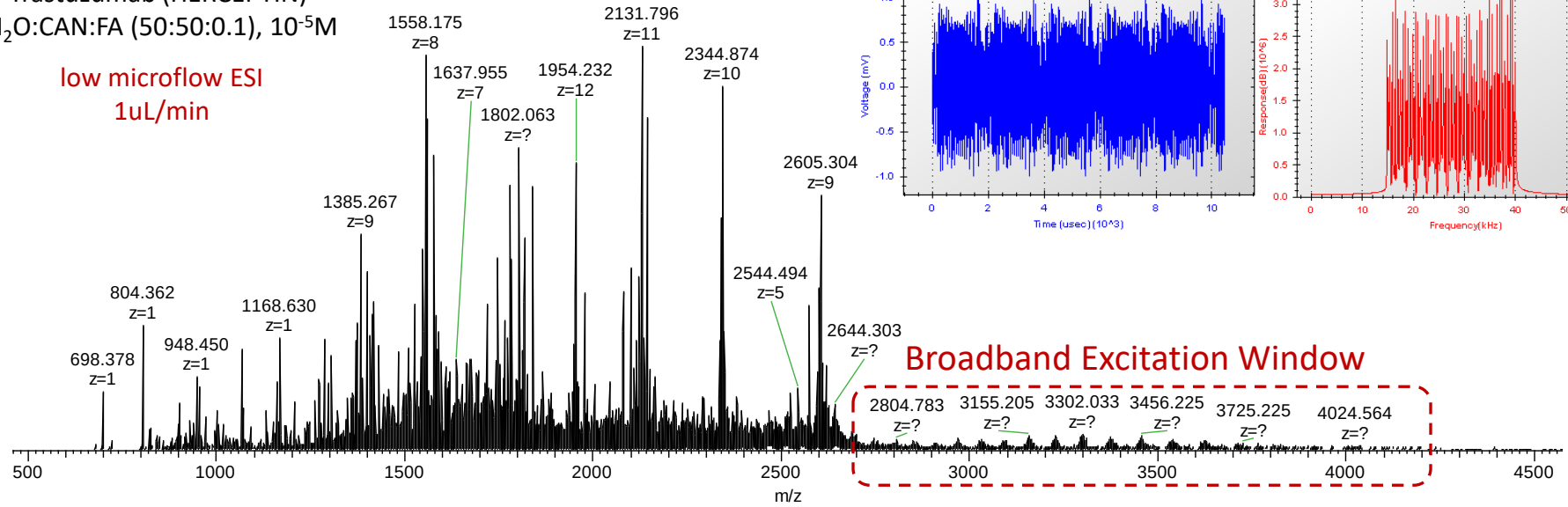
ECnoD & Broadband CID

Denatured ESI

Trastuzumab (HERCEPTIN)

H_2O:CAN:FA (50:50:0.1), 10^{-5}M

low microflow ESI
1uL/min



Broadband Excitation Window

25ms Electron Capture (1eV electron energy)

10ms Broadband Excitation CID (RF=600kHz, ω = 15-40 kHz, δω=500Hz, gain=2500mV)

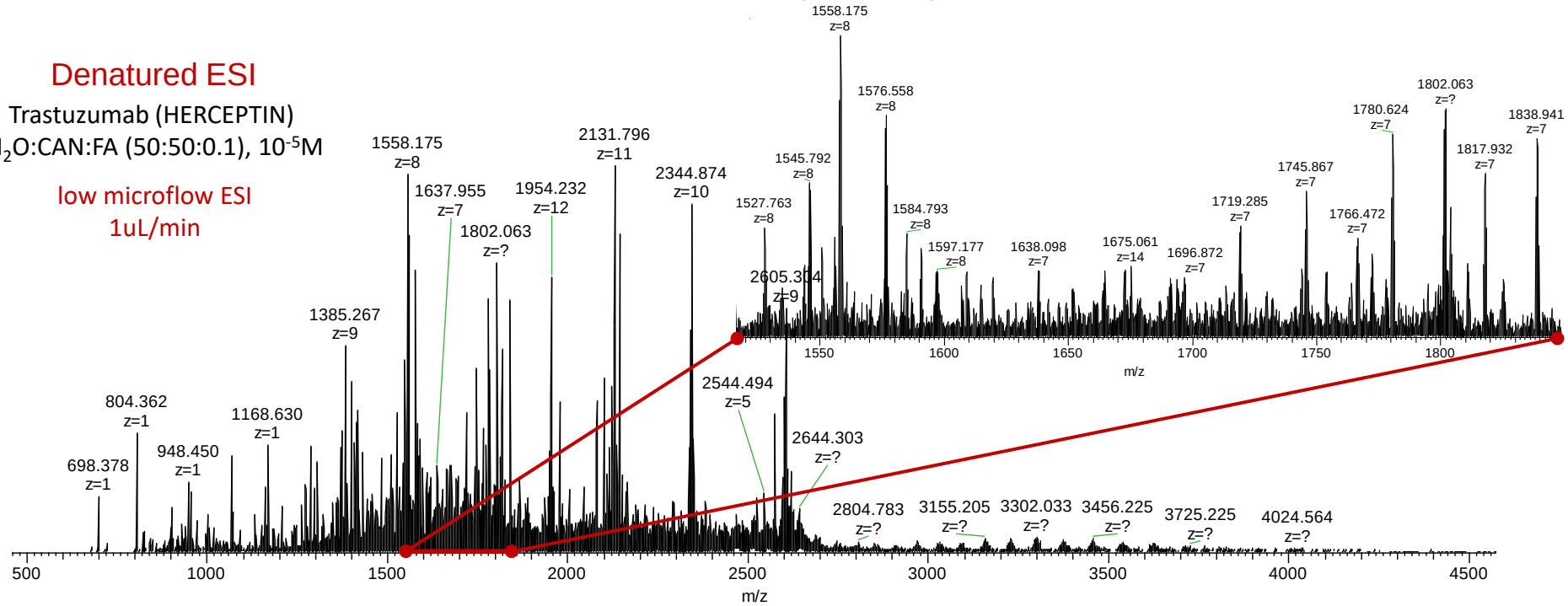
ECnoD & Broadband CID

Denatured ESI

Trastuzumab (HERCEPTIN)

H_2O:CAN:FA (50:50:0.1), 10^{-5}M

low microflow ESI
1uL/min

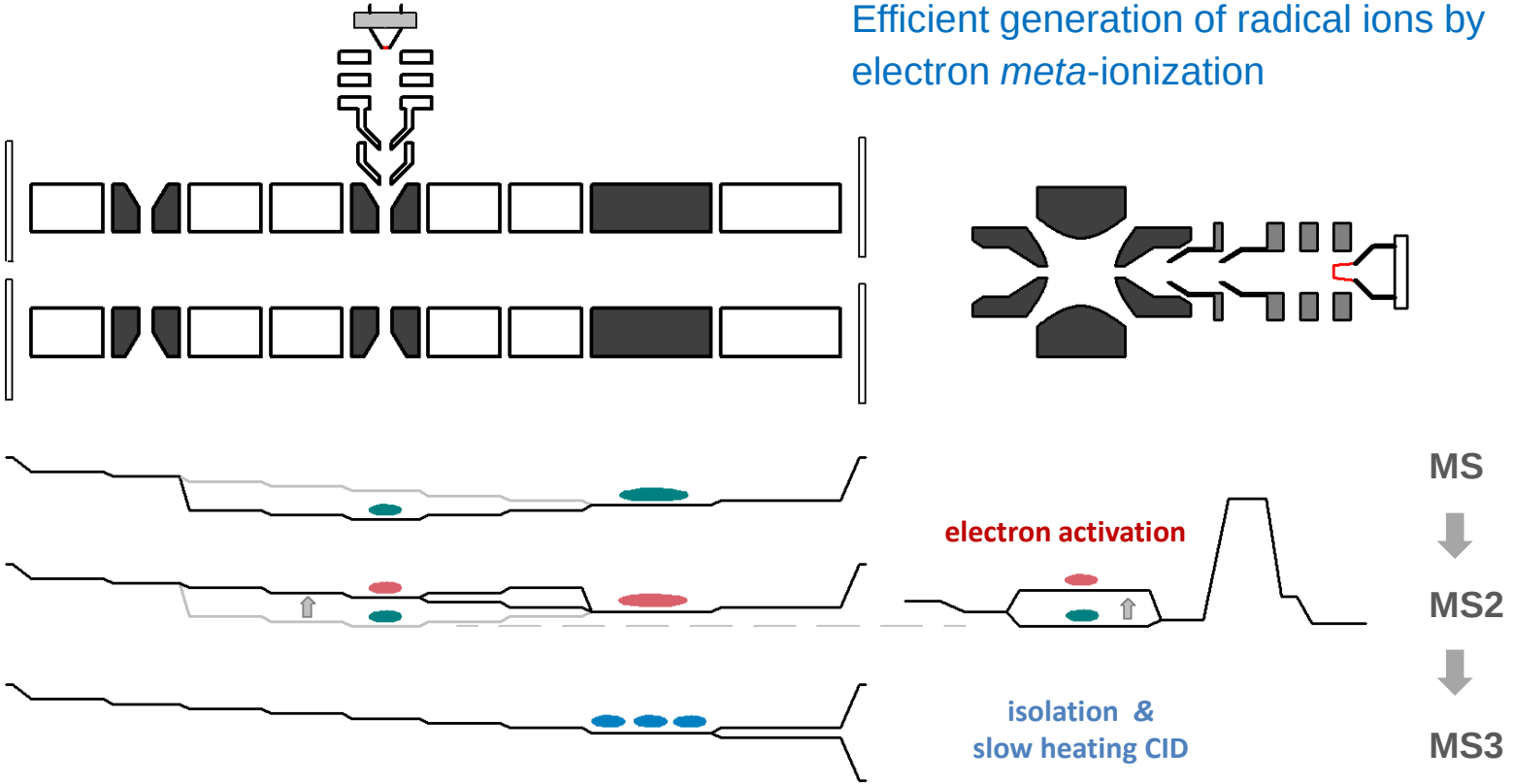


25ms Electron Capture Dissociation (1eV electron energy)

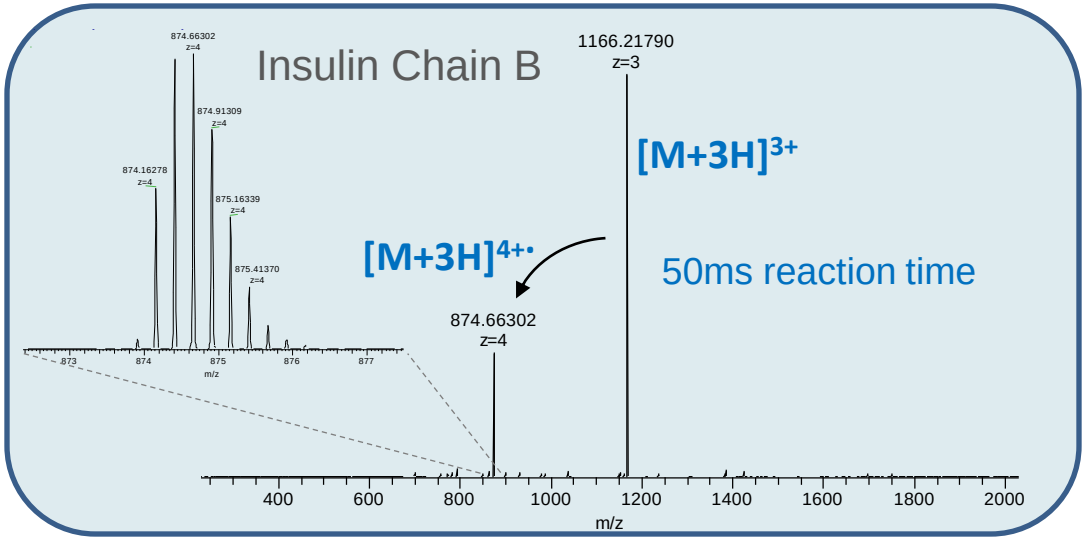
10ms Broadband Excitation (RF=600kHz, ω = 15-40 kHz, δω=500Hz, gain=2500mV)

Multiple-Stage Tandem Mass Spectrometry

Efficient generation of radical ions by electron *meta*-ionization



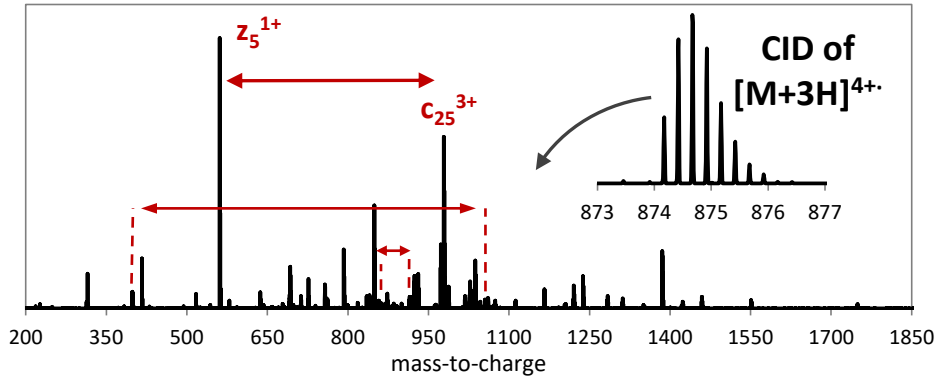
Multiple-Stage Tandem Mass Spectrometry



Electron *meta*-Ionization

$$[M+3H]^{3+} + e^- \rightarrow [M+3H]^{4+\bullet} + 2e^-$$

Slow heating
CID



b-y & a primary fragment ions identical to CID of $[M+4H]^{4+}$

F V N Q H L C G S H L V E A L Y L V C G E R G F F

Y T P K A

preferential c-z• cleavages on aromatic residues for $[M+3H]^{4+\cdot}$

F V N Q H L C G S H L V E A L Y L V C G E R G F F

Y T P K A

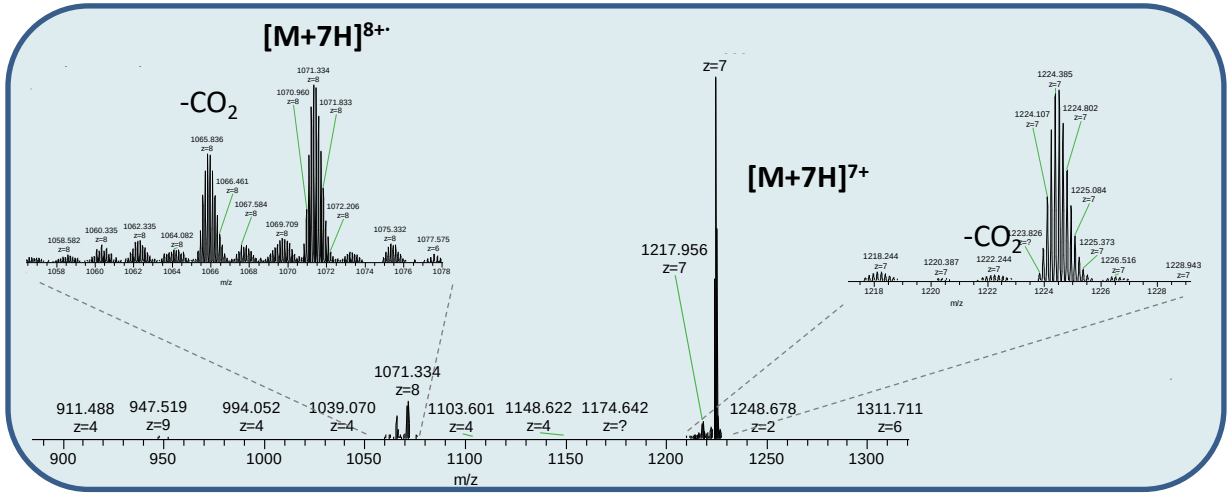
Multiple-Stage Tandem Mass Spectrometry

Ubiquitin

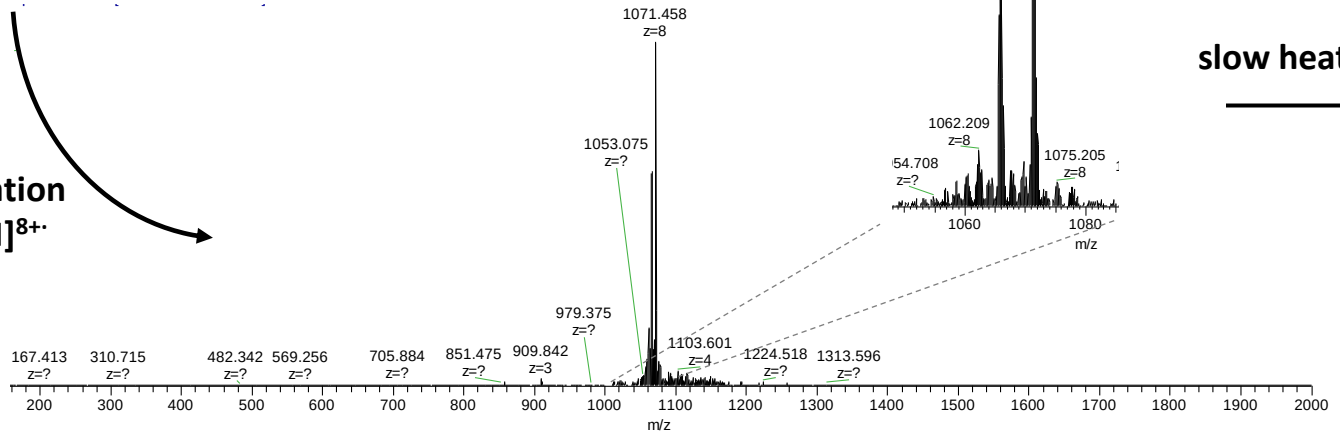
Electron *meta*-Ionization

$[M+7H]^{7+} + e^- \longrightarrow [M+7H]^{8+\cdot} + 2e^-$

~ 40eV electrons



Q2 isolation
$[M+7H]^{8+\cdot}$



slow heating CID

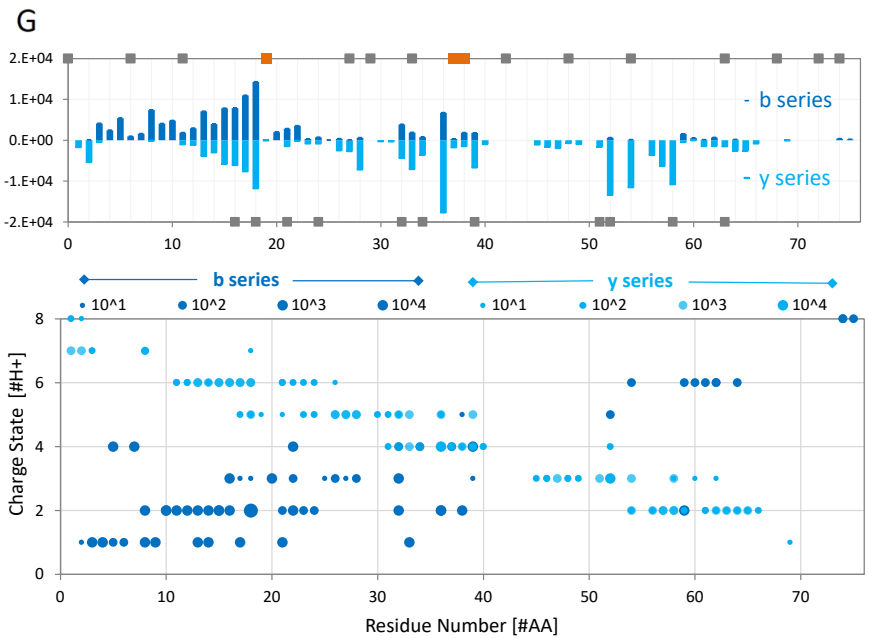
Multiple-Stage Tandem Mass Spectrometry

$[M+7H]^{8+\cdot}$

MQIFVKTLTGKTITLEVEPSDTIEN

VKAKIQDKEGIPPDQQRLIFAGKQL

EDGRTLSDYNIQKESTLHLVLRLRG

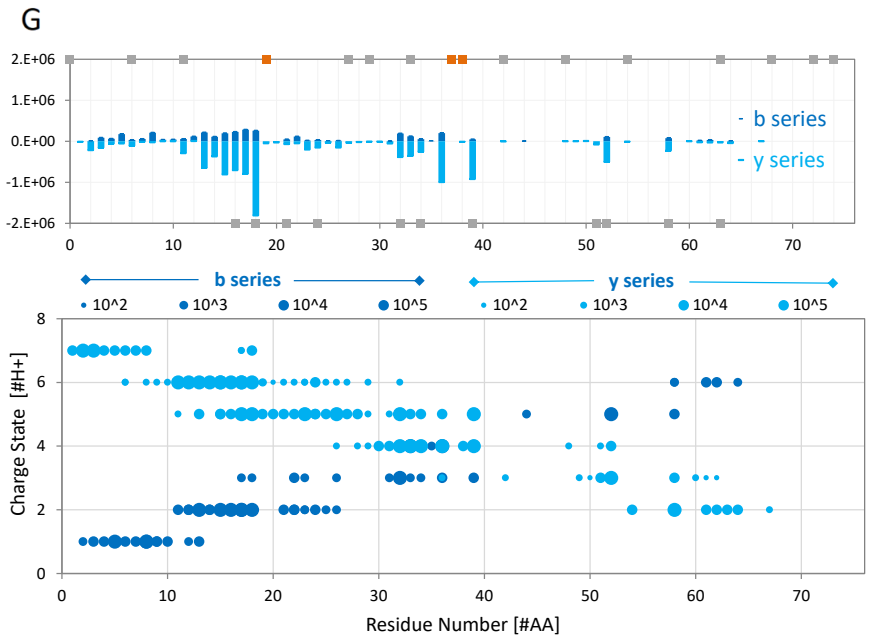


$[M+8H]^{8+}$

MQIFVKTLTGKTITLEVEPSDTIEN

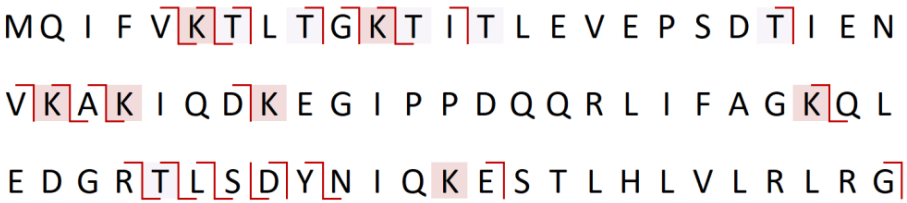
VKAKIQDKEGIPPDQQRLIFAGKQL

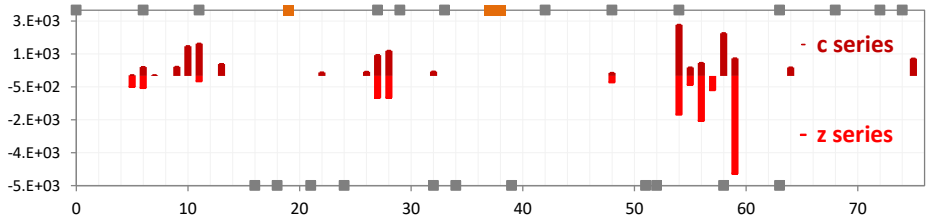
EDGRTLSDYNIQKESTLHLVLRLRG

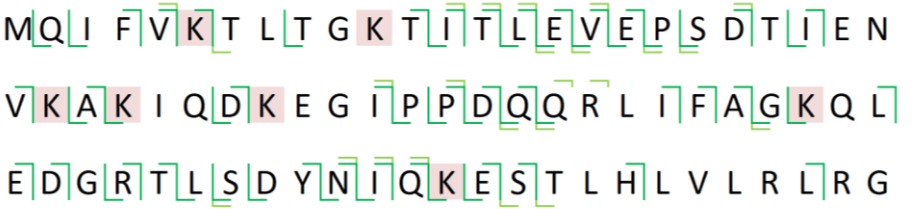


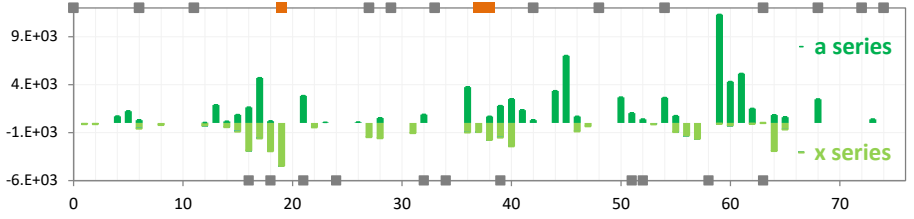
Multiple-Stage Tandem Mass Spectrometry

Hydrogen deficient protein ion : **$[M+7H]^{8+\cdot}$**









preferential c-z$^{\bullet}$ and a-x cleavages for $[M+7H]^{8+\cdot}$

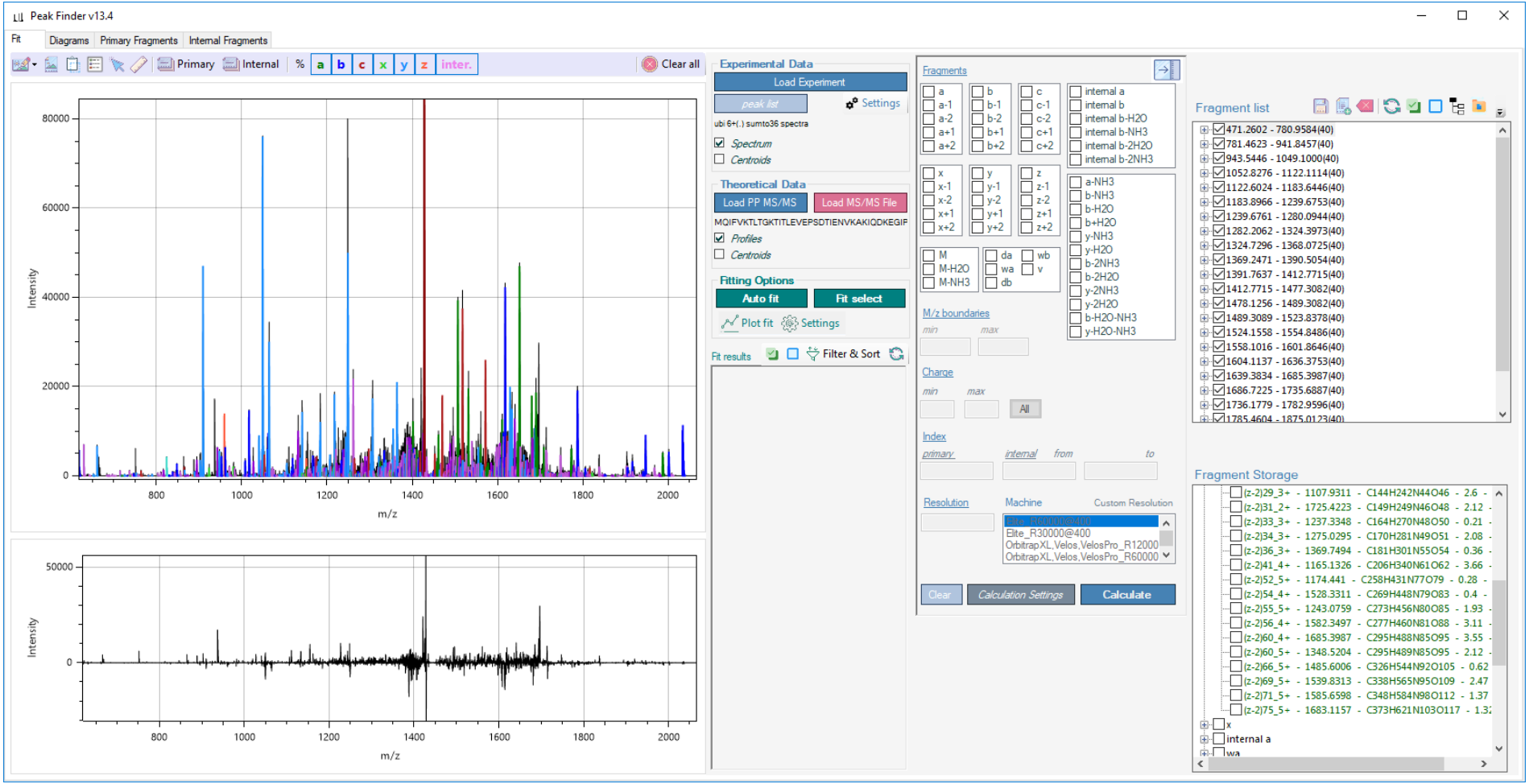
Primary fragment types, complementarity and H atom rearrangements

-3H•	-2H•	-H•	0	+H•
		b (mMass)	b•	b'
		secondary losses	y•	y'
		a (mMass)		
		z (mMass)	z•	z'
		c	c•	c' (mMass)
		a (mMass)	a•	a'
		x (mMass)	x•	
	z▪	z (mMass)	z•	
			c•	c' (mMass)
b◊	b▪	b (mMass)	b•	b'
		y	y•	y'
	z▪	z (mMass)	z•	z'
		c	c•	c' (mMass)

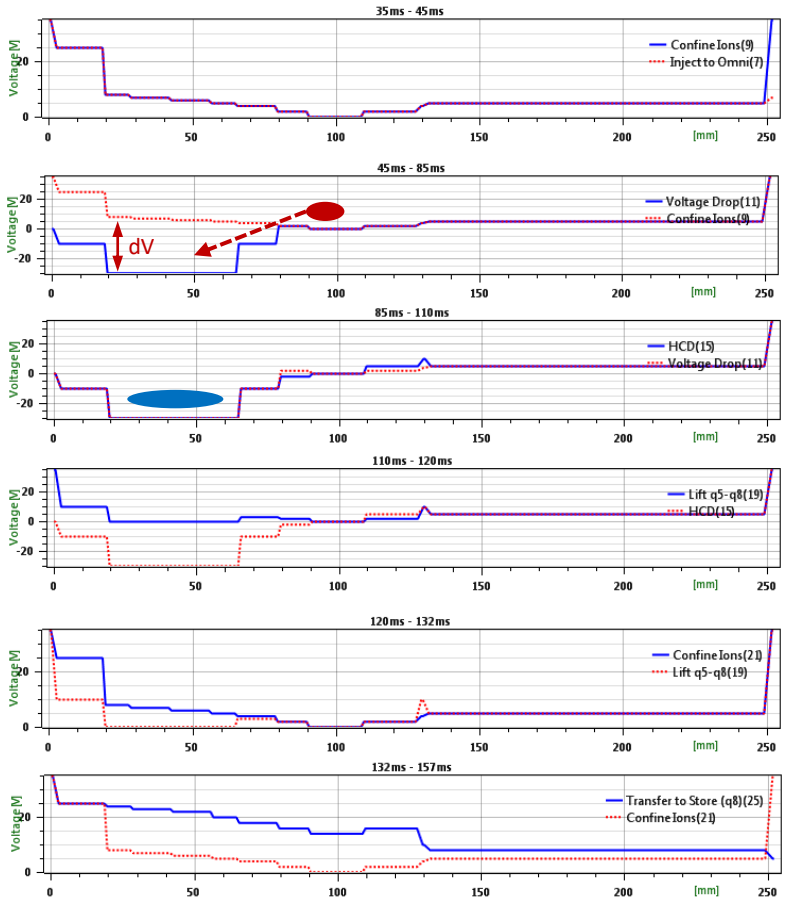
complementary fragments observed

Experiment type	Precursor mass
CID of $[M+nH]^{n+}$	$[M+nH]^{n+}$ $b + y' = b^{\bullet} + y^{\bullet}$
ECD of $[M+nH]^{n+}$	$[M+(n+1)H]^{n+\bullet}$ $\mathbf{c' + z^{\bullet}} = c^{\bullet} + z^{\bullet} + H^{\bullet}$
	$[M+(n+1)H]^{n+\bullet}$ **?**
MS3 CID of $[M+nH]^{(n+x)+\bullet}$	$[M+nH]^{(n+1)+\bullet}$ $\mathbf{c' + z▪} = c^{\bullet} + z^{\bullet} - H^{\bullet}$ $\mathbf{y' + b▪} = y^{\bullet} + b = y^{\bullet} + b^{\bullet} - H^{\bullet}$ $[M+nH]^{(n+2)+\bullet}$ $\mathbf{y' + b^{◊}} = y + b = y^{\bullet} + b^{\bullet} - 2H^{\bullet}$
MS3 ECD of $[M+nH]^{(n+1)+\bullet}$	$[M+nH]^{n+}$ or $[M+nH]^{n+\bullet\bullet}$ $\mathbf{c' + z} = c^{\bullet} + z^{\bullet}$

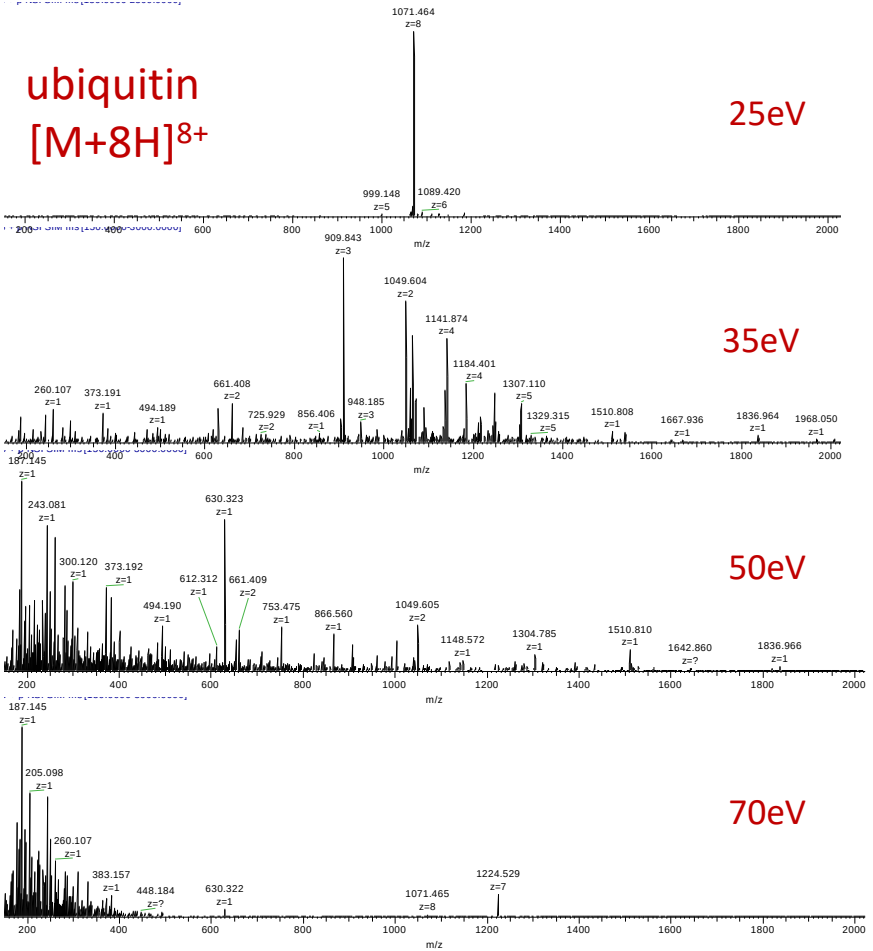
Accelerated Manual Processing Software for Deep Data Analysis



WP2 – Implementation of Activation Techniques



High Energy Collision Induced Dissociation in the Omnitrap

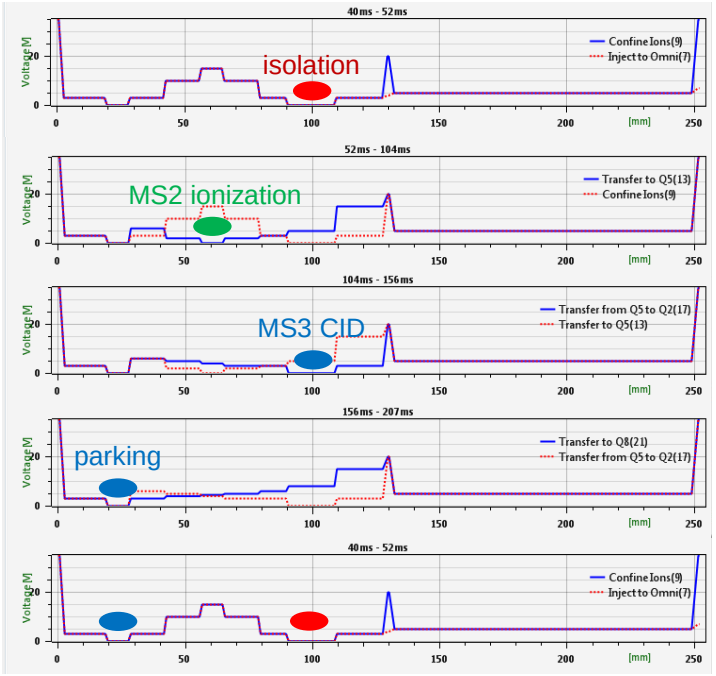


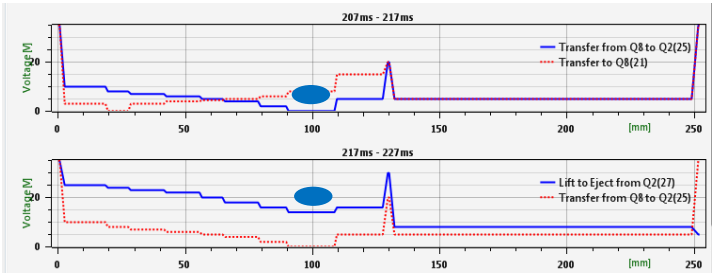
WP2 – Implementation of Activation Techniques

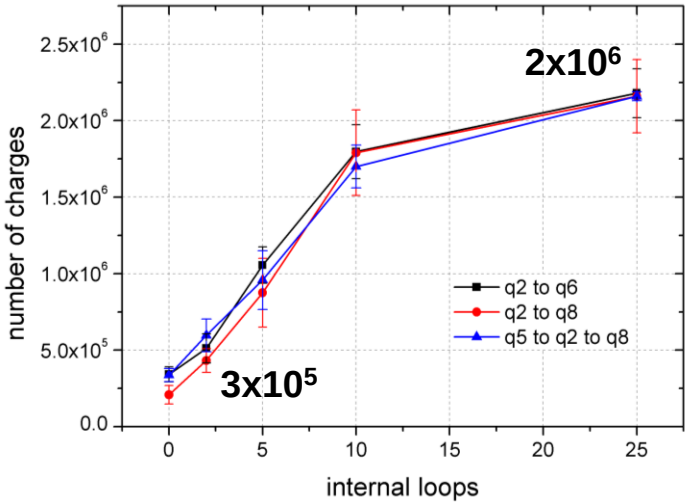
Ion Accumulation in MS3 Mode

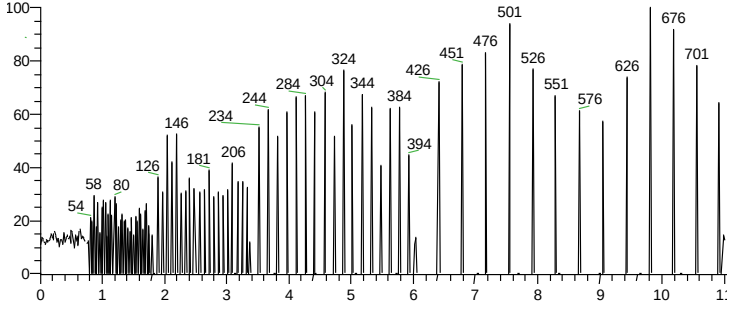
internal loop

#	Instruction	Duration [ms]	Exp.Time [ms]
1	Trigger In	Set	0
2	Digital RF [KHz]	900	0
3	Delay	10	0
4	Normal Q2	->	10
5	Gas Pulse 2 [µs]	285	10
6	Delay	5	10
7	Inject to Omni	->	15
8	Delay	25	15
9	Confine Ions	->	40
10	Delay	10	40
11	Gas Pulse 1 [µs]	155	50
12	Delay	2	50
13	Transfer to Q5	->	52
14	Delay	50	52
15	Gas Pulse 2 [µs]	285	102
16	Delay	2	102
17	Transfer from Q5 to...	->	104
18	Delay	50	104
19	Gas Pulse 1 [µs]	155	154
20	Delay	2	154
21	Transfer to Q8	->	156
22	Delay	50	156
23	Gas Pulse 2 [µs]	285	206
24	Delay	1	206
25	Transfer from Q8 to...	->	207
26	Delay	10	207
27	Lift to Eject from Q2	->	217
28	Delay	10	217
29	Eject to HCD cell	->	227
30	Delay	50	227
31	Digital RF [KHz]	0	277
32	Delay	15	277
33	Normal Q2	->	292

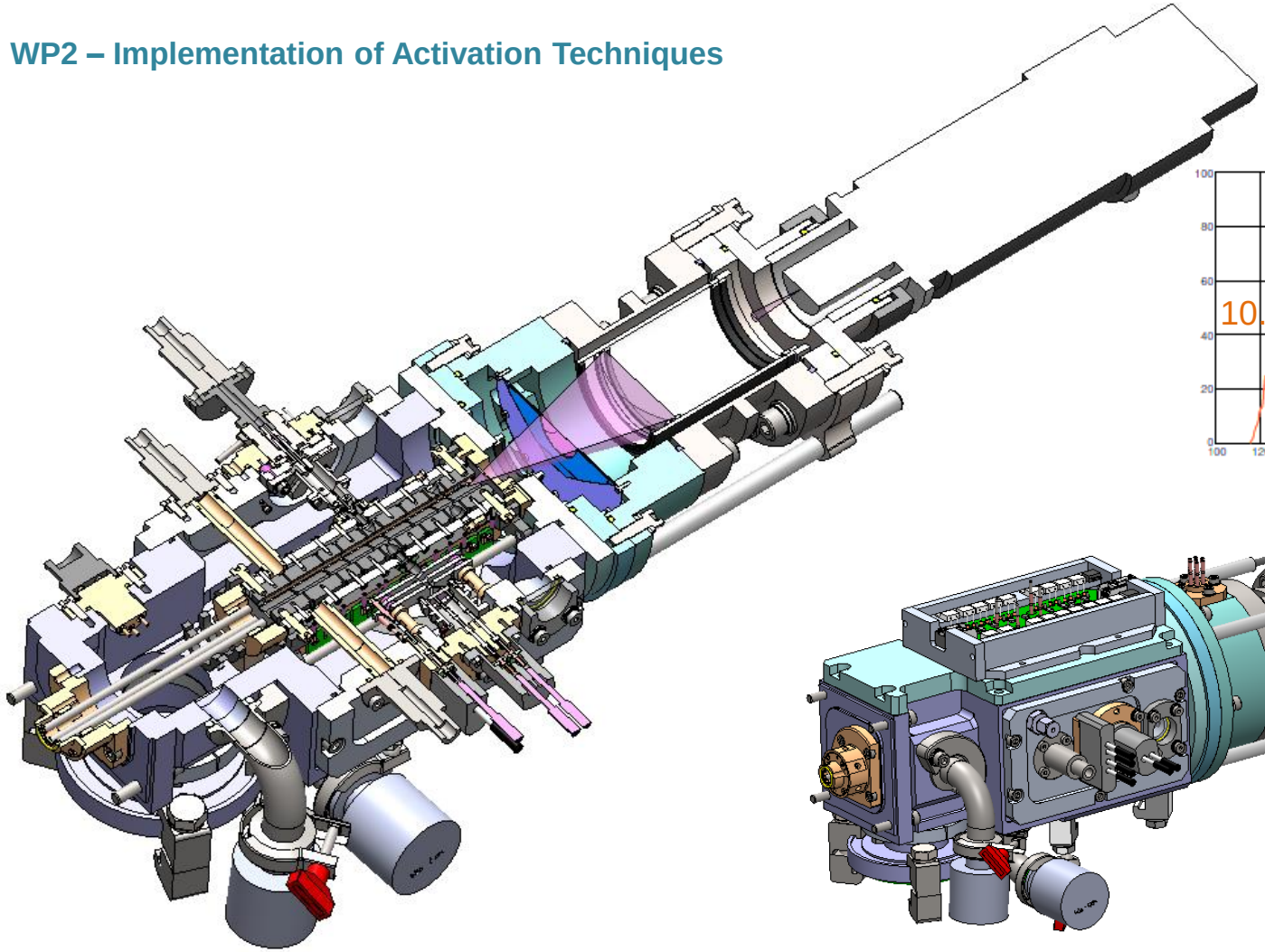


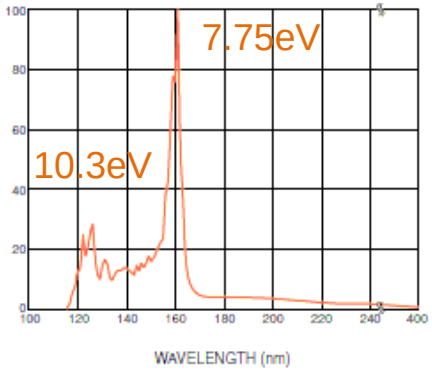




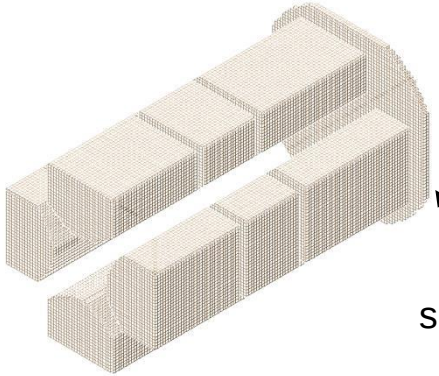


VUV Photo-dissociation

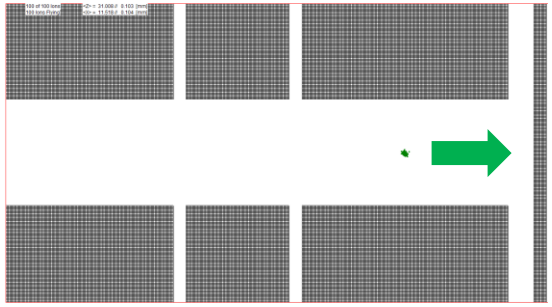


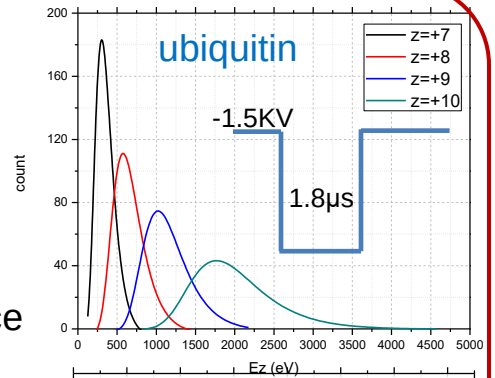


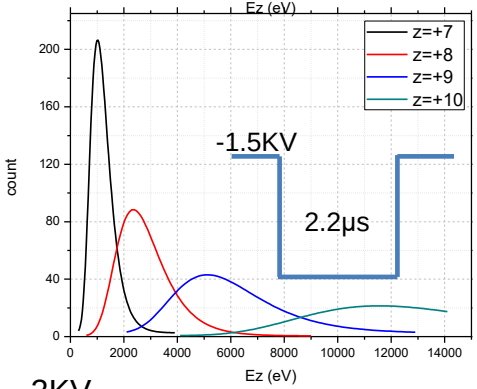
WP2 – Implementation of Activation Techniques

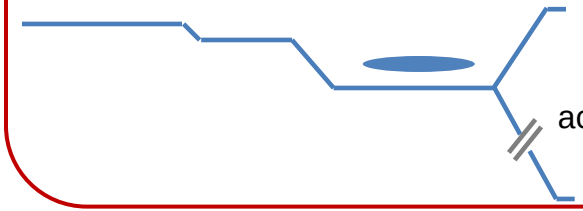


SID surface





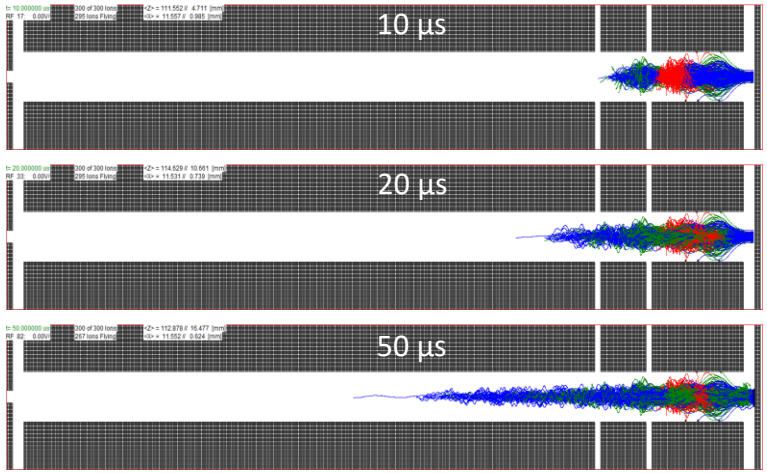




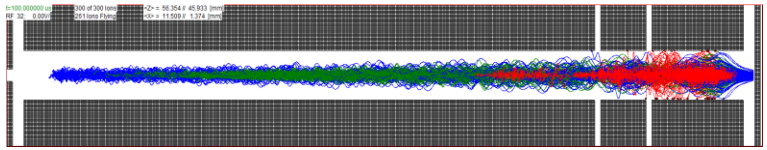
~2KV
~2µs
acceleration
pulse

Surface Induced Dissociation

recoil energy Ez= -400eV



$5x10^{-2}$ mbar



$3x10^{-2}$ mbar

+100V