



Charge state separation mass spectrometry on QTOF platform for top-down protein analysis

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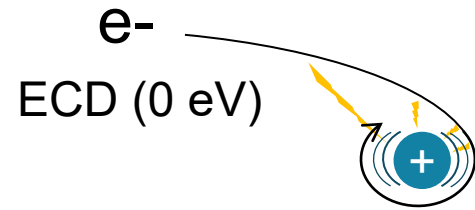
June 09, 2022, ASMS, Minneapolis

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- Resolution: Quadrupole < TOF ($m/\Delta m \sim 30,000\text{-}50,000$) < Fourier transform (FT)
- Ion transmission enhanced by Zeno trap pulsing
- Advantage: Fast TOF MS cycle of 1.5 kHz with Zeno trap pulsing
- Applications using high-sensitivity performance:
 - Top-down protein sequencing using electron capture dissociation (ECD)

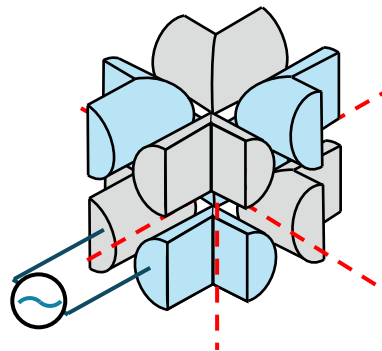
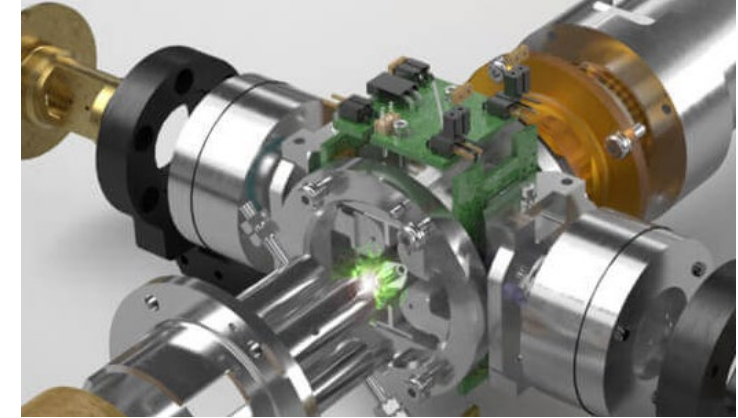
EAD device using a branched RF ion trap

free electron

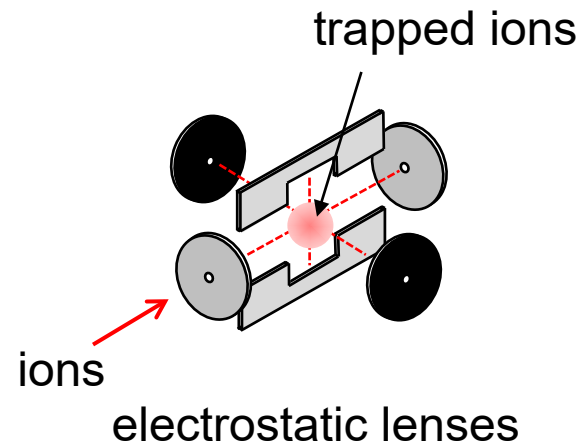


radical state

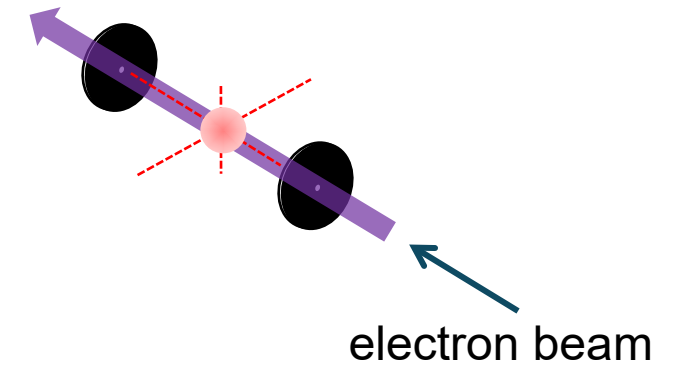
fragments



RF ion trap potential



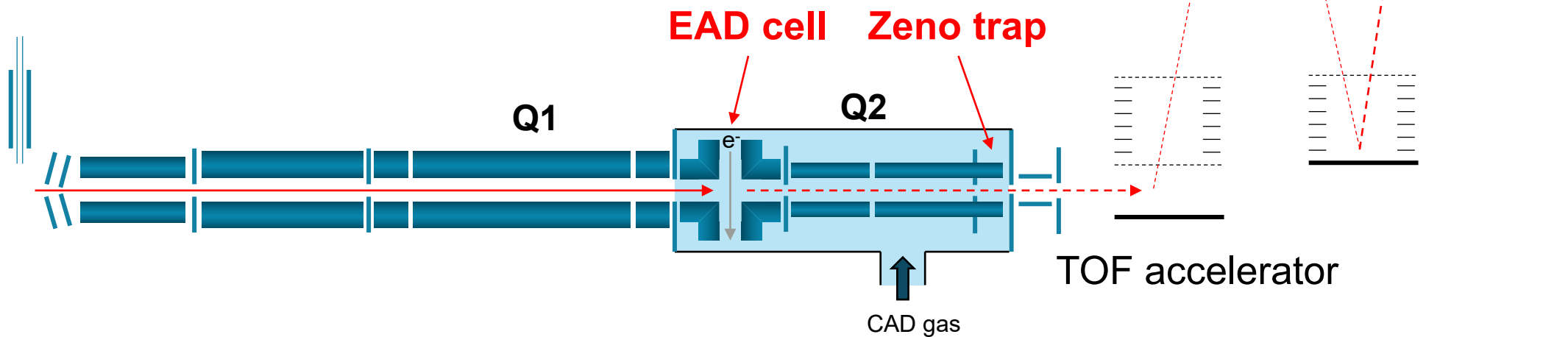
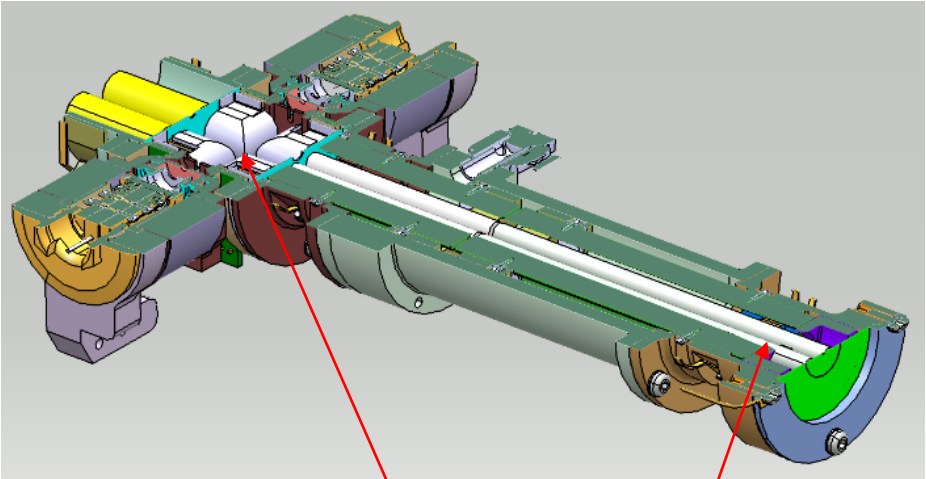
magnetic field



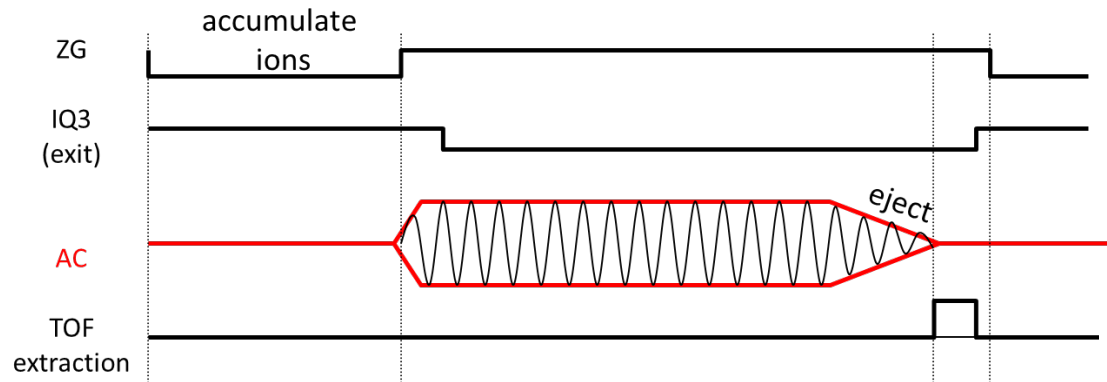
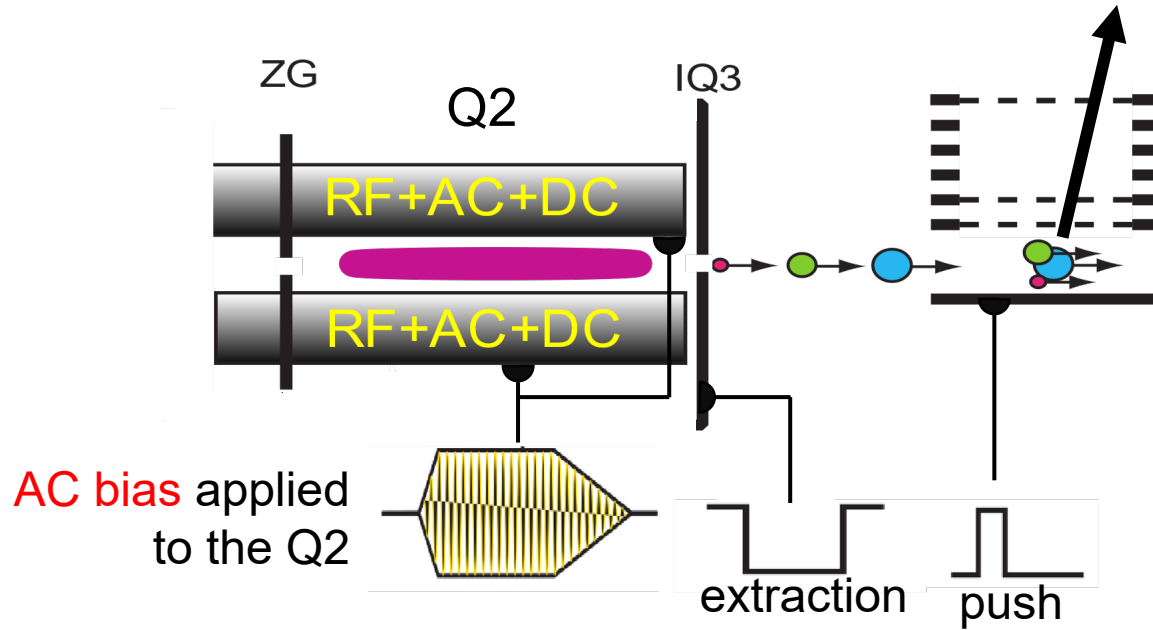
ref. T. Baba et al., *Anal. Chem.* 2014
T. Baba et al., *ASMS 2014*, Oral

Zeno trap pulsing for ion transmission enhancement

Q2 unit for both EAD and CAD, including the Zeno trap



PRINCIPLE: ORDERED ION EJECTION FROM Q2



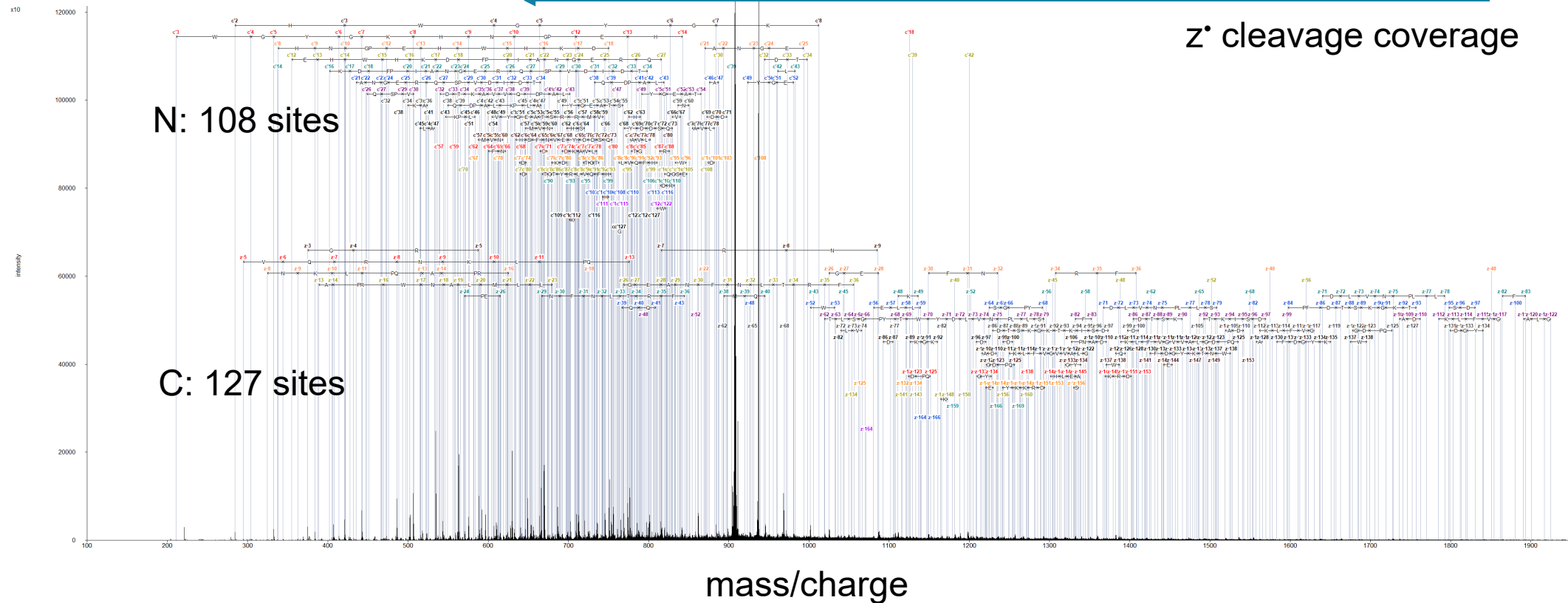
- (1) Axial trapping by **pseudo potential by the AC bias**
*AC "bias": The same AC phase on all four rods
- (2) **m/z ordered ion ejection** by combination of
the lamping AC and the extraction field on IQ3
Spatial focusing of wide range of m/z in the TOF accelerator
- (3) Acceleration of the focused ions

Top-down sequencing of medium-size protein, carbonic anhydrase 2 (CA2)

ACCUMULATION FOR 4 MIN → CLEAVAGE COVERAGE: 90.2%

c' cleavage coverage

SHHVGGRHNGP²HWKDFP¹ANG²RS²PV¹DT¹AVV¹DPAL¹KPL¹ALV¹GE¹A¹SR¹MM¹NN¹GH¹SP¹NE¹V¹DS¹SD¹RAVL¹RD¹GL¹PT¹GT¹RL¹V¹GF¹HL¹VSS¹SD¹SS¹EL¹TV¹RR¹VA¹EL¹HL¹VH¹NT¹RY¹GF¹TA¹AQ¹QP¹DGL¹AV¹VG¹EL¹KV¹GD¹AN¹PA¹LQ¹KV¹LD¹AL¹DSL¹KT¹KG¹ST¹DF¹NP¹DF¹PS¹LL¹PN¹VL¹DY¹WT¹YPS¹LIT¹IP¹LL¹ES¹VT¹WL¹KE¹PI¹SV¹SS¹QML¹K¹F¹TL¹NF¹NA¹E¹GE¹PE¹LL¹ML¹AN¹WP¹AO¹PL¹KN¹RO¹VG¹FP¹K



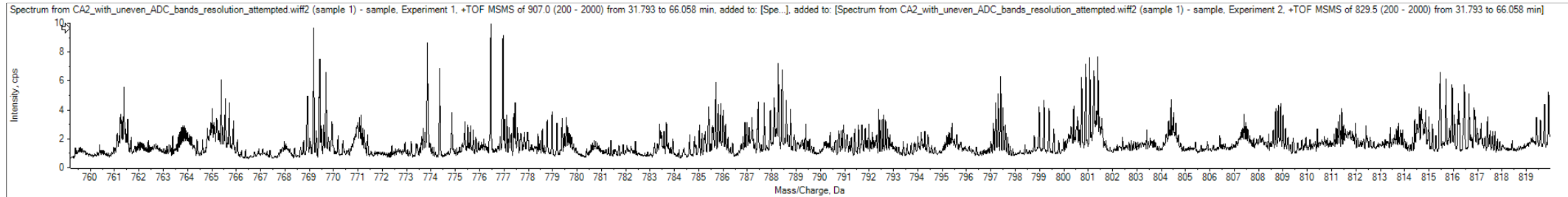
z' cleavage coverage

ref. T. Baba et al. JASMS 2021

T. Baba et al. ASMS 2021

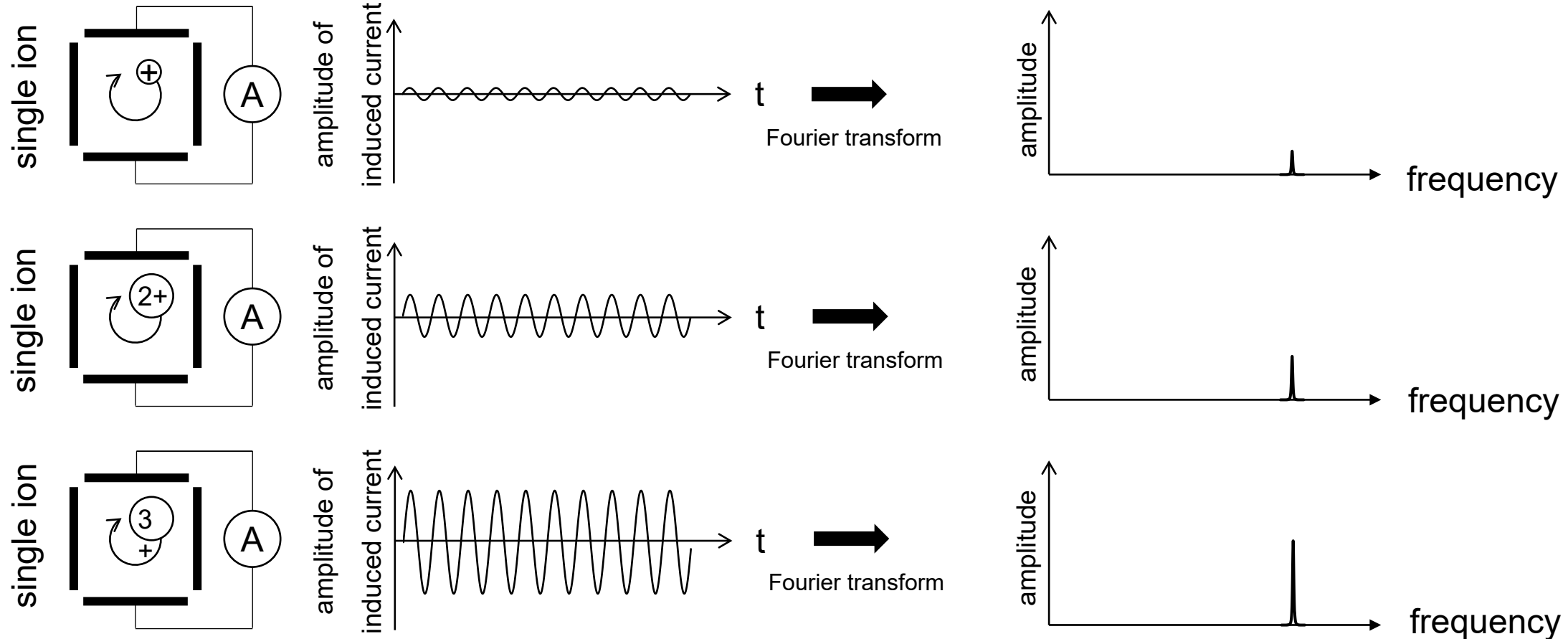
Opportunity for improvement

OVERLAPPED ^{13}C PROFILES OF ECD PRODUCTS WITH MULTIPLE CHARGE STATES



- By charge state separation
 - Hidden highly charged peaks should emerge behind the lower charged peaks

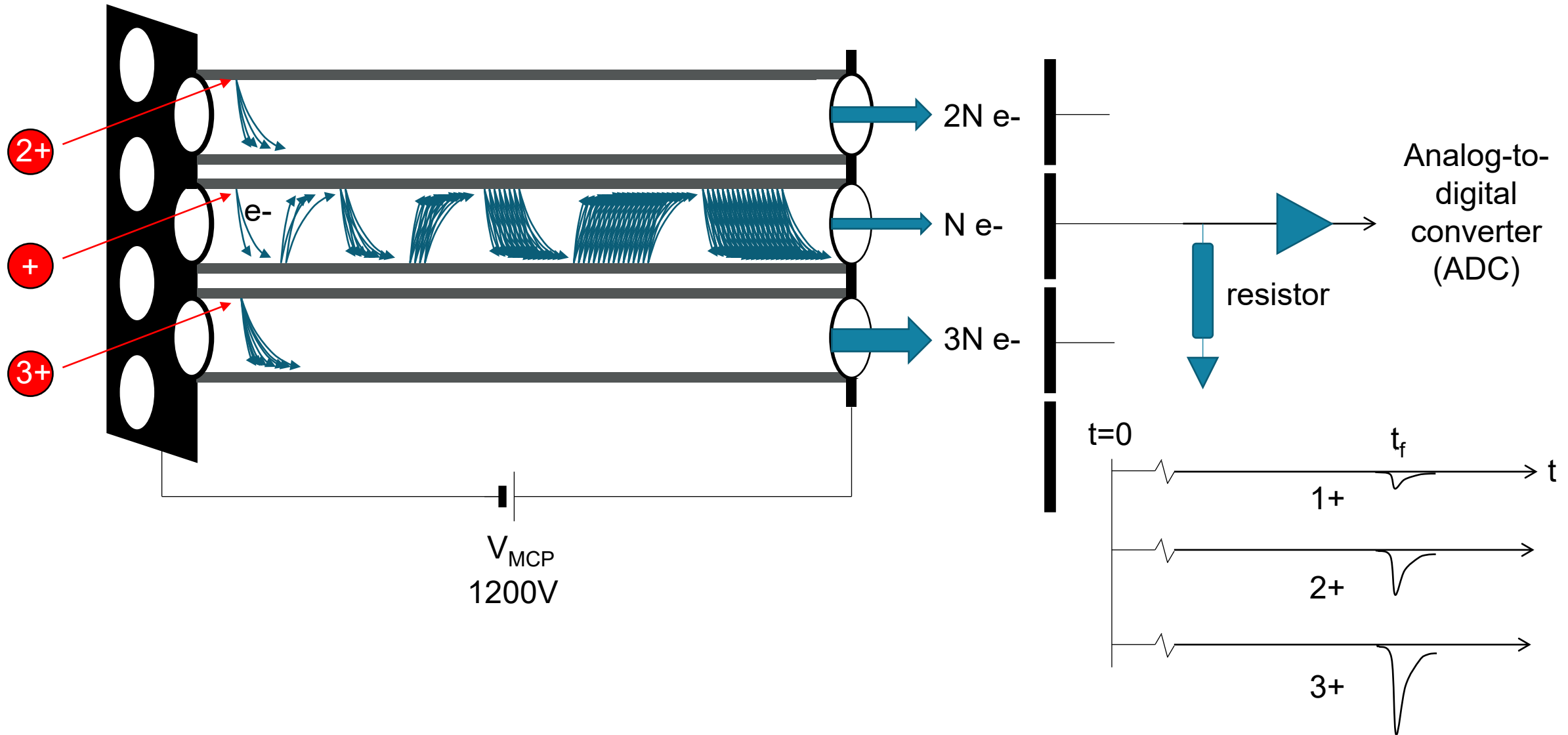
Single ion in FT-MS (FT-ICR, Orbitrap)



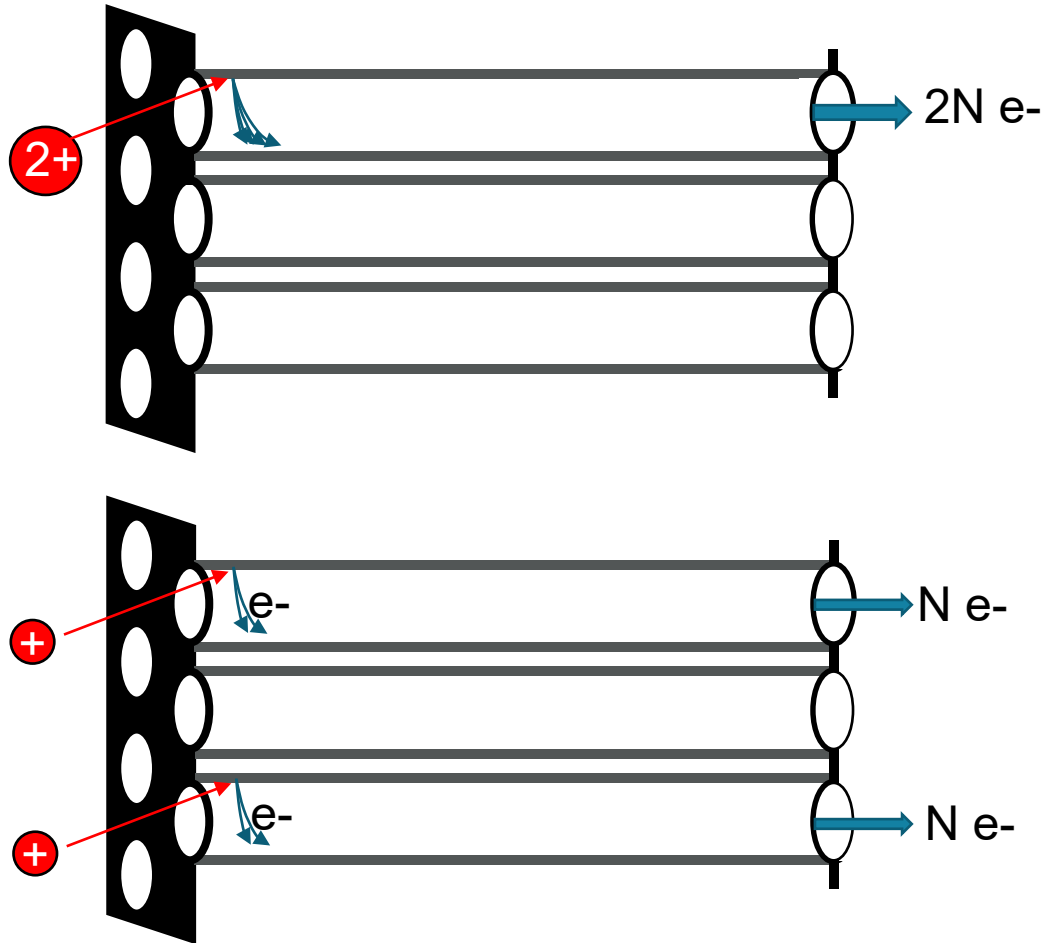
One ion per m/z

High-resolution: slow scan ($\sim$ sec)

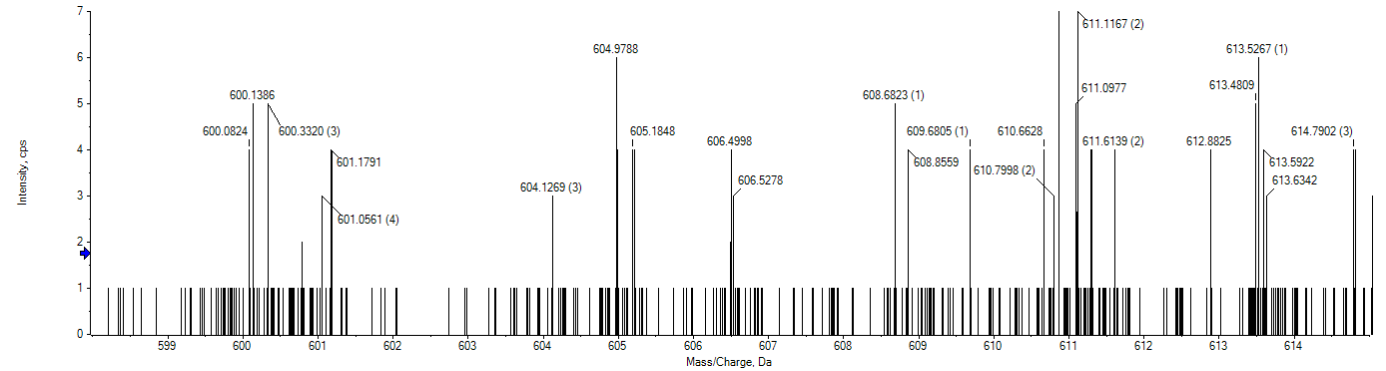
Multi-channel plate (MCP) and response



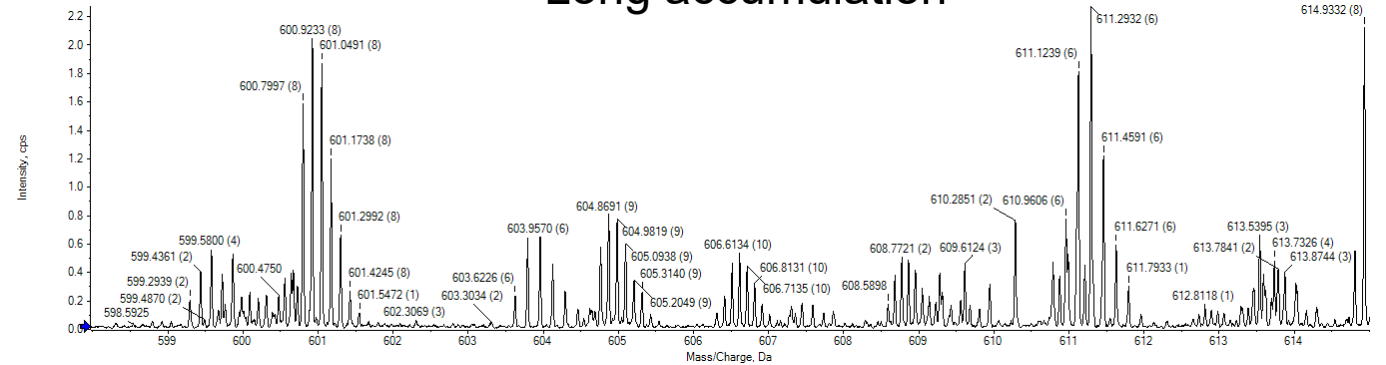
1 ion per TOF cycle assumption automatically satisfied in top-down analysis



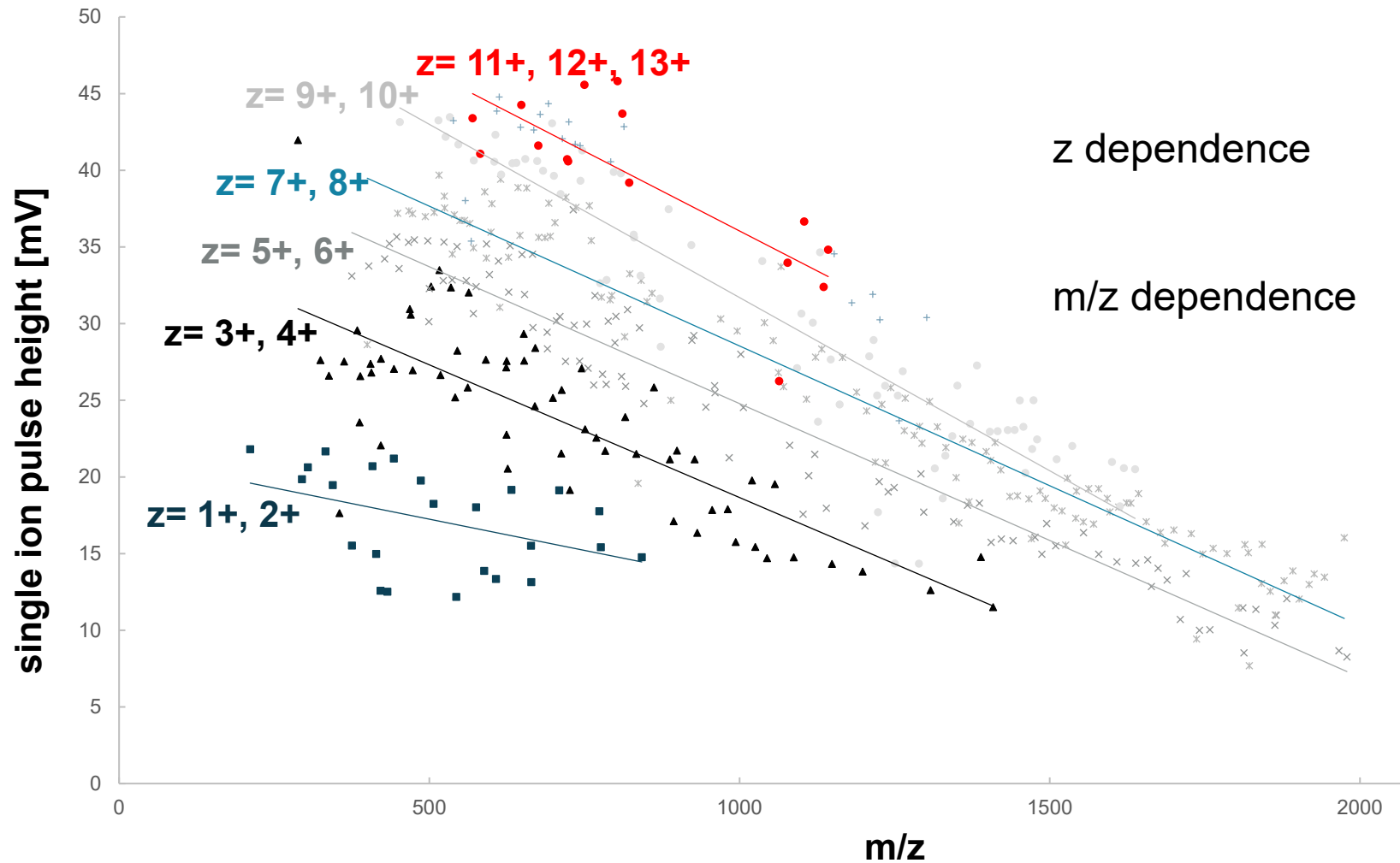
Sum of 1500 TOF cycles/sec



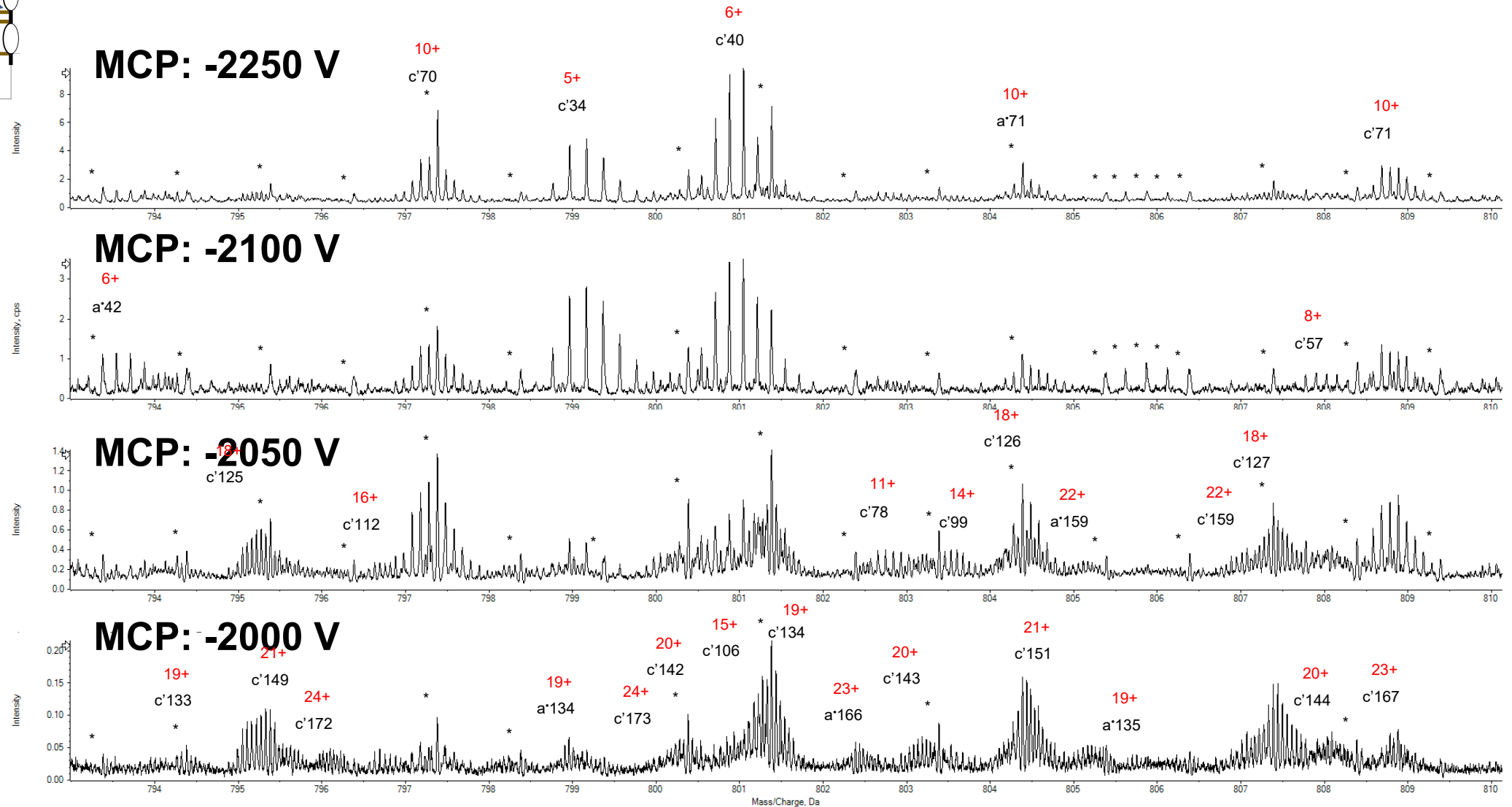
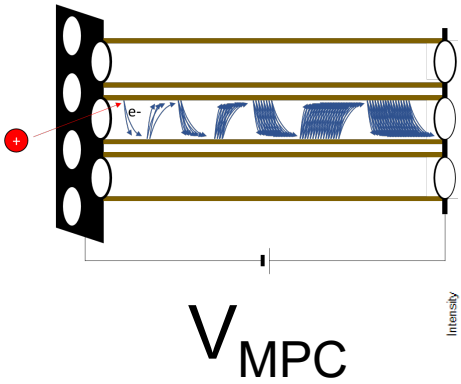
Long accumulation



MCP pulse height of single ion vs. m/z and z



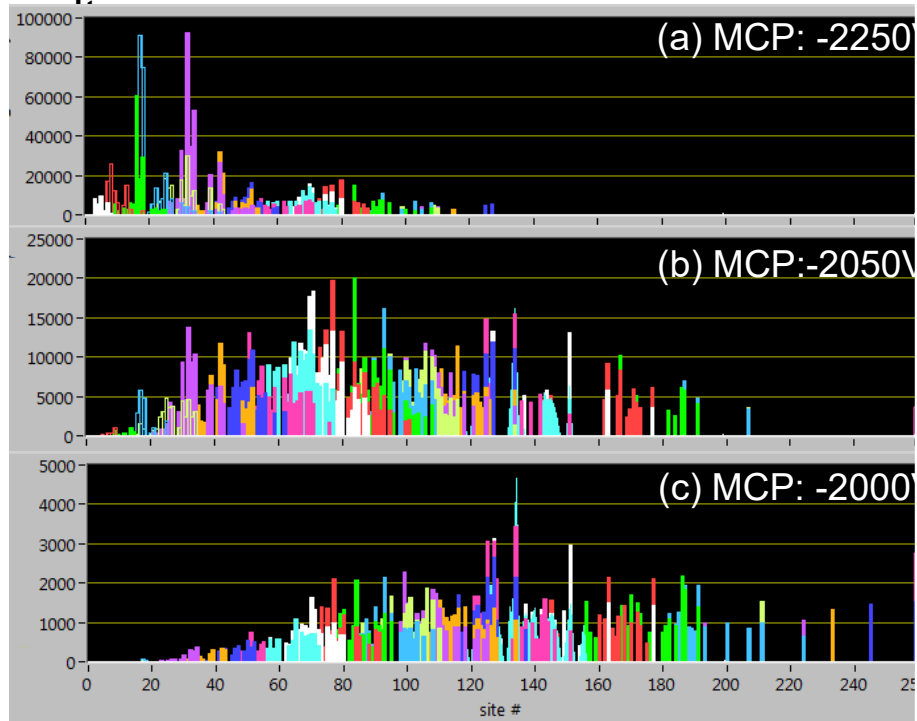
Charge state separation by MCP gain change



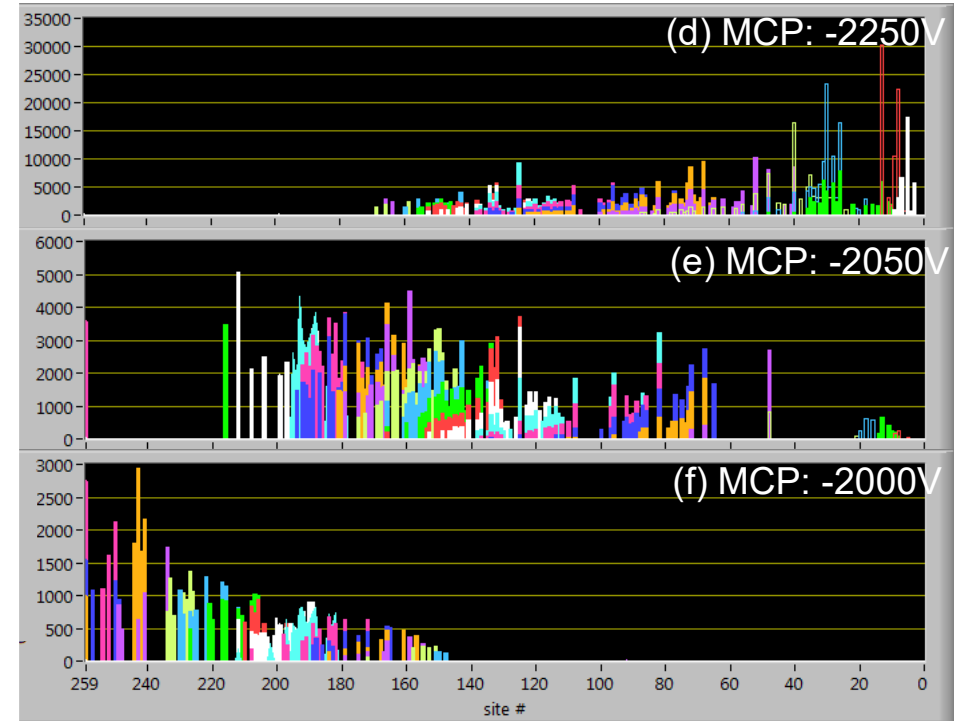
Detected cleavage patterns

CA2 isozyme
bovine
ECD 0 eV

c'



z• intensity



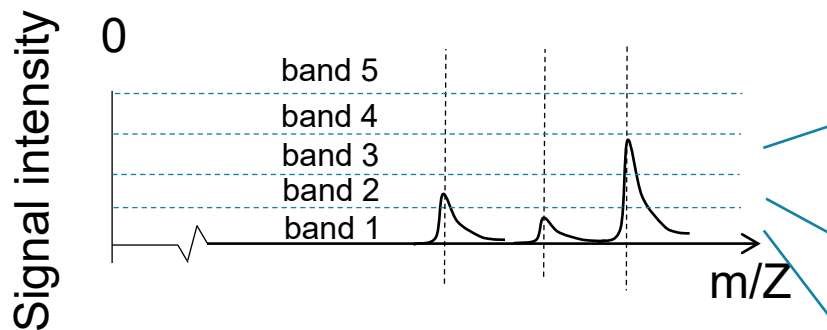
SHWVGRNGP2HHHDFPIANGERQSPVDITKAVVQDPALPLALVYGEATSRMWNNGHSPNV2YDSSDRAVLRDGLTGTIRLVQPFHVGSSDQDGEPIVQKLVYALHLVRRITZVGEFTAAQDPGLAVVQVFLKVQDANPALQKVLDALESLIKTKGKSTDEPNFDPQSLLPNVLDYVITPGSLITPPLESYTVLVLKEPISVSSQOMLKERTLNENAECEPELLMANVRAAPLKNRQVRGEPK

- Need multiple data acquisitions using different MCP voltages
- Low MCP voltage needs extremely long accumulation → slow

Data 2017/11/22

Analysis 2018/01/26

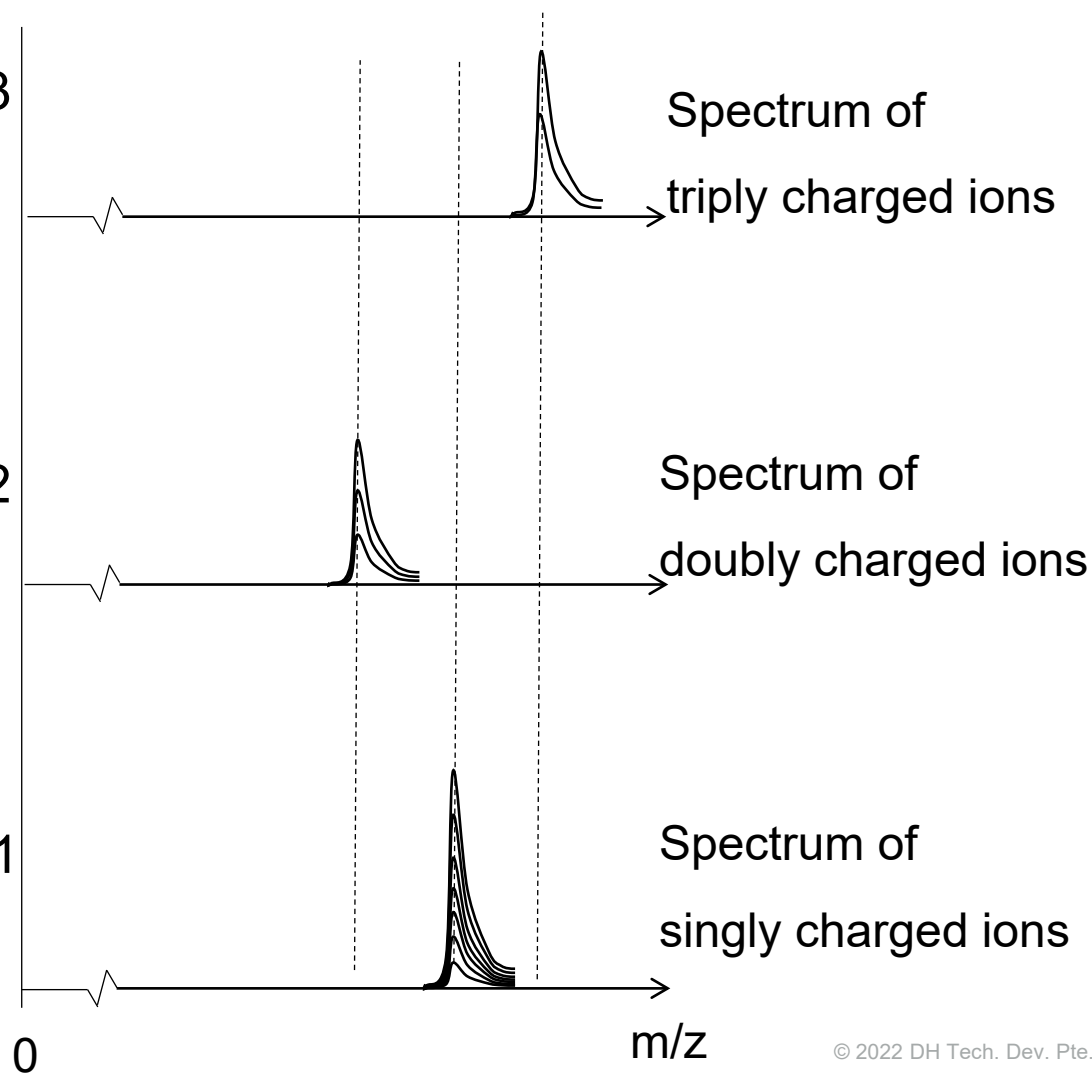
Single TOF scan spectrum



1500 spectra/sec

Multiple spectra accumulation in banded memory buffers

Band 3

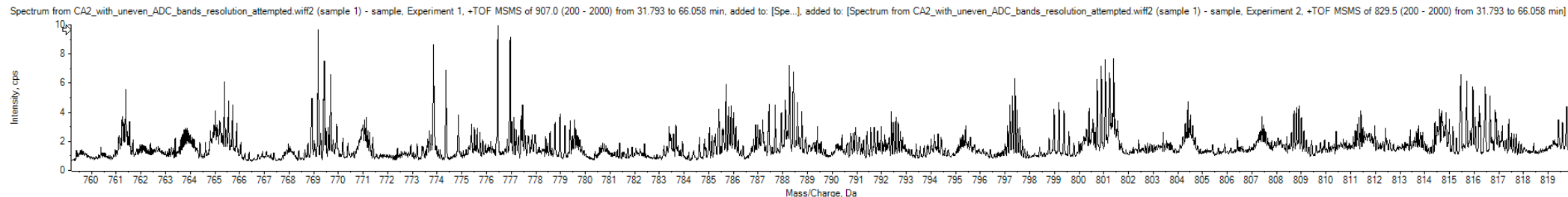


Band 2

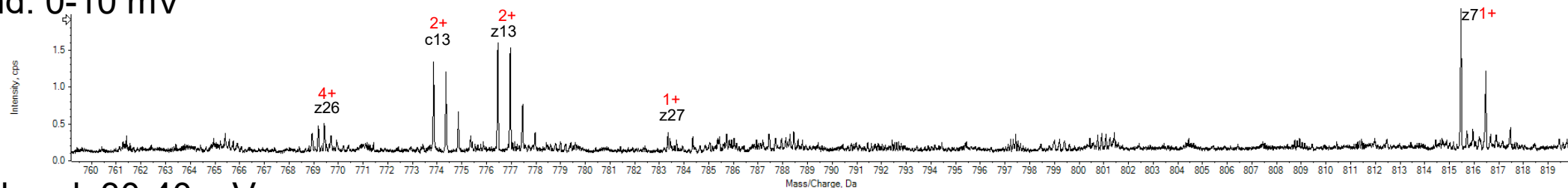
Band 1

ADC banded top-down ECD spectra

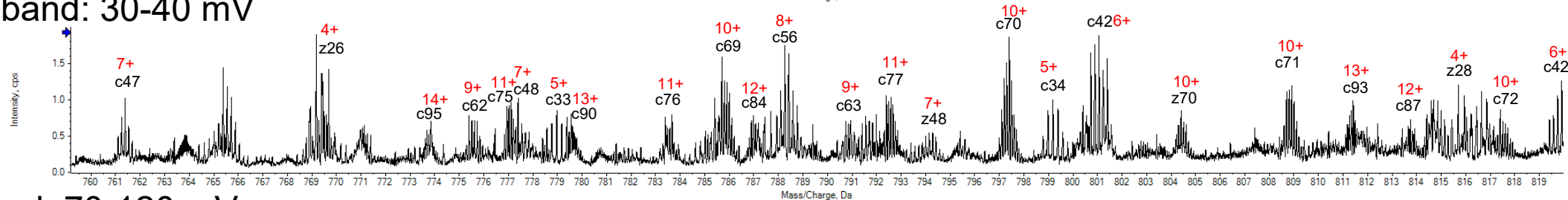
Conventional (no charge state separation)



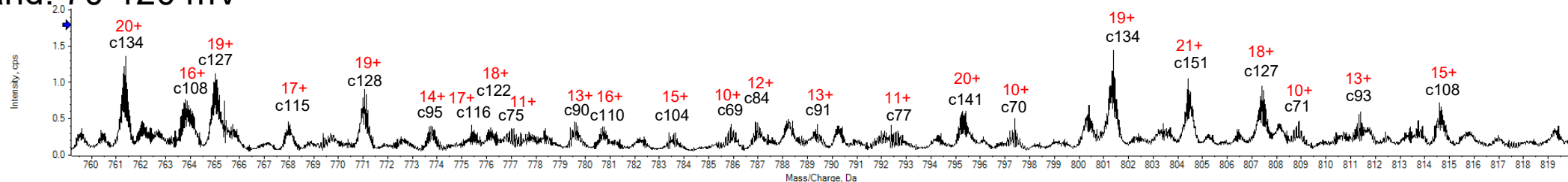
low band: 0-10 mV



middle band: 30-40 mV



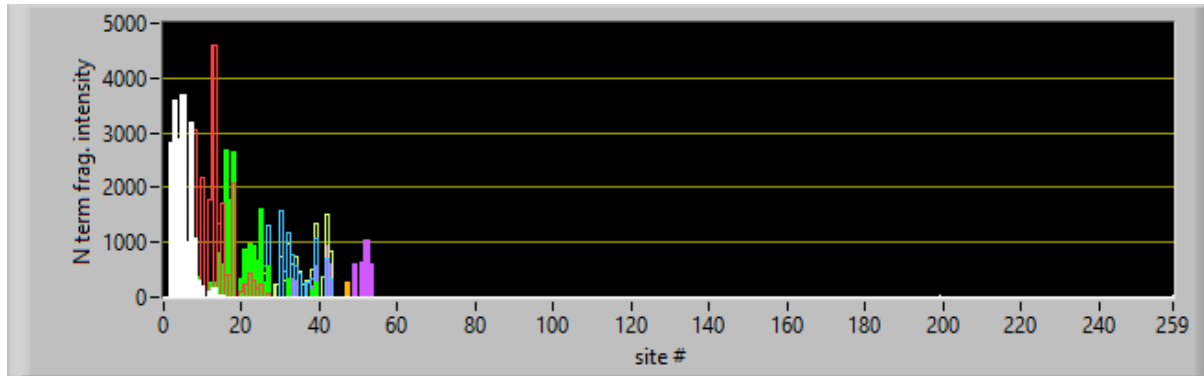
high band: 70-120 mV



Results of sequencing in ADC banding

Low ADC band

c' fragment intensities

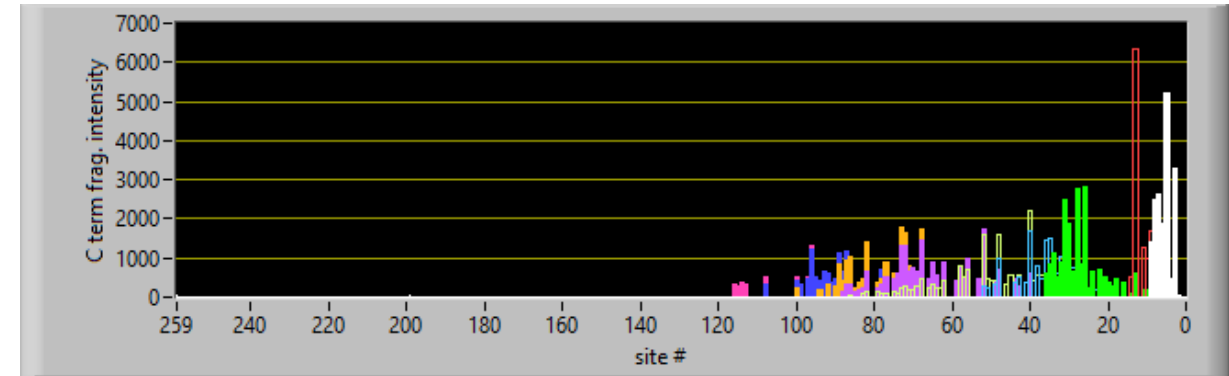


short

dissociation site #

long

z• fragment intensities



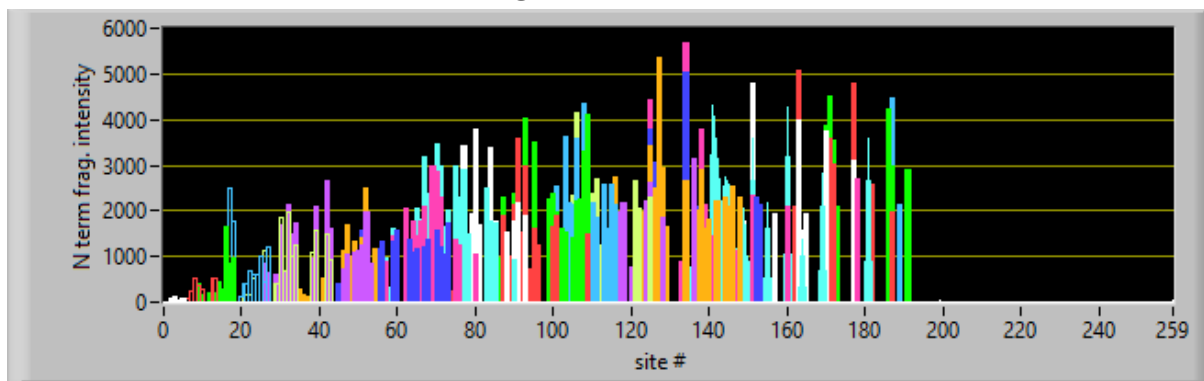
long

dissociation site #

short

High ADC band

c' fragment intensities

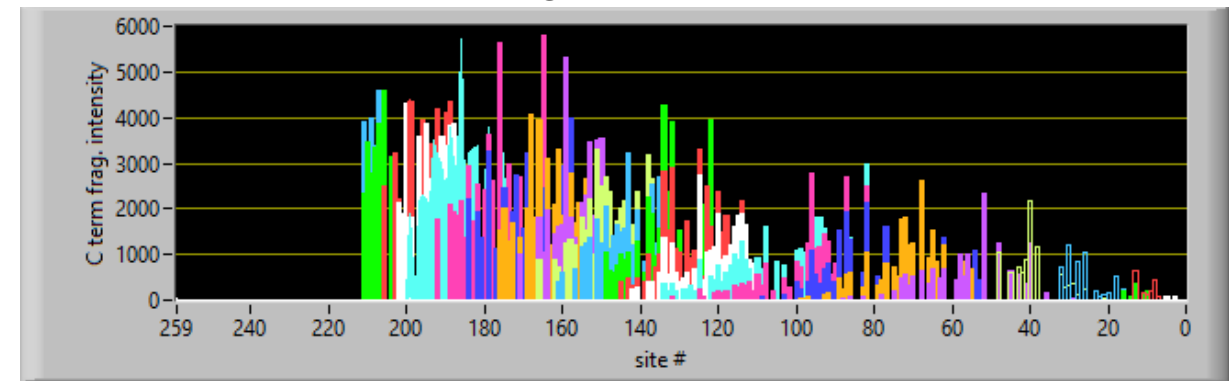


short

dissociation site #

long

z• fragment intensities



long

dissociation site #

short

CHARGE STATE SEPARATION MASS SPECTROMETRY IN TOF PLATFORM CAPABLE OF ECD

- Charge state separation of ECD products by charge-state dependent MCP response
- Wider cleavage coverage in each N/C terminal fragment
- High sensitivity achieved with ADC banding approach
- MCP voltage strategy can be performed using the ZenoTOF 7600 system
- ADC banding is no available in the ZenoTOF 7600 system

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