Strategies for the analysis of proteins and protein-RNA complexes of the HIV-1 psi-site using ESI-FTMS

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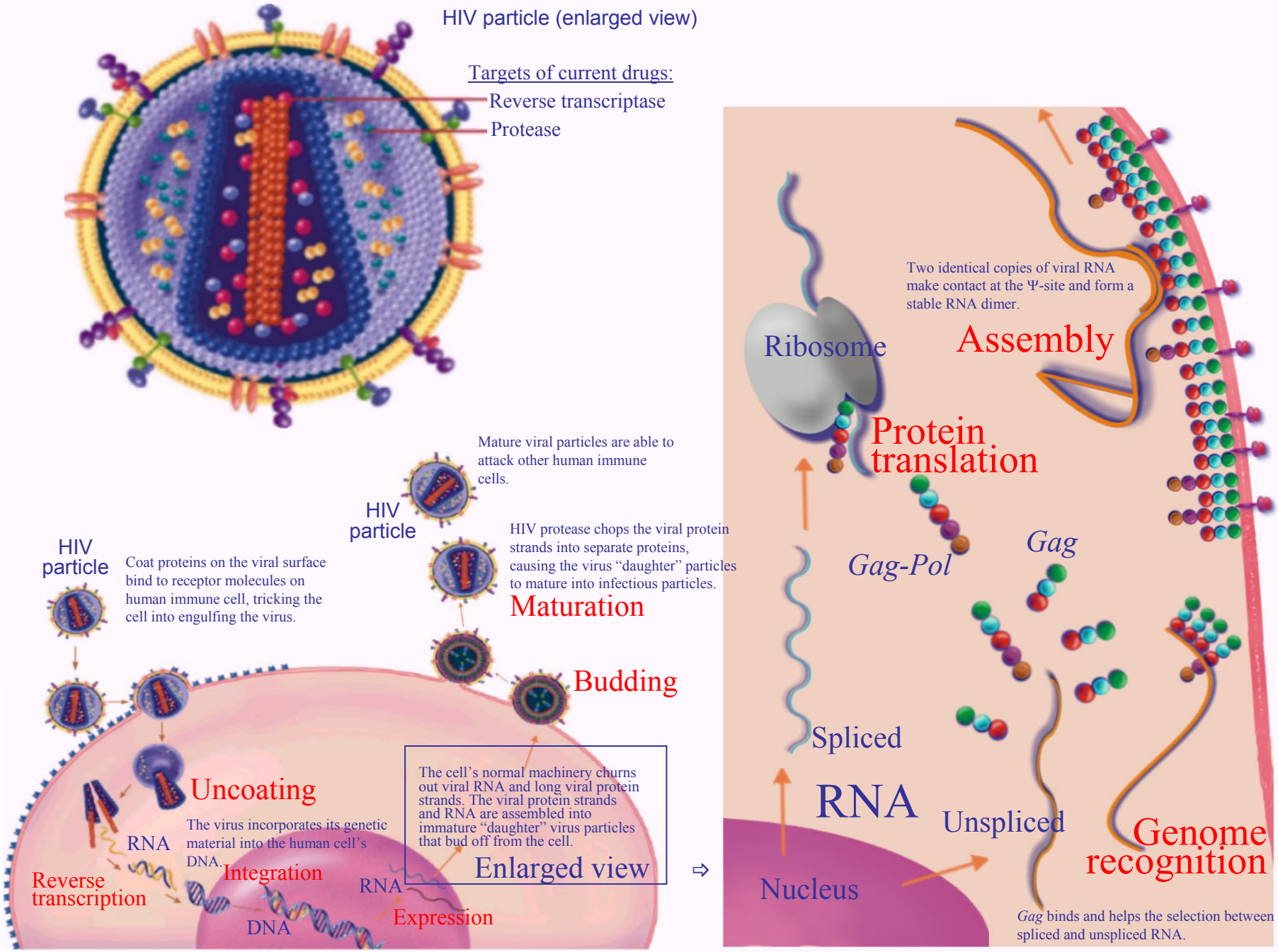
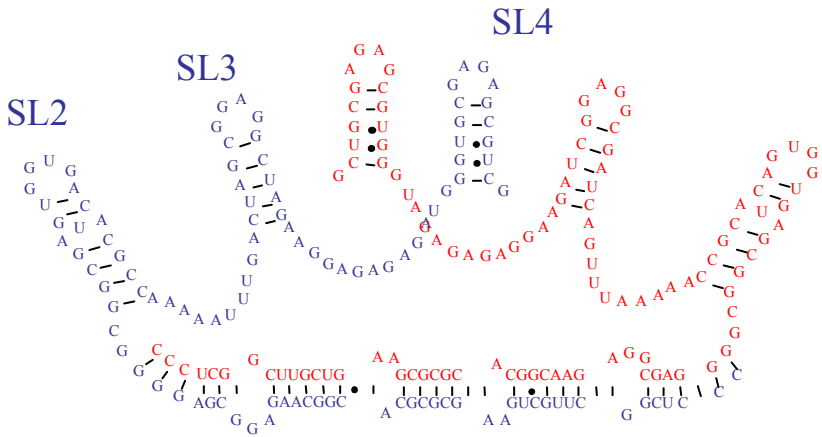
November 18, 2003



Partial adaptation from "The Structure of Life", NIH publication No. 01-2778, National Institute of General Medical Sciences, Nov. 2000

HIV-1 Ψ-site

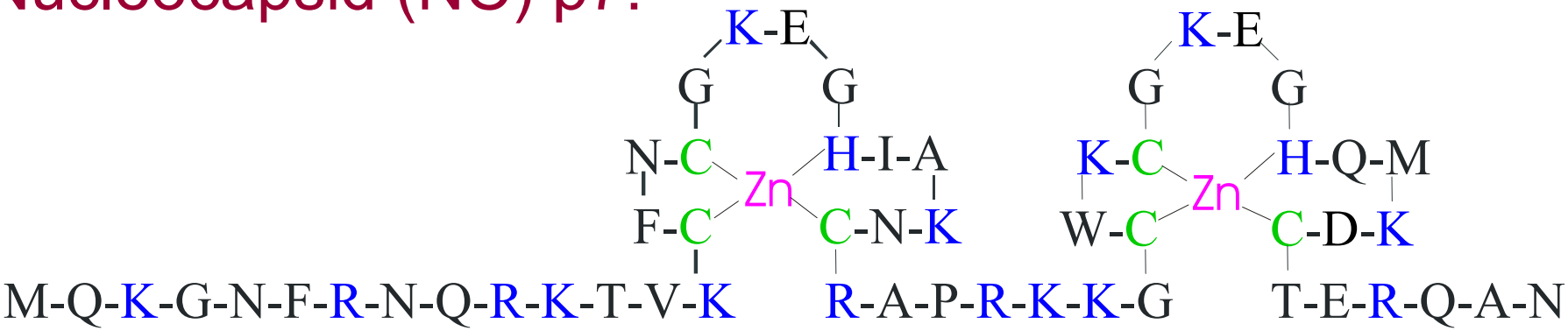


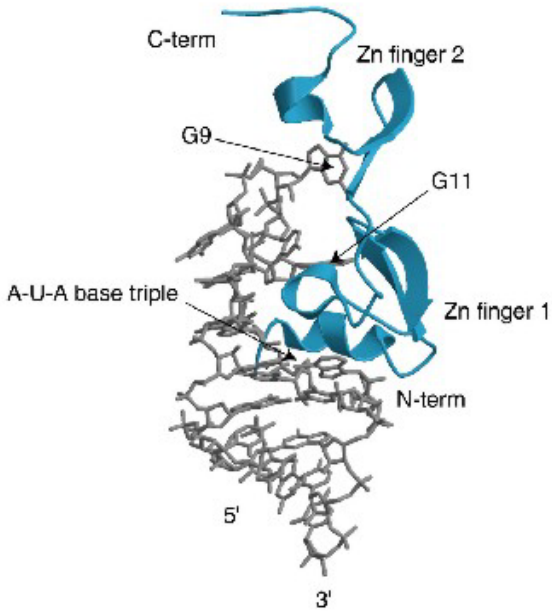
Ψ-RNA

120 nt, ~38 kDa / strand

- Highly conserved ~120 nt kDa region (38 kDa)
- Involved in genome recognition and packaging (mediated by SL1)
- Specifically recognized by Gag/NC
- Discriminates against cellular RNA and spliced viral RNA
  - ⇨3D structure is the fundamental determinant of Ψ activity

Nucleocapsid (NC) p7:



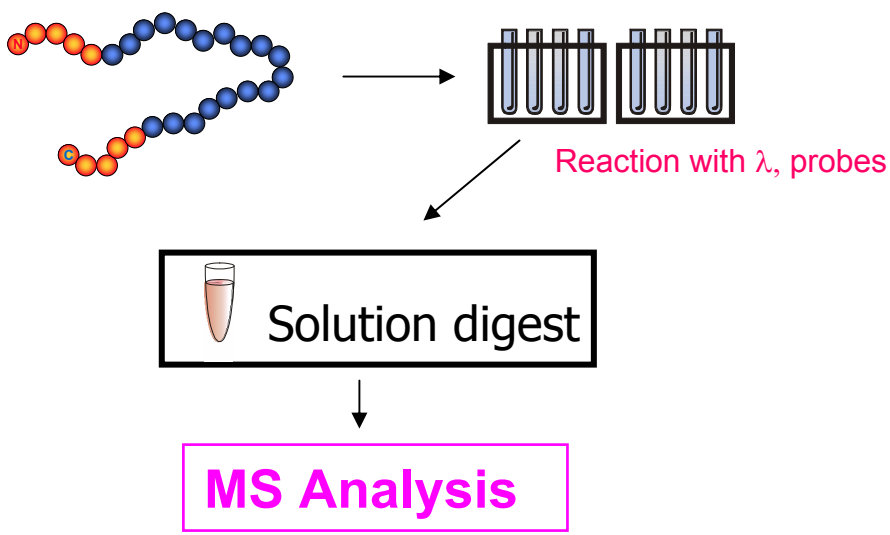


- Nucleic acid chaperone protein
- Part of Gag polyprotein
- 55 amino acid protein; highly basic
- 2 CCHC zinc finger motifs that are required for specific binding to Ψ-RNA
- Mutation of Zn-finger domains renders viral particle non-infectious
- 3-D Structure of RNA:NC complex key to understanding mechanism

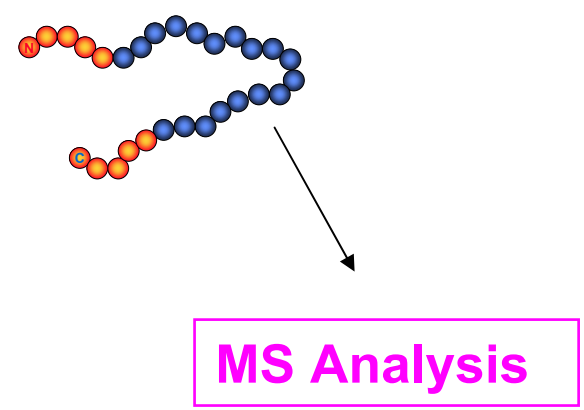
Structural investigation using crosslinking methods and mass spectrometric detection

- Footprinting
- Photo-crosslinking
- Bifunctional crosslinkers
- Simulated annealing and molecular modeling

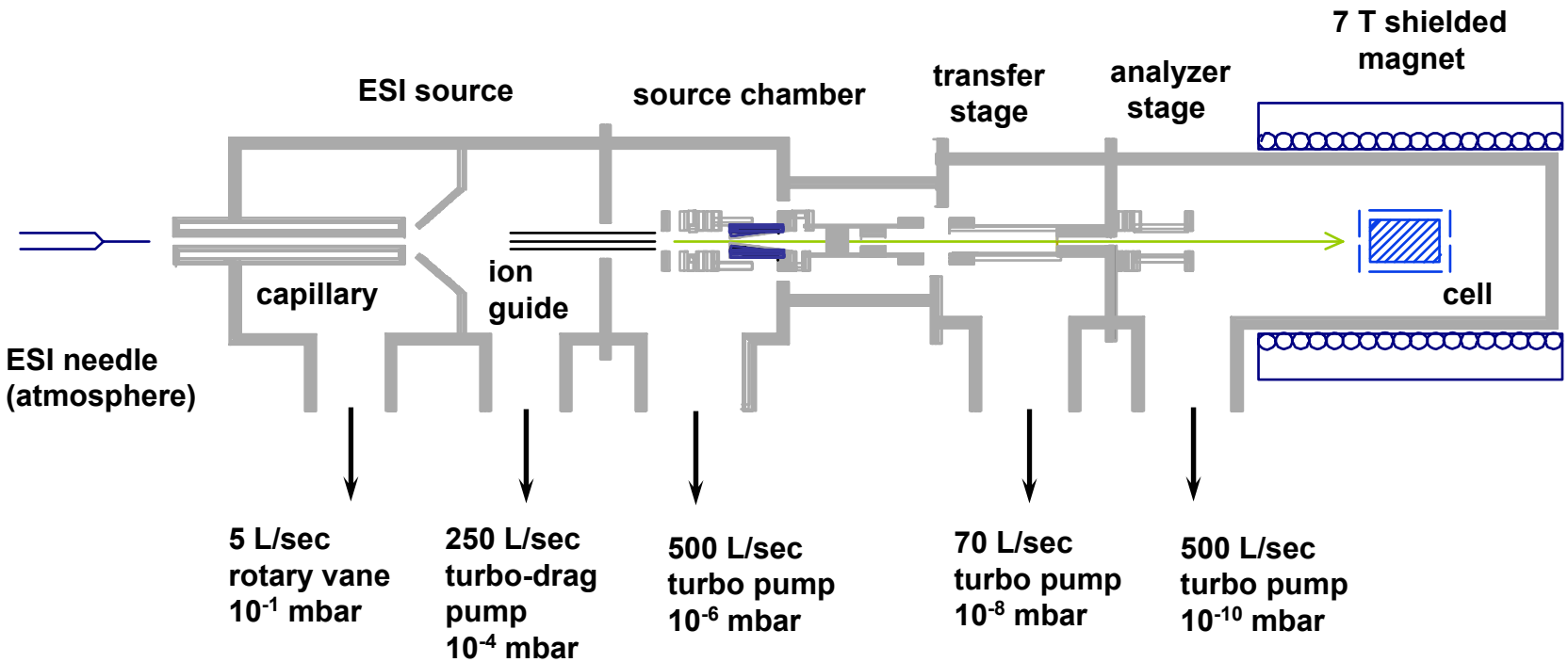
Bottom up



Top-down



FTMS Instrument Diagram

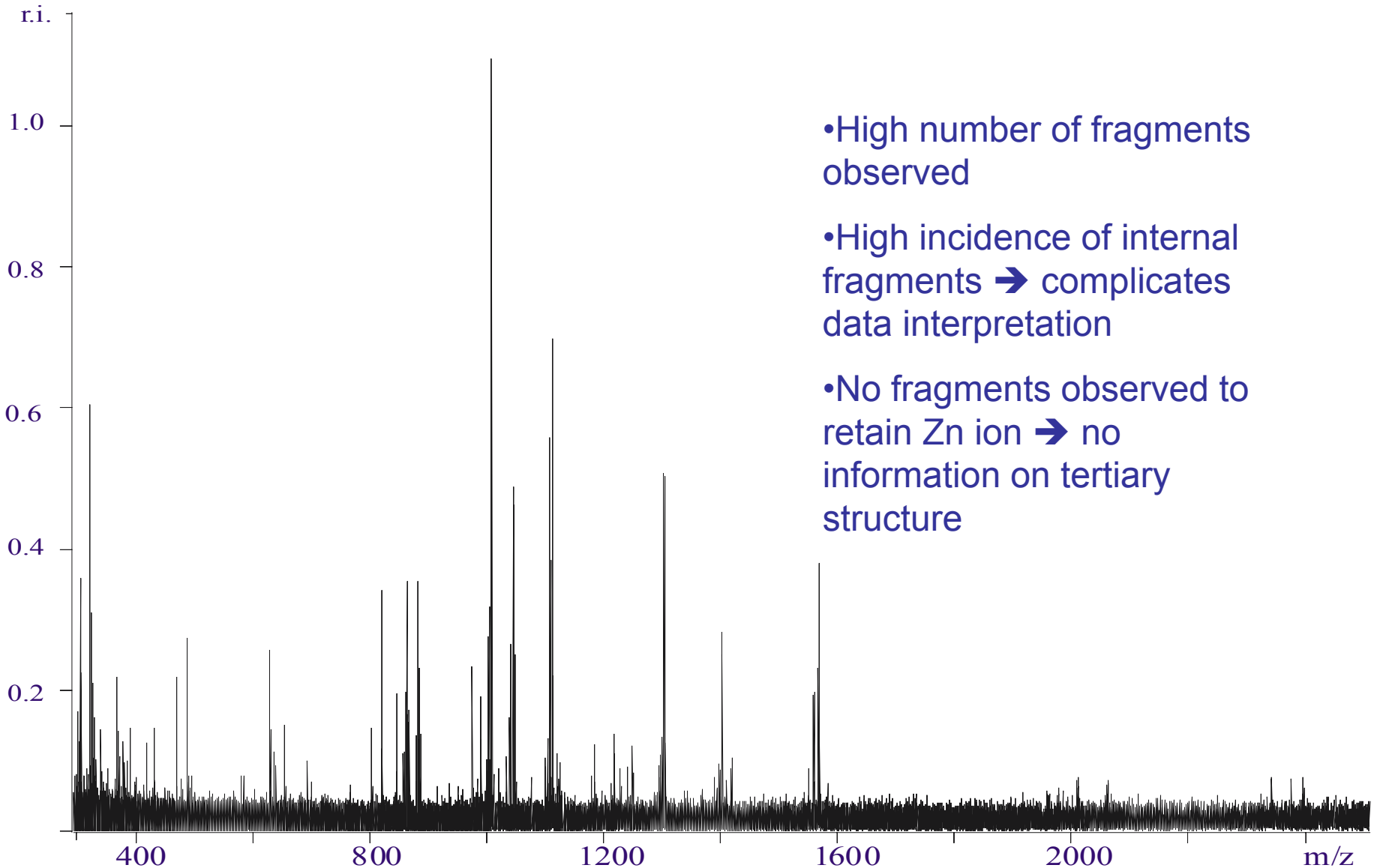


c

Tandem MS by FT-ICR (MS/MS, MS^n)

- SORI-CID —Collision-Induced Dissociation. Ion excited against a background of neutral gas.
  - High incidence of internal fragments
  - Chelated metal commonly lost from metalloproteins
- BIRD — Blackbody Infrared Radiative Dissociation. Blackbody radiation emitted from the walls of the trap in the form of heat is absorbed by the ion until fragmentation occurs.
  - Need a very well-characterized temperature detection system
- IRMPD — Infra-red Multi-Photon Dissociation. Low-intensity IR laser fired at sample multiple times until fragmentation occurs.
  - Requires laser, additional optics
- ECD—Electron Capture Dissociation. Low-energy electrons fired at ion, capture of electron results in fragmentation of backbone bond.
  - Internal filament aligned behind cell
  - Electrons ~0.5 eV

SORI-CID of holo-NC:

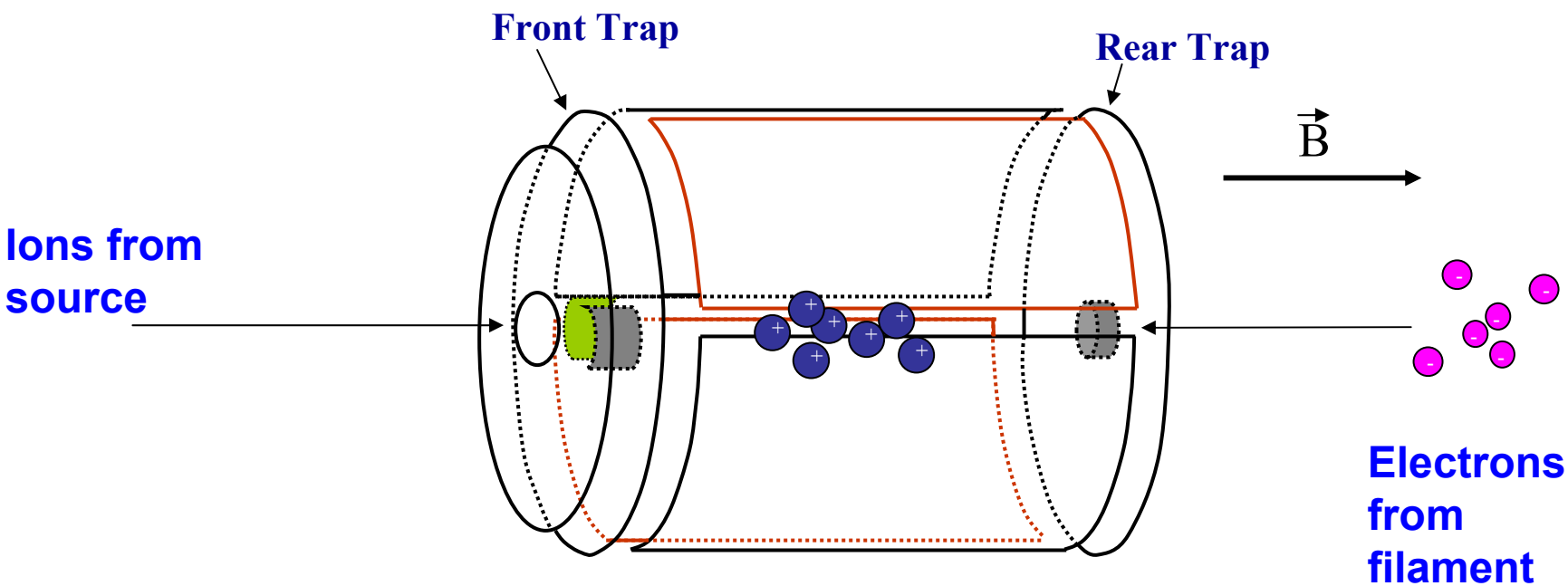


- High number of fragments observed
- High incidence of internal fragments ➔ complicates data interpretation
- No fragments observed to retain Zn ion ➔ no information on tertiary structure

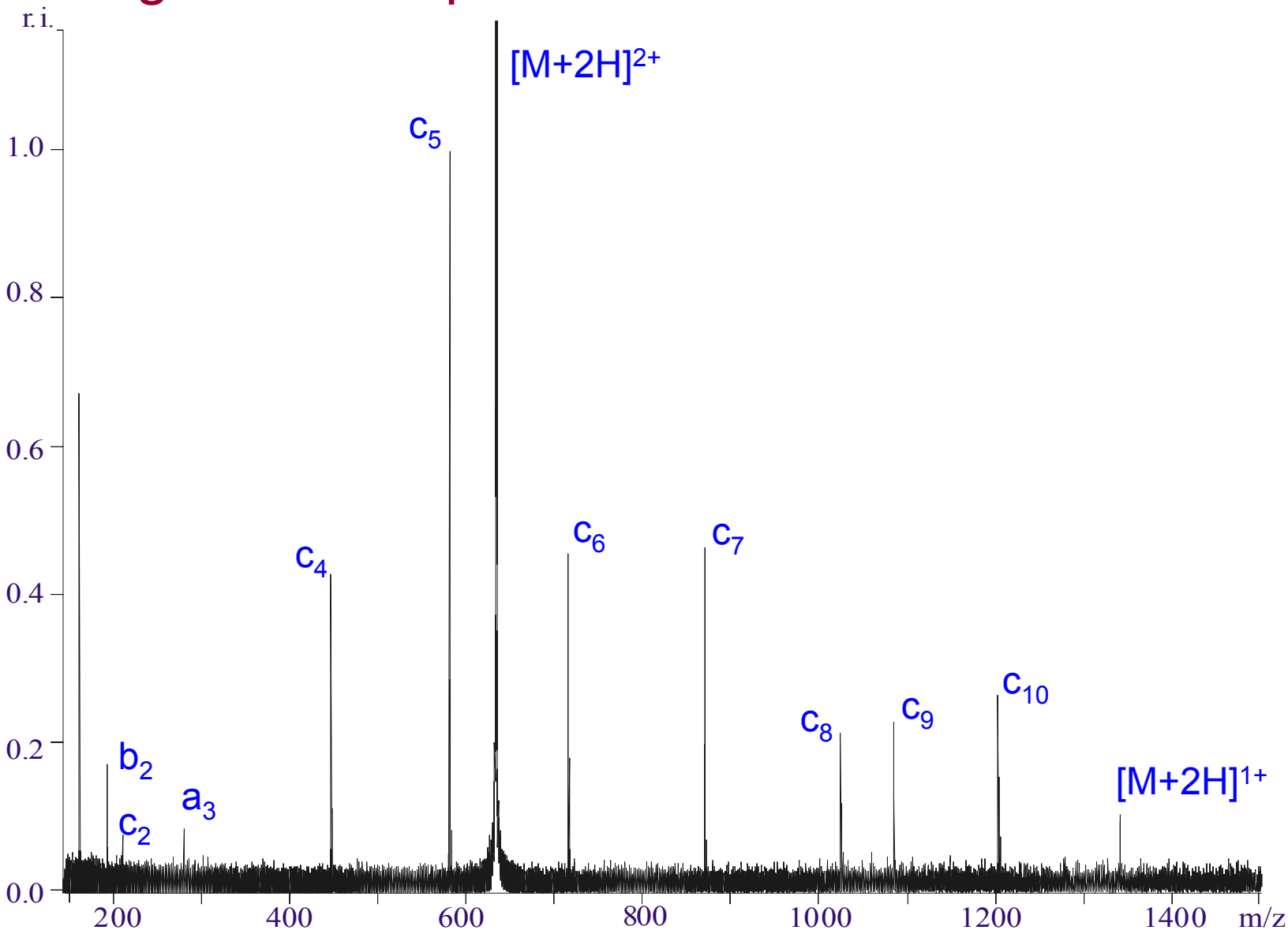
Why ECD?

- Most other slow-heating methods (SORI-CID, IRMPD, BIRD) occur by ergodic processes
  - Energy deposited in molecule is randomized prior to fragmentation
  - Typically, weakest bonds broken first
    - PTMs usually lost
    - Most common cleavage at amide bond
    - Little or no information on tertiary structure
- ECD is non-ergodic
  - Fragmentation occurs prior to energy randomization
    - Non-selective process—most inter-residue bonds broken
    - PTMs often still intact
    - Side chains stay intact—possible to obtain tertiary structure information (intramolecular H-bonds not disrupted)
    - Easily used with other techniques (usu. CID, IRMPD)

ECD in Bruker ICR cell:

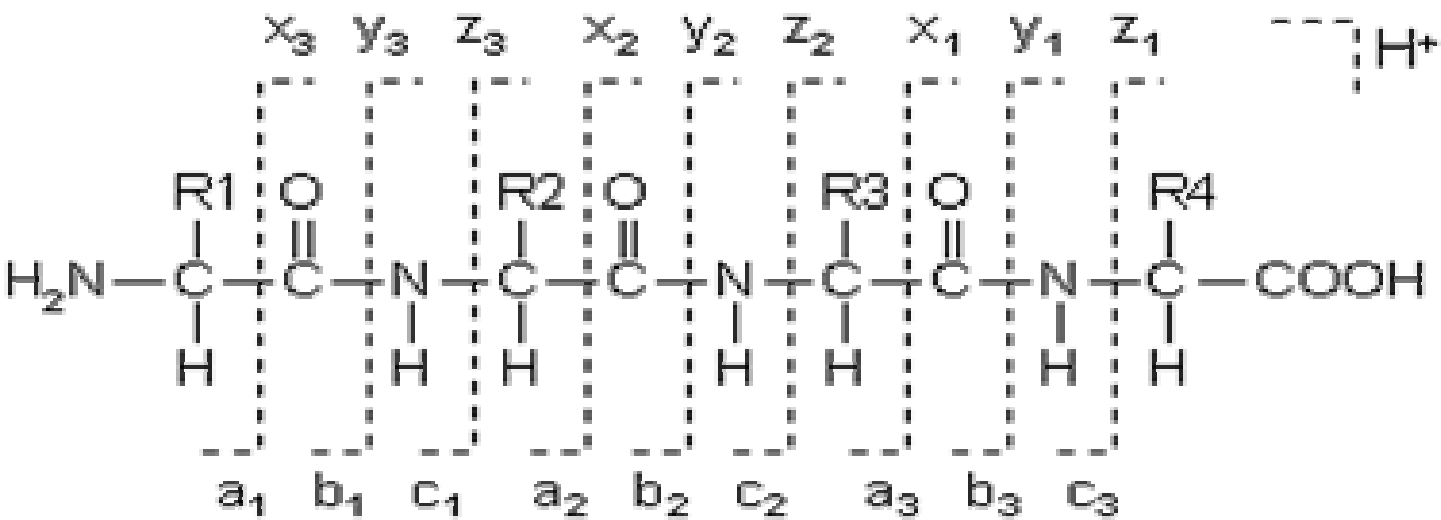


ECD of peptides produces distinctive fragmentation pattern

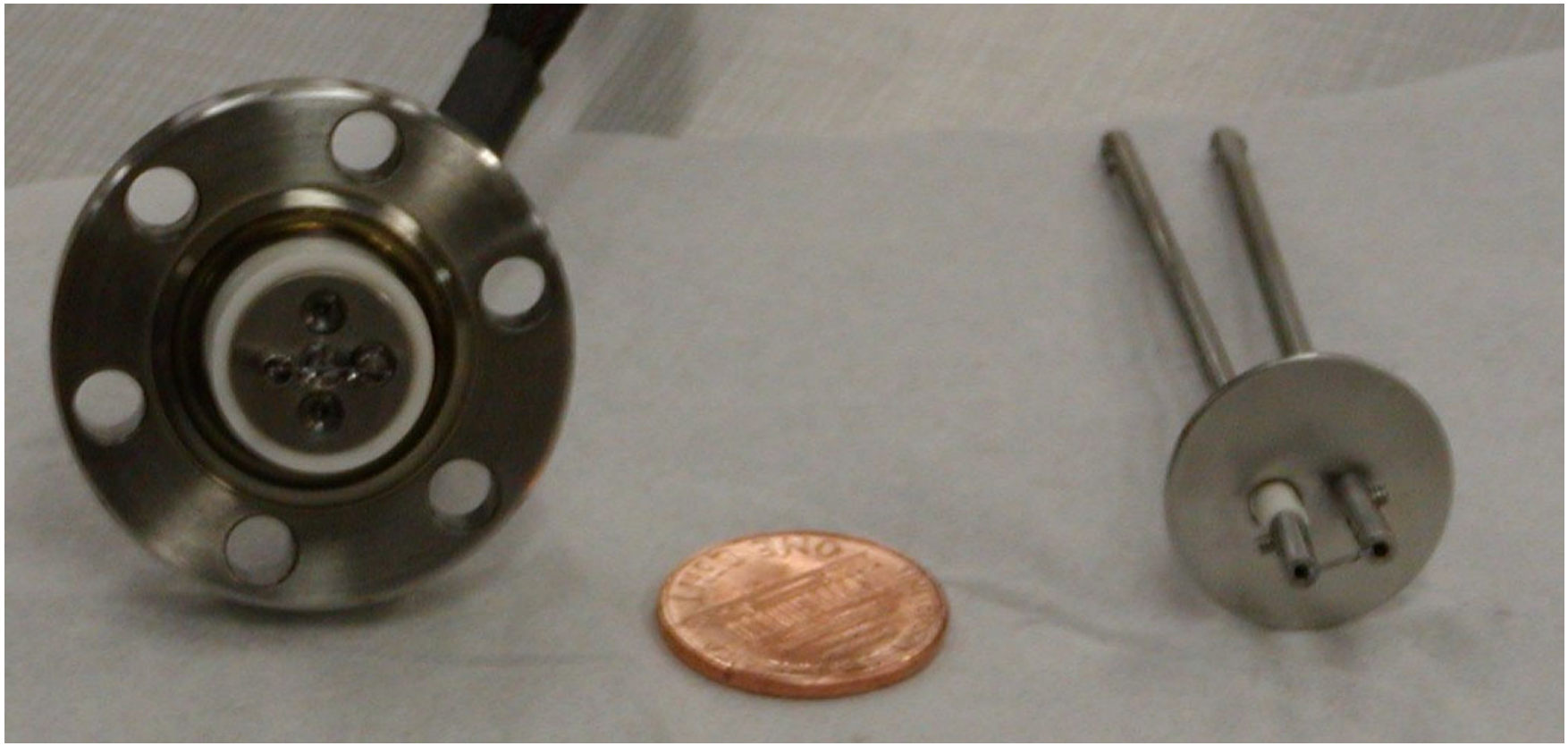


Peptides fragment in a characteristic manner:

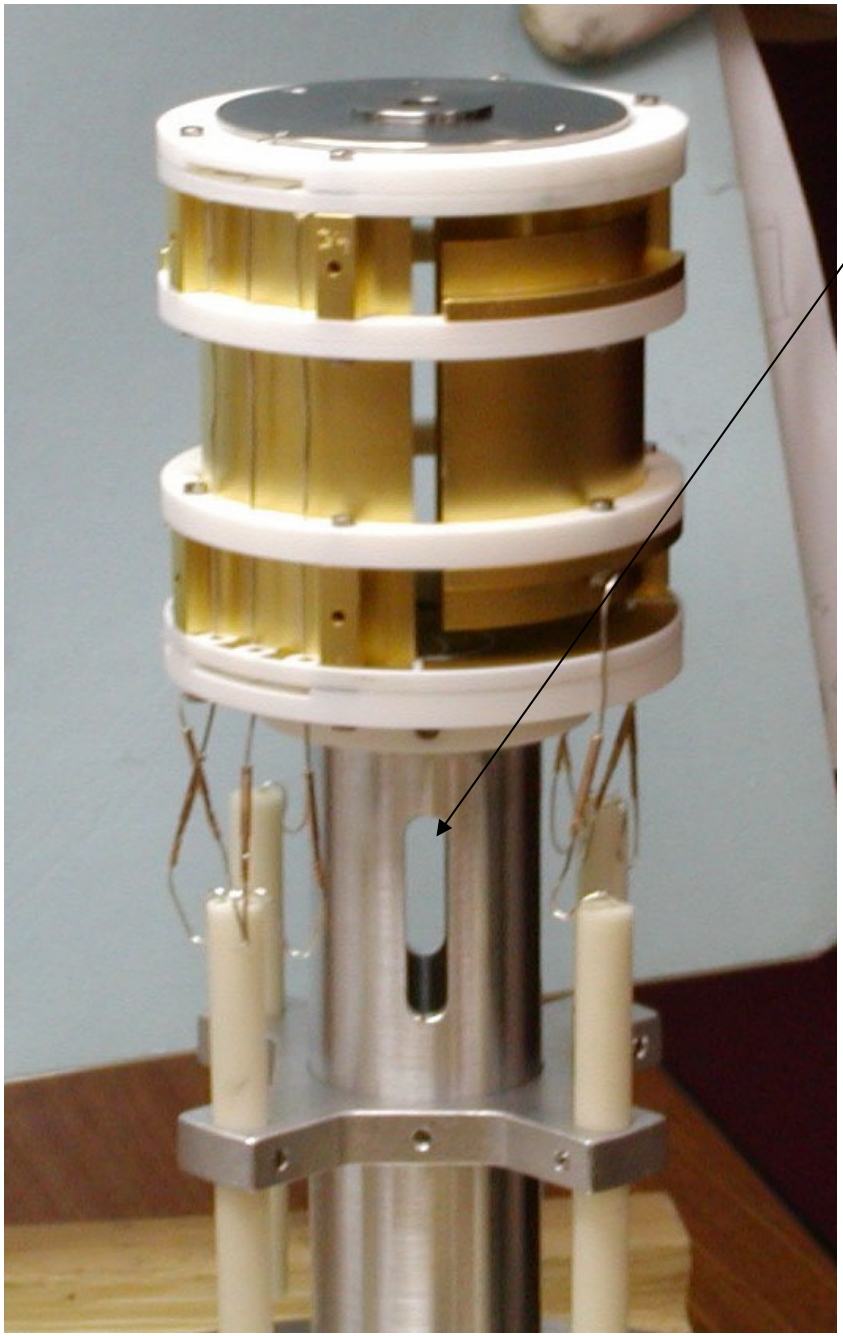
- ECD produces predominantly c, z· ions
  - Some a·, y fragments also observed
  - Amino acid side chains typically not altered



- CID, IRMPD produce predominantly b, y ions—molecule fragments at the weakest point—amide bond
  - PTMs usually lost before amide bond breaks

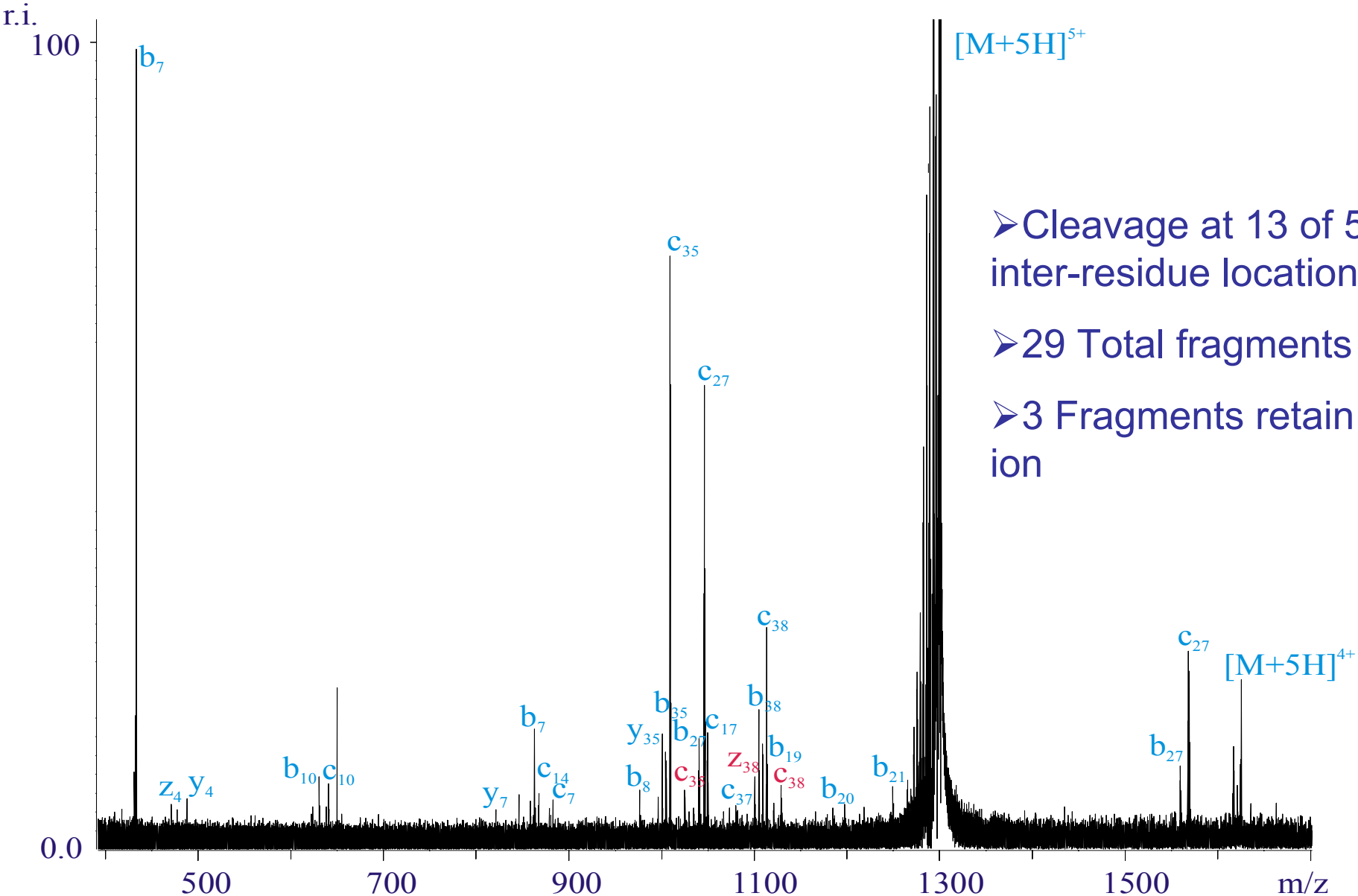


- Emission of e^- low using standard filament—need to increase no. of e^- to observed significant fragmentation
- New filament sits ~ 10 cm closer to cell than old filament



- Filament sits directly behind rear trap (~1.8 cm)
- Increases our success with ECD by ~100-fold!

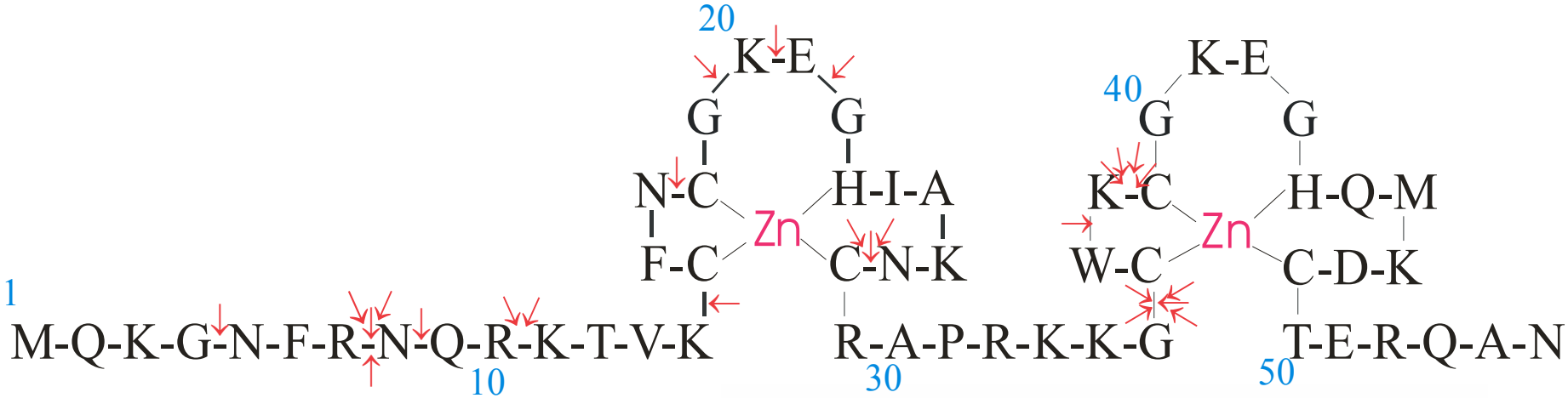
ECD of Holo-NC



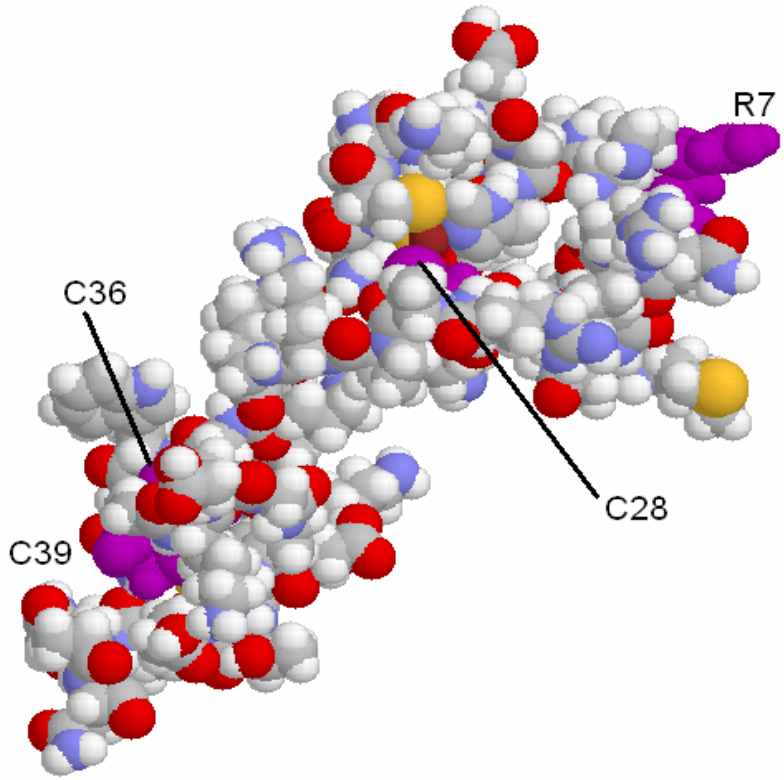
- Cleavage at 13 of 55 inter-residue locations
- 29 Total fragments
- 3 Fragments retain Zn ion

Characteristic features of ECD:

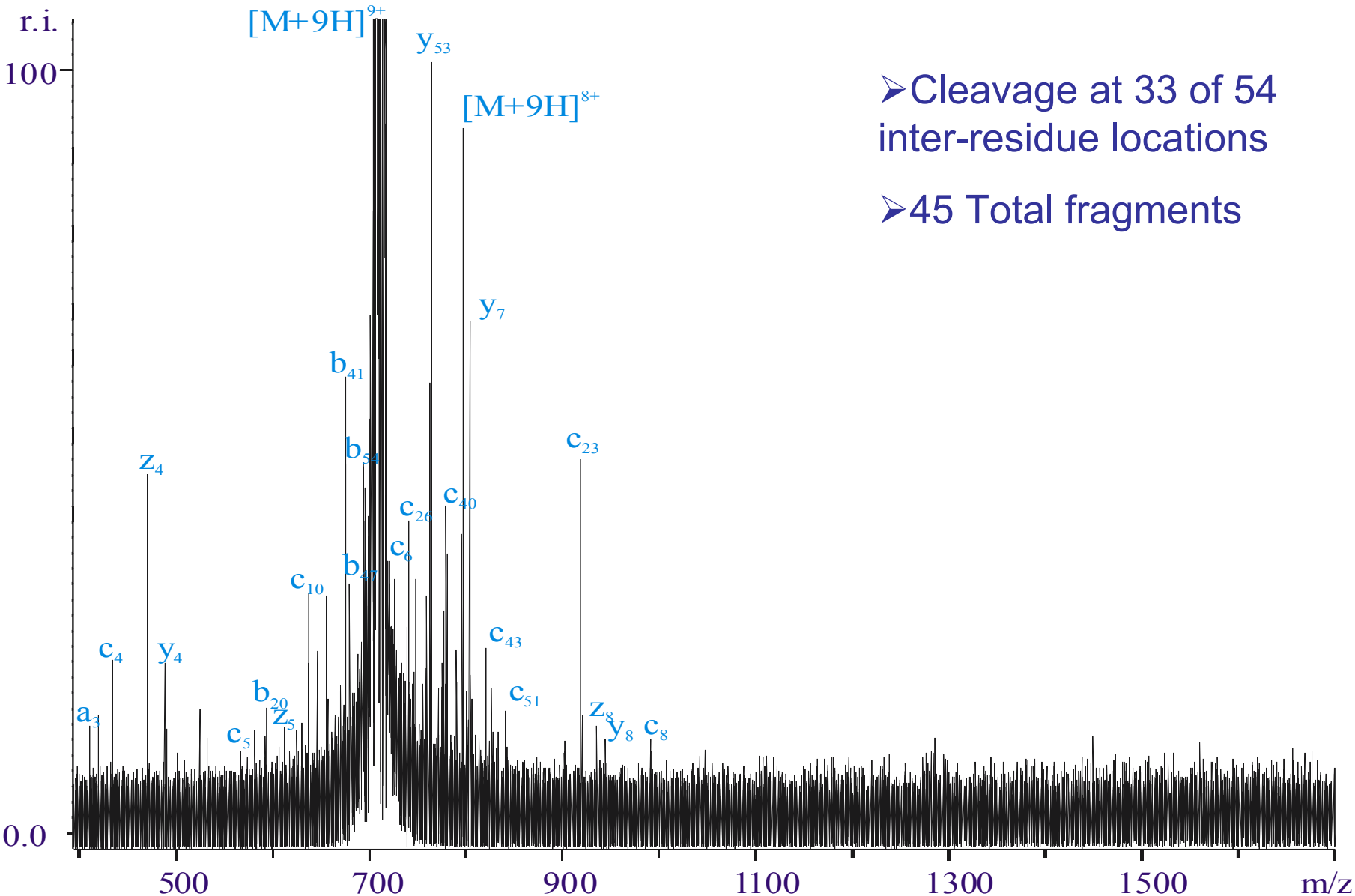
- Cleavage occurs predominantly in central 75% of protein or polymer
  - CID, SORI, or IRMPD used to fragment end pieces
- PTMs typically not cleaved
  - Ideal for locating sites of phosphorylation, glycosylation
- Side chains relatively unaffected
  - Tertiary non-covalent structure is not lost
  - Reflects protein folding/unfolding—Ubiquitin studies
- Sites of high H- affinity tend to be preferentially cleaved
  - S-S bonds broken and backbone site cleaved by capture of one e-
  - Do chelated metal centers show the same behavior?
  - Can ECD be used to characterize metalloproteins?



- 19 of 29 fragments observed from ECD of Holo-NC localized around 4 residues
- C28, C36, C39 directly involved in Zn chelation
- No fragmentation in C-terminal or linker regions

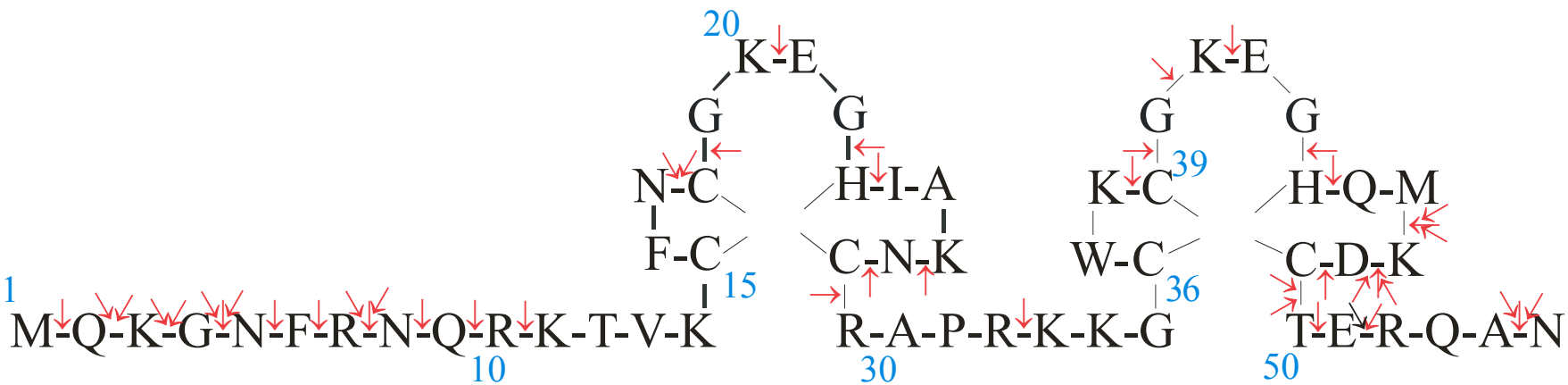


ECD of Apo-NC



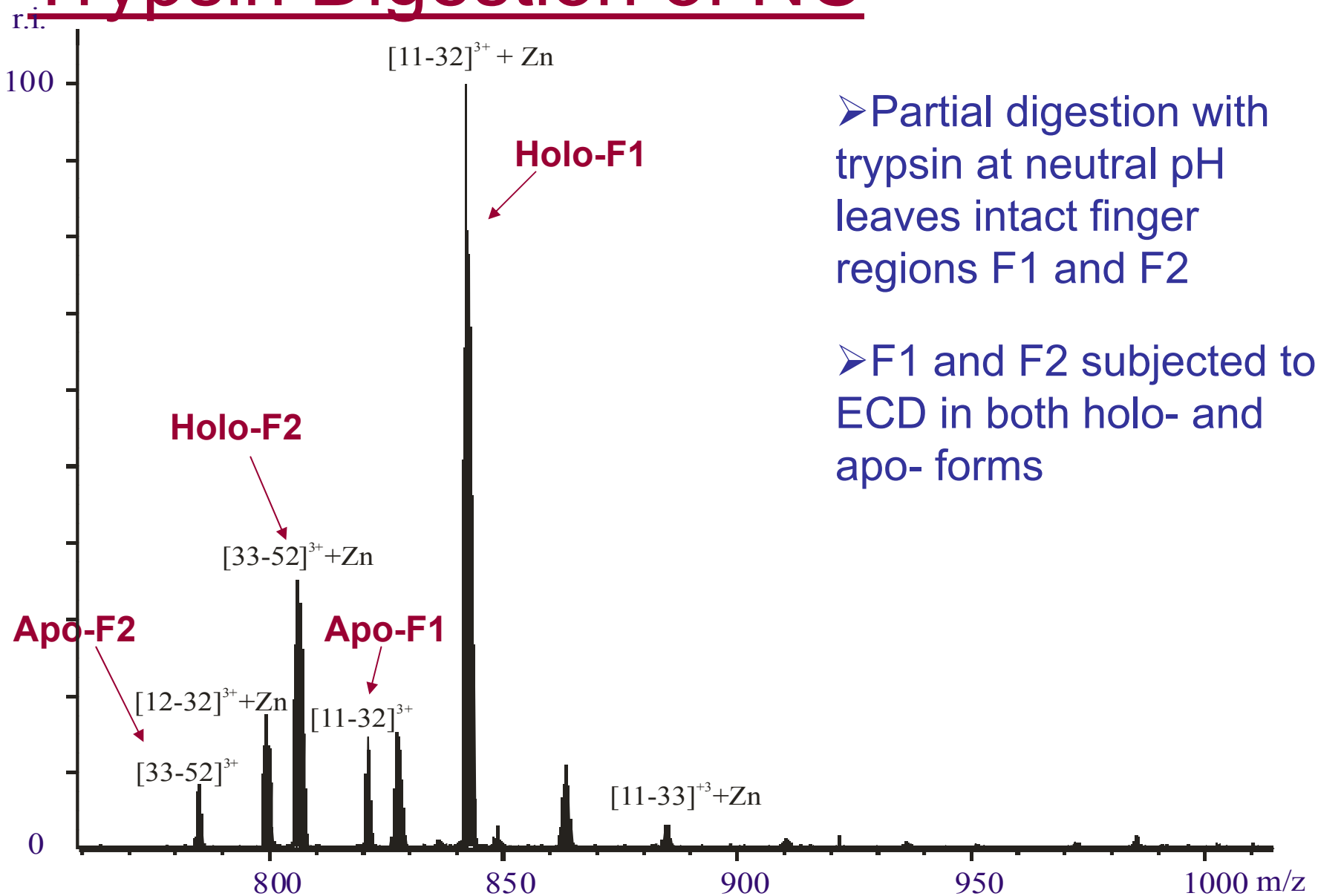
- Cleavage at 33 of 54 inter-residue locations
- 45 Total fragments

ECD of Apo-NC shows distinctly different pattern of fragmentation

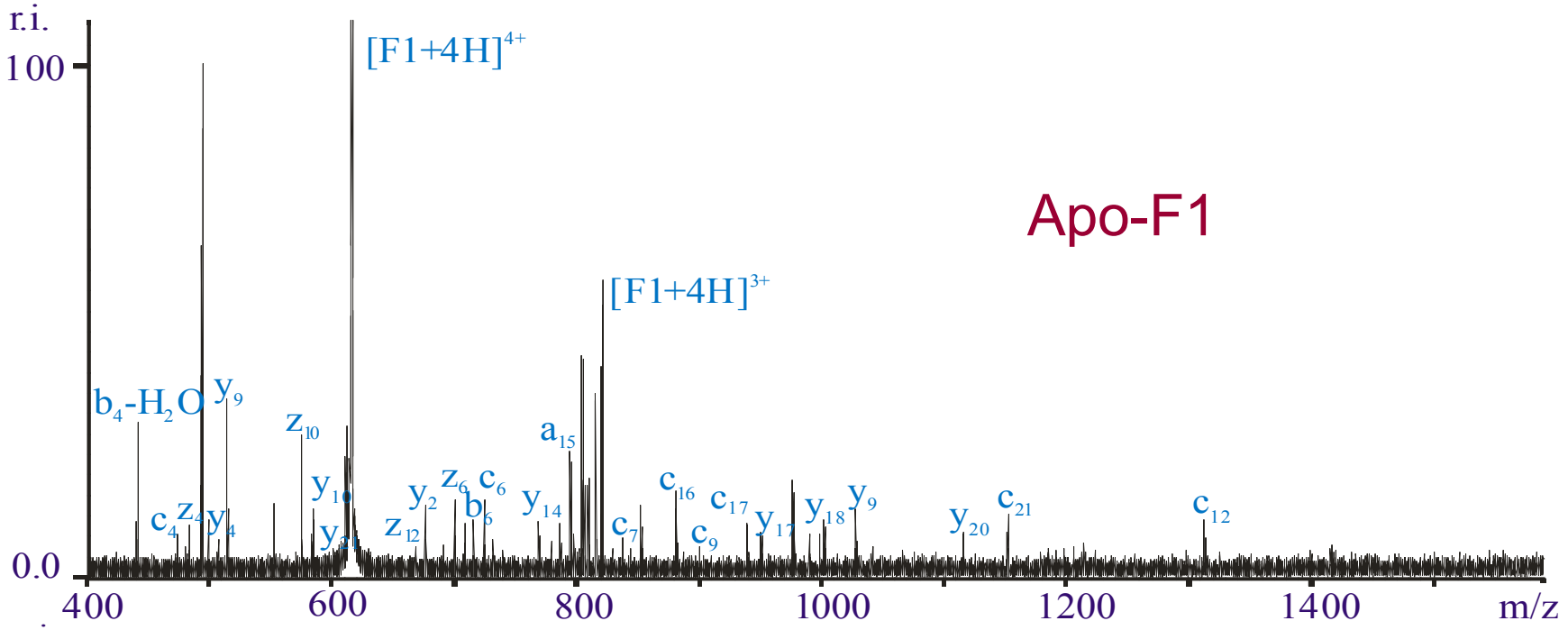


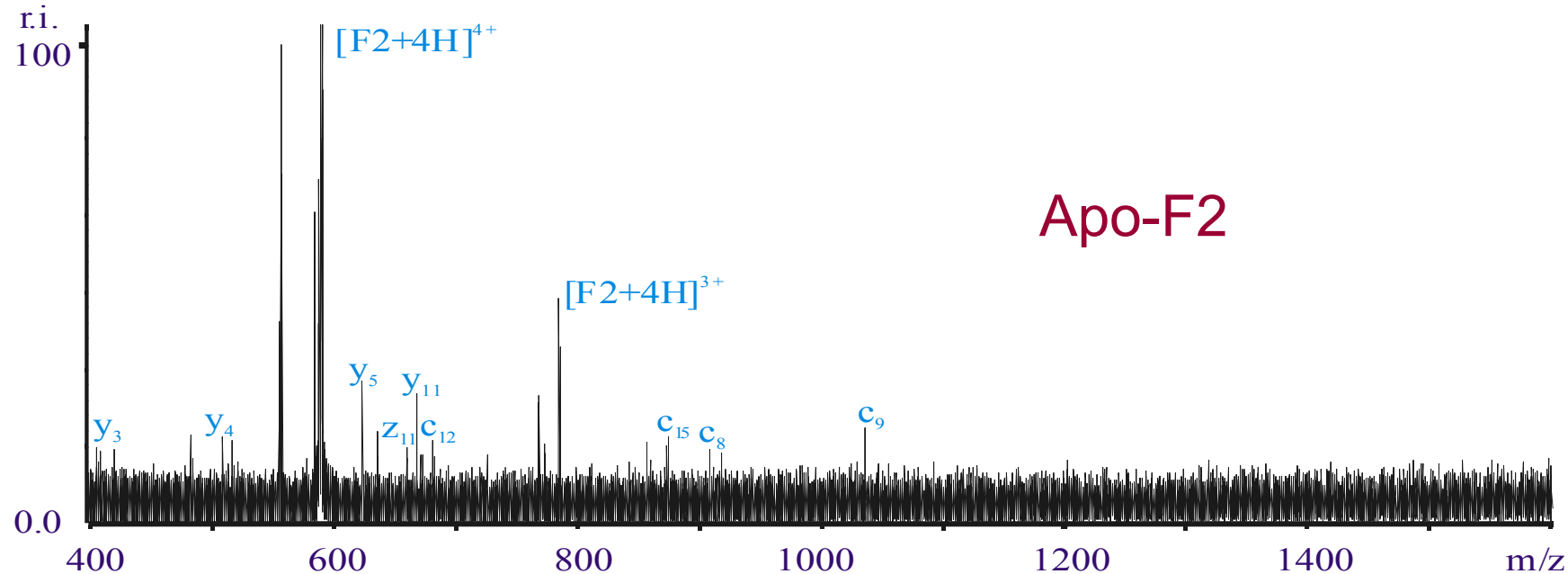
- More complete sequence coverage than for holo-NC
- Extensive fragmentation in N-, C-terminal regions
- Did not show high frequency of cleavage around same residues as holo-NC

Trypsin Digestion of NC

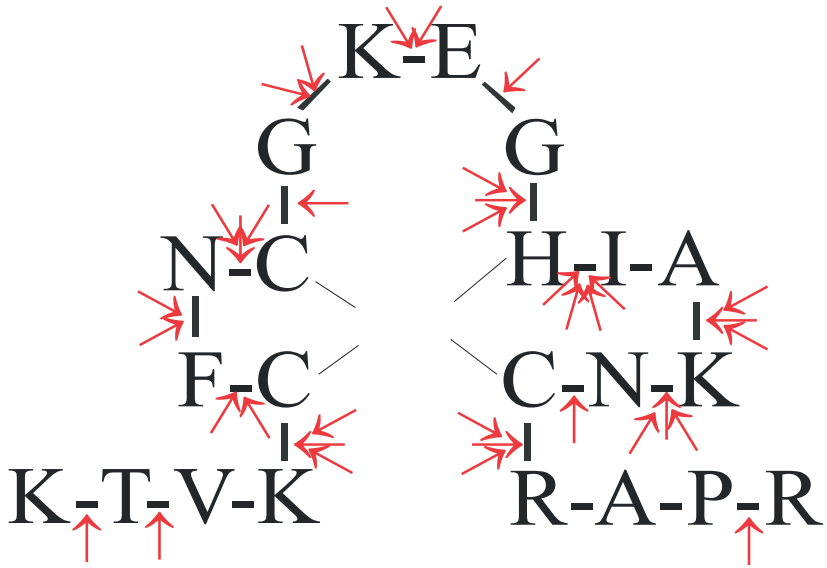


- Partial digestion with trypsin at neutral pH leaves intact finger regions F1 and F2
- F1 and F2 subjected to ECD in both holo- and apo- forms

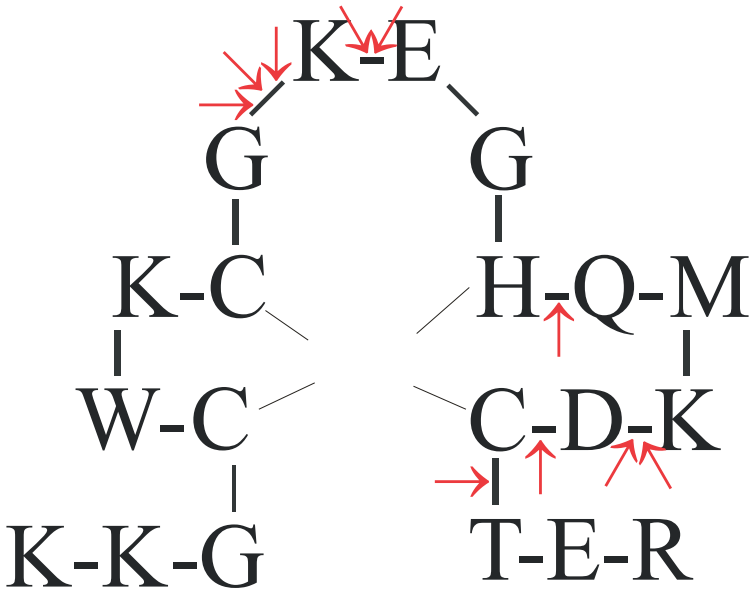

r.i.
100
0.0
[F1+4H]4+
[F1+4H]3+
Apo-F1
b4-H2O
y9
z10
a15
y10
y2
z6
c6
b6
y14
c16
c17
y17
y18
y9
y20
c21
c12
c4
z4
y4
z12
c7
c9
400
600
800
1000
1200
1400
m/z


r.i.
100
0.0
[F2+4H]4+
Apo-F2
[F2+4H]3+
y5
y11
y3
y4
z11
c12
c15
c8
c9
400
600
800
1000
1200
1400
m/z

- Cleavage at 17 of 21 inter-residue locations
- 29 Total fragments observed

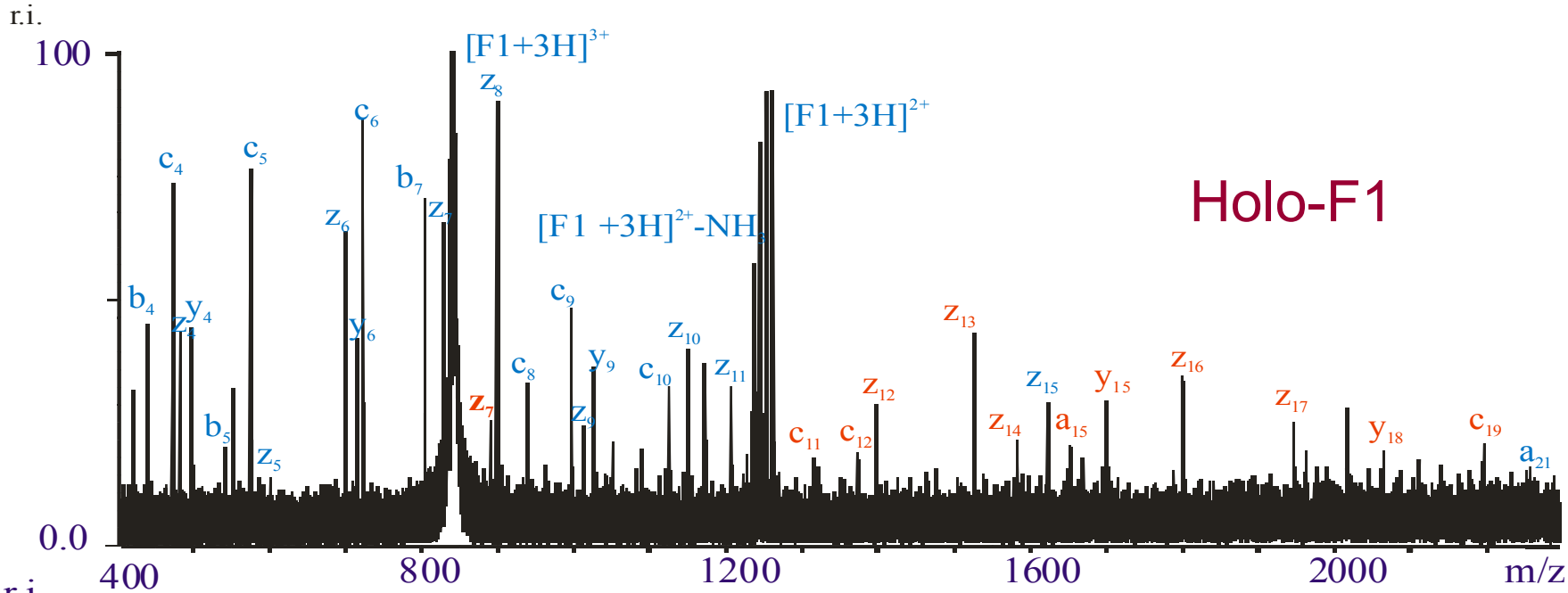


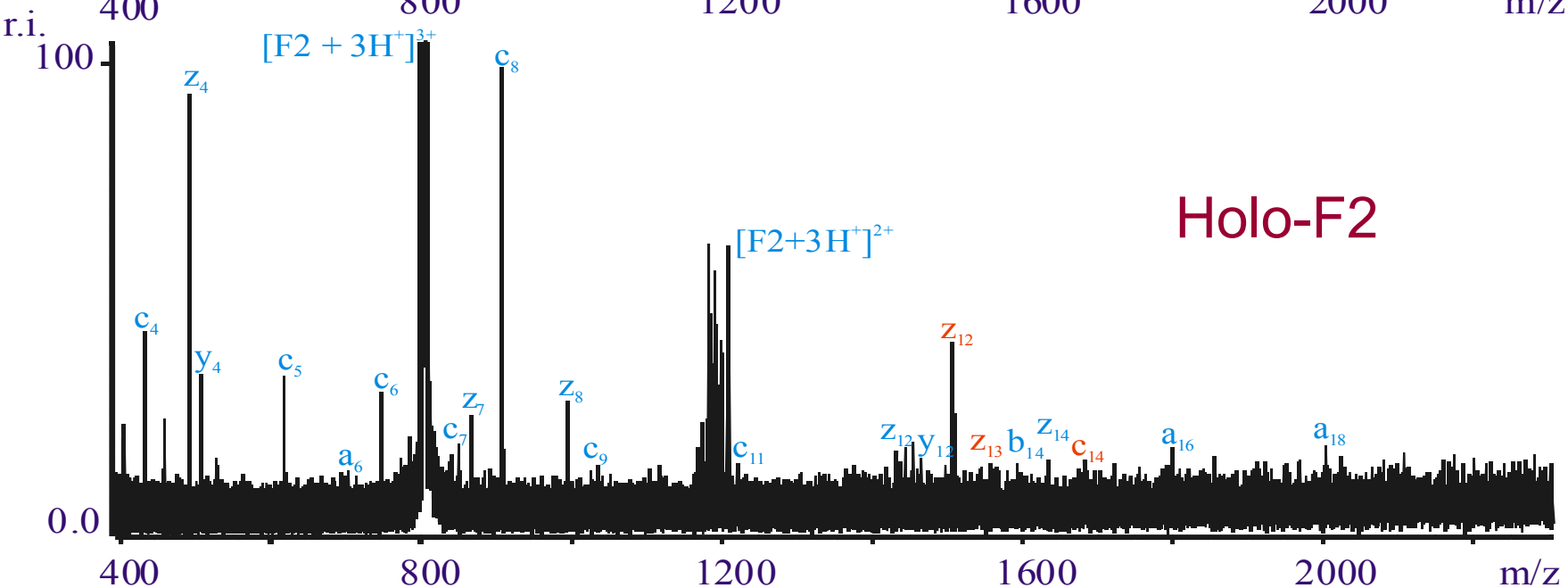
Apo-F1

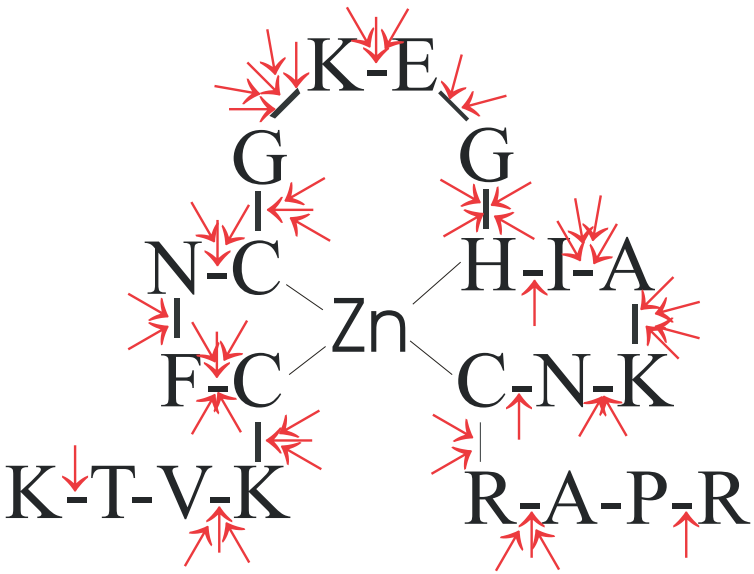


Apo-F2

- Cleavage at 6 of 20 inter-residue locations
- 10 Total fragments observed


r.i.
100
0.0
[F1+3H]3+
[F1+3H]2+
[F1 +3H]2+-NH3
Holo-F1
400
800
1200
1600
2000
m/z

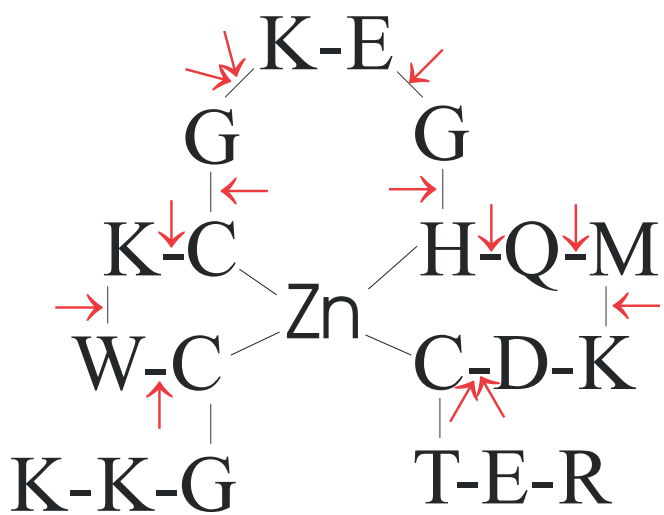

r.i.
100
0.0
[F2 + 3H+]3+
[F2+3H+]2+
Holo-F2
400
800
1200
1600
2000
m/z



Holo-F1

- Cleavage at 19 of 21 inter-residue locations
- Over 50 fragments observed, >18 retained Zn ion

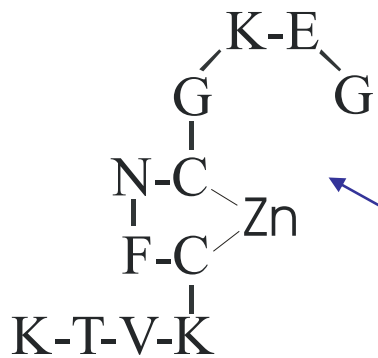
- Cleavage at 11 of 20 inter-residue locations
- 13 total fragments observed, only 3 retained Zn ion



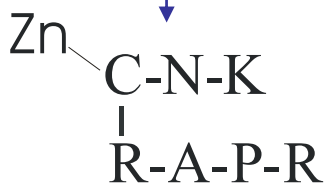
Holo-F2

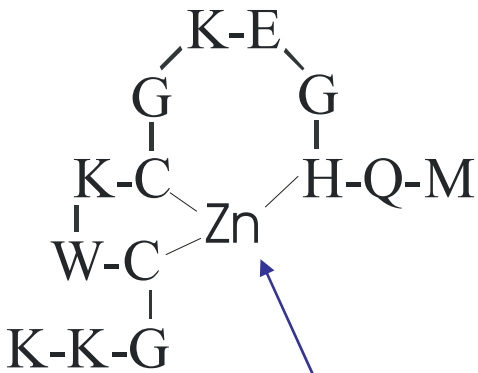
Is there a "minimum structure" required for the retention of Zn by ECD fragment ions?

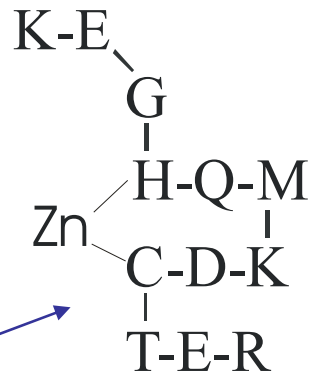
- F1 and F2 show distinct differences in their ability to retain Zn ion following ECD
- Solution-phase data indicate Zn ejected from F2 more rapidly than from F1
- Do ECD data indicate differences in how Zn is lost in the gas phase?



Smallest F1 fragments observed to retain Zn ion



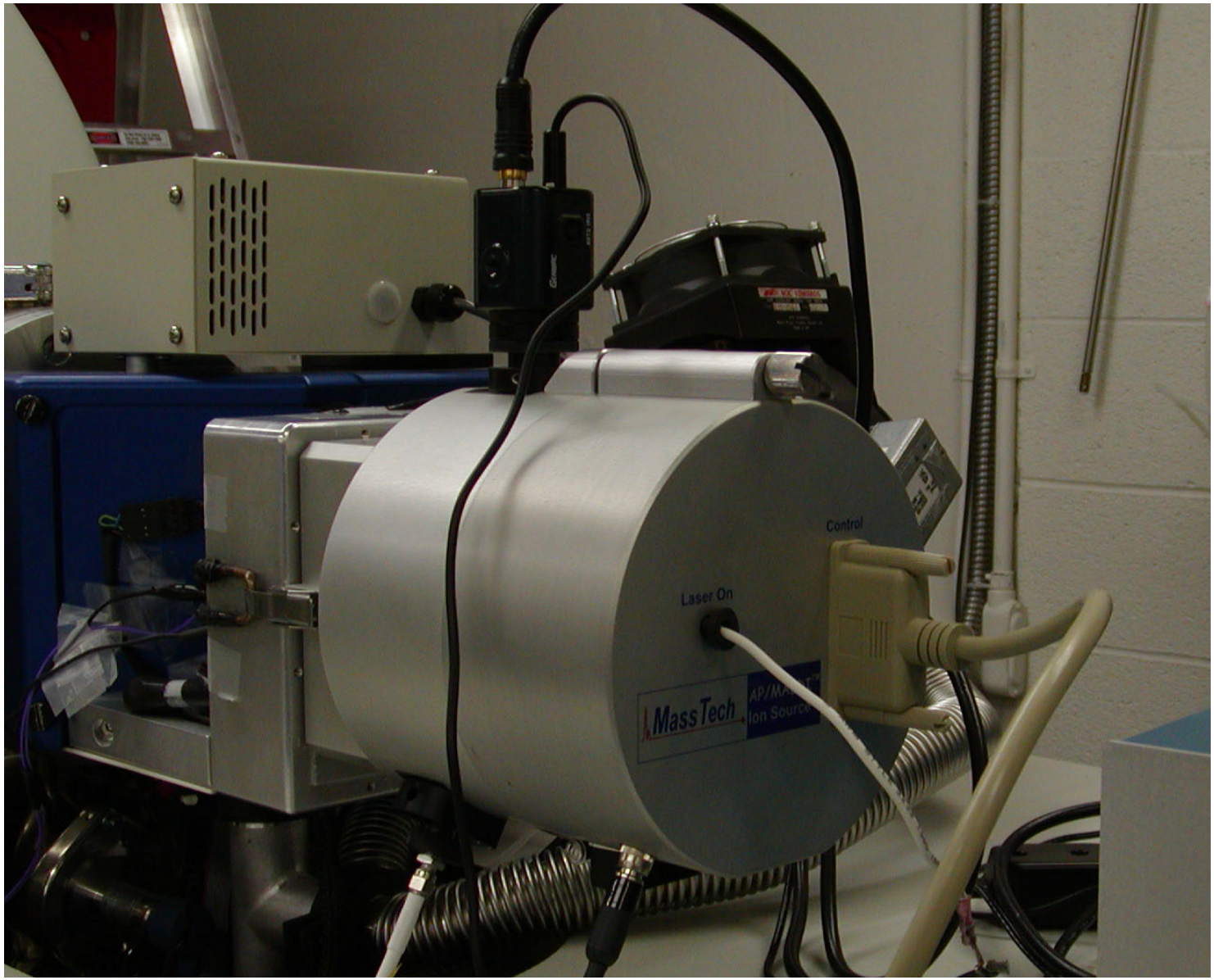


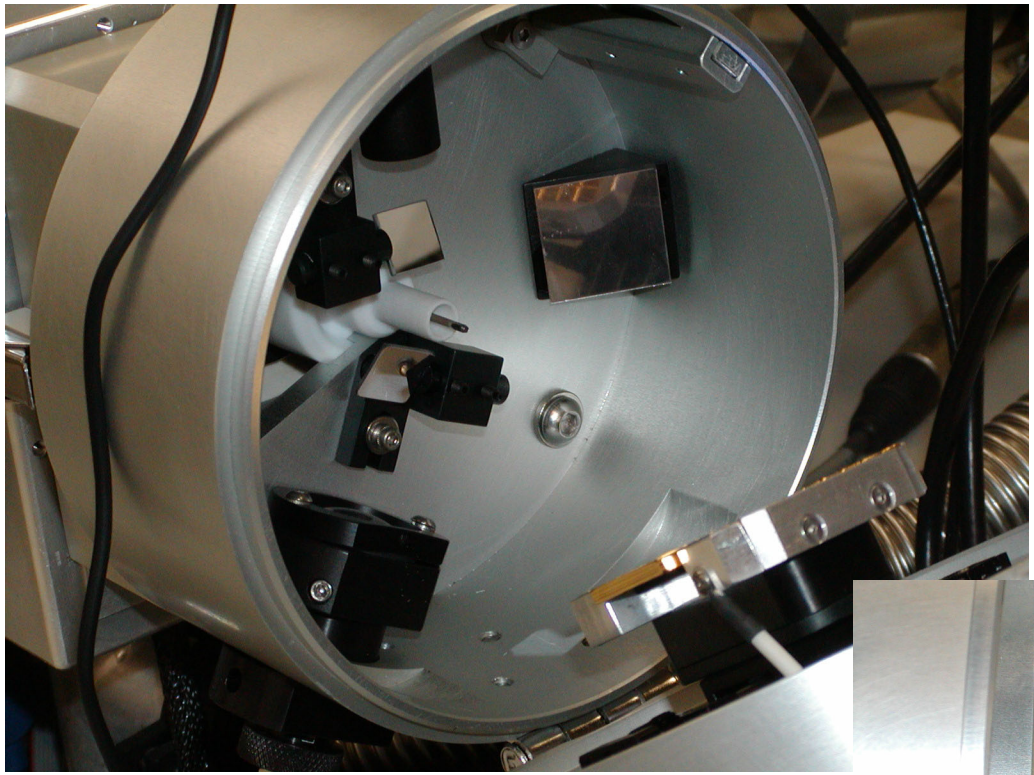


Smallest F2 fragments observed to retain Zn ion

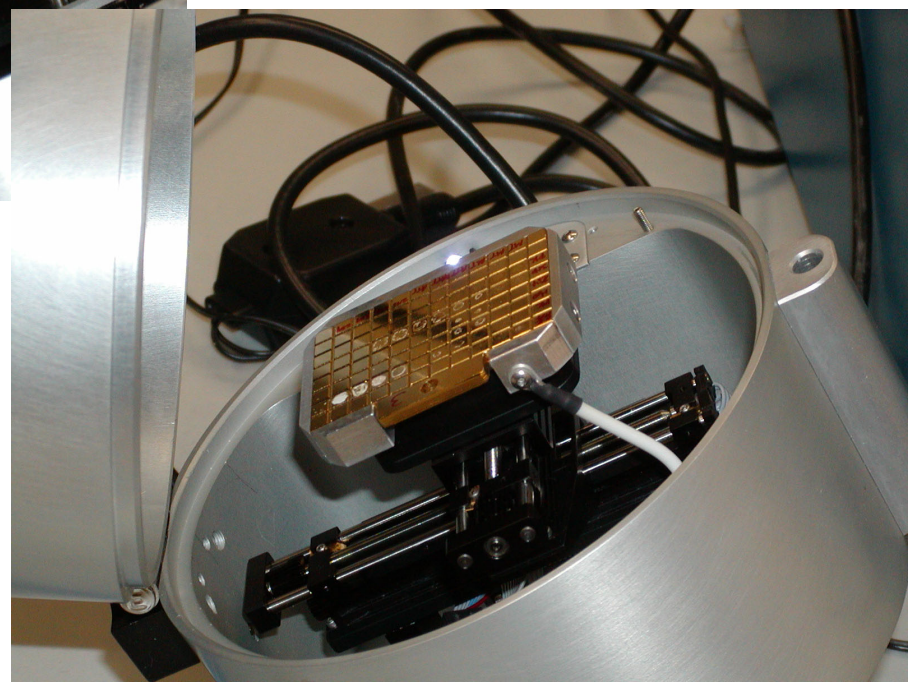
Atmospheric Pressure MALDI

- MALDI commonly used as an alternative to ESI
- AP MALDI interface recently developed for ion trap (Laiko, 1998)
  - Becoming increasingly popular for protein and digest analysis
  - No need for TOF detector
- Similar interface develped for FT-ICR (MassTech, Columbia, MD)

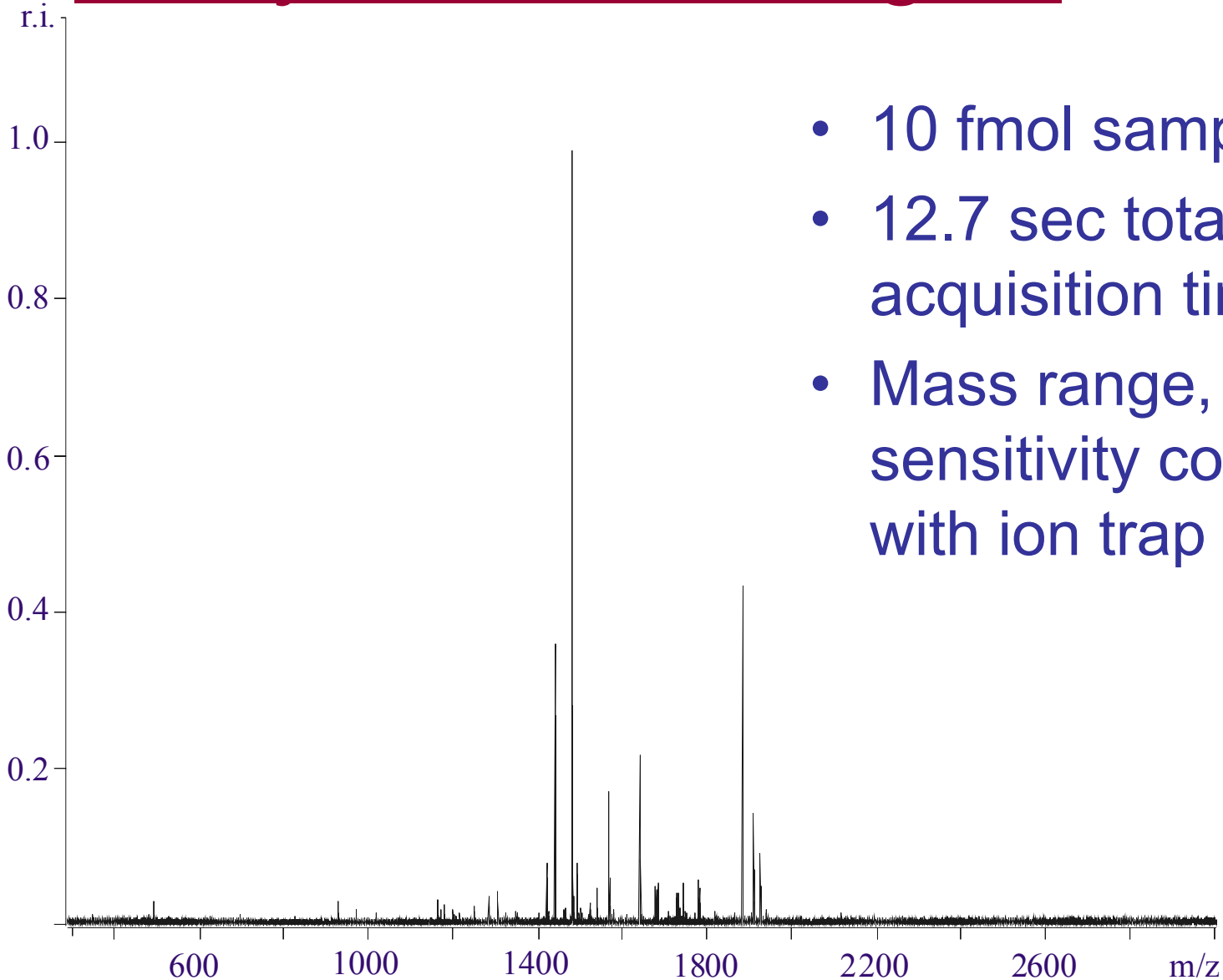

Control
Laser On
MassTech



- 337nm N_2 laser, interfaced via 400 μm fiber optic
- High-resolution CCD camera allows manual adjustment of target
- Sample target placed on a computer-controlled x,y stage



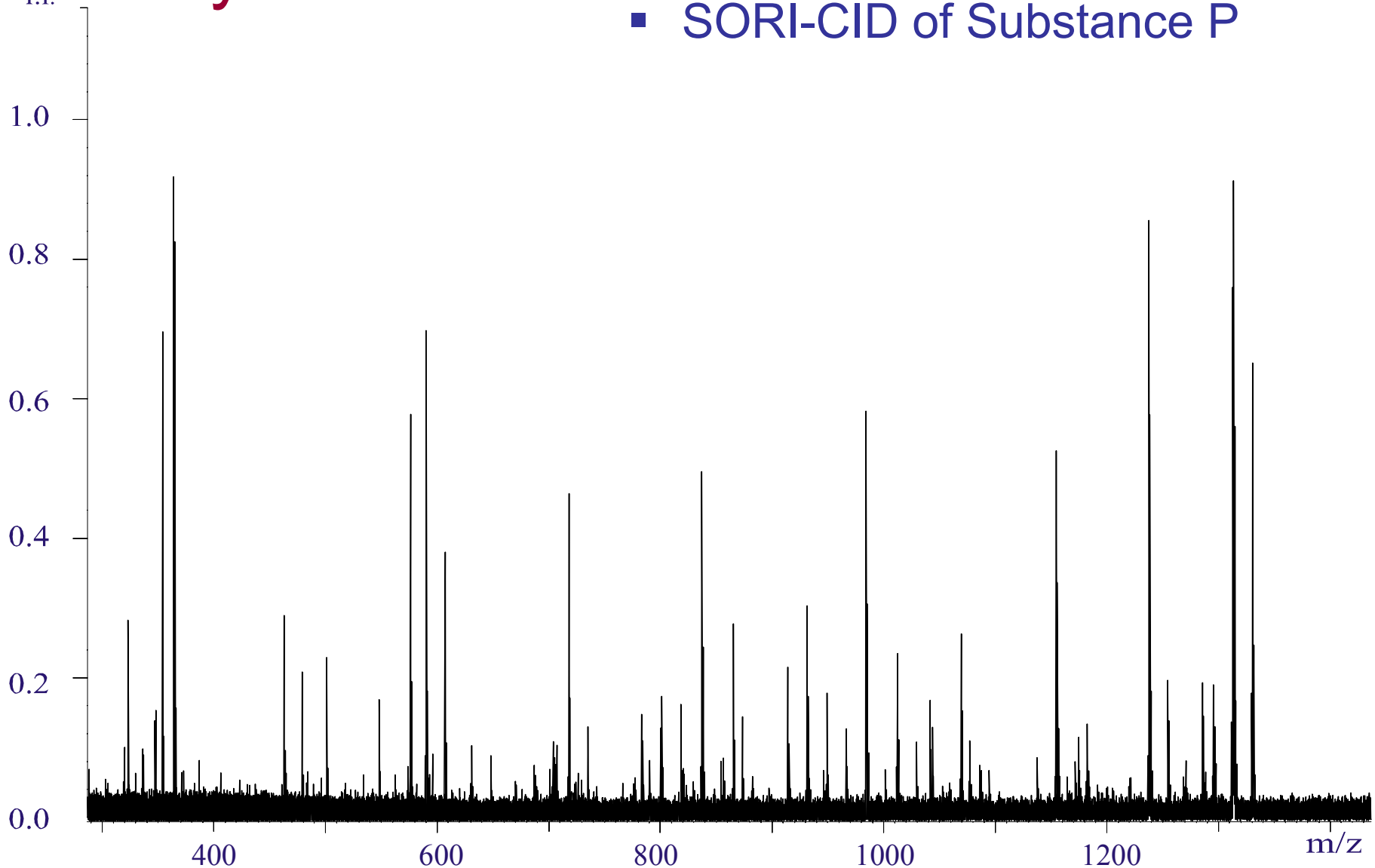
Analysis of BSA digest



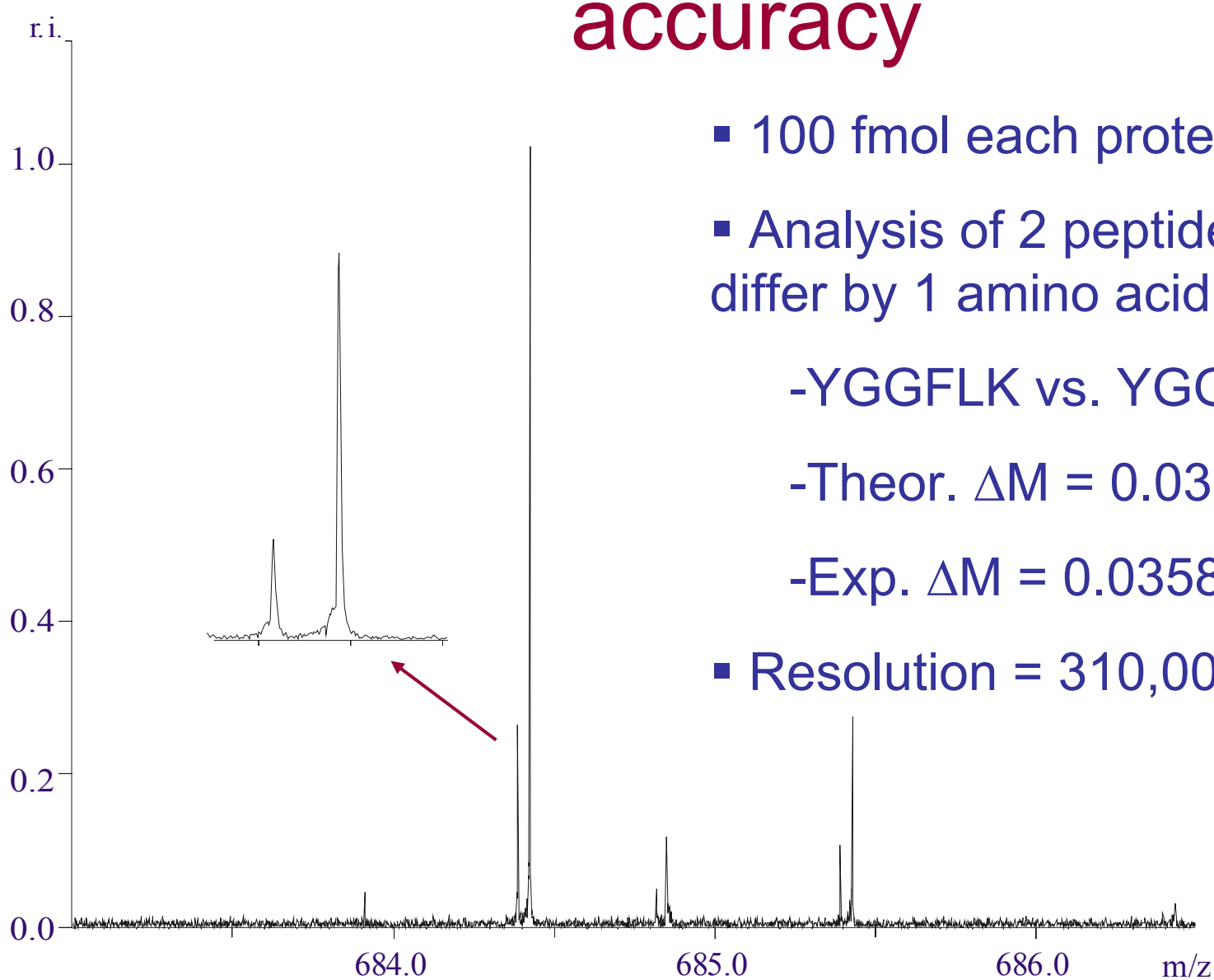
- 10 fmol sample spot,
- 12.7 sec total acquisition time
- Mass range, sensitivity comparable with ion trap

MALDI samples amenable to tandem analysis

- SORI-CID of Substance P

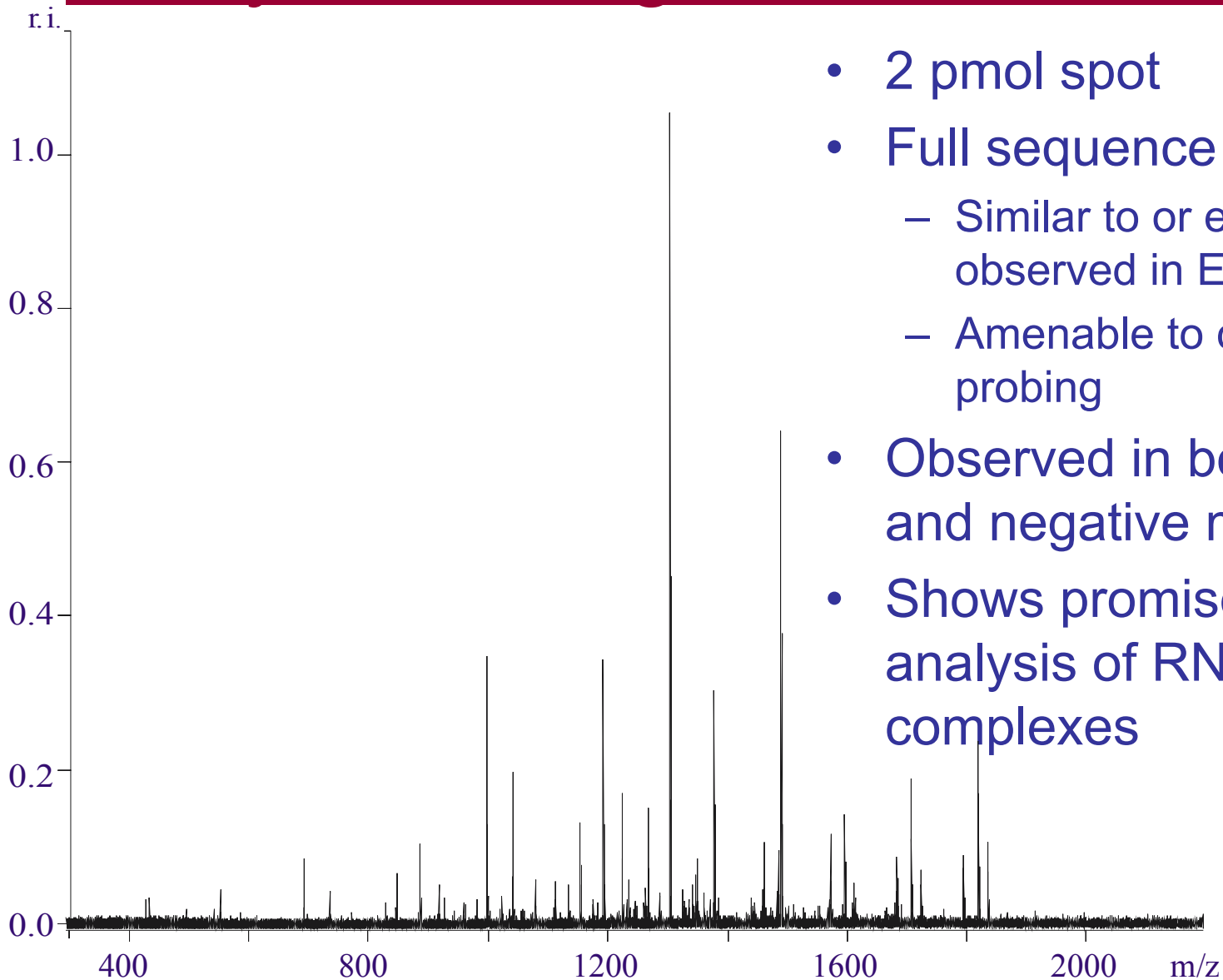


Superior resolution and mass accuracy



- 100 fmol each protein
- Analysis of 2 peptides that differ by 1 amino acid
  - -YGGFLK vs. YGGFLQ
  - -Theor. ΔM = 0.0364 Da
  - -Exp. ΔM = 0.0358 Da
- Resolution = 310,000

Analysis of Oligonucleotide digests:



- 2 pmol spot
- Full sequence coverage
  - Similar to or exceeding that observed in ESI
  - Amenable to chemical probing
- Observed in both positive and negative mode
- Shows promise for analysis of RNA-protein complexes

Advantages:

- Simple, rapid switching from ESI to MALDI without breaking vacuum
- Low sample consumption (fmol to amol consumed)
- Increased resolution and mass accuracy associated with FTMS while achieving sensitivity and LODs similar to ion trap
- Sample prep (matrices, sample levels) similar to traditional (vacuum) MALDI
- Ionization more gentle than vacuum MALDI

Acknowledgements

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