

Mass spectrometric analysis of proteins using Electron Transfer Dissociation

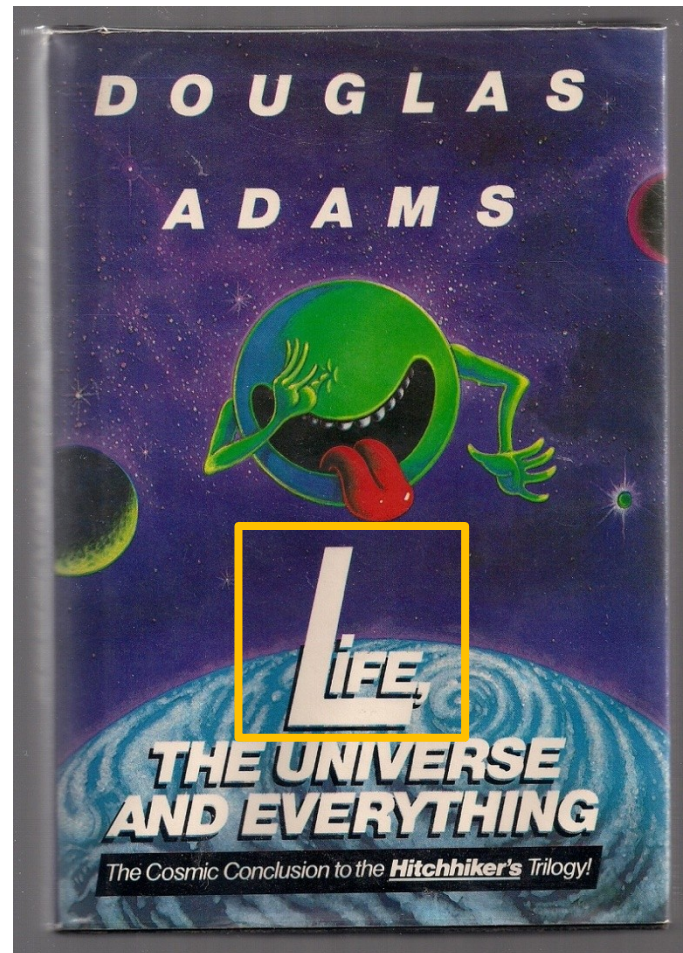
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Introduction



Overview

- » Biochemistry recap
 - » Central dogma
 - » Protein structure
- » Mass spectrometry
- » Bottom-up vs. top-down
 - » Native vs. denatured
- » Electron transfer dissociation (ETD)
 - » Case study: alcohol dehydrogenase
 - » Effect of ETD on isotopic distribution (bioinformatics)

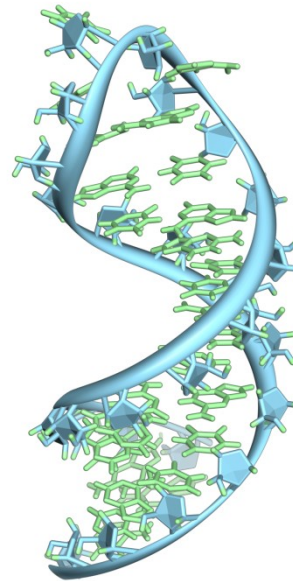
Basic biochemistry

DNA



mRNA

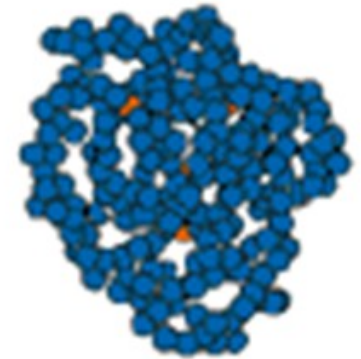
Transcription



Translation



Protein



Genome

Transcriptome

Proteome

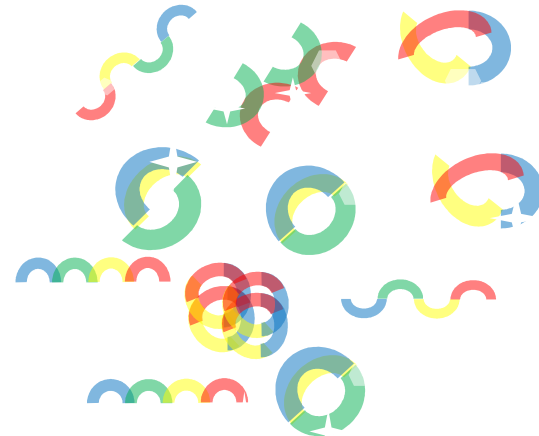
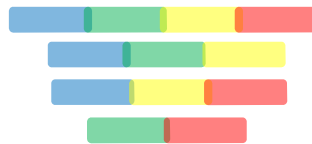
Fraser, C.M. et al.: The minimal gene complement of *Mycoplasma genitalium*. *Science* 270(5235), 397-404 (1995)
 Adams, M.D. et al.: The genome sequence of *Drosophila melanogaster*. *Science* 287(5461), 2185-2195 (2000)
 Lander, E. S. et al.: Initial sequencing and analysis of the human genome. *Nature*, 409(6822), 860-921 (2001)
 Venter, J. C. et al.: The sequence of the human genome. *Science*, 291(5507), 1304-1351 (2001)
 Venter, J.C.: A Part of the Human Genome Sequence. *Science*, 299(5610), 1183-1184 (2003)

Basic biochemistry – information content

Genome
 $2,3 \times 10^4$

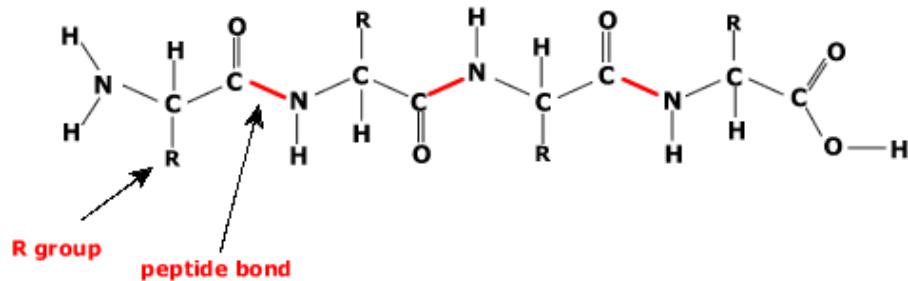
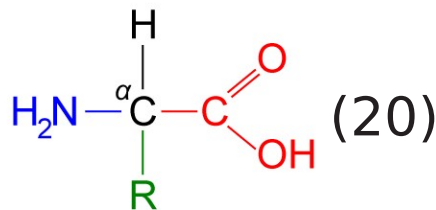
Transcriptome
 $> 10^6$

Proteome
 $> 10^8$



Protein structure

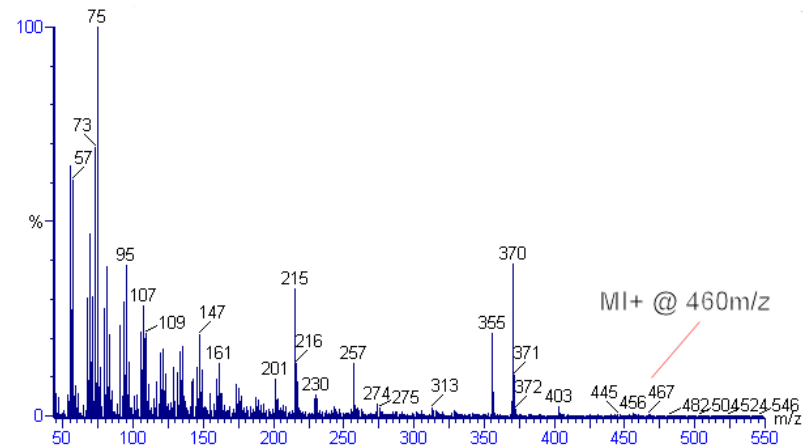
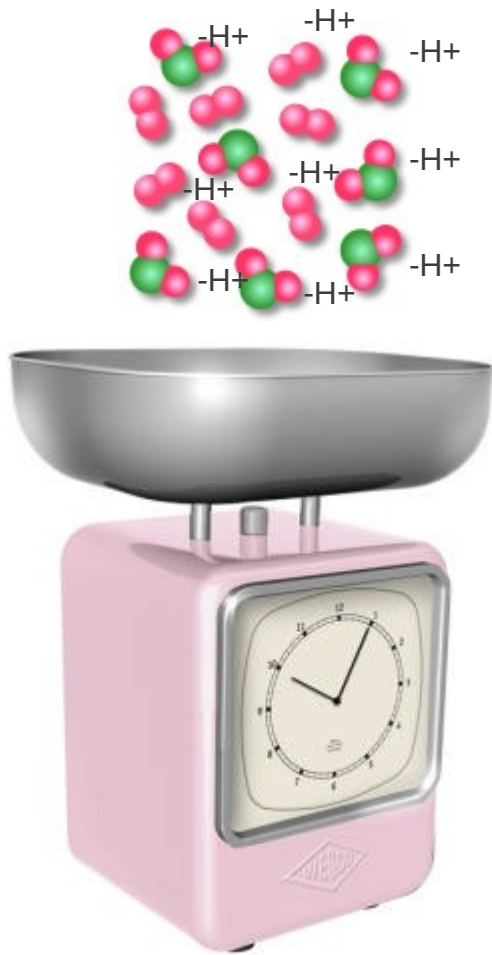
- » (Large) linear polymer (chain) of amino acids





- » Protein = amino acid sequence
 - + post-translational modifications (PTMs)
 - + 3D structure
 - + protein-protein/protein-ligand complexes
- » How do we analyze this? → Mass spectrometry!

Mass spectrometry

H⁺ = proton ('handle' on molecule)
↓



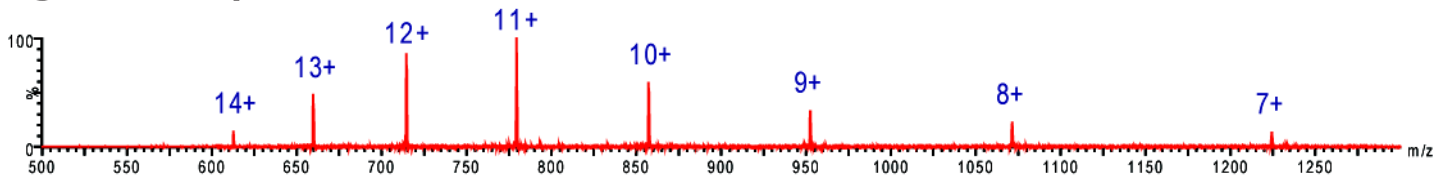
 : (m/z, ~ #)

 : (m/z, ~ #)

Charge state distributions

» Number of protons (charges) placed on protein is not a single value, but a distribution!

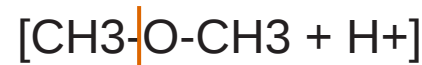
» e.g. ubiquitin (76 amino acids, 8.6 kDa):



Tandem mass spectrometry



$$m/z = 46.07 \text{ Th}$$

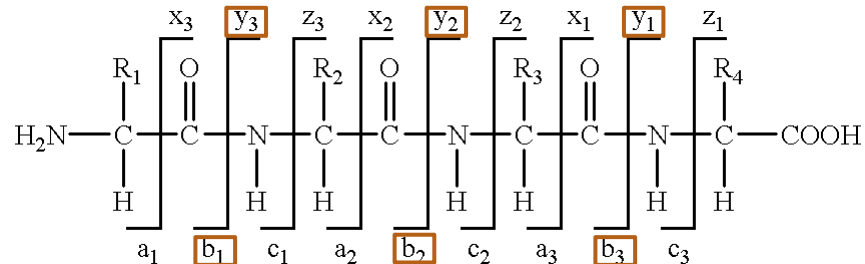


$$m/z = 46.07 \text{ Th}$$



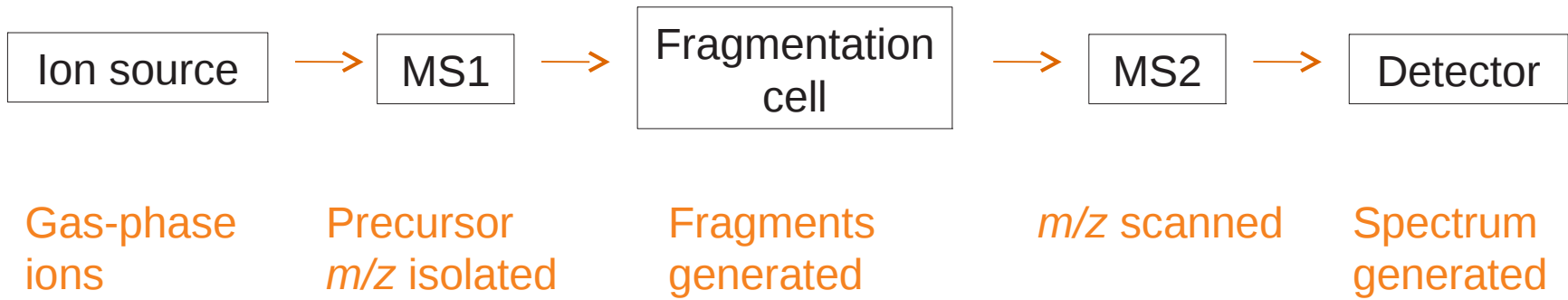
- » Mass analysis: vacuum
- » Collision-induced dissociation (CID): let ions collide with inert gas molecules

- » Internal energy increases
- » Weakest bond(s) break

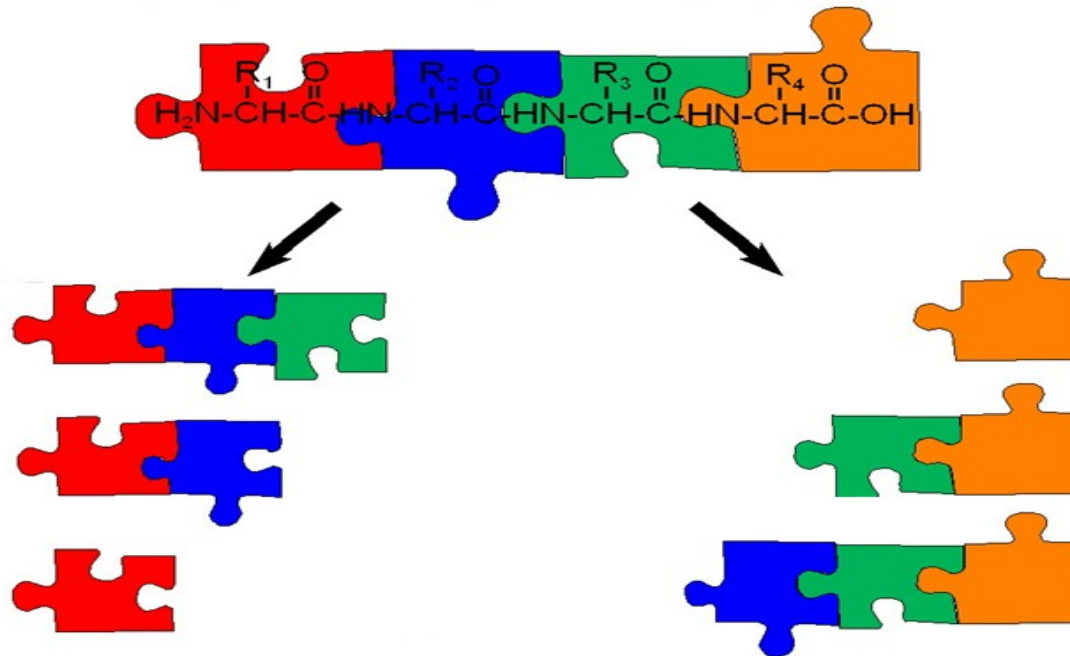


- » Mass of precursor and fragments reveal molecular structure

Tandem mass spectrometry

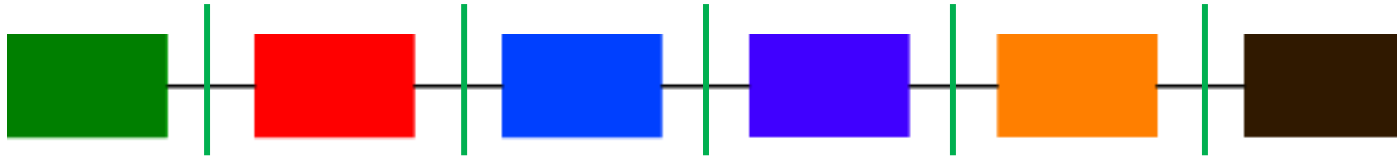


Tandem mass spectrometry

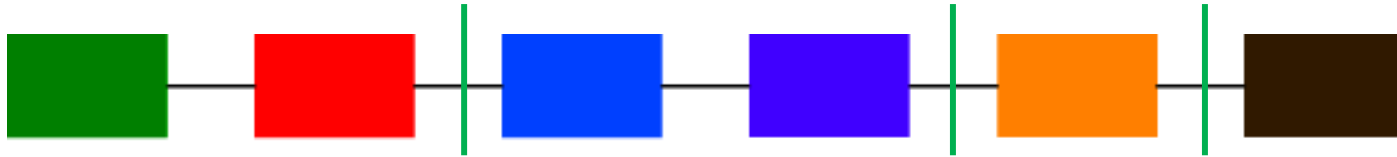


- » Theory: $\Delta M \rightarrow$ amino acid sequence
- » Practice: internal energy buildup \rightarrow side reactions
 - » e.g. loss of PTMs, protein complexes, ligands, etc.

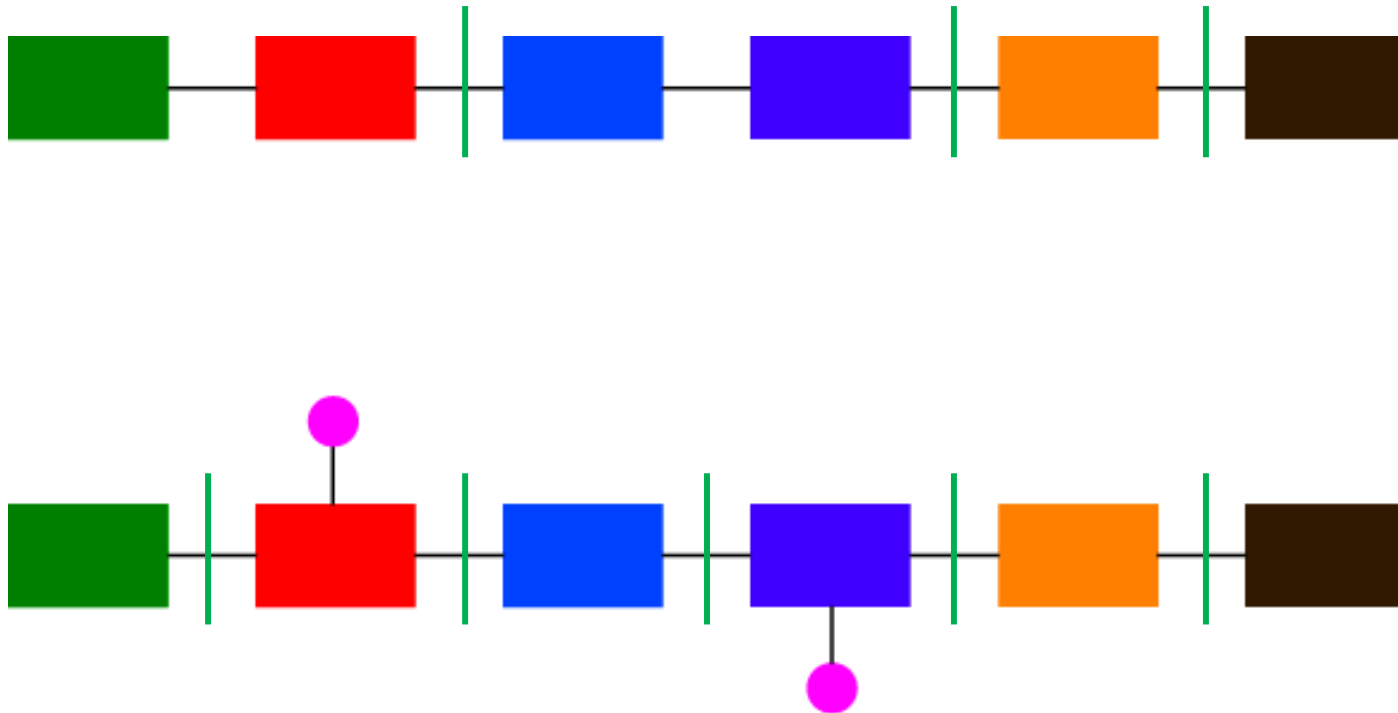
CID side reactions



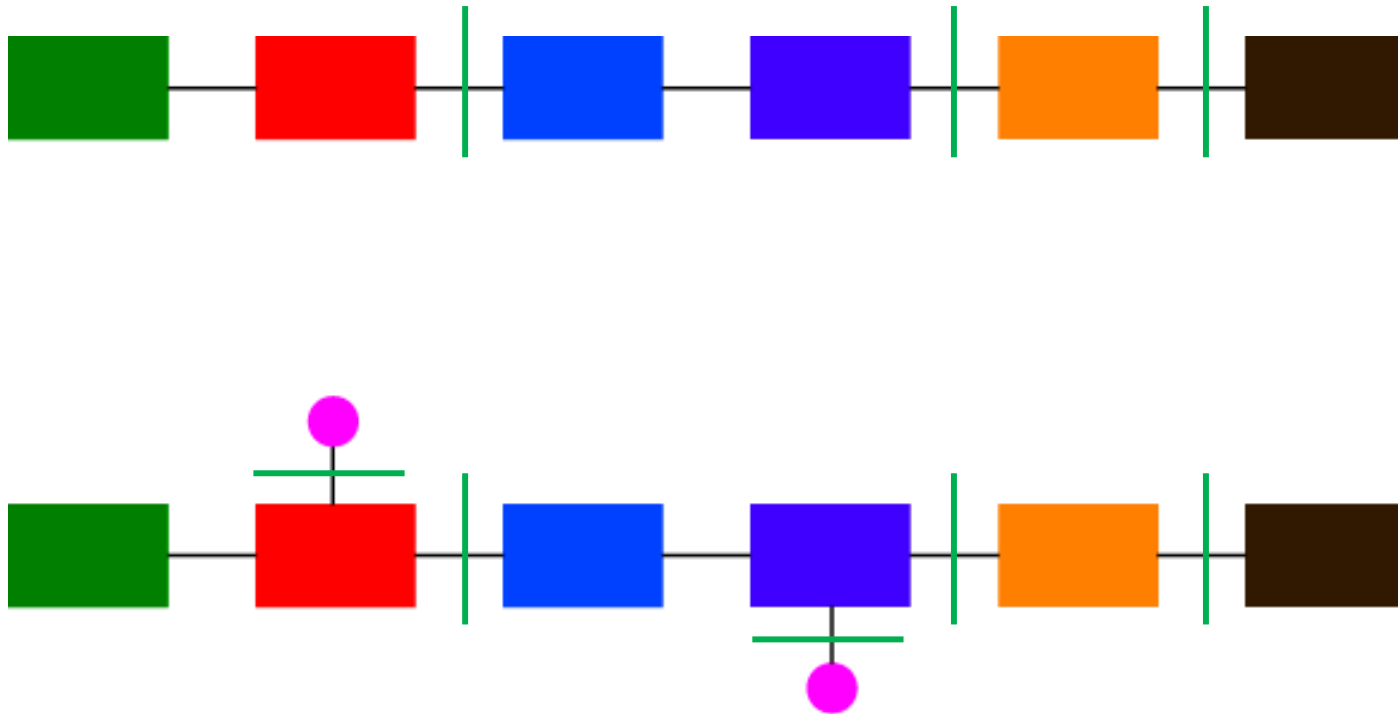
CID side reactions



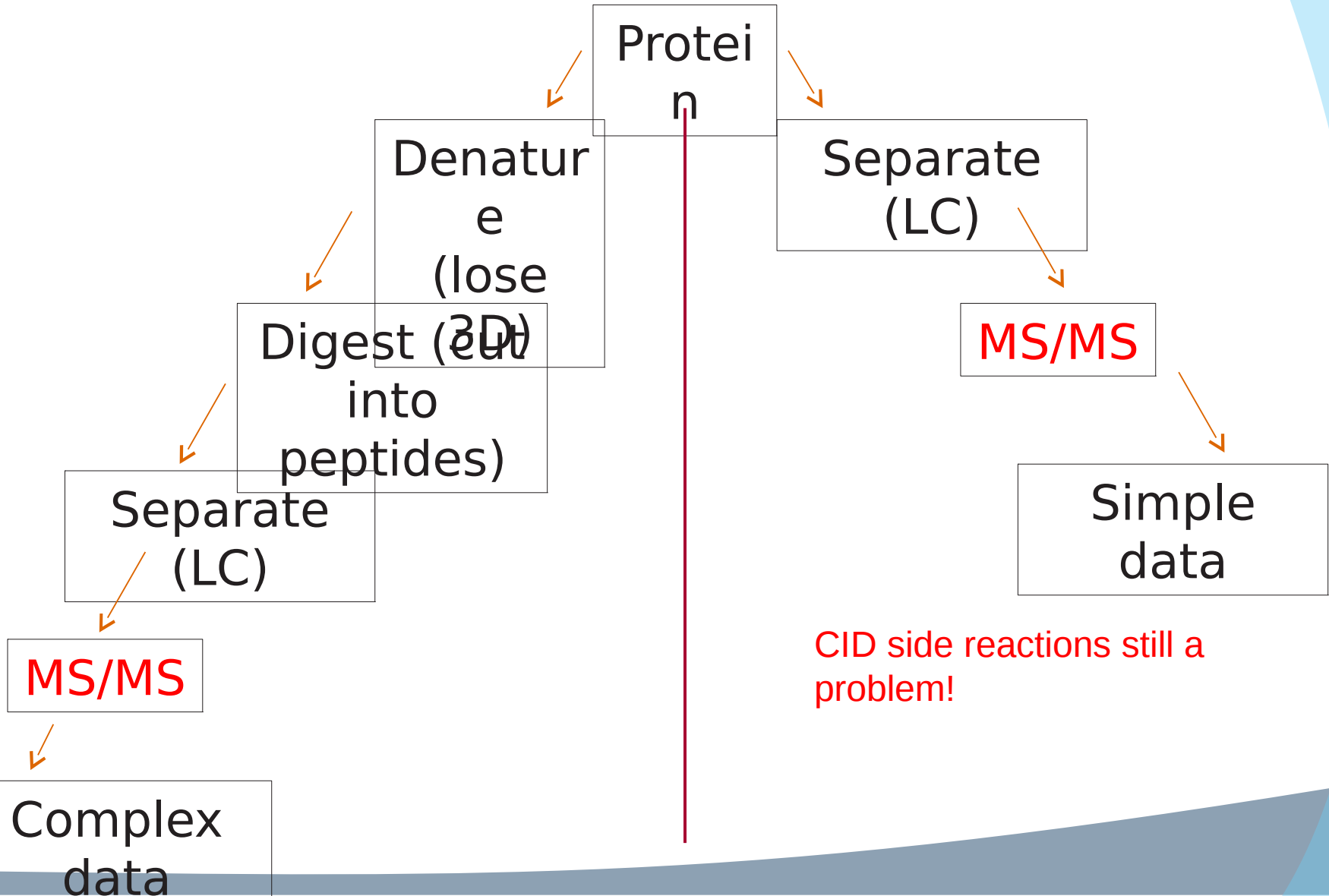
CID side reactions



CID side reactions



Bottom-up/top-down

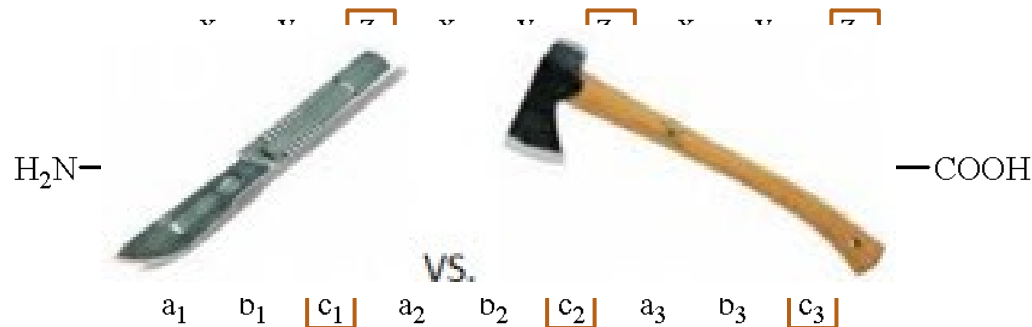


Native/denatured proteins

- » Native: keep 3D structure (including complexes) intact
- » Why?
 - » Study protein conformations
 - » Study protein/protein or protein/ligand interactions directly
- » How?
 - » Solution phase: no acids/organic solvents
 - » Ionization: 'soft' methods (electrospray ionization/ESI)
 - » Careful control of voltages/pressures in gas phase (minimal internal energy buildup)
- » Can we still perform fragmentation?

Electron capture/transfer dissociation

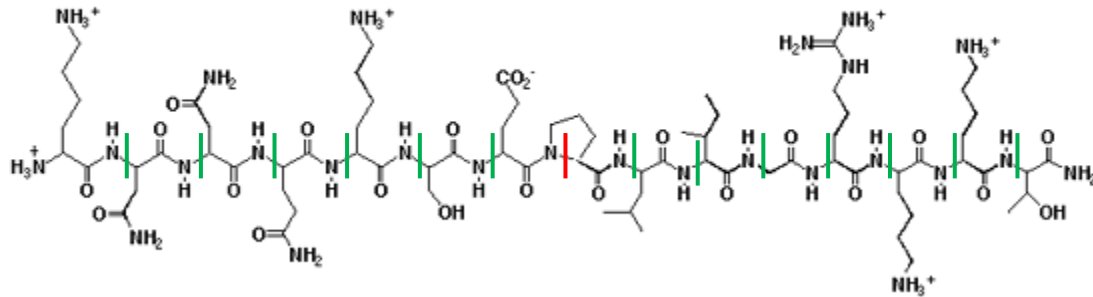
- » CID: gradually increase internal energy by 10³ collisions
- » ECD/ETD: $H^+ + e^- \rightarrow H^\bullet$ (radical causes fragmentation)



- » Advantage: very specific reaction \rightarrow minimal side reactions
- » Also: limited preference for certain residues (exception: proline)

Electron capture/transfer dissociation

» Proline:



» Difference ECD/ETD: source of e-

» ECD: electron beam

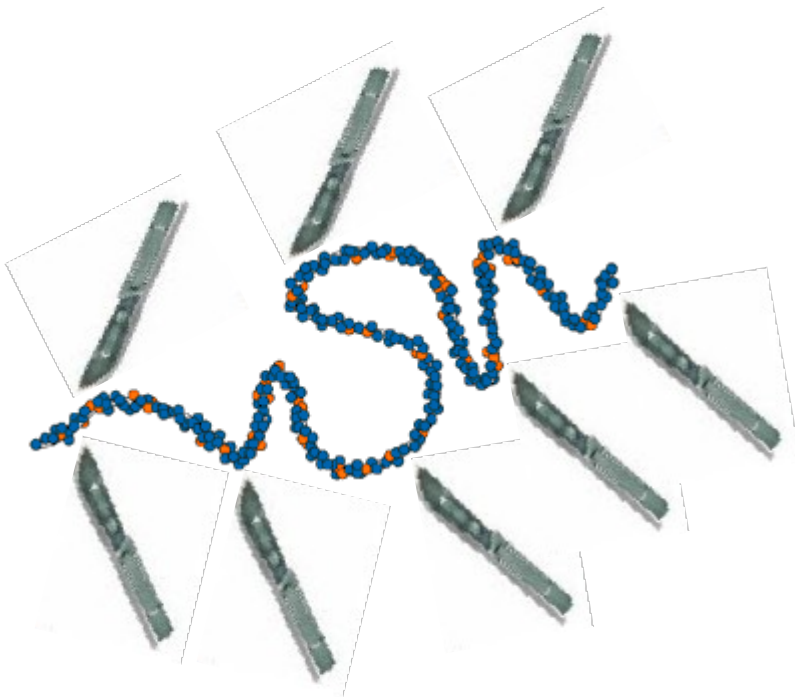
» ETD: radical anion

» ECD: $Mz+ + e^- \rightarrow [M_{\bullet}(z-1)+]^* \rightarrow \text{fragments}$

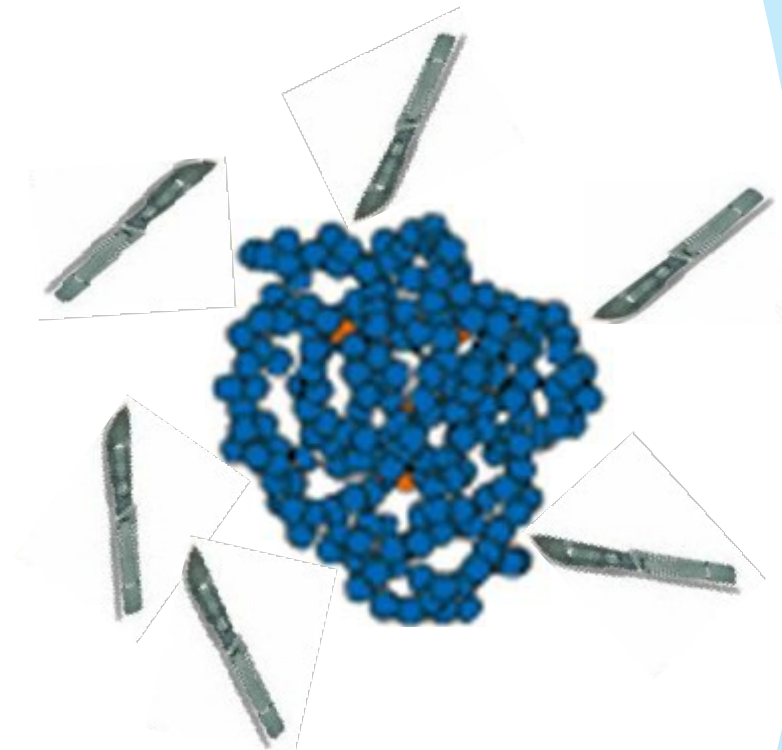
» ETD: $Mz+ + A_{\bullet}^- \rightarrow [M_{\bullet}(z-1)+]^* + A \rightarrow \text{fragments}$

Electron transfer dissociation

- » Is ETD gentle enough to perform on native proteins?



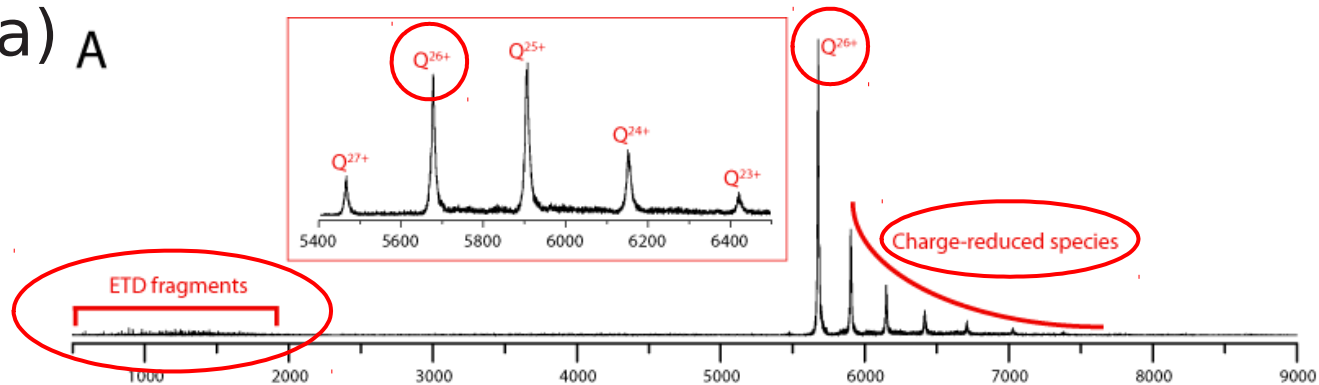
Sequence + PTMs



3D info?

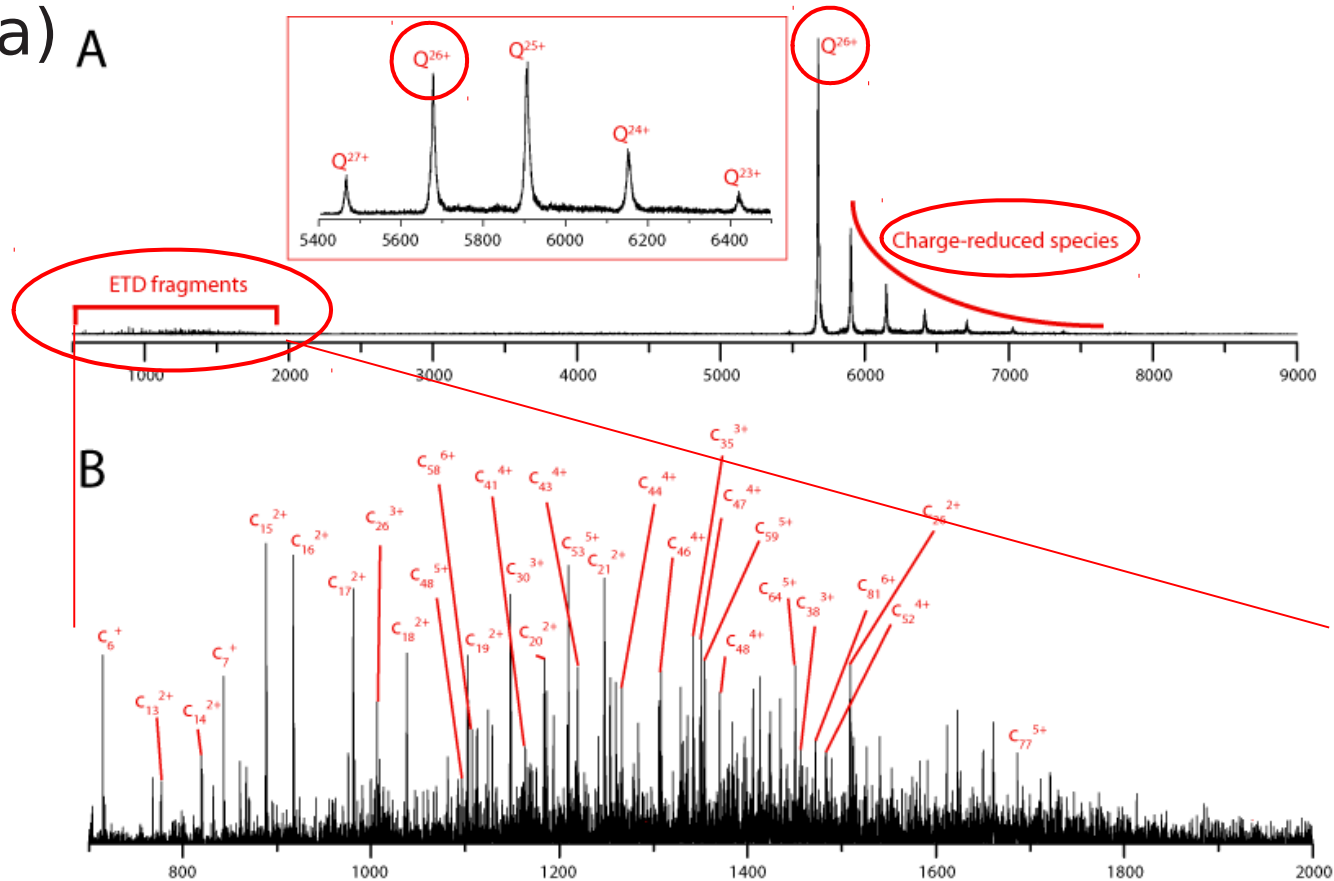
ETD case study: ADH

- » Alcohol dehydrogenase: tetramer (147 000 Da) A



ETD case study: ADH

» Alcohol dehydrogenase: tetramer (147 000 Da) A



ETD case study: ADH

A

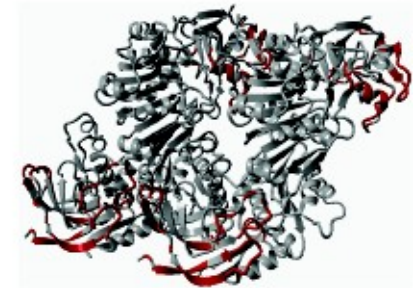
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 HDGSFQEYAT ADAVQAAHIP QGTDLAEVAP VLCAGITVYK ALKSANLMAG HWVAISGAAG
 GLGSLAVQYA KAMGYRVLGI DGEGGKEELF RSIGGEVFID FTKEKDIVGA VLKATDGGAH
 GVINVSVSEA AIEASTRYVR ANGTTVLVGM PAGAKCCSDV FNQVVKISIS VGSYVGNRAD
 TREALDFFAR GLIKSPIKVV GLSTLPEIYE KMEKGQIVGR YVVDTSK

ETD case study: ADH

A

```
ac-SIPETQKGV I FYESHGKLEY KDIPV PKPKA NELLINVKYS GVCHTDLHAW HGDWPLPVKL
PLVGGHEGAG VVVG MGENVK GWKIGDYAGI KWLNGSCMAC EYCELGNESN CPHADLSGYT
HDGSFQEYAT ADAVQAAHIP QGTDLAEVAP VLCAGITVYK ALKSANLMAG HWVAISGAAG
GLGSLAVQYA KAMGYRVLGI DGEGEKEELF RSIGGEVFID FTKEKDIVGA VLKATDGGAH
GVINVSVSEA AIEASTRYVR ANGTTVLVGM PAGAKCCSDV FNQVVKSI SI VGSYVGNRAD
TREALDFFAR GLIKSPIKVV GLSTLPEIYE KMEKGQIVGR YVVDTSK
```

B



- » Fragmentation occurs at surface of native tetramer (confirmed through calculations)
- » Current research: can we extend this to other proteins/complexes?

Isotopic distributions in ETD-MS

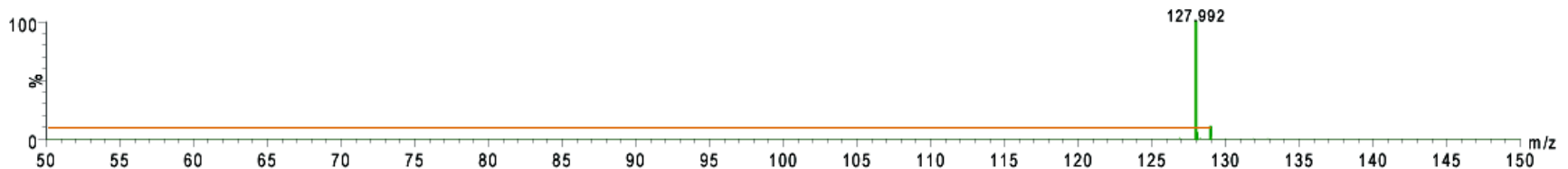


Isotopic distributions in MS

- » Elements naturally occur as different isotopes (same # of protons, different # of neutrons)
- » C: 98.93% ^{12}C , 1.07% ^{13}C
- » H: 99.99% ^1H , 0.01% ^2H
- » O: 99.76% ^{16}O , 0.04% ^{17}O , 0.20% ^{18}O
- » N: 99.63% ^{14}N , 0.37% ^{15}N
- » S: 94.93% ^{32}S , 0.76% ^{33}S , 4.29% ^{34}S , 0.02% ^{36}S
- » Monoisotopic (lightest) peak, $M+1$, $M+2$, etc. determined by statistics

Isotopic distributions in MS

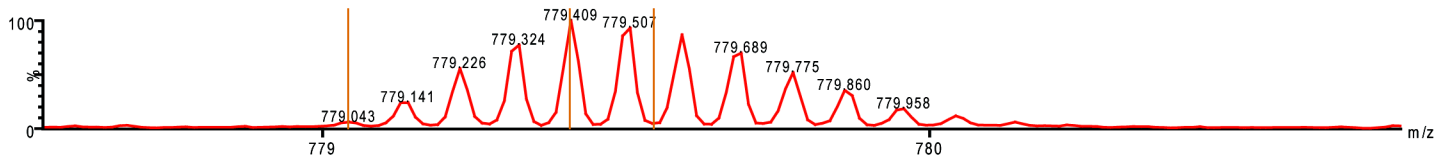
- » 1,4-dicyanobenzene (C₈H₄N₂):
 chance of M+1 $\sim 1 - 0.98938 = 8.2\%$
 $IM+1/IM \sim 8.2/98.93 = 8.3\%$



- » Monoisotopic peak = most abundant peak
- » Monoisotopic mass \sim average mass

Isotopic distributions in MS

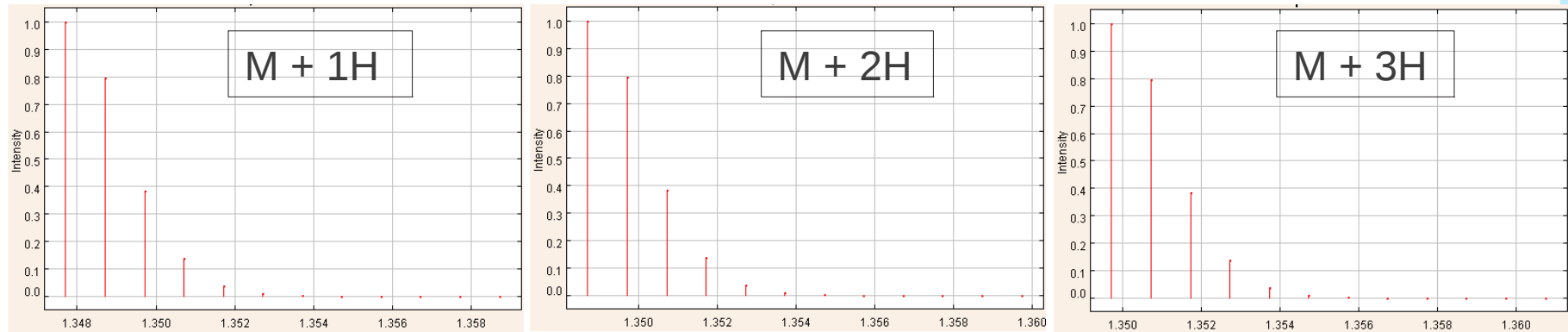
- » Small protein (ubiquitin, 76 amino acids, 8.6 kDa):
hundreds of C → monoisotopic no longer most likely



- » Monoisotopic peak != most abundant peak
- » Monoisotopic mass !~ average mass

Isotopic distributions in ETD-MS

- » In ESI-MS: measured mass = $M_{\text{protein}} + (n \times MH)$
 - » H: 99.99% $^1H \rightarrow$ distribution same shape, shifted to the right
 - » Example: substance P (11 amino acids, 1.3 kDa)

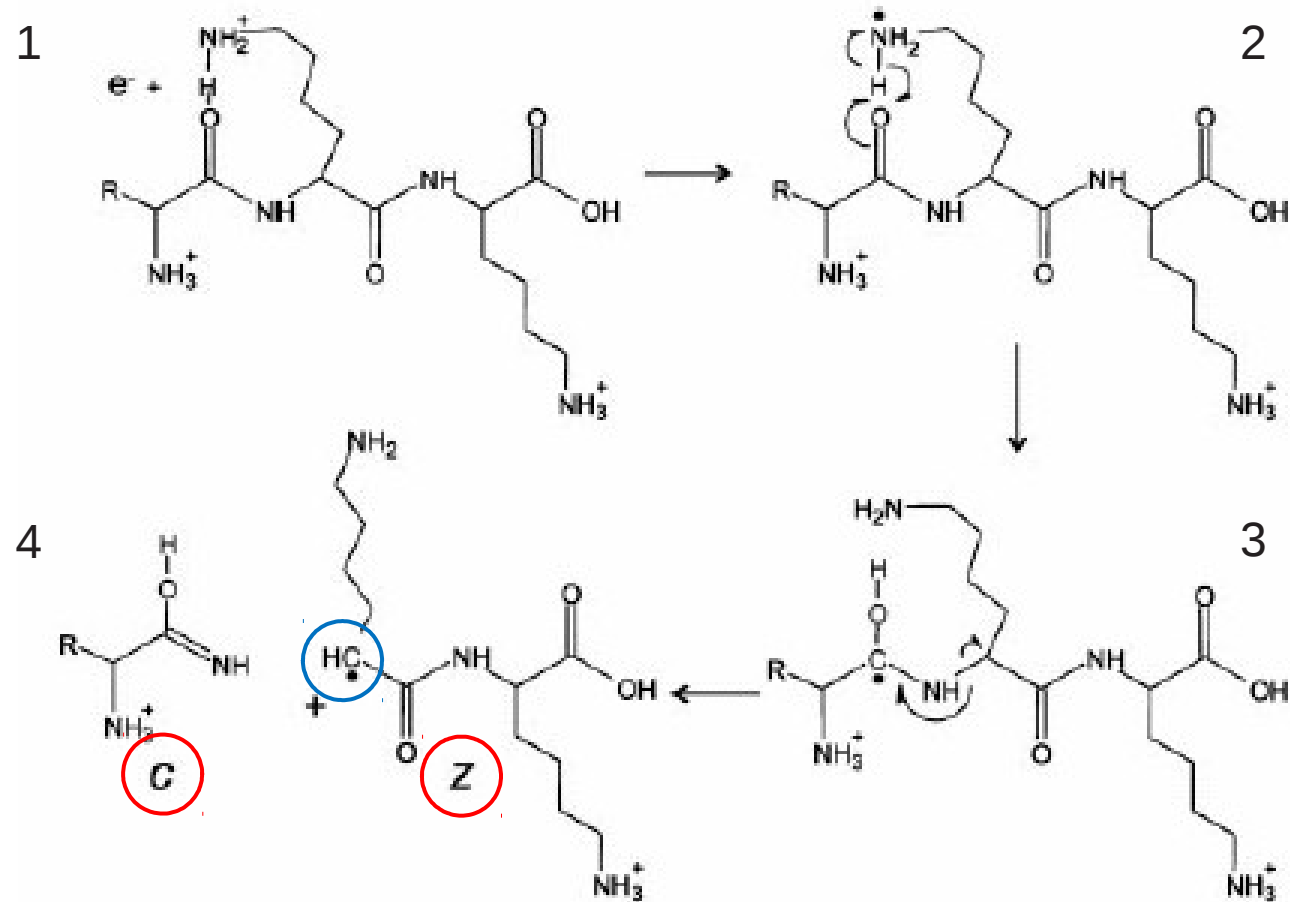


- » Different charge states \rightarrow appear in different m/z regions
- » No problem as long as 'extra' H are protons H^+

Isotopic distributions in ETD-MS

- » ECD/ETD: $H^+ + e^- \rightarrow H^\bullet$ (radical causes fragmentation)
 - » Potential problem!
- » $[M + nH]^{n+} + A^\bullet \rightarrow [c + xH]^x + [z + (n-x)H]^\bullet (n-x-1)^+ + A$ ETD
- » $[M + nH]^{n+} + A^\bullet \rightarrow [M + (n-1)H]^{(n-1)+} + HA$ PTR
- » $[M + nH]^{n+} + A^\bullet \rightarrow [M + nH]^\bullet (n-1)^+ + A$ ETnoD
- » Contribution (probability) of each reaction not known
- » Mix of these reactions distorts isotopic distribution
 - » And it gets worse...

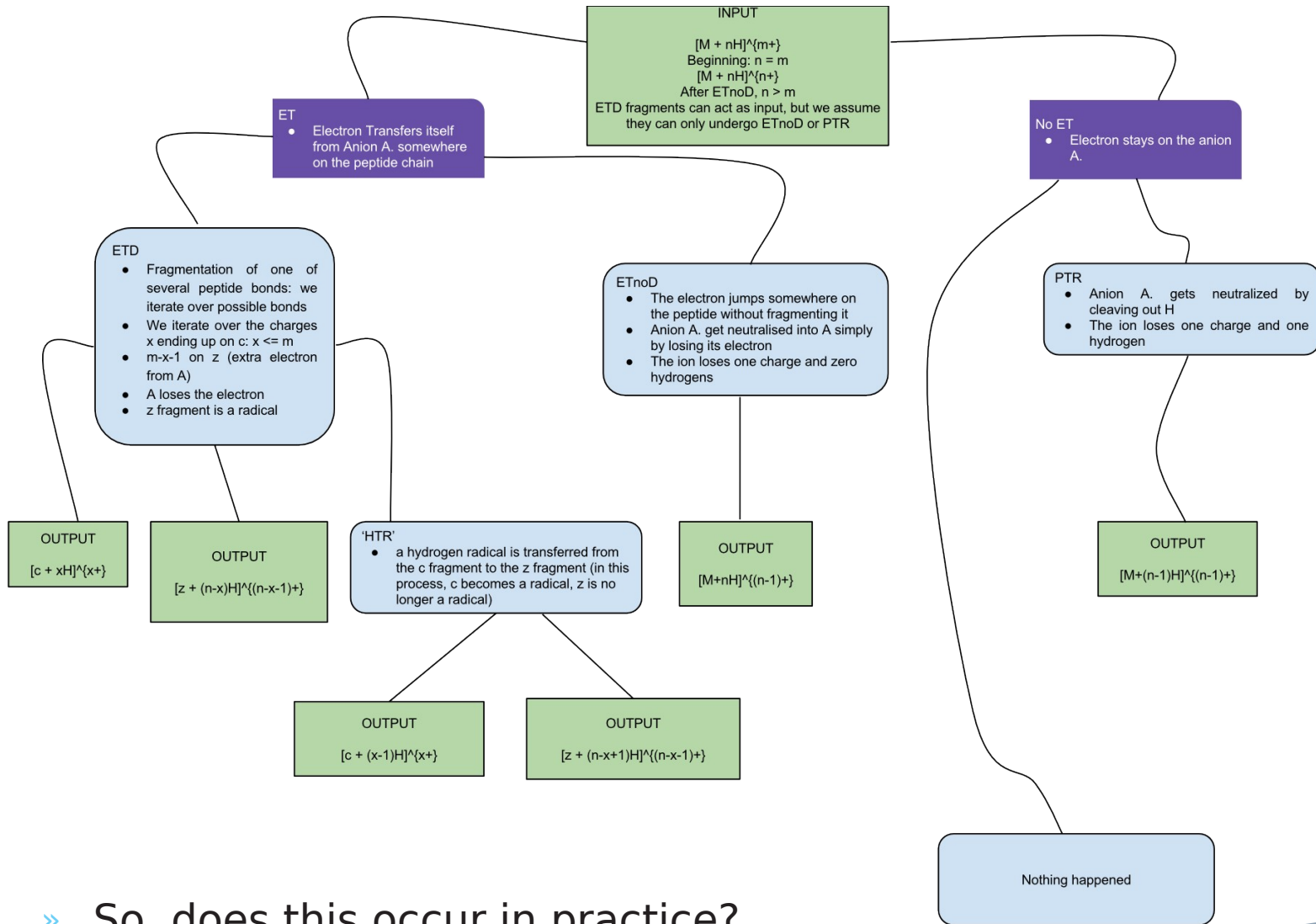
ETD mechanism in detail



ETD mechanism in detail

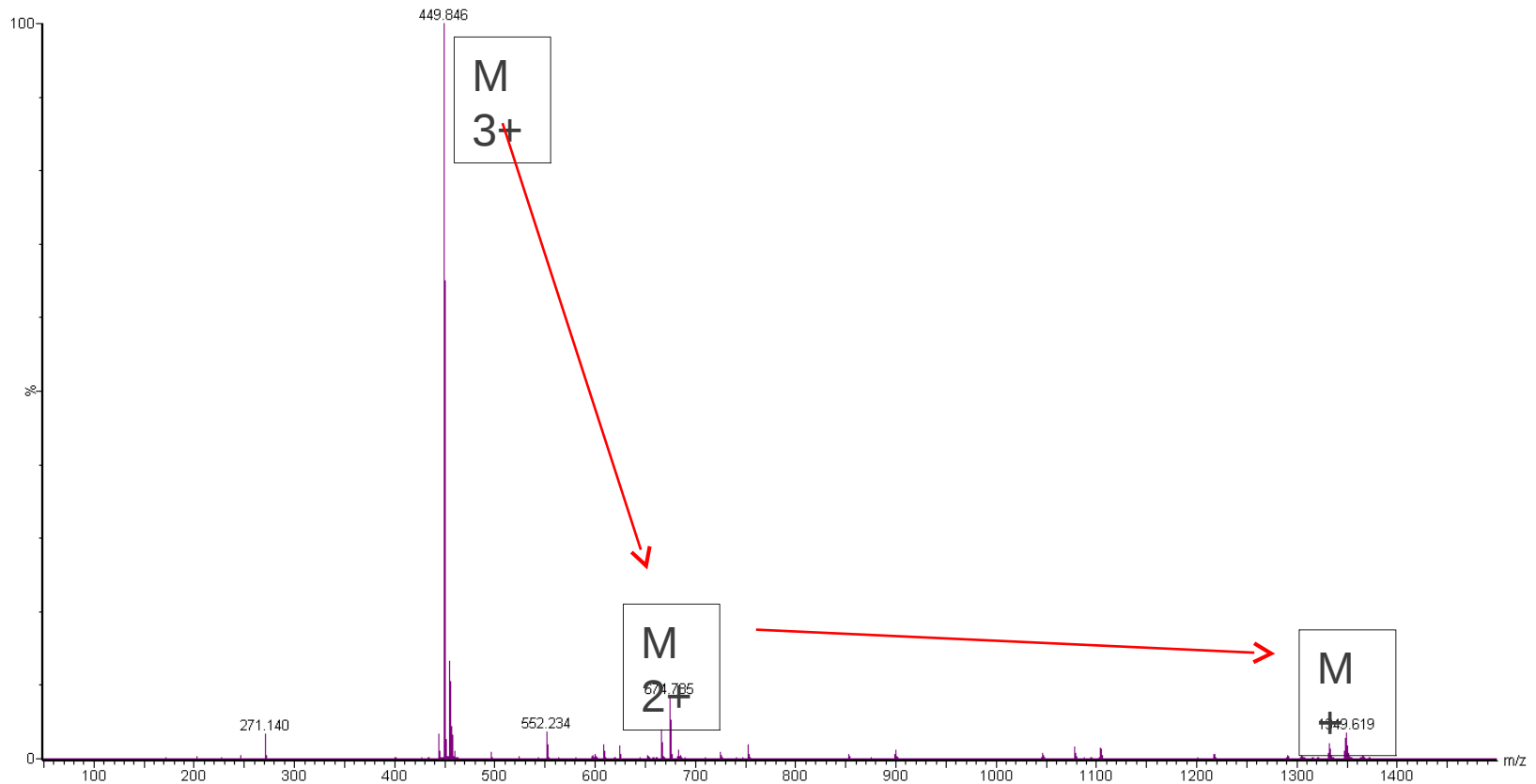
- » Possible step 5: H_n migrates from c to z fragment
- » $[c + xH]^{x+} [z + (n-x)H]^{(n-x-1)+} + [c + (x-1)H]^{x+} [z + (n-x+1)H]^{(n-x-1)+}$

ETD mechanism in detail



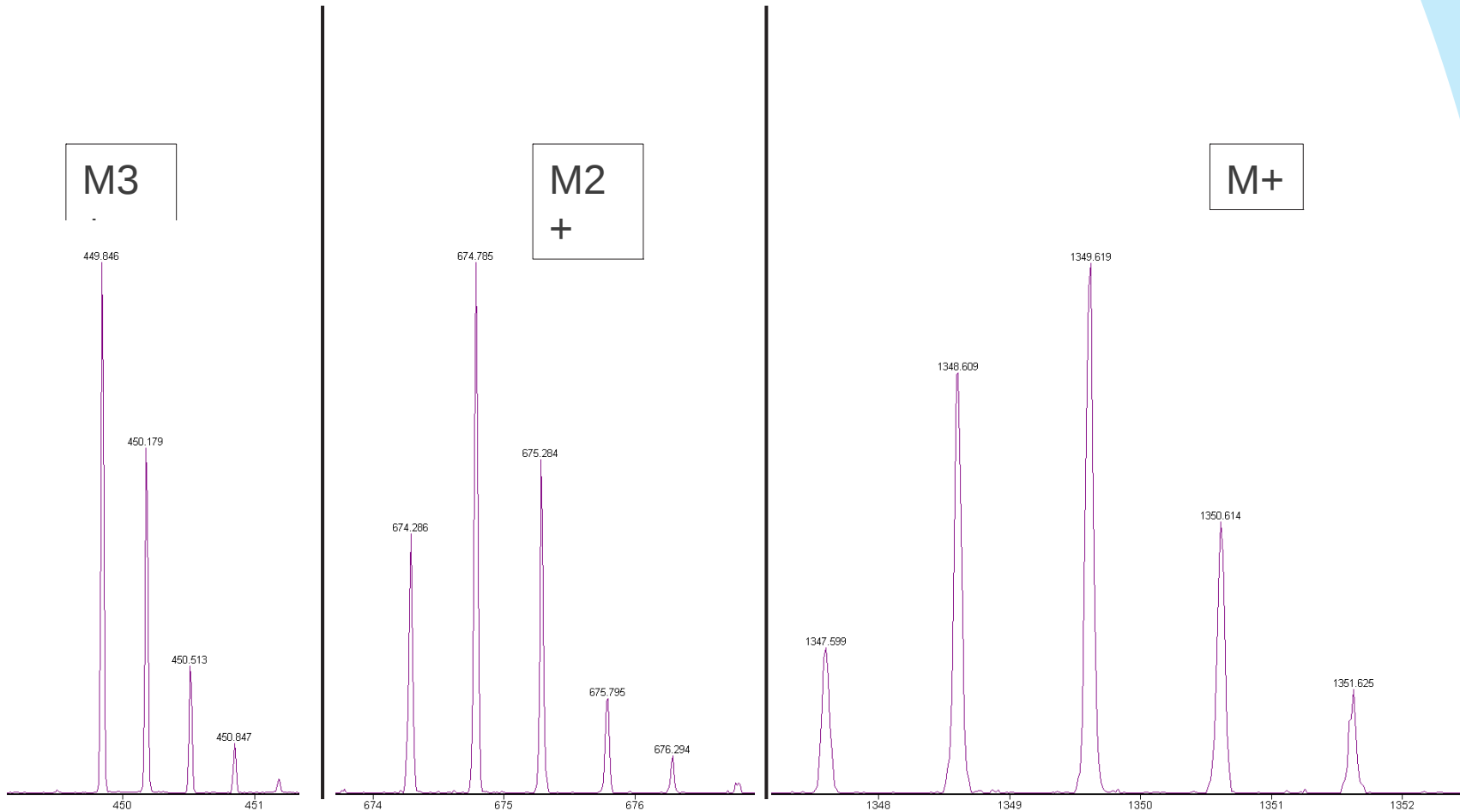
» So, does this occur in practice?

Charge reduction of substance P

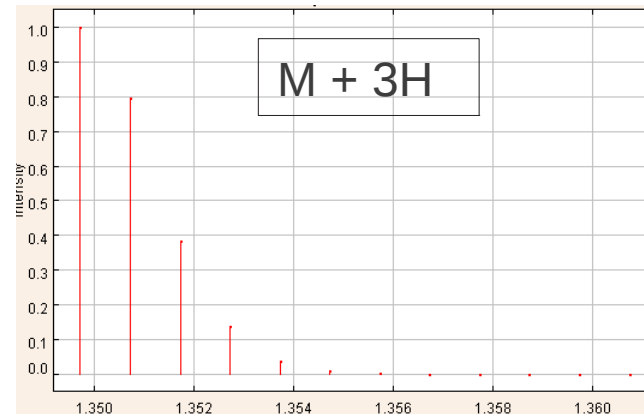


» PTR or ETnoD? → Look at isotopic distribution

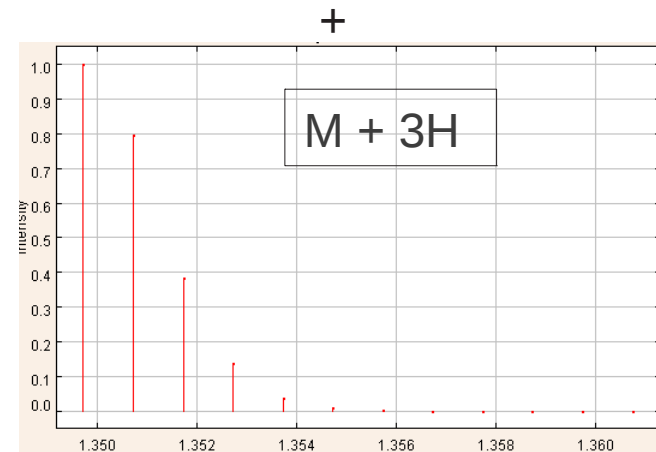
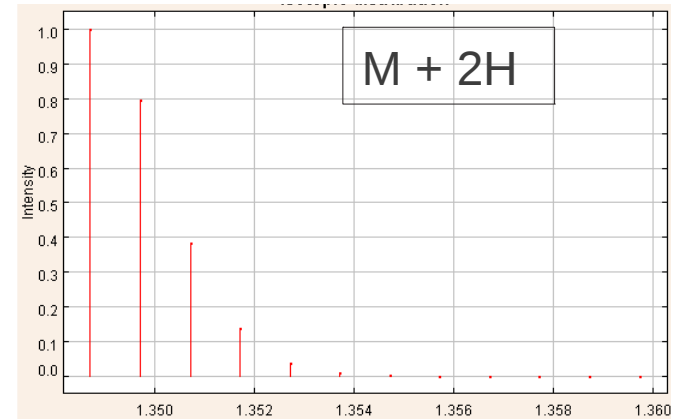
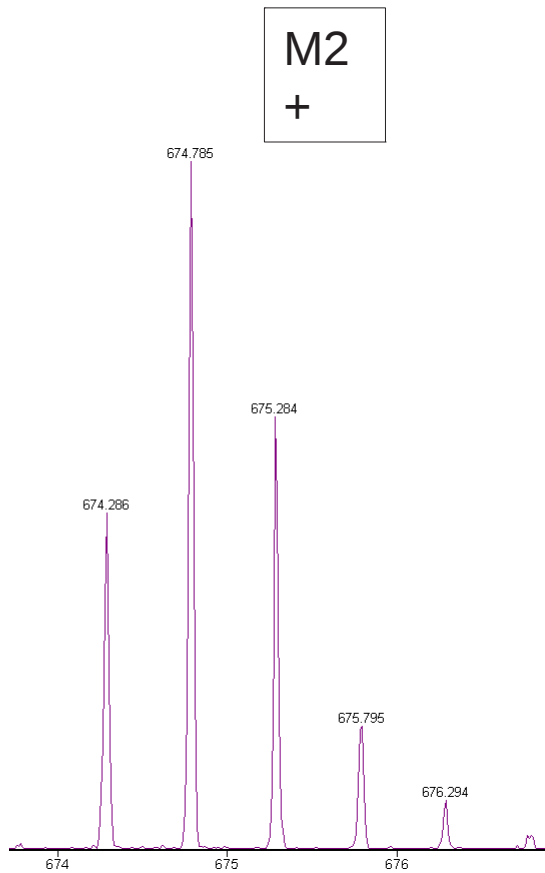
Charge reduction of substance P



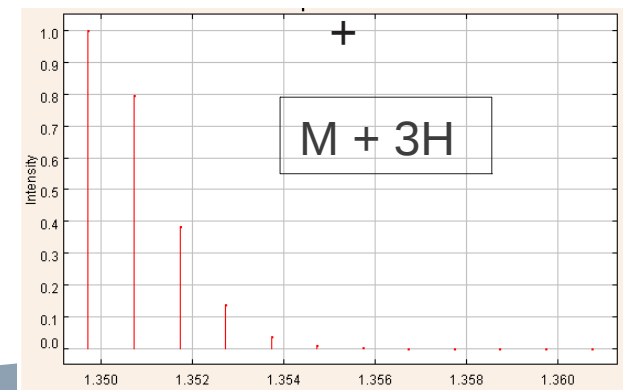
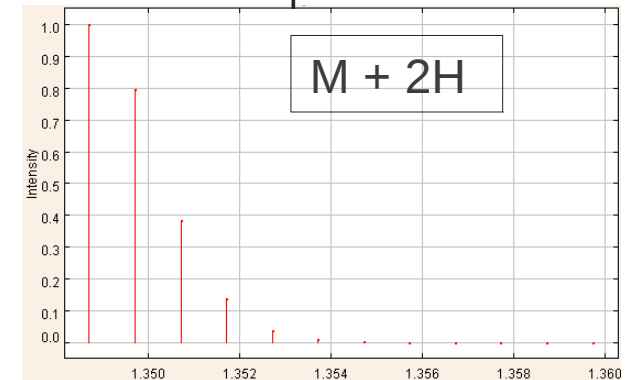
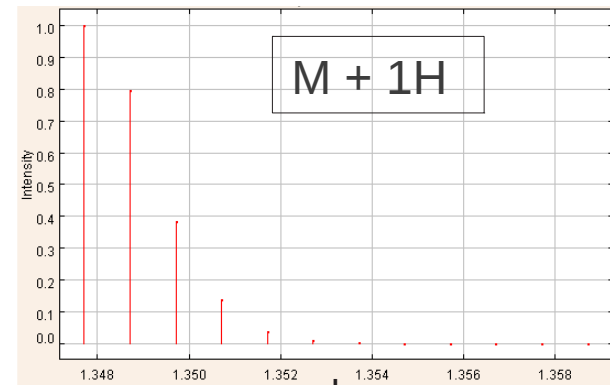
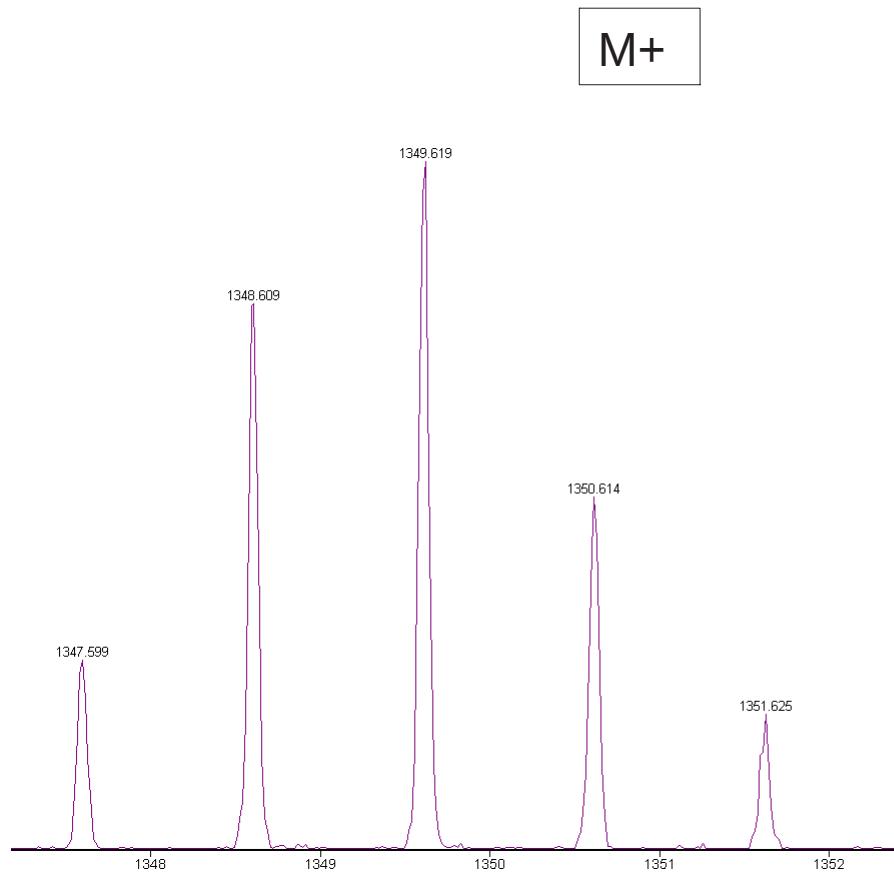
Charge reduction of substance P



Charge reduction of substance P



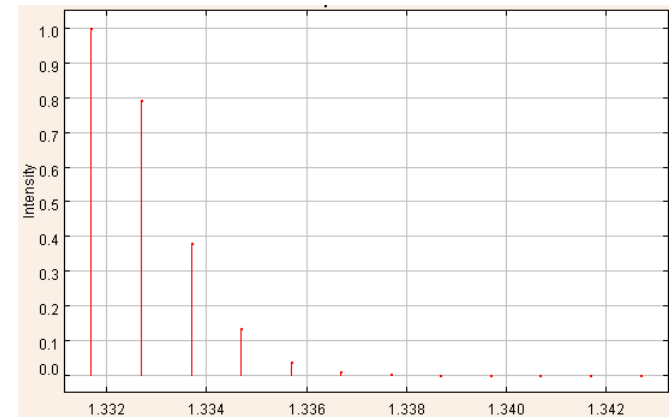
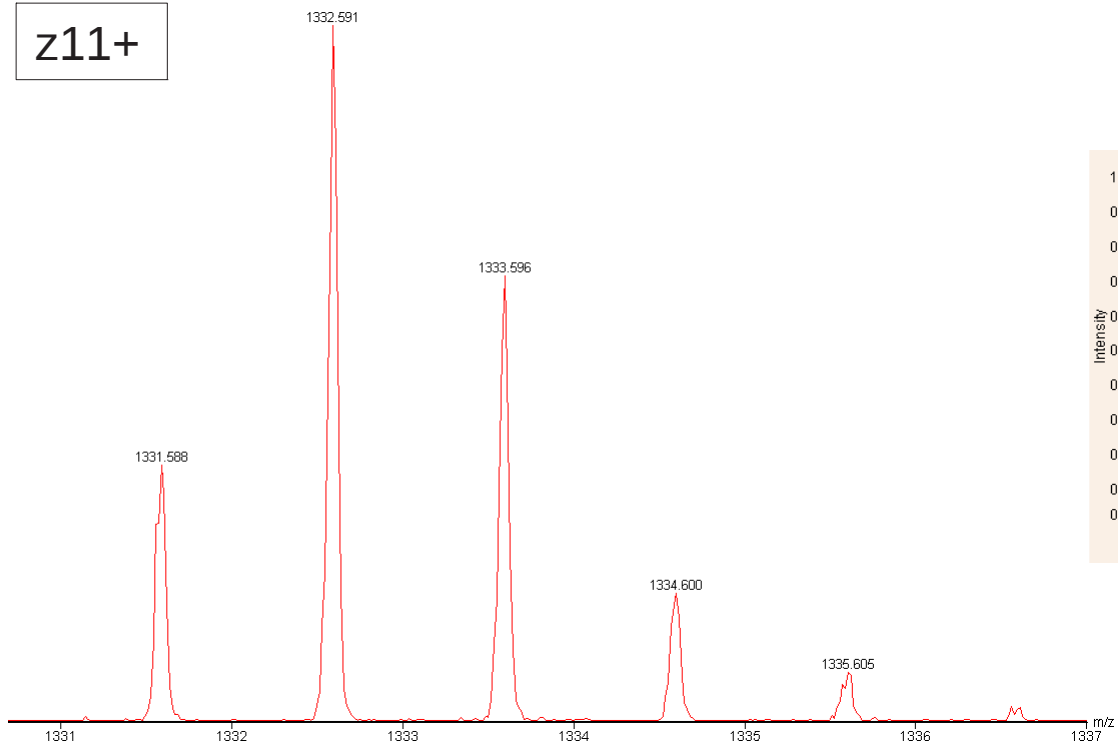
Charge reduction of substance P



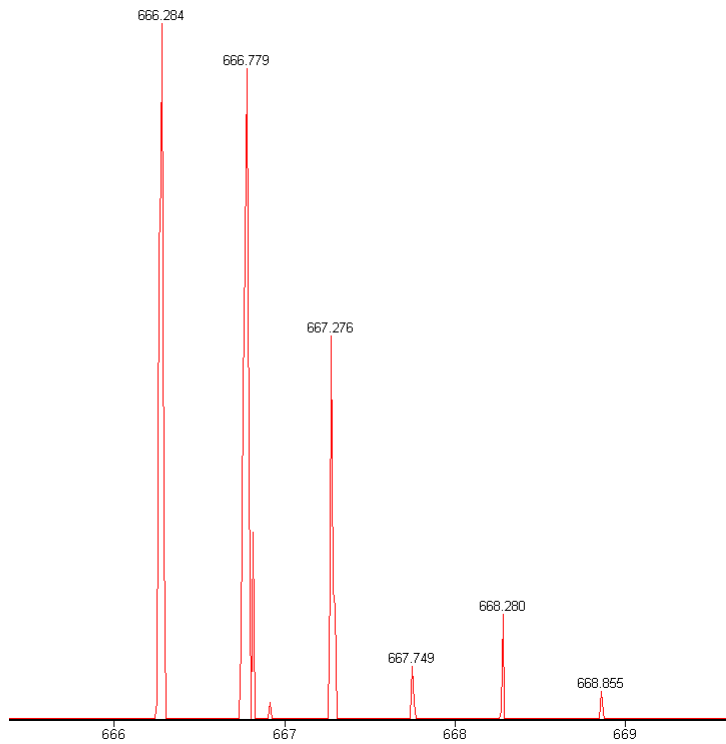
» Both PTR and ETnoD occur!

ETD fragmentation of substance P

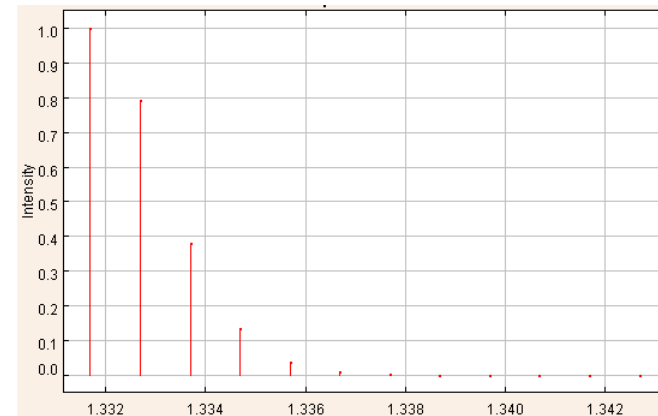
z11+



ETD fragmentation of substance P



z112+

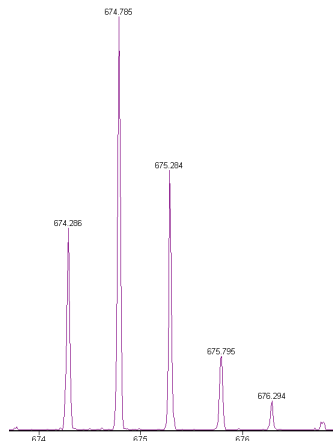


Isotopic distributions in ETD-MS

- » Substance P (1.3 kDa): 3+ precursor used
 - » Maximum of 2 ET steps
- » Ubiquitin (8.6 kDa): 10 – 12+ precursors often used
- » ADH tetramer: (147 kDa): 26+ precursor used
 - » Most observed c fragments heavier than expected
- » ...
- » Can get very complex → bioinformatics required

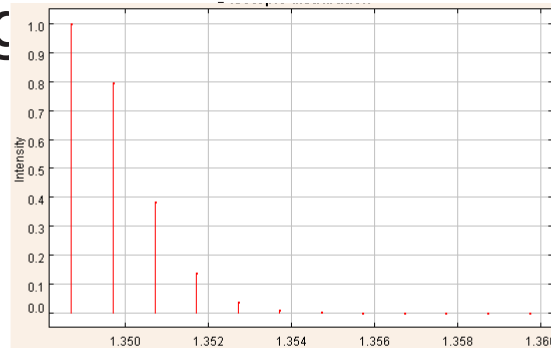
Goals of the project

- » Deconvolute observed isotopic distributions and determine contribution of each component
- » Learn how important each of the possible reactions is and gain insight into the (multistep) ETD process



assigned

$= \alpha$



$+ \beta$

