Mass spectra of peptides and proteins - and LC analysis of proteomes

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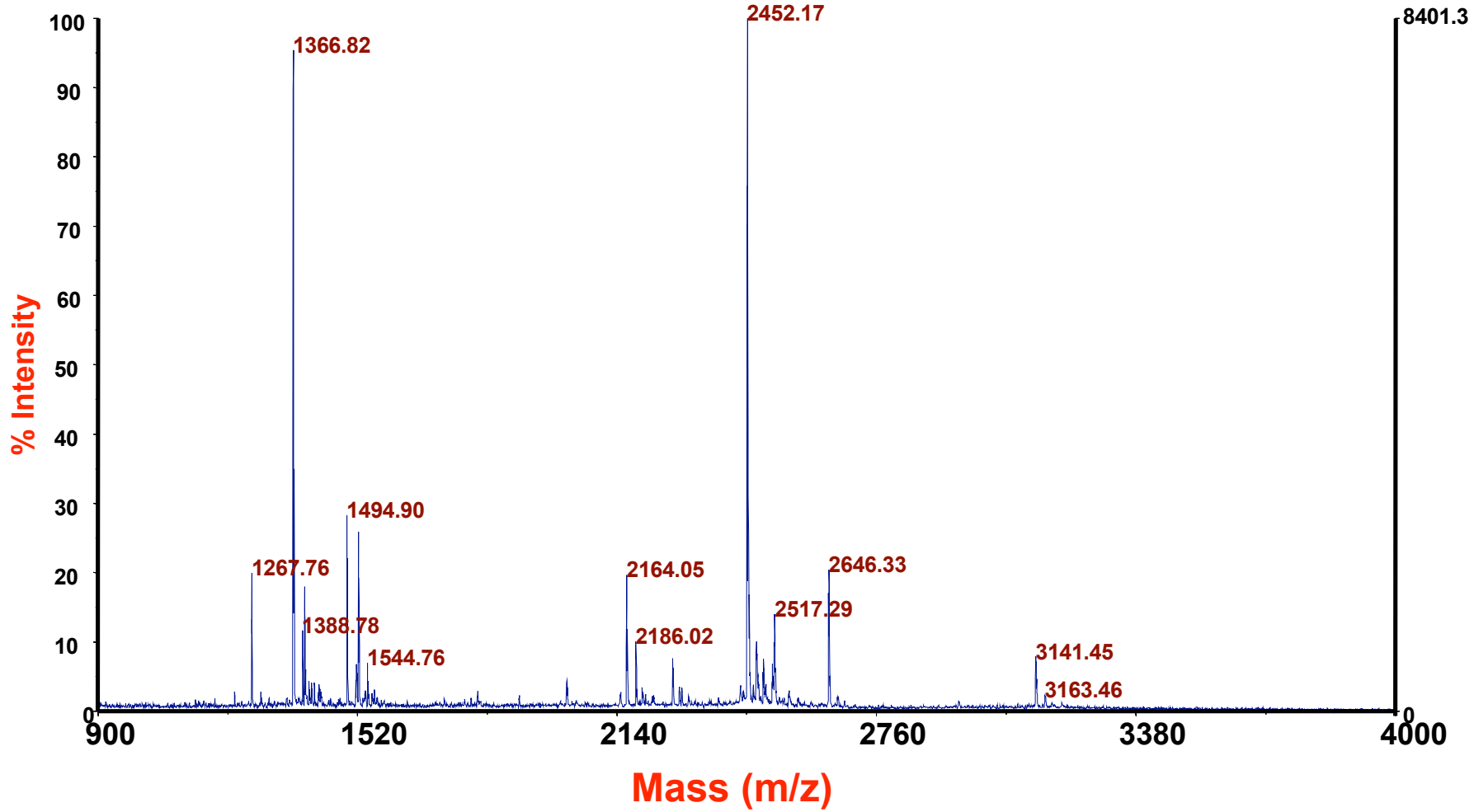
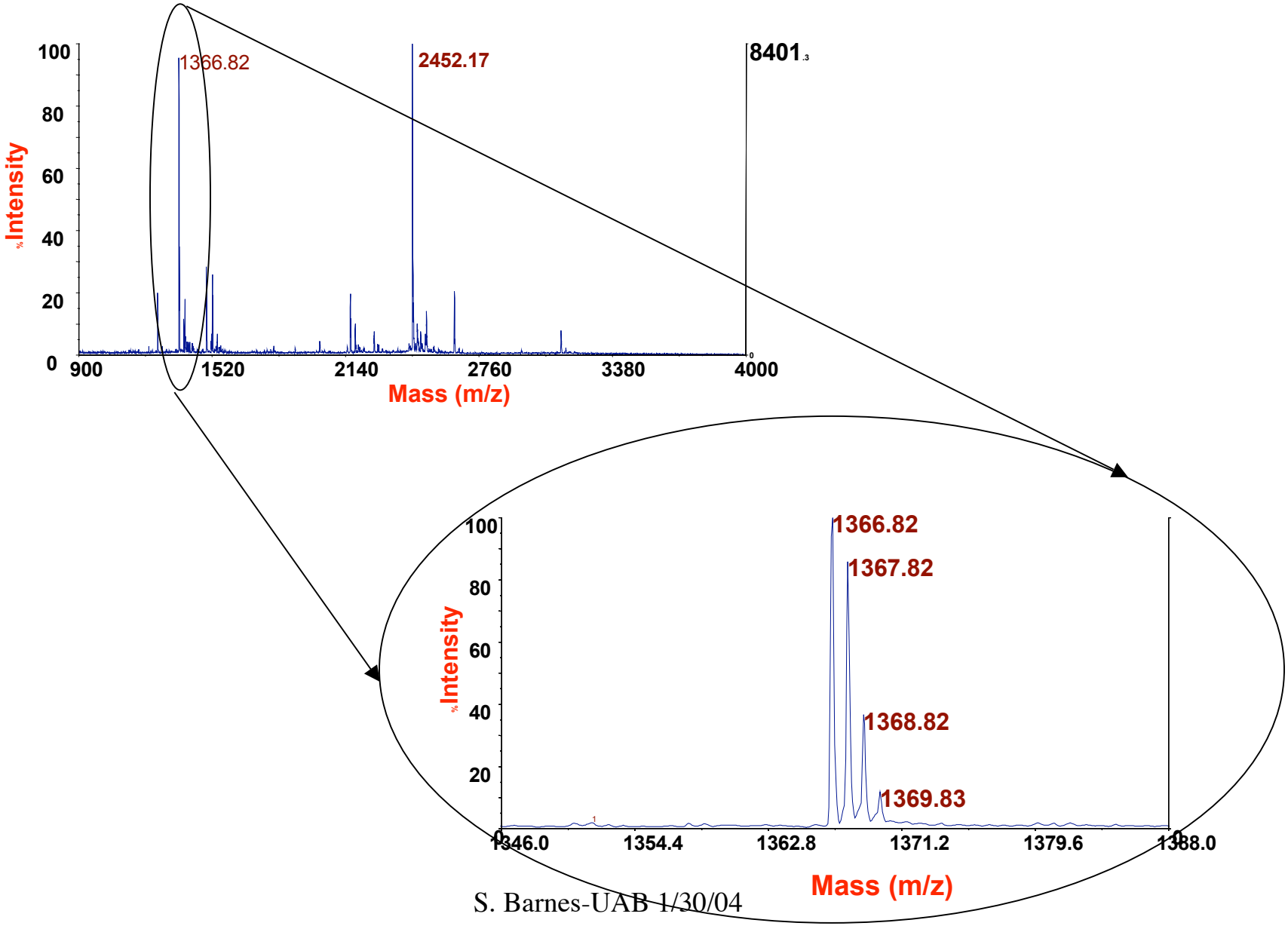
Overview

- A mass spectrum
- Electrospray MS
  - Analysis of intact proteins
  - Molecular weight calculations
  - Max Entropy
- Peptides
  - Purity
- Integration of MS with LC and CE
  - Multidimensional LC
- Tandem MS
  - Identifying modification sites

A mass spectrum of several peptides from a tryptic digest



Isotope profile of individual peptide ion



How to represent the mass of compound?

- **At low mass resolution (where the isotope peaks cannot be resolved) what is observed is the *average mass***

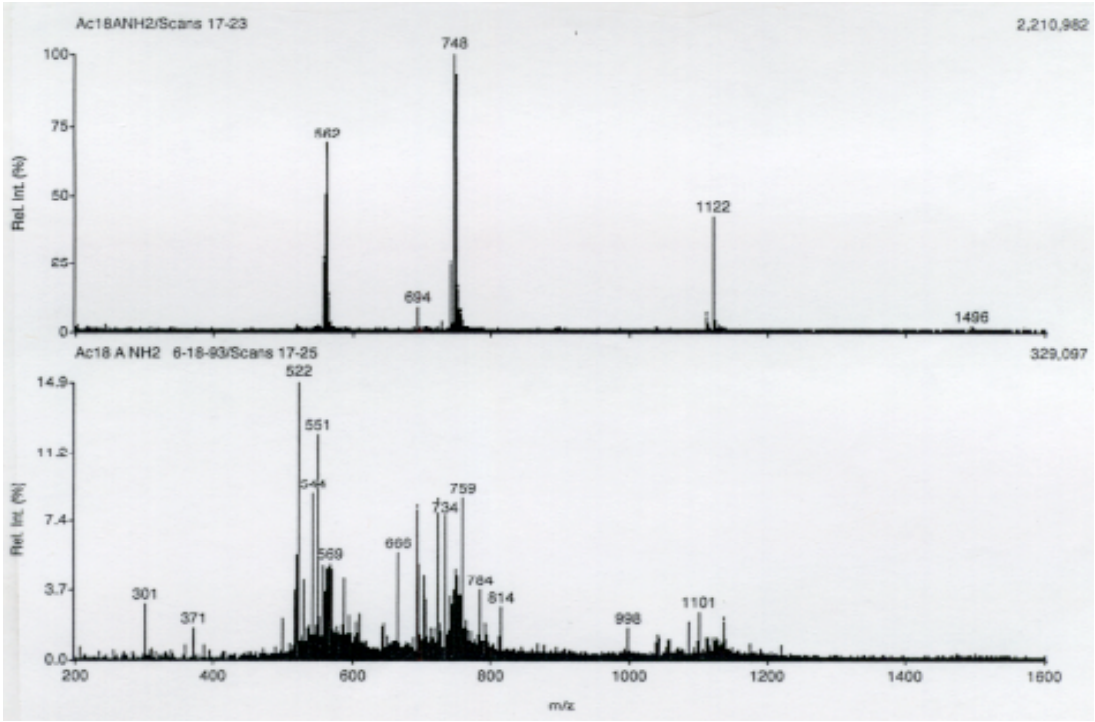
- **At high resolution where the isotopic peaks are fully resolved, then we can determine the *monoisotopic mass* for each one**

Take home question (due Feb 6th)

1. **What is the monoisotopic mass of human cytochrome C?**
   - **Hint: workout the empirical formula of hCyt C**

2. **What is the molecular weight of the most abundant species of human Cyt C?**
   - **Hint: assume that the abundance of ^{13}C is 1.00% of total carbon atoms - do not worry about ^{2}H or other isotopes**

ESI-MS and purity of peptides

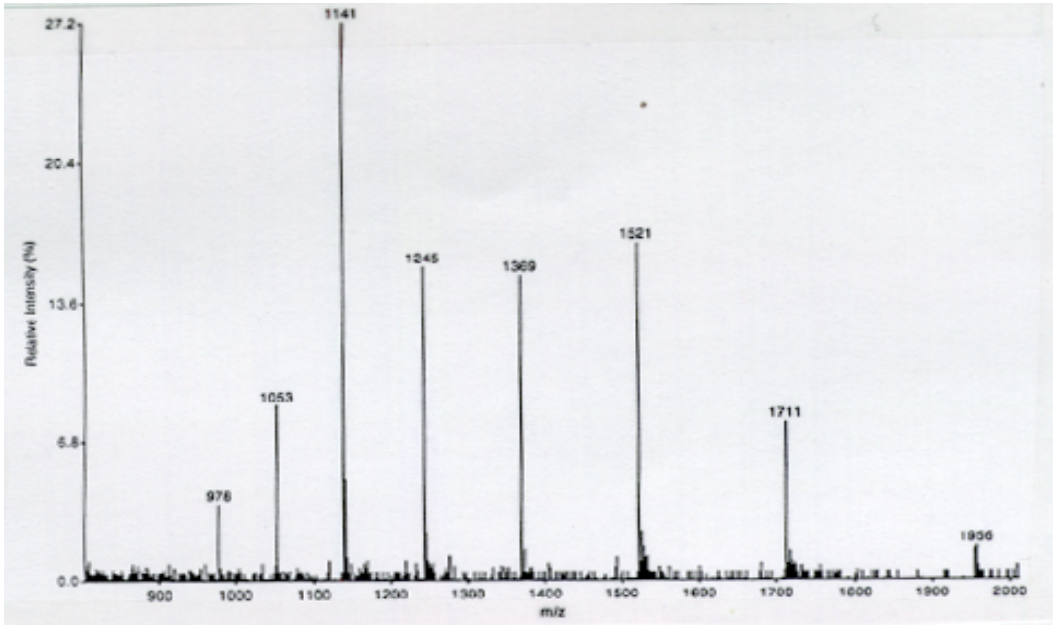


Guarantees of purity based on observation of "a single peak by reverse-phase HPLC" and by "it gave the correct sequence when analyzed by Edman degradation" are hollow.The lower spectrum was of a "pure" HPLC peak. The method of purification was amended and the upper spectrum was obtained

Ionizing proteins and peptides

- $^{+}H_3NCHR_1CO(NHCHR_nCO)_nNHCHR_2COOH$ is the ion that's found in dilute acid solution
- If there are internal basic residues, then the ions will be of the form $[M+nH]^{n+}$, where n = 1, 2, etc.
- A tryptic peptide will have a N-terminal amino group and an amino group from Arg or Lys
  - If the peptide has a mol. wt. of 1000 Da, then the singly charged ion will have a *m/z* of 1001, whereas the doubly charged ion has a *m/z* of 501

ESI mass spectrum of ribonuclease



Cumulative MW estimate = 13,680.29

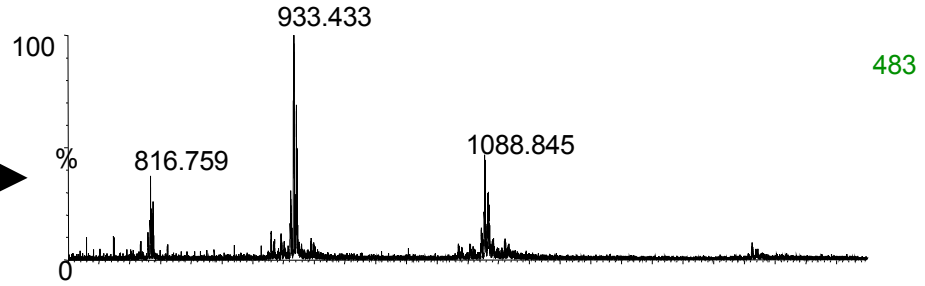
SD = 2.94

Peak (m/z)	Intensity	Charge (est.)	Mol. Wt. (Est.)
978.00	7,778	14.00000	13,677.89
1,053.00	18,532	13.02656	13,675.90
1,141.00	59,087	11.95446	13,679.91
1,245.00	33,275	10.96146	13,683.91
1,369.00	32,390	10.03219	13,679.92
1,521.00	35,668	8.99995	13,679.93
1,711.00	16,624	7.99996	13,679.94
1,956.00	3,333	6.97955	13,684.94

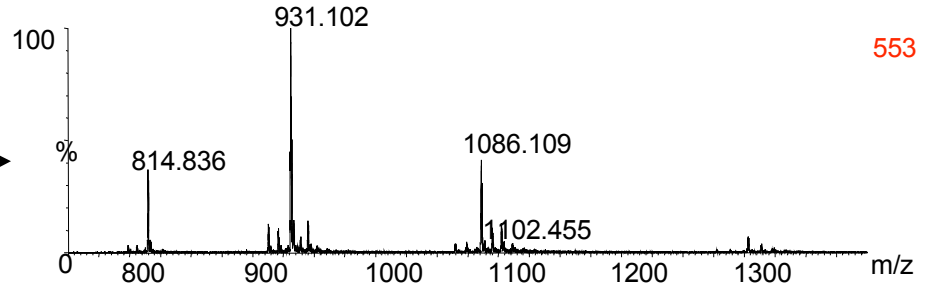
Calculation of molecular weights and ion states

- **For two ions in a series for a peptide of molecular weight M, the lower *m/z* value (x) will be for the n+1 ion state and the larger *m/z* value (y) will be for the n+ ion state.**
  - **(1) $(M+n)/n = y$**
  - **(2) $(M+n+1)/(n+1) = x$**
- **Hence**
  - **(3) $M+n = ny$ and $M = ny-n$**
  - **(4) $M+n+1 = (n+1)x$ and $M = (n+1)x-(n+1)$**
- **Hence**
  - **$ny-n = (n+1)x - (n+1)$**
  - **$ny-n-xn+n = x-1$**
  - **$n(y-x) = x-1$**
  - **$n = (x-1)/(y-x)$**
- **The value of n can then be substituted in equation (1) to obtain the molecular weight of the peptide**

Oxidized Aprotinin ESI mass spectrum



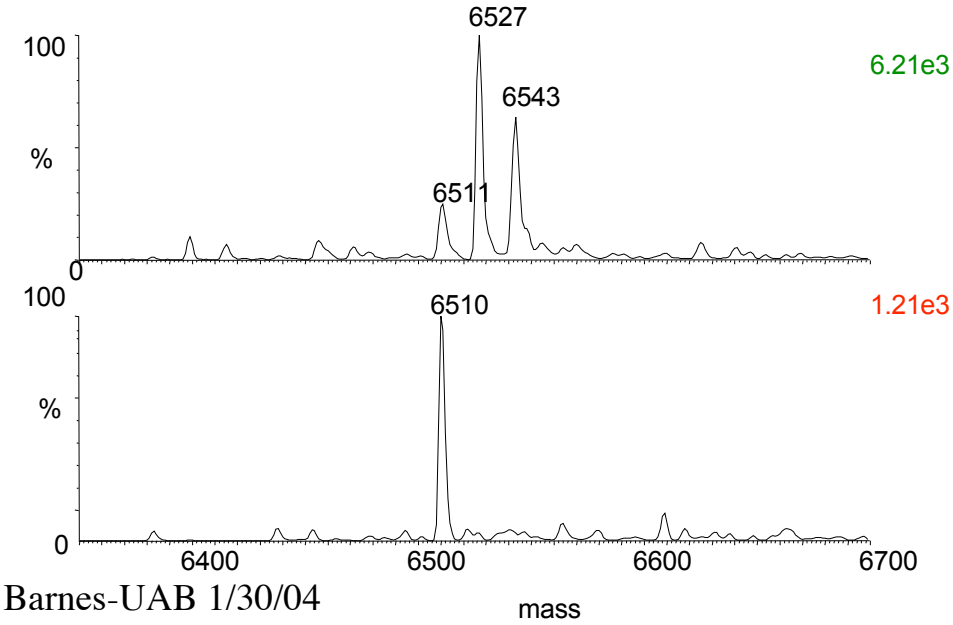
Control Aprotinin ESI mass spectrum



Deconvoluted mass spectra

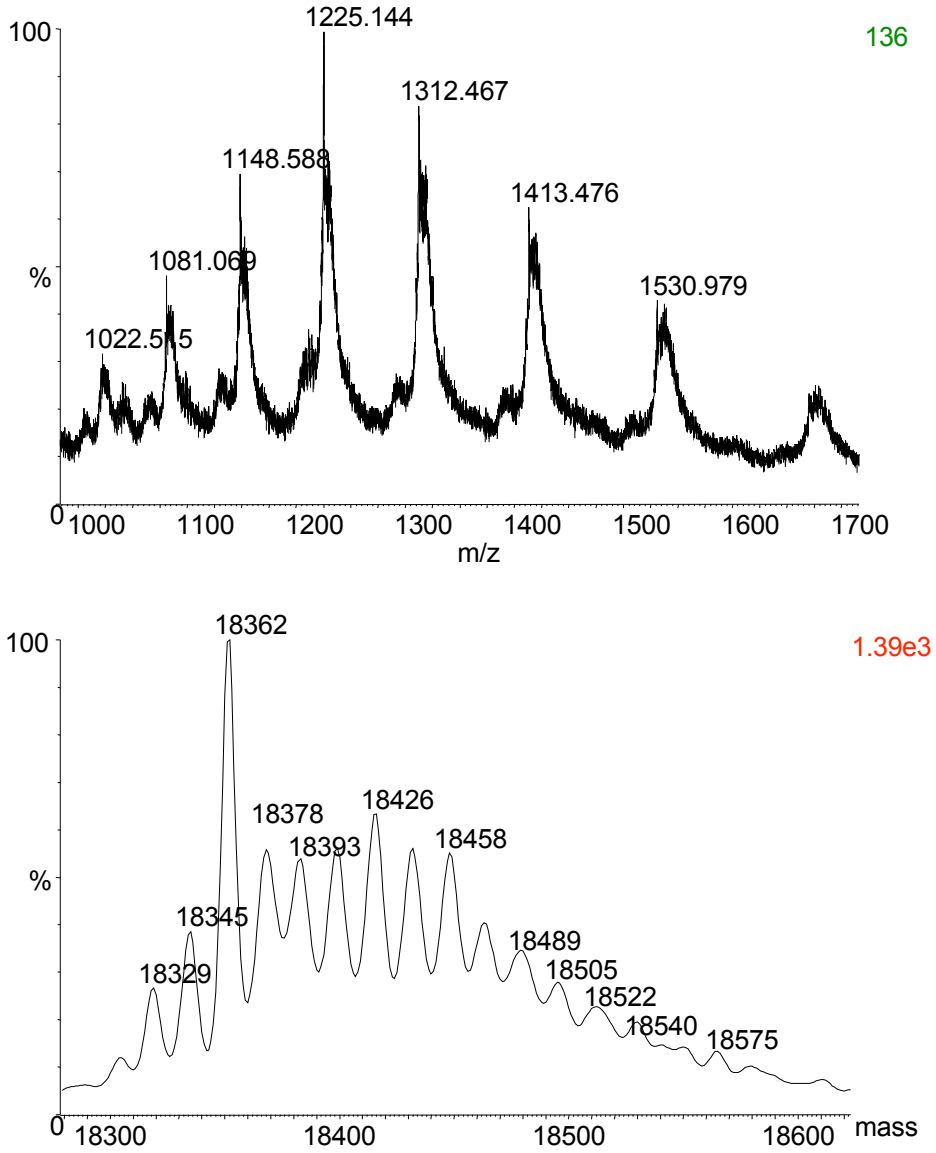
Oxidized

Control



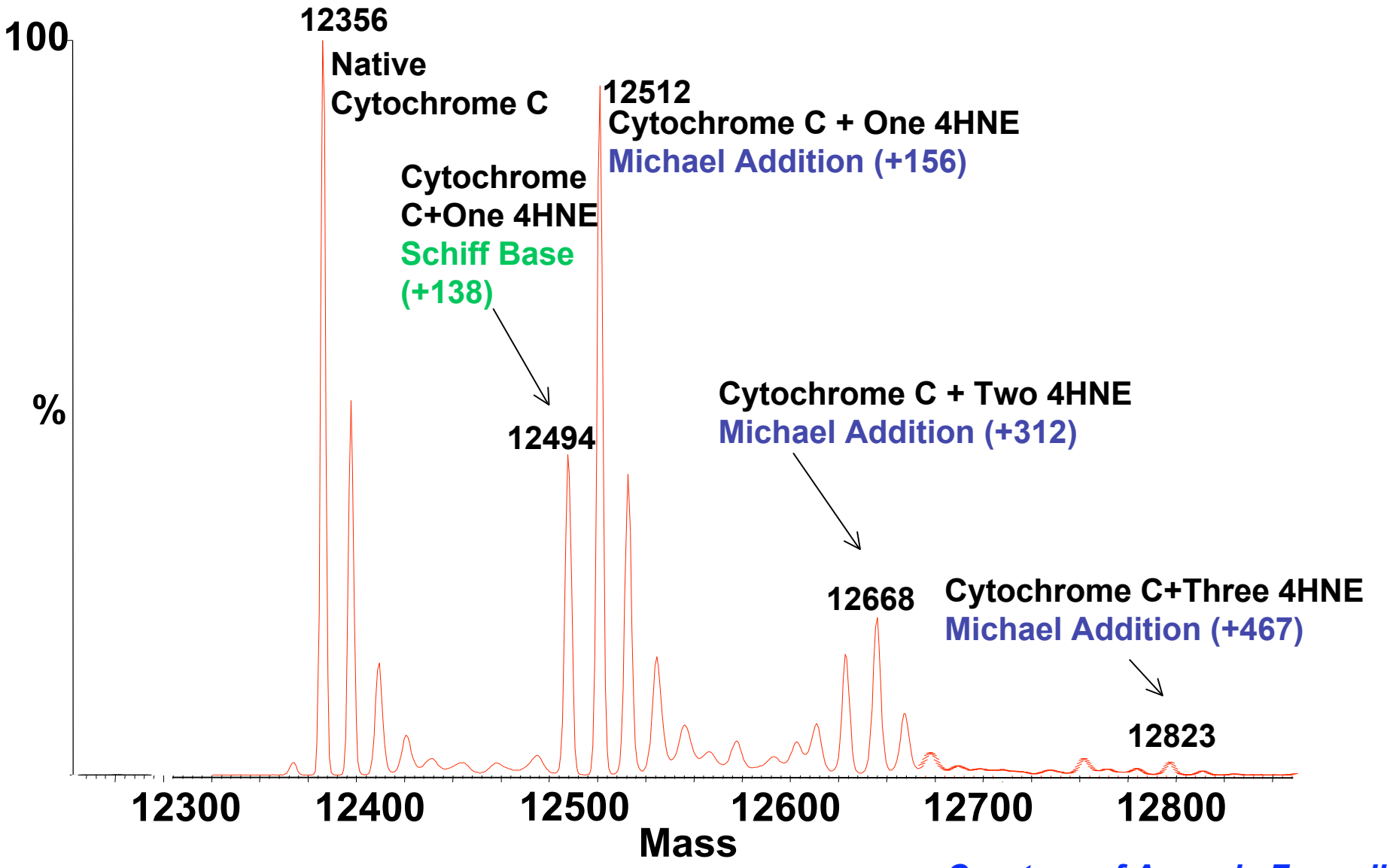
Junlong Shao

Deconvolution of oxidized forms of β-lactoglobulin



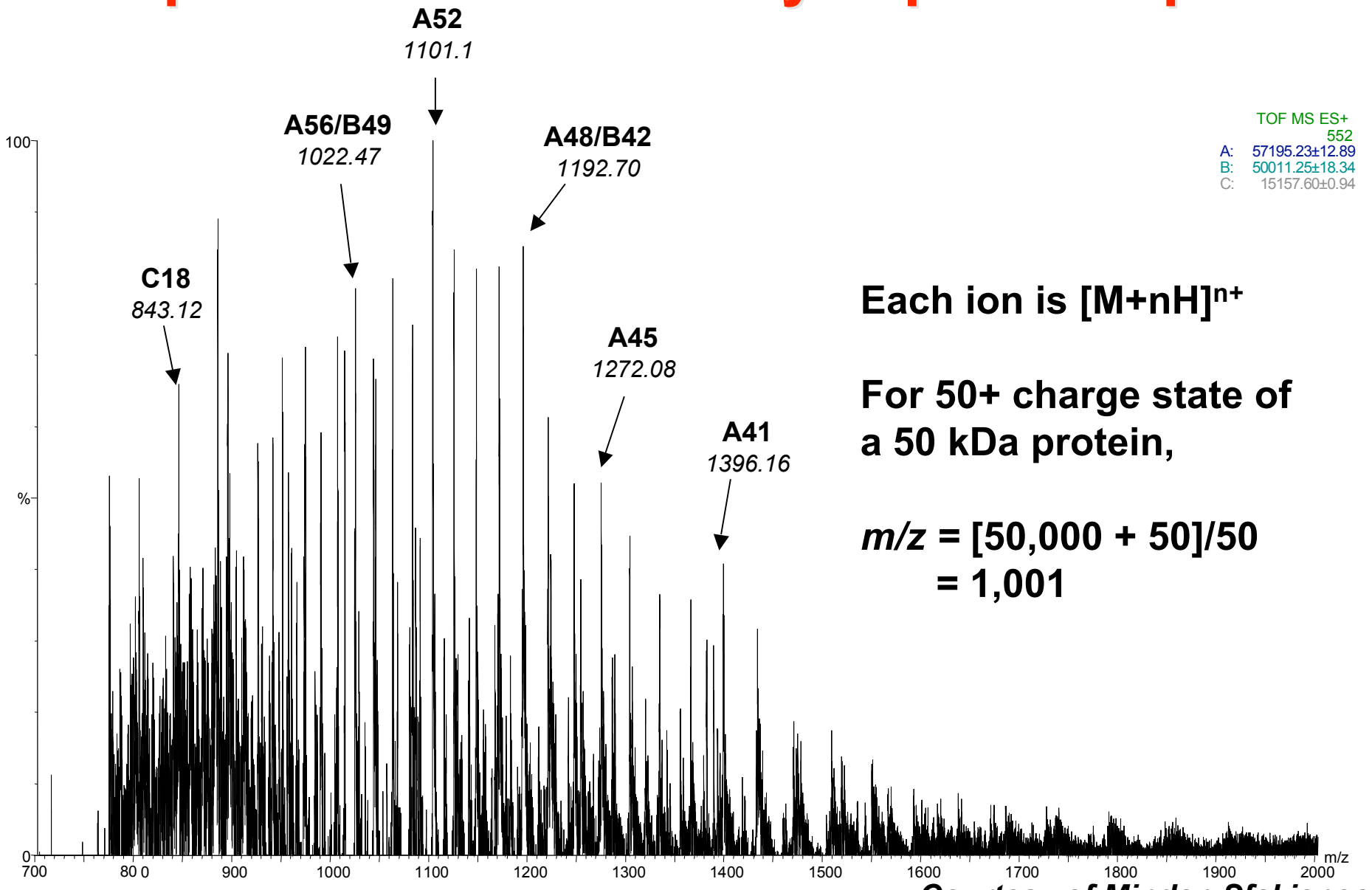
Junlong Shao

LC/MS of 4HNE-Modified Cytochrome C



Courtesy of Amanda Foxwell

ESI spectrum of bacterially expressed protein



Each ion is $[M+nH]^{n+}$

For 50+ charge state of a 50 kDa protein,

***m/z* = [50,000 + 50]/50**
= 1,001

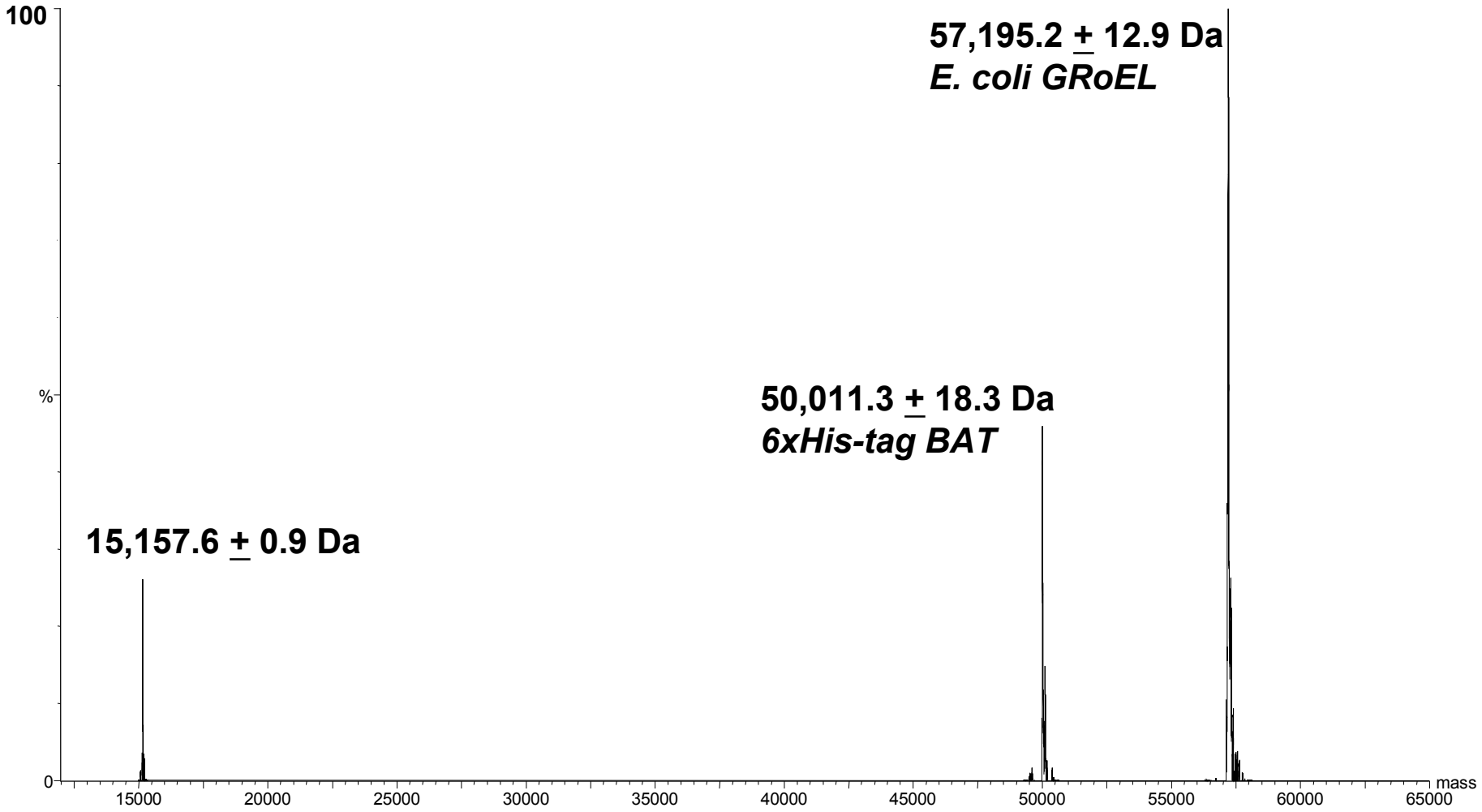
Courtesy of Mindan Sfakianos

Deconvolution of MS data

- When several proteins are present, then their multiply charged ion clusters overlap

- Can this be overcome? - yes, use the MaxEntropy program provided by Micromass

MaxEnt deconvolution of MWs



Courtesy of Mindan Sfakianos

Summary of determining MW by ESI

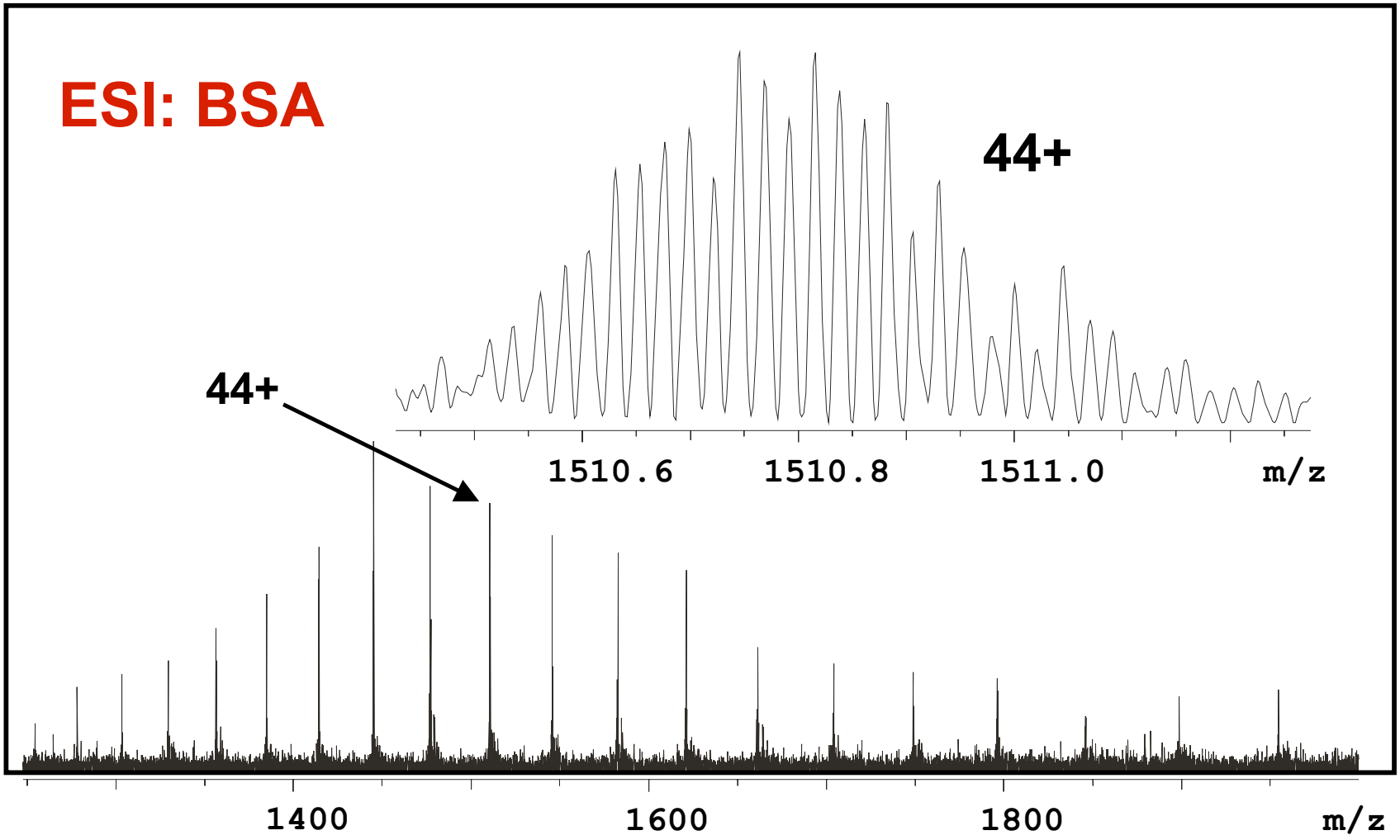
- **The multiple charge states of a protein allow:**
  - **Mol Wt of large proteins to be estimated**
  - **accurate estimation of mol wt (super SDS-PAGE gel)**
- **Important to remember that the protein sample must be free of salt**
  - **Typically, a sample is cleaned up on a short reverse-phase column prior to electrospray**
  - **Alternative, use ammonium acetate as buffer**

Use of FT-MS in ESI of proteins

- **The very high resolving power of FT-MS enables a direct measure of charge state of an individual ion since each peptide or polypeptide will have several/many isotope peaks**

- **The distance in Da between successive isotope peaks of a multiply charged ion is the reciprocal of the number of charges**

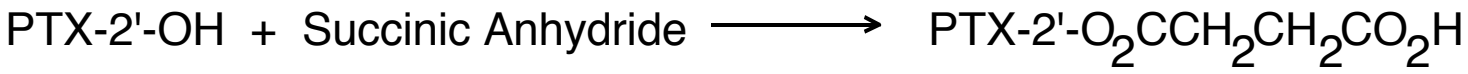
Bovine Serum Albumin (66 kDa)
4.7 T Actively Shielded Magnet



Bruker Daltonics

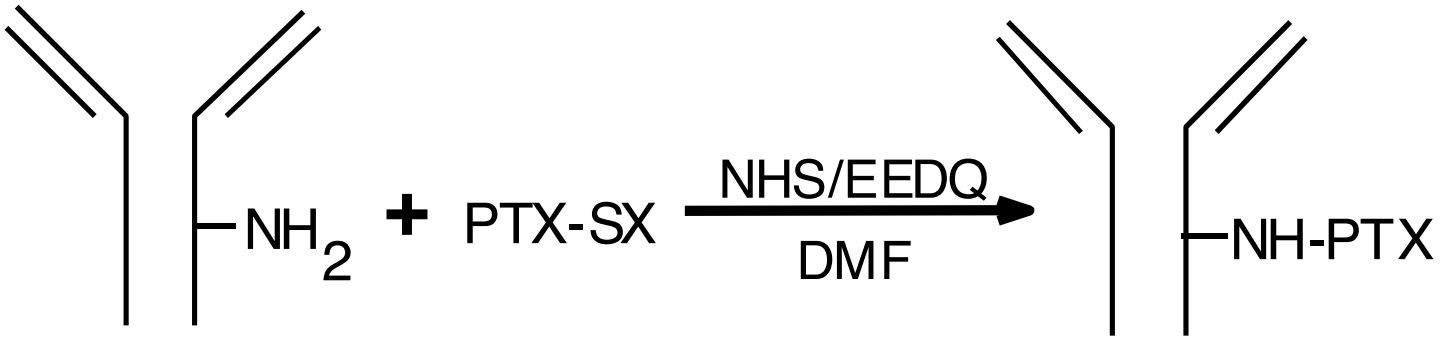
Chemically modifying an antibody

Scheme 1



PTX-SX

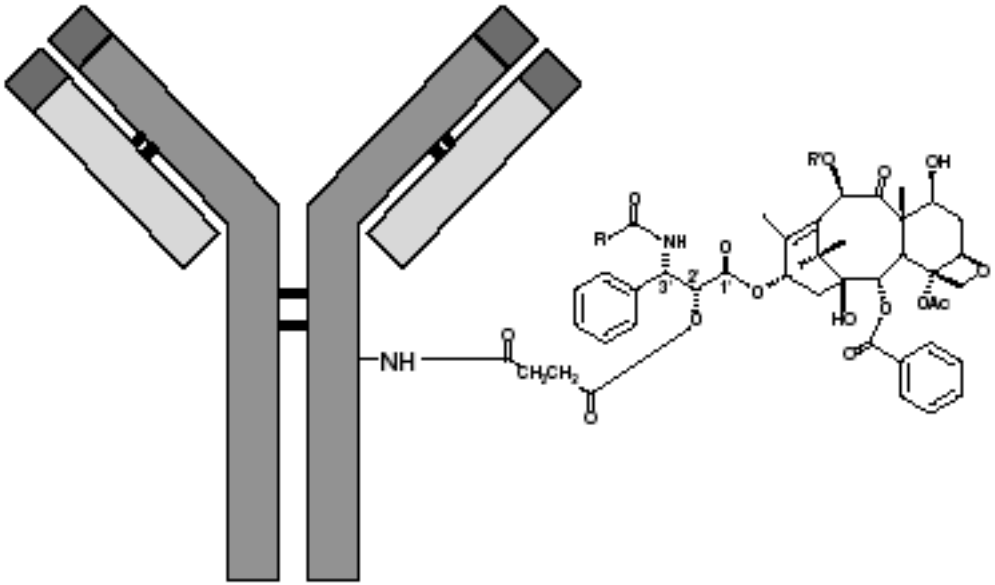
Scheme 2



MAb **PTXMAb**

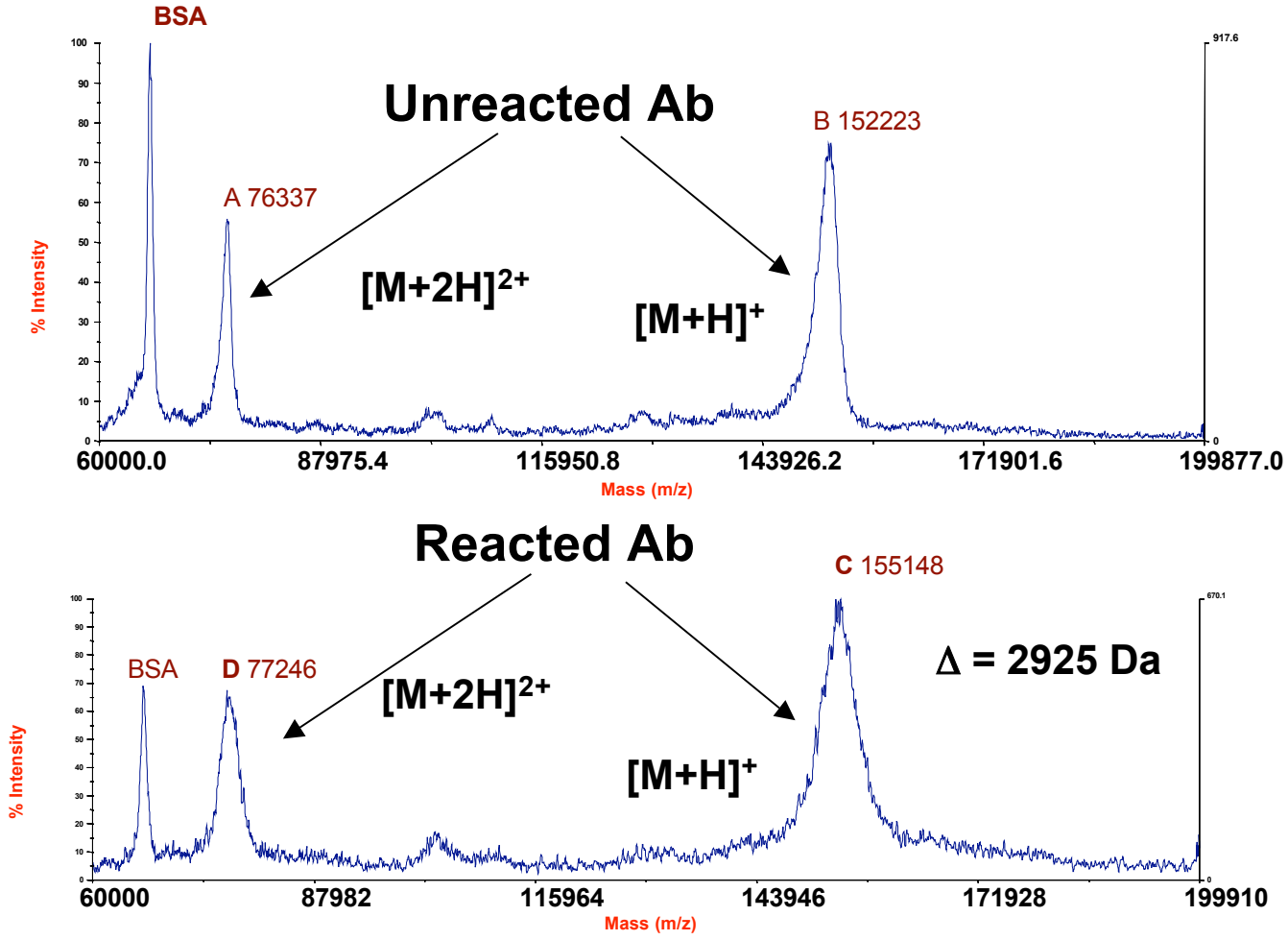
Ahmad Safavy

Structure of modified antibody



Ahmad Safavy

Modification of an antibody by MALDI-TOF

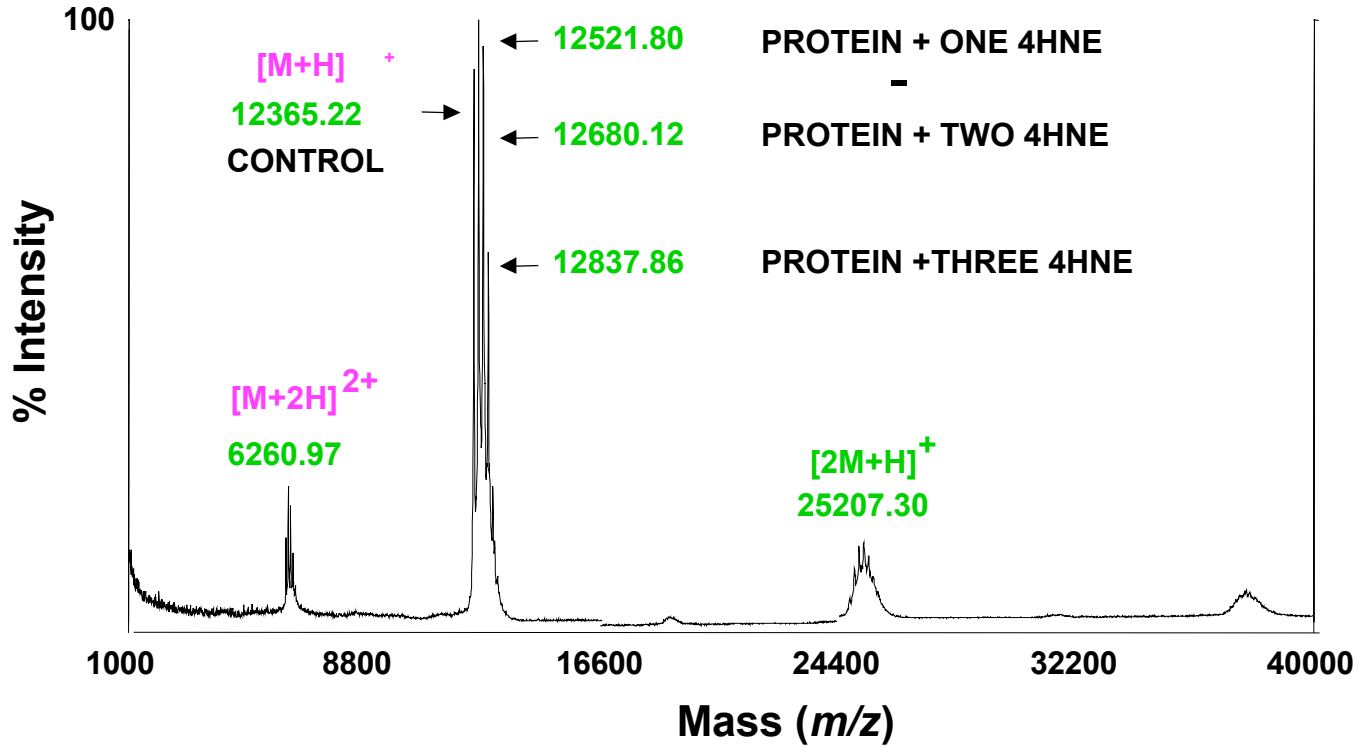


Ahmad Safavy

Example of MS to detect a amino acid modification

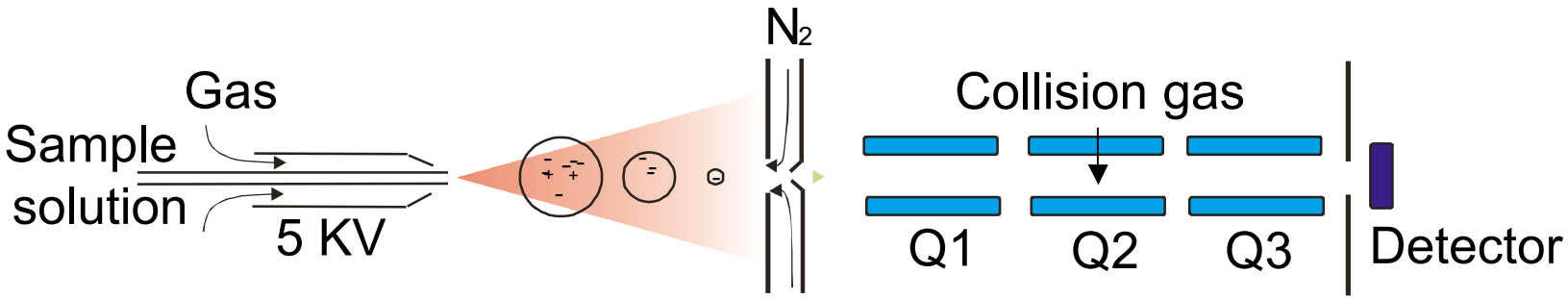
- Cytochrome c, a mitochondrial enzyme, was reacted with 4-hydroxynonenal, an aldehyde formed by oxidation of long chain, unsaturated fatty acids
- Site of attachment believed to be on lysine groups (to form a Schiff's base)
- However, increase in MW consistent with Michael addition
- Protein hydrolyzed with trypsin

Cytochrome C Modified by HNE
MALDI-TOF Mass Spectrum

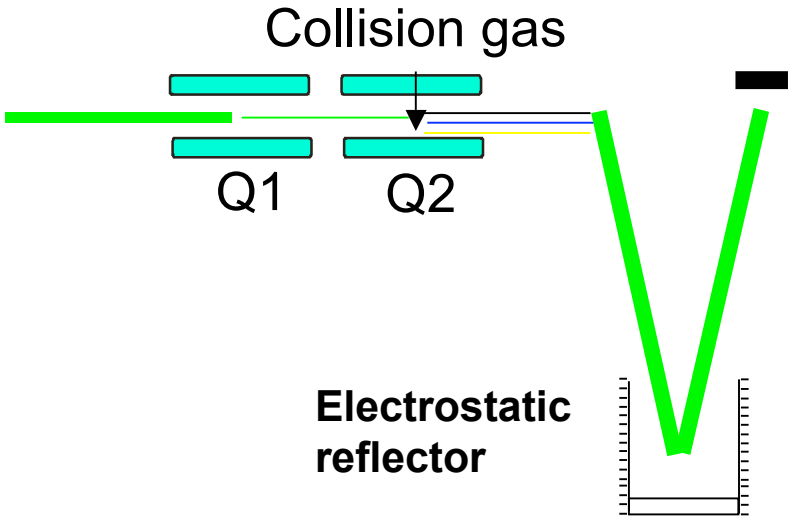


MALDI spectra usually contain only the **molecular ion $[M+H]^+$**. This is an advantage since it maximizes the signal, but is a disadvantage in that it gives no clues as to structure.

Triple quad versus Q-tof and sensitivity



The quadrupole analyzer (Q3) is slow and insensitive - it's a filter - thus throws away large amounts of data

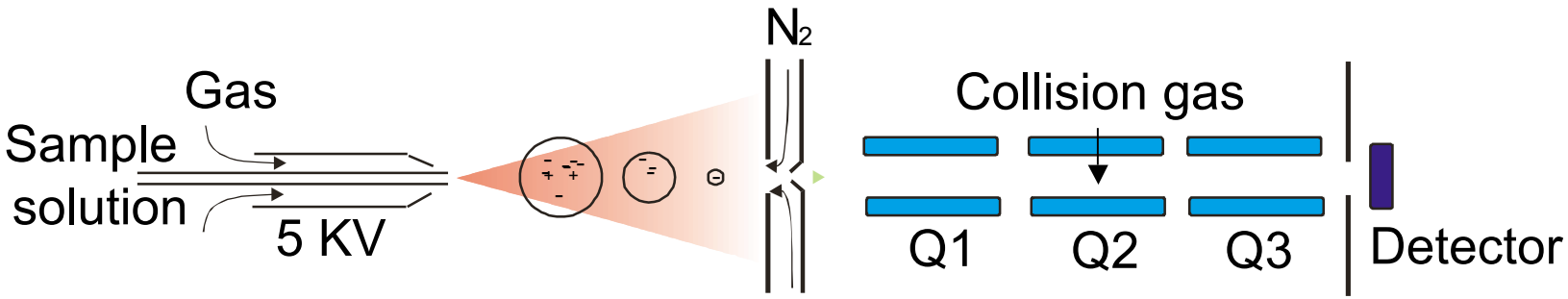


TOF detector collects all ions generated and yields fmol rather than pmol sensitivity

Also gives far greater mass accuracy - from 1000 ppm on the triple quad to <20 ppm on the Q-tof

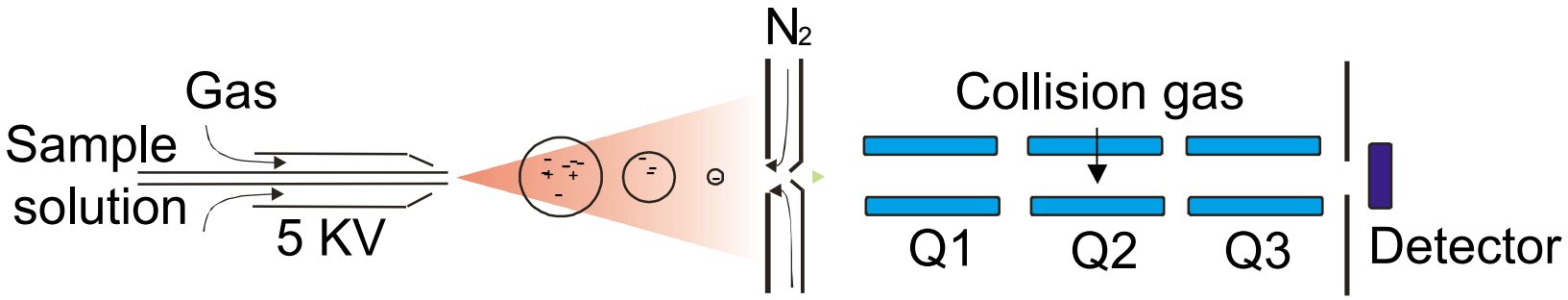
Crucially important for automated interpretation of MS-MS spectra to yield amino acid sequence

Tandem mass spectrometry on a triple quadrupole instrument



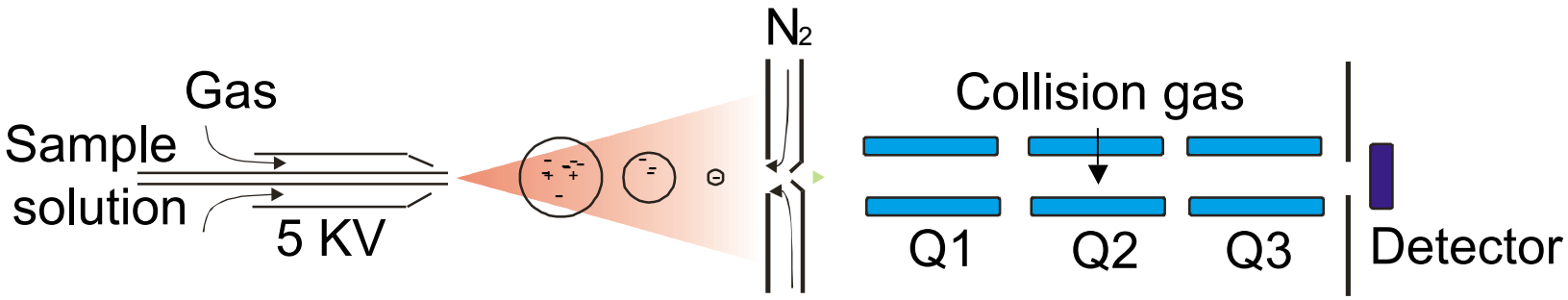
- **Daughter ion spectra**
- **Parent ion spectra**
- **Multiple reaction ion scanning**

Daughter ion tandem MS



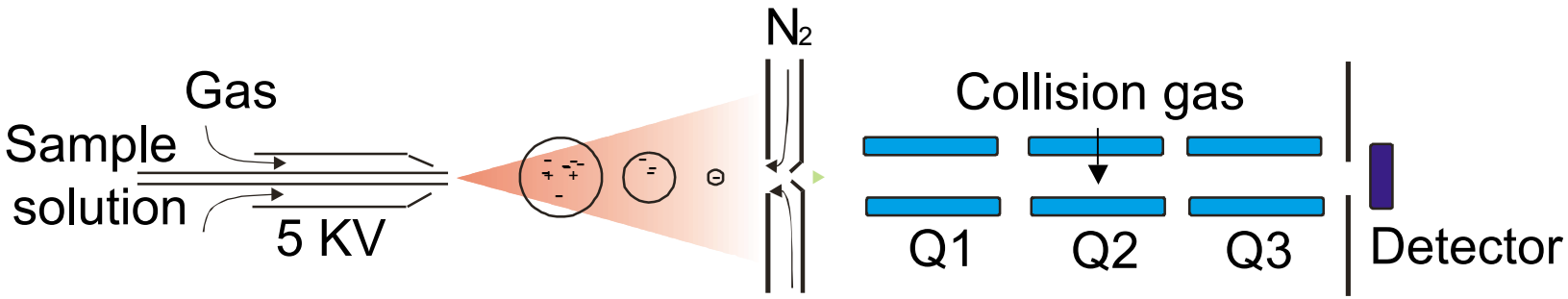
The molecular ion is selected in Q1, collided with Argon gas in Q2, and "daughters" analyzed in Q3

Parent ion tandem MS



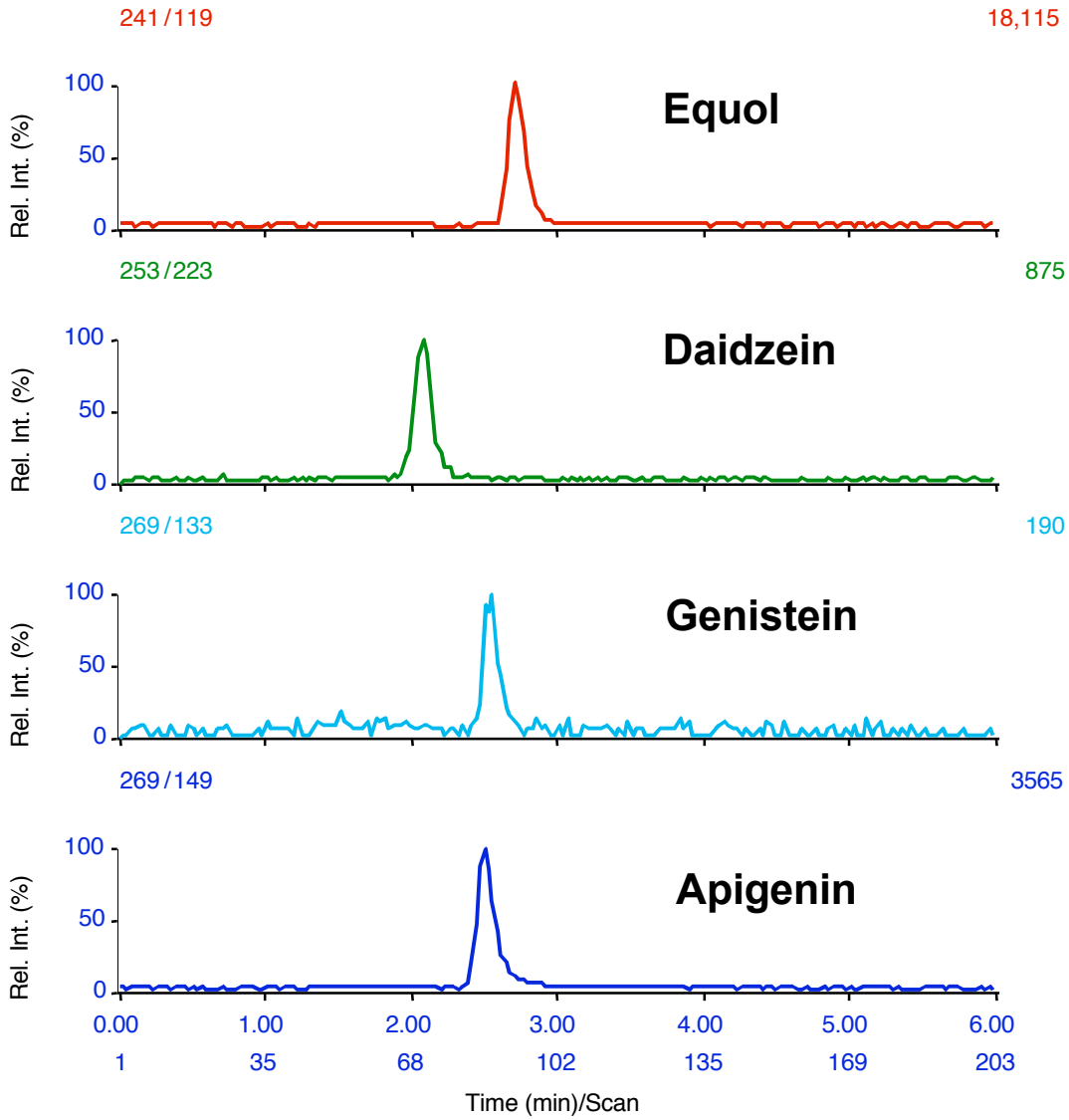
All molecular ions allowed into Q1, collided in Q2, and a selected daughter ion measured in Q3. This could be an O-GlcNAc fragment (*m/z* 204). The parent peptide ions containing an O-GlcNAc will be revealed. Very difficult to do this experiment on a Q-tof.

Multiple reaction ion monitoring (MRM)



A single molecular ion selected in Q1, collided in Q2 and a selected daughter ion measured in Q3. Up to 8 pairs of parent/daughter ions can be measured. This is a quantitative analysis. Can't be done on a Q-tof.

MRM analysis of isoflavones in dog plasma

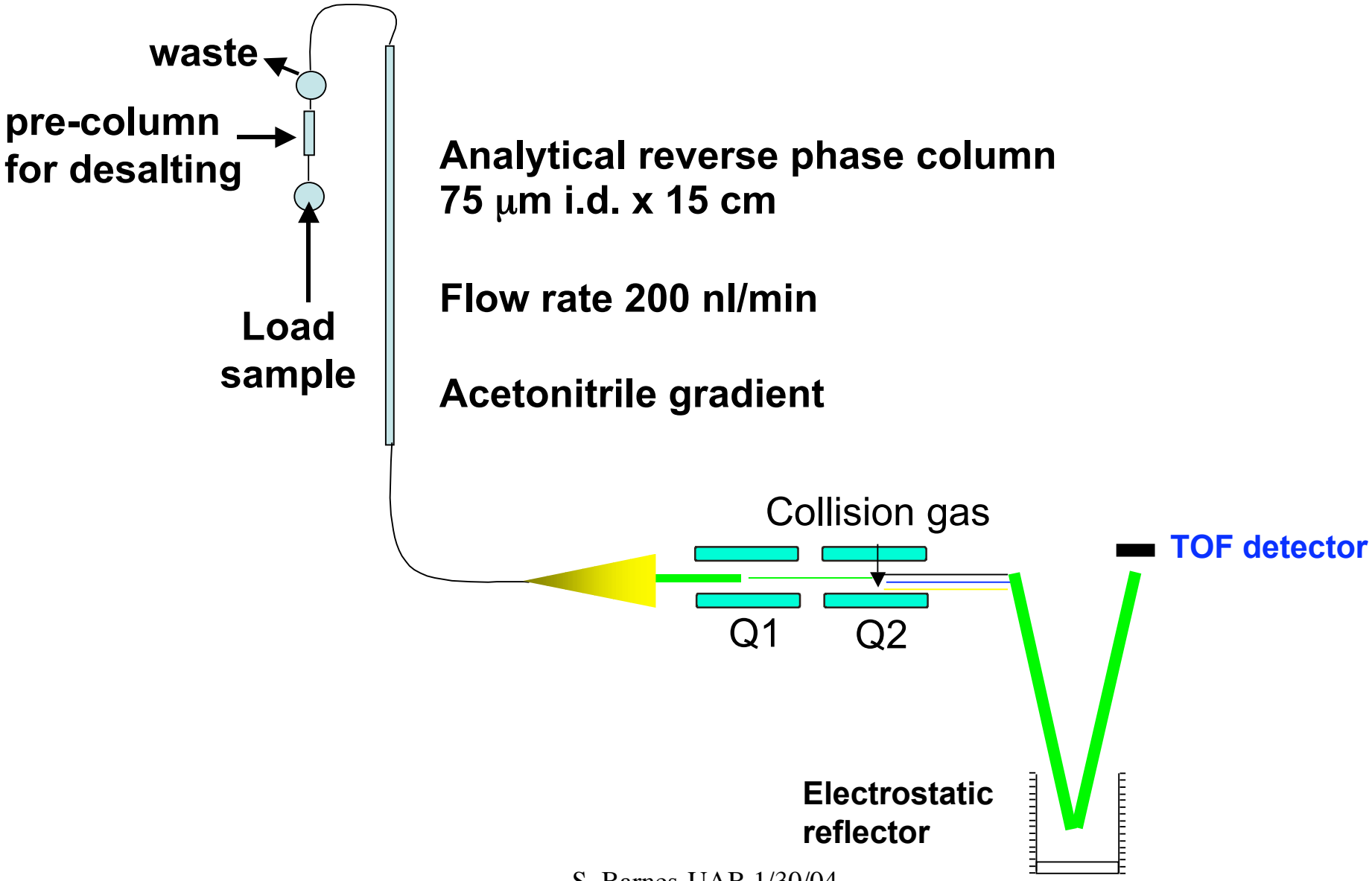


The combination of parent ion and daughter ion allows specific detection of each of these isoflavonoids, even when they are not chromatographically separable

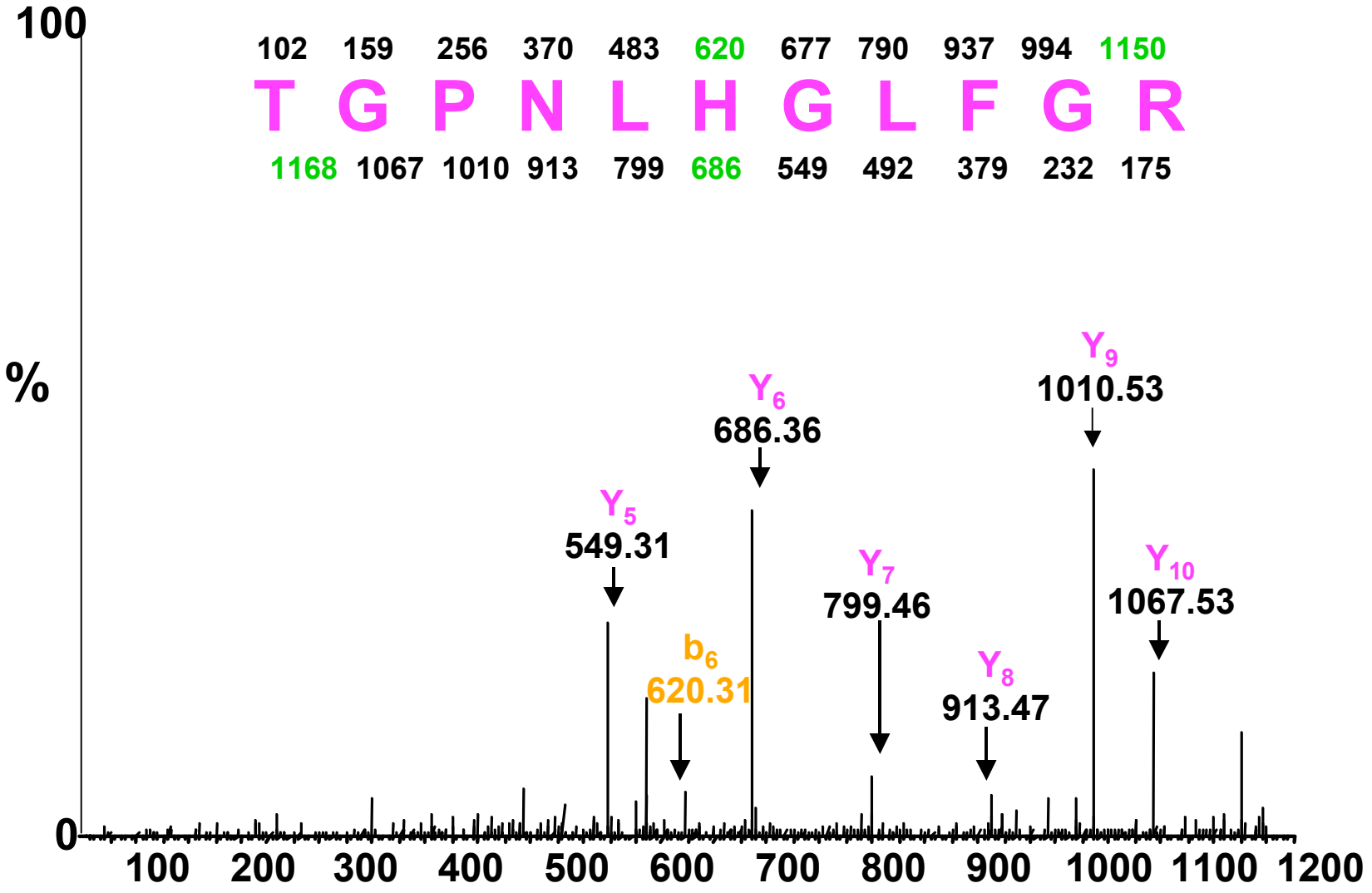
The figure in the right hand corner is the full scale intensity for each channel

Apigenin is an internal standard and is an isomer of genistein

LC-MS of peptide mixtures

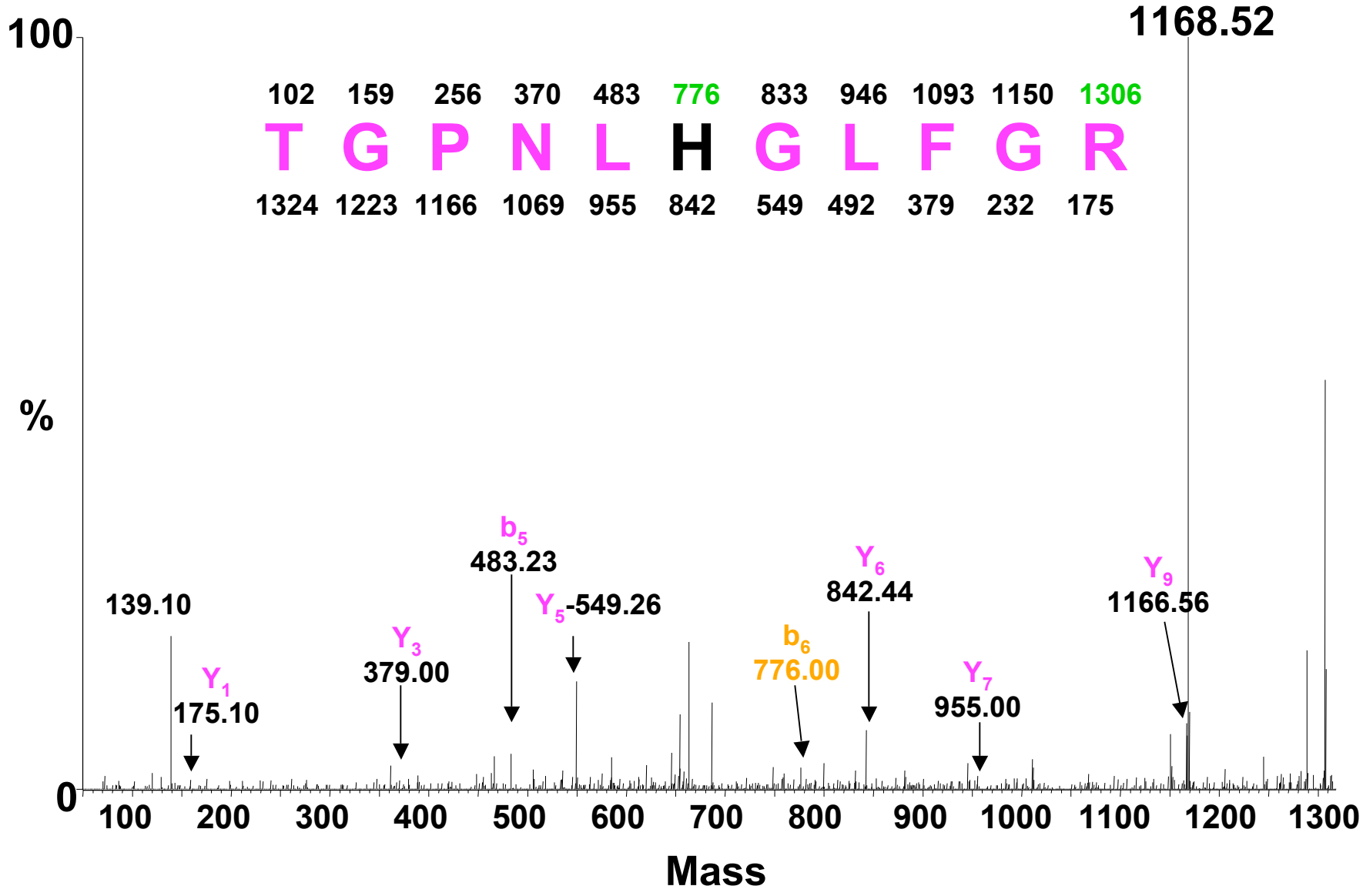


Qtof Tryptic Digest of Control Peptide



Amanda Foxwell

Qtof Tryptic Digest of Modified Peptide



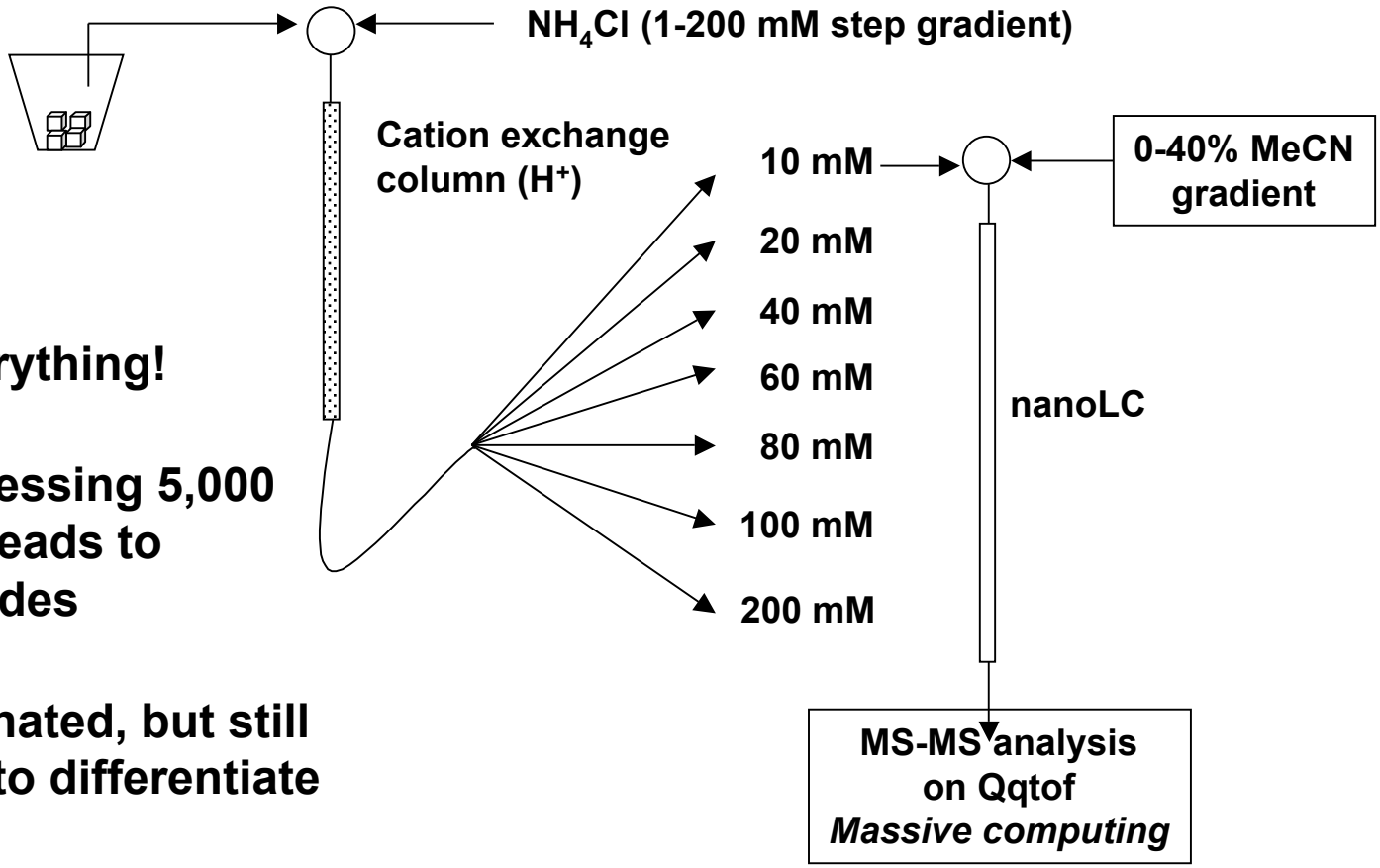
Amanda Foxwell

Conclusions of experiment

- **A peptide was identified that increased its molecular weight by 156 Da**

- **Tandem MS revealed that the 4-HNE was attached to His-33 to form a Michael adduct**

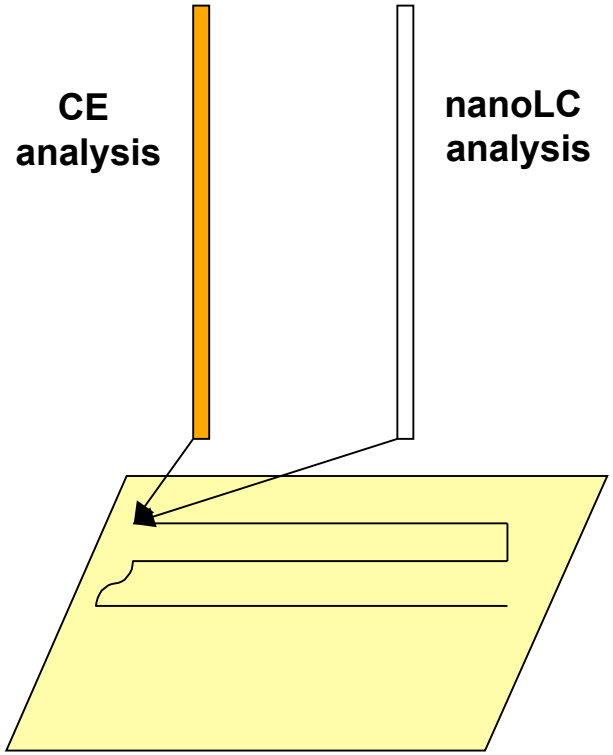
MUDPIT - MUlti-Dimensional Protein Identification Technology



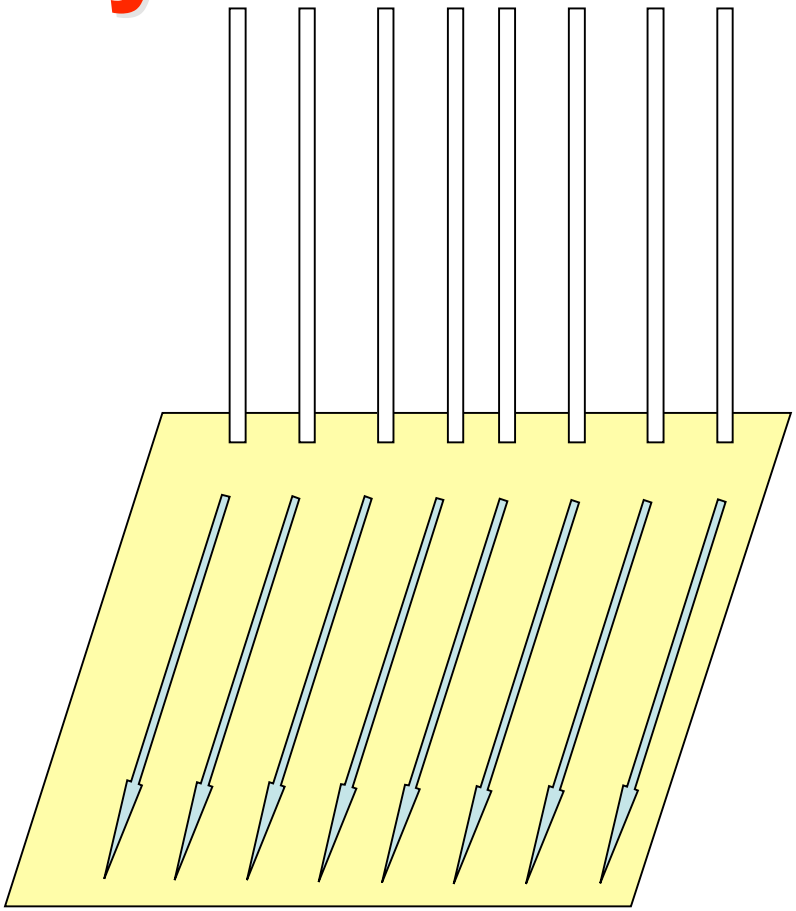
- Hydrolyze everything!
- For a cell expressing 5,000 proteins, this leads to >100,000 peptides
- Can be fractionated, but still 10,000-20,000 to differentiate
- Enormous bioinformatics problem

John Yates

Connecting CE and LC to MALDI analysis



Creates 20 mm wide tracks that can be scanned by MALDI laser for MS analysis



Parallel capture of effluents of 8 nanoLC separations on Mylar - can be scanned simultaneously by fast laser

Pros/Cons of laying down LC or EC separations on matrix plate

- Allows off-line analysis both in real time and then in a retrospective mode
- MALDI-TOF analysis is very fast
- Can do TOF-TOF MS-MS analysis
- BUT what happens chemically on the acidic environment on the surface of the plate during storage?
- Also, can the laser beam cause chemical changes?