Comparison of mass spectrometers performances

Instrument	Mass resolution	Mass accuracy	Sensitivity
Quadrupole	1×10^3	0.1 Da*	0.5-1.0 pmol
DE-MALDI	2×10^4	20 ppm	1-10 fmol peptide 1-5 pmol protein
Ion trap	1×10^3	0.1 Da*	10-20 fmol
FT-ICR	1×10^6	<1 ppm	20 amole

*depends on the mass window being used

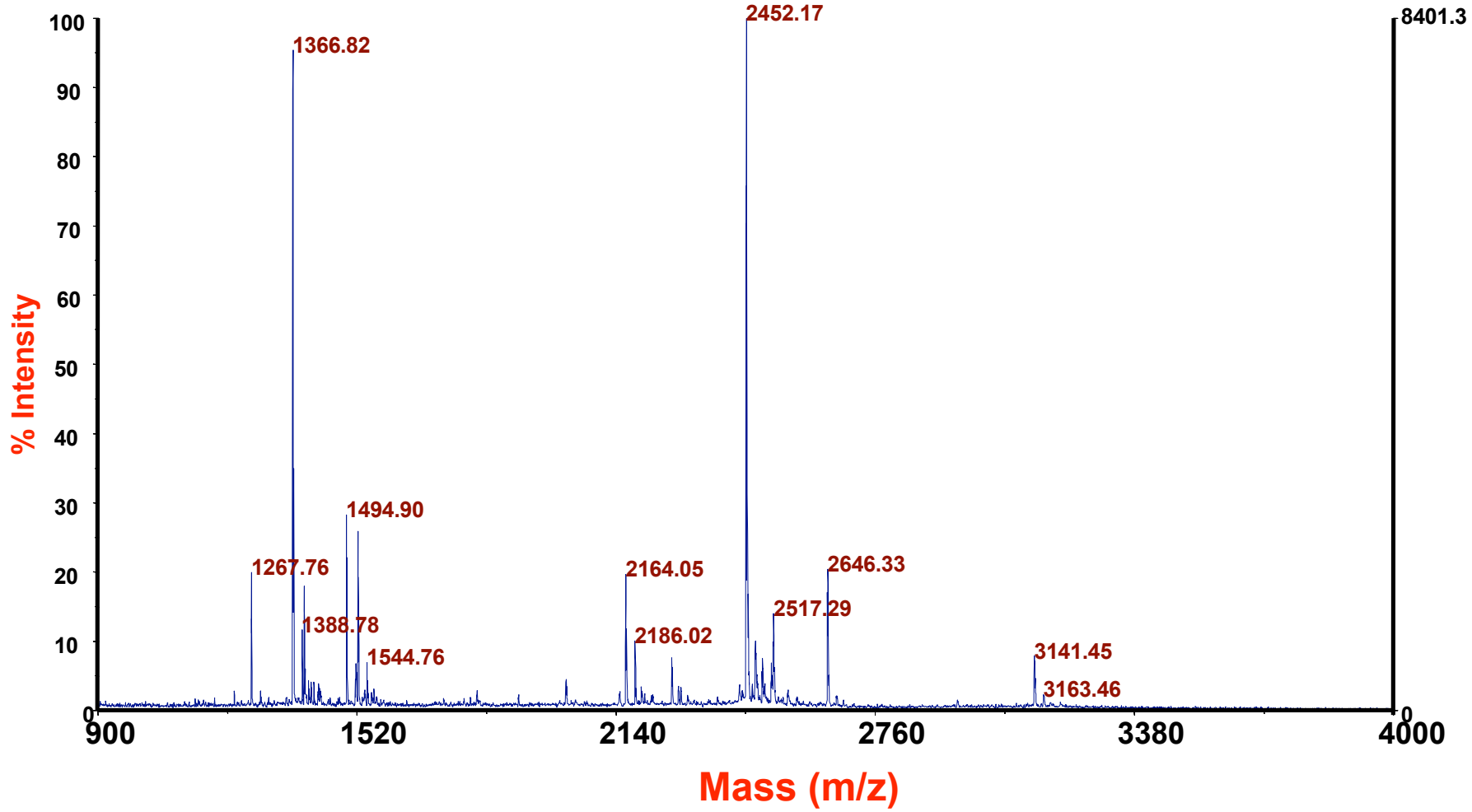
Mass spectra of peptides and proteins - and LC analysis of proteomes

Stephen Barnes, PhD

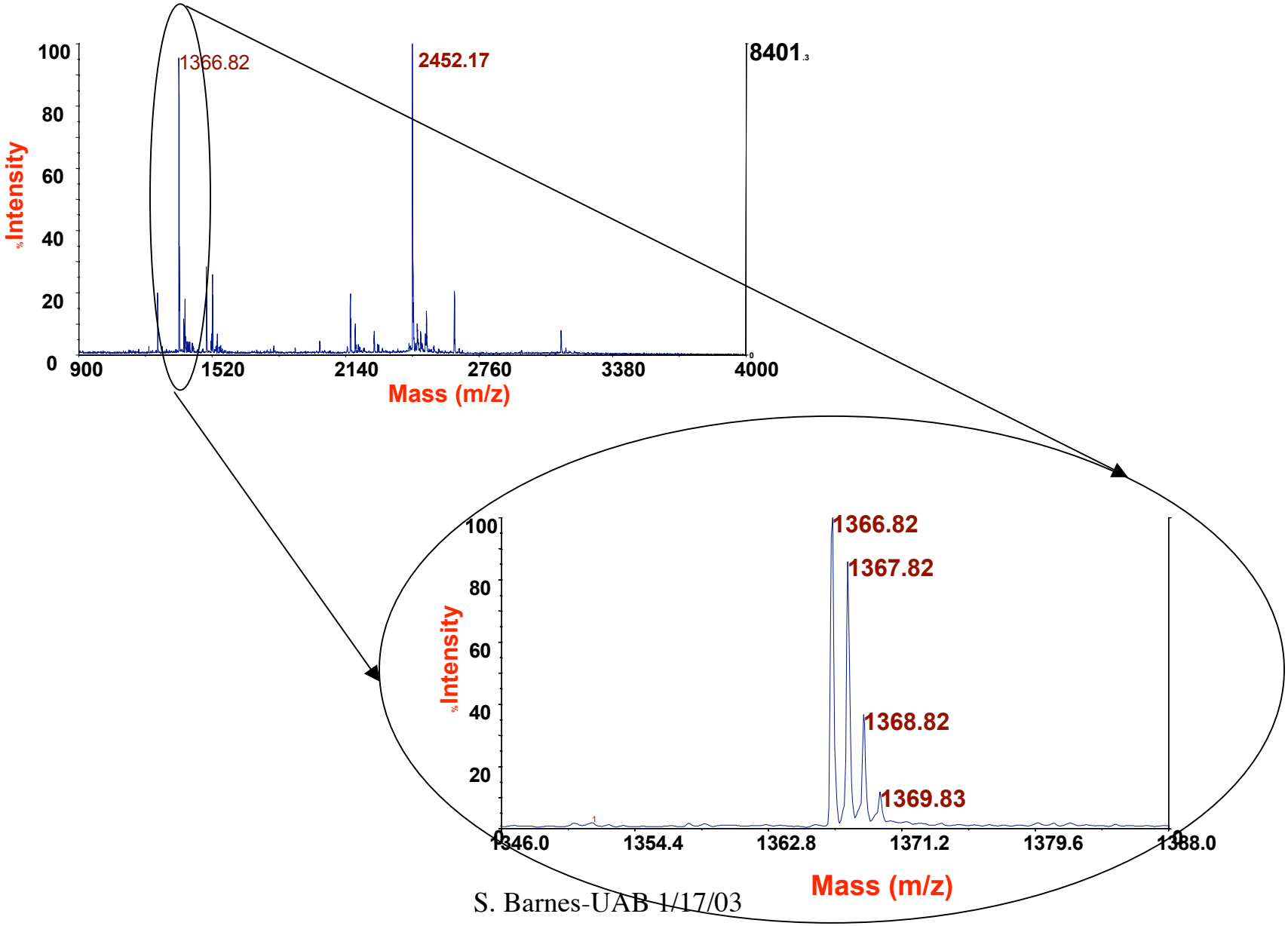
Overview

- 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
- MALDI spectra
  - Tryptic fingerprinting

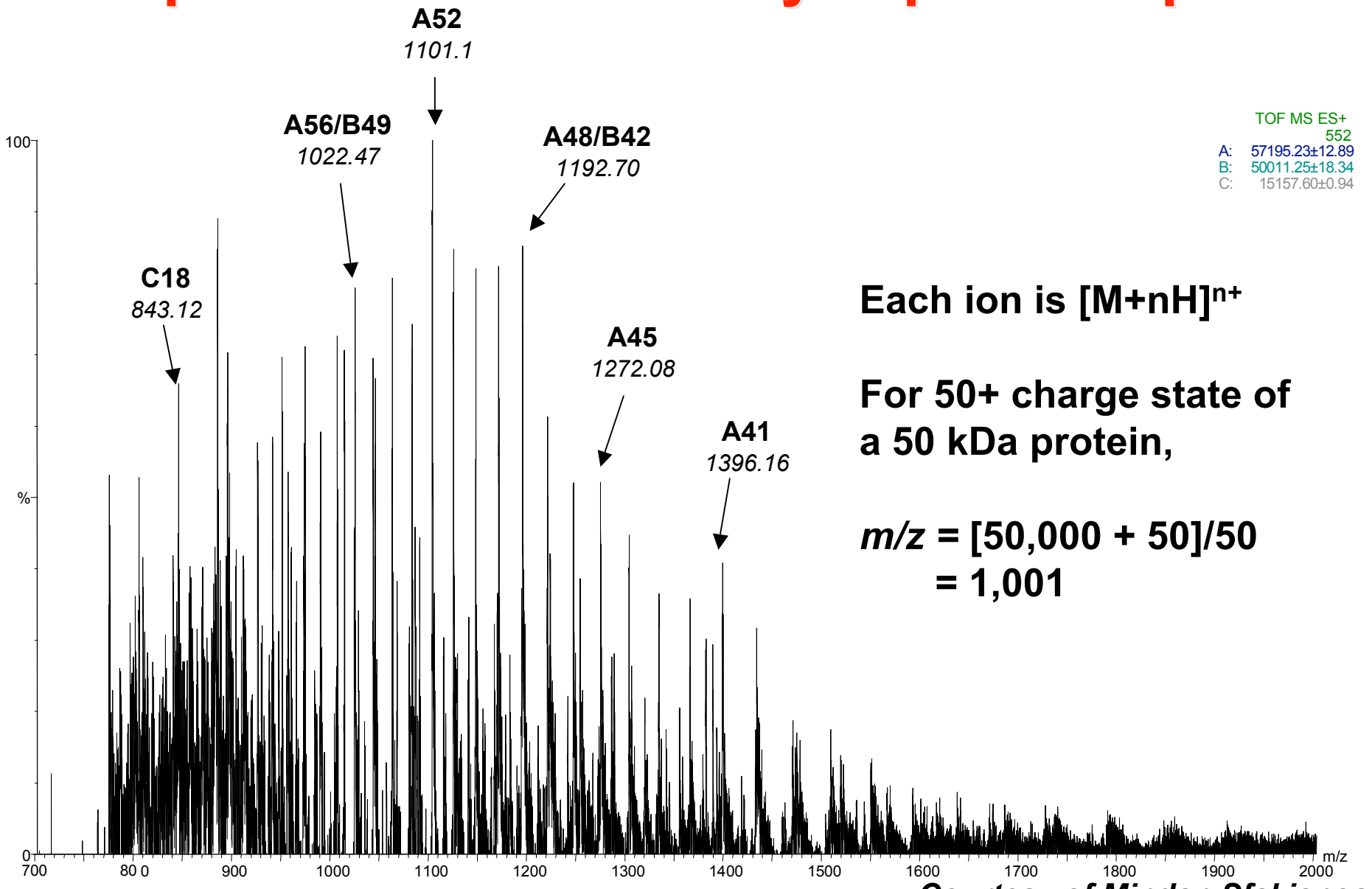
A mass spectrum of several peptides from a tryptic digest



Isotope profile of individual peptide ion



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

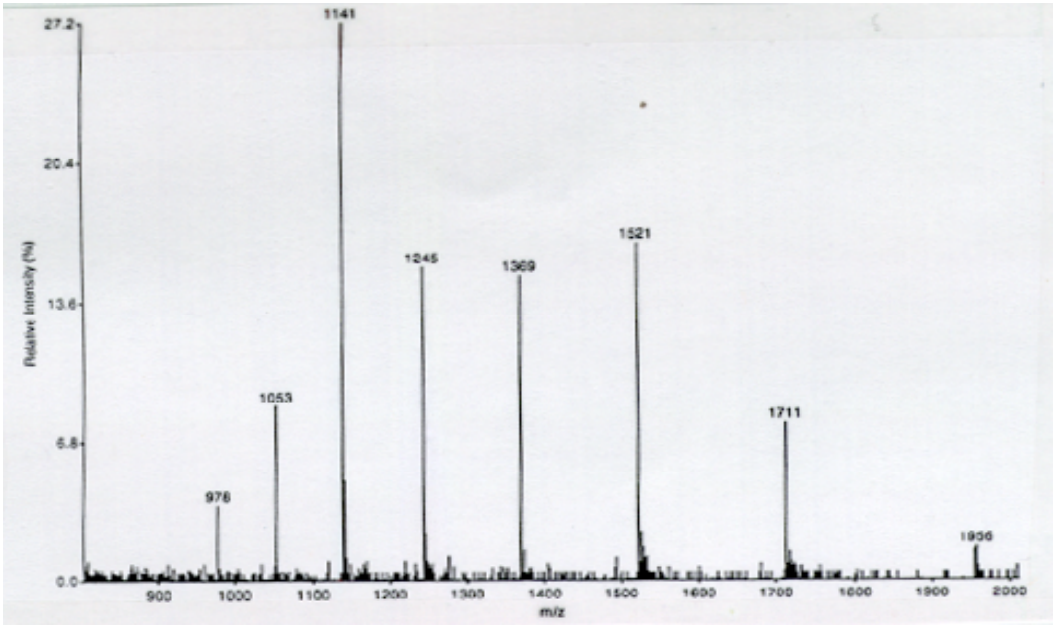
Ionizing proteins and peptides

- $^{+}H_3NCHR_1CO(NHCHR_nCO)_nNHCHR_2COOH$ 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

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**

ESI mass spectrum of ribonuclease



Cumulative MW estimate = 13,680.29

SD = 2.94

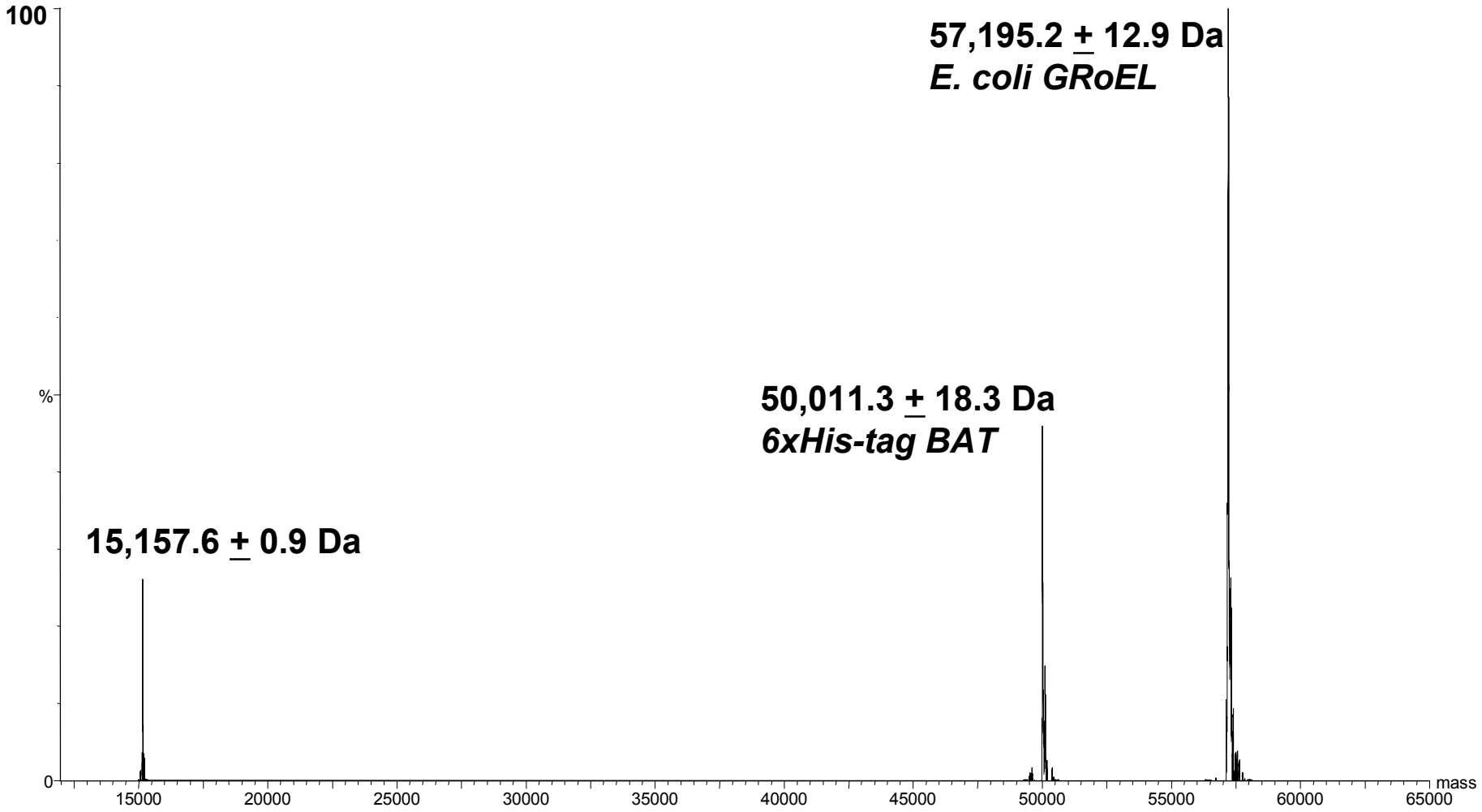
Peak (m/z)	Intensity	Charge (est.)	Mol. Wt. (Est.)
978.00	7,778	14.00000	13,677.89
1,053.00	18,532	13.02656	13,675.90
1,141.00	59,087	11.95446	13,679.91
1,245.00	33,275	10.96146	13,683.91
1,369.00	32,390	10.03219	13,679.92
1,521.00	35,668	8.99995	13,679.93
1,711.00	16,624	7.99996	13,679.94
1,956.00	3,333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

Deconvolution of MS data

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

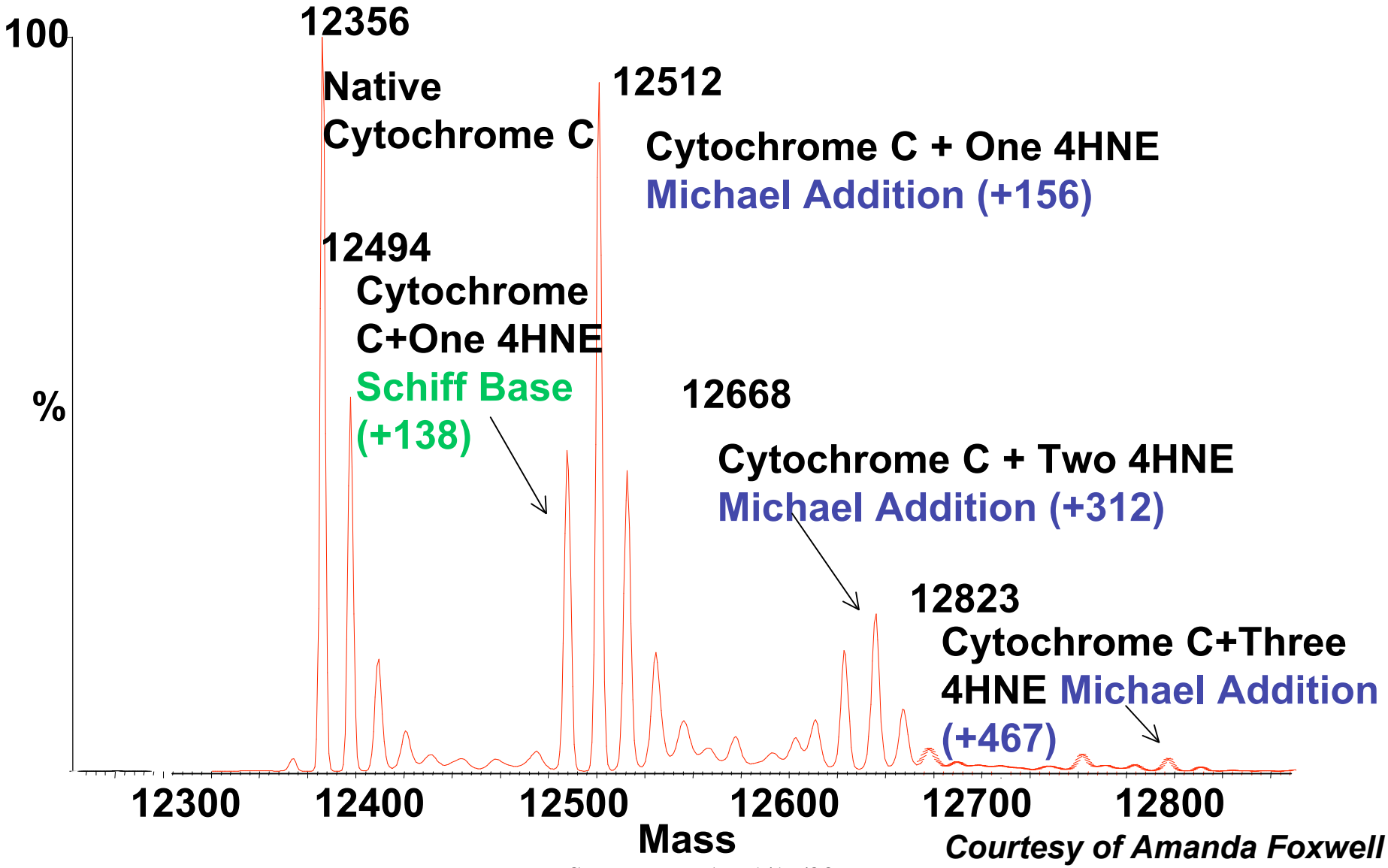
- Can this be overcome - yes, use the MaxEntropy program provided by micromass

MaxEnt deconvolution of MWs



Courtesy of Mindan Sfakianos

LC/MS of 4HNE-Modified Cytochrome C



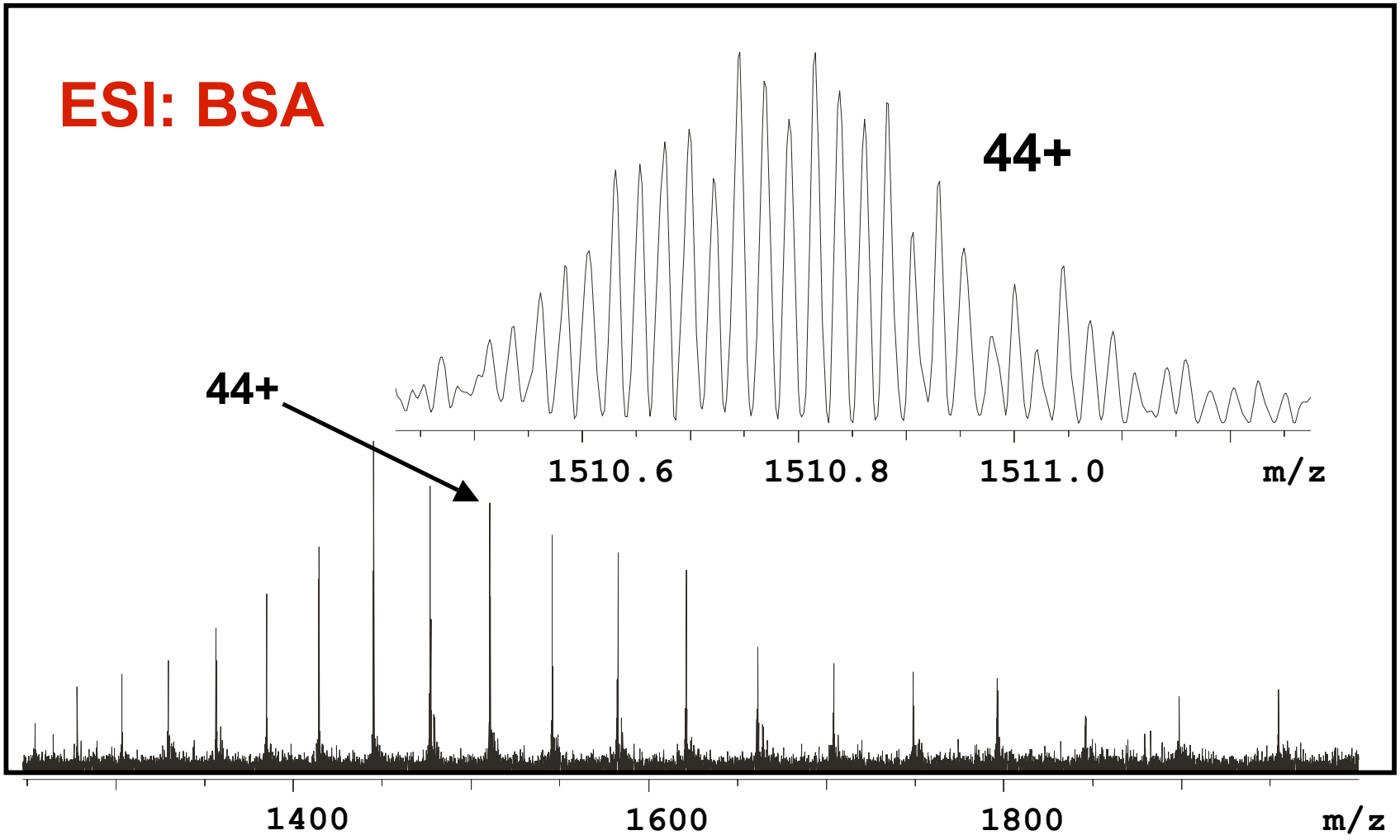
Courtesy of Amanda Foxwell

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**

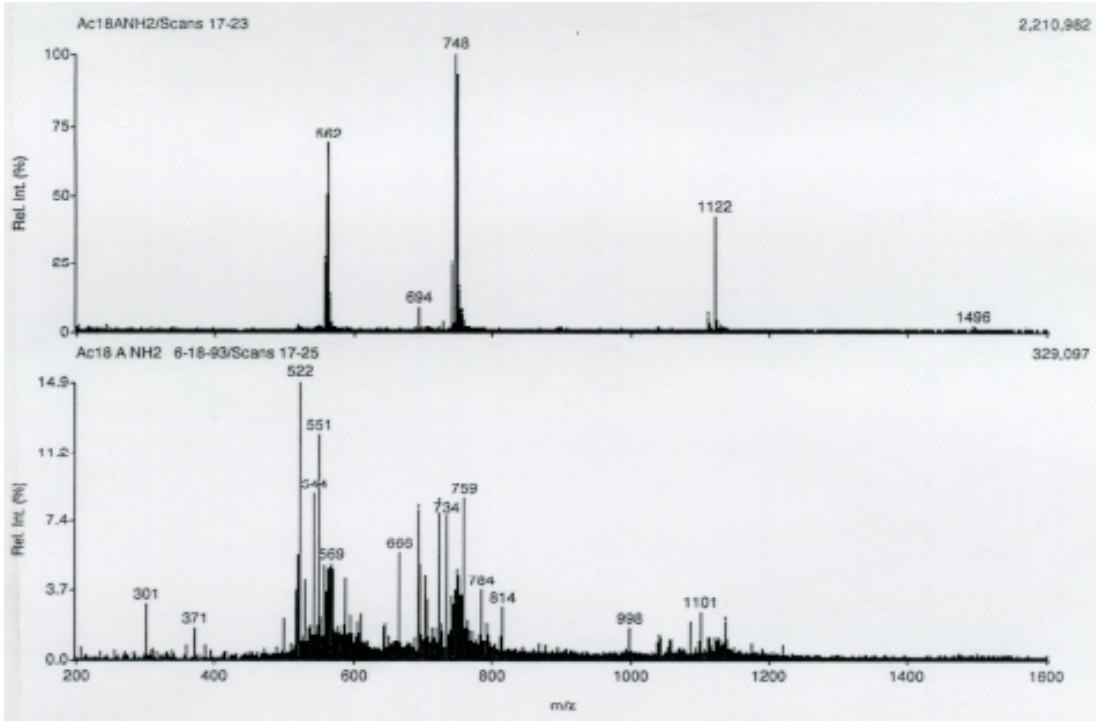
- **The distance in Da between successive isotope peaks of a multiply charged ion is the reciprocal of the number of charges**

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



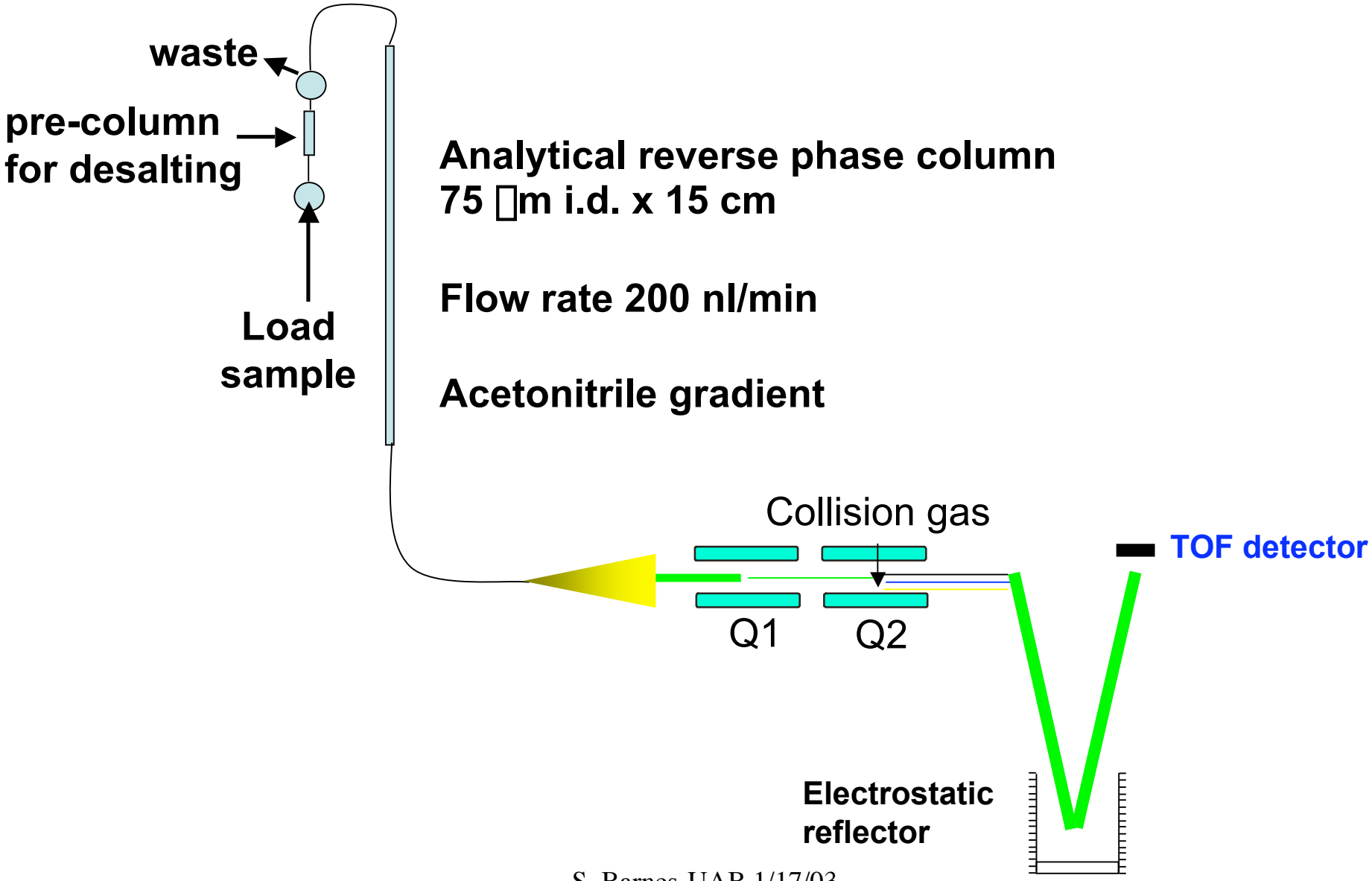
Bruker Daltonics

ESI-MS and purity of peptides



Guarantees of purity based on observation of "a single peak by reversed-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

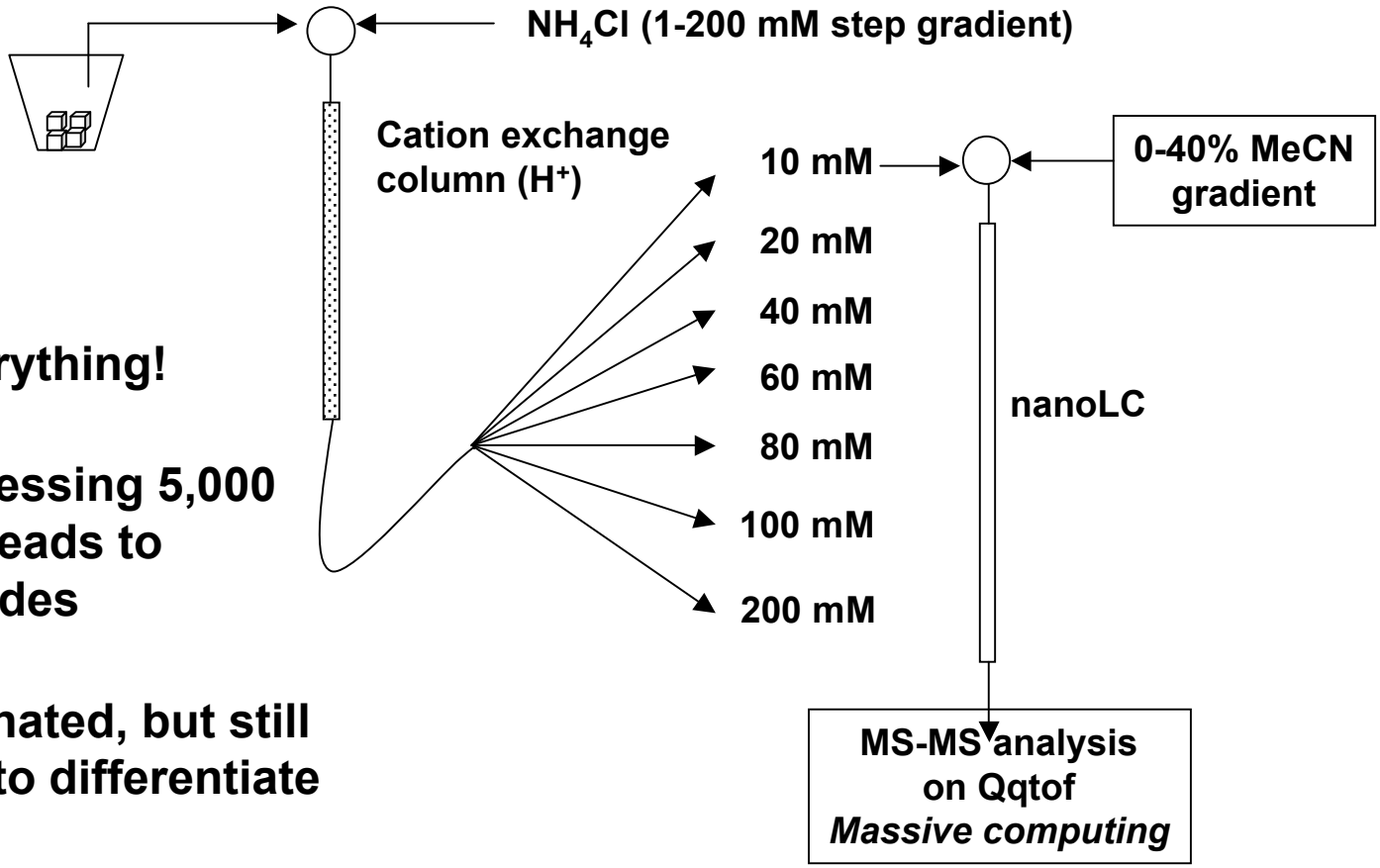
LC-MS of peptide mixtures



Tandem mass spectrometry on a triple quadrupole instrument

- **Daughter ion spectra**
  - **The molecular ion is selected in Q1, collided with Ar gas in Q2, and "daughters" analyzed in Q3**
- **Parent ion spectra**
  - **All molecular ions allowed into Q1, collided in Q2, and a selected daughter ion measured in Q3**
- **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**

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

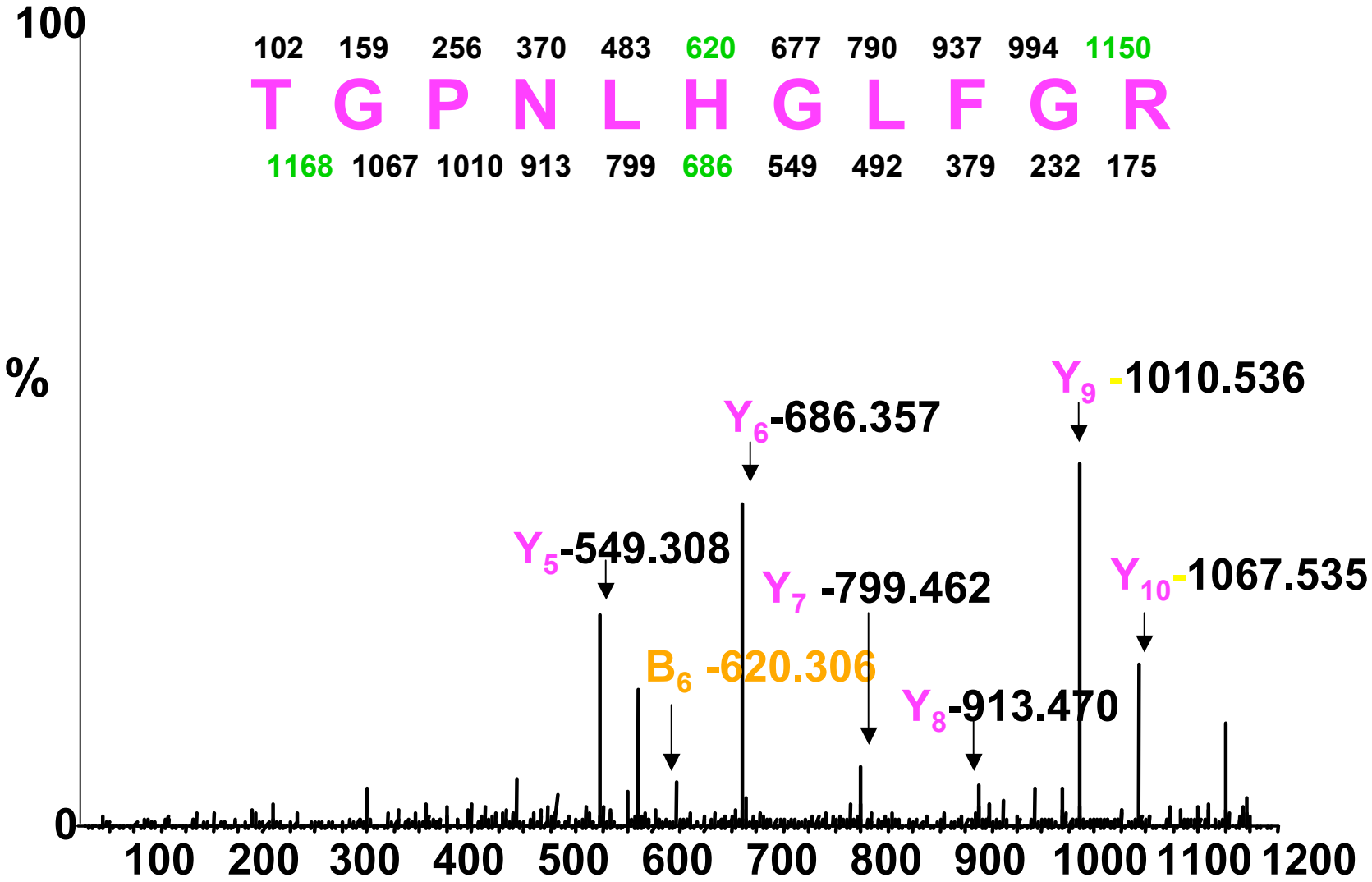
Pros/Cons of laying down LC or EC separations on matrix plate

- **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?**

Example of MS-MS to detect a modification

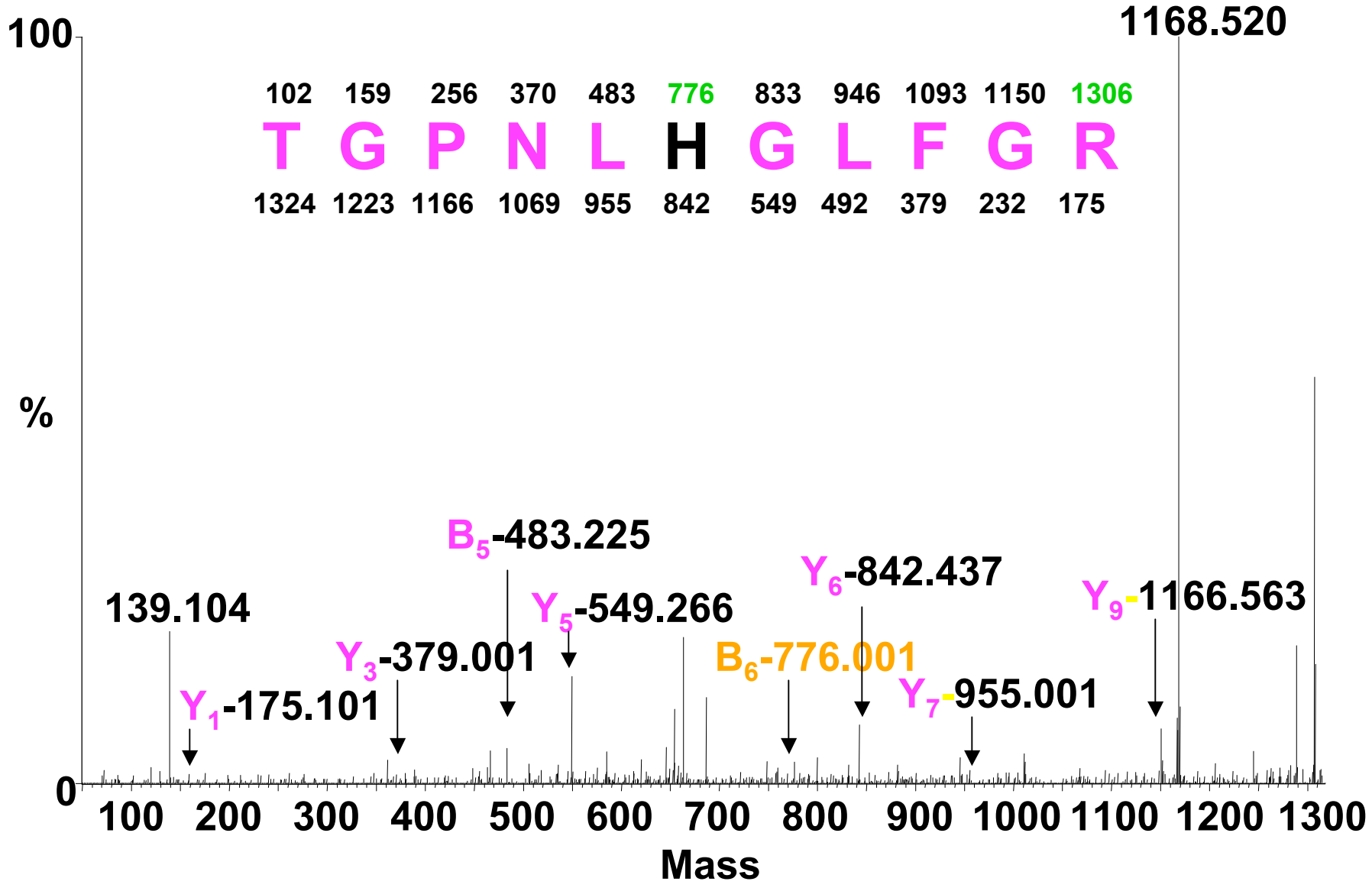
- Cytochrome c, a mitochondrial enzyme was reacted with 4-hydroxynonenal, an aldehyde formed by oxidation of long chain, unsaturated fatty acids
- Site of attachment believed to be on lysine groups (to form a Schiff's base)
- However, increase in MW consistent with Michael addition
- Protein hydrolyzed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

Qtof Tryptic Digest of Control Peptide



Amanda Foxwell

Qtof Tryptic Digest of Modified Peptide



Amanda Foxwell

Conclusions of experiment

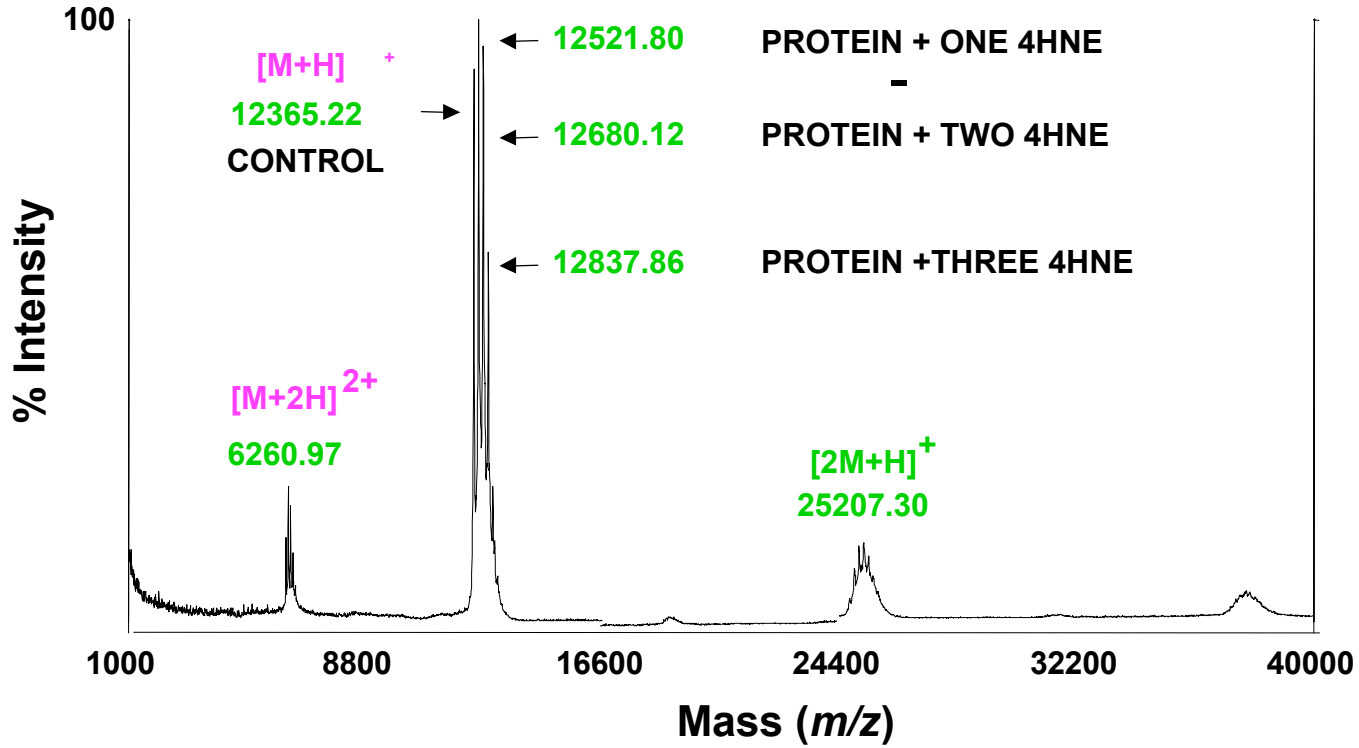
- **A peptide was identified that increased its molecular weight by 156 Da**

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

MALDI-TOF MS

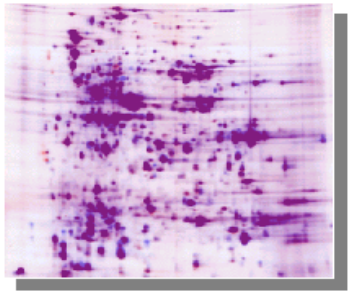
- **Whole proteins**
- **Tryptic fingerprinting**
- **MS-MS**

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.

Protein analysis by MALDI 2003



Eppendorf tube

destain

Speed-Vac

trypsin

1:20

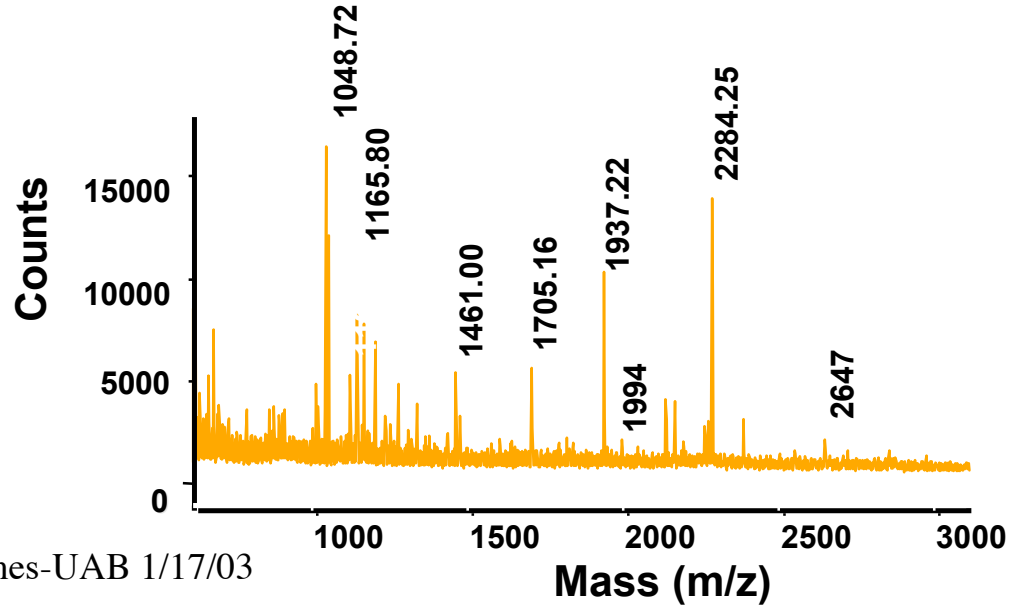
Incubate

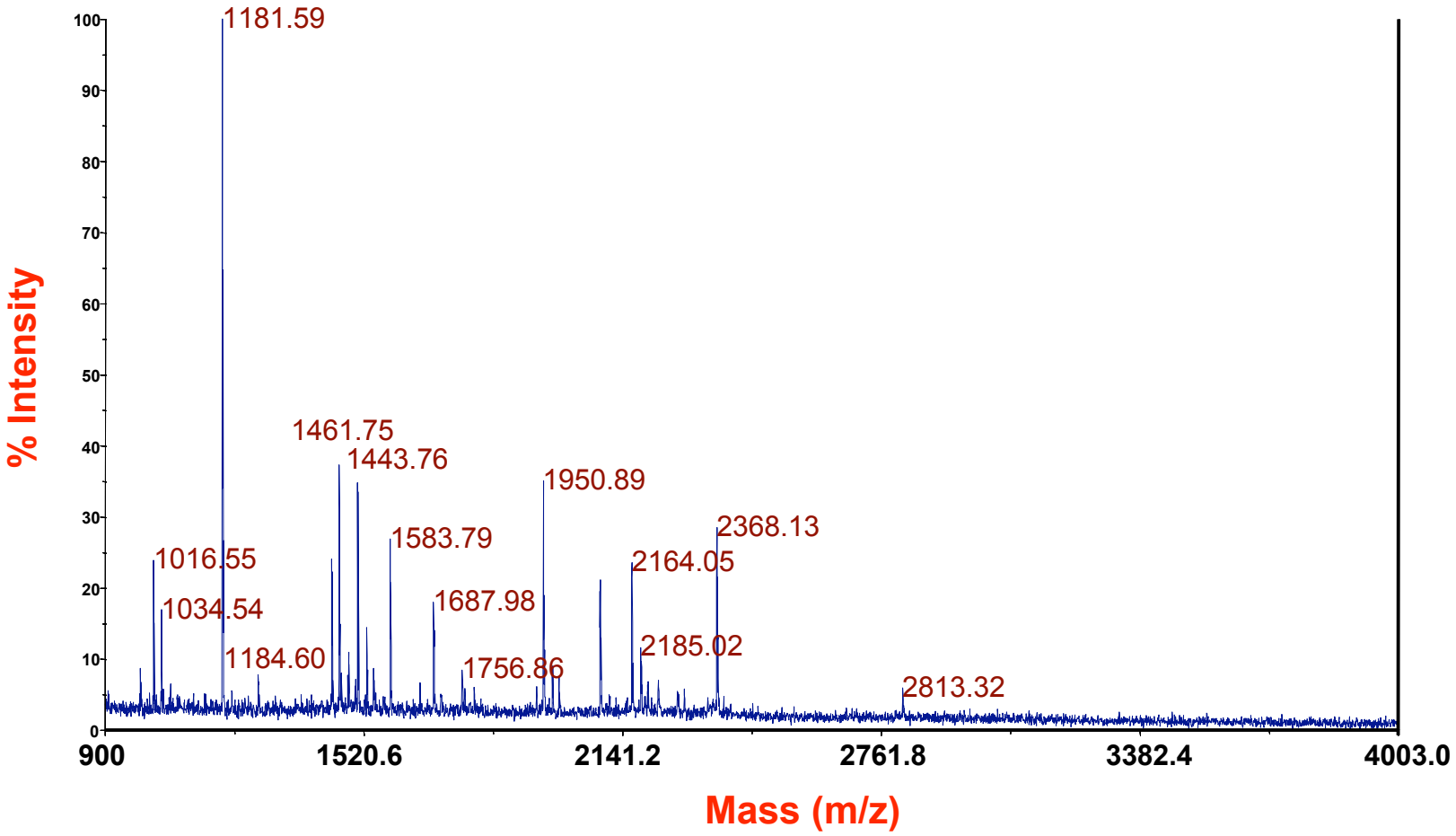
overnight

Water Bath 37°C

MALDI plate

Desalting Ziptip





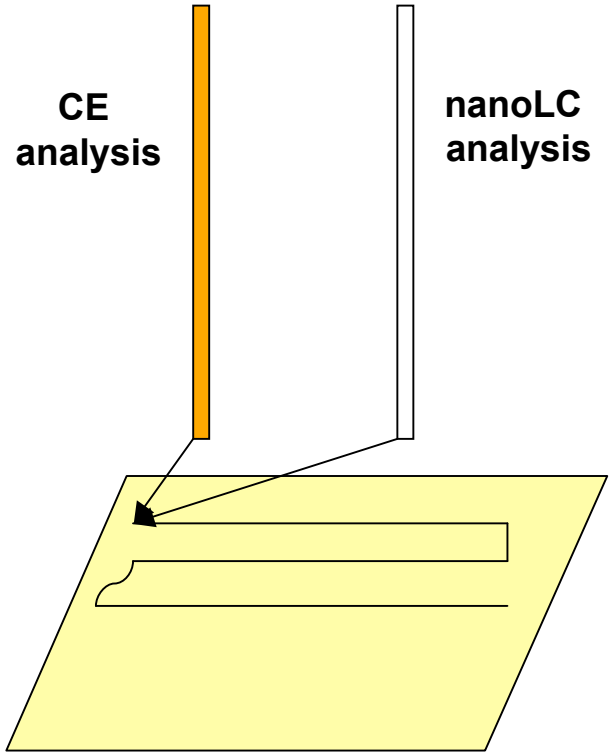
S. Barnes-UAB 1/17/03

MASCOT Search

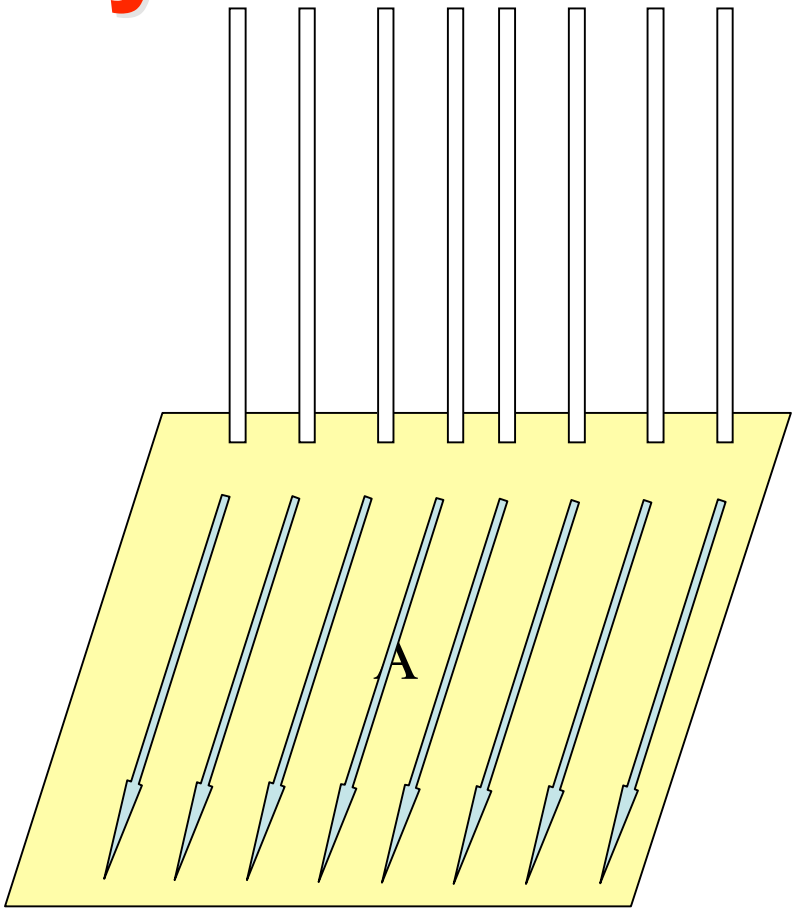
1016.55
1034.54
1181.59
1461.75
1443.76
1687.98
1950.80
2368.13
2813.32

Trypsin Digest of porin-P1; Voltage-dependent anion-selective channel

Connecting CE and LC to MALDI analysis

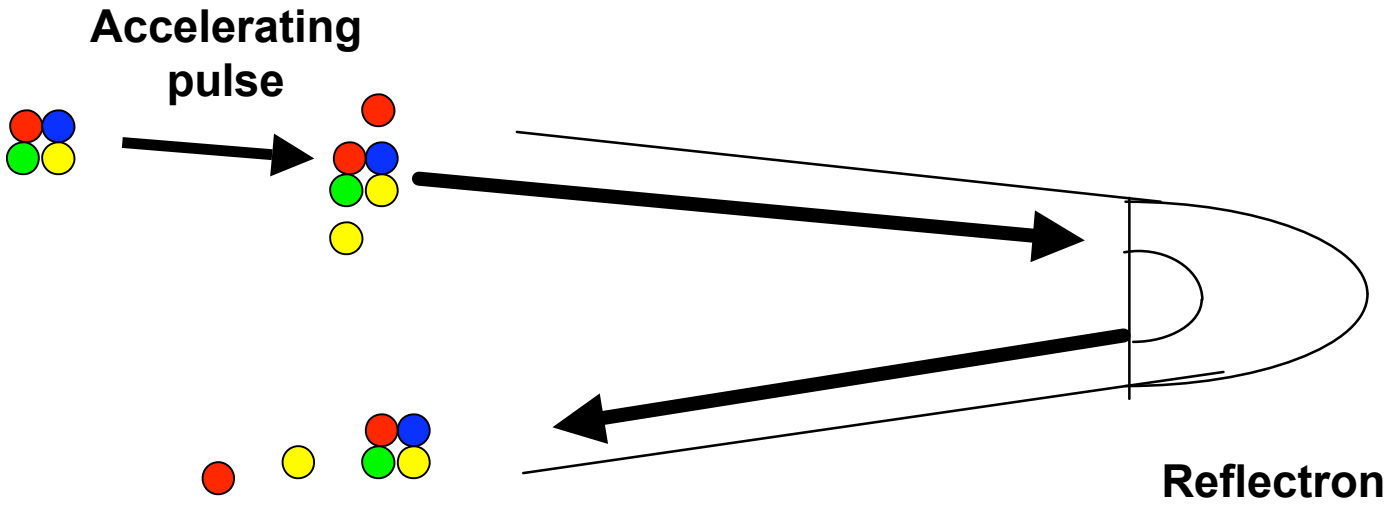


Creates 20 mm wide tracks that can be scanned by MALDI laser for MS analysis



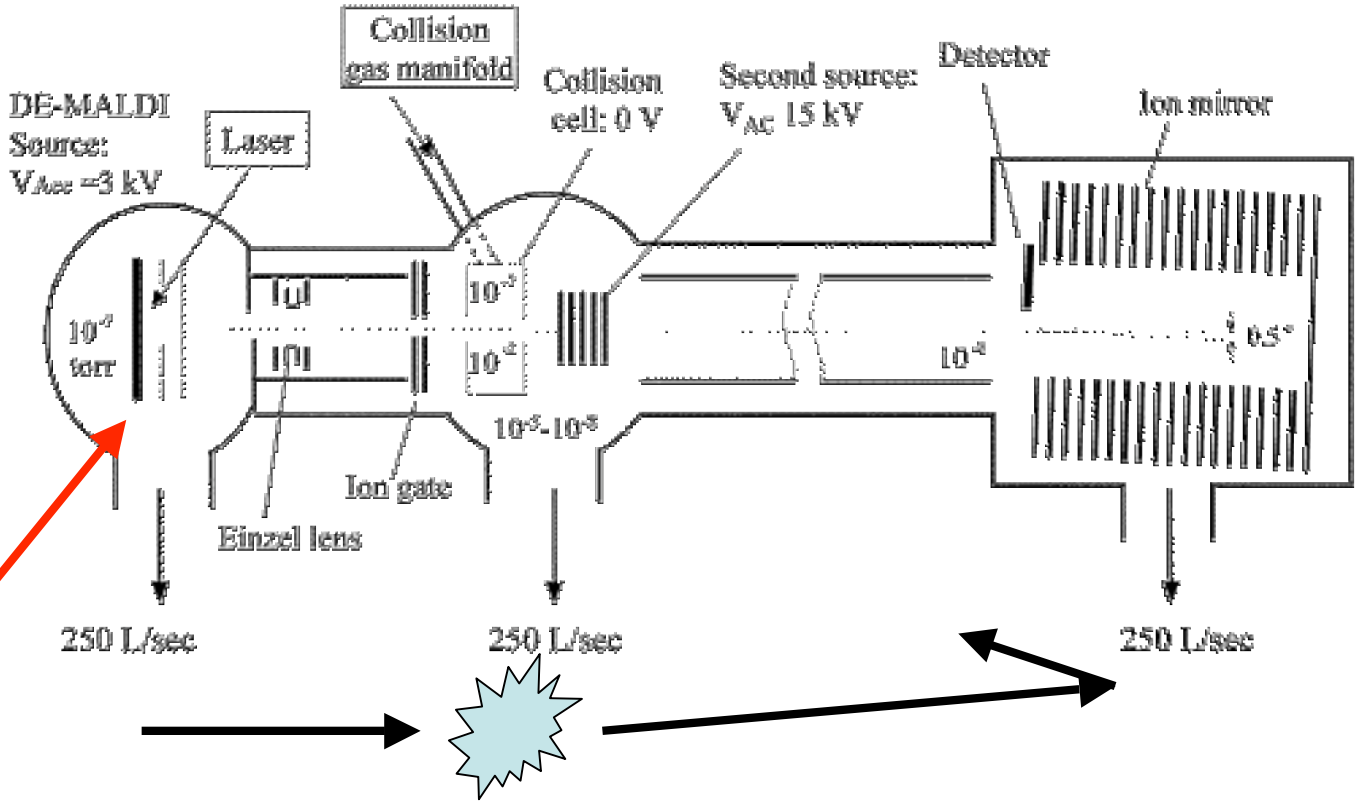
Parallel capture of effluents of 8 nanoLC separations on Mylar - can be scanned simultaneously by fast laser

Post-source decay experiments in a TOF-mass spectrometer



Daughter fragment ions formed in the drift region are separated by the reflector. Suitable resolution only occurs over a limited range of *m/z* values. This can be overcome by recording individual spectra over a wide range of voltage settings (10-12) for the reflector. Alternatively, a curved applied voltage can be used to obtain the daughter ion spectrum in a single experiment.

TOF-TOF - high speed MSMS



ion generation

Decomposition of -NO_2 group in MALDI-TOF MS