# Application of mass spectrometry to the analysis and identification of peptides, proteins and other biological molecules

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**6-4681**

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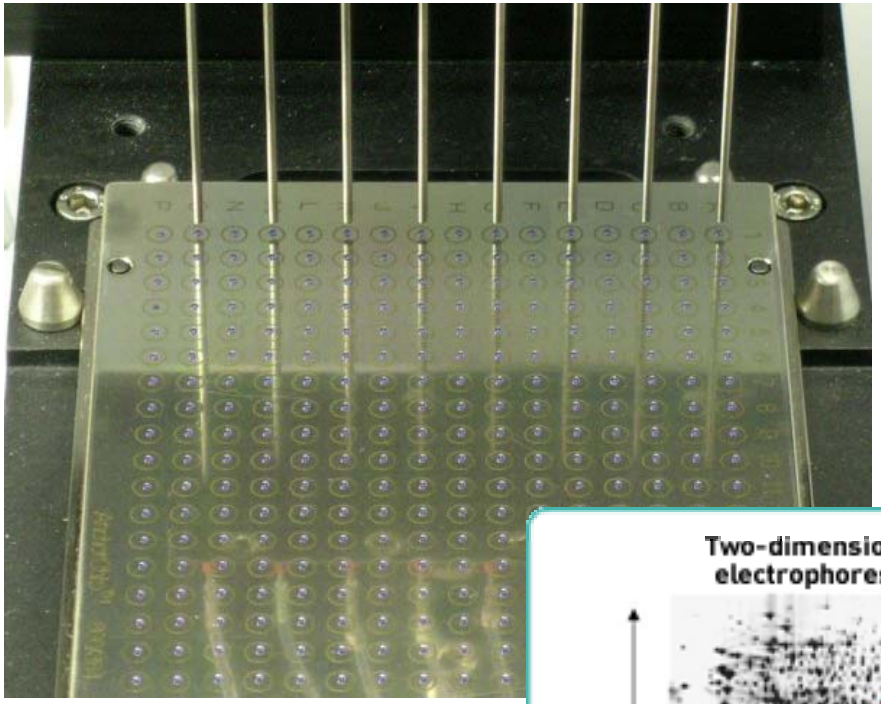
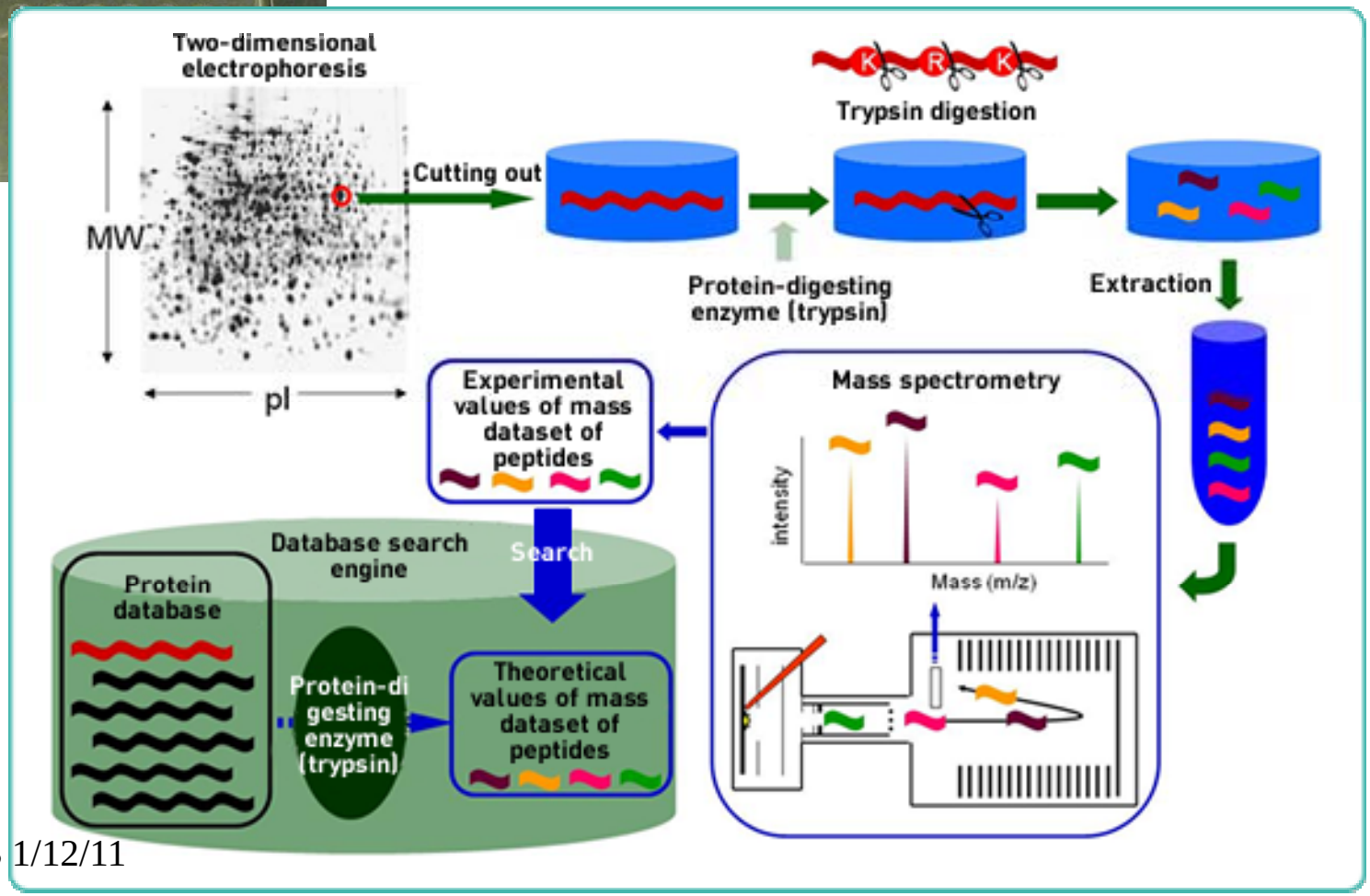
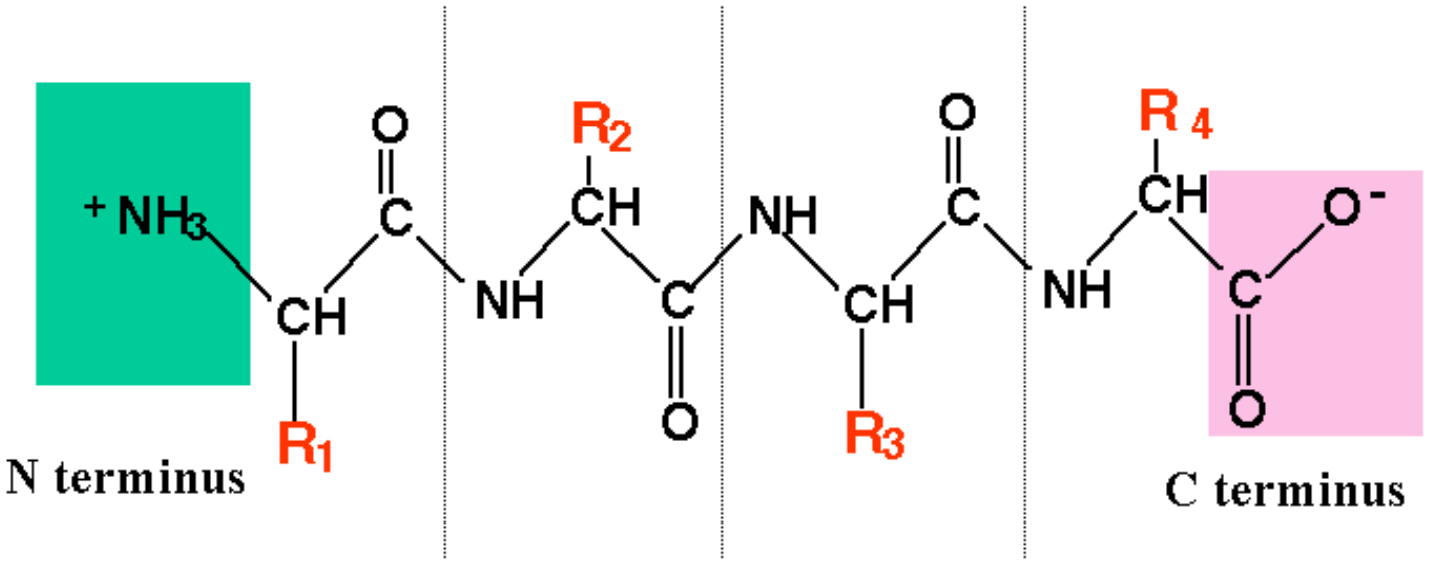
# Overview

- **MALDI-TOF MS**
  - **Peptide mass fingerprinting**
- **Electrospray MS**
  - **Analysis of intact proteins**
  - **Molecular weight calculations**
  - **Max Entropy for MW estimation**
- **Peptide analysis**
  - **Purity - ESI-MS is a revelation**
- **Integration of MS with LC and CE**
  - **Multidimensional LC of peptides**
- **Tandem MS**
  - **Identifying peptide amino acid sequences**

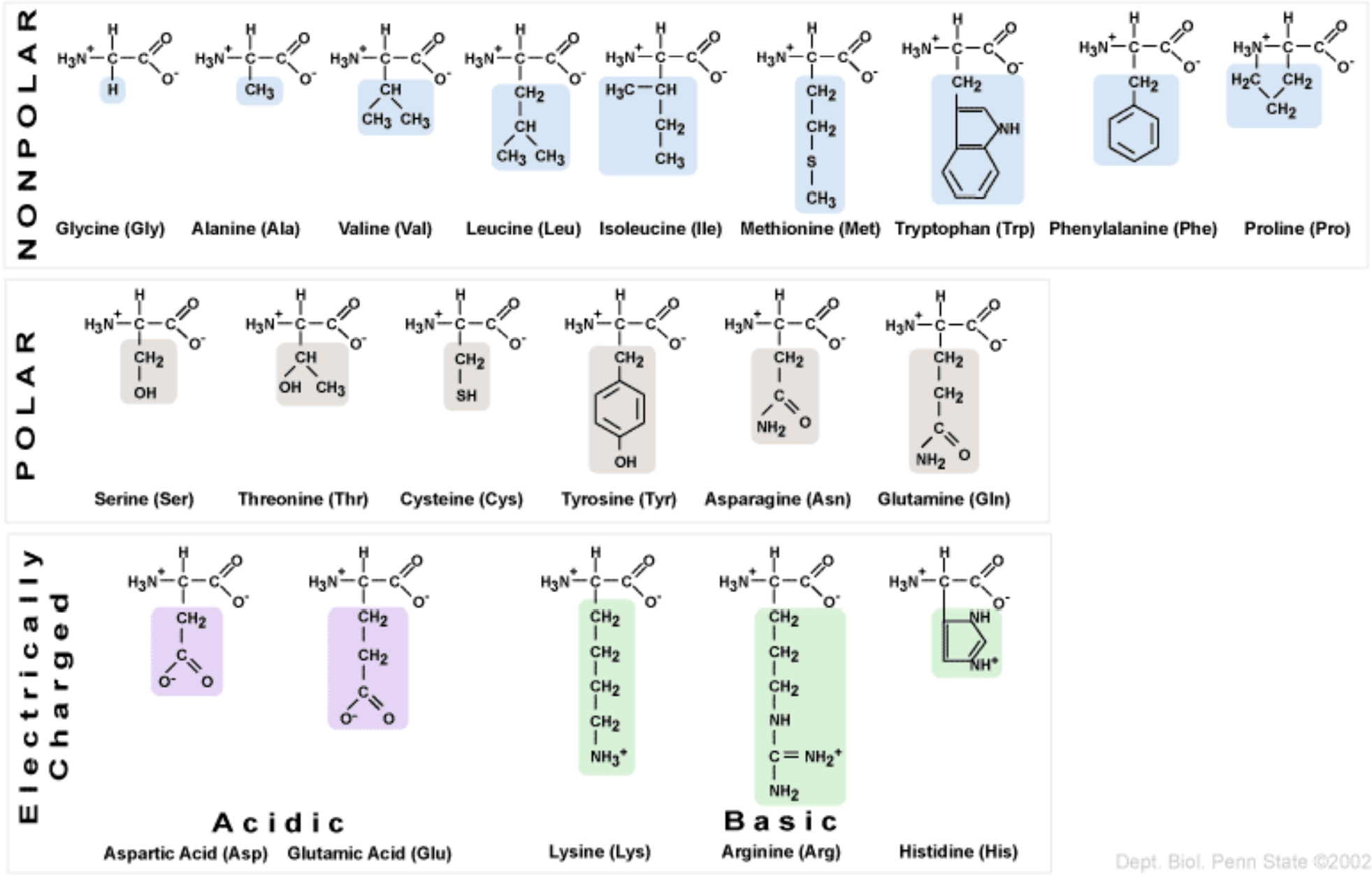


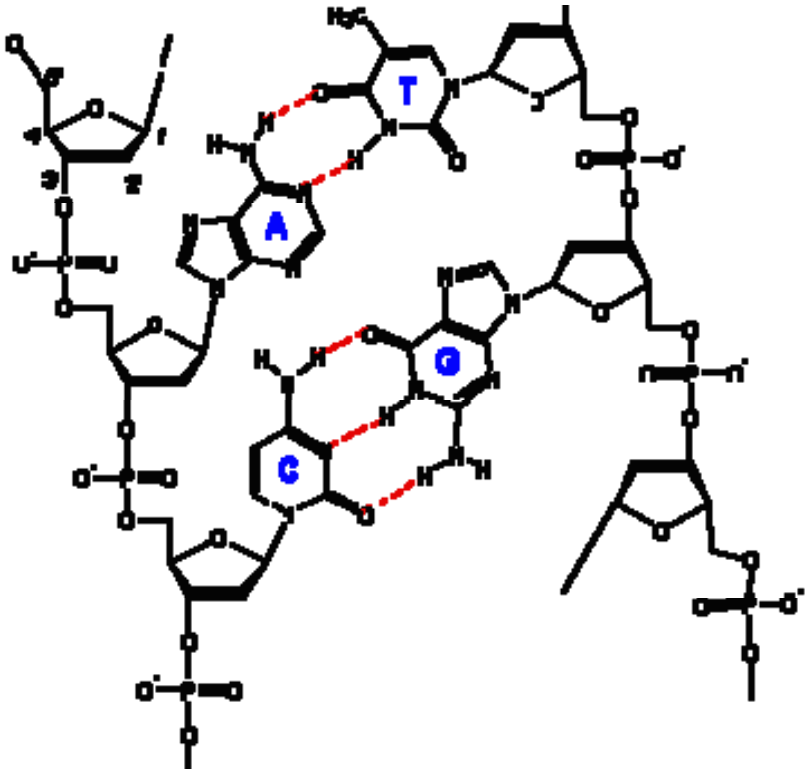

Two-dimensional electrophoresis
MW
pI
Cutting out
Trypsin digestion
K
R
K
Protein-digesting enzyme (trypsin)
Extraction
Experimental values of mass dataset of peptides
Mass spectrometry
intensity
Mass (m/z)
Database search engine
Search
Protein database
Protein-digesting enzyme (trypsin)
Theoretical values of mass dataset of peptides

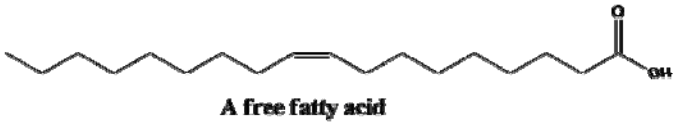
# Peptide = chain of amino acids

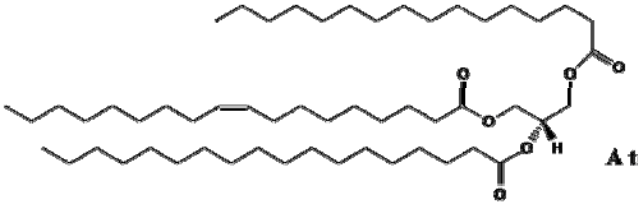


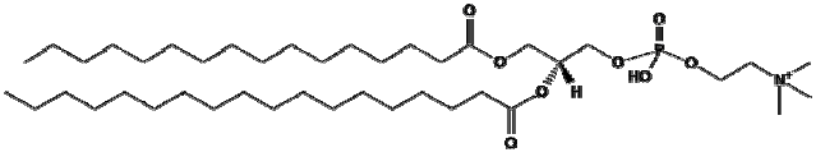

polypeptide chain

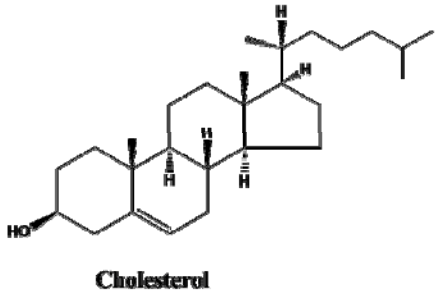

NONPOLAR
Glycine (Gly)
Alanine (Ala)
Valine (Val)
Leucine (Leu)
Isoleucine (Ile)
Methionine (Met)
Tryptophan (Trp)
Phenylalanine (Phe)
Proline (Pro)
POLAR
Serine (Ser)
Threonine (Thr)
Cysteine (Cys)
Tyrosine (Tyr)
Asparagine (Asn)
Glutamine (Gln)
Electrically Charged
Acidic
Aspartic Acid (Asp)
Glutamic Acid (Glu)
Basic
Lysine (Lys)
Arginine (Arg)
Histidine (His)
Dept. Biol. Penn State ©2002

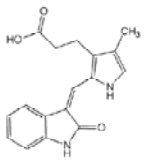




A free fatty acid


A triglyceride


A phospholipid


Cholesterol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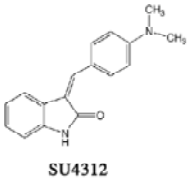
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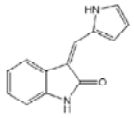
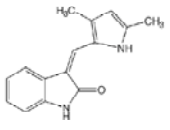

Oxindole I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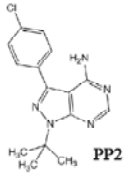
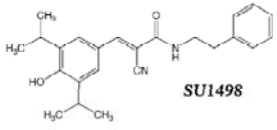

PP2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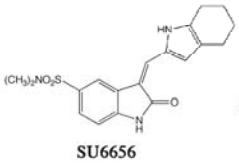
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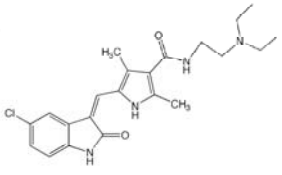
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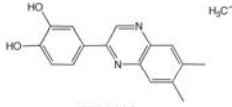
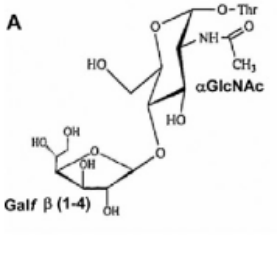
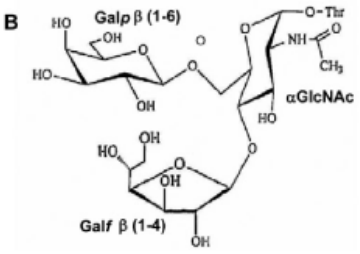
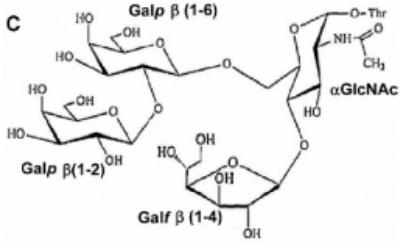
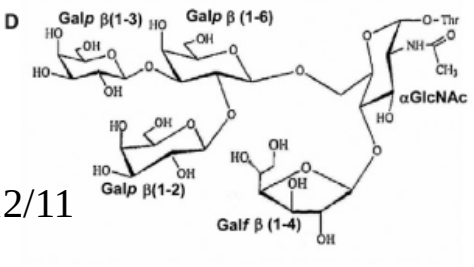
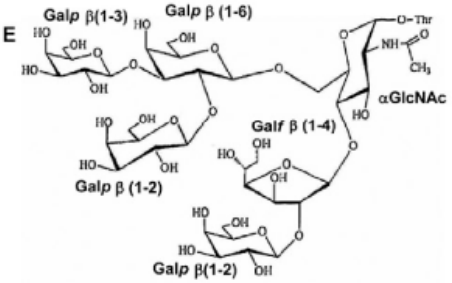

SU1433
(AG1433)

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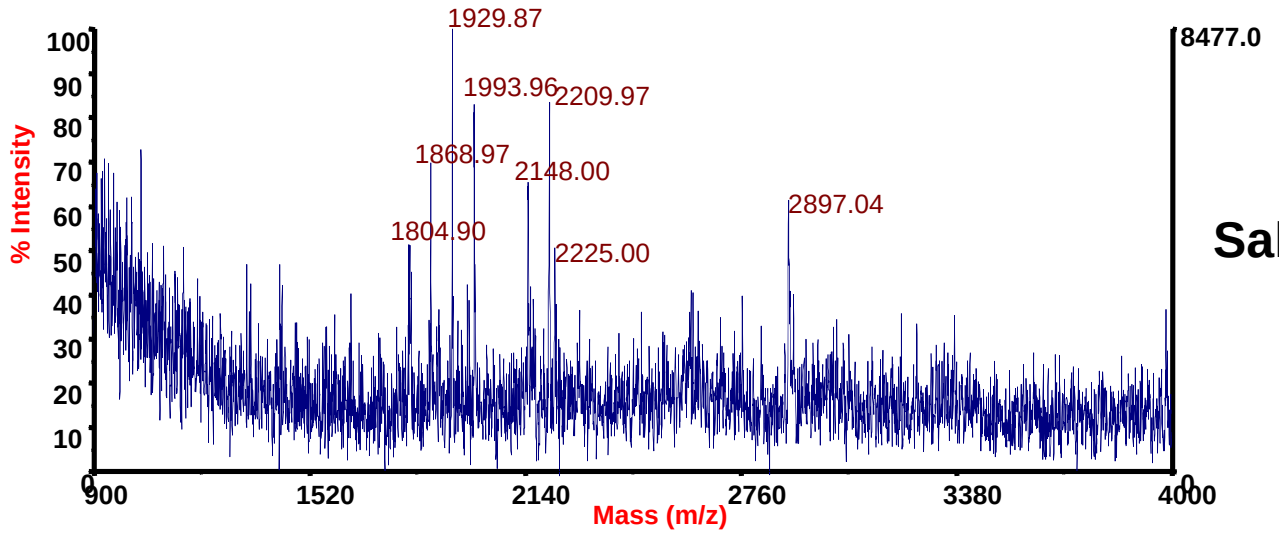
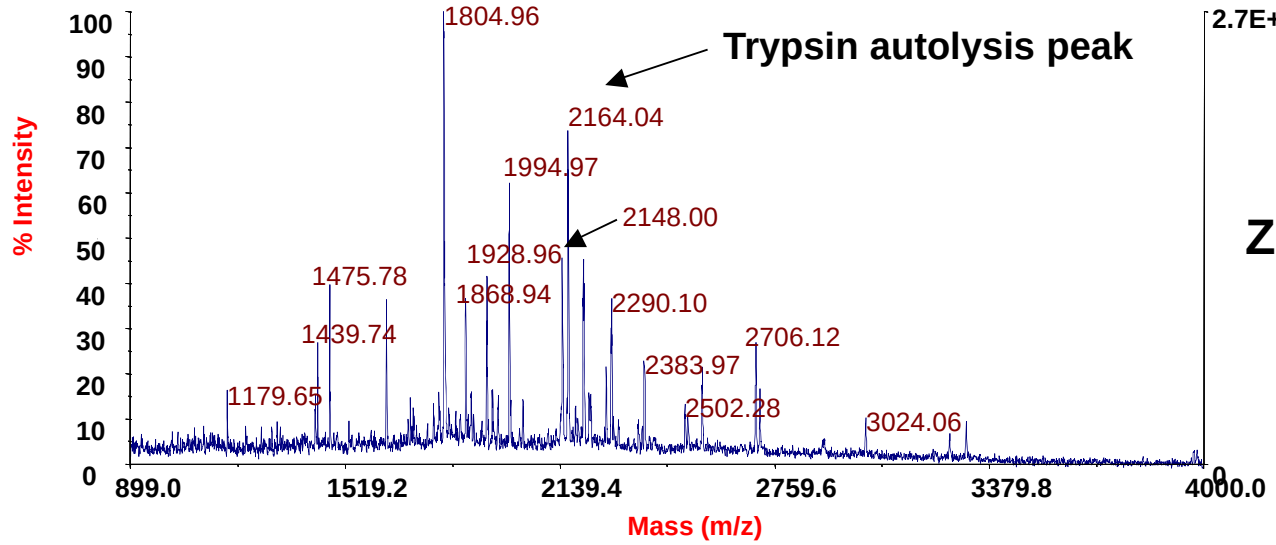
# Benefit of removing salt from tryptic digest




**Salt Contamination**




**ZipTip (desalting)**

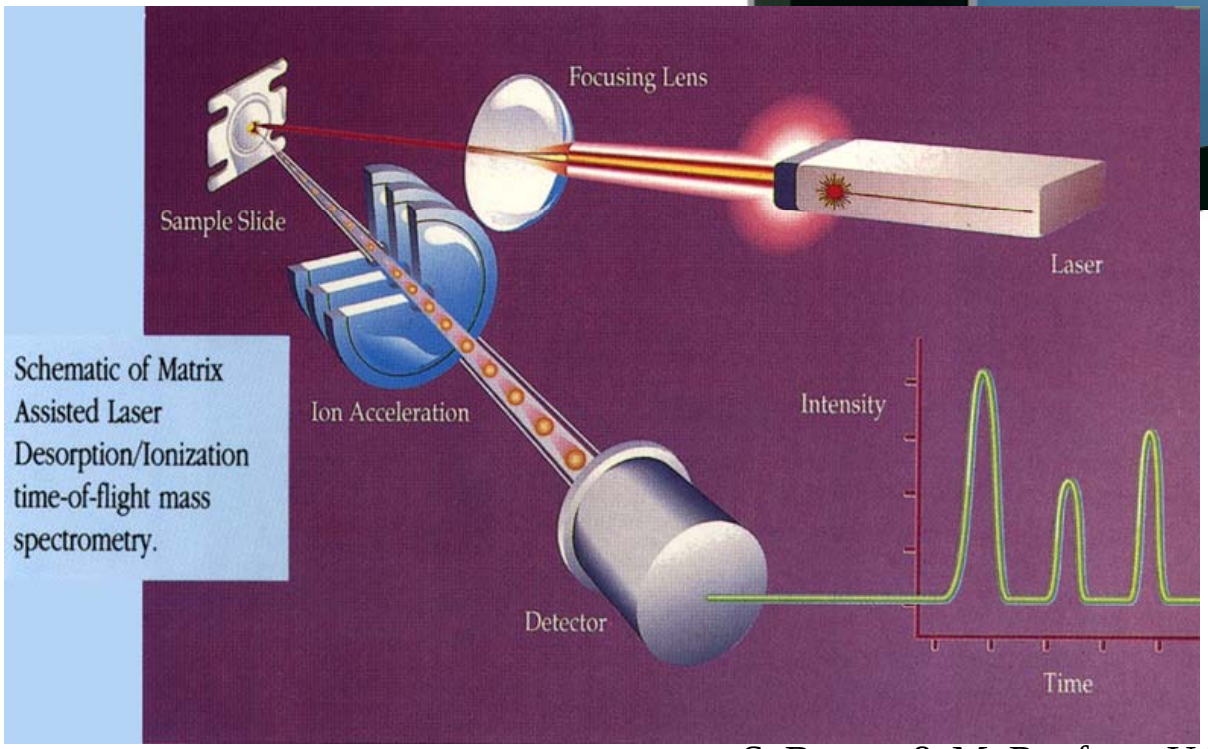
# Factors from conventional experiments that impact MALDI-TOF analysis

- **Tolerance of buffers/chemicals used in sample preparation**
  - **NaCl up to 150 mM**
  - **Urea up to 2-3 M (carbamoylation can occur!)**
  - **Guanidinium-HCl up to 2 M**
- **Tolerance of detergents**
  - **SDS up to 0.05%**
- **Staining Protocols**
  - **Whole proteins form adducts with Coomassie**
  - **Silver staining modifies selected peptides**

# Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS)

- **Advantages of MALDI-TOF**
  - **More tolerant to common buffers than ESI, but…**
  - **High degree of sensitivity, moderate mass accuracy, and mass resolution**
  - **High mass compounds, i.e. proteins, PEG…**

- **Common Applications of MALDI-TOF**
  - **Masses of large proteins and other compounds**
  - **Enzymatic digestion profiles of proteins to establish their identity**
  - **Peptide sequencing (TOF-TOF)**
  - ***In situ* protein/peptide imaging**

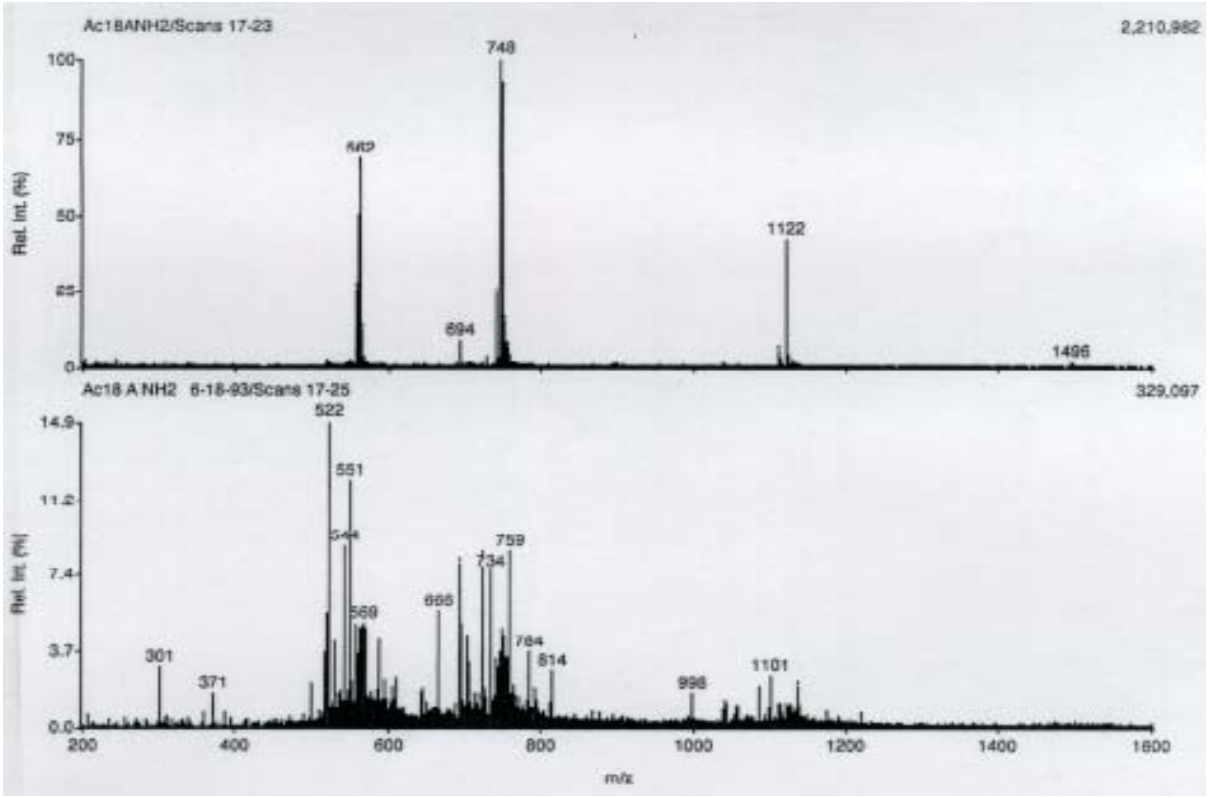




Schematic of Matrix Assisted Laser Desorption/Ionization time-of-flight mass spectrometry.

# Electrospray ionization

- **ESI-MS is very sensitive to the presence of electrolyte species -**
  - **these ionize more easily than solutes and may also form adducts with solutes**
- **In ESI-MS, multiple charge states are possible**
  - **These lead to more accurate MWs**
- **This is a softer ionization than MALDI where the UV laser at 337 nm alters the chemistry of modifications such as Tyr-$NO_2$ and Cys-SNO**

# ESI-MS and purity of peptides

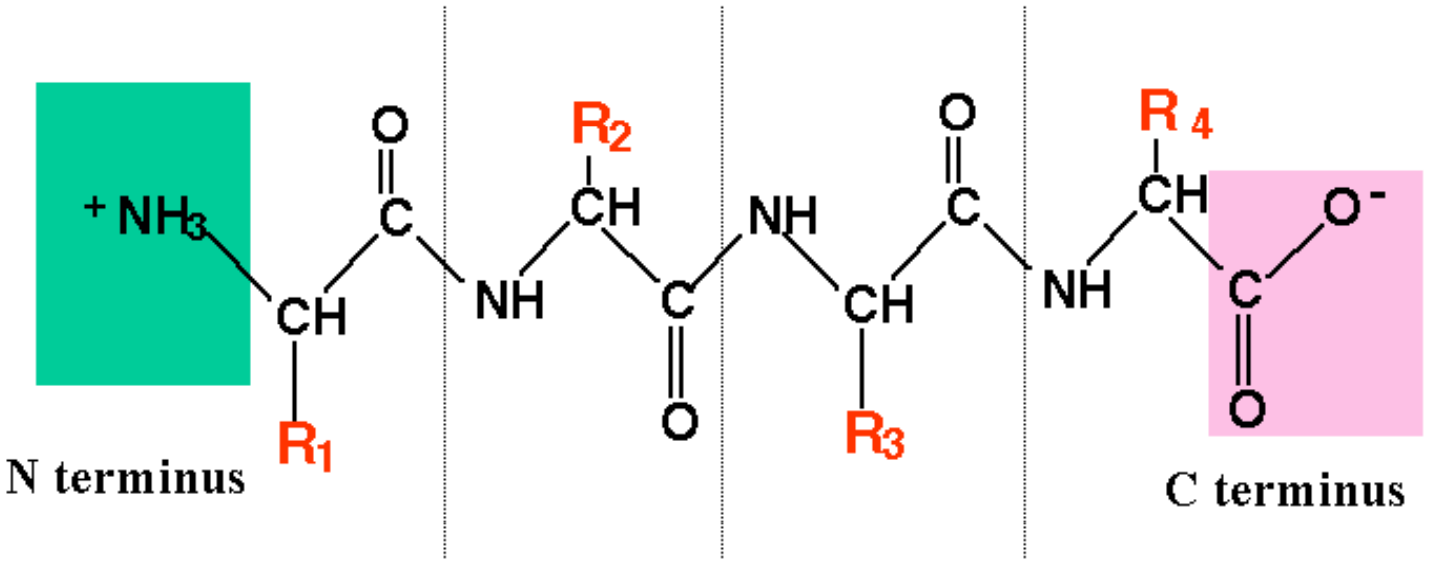



**Guarantees of purity based on observation of "a single peak by reverse-phase HPLC" and by "it gave the correct sequence when analyzed by Edman degradation" are hollow. The lower spectrum was of a "pure" HPLC peak. The method of purification was amended and the upper spectrum was obtained**

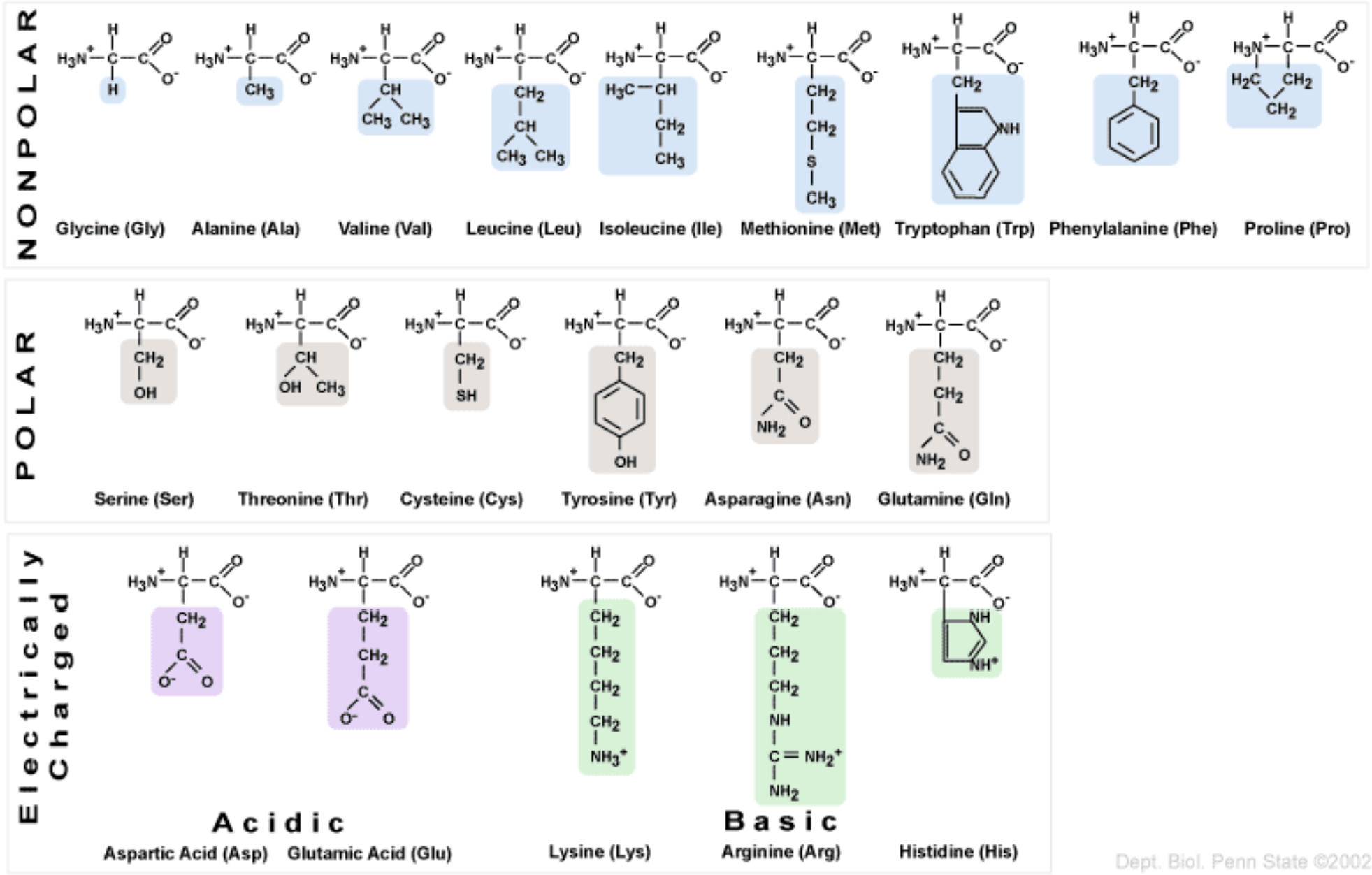
# Ionizing proteins and peptides

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

# Peptide = chain of amino acids




polypeptide chain


NONPOLAR
Glycine (Gly)
Alanine (Ala)
Valine (Val)
Leucine (Leu)
Isoleucine (Ile)
Methionine (Met)
Tryptophan (Trp)
Phenylalanine (Phe)
Proline (Pro)
POLAR
Serine (Ser)
Threonine (Thr)
Cysteine (Cys)
Tyrosine (Tyr)
Asparagine (Asn)
Glutamine (Gln)
Electrically Charged
Acidic
Aspartic Acid (Asp)
Glutamic Acid (Glu)
Basic
Lysine (Lys)
Arginine (Arg)
Histidine (His)
Dept. Biol. Penn State ©2002

# Peptide mass fingerprinting

- **This method was developed because of the availability of predicted protein sequences from genome sequencing**
- **Proteins did not have to have been previously sequenced - only that the open reading frame in the gene is known - the rest is a virtual exercise in the hands of statisticians, bioinformaticists and computers**
- **However, remember the matching is only as good as the database content - this can change**

# Protein analysis by MALDI 2010

Eppendorf tube

destain

Speed-Vac

trypsin 1:20

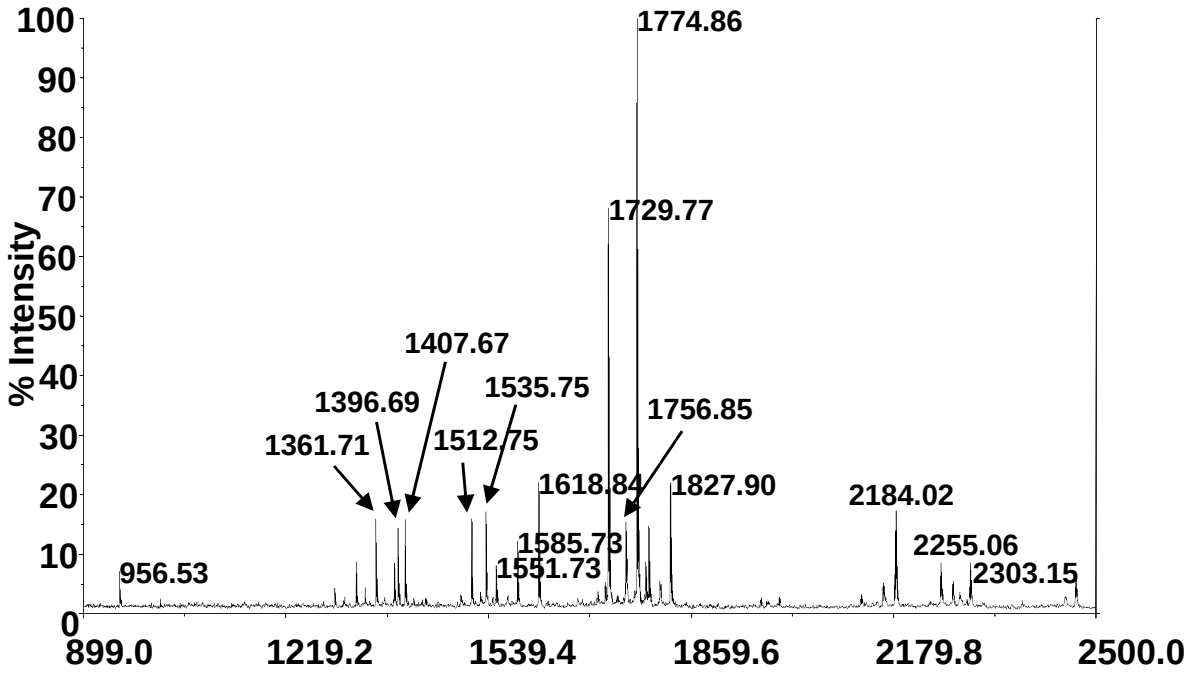
Incubate overnight

Water Bath 37°C

Peptide extraction

MALDI plate

Desalting Ziptip



# Proteolytic enzymes used to hydrolyze proteins

**The choice of enzyme largely depends on the nature of the amino acid sequence and the specific issue that is being addressed**

- **Trypsin - *cleaves at arginine and lysine residues***
- **Chymotrypsin - *cleaves hydrophobic residues***
- **Arg-C *- cleaves at arginine residues***
- **Glu-C *- cleaves at aspartate/glutamic acid residues***
- **Lys-C *- cleaves at lysine residues***
- **V8-protease *- cleaves at glutamic acid residues***
- **Pepsin *- cleaves randomly but consistently, at acid pH***

***See* http://www.abrf.org/JBT/1998/September98/sep98m_r.html**

# Searching databases with peptide masses to identify proteins

**Best site is at www.matrixscience.com**

**The program (MASCOT) can search the OWL or NCBI databases using a set of tryptic peptide masses, or the fragment ions (specified or unspecified) of peptides**

**Presents the expected set of tryptic peptides for each matched protein**

# Choice of peptidase

- **Analogous to DNA restriction enzymes**
- **Tryptic peptide fingerprinting may identify, not one, but several highly related protein candidates (e.g., actins)**
- **Inspection of the sequences may reveal that there is a difference at one residue that distinguishes between two candidates.**
- **If for instance it is a glutamate, then use of Glu-C or V8-protease may enable the two proteins to be correctly identified**
- **INSPECT sequences carefully**

# Sequence of β-lactoglobulin

MKCLLLALAL TCGAQALIVT QTMKGLDIQK
VAGTWYSLAM AASDISLLDA QSAPLRVYVE
ELKPTPEGDL EILLQKWENG ECAQKKIIAE
KTKIPAVFKI DALNENKVLV LDTDYKKYLL
FCMENSAEPE QSLACQCLVR TPEVDDEALE
KFDKALKALP MHIRLSFNPT QLEEQCHI

GLDIQK CLLLALALTCGAQALIVTQTMK VYVEELK

MK VAGTWYSLAMAASDISLLDAQSAPLR TK

ALK YLLFCMENSAEPEQSLACQCLVR K K

WENGECAQK PTPEGDLEILLQK VLVLDTDYK

FDK IPAVFK ALPMHIR IIAEK

TPEVDDEALEK IDALNENK LSFNPTQLEEQCHI

# Peptides from digestion with Glu-C

MKCLLLALALTCGAQALIVTQTMKGLD

IQKVAGTWYSLAMAASD ISLLD AQSAPLRVYVE

E LKPTPE GD LE ILLQKWE NGE CAQKKIIAE

KTKIPAVFKID ALNE NKVLVLD TD YKKYLLFCME

NSAE PE QSLACQCLVRTPE VD D E ALE KFD

KALKALPMHIRLSFNPTQLE E QCHI

# Amino acid residue masses

| | | | |
|---|---|---|---|
| Alanine | 71.037 | Leucine | 113.084 |
| Arginine | 156.101 | Lysine | 128.094 |
| Asparagine | 114.043 | Methionine | 131.040 |
| Aspartic acid | 115.027 | Phenylalanine | 147.068 |
| Cysteine | 103.009 | Proline | 97.053 |
| Glutamic acid | 129.043 | Serine | 87.032 |
| Glutamine | 128.058 | Threonine | 101.048 |
| Glycine | 57.021 | Tryptophan | 186.079 |
| Histidine | 137.059 | Tyrosine | 163.063 |
| Isoleucine | 113.084 | Valine | 99.068 |

**The m/z value of a peptide $[M+H]^+$ is the sum of the residue masses plus 18.015 for $H_2O$ plus 1.008. So, what is it for ISLLD?**

**113.084 + 87.032 + 113.084 +113.084 + 115.027 + 18.015 + 1.008 = 560.334**

# Expected peptides from trypsin and Glu-C digestion of bovine β-lactoglobulin

| | |
|---|---|
| **837.4764** | **800.4876** |
| **916.4734** | **929.5455** |
| **1064.4466** | **1003.5605** |
| **1065.5827** | **1232.6634** |
| **1245.5845** | **1259.7722** |
| **1658.7843** | **1337.6632** |
| **2275.2586** | **1447.7032** |
| **2313.2588** | **1811.8996** |
| **2647.2023** | **2307.3006** |
| **2707.3760** | **2819.5265** |

Assumes all cuts are complete, there is no oxidation of Met residues, and Cys residues are unmodified

# MASCOT Peptide Mass Fingerprint

| | | | |
|---|---|---|---|
| **Your name** | Stephen Barnes | **Email** | sbarnes.uab@gmail.com |
| **Search title** | beta globulin test | | |
| **Database** | NCBInr | | |
| **Taxonomy** | All entries | | |
| **Enzyme** | Trypsin | **Allow up to** | 0 missed cleavages |
| **Fixed modifications** | Acetyl (K)<br>Acetyl (N-term)<br>Acetyl (Protein N-term)<br>Amidated (Protein C-term)<br>Amidated (C-term) | **Variable modifications** | Acetyl (K)<br>Acetyl (N-term)<br>Acetyl (Protein N-term)<br>Amidated (Protein C-term)<br>Amidated (C-term) |
| **Protein mass** | kDa | **Peptide tol. ±** | 1.0 Da |
| **Mass values** | ◉ $MH^+$ ○ $M_r$ ○ $M\text{-}H^-$ | **Monoisotopic** | ◉ **Average** ○ |
| **Data file** | Choose File no file selected | | |
| **Query** NB Contents of this field are ignored if a data file is specified. | 837.4764<br>916.4734<br>1064.4466<br>1065.5827<br>1245.5845<br>1658.7843 | | |
| **Overview** | ☐ | **Report top** | 20 hits |
| | Start Search ... | | Reset Form |

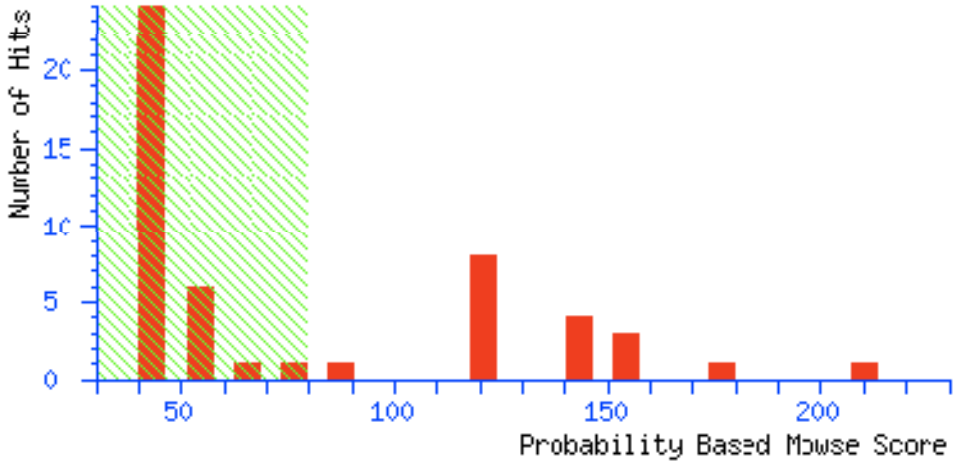
**Enter the ions here in this box**

# Mascot Search Results

```
User            : Stephen Barnes
Email           : sbarnes.uab@gmail.com
Search title    : beta globulin test
Database        : NCBInr 20061230 (4378862 sequences; 1508892933 residues)
Timestamp       : 1 Jan 2007 at 02:17:51 GMT
Top Score       : 210 for gi|87196497, lactoglobulin, beta [Bos taurus]
```

## Probability Based Mowse Score

Protein score is -10*Log(P), where P is the probability that the observed match is a random event.
Protein scores greater than 79 are significant (p<0.05).




## Protein Summary Report

Format As | Protein Summary | Help

Significance threshold p< 0.05 | Max. number of hits 20

# Protein records provided by MASCOT search

| | Accession | Mass | Score | Description |
|---|---|---|---|---|
| 1. | gi\|87196497 | 19870 | 210 | lactoglobulin, beta [Bos taurus] |
| 2. | gi\|4388846 | 18269 | 179 | Chain , Bovine Beta-Lactoglobulin Complexed With Palmitate, Lattice Z |
| 3. | gi\|223780 | 18165 | 152 | lactoglobulin beta |
| 4. | gi\|72079 | 18255 | 152 | beta-lactoglobulin - water buffalo |
| 5. | gi\|520 | 19908 | 150 | beta-lactoglobulin [Bos taurus] |
| 6. | gi\|20178290 | 20010 | 148 | Beta-lactoglobulin precursor (Beta-LG) |
| 7. | gi\|165839 | 19934 | 148 | beta-lactoglobulin |
| 8. | gi\|2194088 | 18297 | 147 | Chain A, Bovine Beta-Lactoglobulin, Lattice X |
| 9. | gi\|110612608 | 19891 | 144 | beta-lactoglobulin [Bubalus bubalis] |
| 10. | gi\|162748 | 17156 | 126 | beta-lactoglobulin |
| 11. | gi\|125912 | 19962 | 125 | Beta-lactoglobulin precursor (Beta-LG) |
| 12. | gi\|7245834 | 18363 | 124 | Chain A, Structural Changes Accompanying Ph-Induced Dissociation Of The |
| 13. | gi\|229460 | 18355 | 124 | lactoglobulin beta |
| 14. | gi\|4388939 | 18355 | 124 | Chain , Structural Basis Of The Tanford Transition Of Bovine Beta-Lacto |
| 15. | gi\|49259423 | 18339 | 124 | Chain X, The Cys121ser Mutant Of Beta-Lactoglobulin |
| 16. | gi\|54037712 | 18139 | 120 | Beta-lactoglobulin (Beta-LG) |
| 17. | gi\|57164367 | 19908 | 117 | beta-lactoglobulin [Ovis aries] |
| 18. | gi\|90108547 | 18264 | 82 | Chain A, Reindeer Beta-Lactoglobulin |
| 19. | gi\|71980384 | 20035 | 80 | beta-lactoglobulin [Rangifer tarandus tarandus] |
| 20. | gi\|26352113 | 13020 | 60 | unnamed protein product [Mus musculus] |

# Comparison of observed and predicted tryptic peptides

gi|87196497 Mass: 19870 Score: 210 Expect: 4.4e-15 Queries matched: 10

lactoglobulin, beta [Bos taurus]

| Observed | Mr(expt) | Mr(calc) | Delta | Start | | End | Miss | Peptide |
|---|---|---|---|---|---|---|---|---|
| 837.4764 | 836.4691 | 836.4691 | 0.0001 | 158 | - | 164 | 0 | K.ALPMHIR.L |
| 916.4734 | 915.4661 | 915.4661 | -0.0000 | 100 | - | 107 | 0 | K.IDALNENK.V |
| 1064.4466 | 1063.4393 | 1063.4393 | 0.0001 | 77 | - | 85 | 0 | K.WENGECAQK.K |
| 1065.5827 | 1064.5754 | 1064.5753 | 0.0001 | 108 | - | 116 | 0 | K.VLVLDTDYK.K |
| 1245.5845 | 1244.5772 | 1244.5772 | 0.0000 | 141 | - | 151 | 0 | R.TPEVDDEALEK.F |
| 1658.7843 | 1657.7770 | 1657.7770 | 0.0000 | 165 | - | 178 | 0 | R.LSFNPTQLEEQCHI.- |
| 2275.2586 | 2274.2513 | 2274.2513 | 0.0000 | 3 | - | 24 | 0 | K.CLLLALALTCGAQALIVTQTMK.G |
| 2313.2588 | 2312.2515 | 2312.2515 | 0.0001 | 57 | - | 76 | 0 | R.VYVEELKPTPEGDLEILLQK.W |
| 2647.2023 | 2646.1950 | 2646.1950 | 0.0001 | 118 | - | 140 | 0 | K.YLLFCMENSAEPEQSLACQCLVR.T |
| 2707.3760 | 2706.3687 | 2706.3686 | 0.0001 | 31 | - | 56 | 0 | K.VAGTWYSLAMAASDISLLDAQSAPLR.V |

# Search against SwissProt database

| | Accession | Mass | Score | Description |
|---|---|---|---|---|
| 1. | LACB_BOVIN | 19870 | 271 | Beta-lactoglobulin precursor (Beta-LG) (Allergen Bos d 5) - Bos taurus (Bovine) |
| 2. | LACB_BUBBU | 20010 | 197 | Beta-lactoglobulin precursor (Beta-LG) - Bubalus bubalis (Domestic water buffalo) |
| 3. | LACB_CAPHI | 19962 | 135 | Beta-lactoglobulin precursor (Beta-LG) - Capra hircus (Goat) |
| 4. | LACB_OVIMU | 18139 | 104 | Beta-lactoglobulin (Beta-LG) - Ovis orientalis musimon (Mouflon) |
| 5. | LACB_SHEEP | 19908 | 102 | Beta-lactoglobulin-1/B precursor (Beta-LG) - Ovis aries (Sheep) |
| 6. | YKH5_YEAST | 52055 | 41 | Hypothetical 52.1 kDa protein in SMY1-MUD2 intergenic region - Saccharomyces cerevi |
| 7. | TCPE_VIBCH | 16247 | 40 | Toxin coregulated pilus biosynthesis protein H (TCP pilus biosynthesis protein tcpH |
| 8. | POLG_DEN22 | 54291 | 39 | Genome polyprotein [Contains: Envelope protein E] (Fragment) - Dengue virus type 2 |
| 9. | PFPI_PYRKO | 18404 | 38 | Intracellular protease 1 (EC 3.2.-.-) (Intracellular protease I) - Pyrococcus kodak |
| 10. | VIRB9_AGRT5 | 32181 | 38 | Protein virB9 precursor - Agrobacterium tumefaciens (strain C58 / ATCC 33970) |
| 11. | VPS71_YEAST | 32001 | 36 | Vacuolar protein sorting-associated protein 71 (SWR complex protein 6) - Saccharomy |
| 12. | YHR7_YEAST | 71812 | 34 | TPR repeat-containing protein YHR117W - Saccharomyces cerevisiae (Baker's yeast) |
| 13. | LGB_LOTJA | 15745 | 34 | Leghemoglobin - Lotus japonicus |
| 14. | YH13_VACCV | 13615 | 34 | Hypothetical 13.6 kDa HindIII-C protein - Vaccinia virus (strain Western Reserve / |
| 15. | MKT1_YEAST | 94435 | 34 | Protein MKT1 - Saccharomyces cerevisiae (Baker's yeast) |
| 16. | H2A1_LILLO | 12165 | 34 | Histone H2A.1 (GcH2A) - Lilium longiflorum (Trumpet lily) |
| 17. | RS12_BRUA2 | 13863 | 33 | 30S ribosomal protein S12 - Brucella abortus (strain 2308) |
| 18. | RS12_BRUAB | 13863 | 33 | 30S ribosomal protein S12 - Brucella abortus |
| 19. | RS12_BRUME | 13863 | 33 | 30S ribosomal protein S12 - Brucella melitensis |
| 20. | RS12_BRUSU | 13863 | 33 | 30S ribosomal protein S12 - Brucella suis |

# Things to consider when doing peptide mass fingerprinting

- **Proteins can be oxidized both biologically (real data) and during the workup**
- **Treat the protein or the peptide digest with a reagent that reacts with Cys sulhydryl groups - e.g., iodoacetamide, iodoacetic acid, N-ethylmaleimide or 4-vinylpyridine. Cysteines may also have reacted with acrylamide in the gel.**
- **Set the options in the fixed or variable modification boxes before searching**
- **Allow for at least one missed cleavage - trypsin does not cut when Lys or Arg are followed by a Pro residue**

# Other web sites for peptide analysis

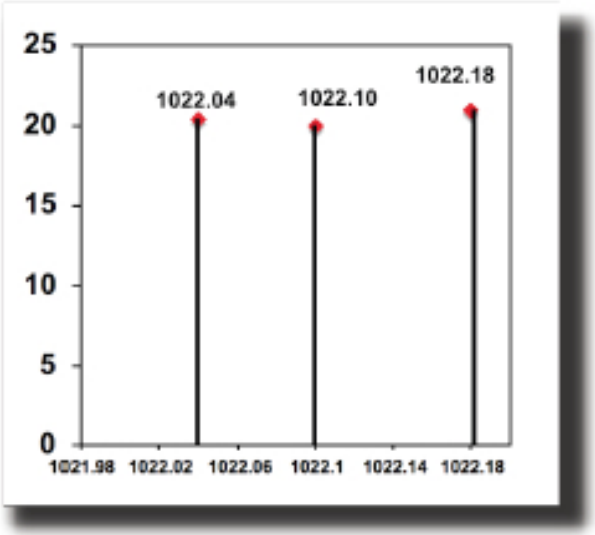
- http://prowl.rockefeller.edu/
  - **Choose ProFound**
- http://prospector.ucsf.edu/
  - **Choose MS-fit**

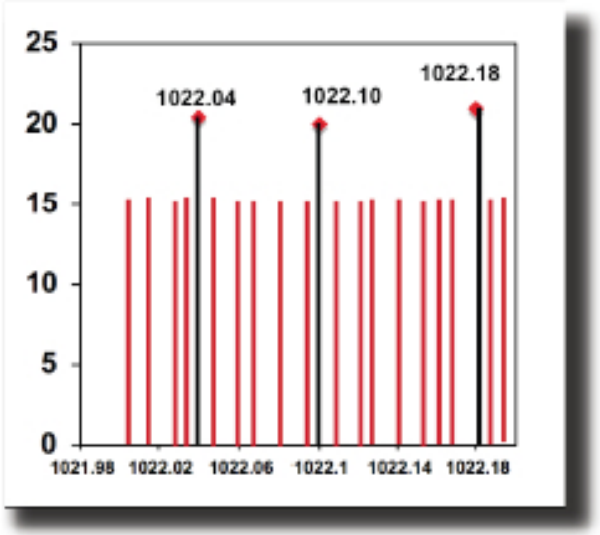
# Further information on identified protein

- **Take the protein identifier number:**
  - **For bovine β-lactoglobulin it is gi|520**
  - **Go to http://www.ncbi.nlm.nih.gov**
  - **Under protein, paste in the gi number**
  - **A link to the protein will appear**
  - **Click on Blink - this is similar to BLAST, but better**
  - **Scroll down the list and select 1CJ5**
  - **Click on image of protein structure**
  - **To view a 3D-image of the protein, first download Cn3D from the NCBI site or RasMol**
    - **Bring a picture of beta-lactoglobulin to the next class**

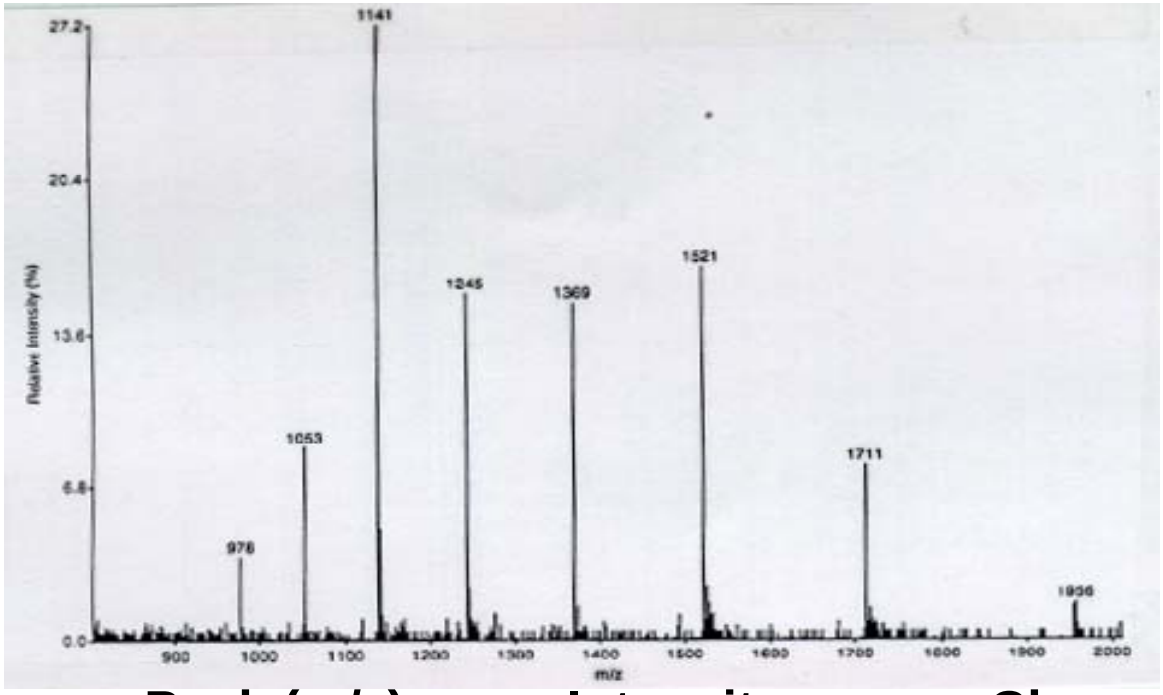
# Blurring of protein space

- **Identification using MALDI-TOF with MASCOT depends on:**
  - Number of peptides recognized as being part of the protein
  - The mass accuracy of the peptides that are recognized
  - Pre-2000, an accuracy of better than 0.05 Da in a 1000 Da peptide (i.e., 50 ppm) was sufficient to distinguish the unknown protein from the other proteins in the databases at that time
  - Now, the protein information space has become more dense and MALDI-TOF is no longer adequate
  - Previously identified proteins may not be correct



# 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 |

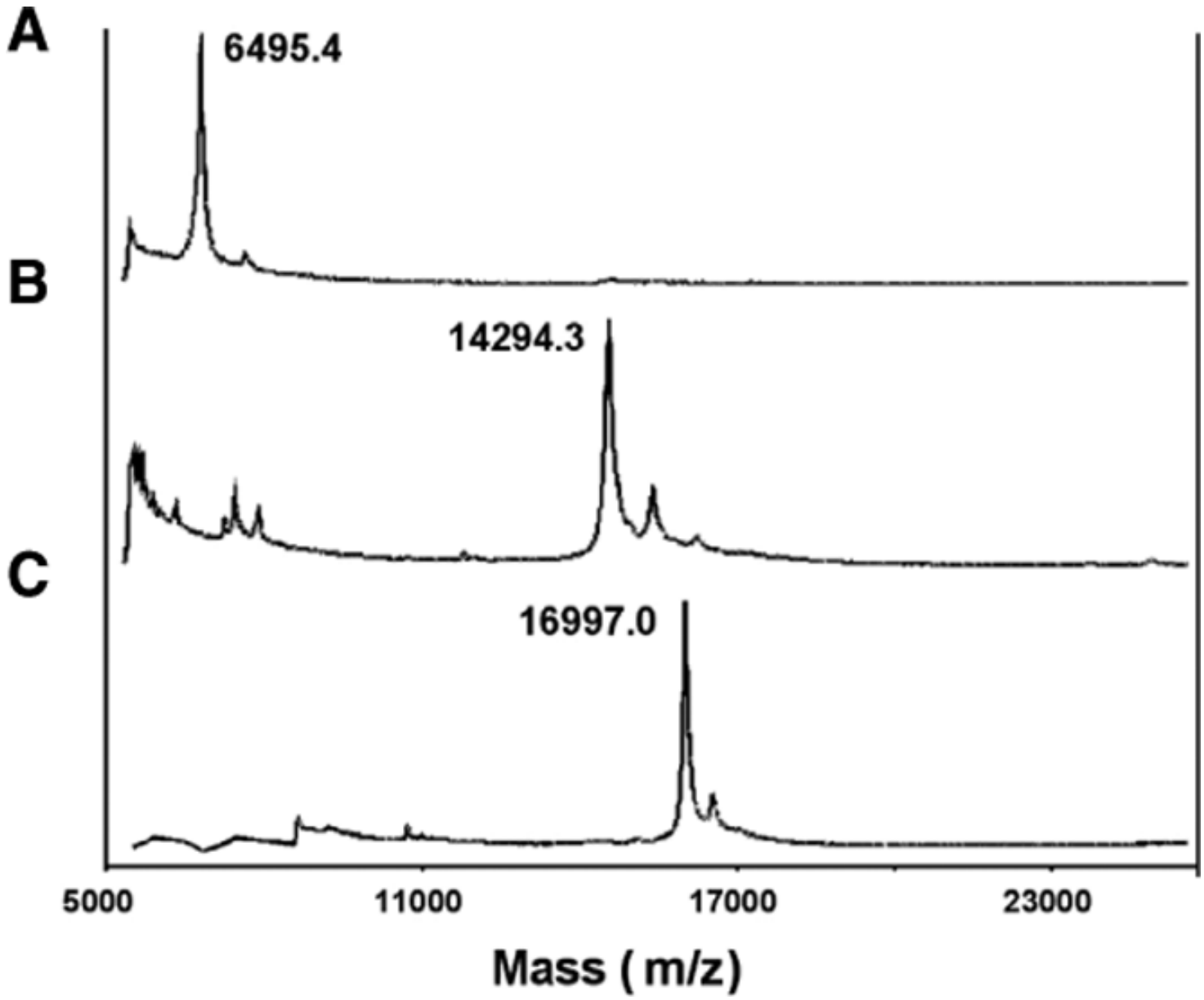
# Calculation of molecular weights and ion states

- **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**

Aprotinin

Lysozyme

Myoglobin

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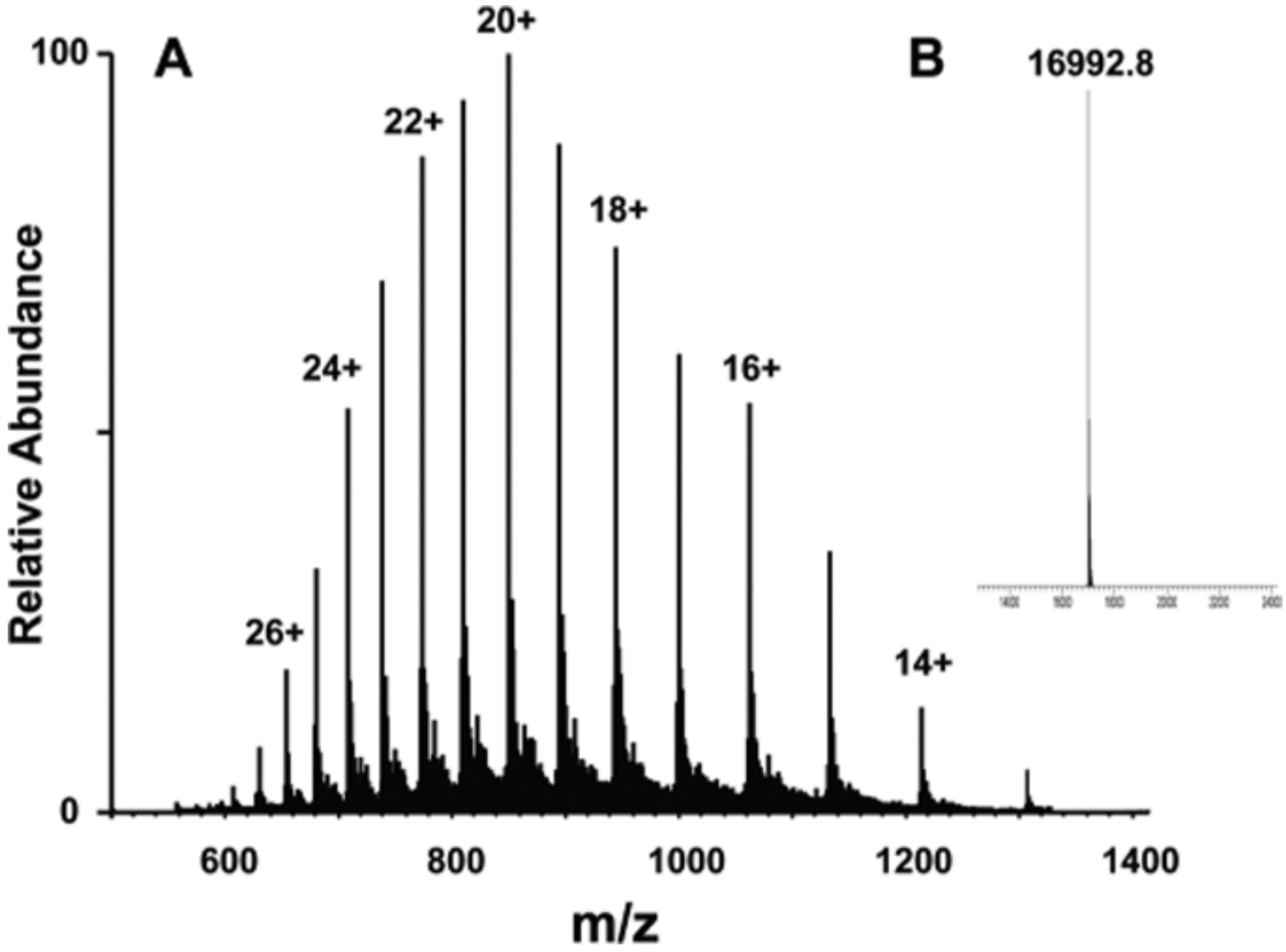
Myoglobin

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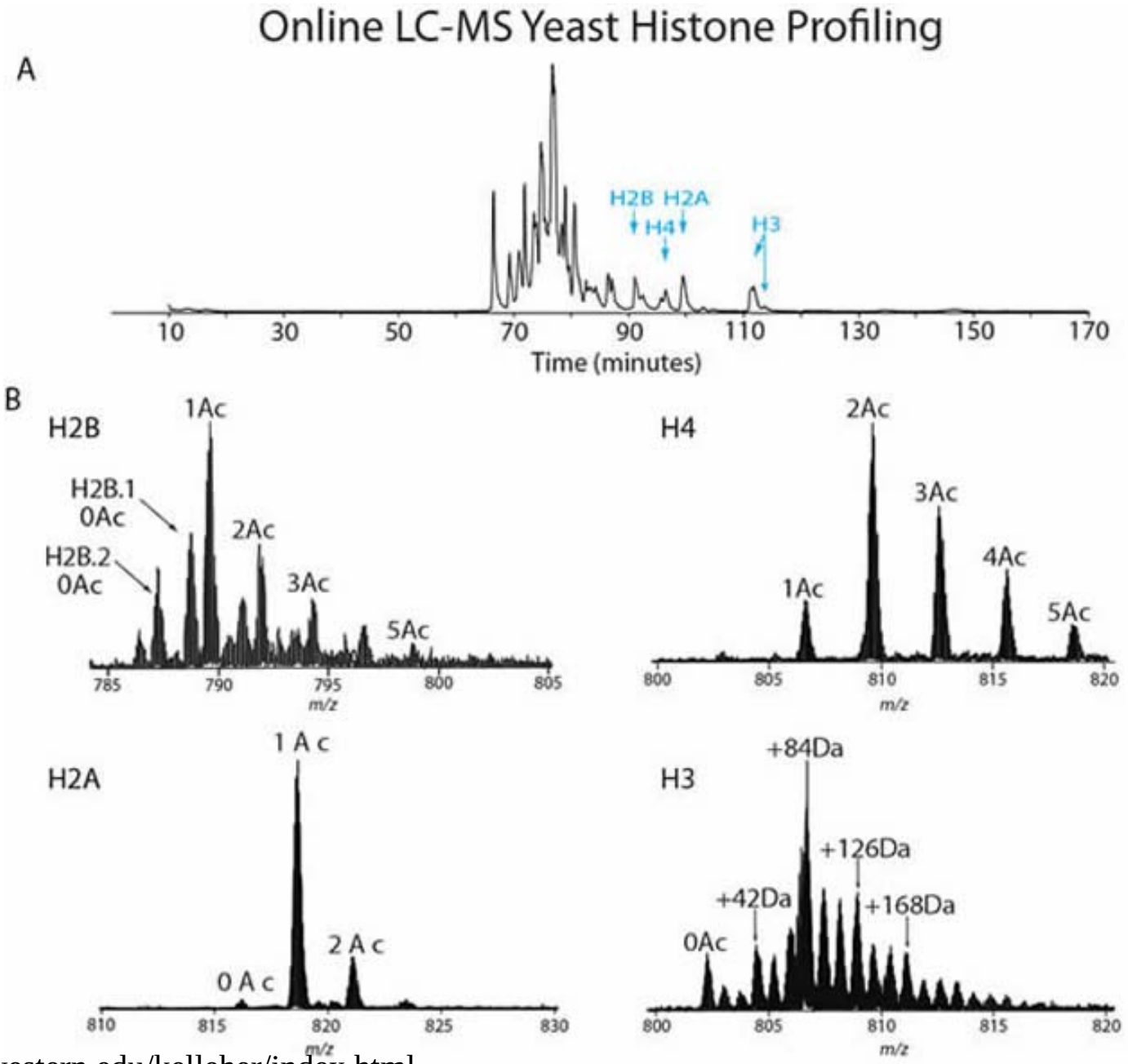
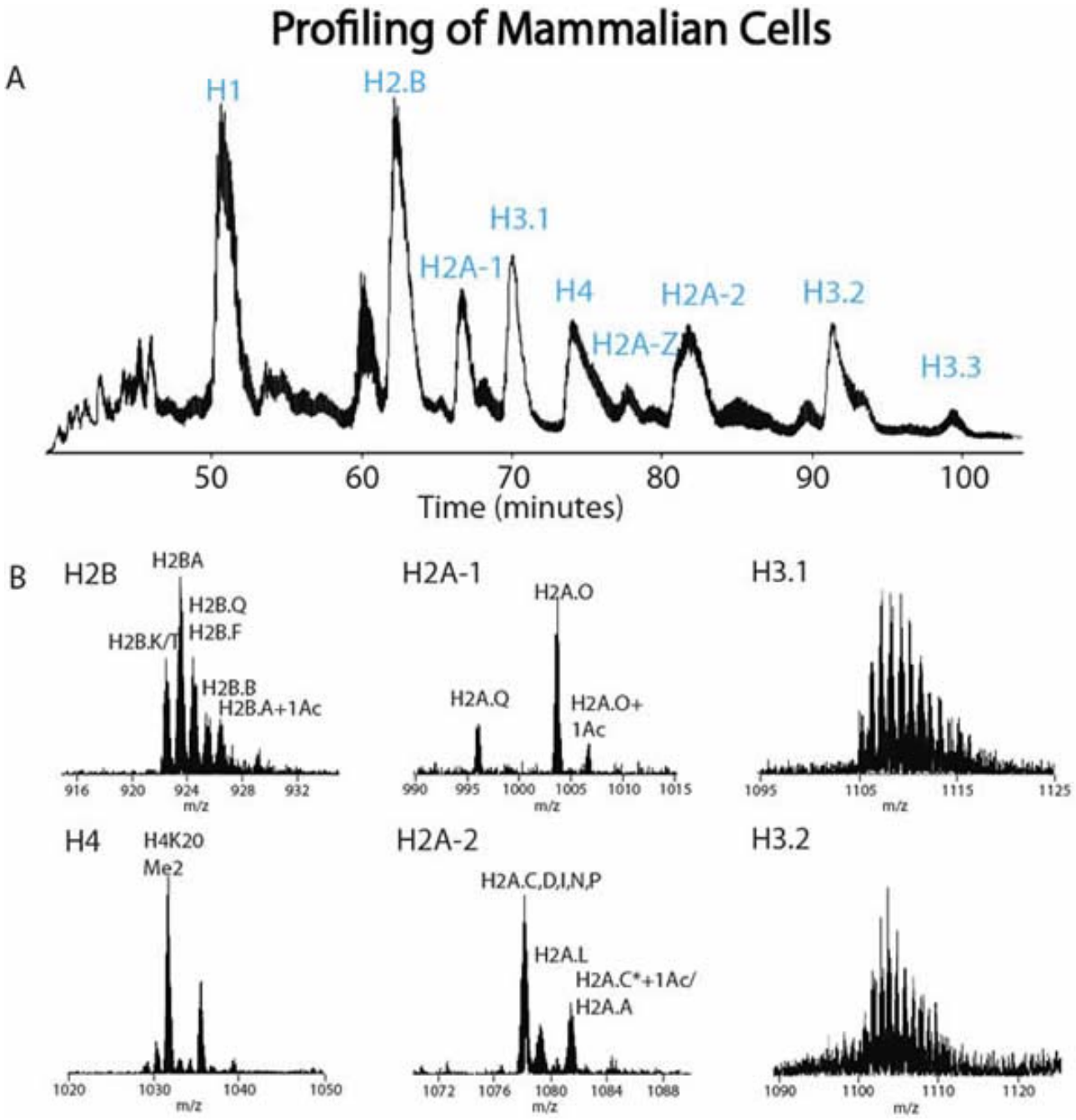



Kelleher, Northwestern Univ.
http://groups.molbiosci.northwestern.edu/kelleher/index.html



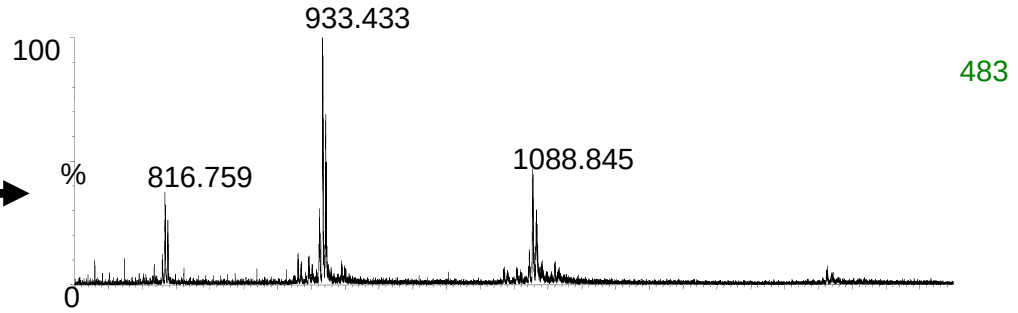

Kelleher, Northwestern Univ.
http://groups.molbiosci.northwestern.edu/kelleher/index.html

# Deconvolution of MS data

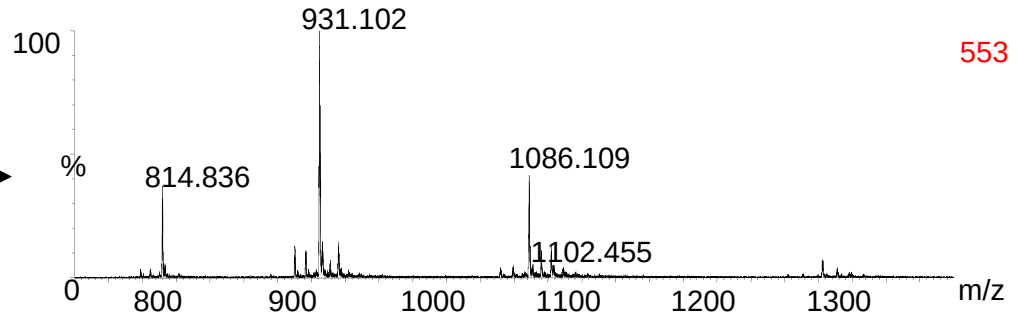
- **When several proteins are present, then their multiply charged ion clusters overlap**

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

Oxidized Aprotinin ESI mass spectrum



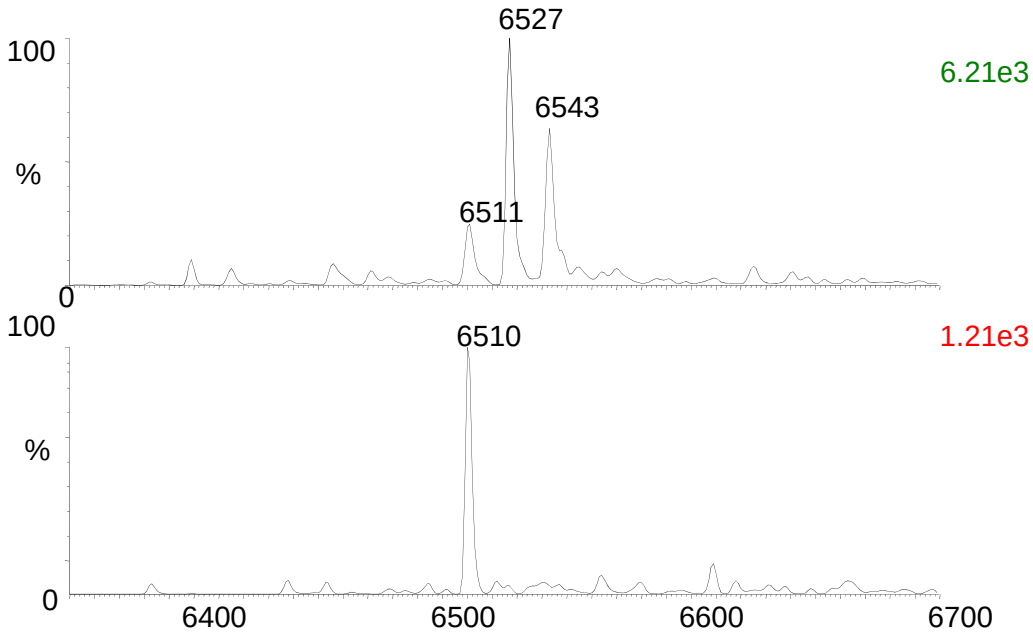

Control Aprotinin ESI mass spectrum




Deconvoluted mass spectra

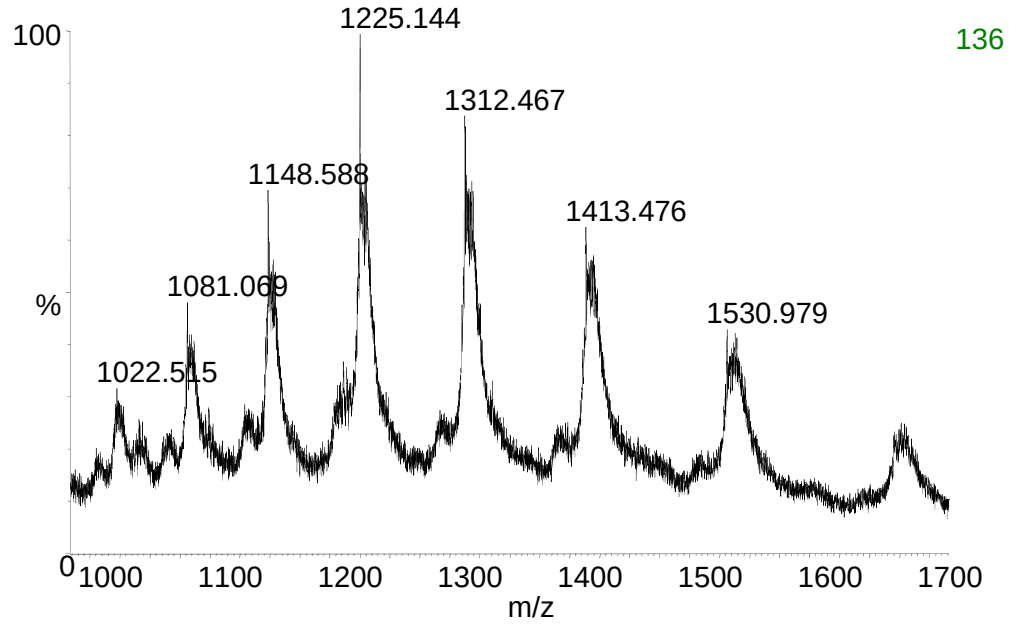
Oxidized

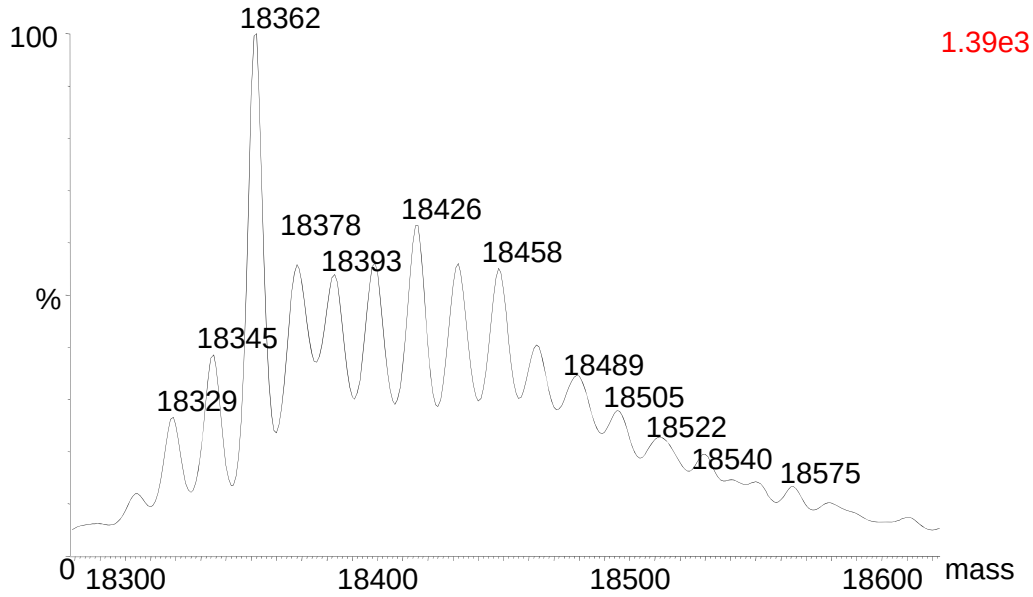
Control




Junlong Shao

# Deconvolution of oxidized forms of β-lactoglobulin

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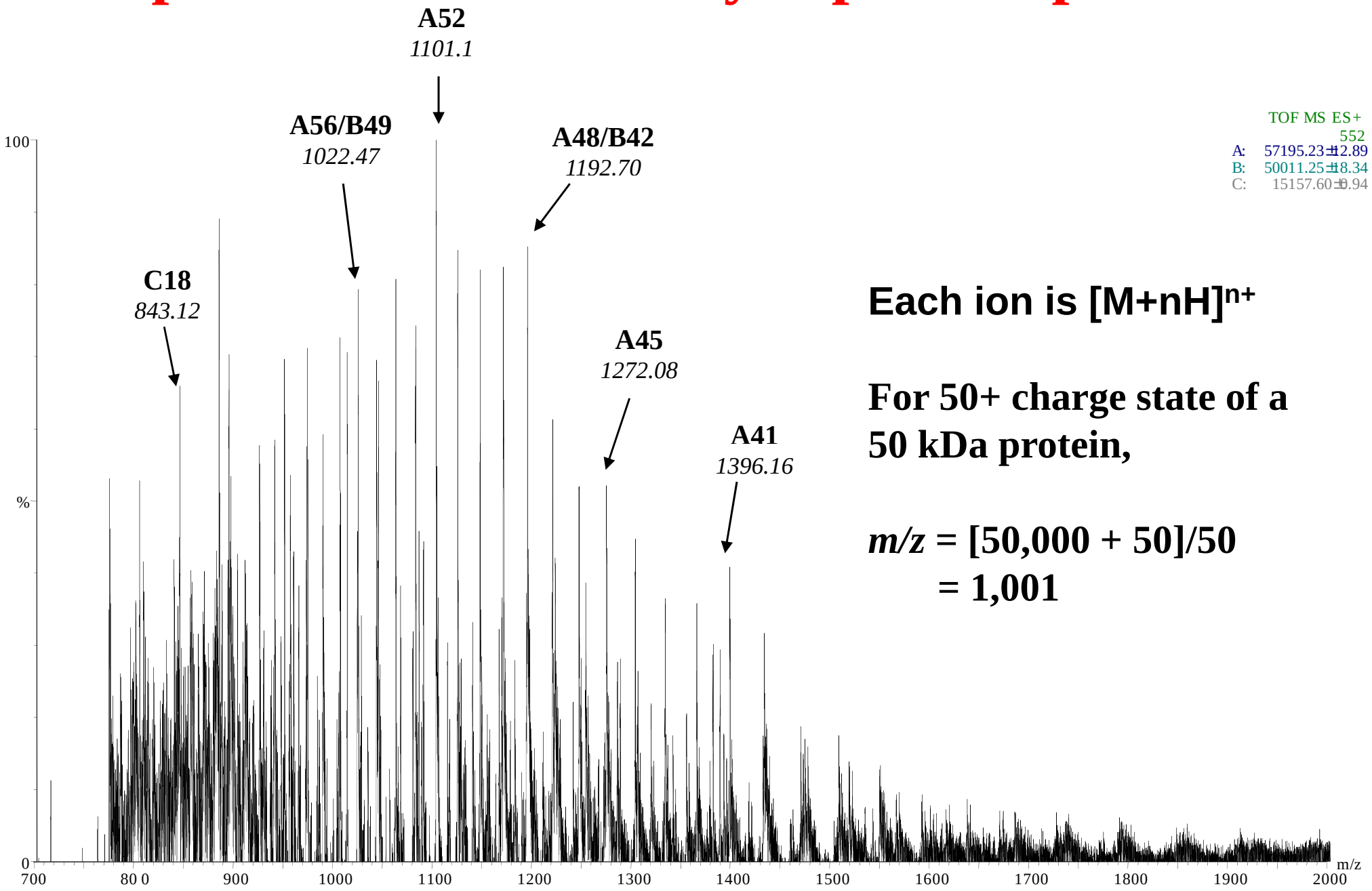
# ESI spectrum of bacterially expressed protein




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

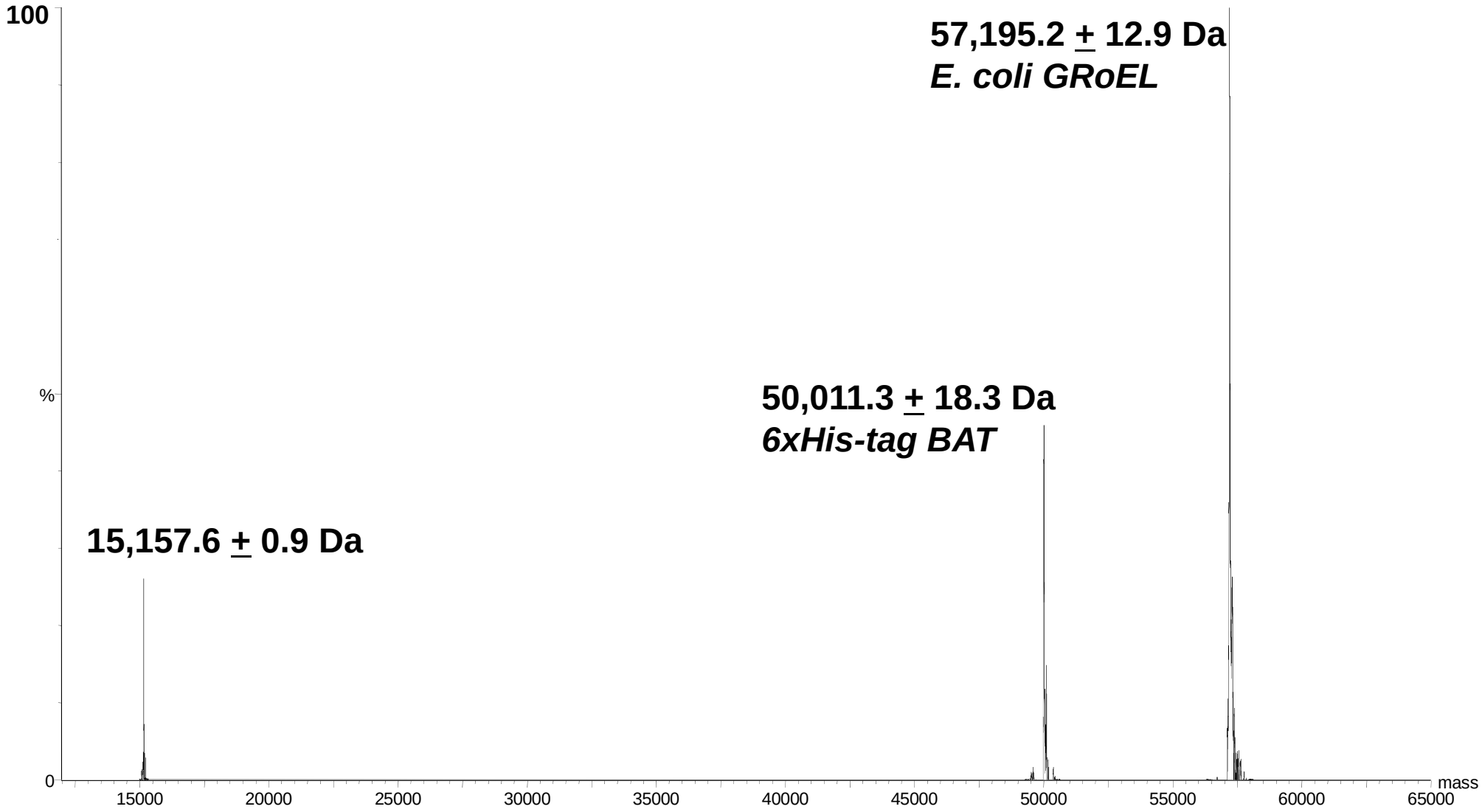
**For 50+ charge state of a 50 kDa protein,**

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



***Courtesy of Mindan Sfakianos***

# *MaxEnt* deconvolution of MWs

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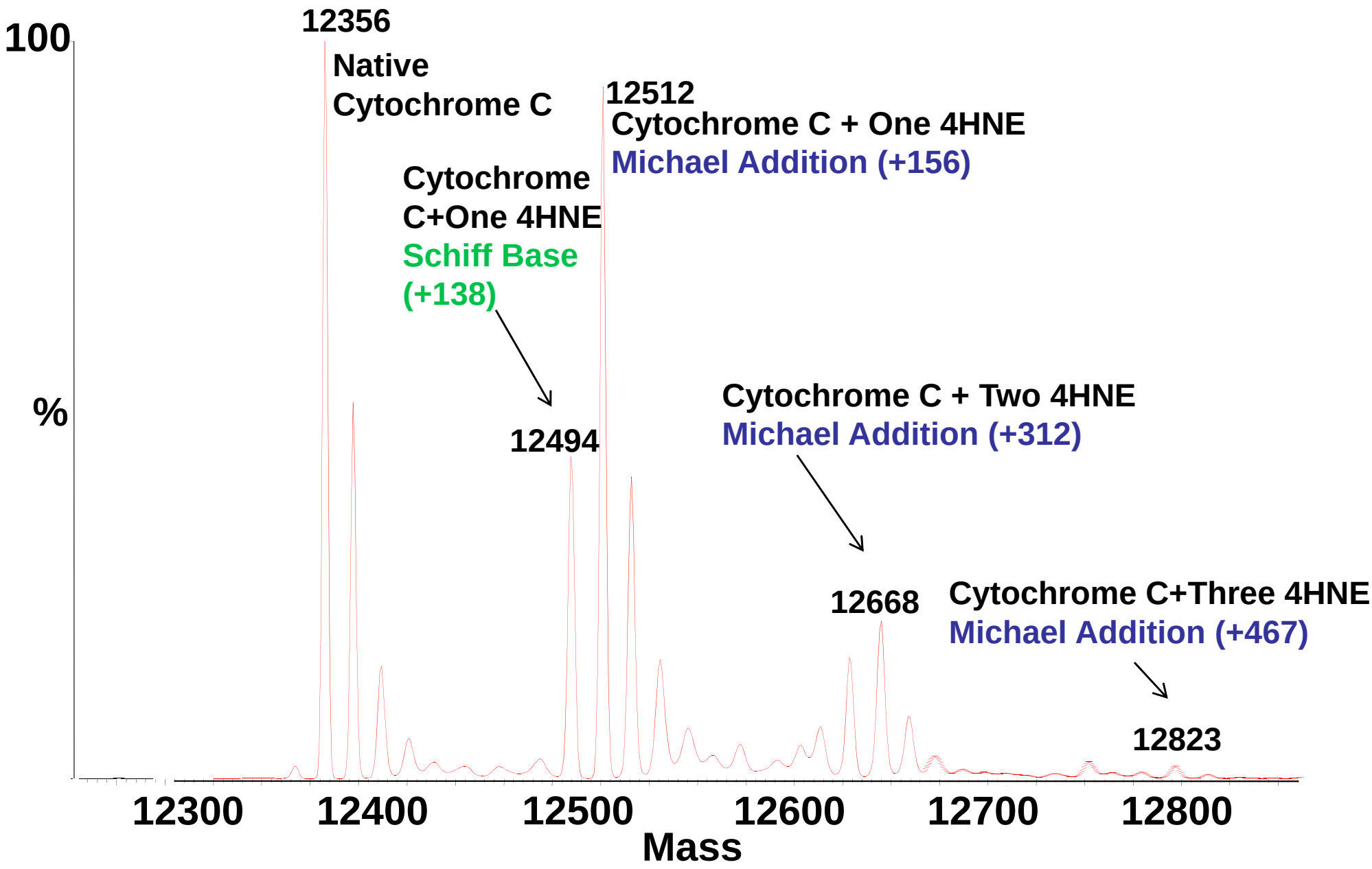
***Courtesy of Mindan Sfakianos***

# ESI-MS of 4HNE-Modified Cytochrome C

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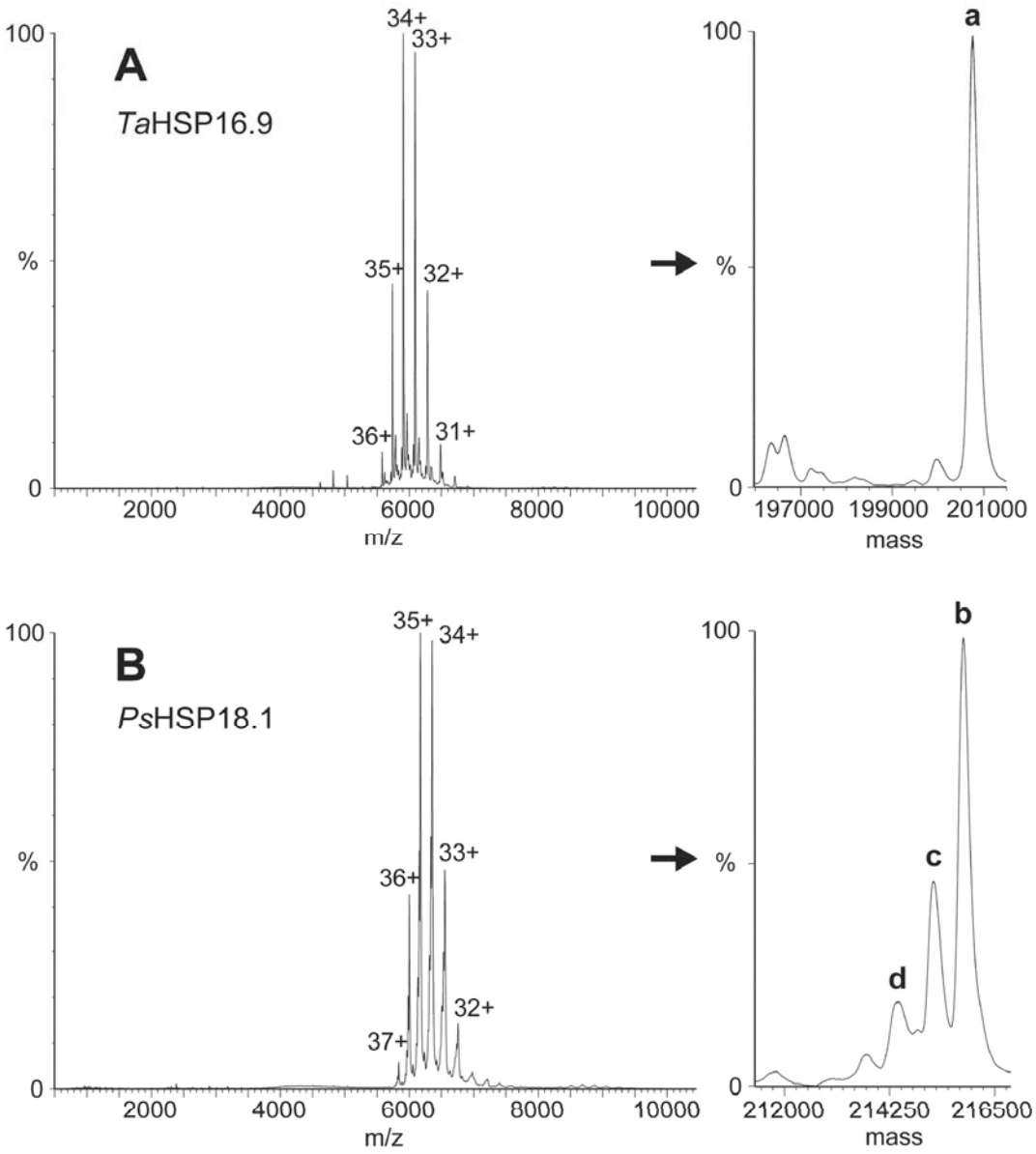
# Summary of determining MW by ESI

- **The multiple charge states of a protein allow:**
  - **Mol Wt of large proteins to be estimated**
  - **It's a 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**

# Studying high molecular weight complexes by ESI

- **Most instrument ESI interfaces have a limited *m/z* range - up to 3,000**
- **In protein complexes water, and hence H+ ions, is "squeezed" out, thereby substantially increasing observed *m/z* values**
- **Interfaces that pass ions with *m/z* values above 10,000 have been designed**

# nanoESI-MS of HMW complexes of small heat shock proteins




**Note the large *m/z* values (6,000-7,000) for the observed ions**

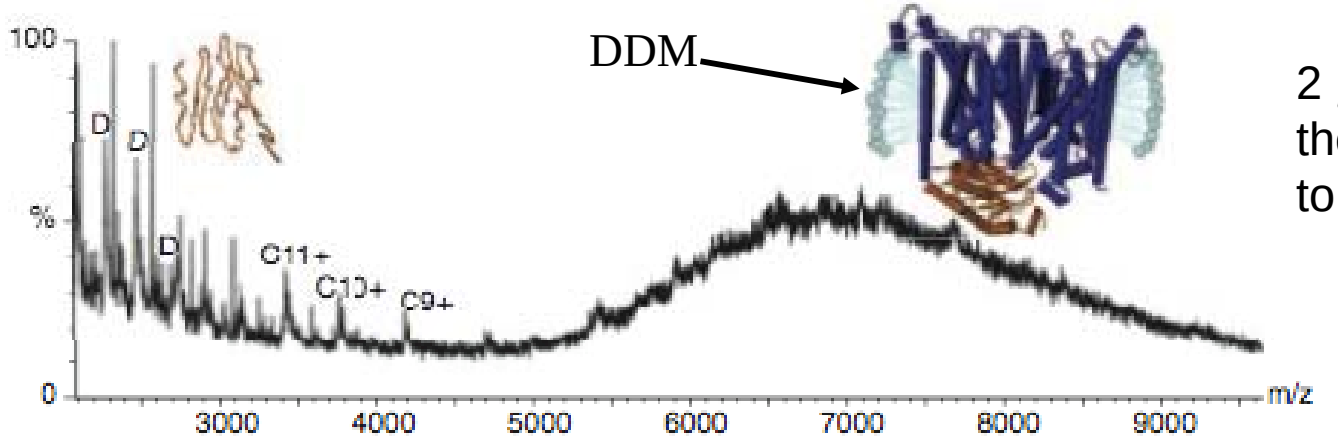
**The ESI data were deconvoluted to reveal the distribution of the masses of the complexes**

**Sobbott et al., J Biol Chem 277:38921**

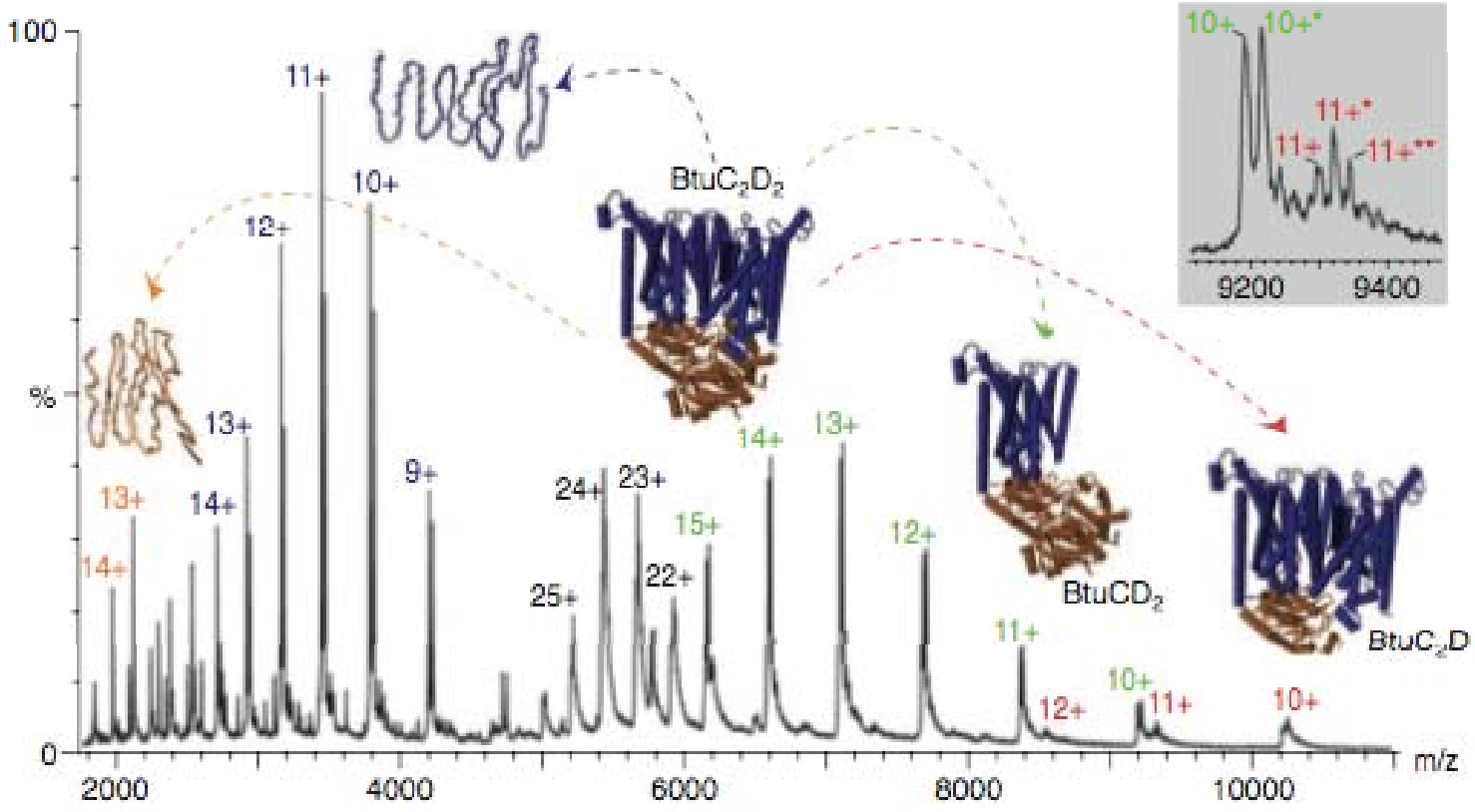
# Studying intact membrane protein complexes by gas-phase mass spectrometry (top-down analysis)

- **Electrosprayed in neutral detergent**
  - **Dodecyl maltoside (DDM)**
- **See Barrera et al., Science 321: 243-246, 2008**
- **Carried out on Waters Qtof II with modification of the ESI interface**
- **Requires high voltage (Δ200 V) to be applied across the interface and collision cell**

# Effect of increasing voltage on $BtuC_2D_2$ membrane complexes observed in nanoMS



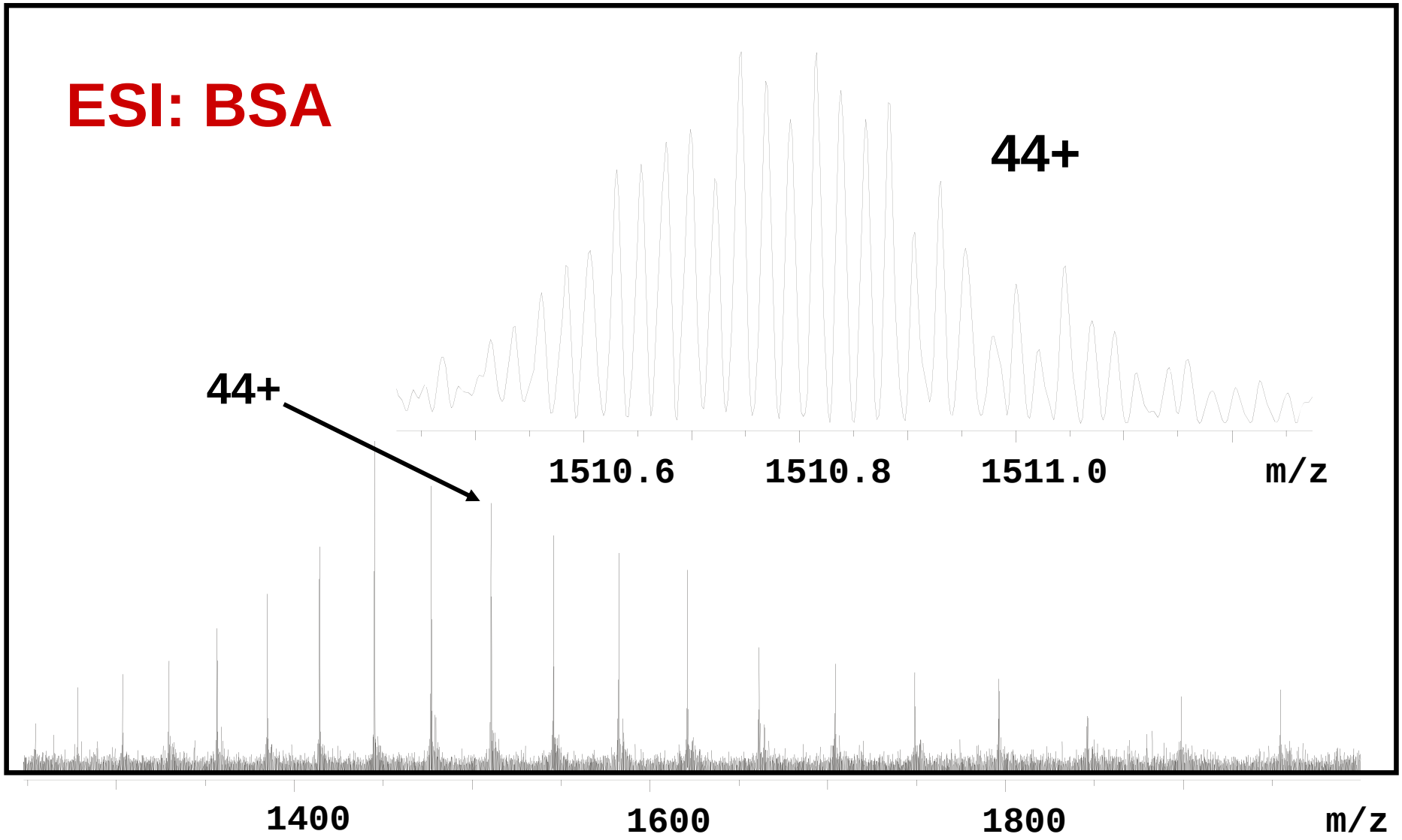

2 μl of 5 μM solution of the complex subjected to nanoelectrospray



# 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

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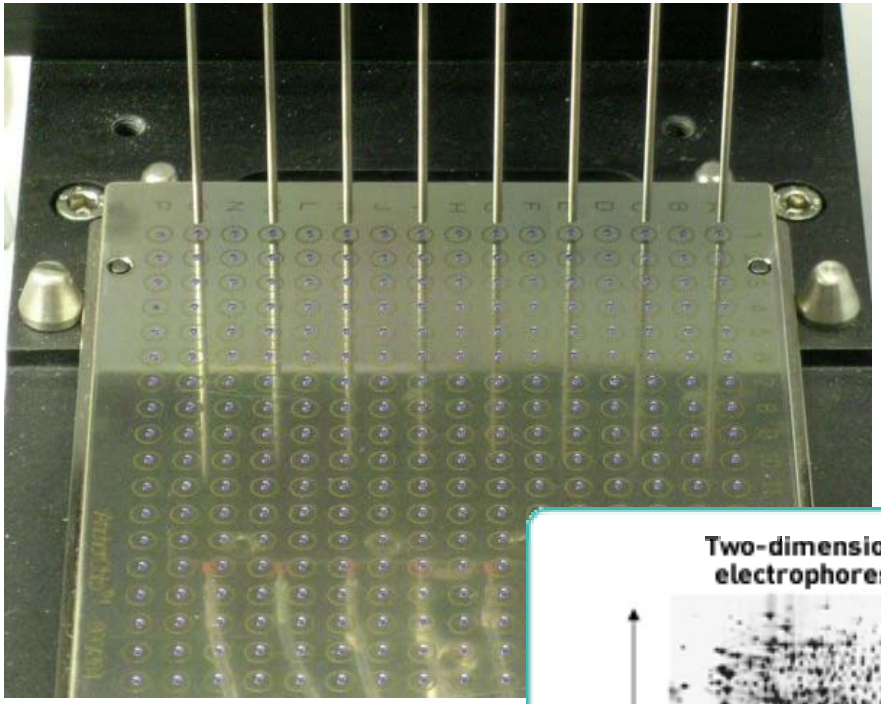
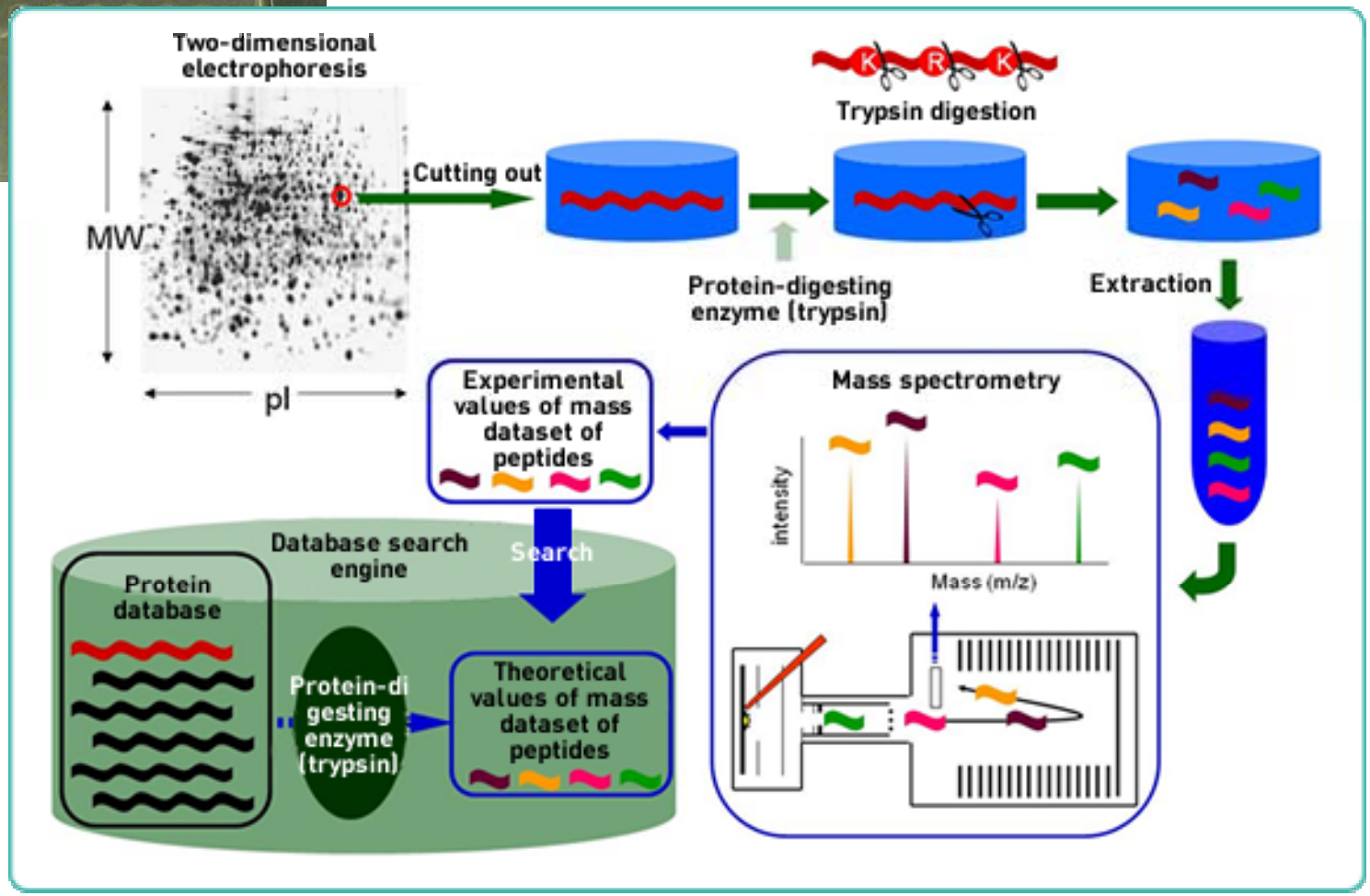
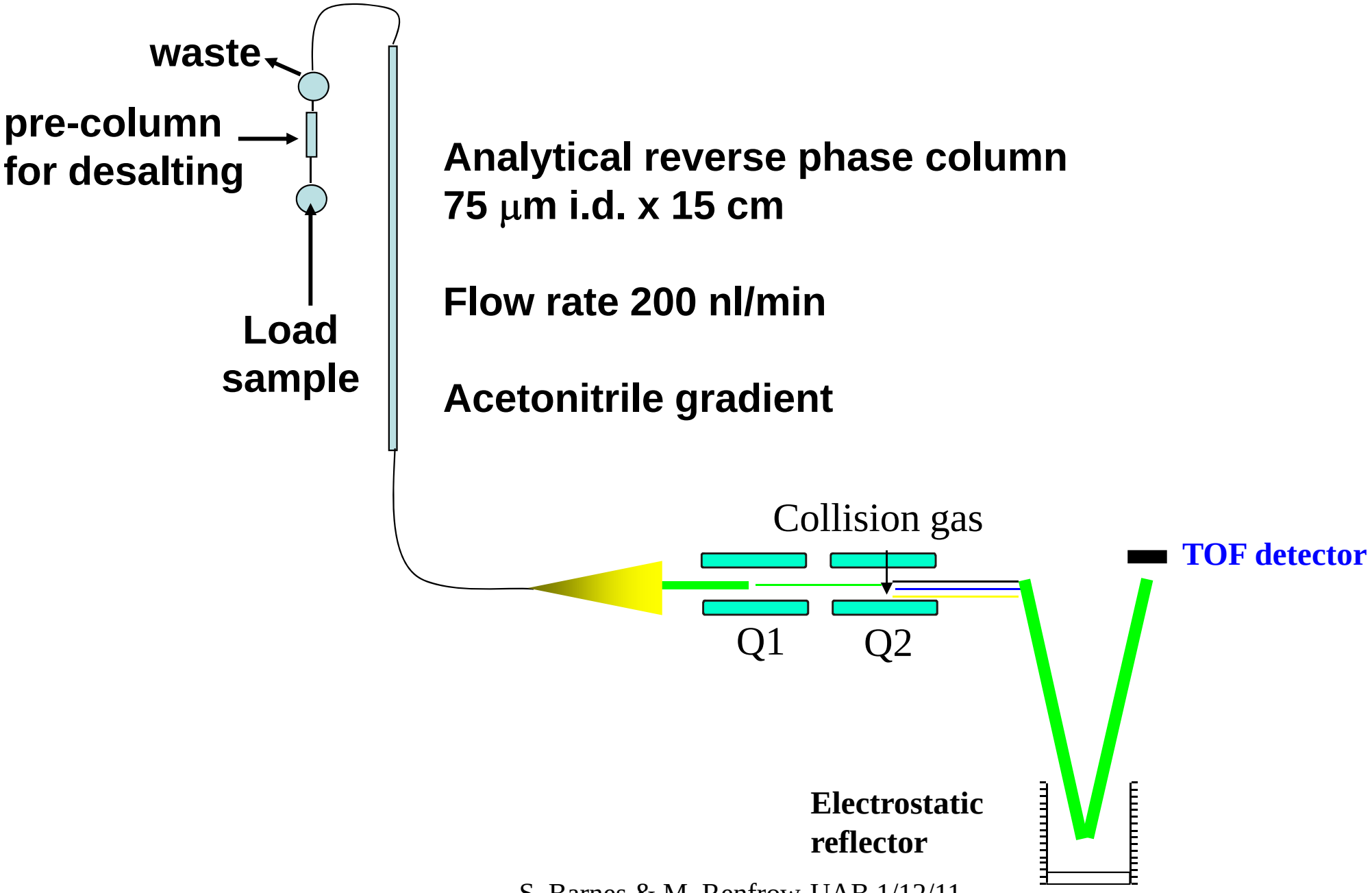
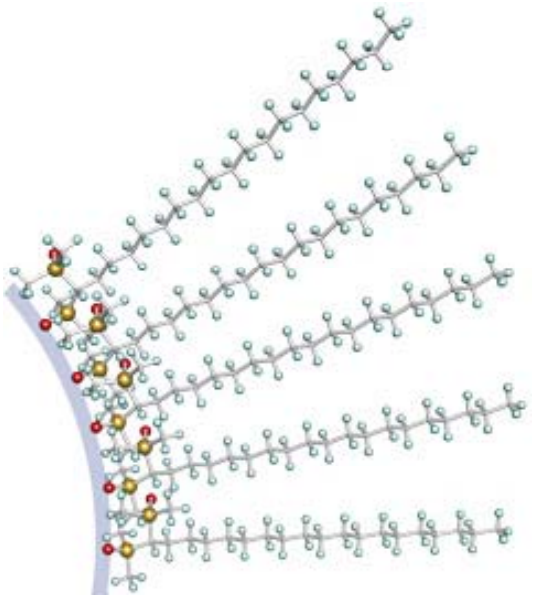
Bruker Daltonics

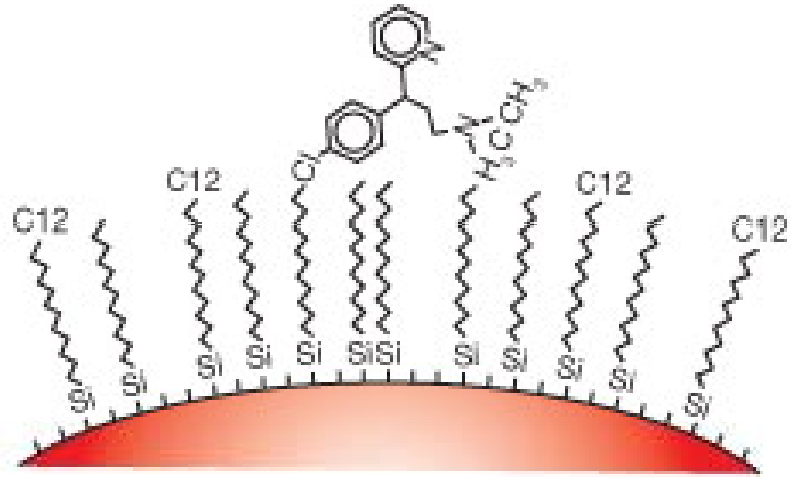


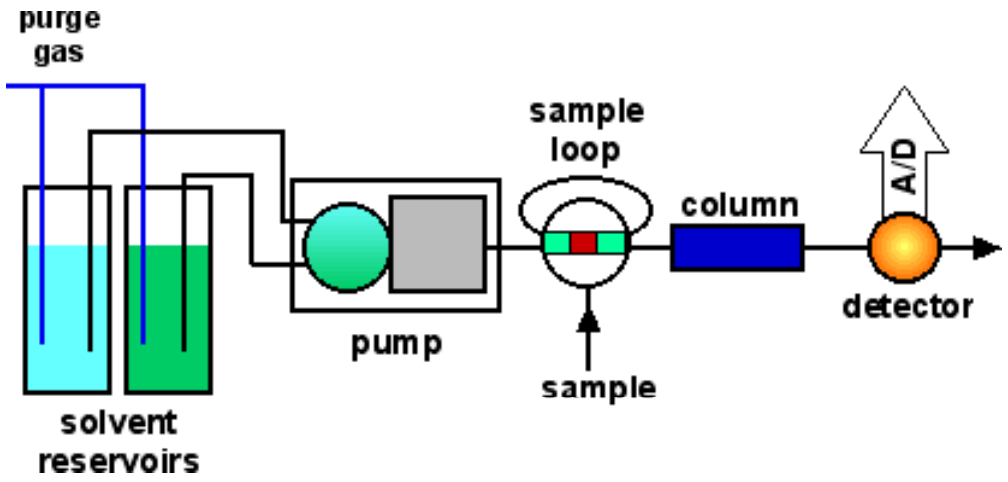

Two-dimensional electrophoresis
MW
pI
Cutting out
Trypsin digestion
K
R
K
Protein-digesting enzyme (trypsin)
Extraction
Experimental values of mass dataset of peptides
Mass spectrometry
intensity
Mass (m/z)
Database search engine
Search
Protein database
Protein-digesting enzyme (trypsin)
Theoretical values of mass dataset of peptides

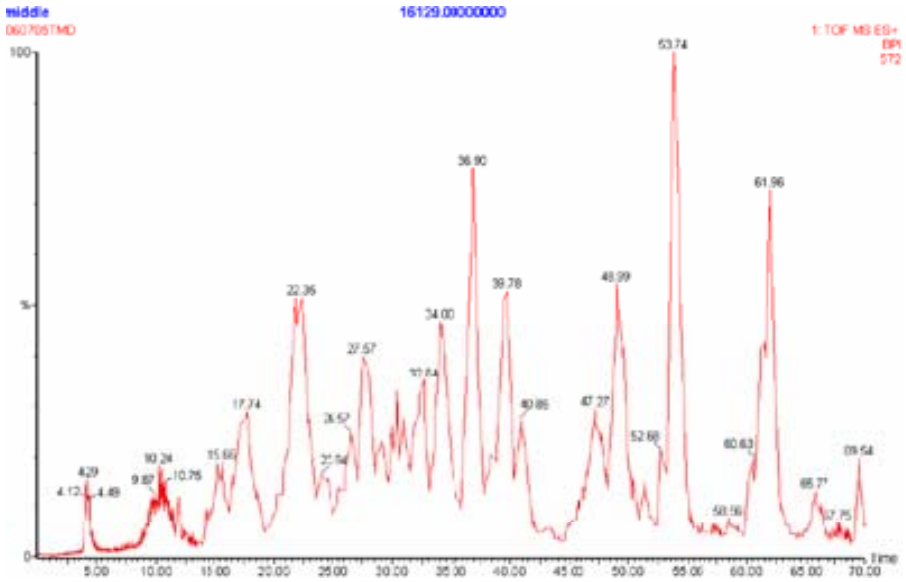
# LC-MS of peptide mixtures






C12
C12
C12
C12
Cl
Si
Si
Si
Si
Si
SiSi
Si
Si
Si
Si
Si


purge
gas
sample
loop
column
A/D
pump
detector
sample
solvent
reservoirs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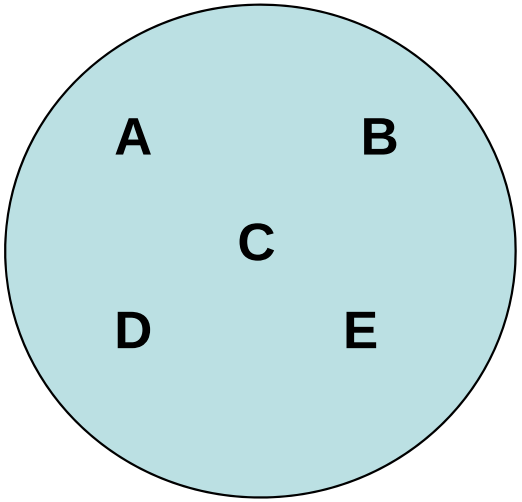
# The MUDPIT approach

## MUlti-Dimensional Protein Identification Technology

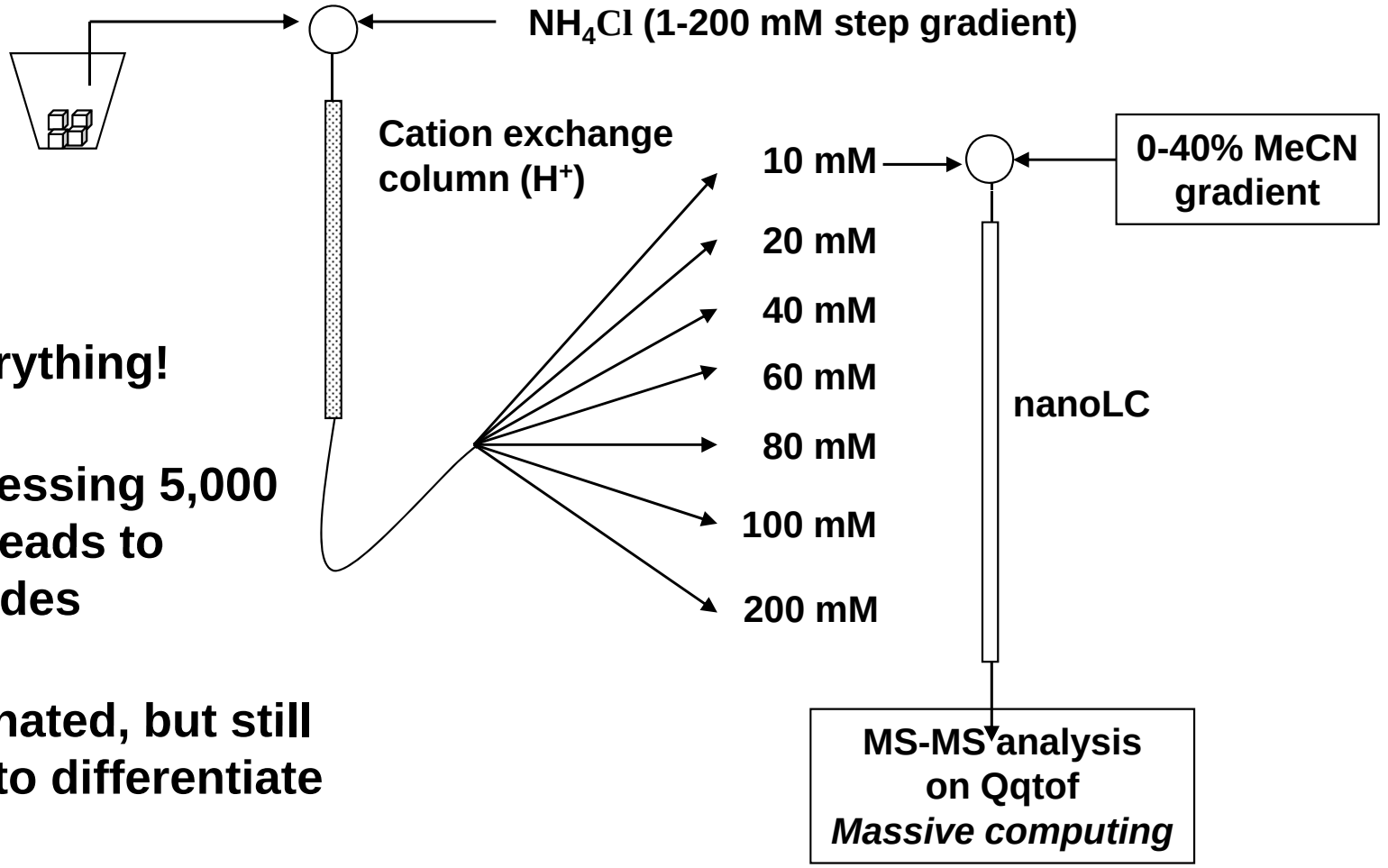



**Digest with trypsin without any protein separation**

**20-50 tryptic peptides per protein (100-250 peptides in this example)**

**Can they be resolved?**

# MUDPIT - MUlti-Dimensional Protein Identification Technology

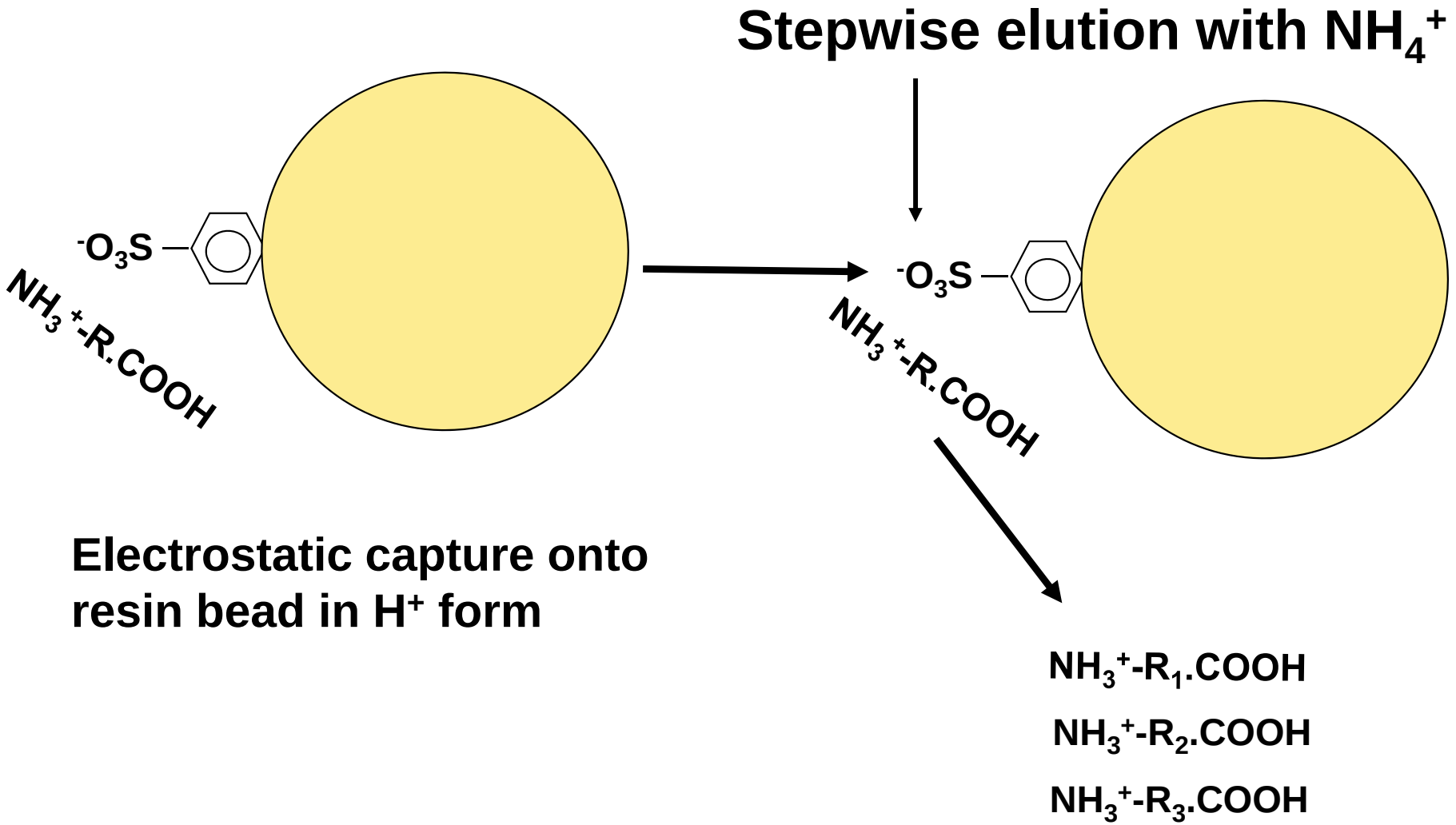



- Hydrolyze everything!
- For a cell expressing 5,000 proteins, this leads to >100,000 peptides
- Can be fractionated, but still 10,000-20,000 to differentiate
- Enormous bioinformatics problem

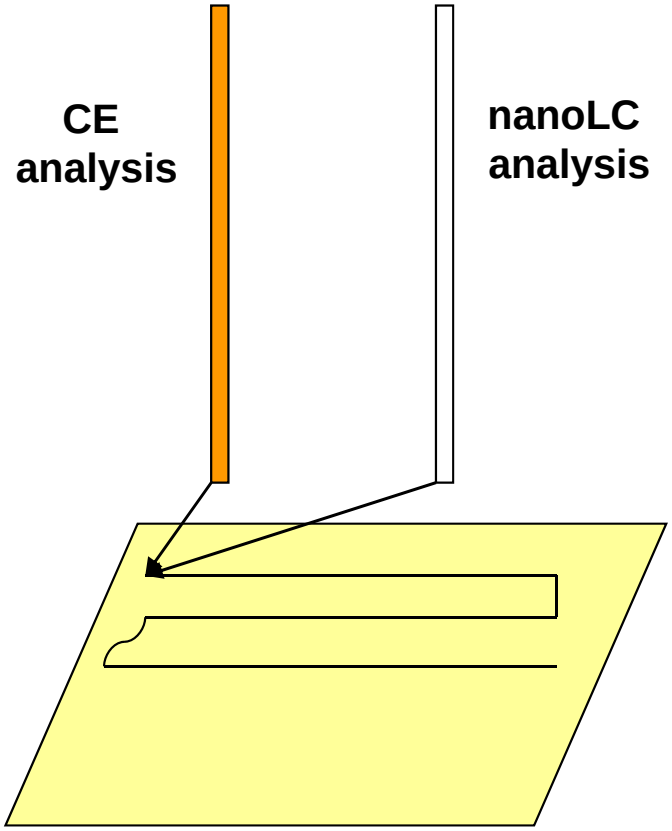


John Yates

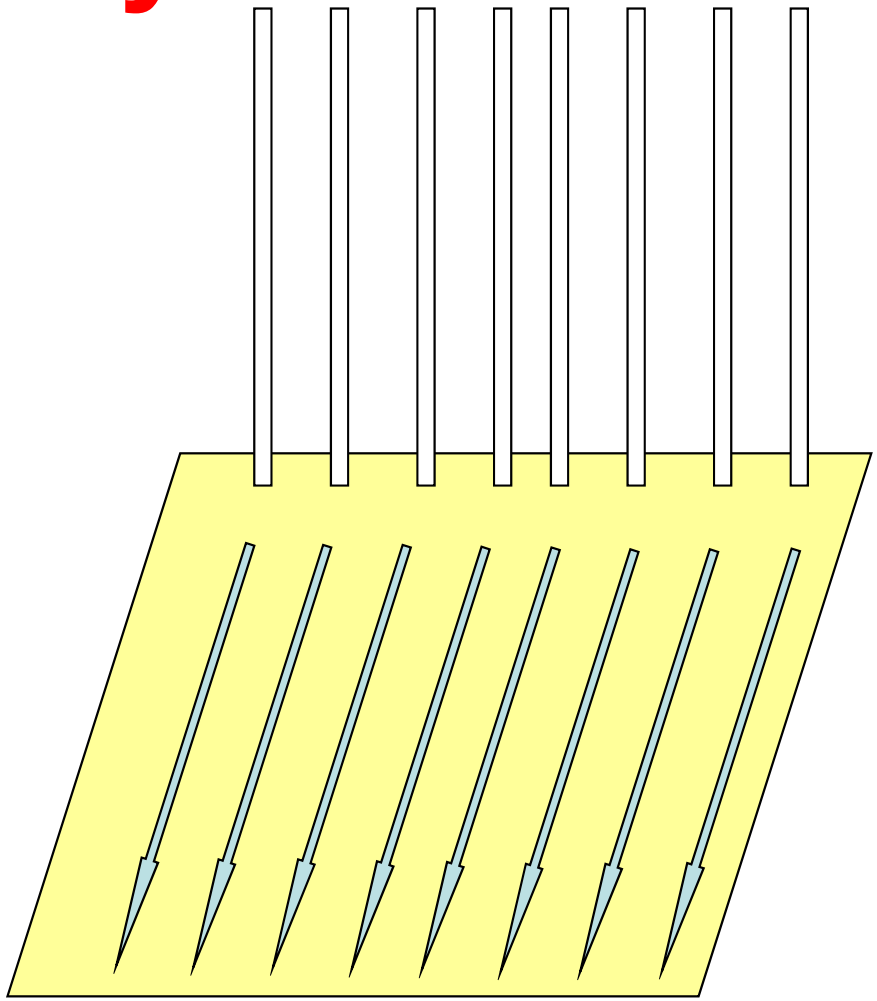
# Cation exchange of peptides



# 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

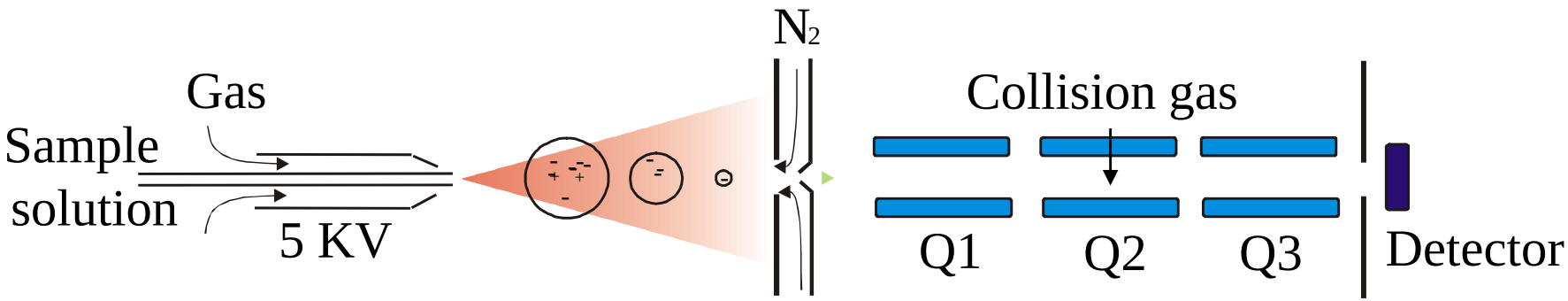
# Pros/Cons of laying down LC or EC separations on matrix plate

- **Allows off-line analysis both in real time and then in a retrospective mode**
- **MALDI-TOF analysis is very fast**
- **Can also 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?**

# Sequencing of peptides

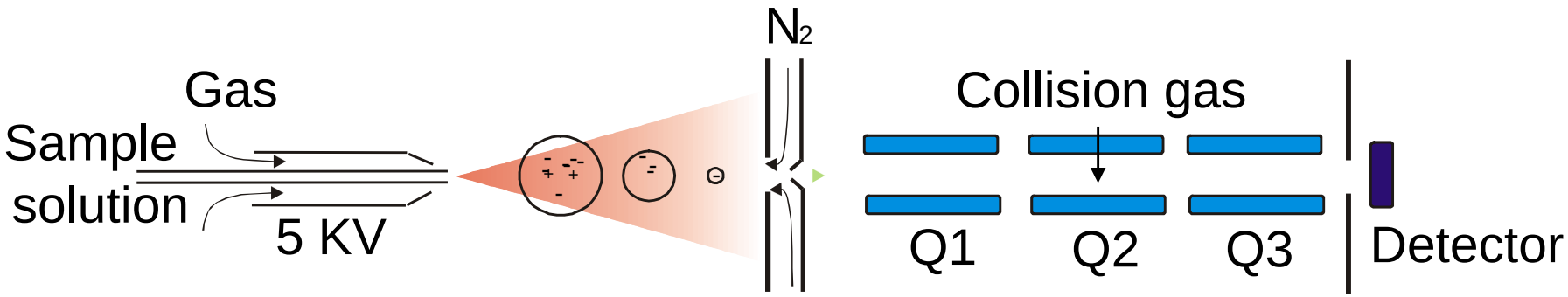
- **Using tandem mass spectrometry in a triple quadrupole, Q-tof, or ion trap instrument, the parent ion is first selected in the first quadrupole**
- **The parent ion is collided with argon gas and it breaks into fragments (daughter ions)**
- **By identifying the daughter ions, the peptide amino acid sequence is inferred**

# Tandem mass spectrometry on a triple quadrupole instrument

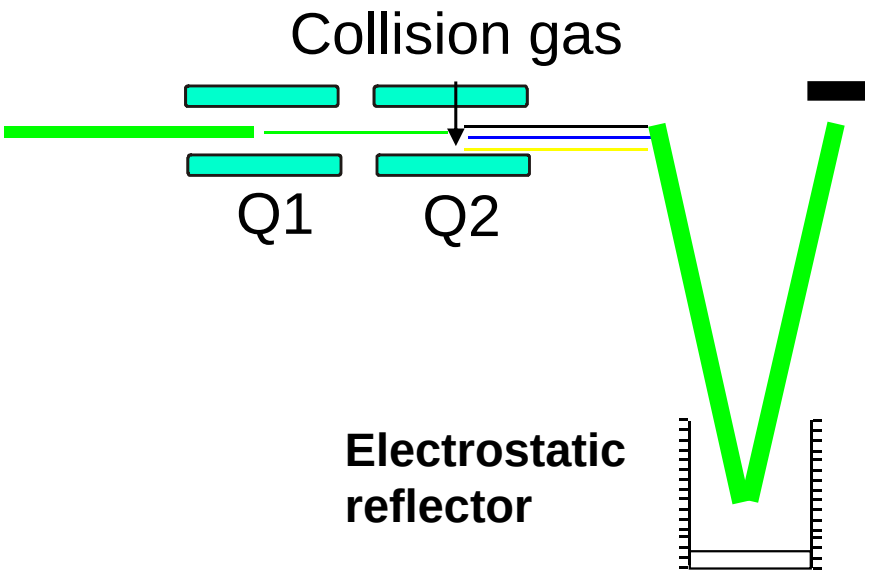



- **Daughter ion spectra**
- **Parent ion spectra**
- **Multiple reaction ion scanning**

# 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**