Peptide Sequencing by Mass Spectrometry

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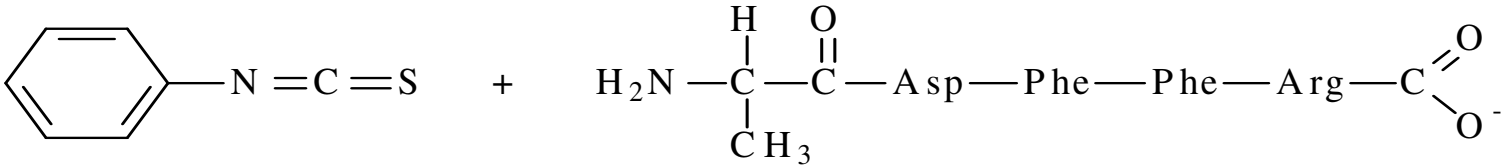
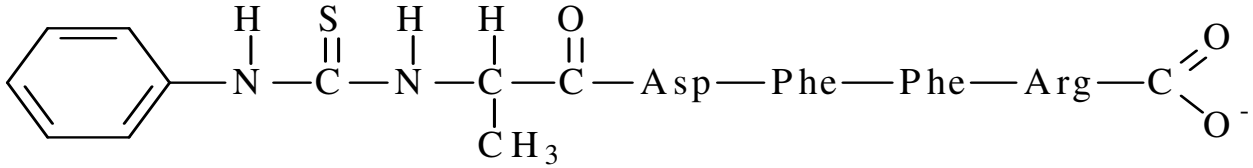
5 April 2005

Edman degradation

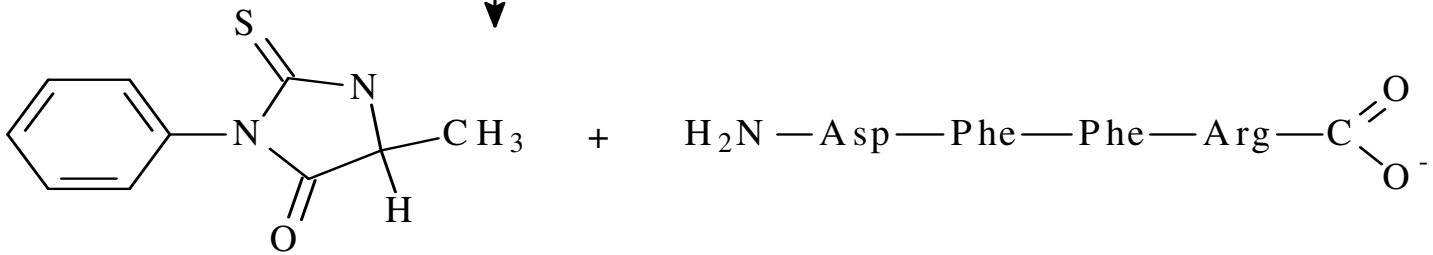
Phenyl isothiocyanate



Labeling



Release



PTH-alanine **Peptide shorthened by one residue**

Edman Degradation v. MS/MS

Protein Identification using Peptide Sequencing

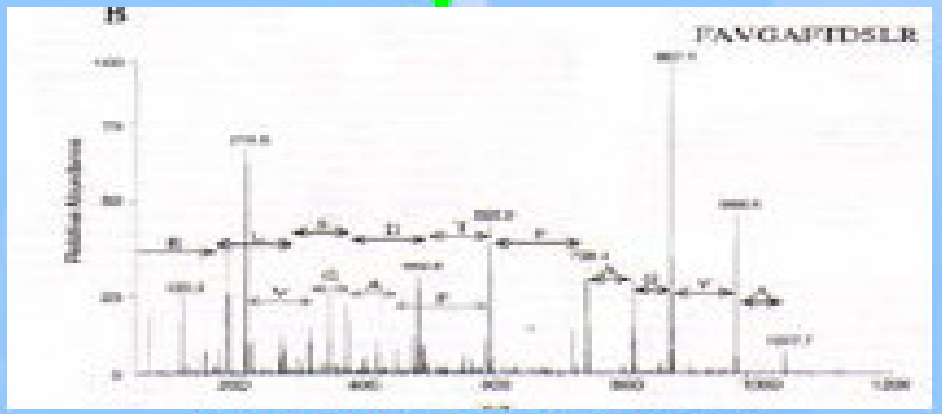
NH_2-Glu-Gly-Ser-Thr-Ser-Pro-Pro-His-Ala-His-Leu-Lys-COOH

Edman-type degradation

1 hr = Glu
1 hr = Gly
1 hr = Ser
.
.
.
1 hr = Lys

Total Time = 12 hours

Tandem mass spectrometry



Total Time = ~1 second

Why study proteins?

- machines that make cells function
- RNA levels do not always accurately predict protein levels
- targets of drugs

Peptide Analysis

- Edman Degradation
- MS
  - More sensitive
  - Can fragment peptides faster
  - Does not require proteins or peptides to be purified to homogeneity
  - Has no problem identifying blocked or modified proteins

Introduction

- MS/MS plays important role in protein identification (fast and sensitive)
- Derivation of peptide sequence an important task in proteomics
- Derivation without help from a protein database ("de novo sequencing"), especially important in identification of unknown protein

Basic lab experimental steps

1. Proteins digested w/ an enzyme to produce peptides
2. Peptides charged (ionized) and separated according to their different m/z ratios
3. Each peptide fragmented into ions and m/z values of fragment ions are measured

- **Steps 2 and 3 performed within a tandem mass spectrometer.**

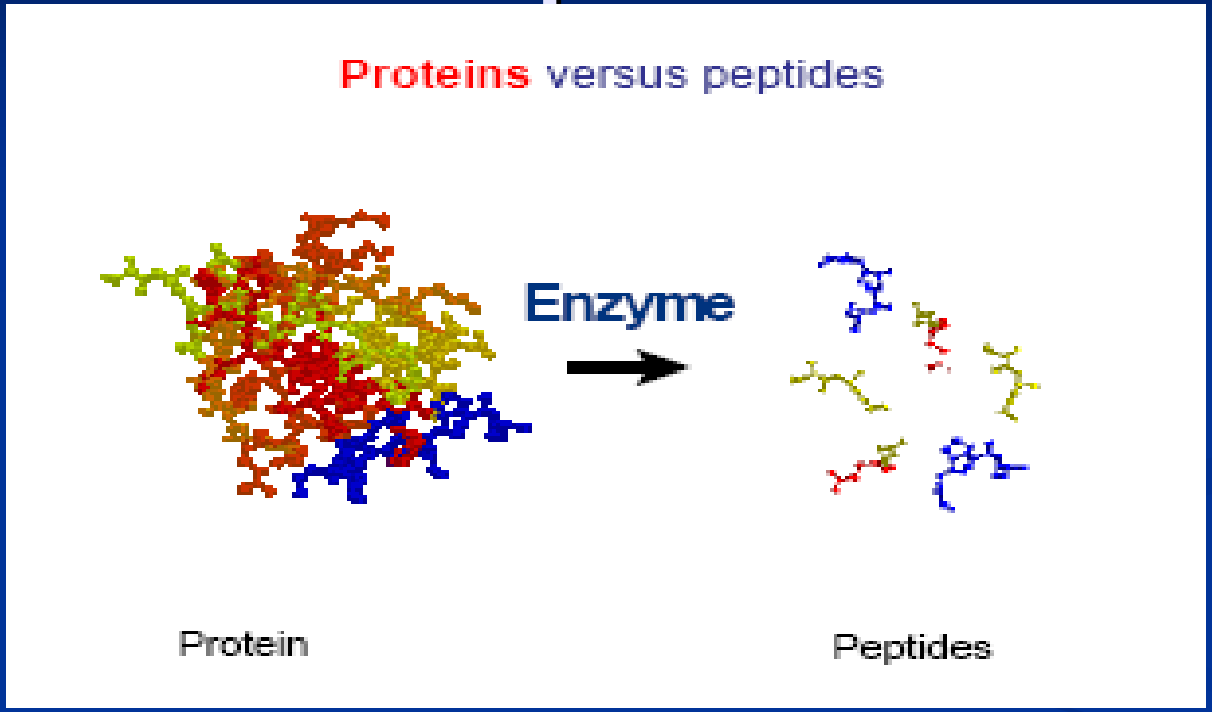
Mass spectrum

- Proteins consist of 20 different types of a. a. with different masses (except for one pair Leu and Ile)
- Different peptides produce different spectra
- Use the spectrum of a peptide to determine its sequence

Objectives

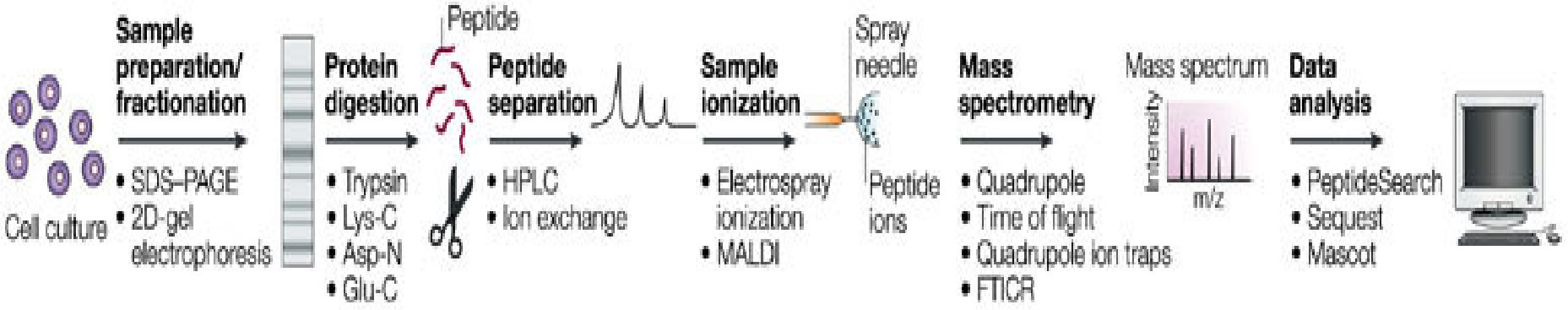
- Describe the steps of a typical peptide analysis by MS (proteomic experiment)
- Explain peptide ionization, fragmentation, identification

Why are peptides, and not proteins, sequenced?



- Solubility under the same conditions
- Sensitivity of MS much higher for peptides
- MS efficiency

MS Peptide Experiment

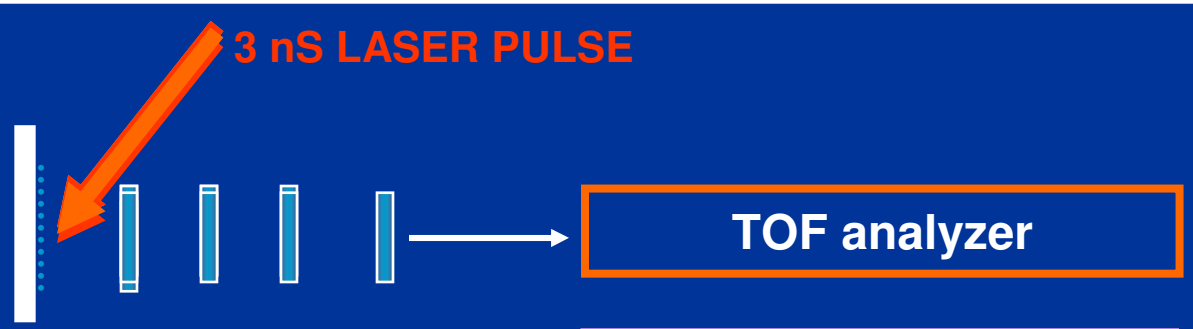


Nature Reviews | Molecular Cell Biology

Choice of Enzyme

Cleaving agent/Proteases	Specificity
A. HIGHLY SPECIFIC	
Trypsin	Arg-X, Lys-X
Endoproteinase Glu-C	Glu-X
Endoproteinase Lys-C	Lys-X
Endoproteinase Arg-C	Arg-X
Endoproteinase Asp-N	X-Asp
B. NONSPECIFIC	
Chymotrypsin	Phe-X, Tyr-X, Trp-X, Leu-X
Thermolysin	X-Phe, X-Leu, X-Ile, X-Met, X-Val, X-Ala

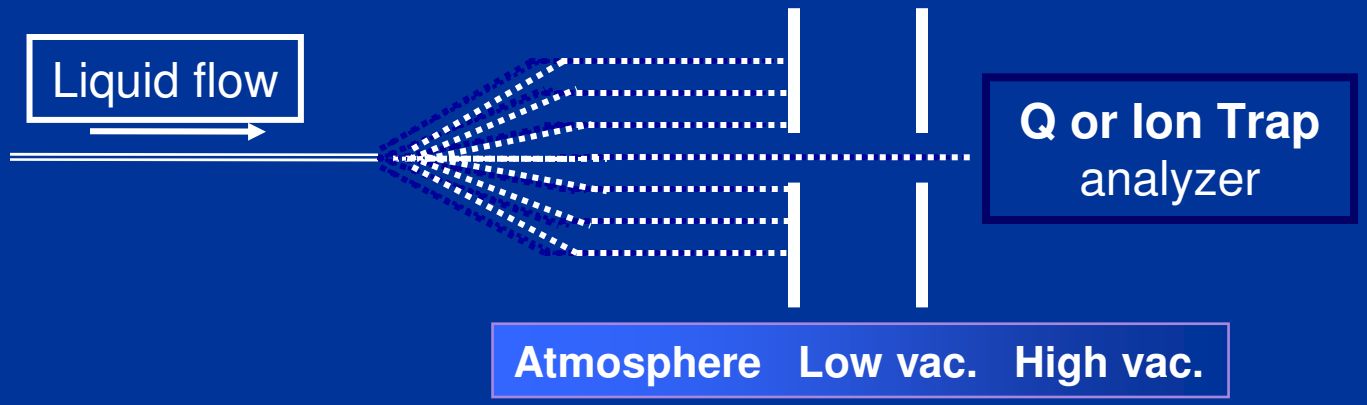
MALDI



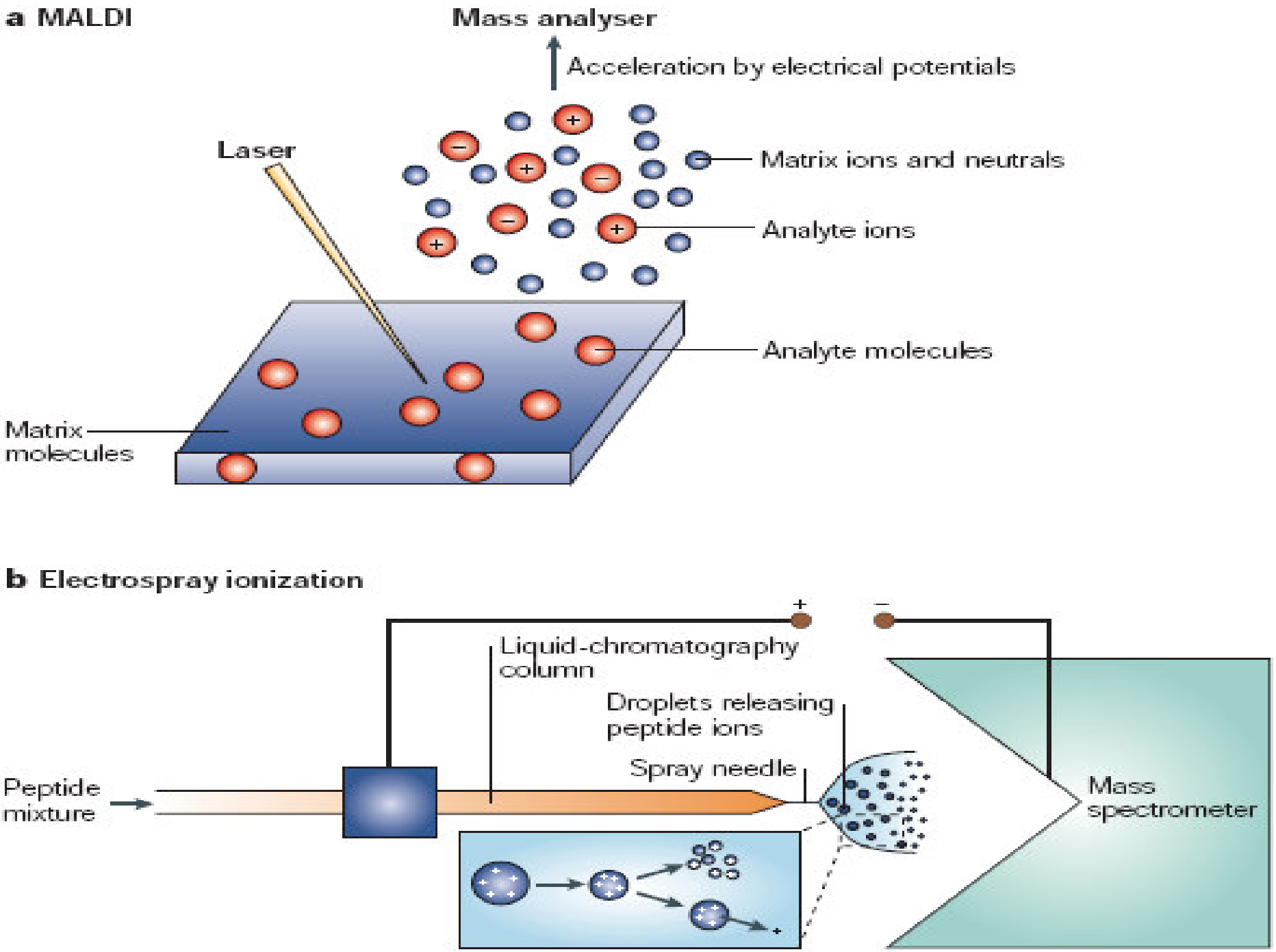
Sample (solid) on target at high voltage/ high vacuum

MALDI is a solid-state technique that gives ions in pulses, best suited to time-of-flight MS.

ESI



ESI is a solution technique that gives a continuous stream of ions, best for quadrupoles, ion traps, etc.


a MALDI
Mass analyser
Acceleration by electrical potentials
Laser
Matrix ions and neutrals
Analyte ions
Analyte molecules
Matrix molecules
b Electrospray ionization
+
−
Liquid-chromatography column
Droplets releasing peptide ions
Spray needle
Peptide mixture
Mass spectrometer

….MALDI or Electrospray ?

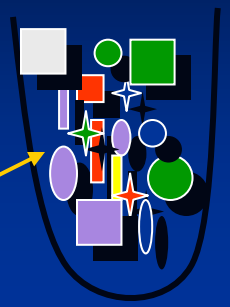
MALDI is limited to solid state, ESI to liquid

ESI is better for the analysis of complex mixture as it is directly interfaced to a separation techniques (i.e. HPLC or CE)

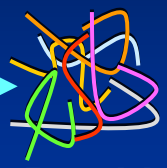
MALDI is more “flexible” (MW from 200 to 400,000 Da)

Protein Identification Strategy

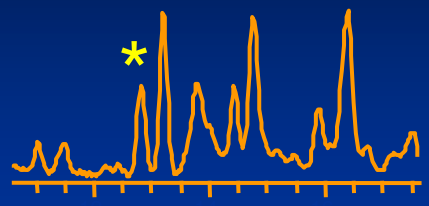
I



Protein mixture

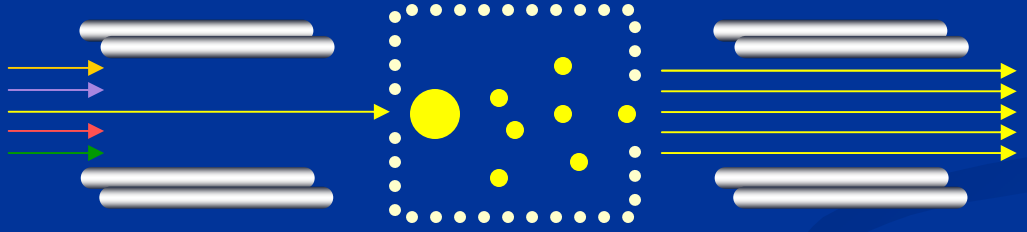


Peptides



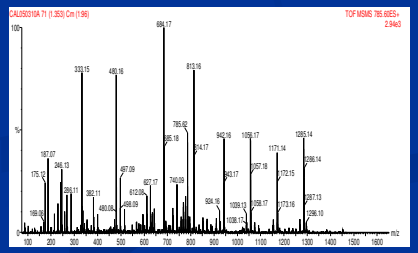
1D, 2D, 3D peptide separation

II



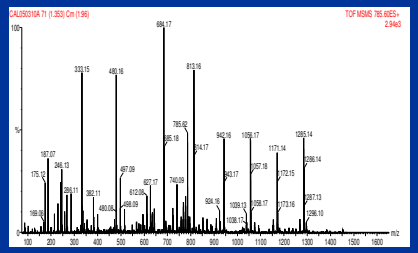
Q1 Q2 Q3

Collision Cell



Tandem mass spectrum

III

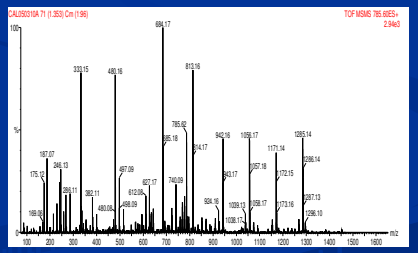


m/z

Theoretical

Correlative sequence database searching

Protein identification



m/z

Acquired

Breaking Protein into Peptides and Peptides into Fragment Ions

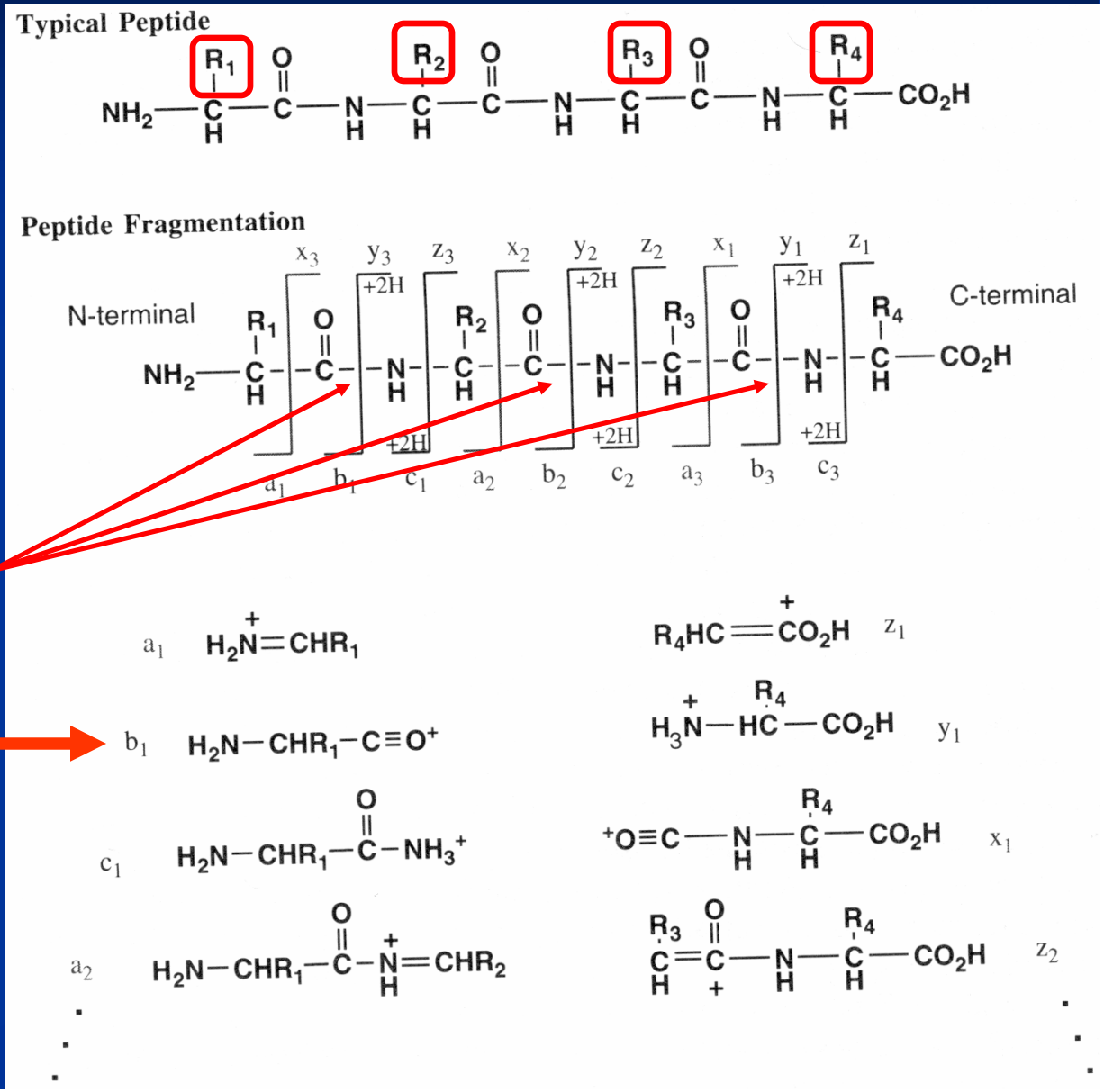
- Proteases, e.g. trypsin, break protein into *peptides*
- MS/MS breaks the peptides down into *fragment ions* and measures the mass of each piece
- MS measure m/z ratio of an ion

Peptide fragmentation

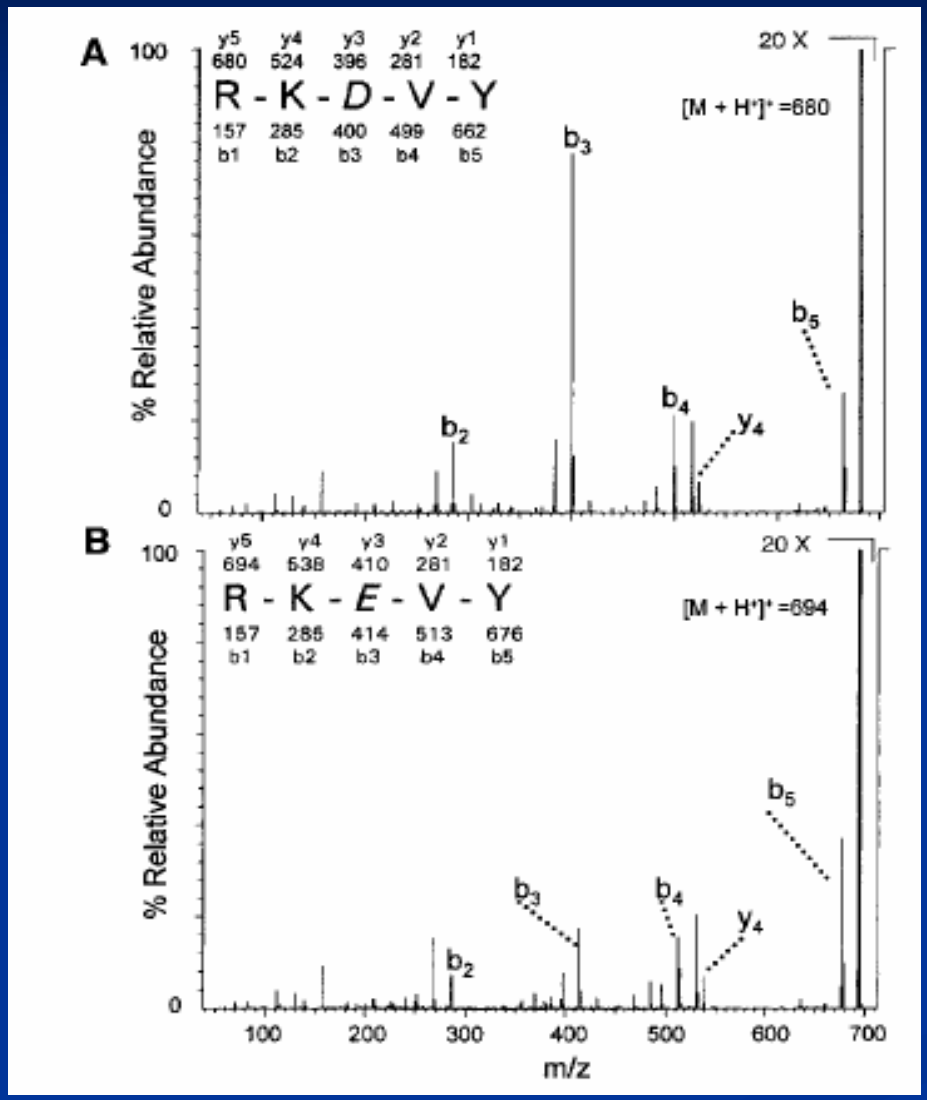
Amino acids differ in their side chains

Weakest bonds

Predominant fragmentation

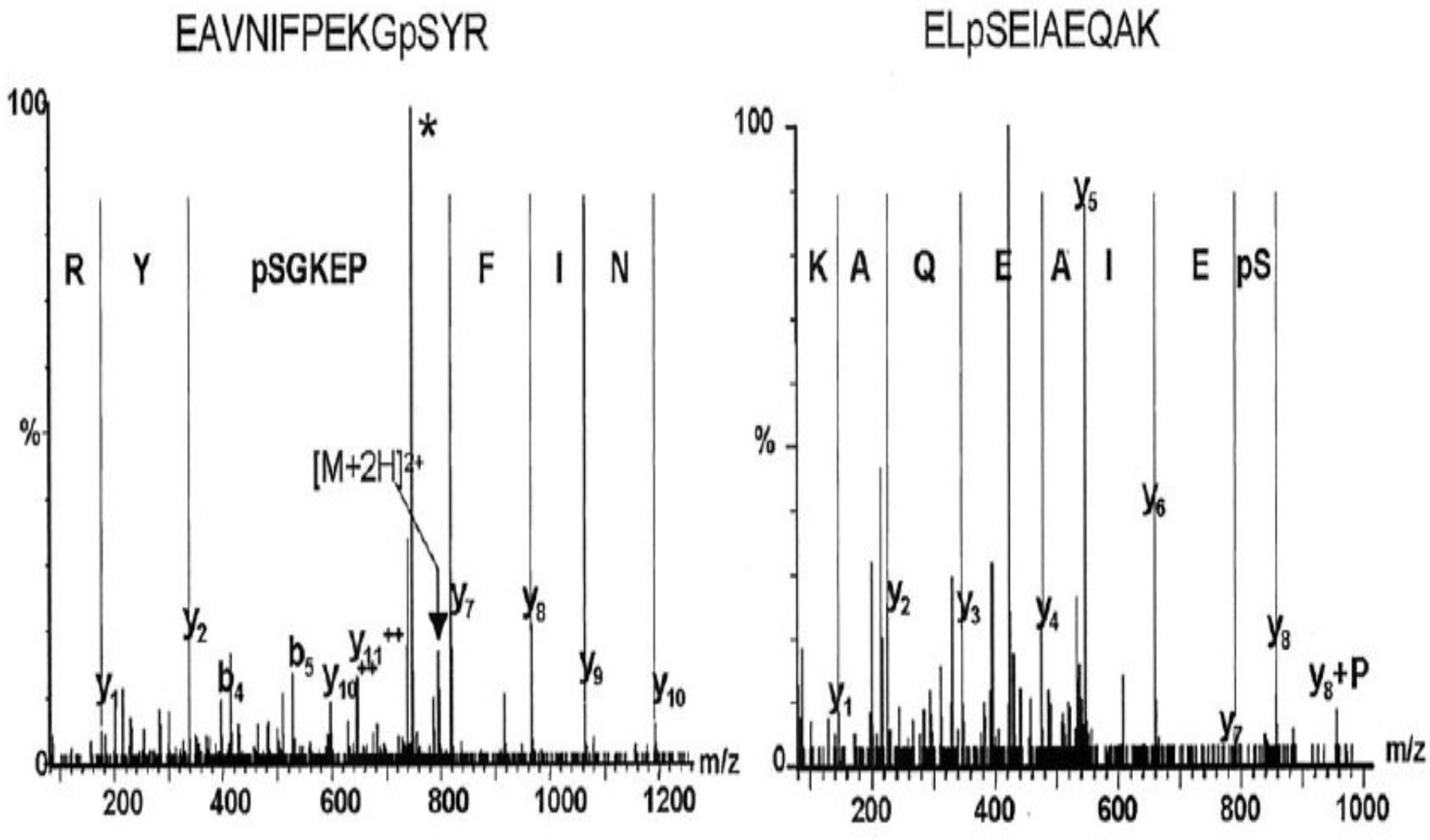


Tendency of peptides to fragment at Asp (D)



C-terminal side of Asp

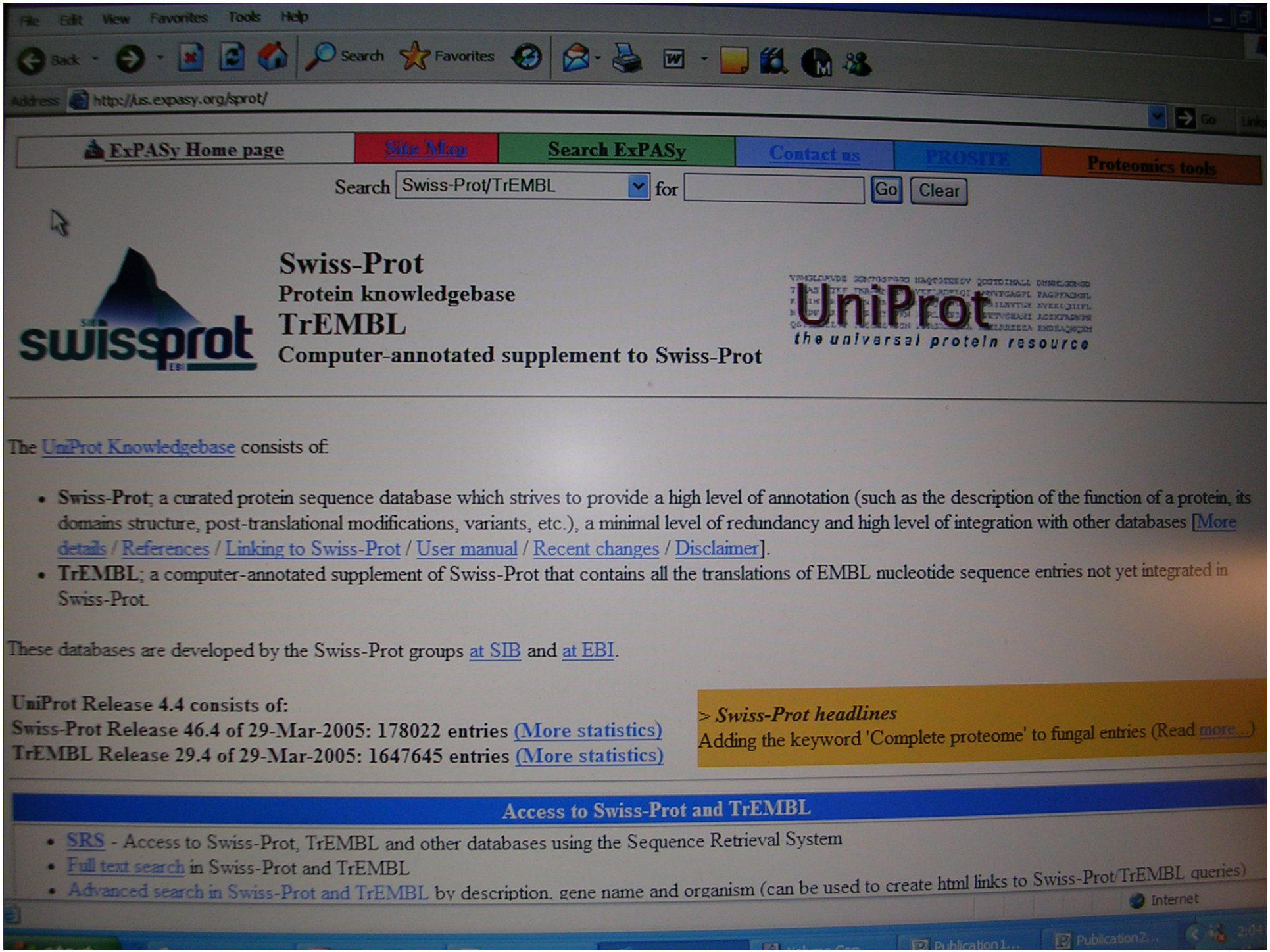
Mass Spectrometry in Proteomics
Ruedi Aebersold* and David R. Goodlett
269 *Chem. Rev.* **2001,** *101,* 269-295



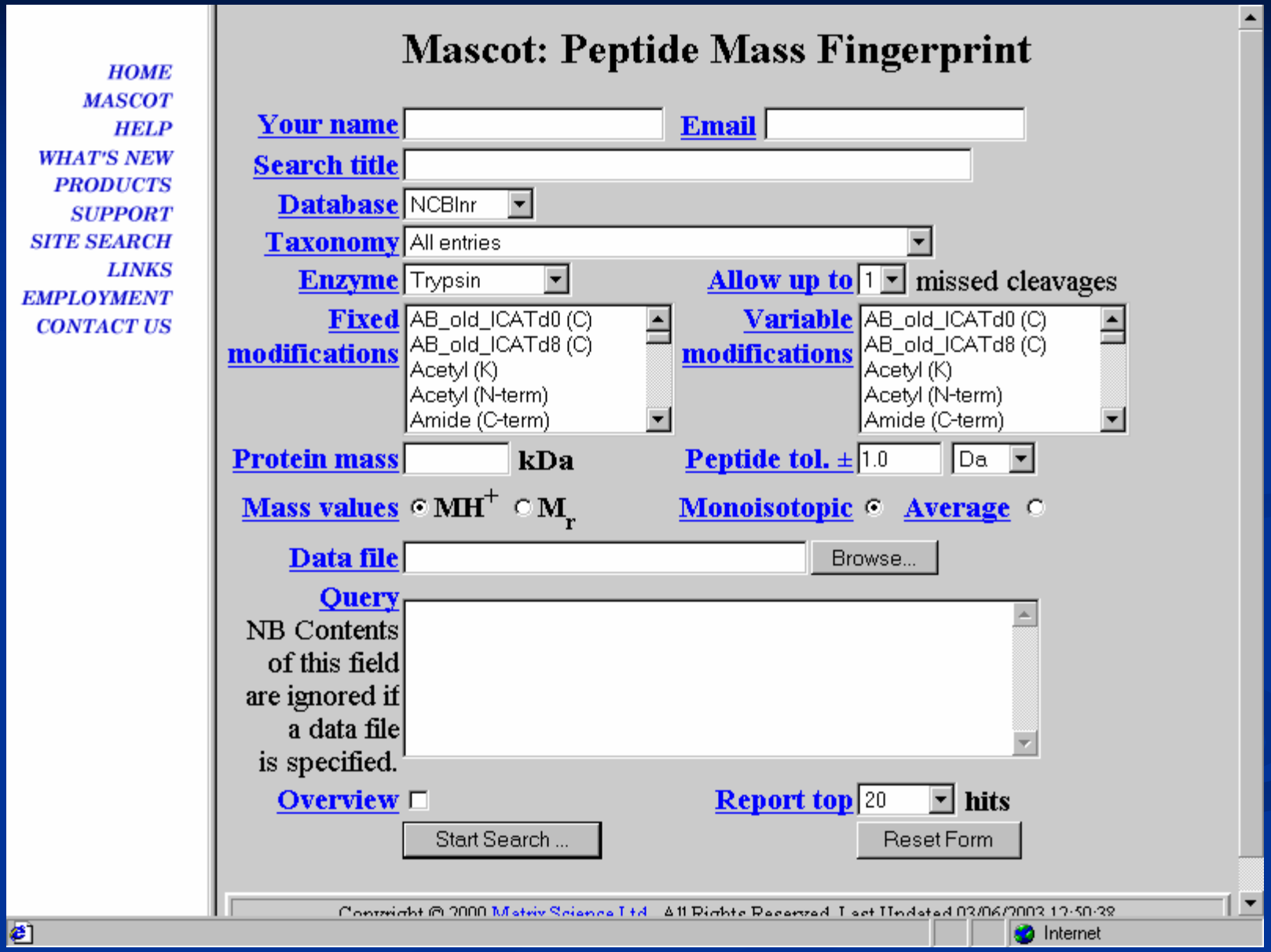
Large-scale Analysis of *in Vivo* Phosphorylated Membrane Proteins by Immobilized Metal Ion Affinity Chromatography and Mass Spectrometry, *Molecular & Cellular Proteomics, 2003, 2.11,* 1234, Thomas S. Nuhse, Allan Stensballe, Ole N. Jensen, and Scott C. Peck

What you need for peptide mass mapping

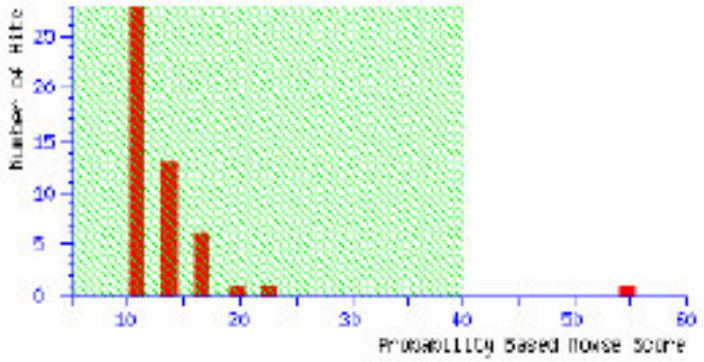
- Peptide mass spectrum
- Protein Database
  - GenBank, Swiss-Prot, dbEST, etc.
- Search engines
  - MasCot, Prospector, Sequest, etc.


File Edit View Favorites Tools Help
Back Search Favorites
Address http://us.expasy.org/sprot/
ExPASy Home page
Site Map
Search ExPASy
Contact us
PROSITE
Proteomics tools
Search Swiss-Prot/TrEMBL for Go Clear
swissprot
Swiss-Prot
Protein knowledgebase
TrEMBL
Computer-annotated supplement to Swiss-Prot
UniProt
the universal protein resource
The UniProt Knowledgebase consists of:
Swiss-Prot; a curated protein sequence database which strives to provide a high level of annotation (such as the description of the function of a protein, its domains structure, post-translational modifications, variants, etc.), a minimal level of redundancy and high level of integration with other databases [More details / References / Linking to Swiss-Prot / User manual / Recent changes / Disclaimer].
TrEMBL; a computer-annotated supplement of Swiss-Prot that contains all the translations of EMBL nucleotide sequence entries not yet integrated in Swiss-Prot.
These databases are developed by the Swiss-Prot groups at SIB and at EBI.
UniProt Release 4.4 consists of:
Swiss-Prot Release 46.4 of 29-Mar-2005: 178022 entries (More statistics)
TrEMBL Release 29.4 of 29-Mar-2005: 1647645 entries (More statistics)
> Swiss-Prot headlines
Adding the keyword 'Complete proteome' to fungal entries (Read more...)
Access to Swiss-Prot and TrEMBL
SRS - Access to Swiss-Prot, TrEMBL and other databases using the Sequence Retrieval System
Full text search in Swiss-Prot and TrEMBL
Advanced search in Swiss-Prot and TrEMBL by description, gene name and organism (can be used to create html links to Swiss-Prot/TrEMBL queries)
Internet

Database search for protein identification



Peptide sequencing using MASCOT



Peptide Summary Report

Switch to Protein Summary Report

To create a bookmark for this report, right click this link: Peptide Summary Report (../data/20021008/FoteIca.dat)

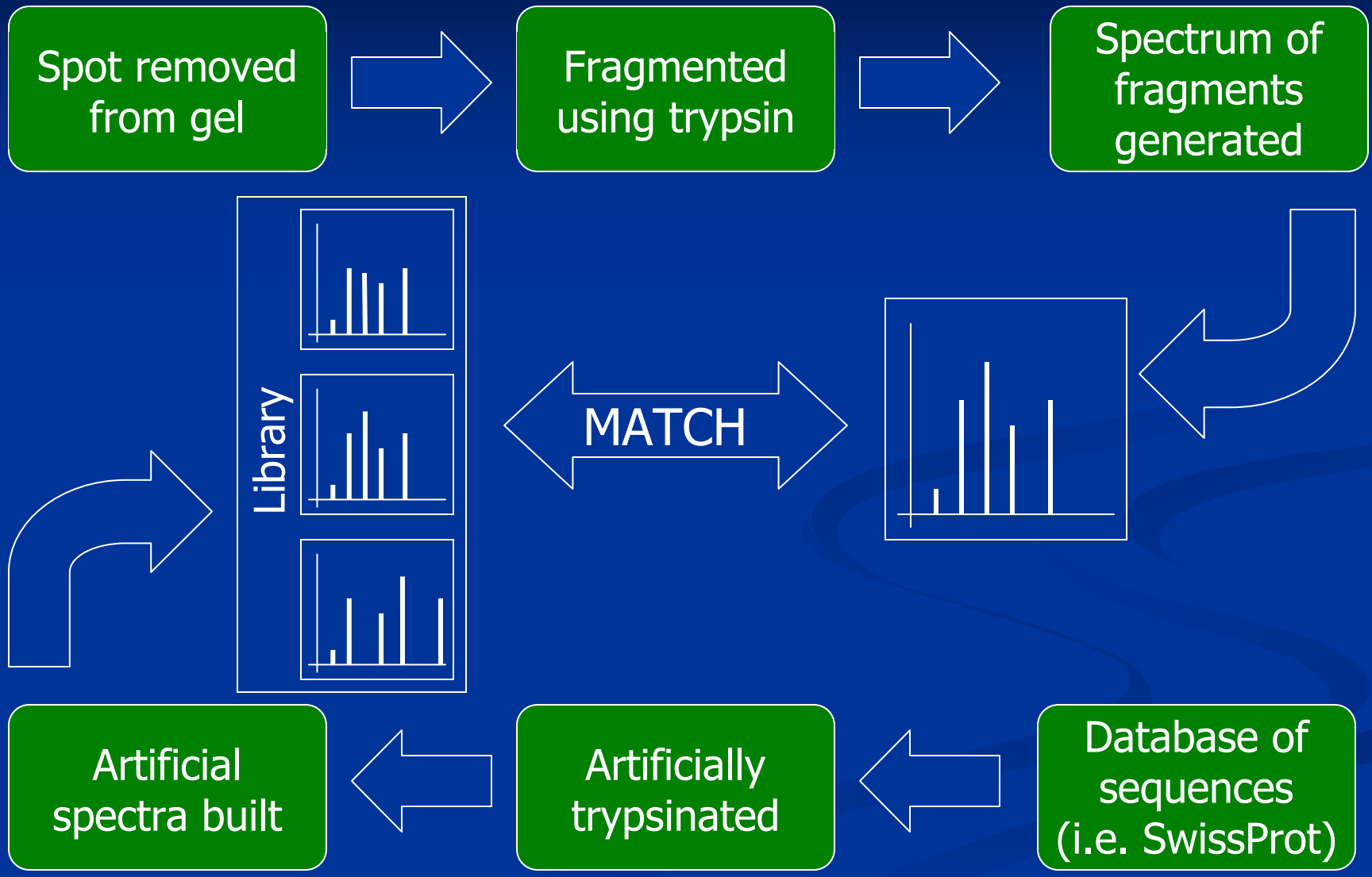
Select All | Select None | Search Selected | ☐ Error tolerant

1. gi|16924319 Mass: 40477 Total score: 55 Peptides matched: 1
(BC017450) Unknown (protein for IMAGE:3538275) [Homo sapiens]
☐ Check to include this hit in error tolerant search

	Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Rank	Peptide
☑	14	895.70	1789.39	1789.88	-0.50	0	55	1	SYELPDGQVITIGNER

Proteins matching the same set of peptides:
gi|4501887 Mass: 41766 Total score: 55 Peptides matched: 1
(NM_001614) actin, gamma 1 propeptide; cytoskeletal gamma-actin; actin, cytoplasmic 2 [Homo sapiens]
gi|16359158 Mass: 41736 Total score: 55 Peptides matched: 1
(BC016045) actin, beta [Homo sapiens]
gi|4885049 Mass: 41992 Total score: 55 Peptides matched: 1
(NM_005159) actin, alpha, cardiac muscle precursor [Homo sapiens]
gi|14714562 Mass: 18762 Total score: 55 Peptides matched: 1

Protein Identification by MS



Conclusions

- MS of peptides enables high throughput identification and characterization of proteins in biological systems
- “de novo sequencing” can be used to identify unknown proteins not found in protein databases

References

H. Steen and M. Mann. “The ABC’s (and XYZ’s) of Peptide Sequencing” Molecular Cell Biology, *Nature Reviews*. 2004, 5, 699.

T. S. Nuhse, A. Stensballe, O. Jensen, and S. Peck. “Large-scale Analysis of *in Vivo* Phosphorylated Membrane Proteins by Immobilized Metal Ion Affinity Chromatography and Mass Spectrometry” *Molecular & Cellular Proteomics, 2003, 2.11,* 1234.

R. Aebersold and D. Goodlett. “Mass Spectrometry in Proteomics” *Chem. Rev.,* 2001, 101, 269.