Mass spectrometry (MS)

By:

Dr. Ashish C Patel,

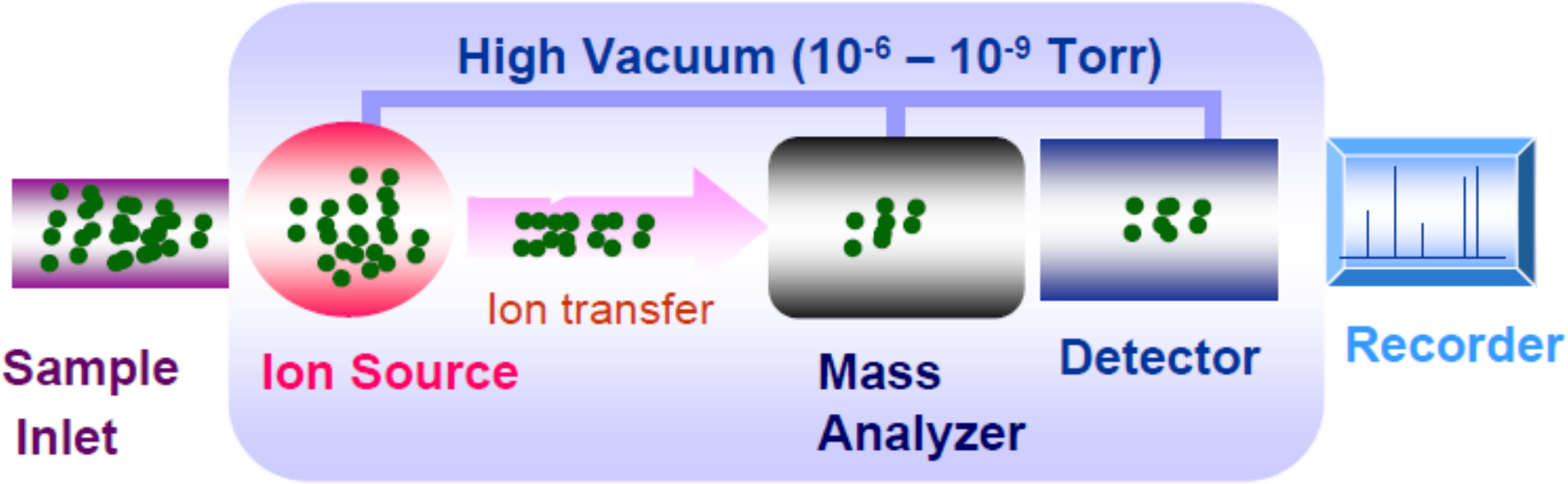
Assistant Professor,

Animal Genetics & Breeding, Veterinary College, Anand

Mass spectrometry (MS)

- Mass spectrometry (MS) measures the mass-to-charge ratio of ions to identify and quantify molecules in simple and complex mixtures.
- MS has become important across a broad range of fields, including proteomics.
- Mass spectrometry is a sensitive technique used to detect, identify and quantitate molecules based on their mass and charge (*m/z*) ratio.
- MS was first used in the biological sciences to trace heavy isotopes through biological systems.
- Later on, MS was used to sequence oligonucleotides and peptides and analyze nucleotide structure.

Basic Components in a Mass Spectrometer



A mass spectrometer is composed of five essential parts:

1. Inlet: introducing samples from ambient room pressure into ion source
2. Ion source: converting sample molecules to ions
3. Mass analyzer: separating ions according to their mass
4. Detector: detecting ions and amplifying the signal
5. Recorder: receiving signal from detector, further amplifying, recording, creating mass spectrum

Sample Inlet Systems

An inlet system is needed to transfer the sample from the atmospheric pressure (760 Torr) into the source as mass spectrometers are operated in vacuum (~10^{-6} - 10^{-9} Torr).

Common Inlet Systems:

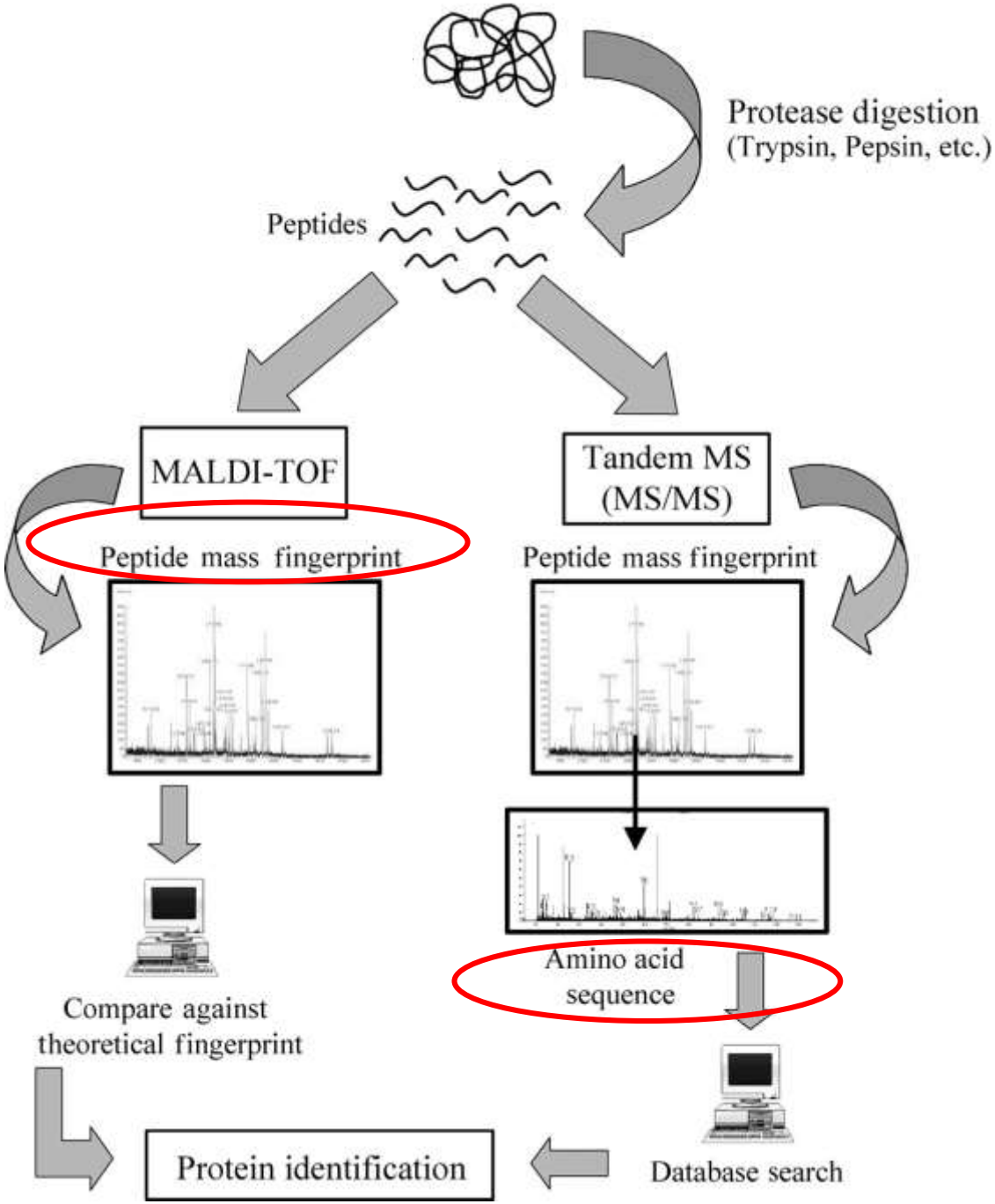
1. Chromatography:
   - gas chromatography (GC)
   - liquid chromatography (LC)
     - HPLC
     - NanoLC
     - CapLC

www.agilent.com

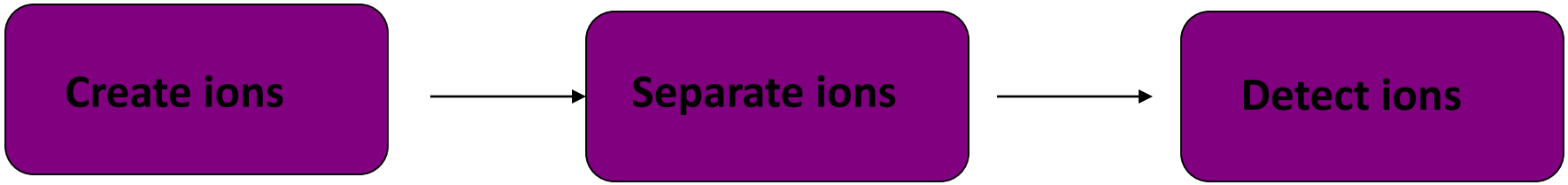
2. Syringe for direct infusion in ESI

3. MALDI probe or MALDI plate

Methods for protein identification



How does a mass spectrometer work?



- **Ionization method**
  - MALDI
  - Electrospray

  (Proteins must be charged and dry)

- Mass analyzer
  - MALDI-TOF
    - MW
  - Triple Quadrapole
    - AA seq
  - MALDI-QqTOF
    - AA seq and MW
  - QqTOF
    - AA seq and protein modif.

- **Mass spectrum**
- **Database analysis**

MS History

- JJ Thomson built MS prototype to measure m/z of electron, awarded Nobel Prize in 1906
- MS concept first put into practice by Francis Aston, a physicist working in Cambridge England in 1919. He Designed to measure mass of elements and Aston Awarded Nobel Prize in 1922
- 1948-52 - Time of Flight (TOF) mass analyzers introduced
- 1955 - Quadrupole ion filters introduced by W. Paul, also invents the ion trap in 1983 (wins 1989 Nobel Prize)
- 1968 - Tandem mass spectrometer appears
- Mass spectrometers are now one of the *MOST POWERFUL ANALYTIC TOOLS IN CHEMISTRY*

Mass spectrometers

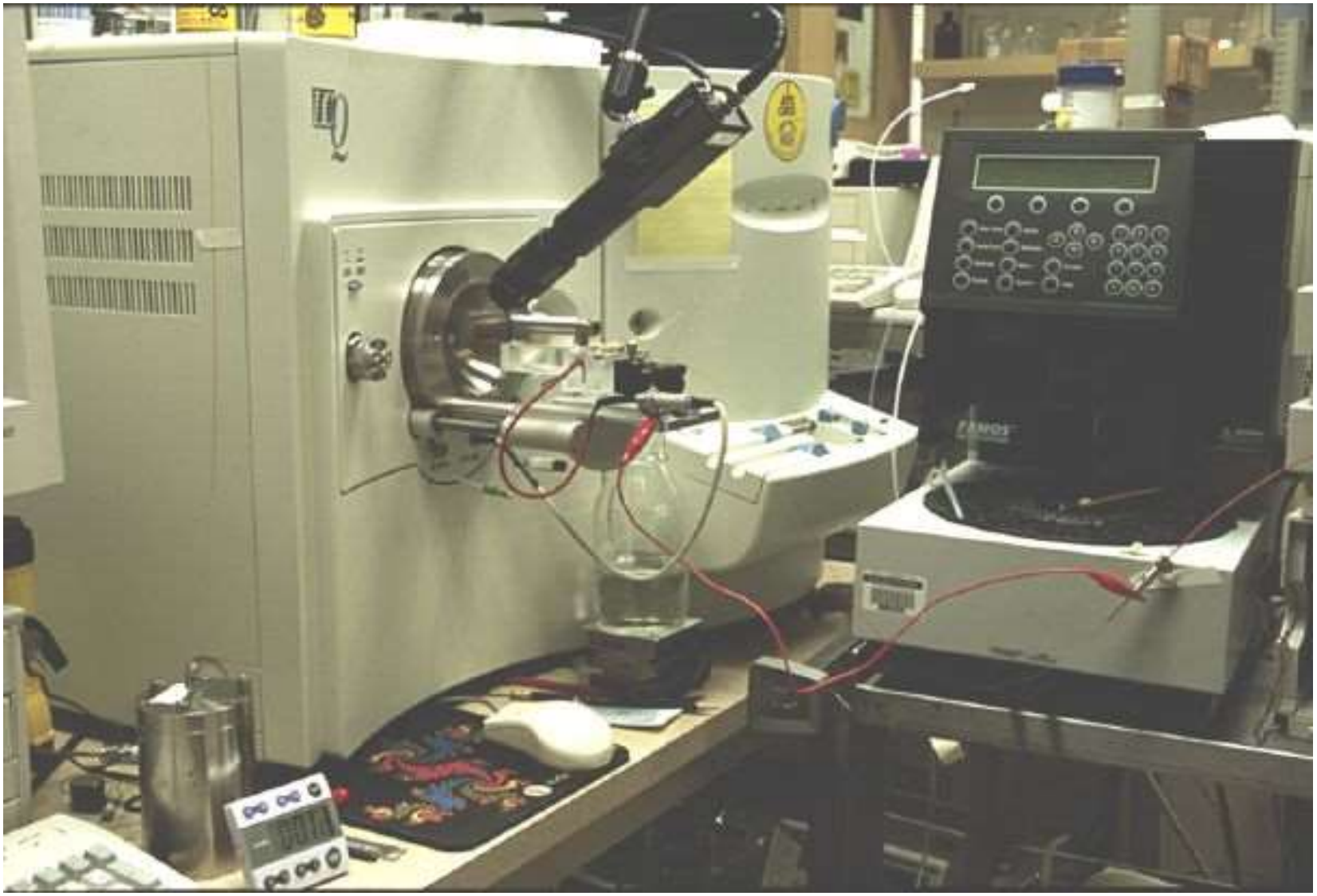
- **Time of flight (TOF) (MALDI)**
  - **Measures the time required for ions to fly down the length of a chamber.**
  - **Often combined with MALDI (MALDI-TOF). Detections from multiple laser bursts are averaged.**
- **Tandem MS- MS/MS**

  -separation and identification of compounds in complex mixtures

  - induce fragmentation and mass analyze the fragment ions.

  - Uses two or more mass analyzers/filters separated by a collision cell filled with Argon or Xenon
- **Different MS-MS configurations**
  - **Quadrupole-quadrupole (low energy)**
  - **Magnetic sector-quadrupole (high)**
  - **Quadrupole-time-of-flight (low energy)**
  - **Time-of-flight-time-of-flight (low energy)**

LC/LC-MS/MS-Tandem LC, Tandem MS



Principle of MS

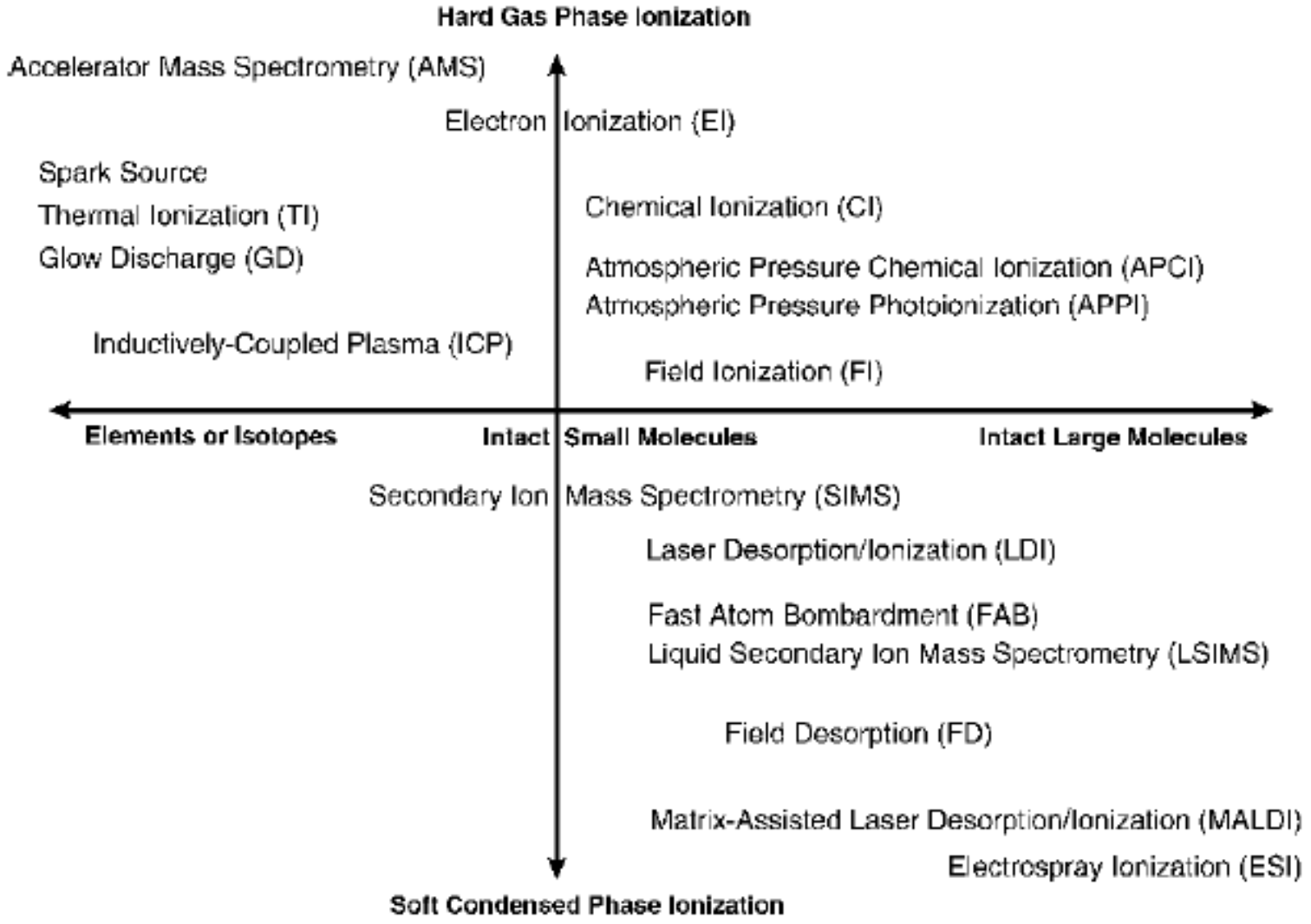
"The basic principle of *mass spectrometry (MS) is to generate ions from either inorganic* or organic compounds by any suitable method, to separate these ions by their *mass-to-charge ratio (m/z) and to detect them qualitatively and quantitatively* by their respective *m/z and abundance.*

The analyte may be ionized thermally, by electric fields or by impacting energetic electrons, ions or photons. These ions can be single ionized atoms, clusters, molecules or their fragments or associates.

Ion separation is effected by static or dynamic electric or magnetic fields."

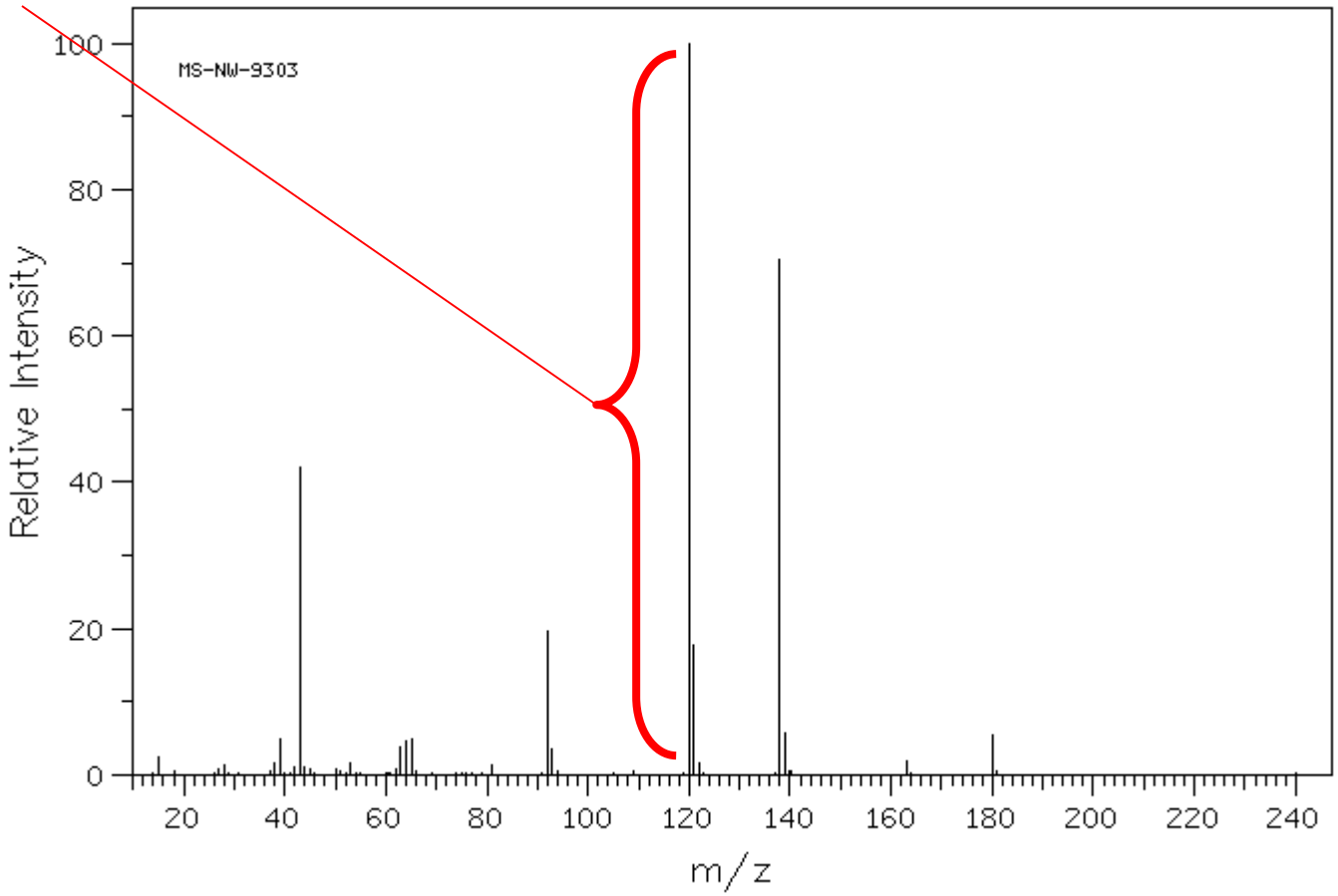
Ionization of a sample can be effected not only by electrons, but also by (atomic) ions or photons, energetic neutral atoms, electronically excited atoms, massive cluster ions, and even electrostatically charged microdroplets can also be used to effect.

The large variety of ionization techniques and their key applications can be roughly classified by their relative hardness or softness and (molecular) mass of suitable analytes.



- All proteins are sorted based on a mass to charge ratio (m/z)
- Molecular weight divided by the charge on the protein

Relative Abundance

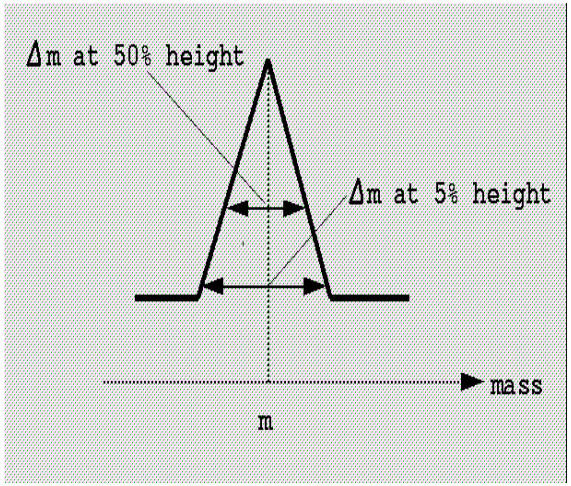


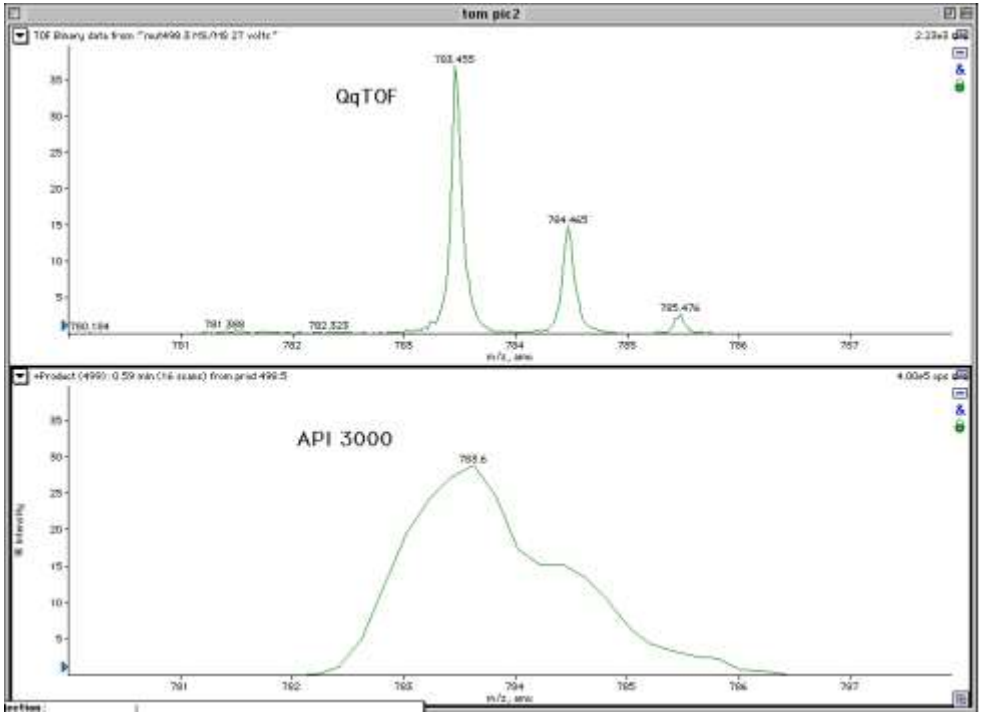
120 m/z-for singly charged ion this is the mass

Resolution & Resolving Power

- Width of peak indicates the resolution of the MS instrument
- The better the resolution or resolving power, the better the instrument and the better the mass accuracy.
- Resolving power is defined as: $\frac{\Delta M}{M}$

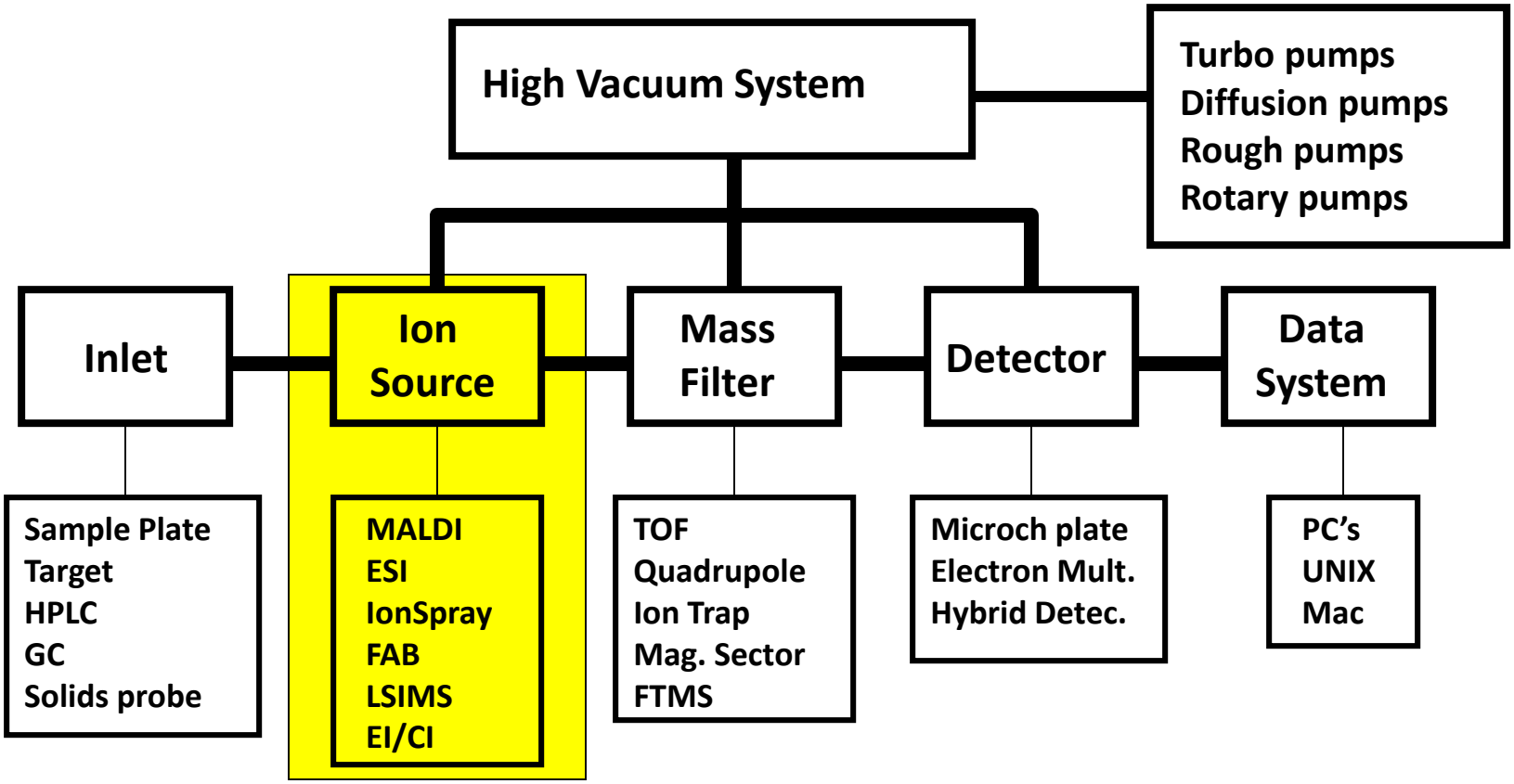
M is the mass number of the observed mass (ΔM) is the difference between two masses that can be separated





- Nuclear magnetic resonance (NMR), infrared (IR) or Raman spectroscopy do allow for sample recovery while mass spectrometry is destructive, i.e., it consumes the analyte.
- The amount of analyte needed is in the low microgram mass spectrometry is the method of choice but other analytical techniques not able to yield analytical information from nanogram amounts of sample.
- The development of macromolecule ionization methods, including electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI), enabled the study of protein structure by MS.
- This allowed to obtain protein mass "fingerprints" that could be matched to proteins and peptides in databases to predict the identity of unknown proteins.
- Methods of isotopic tagging led to the quantitation of target proteins both in relative and absolute quantities.
- Technological advancements have resulted in methods that analyze samples in solid, liquid or gas states.
- The sensitivity of current mass spectrometers allows one to detect analytes at concentrations in the attomolar range (10^{-18}) (*Forsgard N. et al., 2010).*

Mass Spectrometer Schematic



Ionization Sources

Basic Type	Name and Acronym	Ionizing Agent
Gas Phase (for Volatile compounds)	Electron Ionization (EI) √	Energetic electrons
	Chemical Ionization (CI) √	Reagent gaseous ions
	Field ionization (FI)	High-potential electrode
Condensed Phase Desorption (for nonvolatile compounds)	Field desorption (FD)	High-potential electrode
	Plasma desorption (PD)	Fission fragment from ^{252}Cf
	Secondary ion mass spectrometry (SIMS)	Energetic beam of ions
	Fast atom bombardment (FAB) √	Energetic atomic beam
	Electrospray ionization (ESI) √	High electrical field
	Atmospheric pressure chemical ionization (APCI) √	High electric field Laser
	Matrix-assisted desorption/ionization (MALDI) √	Laser Beam

- All mass spectrometers have an ion source, a mass analyzer and an ion detector.
- The nature of ionization components based on the type of mass spectrometer, the type of data required, and the physical properties of the sample.
- Samples are loaded into the mass spectrometer in liquid, gas or dried form and then vaporized and ionized by the ion source.
- The ions encounter electric and/or magnetic fields from mass analyzers, which deflect the paths of individual ions based on their mass and charge (*m/z*).

Different Ionization Methods

- **Electron Impact (EI - Hard method)**
  - **small molecules, 1-1000 Daltons, structure**
- **Fast Atom Bombardment (FAB – Semi-hard)**
  - **peptides, sugars, up to 6000 Daltons**
- **Electrospray Ionization (ESI - Soft)**
  - **peptides, proteins, up to 200,000 Daltons**
- **Matrix Assisted Laser Desorption (MALDI-Soft)**
  - **peptides, proteins, DNA, up to 500 kD**

Electron Impact Ionization

- Sample introduced into instrument by heating it until it evaporates
- Gas phase sample is bombarded with electrons coming from rhenium or tungsten filament.
- Molecule is “shattered” into fragments and fragments sent to mass analyzer

Why Can't Use EI For Analyzing Proteins

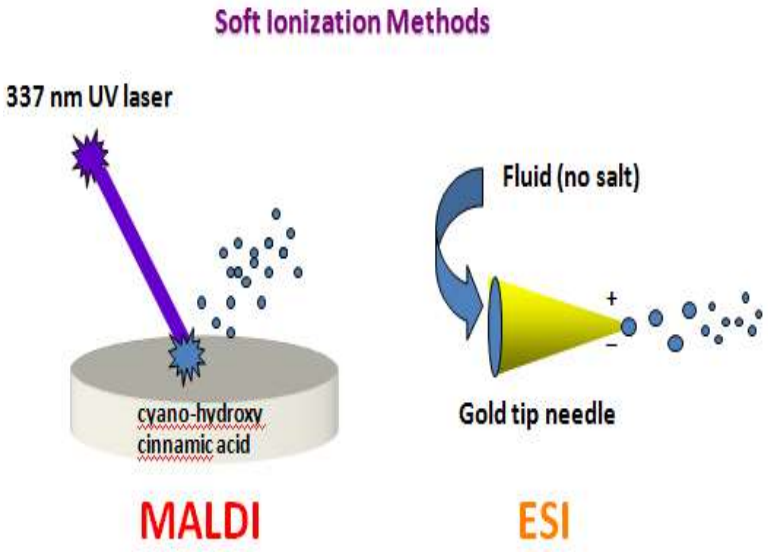
- EI shatters chemical bonds
- Any given protein contains 20 different amino acids
- EI would shatter the protein into not only into amino acids but also amino acid sub-fragments and even peptides of 2,3,4… amino acids
- Result of 10,000's of different signals from a single protein is too complex to analyze.

Soft ionization techniques keep the molecule of interest fully intact

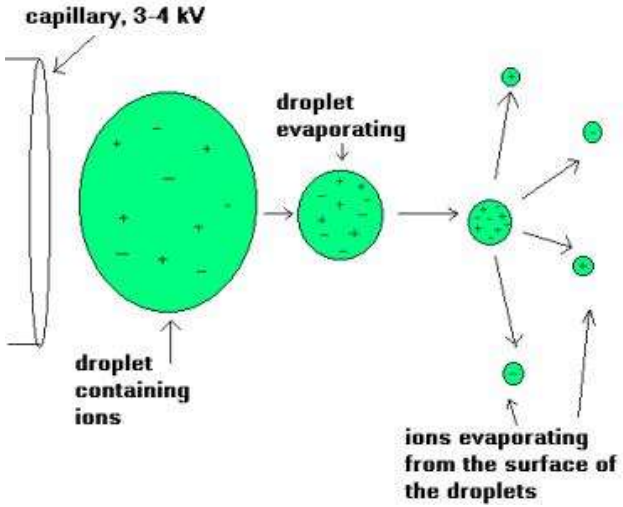
Electro-spray ionization first conceived in 1960's by Malcolm Dole.

MALDI first introduced in 1985 by Franz Hillenkamp and Michael Karas (Frankfurt)

Made it possible to analyze large molecules via inexpensive mass analyzers such as quadrupole, ion trap and TOF

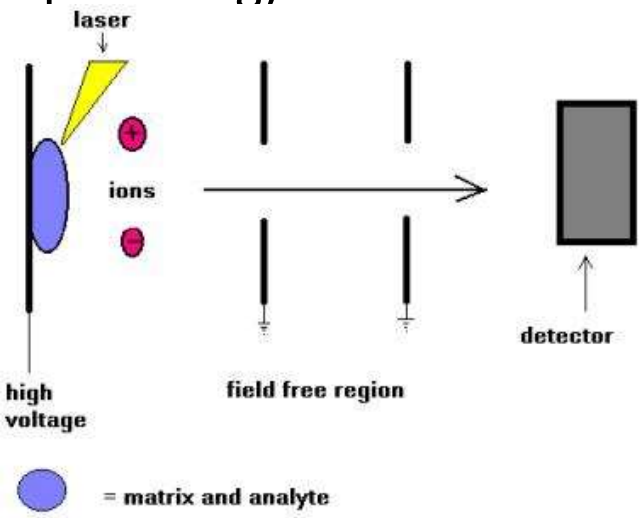


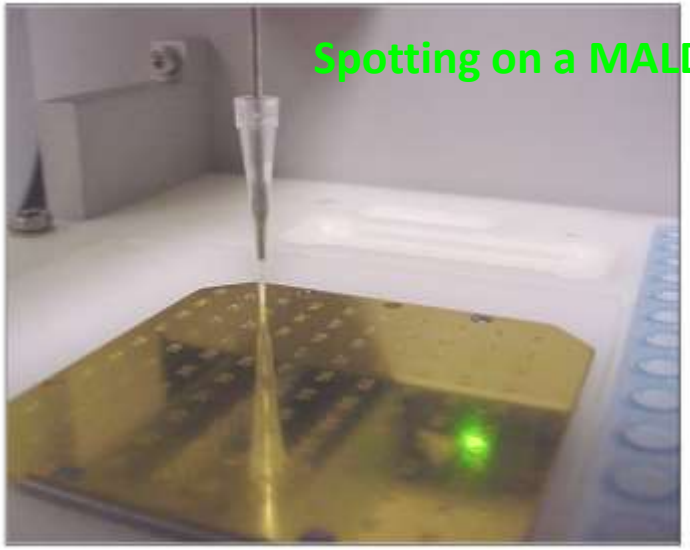
- **Electrospray mass spectrometry (ESI-MS)**
  - Liquid containing analyte is forced through a steel capillary at high voltage to electrostatically disperse analyte. Charge imparted from rapidly evaporating liquid.
- If the sample has functional groups that readily accept H+ (such as amide and amino groups found in peptides and proteins) then positive ion detection is used-PROTEINS
- If a sample has functional groups that readily lose a proton (such as carboxylic acids and hydroxyls as found in nucleic acids and sugars) then negative ion detection is used-DNA



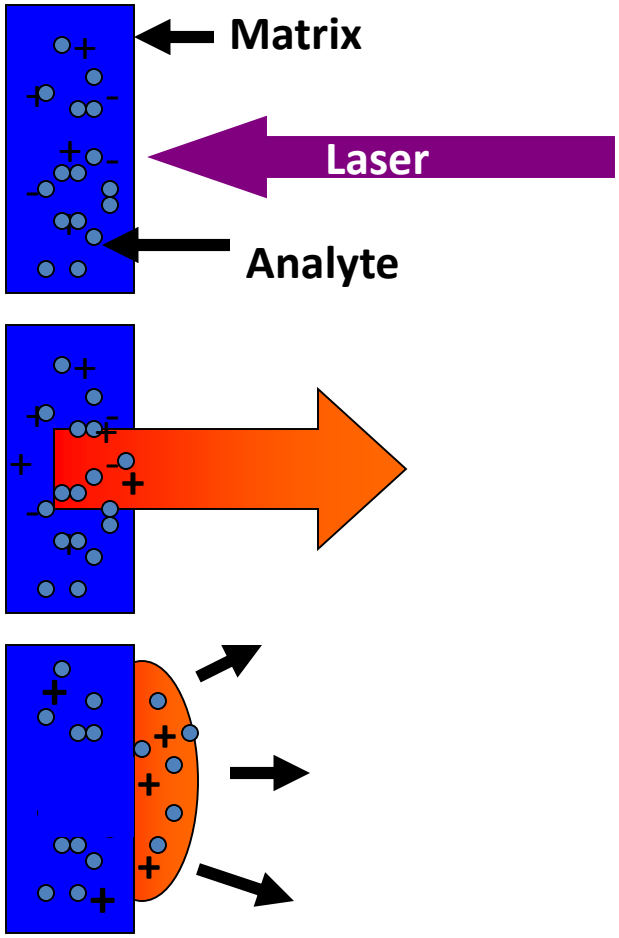
MALDI

- **Matrix-assisted laser desorption ionization (MALDI)**
  - Analyte (protein) is mixed with large excess of matrix (small organic molecule)
  - Irradiated with short pulse of laser light. Wavelength of laser is the same as absorbance max of matrix.
- Sample is ionized by bombarding sample with laser light
- Sample is mixed with a UV absorbant matrix
- Light wavelength matches that of absorbance maximum of matrix so that the matrix transfers some of its energy to the analyte (leads to ion sputtering)





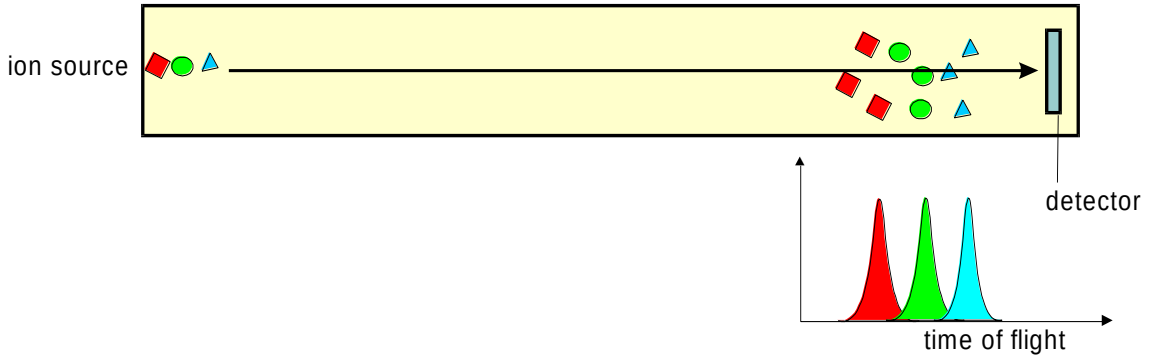
MALDI Ionization



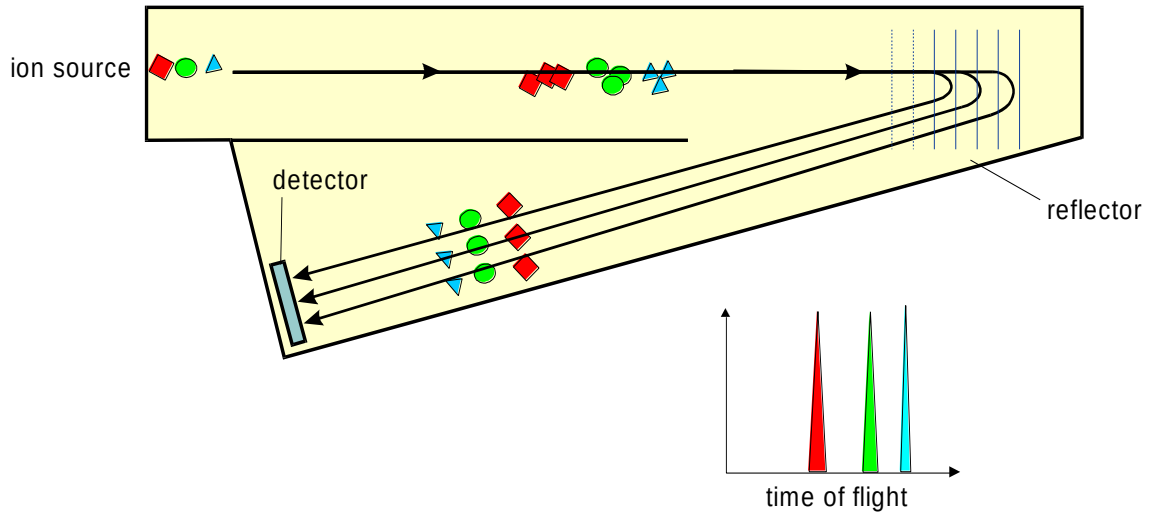
- Absorption of UV radiation by chromophoric matrix and ionization of matrix
- Dissociation of matrix, phase change to super-compressed gas, charge transfer to analyte molecule
- Expansion of matrix at supersonic velocity, analyte trapped in expanding matrix plume (explosion/"popping")

Principal for MALDI-TOF MASS

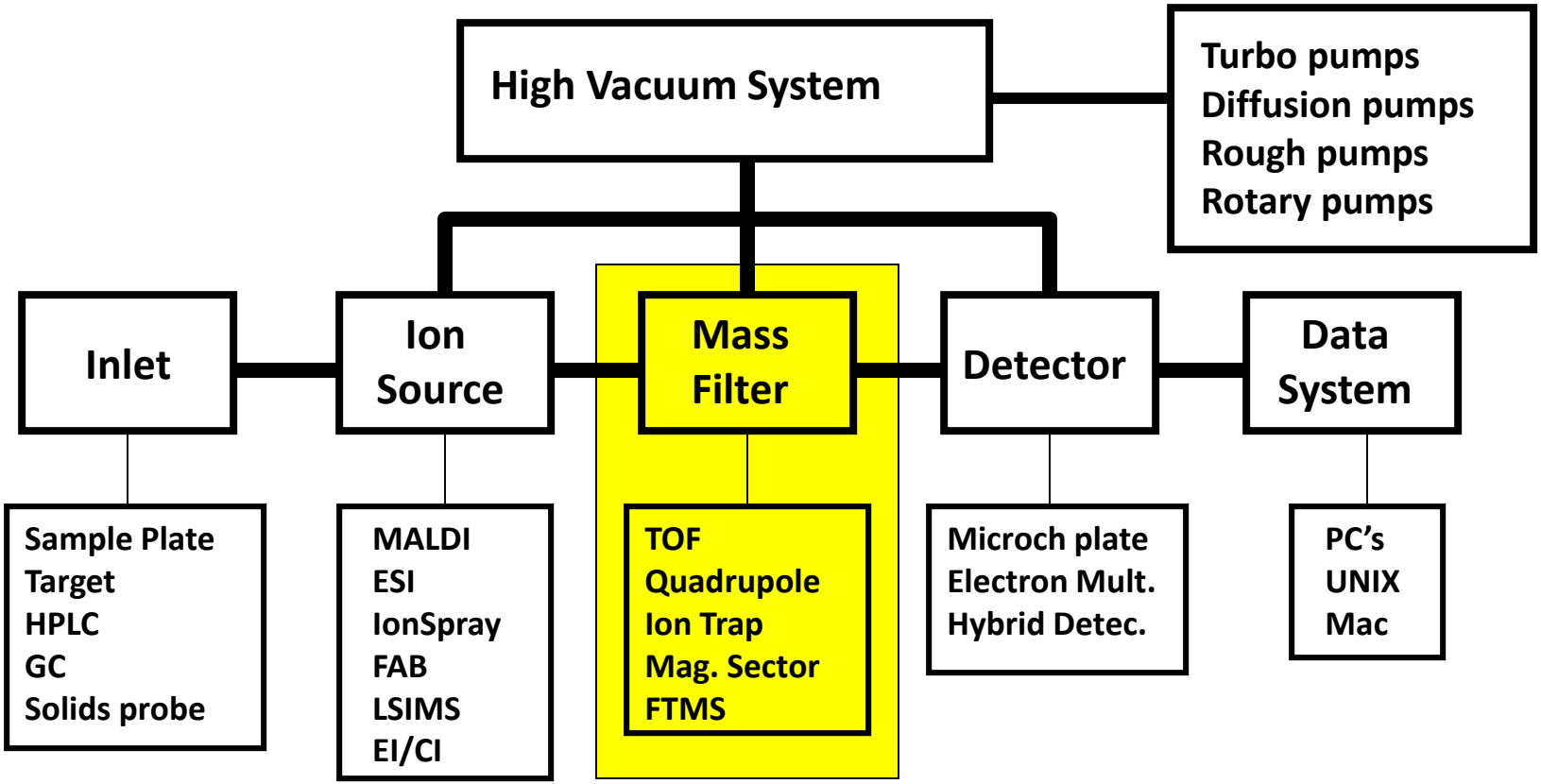
Linear Time Of Flight tube



Reflector Time Of Flight tube



Mass Spectrometer Schematic



- Commonly used mass analyzers include time-of-flight [TOF], orbitraps, quadrupoles and ion traps, and each type has specific characteristics.
- Mass analyzers can be used to separate all analytes in a sample.
- Ions that have prevent by the mass analyzers then hit the ion detector.
- This entire process is performed under an extreme vacuum (10^{-6} to 10^{8} torr) to remove gas molecules, neutrals, and contaminating non-sample ions, which can run with sample ions and alter their paths or produce non-specific reaction products .
- Newer orbitrap technology captures ions around a central spindle electrode and then analyzes their m/z values as they move across the spindle
- Orbitrap technology can achieve extremely high sensitivity and resolution.
- Mass spectrometers are connected to computers with software that analyzes the ion detector data and produces graphs that organize the detected ions by their individual *m/z* and relative abundance.

Different Mass Analyzers

- **Magnetic Sector Analyzer (MSA)**
  - High resolution, exact mass
- **Quadrupole Analyzer (Q)**
  - Low resolution, fast, cheap
- **Time-of-Flight Analyzer (TOF)**
  - No upper m/z limit, high throughput
- **Ion Trap Mass Analyzer (QSTAR)**
  - Good resolution, all-in-one mass analyzer
- **Ion Cyclotron Resonance (FT-ICR)**
  - Highest resolution, exact mass, costly

Different Types of MS

ESI-QTOF

Electrospray ionization source + quadrupole mass filter + time-of-flight mass analyzer

MALDI-QTOF

Matrix-assisted laser desorption ionization + quadrupole + time-of-flight mass analyzer

Both separate by MW and AA seq

Different Types of MS

- **GC-MS - Gas Chromatography MS**
  - separates volatile compounds in gas column and ID's by mass
- **LC-MS - Liquid Chromatography MS**
  - separates delicate compounds in HPLC column and ID's by mass
- **MS-MS - Tandem Mass Spectrometry**
  - separates compound fragments by magnetic field and ID's by mass
- **LC/LC-MS/MS-Tandem LC and Tandem MS**
  - Separates by HPLC, ID's by mass and AA sequence

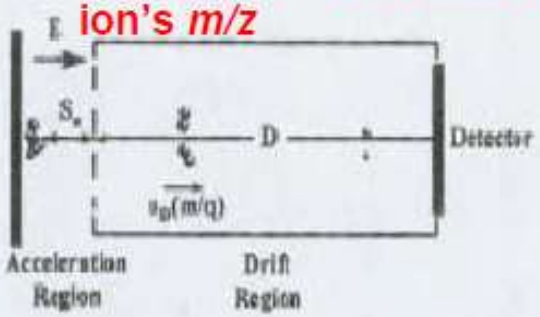
Mass Analyzer - Basic Types

(a). Magnetic Sector

Magnetic field affect radius of curvature of ions -> m/z

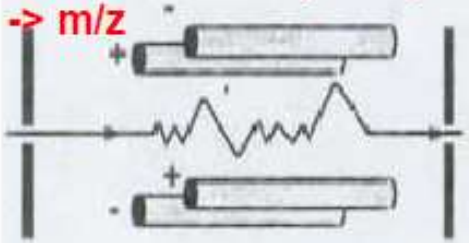
(b). Time of Flight (TOF)

Flight time - correlated directly with ion's *m/z*

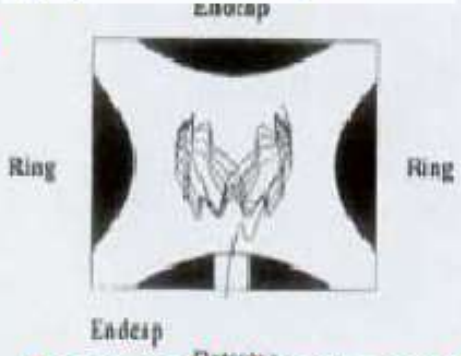


(c). Quadupole

Scan radio frequency field -> m/z



(d). Ion Trap



Scan radio frequency field

(e). Ion Cyclotron Resonance (ICR)

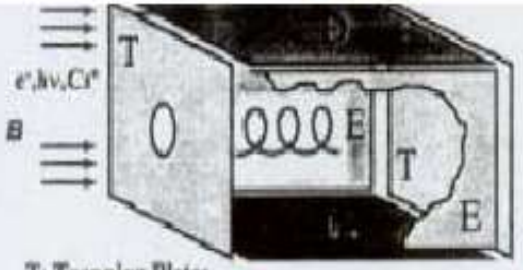
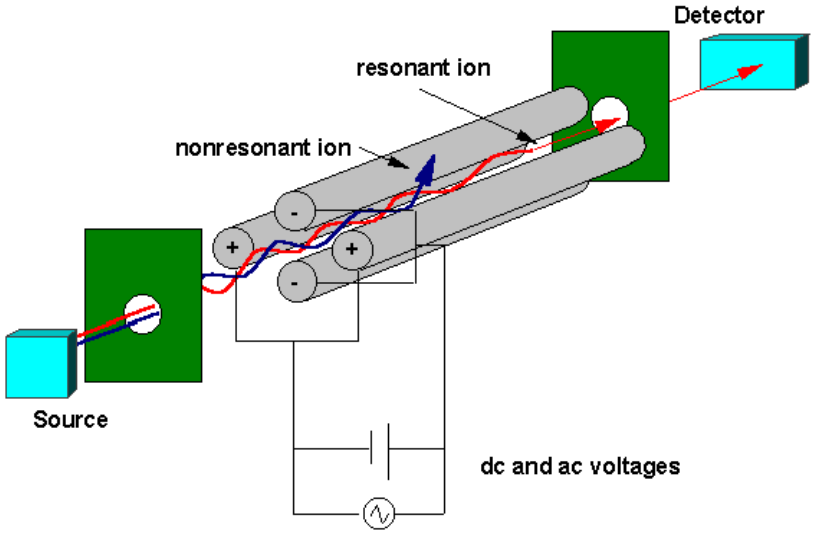


Image current

= Ion cyclotron frequency -> m/z

Quadrupole Mass Analyzer

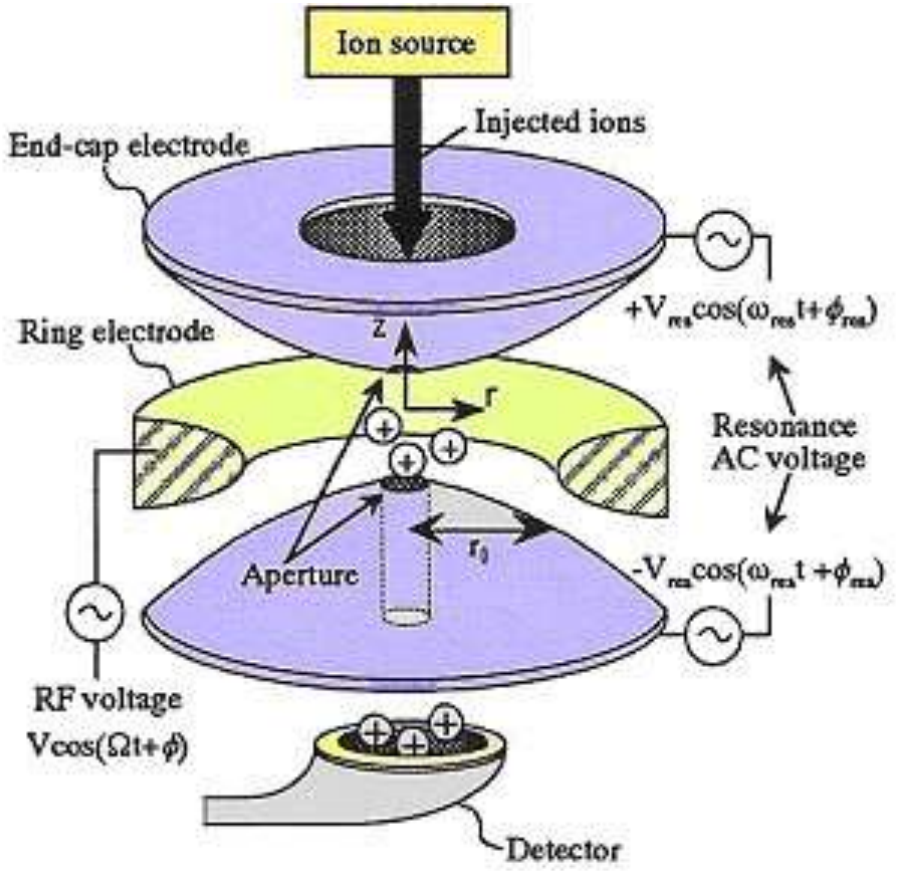
- A quadrupole mass filter consists of four parallel metal rods with different charges
- Two opposite rods have an applied + potential and the other two rods have a – potential
- The applied voltages affect the path of ions traveling down the flight path
- For given dc and ac voltages, **only ions of a certain mass-to-charge ratio pass through the quadrupole filter** and all other ions are thrown out of their original path



Ion Trap Mass Analyzer

- Ion traps are ion trapping devices that make use of a three-dimensional quadrupole field to trap and mass-analyze ions

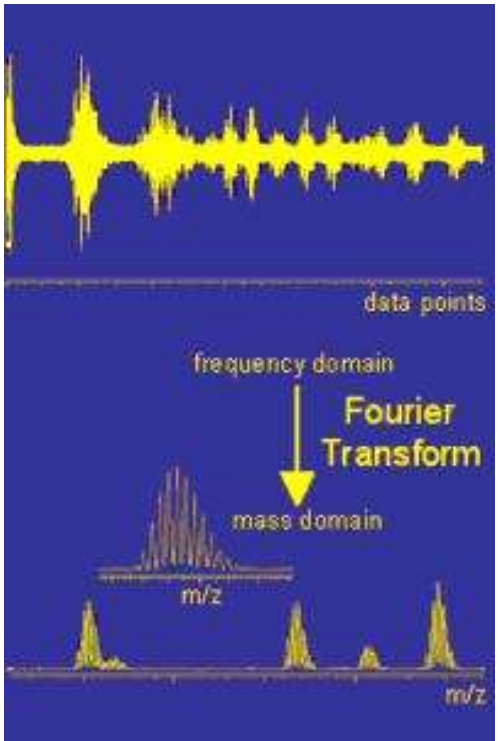
- Offer good mass resolving power



FT-ICR

Fourier-transform ion cyclotron resonance

- Uses powerful magnet (5-10 Tesla) to create a miniature cyclotron
- Originally developed in Canada (UBC) in 1974
- FT approach allows many ion masses to be determined simultaneously.
- Has higher mass resolution than any other MS analyzer available



Current Mass Spec Technologies

- **Proteome profiling/separation**
  - **2D SDS PAGE** - identify proteins
  - **2-D LC or LC** - high throughput analysis of lysates

  (LC = Liquid Chromatography)
  - **2-D LC/MS** (MS= Mass spectrometry)

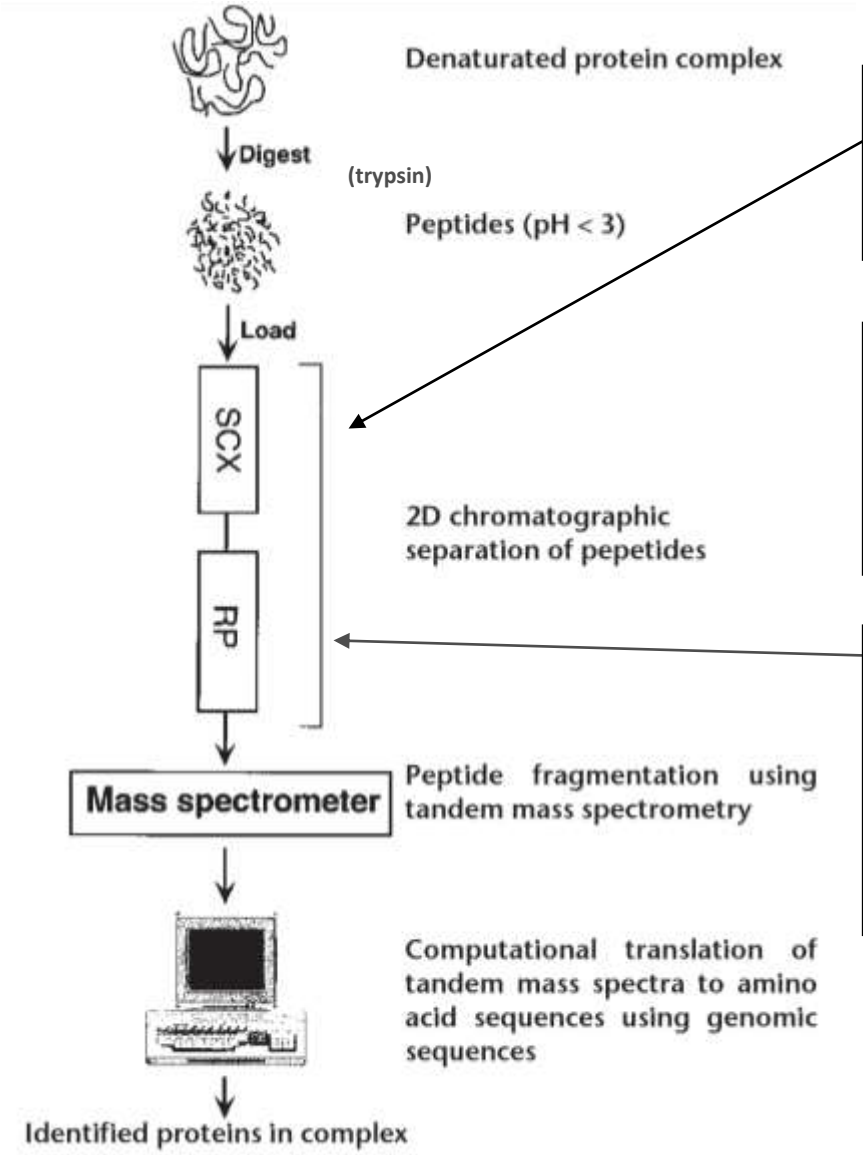
- **Protein identification**
  - Peptide mass fingerprint
  - Tandem Mass Spectrometry (MS/MS)

- **Quantative proteomics**
  - ICAT (isotope-coded affinity tag)
  - ITRAQ

2D – LC or LC

Study protein complexes without gel electrophoresis

Complex mixture is simplified prior to MS/MS by 2D LC

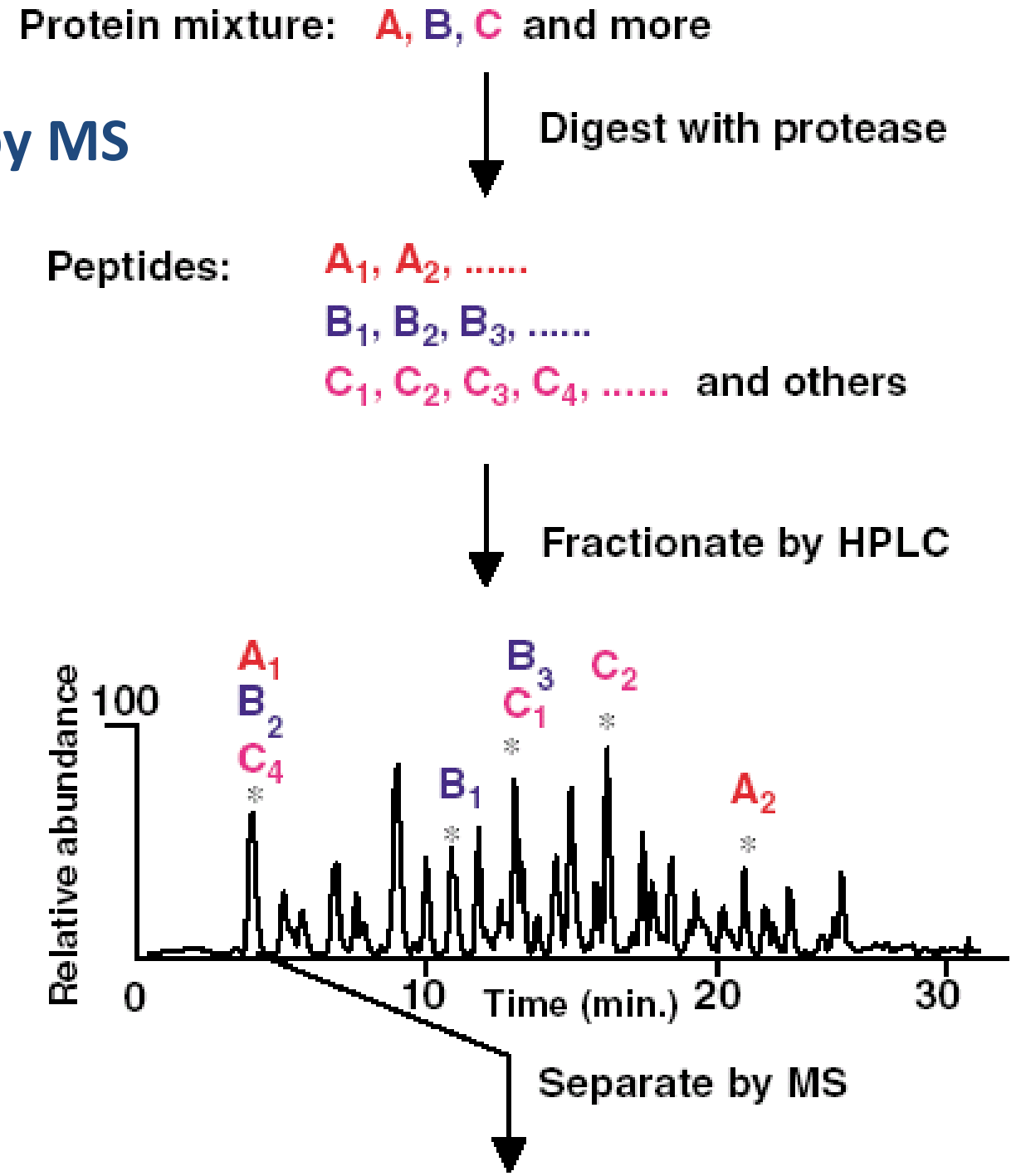


Peptides all bind to cation exchange column

Successive elution with increasing salt gradients separates peptides by charge

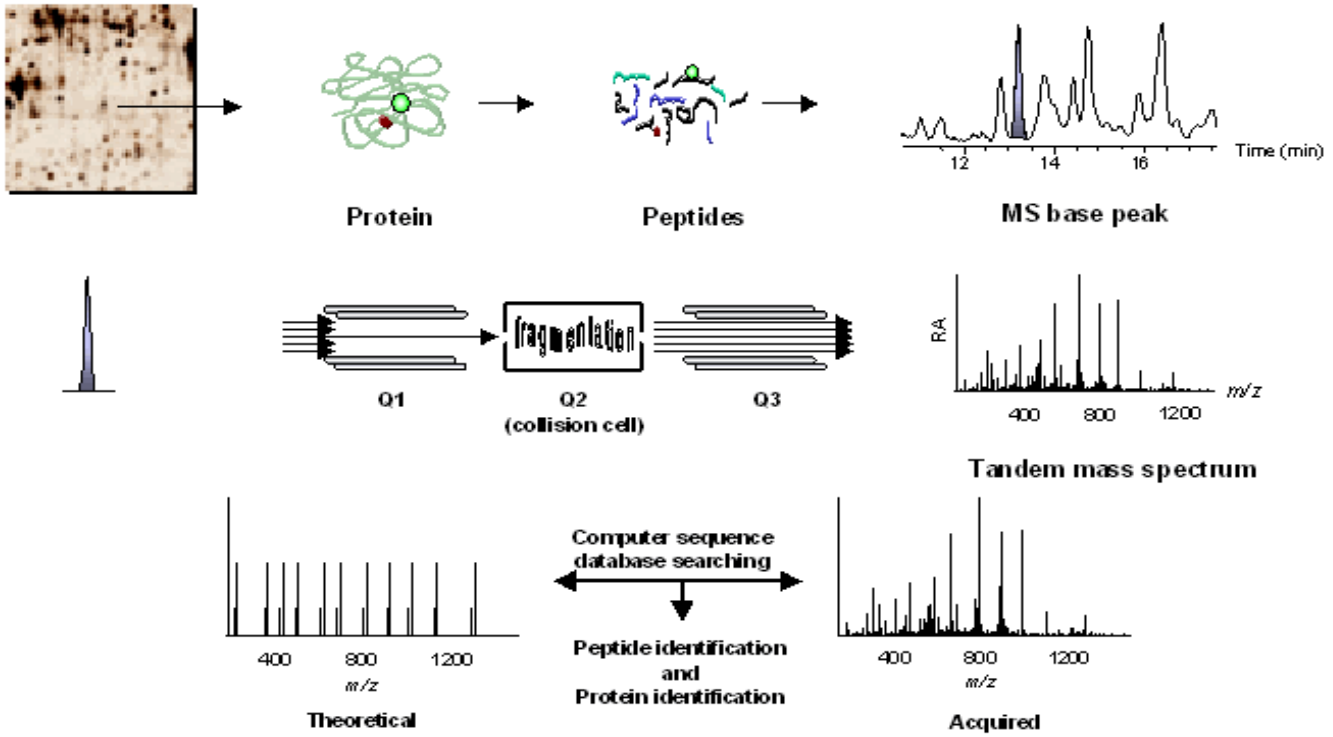
Peptides are separated by hydrophobicity on reverse phase column

2D – LC followed by MS

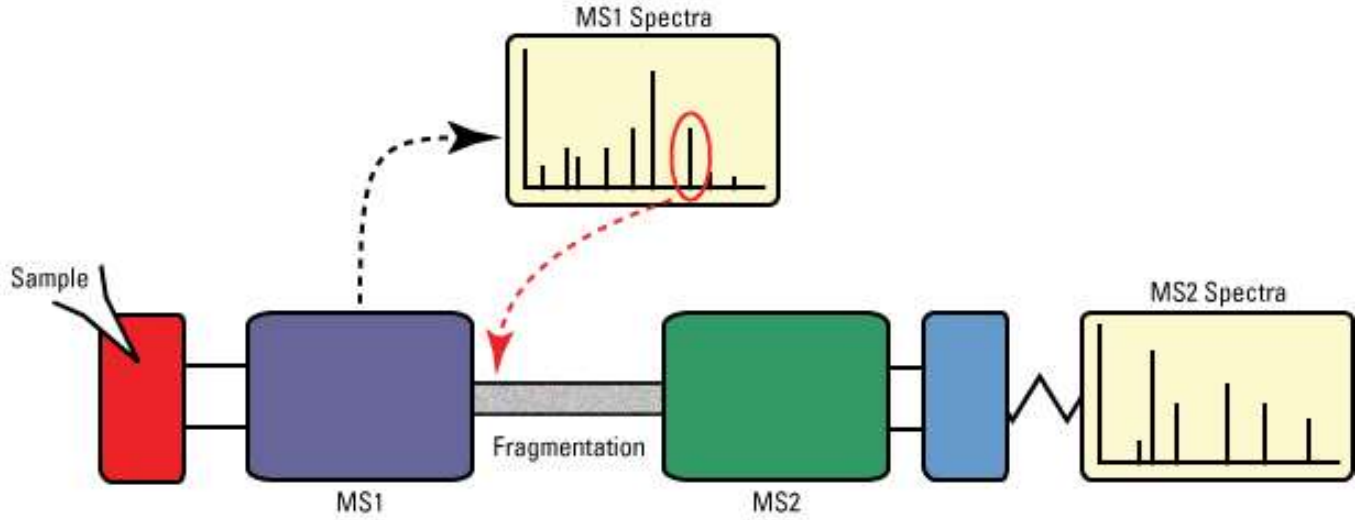


Tandem Mass Spectrometry

- Purpose is to fragment ions from parent ion to provide *structural information* about a molecule
- Also allows mass separation and AA identification of compounds in complex mixtures
- Uses two or more mass analyzers/filters separated by a collision cell filled with Argon or Xenon
- Collision cell is where selected ions are sent for further fragmentation



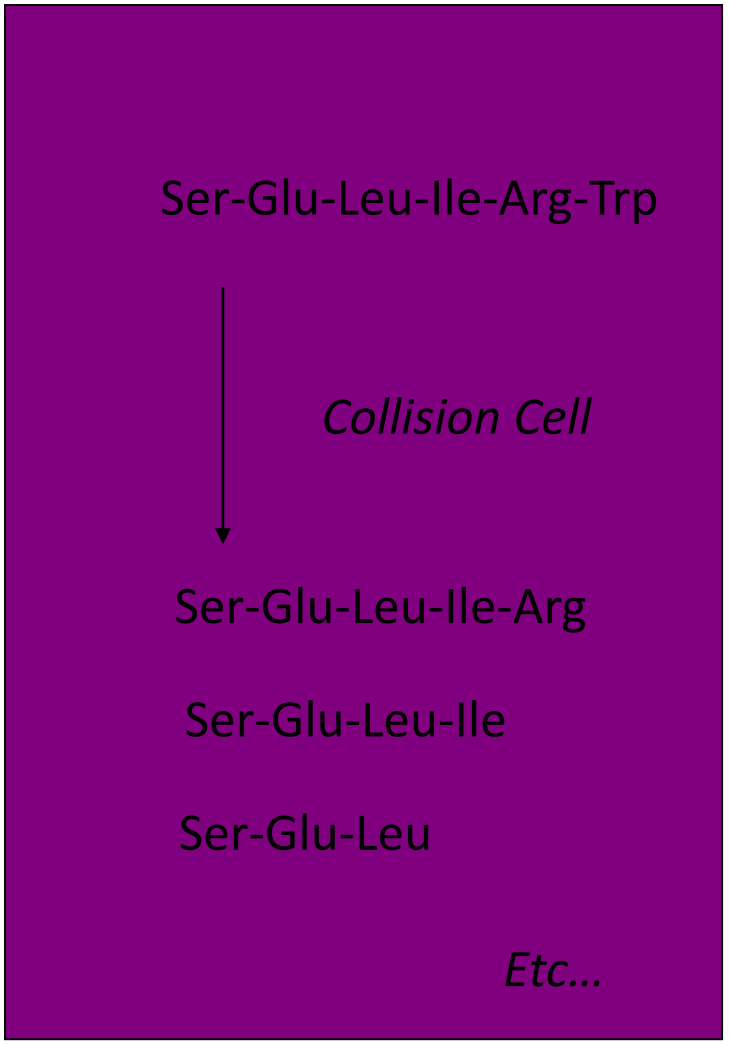
- **In Tandem mass spectrometry (MS/MS)**, distinct ions of interest are selected based on their *m/z* from the first round of MS and are fragmented by a number of methods of dissociation.
- One such method involves colliding the ions with a stream of inert gas, which is known as collision-induced dissociation (CID) or higher energy collision dissociation (HCD). Other methods of ion fragmentation include electron-transfer dissociation (ETD) and electron-capture dissociation (ECD).
- These fragments are then separated based on their individual *m/z* ratios in a second round of MS. MS/MS (i.e., tandem mass spectrometry) is commonly used to sequence proteins and oligonucleotides and these can be match with databases such as IPI, RefSeq and UniProtKB/Swiss-Prot.
- These sequence fragments can then be organized *in silico* into full-length sequence predictions.



- Diagram of tandem mass spectrometry (MS/MS).
- A sample is injected into the mass spectrometer, ionized, accelerated and analyzed by mass spectrometry (MS1).
- Ions from the MS1 spectra are then selectively fragmented and analyzed by a second stage of mass spectrometry (MS2) to generate the spectra for the ion fragments.

How Tandem MS sequencing works

- Use Tandem MS: two mass analyzers in series with a collision cell in between
- Collision cell: a region where the ions collide with a gas (He, Ne, Ar) resulting in fragmentation of the ion
- Fragmentation of the peptides occur in a predictable fashion, mainly at the peptide bonds
- The resulting daughter ions have masses that are consistent with known molecular weights of dipeptides, tripeptides, tetrapeptides…



Advantages of Tandem Mass Spec

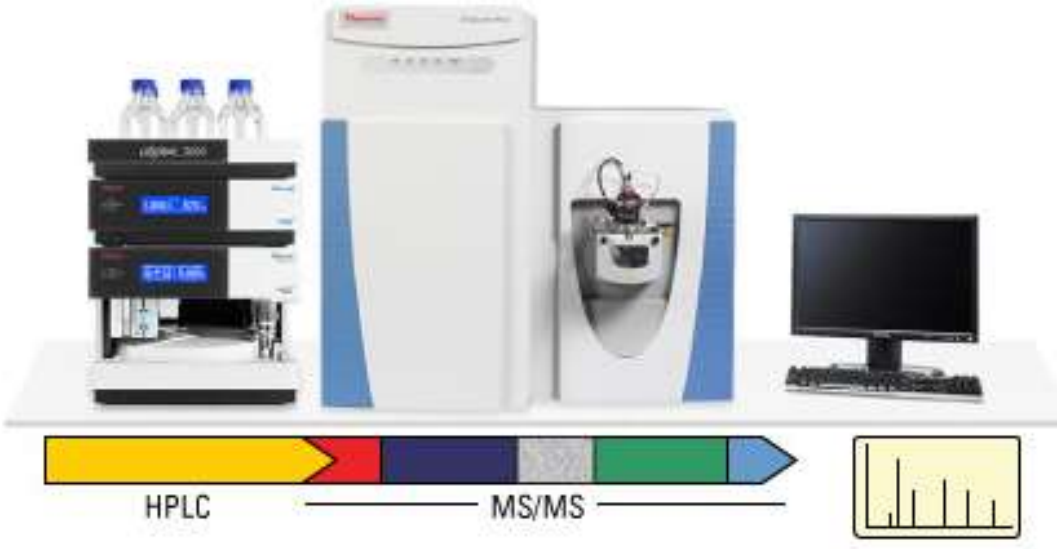
FAST
No Gels
Determines MW and AA sequence
Can be used on complex mixtures-including low copy
Can detect post-translational modifation.
High-thoughput capability

Disadvantages of Tandem Mass Spec

Very expensive-Campus
Hardware: $1000
Setup: $300
1 run: $1000
Requires sequence databases for analysis

- **Gas chromatography (GC)** and **liquid chromatography (LC)** are common methods of pre-MS separation that are used when analyzing complex gas or liquid samples by MS, respectively.
- LC-MS is typically applied to the analysis of thermally unstable and nonvolatile molecules (e.g., sensitive biological fluids),
- while GC-MS is used for the analysis of volatile compounds such as petrochemicals.
- LC-MS and GC-MS also use different methods for ionization of the compound.
- In LC-MS, the sample may be ionized directly by electrospray ionization (EI) and in GC-MS, the sample may be ionized directly or indirectly via EI.
- **High performance liquid chromatography (HPLC)** is the most common separation method to study biological samples by MS or MS/MS (termed **LC-MS** or **LC-MS/MS**, respectively), because the majority of biological samples are liquid and nonvolatile.

- LC columns have small diameters (e.g., 75 µm; nanoHPLC) and low flow rates (e.g., 200 nL/min), which are ideal for minute samples.
- Additionally, "in-line" liquid chromatography (LC linked directly to MS) provides a high-throughput approach to sample analysis, enabling multiple analytes to elute through the column at different rates to be immediately analyzed by MS.
- For example, 1-5 peptides in a complex biological mixture can be sequenced per second by in-line LC-MS/MS .



Quantitative Proteomics

- While mass spectrometry can detect very low analyte concentrations in complex mixtures, MS is not inherently quantitative because of the considerable loss of peptides and ions during analysis.
- Therefore, peptide labels or standards are parallelly analyzed with the sample and act as a reference point for both relative or absolute analyte quantitation, respectively.
- Commercial products are now available that allow the detection and quantitation of multiple proteins in a single reaction, demonstrating the high-throughput and global analytical platform that MS has become in the field of proteomics.
- **In Relative quantitation** approaches, proteins or peptides are labeled with stable isotopes that give them distinct mass shifts over unlabeled analytes.

- This mass difference can be detected by MS and provides a ratio of unlabeled to labeled analyte levels.
- These approaches are often used in discovery proteomics, where many proteins are identified across a broad dynamic range using different-sized labels.
- **Absolute quantitation** is performed in targeted proteomic experiments and increases the sensitivity of detection for a limited number of target analytes.

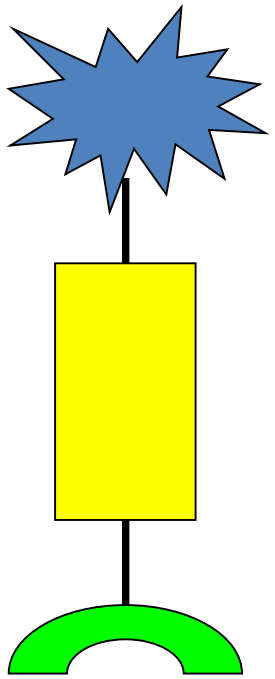
ISOTOPE-CODED AFFINITY TAG (ICAT): a quantitative method

- **Label protein samples with heavy and light reagent**
- **Reagent contains affinity tag and heavy or light isotopes**

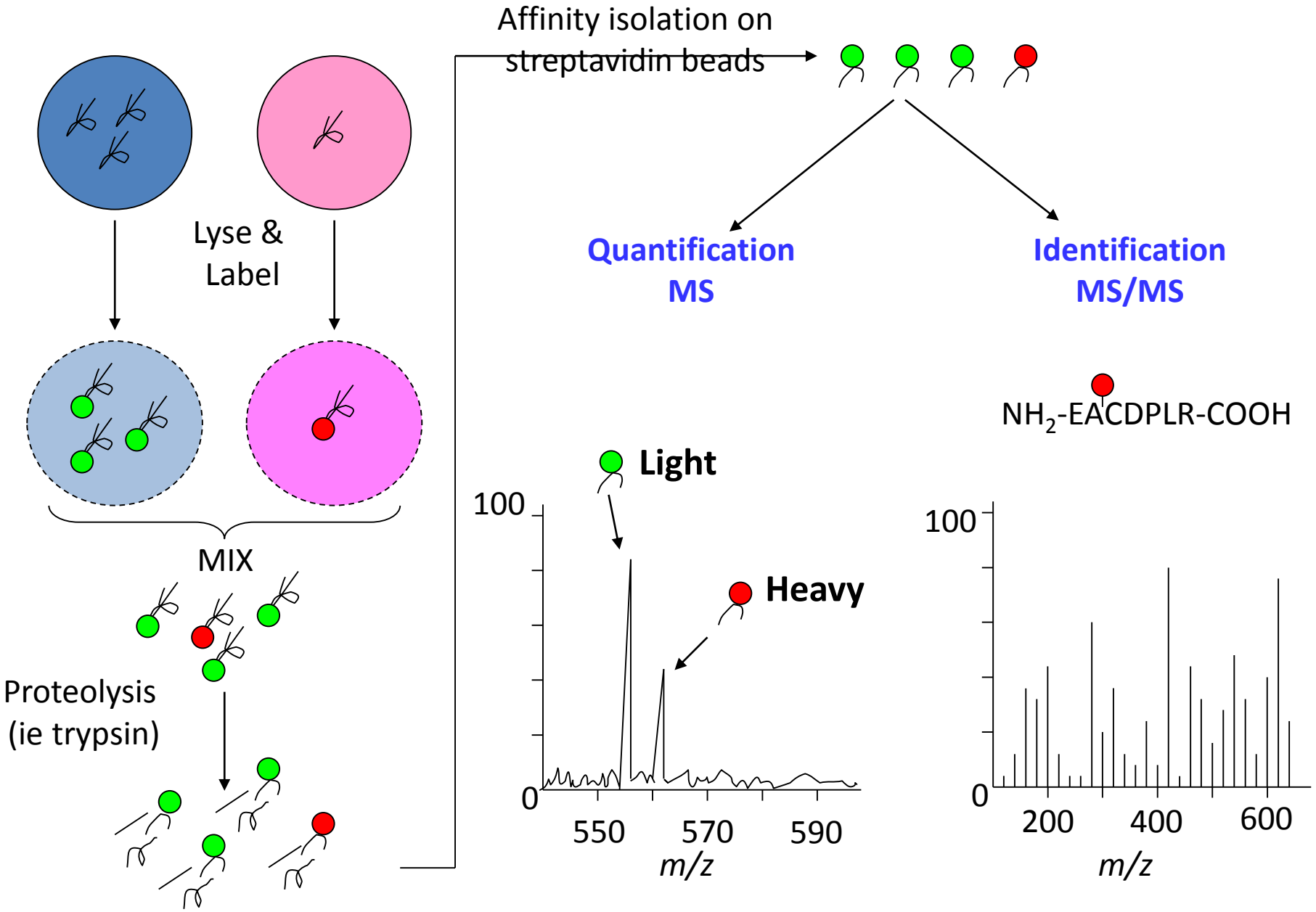
Chemically reactive group: forms a covalent bond to the protein or peptide

Isotope-labeled linker: heavy or light, depending on which isotope is used

Affinity tag: enables the protein or peptide bearing an ICAT to be isolated by affinity chromatography in a single step



How ICAT works?

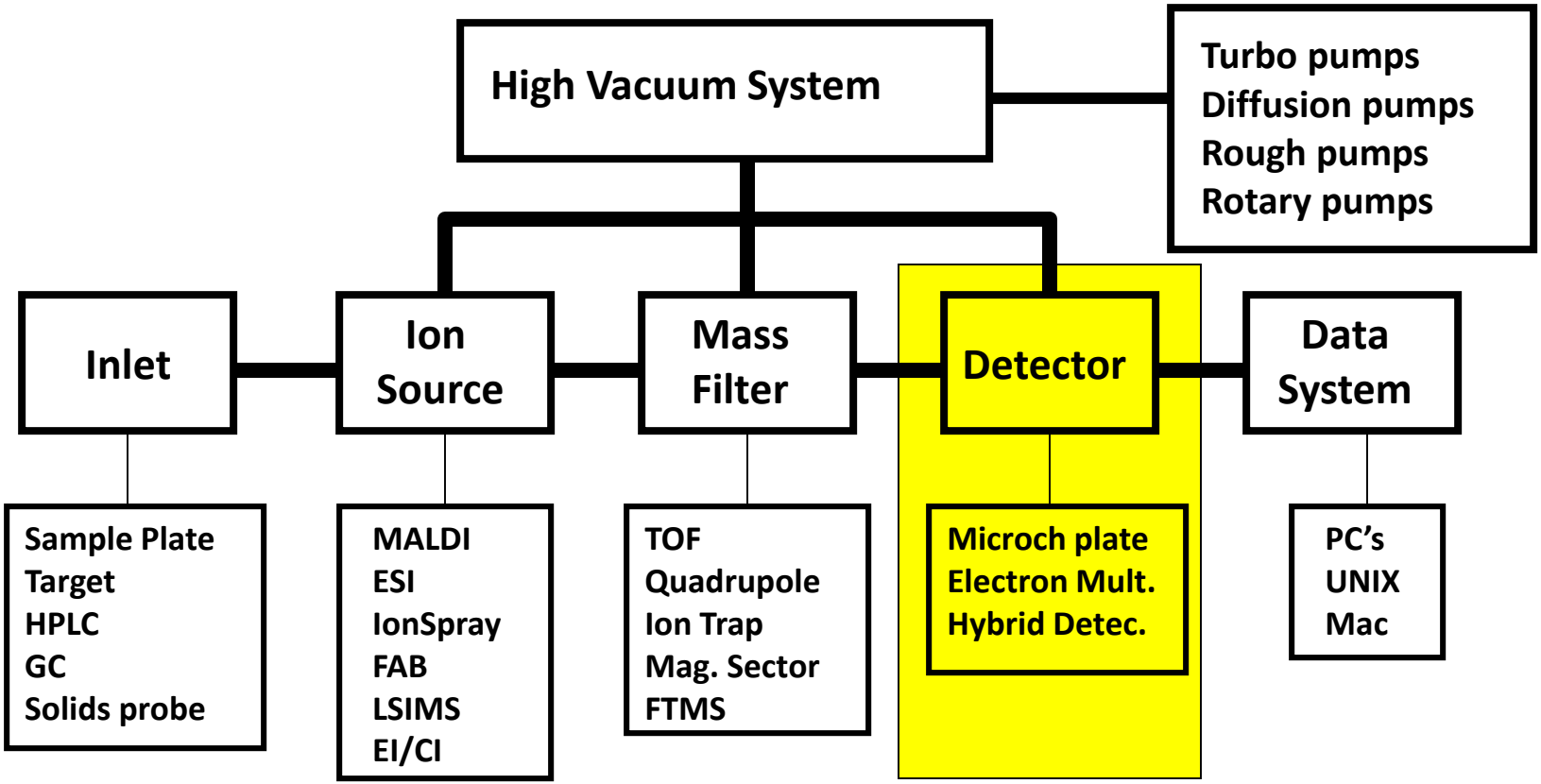


ICAT
Advantages vs. Disadvantages

- Estimates relative protein levels between samples with a reasonable level of accuracy (within 10%)
- Can be used on complex mixtures of proteins
- Cys-specific label reduces sample complexity
- Peptides can be sequenced directly if tandem MS-MS is used

- Non specificity
- Slight chromatography differences
- Expensive
- Tag fragmentation
- Meaning of relative quantification information
- No presence of cysteine residues or not accessible by ICAT reagent

Mass Spectrometer Schematic



Mass Detectors

Mass detector detects a current signal generated from the incident ions. Different mass detectors are used to detect ions depending on the type of mass spectrometer.

Most Commonly Used Detectors:

- Electron Multiplier
- Faraday Cup
- Photomultiplier Conversion Dynode
- High-Energy Dynode Detector (HED)
- Array Detector e.g. Microchannel Plate (MCP)
- Charge (or Inductive) Detector e.g. FTMS (detect image current)

DETECTORS

- Detection of ions is based upon their charge or momentum.
- For large signals a faraday cup is used to collect ions and measure the current.
- Older instruments used photographic plates to measure the ion abundance at each mass to charge ratio.
- A detector is selected for it‘s speed, dynamic range, gain, and geometry.

Channeltron

- A channeltron is a horn-shaped continuous dynode structure that is coated on the inside with a electron emissive material.
- An ion striking the channeltron creates secondary electrons that have an avalanche effect to create more secondary electrons and finally a current pulse.

Daly detector

- A Daly detector consists of a metal knob that emits secondary electrons when struck by an ion.
- The secondary electrons are accelerated onto a scintillator that produces light that is then detected by a photomultiplier tube.

Electron multiplier tube (EMT)

- Similar in design to photomultiplier tubes and consist of a series of dynodes that eject secondary electrons when they are struck by an ion. Multiply the ion current and can be used in analog or digital mode.

Faraday cup

- Metal cup is placed in the path of the ion beam and is attached to an electrometer, which measures the ion-beam current.
- Since a Faraday cup can only be used in an analog mode it is less sensitive than other detectors that are capable of operating in pulse-counting mode.

General Comparison of Mass Detectors

Detector	Advantages	Disadvantages
Faraday Cup	•Good for checking ion transmission and low sensitivity measurements	•Low amplification (≈10)
Photomultiplier Conversion Dynode (Scintillation Counting)	•Robust •Long lifetime (>5 years) •Sensitive (≈gains of 10^6)	•Cannot be exposed to light while in operation
Electron Multiplier	•Robust •Fast response •Sensitive (≈gains of 10^6)	•Shorter lifetime than scintillation counting (~3 years)
High Energy Dynodes with electron multiplier	•Increases high mass sensitivity	•May shorten lifetime of electron multiplier
Array	•Fast and sensitive	•Reduces resolution •Expensive
Charge Detection	• Detects ions independent of mass and velocity	•Limited compatibility with most existing instruments

MS-MS & Proteomics

Advantages

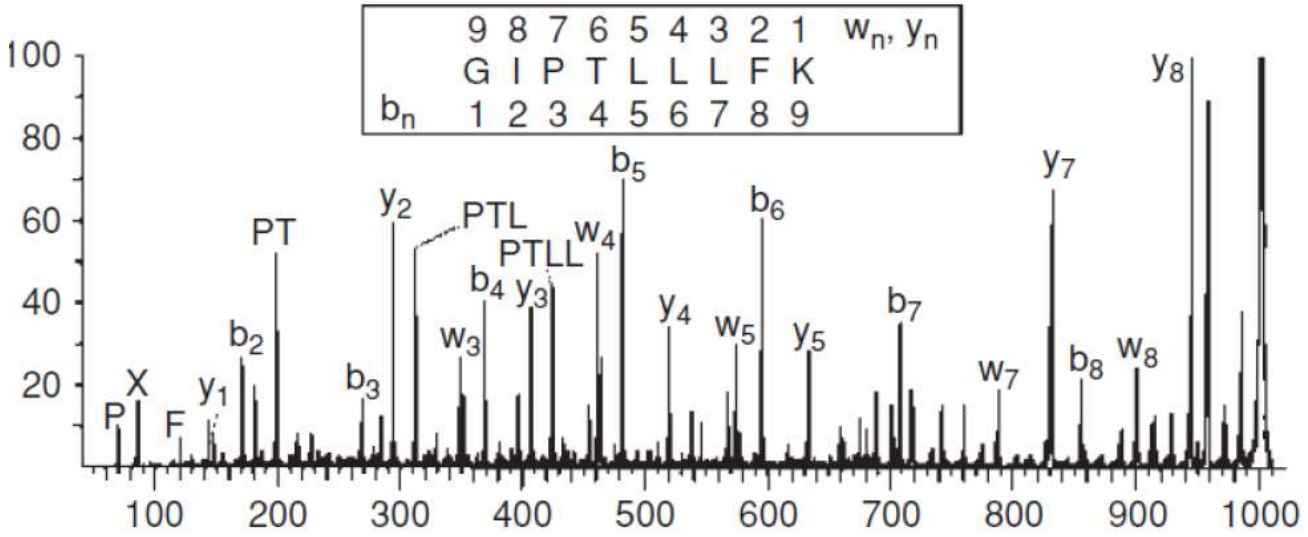
- Provides precise sequence-specific data
- More informative than Peptide mass fingerprinting methods (>90%)
- Can be used for de-novo sequencing (not entirely dependent on databases)
- Can be used to identify post-trans. modifications

Disadvantages

- Requires more handling, refinement and sample manipulation
- Requires more expensive and complicated equipment
- Requires high level expertise
- Slower, not generally high throughput

Data Interpretation:

- The mass difference between consecutive ions within a series allows the identity of the consecutive amino acids to be determined.
- MS/MS fragment spectrum of a peptide with sequence Gly–Ile–Pro–Thr–Leu–Leu–Leu–Phe–Lys is shown below and complete series of bn ions, thus allowing one to deduce the peptide sequence from the N-terminal acid to the C-terminal acid, whereas the series of yn ions allows identification of the sequence in the reverse direction.



- Mass difference of 97 Da between peak b2 and b3 indicates amino acid in position 3 is proline 147 Da difference between peaks y1 and y2 indicates amino acid in the next-to-last position is a phenylalanine.
- The m/z values of ions w3, w4, w5 and w8 imply that the amino acid in positions 3, 4 and 5 starting from the C-terminal side are leucines, whereas the amino acid in position 8 is an isoleucine
- The presence of a proline induces the formation of internal fragments labelled PT, PTL and PTLL that allow one to verify the deduced sequence. The peaks labelled P, F and X represent the immonium ions and indicate the presence of proline, phenylalanine and leucine and/or isoleucine.
- Several algorithms have been developed to interpret tandem mass spectra of peptides and searches the database to find the best sequence that matches the spectrum.
- De novo spectral interpretation involves automatically interpreting the spectra using the table of amino acid masses.

MS/MS for peptide sequencing

Peptide: ^{N}**ABCDEFG**^{C}

MSMS

A BCDEFG

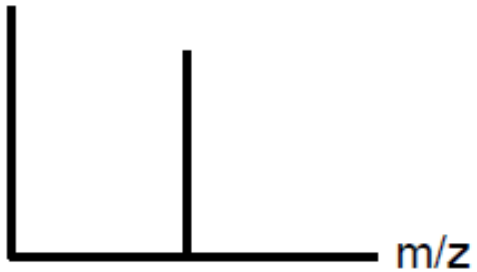
AB CDEFG

ABC DEFG

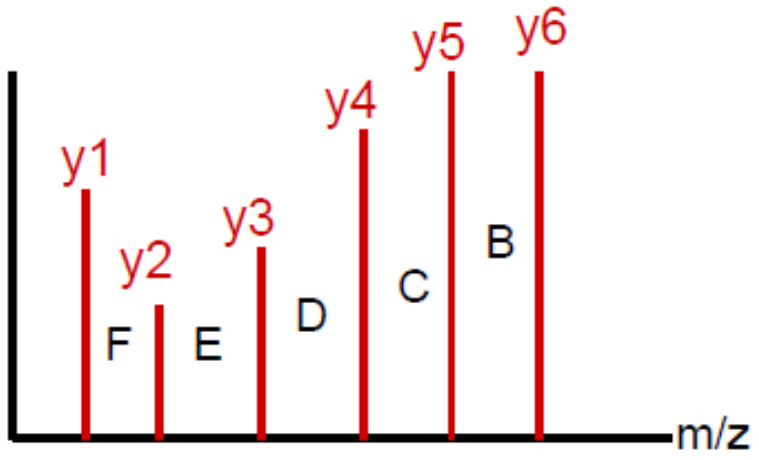
ABCDE FG

ABCDEF G

B_n ions y_n ions



MSMS



Applications of MS

Field of Study	Applications
Proteomics	Determine protein structure, function, folding and interactions
	Identify a protein from the mass of its peptide fragments
	Detect specific post-translational modifications throughout complex biological mixtures
	Quantitate (relative or absolute) proteins in a given sample
	Monitor enzyme reactions, chemical modifications and protein digestion
Drug Discovery	Determine structures of drugs and metabolites
	Screen for metabolites in biological systems
Clinical Testing	Perform forensic analyses such as confirmation of drug abuse
	Detect disease biomarkers (e.g., newborns screened for metabolic diseases)
Genomics	Sequence oligonucleotides
Environment	Test water quality or food contamination
Geology	Measure petroleum composition and Perform carbon dating

Limitations of MS

- It doesn't directly give structural information
- It Needs pure compounds
- It is difficult with non-volatile compounds
- A mass spectrometer cannot distinguish between isomers of a compound. If isomers are compounds having the same molecular formula but different structural formula; these are termed as cis and trans isomers.
- Mass spectrometers cannot distinguish between optical isomers. Optical isomers are non-superimposible mirror images of each other and are termed as enantiomers. For example, alanine is an amino acid that is composed of (+) and (–) forms. Enantiomers react differently to plane polarized light.
- Mass spectrometers cannot distinguish between ortho(o-), meta(m-), and para(p-) substituents of aromatic compounds.
- Mass spectrometers fail to identify similar fragmented ions in hydrocarbons. The ionization process sometimes produces too much fragmentation, so we cannot determine whether highest mass ion is a molecular ion of hydrocarbons.

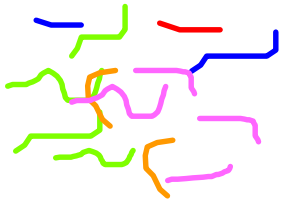
Shotgun Proteomics: Multidimensional Protein Identification Technology (MudPIT)

General Strategy for Proteomics Characterization

Fractionation & Isolation

2-DE

Liquid Chromatography



Peptides

Mass Spectrometry

MALDI-TOF MS
μ-(LC)-ESI-MS/MS

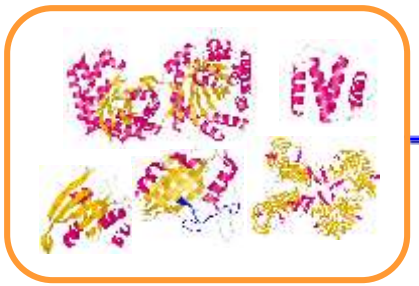
Database Search

Characterization

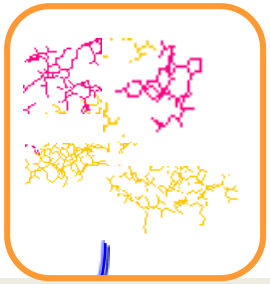
- Identification
- Post Translational modifications
- Quantification

Overview of Shotgun Proteomics: MudPIT

Protein Mixture



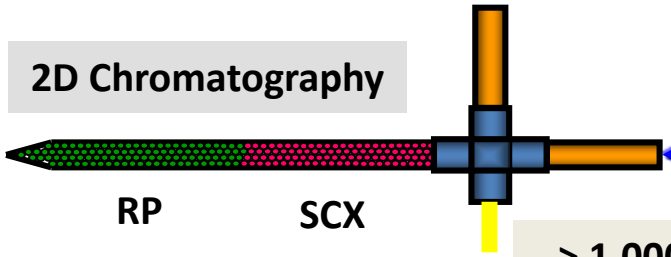
Digestion



Peptide Mixture

Tandem Mass Spectrometer

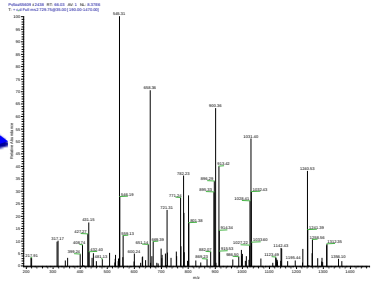
2D Chromatography

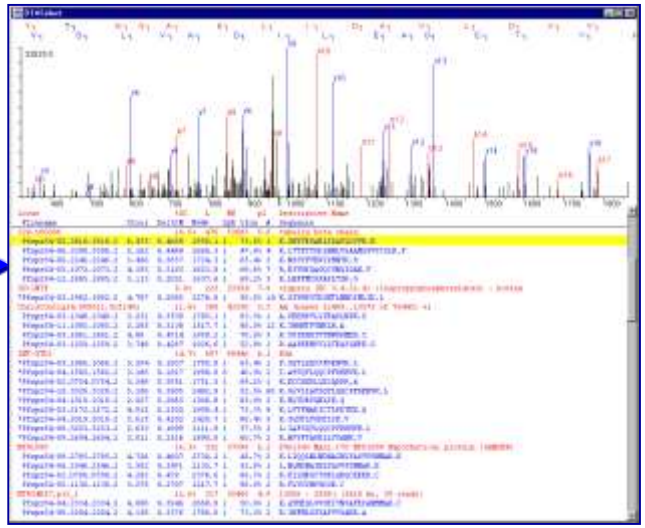


> 1,000 Proteins Identified

SEQUEST®
DTASelect & Contrast

MS/MS Spectrum





Summary of MudPIT

- It is an automated and high throughput technology.
- It is a totally unbias method for protein identification.
- It identifies proteins missed by gel-based methods (i.e. (low abundance, membrane proteins etc.)
- Post translational modification information of proteins can be obtained, thus allowing their functional activities to be derived or inferred.

2-DE *vs* MudPIT

- Widely used, highly commercialized
- High resolving power
- Visual presentation

- Limited dynamic range
- Only good for highly soluble and high abundance proteins
- Large amount of sample required

- Highly automated process
- Identified proteins with extreme pI values, **low abundance and those from membrane**
- Thousands of proteins can be identified

- Expensive
- Computationally intensive
- Quantitation

Thank You