Mass Spectrometry 101

Hackert - BCH 370

Based in part on material from
"An Introductory Lecture On Mass Spectrometry Fundamentals"
Presented to the Sandler Mass Spectrometry Users' Group,
University of California San Francisco, and
"Fundamentals of Mass Spectrometry – Based Proteomics"
by Doug Sheeley – Division of Biomedical Technology, National Center for Research Resources

What does a mass spectrometer do?

1. It measures mass **(m/z)** better than any other technique.

2. It can give information about chemical structures.

What are mass measurements good for?

To identify:

metabolites, synthetic organic chemicals
peptides, proteins, recombinant proteins,
oligonucleotides, polymers, drug candidates
→ sequencing!

What are the essential parts of a mass spec?

Ion source / Analyzer / Detector (databases)

The History of Mass Spectrometry

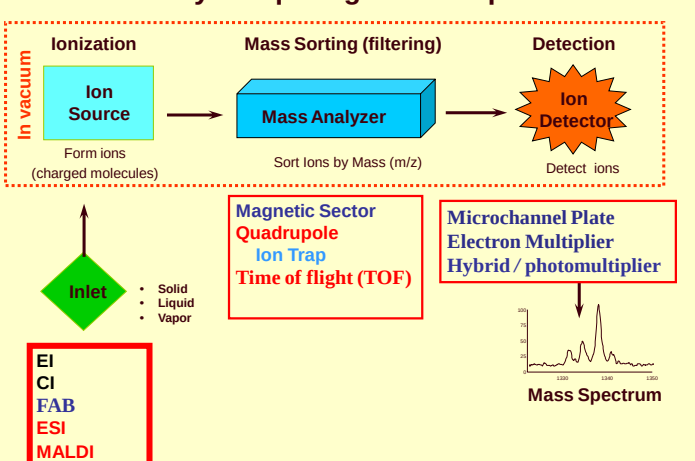
The Five Mass Spectrometry Nobel Prize Pioneers

Joseph John Thomson	Francis William Aston	Wolfgang Paul	John Bennet Fenn	Koichi Tanaka
1906 Nobel Prize for Physics "in recognition of the great merits of his theoretical and experimental investigations on the conduction of electricity by gases"	1922 Nobel Prize for Chemistry "for his discovery, by means of his mass spectrograph, of isotopes, in a large number of non-radioactive elements, and for his enunciation of the whole-number rule"	1989 Nobel Prize for Physics "for the development of the ion trap technique"	2002 Nobel Prize for Chemistry "for the development of soft desorption ionisation methods (ESI) for mass spectrometric analyses of biological macromolecules"	2002 Nobel Prize for Chemistry "for the development of soft desorption ionisation methods (MALDI) for mass spectrometric analyses of biological macromolecules"

~1897 1919 1946 1953 1956 1968 1985
Isotopes TOF Quad GC/MS ESI MALDI

MS Timeline - http://masspec.scripps.edu/mshistory/timeline/timeline.php

Summary: acquiring a mass spectrum



Mass Spectrometry – Focus on Proteomics

Source: produces charged particles (ions)

- **Electron Impact (EI) - Hard (fragments) / 1000 Da**
- Chemical Ionization (CI) – (methane / isobutane / ammonia)
- Fast Atom Bombardment (FAB) – 6keV xenon atoms
- **Electrospray Ionization (ESI) - Soft / 200,000 Da (200 kDa)**
- **Matrix-Assisted Laser Desorption Ionization - Soft / 500kDa**

Mass Spectrometry

Introductory Example: mass spectrum of water

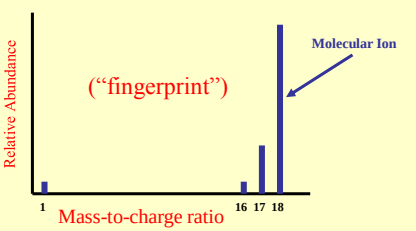
- **Electron Impact (EI) - Hard (fragments) / 1000 Da**

H_2O + fast electron → $[H_2O]+$ + 2 electrons

+ fragments ([OH]+, O+, H+)

Fragmentation pattern

$[H_2O]+$	18
[OH]+	17
O+	16
H+	1



How is mass defined?

Assigning numerical value to the intrinsic property of "mass" is based on using **carbon-12, ^{12}C**, as a reference point.

One unit of mass is defined as a **Dalton (Da).**

One Dalton is defined as 1/12 the mass of a single carbon-12 atom.

Thus, **one ^{12}C atom has a mass of 12.0000 Da**.

Dalton - atomic mass unit (symbol: **Da**) **:** A unit of mass used to express the mass of atomic and subatomic particles, equal to 1/12 the rest mass of an unbound atom of **carbon-12** atom in its nuclear and electronic ground state and has a value of $1.660538782\times10^{-27}$ kg. One Da is *approximately* equal to the mass of a proton or a neutron. *Note: a Dalton is a "non-SI" unit whose value must be measured.*

Isotopes

Most elements have more than one stable isotope.

For example, most carbon atoms have a mass of 12 Da, but in nature, **1.1% of C atoms have an extra neutron, making their mass 13 Da.**

Why do we care?

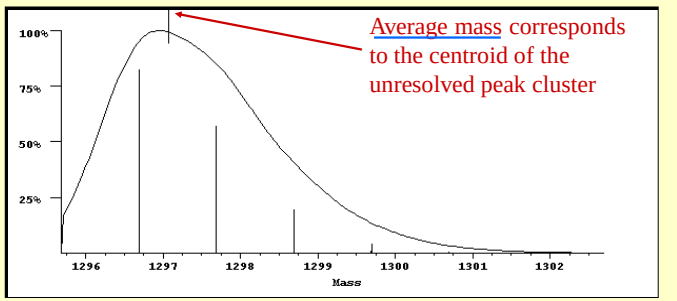
Mass spectrometers can "see" isotope peaks, *if* their resolution is high enough.

If a MS instrument has resolution high enough to resolve these isotopes, better mass accuracy is achieved.

Stable isotopes of most abundant elements of peptides

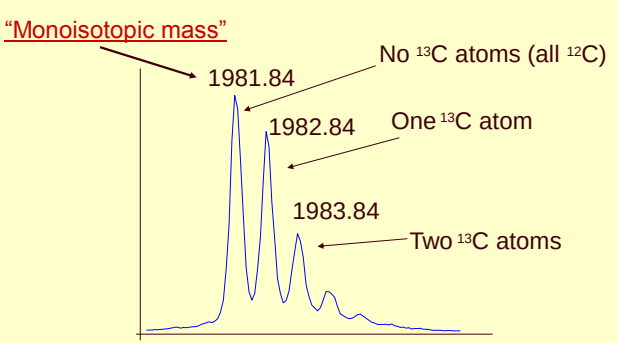
Element	Mass	Abundance
H	1.0078 2.0141	99.985% 0.015
C	12.0000 13.0034	98.89 1.11
N	14.0031 15.0001	99.64 0.36
O	15.9949 16.9991 17.9992	99.76 0.04 0.20

Average mass

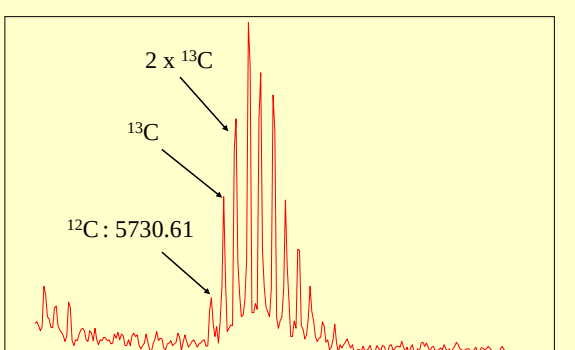


When the isotopes are not resolved, the centroid of the envelope corresponds to the weighted average of all the the isotope peaks in the cluster, which is the same as the average or chemical mass.

Mass spectrum of peptide with 94 C-atoms (19 amino acid residues)



Mass spectrum of insulin



Insulin has 257 C-atoms. Above this mass, the monoisotopic peak is too small to be very useful, and the average mass is usually used.

Mass measurement accuracy depends on resolution

(Mass assignment is easier with higher resolution)

SELECTED COMPARISONS OF MOLECULAR WEIGHTS AND PRECISE MASSES

Molecular Formula (MF)	Molecular Weight (MW) (g/mole)	Precise Mass
C_3H_8O	60.1	60.05754
$C_2H_8N_2$	60.1	60.06884
$C_2H_4O_2$	60.1	60.02112
CH_4N_2O	60.1	60.03242

(from Pavia, Lampman, Kritz and Vyvyan)

How do mass spectrometers get their names?

Types of ion sources:

- **Electrospray** (ESI) - **Soft / 200kDa**
- **Matrix Assisted Laser Desorption Ionization** (MALDI) ~ **500kDa**

Types of mass analyzers:

- **Quadrupole (Quad, Q)**
- **Ion Trap**
- **Time-of-Flight (TOF)**

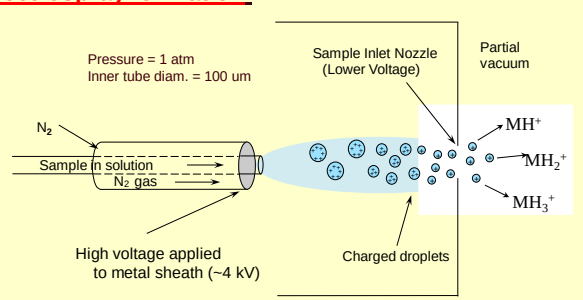
-Either source type can work with either analyzer type: "MALDI-TOF," "ESI-Quad."

-Analyzers can be combined to create "hybrid" instruments. ESI-QQQ, **MALDI QQ TOF**, Q Trap

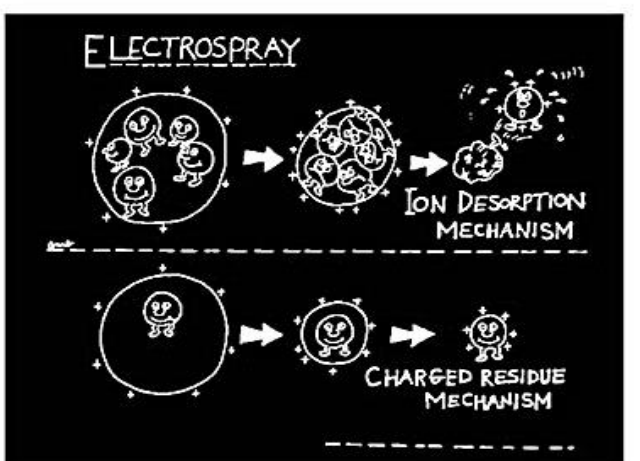
Ion Sources make ions from sample molecules

(Ions are easier to detect than neutral molecules.)

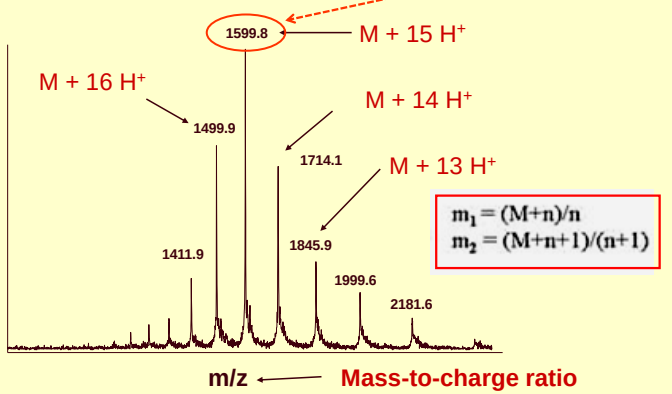
Electrospray ionization:



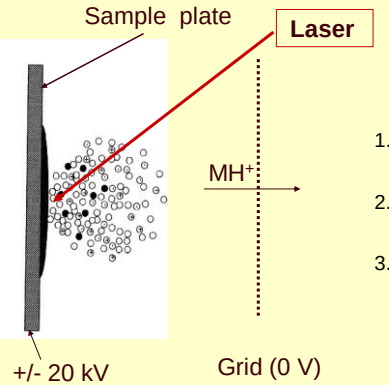
Very gentle / solvent evaporates / multiple charges



ESI Spectrum of Trypsinogen (MW 23,983)

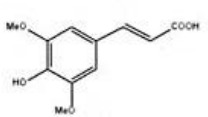


MALDI: Matrix Assisted Laser Desorption Ionization

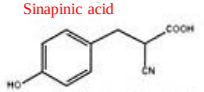


1. Sample is mixed with matrix (X) and dried on plate.
2. Laser flash ionizes matrix molecules.
3. Sample molecules (M) are ionized by proton transfer: $XH^+ + M \rightarrow MH^+ + X$.

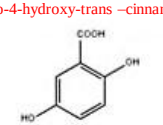
MALDI-TOFMS



Sinapinic acid



Cyano-4-hydroxy-trans –cinnamic acid



2,5-Dihydroxybenzoic acid

the three most commonly used matrices

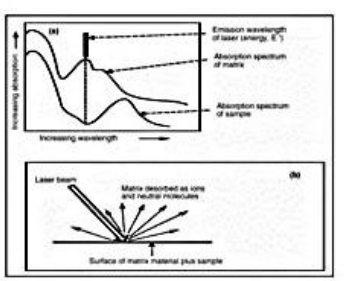
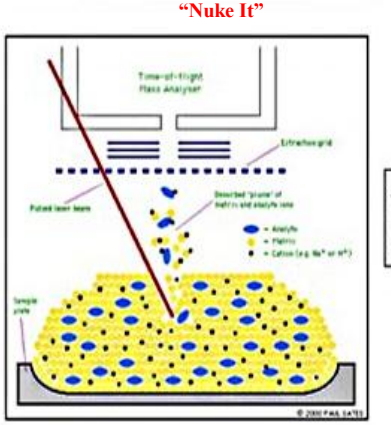


Figure 3 In a MALDI experiment, the sample is mixed or dissolved in a matrix material, which has an absorption spectrum that matches the laser wave length of energy, *E*. The sample may not have a matching absorption peak (a) but this is not important because the matrix material absorbs the radiation, some of which is passed on to the dissolved sample.
Neutral molecules and ions from both sample and matrix material are desorbed (b).

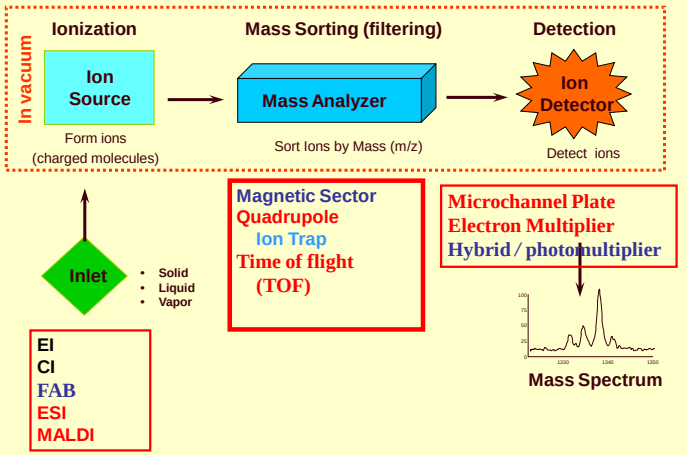
MALDI (Matrix Assisted Laser Desorption Ionization)

"Nuke It"



Analyte:
10 – 1000 fmol
1 – 500 kDa

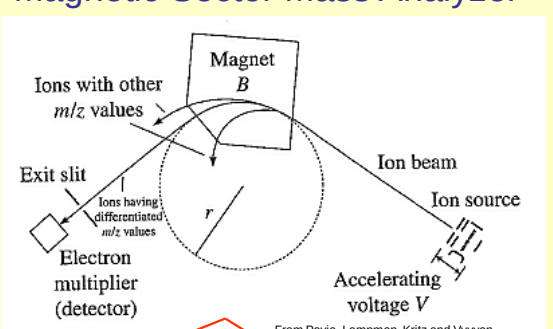
Summary: acquiring a mass spectrum



Mass analyzers separate ions based on their mass-to-charge ratio (m/z)

- Operate under **high vacuum** (keeps ions from bumping into gas molecules)
- Actually measure **mass-to-charge ratio** of ions **(m/z)**
- Key specifications are resolution, mass measurement accuracy, and sensitivity.
- Several kinds exist: for bioanalysis, quadrupole, time-of-flight and ion traps are most used.

Magnetic Sector Mass Analyzer



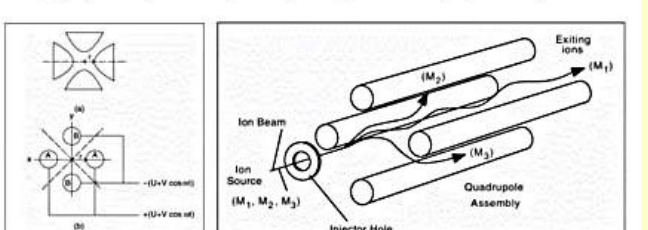
From Pavia, Lampman, Kritz and Vyvyan

$$\frac{1}{2}mv^2 = zV \quad \text{and} \quad r = \frac{mv}{zB} \longrightarrow \frac{m}{z} = \frac{B^2r^2}{2V}$$

Mass Analyzers: The Quadrupole Mass Filter

A potential of ~100-1000 V is applied alternately to the opposing pairs of rods at a frequency of a few MHz. At a specific combination of DC & RF, an m/z has a stable trajectory through the rods, and all other m/z are lost. The mass range is scanned as the voltages are swept from min to max, but at constant DC/RF ratio.

Faster Scanning than sector instruments (but not as fast as ion traps or TOF).
Mass Range generally m/z 0-2000 or 0-4000.
Facile MS/MS using Triple Quadrupole (Q-q-Q) analyzer.
Exquisitely sensitive in selected ion monitoring (both analyzers parked at one m/z).
Largely replaced by the ion trap and hybrid Q-q-TOF for biopolymer analysis.

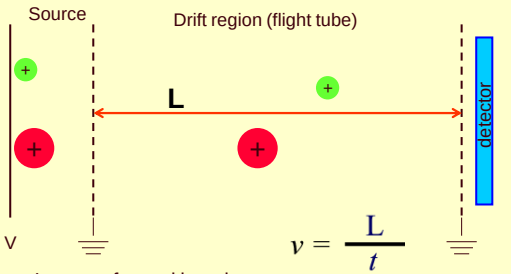


Quadrupoles have variable ion transmission modes

mass scanning mode

single mass transmission mode

Time-of-flight (TOF) Mass Analyzer

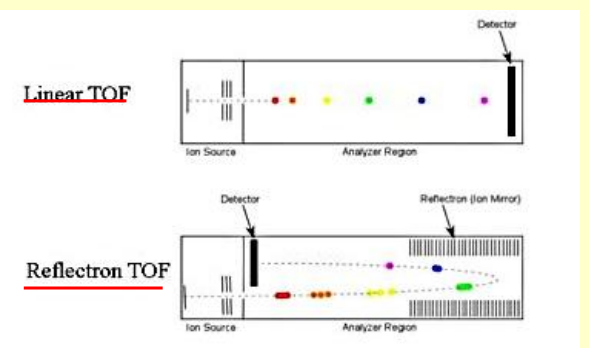


- Ions are formed in pulses.
- The drift region is field free.
- Measures the time for ions to reach the detector.
- Small ions reach the detector before large ones.

Time-of-flight (TOF) Mass Analyzer

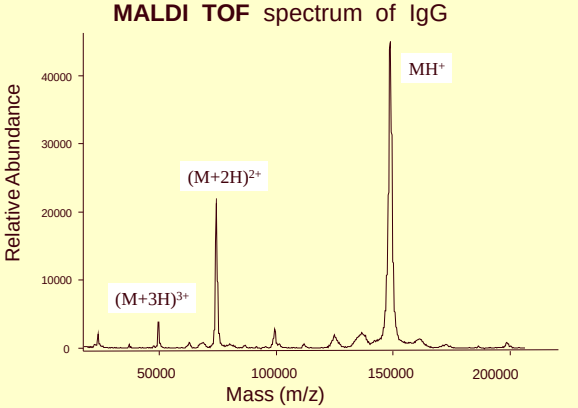
$$v = \frac{L}{t}$$

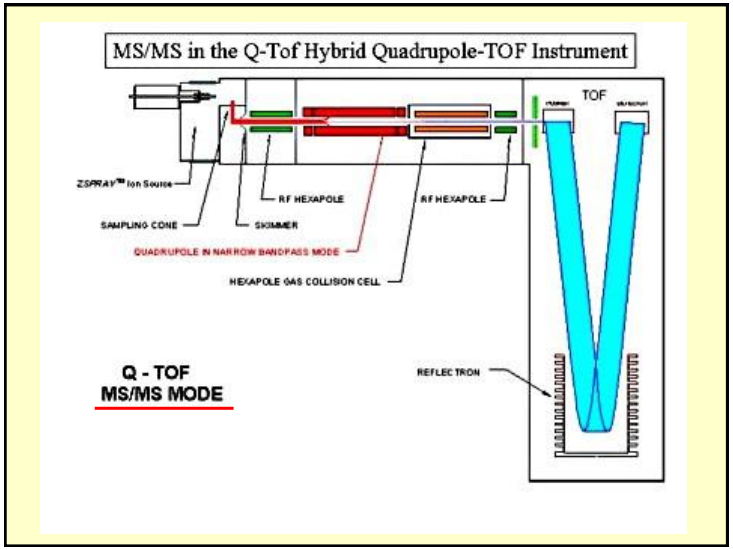
$$zV = \frac{mv^2}{2} = \frac{mL^2}{2t^2} \quad \text{or} \quad \frac{m}{z} = \frac{2Vt^2}{L^2}$$



The mass spectrum shows the results

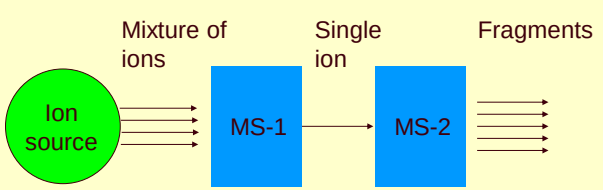
MALDI TOF spectrum of IgG



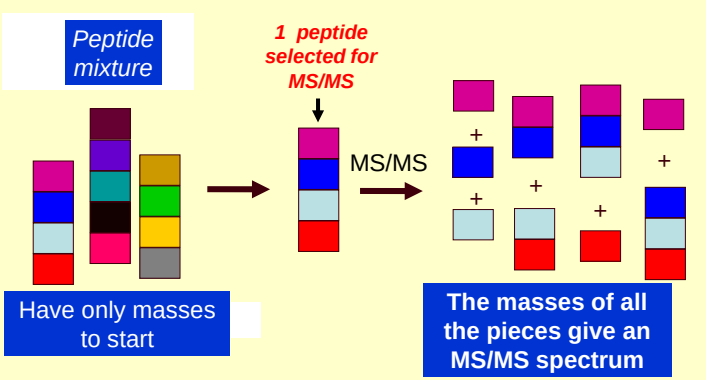


What is MSMS?

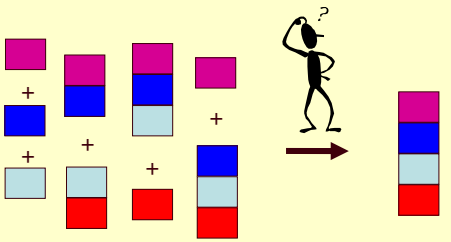
MS/MS means using two mass analyzers (combined in one instrument) to select an analyte (ion) from a mixture, then generate fragments from it to give structural information.



MS/MS



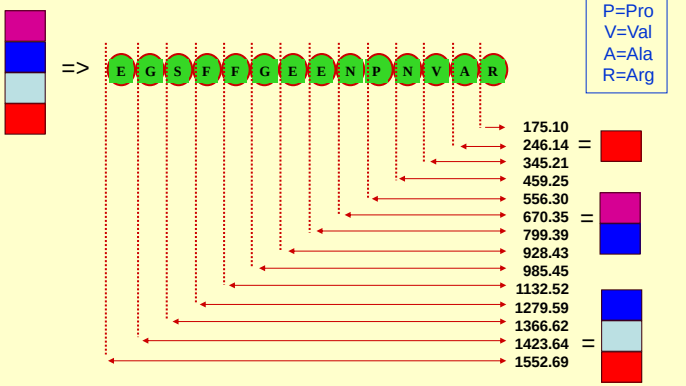
Interpretation of an MSMS spectrum to derive structural information is analogous to solving a puzzle



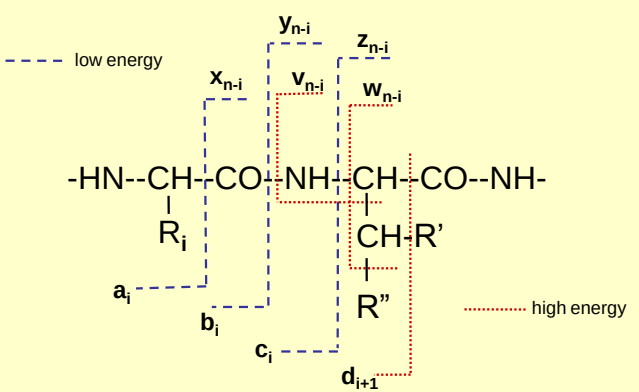
Use the fragment ion masses as specific pieces of the puzzle to help piece the intact molecule back together

Peptide Fragmentation

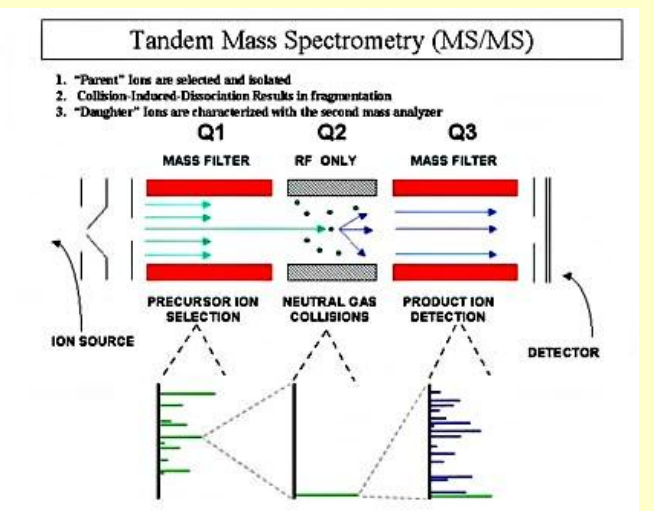
E=Glu
G=Gly
S=Ser
F=Phe
N=Asn
P=Pro
V=Val
A=Ala
R=Arg



Cleavages Observed in MS/MS of Peptides

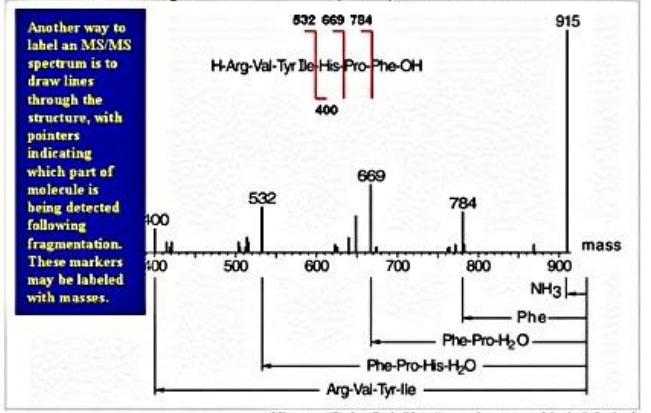


CID (Collision InDuced) Spectra – adds **sequence data** to **mass mapping** for improved database identification!



MS/MS of Angiotensin III:
selection and fragmentation of the (M+H)$^+$ molecular ion at m/z932

Another way to label an MS/MS spectrum is to draw lines through the structure, with pointers indicating which part of molecule is being detected following fragmentation. These markers may be labeled with masses.



Micromass "Back to Basics" http://www.micromass.co.uk/basics/index.html

insight review articles

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Mass spectrometry-based proteomics

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Recent successes illustrate the role of mass spectrometry-based proteomics as an indispensable tool for molecular and cellular biology and for the emerging field of systems biology. These include the study of protein–protein interactions via affinity-based isolations on a small and proteome-wide scale, the mapping of numerous organelles, the concurrent description of the malaria parasite genome and proteome, and the generation of quantitative protein profiles from diverse species. The ability of mass spectrometry to identify and, increasingly, to precisely quantify thousands of proteins from complex samples can be expected to impact broadly on biology and medicine.

Note: HT Proteomics is restricted to those species where a sequence database exists!

Report

Practical Proteomics 1-2/2006

Proteomics Education, an Important Challenge for the Scientific Community: Report on the Activities of the EuPA Education Committee

European Proteomics Association (EuPA)

EuPA Tutorial Program (preliminary draft)
Fundamentals and Core Techniques

Protein Chemistry	
	Amino acid chemistry/functionality
	PTM natural chemical/enzymatic modifications
	PTM un-natural chemical/enzymatic modifications
	Protein function families: E.C; GO classification
	X-ray principles
	NMR principles
	Protein substructure principles
	Protein structure families
	Membrane protein structure/function
	Extracellular protein structure/function
Protein-protein Interaction	
	Protein complex isolation & examples
	MS-TAP approach to complexes
	Two-hybrid approach
	Biacore, microcalorimetry & CD, FT, ...
DNA/RNA Techniques	
	DNA cloning & sequencing
	RNA structure determination
	Microarray formats
	SAGE
	SNP, methylation, CGH analysis
Separation Science	
	Affinity chromatography
	Free flow electrophoresis
	CZE
	Centrifugation
	HPLC
	2D-PAGE
Protein Expression	
	Antibody generation and use
	Phage display
	Protein arrays
	Tissue arrays
	HT cloning & expression library structure
	HT crystallisation

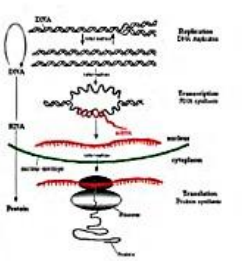
MS Basics	
	MALDI ionisation
	ESI ionisation
	TOF
	Quads
	Ion-trap, linear & 3D
	FT-ICR, Orbitrap
	Detectors
	Scan modes
Metabolomics	
	GC-MS approaches & derivatisation chemistry
	ESI-MS approaches & derivatisation chemistry
	NMR approaches
	Pathway analysis & modelling EcoCYC
Applied Technologies	
	Microfluidics
	Automation
	Fluorescent labeling, DNA sequencing, microarr
Bioinformatics/Systems Biology	
	Sequence homology searching
	Protein id by MALDI
	Protein id by MS/MS
	ID verification principles, Prophet, *etc.*
	Array analysis
	Database structure
	Relevant stat applications
	Advanced data mining techniques
	Web databases
	Experimental design principles

The Proteome

All an organism's cells carry the same Genome, and it is Static. Genomes do not describe function. They are a parts list.

Different cells express different proteins. The type and quantity of this expression changes.

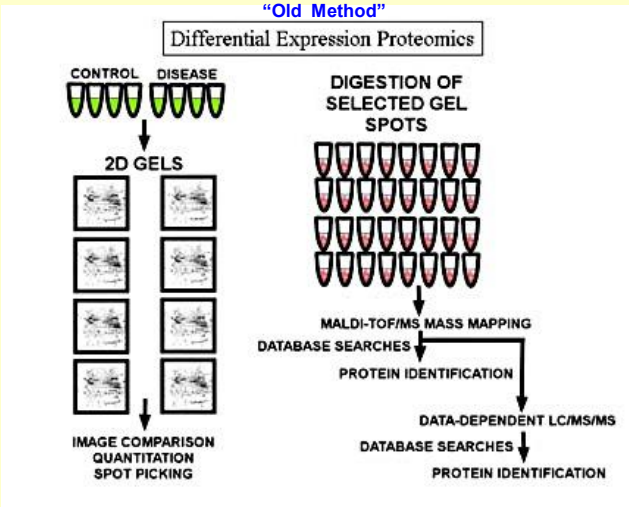
The Proteome is Dynamic. It is the total of all proteins expressed by a particular *cell* at a given *time*, under specific *conditions*.



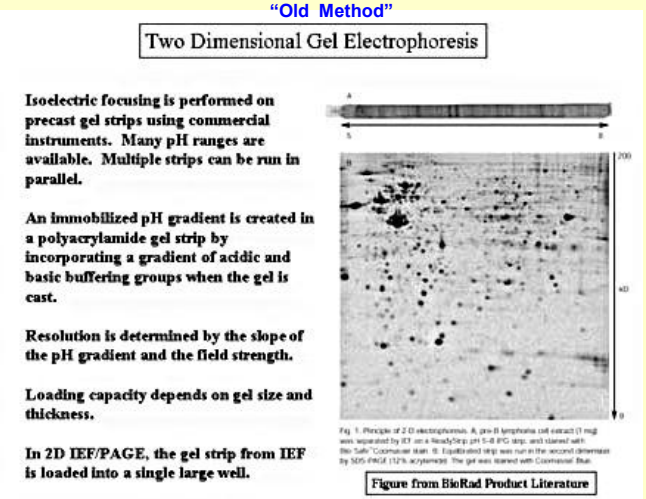
The Central Dogma of Molecular Biology

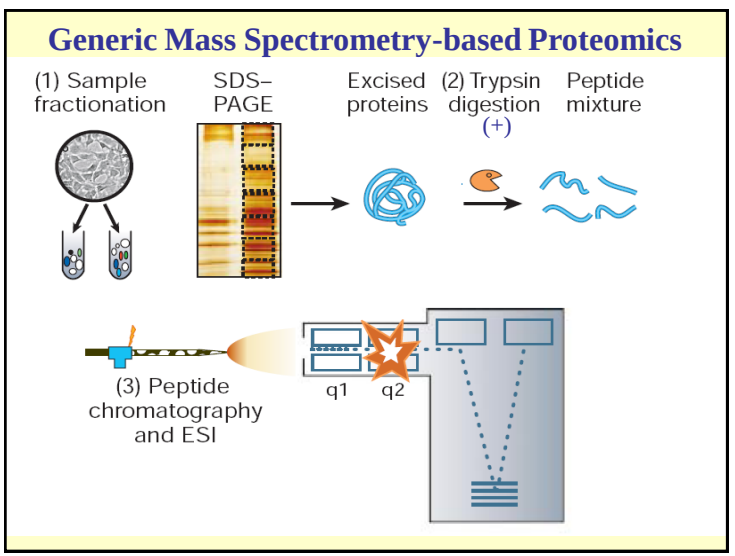
A Proteome cannot be studied the way a Genome is sequenced. There has to be a specific biological question behind an experiment. The questions may be either very broad or strictly defined.

"Old Method"



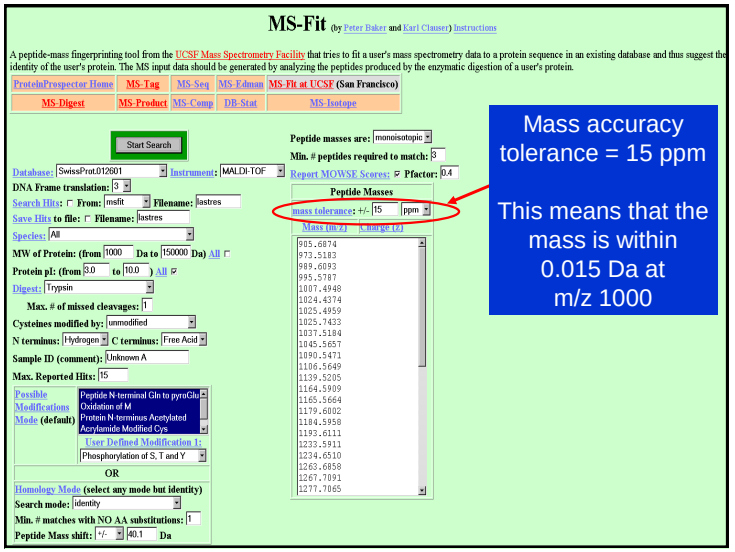
"Old Method"

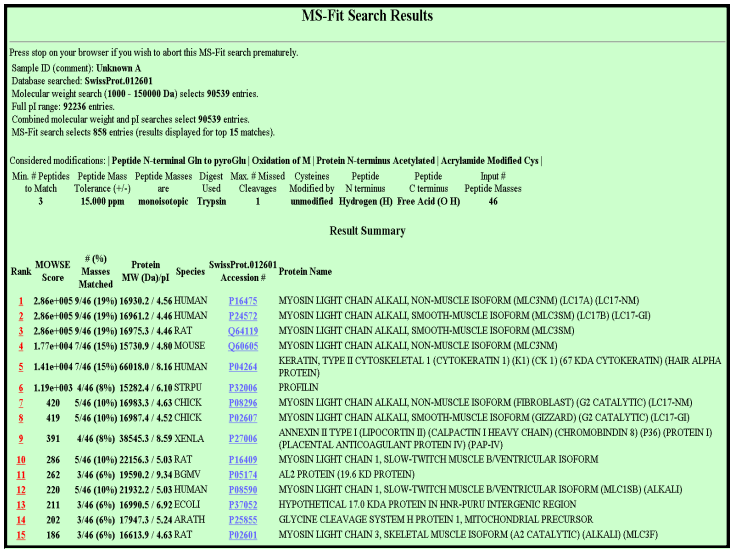




With the new genomic data bases of model species, such as *Esherichia coli, Saccharomyces cerevisae,* mouse, and human, the sequences of many/most proteins of biological interest will in principle be known, and the problem of characterizing a protein primary structure will be reduced to identifying it in the data base.

Within the past few years research groups have demonstrated how MS can be used for identification of proteins in sequence data bases. One approach is to **cleave the protein with a sequence-specific proteolytic enzyme**, **measure molecular weight** values for the resulting peptide mixture by mass spectrometry, and **search a sequence data base for proteins that should yield these values**. **Search algorithms** can utilize low resolution tandem mass spectra of selected peptides (<3 kDa) from the protein degradation. Yates and coworkers compared the MS/MS sequence data to the sequences predicted for each of the peptides that would be generated from each protein in the data base. **In the PEPTIDESEARCH sequence tag approach of Mann and Wilm, a partial sequence of 2–3 amino acids is assigned from the fragment mass differences in the MS/MS spectrum.** This partial sequence and its mass distance from each end of the peptide (based on the masses of the fragment and molecular ions) are used for the data base search. Often, **a single sequence tag retrieved only the correct protein from the data base**.



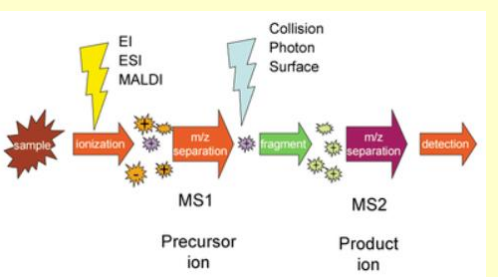


The MudPIT Breakthrough

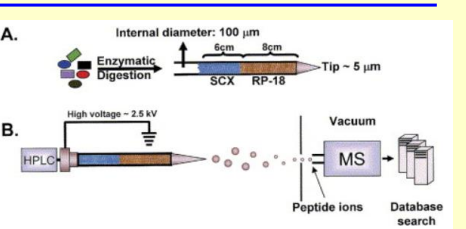
Traditional proteomics methodologies separate complex protein samples by **isoelectric point and molecular weight using 2-dimensional gels**. Patterns can be compared between samples, but to determine which protein is changing requires **isolating individual protein spots, proteolyzing these, and analyzing the mass of each peptide by Matrix-assisted laser desorption/ionization-time of flight** (MALDI-TOF) mass spectrometry. The measured peptide masses are searched against the predicted mass values for theoretical digestion of proteins in a sequence database, and the protein is identified by a statistically significant number of matches.

Multidimensional Protein Identification Technology (MudPIT) eliminates gel separations. Instead, **biochemical fractions containing many proteins are directly proteolyzed and the enormous number of peptides generated, are separated by 2-dimensional liquid chromatography before entering the mass spectrometer**. Instead of MALDI-TOF, the procedure employs tandem mass spectrometry so that, **after the mass of a peptide is measured, the peptide is fragmented using a collision-induced dissociation cell and the masses of the fragmentation products are determined. Considerable computational effort can typically transform this data into an amino acid sequence.** Thus **one peptide is often sufficient to identify a protein**, a sensitivity advantage that enables identification of minor proteins in a biological fraction that can not be visualized on 2-dimensional gels. Recent studies have **identified 1,000 to 2,000 proteins in a single fraction with MudPIT**.

MS/MS

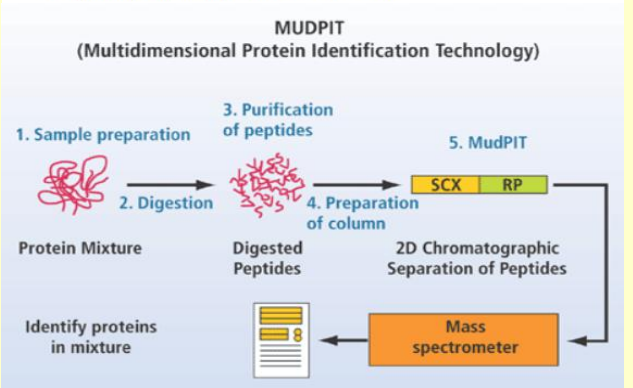


MUDPIT



A state of the art setup

- MudPIT (multi-dimensional protein identification technology)
- Originally developed at Yates lab



Modern mass spec based proteomics

(Because nucleic acids are overrated)

From: http://www.stats.ox.ac.uk/__data/assets/pdf_file/0020/5960/Proteomics_final.pdf

Find pdf link on course web site - Proteomics - Syllabus and Lecture Notes

What is proteomics?

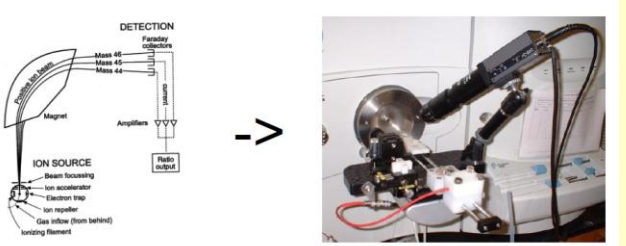
Dictionary definition:

- Proteomics is the systematic characterization of all the proteins in an organism, their abundance, localization, structure, modifications, function and interactions.
- Most researchers take a narrower view
  - Protein-protein interactions
  - Quantitative proteomics
  - Functional proteomics
- Various technogogies can be applied
  - Our focus: LC-MS/MS

Development of the technology

(From the deflection of "canal rays" to MudPIT)

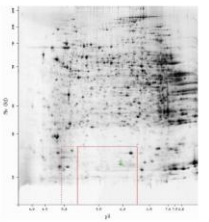
- Protein mass spectrometry
- Protein separation
- Data analysis

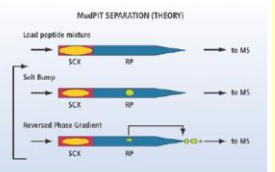


http://www.stats.ox.ac.uk/__data/assets/pdf_file/0020/5960/Proteomics_final.pdf

Protein separation

- 2D gel based approaches
  - low sensitivity (staining)
  - extensive sample handling
  - difficult to reproduce
  - no sympathy for the gel
- Chromatography based approaces
  - Washburn *et al.* (MudPIT), 2001
    - on-line
    - semi quantitative
    - more sensitive
    - high throughput



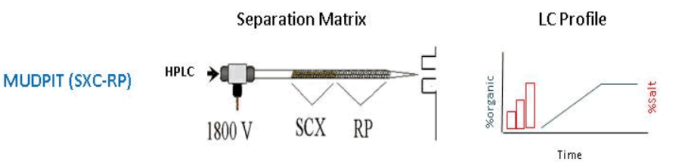


Protein Sequencing: MUDPIT

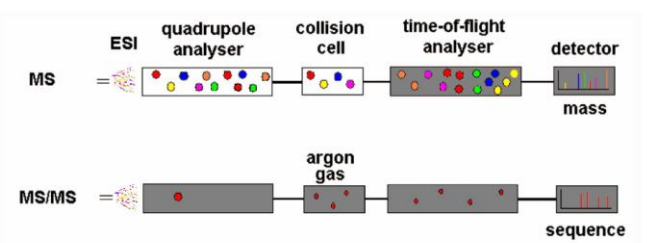
Mass Spectrometry and Complex Protein Mixture Sequencing (1-3000 proteins)

MUDPIT (multidimensional protein identification technology) is the method of choice for complex protein sample analysis in which more elaborate separation techniques are needed. For MUDPIT analysis strong cation exchange resin (SCX), which binds positively charged compounds, is packed in tandem to the RP-C18 resin. SCX resin first binds all peptides before they encounter the C18 material. The peptides are then eluted off the SCX using ammonium salts in a stepwise manner, starting at low salt concentration step and ending with a high salt concentration step. After each salt step some peptides will be released from the SCX resin and will bind the RP-C18 material. This salt step is then followed by a cycle of organic gradient to elute off the peptides from the C18 resin and into the mass spectrometer for sequencing. For more complex samples more salt steps can be added to the method to increase the separation capability of the setup.

Recent method developments in peptide separation are using alternative separation strategies to SCX to improve peak separation and hence increase peptide identifications for Mudpit. One encouraging method is the use of "**high-pH-reverse-phase**" separation. Similar to low pH reverse phase separation techniques currently used in LC-MS, the high pH equally generates high peak resolution for peptides. We have observed that the use of this method in replacement of SCX in Mudpit analysis increases peptide identifications in similar Mudpit runs by the **factor of two**.



Methodological background Quadrupole-TOF (MS/MS)



Operates on either MS or MS/MS mode

Data Analysis

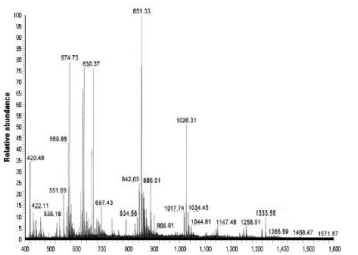
- Reducing raw data to manageable levels.
- Analysis
- Algorythms
- How to estimate the quality of data

Reducing raw data to manageable levels

- Preprocessing
  - Peak detection, peak labeling, baseline correction
  - Data reduction
    - noise removal, smoothing
  - Normalization
  - Deconvolution
    - Ion charge state recognition (isotope patterns)
  - Peak alignment

Analysis

- Database search, Mann and Yates
- High throughput data
- High noise
- Computationally intense
- Variety of software



Algorithms

Examples:

- SEQUEST (Yates 1995)
- Mascot
- ProLuCID
- Specral network analysis (Bandeira 2007)

SEQUEST

Basic concept published by Yates et al. in 1995.

- Reverse pseudospectral library search.
- Protein sequences analysed sequentially through entire database.
- Preliminary scoring equation:

$$S_p = (\sum i_m) n_i (1+\beta)(1+\varrho)/\eta_\tau$$

- Cross correlation by Fourier transforming gives final score.
- Detects modified amino acids by testing alternative masses for all possible modification sites.
- Descriptive model.

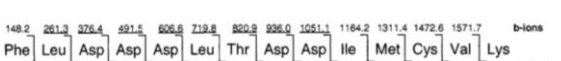


Figure 1. Schematic of the approach used by the computer algorithm to match tandem mass spectra of modified peptides to sequences in the protein database.

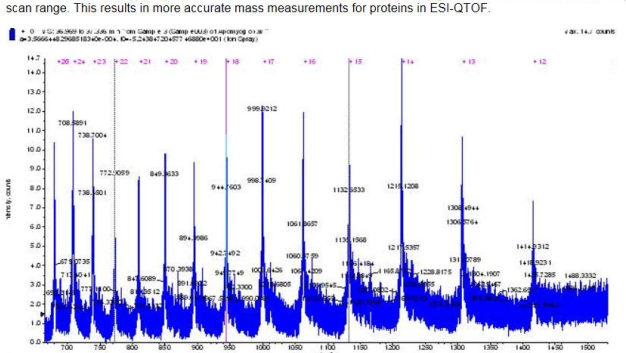
Applications off protein mass spec

- Post translational modifications
- Protein interactions
- Disease genes and Biomarkers
- Stem cell characterization
- Alternative to microarrays
  - mRNA changes may not be physiologically relevant
  - mRNA may not be present in tissue of interest (blood)

- Field is young and moves fast
- MudPIT setups are becoming commercially available
- High demand (everybody wants so be friends with the mass spec guy)

Protein Mass Determination: ESI-QTOF

Mass spectrometry is an ideal method for protein mass determination. The two primary methods for ionization of proteins are electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI). In ESI ionization proteins are ionized in solution and carry multiple charge state (see figure below which shows a ESI-QTOF scan of myoglobin protein). The advantage of using ESI-QTOF analysis for protein mass determination is that due to the high charge state of proteins their m/z measurements is typically less than 2000 and the TOF detector has a very good resolution and mass accuracy in this scan range. This results in more accurate mass measurements for proteins in ESI-QTOF.



Protein Mass Determination: MALDI-TOF

Mass spectrometry is an ideal method for protein mass determination. The two primary methods for ionization of proteins are electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI). In a MALDI source, proteins typically carry a charge state of +1 and since mass spectrometers measure m/z (mass/charge state) proteins typically appear with their mass plus the mass of a single hydrogen ion in a MALDI TOF spectrum. The disadvantage of using MALDI TOF analysis for protein mass determination is the limitations of the TOF detector in the high m/z scan range. TOF reading in high m/z region have a lower resolution and mass accuracy.

Protein Sequencing: Reverse Phase C18

Mass Spectrometry and Protein Sequencing

Most mass spectrometers that are used for protein sequencing are equipped with collision cells that are ideal for sequencing peptides that are under 4 KDa range. Mainly for this reason proteins need to be cut using proteases that will result in peptide fragments that on average are less than 4KDa. Once these peptides are generated they need to be fractionated before ionization into mass spectrometer. This is mainly to allow mass spectrometers time to analyze different peptides that are present in a mixture. The fractionation schemes can be carried out online or offline. The advantage of online schemes are that they require very little sample material which ultimately increases sensitivity of the system. For MALDI-TOF peptide sequencing, peptides will need to be fractionated offline for complex protein mixtures.

Reverse Phase (RP) C18 Resin Liquid Chromatography

The simplest fractionation strategy for low protein complexity samples is the reverse phase (RP) C18 resin liquid chromatography. In this separation scheme peptides bind the C18 resin based on their hydrophobicity. They are then eluted off the C18 resin using an organic reagent.

Modern Mass Spectrometry-Based Structural Proteomics

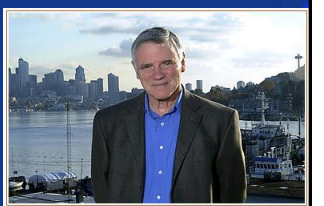
Evgeniy V. Petrotchenko*,
Christoph H. Borchers

Abstract:

Recent developments in the modern mass spectrometry of proteins and peptides have resulted in significant progress in **structural proteomics** techniques for studying protein structure. A variety of protein structural questions, ranging from defining **protein interaction networks** to the study of **conformational changes** and the structure of single proteins, can be addressed using multiple **mass spectrometry-based structural proteomics approaches**. Each technique provides specific structural information which can be used as **experimental structural constraints in protein structure modeling**. Here, we describe recent developments in **limited proteolysis, surface modification, hydrogen–deuterium exchange, ion mobility, and cross-linking—all combined with modern mass spectrometric techniques—for the studying protein structure.**

Systems Biology and Systems Medicine: Predictive, Personalized, Preventive and Participatory (P4)

Lee Hood
Institute for Systems Biology, Seattle



Dr. Leroy Hood
M.D., Johns Hopkins School of Medicine, 1964
Ph.D., Biochemistry, California Institute of Technology, 1968

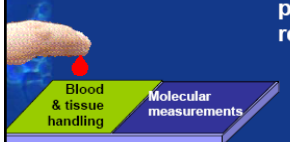
Note: The following (blue) slides were edited from a presentation by Lee Hood of the Inst. for Systems Biology to NIST on the P4 Medicine found at:

http://www.itl.nist.gov/Healthcare/conf/presentations/LH%20NIST%209-24-07.pdf

A similar lecture on P4 Medicine was presented by Dr. Hood at the 2007 Welch Conference – "From Atoms to Cells"

In vitro diagnostics

Quantitate 1000-2000 organ-specific proteins to:
identify disease;
stratify disease;
progression of disease;
response of disease to therapy etc.



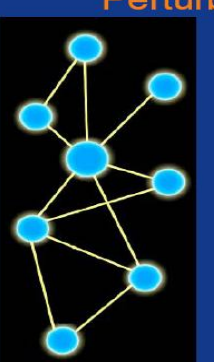
→ 10^4 molecules/cell

Our sensitivity: TNFα or MIP2.
50-100pg/ml in 1nl
Amount: 100pg*10^{-6}ml
→ 10^{-16} g (~femtograms)

Fundamental Materials/Chemical Issues
- Scalable & Simple Detection Technologies
- Multiple Functions Integrated onto Microfluidics Chips
- Protein Capture Agents
- Manufacturability

What is Systems Medicine?

Disease Arises from Disease Perturbed Networks



Non-Diseased

Diseased

dynamics of pathophysiology

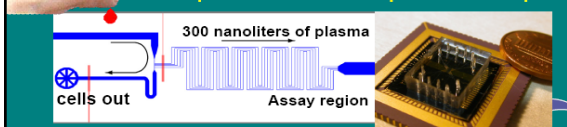
diagnosis

therapy

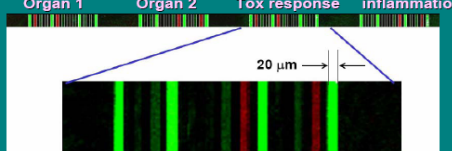
prevention

DEAL for *In vitro* molecular diagnostics:
Integrated biology/chemistry/nanotech/microfluidics platforms

Separate plasma & rapidly quantitate protein biomarker panels to:
- **Profile health status of individual organs**
- **Detect disease prior to clinical symptoms**
- **Select appropriate therapies or combination therapies**
- **Profile positive & adverse responses to therapies**



Large panel of protein biomarkers measured in a single microfluidics channel (15 min assay time)



Jim Heath, et al

DEAL = DNA-Encoded Antibody Library

Predictive, Preventive, Personalized and Participatory Medicine (P4)

- Predictive:
  - Probabilistic health history--DNA sequence
  - Biannual multi-parameter blood protein measurements
  - In vivo diagnostic measurements to stage and localize disease
- Preventive:
  - Design of therapeutic and preventive drugs via systems approaches
- Personalized:
  - Unique individual human genetic variation mandates individual treatment
- Participatory:
  - Patient understands and participates in medical choices

Patient and physician education