Mass Spectrometry 101

Hackert - CH 370 / 387D

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:

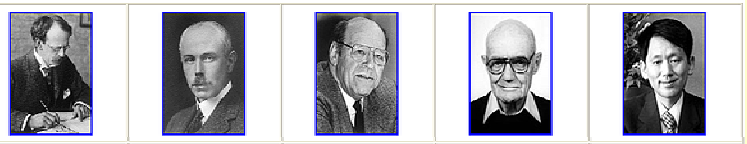
metabolites, synthetic organic chemicals
peptides, proteins, recombinant proteins,
oligonucleotides, polymers, drug candidates
→ sequencing!

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
1906 Nobel Prize for Physics
"in recognition of the great merits of his theoretical and experimental investigations on the conduction of electricity by gases"

Francis William Aston
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"

Wolfgang Paul
1989 Nobel Prize for Physics
"for the development of the ion trap technique"

John Bennet Fenn
2002 Nobel Prize for Chemistry
"for the development of soft desorption ionisation methods (ESI) for mass spectrometric analyses of biological macromolecules"

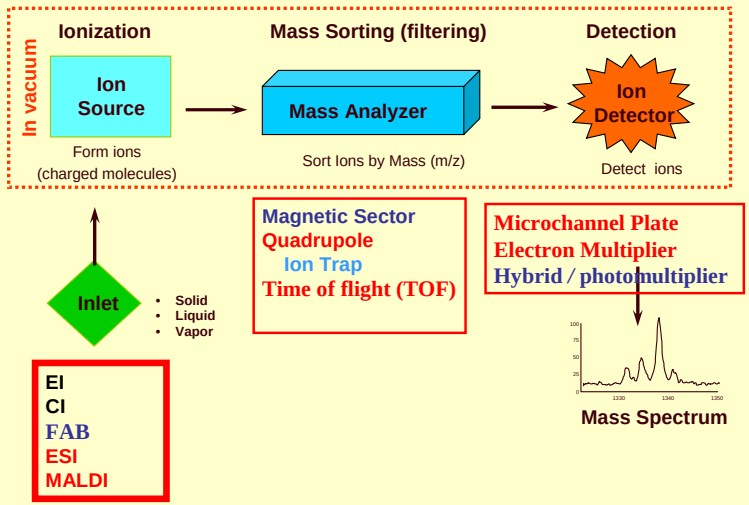
Koichi Tanaka
2002 Nobel Prize for Chemistry
"for the development of soft desorption ionisation methods (MALDI) for mass spectrometric analyses of biological macromolecules"

1989

~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 / 200kDa**
- **Matrix-Assisted Laser Desorption Ionization - Soft / 500kDa**

Mass Spectrometry

Introductory Example: mass spectrum of water

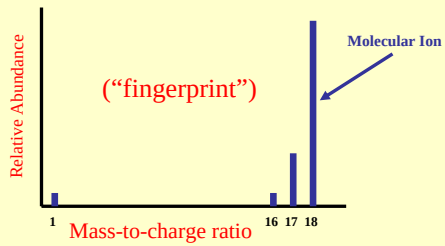
- **Electron Impact (EI) - Hard (fragments) / 1000 Da**

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**.

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?

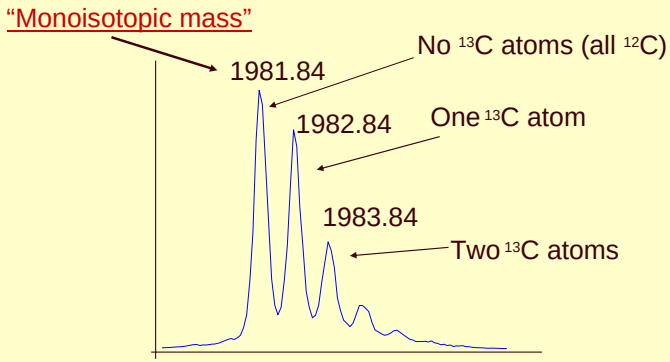
Mass spectrometers can "see" isotope peaks if their resolution is high enough.

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

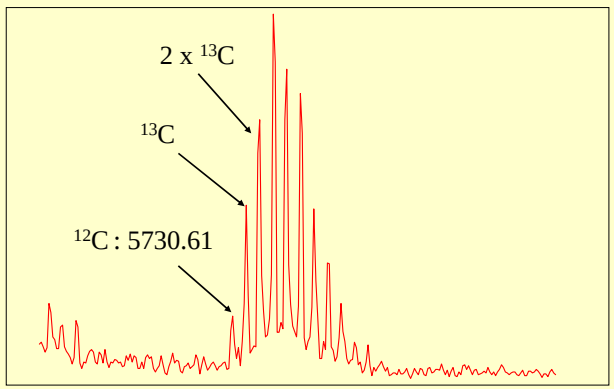
Stable isotopes of most abundant elements of peptides

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

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

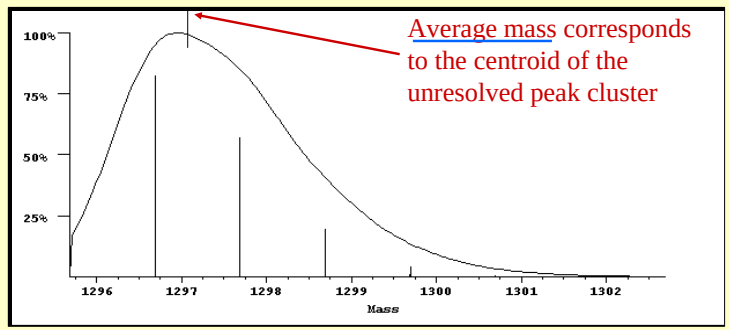


Mass spectrum of insulin



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.

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 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_9O	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)**

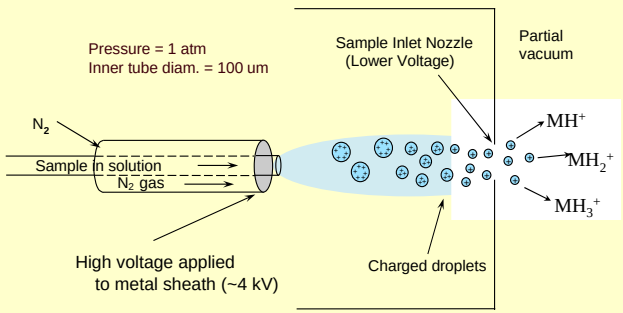
-Either source type can work with either analyzer type: "MALDI-TOF," "ESI-Quad."

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

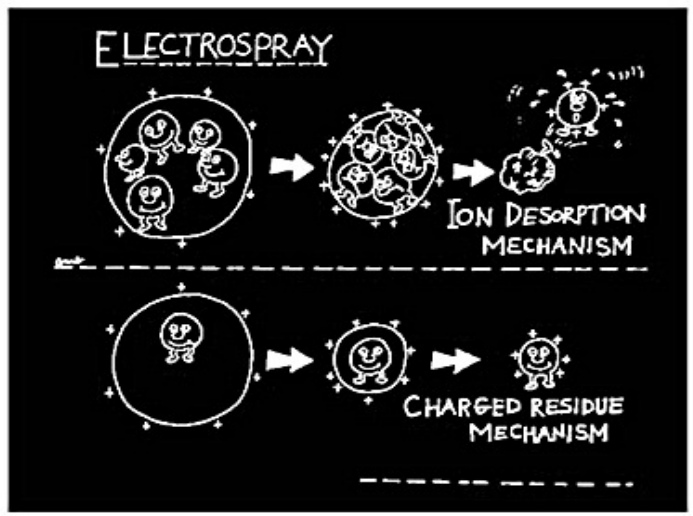
Ion Sources make ions from sample molecules

(Ions are easier to detect than neutral molecules.)

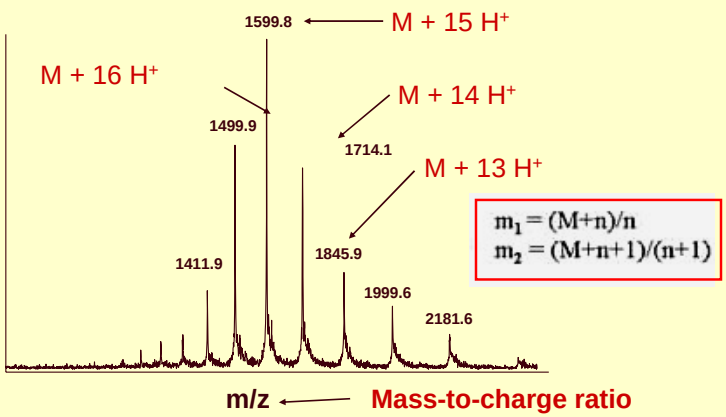
Electrospray ionization:



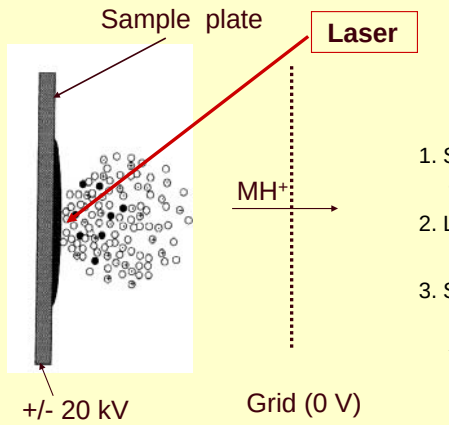
Very gentle / solvent evaporates / multiple charges



ESI Spectrum of Trypsinogen (MW 23,983)

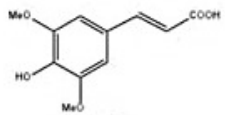


MALDI: Matrix Assisted Laser Desorption Ionization

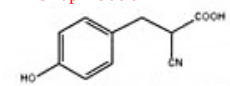


1. Sample is mixed with matrix (X) and dried on plate.
2. Laser flash ionizes matrix molecules.
3. Sample molecules (M) are ionized by proton transfer: $XH^+ + M \rightarrow MH^+ + X$.

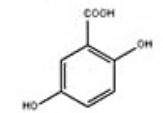
MALDI-TOFMS



Sinapinic acid



Cyano-4-hydroxy-trans –cinnamic acid



2,5-Dihydroxybenzoic acid

the three most commonly used matrices

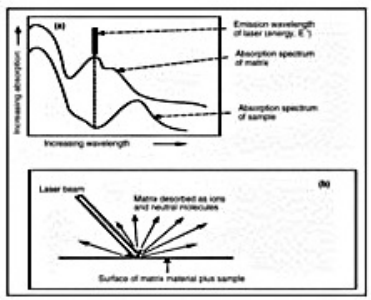
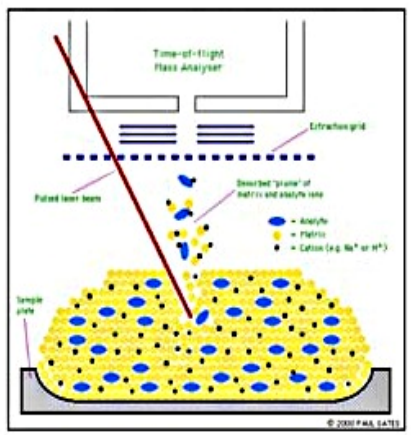


Figure 3 In a MALDI experiment, the sample is mixed or dissolved in a matrix material, which has an absorption spectrum that matches the laser wave length of energy, *E'*. The sample may not have a matching absorption peak (a) but this is not important because the matrix material absorbs the radiation,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

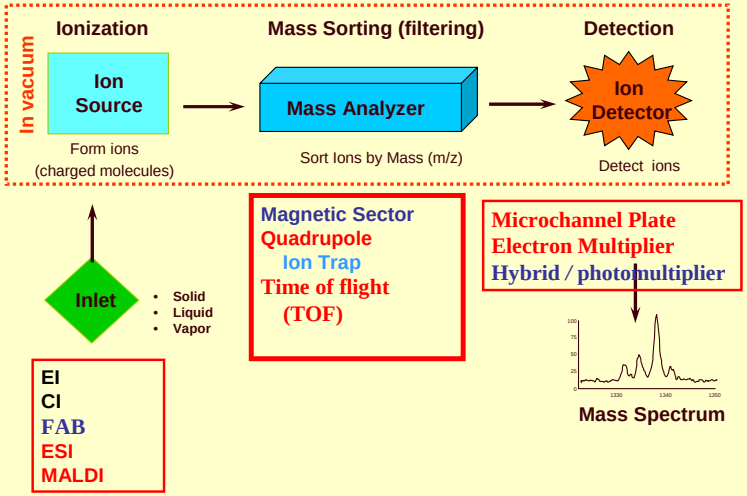
MALDI (Matrix Assisted Laser Desorption Ionization)

"Nuke It"



Analyte:
10 – 1000 fmol
1 – 500 kDa

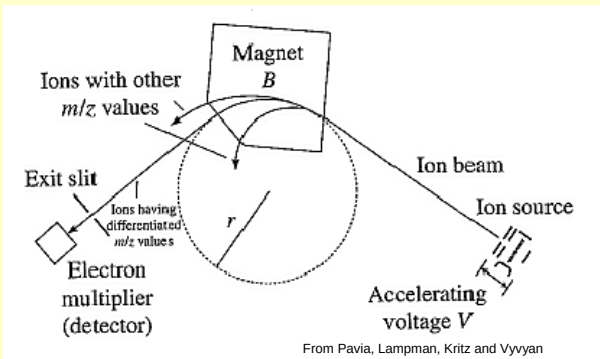
Summary: acquiring a mass spectrum



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



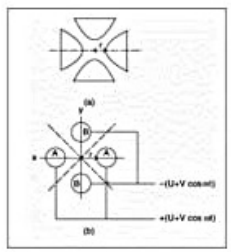
From Pavia, Lampman, Kritz and Vyvyan

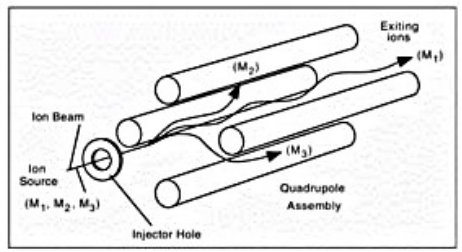
$$\frac{1}{2}mv^2 = zV \qquad r = \frac{mv}{zB} \qquad \frac{m}{z} = \frac{B^2r^2}{2V}$$

Mass Analyzers: The Quadrupole Mass Filter

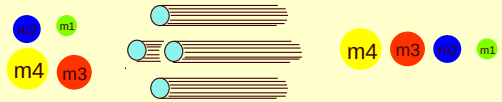
A potential of ~100-1000 V is applied alternately to the opposing pairs of rods at a frequency of a few MHz. At a specific combination of DC & RF, an m/z has a stable trajectory through the rods, and all other m/z are lost. The mass range is scanned as the voltages are swept from min to max, but at constant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

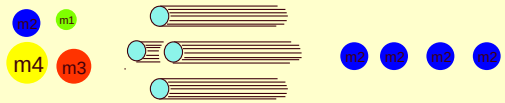




Quadrupoles have variable ion transmission modes

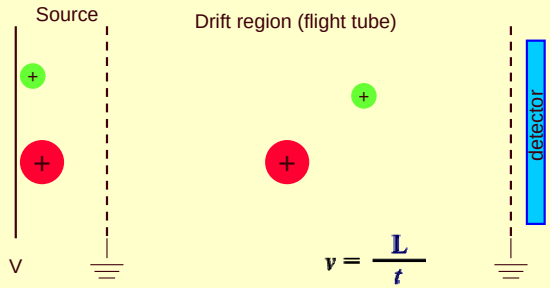


mass scanning mode



single mass transmission mode

Time-of-flight (TOF) Mass Analyzer

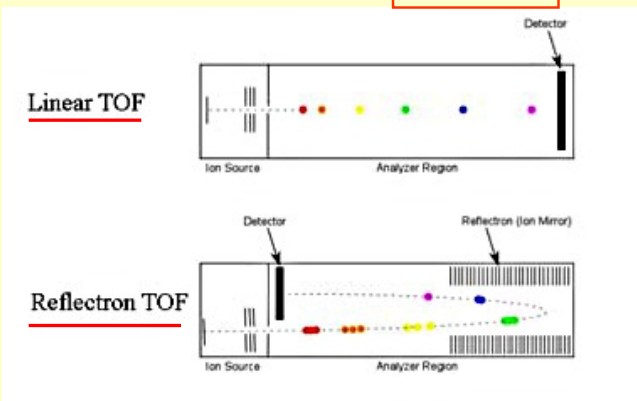


- Ions are formed in pulses.
- The drift region is field free.
- Measures the time for ions to reach the detector.
- Small ions reach the detector before large ones.

Time-of-flight (TOF) Mass Analyzer

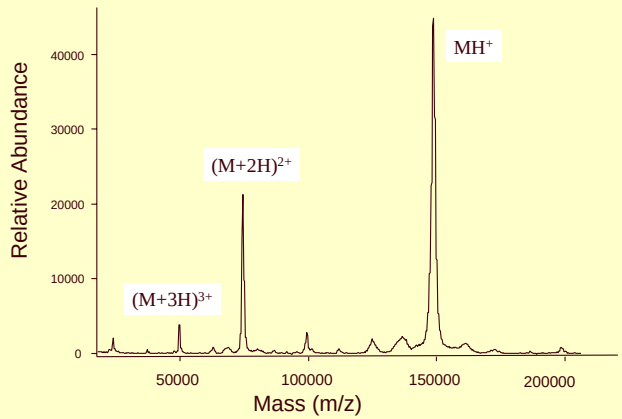
$$v = \frac{L}{t}$$

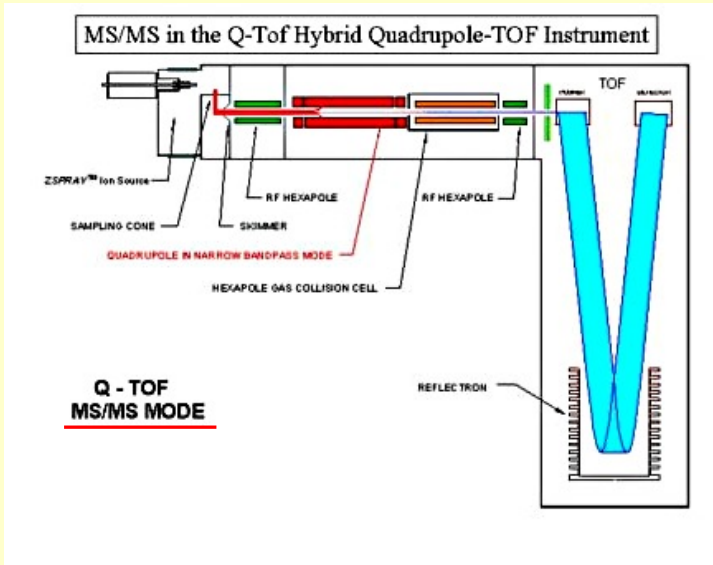
$$zV = \frac{mv^2}{2} = \frac{mL^2}{2t^2} \quad \text{or} \quad \frac{m}{z} = \frac{2Vt^2}{L^2}$$



The mass spectrum shows the results

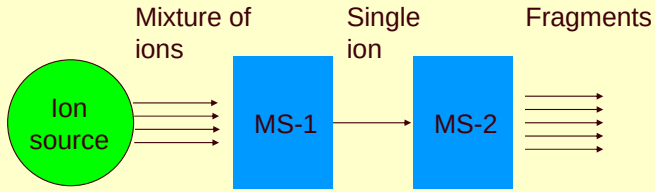
MALDI TOF spectrum of IgG

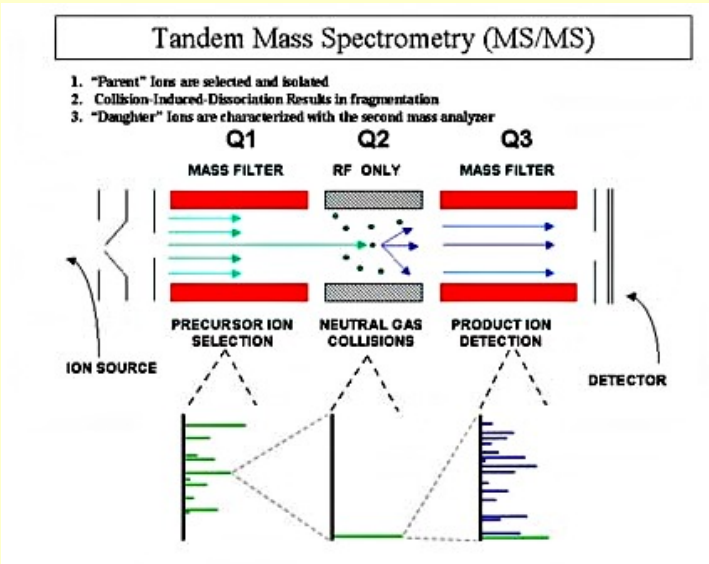




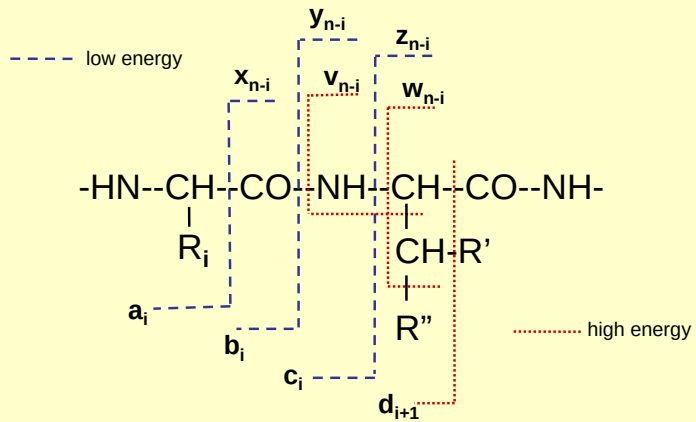
What is MSMS?

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.



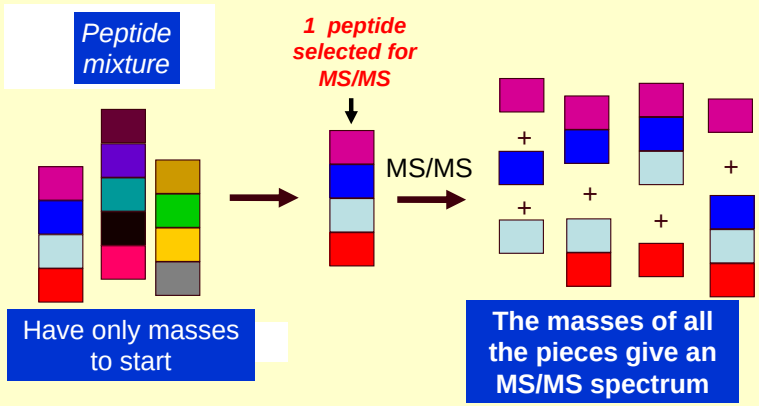


Cleavages Observed in MS/MS of Peptides

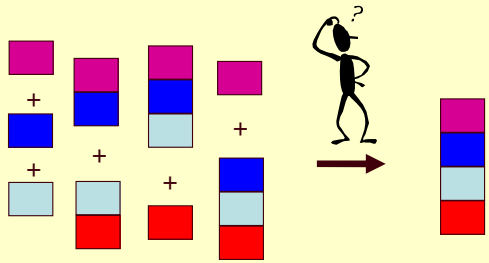


CID (Collision InDuced) Spectra – adds **sequence data** to **mass mapping** for improved database identification!

What is MS/MS?

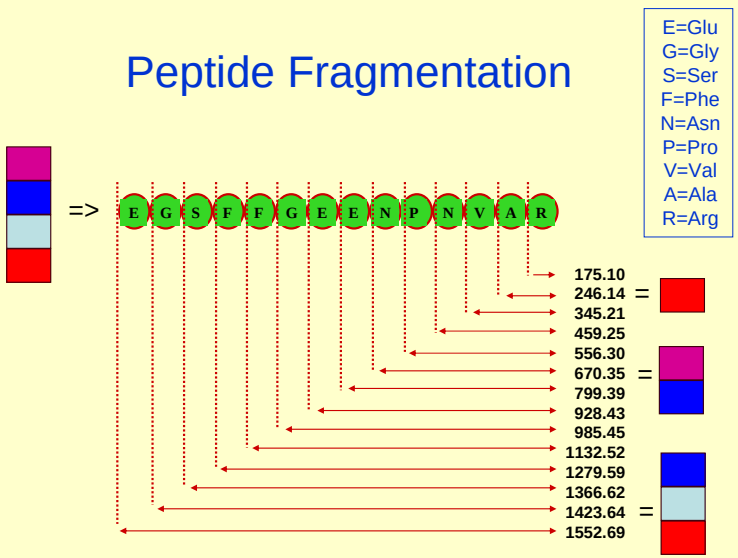


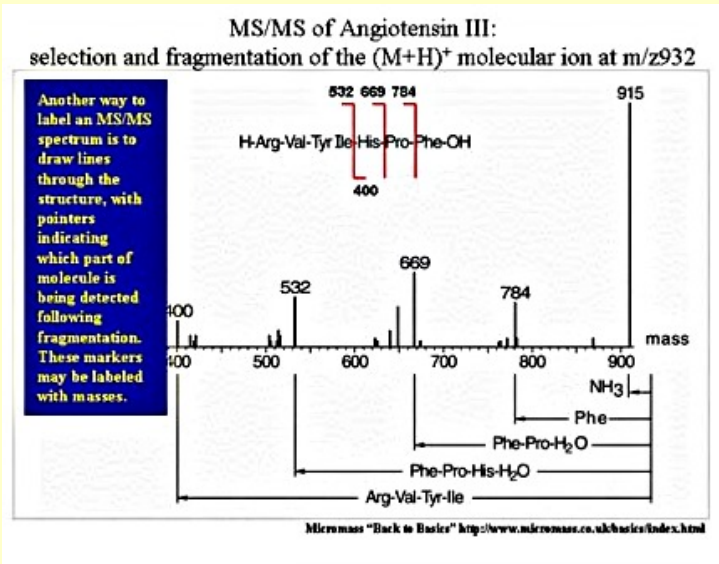
Interpretation of an MSMS spectrum to derive structural information is analogous to solving a puzzle



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

Peptide Fragmentation





insight review articles

NATURE | VOL 422 | 13 MARCH 2003 | www.nature.com/nature

Mass spectrometry-based proteomics

Ruedi Aebersold* & Matthias Mann†

*Institute for Systems Biology, 1441 North 34th Street, Seattle, Washington 98103-8904, USA (e-mail: raebersold@systemsbiology.org)
†Center for Experimental BioInformatics (CEBI), Department of Biochemistry and Molecular Biology, University of Southern Denmark, Campusvej 55, DK-5230 Odense M, Denmark (e-mail: mann@bmb.sdu.dk)

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

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

European Proteomics Association (EuPA)

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

Mass Spec

Applications in Proteomics and Systems Biology

Proteomics: *From Technology Development to Biomarker Applications*

Co-sponsored by HUPO, AOHUPO and KHUPO

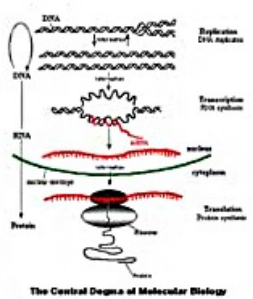
CH370 - Hackert

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.

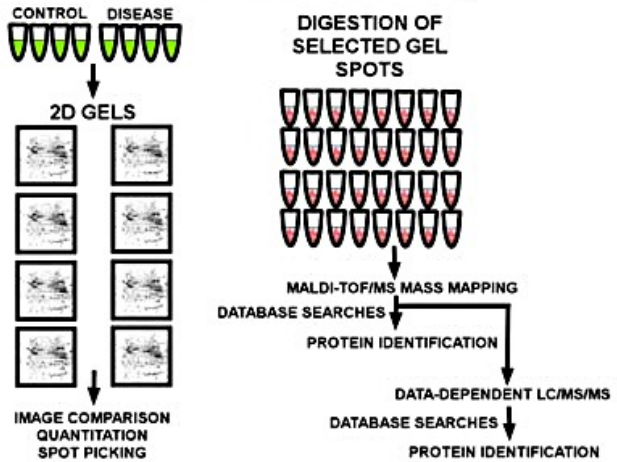
The Proteome is Dynamic. It is the total of all proteins expressed by a particular *cell* at a given *time*, under specific *conditions*.



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.

Differential Expression Proteomics



Two Dimensional Gel Electrophoresis

Isoelectric focusing is performed on precast gel strips using commercial instruments. Many pH ranges are available. Multiple strips can be run in parallel.

An immobilized pH gradient is created in a polyacrylamide gel strip by incorporating a gradient of acidic and basic buffering groups when the gel is cast.

Resolution is determined by the slope of the pH gradient and the field strength.

Loading capacity depends on gel size and thickness.

In 2D IEF/PAGE, the gel strip from IEF is loaded into a single large well.

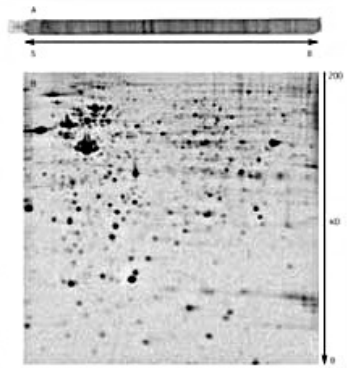
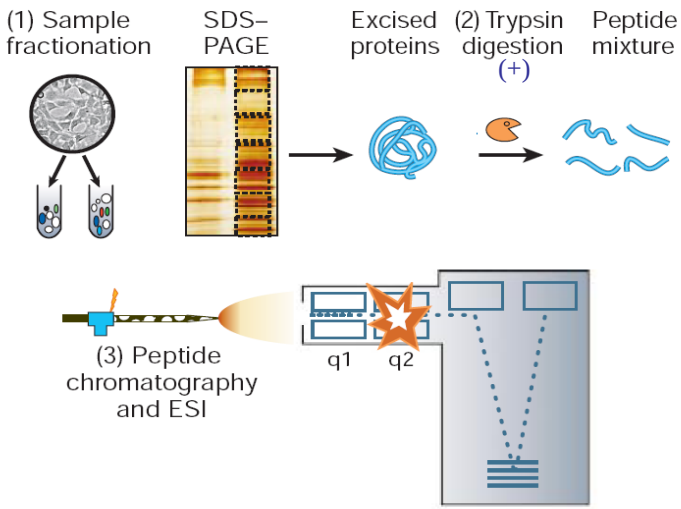


Figure from BioRad Product Literature

Generic Mass Spectrometry-based Proteomics



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**.

MS-Fit (by Peter Baker and Karl Clauser) Instructions

A peptide-mass fingerprinting tool from the UCSF Mass Spectrometry Facility that tries to fit a user's mass spectrometry data to a protein sequence in an existing database and thus suggest the identity of the user's protein. The MS input data should be generated by analyzing the peptides produced by the enzymatic digestion of a user's protein.

ProteinProspector Home | MS-Tag | MS-Seq | MS-Edman | MS-Fit at UCSF (San Francisco)
MS-Digest | MS-Product | MS-Comp | DB-Stat | MS-Isotope

Start Search

Peptide masses are: monoisotopic
Min. # peptides required to match: 3
Database: SwissProt.012601 Instrument: MALDI-TOF Report MOWSE Scores: Pfactor: 0.4
DNA Frame translation: 3
Search Hits: From: msfit Filename: lastres
Save Hits to file: Filename: lastres
Species: All
MW of Protein: (from 1000 Da to 150000 Da) All
Protein pI: (from 3.0 to 10.0) All
Digest: Trypsin
Max. # of missed cleavages: 1
Cysteines modified by: unmodified
N terminus: Hydrogen C terminus: Free Acid
Sample ID (comment): Unknown A
Max. Reported Hits: 15

Possible Modifications Mode (default): Peptide N-terminal Gln to pyroGlu; Oxidation of M; Protein N-terminus Acetylated; Acrylamide Modified Cys
User Defined Modification 1: Phosphorylation of S, T and Y

OR

Homology Mode (select any mode but identity)
Search mode: identity
Min. # matches with NO AA substitutions: 1
Peptide Mass shift: +/- 40.1 Da

Peptide Masses

mass tolerance: +/- 15 ppm

Mass (m/z) | Charge (z)

905.6874
973.5183
989.6093
995.5787
1007.4948
1024.4374
1025.4959
1025.7433
1037.5184
1045.5657
1090.5471
1106.5649
1139.5205
1164.5909
1165.5664
1179.6002
1184.5958
1193.6111
1233.5911
1234.6510
1263.6858
1267.7091
1277.7065

Mass accuracy tolerance = 15 ppm

This means that the mass is within 0.015 Da at m/z 1000

MS-Fit Search Results

Press stop on your browser if you wish to abort this MS-Fit search prematurely.

Sample ID (comment): **Unknown A**
Database searched: **SwissProt.012601**
Molecular weight search (**1000 - 150000 Da**) selects **90539** entries.
Full pI range: **92236** entries.
Combined molecular weight and pI searches select **90539** entries.
MS-Fit search selects **858** entries (results displayed for top **15** matches).

Considered modifications: | **Peptide N-terminal Gln to pyroGlu** | **Oxidation of M** | **Protein N-terminus Acetylated** | **Acrylamide Modified Cys** |

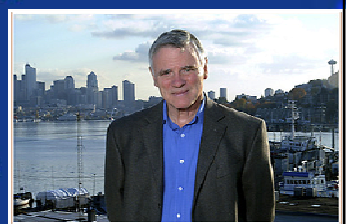
Min. # Peptides to Match	Peptide Mass Tolerance (+/-)	Peptide Masses are	Digest Used	Max. # Missed Cleavages	Cysteines Modified by	Peptide N terminus	Peptide C terminus	Input # Peptide Masses
3	15.000 ppm	monoisotopic	Trypsin	1	unmodified	Hydrogen (H)	Free Acid (O H)	46

Result Summary

Rank	MOWSE Score	# (%) Masses Matched	Protein MW (Da)/pI	Species	SwissProt.012601 Accession #	Protein Name
1	2.86e+005	9/46 (19%)	16930.2 / 4.56	HUMAN	P16475	MYOSIN LIGHT CHAIN ALKALI, NON-MUSCLE ISOFORM (MLC3NM) (LC17A) (LC17-NM)
2	2.86e+005	9/46 (19%)	16961.2 / 4.46	HUMAN	P24572	MYOSIN LIGHT CHAIN ALKALI, SMOOTH-MUSCLE ISOFORM (MLC3SM) (LC17B) (LC17-GI)
3	2.86e+005	9/46 (19%)	16975.3 / 4.46	RAT	Q64119	MYOSIN LIGHT CHAIN ALKALI, SMOOTH-MUSCLE ISOFORM (MLC3SM)
4	1.77e+004	7/46 (15%)	15730.9 / 4.80	MOUSE	Q60605	MYOSIN LIGHT CHAIN ALKALI, NON-MUSCLE ISOFORM (MLC3NM)
5	1.41e+004	7/46 (15%)	66018.0 / 8.16	HUMAN	P04264	KERATIN, TYPE II CYTOSKELETAL 1 (CYTOKERATIN 1) (K1) (CK 1) (67 KDA CYTOKERATIN) (HAIR ALPHA PROTEIN)
6	1.19e+003	4/46 (8%)	15282.4 / 6.10	STRPU	P32006	PROFILIN
7	420	5/46 (10%)	16983.3 / 4.63	CHICK	P09296	MYOSIN LIGHT CHAIN ALKALI, NON-MUSCLE ISOFORM (FIBROBLAST) (G2 CATALYTIC) (LC17-NM)
8	419	5/46 (10%)	16987.4 / 4.52	CHICK	P02607	MYOSIN LIGHT CHAIN ALKALI, SMOOTH-MUSCLE ISOFORM (GIZZARD) (G2 CATALYTIC) (LC17-GI)
9	391	4/46 (8%)	38545.3 / 8.59	XENLA	P27006	ANNEXIN II TYPE I (LIPOCORTIN II) (CALPACTIN I HEAVY CHAIN) (CHROMOBINDIN 8) (P36) (PROTEIN I) (PLACENTAL ANTICOAGULANT PROTEIN IV) (PAP-IV)
10	286	5/46 (10%)	22156.3 / 5.03	RAT	P16409	MYOSIN LIGHT CHAIN 1, SLOW-TWITCH MUSCLE B/VENTRICULAR ISOFORM
11	262	3/46 (6%)	19590.2 / 9.34	BGMV	P05174	AL2 PROTEIN (19.6 KD PROTEIN)
12	220	5/46 (10%)	21932.2 / 5.03	HUMAN	P08590	MYOSIN LIGHT CHAIN 1, SLOW-TWITCH MUSCLE B/VENTRICULAR ISOFORM (MLC1SB) (ALKALI)
13	211	3/46 (6%)	16990.5 / 6.92	ECOLI	P37052	HYPOTHETICAL 17.0 KDA PROTEIN IN HNR-PURU INTERGENIC REGION
14	202	3/46 (6%)	17947.3 / 5.24	ARATH	P25855	GLYCINE CLEAVAGE SYSTEM H PROTEIN 1, MITOCHONDRIAL PRECURSOR
15	186	3/46 (6%)	16613.9 / 4.63	RAT	P02601	MYOSIN LIGHT CHAIN 3, SKELETAL MUSCLE ISOFORM (A2 CATALYTIC) (ALKALI) (MLC3F)

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

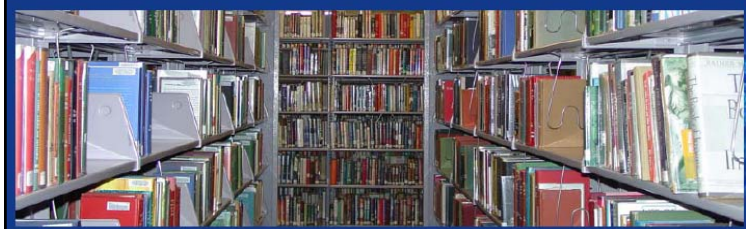
Note: The following (blue) slides were edited from a presentation by Lee Hood of the Inst. for Systems Biology to NIST on the P4 Medicine found at:

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"

Institute for Systems Biology

Biology is an Informational Science



Two Types of Biological Information:

Digital (genome / DNA sequences)

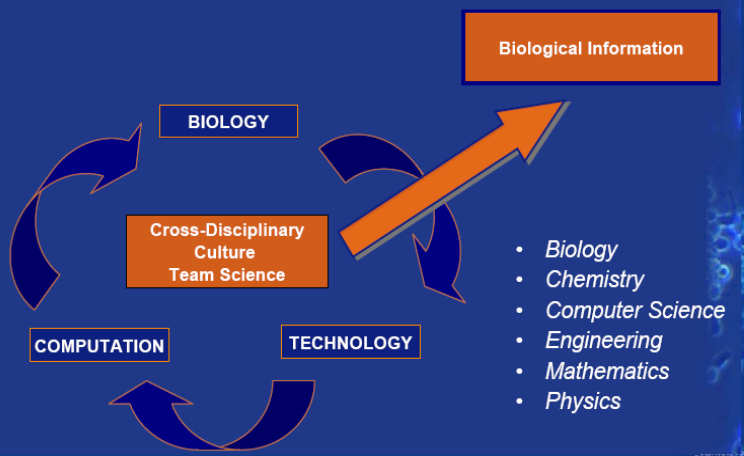
CCAGGAGGTT GCTTCTTCCA
GCTCCCAGCT GCTGTGAGTG
CACTTCTGGT GCCCACTGTG
GCCTCCTGGG GAGCTGCTGA

Environmental

- Biological networks capture, transmit, integrate, dispense and execute biological information.
- Biological information is hierarchical and multiscalar--DNA, RNA, protein, interactions, networks, cells, organs, individuals, ecologies.

Institute for Systems Biology

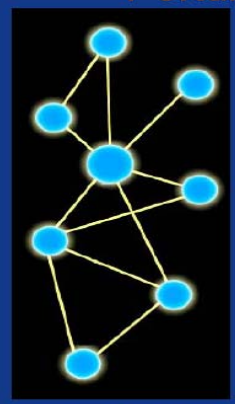
Agenda: Use biology to drive technology and computation.
Need to create a cross-disciplinary culture.

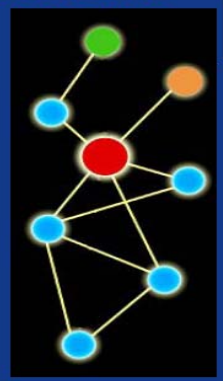


Systems Biology

What is Systems Medicine?

Disease Arises from Disease Perturbed Networks





Non-Diseased

Diseased

dynamics of pathophysiology

diagnosis

therapy

prevention

DEGs Encoding Known and Novel Prion Disease Phenotypes

- 7400 Differentially Expressed Genes (DEGs) in 5 inbred strains upon prion perturbation.
- Biological filters reduce to 924 core DEGs for prion disease
- 253/924 DEGs encode known disease phenotypes
- 671/924 DEGs encode novel disease phenotypes

Systems Biology

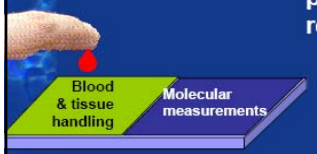
Organ-Specific Blood Proteins Will Make the Blood a Window into Health and Disease

- Perhaps 50 major organs or cell types--each secreting protein blood molecular fingerprint.
- The levels of each protein in a particular blood fingerprint will report the status of that organ. Probably need 10-50 organ-specific proteins per organ.
- Need to quantify 500-2500 blood proteins from a droplet of blood.
- Key point: changes in the levels of organ-specific markers will assess all diseases or environmental challenges for a particular organ

Systems Biology

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

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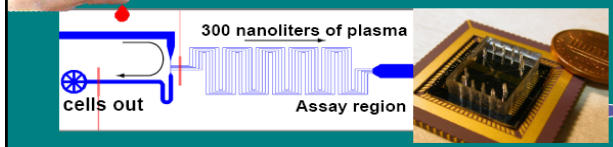
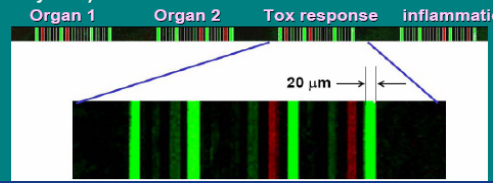
DEAL for *In vitro* molecular diagnostics:
Integrated biology/chemistry/nanotech/microfluidics platforms

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

Institute for Systems Biology

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

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