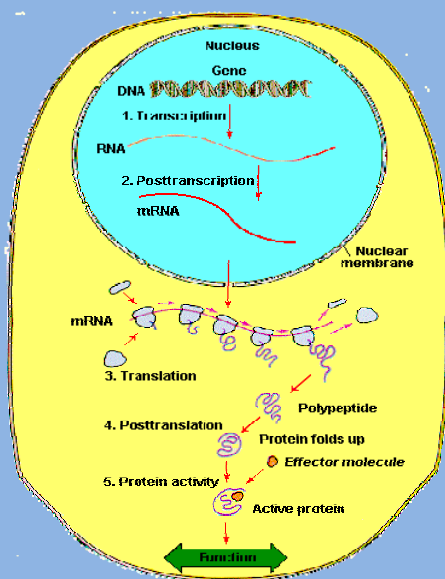


Protein analysis by mass spectrometry

Nick Polfer

Department of Chemistry
University of Florida

Proteins in biology



DNA encodes protein information



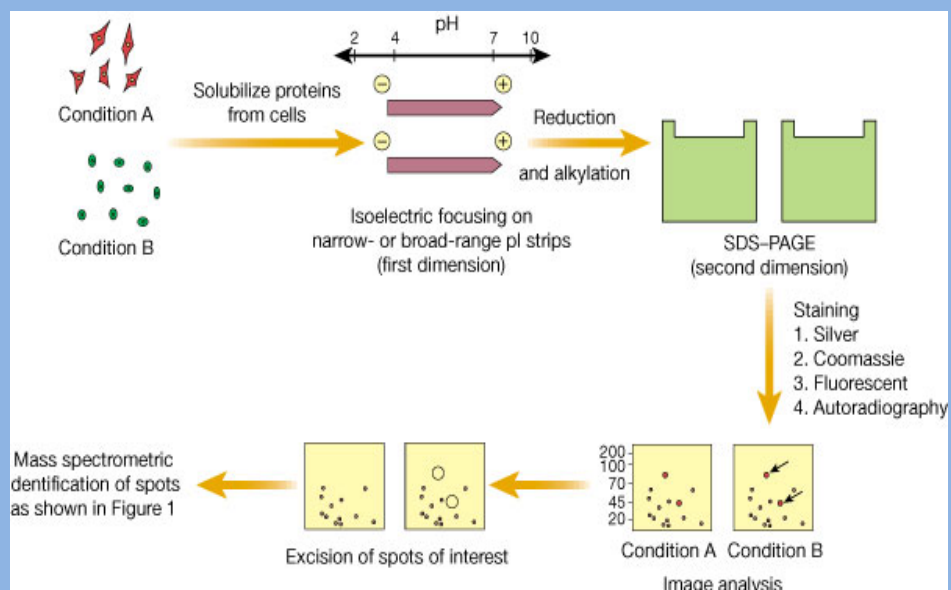
Natural History Museum (UF)

Challenges

- highly complex (homo sapiens: 35,000 genes $\Rightarrow$ 10^6 proteins)
- dynamic protein expression
- very low sample amount (10-1,000 copies per cell)
- cannot amplify, such as with DNA (i.e., polymerase chain reaction)

$\Rightarrow$ need ultra-sensitive and quantitative analytical techniques

Separation of



Mass spectrometer



Thermo-Fisher



Bruker



Synopsis

- how do we measure the mass of molecules?
- what techniques are involved?
- what do mass spectra look like?
- how is peptide/protein analysis carried out?

Weighing mass



Gravitational force acts on spring

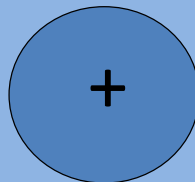


Down to μg (10^{-6} g)

Weighing molecules

Atomic mass unit
(a.m.u.): 1.66×10^{-24} g

Ionize molecules



$$F = ma = q(E + vB)$$

q = electric charge
 E = electric field
 v = ion velocity
 B = magnetic field

Ignore gravitational force

$F_{\text{ele}} = qE = 10^{-19} \times E$
$F_{\text{grav}} = mg = 10^{-27} \times 9.8$

Mass spectrometer

Make ions Analyze mass

Ionize sample
without
destroying it

Need
vacuum:
⇒ **pumps**

Electrical power
supplies,
magnets,
computer, etc.

Smallest charged particle: electron

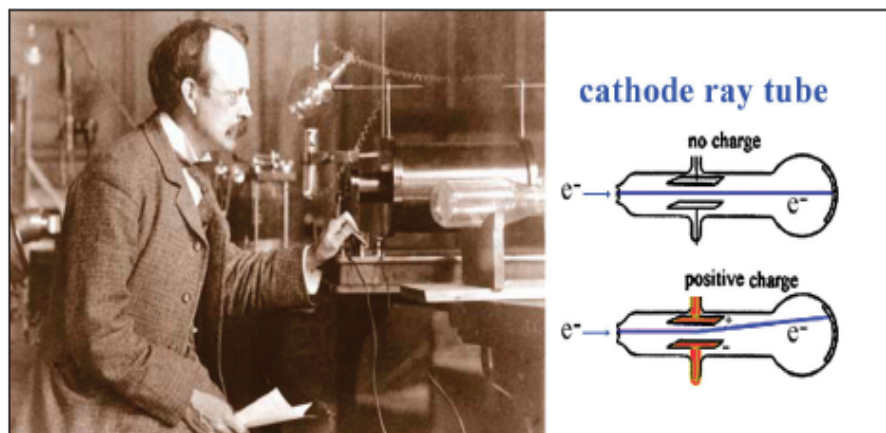


FIGURE 1: J. J. Thomson and a cathode ray tube used to perform some of the first m/z measurements. Deflection of the electron was observed once the electric field was turned on.

Sir Joseph John Thompson – discovery of the electron - 1897

Siuzdak; *Today's Chemist at Work*, 2003, 47.

Contribution to science

Nobel Prize Winners



J.J. Thomson
1906 Physics



Francis W. Aston
1922 Chemistry



Wolfgang Paul
1989 Physics



Hans G. Dehmelt
1989 Physics



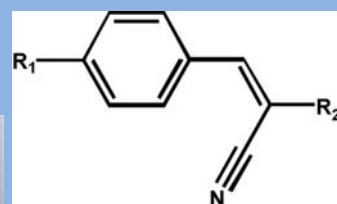
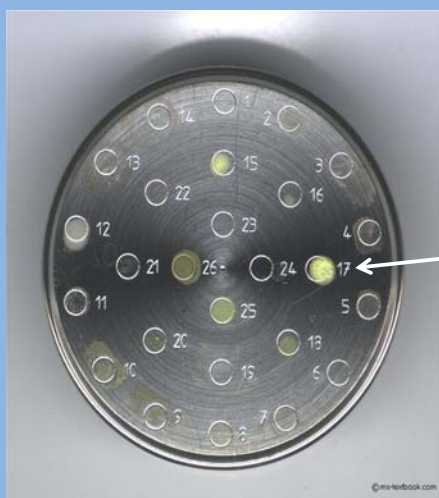
John B. Fenn
2002 Chemistry



Koichi Tanaka
2002 Chemistry

“for their development of soft desorption ionization methods for mass spectrometric analyses of biological macromolecules”

Prepare sample

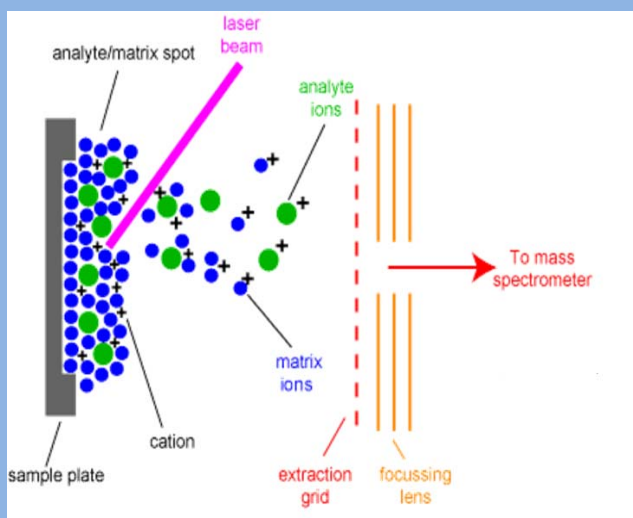


α -cyano-4 hydroxycinnamic acid (CHCA)

Matrix crystals

Matrix-Assisted Laser Desorption/Ionization MALDI

Tanaka (Nobel Prize)



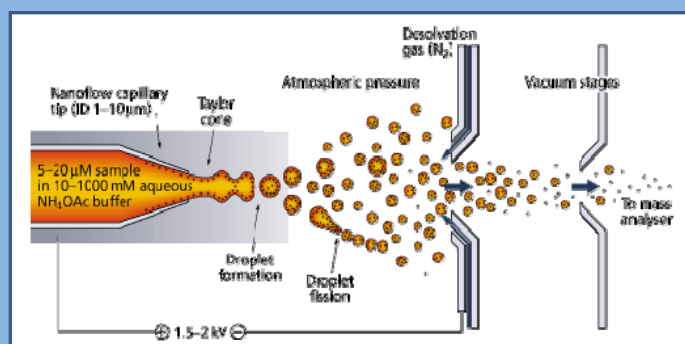
Role of matrix is to absorb laser energy and to transfer proton to analyte molecule



Molecule + proton

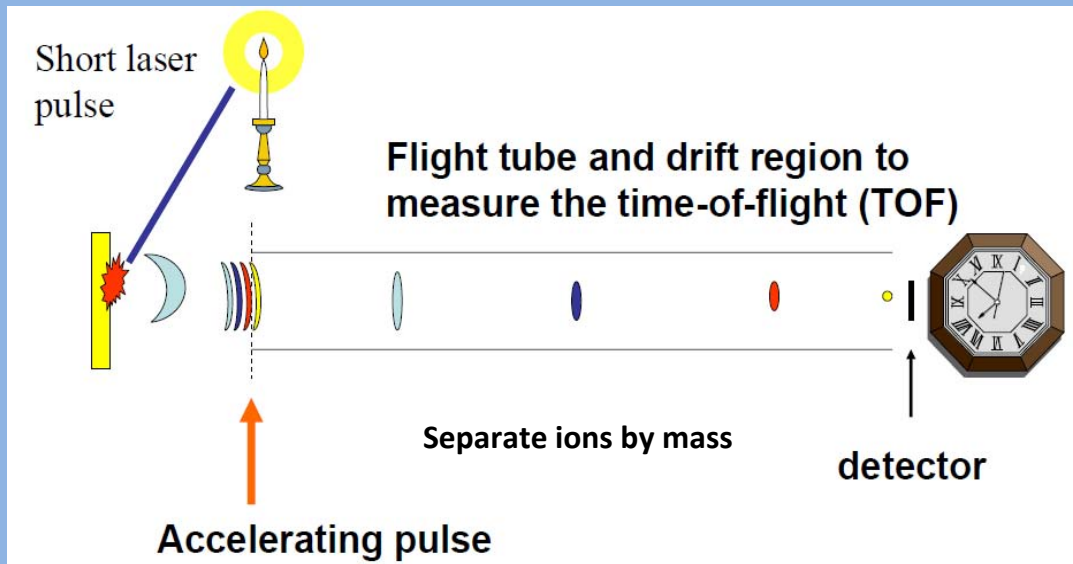
<http://www.chm.bris.ac.uk/ms/theory/maldi-ionisation.html>

Ions from solution: ElectroSpray Ionization ESI



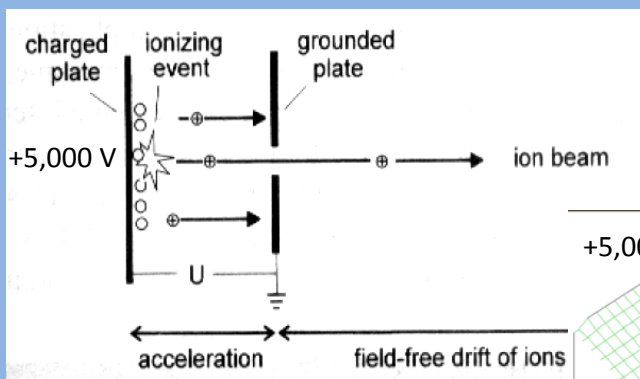
Royal Society of Chemistry website

MALDI-Time-of-Flight (TOF)



Principles of MALDI MALDI-TOF mass spectrometry Stephen Barnes, Ph.D.
Purdue-UAB Botanical Center Workshop 2002 Mass Spectrometry Methods in Botanicals Research

Accelerate ions – measure arrival time



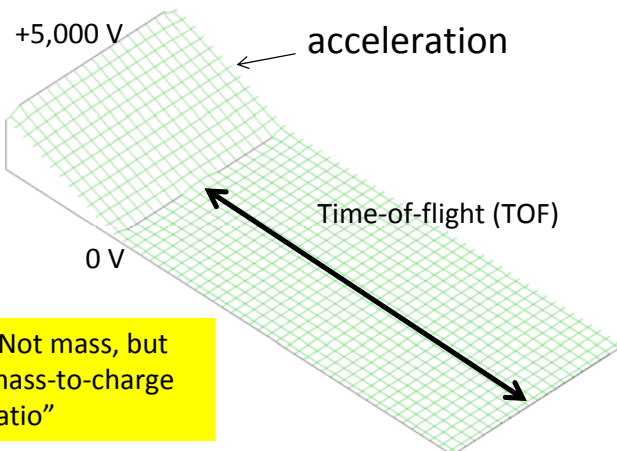
$$E_{electric} = e \cdot z \cdot U_{accel} = \frac{1}{2} m \cdot v^2 = K.E.$$

potential energy = kinetic energy

$$v = \sqrt{2eU} \sqrt{\frac{z}{m}}$$

$$TOF \propto \sqrt{\frac{m}{z}}$$

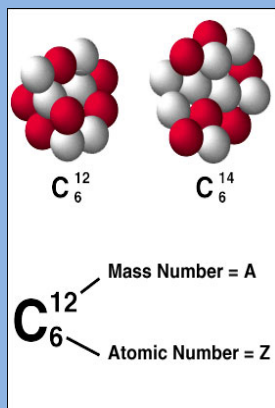
"Not mass, but mass-to-charge ratio"



Mass

CHNOS(P)

1 atomic mass
unit (a.m.u.)
= $^{12}\text{C}/12$

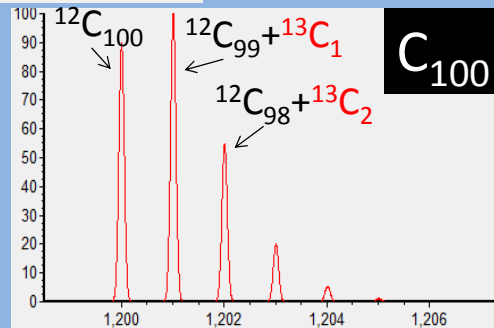
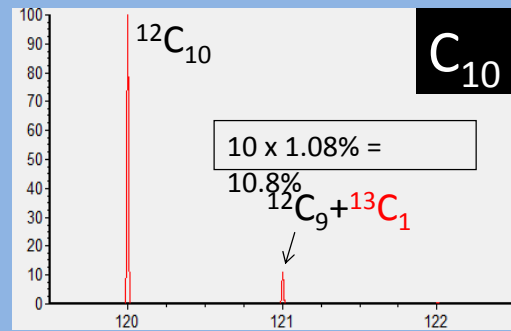
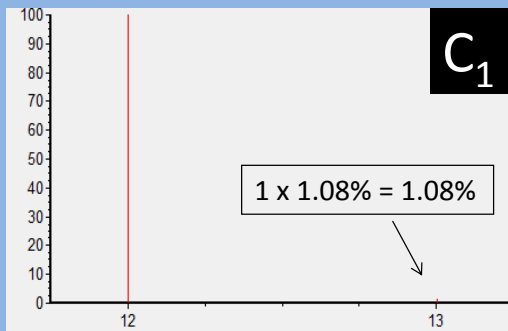


1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1 H 1.008	2 He 4.003	3 Li 6.941	4 Be 9.012	5 B 10.81	6 C 12.01	7 N 14.01	8 O 16.00	9 F 19.00	10 Ne 20.18	11 Na 22.99	12 Mg 24.31	13 Al 26.98	14 Si 28.09	15 P 30.97	16 S 32.07	17 Cl 35.45	18 Ar 39.95
19 K 39.10	20 Ca 40.08	21 Sc 44.96	22 Ti 47.88	23 V 50.94	24 Cr 52.00	25 Mn 54.94	26 Fe 55.85	27 Co 58.93	28 Ni 58.69	29 Cu 63.55	30 Zn 65.39	31 Ga 69.72	32 Ge 72.61	33 As 74.92	34 Se 78.96	35 Br 79.90	36 Kr 83.80
37 Rb 85.47	38 Sr 87.62	39 Y 88.91	40 Zr 91.22	41 Nb 92.91	42 Mo 95.94	43 Tc 98.91	44 Ru 101.1	45 Rh 102.9	46 Pd 106.4	47 Ag 107.9	48 Cd 112.4	49 In 114.8	50 Sn 118.7	51 Sb 121.8	52 Te 127.6	53 I 126.9	54 Xe 131.3
55 Cs 132.9	56 Ba 137.3	57 La 175.0	58 Ce 175.0	59 Pr 175.0	60 Nd 175.0	61 Pm 175.0	62 Sm 175.0	63 Eu 175.0	64 Gd 175.0	65 Tb 175.0	66 Dy 175.0	67 Ho 175.0	68 Er 175.0	69 Tm 175.0	70 Yb 175.0	71 Lu 175.0	72 Hf 175.0
87 Fr 223.0	88 Ra 226.0	89 Ac 227.0	90 Th 232.0	91 Pa 231.0	92 U 238.0	93 Np 237.0	94 Pu 244.1	95 Am 243.1	96 Cm 247.1	97 Bk 247.1	98 Cf 251.1	99 Es 252.0	100 Fm 257.1	101 Md 258.1	102 No 259.1	103 Lr 261.1	104 Hf 261.1

Isotopes

Element	Isotope	Abundance	Mass
Hydrogen	^1H	100	1.007825
	^2H	0.0115	2.014101
Carbon	^{12}C	100	12.000000
	^{13}C	1.08	13.003355
Nitrogen	^{14}N	100	14.00307
	^{15}N	0.369	15.000109
Oxygen	^{16}O	100	15.994915
	^{17}O	0.038	16.999132
	^{18}O	0.205	17.999116

Isotopes: influence on mass



IsoPro

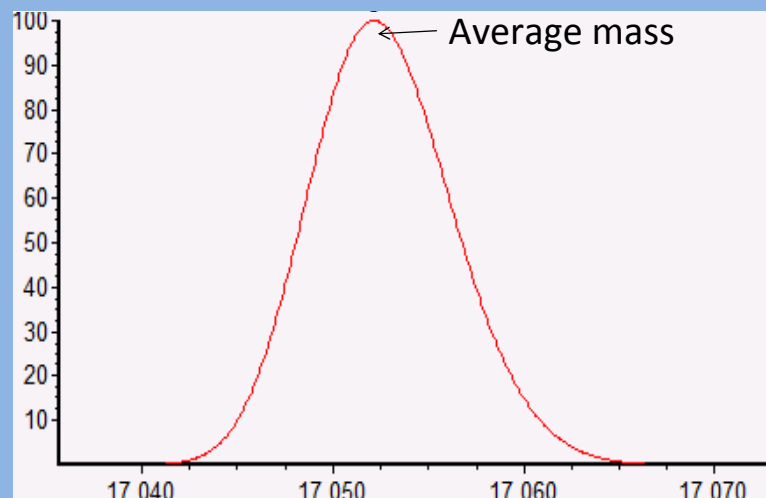
<http://sites.google.com/site/isoproms/>

Whole protein

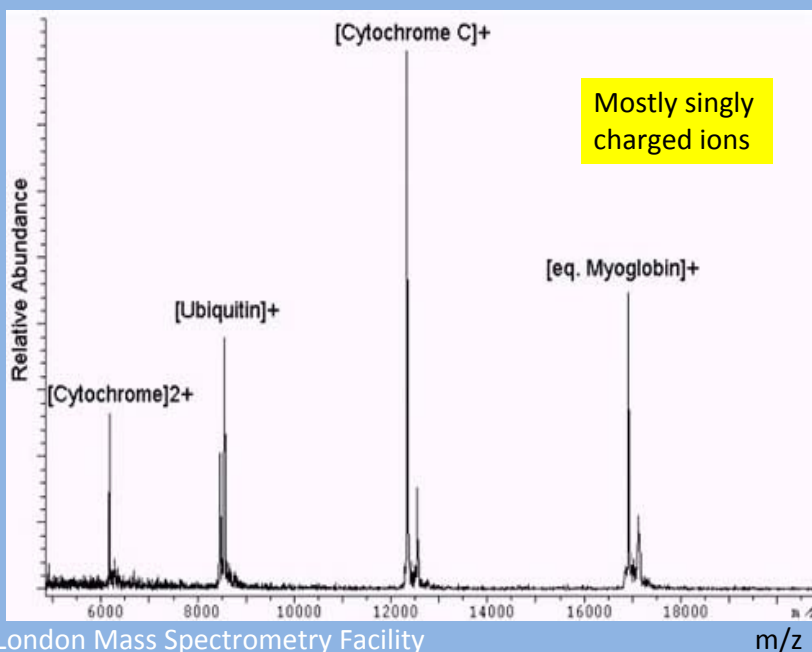
Bovine myoglobin

$C_{769} H_{1215} N_{209} O_{221} S_4$

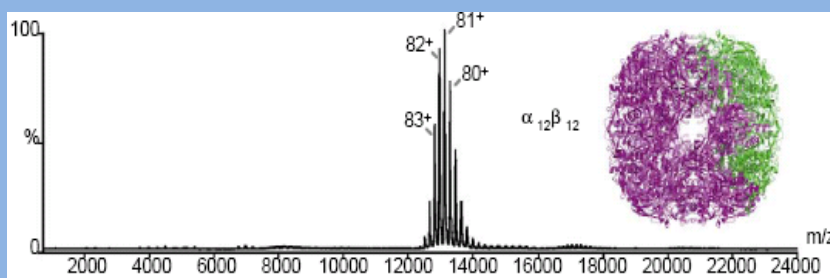
Where is peak
composed of
 $^{12}C, ^1H, ^{14}N, ^{16}O, ^{32}S$?



MALDI mass spectrum



Electrospray ionization (ESI)



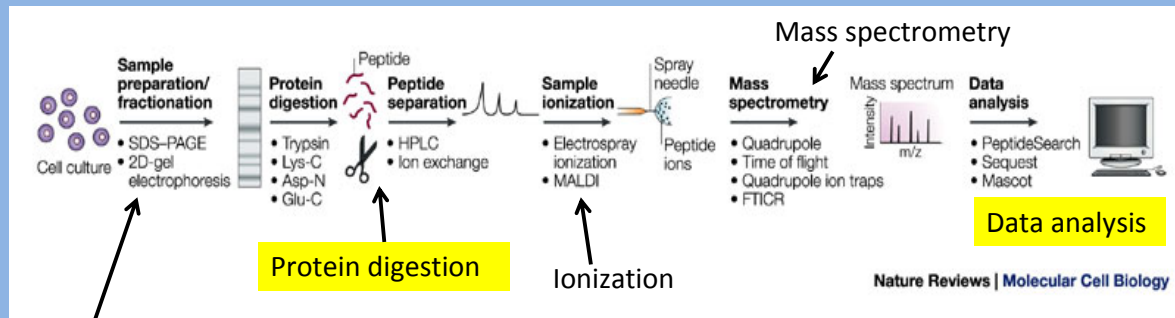
Fenn (Nobel Prize)

$$\cancel{m/z} \times \cancel{z} = m$$

$$13,000 \times 80 = 1,000,000 !!!$$

Multiply charged ions

Protein identification



Stein, Mann; Nature Rev. Mol. Cell Biol. 2004, 5, 699.

Protein sequence

A) Primary Sequence of Horse Myoglobin

GLSDGEWQQVLNVWGK|VEADIAGHGQEVLI|LFTGHPETLEK|FDK|FKHLKTEAEMKA
 SEDLKKGHTVLTALGGILKKKGHHAEELKPLAQSHATKHKIPIKYLEFISDAIIHVLHSK
 HPGDFGADAQGAMTKALELFRNDIAAKYKELGFQG

Enzyme to digest protein into peptides – *trypsin* selectively cleaves at K and R (lysine and arginine)

B) Example of a Mass Map for Myoglobin

Sequence	Monoisotopic Mass
ASEDLK	661.328
ASEDLKK	789.423
LFTGHPETLEK	1270.655
GLSDGEWQQVLNVWGK	1814.895

List of peptide masses that reflect protein = **peptide mass fingerprinting (PMF)**

Aebersold, Goodlett; Chem. Rev. 2001, 101, 269.

Protein database identification

- Contain protein sequences of all known proteins
- Compare experimental peptide masses to *in silico* peptide masses



Software calculates ALL possible enzymatic peptide masses

Online tools and databases

Molecular Detective <http://www.moleculardetective.org/> Dr. Chen, UF

UniProt

<http://www.ebi.ac.uk/uniprot/>

- TrEMBL

Non-annotated database

- SWISS-PROT

Annotated database

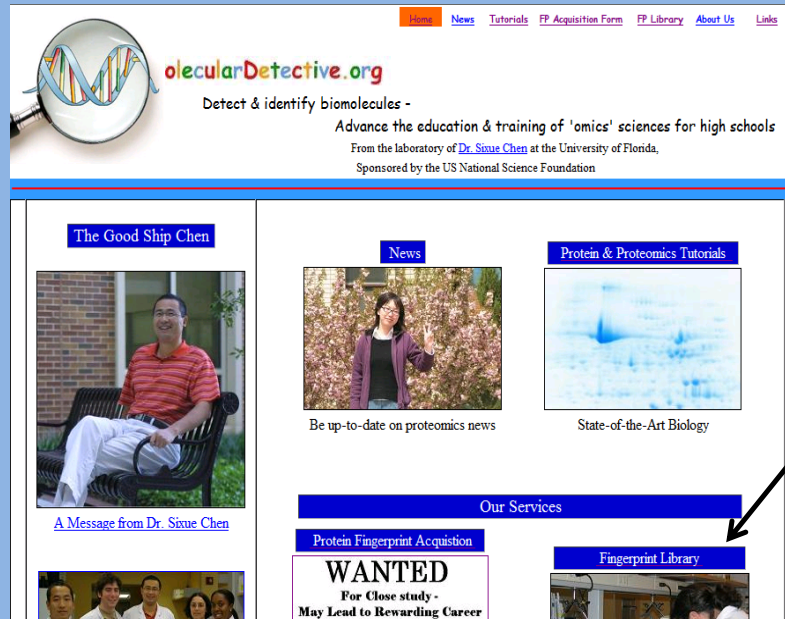
Protein Data Bank

<http://www.pdb.org/pdb/home/home.do>

Other Protein Databases

<http://www.ebi.ac.uk/Databases/protein.html>

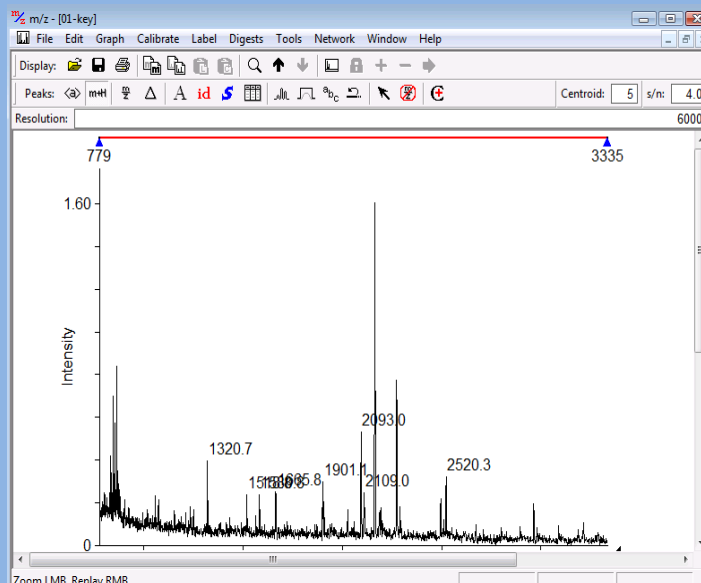
Workshop (Chen)



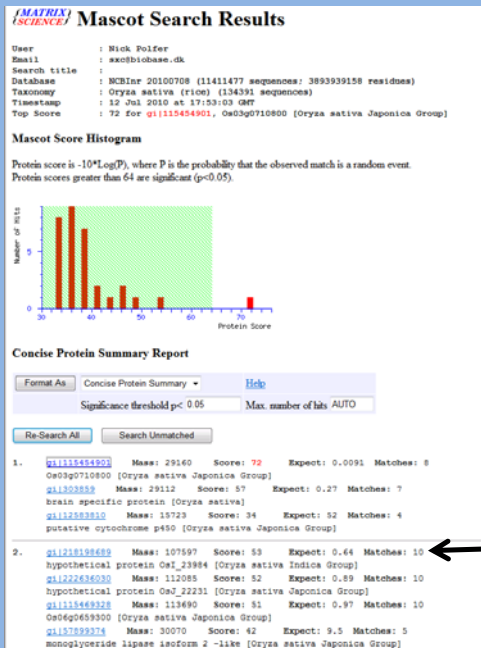
Fingerprint Library

Workshop (Chen)

1. Download and install Moverz Bioinformatics Software
2. Download 'Mass Spectra Examples from Rice Pollen Grain Proteins'
3. Open file



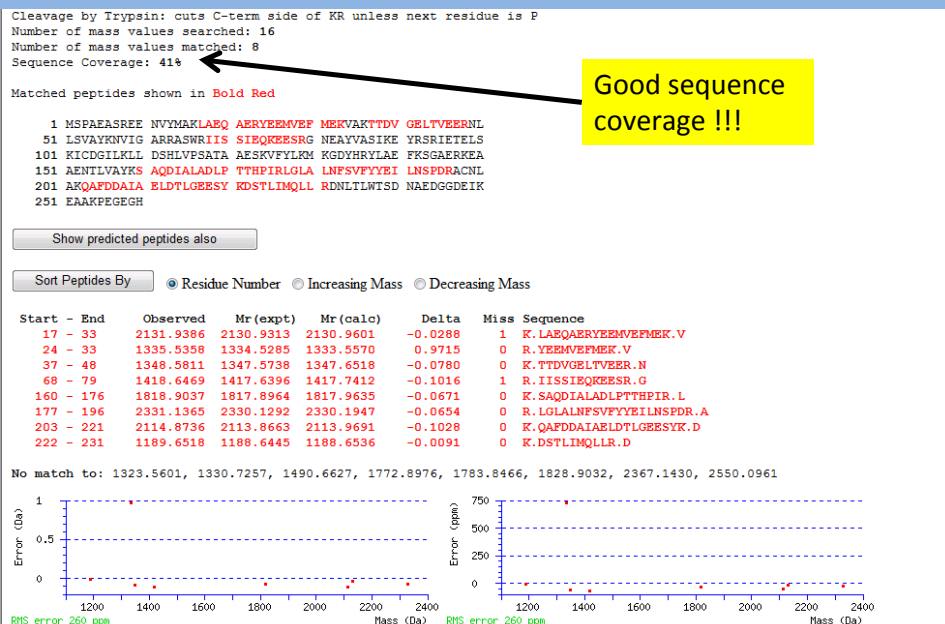
Workshop (Chen)



- Score: should be > 65 (green box)
- Matches: number of peptides identified
- Mass of protein

Why is score here lower?

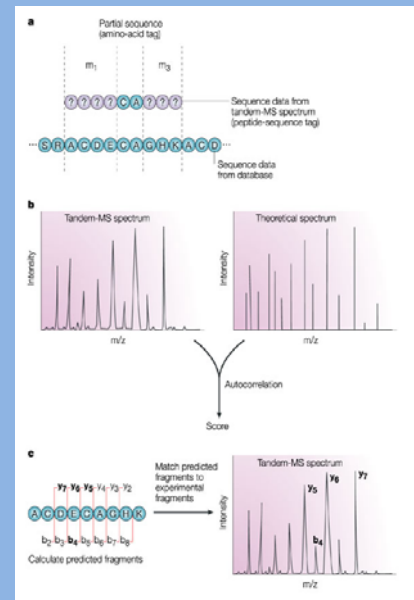
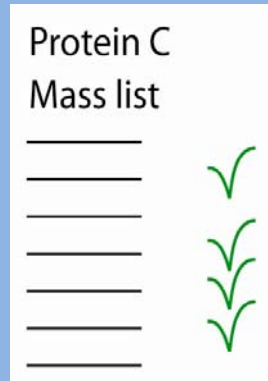
Workshop (Chen)



Good sequence coverage !!!

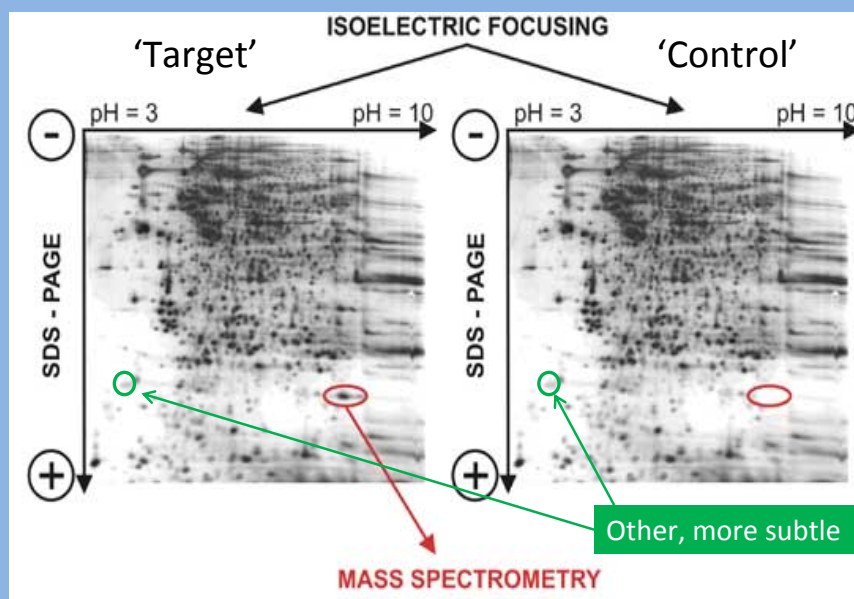
Workshop (Polfer) – confirm peptide sequence

How can we confirm peptide sequence?



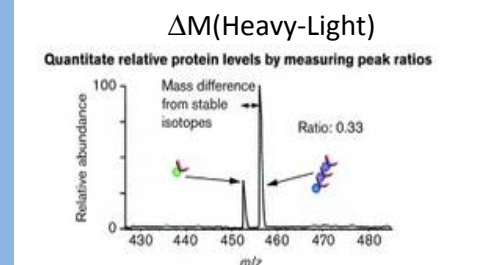
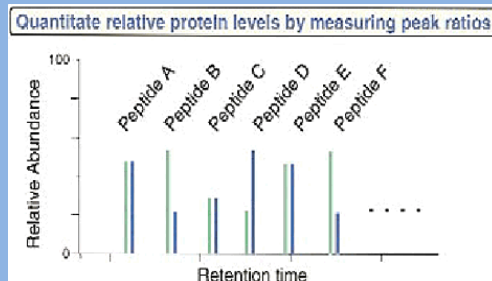
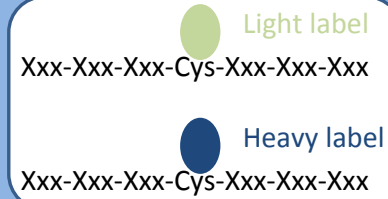
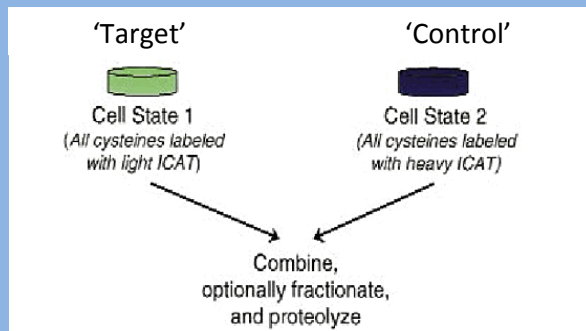
Stein, Mann; Nature Rev. Mol. Cell Biol. 2004, 5, 699.

Gel electrophoresis



<http://biol.lf1.cuni.cz/ucebnice/en/proteomics.htm>

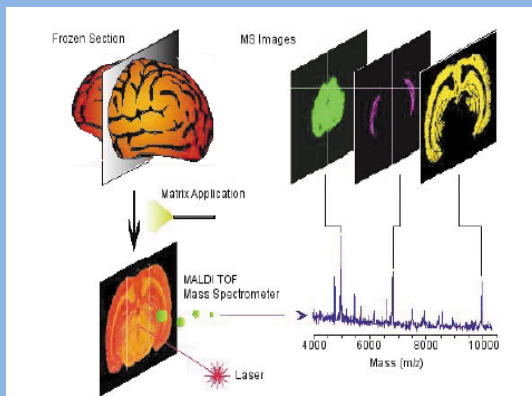
Quantification: label peptides



Aebersold and co-workers; *Nature Biotechnology*, 1999, 17, 994.

MALDI application: imaging

Tissue sections



Spatial distribution of peptides/proteins

Caprioli, *Nature Medicine*, 2001, 7, 493.

Human glioblastoma (form of brain cancer)

