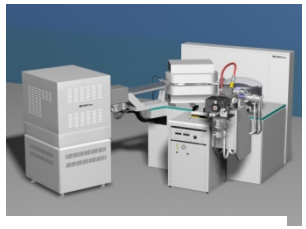
Chap 5 Mass Spectrometry

Biochemistry and Molecular Biology

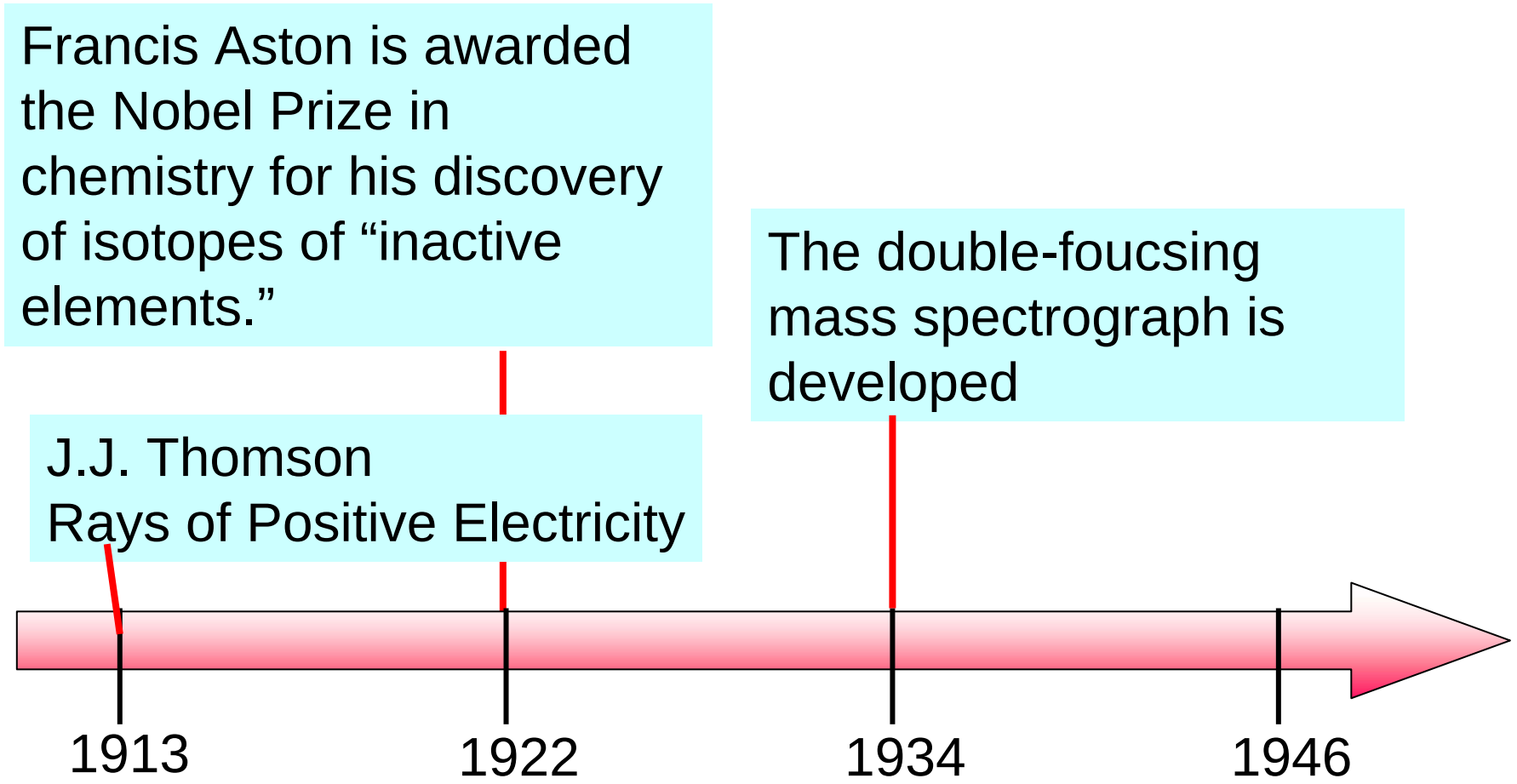
- 9.1 Introduction
- 9.2 Ionization
- 9.3 Mass Analysis
- 9,5 Structural Information by Tandem Mass Spectrometry
- 9.7 Computing and Database Analysis

Biological Mass Spectrometry



A key tool for Protein.
Peptides. Carbohydrate.
DNA……and more

Evolution of Mass Spectrometry-1



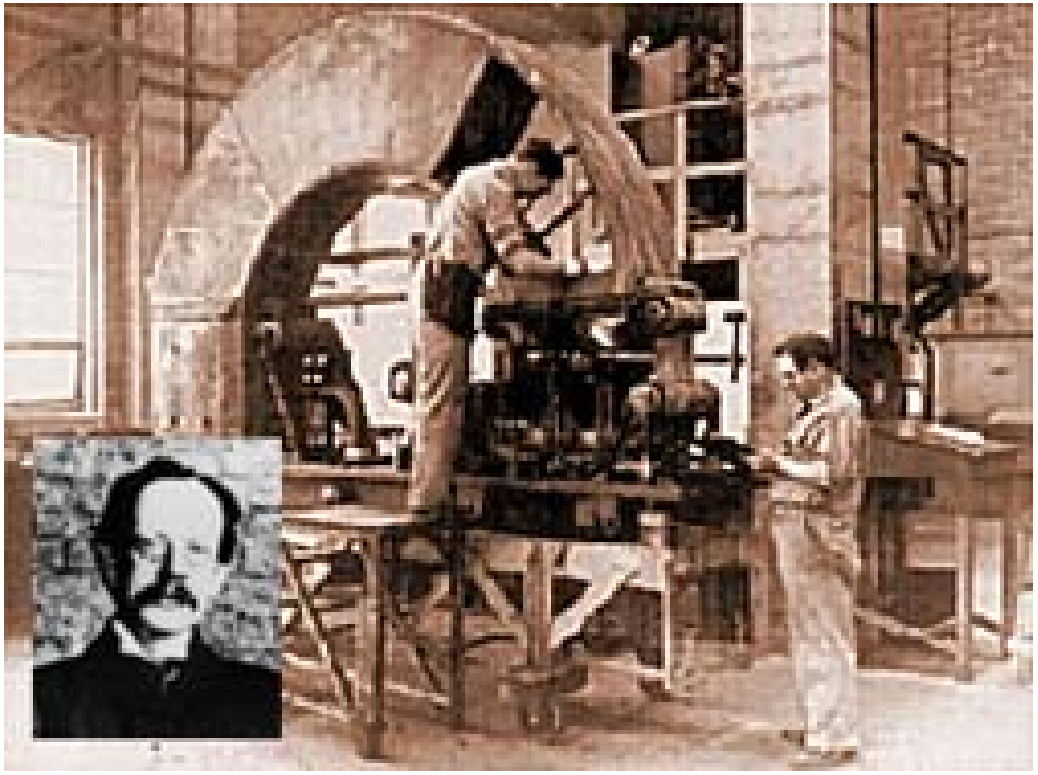
Back to the history……

J.J. Thomson

- J.J. Thomson observes a line at mass 22 in the spectrum of neon.
- J.J. Thomson delivers his Bakerian Lecture, “Rays of Positive Electricity” to the Royal Society of London.

Back to the history……..

The **first** mass spectrometer - parabola spectrograph



1912 J. J. Thomson

Used magnetic field and recorded the resultant spatial dispersion of ions on photographic plates.

- Slow
- instability of magnet
- Low detection sensitivity

- ***Francis Aston*** is awarded the Nobel Prize in chemistry for his discovery of isotopes of "inactive elements."

Francis Aston

1																	18
1 H 1.0079	2											13	14	15	16	17	2 He 4.0026
3 Li 6.941	4 Be 9.0122											5 B 10.811	6 C 12.011	7 N 14.007	8 O 15.999	9 F 18.998	10 Ne 20.180
11 Na 22.990	12 Mg 24.305	3	4	5	6	7	8	9	10	11	12	13 Al 26.982	14 Si 28.086	15 P 30.974	16 S 32.065	17 Cl 35.453	18 Ar 39.948
19 K 39.098	20 Ca 40.078	21 Sc 44.956	22 Ti 47.867	23 V 50.942	24 Cr 51.996	25 Mn 54.938	26 Fe 55.845	27 Co 58.933	28 Ni 58.693	29 Cu 63.546	30 Zn 65.409	31 Ga 69.723	32 Ge 72.64	33 As 74922	34 Se 78.96	35 Br 79.904	36 Kr 83.798
37 Rb 85.468	38 Sr 87.62	39 Y 88.906	40 Zr 91.224	41 Nb 92.906	42 Mo 95.94	43 Tc (98)	44 Ru 101.07	45 Rh 102.91	46 Pd 106.42	47 Ag 107.87	48 Cd 112.41	49 In 114.82	50 Sn 118.71	51 Sb 121.76	52 Te 127.60	53 I 126.90	54 Xe 131.29
55 Cs 132.91	56 Ba 137.33	57-71 *	72 Hf 178.49	73 Ta 180.95	74 W 183.84	75 Re 186.21	76 Os 190.23	77 Ir 192.22	78 Pt 195.08	79 Au 196.97	80 Hg 200.59	81 Tl 204.38	82 Pb 207.2	83 Bi 208.98	84 Po (209)	85 At (210)	86 Rn (222)
87 Fr (223)	88 Ra (226)	89-103 #	104 Rf (261)	105 Db (262)	106 Sg (266)	107 Bh (264)	108 Hs (277)	109 Mt (268)	110 Ds (281)	111 Rg (272)	112 Uub (285)	113 Uut (284)	114 Uuq (289)	115 Uup (288)			

* Lanthanide series	57 La 138.91	58 Ce 140.12	59 Pr 140.91	60 Nd 144.24	61 Pm (145)	62 Sm 150.36	63 Eu 151.96	64 Gd 157.25	65 Tb 158.93	66 Dy 162.50	67 Ho 164.93	68 Er 167.26	69 Tm 168.93	70 Yb 173.04	71 Lu 174.97
# Actinide series	89 Ac (227)	90 Th 232.04	91 Pa 231.04	92 U 238.03	93 Np (237)	94 Pu (244)	95 Am (243)	96 Cm (247)	97 Bk (247)	98 Cf (251)	99 Es (252)	100 Fm (257)	101 Md (258)	102 No (259)	103 Lr (262)

Advances in Modern Mass Spectrometry

Limitations of traditional MS on biological applications

- High molecular weight >50,000
- Amount of Sample $< 10^{-12} - 10^{-15}$ mole

Intact Molecule

Non-covalent Complex

Electro**S**pray **I**onization MS

Matrix **A**ssisted **L**aser **D**esorption **I**onization MS

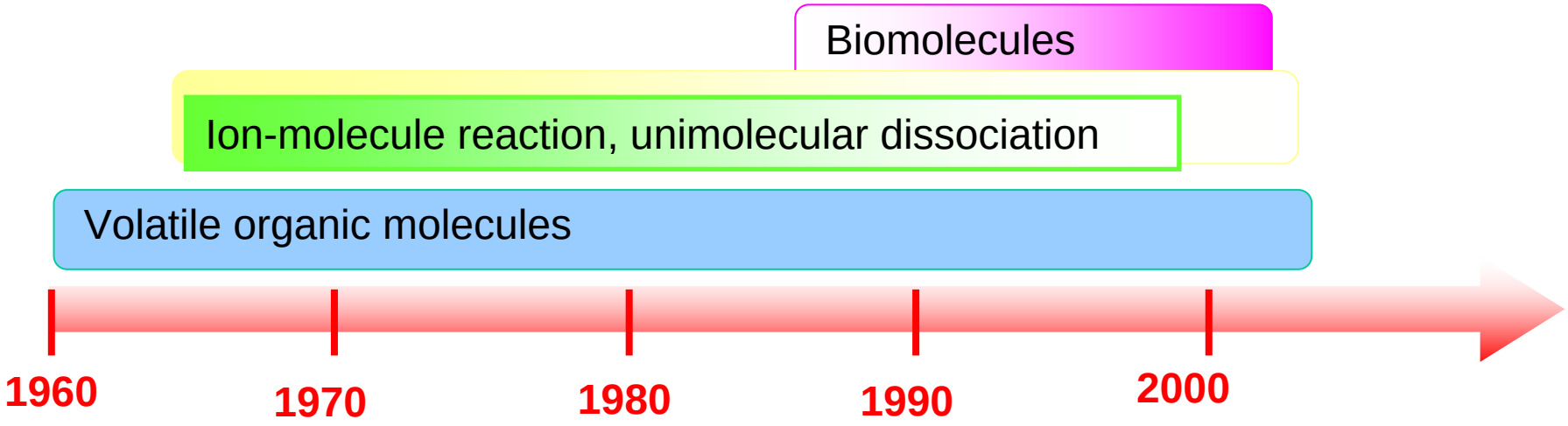
John B. Fenn

ESI

Koichi Tanaka

MALDI

Evolution of Mass Spectrometry-2



Human genome Project
(planned in 1988, started in 1990)

2003, 4, 14

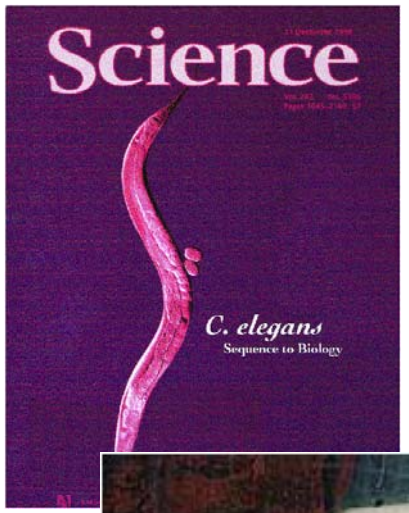
Lead by Department of Energy, USA

- Estimated 30000 genes
- The genome is nearly the same (99.9%) for individual

Genomics（基因體學）

1953: Watson and Crick: DNA double helix

C. Elegans (1998)



Rattus norvegicus

Danio rerio

Anopheles gambiae (2002

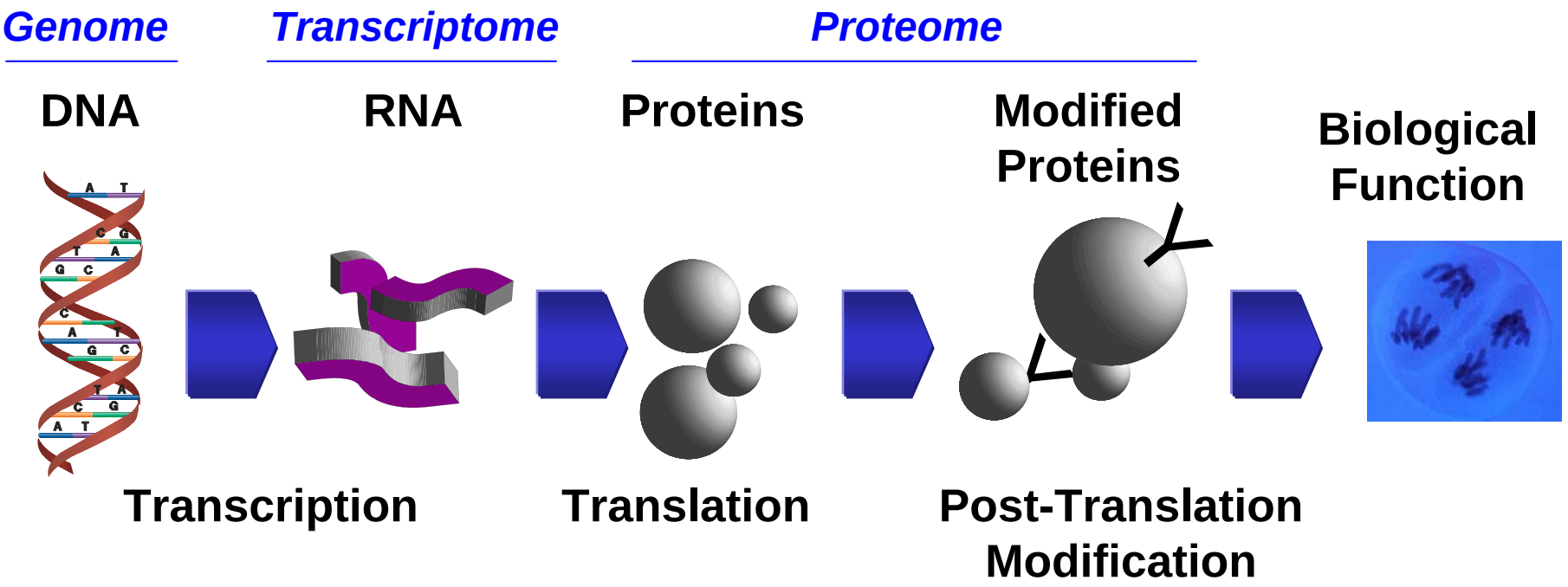
Fugu rubripes (2

Homo sapiens (2001)

Drosophila melanogaster (2000)

Mus musculus (2002)

從基因體到蛋白質體的研究



PROTEin complement to a gen**OME**

Entire protein complement in a given cell, tissue or organism

PR TE MICS

蛋白質體學

Marc Wilkins
The University of New South Wales (UNSW), Sydney, Australia

Protein activity, modifications, localizations, amd interactions of proteins in complexes

Proteomics can be defined as *the qualitative and quantitative comparison of proteomes under different conditions to further unravel biological processes*

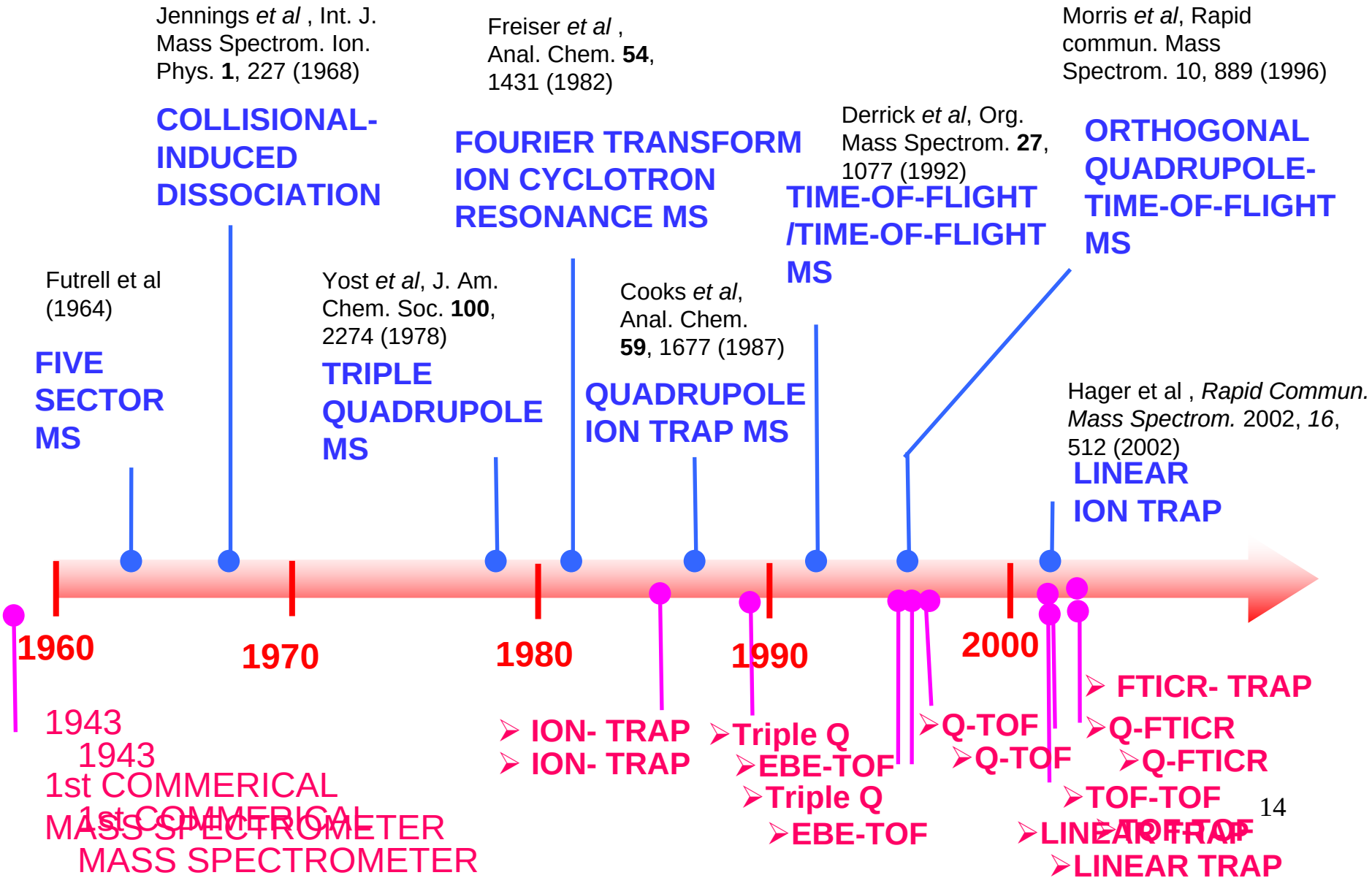
Protein chemistry and proteomics

Protein Identification

(蛋白質鑑定)

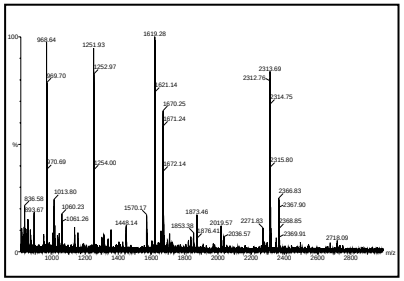
Protein chemistry	Proteomics
Individual proteins	Complex mixtures
Complete sequence analysis	Partial sequence analysis
Emphasis on structure and function	Emphasis on identification by database matching
Structual biology	Systems biology

生物分子之應用促使質譜技術高度發展



What is mass spectrometry?

$M \xrightarrow{\text{Ionization}} M^+ \text{ or } M^-$



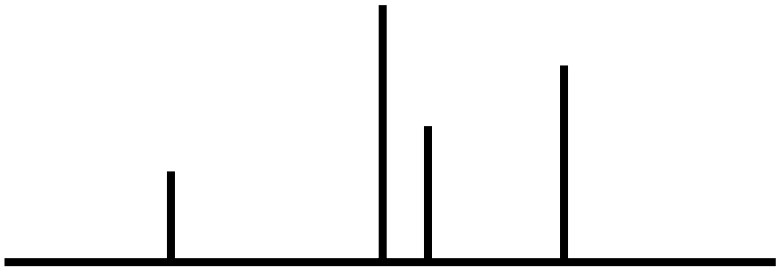
Mass-to-charge Ratio (***m/z***)

MS is an analytical tool that measure the molecular weight of molecules based on the motion of charged particle in an electrical or magnetic field.

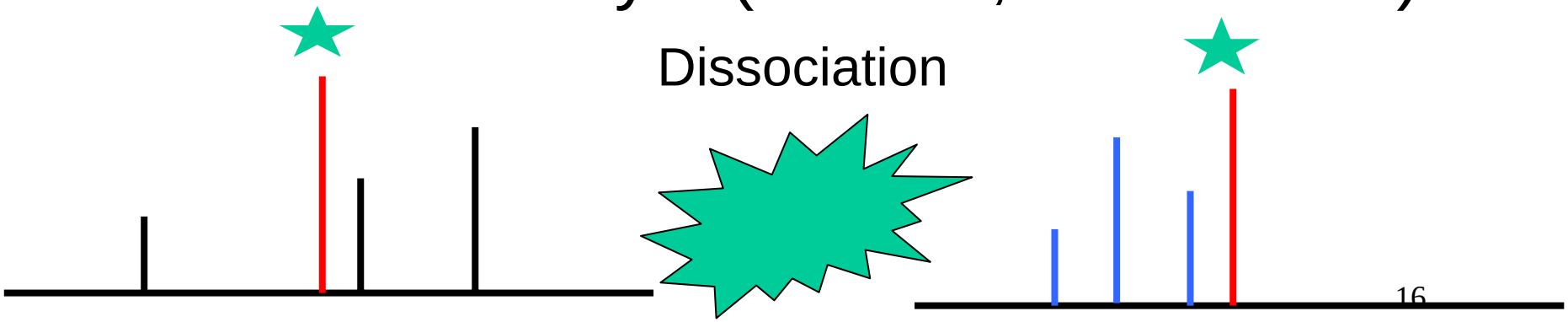
Two different ways of measurement

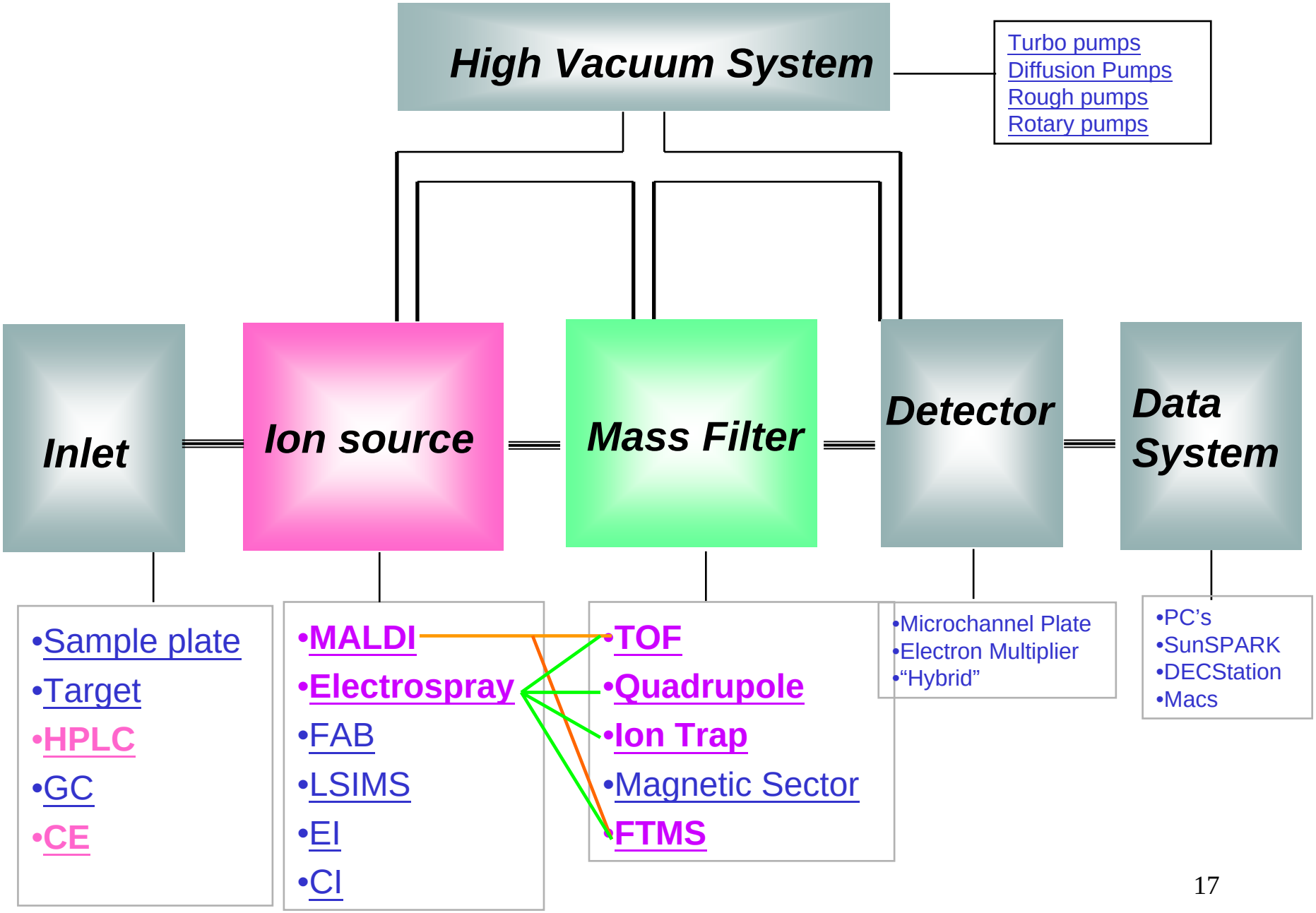
- Composition of analyte (MS)

  e.g. Peptide mass fingerprinting



- Structure of analyte (MS/MS, tandem MS)




High Vacuum System
Turbo pumps
Diffusion Pumps
Rough pumps
Rotary pumps
Inlet
Ion source
Mass Filter
Detector
Data System
•Sample plate
•Target
•HPLC
•GC
•CE
•MALDI
•Electrospray
•FAB
•LSIMS
•EI
•CI
•TOF
•Quadrupole
•Ion Trap
•Magnetic Sector
•FTMS
•Microchannel Plate
•Electron Multiplier
•"Hybrid"
•PC's
•SunSPARK
•DECStation
•Macs

Ionization Methods

- Electron Impact (EI)
- Fast Atom Bombardment (FAB)
- Electrospray Ionization (ESI)
- Matrix-Assisted Laser Desorption Ionization (MALDI)

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Intact Molecule

Non-covalent Complex

Electro**S**pray **I**onization MS

Matrix **A**ssisted **L**aser **D**esorption **I**onization MS

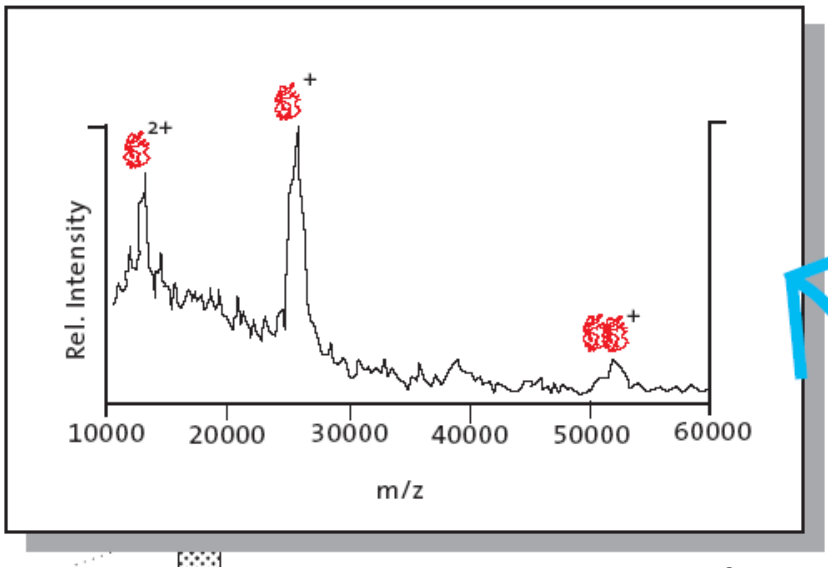
2002 Nobel Prize in Chemistry

Soft Laser Desorption (SLD)

A breakthrough for the laser desorption method in its application to large biomolecules was reported at a symposium in Osaka in 1987, when Koichi Tanaka at the Shimadzu Corp. in Kyoto presented results of a mass spectrometric analysis of an intact protein (12,384 Da).

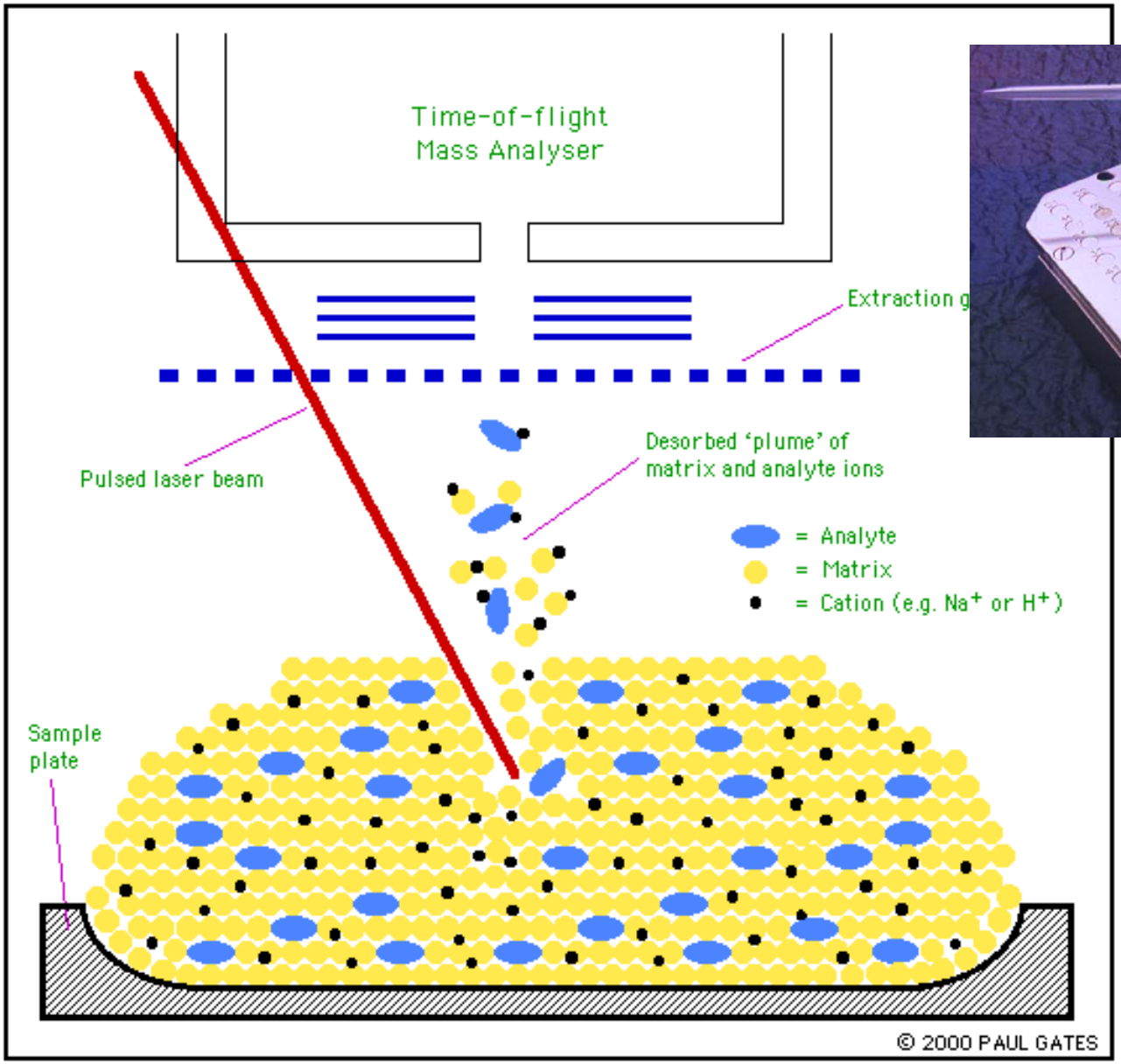
Koichi Tanaka
Shimadzu Corp.

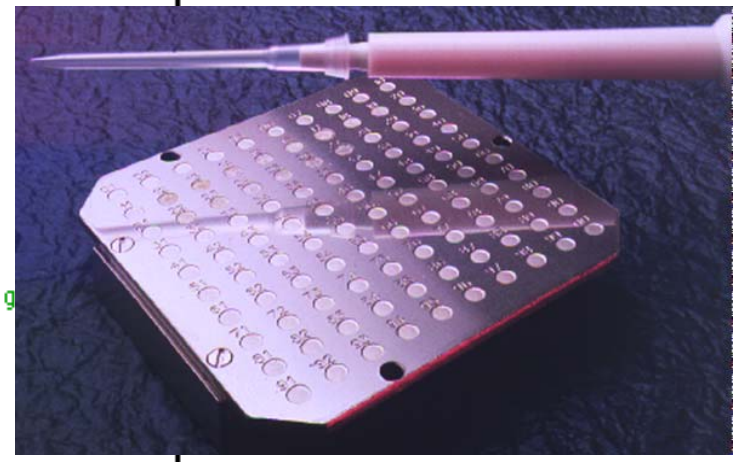
In two publications and lectures in 1987-1988, Tanaka presented ionisation of proteins such as chymotrypsinogen (25,717 Da), carboxypeptidase-A (34,472 Da) and cytochrome c



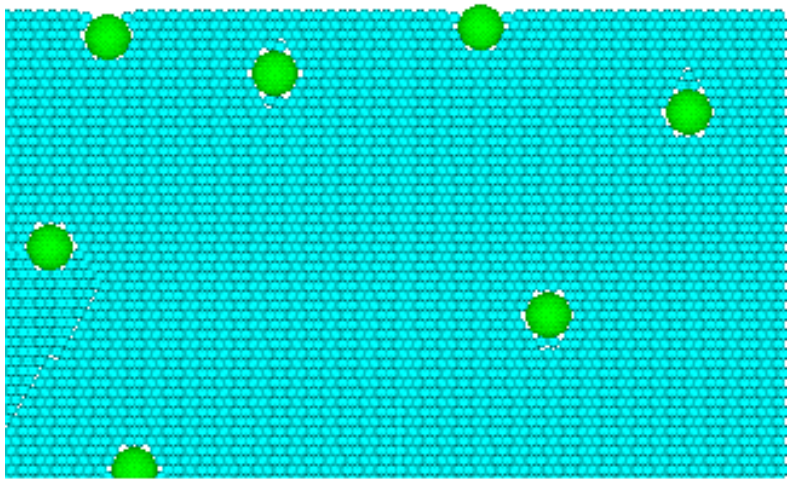
1. Second Japan-China Joint Symposium on Mass Spectrometry.
2. Mass Spectroscopy (Japan). 36, (1988) 59.
3. Rapid Commun. Mass Spectrom. 2 (1988) 151-153.

*M*atrix-*A*ssisted *L*aser *D*esorption *I*onization (*MALDI*)





*M*atrix-*A*ssisted *L*aser *D*esorption *I*onization (*MALDI*)



Analyte

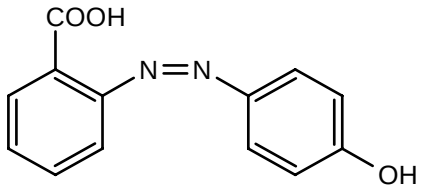
Metal Ion

Matrix

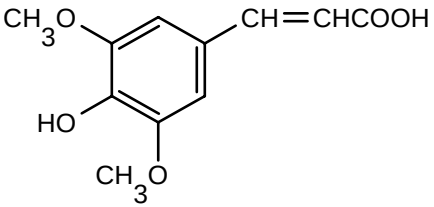
http://www.ionsource.com

Matrix selection

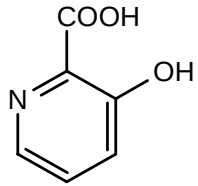
α-Cyano-4-hydroxy-cinnamic acid (CHCA)	Peptides<10kDa
Sinapinic Acid	Proteins >10kDa
2,5-Dihydroxybenzoic acid (DHB)	Neutral Carbohydrates, Synthetic Polymers
"Super DHB"	Proteins, Glycosylated proteins
3-Hydroxypicolinic acid	Oligonucleotides
HABA	Proteins, Oligosaccharides



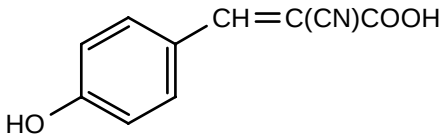
2-(4-hydroxyphenylazo)-benzoic acid (HABA)



Sinapinic acid (3,5-Dimethoxy-4-hydroxy cinnamic acid)

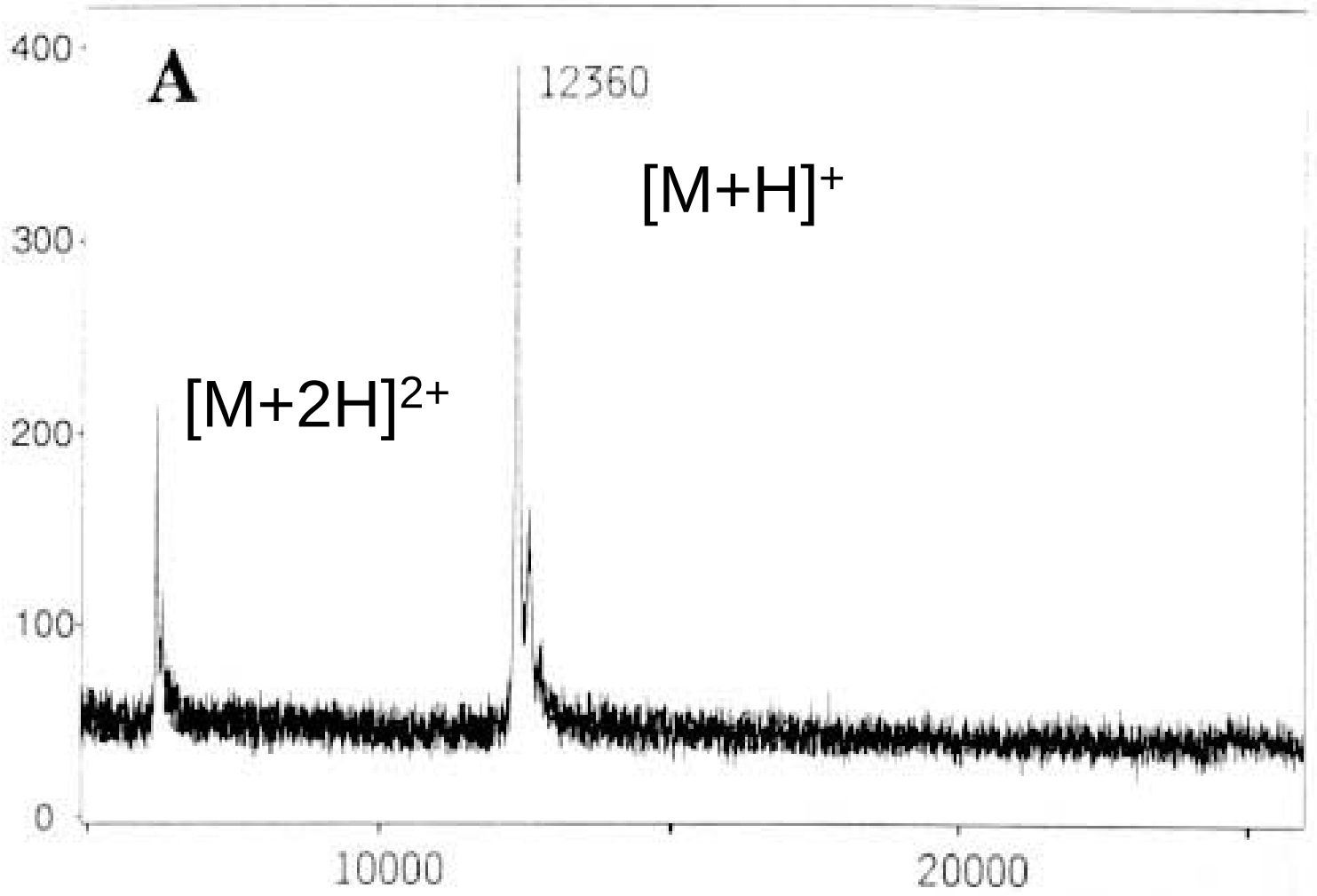


3-hydroxypicolinic acid (3-HPA)



α-cyano-4-hydroxycinnamic acid

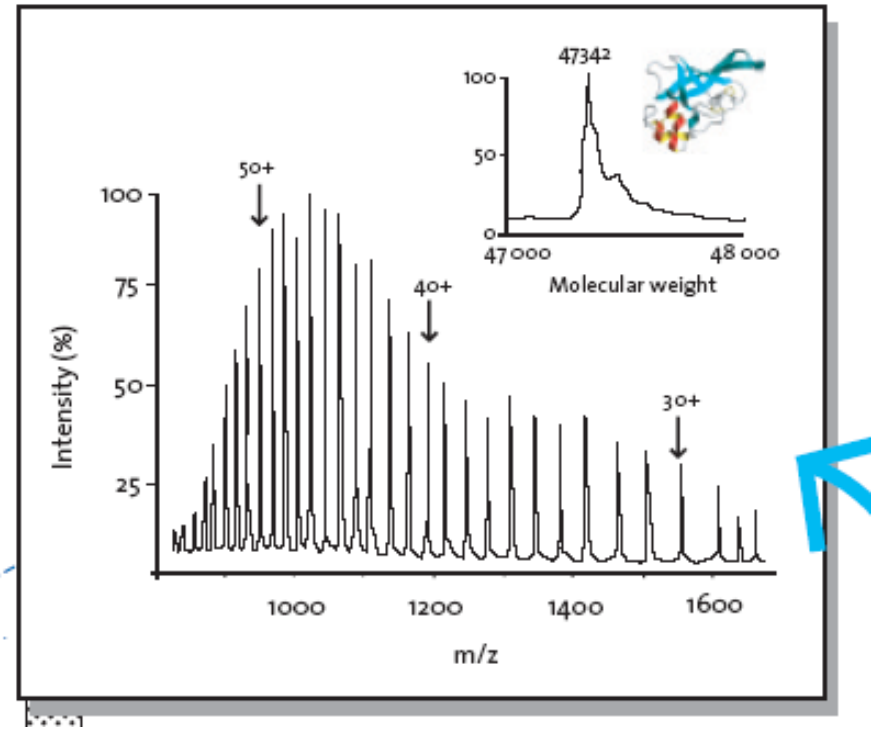
A typical mass spectra



Electrospray Ionisation

John Fenn

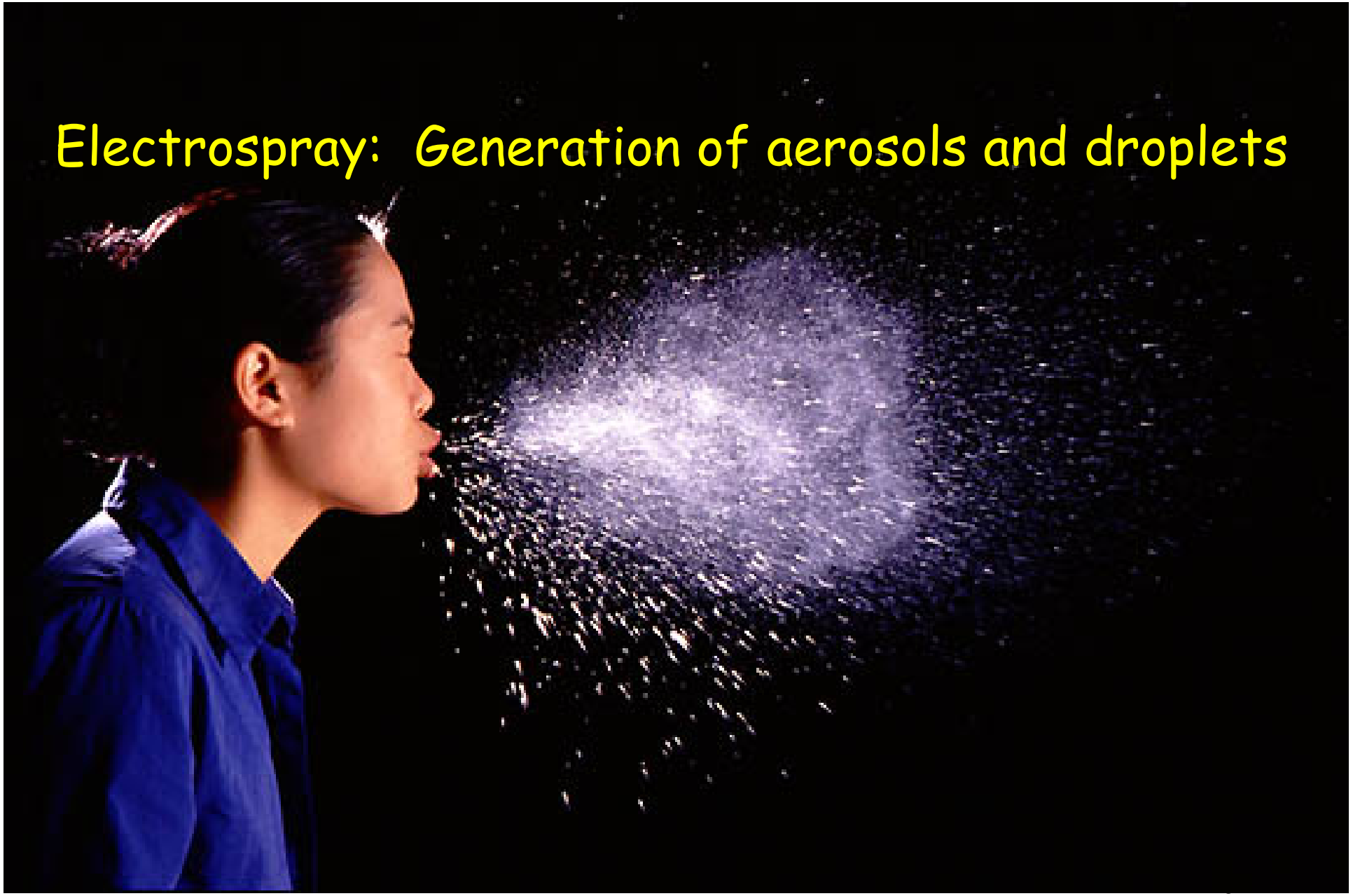
the charged droplets evaporate to a point where the number of repulsive electrostatic charges on the surface becomes so large relative to the droplet size that an explosion (”Rayleigh explosion”) occurs. This produces a number of smaller droplets that also have a surface containing electrostatic charges.



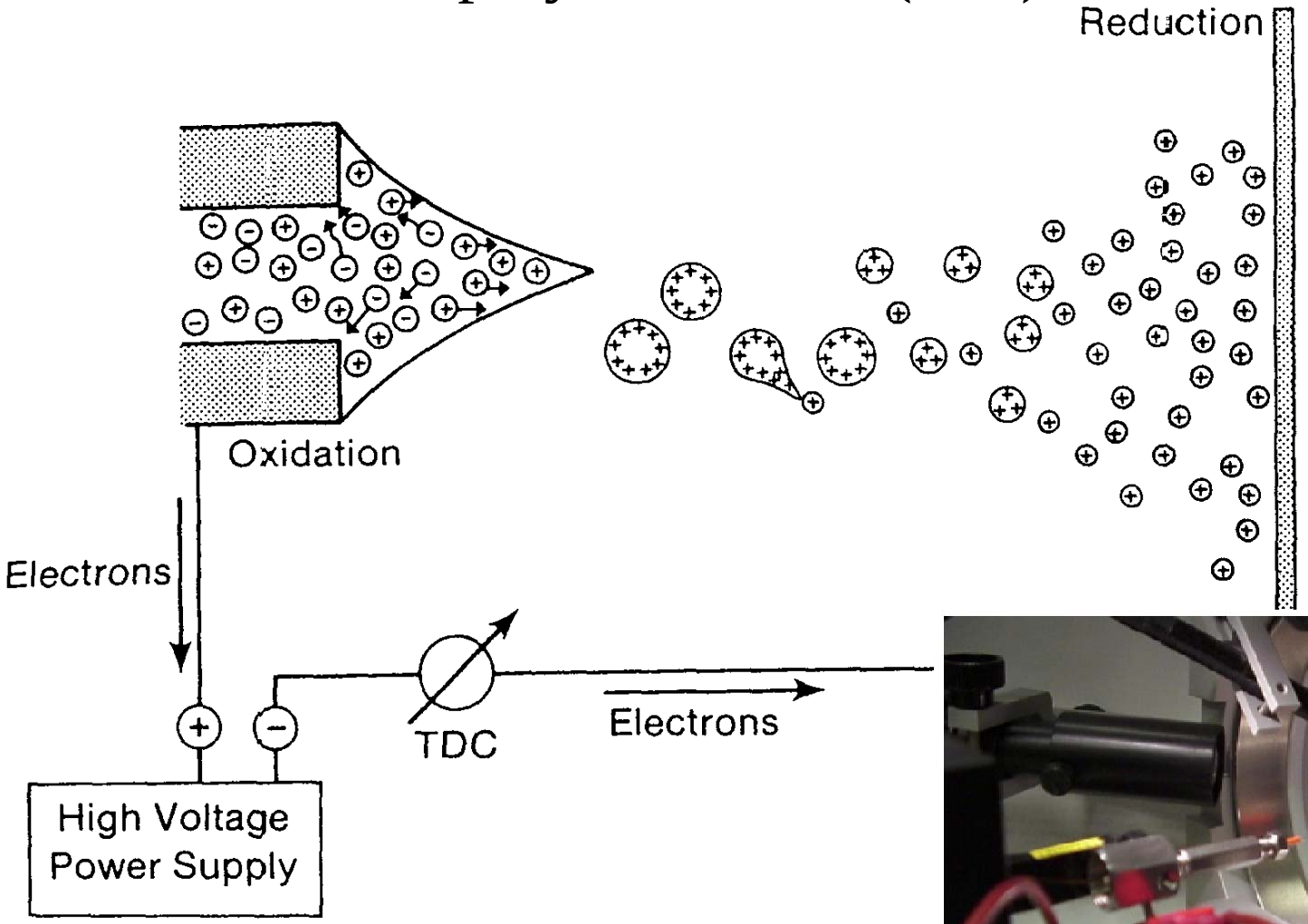
The well-defined breakthrough of ESI came in 1988 at a symposium in San Francisco, when John Fenn presented an identification of polypeptides and proteins of molecular weight 40 kDa

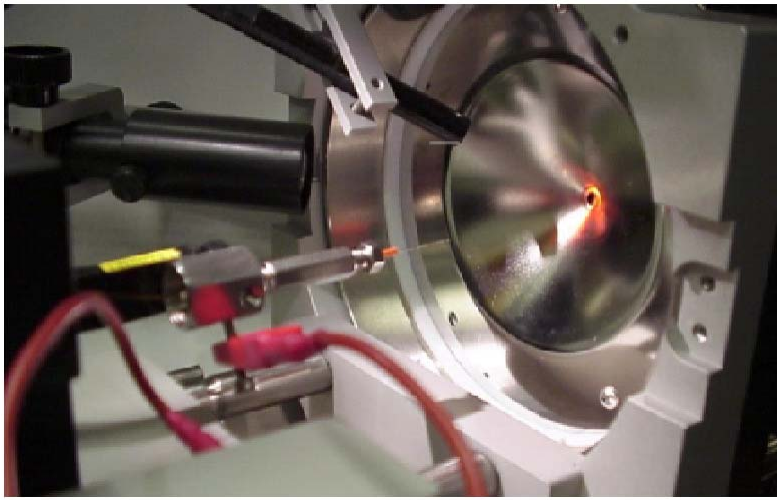
Proc 36th Annual Conference, Am. Soc. for Mass Spectrom., San Francisco, 5-10 June 1988, p. 773.

Electrospray: Generation of aerosols and droplets

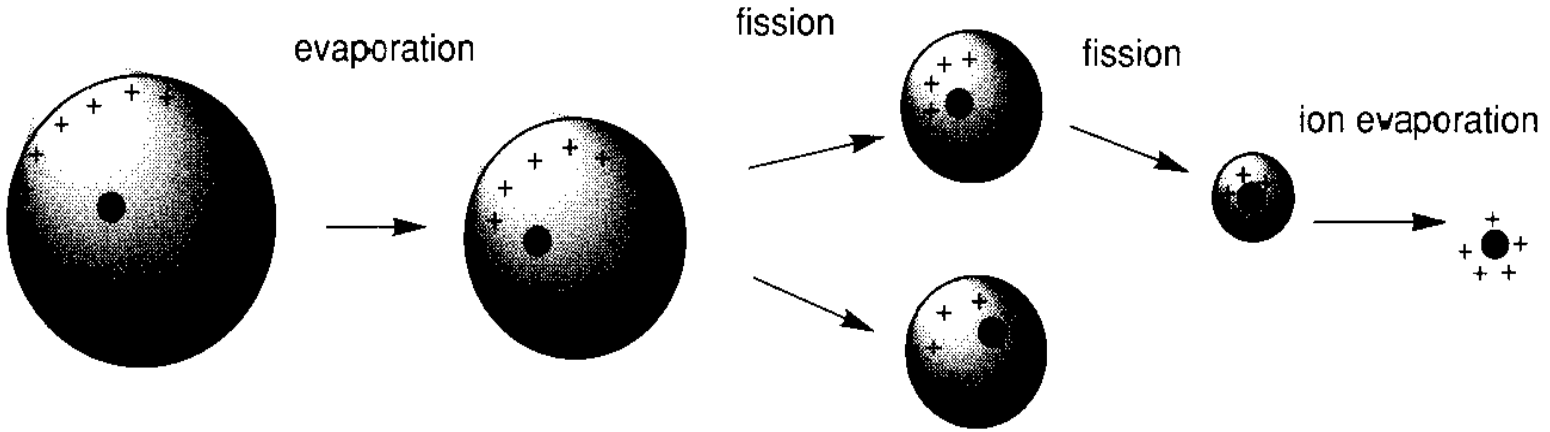


*E*lectro*S*pray *I*onization (**ESI**)



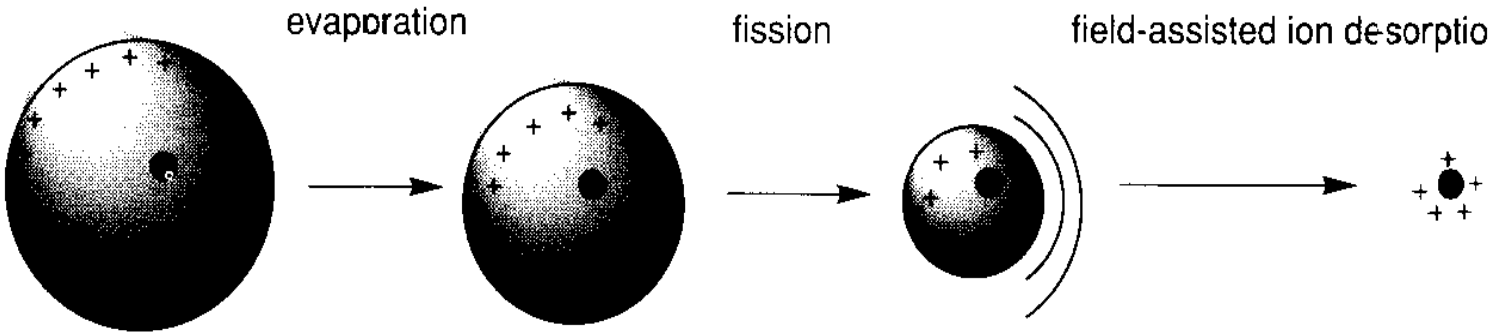


Charge Residue Model



(a)

Coulomb repulsion : Charge repulsion > surface tension

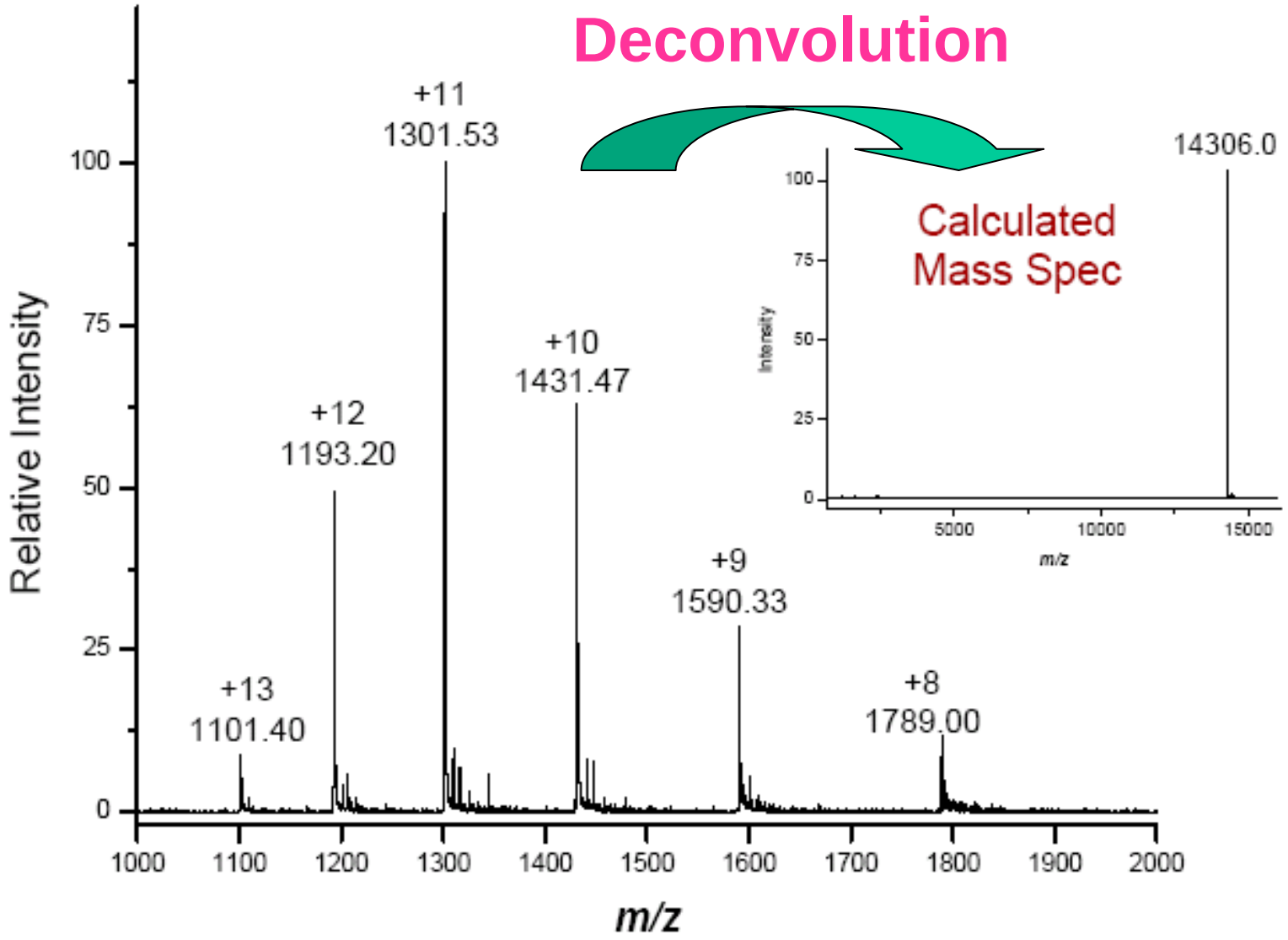


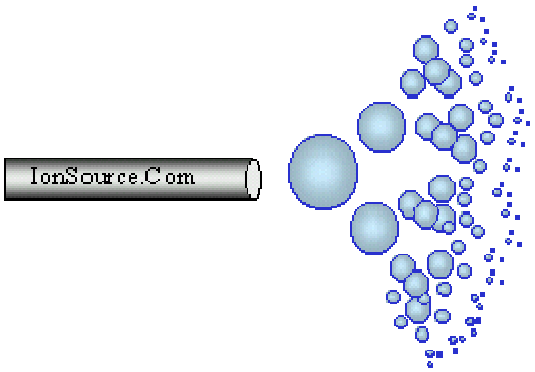
(b)

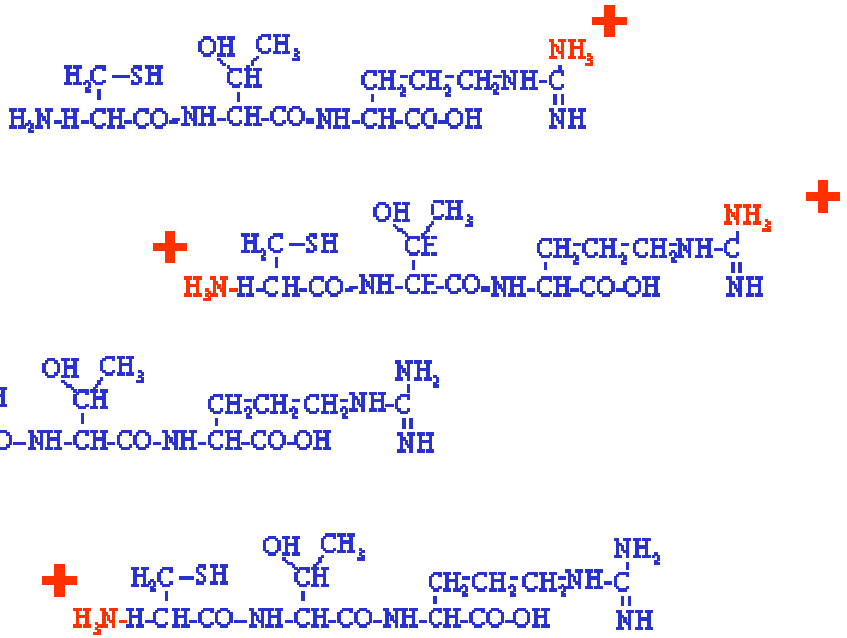
Ion Desorption Model

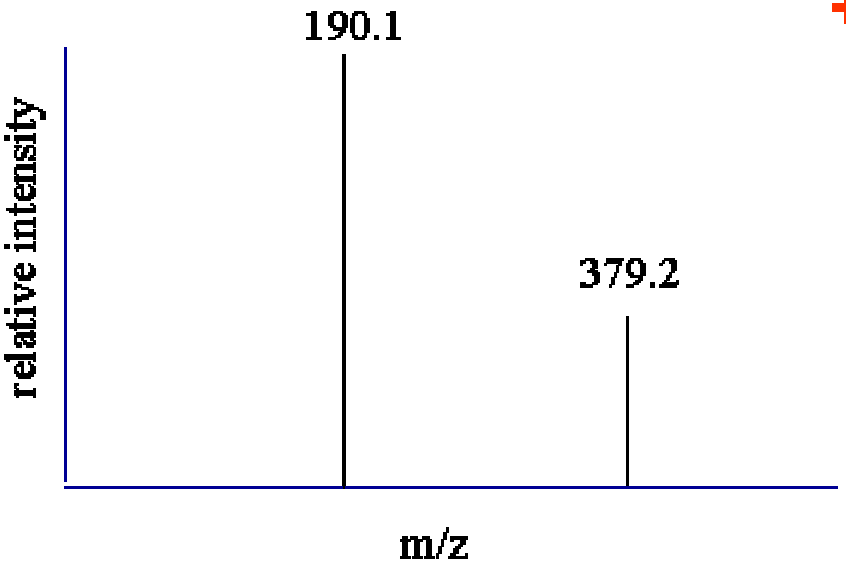
Ion desorption from the droplet surface

Multiple-charged ESI spectra

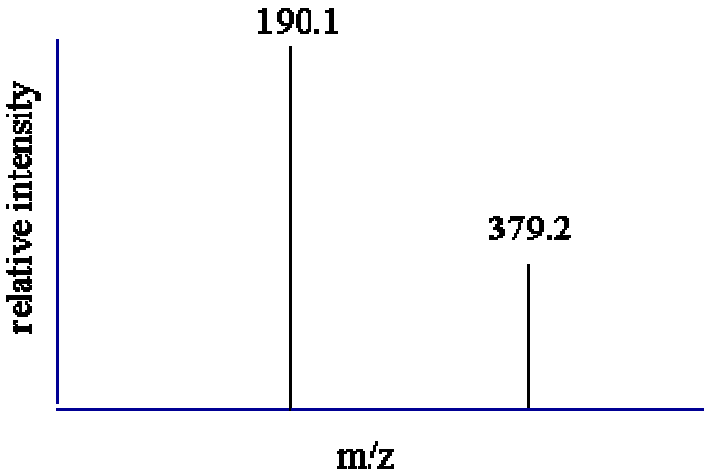



IonSource.Com




190.1
379.2
relative intensity
m/z

Deconvolution



Step 1: assume 190.1 = mass/charge

379.2 = mass/charge

Step 2: assume the two peaks are related

190.1 = [m+(z+1)]/(z+1)

379.2=m+z/z

Step 3: solve m and z

m=378.2, z=1

Charge state	Calculation	Unprotonated mass
+1	(379.2-1)*1	378.2
+2	(190.1-1)*2	378.2
	average	378.2

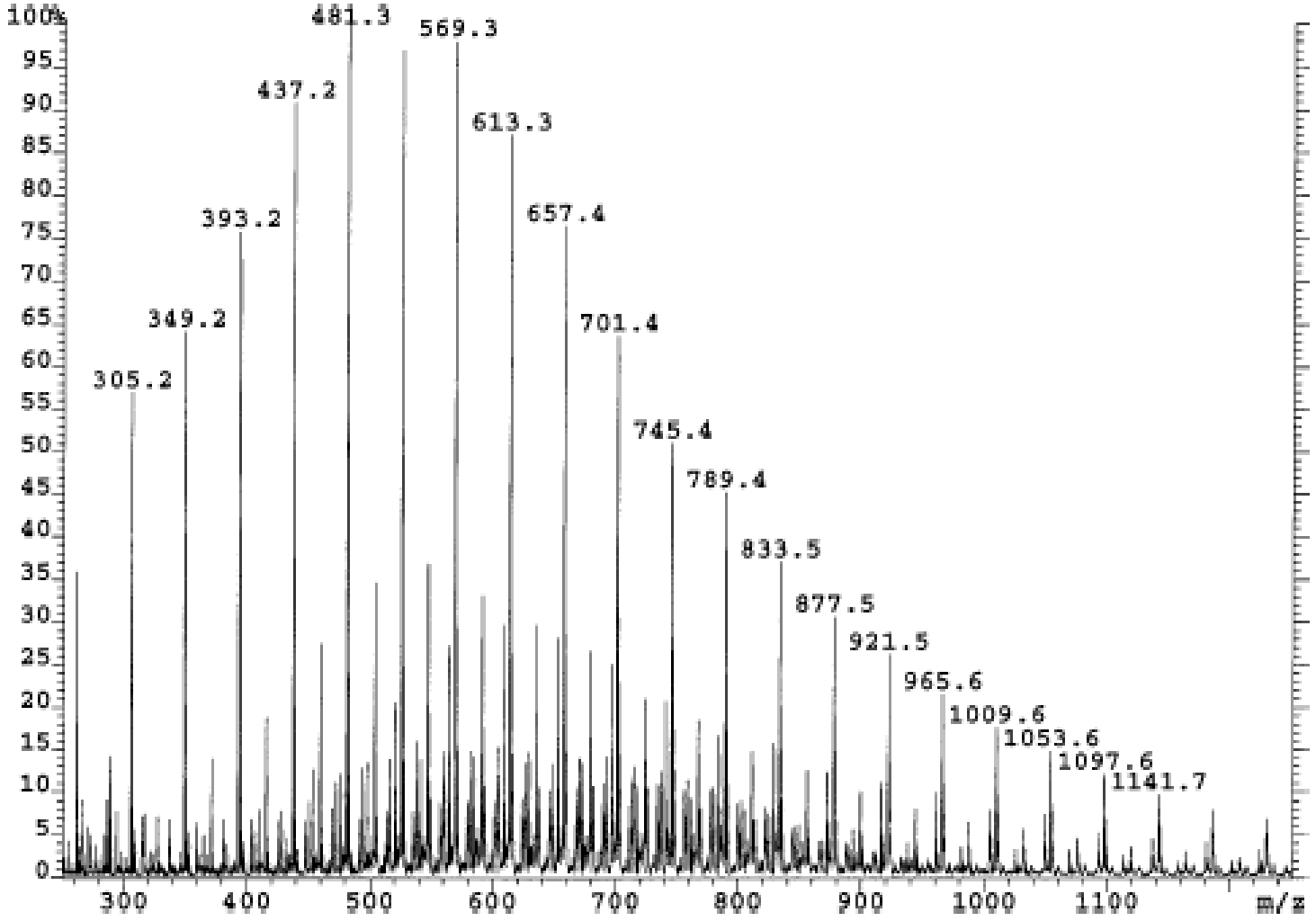
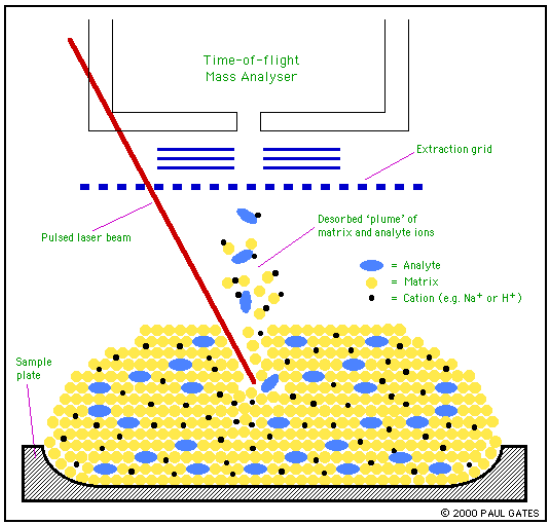
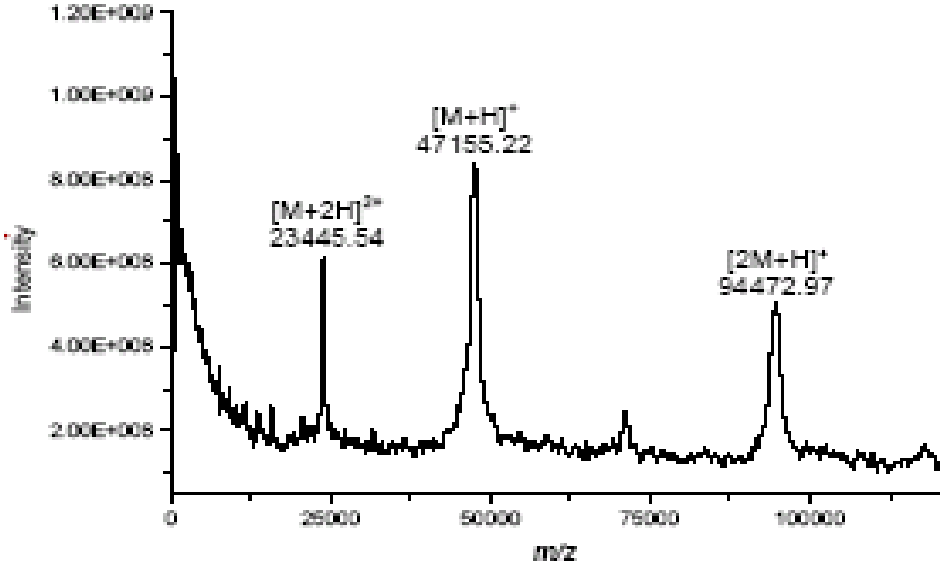


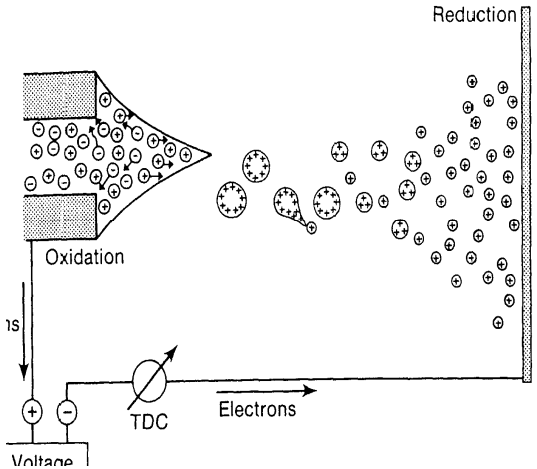
Figure 2 Positive-ion ES mass spectrum of a PEG mixture

Molecular Weight of Proteins

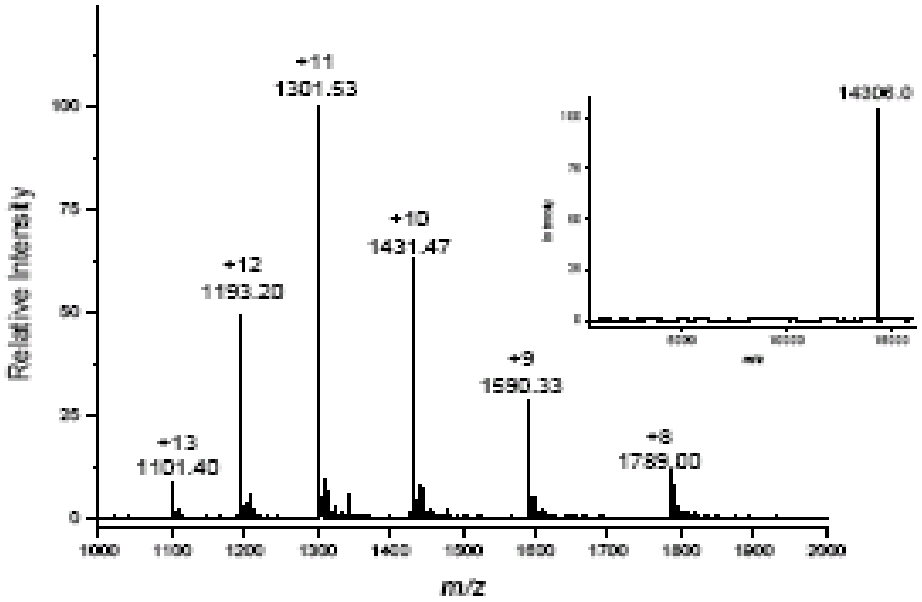


MALDI



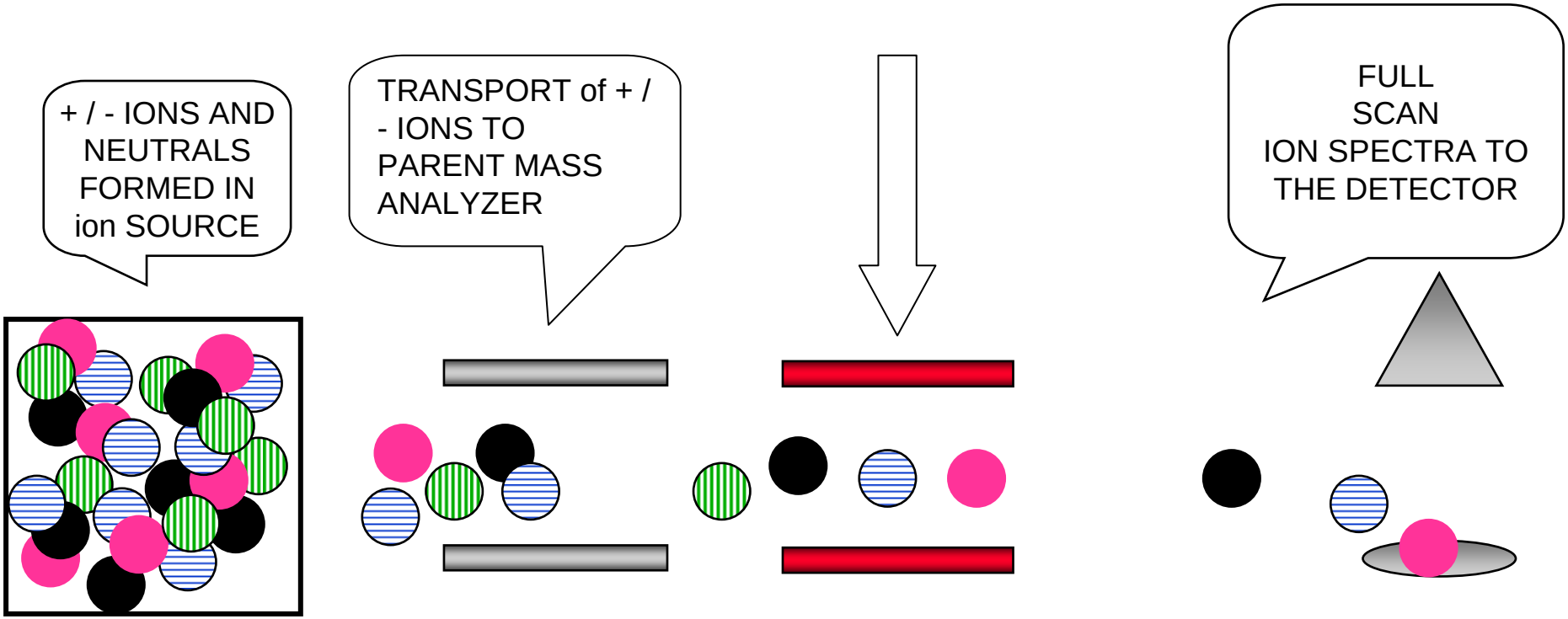


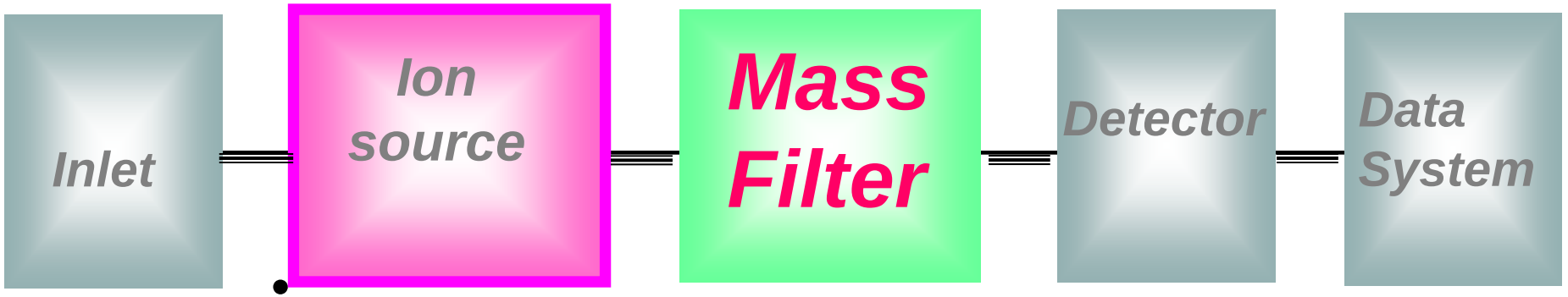
ESI



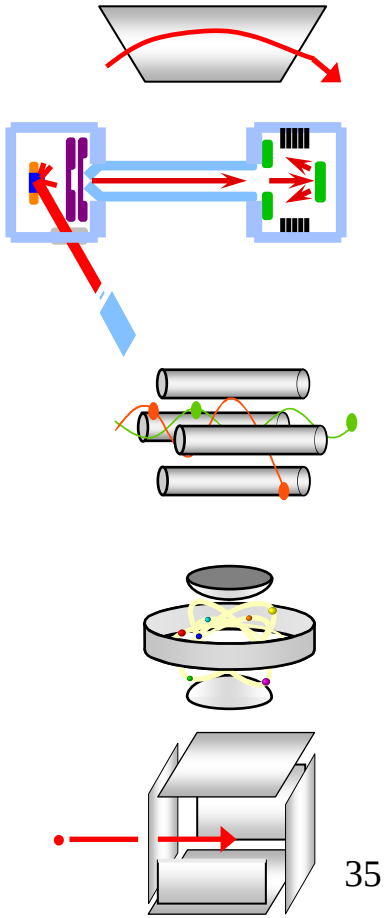
Mass Analysis

Ions are separated according to their mass-to-charge (*m/z*)

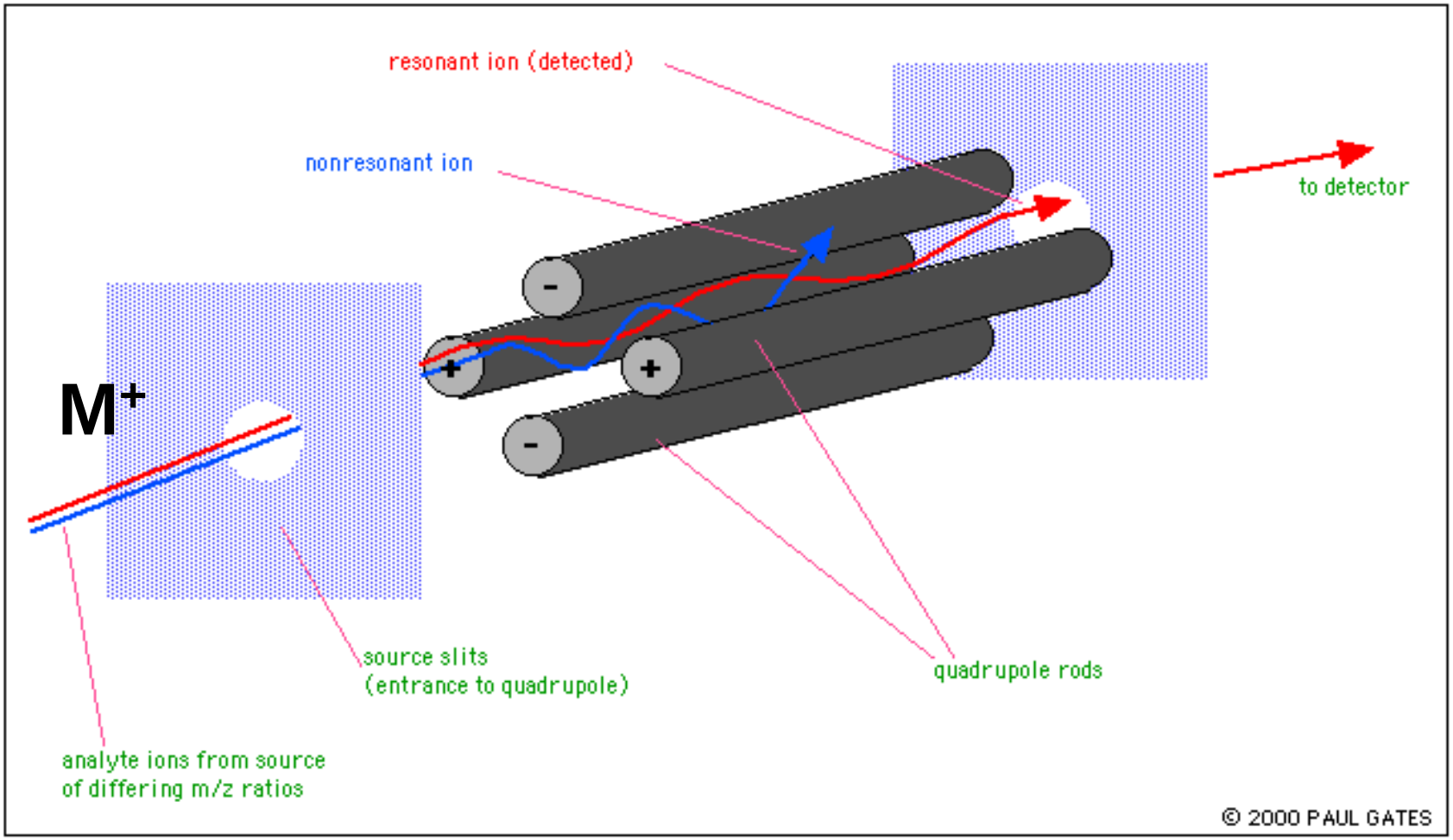




- Sector Instruments
- Time-of-flight Analyzer
- Quadrupole Mass Filter
- Ion-Trap Instrument
- FT-ICR



Quadrupole Mass Filter (m/z -4000)



$a_u = a_x = -a_y = 4zU / m\omega^2 r_o^2$

$q_u = q_x = -q_y = 2zV / m\omega^2 r_o^2$

$a/q = 2U/V$, others fixed

Length, diameter, kinetic energy -→m/e range, resolution

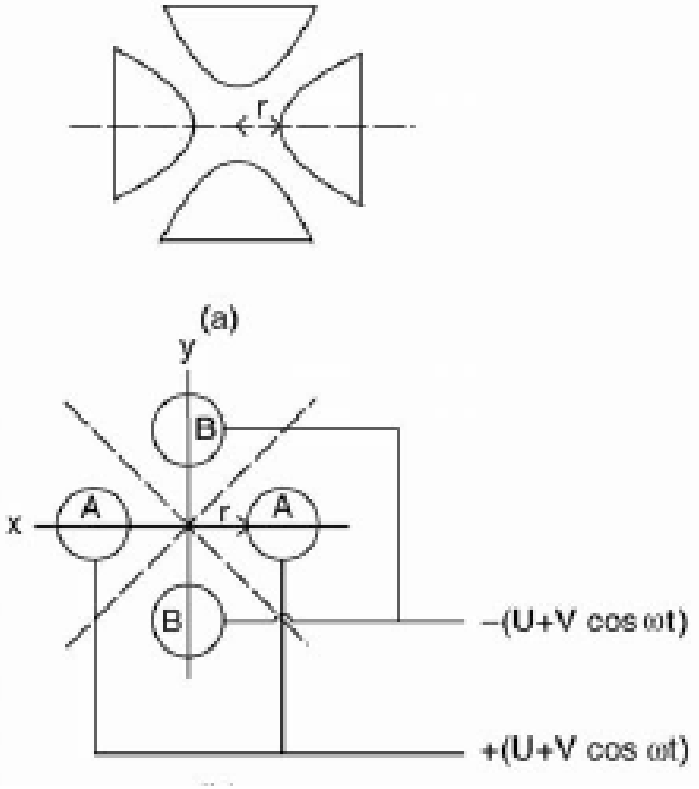
Principles of Quadrupole Mass Filter

1. A potential of ~100-1000 V (DC) is applied alternately to the opposing pairs of rods at a frequency of a few MHz (RF).
2. At a specific combination of DC & RF, an m/z has a stable trajectory through the rods, and all other m/z are lost.
3. The mass range is

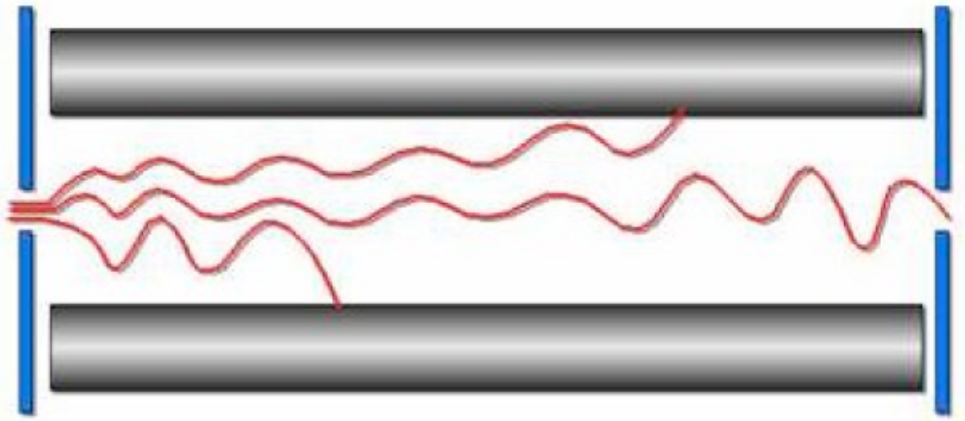
scanned as the voltages

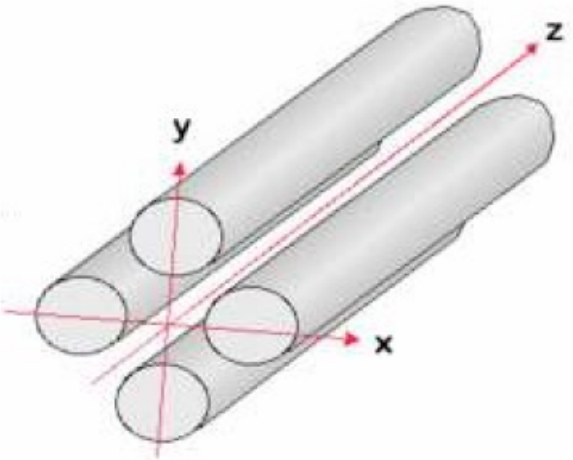
are swept from min m/z to max m/z,

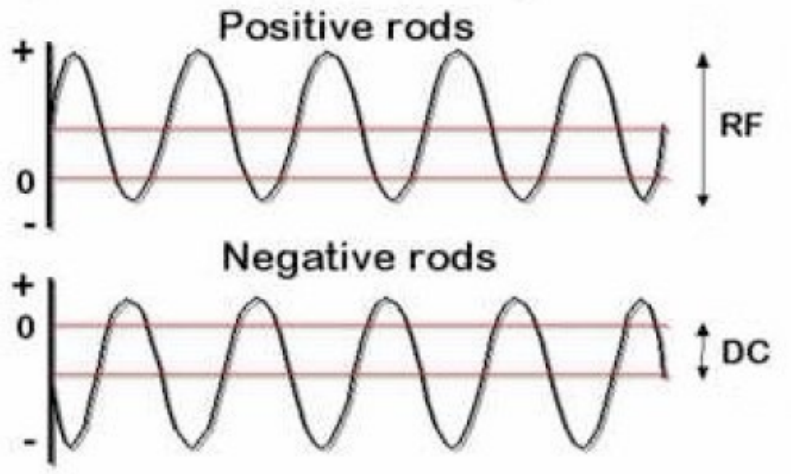
but at constant DC/RF ratio.



Elements of a quadrupole analyzer



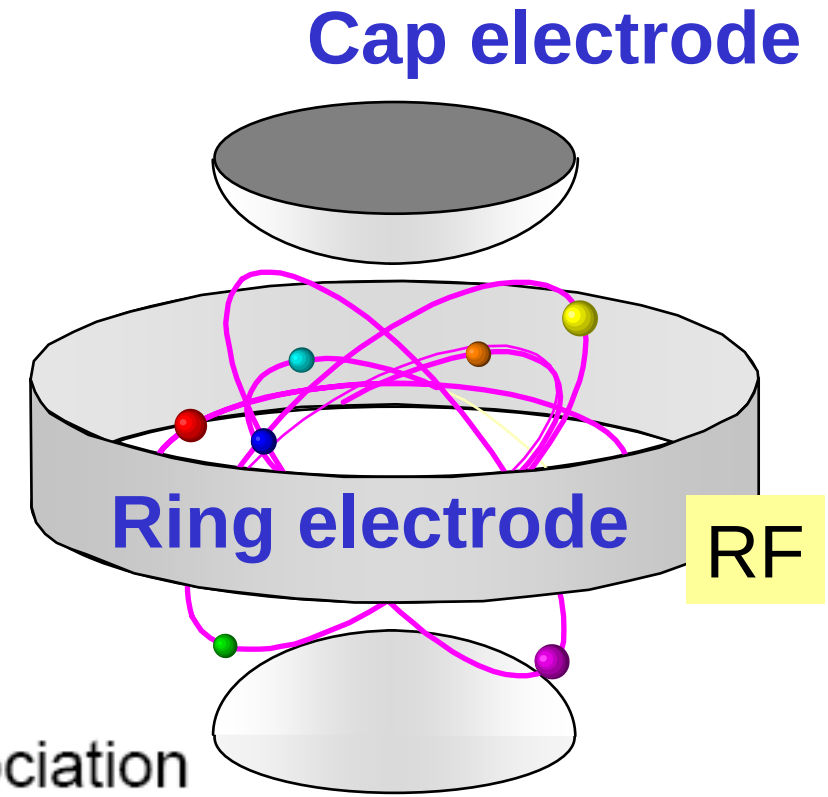




S Barnes-UAB 1/27/04

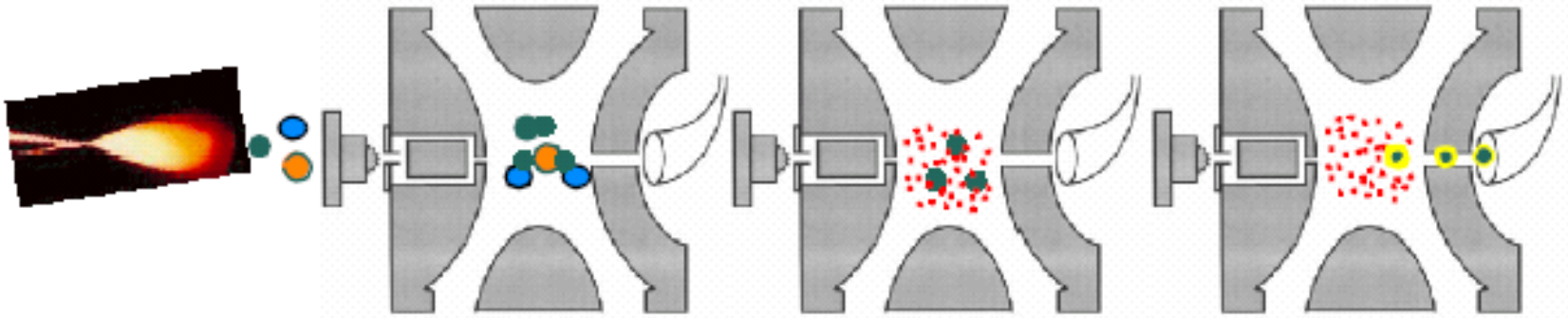
Ion-Trap Analyzer

- Principle very similar to quadrupole
- Ions stored by RF & DC fields
- Scanning field can eject ions of specific m/z



MS^n

Collisions with gas msec dissociation

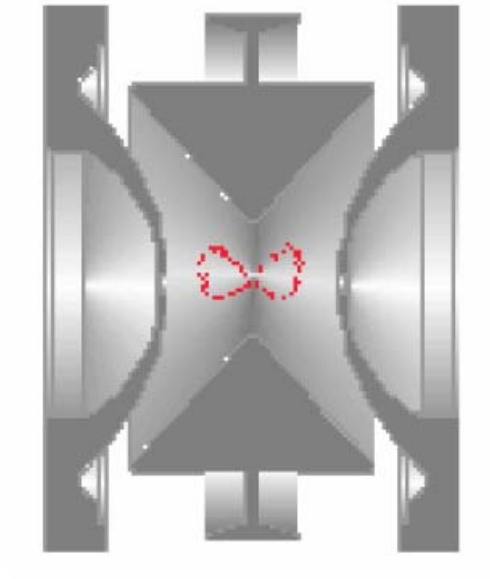


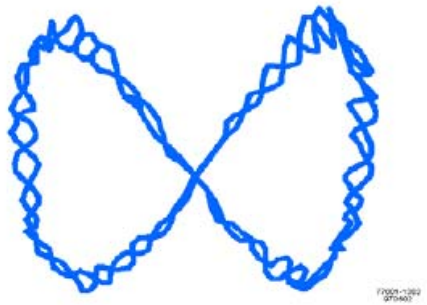
Principle of Ion Trap Analyzer

1. Ions are focused using an electrostatic lensing system into the ion trap.
2. An electrostatic ion gate pulses open (-V) and closed (+V) to inject ions into the ion trap.
3. Collisions with helium dampens the kinetic energy of the ions and serve to quickly contract trajectories toward the center of the ion trap, enabling trapping of injected ions.
4. Trapped ions are further focused toward the center of the trap through the use of an oscillating potential, called the fundamental **rf** , applied to the ring electrode.
5. An ion will be stably trapped depending upon the values for the mass and charge of the ion, the size of the ion trap (r), the oscillating frequency of the fundamental rf (w), and the amplitude of the voltage on the ring electrode (V).

See more details in
http://www.abrf.org/ABRFNews/1996/September1996/sep96iontrap.html

http://www.chem.wm.edu/dept/faculty/jcpout/faculty.html

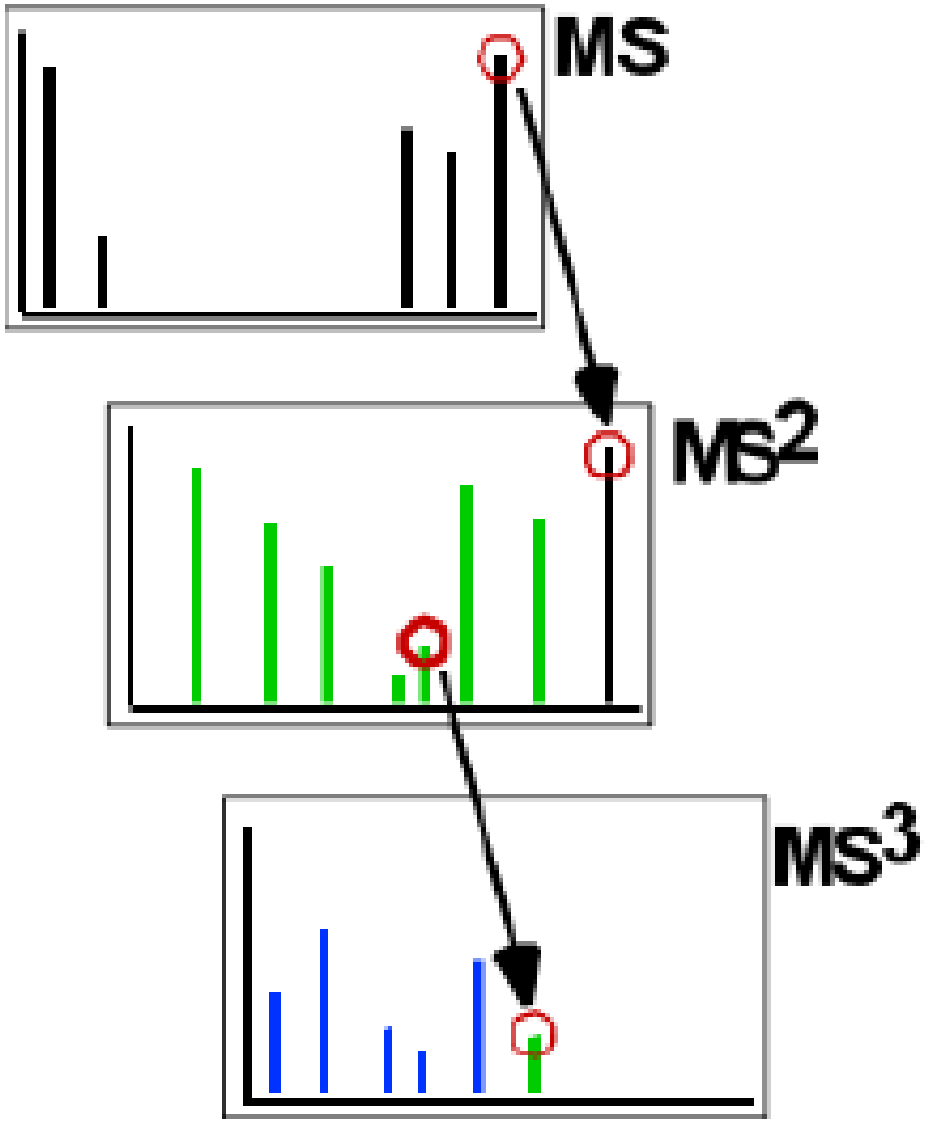




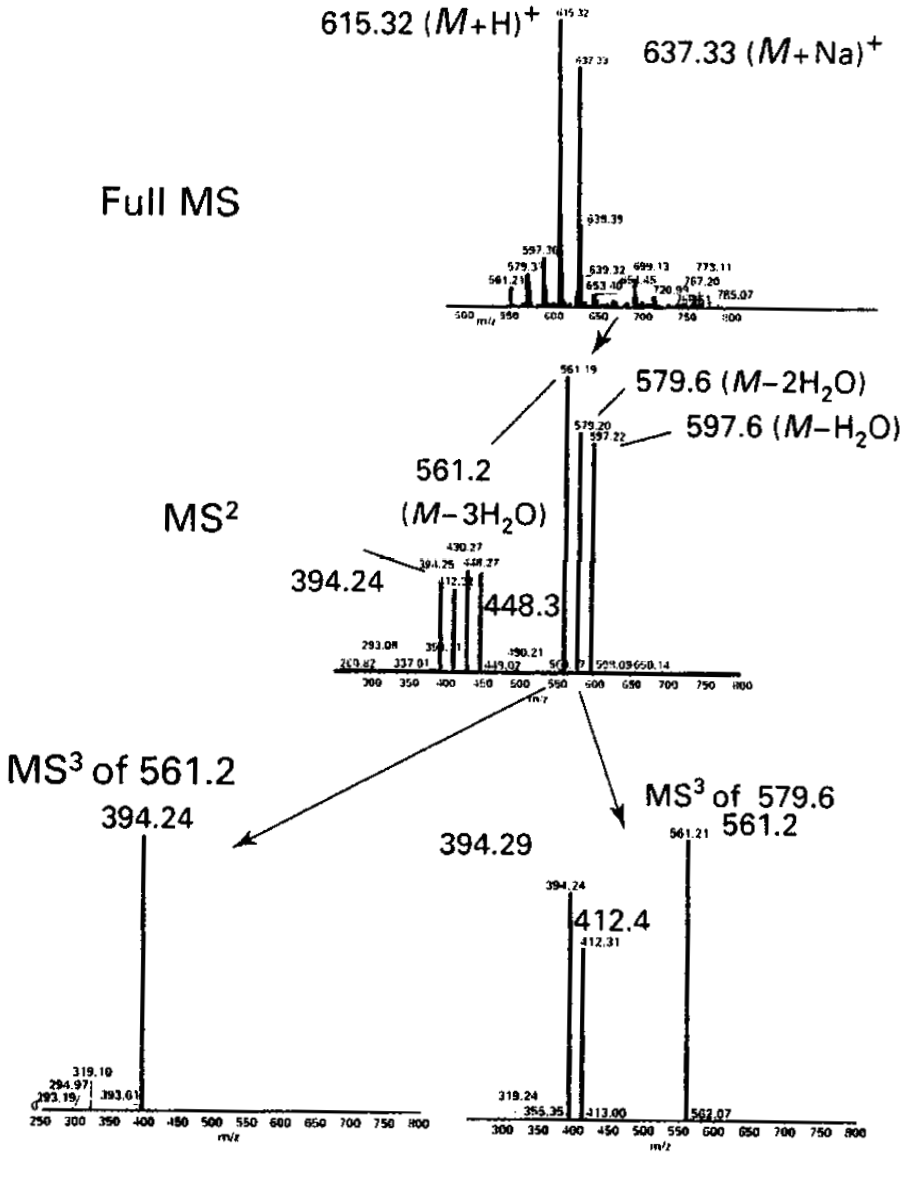
Ion path in a trap

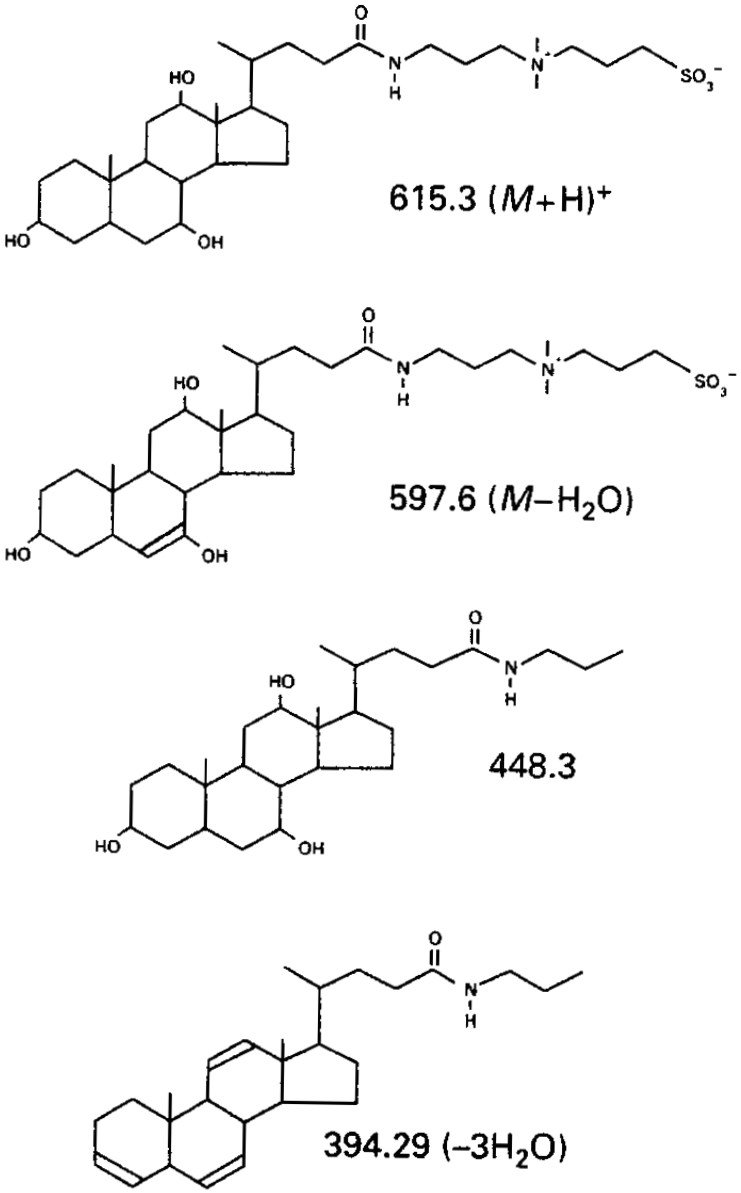
Multiple MS/MS (fragmentation) Capability

- ✓Facile MSn
- ✓ Very Sensitive
- ✓Fast Scanning
- ✓Small
- ✓Inexpensive



Multiple MS/MS (MS^n) for Structural Determination





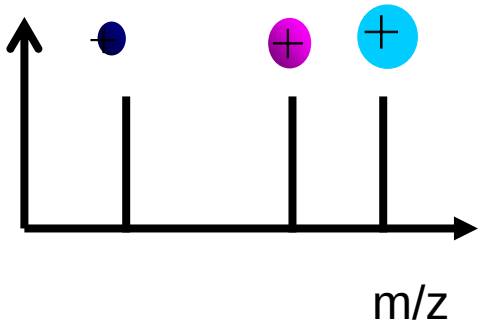
Time-of-flight Analyzer

- Ions are generated in the source zone of the instrument.
- A potential (V) is applied across the source to extract and accelerate the ions from the source into the field-free 'drift' zone of the instrument.
- Ions travel with velocity **v = d/t** ; d:tube distance, t:time
- All ions produced will leave the source at the same time with the same kinetic energy (**KE = ½ *m*v^2 = *z*V**), due to their having been accelerated through the same potential difference (ideally).
- The time-of-flight of the ions produced will only be dependent on the mass and the charge of the produced ion.

$$m/z = [2\ t^2\ V] / d^2$$

- The larger the ion, the slower its velocity and thus the longer it takes to traverse the field-free drift zone.

Mass spectrum



$$t = \left(\frac{m}{2zeEs} \right)^{\frac{1}{2}} D$$

t= 100 μ second for mass 50 (small moleculae)

1000 μ second for mass 5,000 (peptide, polymer…)

3000 μ second for mass 50,000 (protein, DNA….)

Calibration of Mass Spectrum

Flight time

$$t = \left(\frac{m}{2zeEs} \right)^{\frac{1}{2}} D$$

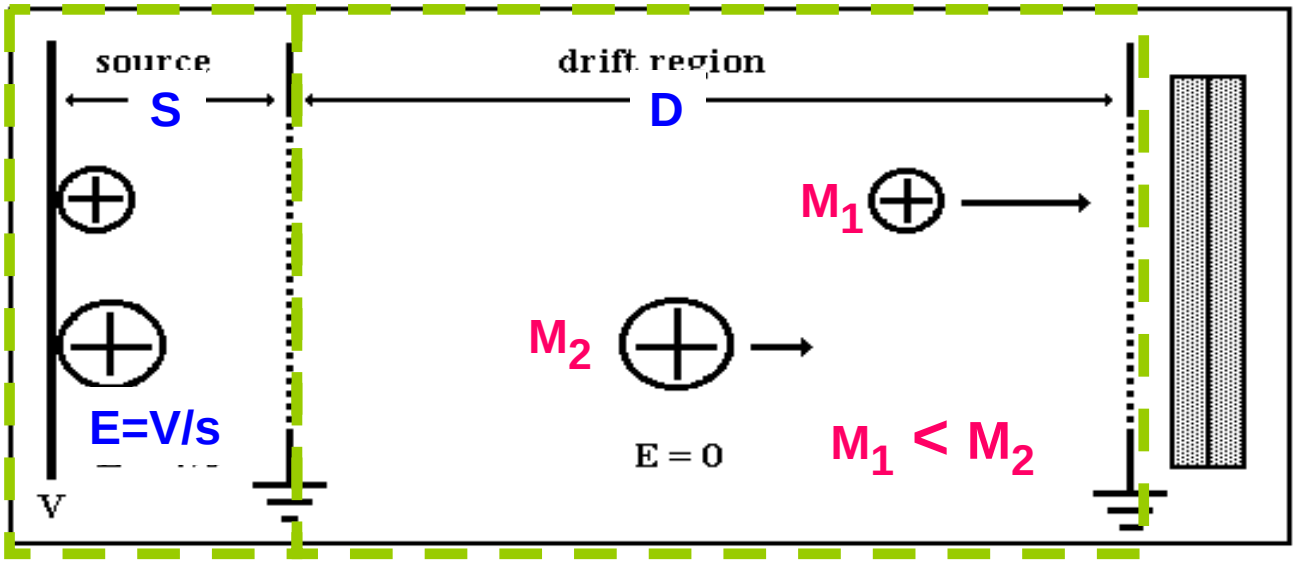
D: drift length

$$t = a(m)^{\frac{1}{2}} + b$$

The mass is independent of any instrumental parameters

- Internal calibration
- External calibration

Mass-to-Charge (m/z) is a Function of Flight Time



In a electric field [Potential Energy] ⇨ **In drift region [Kinetic Energy]**

$$E = \frac{V}{s}$$ *where V = accelerating voltage*

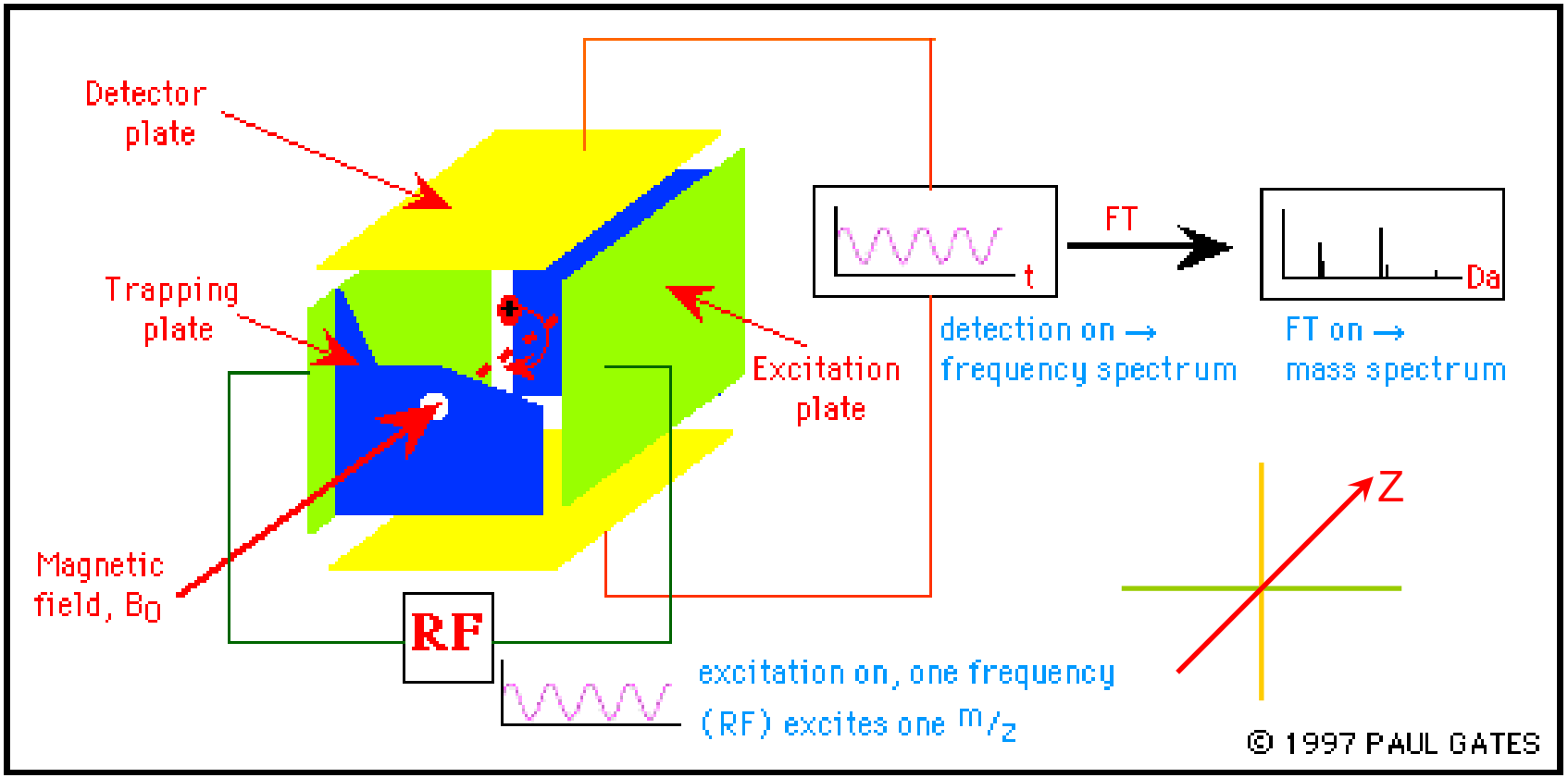
$$\frac{1}{2}mv^2 = qV = zeEs \longrightarrow v = \left(\frac{2zeEs}{m}\right)^{\frac{1}{2}}$$

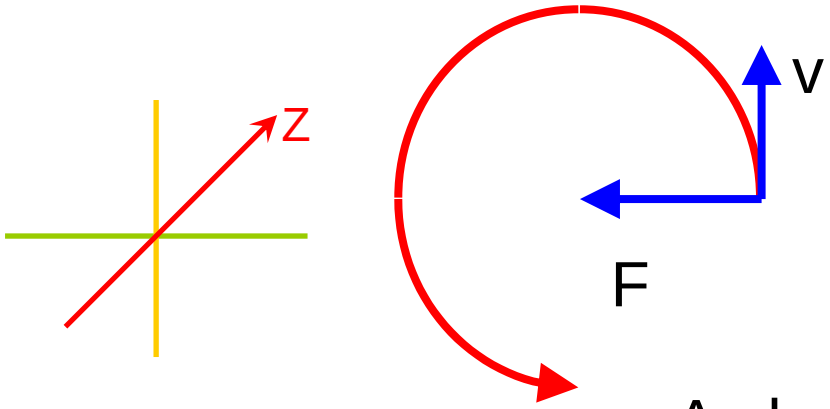
→ **Flight time** $$t = \left(\frac{m}{2zeEs}\right)^{\frac{1}{2}} D$$

Relation between m/z and t

$$\left(\frac{m}{z}\right) = \left(\frac{2eEs}{D^2}\right)t^2$$

Fourier Transform Ion Cyclotron Resonance Analyzer, FTICR





F=z v x B

F: Magnetic force on the ion

z: Charge of the ion

v: Velocity of the ion

A charged particle (ion) will rotate around the magnetic field line of a homogeneous magnetic field in a circula motion.

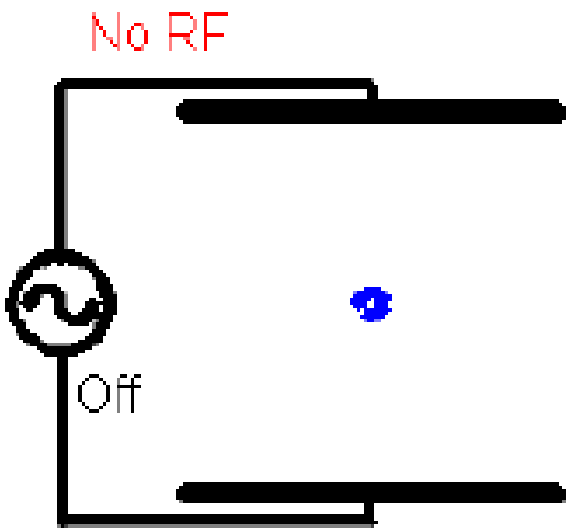
B: Magnetic Field

1. Ions in a magnetic field move in circular orbits characteristic of their m/z values.
2. If energy is provided at a frequency equal to their precession frequency, and in a direction perpendicular to their plane of precession, the ions will absorb the energy, enabling them to be detected.

$$\omega_c = \frac{z_i \mathbf{B}_0}{2\pi m_i}$$

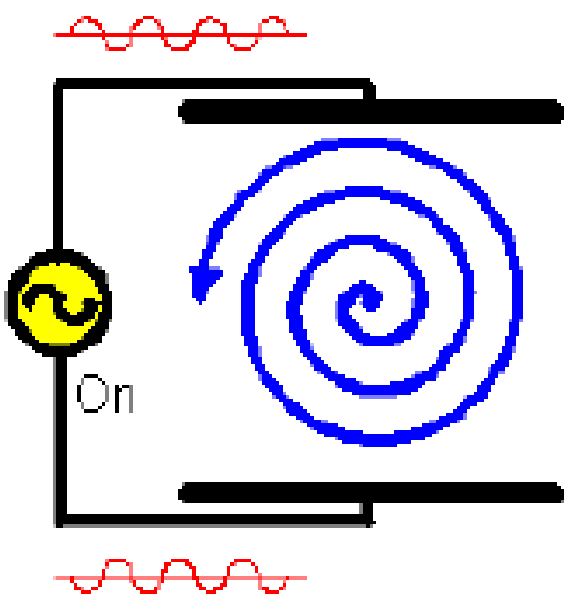
$$\frac{m_i}{z_i} = \frac{\mathbf{B}_0}{2\pi\omega_c}$$

Step 1



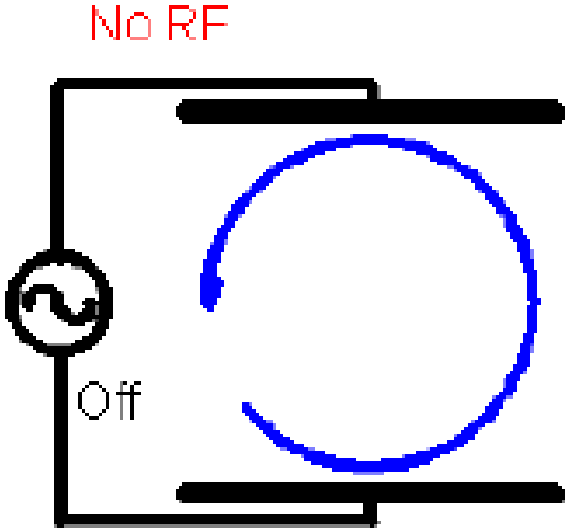
Thermal Ions cyclotron at a frequency dependent on their m/z at a smaller orbit radius

Step 2



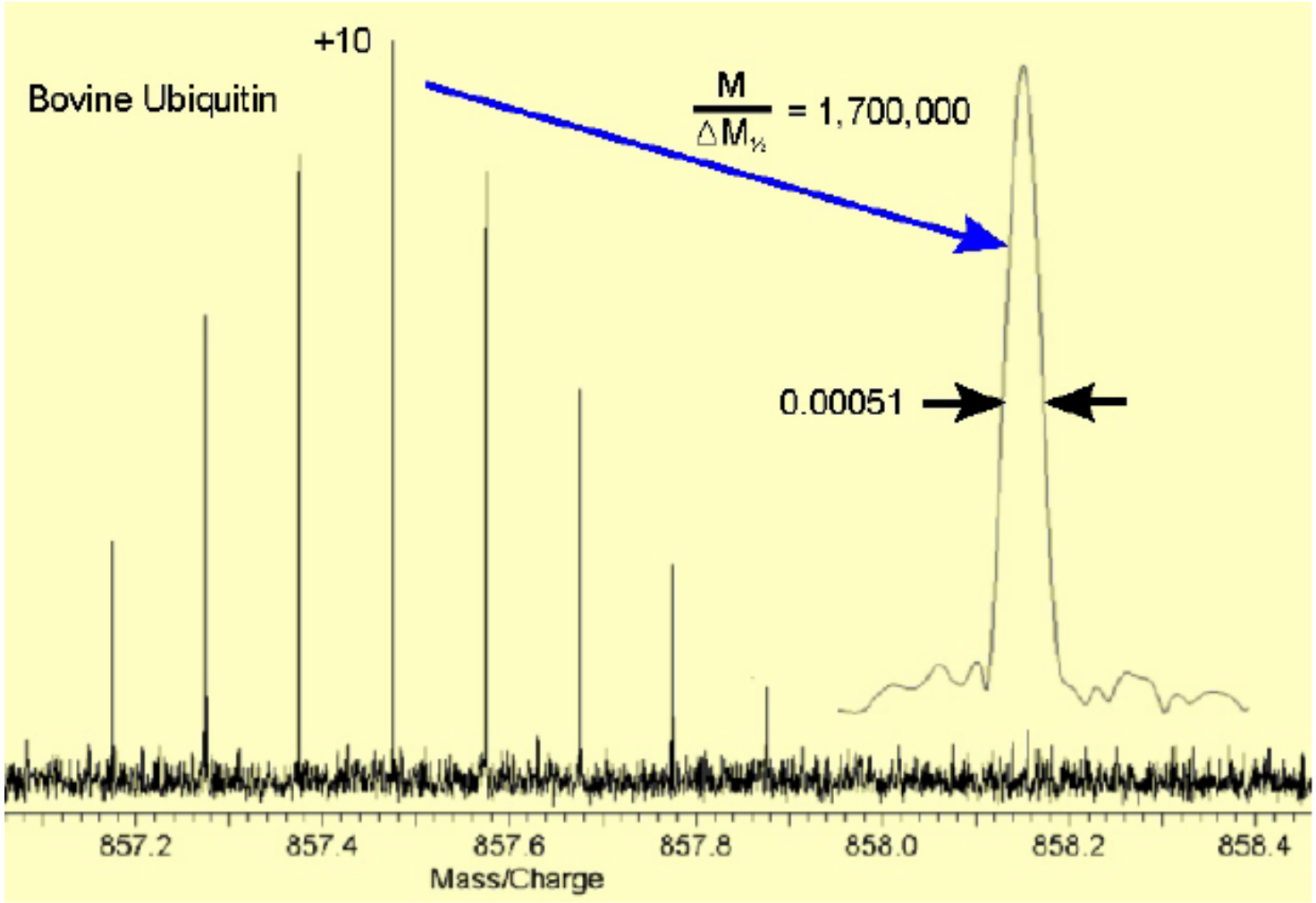
Applying a RF signal at the cyclotron frequency resonantly accelerate the ions to larger orbit radius

Step 3



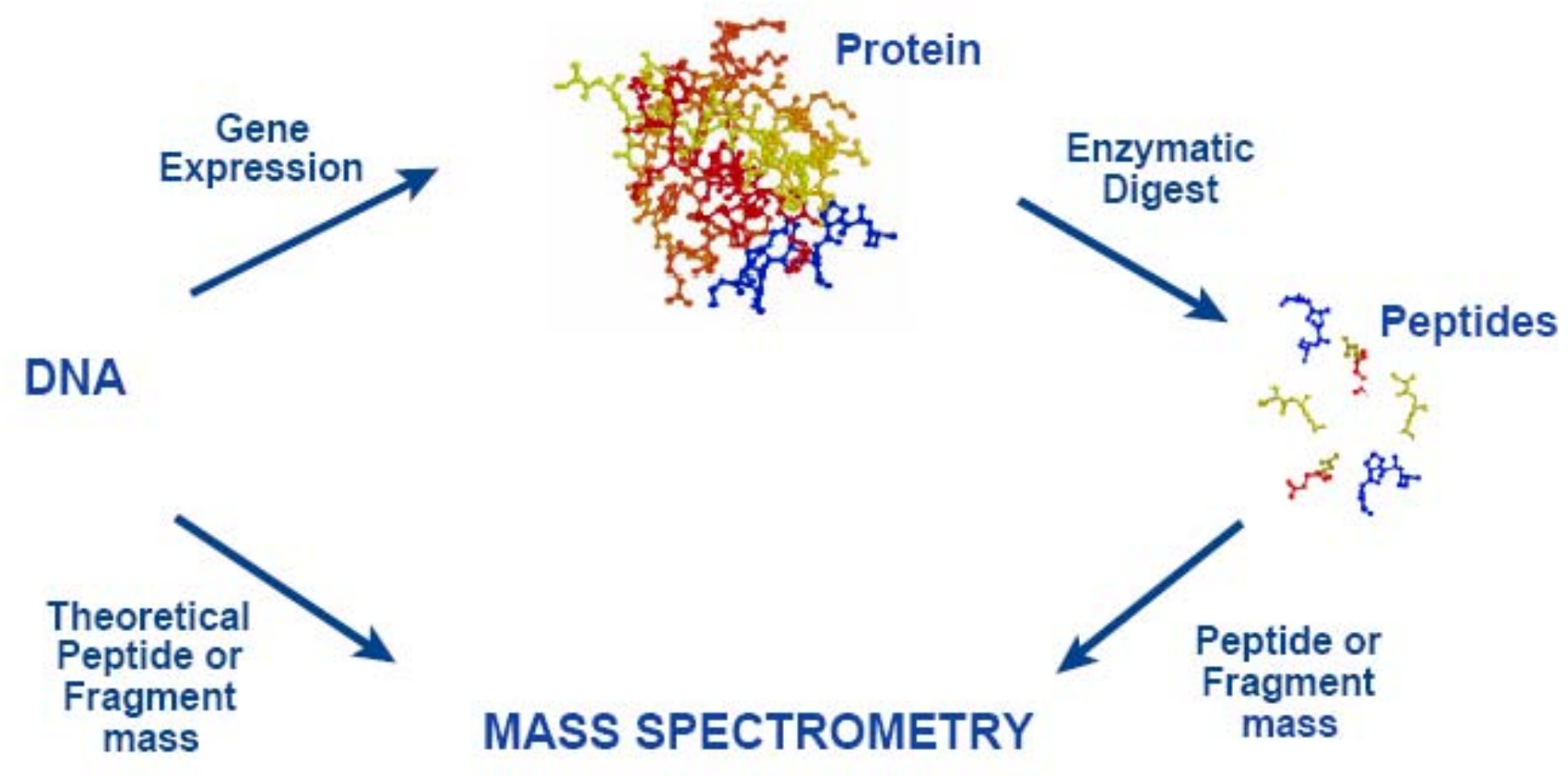
Without collisions, the accelerated ions continue to cyclotron at the larger orbit radius at the same frequency

- ✓Extremely High Resolution
- ✓MS^n capability
- ✓Must Operate at very good vacuum
- ✓Superconducting Magnet
- ✓Difficult to operate
- ✓Becoming increasingly reliable

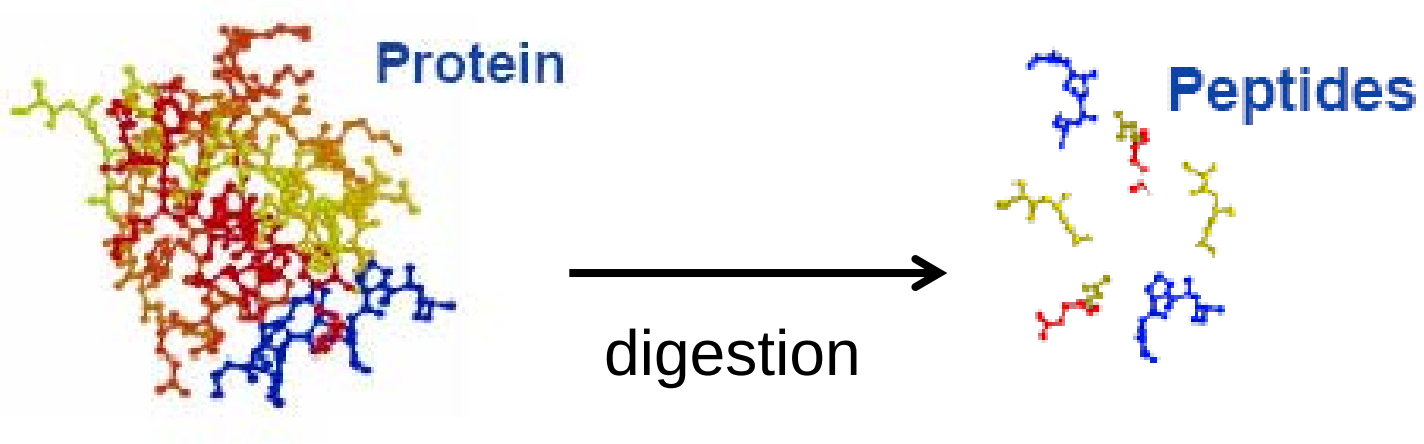


Principle of Protein Identification and Quantification

Peptide and Protein Analysis



Mass Spectrometry Methods



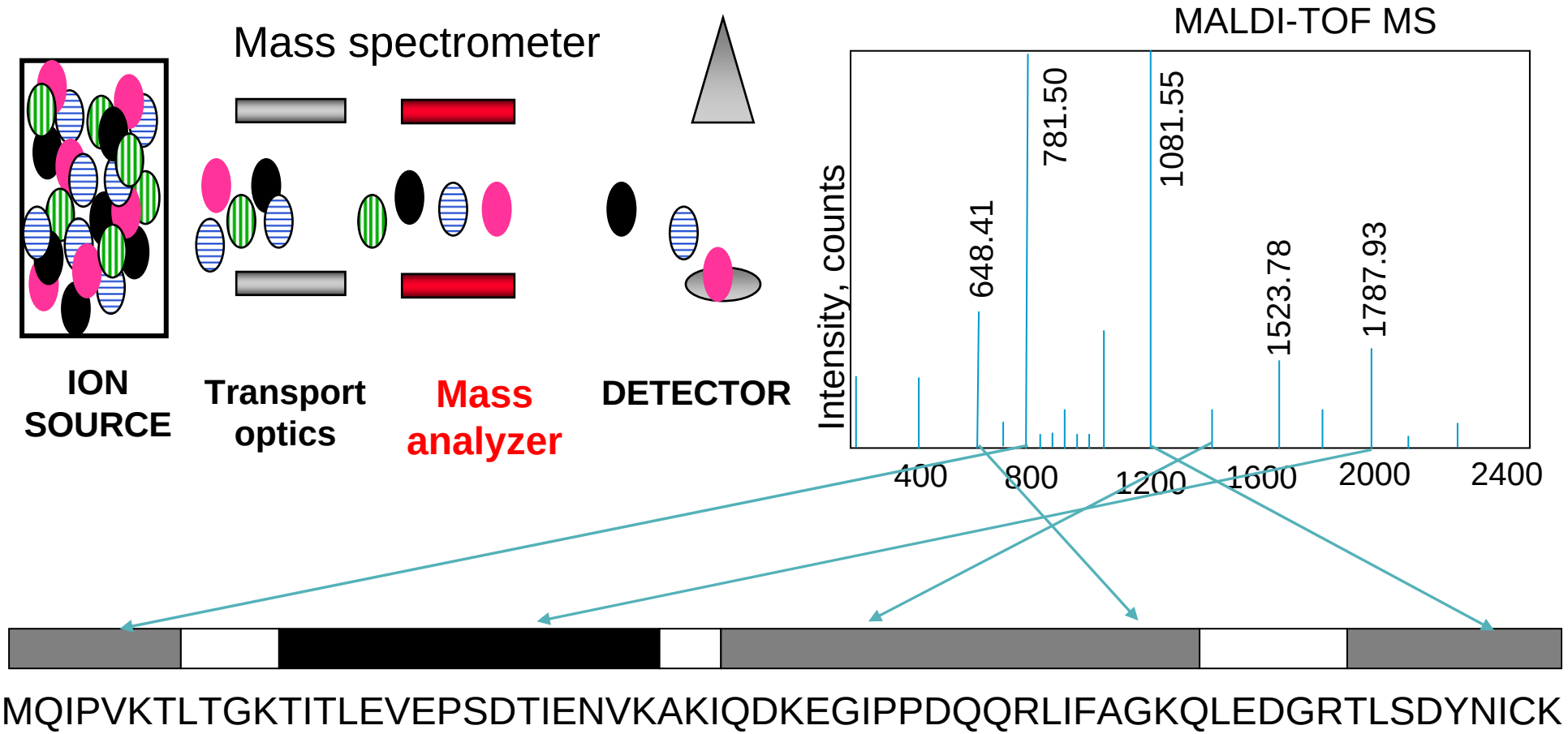
Molecular weight

Identification
Sequence information

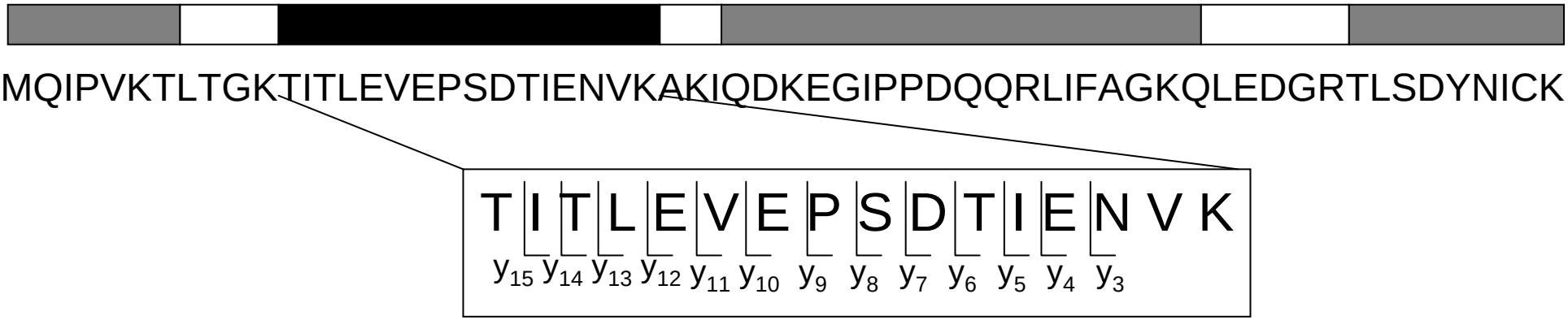
- ✓ MALDI TOF MS
- ✓ ESI MS

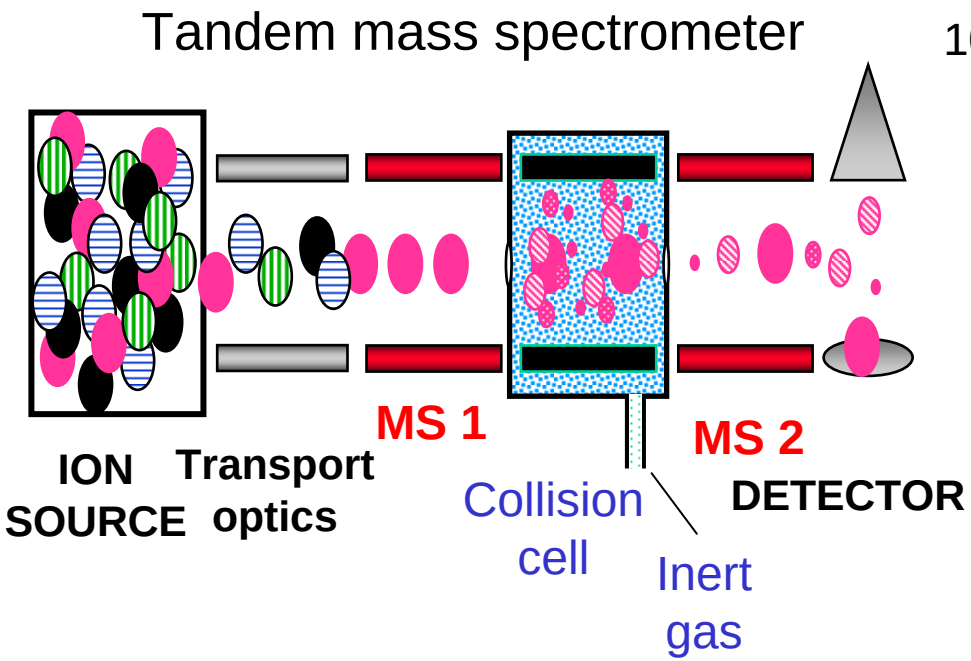
- ✓Peptide mass fingerprinting (MALDI TOF MS)
- ✓Peptide Sequencing (Tandem MS)

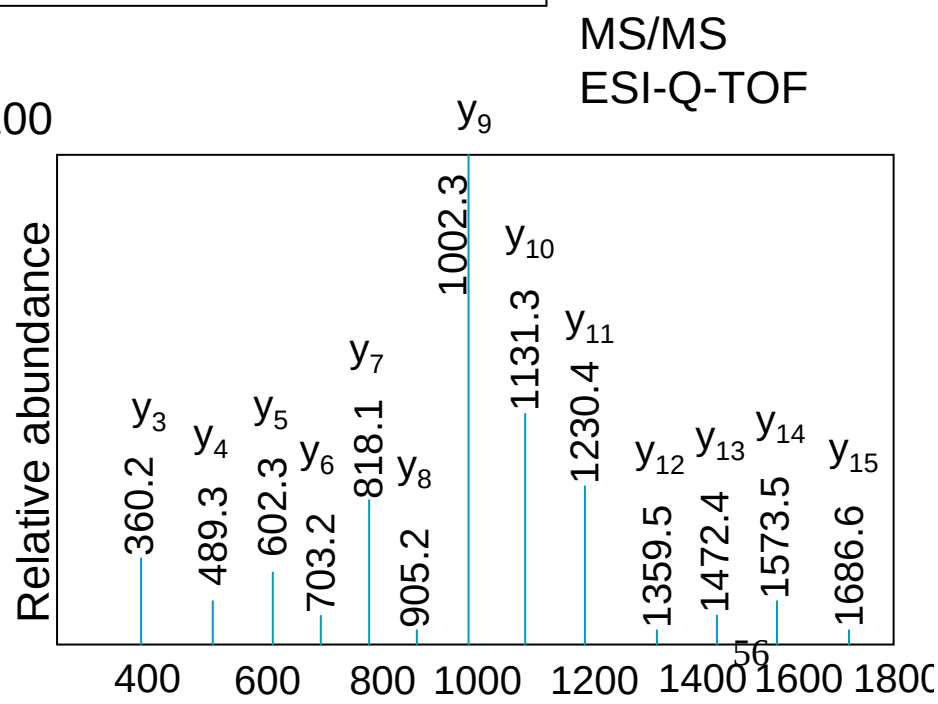
Two Ways of Measurement—
1. Peptide Mass Fingerprint (by M.W. measurement)



Two Ways of Measurement—
2. Peptide Sequencing (by MS/MS, or Tandem MS)







Peptide Mass Fingerprint

Left to right:

William J. Henzel; ***Protein Chemistry;*** Genentech

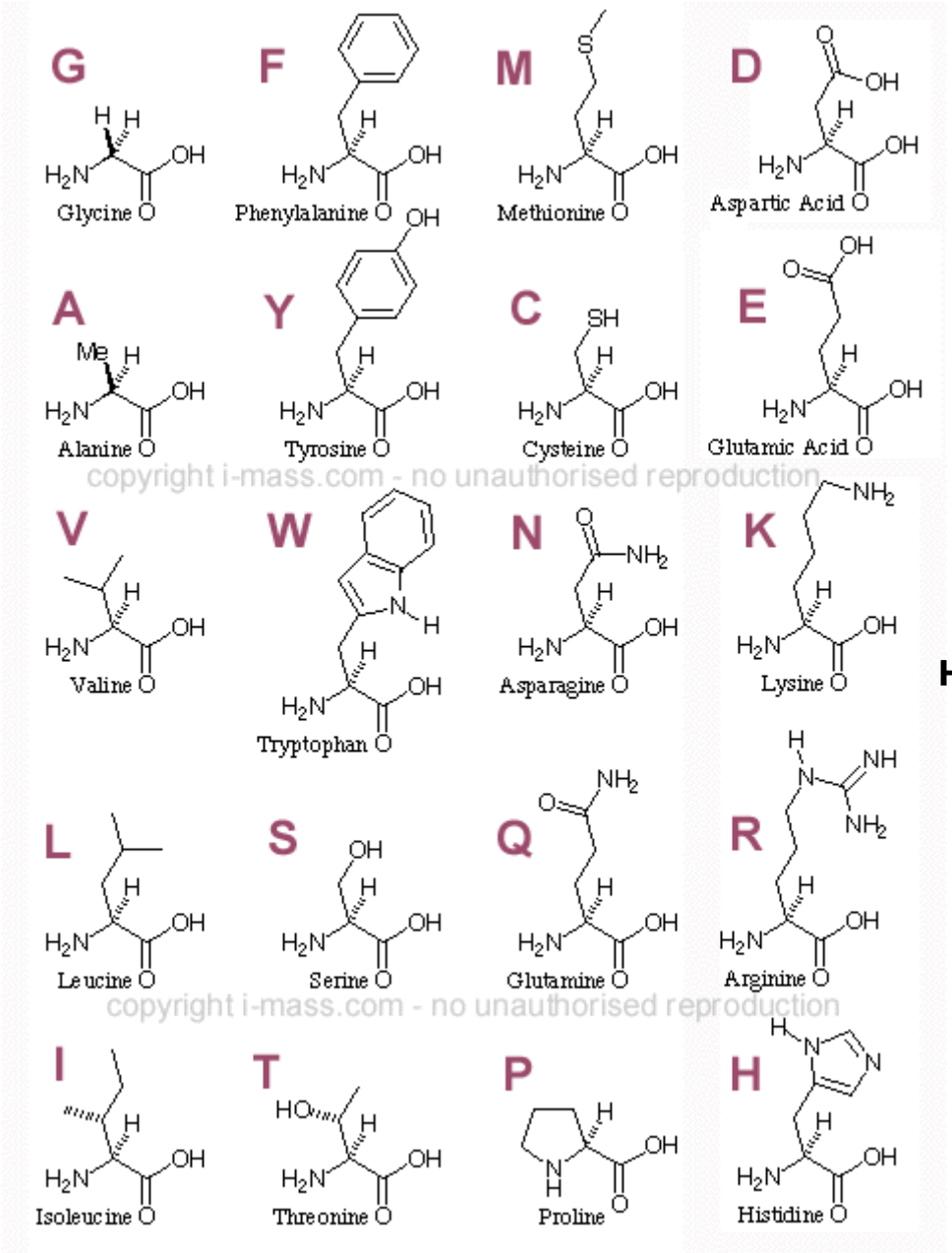
John T. Stults; ***Analytical Chemistry***; Genentech

Colin Watanabe; ***Software Engineer;*** Genentech

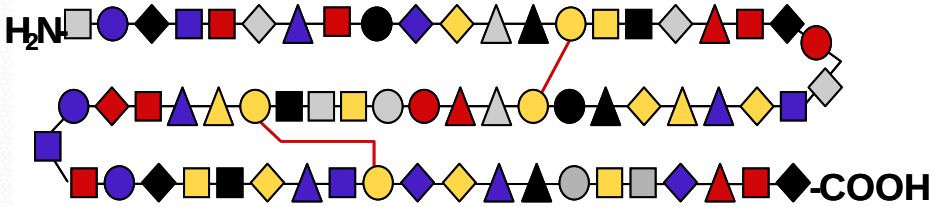
- This methodology was the first to allow protein identification without the need for time-consuming Edman sequencing or immunoaffinity probes..

- Landmark Study(1993)

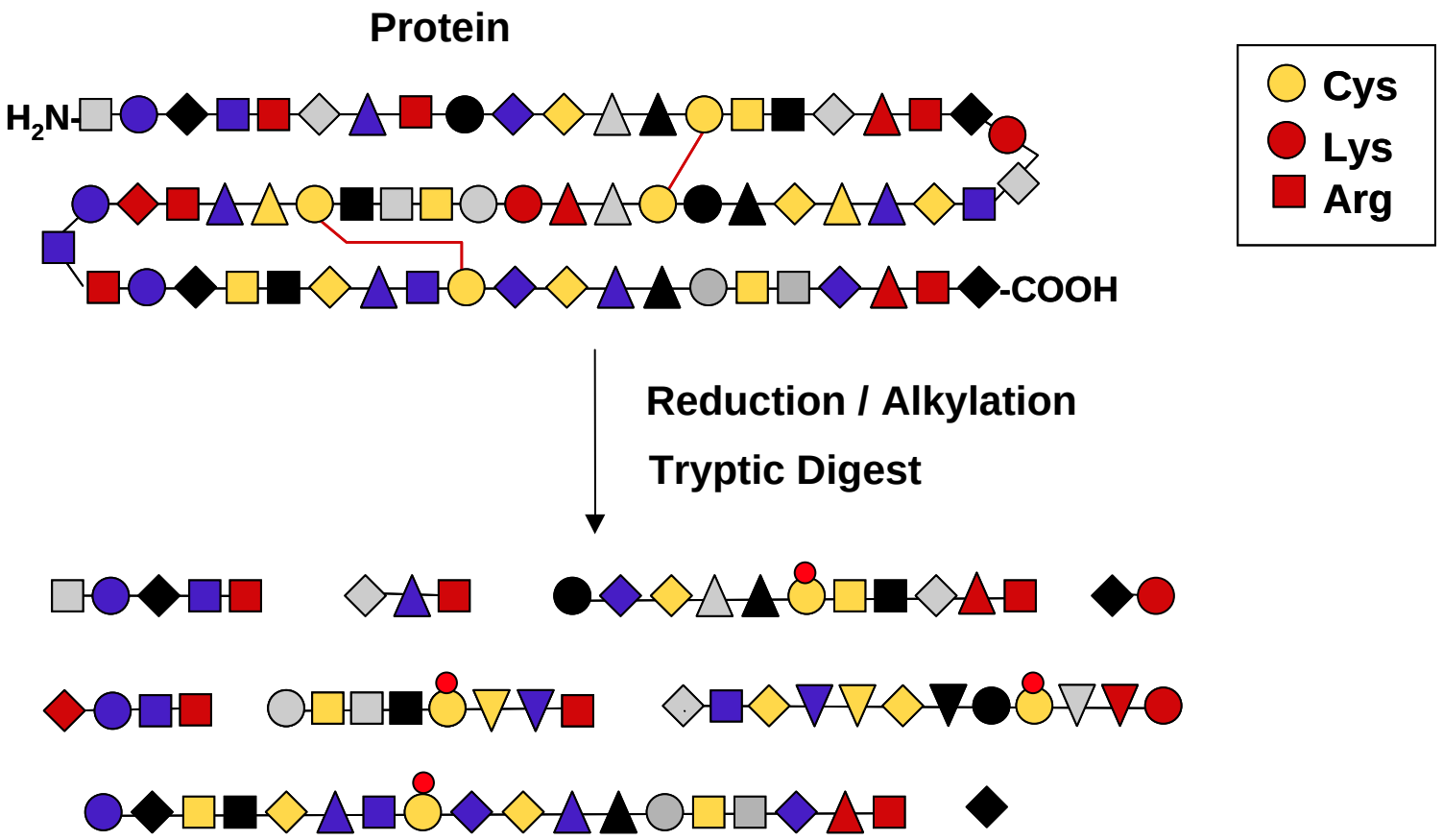
MS approaches alone could be used to analyze proteins from 2DE



Peptide Mass Fingerprinting



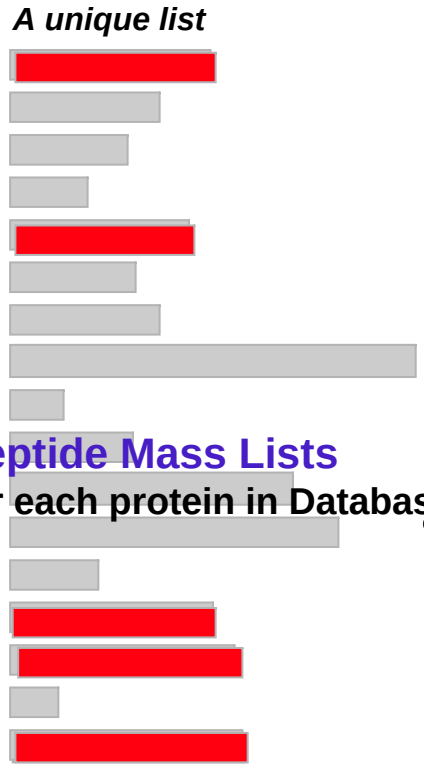
Step 1: Enzyme digestion or chemical fragmentation



Every protein generate a set of unique peptides

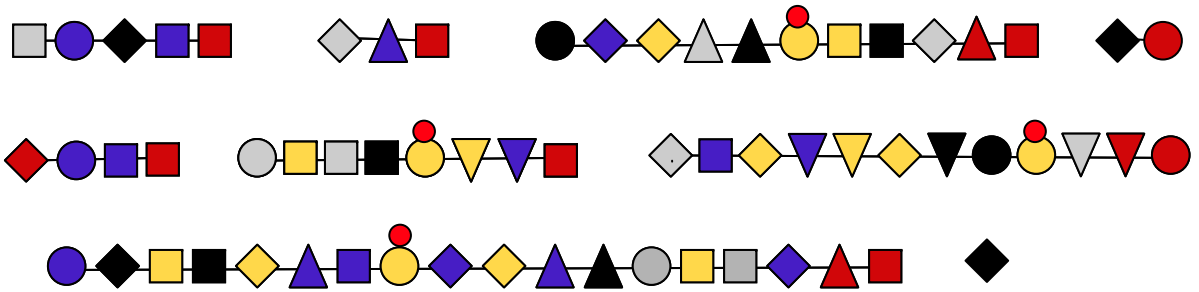
每一個蛋白質有一套獨特的peptide碎片

Step 2: Mass spectrometry analysis



Peptide Mass Lists

for each protein in Database

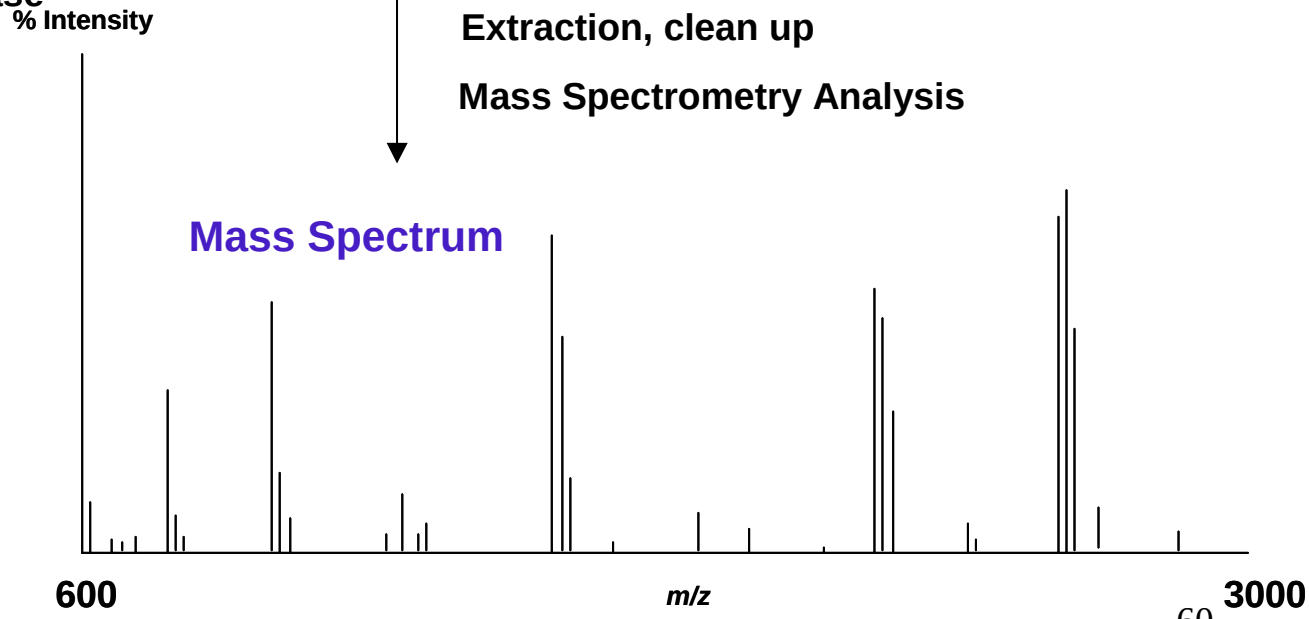


Peptide Mixtures

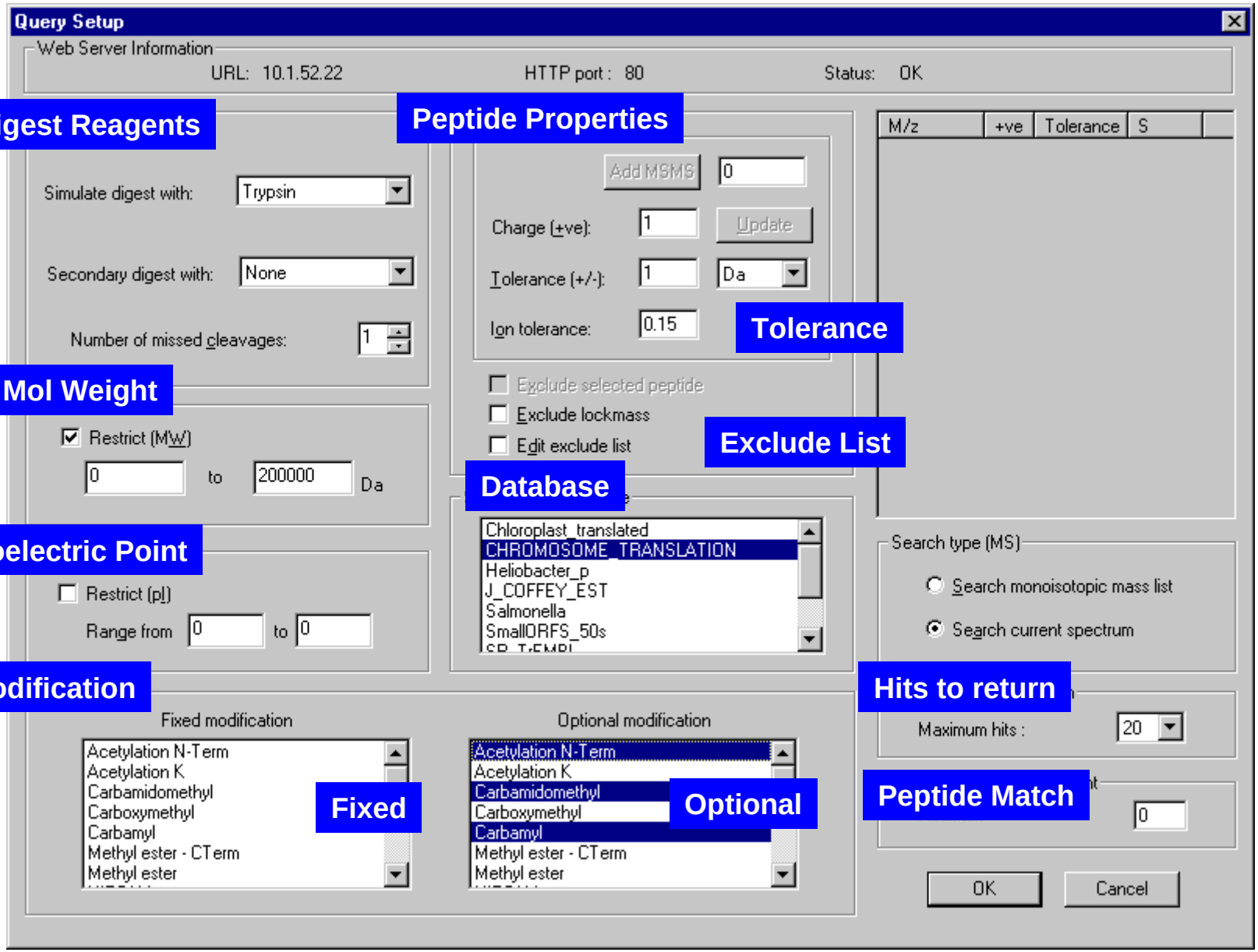
Extraction, clean up

Mass Spectrometry Analysis

Mass Spectrum



Step 3: Database match



Step 3: In-Silica digestion

Protein Sequence

MVYIIAEIGC NHNGDINLAK KMVDVAVSCG
VDAVKFQTFK AEKLISKFAP KAEYQKATTG
TADSQLEMTK RLELSFEEYL EMRDYAISKG
VETFSTPFDE ESLEFLISTD MPIYKIPSGE
ITNLPYLEKI GKQQKKVILS TGMAVMEEIH
QAVNILRQNG TTDISILHCT TEYPTPYPSL
NLNVIHTLKD EFKDLTIGYS DHSIGSEVPI
AAAAMGAEVI EKHFTLDTNM
EVPDHKASAT
PDILAALVKG FALLNQALGR FEKIPDPVEE
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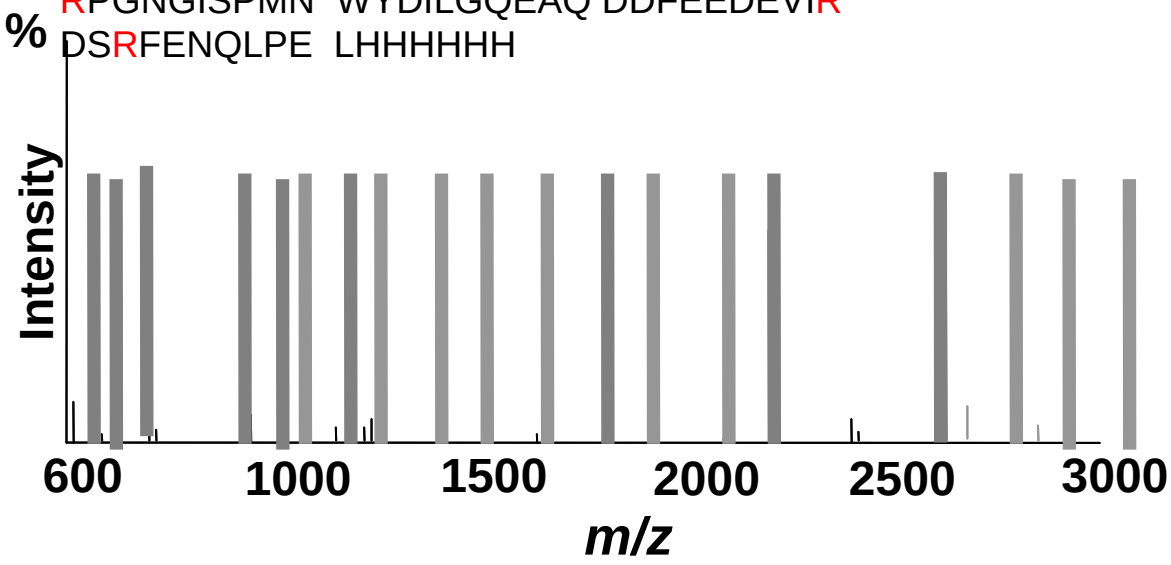
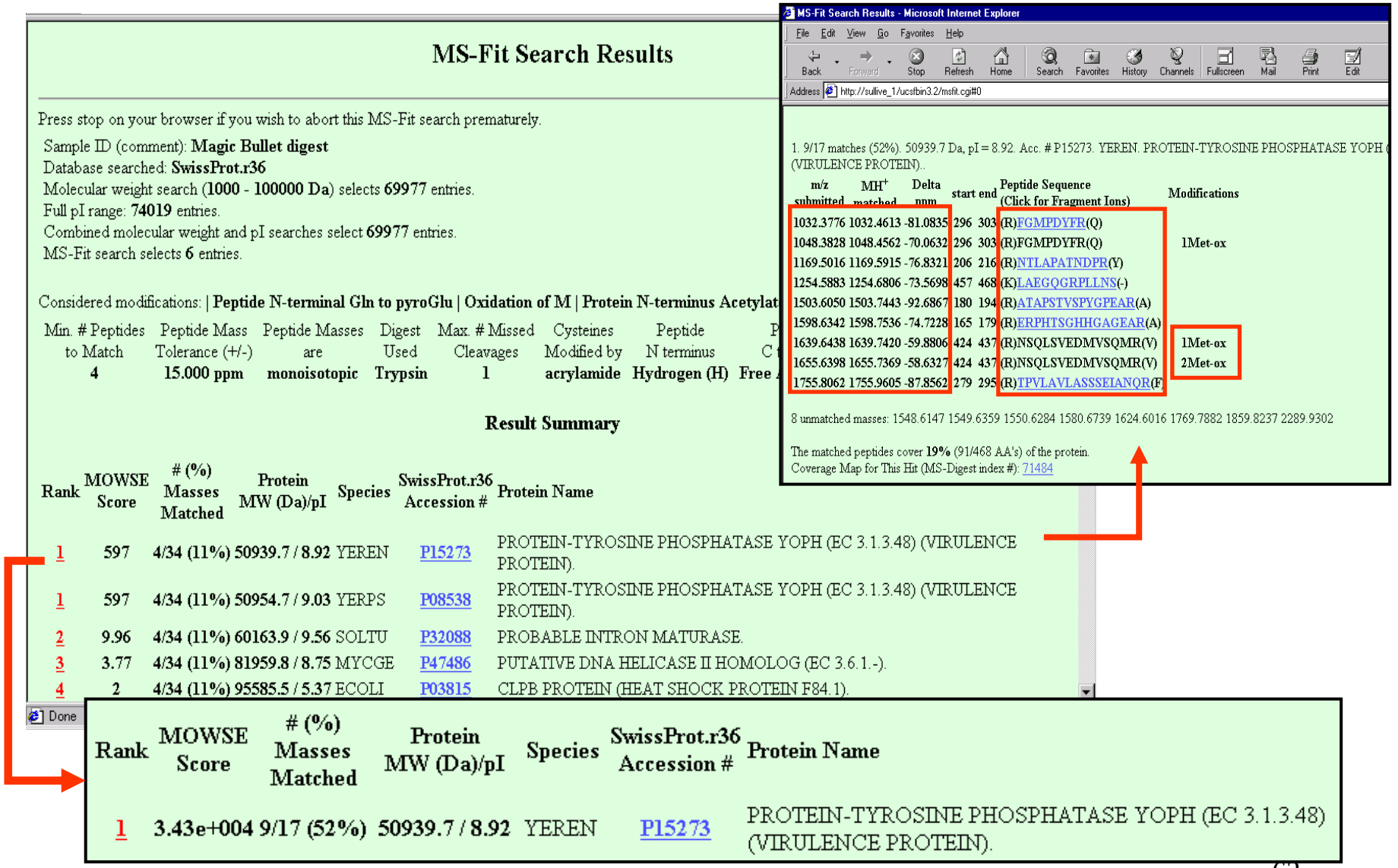
RPGNGISPMN WYDILGQEAQ DDFEEDEVIR
DSRFENQLPE LHHHHHH

Digestion



Mass	Peptide Sequence
3583.8	QNGTTDISILHCTTEYPTPY PSLNLNVIHTLK
3493.6	RPGNGISPMNWYDILGQEAQ DDFEEDEVIR
2995.4	GVETFSTPFDEESLEFLIST DMPIYK
2944.5	DLTIGYSDHSIGSEVPIAAA AMGAEVIEK
2324.2	VILSTGMAVMEEIHQAVNIL R
2188.1	MVYIIAEIGCNHNGDINLAK
1811.8	FENQLPELHHHHHH
1683.8	HFTLDTNMEVPDHK
1573.8	IPSGEITNLPYLEK
1558.7	LELSFEEYLEMR
1453.7	ATTGTADSQLEMTK
1392.7	MVDVAVSCGVDAVK
1351.7	GDIYSIENITVK
1269.7	ASATPDILAALVK
1159.7	GFALLNQALGR
954.63	SVVALKPIK
926.48	IPDPVEEK
696.36	DYAISK
670.36	FQTFK
638.31	AEYQK
538.25	DEFK

Search Result



MS-Fit Search Results

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

Sample ID (comment): **Magic Bullet digest**
Database searched: **SwissProt.r36**
Molecular weight search (**1000 - 100000 Da**) selects **69977** entries.
Full pI range: **74019** entries.
Combined molecular weight and pI searches select **69977** entries.
MS-Fit search selects **6** entries.

Considered modifications: | **Peptide N-terminal Gln to pyroGlu** | **Oxidation of M** | **Protein N-terminus Acetylat**

Min. # Peptides to Match	Peptide Mass Tolerance (+/-)	Peptide Masses are	Digest Used	Max. # Missed Cleavages	Cysteines Modified by	Peptide N terminus	P C
4	**15.000 ppm**	**monoisotopic**	**Trypsin**	**1**	**acrylamide**	**Hydrogen (H)**	**Free**

Result Summary

Rank	MOWSE Score	# (%) Masses Matched	Protein MW (Da)/pI	Species	SwissProt.r36 Accession #	Protein Name
1	597	4/34 (11%)	50939.7 / 8.92	YEREN	P15273	PROTEIN-TYROSINE PHOSPHATASE YOPH (EC 3.1.3.48) (VIRULENCE PROTEIN).
1	597	4/34 (11%)	50954.7 / 9.03	YERPS	P08538	PROTEIN-TYROSINE PHOSPHATASE YOPH (EC 3.1.3.48) (VIRULENCE PROTEIN).
2	9.96	4/34 (11%)	60163.9 / 9.56	SOLTU	P32088	PROBABLE INTRON MATURASE.
3	3.77	4/34 (11%)	81959.8 / 8.75	MYCGE	P47486	PUTATIVE DNA HELICASE II HOMOLOG (EC 3.6.1.-).
4	2	4/34 (11%)	95585.5 / 5.37	ECOLI	P03815	CLPB PROTEIN (HEAT SHOCK PROTEIN F84.1).

Done

MS-Fit Search Results - Microsoft Internet Explorer

File Edit View Go Favorites Help

Back Forward Stop Refresh Home Search Favorites History Channels Fullscreen Mail Print Edit

Address http://sullive_1/ucsfbin3.2/msfit.cgi#0

1. 9/17 matches (52%). 50939.7 Da, pI = 8.92. Acc. # P15273. YEREN. PROTEIN-TYROSINE PHOSPHATASE YOPH (VIRULENCE PROTEIN)..

m/z submitted	MH+ matched	Delta ppm	start	end	Peptide Sequence (Click for Fragment Ions)	Modifications
1032.3776	1032.4613	-81.0835	296	303	(R)FGMPDYFR(Q)	
1048.3828	1048.4562	-70.0632	296	303	(R)FGMPDYFR(Q)	1Met-ox
1169.5016	1169.5915	-76.8321	206	216	(R)NTLAPATNDPR(Y)	
1254.5883	1254.6806	-73.5698	457	468	(K)LAEGQGRPLLNS(-)	
1503.6050	1503.7443	-92.6867	180	194	(R)ATAPSTVSPYGPEAR(A)	
1598.6342	1598.7536	-74.7228	165	179	(R)ERPHTSGHHGAGEAR(A)	
1639.6438	1639.7420	-59.8806	424	437	(R)NSQLSVEDMVSQMR(V)	1Met-ox
1655.6398	1655.7369	-58.6327	424	437	(R)NSQLSVEDMVSQMR(V)	2Met-ox
1755.8062	1755.9605	-87.8562	279	295	(R)TPVLAVLASSSEIANQR(F)	

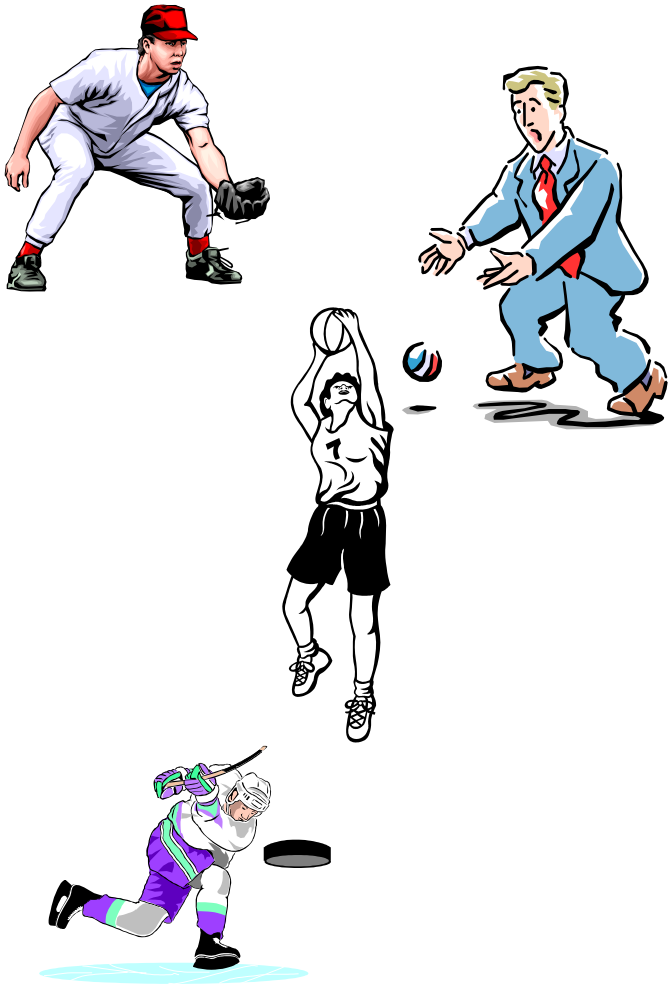
8 unmatched masses: 1548.6147 1549.6359 1550.6284 1580.6739 1624.6016 1769.7882 1859.8237 2289.9302

The matched peptides cover **19%** (91/468 AA's) of the protein.
Coverage Map for This Hit (MS-Digest index #): 71484

Rank	MOWSE Score	# (%) Masses Matched	Protein MW (Da)/pI	Species	SwissProt.r36 Accession #	Protein Name
1	3.43e+004	9/17 (52%)	50939.7 / 8.92	YEREN	P15273	PROTEIN-TYROSINE PHOSPHATASE YOPH (EC 3.1.3.48) (VIRULENCE PROTEIN).

Peptide Mass Fingerprinting (PMF)

蛋白質身份鑑定



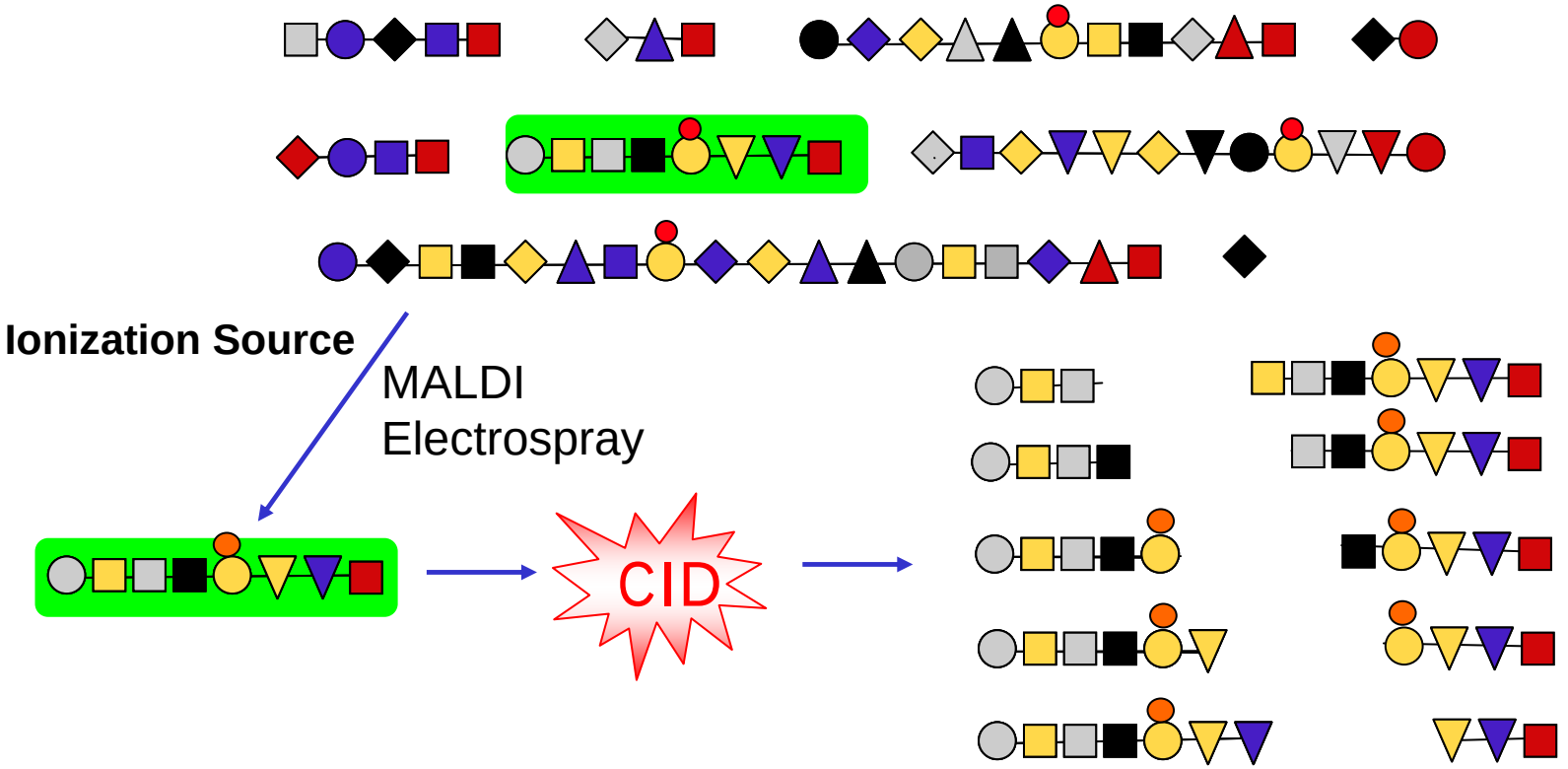
In-Gel Digestion

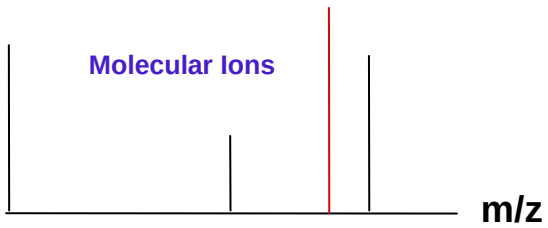
Extract peptides; mass analyze

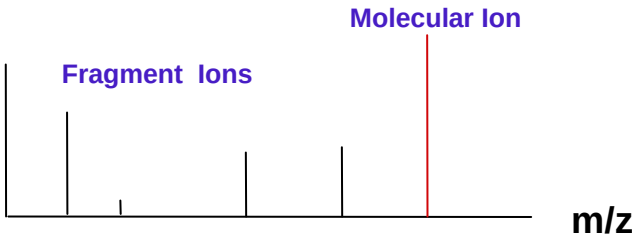
Each protein has a unique peptide mass list

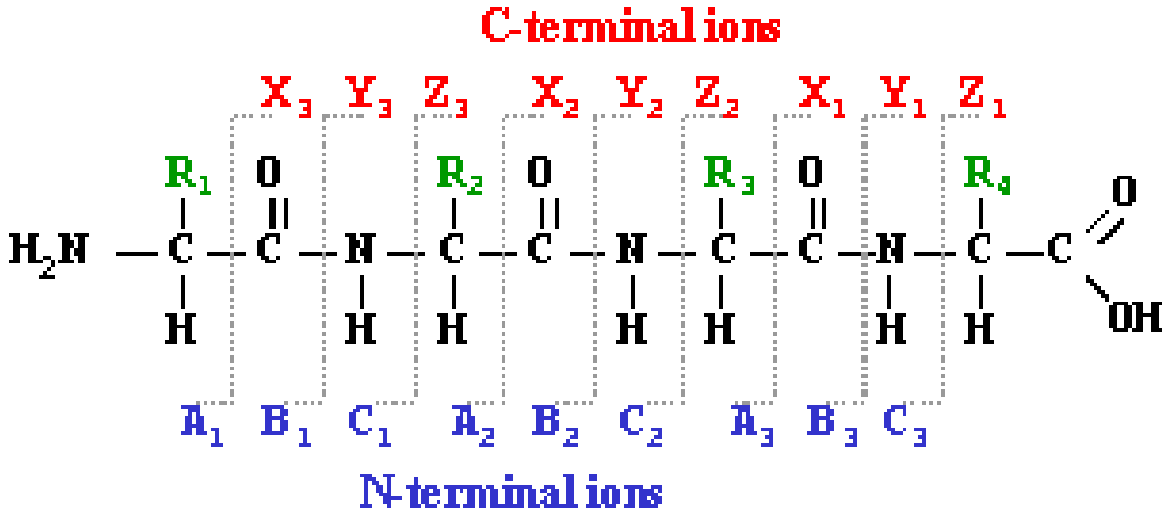
Database search

Tandem Mass Spectrometry (MS/MS)









Peptide Fragmentation

a,b,c – N-terminal side

x,y,z – C-terminal side

S-P-A-F-D-S-I-M-A-E-T-L-K

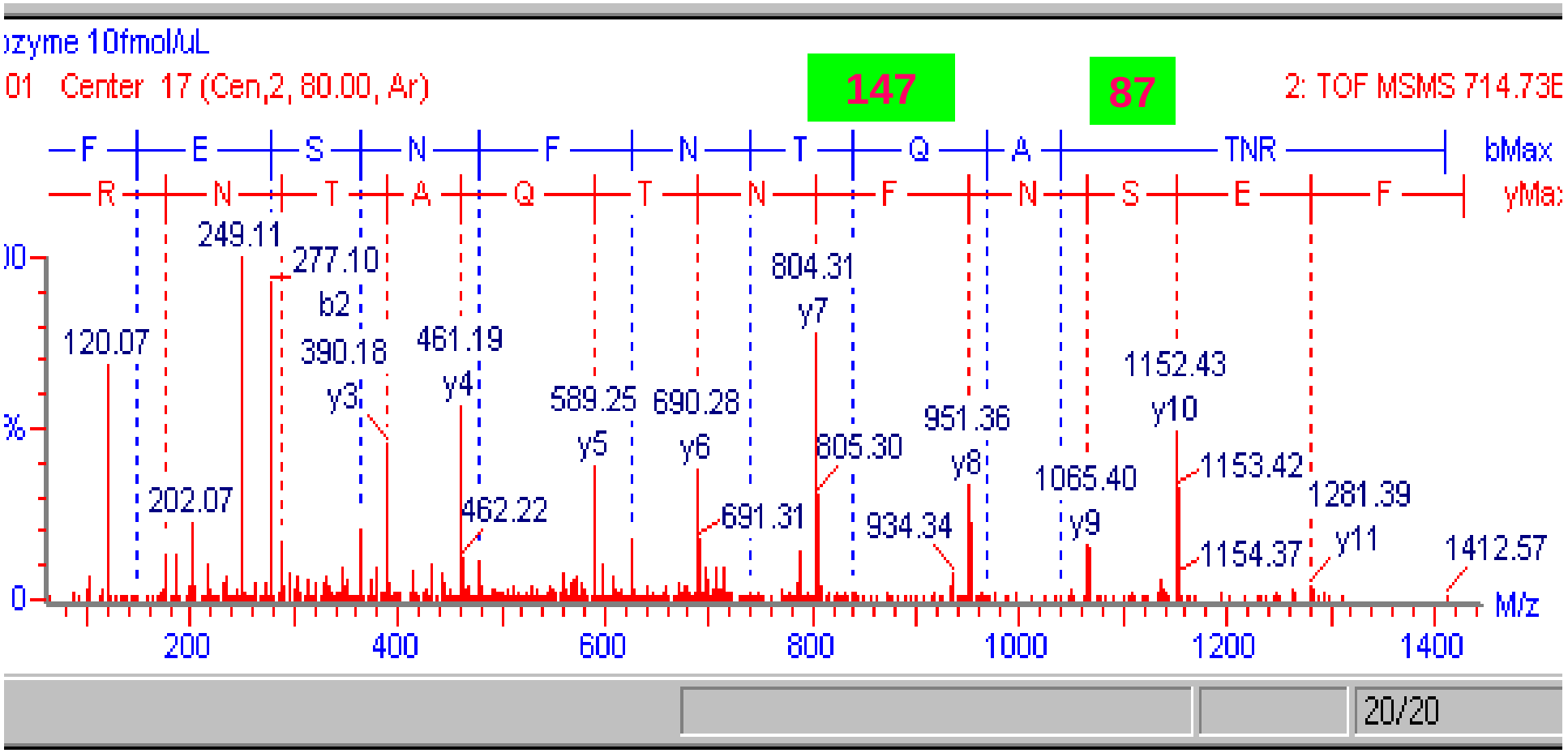
(protonated mass 1410.6)

mass+	b-ions	y-ions	mass+
88.1	S	PAFDSIMAETLK	1323.6
185.2	SP	AFDSIMAETLK	1226.4
256.3	SPA	FDSIMAETLK	1155.4
403.5	SPAF	DSIMAETLK	1008.2
518.5	SPAFD	SIMAETLK	893.1
605.6	SPAFDS	IMAETLK	806.0
718.8	SPAFDSI	MAETLK	692.3
850.0	SPAFDSIM	AETLK	561.7
921.1	SPAFDSIMA	ETLK	490.6
1050.2	SPAFDSIMAE	TLK	361.5
1151.3	SPAFDSIMAET	LK	260.4
1264.4	SPAFDSIMAETL	K	147.2

Residue Mass of Amino Acids

Table9.2	Symbols and residue masses of the protein amino acids		
Name	Symbol	Residue mass	Side-chain
Alanine	A,Ala	71.079	CH_3-
Arginine	R,Arg	156.188	HN=C(NH_2)-N-(CH_2)$_3$
Asparagine	N,Asn	114.104	H_2N-CO-CH_2-
Aspartic acid	D,Asp	115.089	HOOC-CH_2-
Cysteine	C,Cys	103.145	HS-CH_2-
Glutamine	Q,Gln	128.131	H_2N-CO-(CH_2)$_2$-
Glutamic acid	E,Glu	129.116	HOOC-(CH_2)$_2$-
Glycine	G,Gly	57.052	H-
Histidine	H,His	137.141	Imidazole-CH_2-
Isoleucine	I,Ile	113.16	CH_3-CH_2-CH(CH_3)-
Leucine	L,Leu	113.16	(CH_3)$_2$-CH-CH_2-
Lysine	K,Lys	128.17	H_2N-(CH_2)$_4$-
Methionine	M,Met	131.199	CH_3-S-(CH_2)$_2$-
Metsulphoxide	Met.SO	147.199	CH_3-S(O)-(CD_2)$_2$-
Phenylalanine	F,Phe	147.177	Phenyl-CH_2-
Proline	P,Pro	97.117	Phrrolidone-CH-
Serine	S,Ser	87.078	HO-CH_2-
Threonine	T,Thr	101.105	CH_3-CH(OH)-
Tryptophan	W,Trp	186.213	ndole-NH-CH=C-CH
Tyrosine	Y,Tyr	163.176	4-OH-Phenyl-CH_2-
Valine	V,Val	99.133	CH_3-CH(CH_2)-

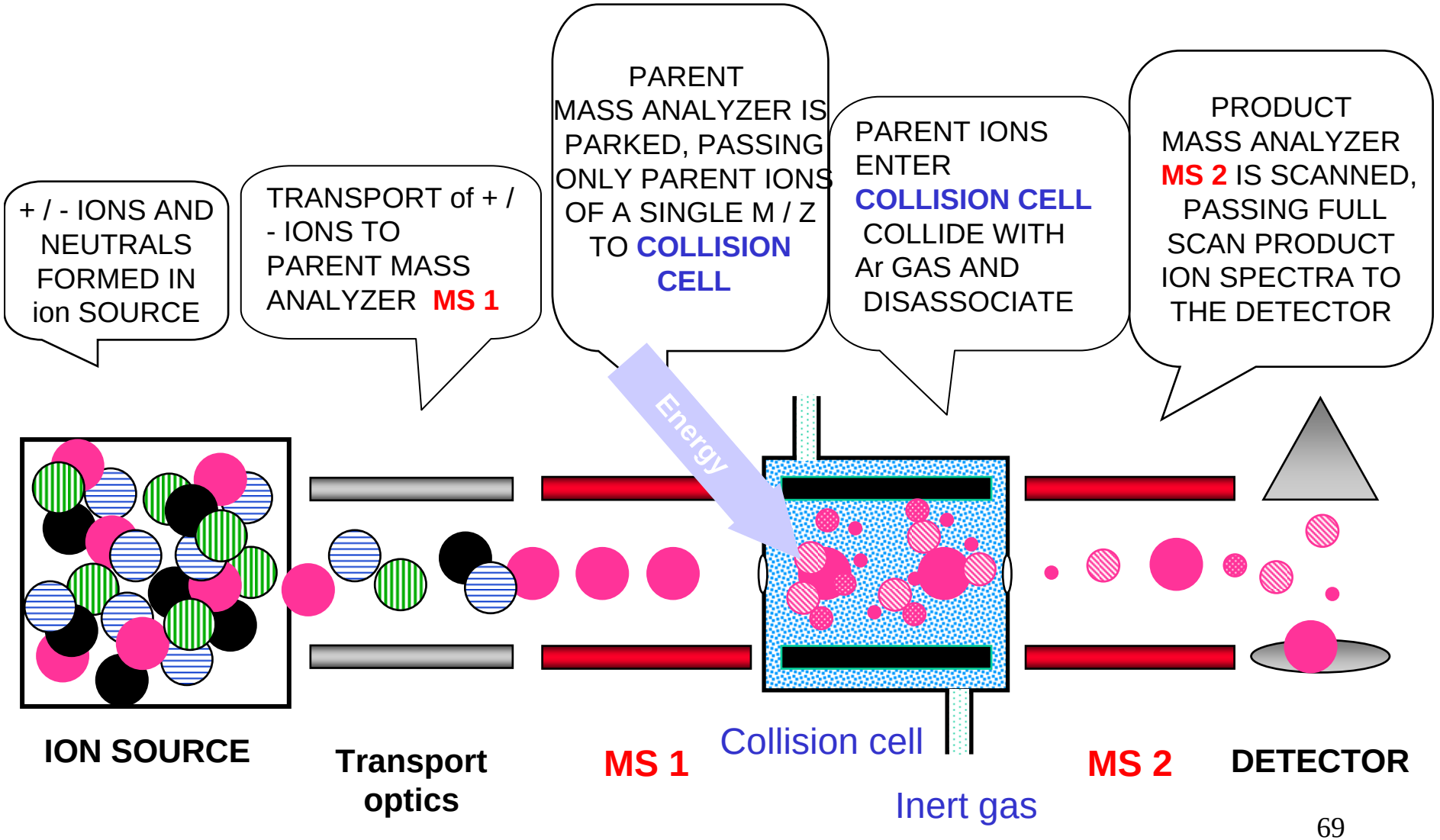
Peptide Sequencing



F (phenylalanine): 147.177

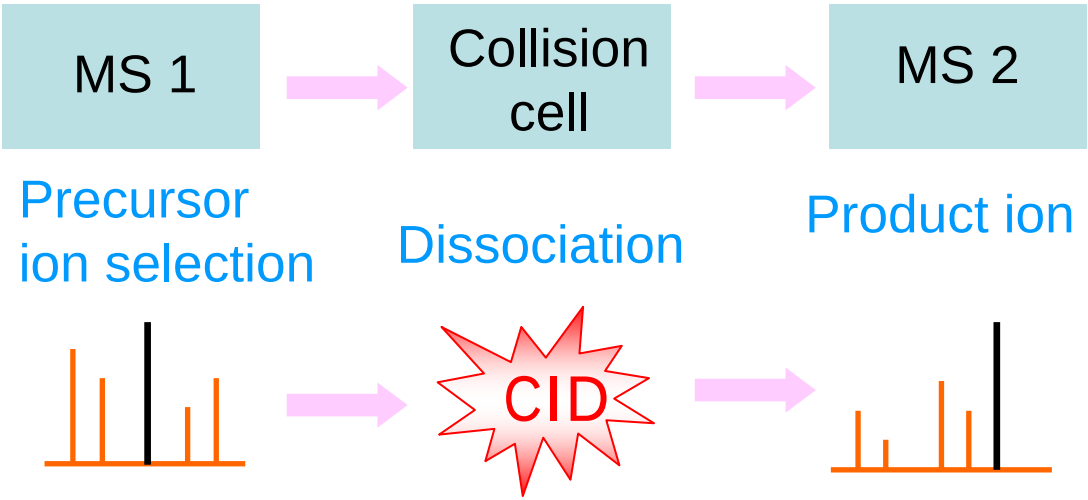
S (Serine) : 87.078

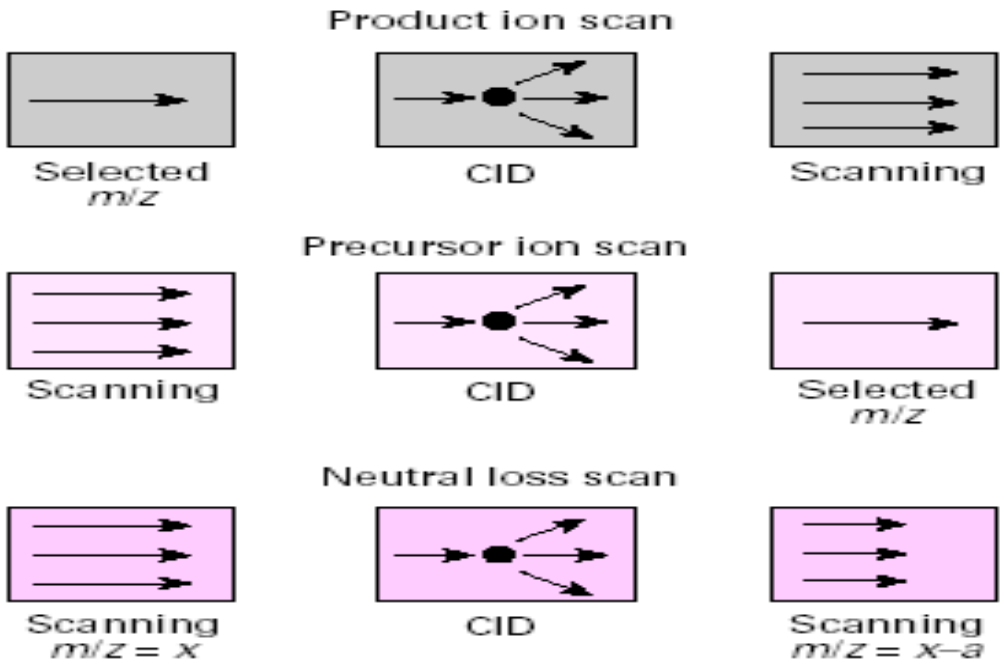
Tandem Mass Spectrometry



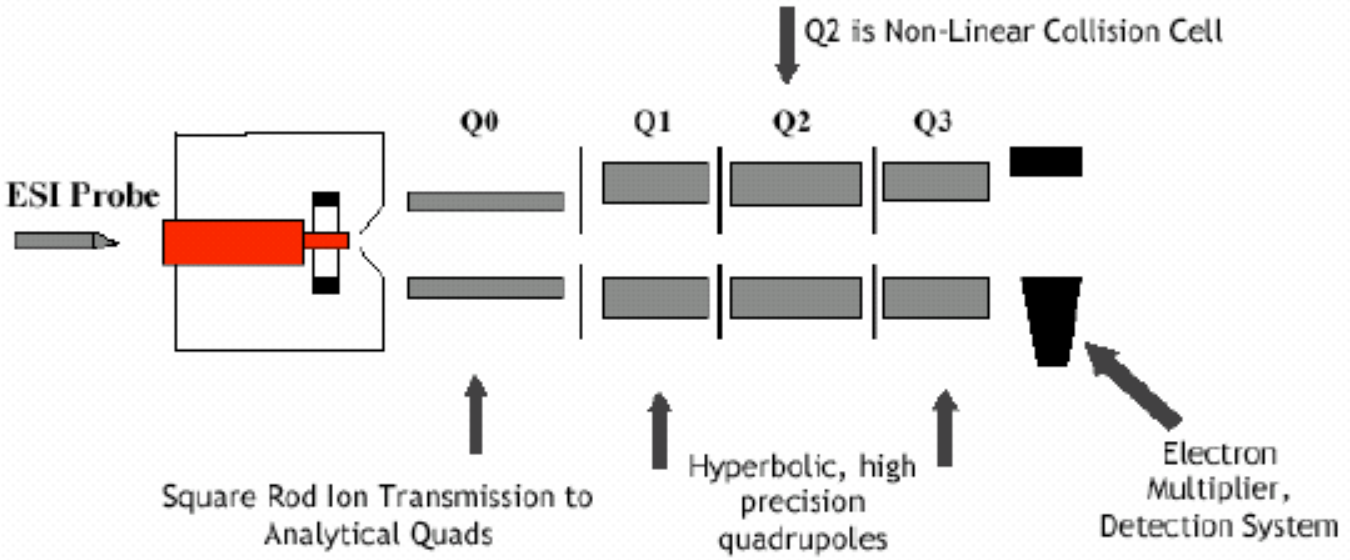
Methods of MS-MS

In space





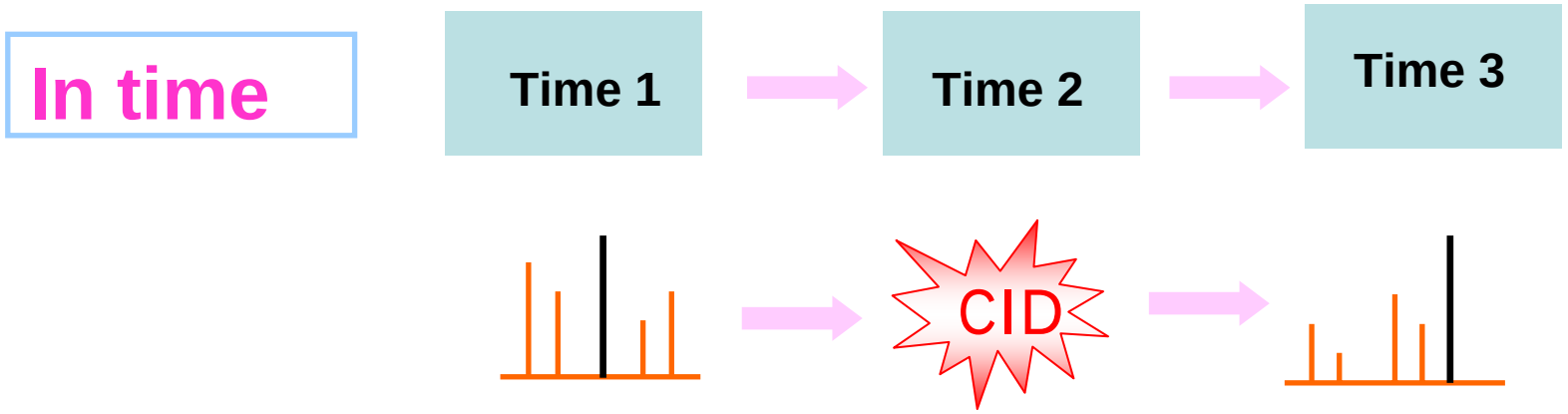
Triple Quadrupole (QQQ)

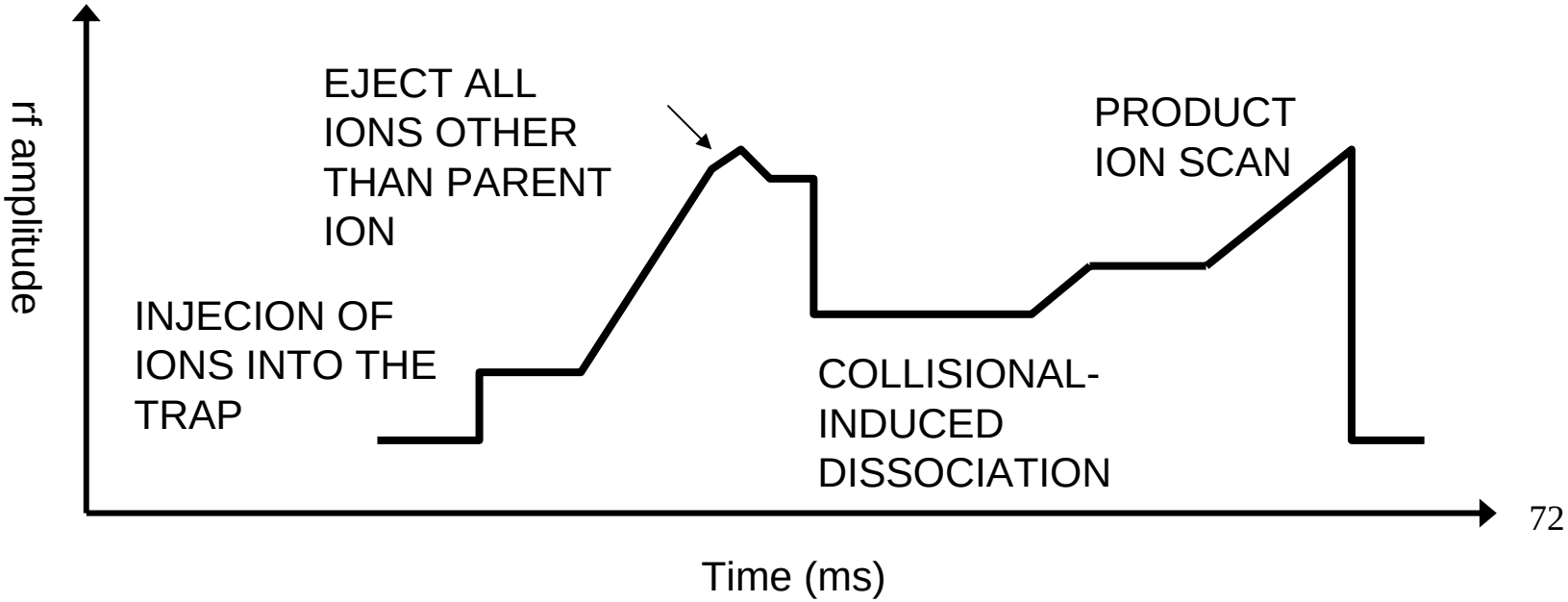


Quad Functions during MS/MS

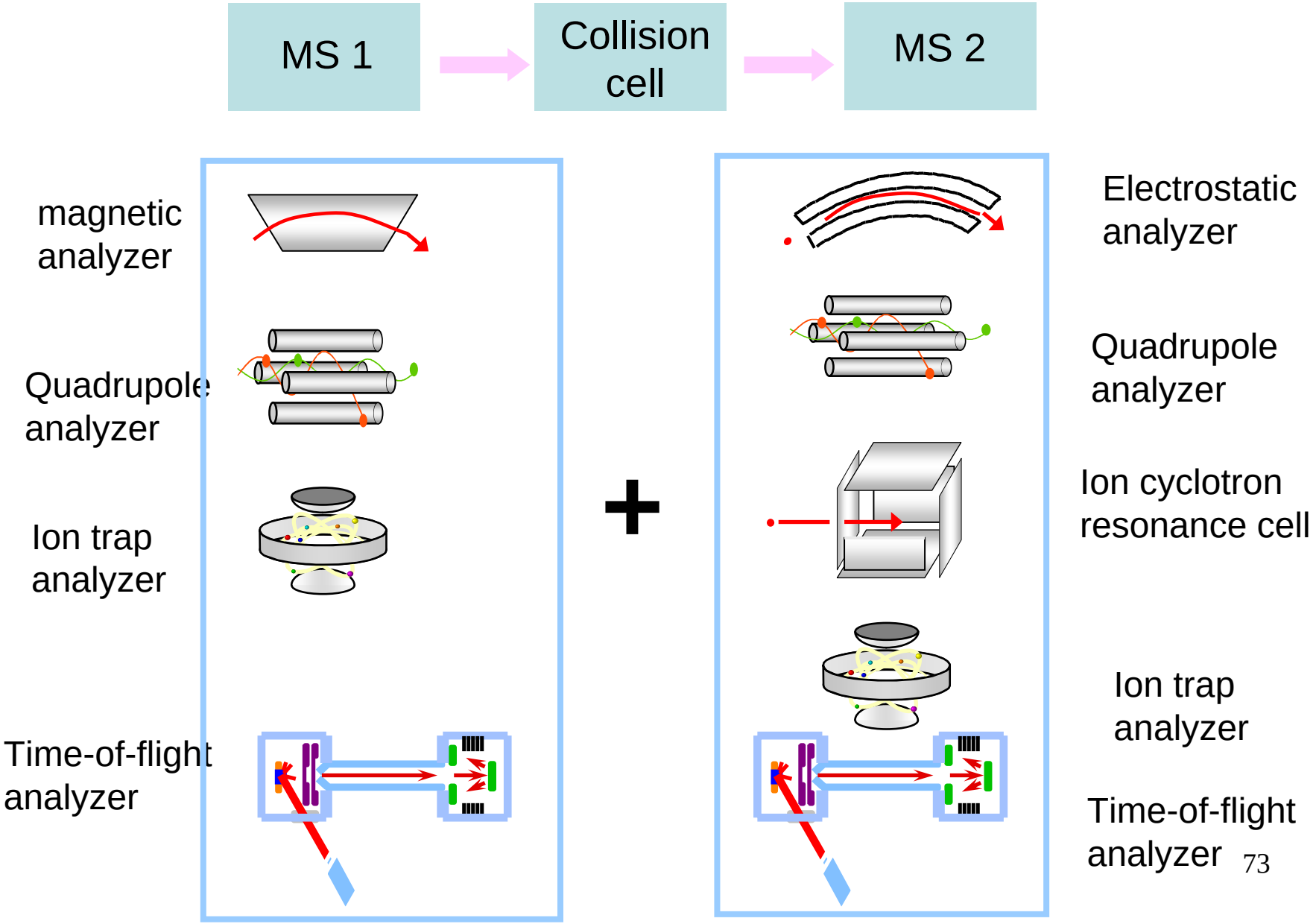
- Q1 scans a preset m/z range or selects ion of interest
- Q2 (collision cell) transmits ion and introduces collision gas into flight path
- Q3 analyses fragment ions generated in Q2

Methods of MS-MS



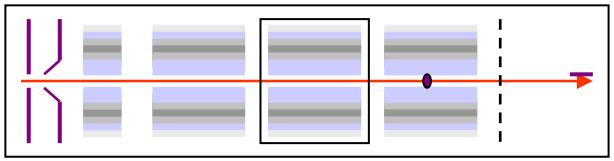


New Types Mass spectrometer



Orthogonal-Quadrupole Time-of-flight Mass Spectrometer, Q-TOF

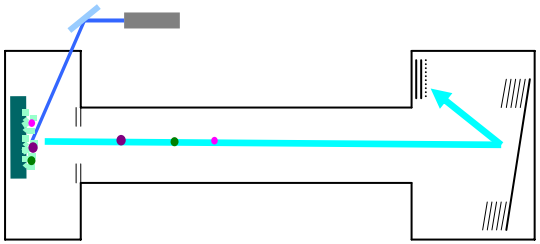
Triple quadruple MS



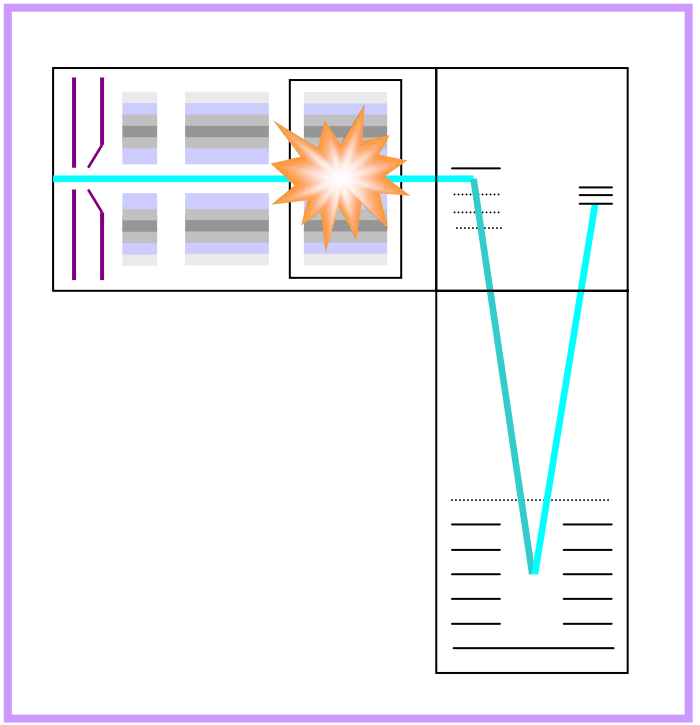
- Good precursor ion selection
- Efficient fragmentation

+

Time-of-flight MS

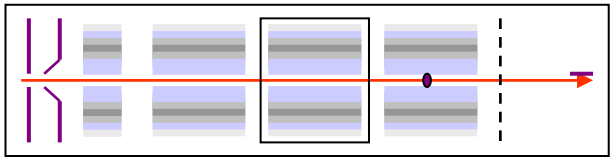


- Good sensitivity
- Good sensitivity
- Good resolution
- Speed

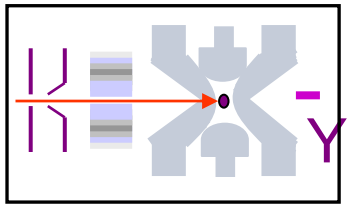


2D Linear Trap Instrument

Triple quadruple MS

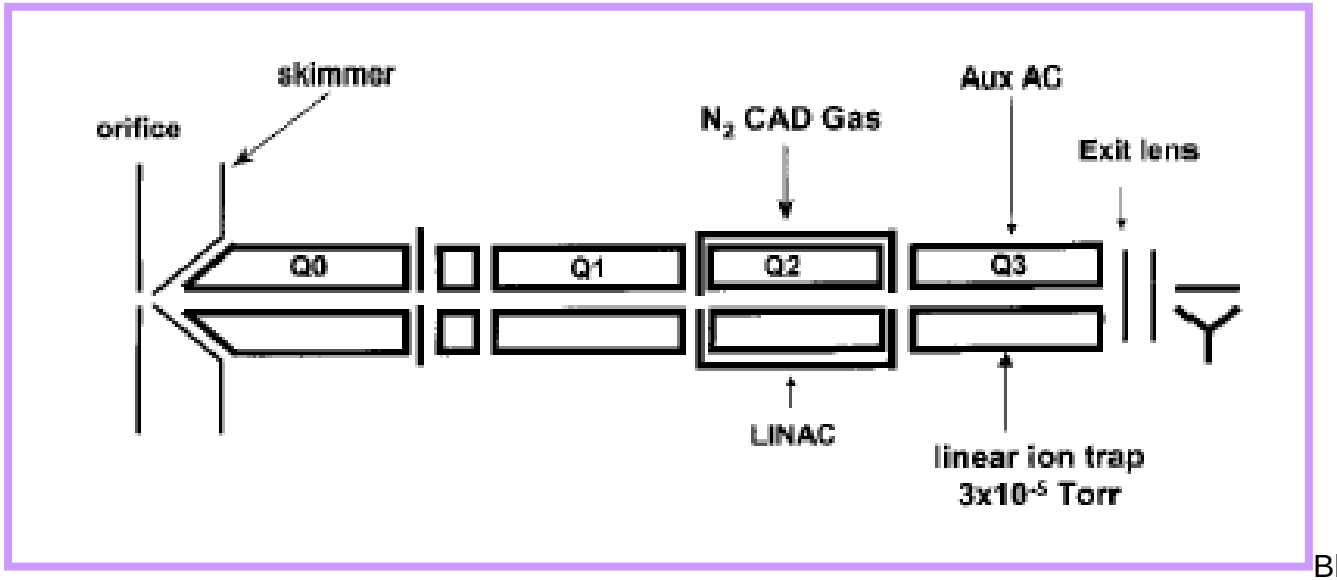


Quadrupole ion trap MS



- 4 scan modes
- Quantitative analysis

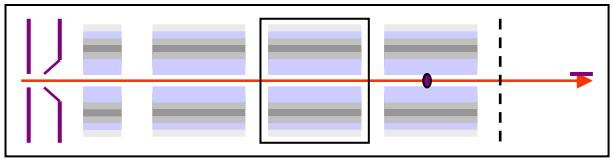
- Good sensitivity
- Sensitive product ion scans



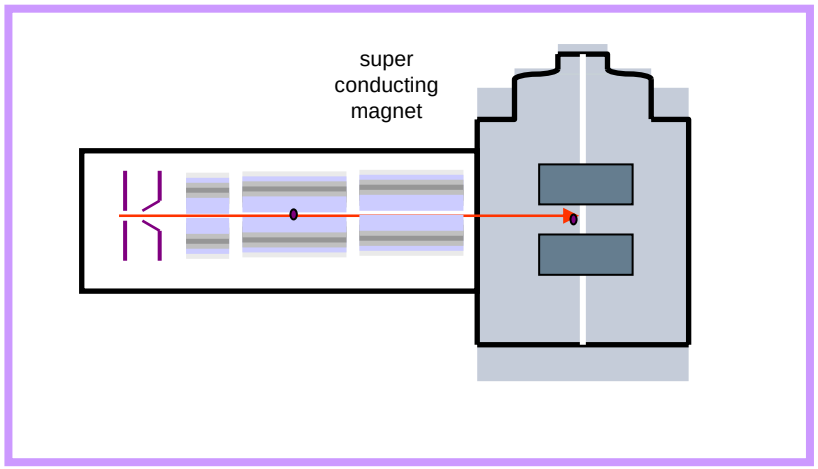
Blanc *et al*, Proteomics, **3,** 859 (2003)

FTICR Trap Instrument

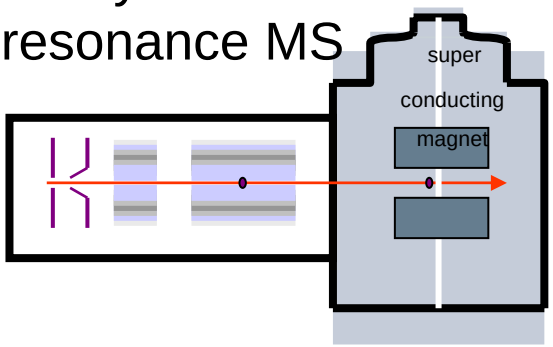
Linear ion trap MS



- Good sensitivity
- MS^n capibility

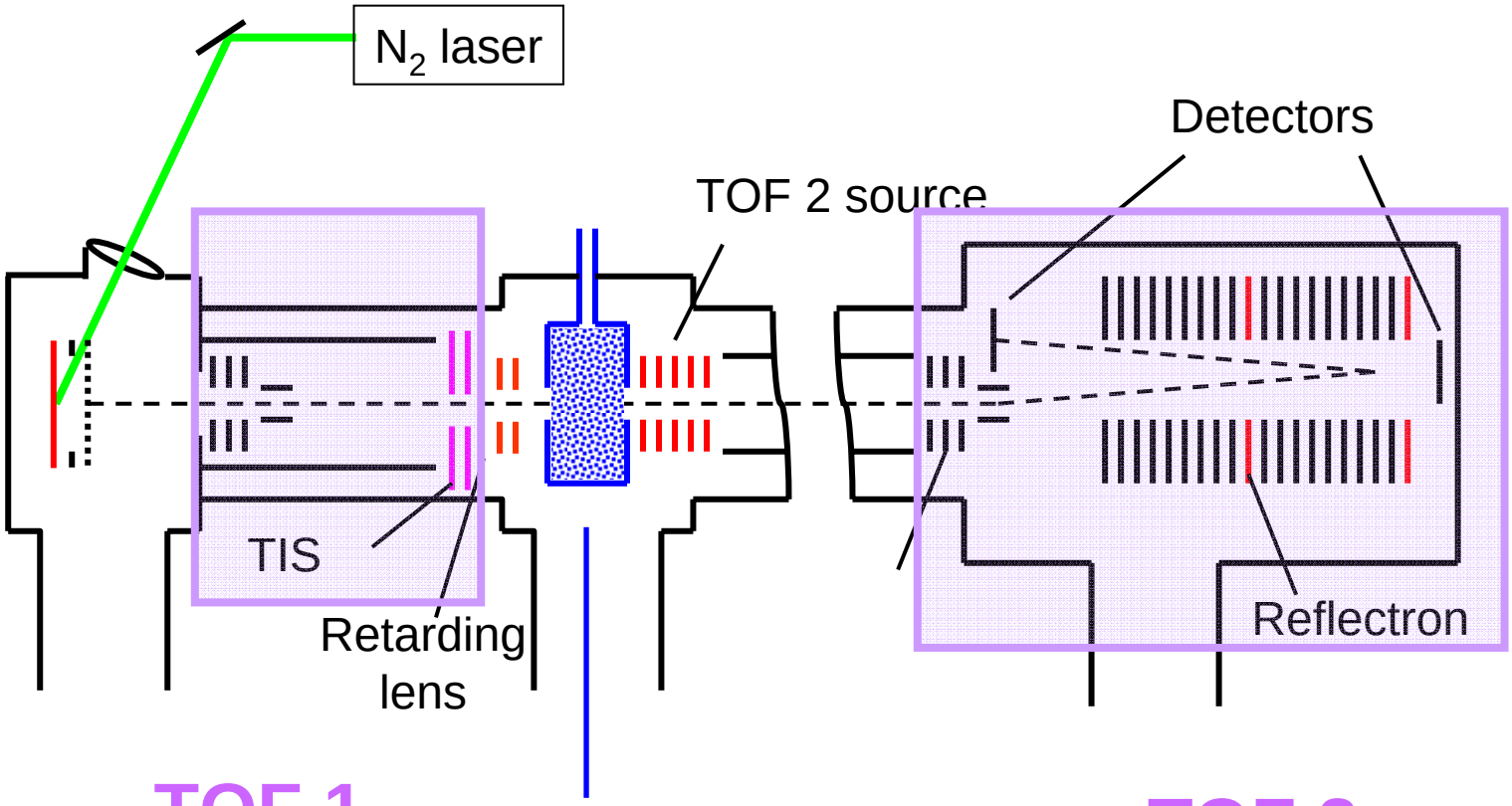


Fourier transform
ion cyclotron
resonance MS



- Accurate Mass
- Ultra-high Resolution
- High dynamic range

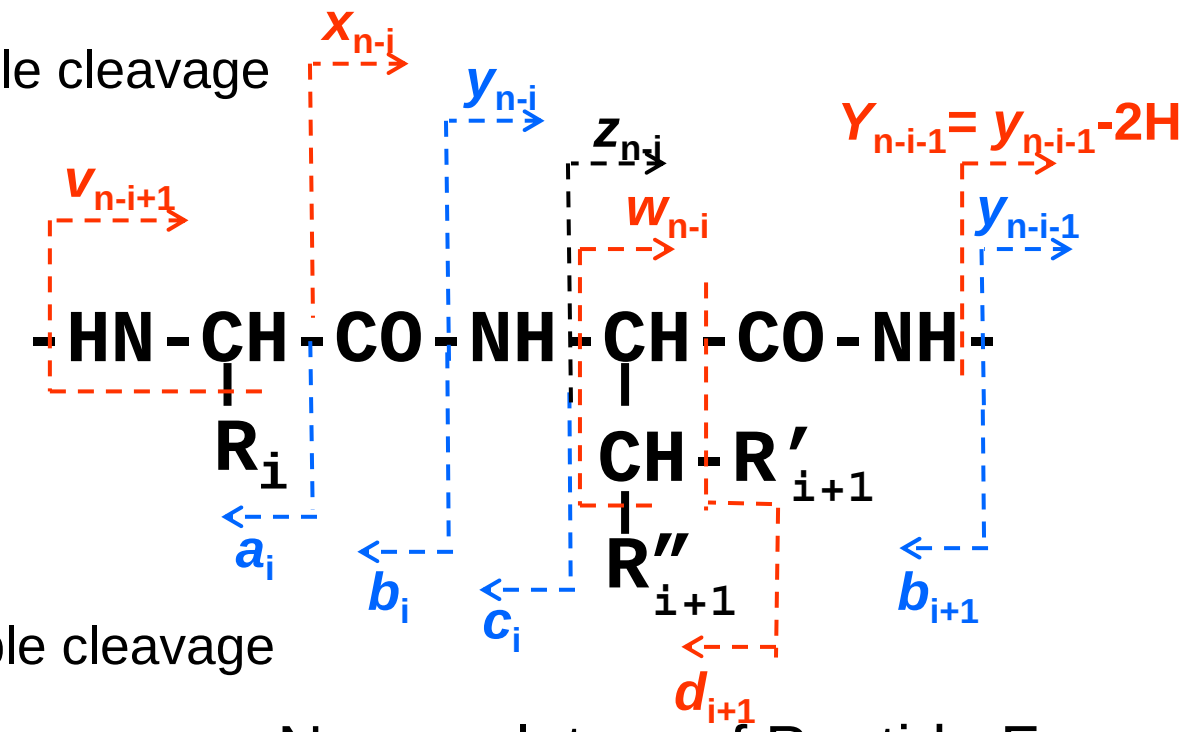
Time-of-flight / time-of-flight Mass Spectrometer, TOF-TOF



Schematics from Applied Biosystemss

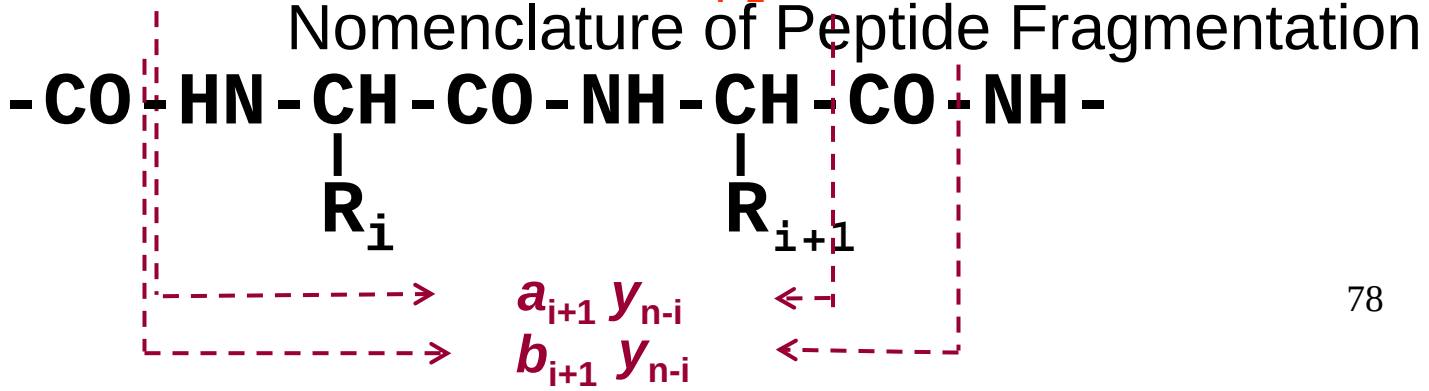
High Energy Peptide Fragment Pattern and Nomenclature

A - Single cleavage



B - Double cleavage

Nomenclature of Peptide Fragmentation



Separation and Fractionation Tools

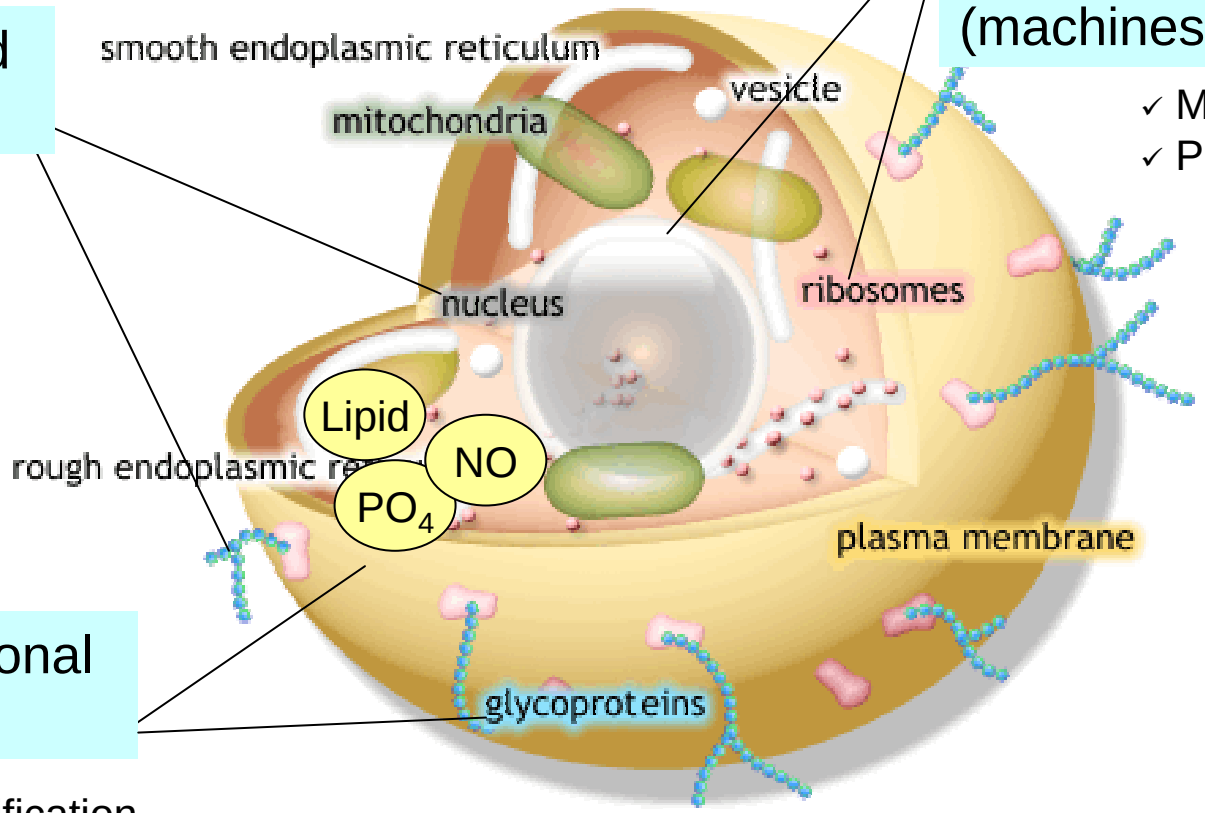
Diverse Properties of Proteins v.s. Mass Spectrometry

- ✓ M.W. measurement
- ✓ Protein identification
- ✓ tandem mass spectrometry

Protein-ligand interaction

Protein Complex (machines)

- ✓ M.W. measurement
- ✓ Protein identification



Post-translational modification

- ✓ Protein identification
- ✓ Tandem mass spectrometry

Number of Proteins per Genome

- Haemophilus 1742
- *E. coli* 4413
- Yeast 6600
- Caenorhabditis 18000
- Drosophila 13000
- Human >100000

What Do We See?

Technology Platform V.S. ***Complex Proteome***

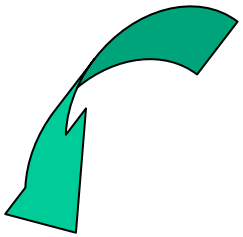
Proteomic Technologies for Biomarker Detection & Discovery

- Gel electrophoresis
  - ✓ Two-dimensional gel electrophoresis (2DE)
  - ✓ Two-dimensional differential gel electrophoresis (DIGE)
- Gel free (Mass spectrometry)
  - ✓ Surface-enhanced laser desorption / ionization TOF MS (SELDI-TOF MS)
  - ✓ Multidimentional protein identification technology (MudPIT)
  - ✓ Isotope coded affinity tag (ICAT)
- Other technologies:
  - Protein microarrays
  - ELISA

A key technical challenge in proteomics

A more complex biological problem

Diverse solution
(10^5 – 10^6 for protein abundance)



Mass-spectrometry strategy for more complex mixture ?

+

Sample prefractionation

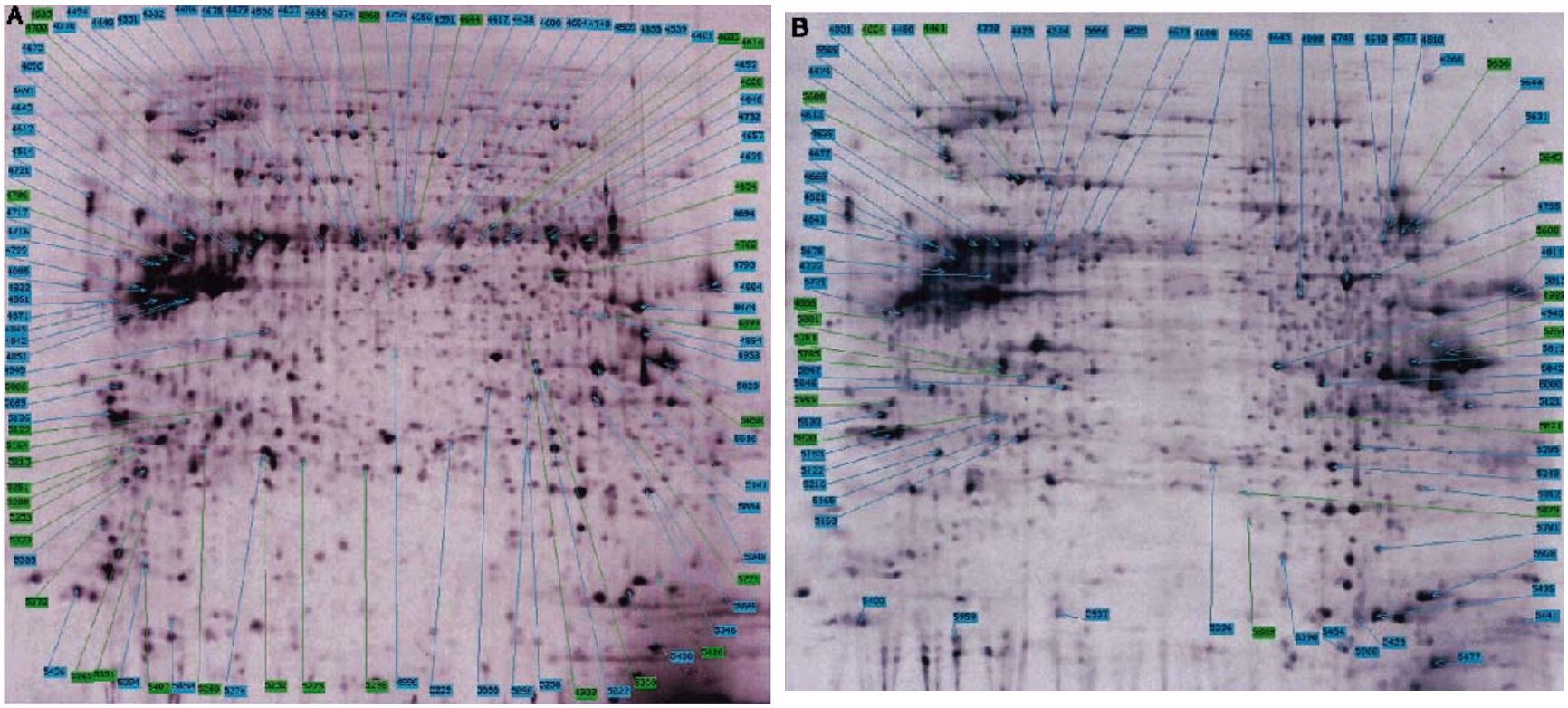
Separation Methods for Protein and Peptides

Method	Basis of separation
Chromatographic methods	
size-exclusion chromatography	molecular weight
ion-exchange chromatography	charge
reverse-phase high-performance chromatograph	hydrophobic interaction between the sample and bonded
hydrophobic-interaction chromatography	salt-promoted adsorption chromatography
affinity chromatography	biomolecular interaction (DNA, ligand, antibody…)
Electrophoretic methods	
one-dimensional gel electrophoresis	molecular weight
two-dimensional gel electrophoresis	1st dimension: charge; 2nd dimension: molecular weigh
gel-free isoelectric focusing	charge
Subcellular fractionation	subcellular fractionation

(二維電泳分離)

Example:

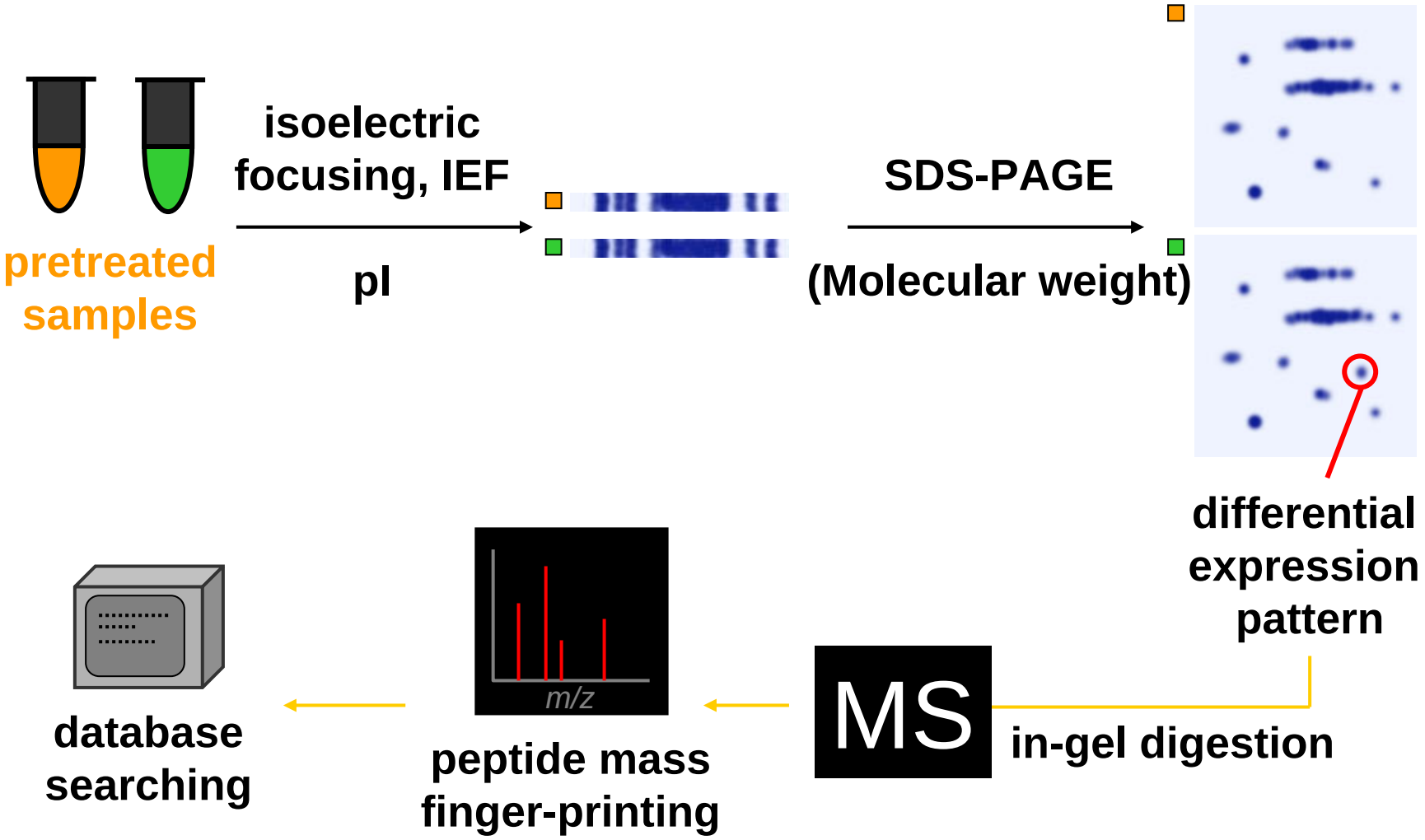
Today's 2D Gel-Based Proteomics

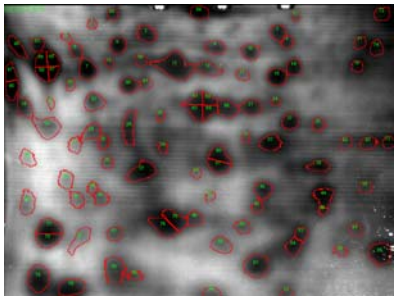


differentially expressed (170)

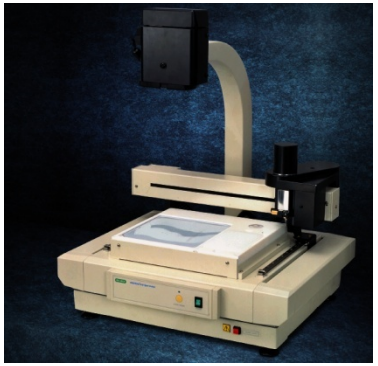
no differences

2D-PAGE & Protein Identification

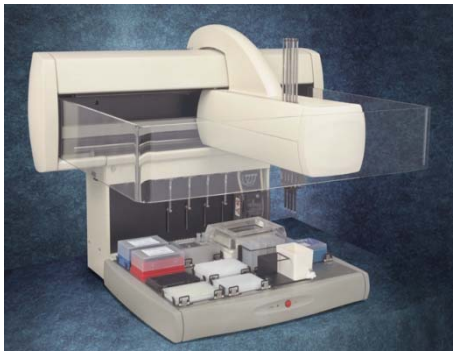




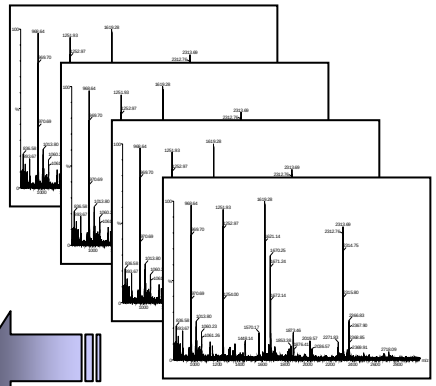
2D Gel



Robotic spot excision



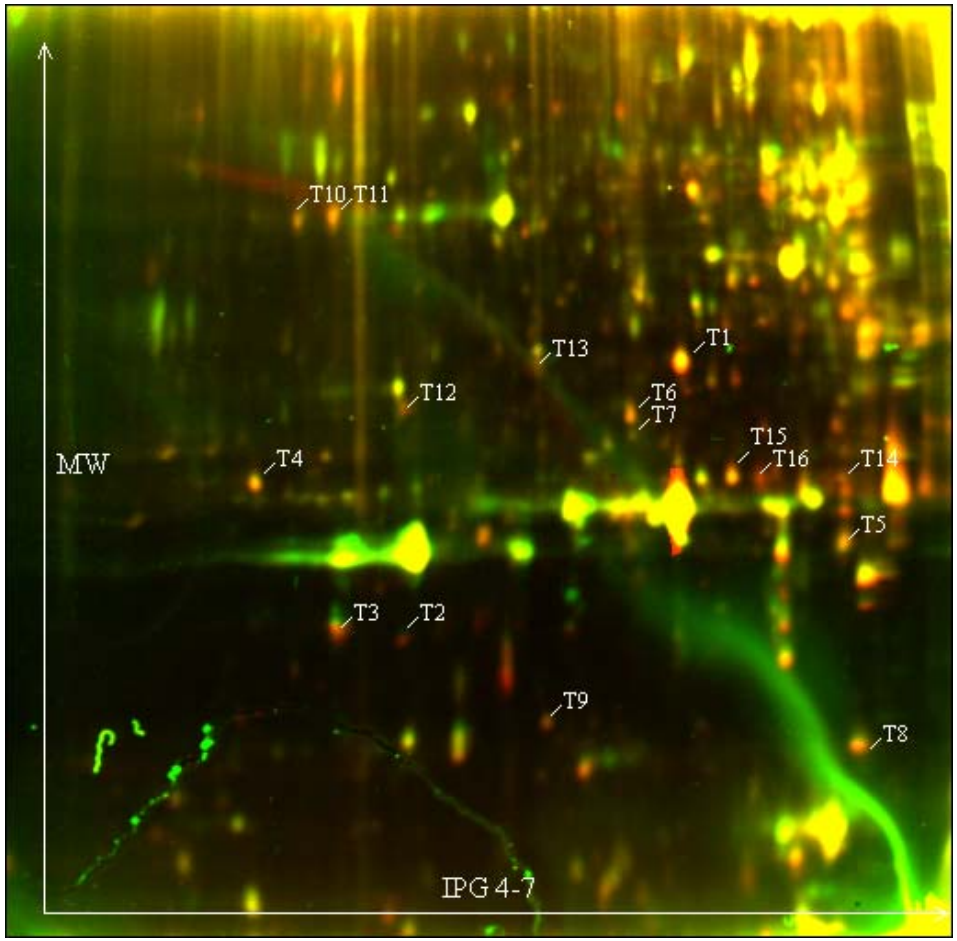
In-Gel Digestion and sample prep for MS



Automated data processing and database searching

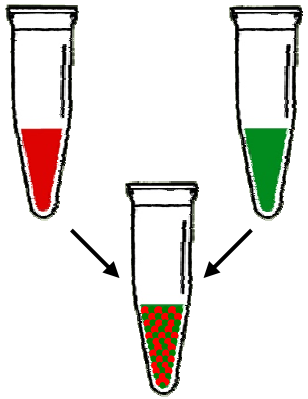
Advanced MS

Differential Gel Electrophoresis (2D-DIGE)



Test labelled with propyl-Cy3

Control labelled with methyl-Cy5



2D-electrophoresis

IEF

SDS-PAGE

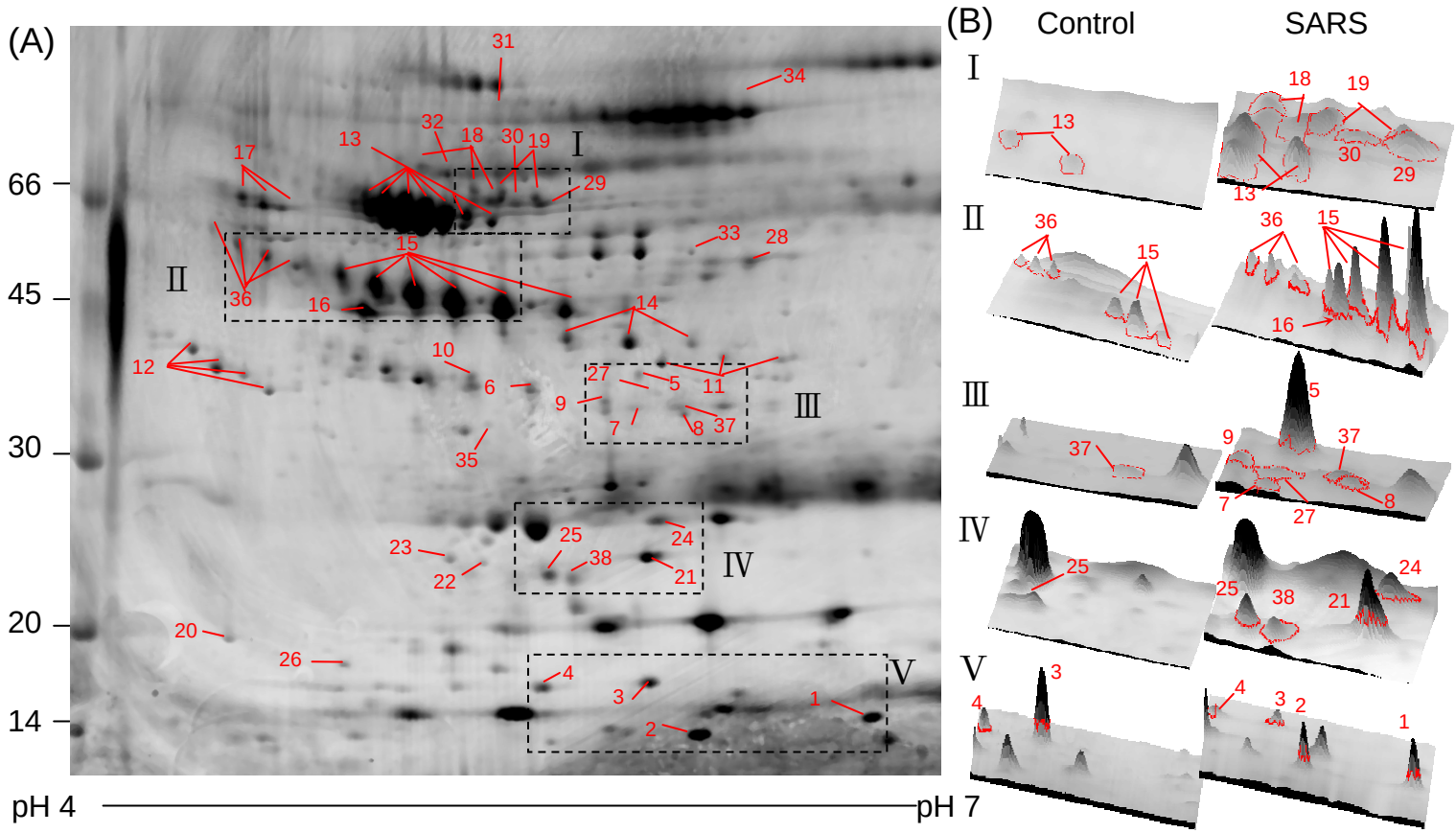
λE Cy3 Image A

λE Cy5 Image B

2D-PAGE & protein identification

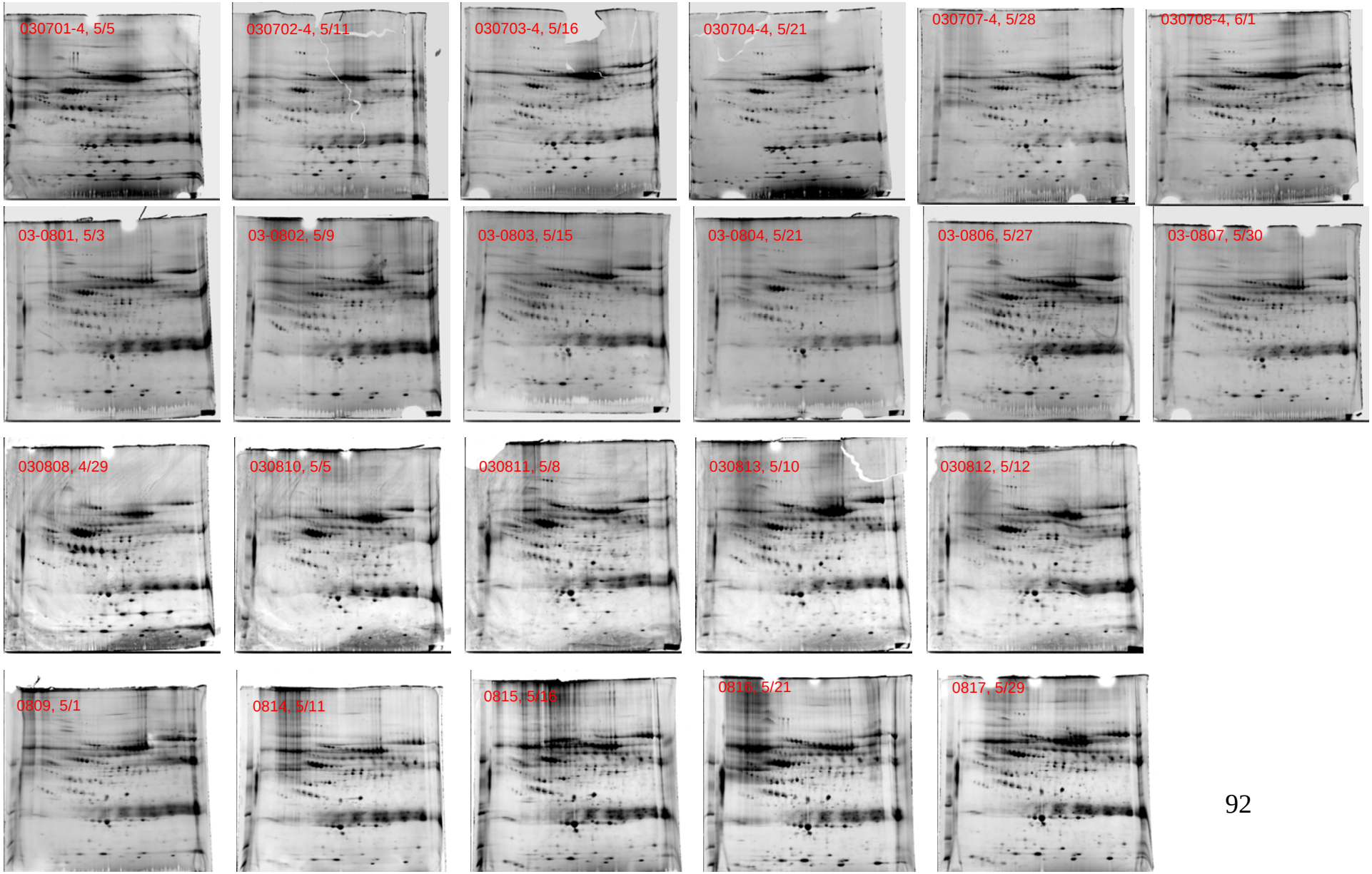
- MALDI-TOF MS is mostly used
- advantages:
  - discovering biomarker
  - high reproducibility
  - semi-quantitative
- drawbacks:
  - low sensitivity, particularly for less-abundant proteins

Plasma proteome of Severe Acute Respiratory Syndrome (SARS) analyzed by 2-DE and MS



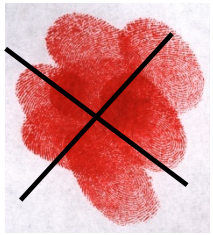
Proc. Natl. **Acad. Sci.** *USA.*, **101**, 17039 (2004)

Protein Profiles of SARS Patients



MALDI

Peptide Mass Fingerprinting (PMF)

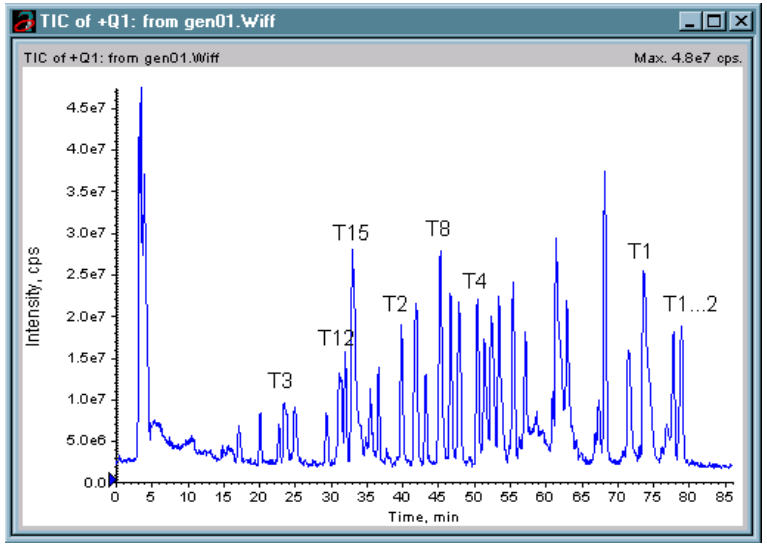


Direct LC-MS/MS

Poor salt tolerance
Ion suppression

液相層析輔助質譜

Ion suppression
Salt tolerance
Sample concentration



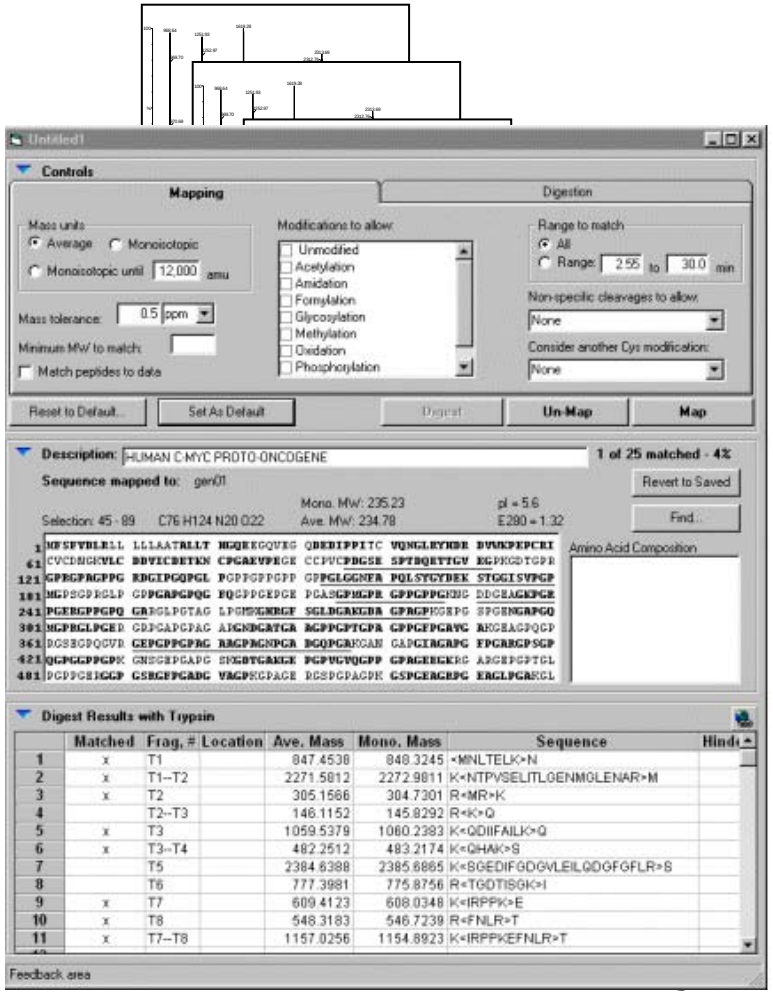
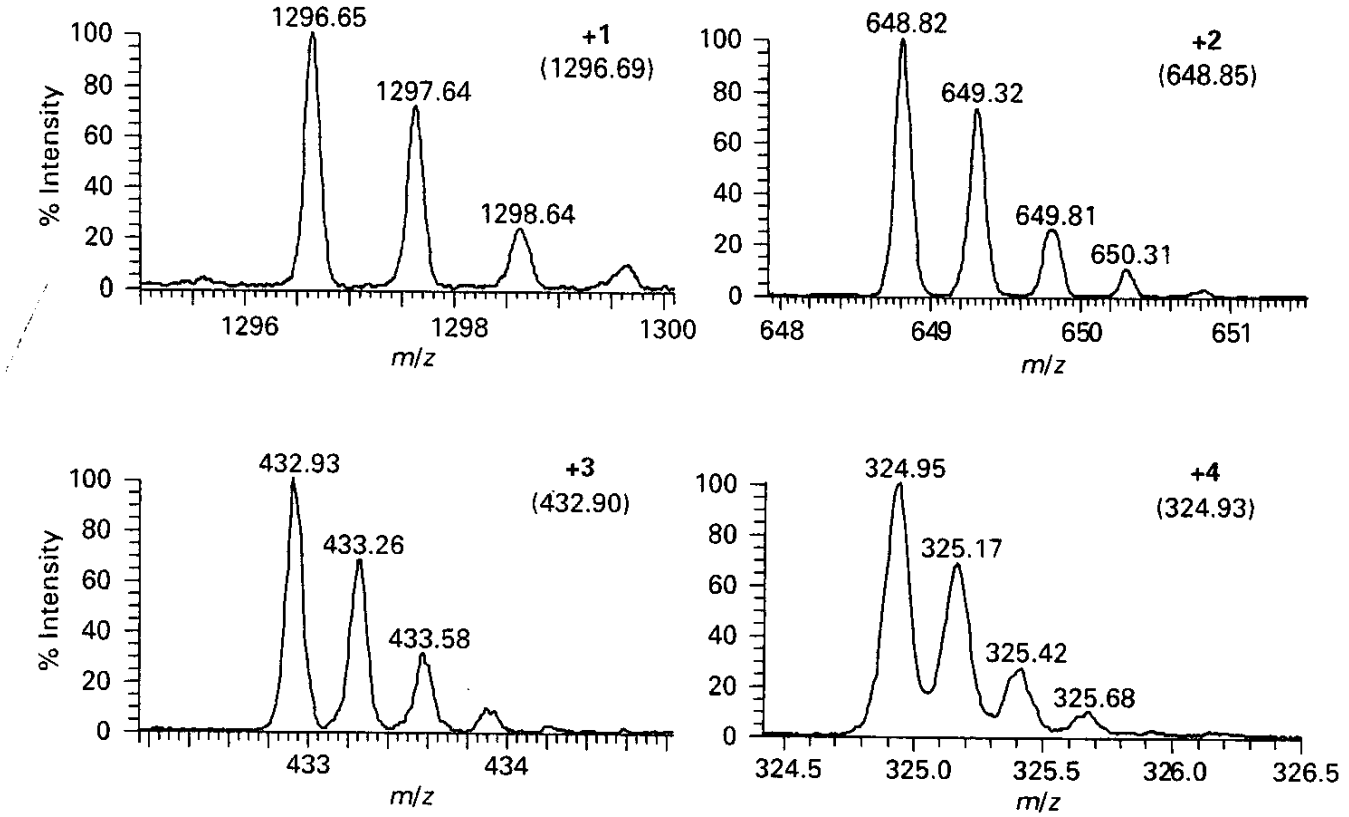
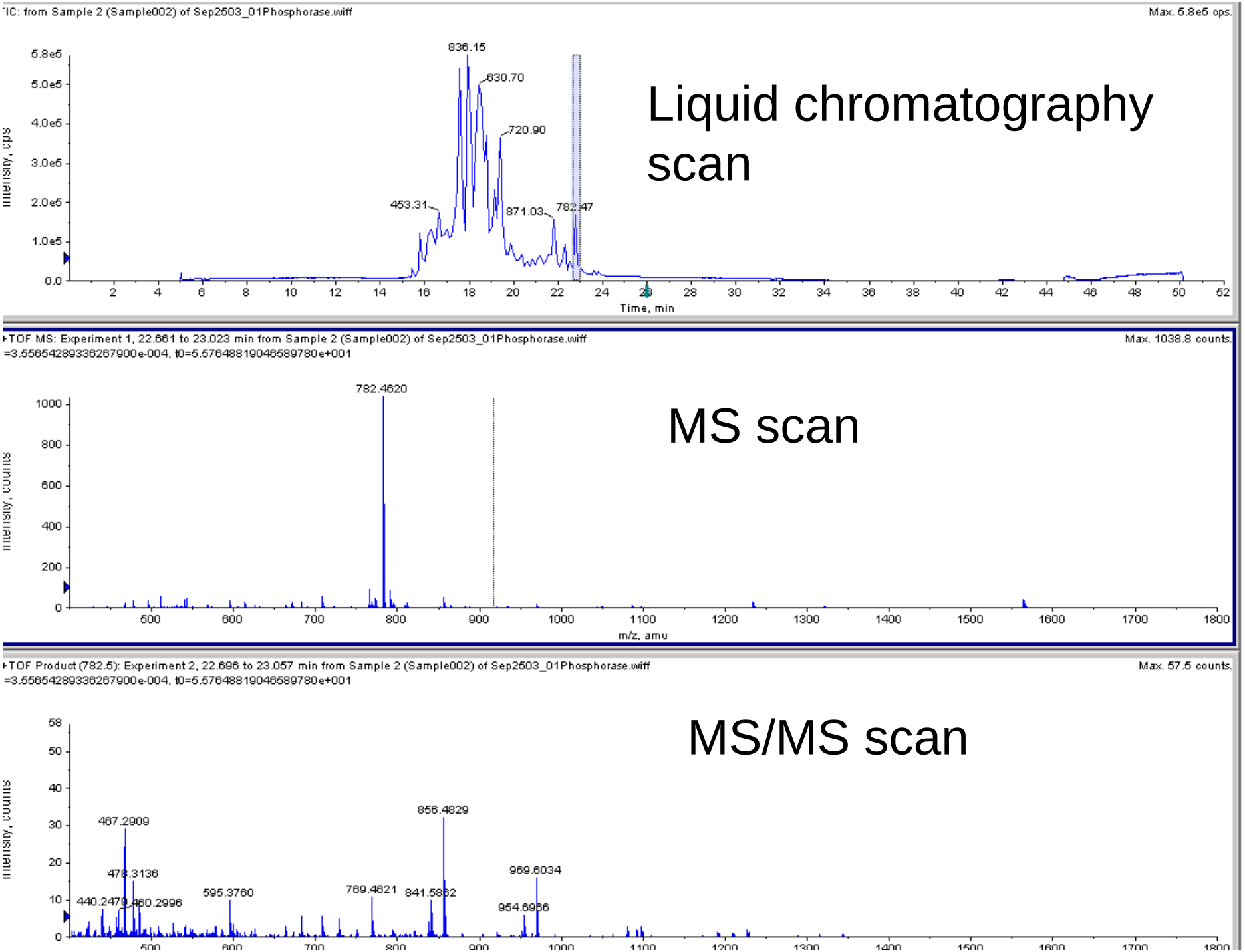
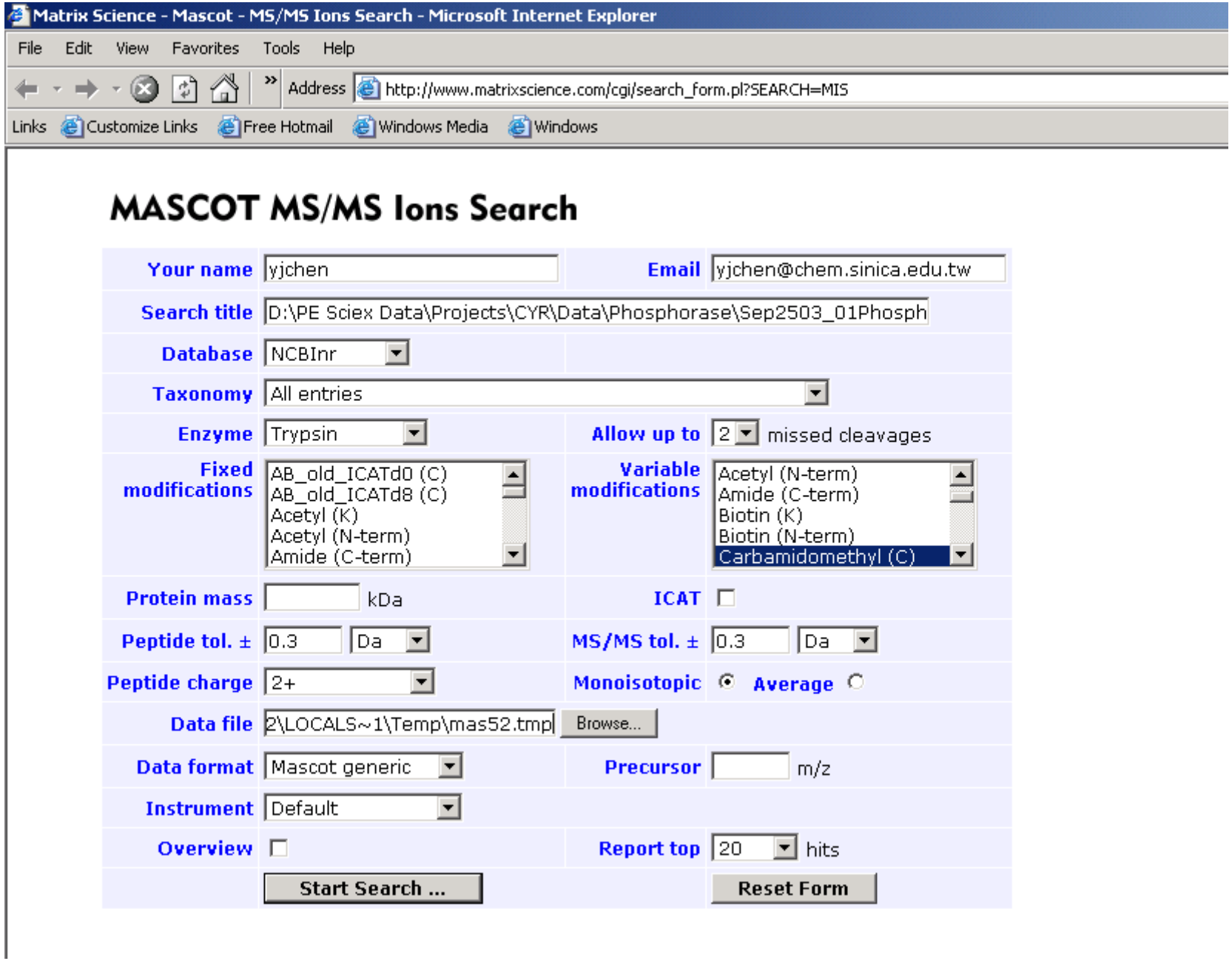


Table9.3	Mass differences due to isotopes in multiply charged p

Charge on peptide	Apparent mass	Mass difference between isotope
Single charge	[(M+H)/1]	1Da
Double charge	[(M+2H)/2]	0.5Da
Triple charge	[(M+3H)/3]	0.33Da
n charges	[(M+nH)/n]	1/nDa




TIC: from Sample 2 (Sample002) of Sep2503_01Phosphorase.wiff
Max. 5.8e5 cps.
836.15
630.70
720.90
453.31
871.03
782.47
Intensity, cps
Time, min
Liquid chromatography scan
TOF MS: Experiment 1, 22.661 to 23.023 min from Sample 2 (Sample002) of Sep2503_01Phosphorase.wiff
=3.5565428933626790 0e-004, t0=5.5764881904658978 0e+001
Max. 1038.8 counts.
782.4620
Intensity, counts
m/z, amu
MS scan
TOF Product (782.5): Experiment 2, 22.696 to 23.057 min from Sample 2 (Sample002) of Sep2503_01Phosphorase.wiff
=3.5565428933626790 0e-004, t0=5.5764881904658978 0e+001
Max. 57.5 counts.
856.4829
467.2909
478.3136
595.3760
769.4621
841.5862
969.6034
954.6956
440.2479
460.2996
Intensity, counts
MS/MS scan


Matrix Science - Mascot - MS/MS Ions Search - Microsoft Internet Explorer
File Edit View Favorites Tools Help
Address http://www.matrixscience.com/cgi/search_form.pl?SEARCH=MIS
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Email yjchen@chem.sinica.edu.tw
Search title D:\PE Sciex Data\Projects\CYR\Data\Phosphorase\Sep2503_01Phosph
Database NCBInr
Taxonomy All entries
Enzyme Trypsin
Allow up to 2 missed cleavages
Fixed modifications
AB_old_ICATd0 (C)
AB_old_ICATd8 (C)
Acetyl (K)
Acetyl (N-term)
Amide (C-term)
Variable modifications
Acetyl (N-term)
Amide (C-term)
Biotin (K)
Biotin (N-term)
Carbamidomethyl (C)
Protein mass kDa
ICAT
Peptide tol. ± 0.3 Da
MS/MS tol. ± 0.3 Da
Peptide charge 2+
Monoisotopic Average
Data file 2\LOCALS~1\Temp\mas52.tmp Browse...
Data format Mascot generic
Precursor m/z
Instrument Default
Overview
Report top 20 hits
Start Search ...
Reset Form

Mascot Search Results

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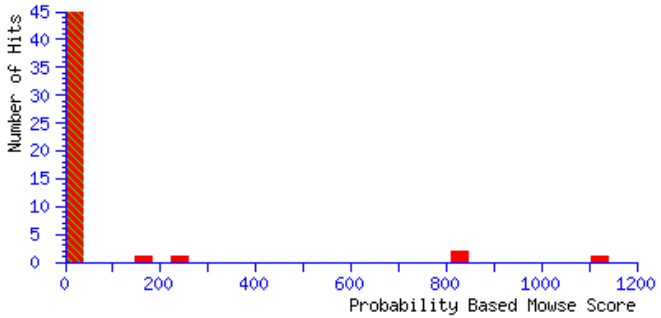
User            : yjchen
Email           : yjchen@chem.sinica.edu.tw
Search title    : D:\PE Sciex Data\Projects\CYR\Data\Phosphorase\Sep2503_01Phosphorase.wiff : Sample002
MS data file    : C:\DOCUME~1\lab212\LOCALS~1\Temp\mas52.tmp
Database        : SwissProt 42.10 (184535 sequences; 83187710 residues)
Timestamp       : 24 Feb 2004 at 12:03:33 GMT
Significant hits: P00489           (PHS2_RABIT) Glycogen phosphorylase, muscle form (EC 2.4.1.1) (Myophosphorylase) Glycogen phosphorylase, musc
                  P11217-00-00-00  (PHS2_HUMAN) Splice isoform Displayed; Variant Displayed; Conflict Displayed; from P11217 Glycogen phosphoryl
                  O18751           (PHS2_SHEEP) Glycogen phosphorylase, muscle form (EC 2.4.1.1) (Myophosphorylase) Glycogen phosphorylase, musc
                  P11216           (PHS3_HUMAN) Glycogen phosphorylase, brain form (EC 2.4.1.1) Glycogen phosphorylase, brain form (EC 2.4.1.1)
                  P06737-00-02-00  (PHS1_HUMAN) Splice isoform Displayed; Variant GSD-VI-VAR_007909; Conflict Displayed; from P06737 Glycogen ph
                  P04191-00-00-00  (ATA1_RABIT) Splice isoform SERCA1B; Variant Displayed; Conflict Displayed; from P04191 Sarcoplasmic/endoplasm
                  Q85FR6           (RPOC_CYAME) Bifunctional DNA-directed RNA polymerase beta' and beta'' chain (EC 2.7.7.6) (PEP) [Includes: DNA
                  P22705           (RPOD_AHASP) DNA-directed RNA polymerase beta' chain (EC 2.7.7.6) (RNAP beta' subunit) (Transcriptase beta' ch
```

Probability Based Mowse Score

Ions score is -10*Log(P), where P is the probability that the observed match is a random event.
Individual ions scores > 38 indicate identity or extensive homology (p<0.05).
Protein scores are derived from ions scores as a non-probabilistic basis for ranking protein hits.



Peptide Summary Report

Switch to Protein Summary Report

To create a bookmark for this report, right click this link: Peptide Summary Report (D:\PE Sciex Data\Projects\CYR\Data\Phosphorase\Sep2503_01Phosphorase.wiff : Sample002)

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

1. P00489 **Mass:** 97097 **Score:** 1121 **Peptides matched:** 31

(PHS2_RABIT) Glycogen phosphorylase, muscle form (EC 2.4.1.1) (Myophosphorylase) Glycogen phospl

☐ Check to include this hit in error tolerant search

	Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
☑	12	490.33	978.64	978.54	0.10	1	18	8.1	1	LPAPDEKIP
☑	19	527.32	1052.63	1052.57	0.06	0	40	0.037	1	VIFLENYR
☑	20	527.80	1053.58	1053.48	0.11	0	65	0.00013	1	TNFDAFPDK
	22	536.84	1071.66	1071.53	0.13	0	11	39	5	EIWGVEPSR
☑	27	559.35	1116.68	1116.56	0.12	0	61	0.00044	1	VAAAFPGDVDR
☑	29	573.32	1144.62	1144.56	0.06	0	45	0.017	1	YEFGIFNQK
☑	40	615.40	1228.78	1228.67	0.11	0	82	3.5e-06	1	GLAGVENVTELK
☑	45	631.83	1261.64	1261.59	0.05	0	42	0.034	1	VFADYEEYVK
☑	46	421.95	1262.82	1262.70	0.12	2	30	0.45	1	QRLPAPDEKIP
☑	63	453.30	1356.89	1356.76	0.13	1	41	0.034	1	GLAGVENVTELKK
☑	73	713.95	1425.89	1425.77	0.11	0	70	4.1e-05	1	HLQIIYEINQR
☑	79	721.89	1441.76	1441.69	0.08	0	25	1.6	1	VLYPNDNFFEGK
☑	82	491.99	1472.95	1472.85	0.10	0	46	0.011	1	WPVHLLETLLPR
☑	83	737.50	1472.98	1472.85	0.13	0	(45)	0.013	1	WPVHLLETLLPR
☑	86	497.29	1488.84	1488.76	0.08	1	20	3.6	1	LDWDKAWEVTVK
☑	100	775.95	1549.88	1549.76	0.12	0	60	0.0004	1	IGEEYISDLDQLR
☑	105	522.98	1565.91	1565.78	0.12	0	(63)	0.0002	1	DFNVGGYIQAVLDR
☑	106	783.98	1565.94	1565.78	0.16	0	70	3.8e-05	1	DFNVGGYIQAVLDR
☑	109	790.98	1579.95	1579.82	0.12	0	(61)	0.00031	1	QIIEQLSSGFFSPK
☑	110	790.98	1579.95	1579.82	0.12	0	74	1.7e-05	1	QIIEQLSSGFFSPK
☑	112	537.32	1608.95	1608.86	0.09	0	45	0.012	1	VHINPNSLFDVQVK
	113	805.49	1608.97	1608.86	0.11	0	(7)	69	4	VHINPNSLFDVQVK
☑	127	560.32	1677.95	1677.86	0.09	1	55	0.0013	1	IGEEYISDLDQLRK
☑	128	840.00	1677.98	1677.86	0.12	1	(48)	0.0076	1	IGEEYISDLDQLRK
	131	565.67	1693.97	1693.81	0.16	1	11	35	2	DFYELEPHKFQNK
	134	570.99	1709.95	1709.86	0.09	1	16	9.3	3	RIYYLSLEFYMGR

Nominal mass (M_r): **97097**; Calculated pI value: **6.76**
NCBI BLAST search of P00489 against nr
Unformatted sequence string for pasting into other applications

Taxonomy: Oryctolagus cuniculus

Variable modifications: Carbamidomethyl (C)
Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
Sequence Coverage: **33%**

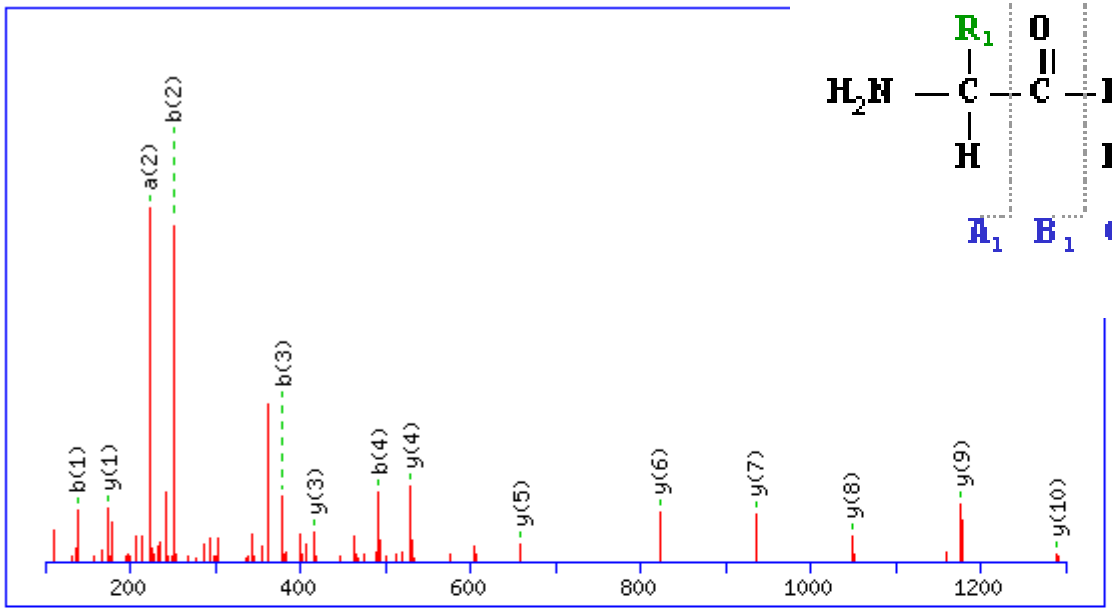
Matched peptides shown in **Bold Red**

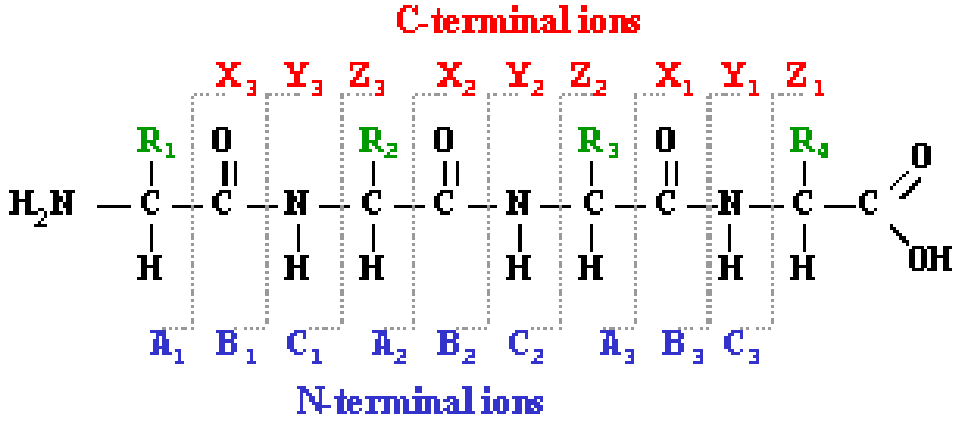
```
     1 SRPLSDQEKR KQISVRGLAG VENVTELKKN FNRHLHFTLV KDRNVATPRD
    51 YYFALAHTVR DHLVGRWIRT QQHYYEKDPK RIYYLSLEFY MGRTLQNTMV
   101 NLALENACDE ATYQLGLDME ELEEIEEDAG LGNGGLGRLA ACFLDSMATL
   151 GLAAYGYGIR YEFGIFNQKI CGGWQMEEAD DWLRYGNPWE KARPEFTLPV
   201 HFYGRVEHTS QGAKWVDTQV VLAMPYDTPV PGYRNNVVNT MRLWSAKAPN
   251 DFNLKDFNVG GYIQAVLDRN LAENISRVLY PNDNFFEGKE LRLKQEYFVV
   301 AATLQDIIRR FKSSKFGCRD PVRTNFDAFP DKVAIQLNDT HPSLAIPELM
   351 RVLVDLERLD WDKAWEVTVK TCAYTNHTVL PEALERWPVH LLETLLPRHL
   401 QIIYEINQRF LNRVAAAFPG DVDRLRRMSL VEEGAVKRIN MAHLCIAGSH
   451 AVNGVARIHS EILKKTIFKD FYELEPHKFQ NKTNGITPRR WLVLCNPGLA
   501 EIIAERIGEE YISDLDQLRK LLSYVDDEAF IRDVAKVKQE NKLKFAAYLE
   551 REYKVHINPN SLFDVQVKRI HEYKRQLLNC LHVITLYNRI KKEPNKFVVP
   601 RTVMIGGKAA PGYHMAKMII KLITAIGDVV NHDPVVGDRL RVIFLENYRV
   651 SLAEKVIPAA DLSEQISTAG TEASGTGNMK FMLNGALTIG TMDGANVEMA
   701 EEAGEENFFI FGMRVEDVDR LDQRGYNAQE YYDRIPELRQ IIEQLSSGFF
   751 SPKQPDLFKD IVNMLMHHDR FKVFADYEEY VKCQERVSAL YKNPREWTRM
   801 VIRNIATSGK FSSDRTIAQY AREIWGVEPS RQRLPAPDEK IP
```

Show predicted peptides also

Sort Peptides By ◉ Residue Number ○ Increasing Mass ○ Decreasing Mass

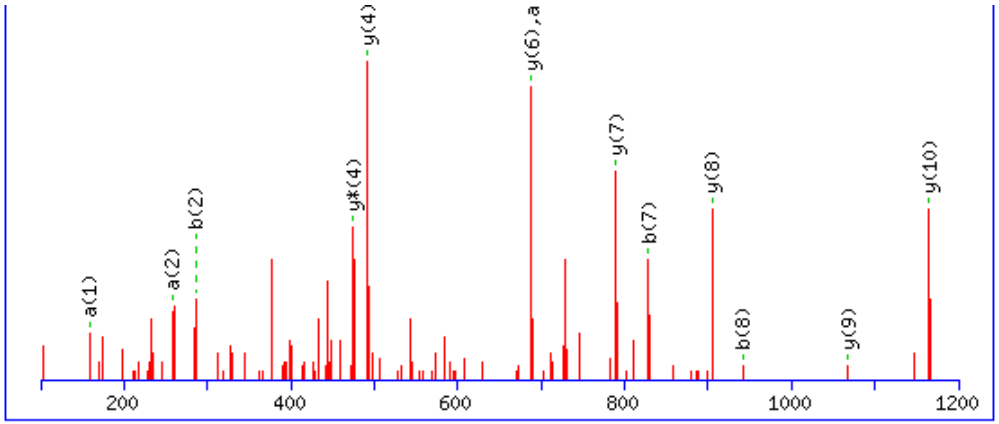
Start - End	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Sequence	
17 - 28	615.40	1228.78	1228.67	0.11	0	GLAGVENVTELK	(Ions score 82)
17 - 29	453.30	1356.89	1356.76	0.13	1	GLAGVENVTELKK	(Ions score 41)
81 - 93	570.99	1709.95	1709.86	0.09	1	RIYYLSLEFYMGR	(Ions score 16)
161 - 169	573.32	1144.62	1144.56	0.06	0	YEFGIFNQK	(Ions score 45)
215 - 234	769.78	2306.32	2306.14	0.18	0	WVDTQVVLAMPYDTPVPGYR	(Ions score 38)
215 - 234	1154.16	2306.31	2306.14	0.17	0	WVDTQVVLAMPYDTPVPGYR	(Ions score 25)
256 - 269	783.98	1565.94	1565.78	0.16	0	DFNVGGYIQAVLDR	(Ions score 70)
256 - 269	522.98	1565.91	1565.78	0.12	0	DFNVGGYIQAVLDR	(Ions score 63)
278 - 289	721.89	1441.76	1441.69	0.08	0	VLYPNDNFFEGK	(Ions score 25)





Monoisotopic mass of neutral peptide Mr(calc): 1425.77
Ions Score: 70 **Expect:** 4.1e-05
Matches (Bold Red): 14/112 fragment ions using 21 most intense peaks

#	a	a^{++}	a*	a^{*++}	b	b^{++}	b*	b^{*++}	Seq.	y	y^{++}	y*	y^{*++}	#
1	110.07	55.54			**138.07**	69.54			H					11
2	**223.16**	112.08			**251.15**	126.08			L	**1289.72**	645.36	1272.69	636.85	10
3	351.21	176.11	334.19	167.60	**379.21**	190.11	362.18	181.59	Q	**1176.64**	588.82	1159.61	580.31	9
4	464.30	232.65	447.27	224.14	**492.29**	246.65	475.27	238.14	I	**1048.58**	524.79	1031.55	516.28	8
5	577.38	289.19	560.36	280.68	605.38	303.19	588.35	294.68	I	**935.49**	468.25	918.47	459.74	7
6	740.45	370.73	723.42	362.21	768.44	384.72	751.41	376.21	Y	**822.41**	411.71	805.38	403.20	6
7	869.49	435.25	852.46	426.73	897.48	449.25	880.46	440.73	E	**659.35**	330.18	642.32	321.66	5
8	982.57	491.79	965.55	483.28	1010.57	505.79	993.54	497.27	I	**530.30**	265.66	513.28	257.14	4
9	1096.61	548.81	1079.59	540.30	1124.61	562.81	1107.58	554.30	N	**417.22**	209.11	400.19	200.60	3
10	1224.67	612.84	1207.65	604.33	1252.67	626.84	1235.64	618.32	Q	303.18	152.09	286.15	143.58	2



Monoisotopic mass of neutral peptide Mr(calc): 2306.14
Ions Score: 38 **Expect:** 0.041
Matches (Bold Red): 13/212 fragment ions using 22 most intense peaks

#	a	a^{++}	a*	a^{*++}	b	b^{++}	b*	b^{*++}	Seq.	y	y^{++}	y*	y^{*++}	#
1	**159.09**	80.05			187.09	94.05			W					20
2	**258.16**	129.58			**286.15**	143.58			V	2121.07	1061.04	2104.04	1052.52	19
3	373.19	187.10			401.18	201.09			D	2022.00	1011.50	2004.97	1002.99	18
4	474.23	237.62			502.23	251.62			T	1906.97	953.99	1889.95	945.48	17
5	602.29	301.65	585.27	293.14	630.29	315.65	613.26	307.13	Q	1805.93	903.47	1788.90	894.95	16
6	701.36	351.18	684.34	342.67	729.36	365.18	712.33	356.67	V	1677.87	839.44	1660.84	830.92	15
7	800.43	400.72	783.40	392.21	**828.43**	414.72	811.40	406.20	V	1578.80	789.90	1561.77	781.39	14
8	913.51	457.26	896.49	448.75	**941.51**	471.26	924.48	462.74	L	1479.73	740.37	1462.70	731.86	13
9	984.55	492.78	967.52	484.27	1012.55	506.78	995.52	498.26	A	1366.65	683.83	1349.62	675.31	12
10	1115.59	558.30	1098.57	549.79	1143.59	572.30	1126.56	563.78	M	1295.61	648.31	1278.58	639.79	11
11	1212.64	606.83	1195.62	598.31	1240.64	620.82	1223.61	612.31	P	**1164.57**	582.79	1147.54	574.27	10
12	1375.71	**688.36**	1358.68	679.84	1403.70	702.36	1386.68	693.84	Y	**1067.52**	534.26	1050.49	525.75	9
13	1490.73	745.87	1473.71	737.36	1518.73	759.87	1501.70	751.36	D	**904.45**	452.73	887.43	444.22	8
14	1591.78	796.39	1574.76	787.88	1619.78	810.39	1602.75	801.88	T	**789.43**	395.22	772.40	386.70	7
15	1688.84	844.92	1671.81	836.41	1716.83	858.92	1699.80	850.41	P	**688.38**	344.69	671.35	336.18	6
16	1787.90	894.46	1770.88	885.94	1815.90	908.45	1798.87	899.94	V	591.32	296.17	574.30	287.65	5
17	1884.96	942.98	1867.93	934.47	1912.95	956.98	1895.92	948.47	P	**492.26**	246.63	**475.23**	238.12	4
18	1941.98	971.49	1924.95	962.98	1969.97	985.49	1952.95	976.98	G	395.20	198.11	378.18	189.59	3

Multi-**d**imensiona **L**iquid **C**hromatography(MDLC) Coupled Directly to MS

Initial Biological Sample Prep and Protein Extration
(organs, tissues, cell types, subcellular components)

|

MDLC

|

Dry Down
Reduce/Alkylate/Digest

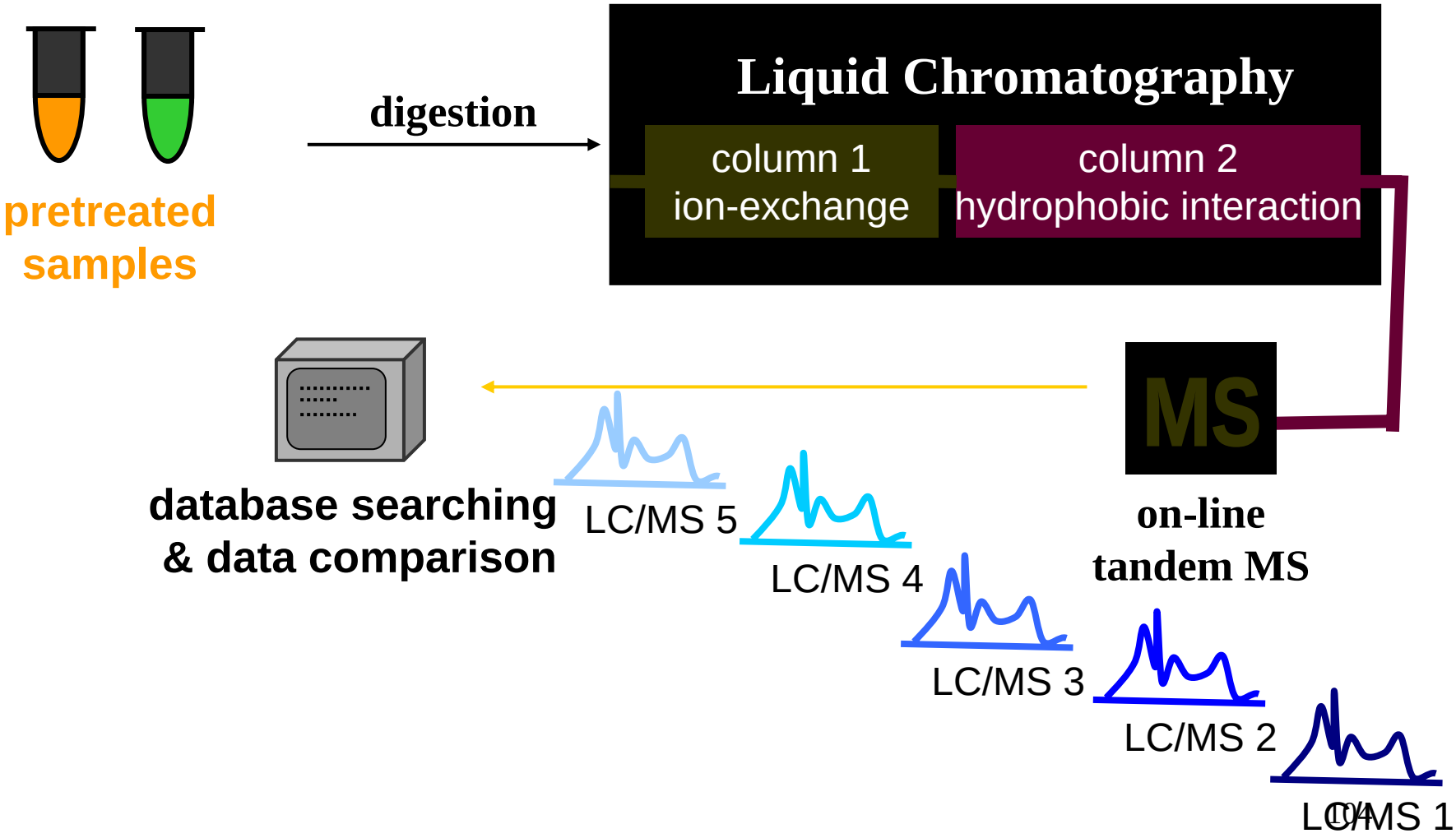
|

Edman Sequencing
Mass Spectrometry

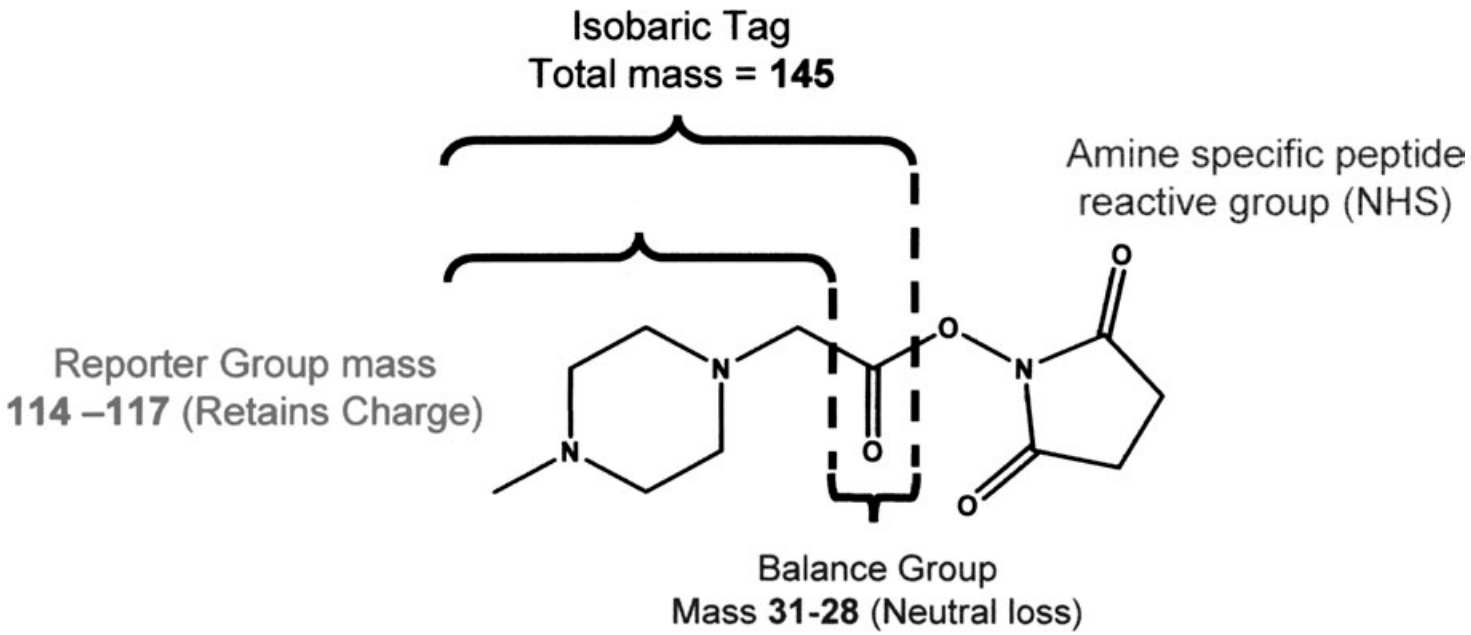
|

Further Analysis and
Characterization

MudPIT (Multidimentional Protein Identification Technology)



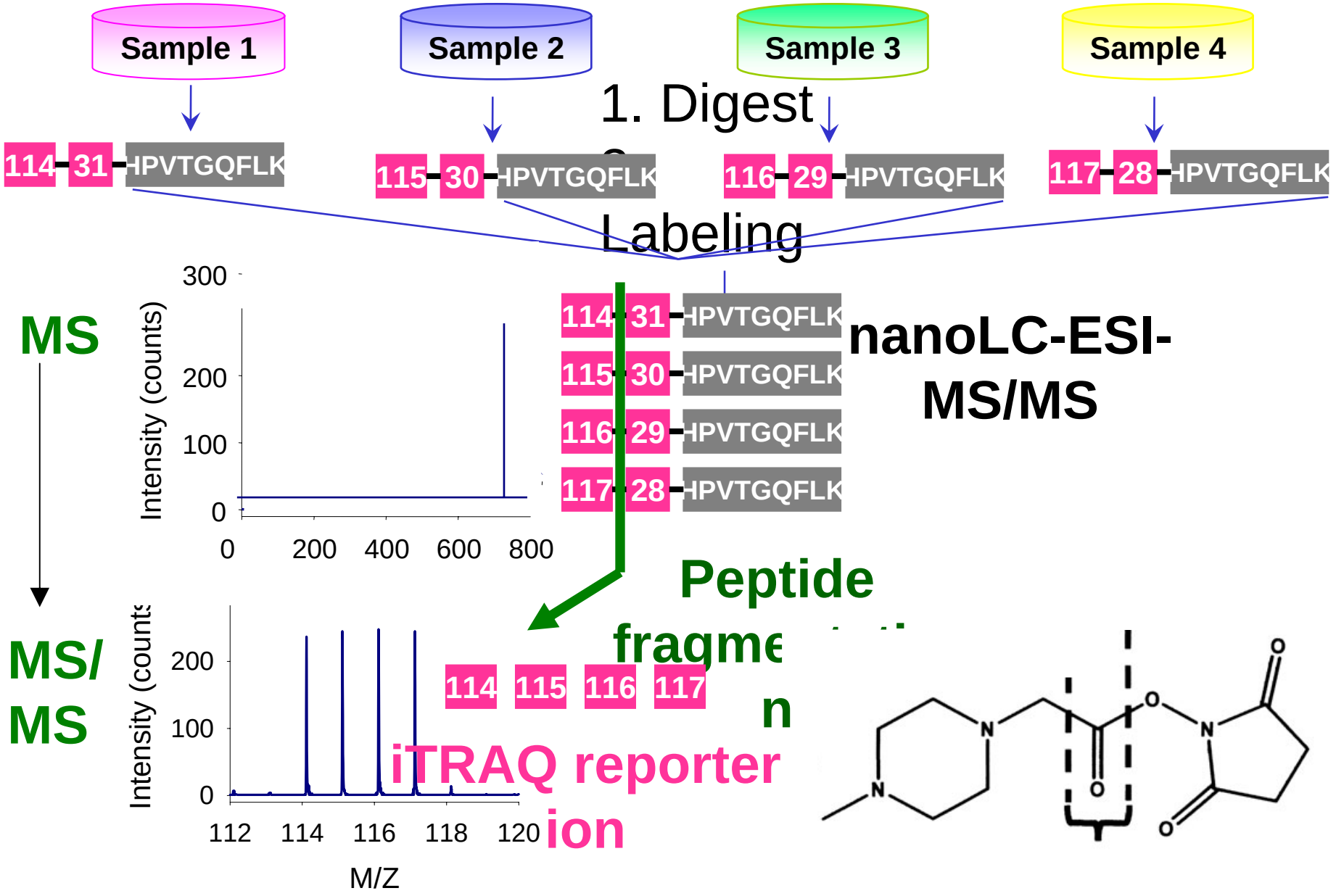
Quantitation by Isotope Labeling (Example: iTRAQ)
Isobaric Tags for Related and Absolute Quantitation



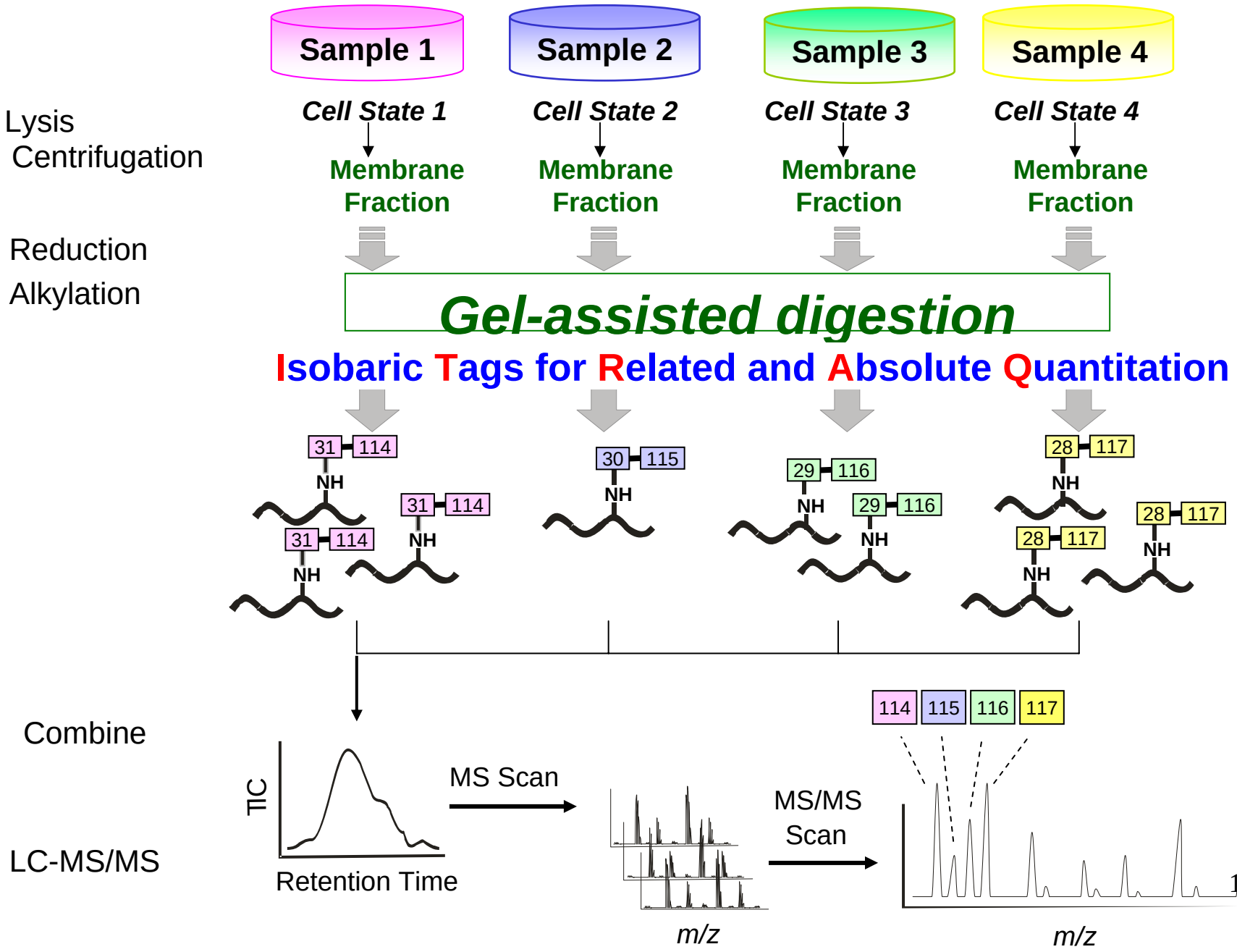
- Multiplex experiment: up to four different biological samples
- Amine-specificity: label all peptides in samples
- Stable isotope reagents: product ion spectrum reveals the difference in abundance from each sample

Journal of Experimental Botany, Vol. 57, No. 7, pp. 1501–1508, 2006

Multiplexed Protein Quantitation by iTRAQ



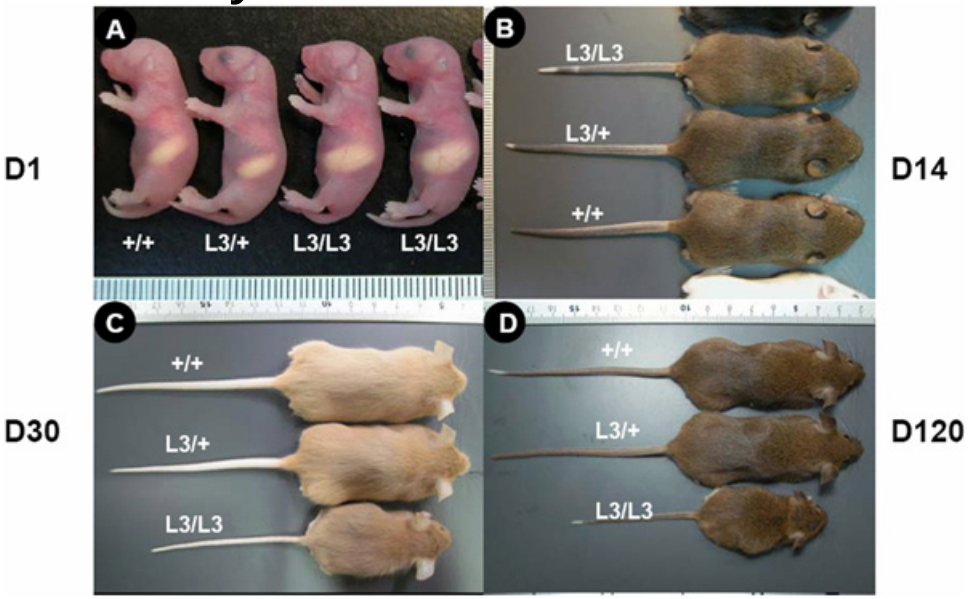
A New Quantitative Strategy for Membrane Proteomics



Autosomal Dominant Polycystic Kidney Disease

ADPKD is one of the most common inherited life-threatening diseases.It affects between 1 in 600 and 1 in 1000 live births in all ethnic groups worldwide.

enlarging renal cysts and a progressive loss of normal kidney tissue



Postnatal growth retardation in Pkd1L3/L3 mutant mice. (A-D) Overall appearances of Pkd1L3/L3 mutant mice and their control littermates at postnatal day 1 (A: D1), day 14 (B: D14), day 30 (C: D30), and day 120 (D: D120) are shown.

Pathogenesis of the renal cyst formation and progression is involved:

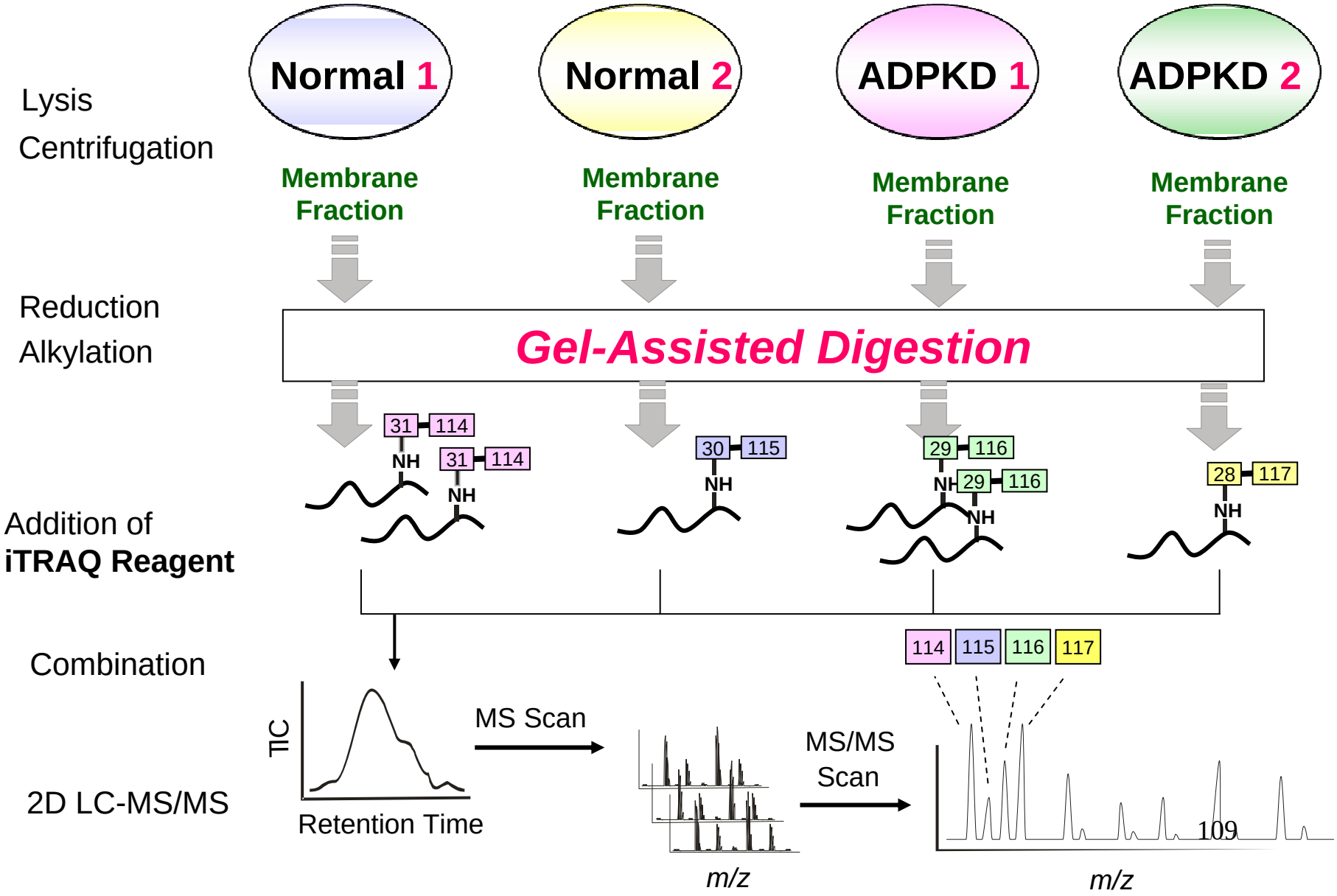
- Alternations in specific membrane protein polarity ?
- Changes in cell-matrix interactions ?

Pkd1 Conditional Knockout

by Dr. Hung LI, IMBS, Academia Sinica

American Journal of Pathology, Vol. 168, No. 1, January 2006

Identification of Differentially Expressed Membrane proteins in ADPKD mice

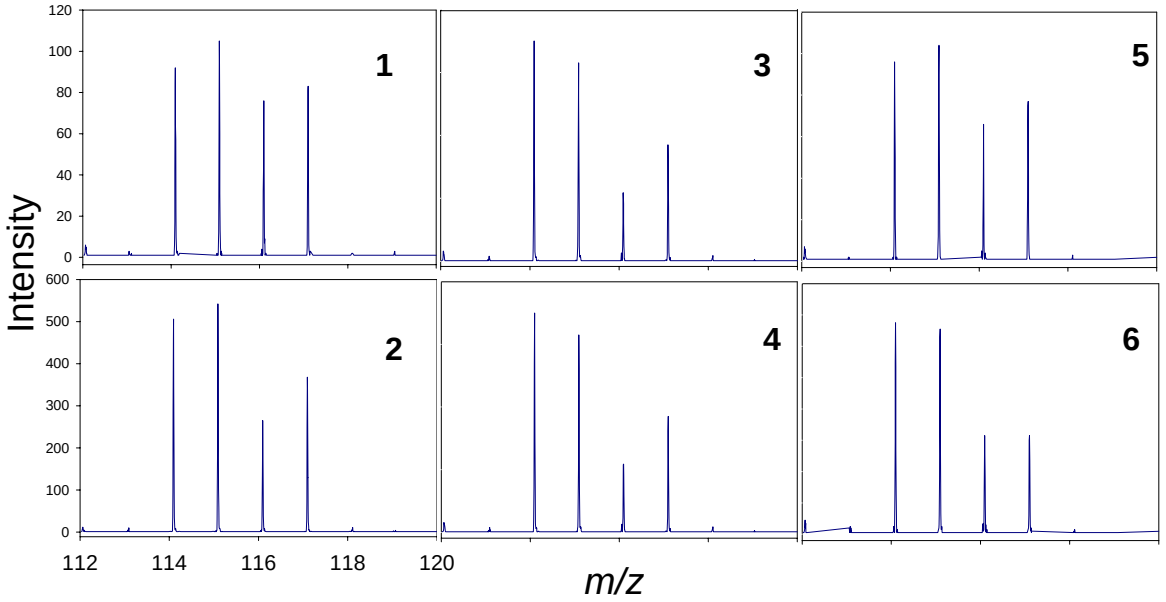


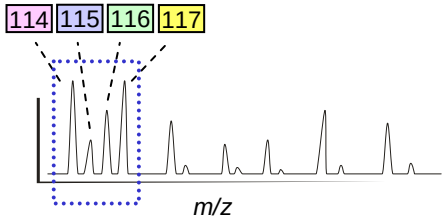
Quantitative Analysis of Membrane Proteins from the ADPKD Mice

845 proteins are quantified (False discovery rate =0)

- ✓ **69** proteins are down-regulated (2-fold)
- ✓ **37** proteins are up-regulated (2-fold)

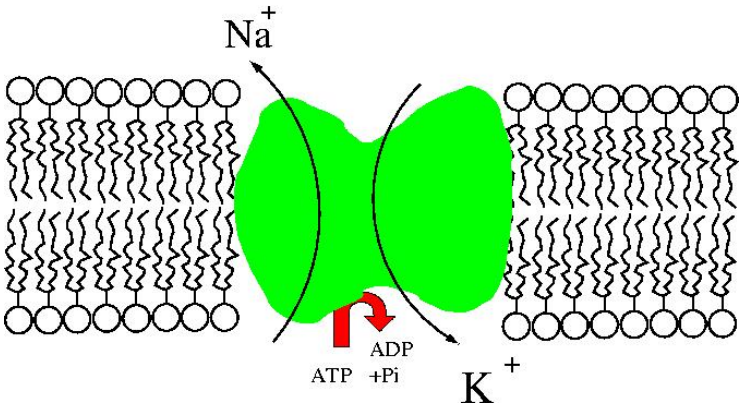
✓ Sodium/potassium-transporting ATPase alpha-1 chain precursor (IPI00311682)

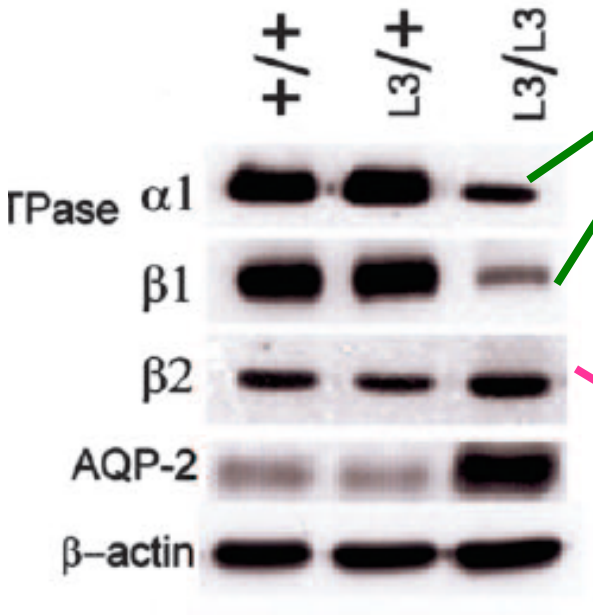




1. **DMTSEELDDILR + iTRAQ (Nterm)**
2. **LIFDNLK + iTRAQ (Nterm); iTRAQ (K)**
3. **DMTSEELDDILR + iTRAQ (Nterm)**
4. **VIMVTGDHPITAK + iTRAQ (Nterm), (K)**
5. **DAFQNAYLELGGLGER.V + iTRAQ (Nterm)**
6. **GVGIISEGNETVEDIAAR.L + iTRAQ (Nterm)**

The **Na^+-K^+ ATPase** is an ion pump that uses the energy from the hydrolysis of ATP to actively pump sodium and potassium ions against their concentration





- **Down-regulation of α1 or β1 subunit**

Diminish the *trans*-epithelial sodium gradients, leading to fluid accumulation in renal cysts.

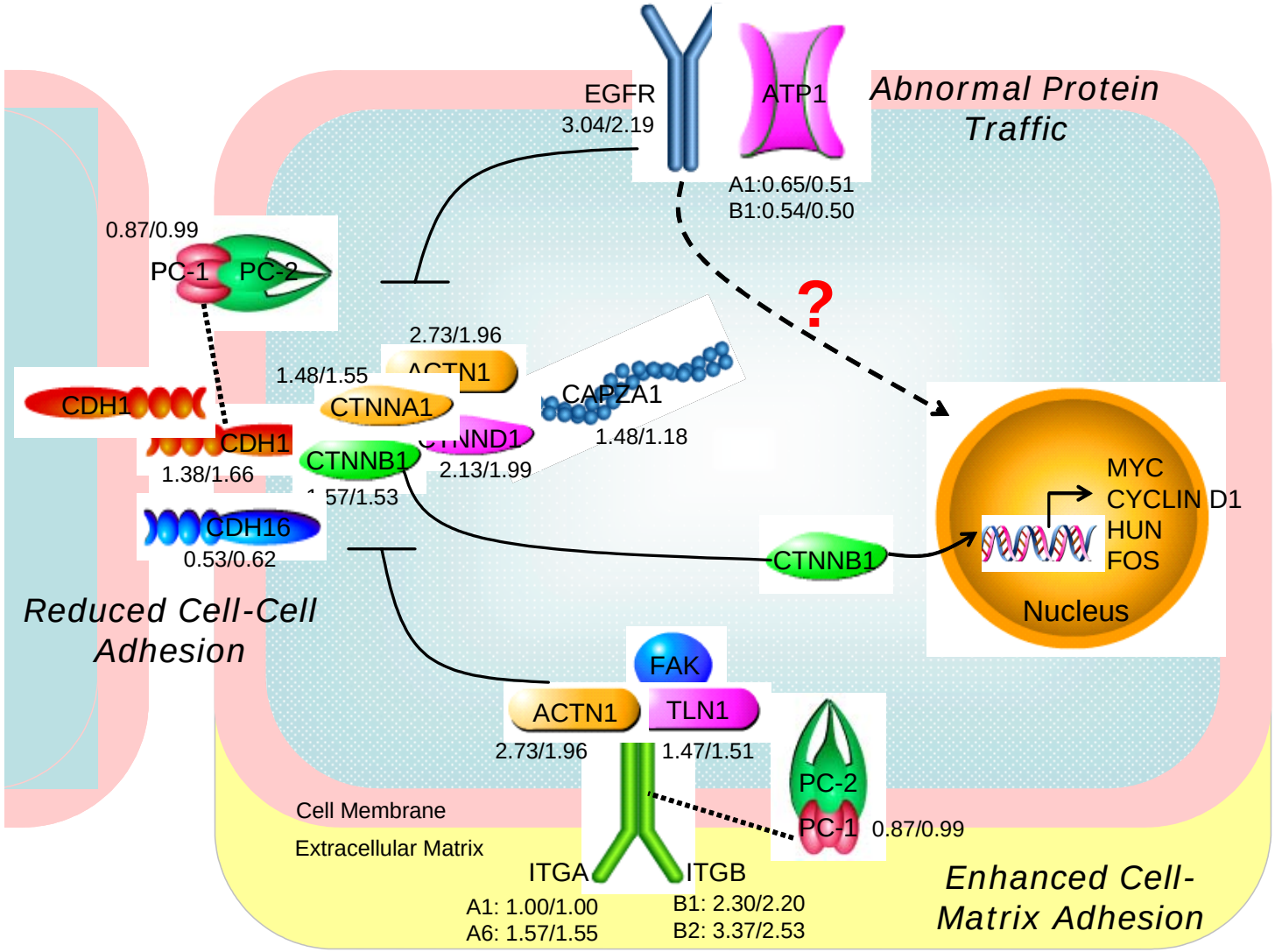
- **Up-regulation of β2 subunit**

Because the β2 subunit is highly expressed in normal fetal kidneys, this result suggests a degree of either undifferentiation or dedifferentiation in the renal cystic epithelium.

Result provided by Dr. Hung, Li, IMB,

Mol. Cell. Proteomics, **2008**, 7, 1983–1997

Systematic Manifestation of the Altered Membrane Proteome in ADPKD



Mol. Cell. Proteomics, **2008**, 7, 1983–1997

List of Potential Drugable Target Proteins

Protein	Drugs	Diseases[b]
Epidermal growth factor receptor	Cetuximab, AEE 788, panitumumab, BMS-599626, ARRY-334543, XL647, canertinib, gefitinib, HKI-272, PD 153035, lapatinib, vandetanib, erlotinib	ADPKD' lung cancer, head and neck cancer, breast cancer, ovarian cancer
Prostaglandin-endoperoxide synthase 1 (cycloxygense)	Acetaminophen/pentazocine, acetaminophen/clemastine/pseudoephedrine, aspirin/butalbital/caffeine, acetaminophen/caffeine/dihydrocodeine	PKD, lung cancer, ovarian cancer
Na^+/K^+ ATPase alpha-1 chain	Cardiotonic steroid	ADPKD, cardiovascular disease, cancer
Fibrinogen beta chain	Thrombin	Congenital afrinogenemia, hemorrhage, hypofibrinogenemia
Fibrinogen gamma chain	Thrombin	Congenital afibrinogenemia, hemorrhage, dysfibrinogenemia, hypofibrinogenemia
Fibrinogen alpha chain	Thrombin	Congenital afibrinogenemia, hypofibrinogenemia, dysfibrinogenemia, liver cancer, hereditary renal amyloidoses
Alcohol dehydrogenase 1C (class I), gamma polypeptide	Fomepizole	Lung cancer
Collagen, type VI, alpha 1	Collagenase	Prostate cancer
Plasminogen	Tissue plasminogen activator, tenecteplase, aprotinin, epsilon-amino caproic acid	Metastasis
Tumor-associated calcium signal transducer 1	Tucotuzumab celmoleukin	Ovarian cancer, prostatic carcinoma
Folate hydrolase (prostate-specific membrane antigen) 1	Capromab pendetide	Oral cancer, head and neck cancer
Dipeptidyl-peptidase 4 (CD26)	Saxagliptin, Talabostat, SYR-322, Sitagliptin	Lung cancer, neoplasia

Imaging Mass Spectrometry

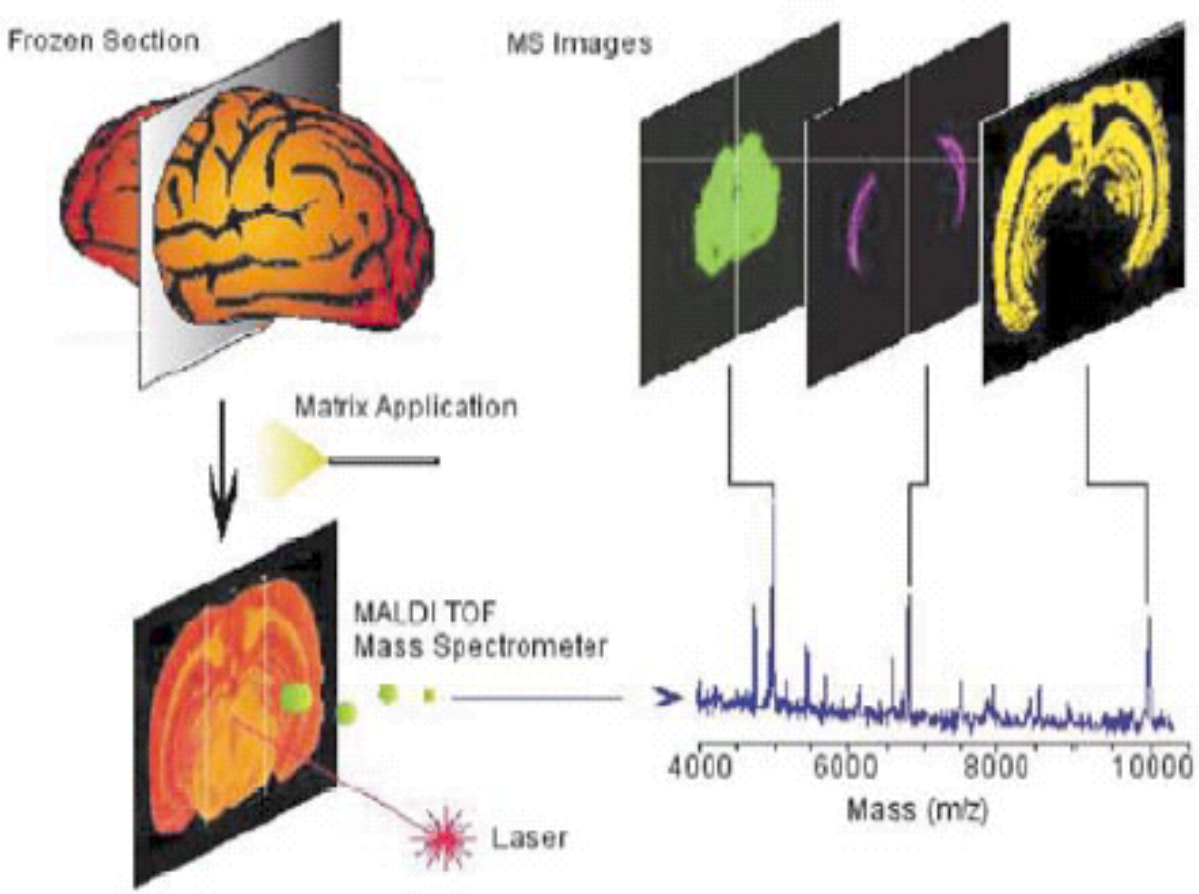


Fig. 1 Methodology developed for the spatial analysis of tissue by MALDI mass spectrometry. Frozen sections are mounted on a metal plate, coated with an UV-absorbing matrix and placed in the mass spectrometer.
A pulsed UV laser desorbs and ionizes analytes from the tissue and their m/z values are determined using a time-of-flight analyzer. From a raster over the tissue and measurement of the peak intensities over thousands of spots, mass spectrometric images are generated at specific molecular weight values.

Nat. Med. **2001**, 7, 493-496

MALDI Imaging Strategies

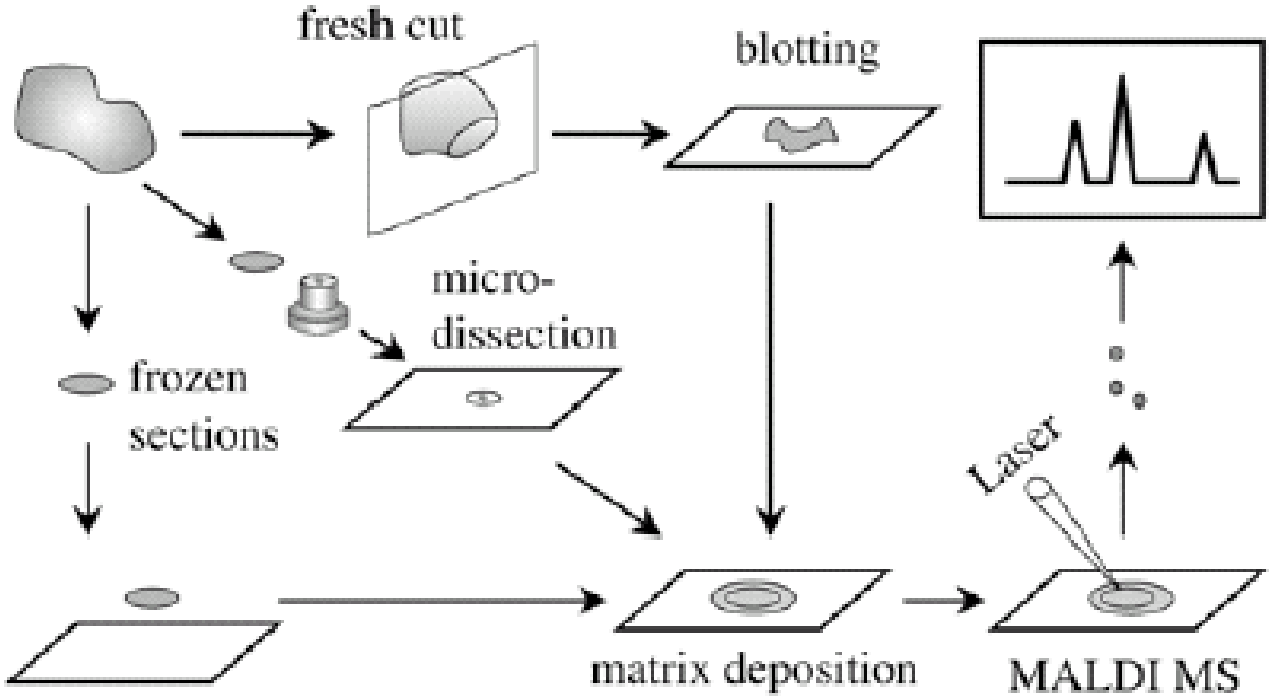
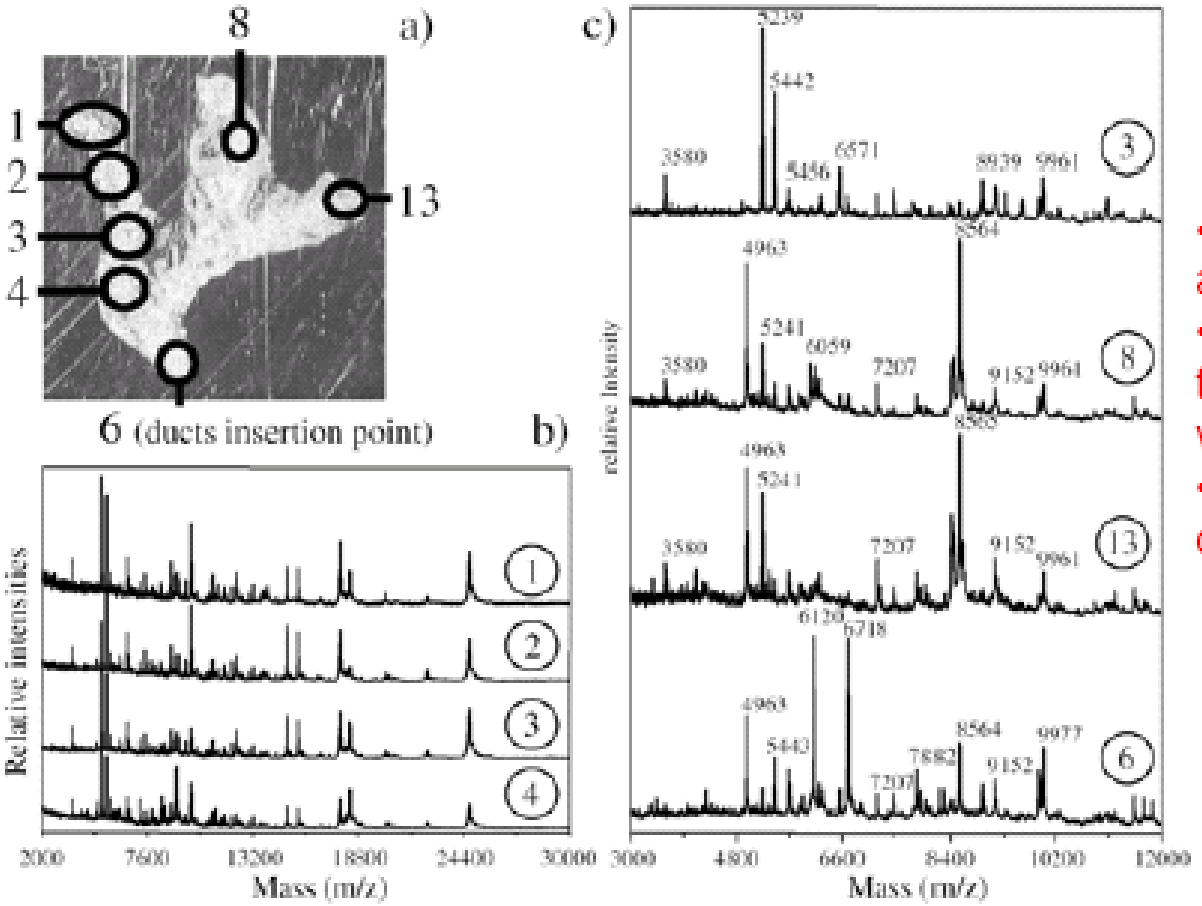


Figure 6. Schematics of the different strategies and sample preparation methods employed to investigate protein populations in tissue samples.

1. Membrane blotting of fresh tissue section, transferring some of the proteins present
2. Direct analysis of a frozen and dried tissue section by coating it with MALDI matrix
3. Laser capture microdissection followed by MALDI MS of selected cell

JMS **2001**, *36*, 355-369

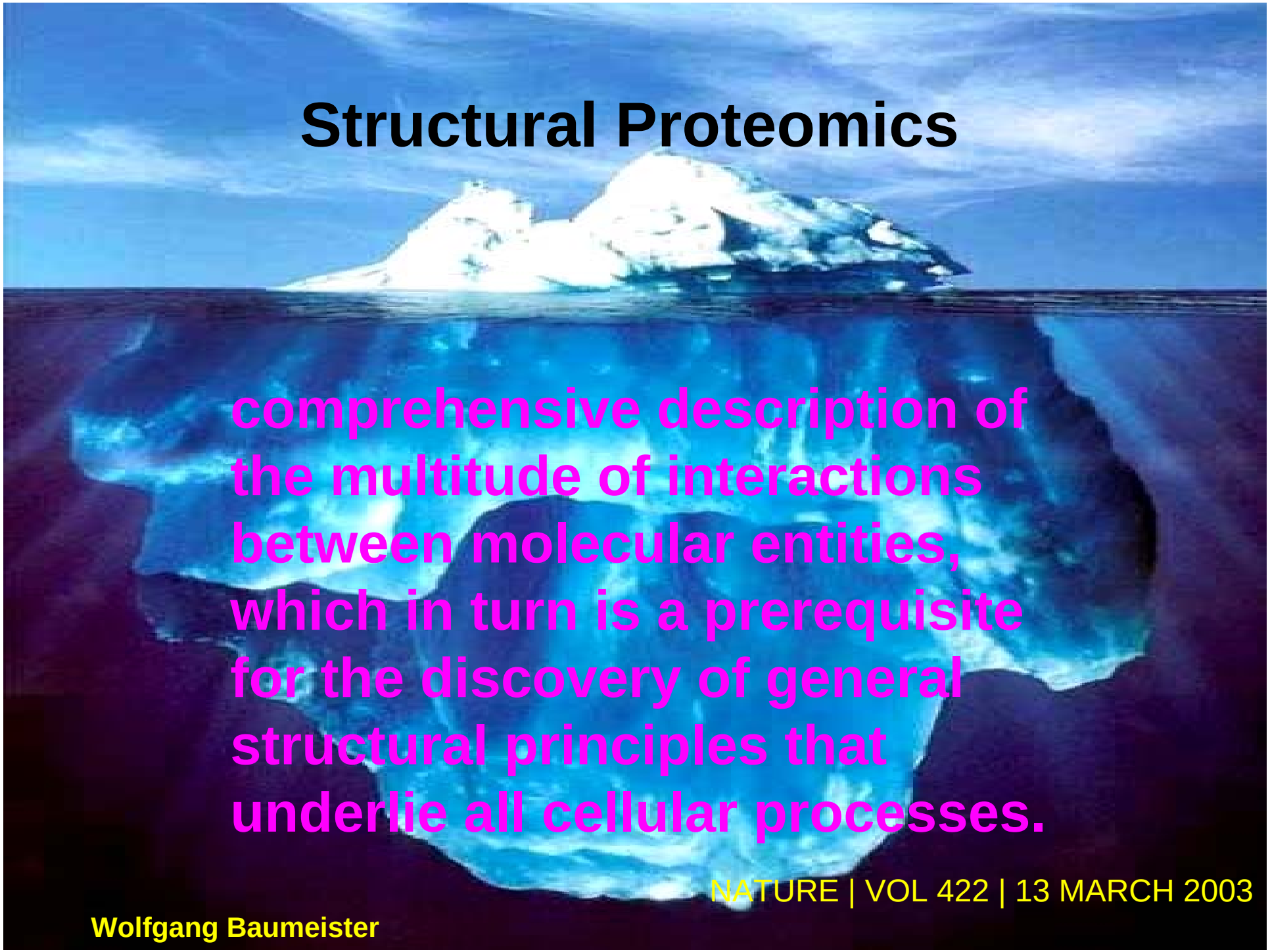
MALDI MS Analysis of Prostate Branches



- Matrix deposited using a narrow capillary
- Within the duct (1-4) the protein profiles are very similar
- Variability seen in other regions

Figure 10. MALDI-MS analysis of a 12 µm tissue section obtained from the mouse anterior prostate: (a) optical image of the section. The drops of matrix are circled. (b) Comparison of the protein profiles obtained in the left branch of the section. (c) Comparison of the protein profiles obtained in all three major branches and also near the point of embranchment.

JMS **2001**, *36*, 355-369


Structural Proteomics
comprehensive description of the multitude of interactions between molecular entities, which in turn is a prerequisite for the discovery of general structural principles that underlie all cellular processes.
NATURE | VOL 422 | 13 MARCH 2003
Wolfgang Baumeister