Mass Spectrometry-based Proteomics

- Introduction to Proteomics
- Introduction of Mass Spectrometry
- Protein Identification by Mass Spectrometry
- Quantitation Strategy

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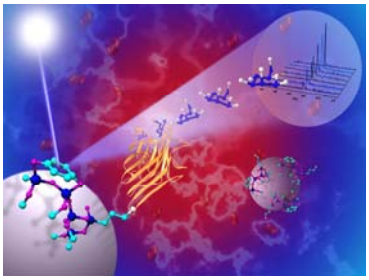
Nanoprobe-based Affinity Mass Spectrometry for Protein Separation



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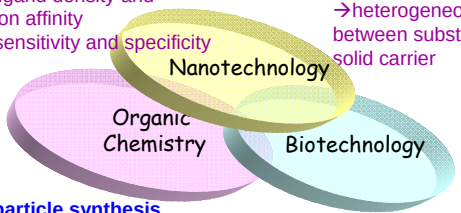
Interdisciplinary Nanotechnology in Biomedical Research

•High surface area to volume ratio
→High ligand density and interaction affinity
→High sensitivity and specificity

•Pseudo-homogeneity in water
→heterogeneous interface between substrate and solid carrier



•Nanoparticle synthesis
→uniform shape and composition
→narrow size distribution

•Surface modification
→high reaction efficiency
→high biocompatibility

Biological challenges
→low abundant protein enrichment
→site-directed protein modification
→low-affinity protein-ligand interaction
→low molecular-weight molecules identification

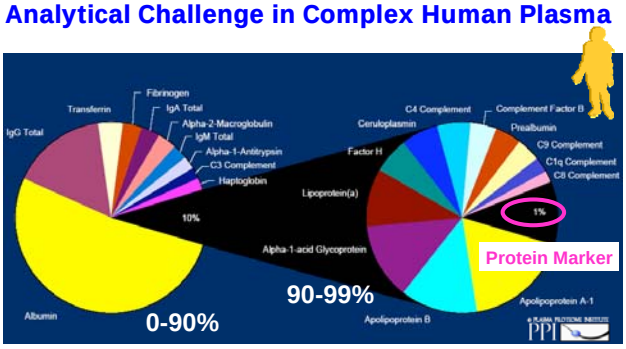
Example:
Immunoassay for Disease Diagnosis

In the post-genome era, the rapid evolution of proteomics research has opened new horizons because it promises to accelerate the discovery of new protein disease markers. The recognition that every disease induces a specific pattern of change in a proteomics microenvironment has important implications on the early detection and progression of diseases. Many clinical diagnostic assays, such as **ELISA**, correlate the concentration of specific protein markers with the onset of disease.

Disease Biomarker for Cancer Diagnostics

Serum Tumor Markers	Primary Clinical Applications	Other Related Cancer Type
Alpha-Fetoprotein (AFP)	Hepatocellular carcinoma (HCC) and germ-cell (nonseminoma) tumor monitoring and diagnosing	
CA 15-3	Breast cancer monitoring[1]	colorectal, liver, lung, ovarian, pancreatic cancer
CA 19-9	Colorectal and pancreatic cancer monitoring	breast, gastric, hepatobiliary, hepatocellular, and ovarian cancer
CA 125	Endometrial and ovarian cancer monitoring[2]	breast, cervical, colorectal, gastrointestinal, lung, pancreatic cancer
Prostate Specific Antigen (PSA)	Prostate cancer monitoring and diagnosing[3]	

Analytical Challenge in Complex Human Plasma



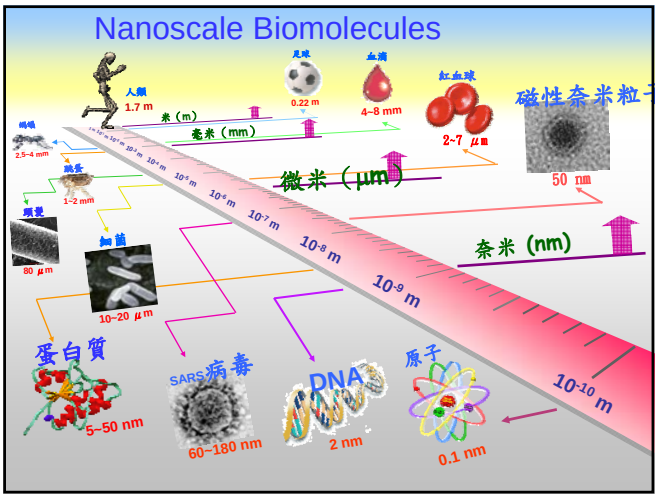
Twenty-two proteins constitute **99%** of the protein content of plasma

Molecular & Cellular Proteomics 2:1096–1103, 2003

www.plasmaproteome.org

Challenge: Low Abundance of Protein Biomarkers

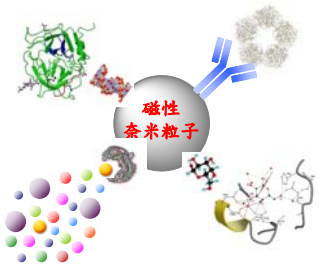
Biomarker	Cancer Type	Concentration [*pmol/liter*]
a-Fetoprotein (AFP)	Hepatoma; testicular	150
PSA	Prostate	140
Carcinoembryonic antigen	Colon; breast; lung; pancreatic	30
Chroriogonadotropin (hCG)	Testicular cancer;	20
Albumin		600,000,000
Immunoglobulins		30,000,000



Advantages of Nanoparticles

- Bio-compatibility
- Unique physical property, which directly related to size, composition, and shape
- Small size (1-100 nm), Large surface-to-volume ratio and globular shape
- Unusually high target binding efficiency– provides more multivalent and three-dimensional interactions between ligands and receptors
- Overall structural robustness

Versatile Nanoparticle-Based Biomolecular Assay



Antibody, Protein, DNA, Small molecule (inhibitor, Drug…etc)

- Nanoparticle can be easily functionalized on surface
- Overall structural robustness

Using Nanoparticles as Bioaffinity Probe

• Chemical Challenges

1) Size distribution
2) Surface modification
3) Surface characterization

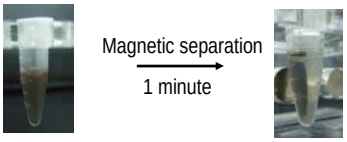
Commonly used nanoparticles

- Gold nanoparticles
  (immunohistochemical staining, biomolecule detection)
- Magnetic nanoparticles
  Iron oxide, FePt … (MRI contrast agent, hyperthermia)
- Polymer nanoparticles
  (gene delivery, drug delivery)
- Semiconductor nanoparticle
  Quantum dots (in vivo imaging)

Why Magnetic Nanoparticles (I) ?

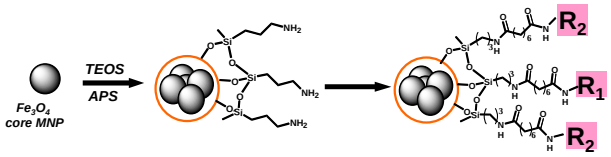
Rapid Magnetic Separation

- Time-effective assay → rapid assay
- Reduce risk on target molecule degradation
- Reduce undesired contamination potentially resulting from centrifugation



Why Magnetic Nanoparticles (II) ?

Simple and effective surface modification



Important Issues for Protein Assay

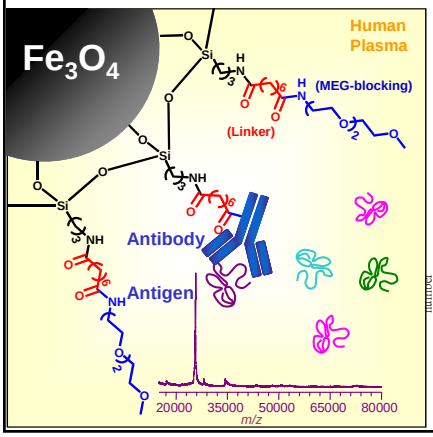
Biological Challenges

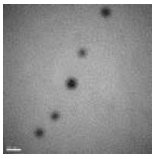
1) Sensitivity for the low-affinity interaction
2) Specificity for the complex system
3) Biocompatibility to the complex bio-condition.

Assay Evaluation

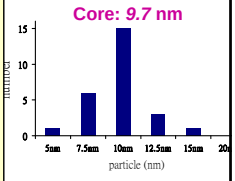
- Precision (Repeatability)
- Accuracy (True value)
- Specificity (no contamination)
- Sensitivity (low abundant target)

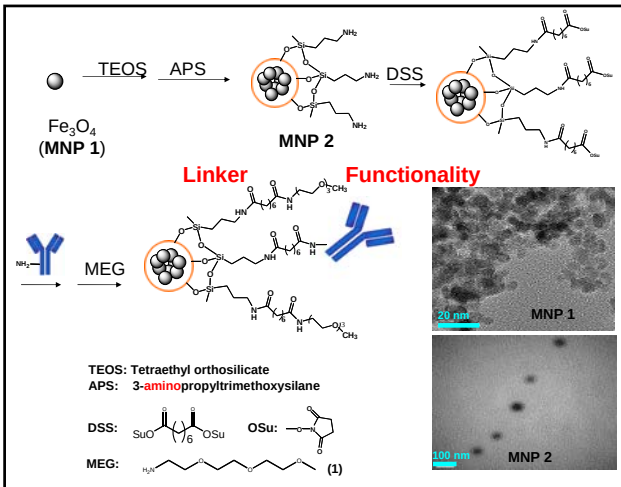
Case Study: Nanoprobe Immunoassay

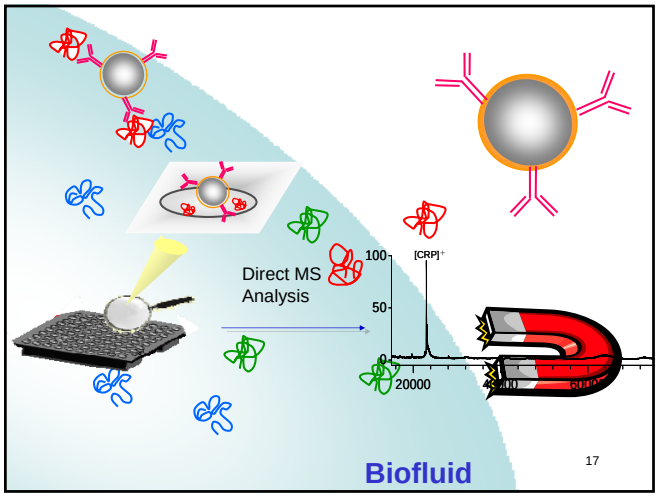




Size-distribution histogram







Why Mass Spectrometry for Protein Detection and Identification?

PROTEOMICS

蛋白質體學

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

Estimated Number of Proteins per Genome

- Haemophilus 1742
- *E. coli* 4413
- Yeast 6600
- Caenorhabditis 18000
- Drosophila 13000
- Human >1000000 (35000 genes)

What Do We See?

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

A Key Technical Challenge in Proteomics

A complex biological problem

↓

Diverse solution

(10^5 – 10^6 for protein abundance)

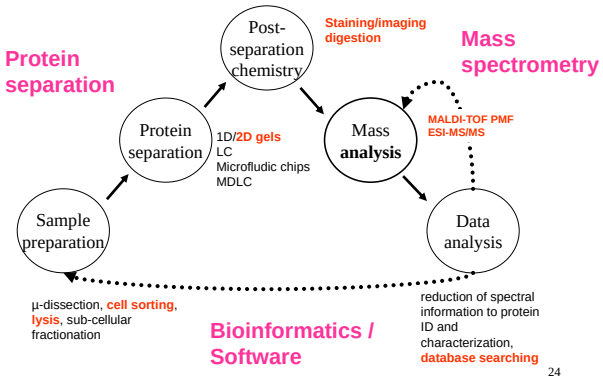
↙

Mass-spectrometry strategy for more complex mixture ?

\+

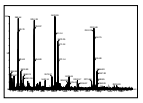
Sample prefractionation

Three Key Technologies in Proteomics



Principle of Mass Spectrometry

What is mass spectrometry?

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

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.

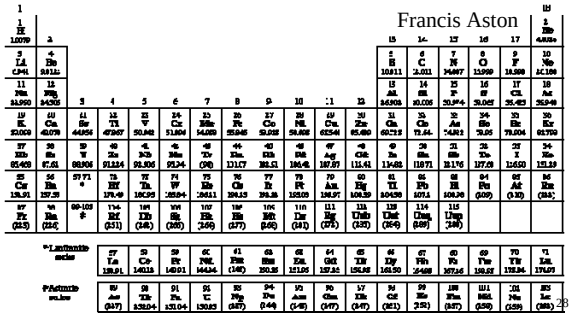
Back to the history……

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.

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

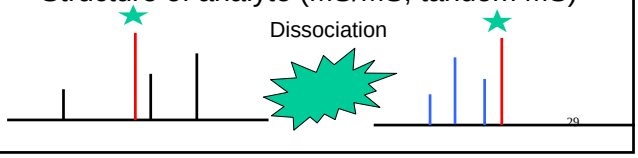
Francis Aston

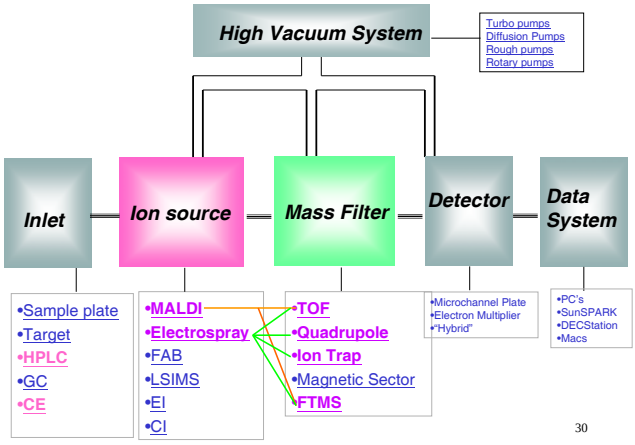


Two different ways of measurement

- Composition of analyte (MS)
  e.g. Peptide mass fingerprinting

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





Ionization Methods

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

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

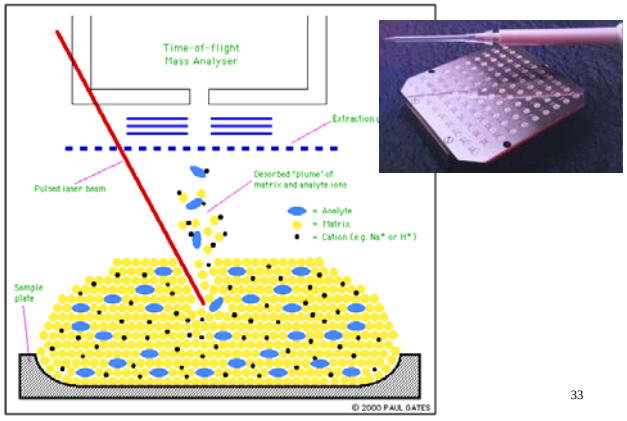
Intact Molecule Non-covalent Complex

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

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

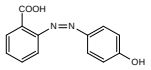
2002 Nobel Prize in Chemistry

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

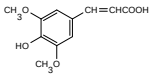


Matrix selection

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

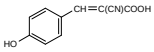


2-(4-hydroxyphenylazo)-benzoic acid (HABA)



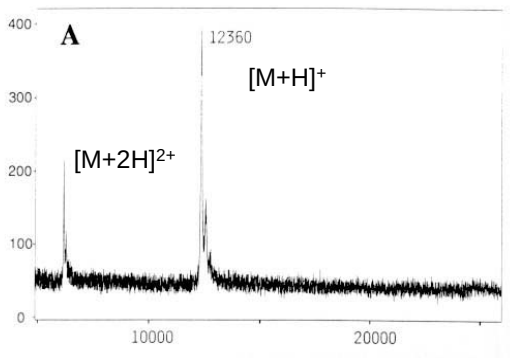
Sinapinic acid (3,5-Dimethoxy-4-hydroxy cinnamic acid)

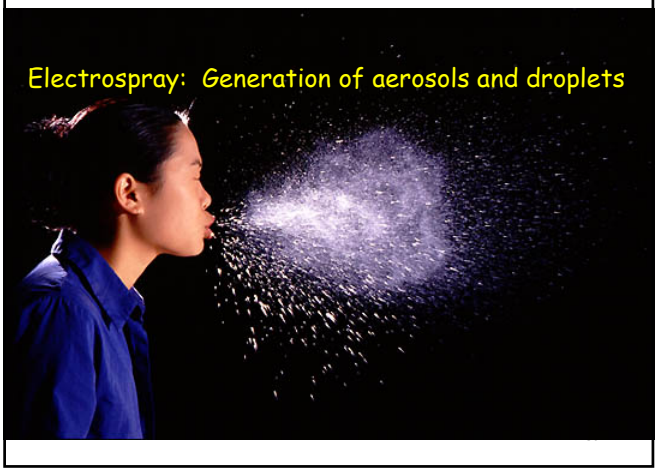
3-hydroxypicolinic acid (3-HPA)



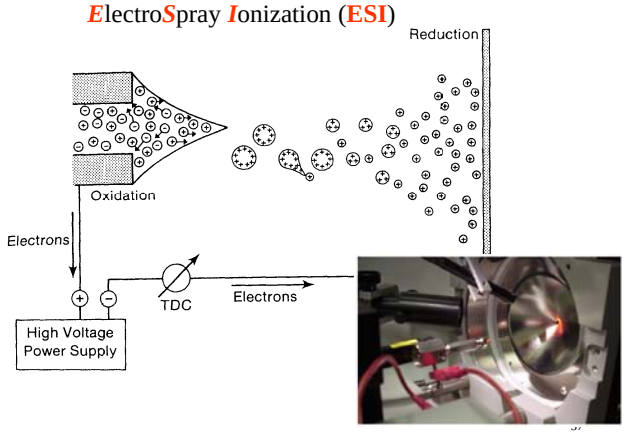
α-cyano-4-hydroxycinnamic acid

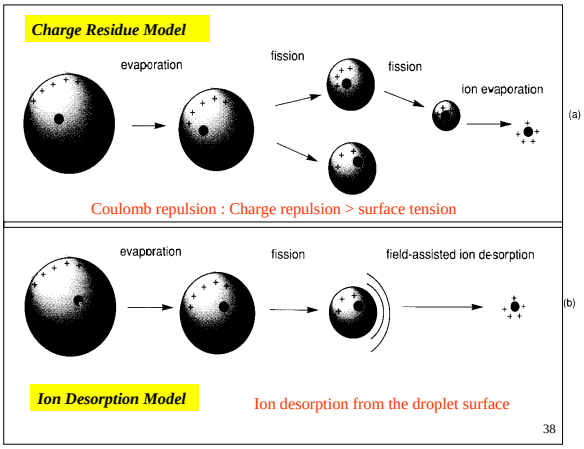
A typical mass spectra





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





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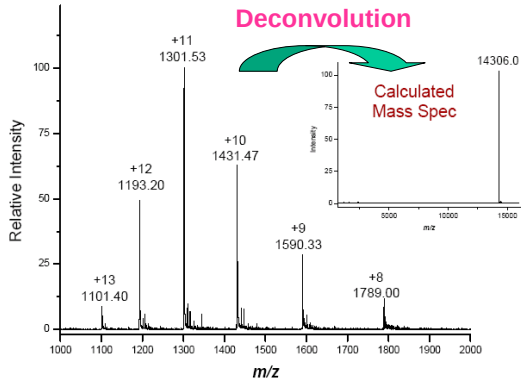
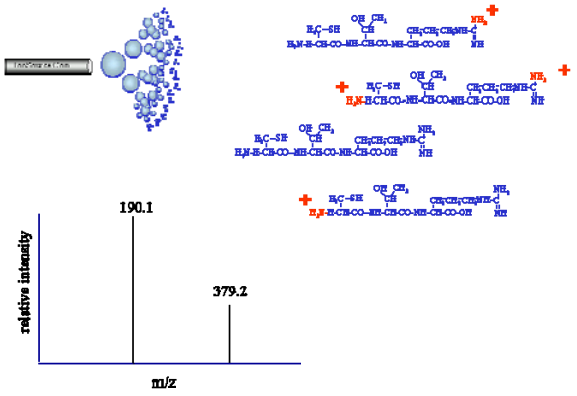
Multiple-charged ESI spectra





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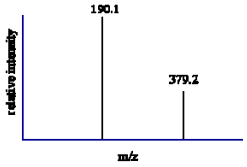
Deconvolution



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

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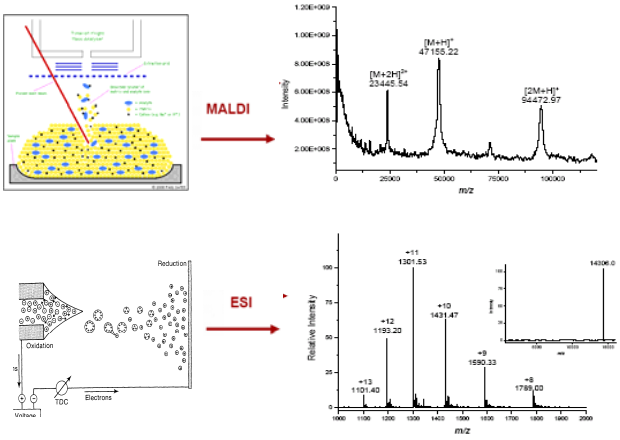
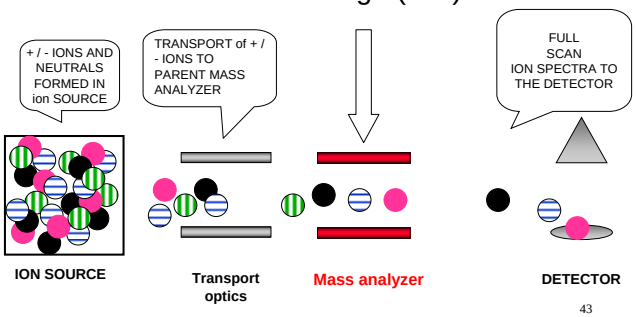
Molecular Weight of Proteins



Mass Analysis

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



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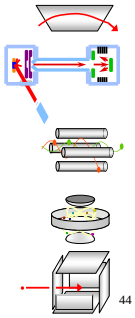
- Sector Instruments
- Time-of-flight Analyzer
- Quadrupole Mass Filter
- Ion-Trap Instrument
- FT-ICR



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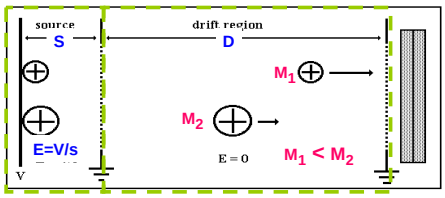
Time-of-flight Analyzer

- 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 *mv*² = *zV***), 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-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

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

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

Relation between m/z and t

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

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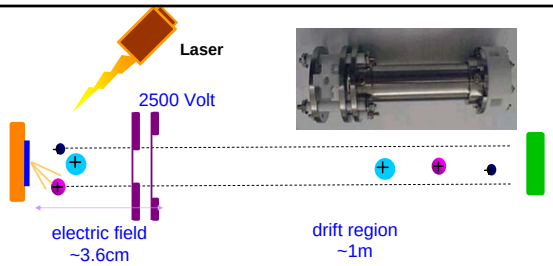
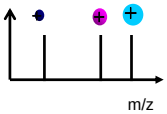


Mass spectrum

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



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….)

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蛋白質定性及定量分析

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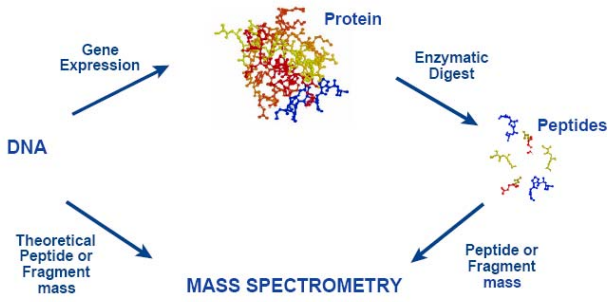
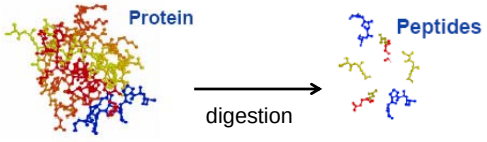
Peptide and Protein Analysis



Mass Spectrometry Methods



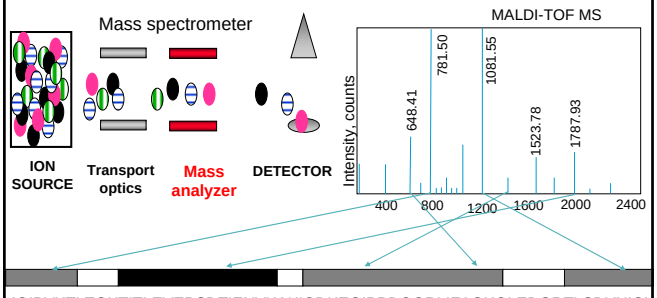
Molecular weight

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)



Peptide Mass Fingerprint

Left to right:

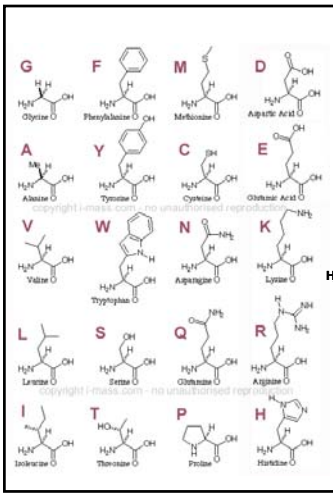
William J. Henzel; ***Protein Chemistry;*** Genentech

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

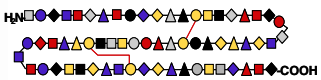
Colin Watanabe; ***Software Engineer;*** Genentech

- This methodology was the first to allow protein identification without the need for time-consuming Edman sequencing or immunoaffinity probes..
- Landmark Study(1993)

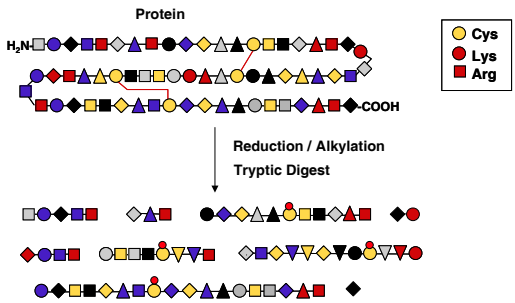
MS approaches alone could be used to analyze proteins from 2DE



Peptide Mass Fingerprinting



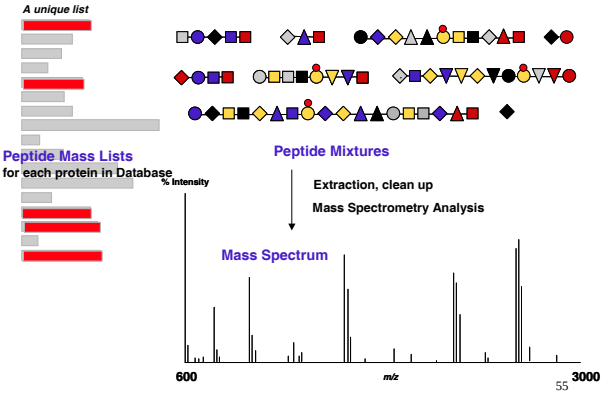
Step 1: Enzyme digestion or chemical fragmentation



Every protein generate a set of unique peptides

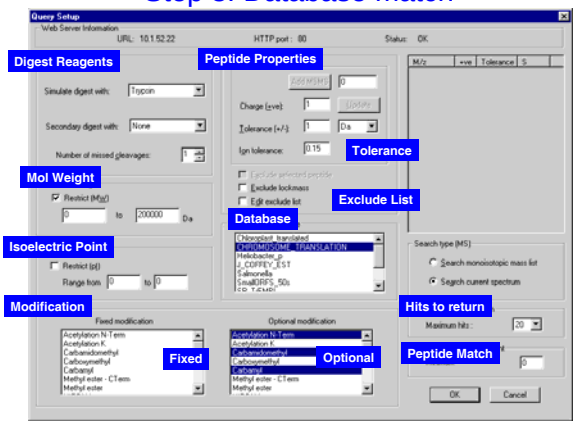
每一個蛋白質有一套獨特的peptide碎片

Step 2: Mass spectrometry analysis



55

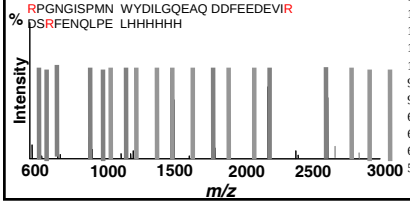
Step 3: Database match



Step 3: In-Silica digestion

Protein Sequence

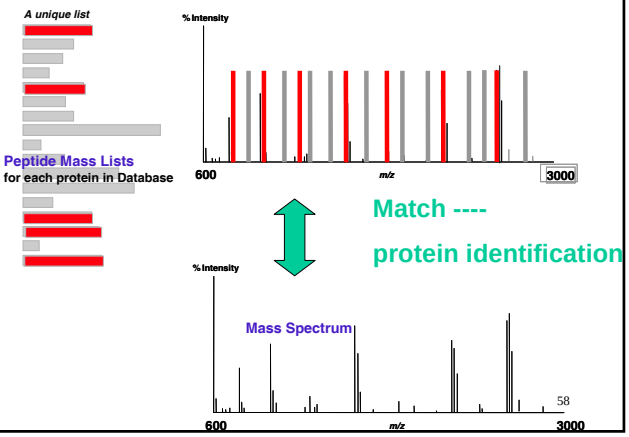
MVYIIAEIGC NHNGDINLAK KMVDVAVSCG
VDAVKFQTFK AEKLISKFAP KAEYQKATTG
TADSQLEMTK RLELSFEEYL EMRDYAISKG
VETFSTPFDE ESLEFLISTD MPIYKIPSGE
ITNLPYLEKI GKQQKKVILS TGMAVMEEIH
QAVNILRQNG TTDISILHCT TEYPTPYPSL
NLNVIHTLKD EFKDLTIGYS DHSIGSEVPI
AAAAMGAEVI EKHFTLDTNM
EVPDHKASAT
PDILAALVKG FALLNQALGR FEKIPDPVEE
KNKIVARKSV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



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

57

Step 4: MS match to Database



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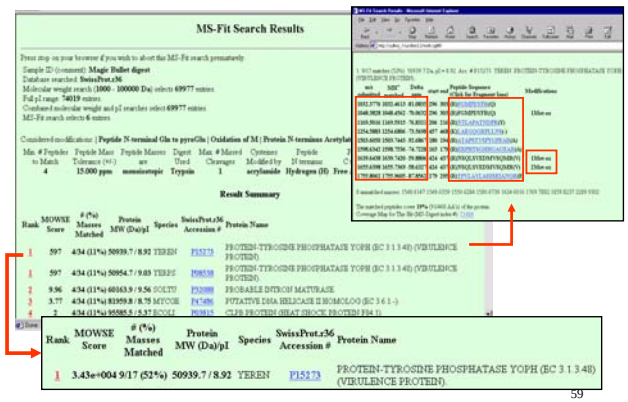
Search Result



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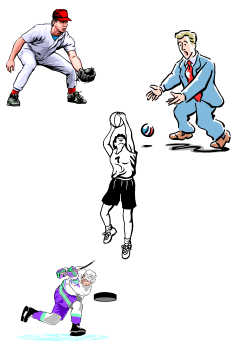
Peptide Mass Fingerprinting (PMF)

蛋白質身份鑑定



In-Gel Digestion

Extract peptides; mass analyze

Each protein has a unique peptide mass list

Database search

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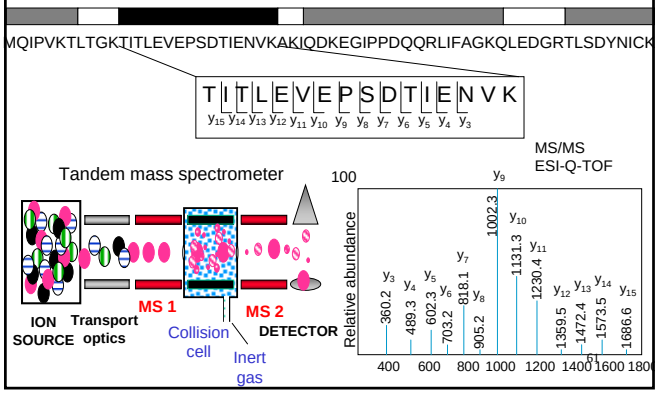
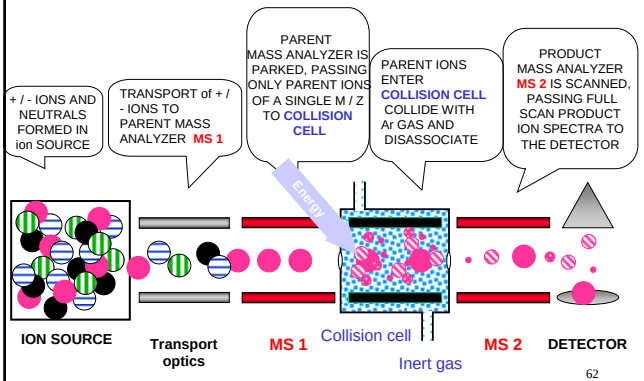
Two Ways of Measurement—
2. Peptide Sequencing (by MS/MS, or Tandem MS)



Tandem Mass Spectrometry



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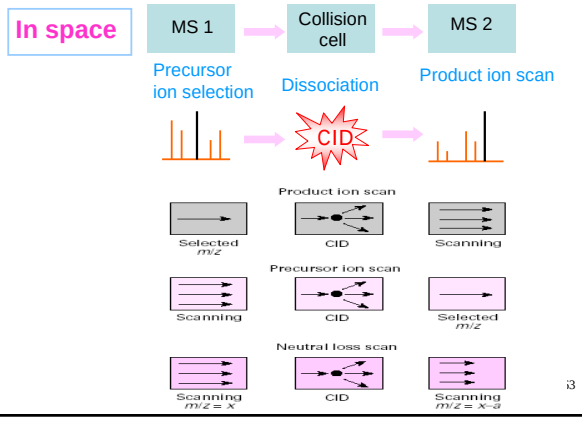
Methods of MS-MS



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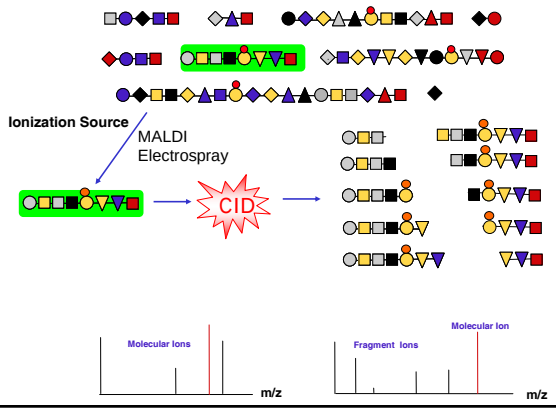
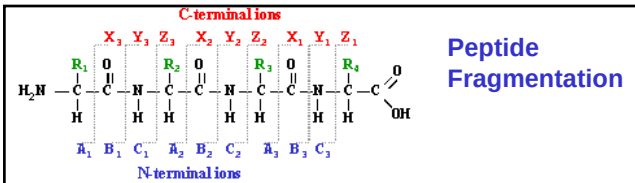
Tandem Mass Spectrometry (MS/MS)



64



Peptide Fragmentation

a,b,c – N-terminal side

x,y,z – C-terminal side

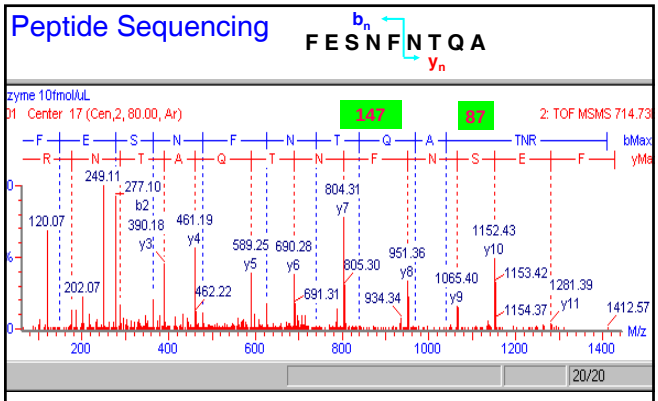
S-P-A-F-D-S-I-M-A-E-T-L-K
(protonated mass 1410.6)

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	$HN{=}C(NH_2)\text{-}N\text{-}(CH_2)_3$-
Asparagine	N,Asn	114.104	$H_2N\text{-}CO\text{-}CH_2$-
Aspartic acid	D,Asp	115.089	$HOOC\text{-}CH_2$-
Cysteine	C,Cys	103.145	$HS\text{-}CH_2$-
Glutamine	Q,Gln	128.131	$H_2N\text{-}CO\text{-}(CH_2)_2$-
Glutamic acid	E,Glu	129.116	$HOOC\text{-}(CH_2)_2$-
Glycine	G,Gly	57.052	H-
Histidine	H,His	137.141	Imidazole-CH_2-
Isoleucine	I,Ile	113.16	$CH_3\text{-}CH_2\text{-}CH(CH_3)$-
Leucine	L,Leu	113.16	$(CH_3)_2\text{-}CH\text{-}CH_2$-
Lysine	K,Lys	128.17	$H_2N\text{-}(CH_2)_4$-
Methionine	M,Met	131.199	$CH_3\text{-}S\text{-}(CH_2)_2$-
Metsulphoxide	Met.SO	147.199	$CH_3\text{-}S(O)\text{-}(CD_2)_2$-
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	$CH_3\text{-}CH(OH)$-
Tryptophan	W,Trp	186.213	Idole-NH-CH=C-CH
Tyrosine	Y,Tyr	163.176	4-OH-Phenyl-CH_2-
Valine	V,Val	99.133	$CH_3\text{-}CH(CH_2)$-

Peptide Sequencing



F (phenylalanine): 147.177
S (Serine) : 87.078

Separation Tool to Resolve Proteome Complexity

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

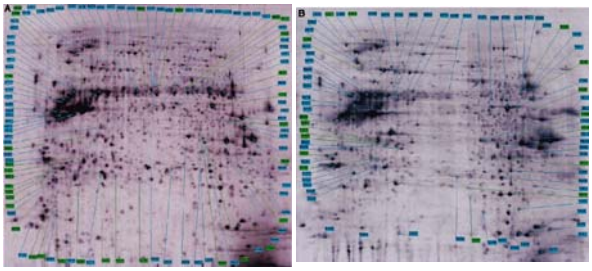
Separation Methods for Protein and Peptides

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 bonde
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:

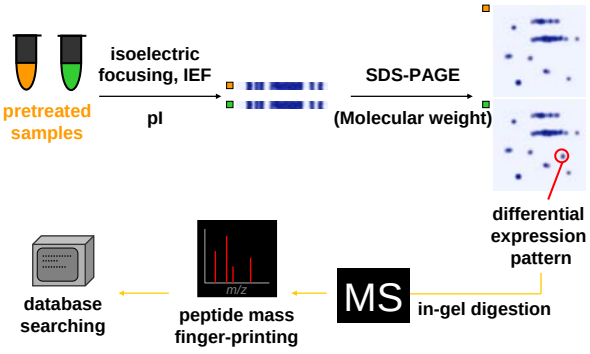
Today's 2D Gel-Based Proteomics



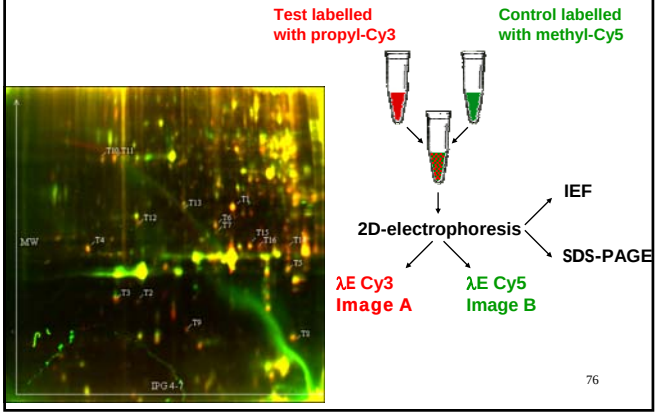
differentially expressed (170)

no differences

2D-PAGE & Protein Identification



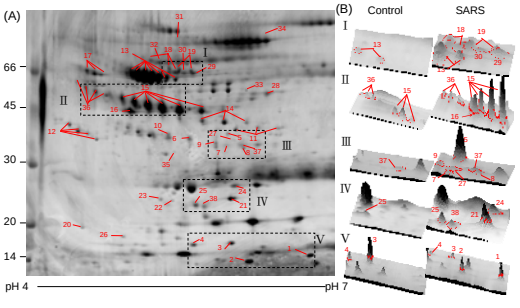
Differential Gel Electrophoresis (2D-DIGE)



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)

Multi-dimensiona Liquid Chromatography(MDLC) Coupled Directly to MS

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

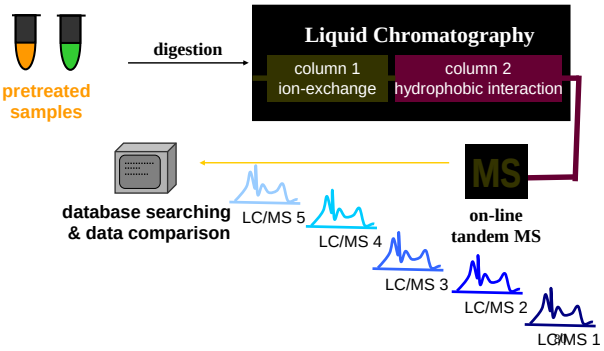
MDLC

Dry Down
Reduce/Alkylate/Digest

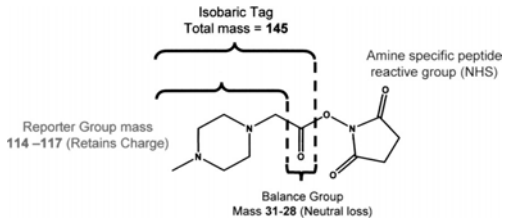
Edman Sequencing
Mass Spectrometry

Further Analysis and
Characterization

MudPIT (Multidimentional Protein Identification Technology)



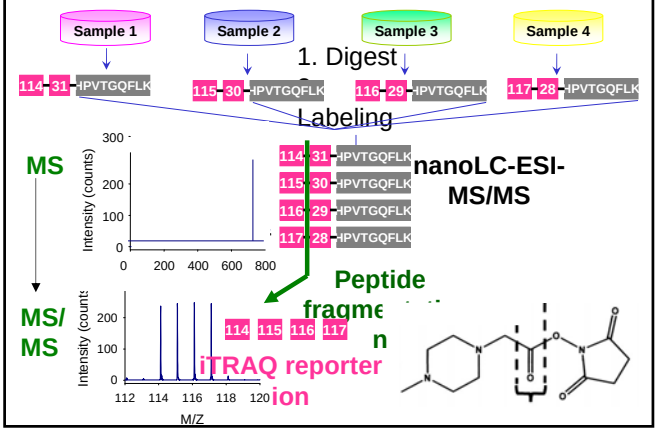
Quantitation by Isotope Labeling (Example: iTRAQ)
Isobaric Tags for Related and Absolute Quantitation



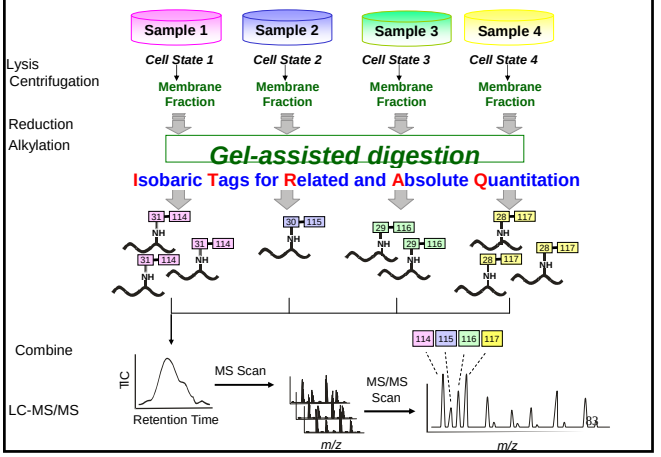
- Multiplex experiment: up to four different biological samples
- Amine-specificity: label all peptides in samples
- Stable isotope reagents: product ion spectrum reveals the difference in abundance from each sample

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

Multiplexed Protein Quantitation by iTRAQ



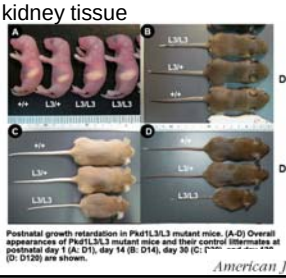
A New Quantitative Strategy for Membrane Proteomics



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: [illegible]) and (D: D120) are shown.

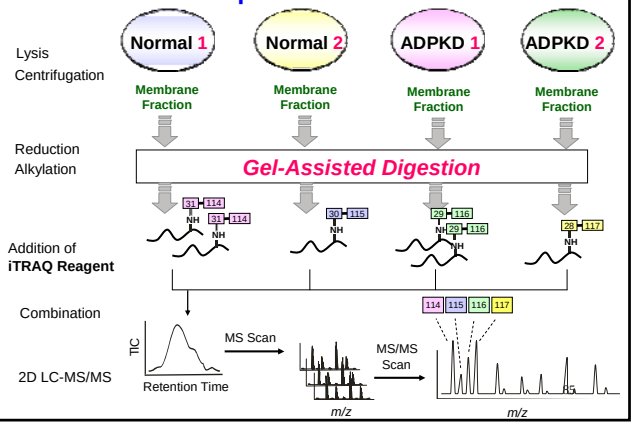
Pathogenesis of the renal cyst formation and progression is involved:

- 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

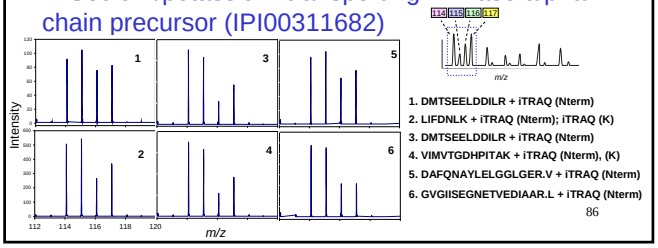


Quantitative Analysis of Membrane Proteins from the ADPKD Mice

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)



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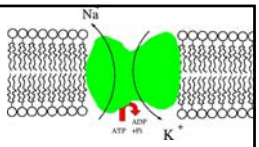
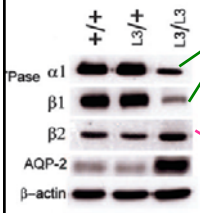
The **Na⁺-K⁺ ATPase** is an ion pump that uses the energy from the hydrolysis of ATP to actively pump sodium and potassium ions against their concentration





- **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

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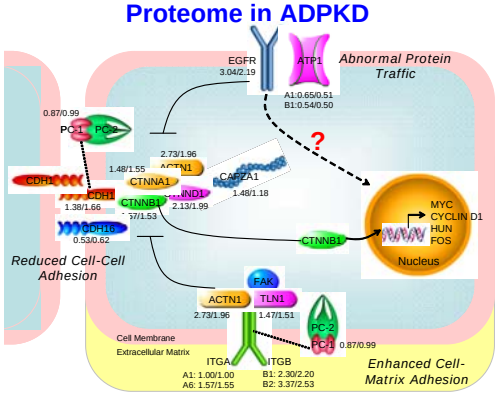
Systematic Manifestation of the Altered Membrane Proteome in ADPKD



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

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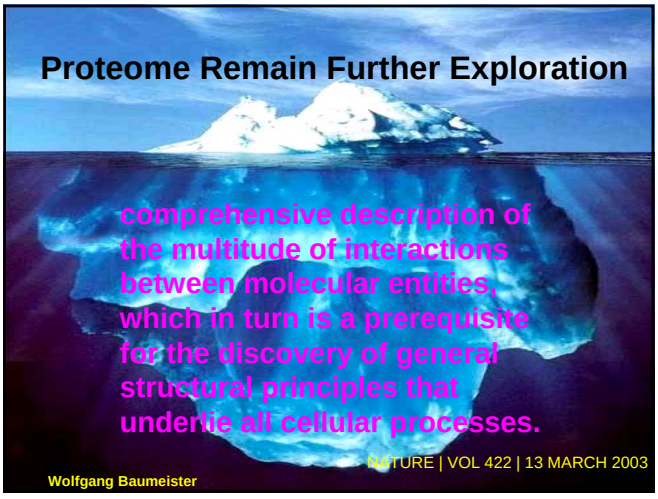
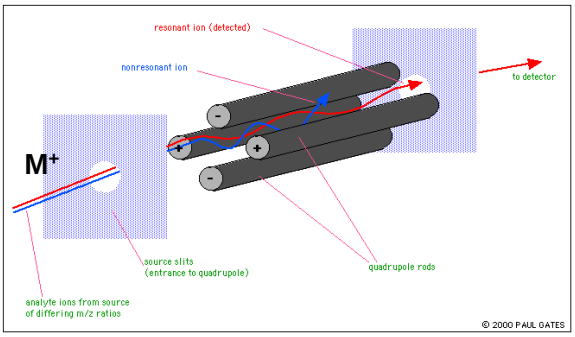
List of Potential Drugable Target Proteins

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 (cyclooxygense)	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

Proteome Remain Further Exploration



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

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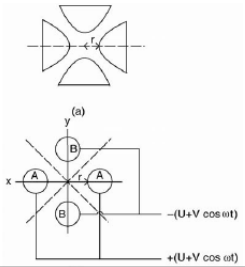
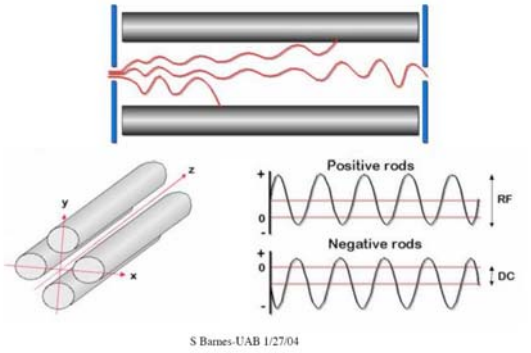
Length, diameter, kinetic energy -→m/e range, resolution

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

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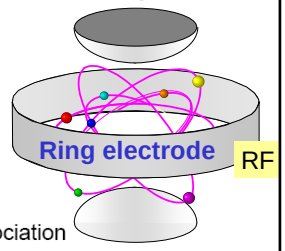
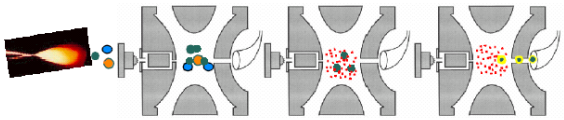
Ion-Trap Analyzer

Cap electrode

- 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



T1 T2 T3

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

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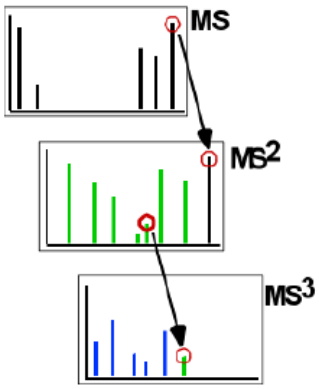
Multiple MS/MS (fragmentation) Capability

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



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