Forschungszentrum Karlsruhe
in der Helmholtz-Gemeinschaft

Universität Karlsruhe
Institut für Organische Chemie

Mass spectrometry II

27 April 2005

Sergii Afonin

Outlook

- History
- Reminder: MS spectrometer
    - ionisation/ion sources: history and place for MALDI
    - ion analysis/analyzators
    - ion detection/detectors
- Hyphenation with other techniques
- MALDI
    - *Ionization process*
    - *Matricies*
    - *Sample preparation*

- Applications

1899 Early Mass Spectrometry

1934 Double focusing analyzer

1946 Time of Flight MS

1947 Preparative Mass Spectrometry

1949 Ion Cyclotron Resonance

1953 Reverse Geometry Double Focusing MS

1953 Quadrupole MS

1956 GC/MS

1956 Identifying Organic Compounds with MS

1966 Chemical Ionization

1966 Peptide Sequencing

1968 Atmospheric Pressure Ionization

1969 Field Ionization

1974 Fourier Transform Ion Cyclotron Resonance

1974 Extraterrestrial Mass Spectrometry

1976 Californium-252 Plasma Desorption MS

1977 Accelerator MS

1978 GC-C-IRMS

1980 Inductively Coupled Argon Plasma MS

1981 Matrix-Assisted Desorption/Ionization

1985 Matrix-Assisted Laser Desorption/Ionization

1989 ESI of Biomolecules

1990 Protein Structure

1991 MALDI Post-Source Decay

1991 Noncovalent Interactions with ESI

1992 Low Level Peptide Analysis

1993 Oligonucleotide Sequencing

1993 Protein Mass Mapping/Fingerprinting

1995 Orthogonal ESI

1996 MS of a Virus

1999 Desorption/ionization without matrix

1999 Isotope-Coded Affinity Tags

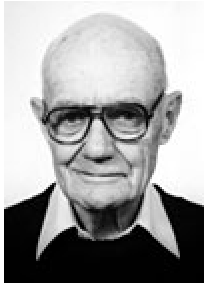
2004 Desorption Electrospray Ionization

The Nobel Prize in Chemistry 2002

"for the development of methods
for identification and structure
analyses of biological
macromolecules"

"for their development of soft desorption ionisation methods for mass spectrometric analyses of biological macromolecules"

"for his development of nuclear magnetic resonance spectroscopy for determining the three-dimensional structure of biological macromolecules in solution"



John B. Fenn

1/4 of the prize

USA

Koichi Tanaka

1/4 of the prize

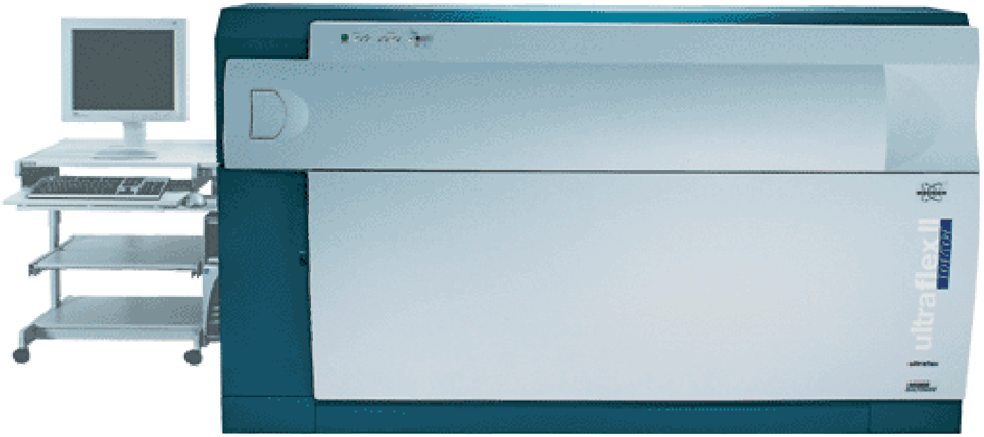
Japan

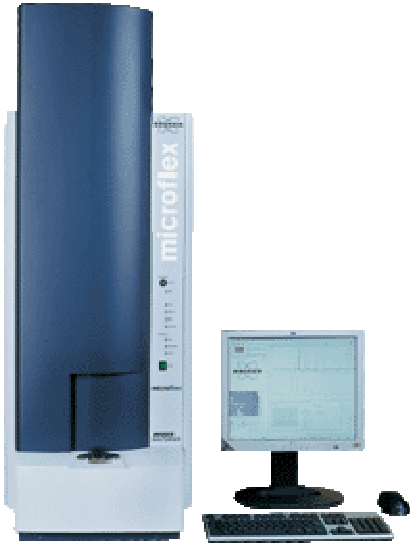
Kurt Wüthrich

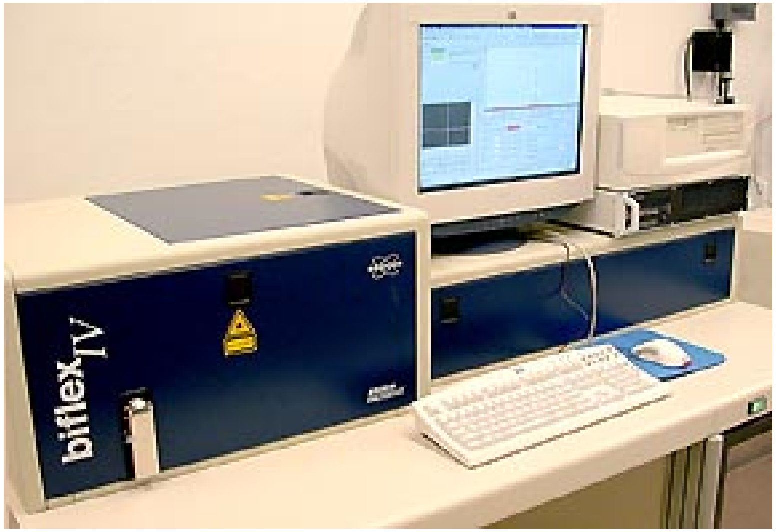
1/2 of the prize

Switzerland

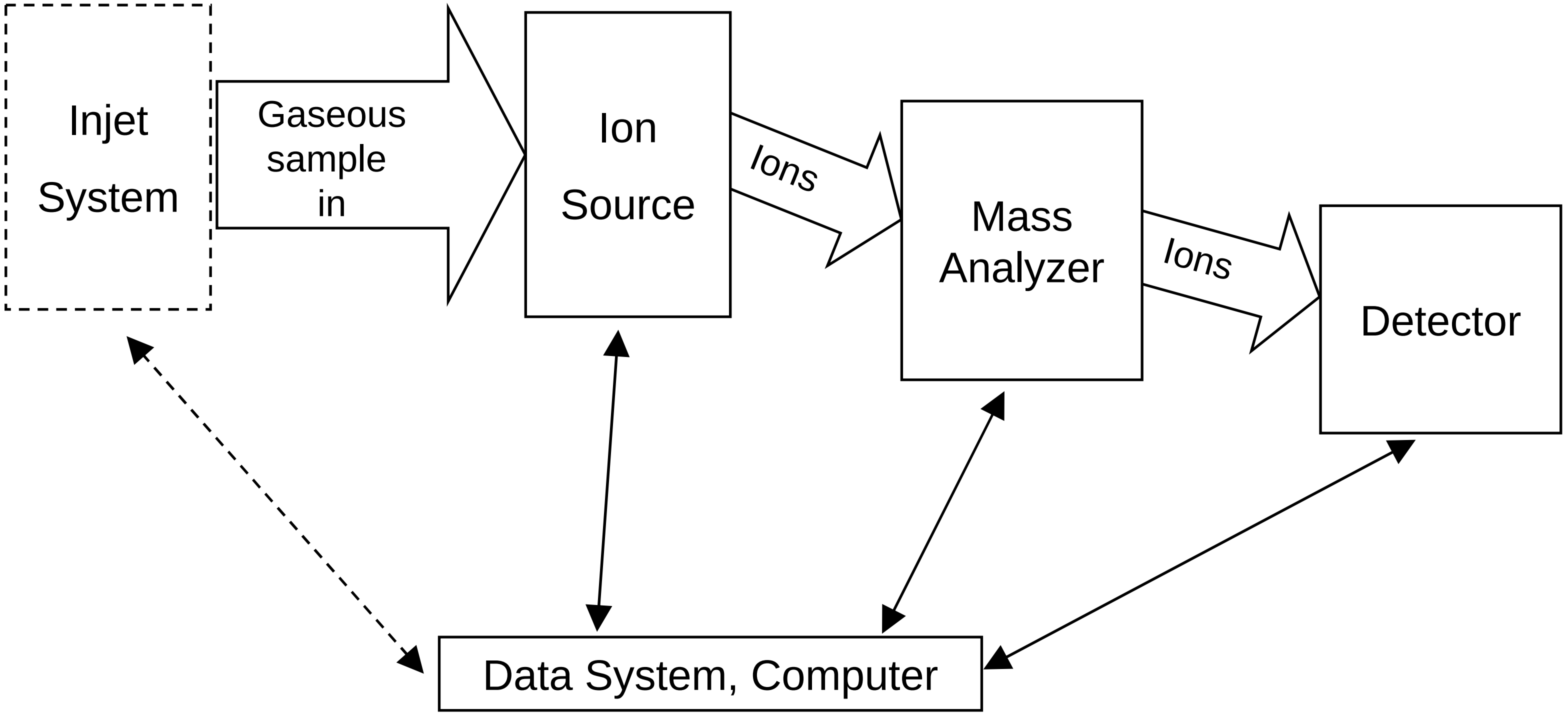
MALDI instruments



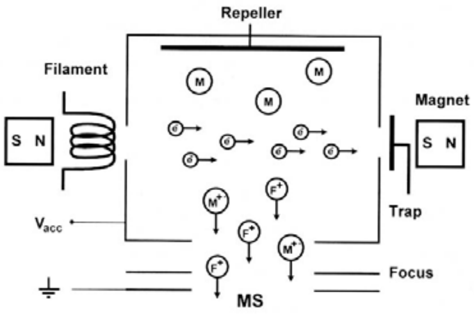




Mass Spectrometer



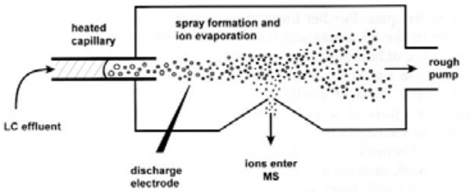
Ionization



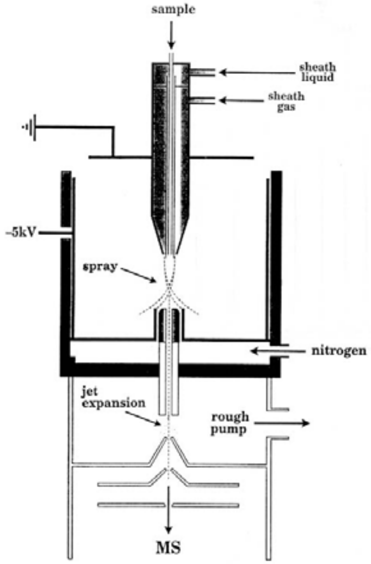
EI/CI

$$M + e^- \rightarrow M^{+\cdot} + 2e^-$$

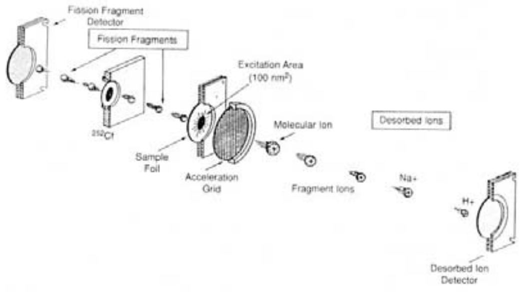
$$CH_4^{+\cdot} + CH_4 \rightarrow CH_5^+ + CH_3\cdot$$
$$M + CH_5^+ \rightarrow MH^+ + CH_4M^+\cdot + 2e^-$$



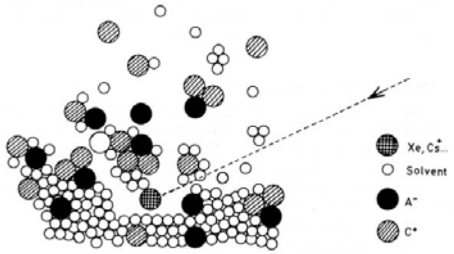
TSI/PB



ESI

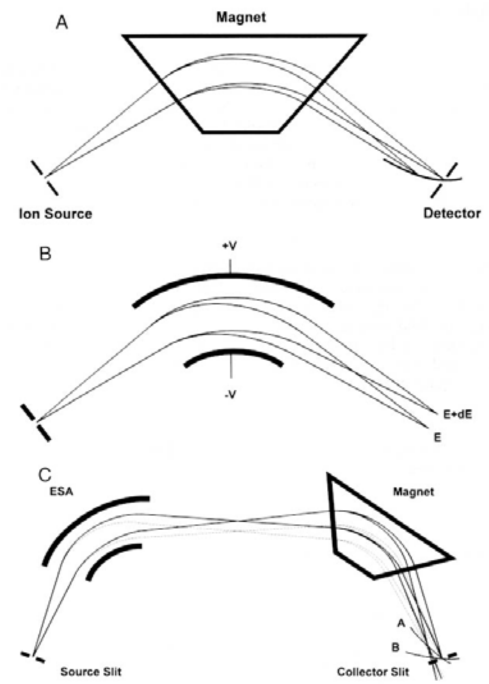


PDMS (PDI)



FAB(FABDI)

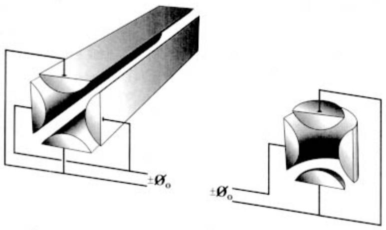
Mass analyzer



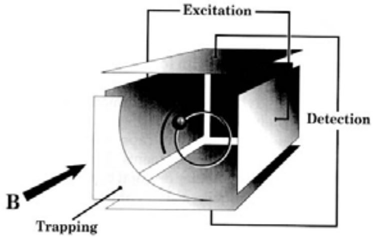
Magnetic sector

Electrostatic analyzer

Double-focusing analyzer

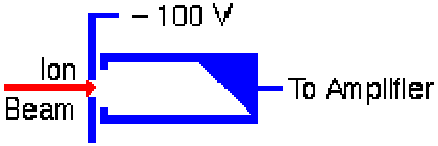


Quadrupol(Q)/Ion trap(IT)

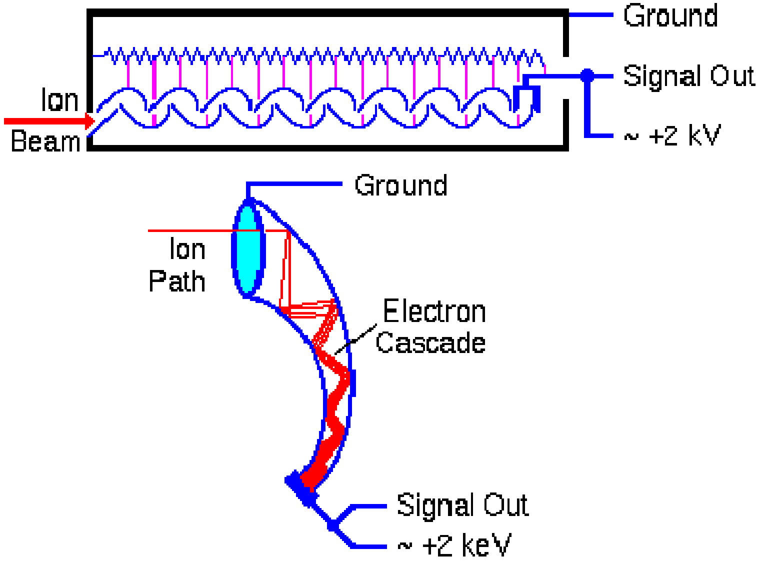


Ion cyclotron resonance(ICR)/FT-MS

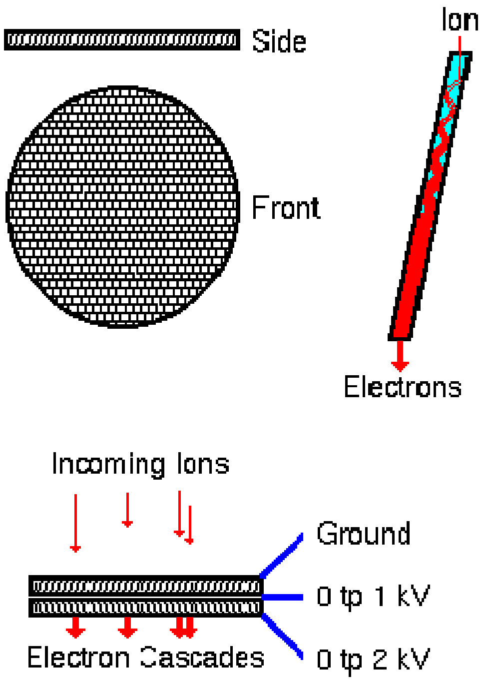
Detector



Faraday Cup



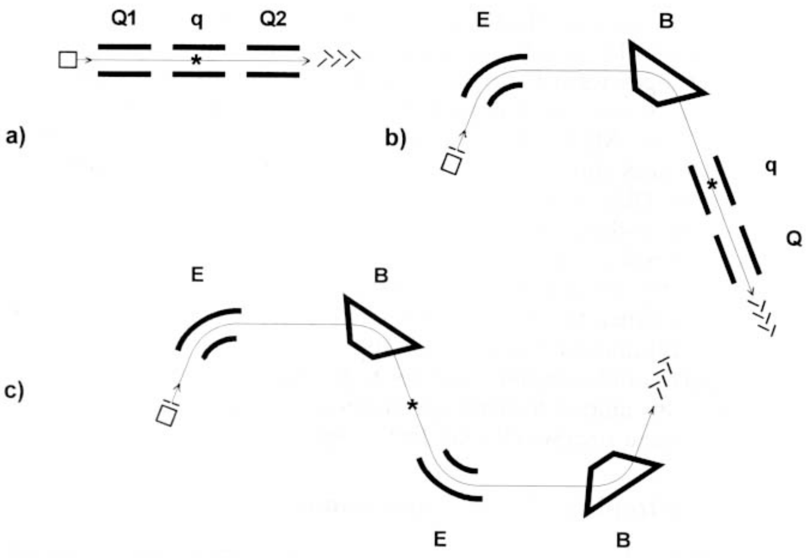
Electron multiplier



Microchannel plate
electron multiplier
(Ion image detector)

MS Hyphenation with other techiques

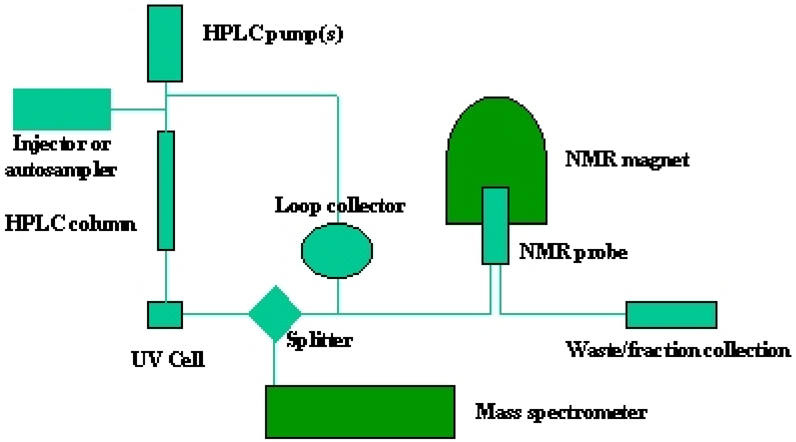
- MS^n
- GC/MS
- LC/MS
- CE/MS
- TLC/MS
- NMR/MS



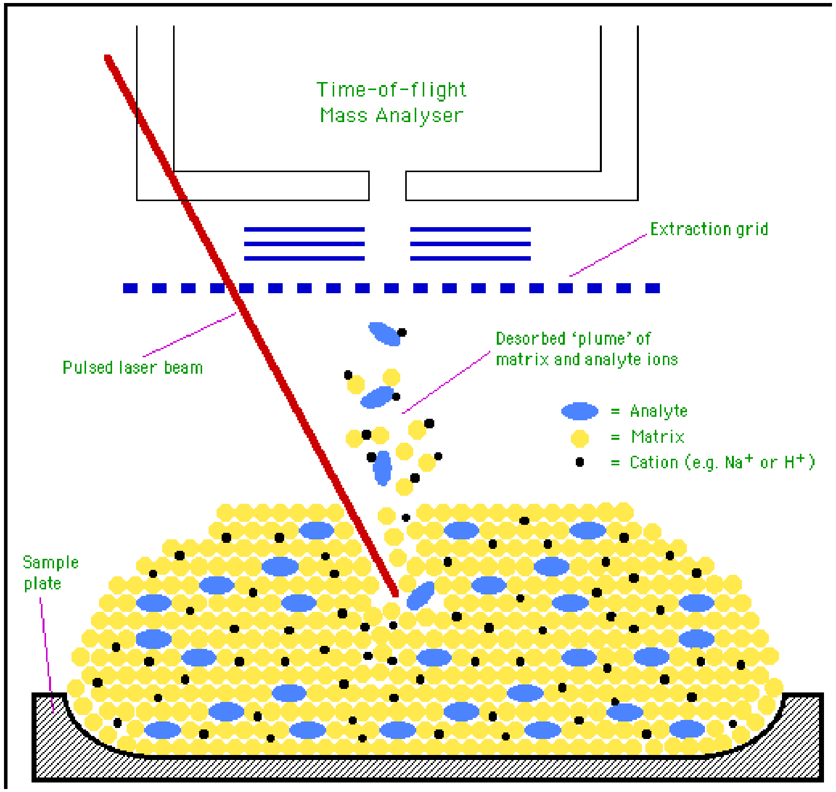
Tamdem MS

QqQ, EB-qQ, EB-EB, etc.

LC/NMR/MS



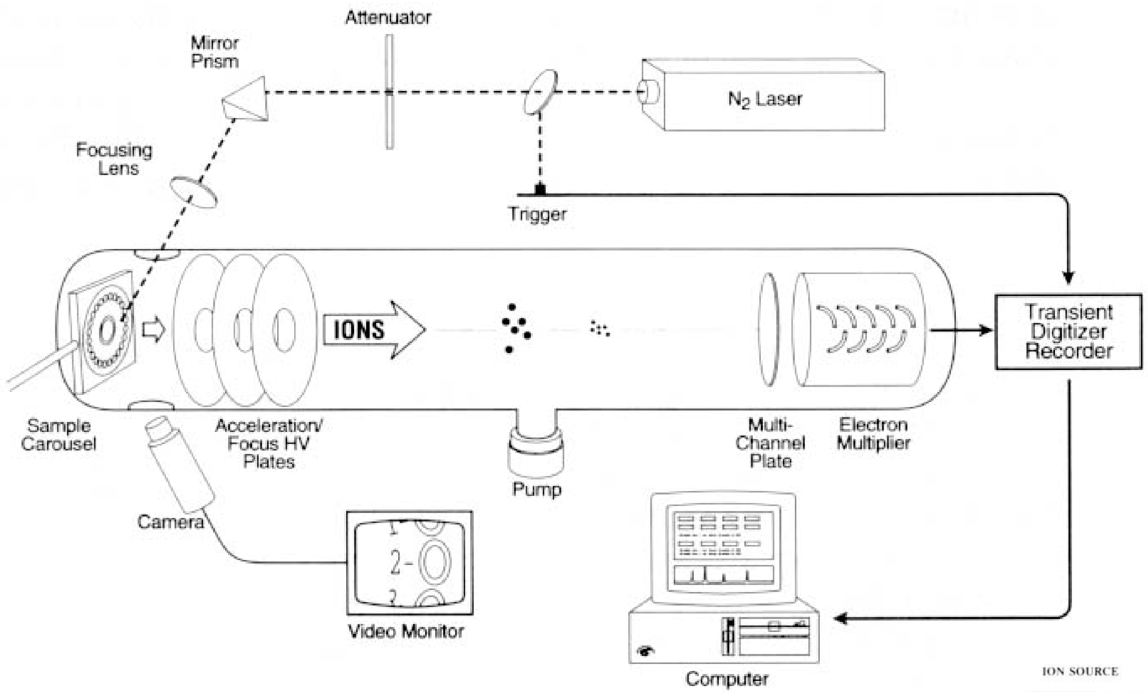
MALDI process



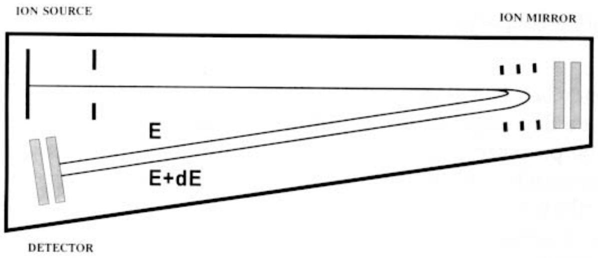
Mechanism	**Name**
$CA \rightarrow C^{+} + A^{-}$	*Dissociation*
$M \rightarrow M^{+\cdot} + e^{-}$	*Radical formation*
$M + nH^{+} \rightarrow [M + nH]^{n+}$	*Proton addition*
$M \rightarrow [M - nH]^{n-} + nH^{+}$	*Proton elimination*
$M + nC^{+} \rightarrow [M + nC]^{n+}$	*Cation addition*
$M + nA^{-} \longrightarrow [M + nA]^{n-}$	*Anion addition*

Hillenkamp et al. 2001

MALDI-TOF

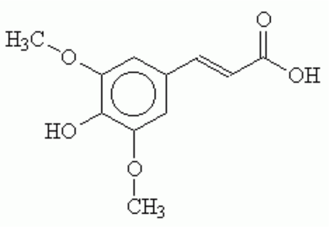


Reflectron

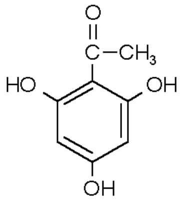


Common MALDI matrices

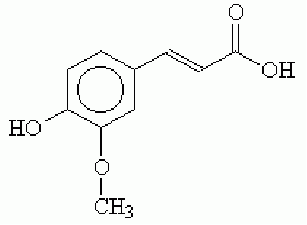
sinapinic acid (**SA**)
(3,5-dimethoxy-4-hydroxycinnamic acid)



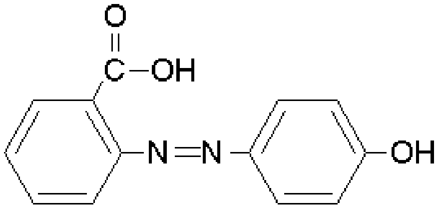
2',4',6'-trihydroxyacetophenone
(**THAP**)



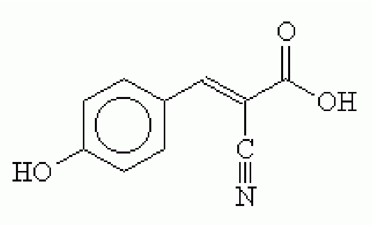
ferulic acid (**FA**)
(4-hydroxy-3-methoxycinnamic acid)



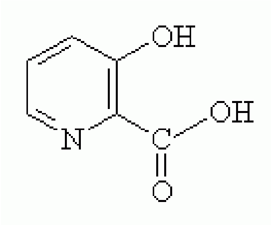
2,-(4-hydroxy-phenlyazo)-benzoic acid
(**HABA**)



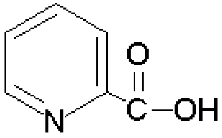
alpha-cyano-4-hydroxycinnamic acid
(**CHCA**)



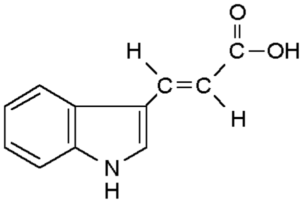
3-hydroxypicolinic acid (**HPA**)
(3-hydroxy-2-pyridinecarboxylic acid)



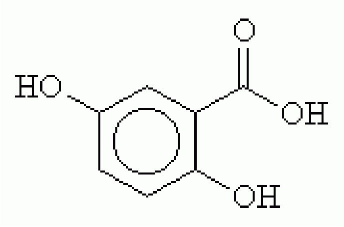
picolinic acid (**PA**)
(2-pyridine carboxylic acid)



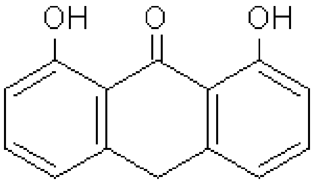
trans-3-indoleacrylic acid (**IAA**)



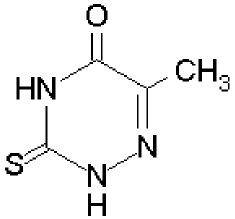
gentisic acid (**DHB**)
(2,5-dihydroxybenzoic acid)



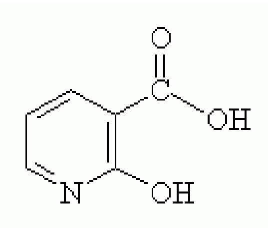
dithranol (**DIT**)



6-aza-2-thiothymine (**ATT**)



nicotinic acid-N-oxide



Matrix selection (properties to consider)

->Solubility
1-100 mM: acidified water, water-acetonitrile mixtures, water-alcohol mixtures, 70% formic acid, etc.

->Spectroscopic properties
light absorption spectrum overlap the frequency of the laser. e.g. UV-MALDI vs. IR-MALDI

->Reactivity
e.g. NO oxidizing agents (react with SH groups)
e.g. NO Aldehydes (reactivity with amino groups)

->Photostability
e.g. nicotinic acid looses; -COOH when photochemically excited leaving reactant pyridyl group

->Volatility
e.g. nicotinic and vanillic acids sublime away in vacuum, CHCA does not

->High crystallization affinity for analytes

->Act as proton donor under irradiation

->Adduction to the analytes
Formation of (M+matrix+H)+

->Fragmentation

NB:Co-Matrices (e.g. common matrices, bumetanide, glutathione, 4-nitroaniline, vanillin, nitrocellulose and L (-) fucose.
):
(1) increase the homogeneity of the matrix/analyte deposit, (2) decrease/increase the amount of fragmentation,
(3) decrease the levels of cationization, (4) increase ion yields, (5) increase precision of quantitation, (6) increase sample-to-sample reproducibility,
(7) increase resolution.

Matrix selection algorithm: trial-and-error (with a few educated guesses)

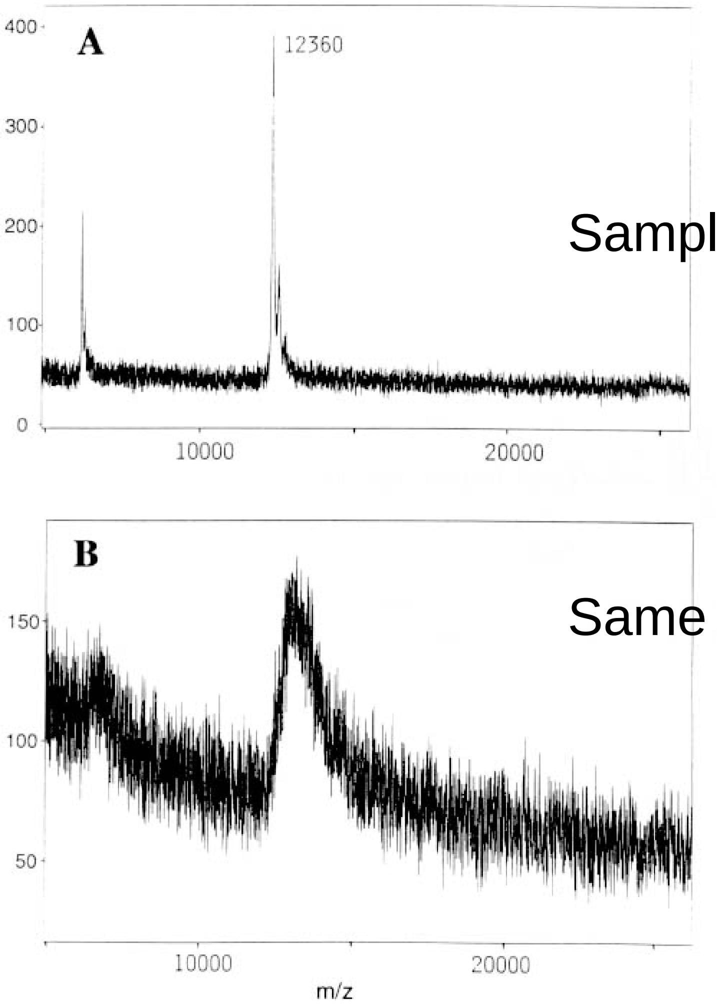
Common MALDI matrix applications

Matrix	*Matrix peaks (Da)*	*Application*
a-cyano-4-hydroxycinnamic acid (CHCA)	172.0, 190.0, 212.0, 294.1,379.1	Peptides (<10 KDa), lipids, carbohydrates
sinapinic acid (SA), or trans-3,5-dimethoxy-4-hydroxycinnamic acid	202.1, 224,1, 225.1	Peptides and large proteins (10-150KDa), glycoproteins, membrane proteins
gentisic acid, or 2,5-dihydroxy benzoic acid (DHB)	137.1, 154.1, 155.0, 273.1	peptides, proteins, carbohydrates, glycoproteins, glycolipids, polymers, lipids, organic molecules
trans-3-indoleacrylic acid (IAA)	170.1, 187.1, 284.1, 329.1, 375.1	synthetic polymers
3-hydroxypicolinic acid (HPA)	96.0, 140.0, 189.0, 235.1, 279.1	Oligonulceotides > 3.5KDa
2,4,6-trihydroxyacetophenone (THAP)	169.1	Oligonucleotides < 3.5KDa
dithranol (DIT)	211.1, 226.1, 227, 1	Polymers and fullerene compounds

MALDI sample preparation

- **Dried-Droplet**
- **Vacuum-Drying**
- **Crushed-crystal**
- **Fast-evaporation**
- **Overlayer (also Two-layer and Seed-layer)**
- **Sandwich**
- **Spin-coating**
- **Slow-crystallization (growing large crsytals)**
- **Electrospray**
- **Matrix-precoated target**

MALDI MS of Cytochrome c



Sample/matrix dried gently in air

Same sample dried rapidly with hot air

Applications

Proteins/peptides
Absolute mass determination
-Conformation determination via 2H/1H exchange
-Post translation modifications
Analysis of complex mixtures
-Link to separation (LC/MS, CE/MS, 2D-GEL: SDS-PAGE, IEF)
-Proteomics (proteome mapping)
-Sequence ordered tryptic maps (digest kinetics)
-Proteolytic/chemical digests → sequencing (PSD, CID: MSn)
-Digest with derivatisation (e.g. sulfonation of the term. K)
-In gel digestion with OG extraction (up to 85%)
-Piger-print peptide fragments mapping
-Hydrolysis in 18O-labeled water: all fragments except the C-terminal are labeled
-Disulfide bond determination (use of reducing agents, e.g.dithiothreitol or dithioerythritol)
-Non-covalent interactions
(protein-protein, protein-ligand, protein-DNA, metal-coordination)

Nucleic acids
Absolute mass determination
-Single nucleotide polymorphism
Analysis of complex mixtures
-Chemical/enzymatic digest of RNA/DNA
-Digests linked to separation
-Derivatization of nucleosides using trimethylsilyl (for EI, CI)

Applications: Mass determination

MS mass vs. molecular mass of a compound

The **nominal molecular mass**
nearest whole integer atomic masses, i.e., C=12, H=1, O=16, N=14, S=32, etc.

The **monoisotopic molecular mass**
exact atomic masses, i.e., C=12.000, H=1.00782, N=14.0031, O=15.9949, and S=31.9721, etc.

The **average molecular mass** accounts for both atomic mass defect and stable isotope natural abundance

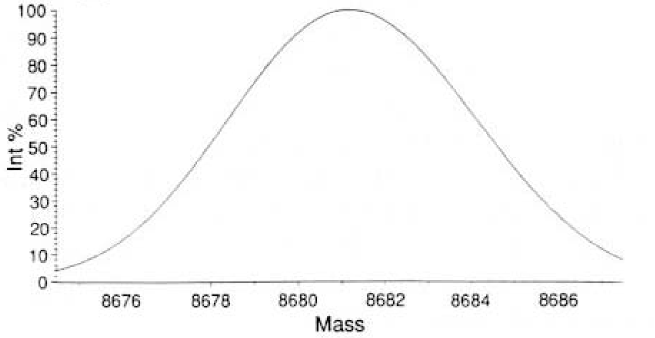
The **peak centroid** position
The **peak maximum** position

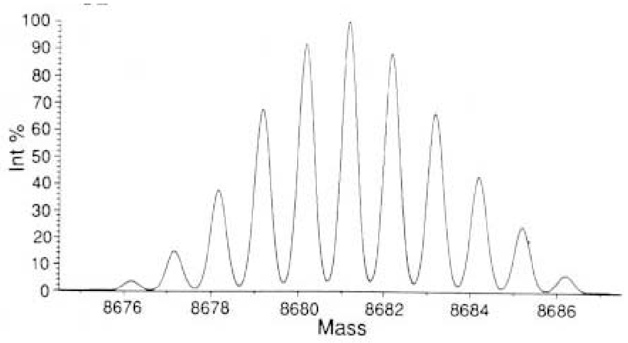
Isotopic masses for nominal mass (m/z) 8681 from proinsuline (in Da)

		Numbers of Atoms										
Mass (m/z)	**Relative Abundance**	**^{12}C**	**^{13}C**	**^{1}H**	**^{2}H**	**^{14}N**	**^{15}N**	**^{16}O**	**^{18}O**	**^{17}O**	**^{32}S**	**^{34}S**
8681.1666	0.6	379	2	586	0	106	1	114	0	0	5	1
.17123	0.6	378	3	586	0	105	2	114	0	0	6	0
.17295	2.2	378	3	586	0	107	0	114	0	0	5	1
.17509	0.5	379	2	586	0	106	1	113	1	0	6	0
.17755	3.4	377	4	586	0	106	1	114	0	0	6	0
.17991	0.4	377	4	586	0	107	0	114	0	0	5	0
.18141	1.9	378	3	586	0	107	0	113	1	0	6	0
.18387	7.4	376	5	586	0	107	0	114	0	0	6	0
.18474	0.6	377	4	586	0	107	0	113	0	1	6	0
.18679	0.1	377	4	585	1	107	0	114	0	0	6	0

Absolute mass determination

e.g. bovine proinsulin : C381 H586 N107 O114 S6.
-monoisotopic mass for the [M+H]+ ion of this protein at m/z 8676.15 but lowest abundant
-eleven different nominal (whole integer) mass peaks can be measured.
-each nominal m/z peak is itself composed of many different isotopic species
(most abundant nominal mass at 8681 Da is itself composed of 10 different isotopic species)
-total pattern contains 62 different isotopic species.





The ***centroid*** of low resolution peak –
average mass of 8681.8 Da for the [M+H]+ = ***average of the resolved isotopic peaks***.

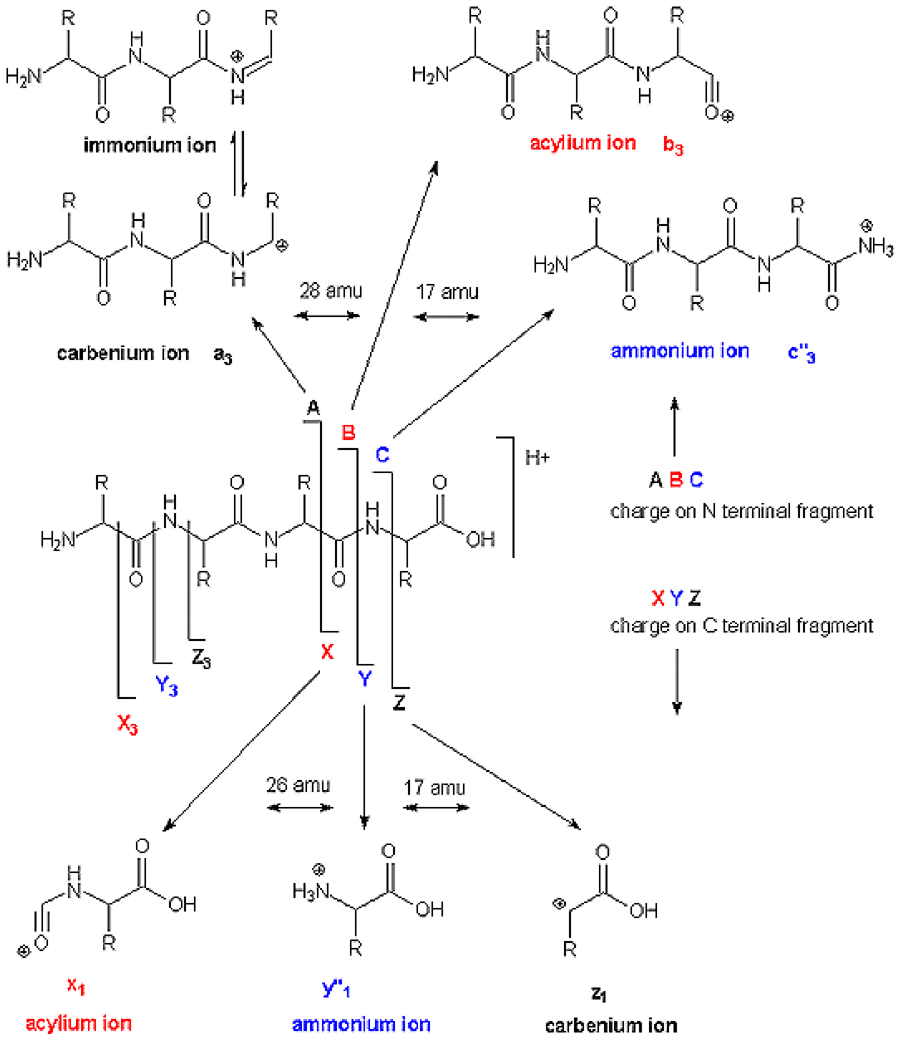
High molecular masses (proteins)
– low resolution
- isotope-**averaged mass** should be used

Low molecular masses (peptides)
- high resolution
- **monoisotopic mass** should be used

Absolute mass determination: proteins

	ESI MS	**PD MS**	**MALDI MS**
upper mass limit (Da)	100,000	25,000	300,000
mass measurement accuracy (internal standard)	0.005% - 0.05%	0.05% - 0.1%	0.05% -0.1%
speed (calibration + analysis)	30 min	15-120	10 min
compatibility with salts	poor	good[b]	good[b]
sensitivity	0.5 picomole	0.5 - 10 picomole	0.1 picomole

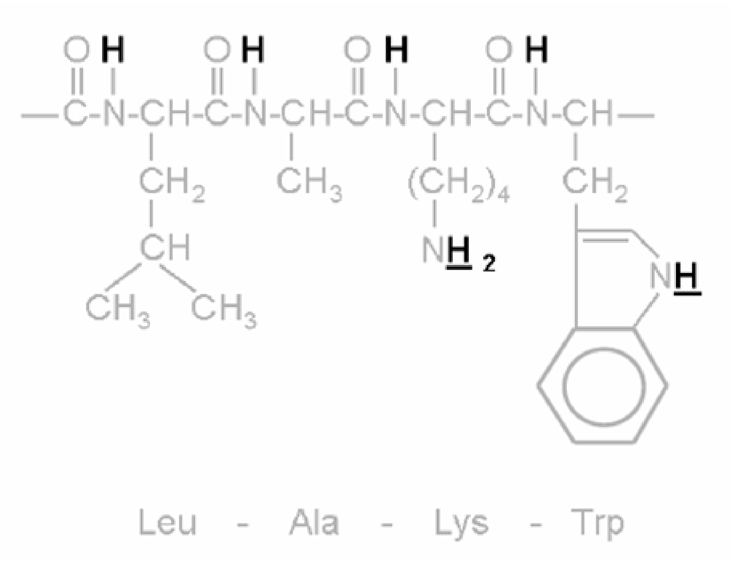
Peptide fragmentation nomenclature

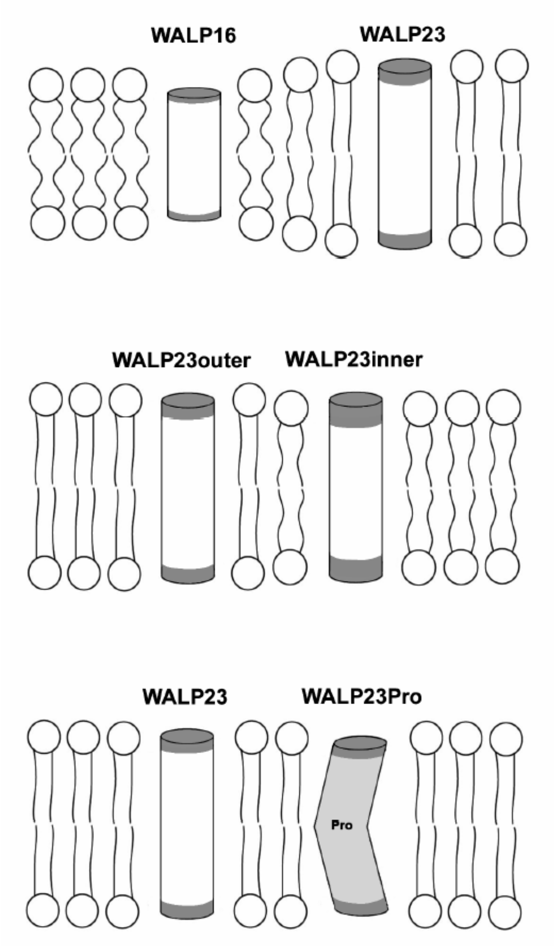


From C-term: x_n, y_n, z_n

From N-term: a_n, b_n, c_n

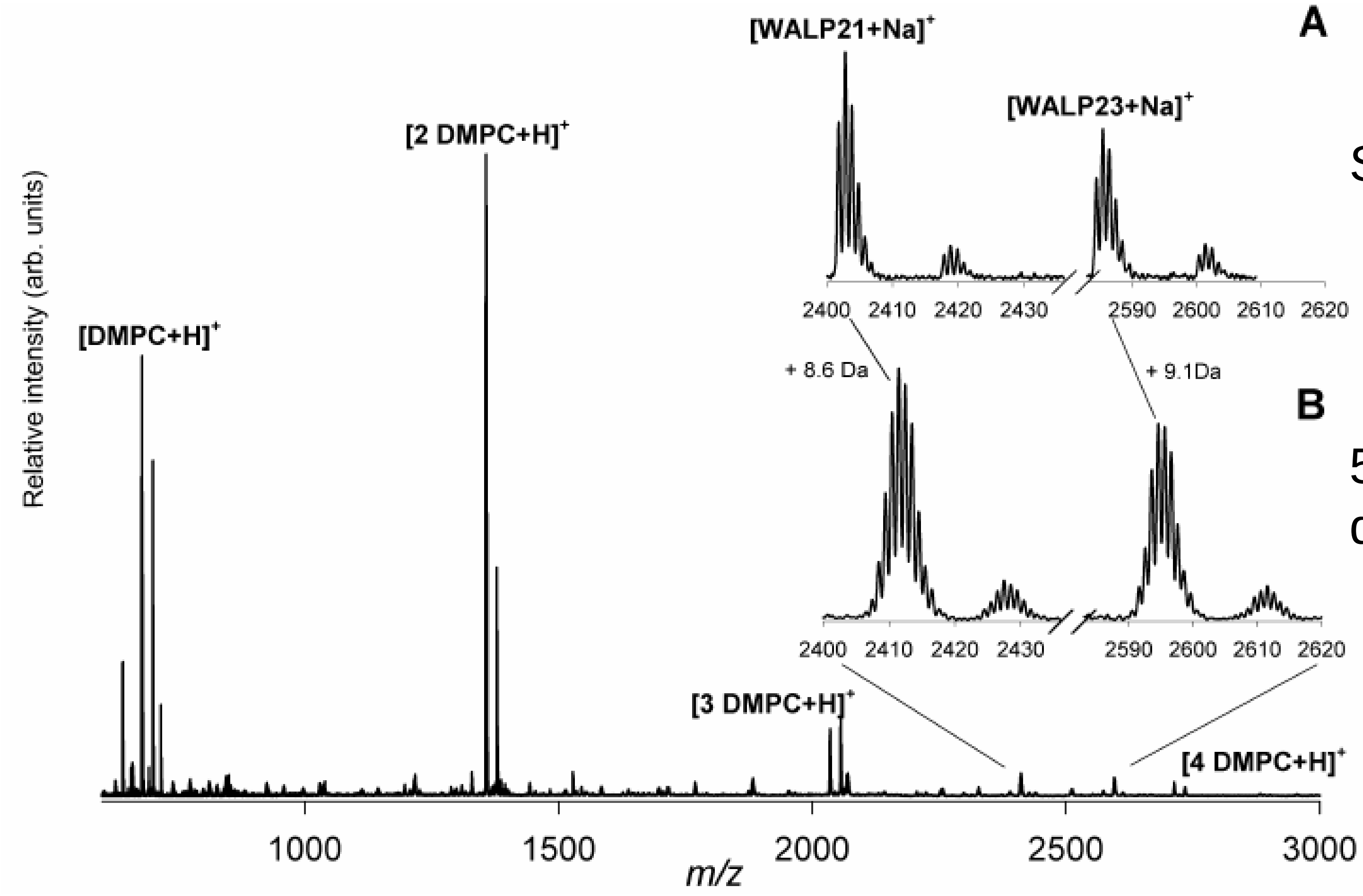
Deuterium exchange on transmembrane peptides (WALPs)





Demmers *et. al* 2001

Deuterium exchange on transmembrane peptides (WALPs)

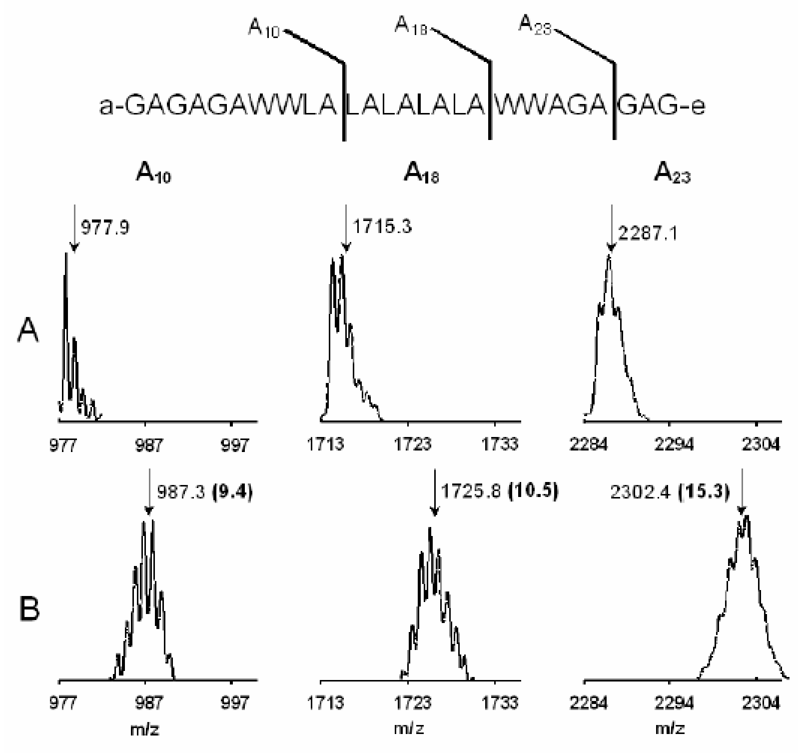


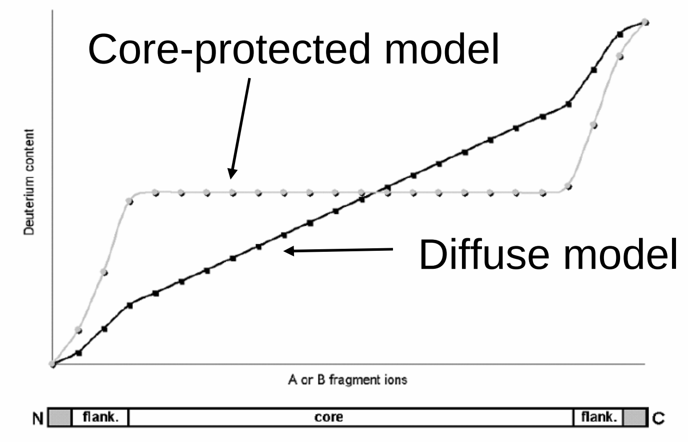
Start

5 min deuteration

Demmers *et. al* 2001

Deuterium exchange on transmembrane peptides (WALPs)



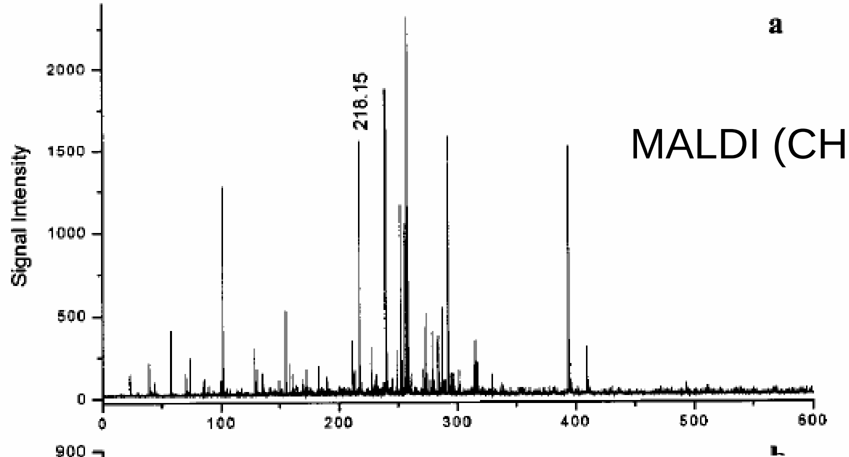


Core-protected model

Diffuse model

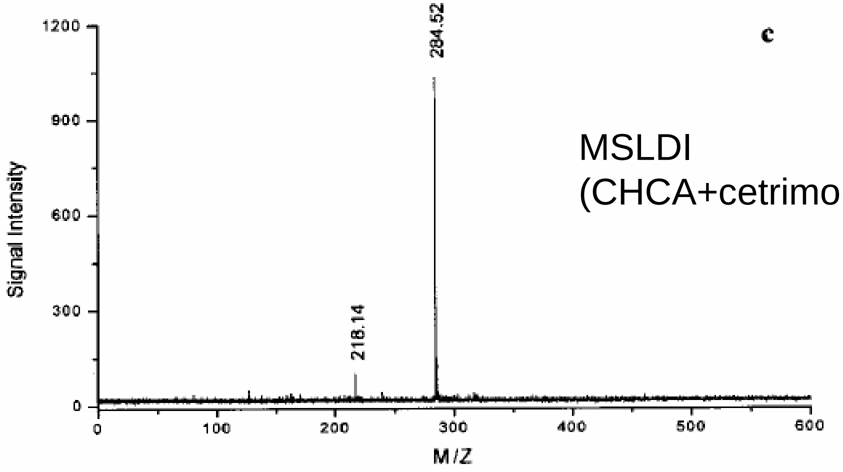
Demmers *et. al* 2001

MALDI of low mass molecules



MALDI (CHCA)

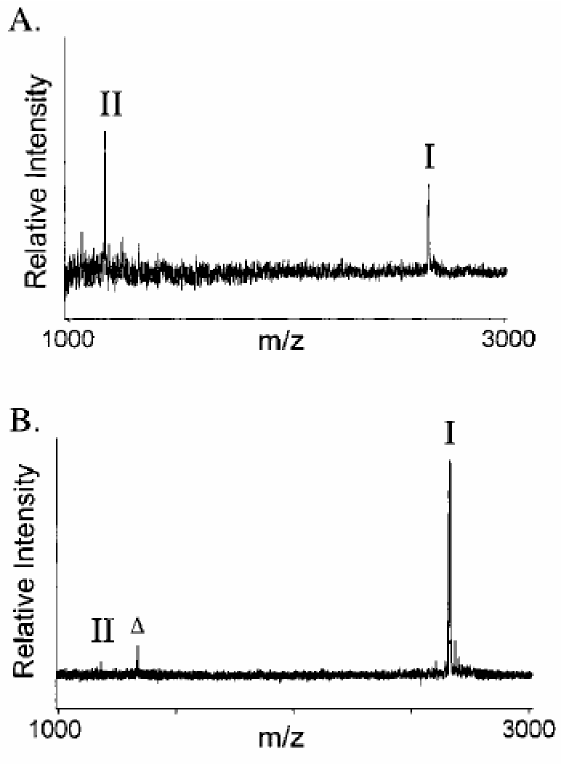
GAA peptide: 217.1Da

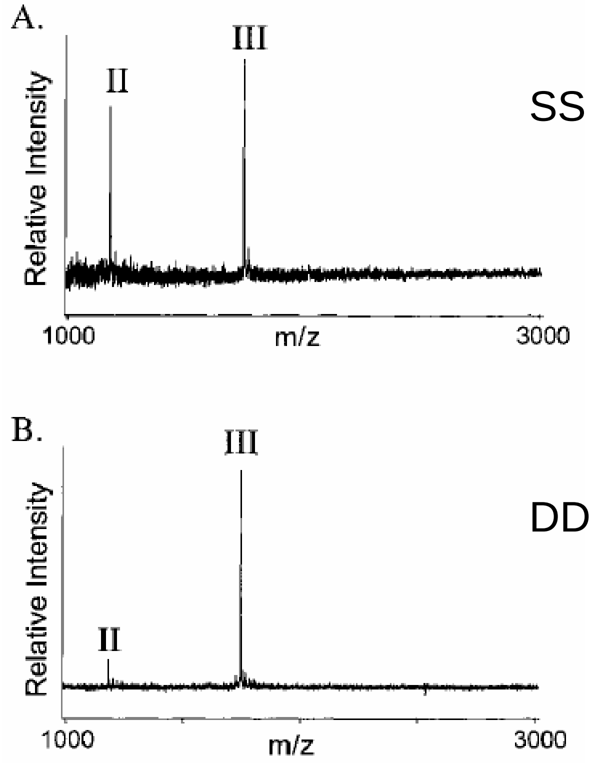


MSLDI
(CHCA+cetrimonium bromide)

Guo et. al, 2002

Towards quantitative MALDI



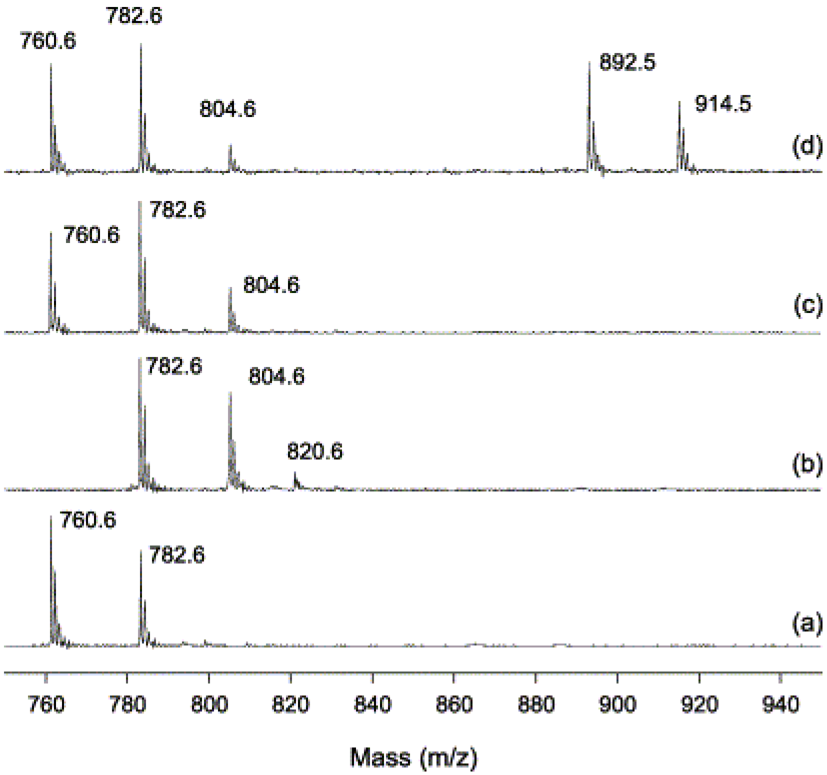


SS sample preparation

DD sample preparation

Wang et. al, 2001

MALDI of lipids: Cationisation



PAPC+POPC **+CsCl**

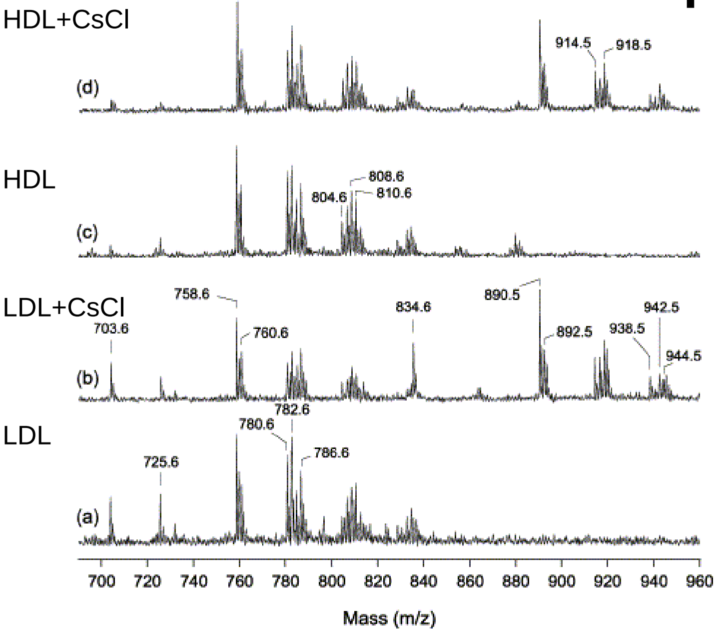
PAPC+POPC

PAPC (16:0/20:4), MW=781.6

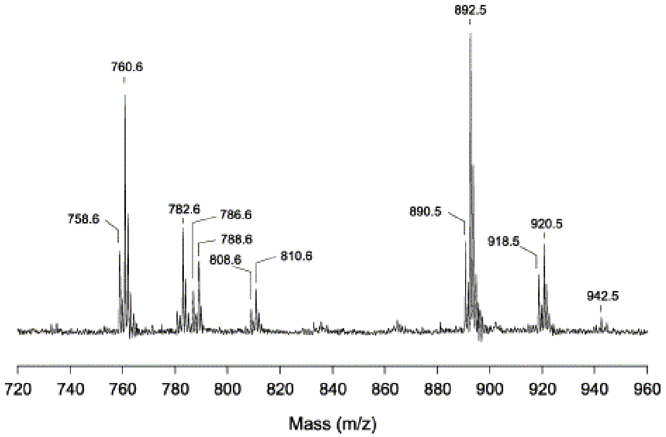
POPC (16:0/18:1), MW=759.6

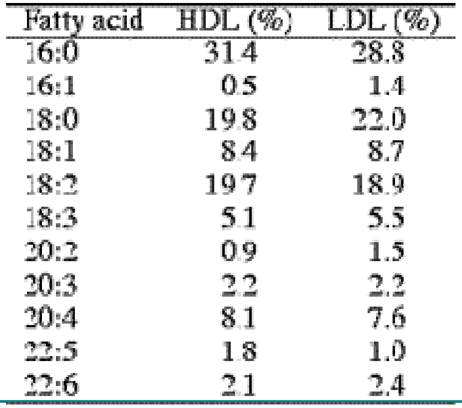
Schiller *et. al, 2001*

MALDI of lipids: mixture analysis

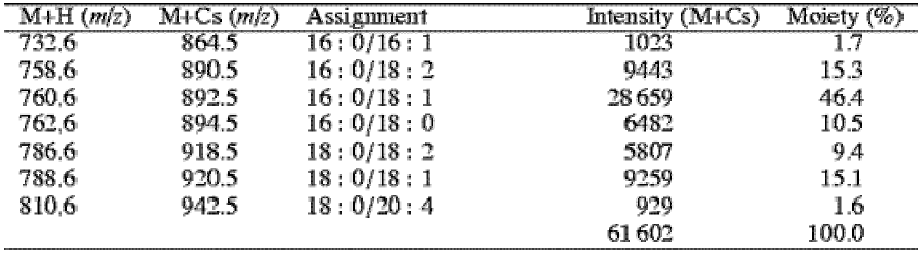


Egg yolk PC





Fatty acid	HDL (%)	LDL (%)
16:0	31.4	28.8
16:1	0.5	1.4
18:0	19.8	22.0
18:1	8.4	8.7
18:2	19.7	18.9
18:3	5.1	5.5
20:2	0.9	1.5
20:3	2.2	2.2
20:4	8.1	7.6
22:5	1.8	1.0
22:6	2.1	2.4

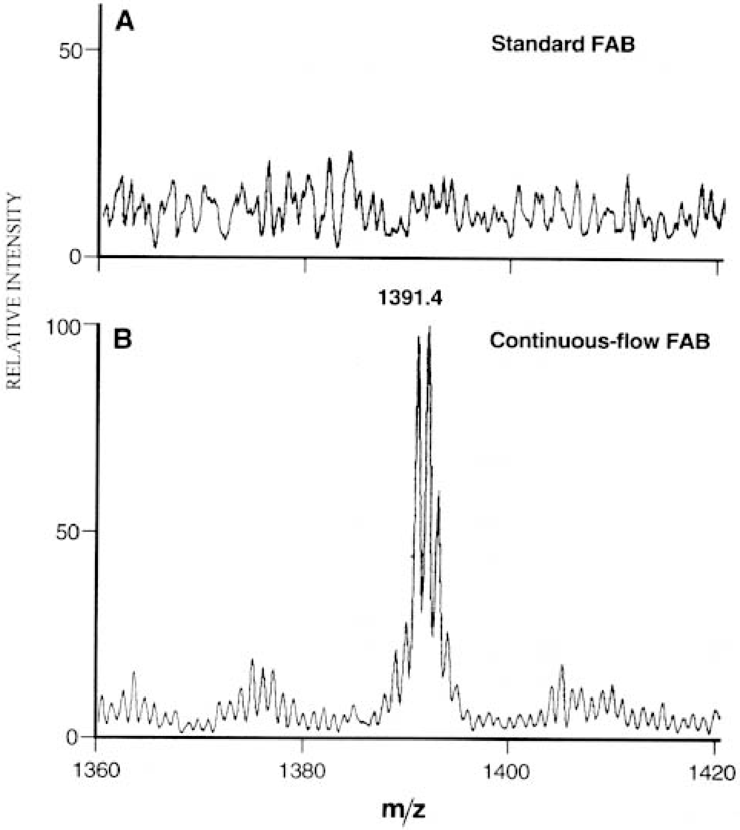


M+H (*m/z*)	M+Cs (*m/z*)	Assignment	Intensity (M+Cs)	Moiety (%)
732.6	864.5	16 : 0/16 : 1	1023	1.7
758.6	890.5	16 : 0/18 : 2	9443	15.3
760.6	892.5	16 : 0/18 : 1	28 659	46.4
762.6	894.5	16 : 0/18 : 0	6482	10.5
786.6	918.5	18 : 0/18 : 2	5807	9.4
788.6	920.5	18 : 0/18 : 1	9259	15.1
810.6	942.5	18 : 0/20 : 4	929	1.6
			61 602	100.0

Schiller *et. al, 2001*

Thanks!

CF-FAB


A
Standard FAB
50
0
RELATIVE INTENSITY
1391.4
100
B
Continuous-flow FAB
50
0
1360
1380
1400
1420
m/z