SCST ADVANCED ANALYTICAL CHEMISTRY

Application of matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS)

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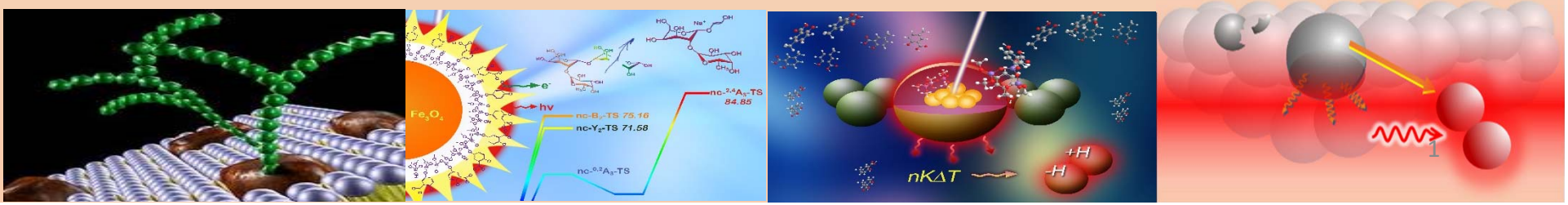


Lecture Outline

Part I: Introduction to mass spectrometry (MS)

- MS spectrum
- Masses in MS
- Accuracy and resolution
- Basic parts
- Principles MS and MS/MS

Part II: MALDI-TOF MS

- Principle
- Sample preparation
- Basic parts (Ion sources and mass analyzers)
- Unique features of MALDI

Part III: Applications of MALDI-TOF MS

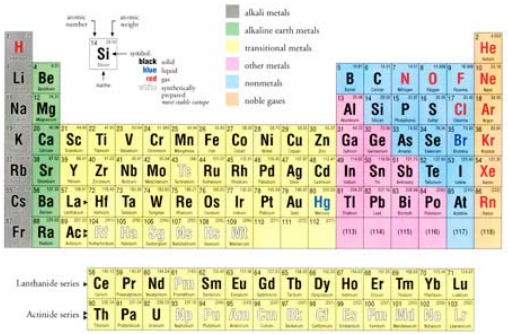
- Small molecules
- Carbohydrates
- Polymers
- Proteins

Part IV: Noble materials can be used/coupled with MALDI MS

- Nanoparticles
- Thin-layer chromatography
- Silica wafer
- Membrane

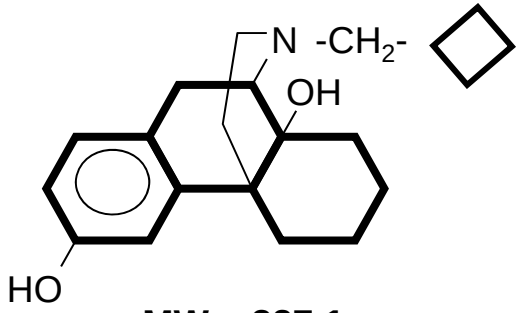
What is Mass Spectrometry (MS)?

- Different elements can be uniquely identified by their mass



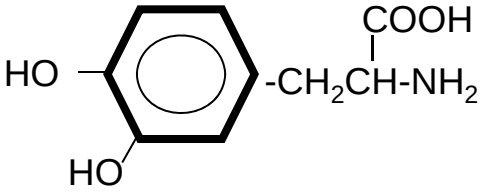
- Different compounds can be uniquely identified by their mass

Butorphanol



MW = 327.1

L-dopa



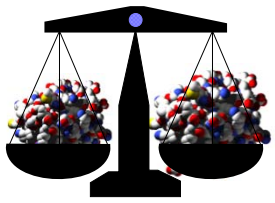
MW = 197.2

Ethanol

CH_3CH_2OH

MW = 46.1

- Analytical method to measure the molecular or atomic weight of samples



What Information does Mass Spectrometry Provide?

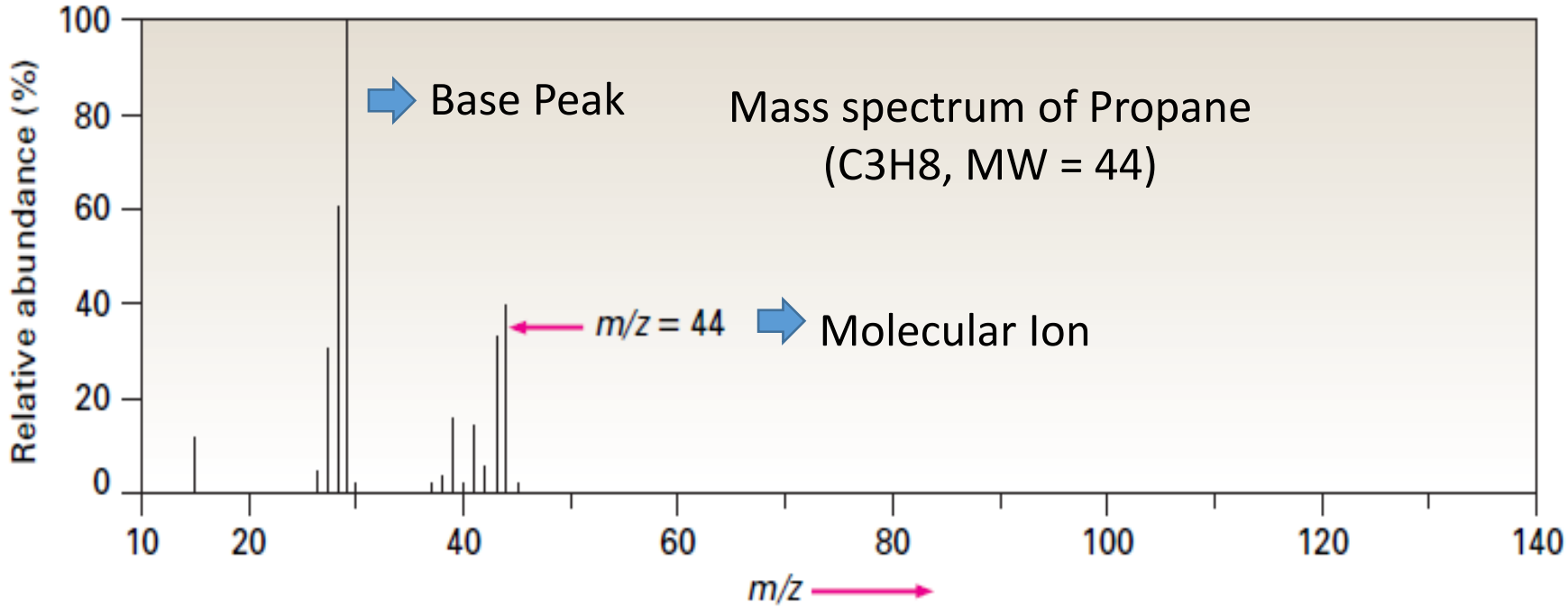
- **Used for measuring the molecular weight (m/z) of a sample**.

- **Large Mass Range** → For large samples such as **biomolecules,** molecular weights can be measured to within an accuracy of **0.01%** of the total molecular weight of the sample *i.e.* within a 4 Daltons (Da) or atomic mass units (amu) error for a sample of 40,000 Da. This is sufficient to allow minor mass changes to be detected, *e.g.* the substitution of one amino acid for another, or a post-translational modification.

- **High Mass Accuracy** → For small **organic or inorganic molecules** the molecular weight can be measured to within an accuracy of **5 ppm**, which is often sufficient to confirm the molecular formula of a compound, and is also a standard requirement for publication in a chemical journal.

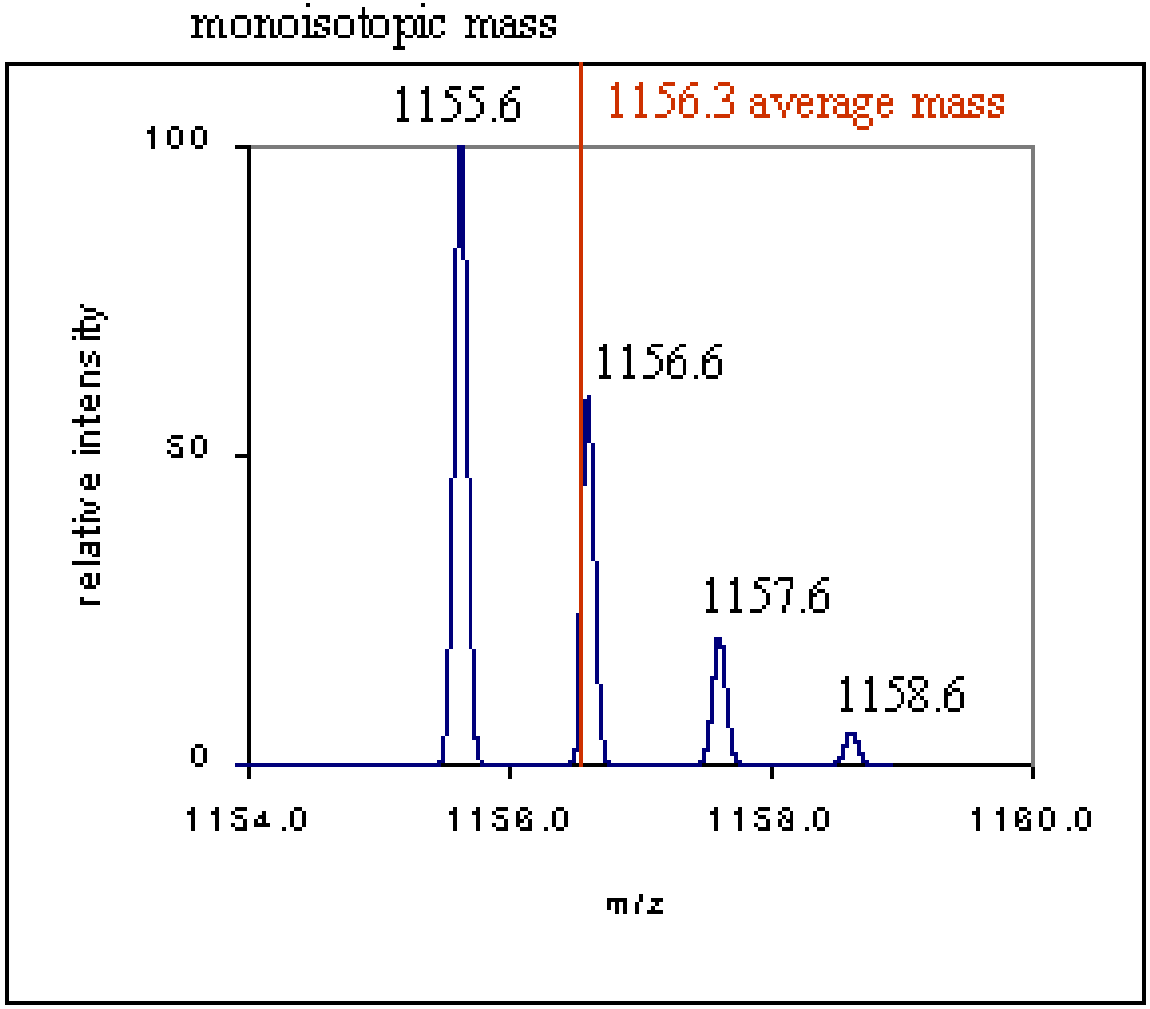
- **Information Rich** → **Structural information** can be generated using certain types of mass spectrometers, usually **tandem mass spectrometers**, and this is achieved by fragmenting the sample and analysing the products generated. This procedure is useful for the structural elucidation of **organic compounds**, for **peptide** or **oligonucleotide** sequencing, and for monitoring the existence of previously characterised compounds in complex mixtures with a high specificity by defining both the molecular weight and a diagnostic fragment of the molecule simultaneously *e.g*. for the detection of specific drug metabolites in biological matrices.

The Mass Spectrum



- A bar graph with **x-axis of m/z** and **y-axis of intensity or relative abundance**
- **Base peak:** the tallest peak with assigned intensity of 100%
- **Parent peak/molecular ion:** unfragmented cation radical , ~ m/z as the molecule

Masses in MS



- Monoisotopic mass is the mass determined using the masses of the most abundant isotopes
- Average mass is the abundance weighted mass of all isotopic components

$^{1}H = 99.9\%$ $^{2}H = 0.02\%$

$^{12}C = 98.9\%$ $^{13}C = 1.1\%$

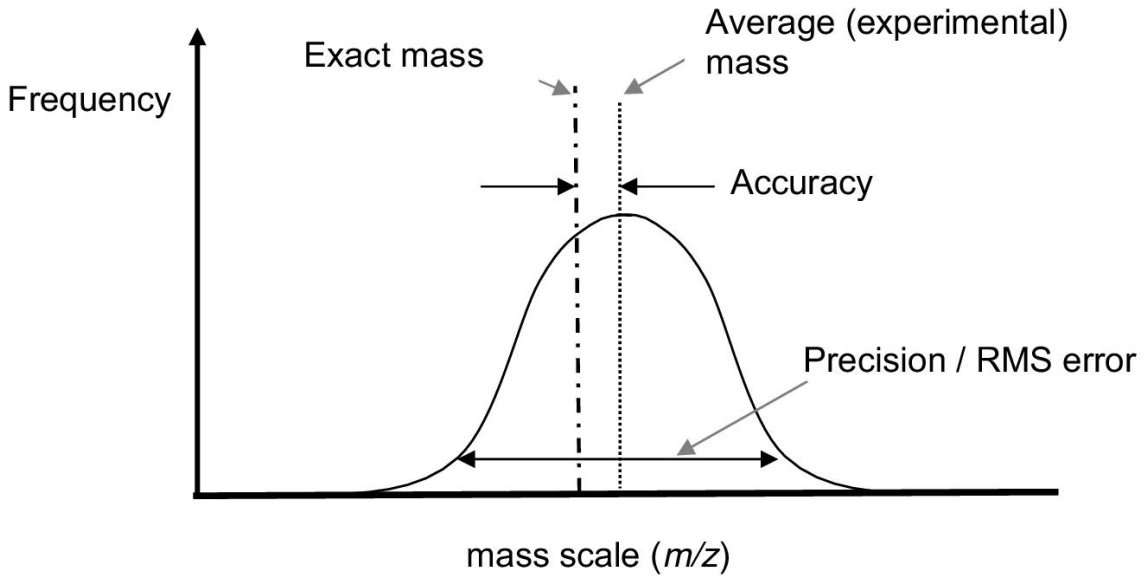
$^{35}Cl = 68.1\%$ $^{37}Cl = 31.9\%$

$$\text{Ave. Atomic Mass} = \sum_{i=1}^{n} (\text{mass}_{(i)})(\text{abundance}_{(i)})$$

Accurate Mass Measurement

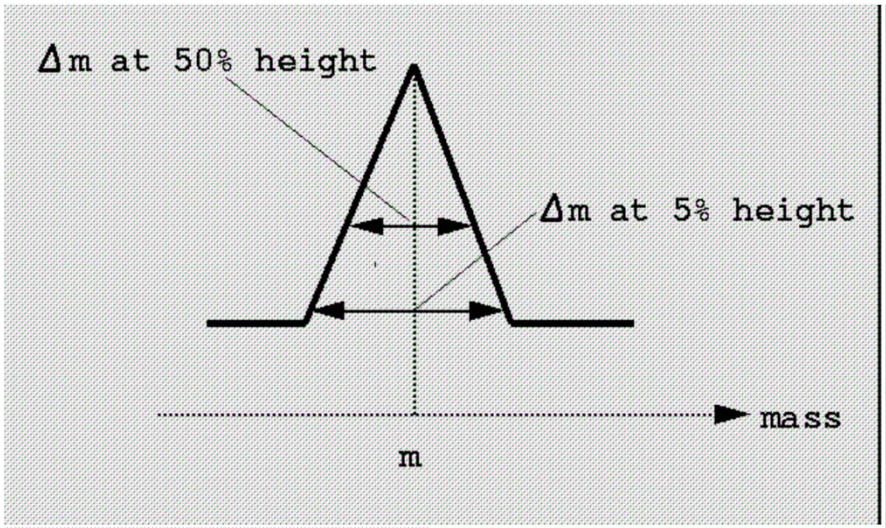
- **Mass accuracy** is a valuable tool for target quantification and confirmation
- **Accurate mass**: the experimentally determined mass of an ion measured to an appropriate degree of accuracy and precision used to determine, or limit the possibilities for, the elemental formula of the ion
- **Exact mass**: is the calculated mass of an ion whose elemental formula, isotopic composition and charge state are known, i.e., it is the theoretical mass.

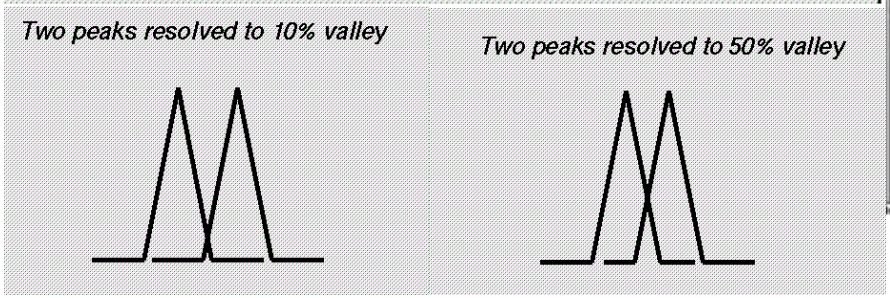
$$\text{Mass error (ppm)} = \frac{(\text{Accurate mass} - \text{Exact mass})}{\text{Exact mass}} \times 10^6$$

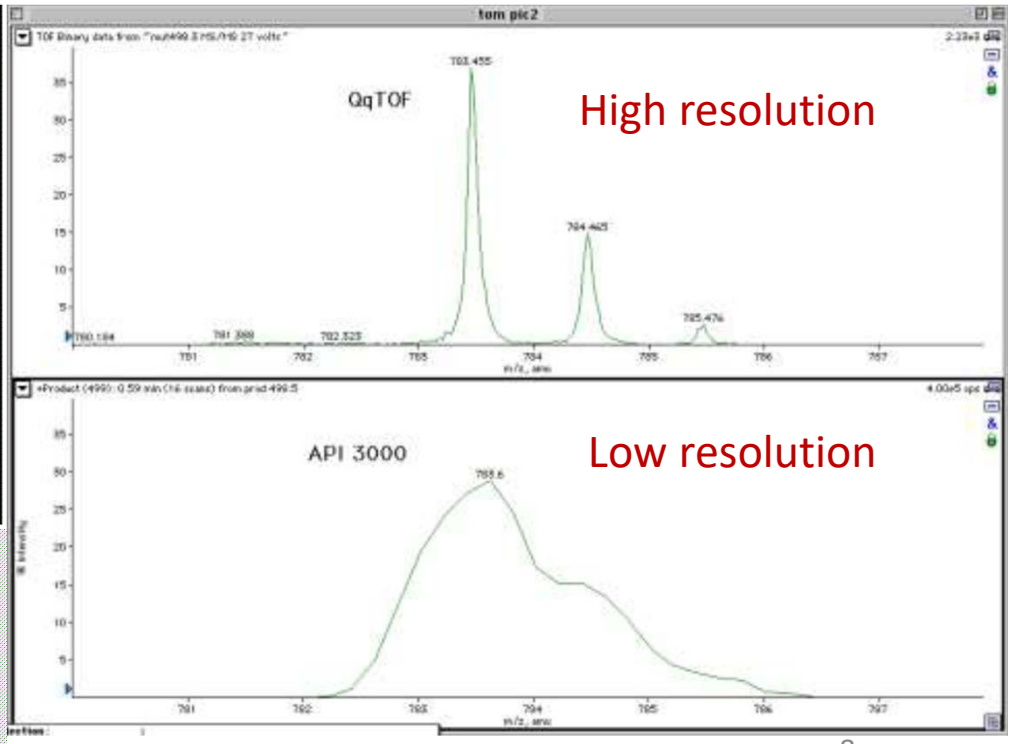


Resolution and Resolving Power

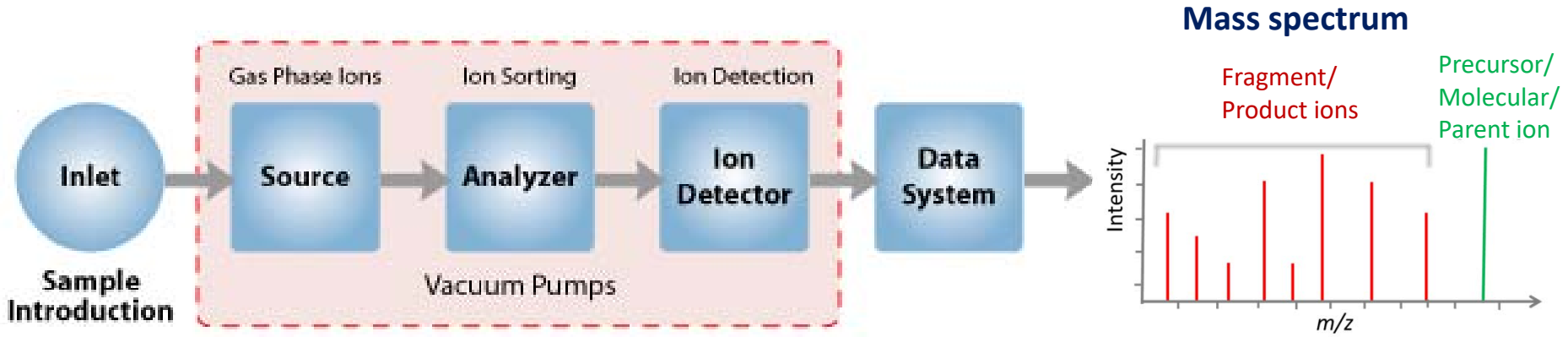
- Width of peak indicates the resolution of the MS instrument
- The better the resolution or resolving power, the better the instrument and the better the mass accuracy.
- Resolving power is defined as: **M/ΔM**
- M is the mass number of the observed mass (ΔM) is the difference between two masses that can be separated







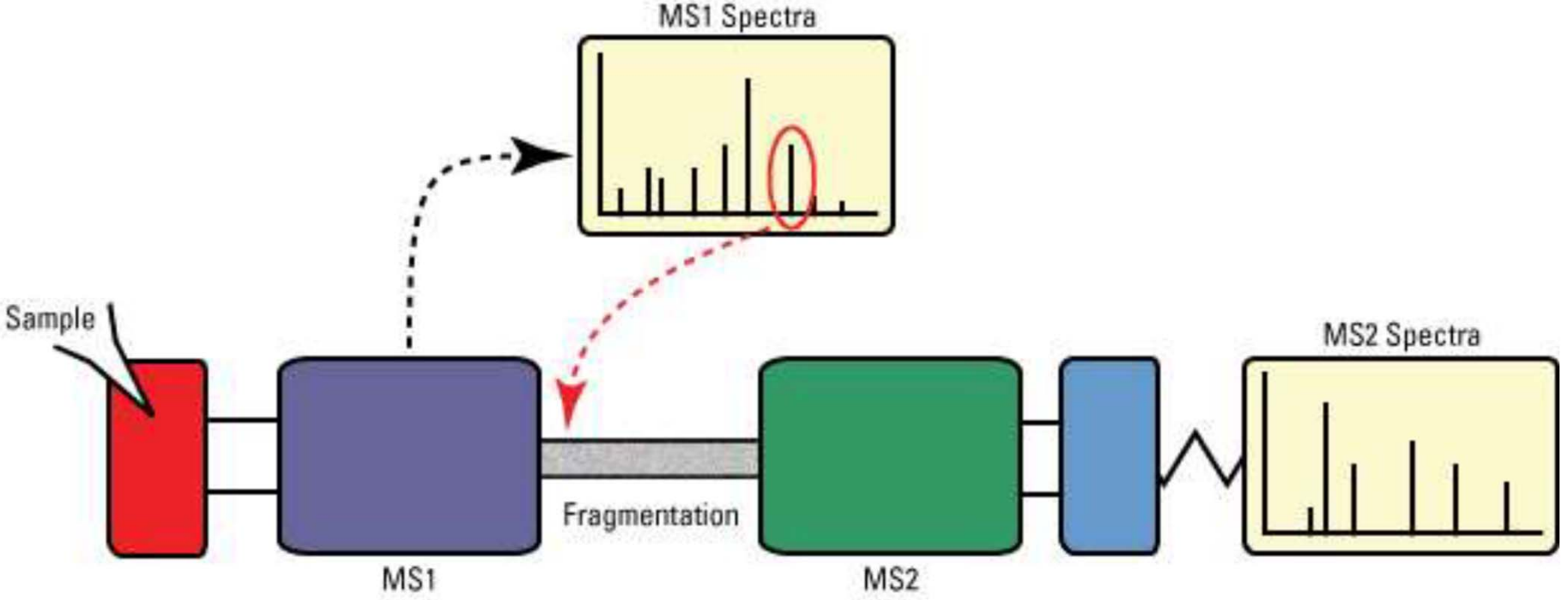
Basic components of a MS



1. **Inlet:** Introducing sample into ion source
2. **Ion source** : samples are ionized and given an electric charge
3. **Mass analyzer**: separates ions according to their mass-to-charge ratio (m/z)
4. **Detector:** where ions are observed and counted
5. **Data System:** receiving signal from detector

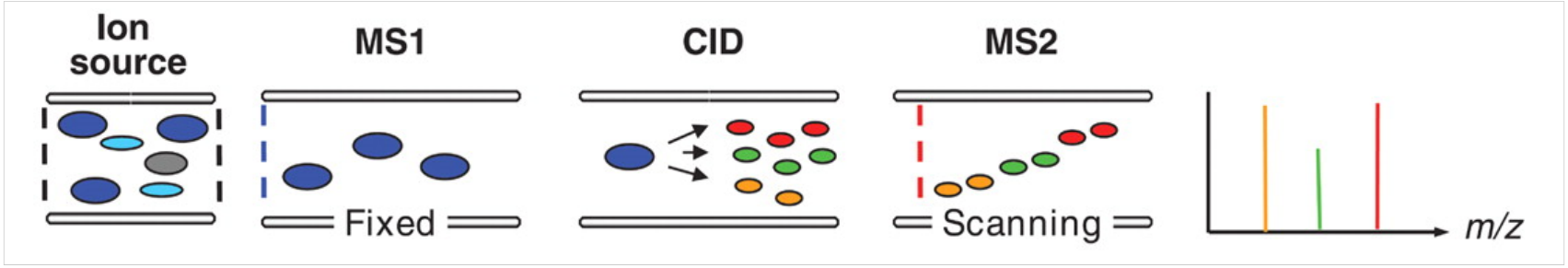
http://www.premierbiosoft.com/tech_notes/mass-spectrometry.html

Principle of Tandem Mass Spectrometry (MS/MS)



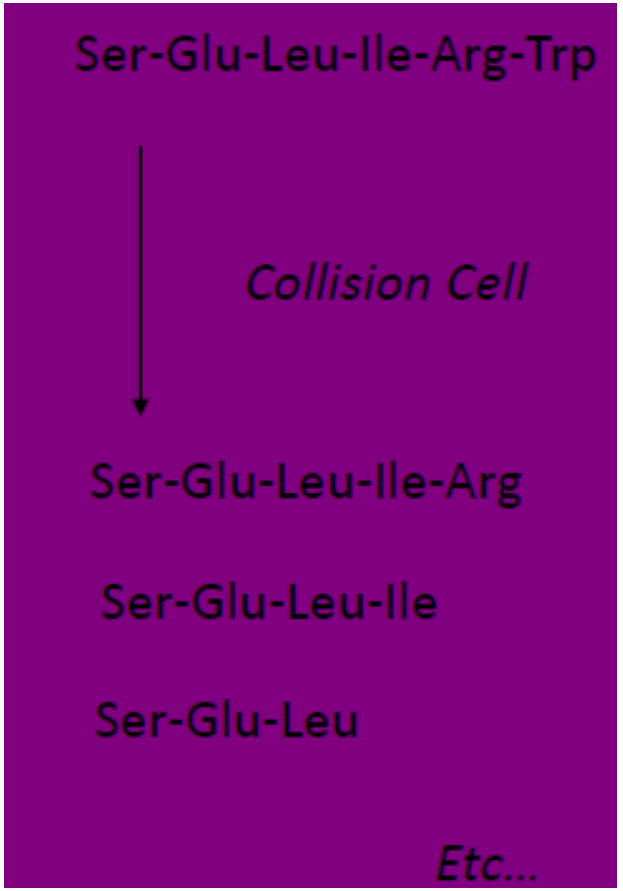
- A sample is injected into the mass spectrometer, ionized, accelerated and analyzed by mass spectrometry (MS1).
- Ions from the MS1 spectra are then selectively fragmented and analyzed by a second stage of mass spectrometry (MS2) to generate the spectra for the ion fragments.

How Tandem MS sequencing works



Science 2006, 312 (5771), 212

- Tandem MS: two mass analyzers in series with a collision cell in between
- Collision cell: a region where the ions collide with a gas (He, Ne, Ar) resulting in fragmentation of the ion
- The resulting daughter ions have masses that are consistent with known molecular weights of dipeptides, tripeptides, tetrapeptides…



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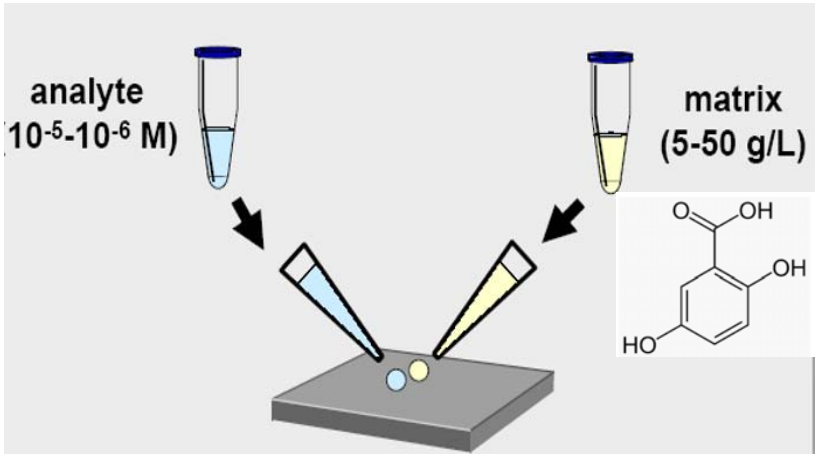
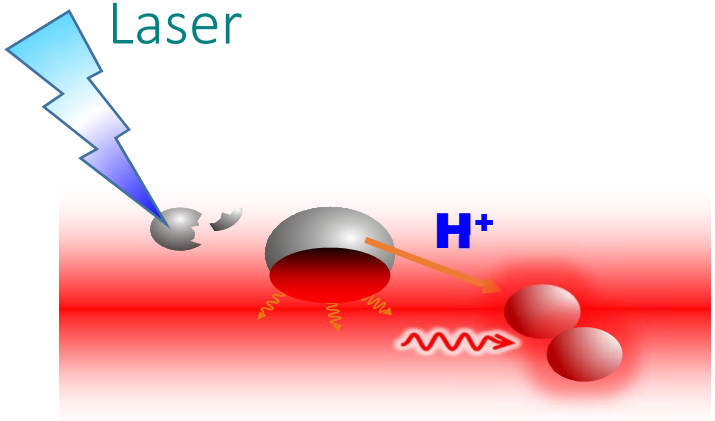
MALDI-TOF MS

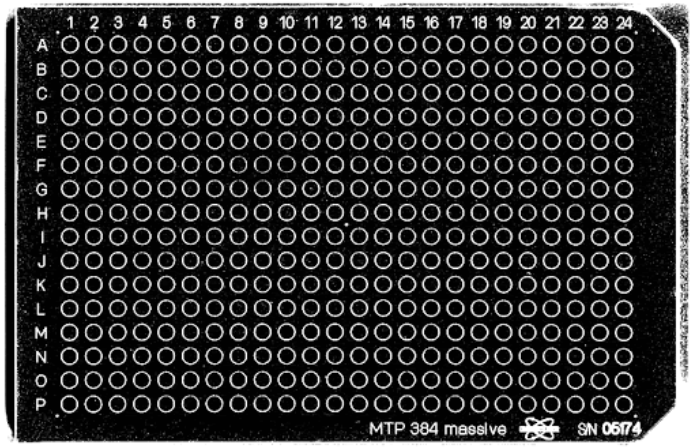

analyte
(10-5-10-6 M)
matrix
(5-50 g/L)
O
OH
OH
HO


Laser
H+

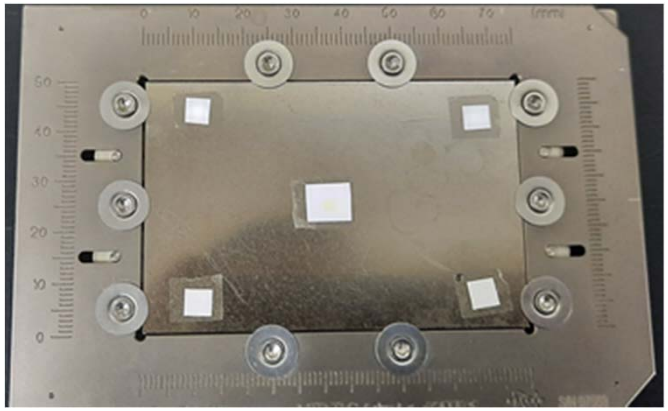
ultrafleXtreme

Common MALDI Sample Inlet

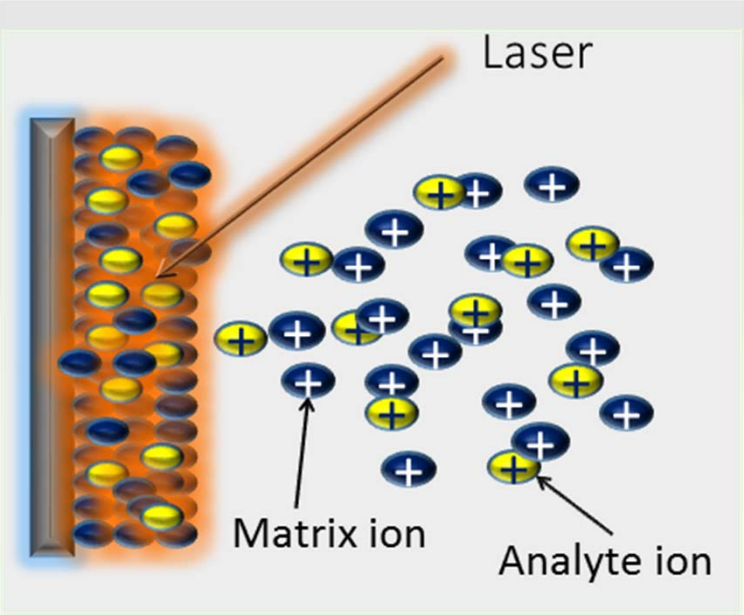
1. MALDI plate



2. MALDI adapter



Matrix-assisted laser desorption Ionization (MALDI)



- Sample is ionized by bombarding sample with laser light
- Sample is mixed with a UV absorbant matrix
- Light wavelength matches that of absorbance maximum of matrix so that the matrix transfers some of its energy to the analyte (leads to ion sputtering)

Spotting:

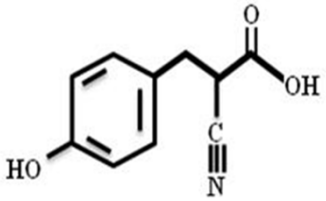
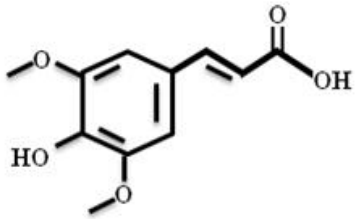
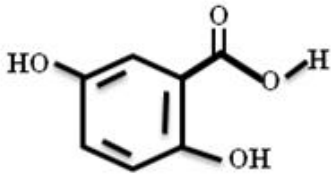
Analyte → dissolved in solvent → mixed with matrix → spot in plate → dry

Mechanism:

- Analyte-matrix crystal → laser beam → matrix absorbs energy → analyte desorption and ionization → P**rotonated analyte cation**

El-aneed, A., Cohen, A., & Banoub, J. (2009). Mass Spectrometry

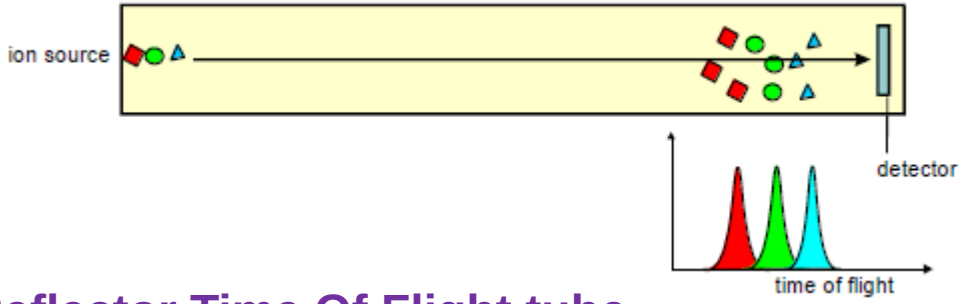
List of some MALDI matrices and their applications

Matrix	Preparation	Excitation Wavelength	Applications
α-cyano-4-hydroxycinnamic acid (CHCA) 	50% Acetonitile, 0.1% TFA in deionized pure water	337 nm, 355 nm	Peptides less than 5000 Da, lipids and nucleic acids
Sinapinic acid (SA) 	30-50% acetonitrile, 0.1% TFA in deionized water	337, 355, 266 nm	Peptides and proteins higher than 5000 Da and sometimes also used
2,5-Dihydroxybenzoic acid (DHB) 	50% acetonitrile, 0.1% TFA in deionized water, 1% Phosphoric acid in deionized water	337, 355, 266 nm	Small molecules and peptides which are not ionized by other matrices

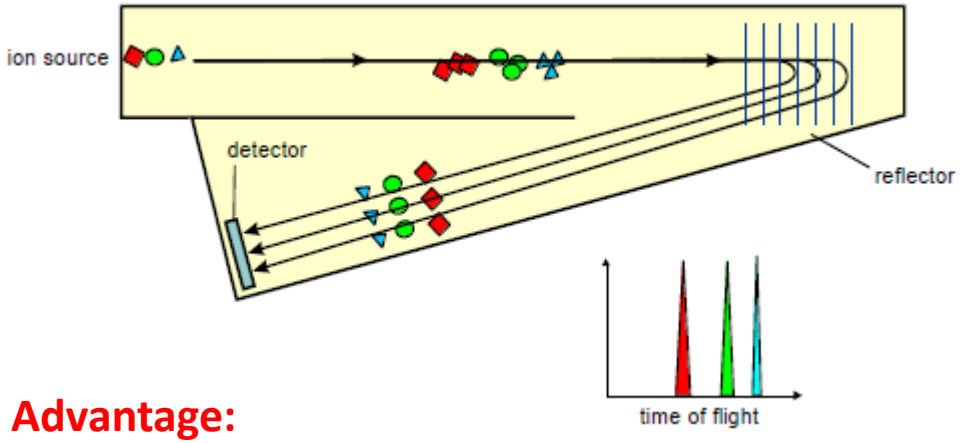
http://iitb.vlab.co.in/?sub=41&brch=118&sim=414&cnt=1

Time of Flight (TOF)

Linear Time Of Flight tube



Reflector Time Of Flight tube



Advantage:

- Switchable modes to increase sensitivity or resolution
- High mass range that can go beyond mega dalton range
- Ex.. 4800 MALDI and Bruker MALDI

Linear TOF

- Relies on the free flight of ionized molecules in a flight tube 0.5-2m long at a fixed potential
- Separates ion's based on the relationship that **m/z is proportional to the square of the time of flight** (t_f)
- $E = ½ (m/z)v2$

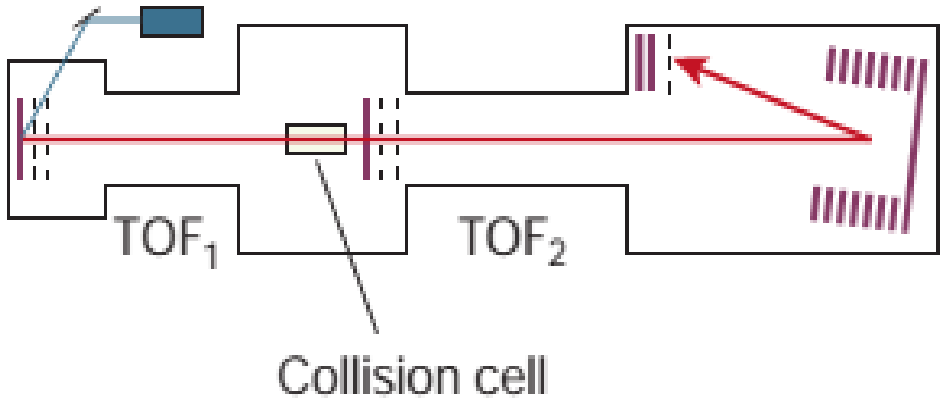
Reflector TOF

- Uses a reflectron (electrostatic ion mirror) to alter the path of the ions inside the tube
- The longer flight path leads to higher mass resolution but compromises the sensitivity
- Most TOFs have both linear and reflector options

Glish, G. L.; Vachet, R. W. The Basics of Mass Spectrometry in the Twenty-First Century. Nat. Rev. Drug Discov. 2003, 2, 140–150.

Time of Flight/Time-of Flight (TOF/TOF)

TOF/TOF



Mass resolution: 10,000-20,000
Mass accuracy (ppm): 10-20^{a}, < 5^{b}
Sensitivity: femtomole
m/z: no upper limit
Scan rate: fast
Dynamic range: 1E4
MS/MS capability: MS/MS
Ion source: MALDI

- Includes a **collision cell between** the **two TOFs**
- Ions of specific m/z are first selected in the first TOF
- Fragmented in the collision cell
- The fragments are separated in the second TOF

Main applications:

- Protein identification from in-gel digestion of gel-separated protein band by peptide mass finger printing or sequence tagging via CID MS/MS

Aebersold, R.; Mann, M. Mass Spectrometry-Based Proteomics. 2003, 422.

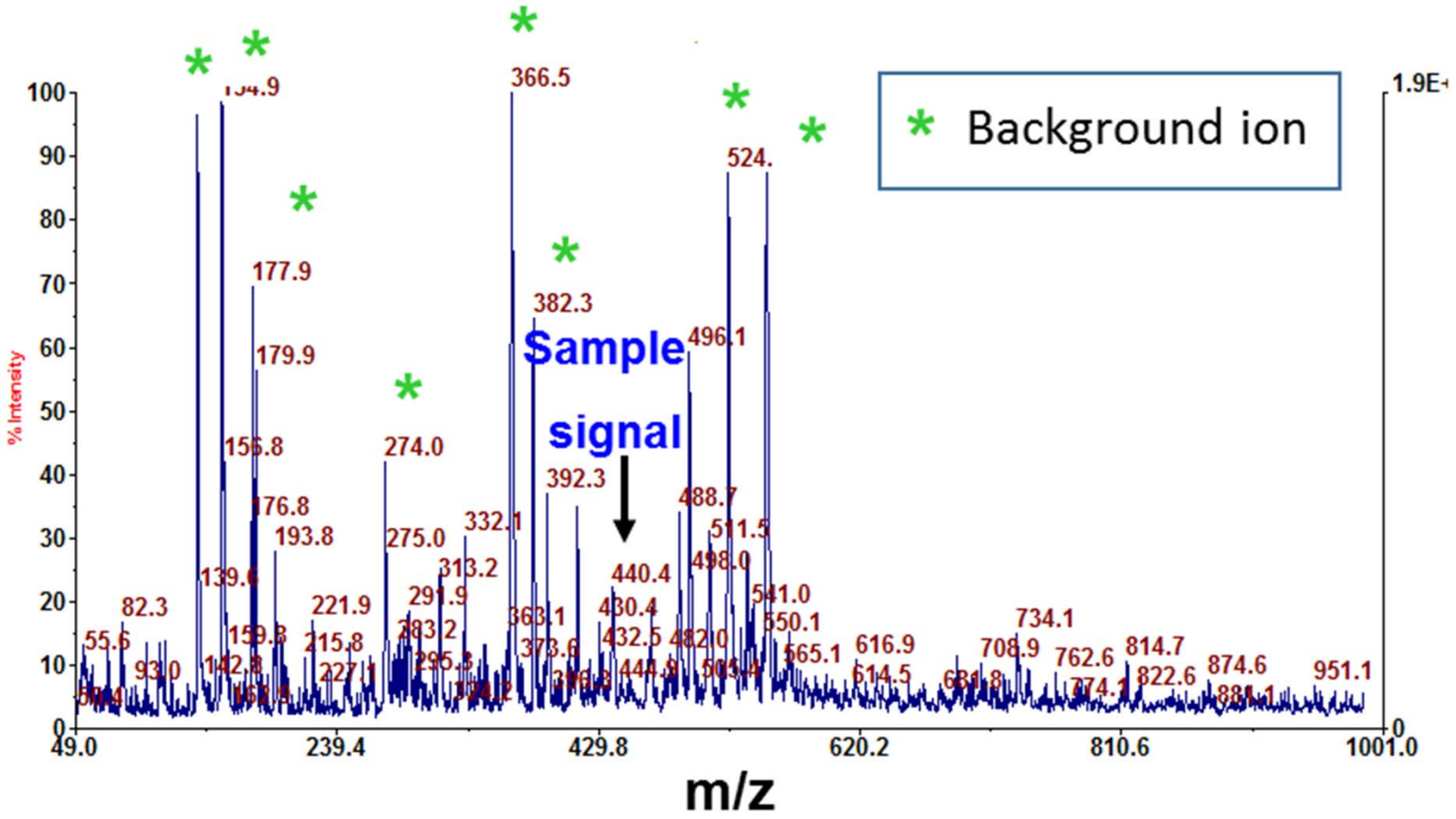
Han, X.; Aslanian, A.; Yates, J. R. Mass Spectrometry for Proteomics. *Curr. Opin. Chem. Biol.* **2008**, *12*, 483–490.

Advantage of MALDI-TOF MS

- ✓ Soft ionization - analyze intact biomolecules and synthetic polymers
- ✓ Broad mass range - analyze a wide variety of biomolecules
- ✓ Relatively tolerant of buffers and salts
- ✓ Fast data acquisition
- ✓ Solid sample
- ✓ Easy to use and maintain, no water or gas hook ups required
- ✓ High sensitivity, resolution and accuracy

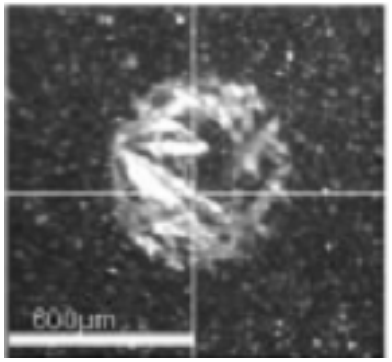
Limitations of MALDI-TOF MS

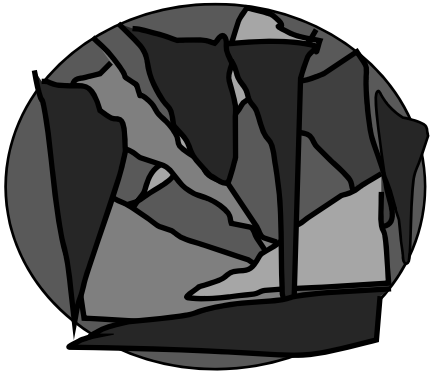
- **Background (matrix peaks) in low mass range (m/z = 600)**

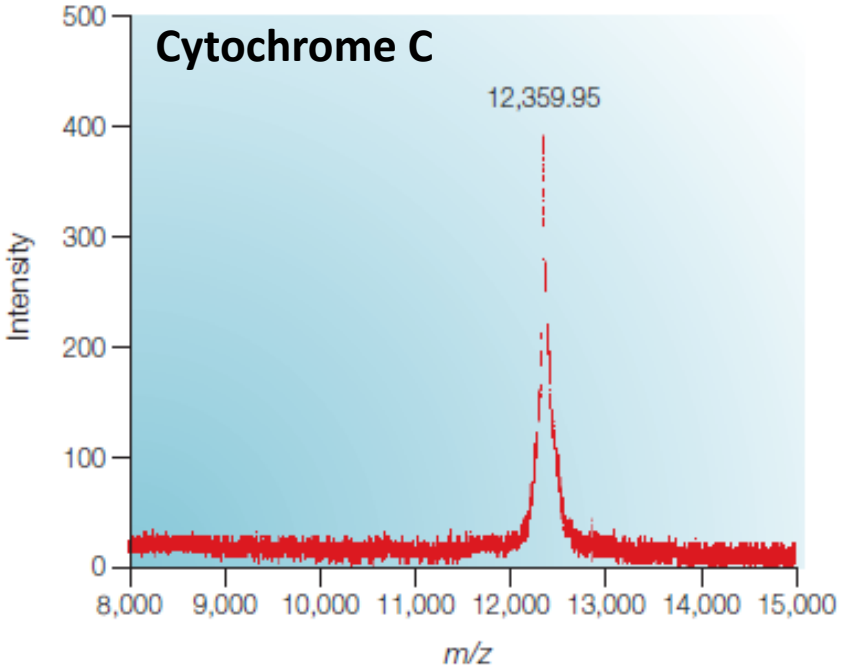


Limitations of MALDI-TOF MS

- **Sweet spot effect make it difficult for quantification**







Large crystals in DHB and sweet spot effect

***sweet spots = dark spots**

Nature Reviews, 2003, Volume 2, 140-150

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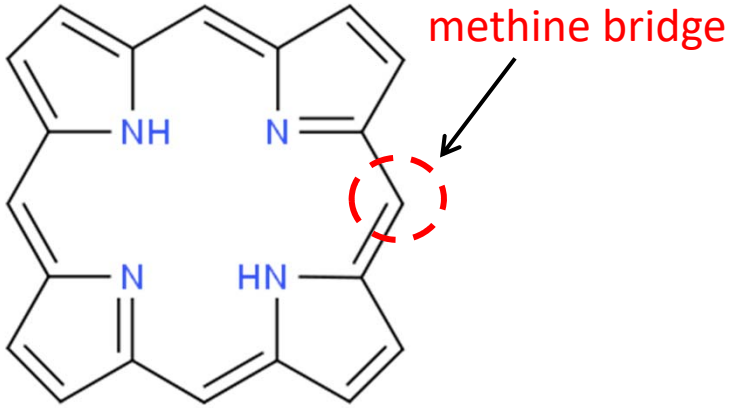
Porphyrins

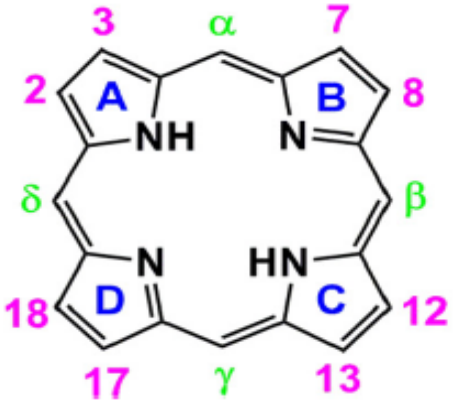
- Heterocyclic molecule formed by linkage of four pyrrole rings through methine bridges
- Porphine is the parent porphyrin



Properties

- High affinity with metals
- Large surface area
- Inherent nanopores
- Excellent stability

❑ Porphyrin Side Chains



M = Methyl ($-CH_3$)

E = Ethyl ($-CH_2CH_3$)

V = Vinyl ($-CH=CH_2$)

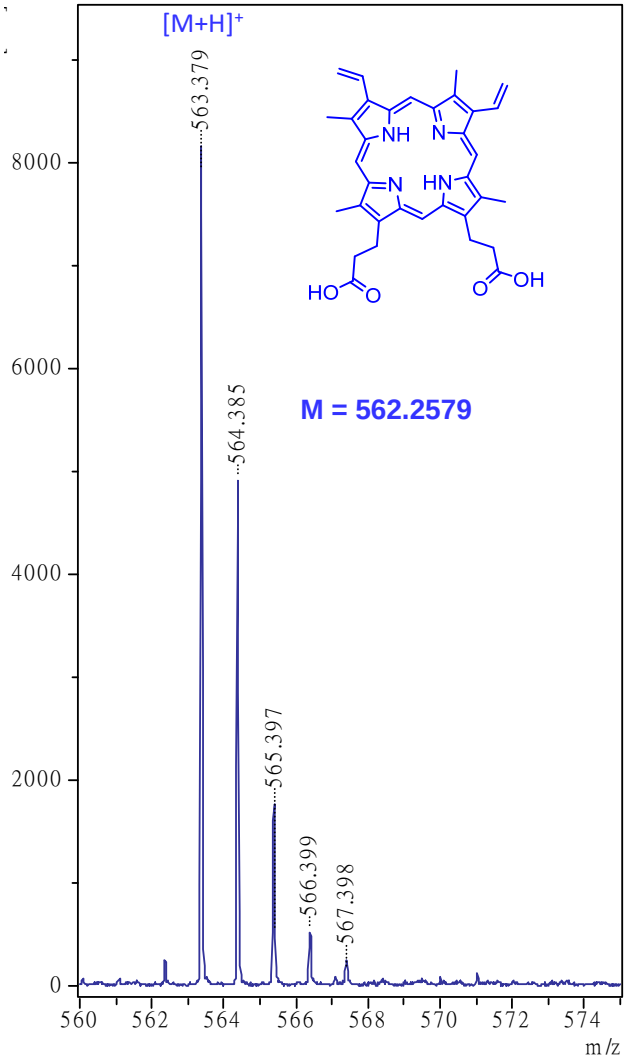
A = Acetyl ($-CH_2COOH$)

P = Propionyl ($-CH_2CH_2COOH$)

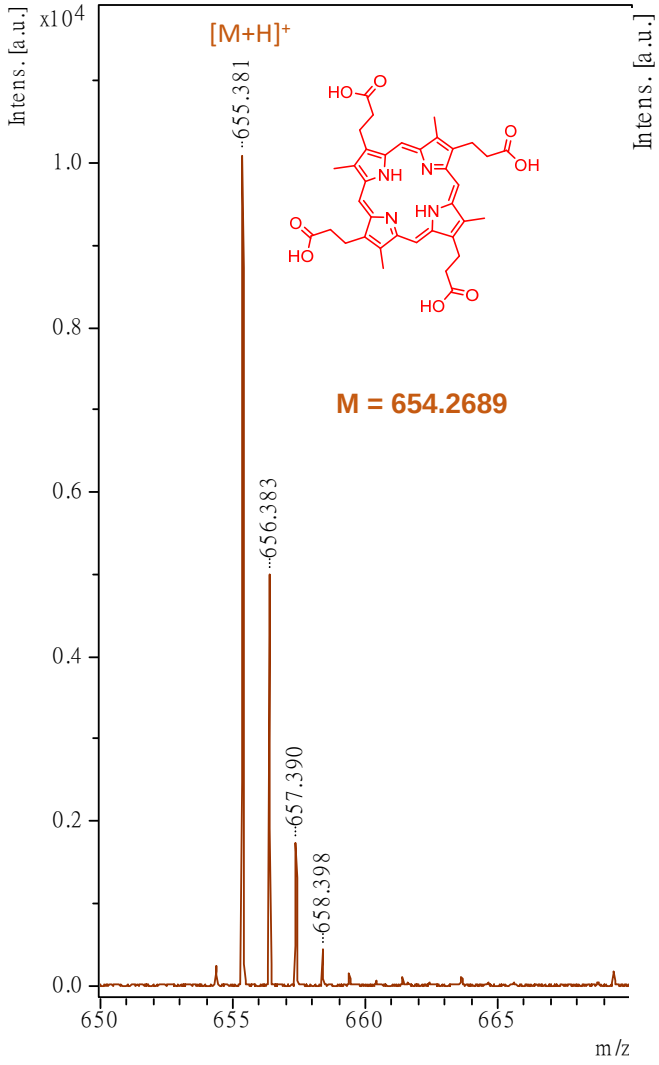
Anal. Biochem. ***2015****, 478, 82–89 and* *Biomed. Chromatogr.* **2006**, **20**, 612–621.

TOF spectra of porphyrins

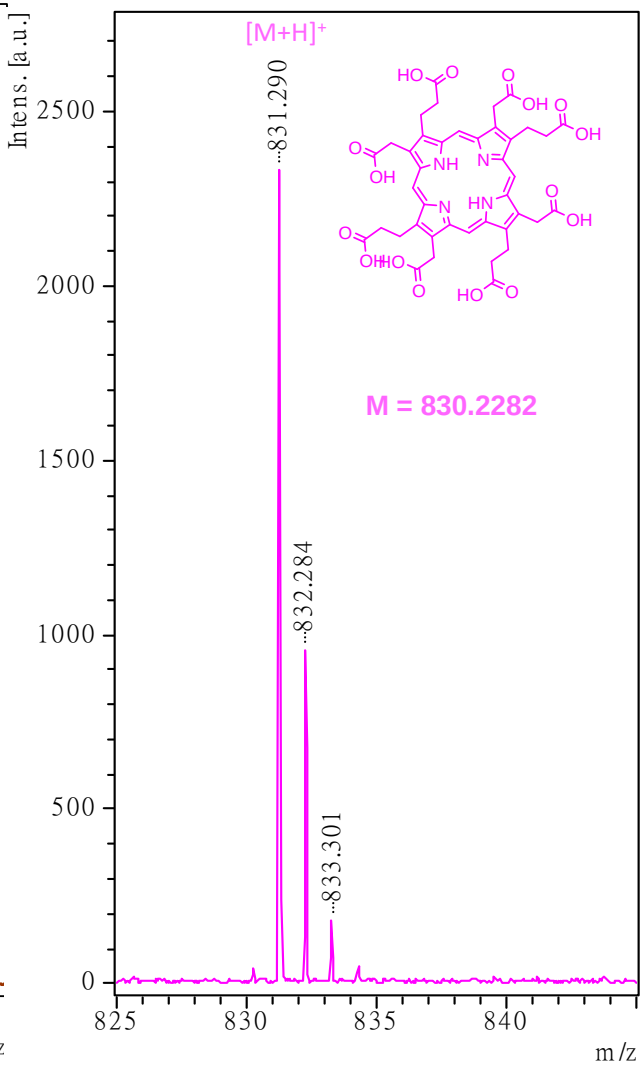
Protoporphyrin IX (PPIX)



Coproporphyrin I (CopI)



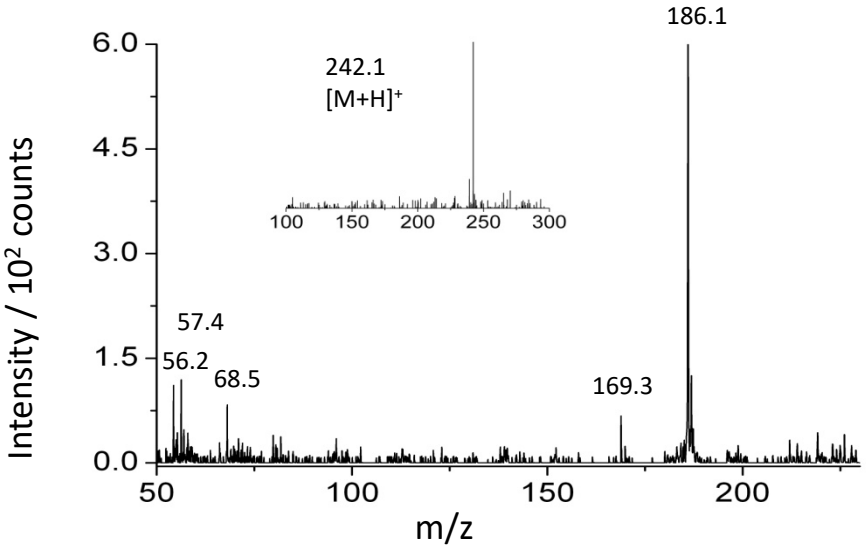
Uroporphyrin I (Urop I)

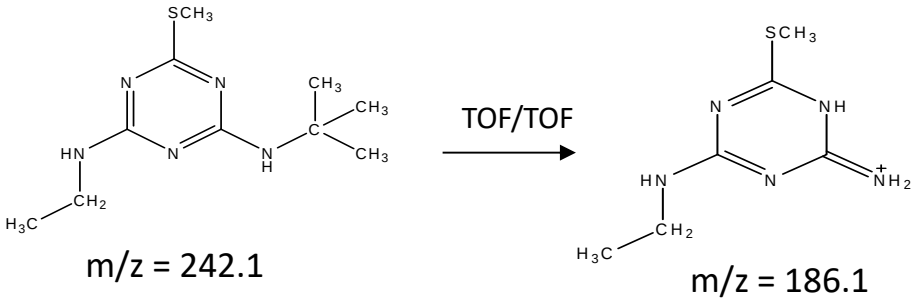


Analysis of Isomer Triazine Compounds

Terbutryn

(a)



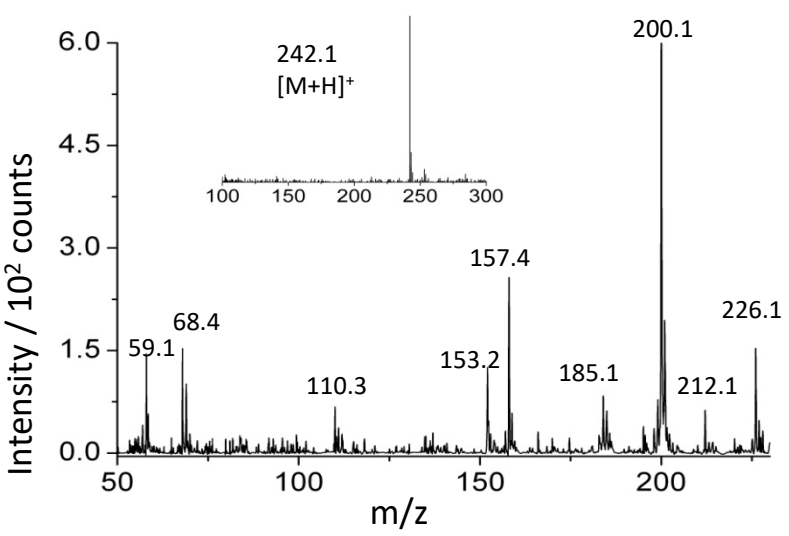


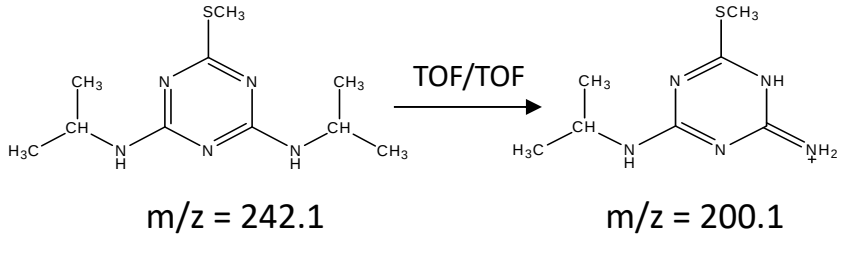
m/z = 242.1

m/z = 186.1

Prometryn

(b)





m/z = 242.1

m/z = 200.1

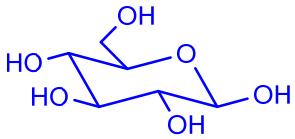
Carbohydrates/Glycans

- Carbohydrates are one of the most abundant molecule found in nature and made by all living organisms

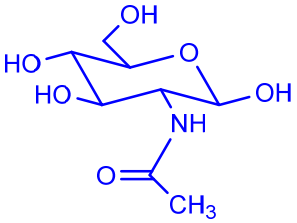
- Based on the number of sugar units, carbohydrates are classified into four different groups: monosaccharides, disaccharides, oligosaccharides and polysaccharides.

Monosaccharides

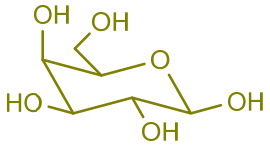
Glucose (Glc)



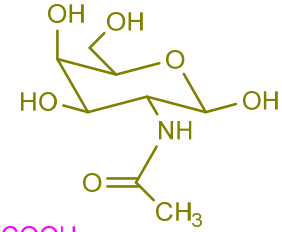
N-acetylglucosamine (GlcNAc)



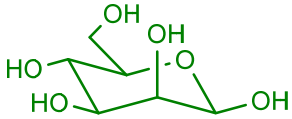
Galactose (Gal)



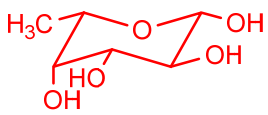
N-acetylgalacosamine (GalNAc)



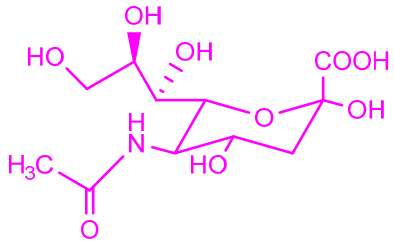
Mannose (Man)



Fucose (Fuc)

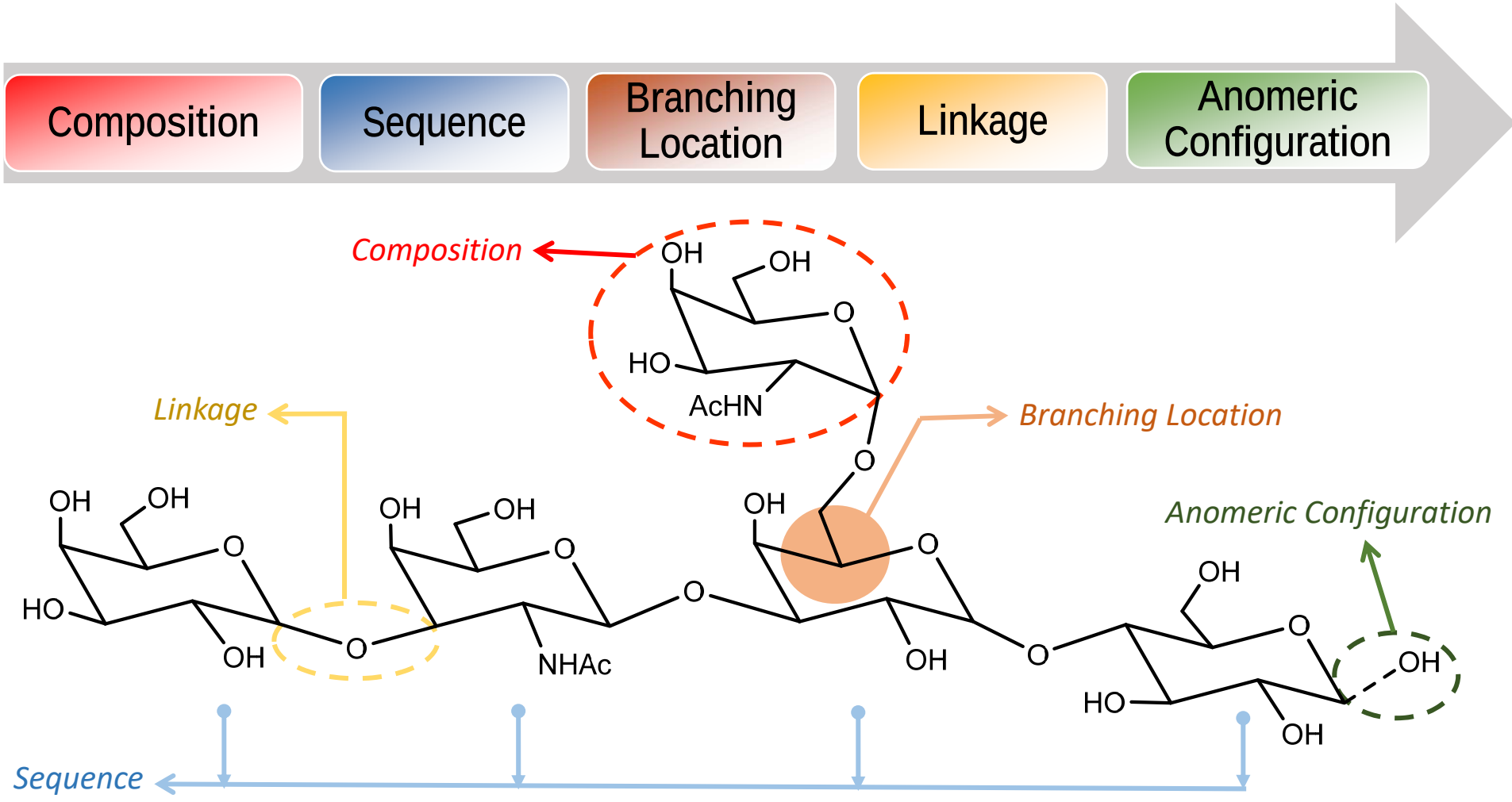


Sialic acid



Structures of carbohydrates

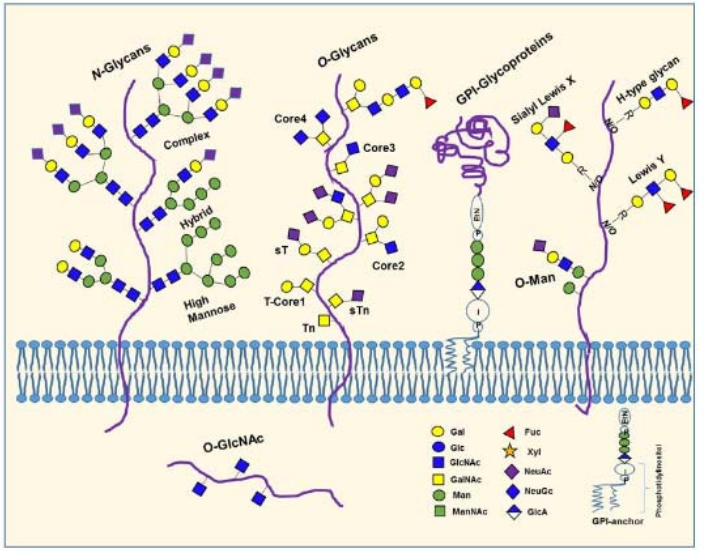
- **A complete structure of carbohydrates include the following information**



Glycan analysis is essential for understanding their roles in biological processes

❑ Glycan structures are closely related to biological function:

- Understanding biological processes
- Disease detection, progression and diagnosis
- Therapeutic strategies



Neoplasia 2018, 20 , 813-825

❑ Examples of important glycans

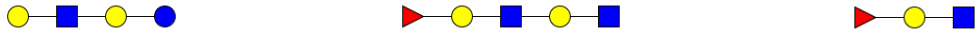
Glycans	Application	References
β-Glucan	Diagnosis and therapeutic monitoring of fungal infections	*Lancet, 1995, 345, 17-20 and Clin. Infect. Dis. 2005, 41, 654-9.*
Heparin	Anticoagulant and Inhibitor of cancer progression	*Handb. Exp. Pharmacol. 2012, 207, 3–22. and Int. J. Oncol. 1995, 6 , 885-8.*
Gangliosides	Cancer progression and immunotherapies	*Biochimie 2003, 85, 455-463 and Front Immunol. 2014, 5, 325.*

Carbohydrate analysis is challenging

❑ Complex structure

(A) Composition

(B) Sequence



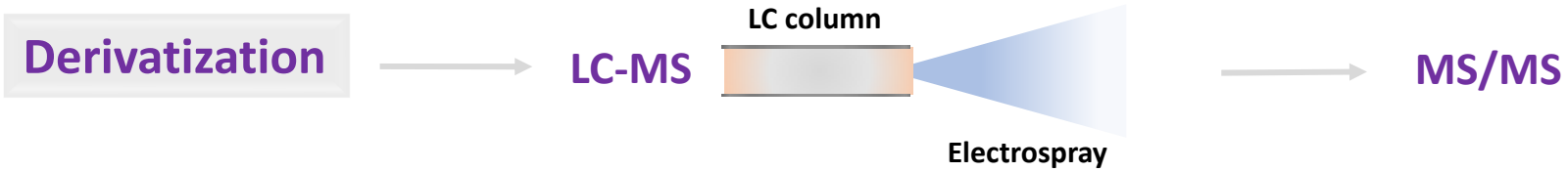
(C) Branching

(D) linkage

❑ Low Ionization efficiency

❑ Incomplete fragmentation

Chemical derivatization followed by tandem mass is conventional method for glycan analysis



❑ Why derivatization?

- ➢ To improve ionization efficiency
- ➢ To facilitate fragmentation in MS/MS
- ➢ To eliminate the labile moieties loss

❑ Common Derivatizations

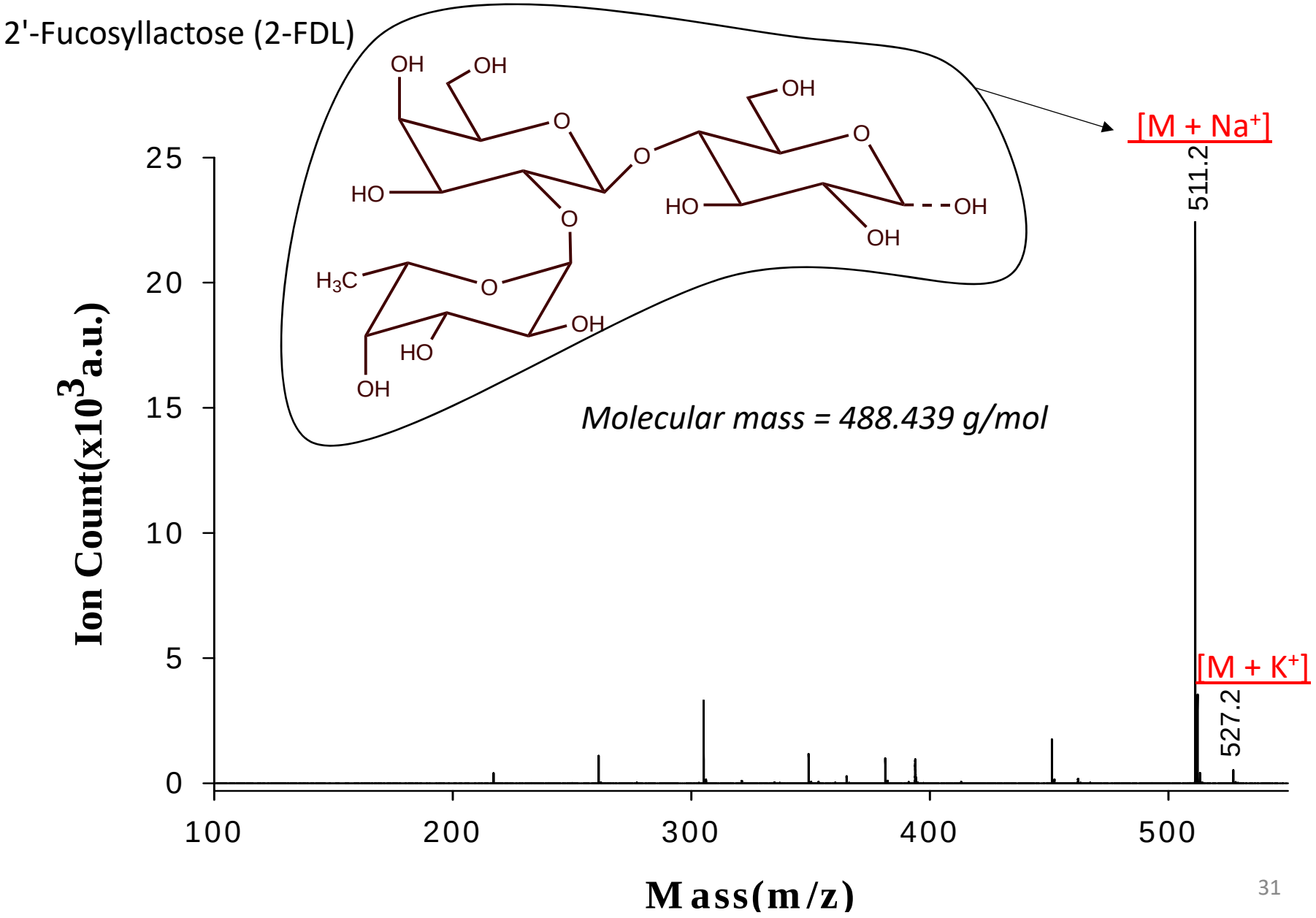
- ➢ Permethylation
- ➢ Peracetylation
- ➢ Reducing end labeling

❑ Limitations

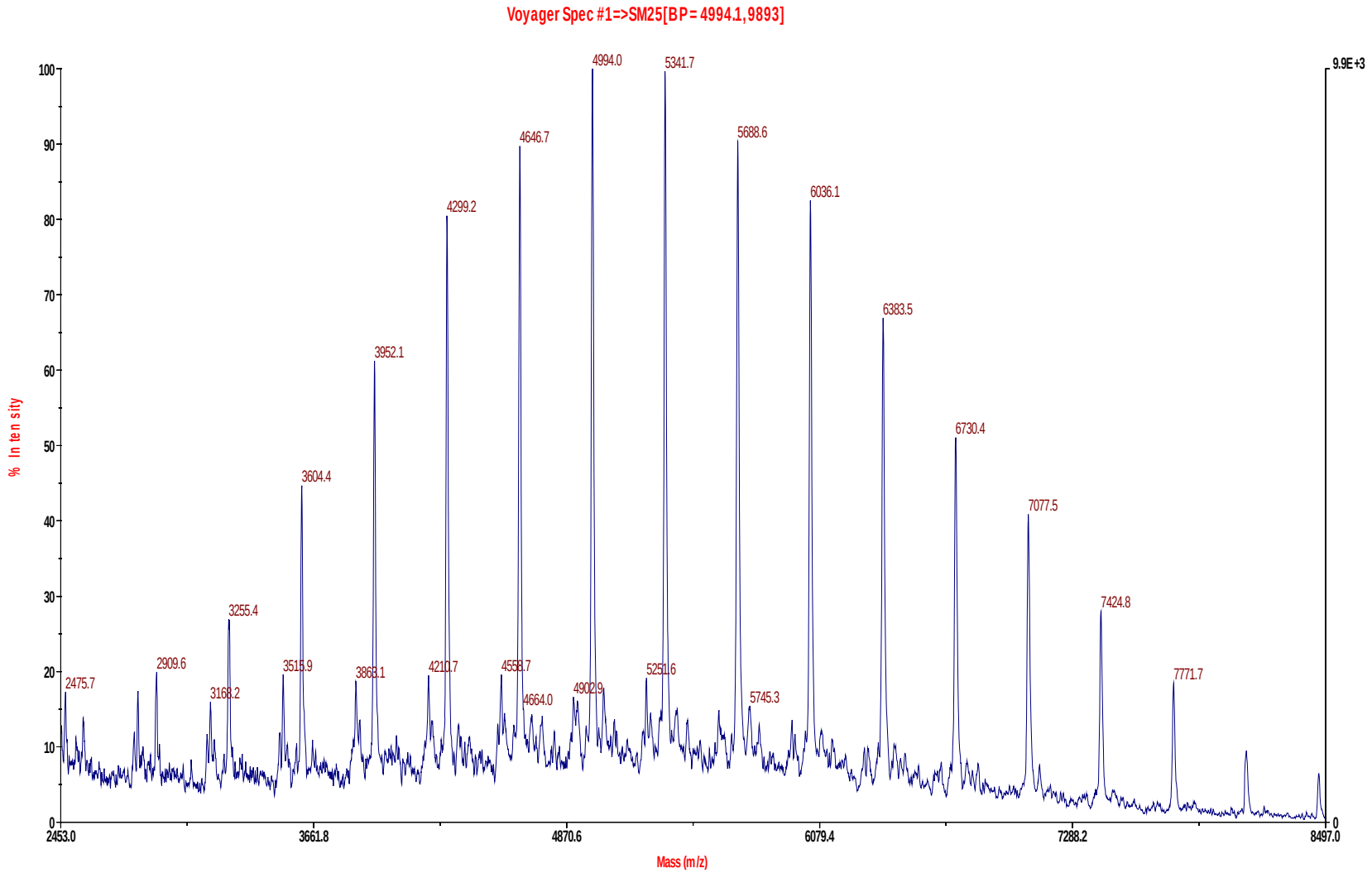
- ✓ *Sample and time consuming*
- ✓ *Risk of contamination and side reaction*
- ✓ *Tedious sample preparation*
- ✓ *Mass shift*

Analyst 2017, 142, 4446-4455

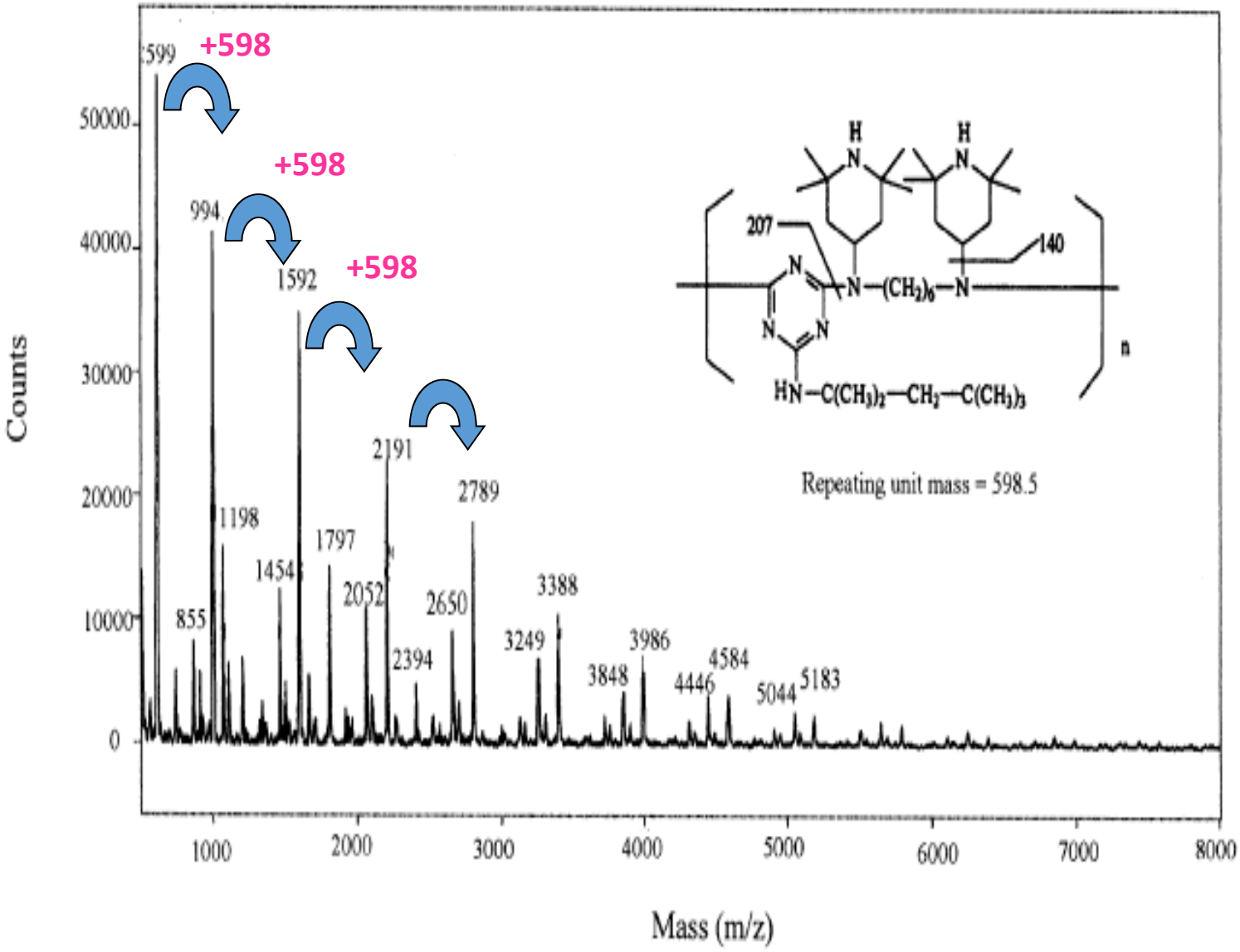
TOF Spectrum of carbohydrates



Identification of Polymer by using MALDI TOF

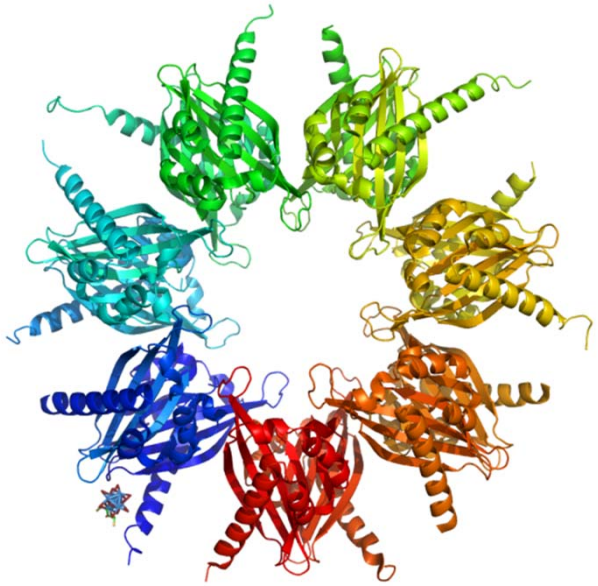


Identification of Polymer by using MALDI TOF



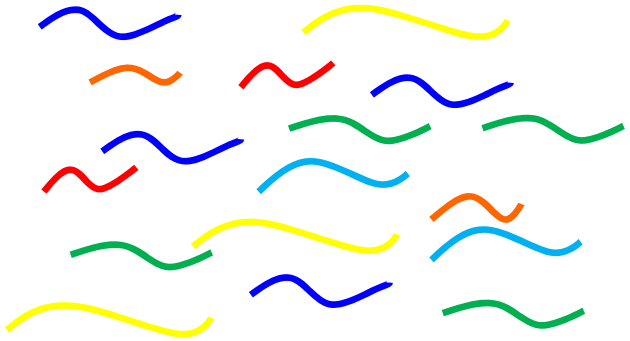
Identification of Protein by using MALDI TOF

Protein



Enzymatic Digestion

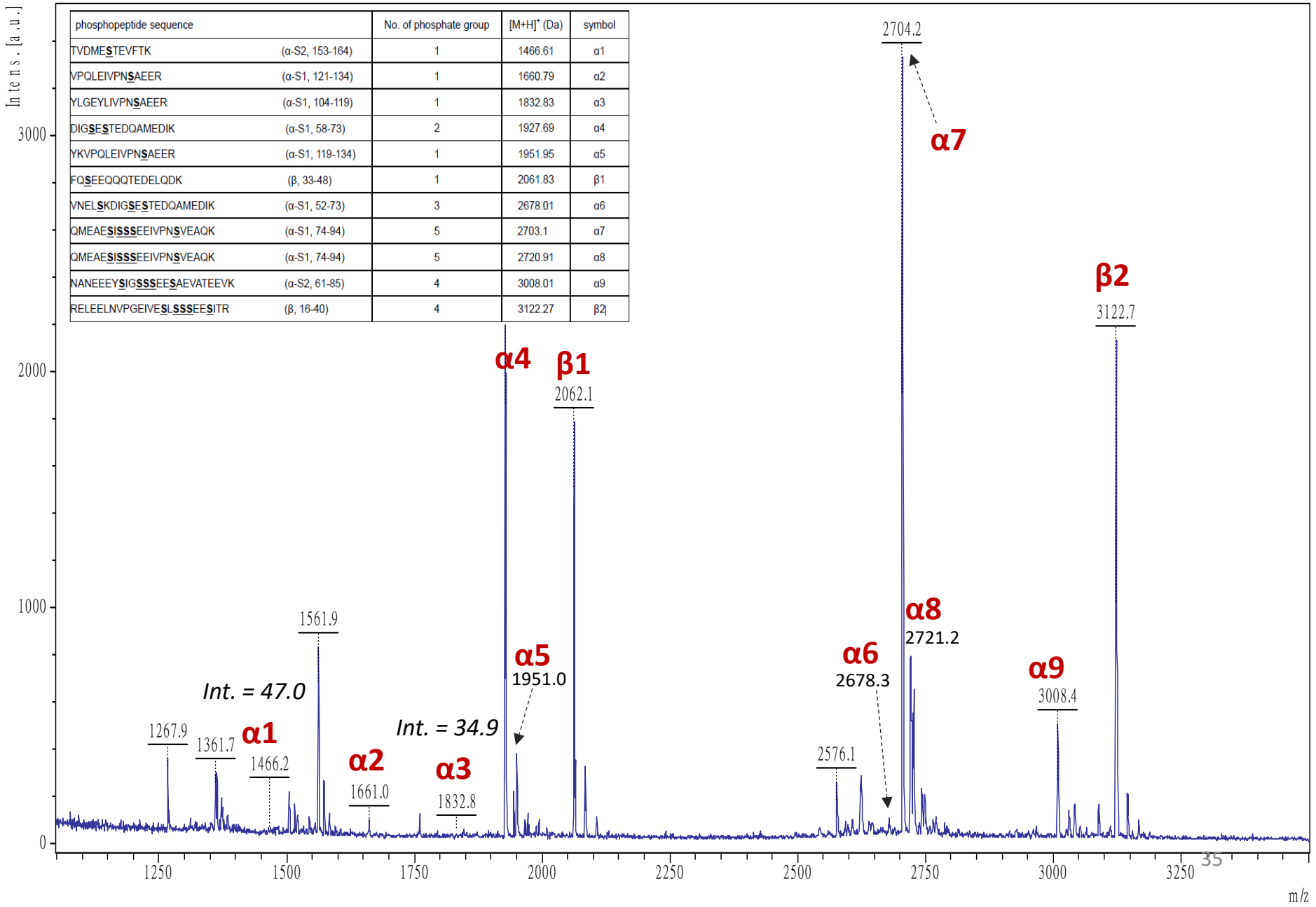
Peptide



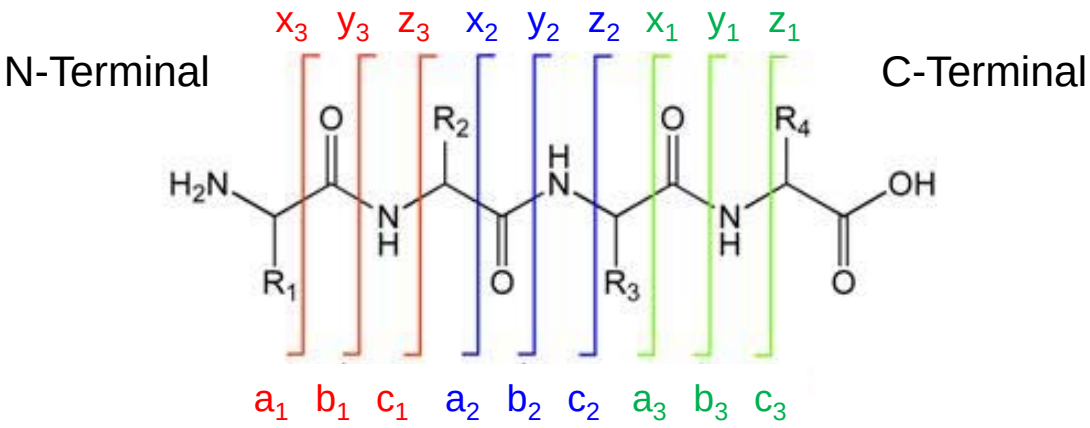
- **Molecular Weight**
  - MALDI MS
- **Sequence information**
  - MALDI MS
    (Peptide mass fingerprinting)

α&β-casein Protein analysis by using MALDI TOF

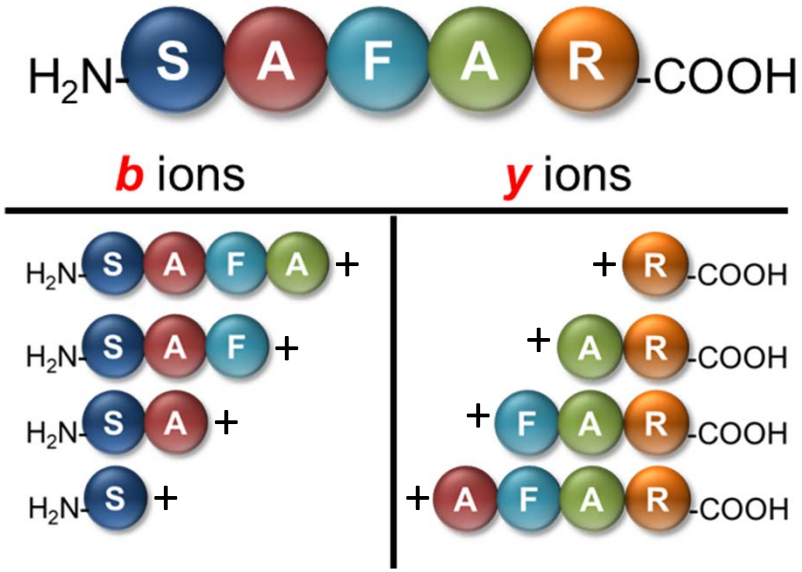
phosphopeptide sequence		No. of phosphate group	$[M+H]^+$ (Da)	symbol
TVDME**S**TEVFTK	(α-S2, 153-164)	1	1466.61	α1
VPQLEIVPN**S**AEER	(α-S1, 121-134)	1	1660.79	α2
YLGEYLIVPN**S**AEER	(α-S1, 104-119)	1	1832.83	α3
DIG**S**E**S**TEDQAMEDIK	(α-S1, 58-73)	2	1927.69	α4
YKVPQLEIVPN**S**AEER	(α-S1, 119-134)	1	1951.95	α5
FQ**S**EEQQQTEDELQDK	(β, 33-48)	1	2061.83	β1
VNEL**S**KDIG**S**E**S**TEDQAMEDIK	(α-S1, 52-73)	3	2678.01	α6
QMEAE**S**I**SSS**EEIVPN**S**VEAQK	(α-S1, 74-94)	5	2703.1	α7
QMEAE**S**I**SSS**EEIVPN**S**VEAQK	(α-S1, 74-94)	5	2720.91	α8
NANEEEY**S**IG**SSS**EE**S**AEVATEEVK	(α-S2, 61-85)	4	3008.01	α9
RELEELNVPGEIVE**S**L**SSS**EE**S**ITR	(β, 16-40)	4	3122.27	β2

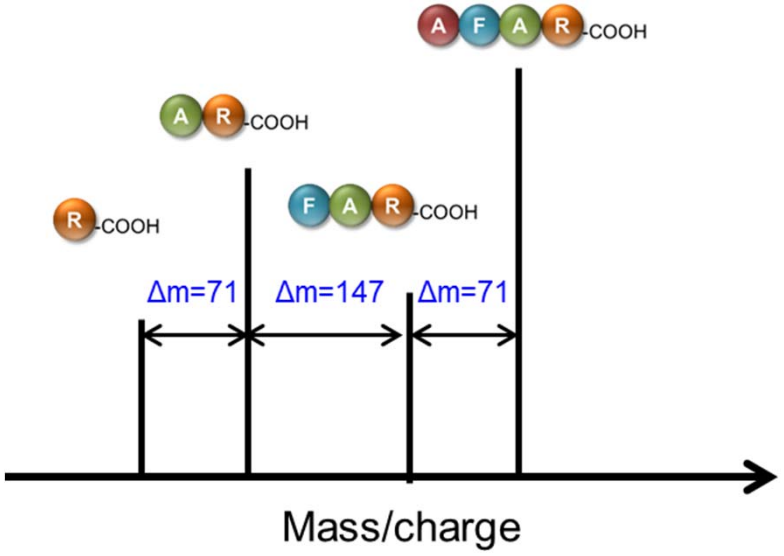


Peptide Fragmentation

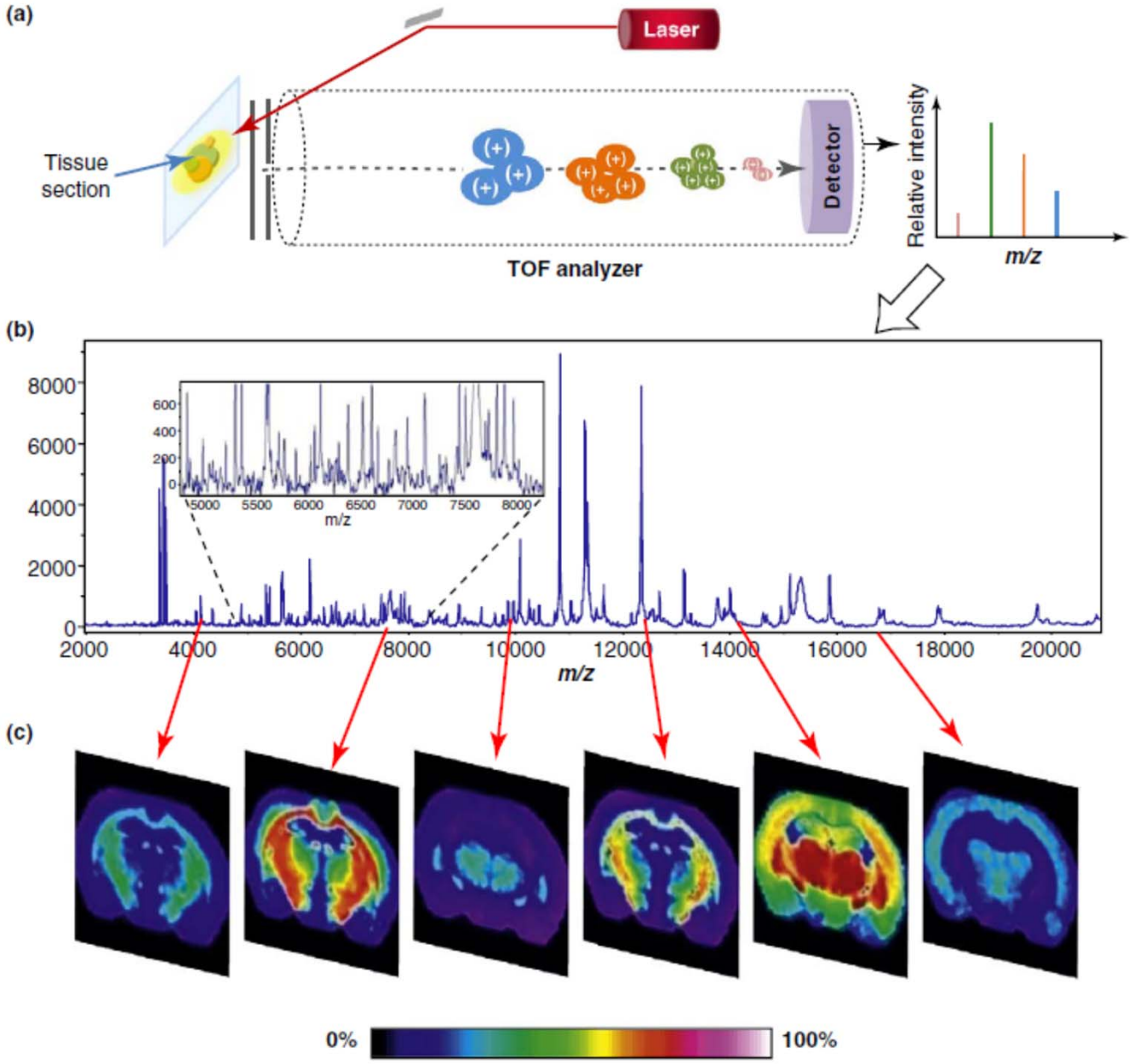


- a,b,c ion - C-Terminal side
- x,y,z ion - N-Terminal side



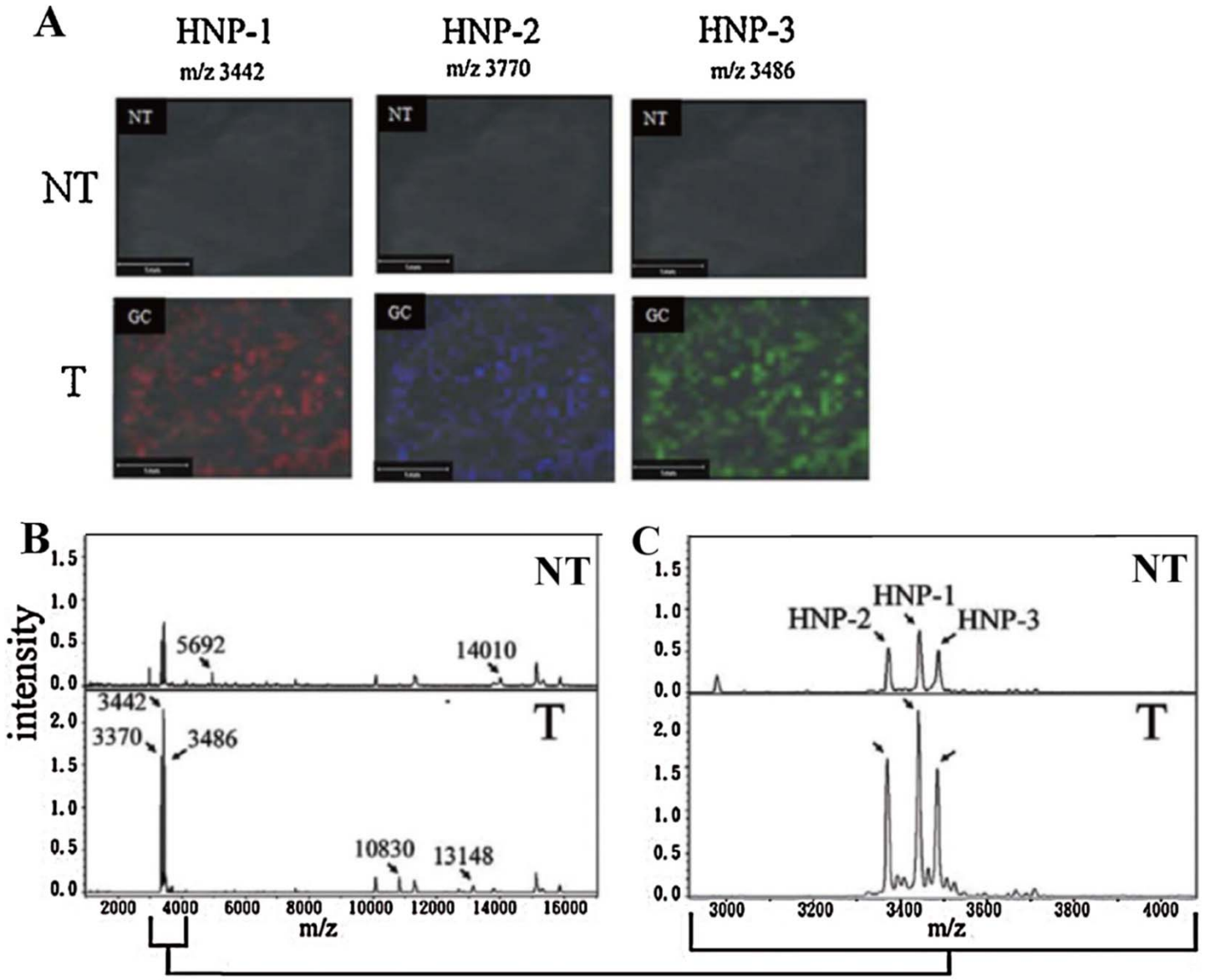


MALDI Imaging



Trends in Biotechnology March 2011, Vol. 29, No. 3

MALDI Imaging



Journal of Pharmaceutical and Biomedical Analysis 95 (2014) 245–255

Lecture Outline

Part I: Introduction to mass spectrometry (MS)

- MS spectrum
- Masses in MS
- Accuracy and resolution
- Basic parts
- Principles MS and MS/MS

Part II: MALDI-TOF MS

- Principle
- Sample preparation
- Basic parts (Ion sources and mass analyzers)
- Unique features of MALDI

Part III: Applications of MALDI-TOF MS

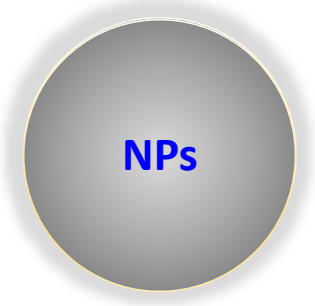
- Small molecules
- Carbohydrates
- Proteins
- Lipids

Part IV: Noble materials can be used/coupled with MALDI MS

- Nanoparticles
- Thin-layer chromatography
- Silica wafer
- Membrane

Nanoparticles as a MALDI matrix

❑ Nanoparticle (NPs) as a MALDI matrix



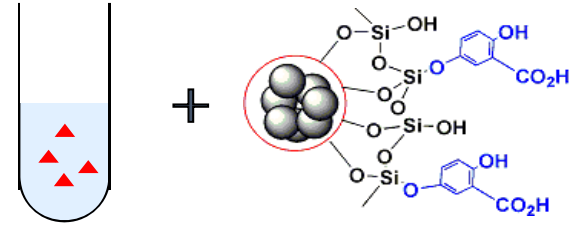
- ➢ *Large surface area-to-volume ratios*
- ➢ *High molar absorption coefficients*
- ➢ *Ready for functionalization*

- ▪ *Strong UV absorption*
- ▪ *Background free*
- ▪ *Enhanced ionization*
- ▪ *Facilitate fragmentation*

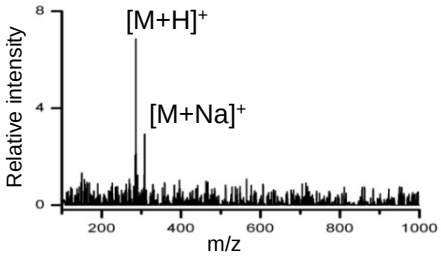
❑ Commonly used nanoparticles

Nanomaterials	Analytes	Reference
Au	Glycopeptide	*Proteomics, 2009, 9, 5046*
Fe_3O_4	Biomolecules, protein, & serum Amyloid	*Anal.Chem., 2008, 80, 6159*
Diamond	protein	*Anal. Chem., 2005, 77, 259*
HgTe	Peptide, protein, & carbohydrate	*Chem. Sci., 2012, 3, 2147*
TiO_2	Carbohydrate in plant tissue	*Anal. Chem., 2010, 82, 5518*
ZnO	Peptide, protein, & small oligosaccharide	*Talanta, 2010, 82, 1594*
Mn_2O_3	Drug & insulin	*Sci. Technol. Adv. Mater. 2009, 10, 034602*

Pharmaceutical Analysis using DHB@MNP MALDI-TOF



Identification



(A)

1 **salicylamide**
MW: 137.14

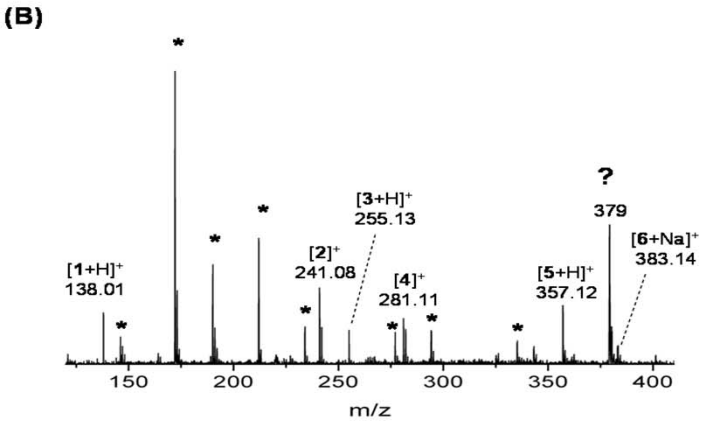
4 **flufenamic acid**
MW: 281.13

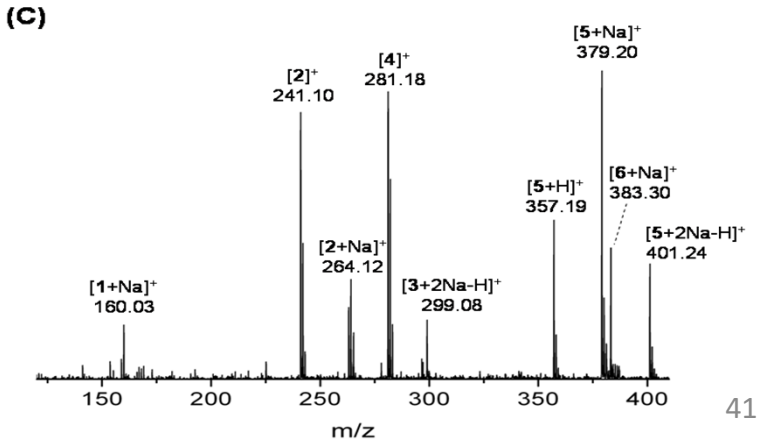
2 **mefenamic acid**
MW: 241.29

5 **sulindac**
MW: 356.13

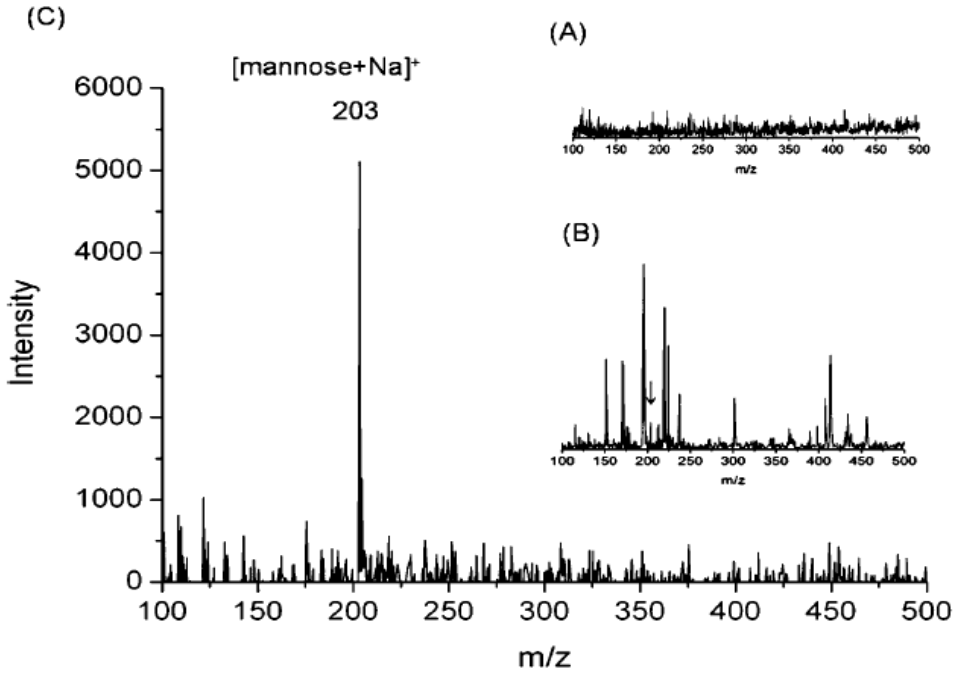
3 **keetoprofen**
MW: 254.28

6 **prednisolone**
MW: 360.44



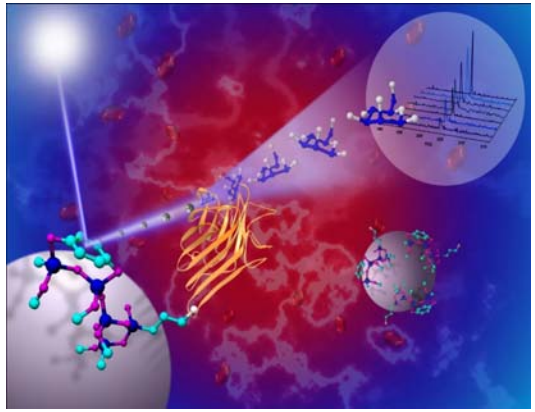


Mannose Extracted by DHB@MNP-Con A from Human Serum



(A)the background signal of DHB@MNP-Con A;
(B) The human serum profile; the black arrow indicates the spiked mannose;
(C) the mass spectrum of mannose [mannose + Na]+ extracted by DHB@MNP-Con A in human serum.

The DHB@MNP-ConA combined with rapid identification of MALDI MS provides a convenient, effective and specific bioassay for small molecule analysis from extremely complex medium.

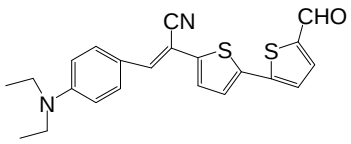


Anal. Chem., 79, 3401, **2007**. IF 6.3

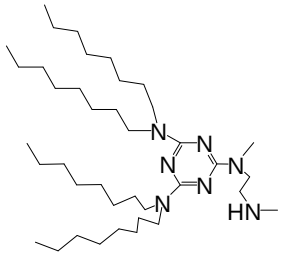
Identification of Small Molecules by Using DHB@MNP Nanomatrix

Category			Analyte (A)			Fragments (B)					
	No.	Fomula	Theory (*m/z*)	Observed (*m/z*)	Int (%)	DHB@MNP (*m/z*)		DHB (*m/z*)			
Solar cell	1	$C_{21}H_{20}N_2S_2$	$[M]^+$ = 364.107	$[M]^+$ = 364.107	100%	349.114		349.112	336.241	321.108	
	2	$C_{22}H_{20}N_2OS_2$	$[M]^+$ = 392.102	$[M]^+$ = 392.102	100%	377.094		377.183	364.138	320.291	
	3	$C_{25}H_{21}N_3O_2S_2$	$[M]^+$ = 459.108	$[M]^+$ = 459.109	100%	444.145	431.076	444.097	431.075	415.069	
	4	$C_{49}H_{41}N_3O_2S_2$	$[M]^+$ = 767.264	$[M]^+$ = 767.266	100%	723.238	457.122	724.160	457.076	438.514	
	5	$C_{41}H_{32}N_2O_2S$	$[M]^+$ = 616.218	$[M]^+$ = 616.220	100%	587.199	572.213	587.216	572.215	549.194	521.203
	6	$C_{31}H_{23}N_3O_2S$	$[M]^+$ = 501.151	$[M]^+$ = 501.152	30%	457.148	289.106	457.139	289.076		
	7	$C_{46}H_{37}N_3O_2S_2$	$[M]^+$ = 727.231	$[M]^+$ = 727.233	100%	698.301	683.220	698.334	683.270	530.125	
	8	$C_{37}H_{42}BrN$	$[M]^+/^{79}Br$ = 579.252	$[M]^+/^{79}Br$ = 579.253	65%	501.348	416.229	556.769	500.733	428.689	415.645
			$[M]^+/^{81}Br$ = 581.249	$[M]^+/^{81}Br$ = 581.250	63%						
Triazine dendrimer	9	$C_{36}H_{69}ClN_4$	$[M + Na]^+$ = 615.511	$[M + Na]^+$ = 615.513	100%	503.395		560.727	492.235	463.527	437.314
	10	$C_{39}H_{79}N_7$	$[M + Na]^+$ = 646.648	$[M + Na]^+$ = 646.648	70%	615.594		615.445	601.722	583.693	517.587
				$[M + Na]^+$ = 668.458	100%						
	11	$C_{81}H_{156}ClN_{17}$	$[M + H]^+$ = 1403.249	$[M + H]^+$ = 1403.254	100%	615.598		780.234	646.768	615.598	
	12	$C_{85}H_{167}N_{19}$	$[M + H]^+$ = 1455.373	$[M + H]^+$ = 1455.379	15%	615.540	194.136	701.373	615.552	503.423	403.290
Light emitting device	13	$C_{33}H_{24}IrN_3$	$[M + H]^+$ = 656.168	$[M + H]^+$ = 656.171	100%	499.584		581.285	527.648	513.619	499.628
	14	$C_{65}H_{57}IrN_4O_2$	$[M]^+$ = 1118.411	$[M]^+$ = 1118.421	100%	1019.366	414.167	1019.266	977.464	414.167	
	15	$C_{39}H_{29}IrN_4O_2S_2$	$[M]^+$ = 842.136	$[M]^+$ = 842.139	15%	743.097		743.112	436.928	273.641	199.571
	16	$C_{61}H_{45}IrN_4O_2$	$[M]^+$ = 1058.317	$[M]^+$ = 1058.320	20%	959.231	385.412	959.234	385.412		

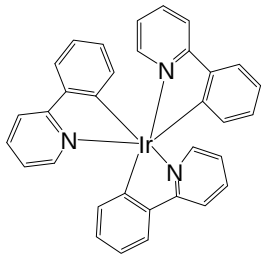
Solar Cell



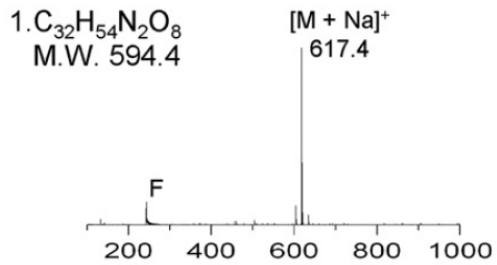
Triazine Dendrimer

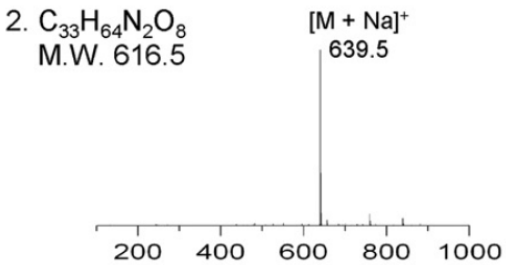


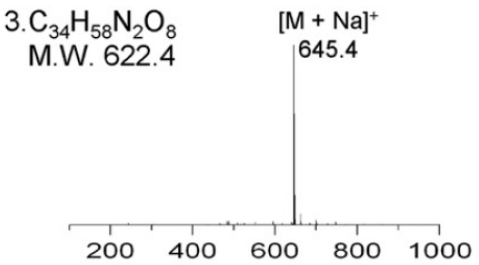
Light Emitting Device

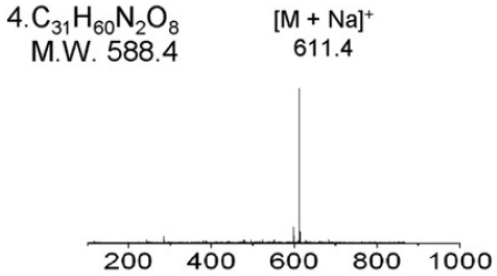


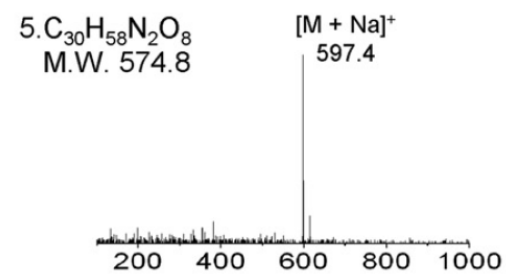
High-throughput for Identification of glycolipids by Using DHB@MNP nanomatrix

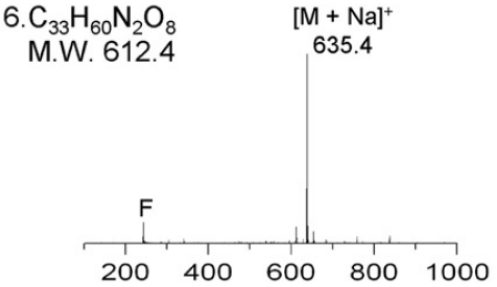


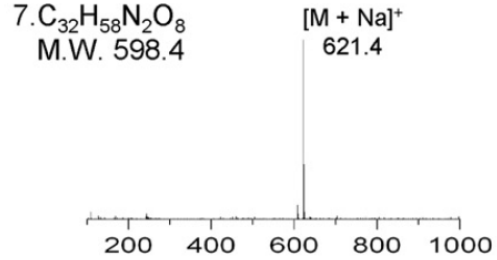


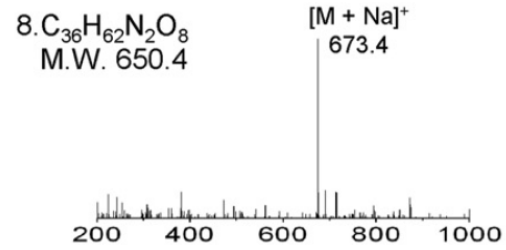


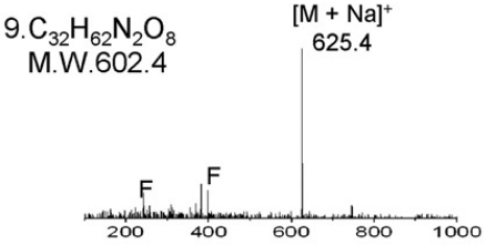


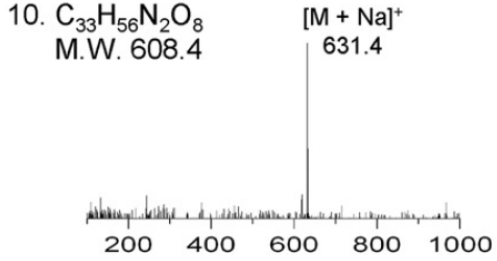


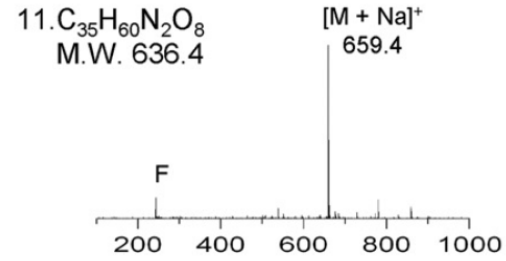


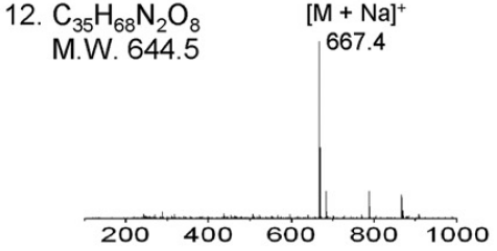


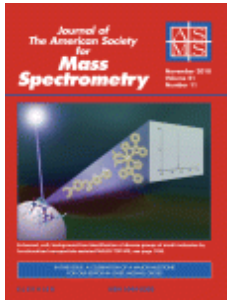








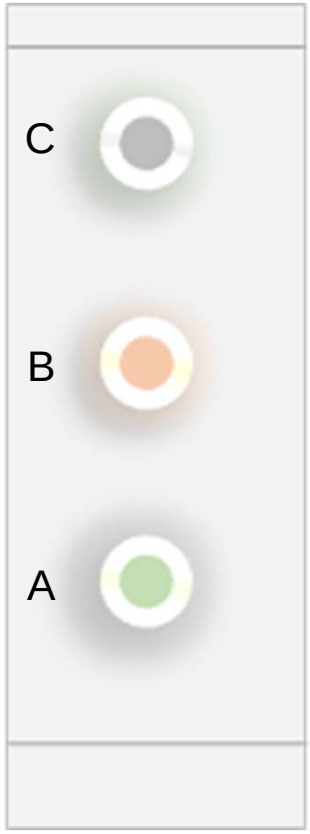




J. Am. Soc. Mass Spectrom., 21, 1930, **2010**. **(Cover Story)** IF 3.2

TLC is alternative tool for separation prior to MALDI-MS

➢ Simple, fast, inexpensive separation techniques



❑ Applications

- Organic synthesis
- Pharmaceutical analysis
- For analytes not compatible with LC column

❑ Challenges for oligosaccharides

- Low separation efficiency
- Chemical derivatization is required
- Separated spot not visualized under UV (UV-inactive)

❖ However, desorption of sample from the TLC silica and direct coupling to TLC-MS are challenging

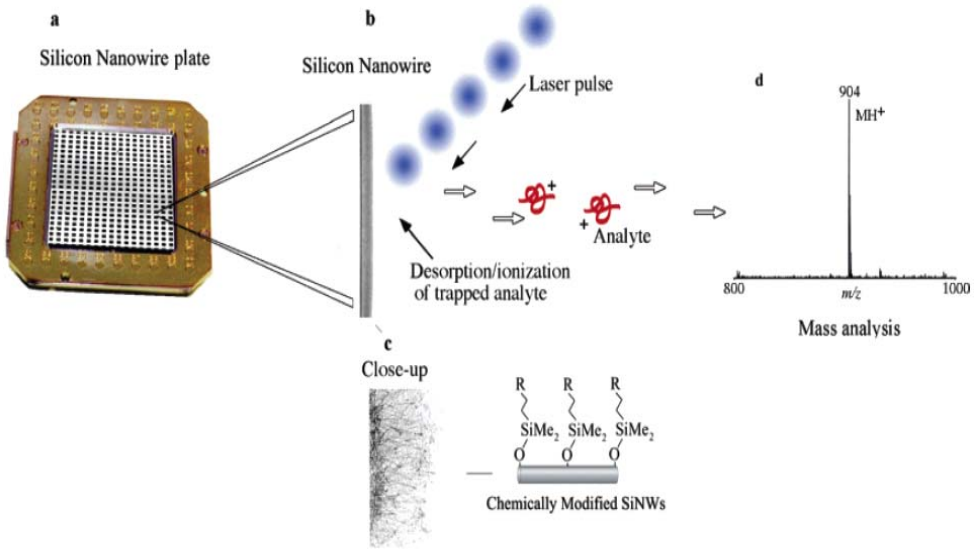
Porous silicon as a sample target for matrix free analysis

❑Techniques of using porous silicon as a sample target for matrix free analysis named Desorption-Ionization on porous silicon (DIOS)

Nature, ***1999***, *399, 243-246*

Features of porous silicon

- ✓ Inert nature
- ✓ High surface area
- ✓ Easily stabilized and functionalized
- ✓ Strong ultraviolet absorption

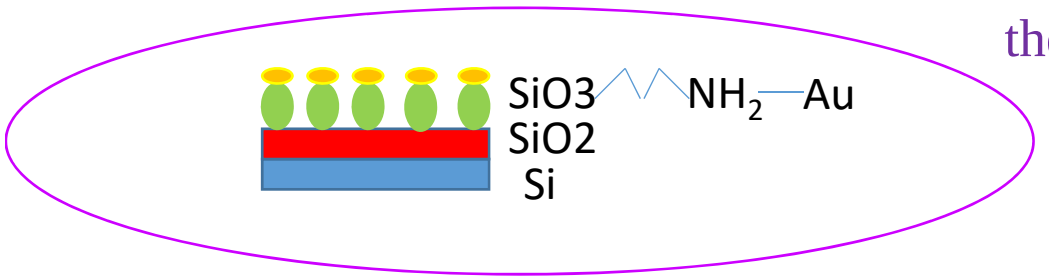


Anal. Chem. ***2005,*** *77, 1641-1646*

Advantage

- ➢ Elimination of matrix background ior
- ➢ Simple sample preparation
- ➢ No Hot-spot problem

❑MSN-Au

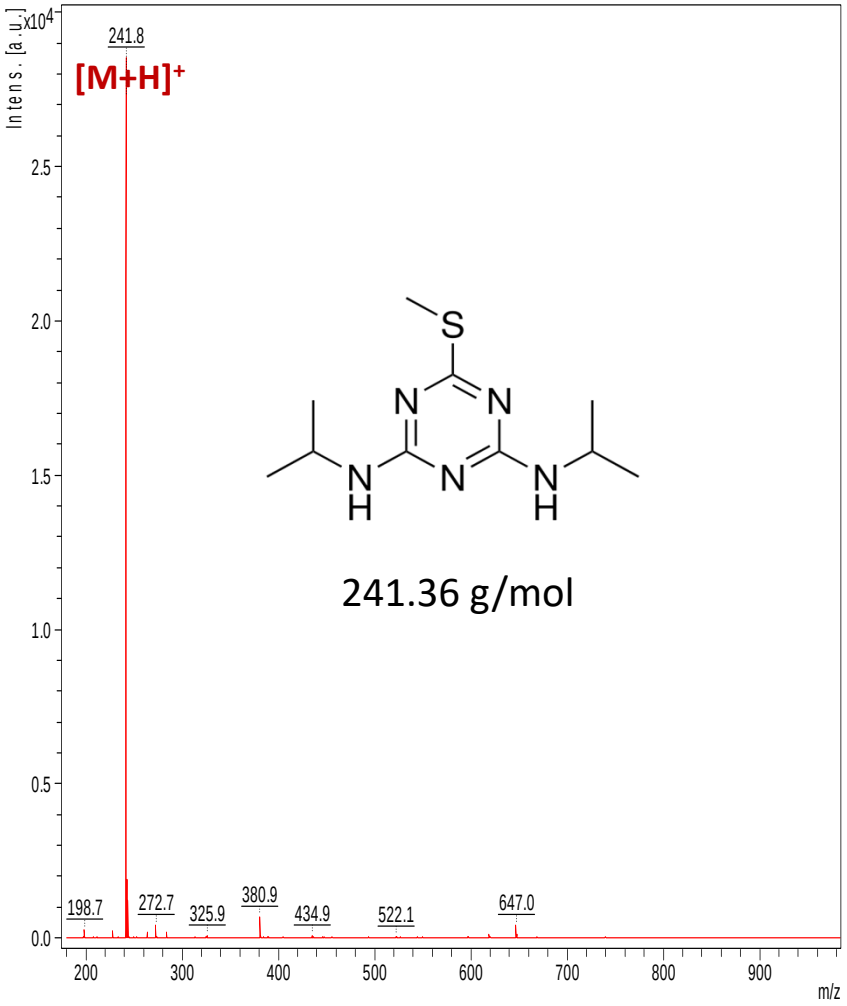


❑ Size-selected gold nanoparticle (AuNP) has been used as a selective matrixes for the ionization of biomolecules

J. Am. Chem. SOC. ***2005****, 127, 5304-5305*

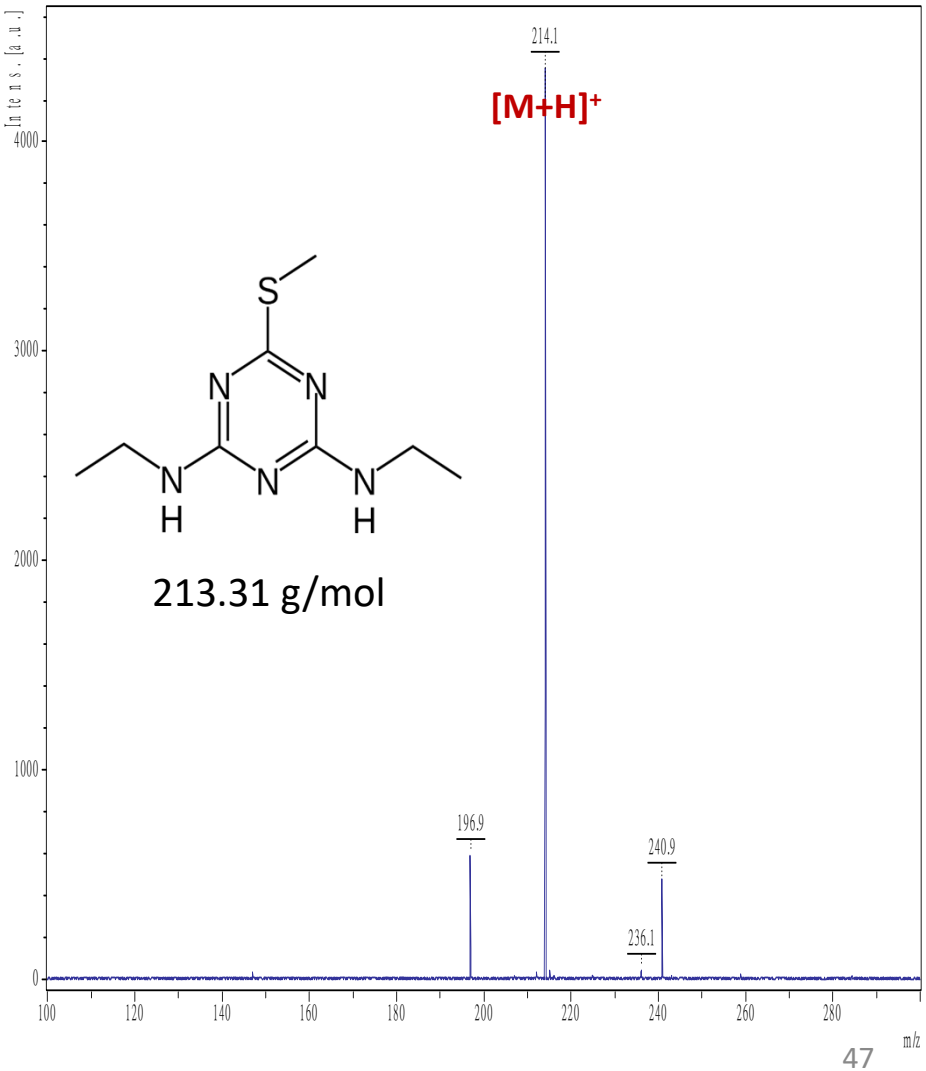
MSN-Au wafer test for small molecule analysis (Promtryn and Simetryn)

Promtryn



241.36 g/mol

Simetryn



213.31 g/mol

MALDI TOF spectra of proteins using PVDF

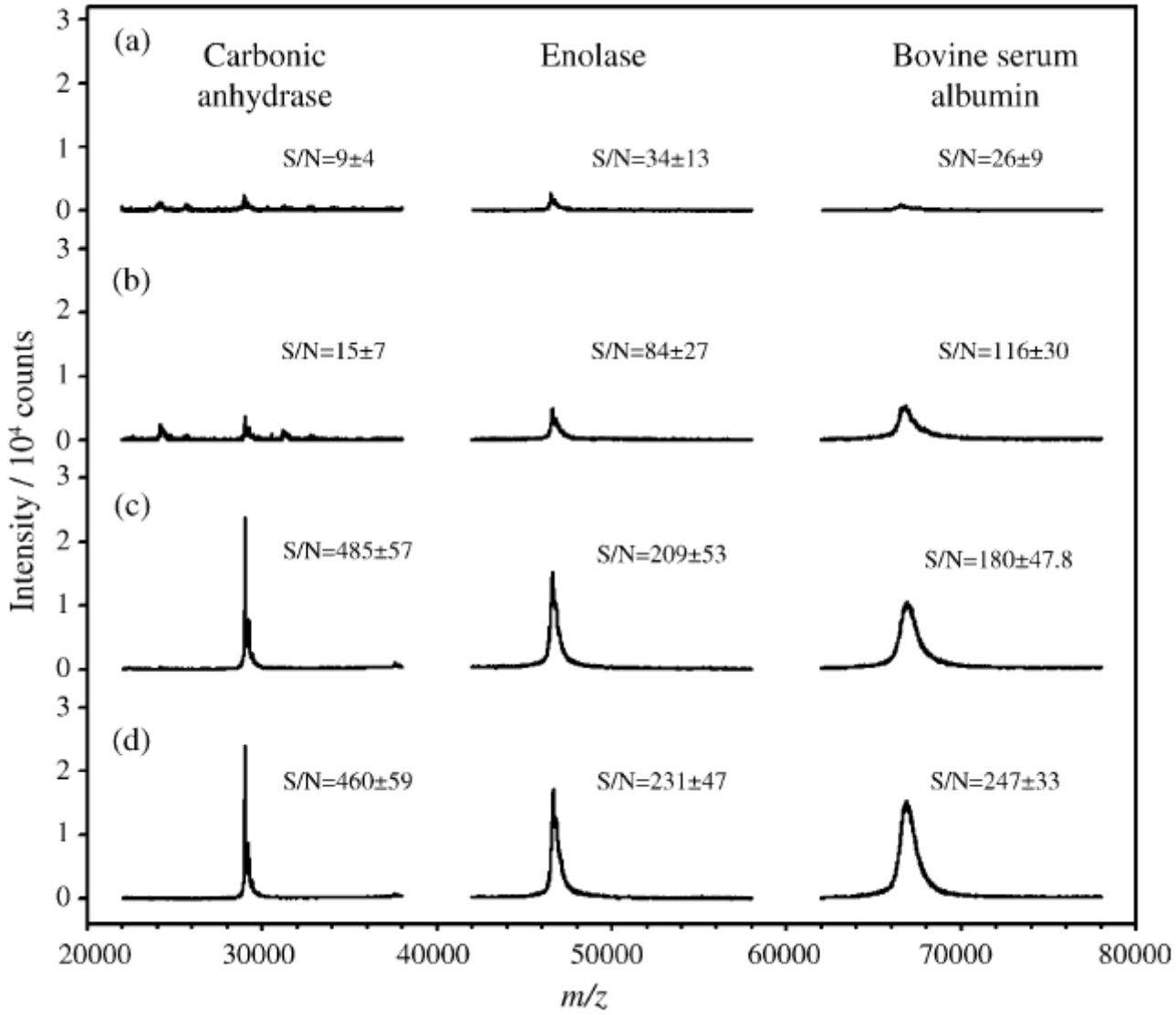


Fig. 1. The effect of on-PVDF membrane washing on MALDI-TOF mass spectra of three proteins, carbonic anhydrase, enolase and bovine serum albumin, containing 250mM Tris, 250mM NaCl, 5mM EDTA, and 5mM DTT using (a) stainless steel plate; (b) PVDF membrane; (c) PVDF membrane followed by on-probe washing with water and (d) PVDF membrane followed by on-probe washing with ammonium bicarbonate.

Analytica Chimica Acta 556 (2006) 237–246

THANK YOU