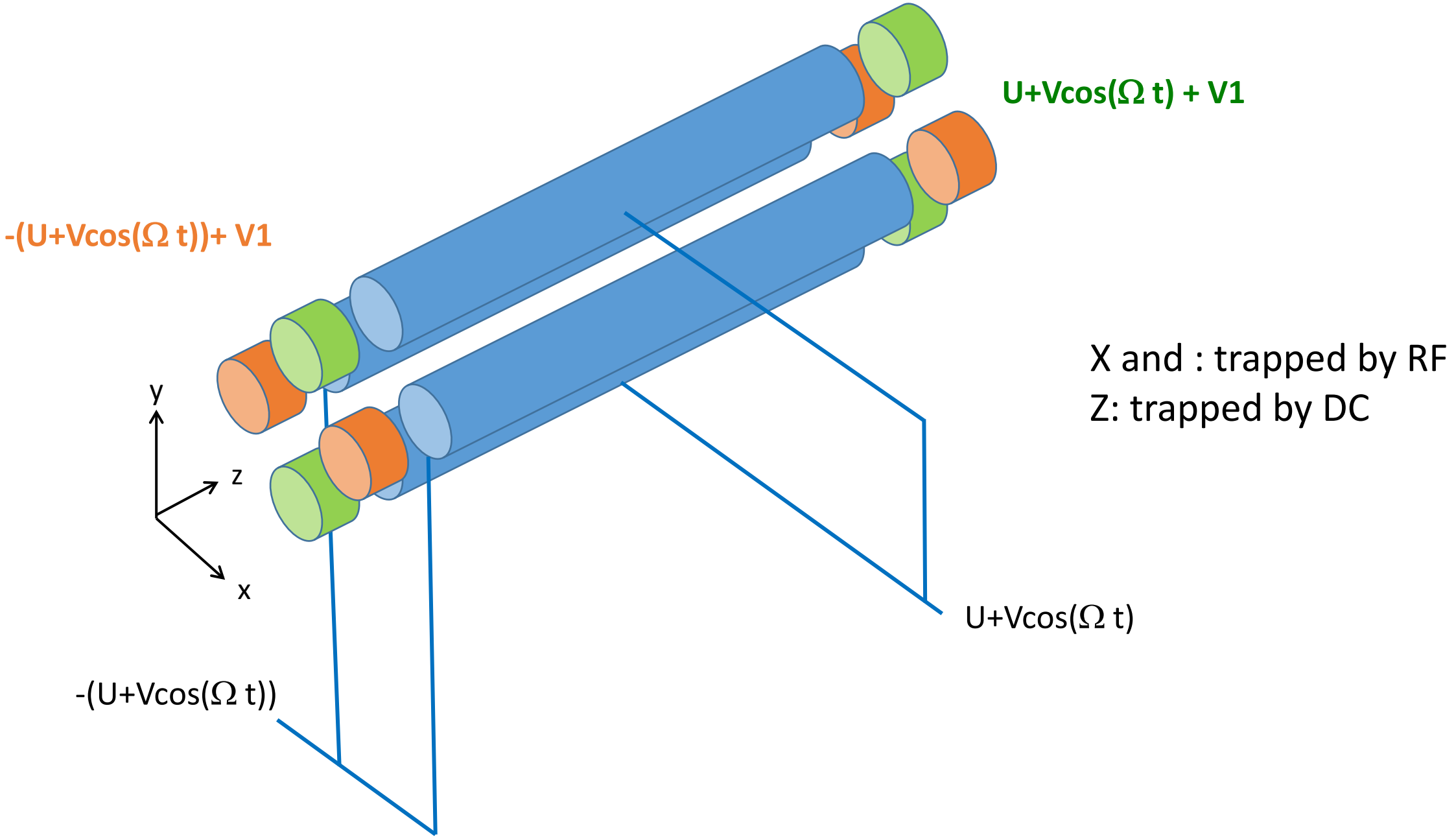
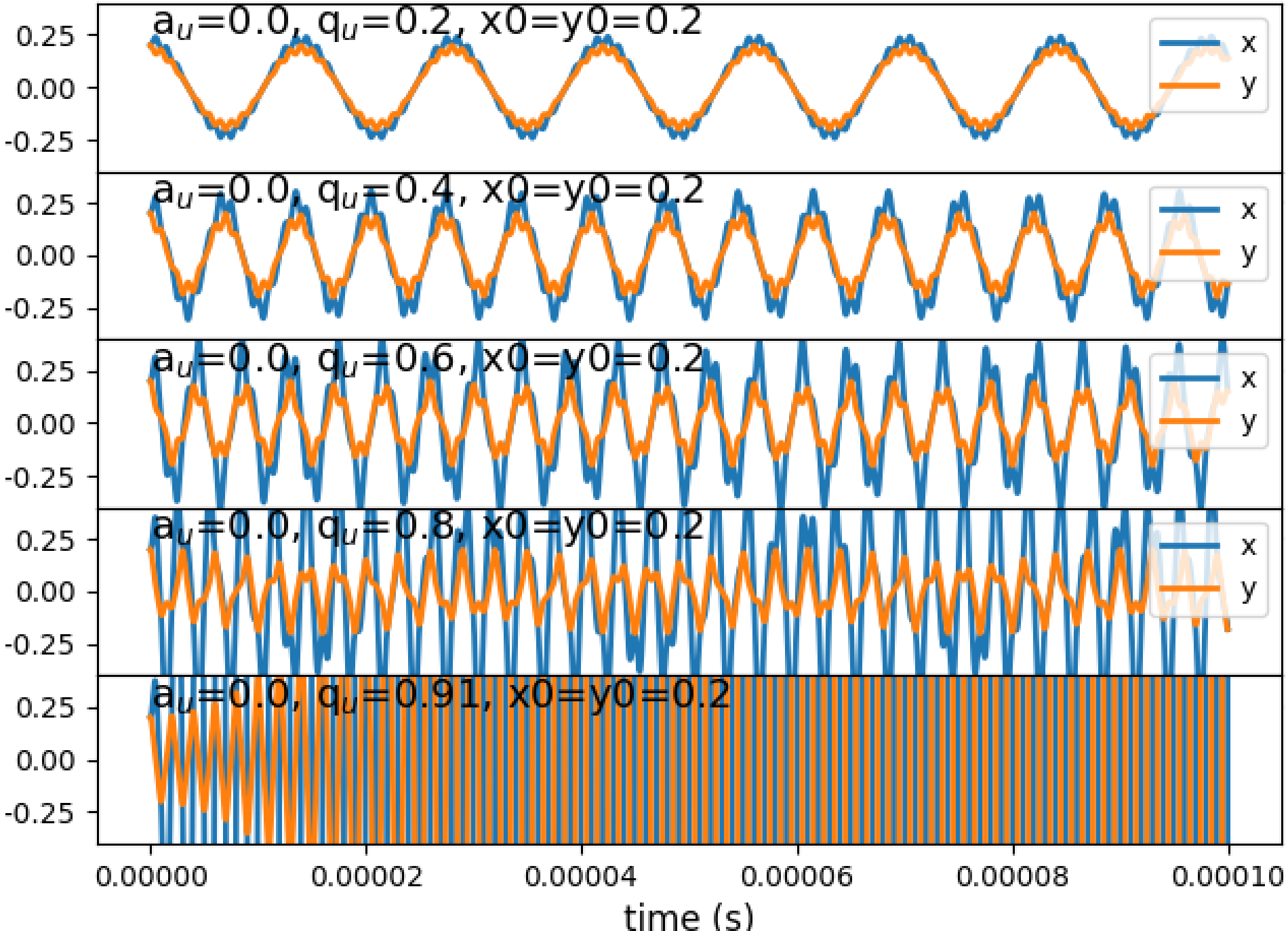
Ion trap mass spectrometer



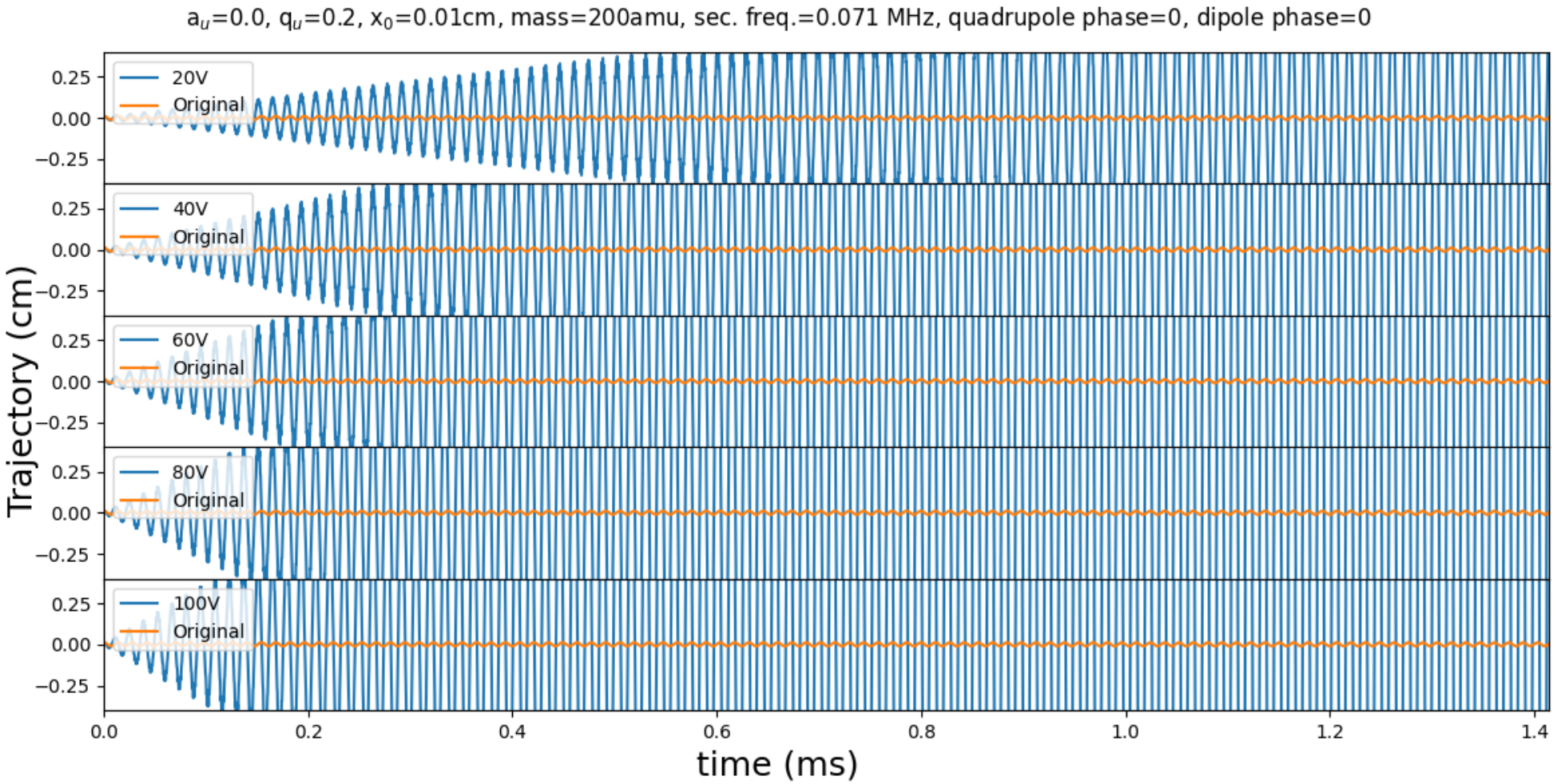
X and : trapped by RF
Z: trapped by DC



Trajectory includes a fast oscillation and A slow oscillation.

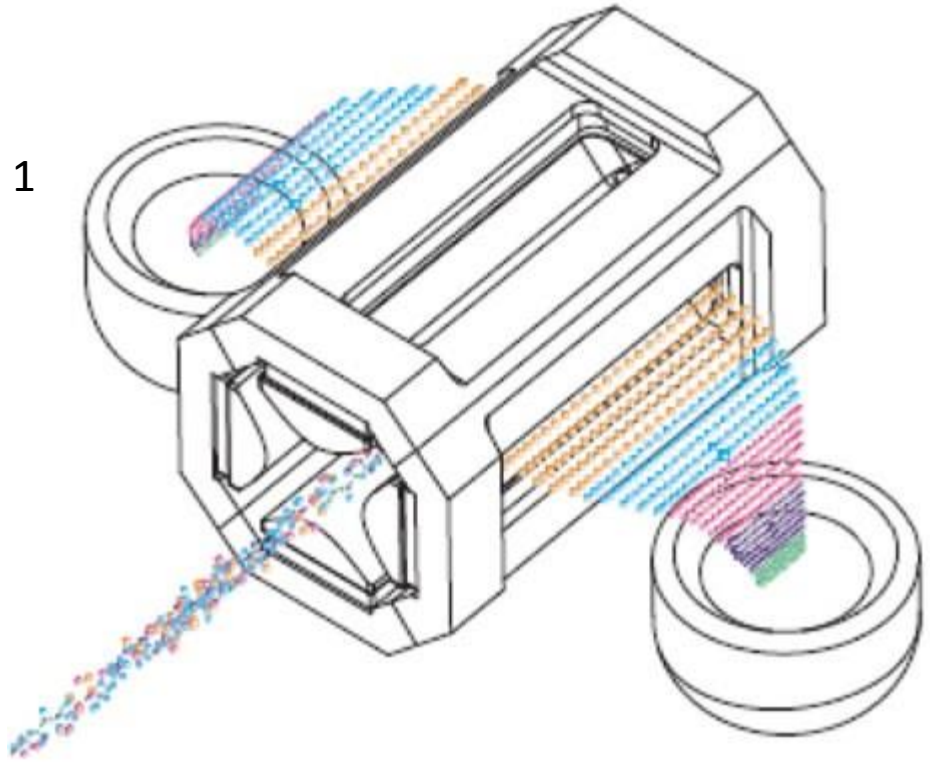
Frequency of slow oscillation is mass dependent.

m/z detection: applied the RF with the frequency of slow oscillation to m/z ions



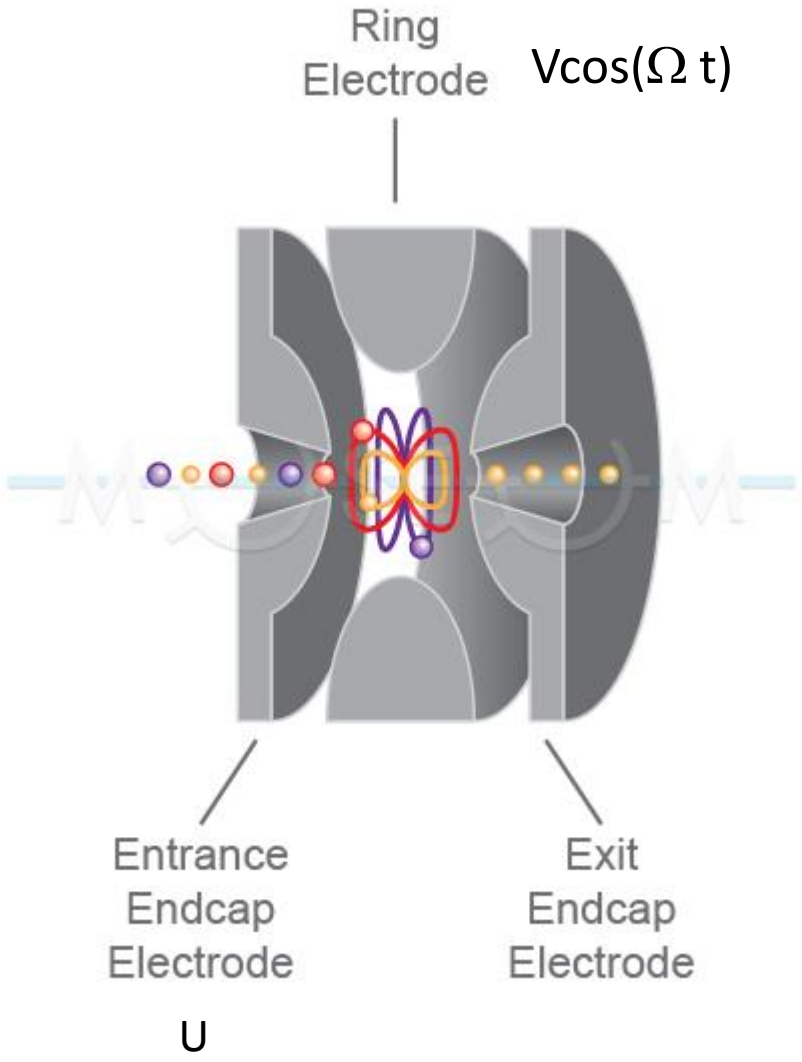
Linear (2D) ion trap

Detector 1



Detector 2

3D ion trap


Ring
Electrode
Vcos(Ω t)
Entrance
Endcap
Electrode
Exit
Endcap
Electrode
U

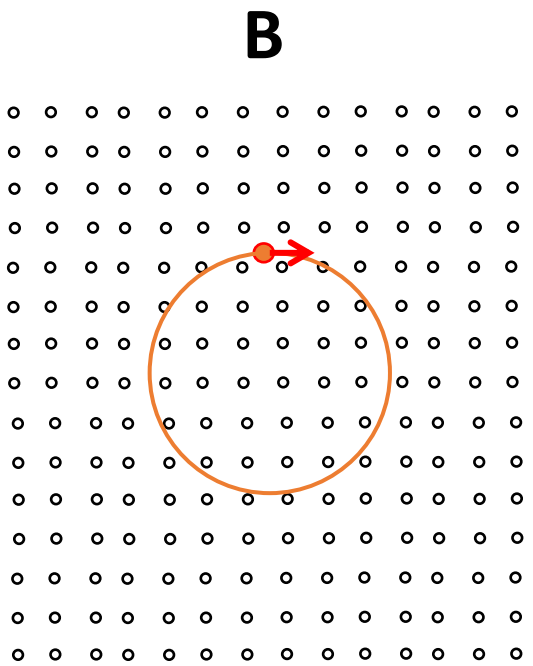
Fourier-transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR-MS)

- The most complex method of mass analysis and detection, although the principle is simple
- Spatial uniform static superconducting high field magnet (typically 4.7 to 13 Tesla)
- Cooled by liquid helium and liquid nitrogen
- 10^{-10}~10^{-11} Torr, 10K
- Resolution: 10^5 to 10^6, and $>10^7$ is possible

$F = qVxB$

$\omega = qB/(2\pi m)$

$m/z = m/q = B/(2\pi\omega)$



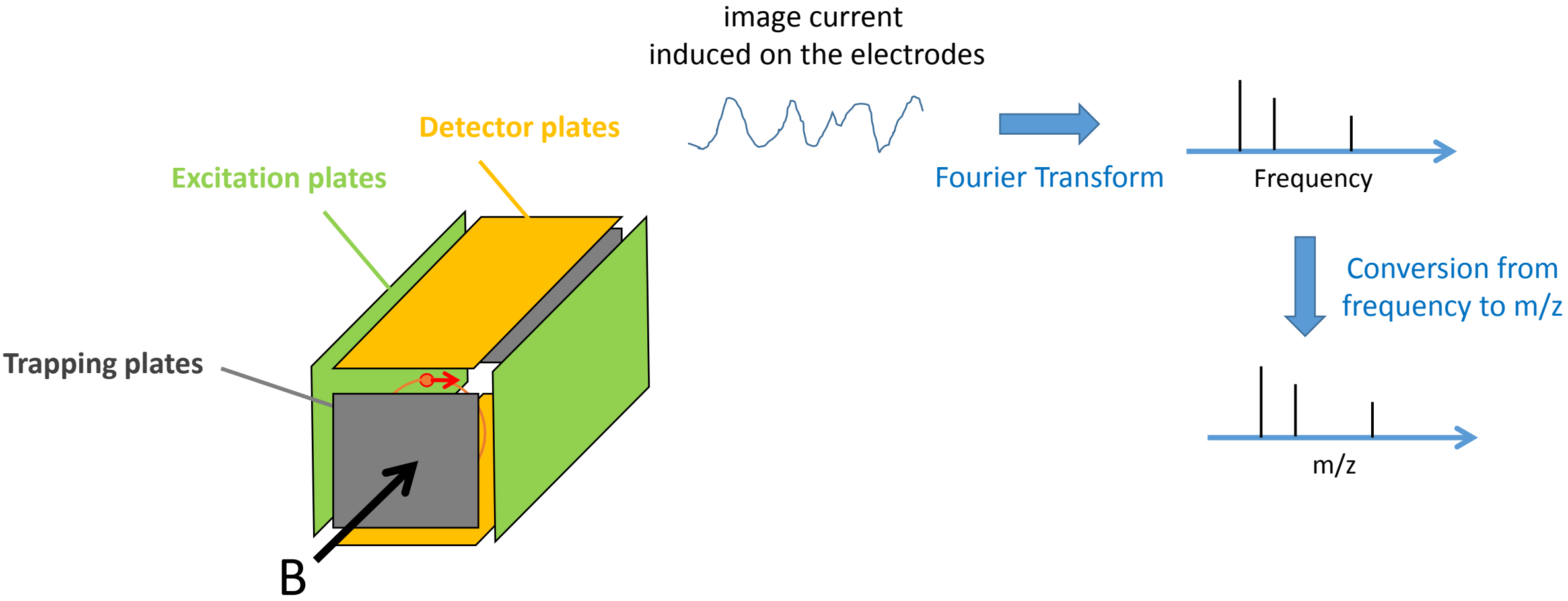
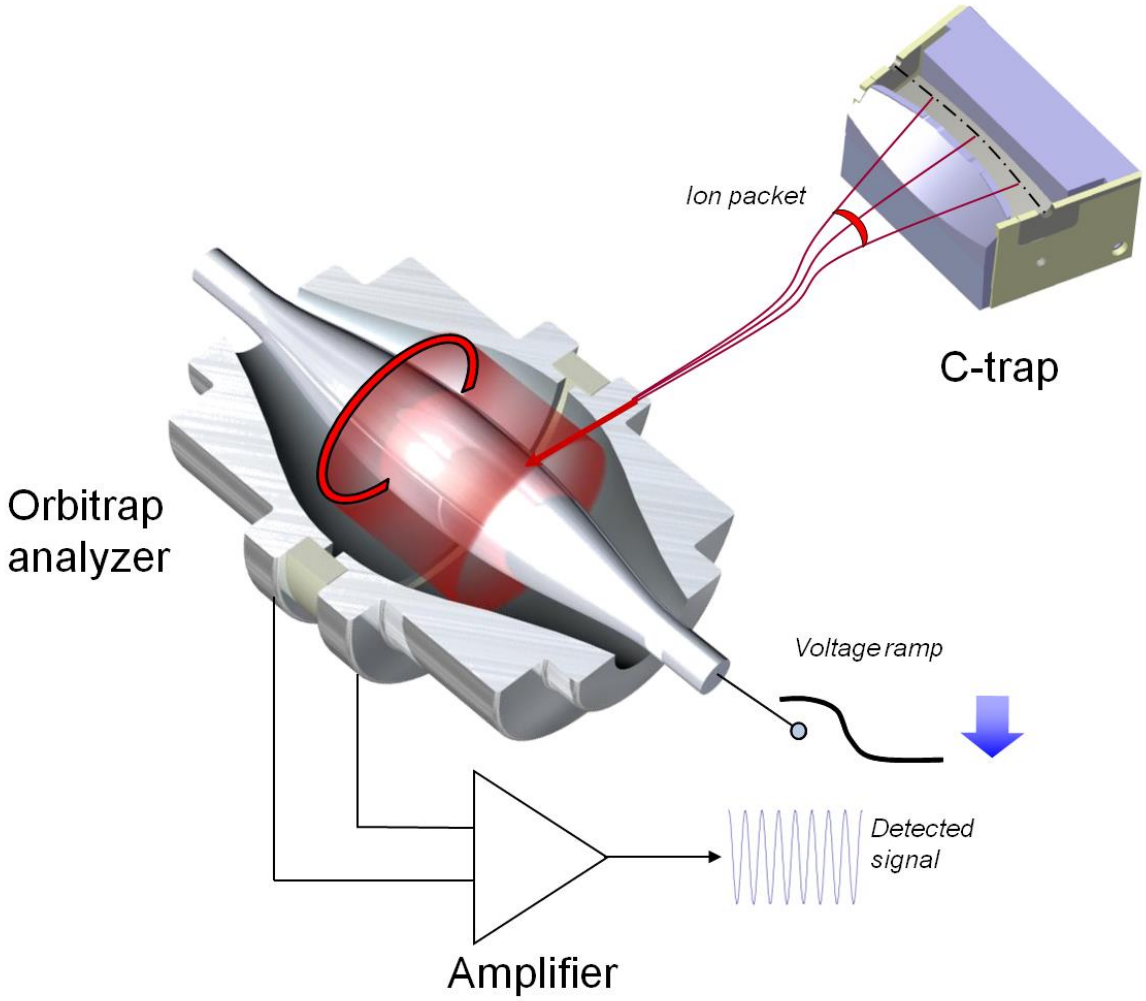


image current
induced on the electrodes
Detector plates
Excitation plates
Trapping plates
B
Fourier Transform
Frequency
Conversion from
frequency to m/z
m/z

Orbitrap mass analyzer

- Electric field
- Resolution 10^4



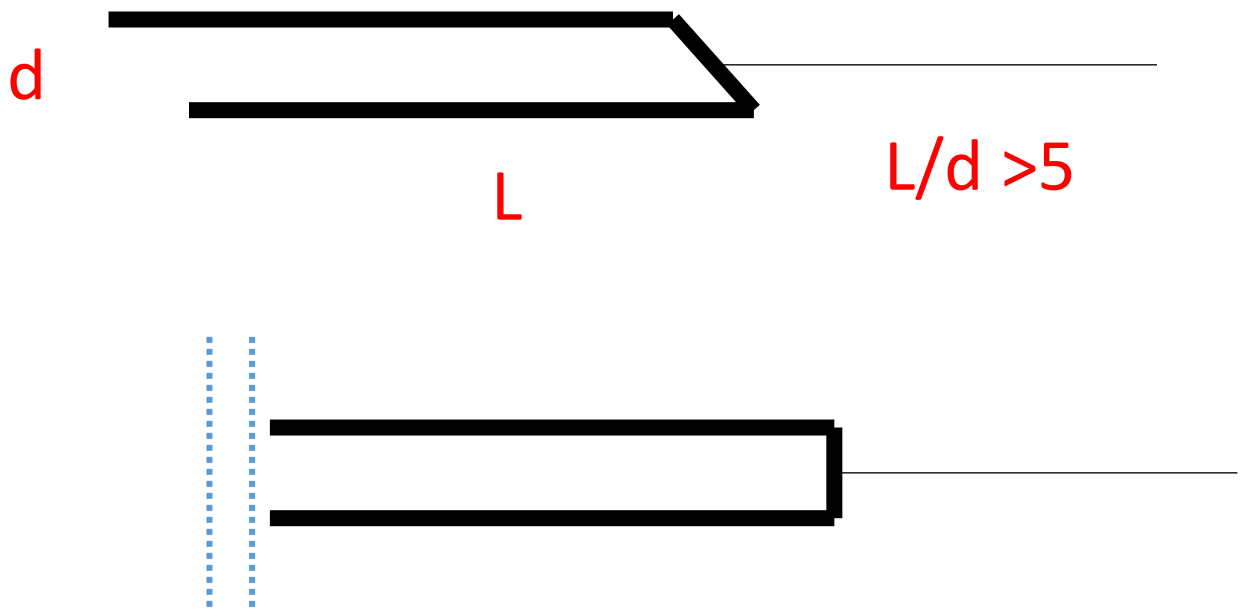
Detectors

1. Charge particle
2. Frequency

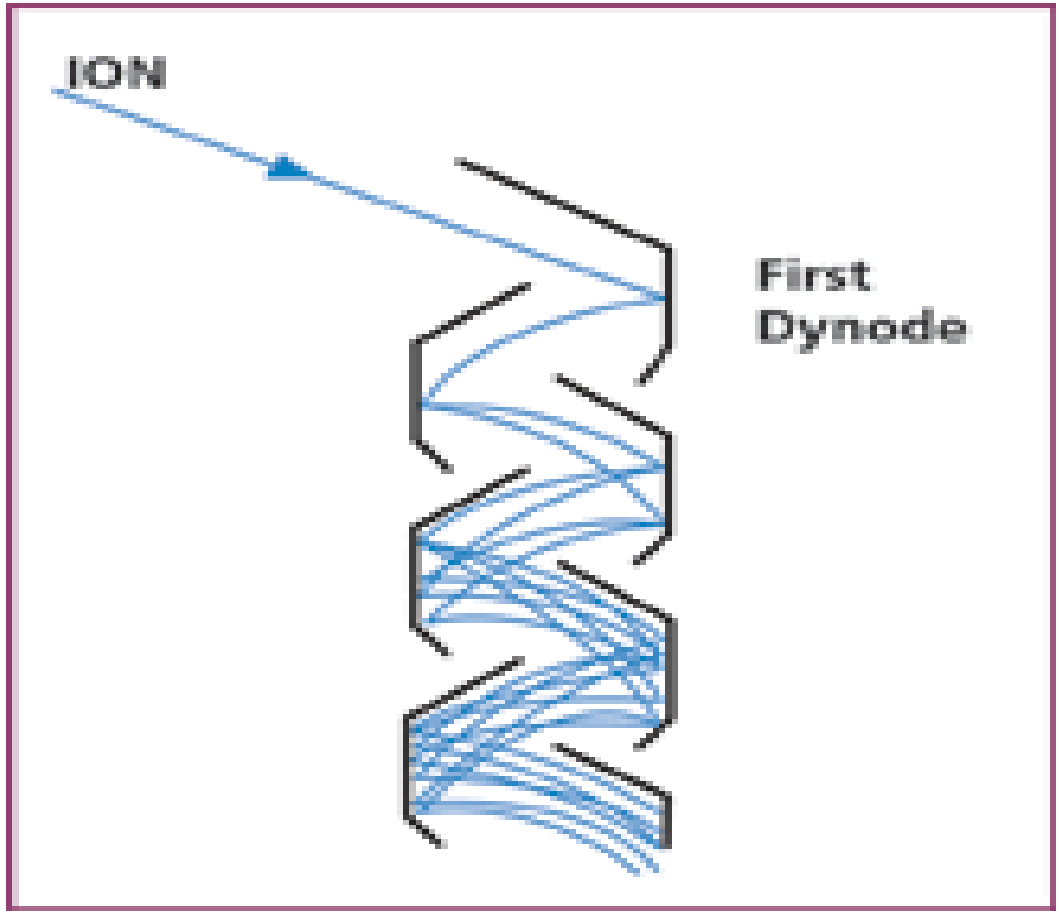
Charge detector:

Faraday-cup: $>10^{-14}$ A or 10^{5} charges

Number of charge particle > noise



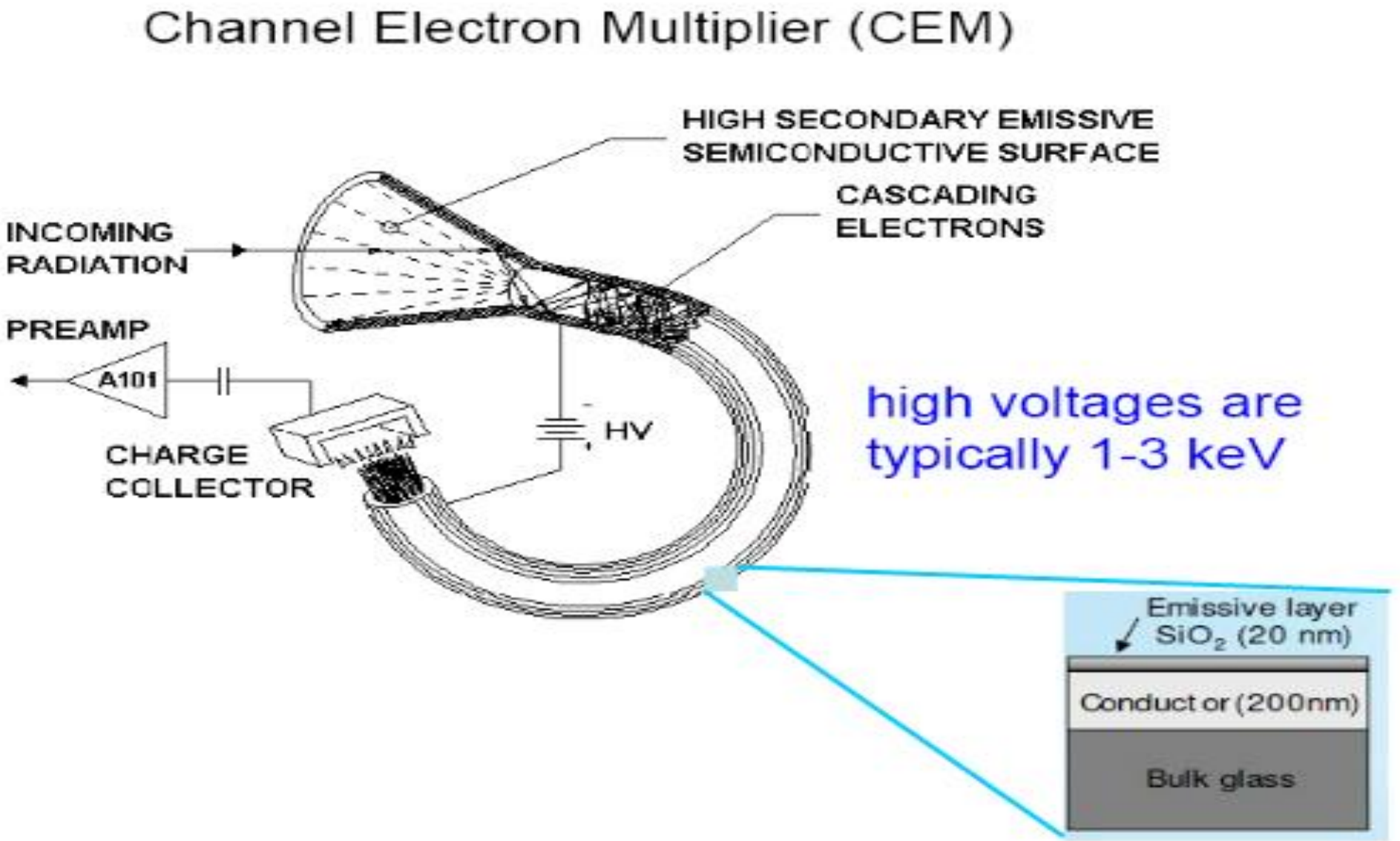
Electron multiplier



Conversion dynode

Electron multiplier

channeltron



Burle channeltron

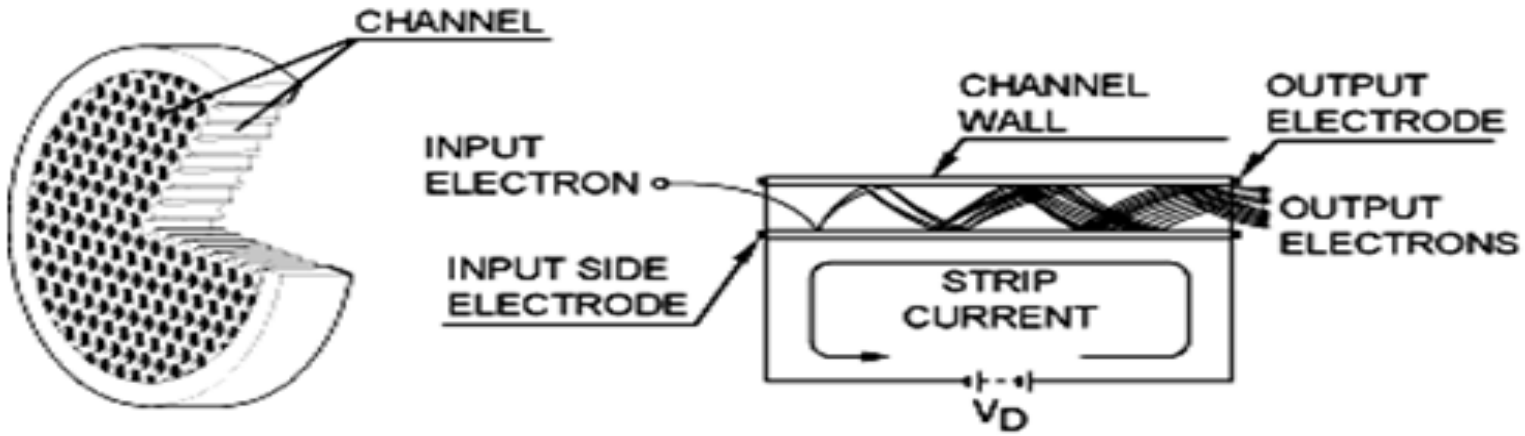
Parameters	Analog	Pulsed
1. Pulse Width		18-20 nSec
2. Rise Time		3-5 nSec
3. Dark Count Rate	<.05 cps	<.05 cps
4. Linearity	~10% of bias current	
5. Bias Current	25-46μA	15-3μA
6. Maximum Count Rate		10^7 cps
7. Gain @ 3000V	$>10^7$	$>10^8$
8. FWHM		<75%

Channeltrons:

- Channeltrons: typical 10^{-5} Torr or lower. Higher pressure operation is observed to increase the background current and can result in shortened life.
- Some channeltrons (Photonics) can operate at 10^{-2} Torr.
- Channeltrons are customarily operated at 1500 to 3000 volts.

Microchannel plate (MCP) detector: high gain, high spatial resolution and high temporal resolution

Single ion, electron, or photon detection

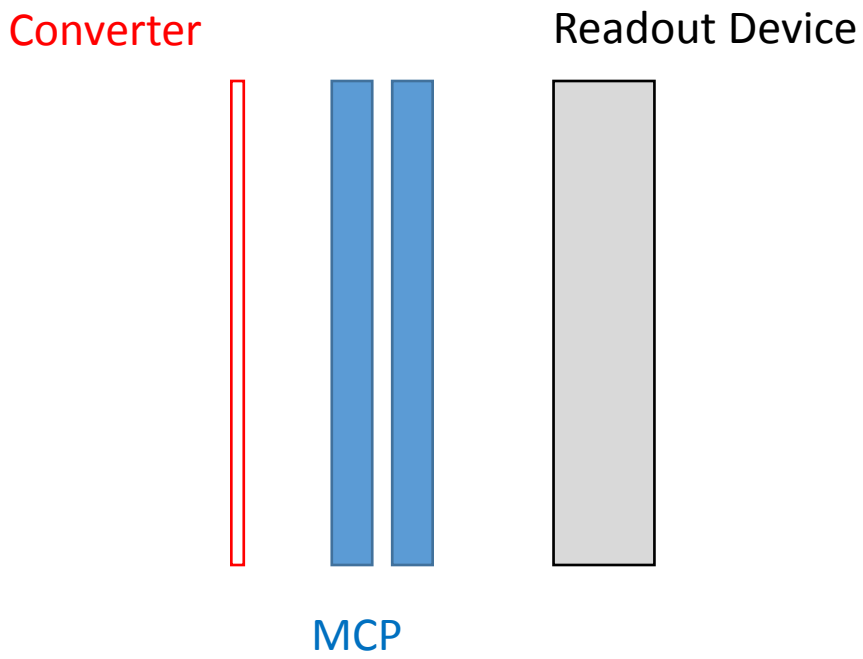


MCP detector

1) A Converter - a mechanism to convert initial particles in photons or electrons,

2) An Assembly of MCPs - a mechanism to amplify initial single electron or photon event into electron pulse.

3) A Readout Device - a mechanism to detect the electron avalanche.



Converter

Photocathodes: for visible and IR radiation.

MCP is directly sensitive to ultraviolet rays (VUV, UV), X-rays

Assembly

Single: low-level light

Double (so-called Chevron or V-stack): TOF-MS ion detector

Triple (Z-stack): image single particles

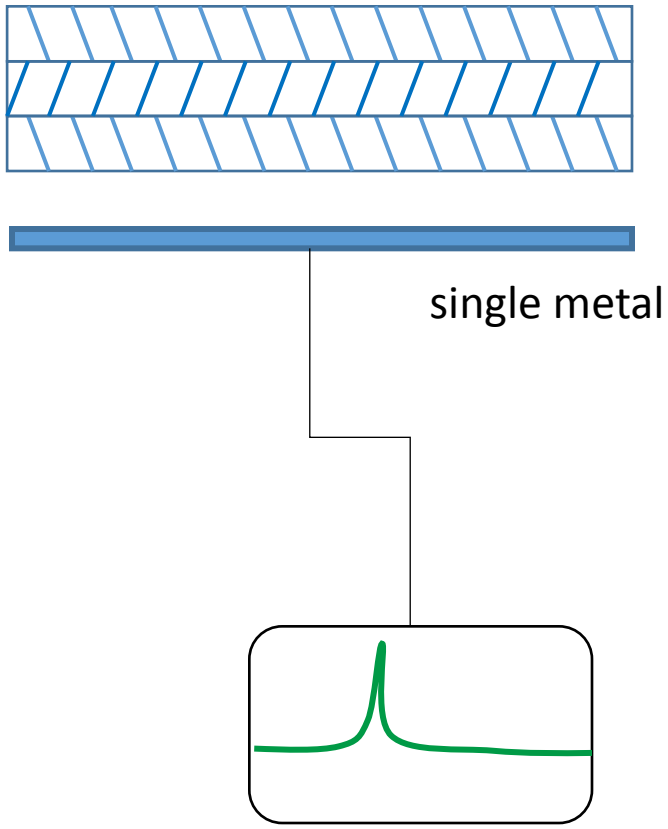
Readout Device

- Detection (e.g. mass-spectrometry): single metal
- Imaging with low temporal resolution: phosphor screen (P20, P22, P46, etc.) coupled with CCD. Gate >10 nanoseconds.
- Imaging with moderate and high temporal resolution: anode configurations have been developed that fall into the following classes:

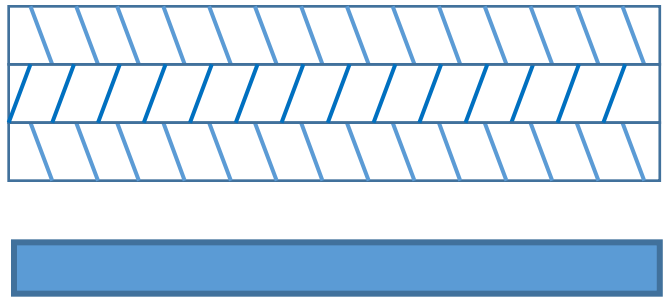
  Resistive anodes (one and two dimensional)

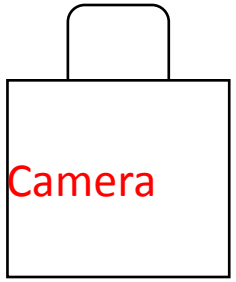
  Wedge and strip designs

  Delay-Line-Readout



phosphor screen



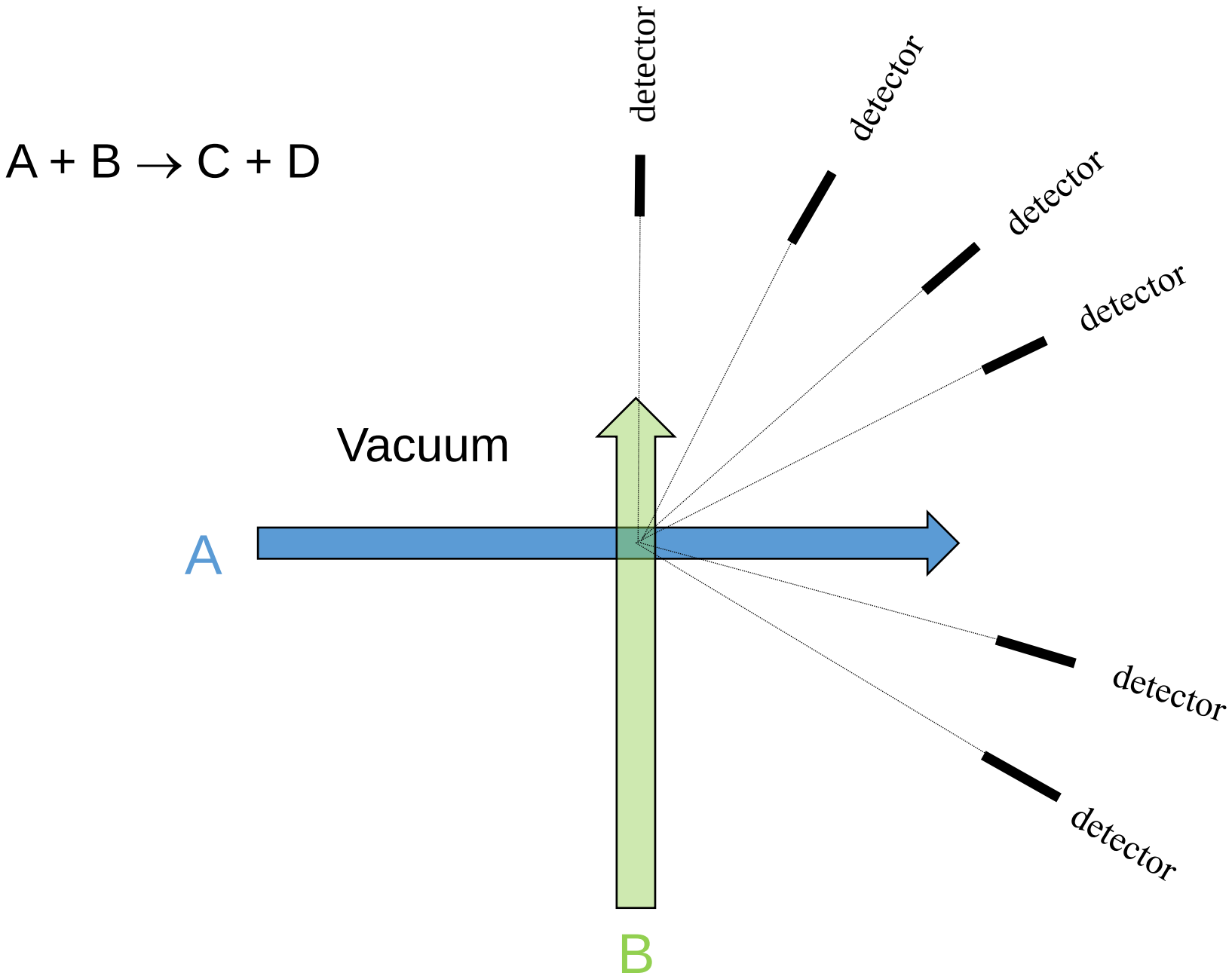


Time resolved image:

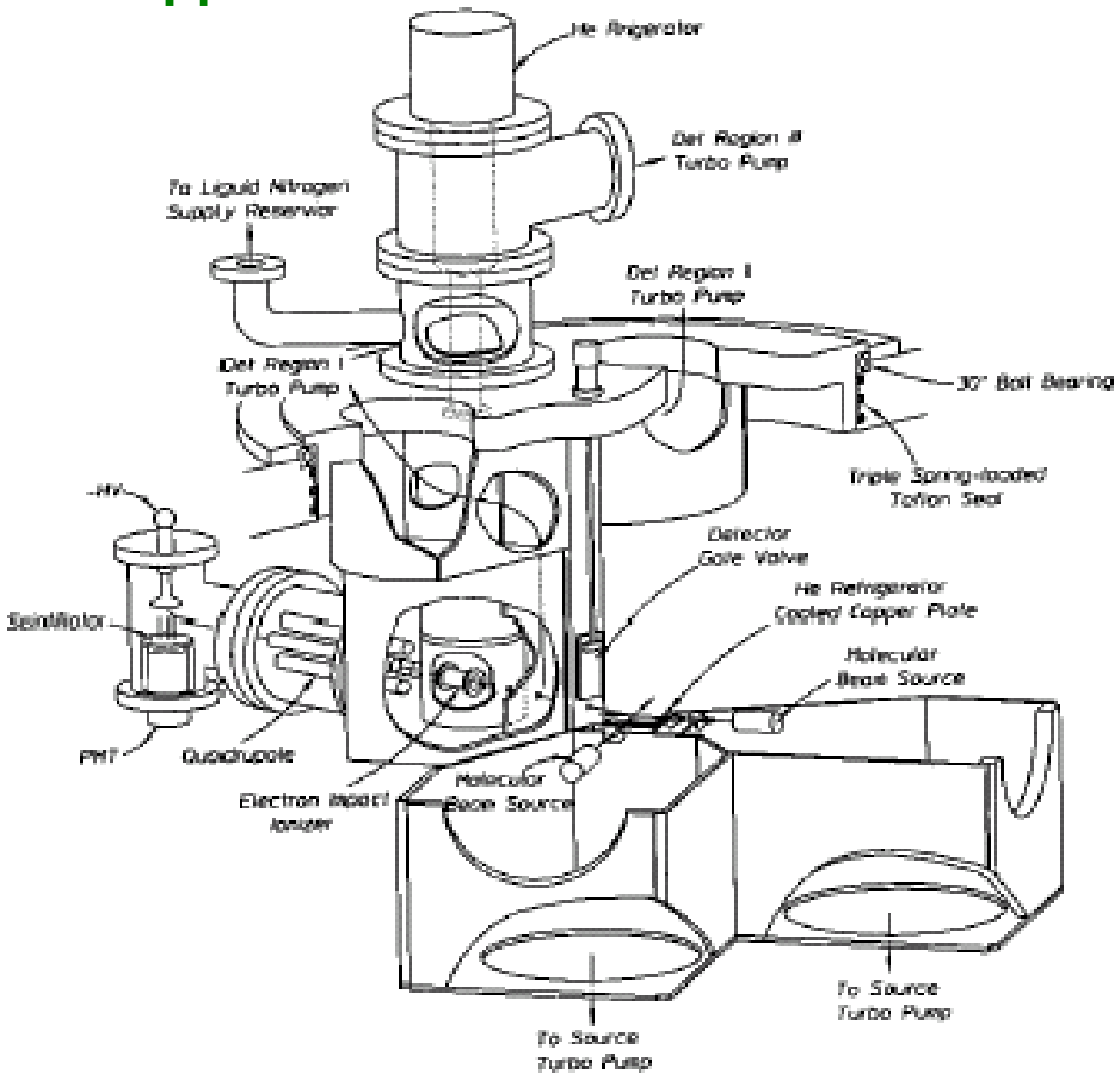
1. Phosphor
2. Camera

Applications

Applications to crossed molecular beam experiments



Crossed molecular beam apparatus



Detection of chemicals
Gas chromatography coupled to mass spectrometry (GC-MS)

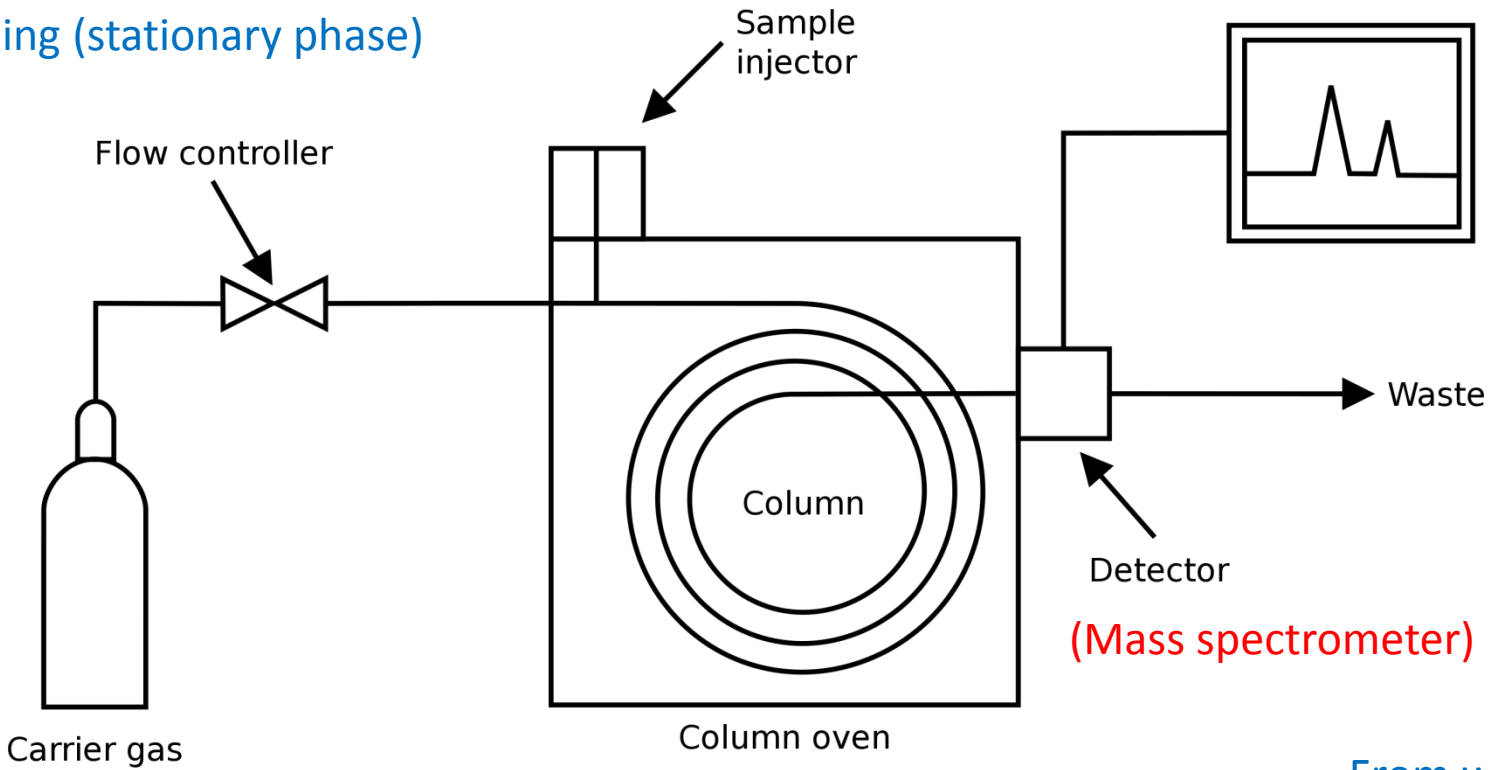
Vaporized sample

Flow continuous with inert or nonreactive gas through column (narrow tube)

Interactions with the column lining or filling (stationary phase)

Chemicals have different retention time

Detected by MS



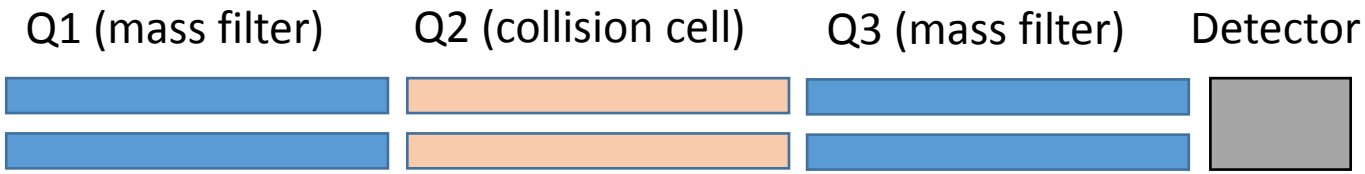
From wiki

Chemicals are separated by GC, and identified by

1. Retention time
2. Mass spectra

Gas chromatography coupled to tandem mass spectrometry (GC-MS/MS) using a triple quadrupole (QqQ) analyzer

- Single stage MS: molecular weight, cannot distinguish isomers
- Tandem MS: isomers (different structures) have different fragmentation

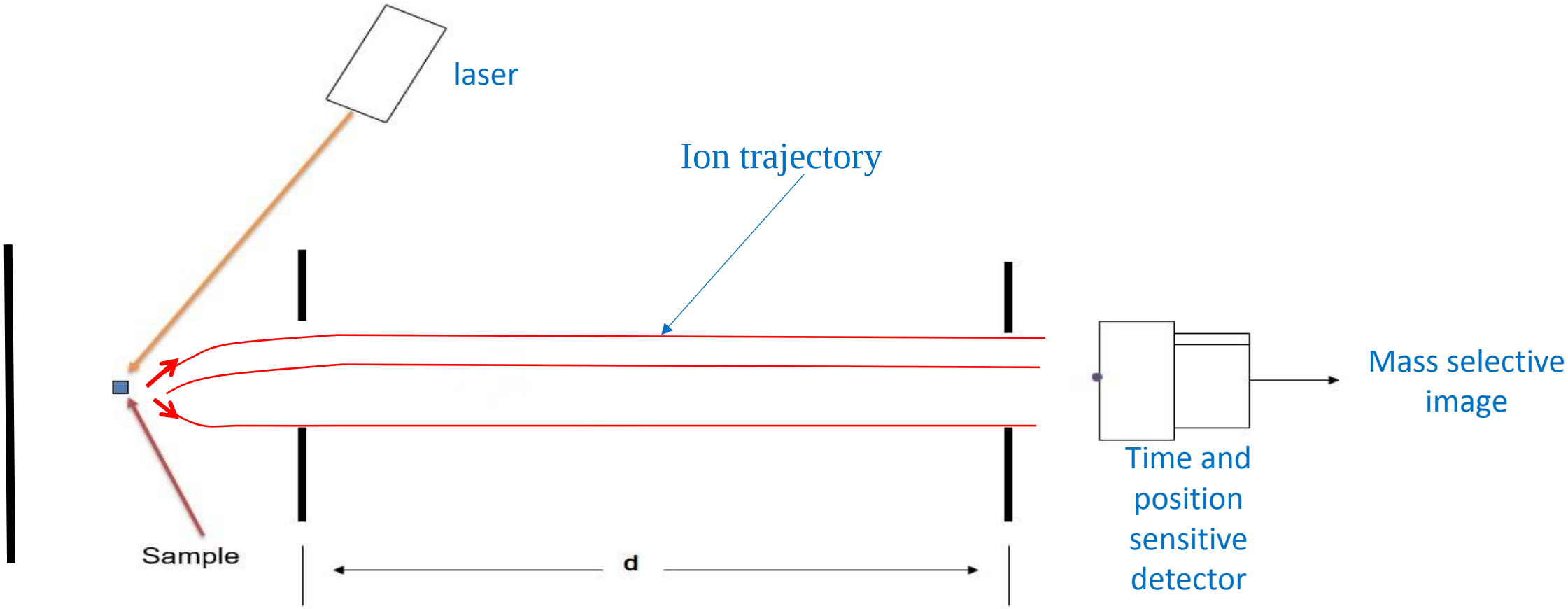


Vaporized chemicals

1. pesticides in food, water, soil, … Sensitivity: 2 µg kg^{-1}
2. volatile organic compounds (VOCs) in air

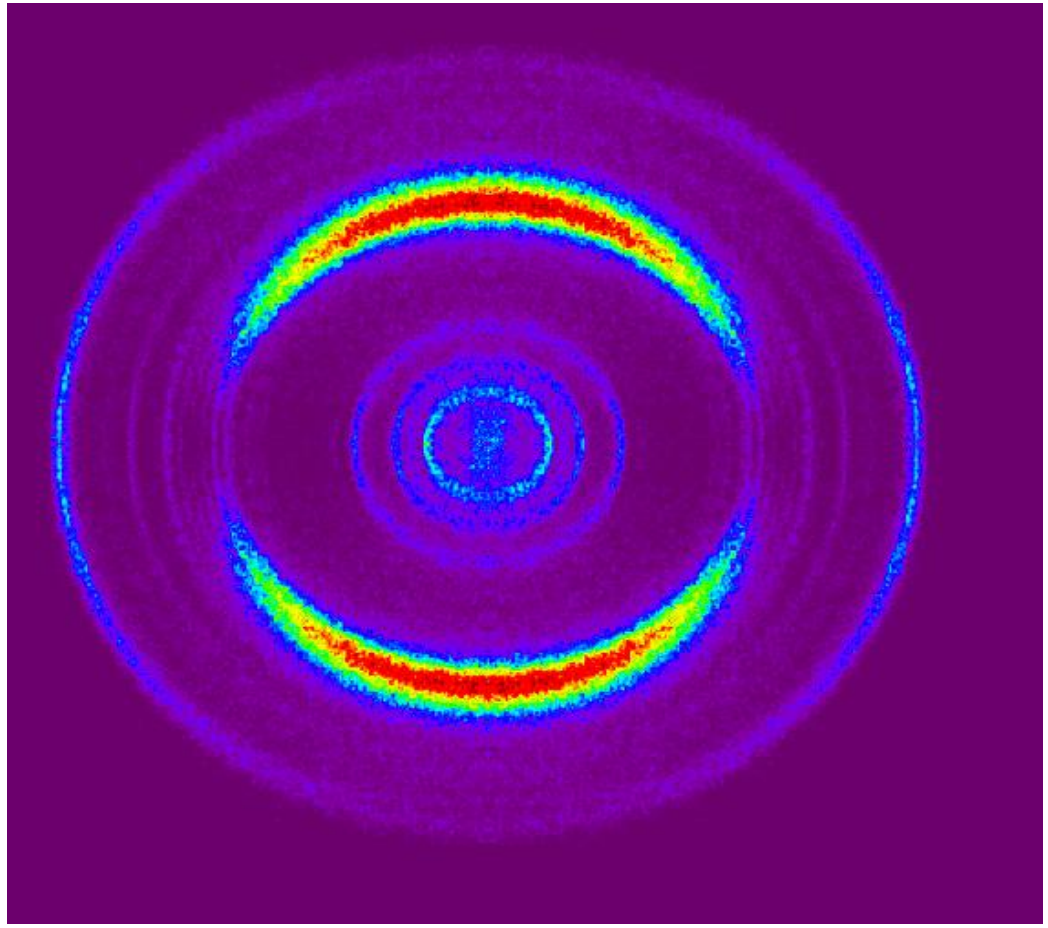
Applications to imaging

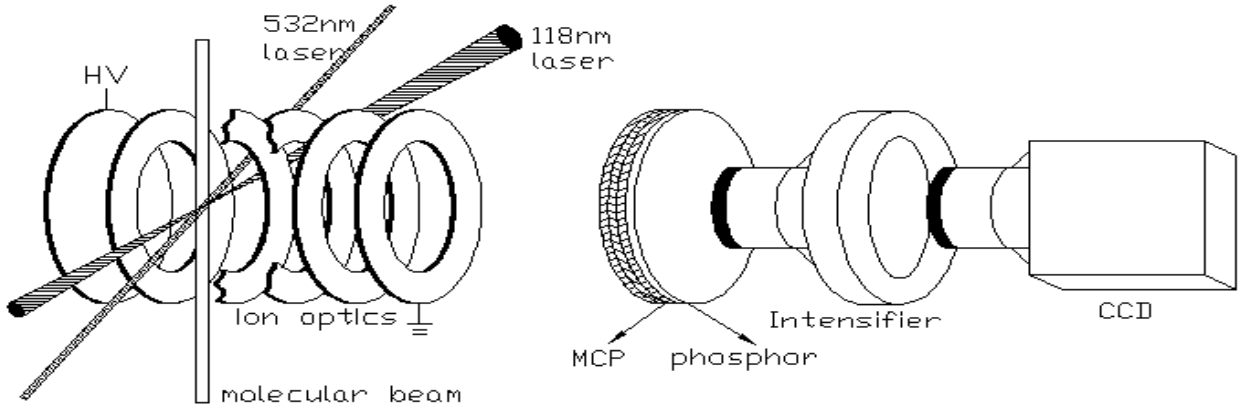
1. Velocity distributions of ions
2. Spatial distributions of chemicals on solid samples (e.g., tissue)


laser
Ion trajectory
Sample
d
Time and position sensitive detector
Mass selective image

Time of Flight mass spectrometer / Velocity map ion imaging

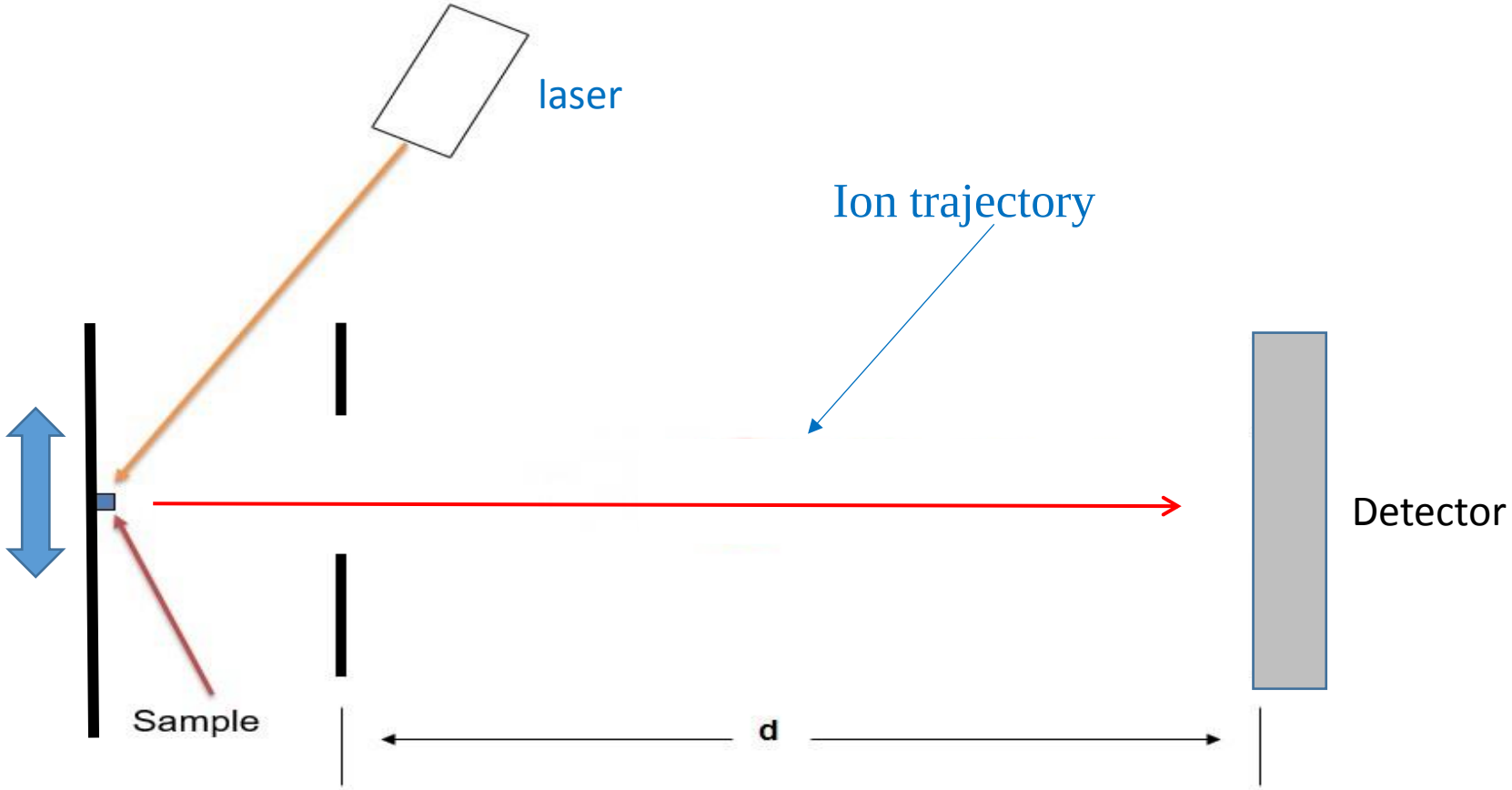
$I_2 + h\nu \rightarrow I$ (quantum state 1) + I (quantum state 2)

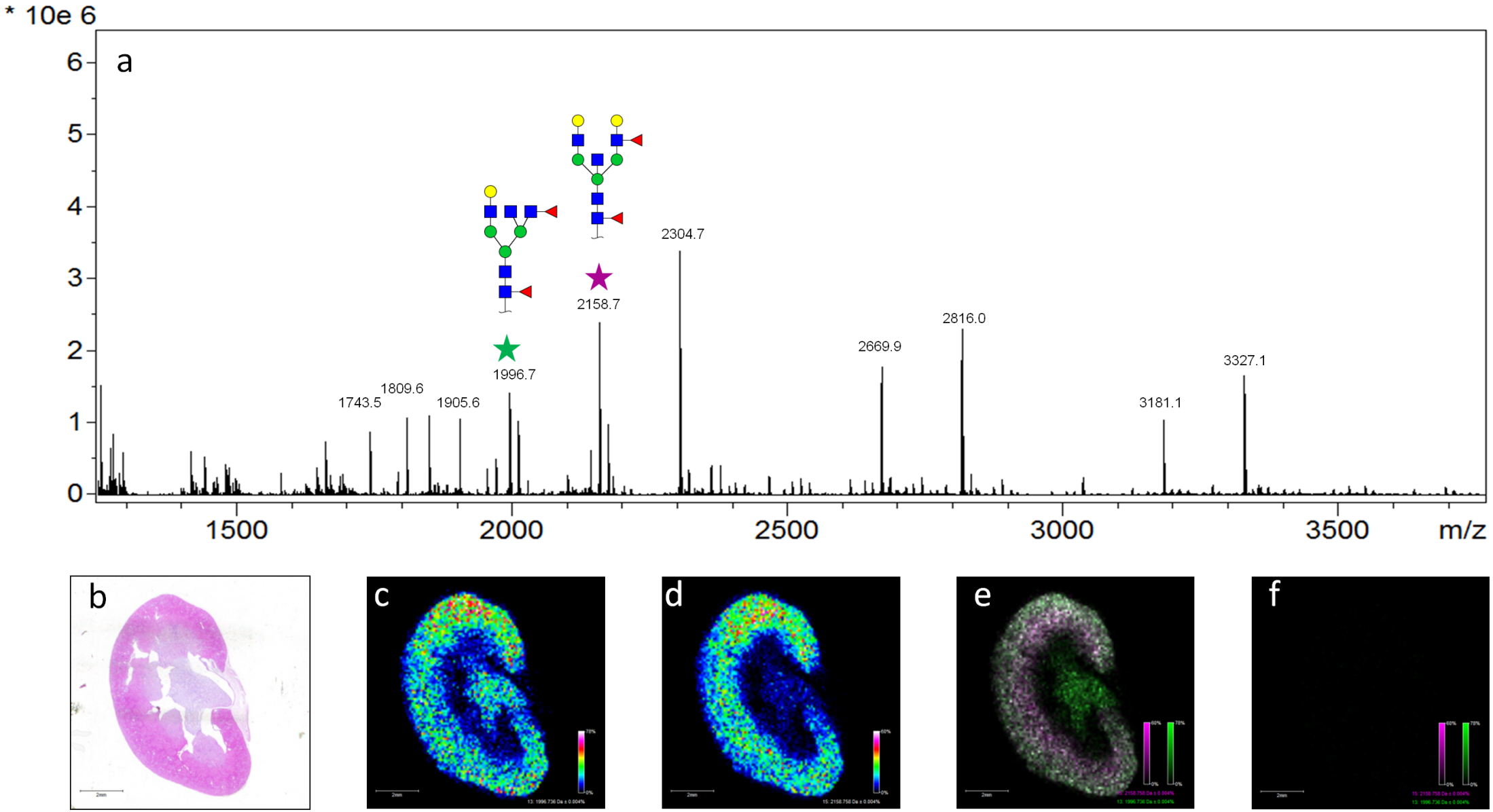




MALDI + TOF mass spectrometer

- Sample plate is moved perpendicular to TOF axis




* 10e 6
a
6
5
4
3
2
1
0
1743.5
1809.6
1905.6
1996.7
2158.7
2304.7
2669.9
2816.0
3181.1
3327.1
1500
2000
2500
3000
3500
m/z
b
c
d
e
f

Application to biomolecules

- Non volatile
- Unstable at high temperature

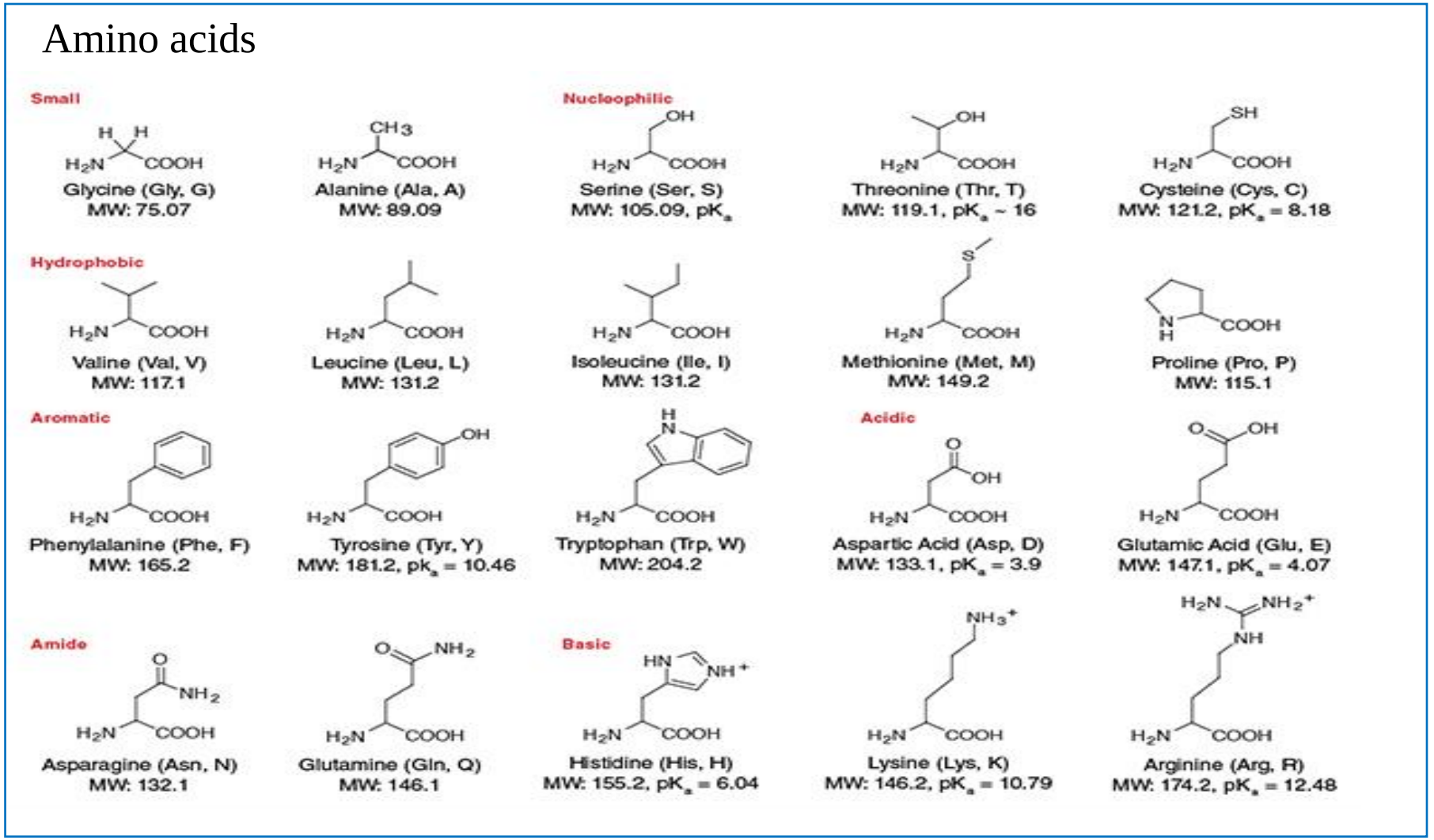
The four major classes of molecules creating life

1. Nucleic acid (核酸)
2. Protein (蛋白質)
3. Carbohydrate (碳水化合物)
4. Lipid (脂質)

} macromolecules

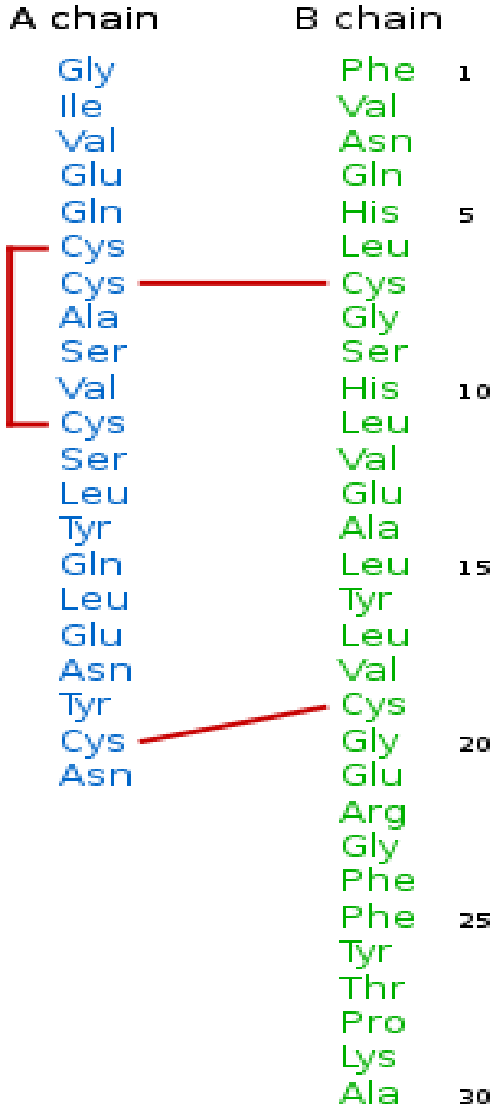
Application to Proteins

Amino acids



Sequence of insulin

胰島素

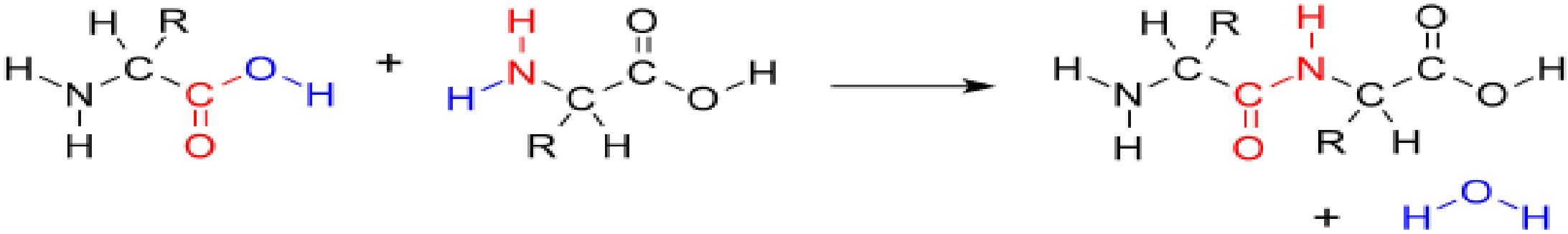


(From Wikipedia)

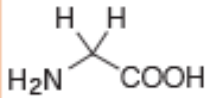
In 1958, Frederick Sanger was awarded a Nobel Prize in chemistry "for his work on the structure of proteins, especially the sequence of insulin (胰島素).

(From Wikipedia)

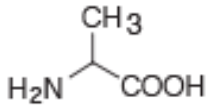
Two consecutive amino acids are linked by a peptide bond.



SMALL

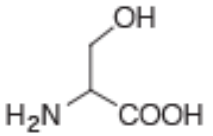


Glycine (Gly, G)
MW: 75.07

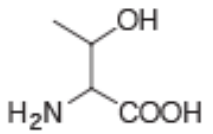


Alanine (Ala, A)
MW: 89.09

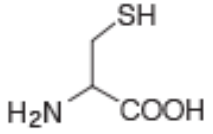
NUCLEOPHILIC



Serine (Ser, S)
MW: 105.09, $pK_a \sim 16$

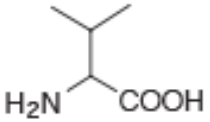


Threonine (Thr, T)
MW: 119.1, $pK_a \sim 16$

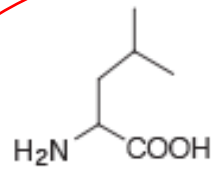


Cysteine (Cys, C)
MW: 121.2, $pK_a = 8.18$

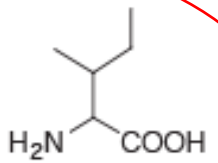
HYDROPHOBIC



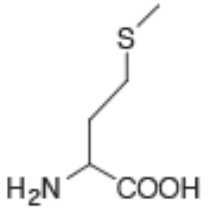
Valine (Val, V)
MW: 117.1



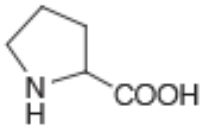
Leucine (Leu, L)
MW: 131.2



Isoleucine (Ile, I)
MW: 131.2

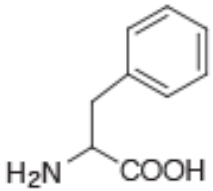


Methionine (Met, M)
MW: 149.2

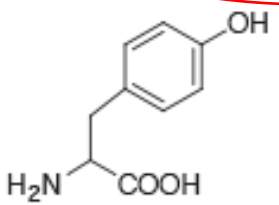


Proline (Pro, P)
MW: 115.1

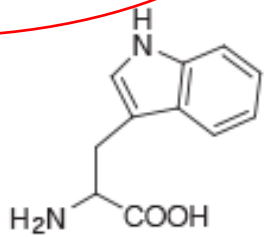
AROMATIC



Phenylalanine (Phe, F)
MW: 165.2

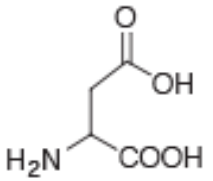


Tyrosine (Tyr, Y)
MW: 181.2, $pK_a = 10.46$

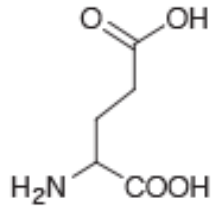


Tryptophan (Trp, W)
MW: 204.2

ACIDIC

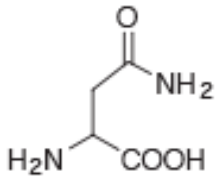


Aspartic Acid (Asp, D)
MW: 133.1, $pK_a = 3.9$

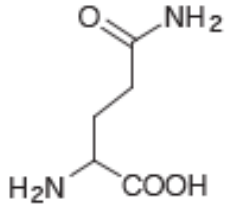


Glutamic Acid (Glu, E)
MW: 147.1, $pK_a = 4.07$

AMIDE

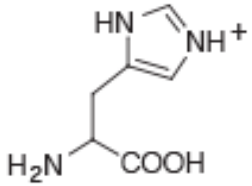


Asparagine (Asn, N)
MW: 132.1

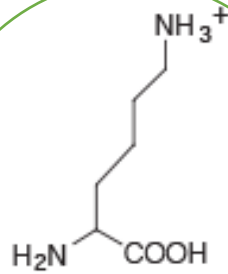


Glutamine (Gln, Q)
MW: 146.1

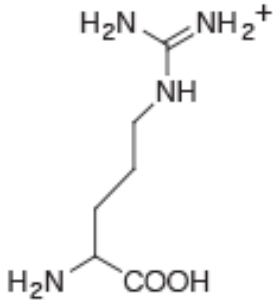
BASIC



Histidine (His, H)
MW: 155.2, $pK_a = 6.04$



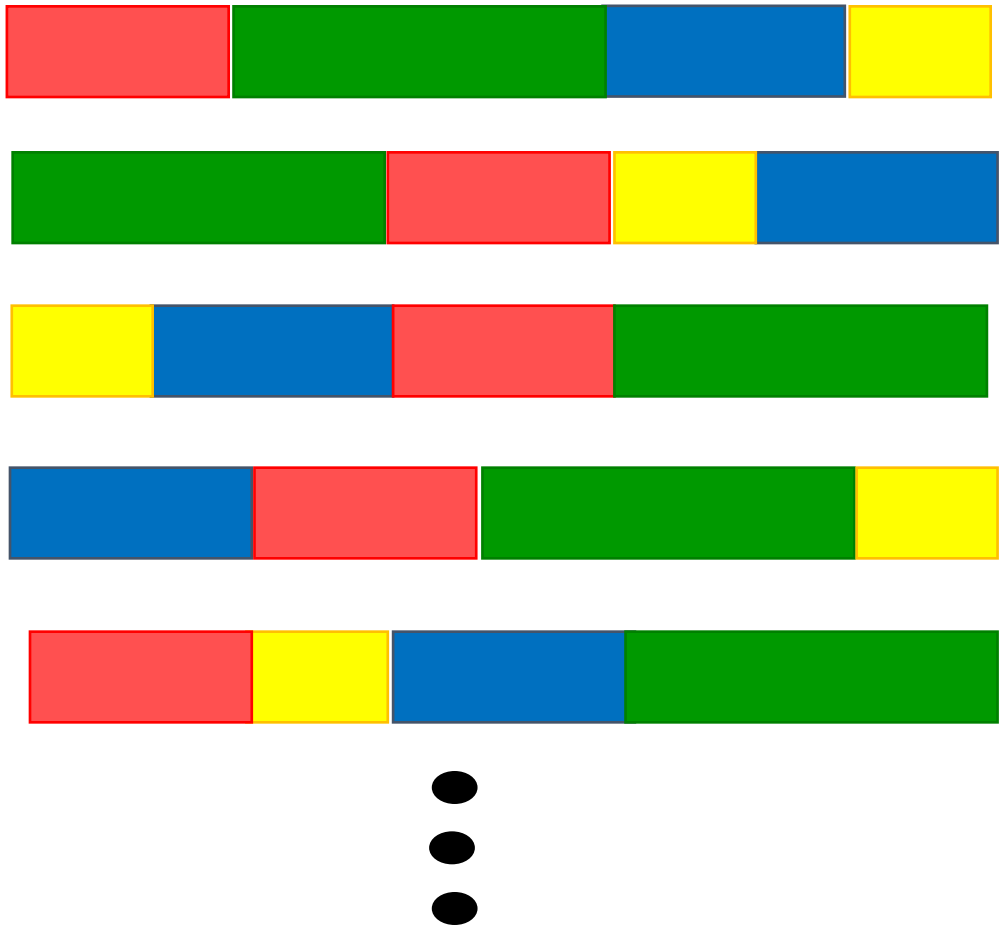
Lysine (Lys, K)
MW: 146.2, $pK_a = 10.79$



Arginine (Arg, R)
MW: 174.2, $pK_a = 12.48$

Protein sequence determined by mass spectrometry

Four amino acids

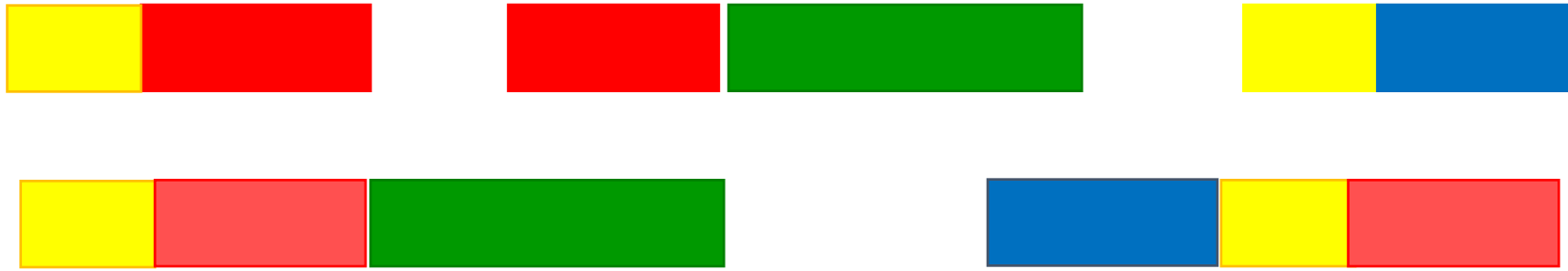


Protein sequencing by mass spectrometry

153.5 = 10 + 20.5 + 42 + 81

CID (collision induced dissociation) mass spectrometry

30.5 101.5 52



Answer:

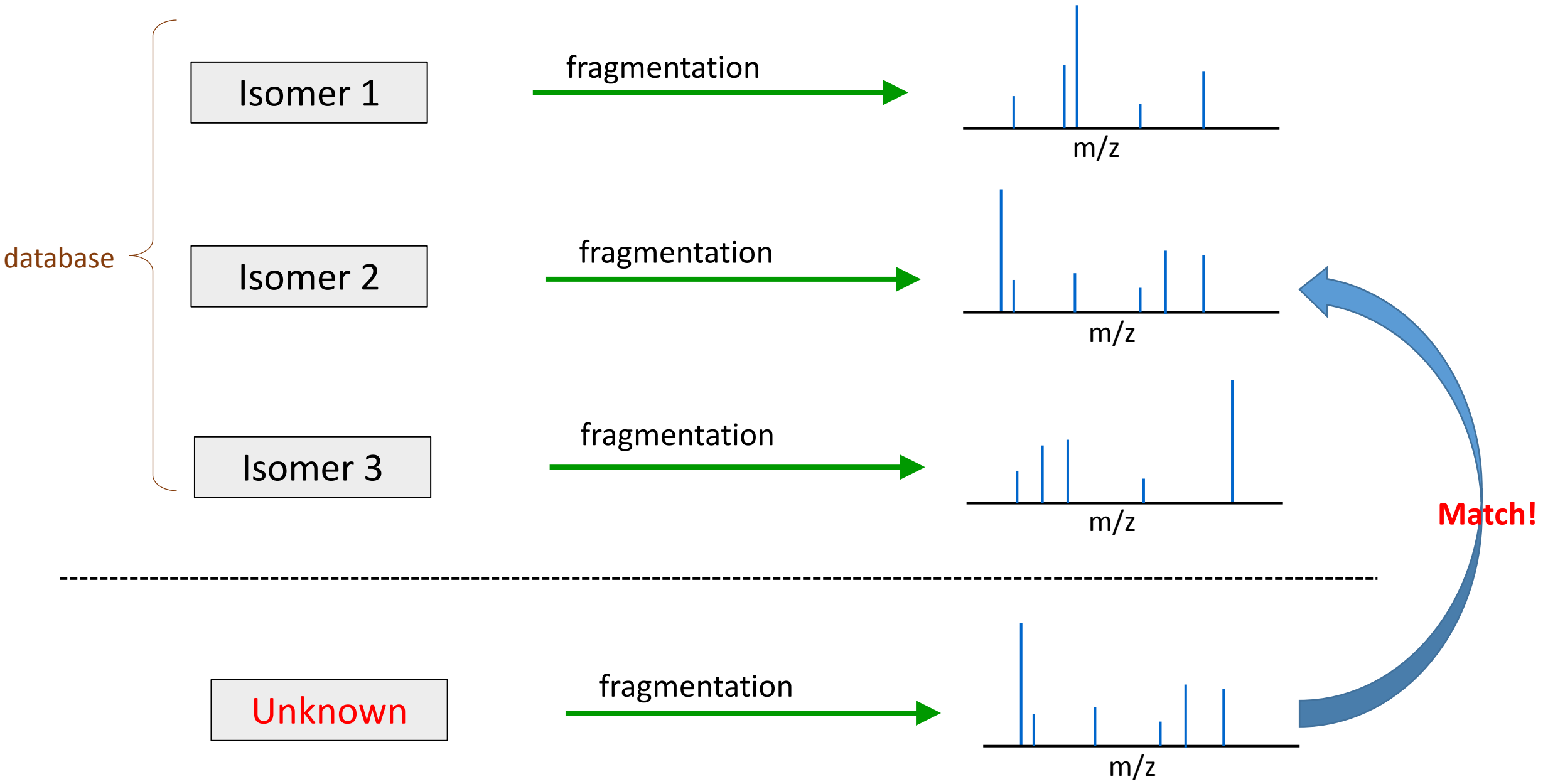
Mass spectrometry

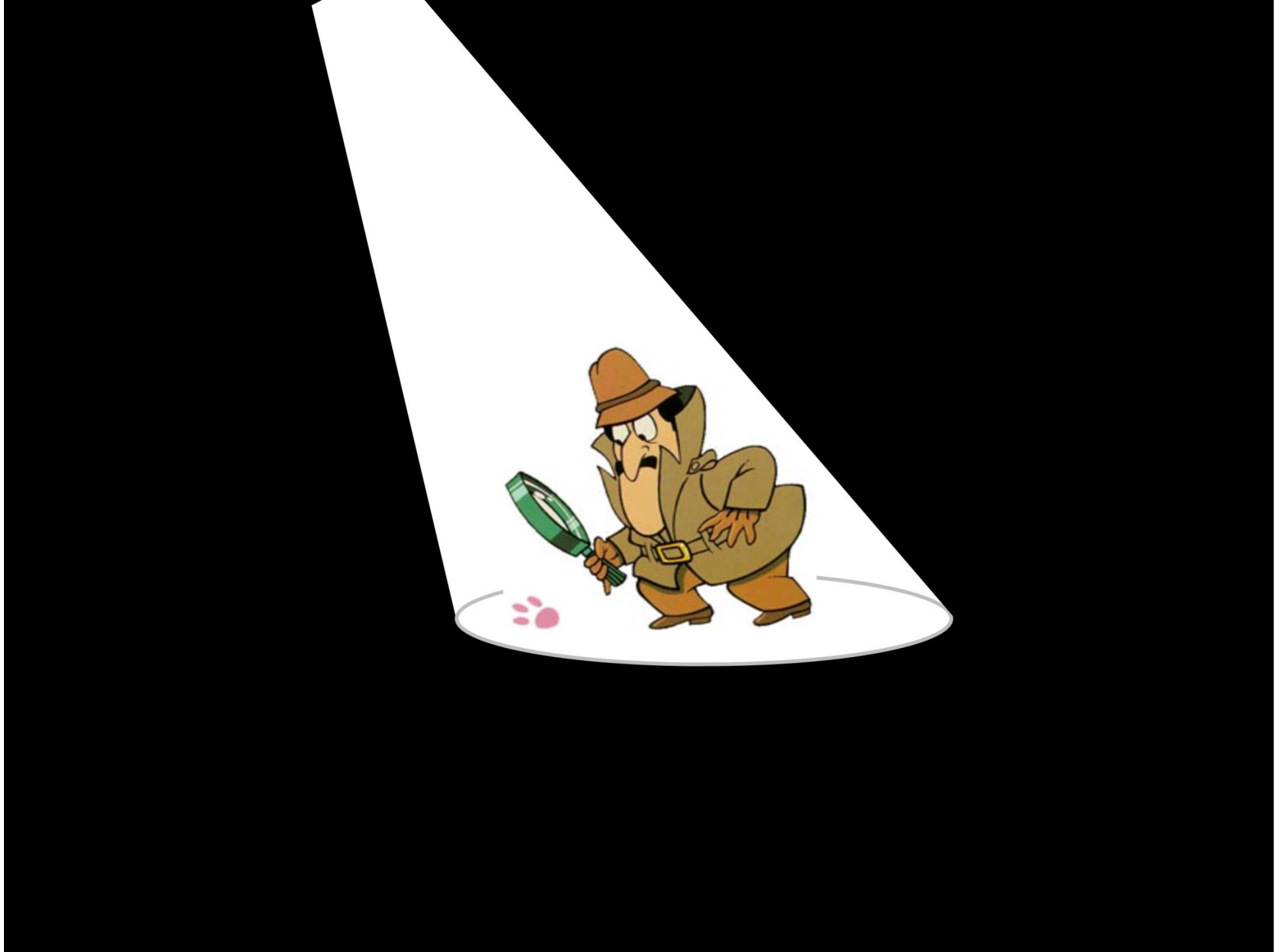
Molecular weight

Different
molecular structures

fragmentation

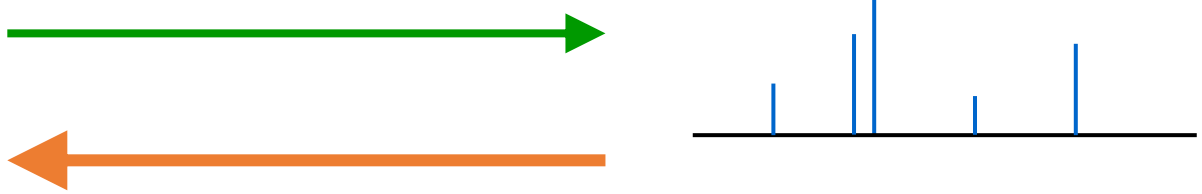
Different
Mass spectra


database
Isomer 1
Isomer 2
Isomer 3
fragmentation
fragmentation
fragmentation
m/z
m/z
m/z
Match!
Unknown
fragmentation
m/z



Unknown

structure



Carbohydrates

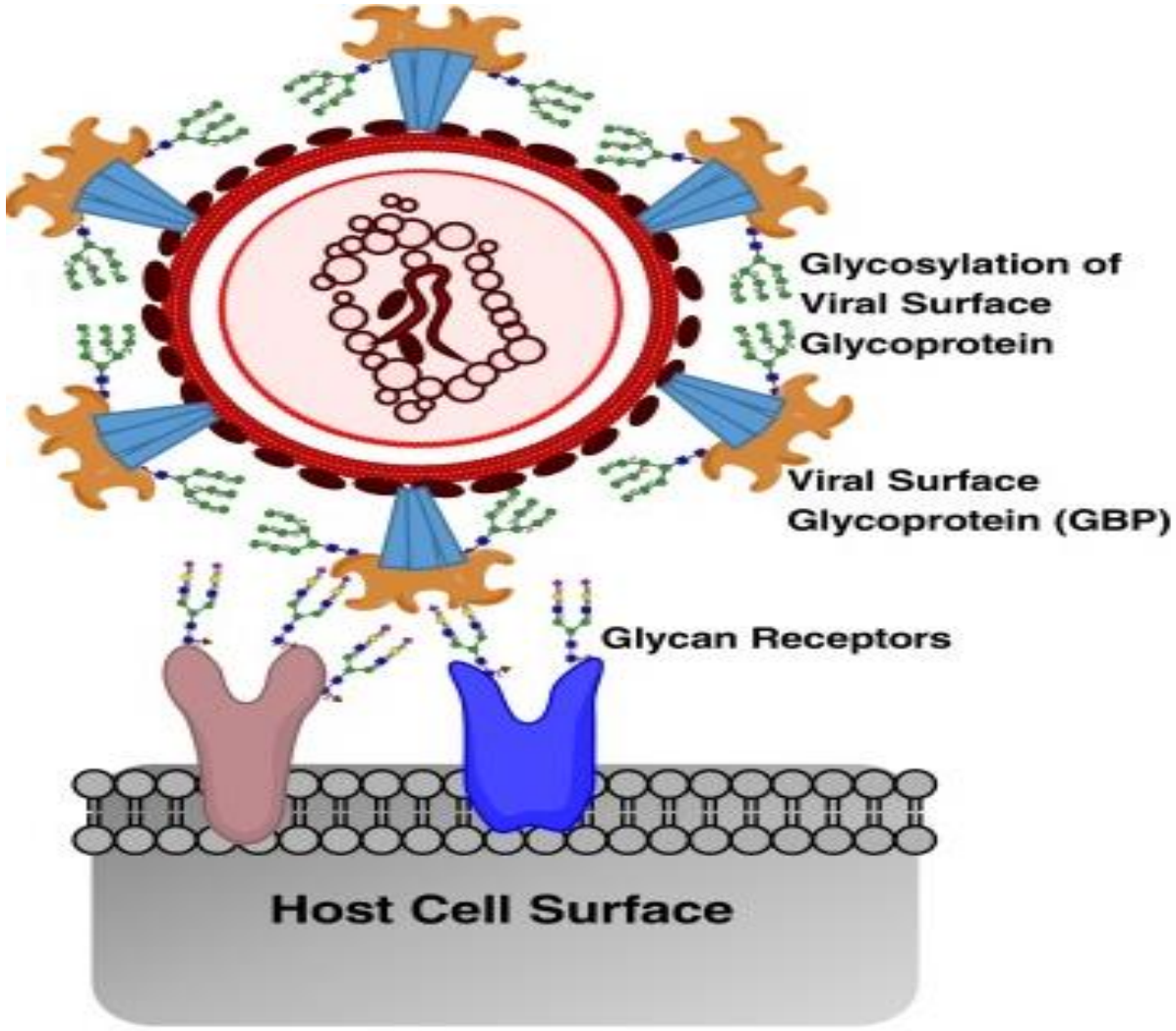
Carbohydrates (glycans, saccharides, sugars)

碳水化合物　　聚醣　　醣類　　糖

Carbohydrates play important roles in the interaction between

- cell-cell
- cell-bacterial
- cell-virus

cell-virus interaction



From: Current Opinion in Structural Biology, 2016, 40, 153

Carbohydrates are macromolecules containing a number of monosaccharides (單醣)

Disaccharides: 2 monomers
雙醣

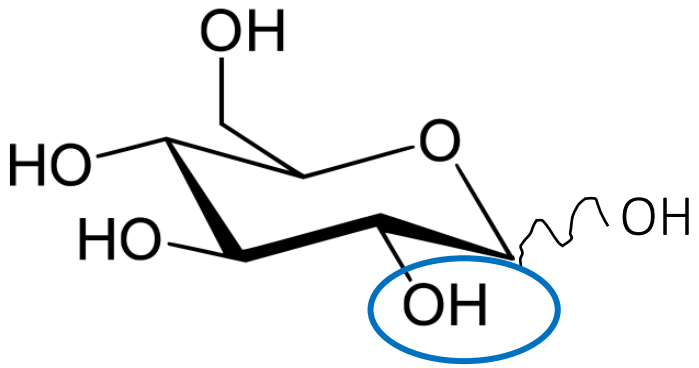
Trisaccharides: 3 monomers
三醣

Oligosaccharides: < 10 monomers
寡糖

Polysaccharides: ≥ 10 monomers
多醣

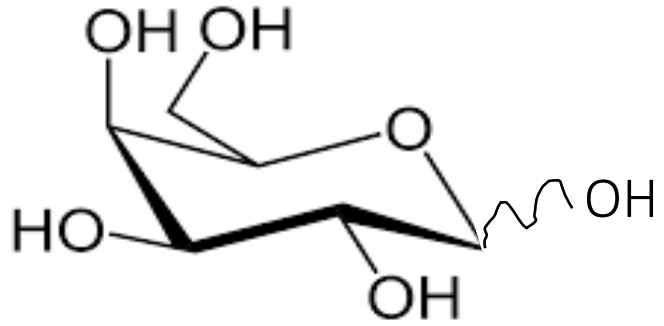
Three common monosaccharides of hexoses (Hex)

六碳糖



D-Glucose

葡萄糖

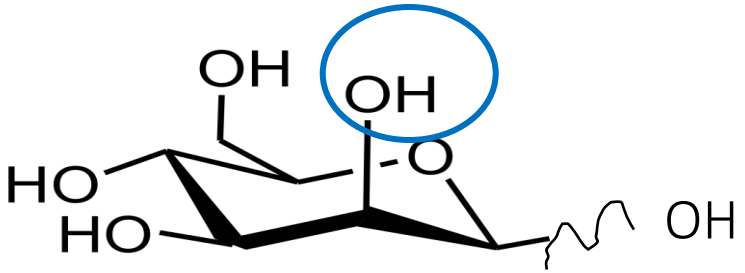


D-Galactose

半乳糖

Isomer

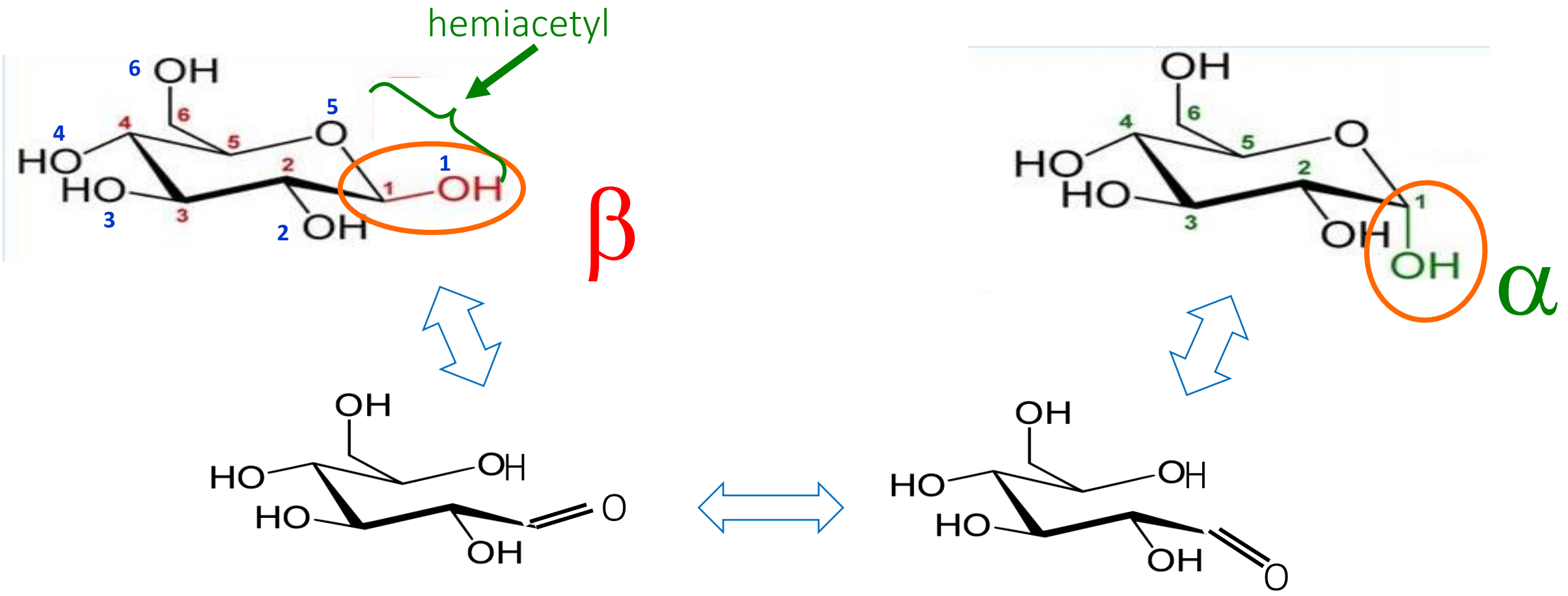
Stereoisomer



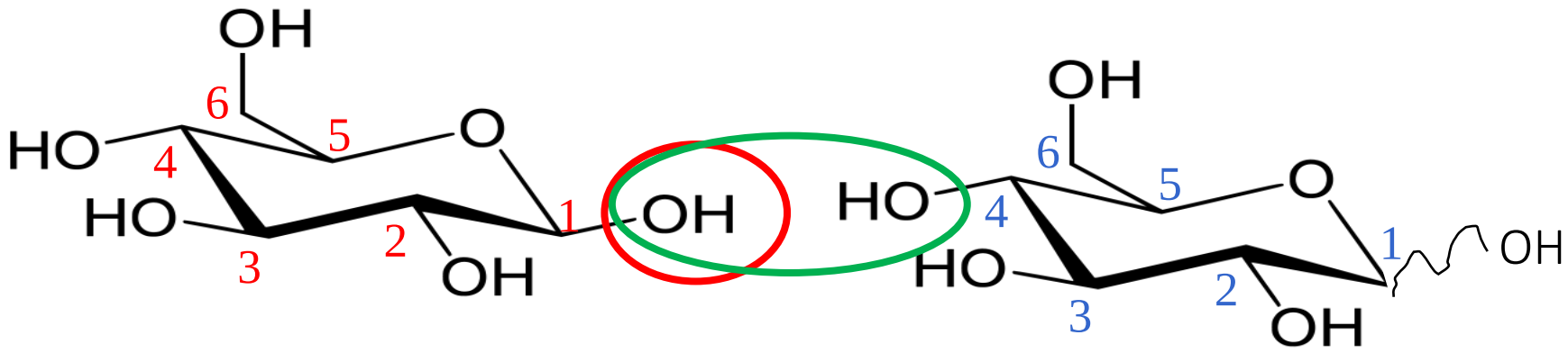
D-Mannose

甘露糖

Anomers (anomeric configurations)



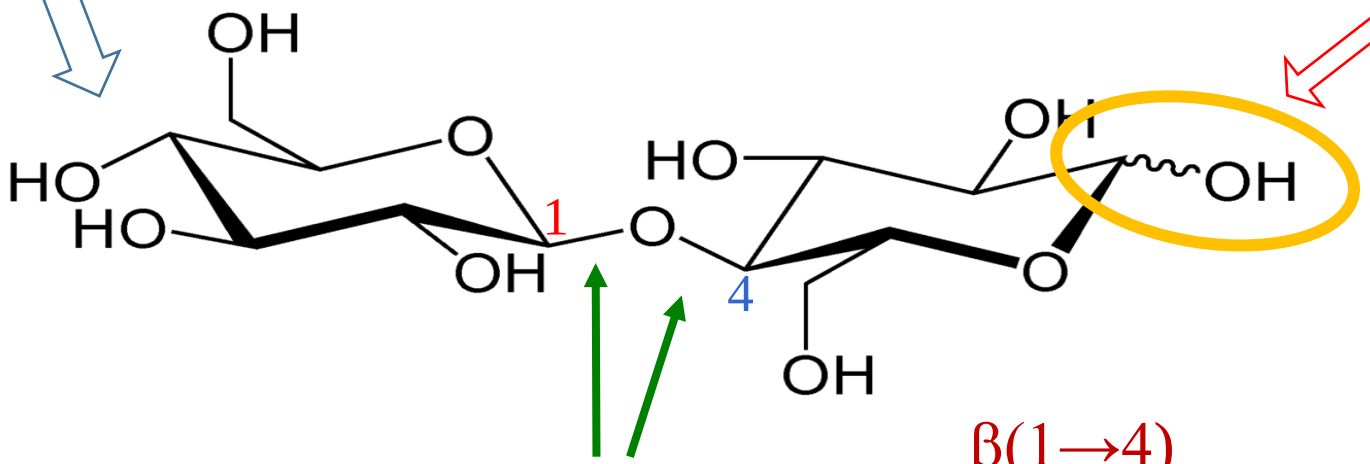
Linkage between monosaccharides



Non-reducing end

$-H_2O$

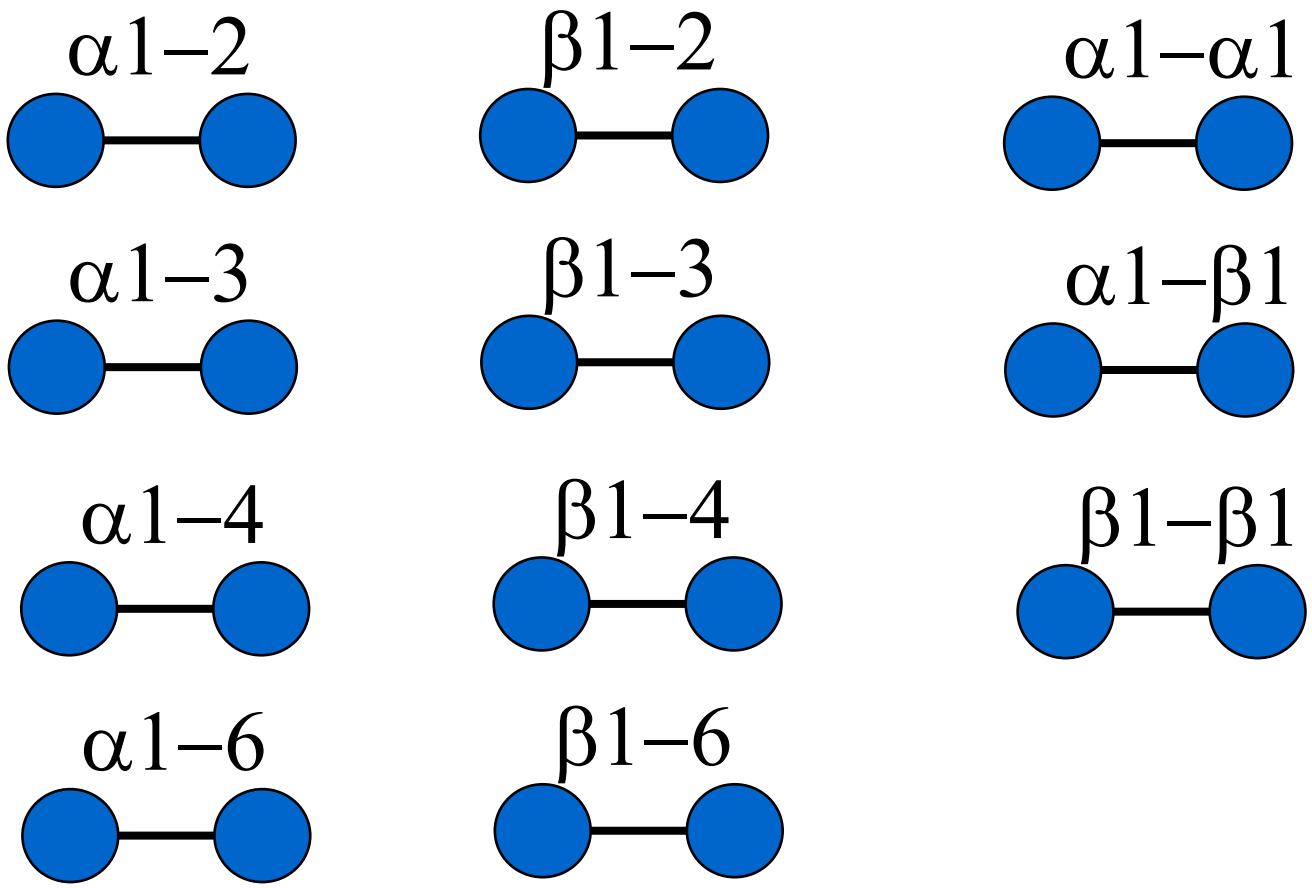
Reducing end



glycosidic bond

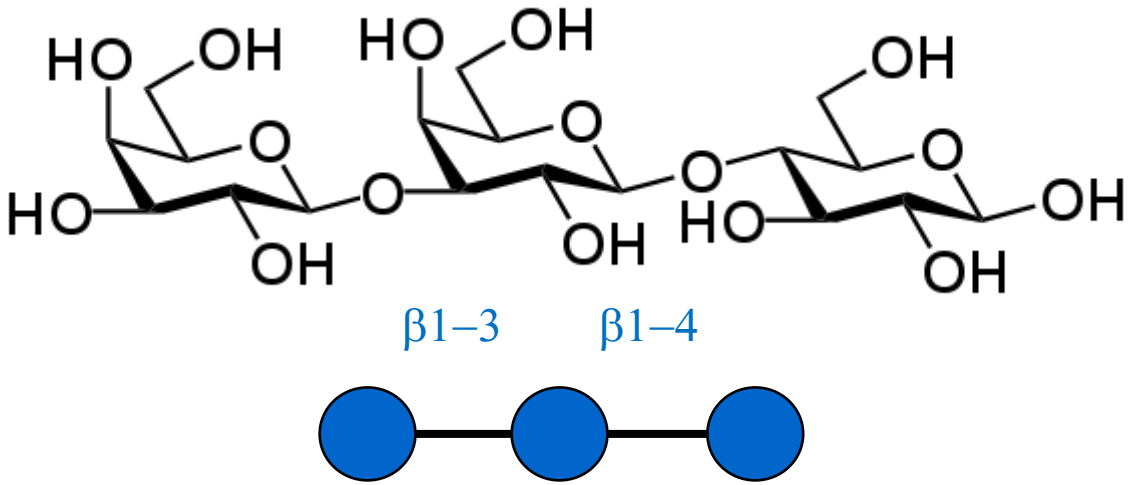
β(1→4) linkage

Glucose disaccharide

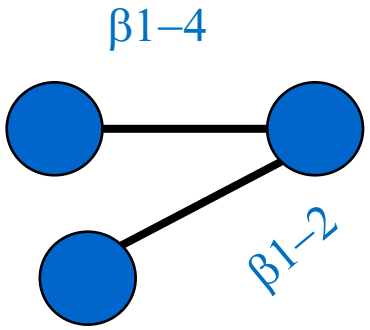


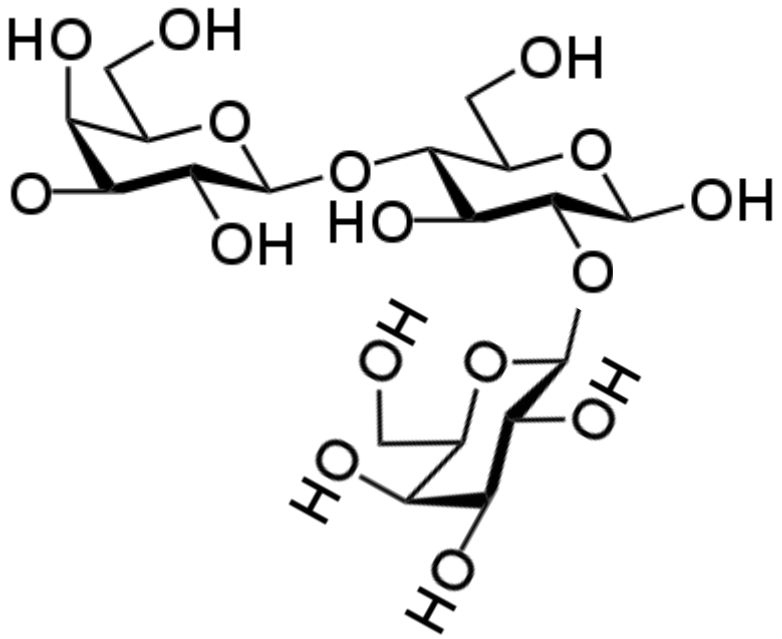
11 isomers

Linear trisaccharides



Branched trisaccharides





Number of isomers

Glucose, Galactose, Mannose

Disaccharide: 99

Trisaccharide: > 2700

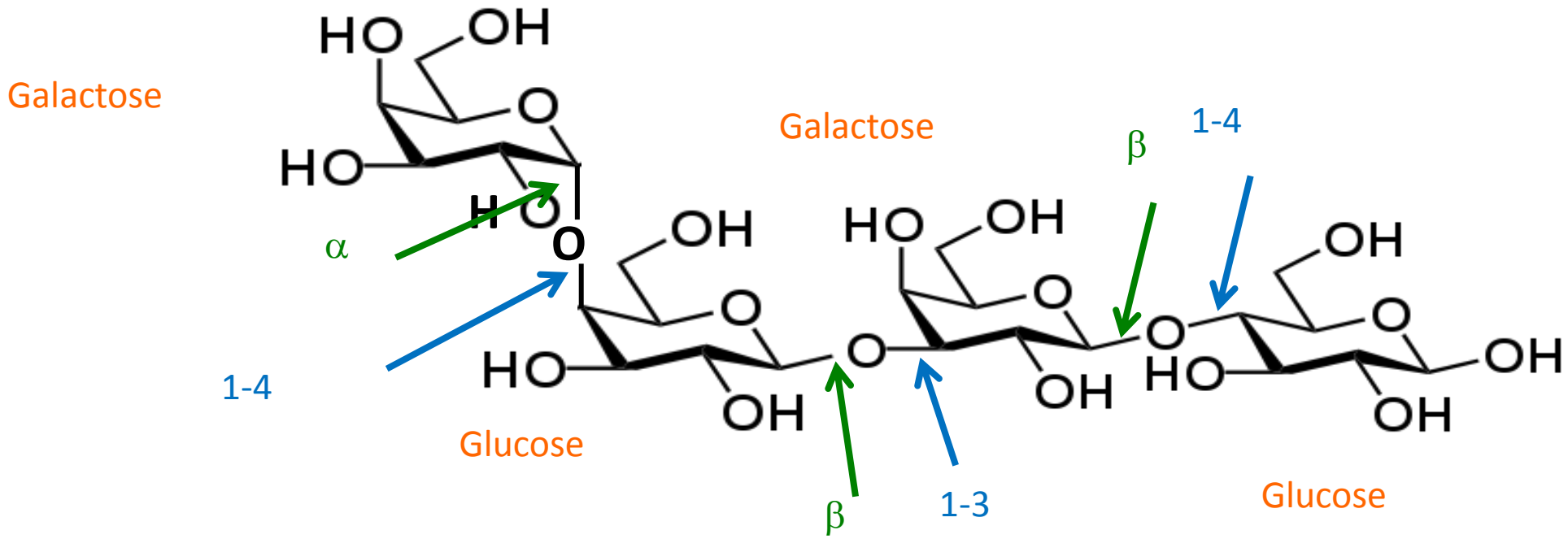
Tetrasaccharide: > 8×10^4

Pentasaccharide: > 2×10^6

Hexosaccharide: > 6×10^7

- Isomer barrier
- Challenge for structural determination, synthesis, separation!

What are the structures to be determined?



- Linkage position
- Anomeric configuration
- Stereoisomer
- Sequence

Traditional methods

1. Chemical reactions + enzyme digestion
2. Nuclear Magnetic Resonance Spectroscopy

Time and sample consuming

Sensitivity

Mass spectrometry : NMR/wet chemistry

10000~1000 : 1

Conventional Mass Spectrometry

- Multistage tandem mass spectroscopy
- CID, HCD, electron transfer, electron attachment, photodissociation, …
- Sample pretreatment: reduction and permethylation
- H^+, Li^+, Na^+, K^+, Cs^+, Mg^+, Cu^+, Pb^+…, e^-, Cl^-, NO_3^-, …

- Linkage position **Yes**
- Anomer **No**
- Stereoisomer **No**
- Sequence **Yes/No**

A report from United States National Academy of Sciences in 2012:

Call for the development of technology over the next 10 years to purify, identify, and determine the structures of all the important glycoproteins, glycolipids, and polysaccharides in any biological sample.

[a] National Research Council (US) Committee on Assessing the Importance and Impact of Glycomics and Glycosciences: Transforming Glycoscience: A Roadmap for The Future. National Academies Press, Washington, DC (2012)

Conventional Mass Spectrometry

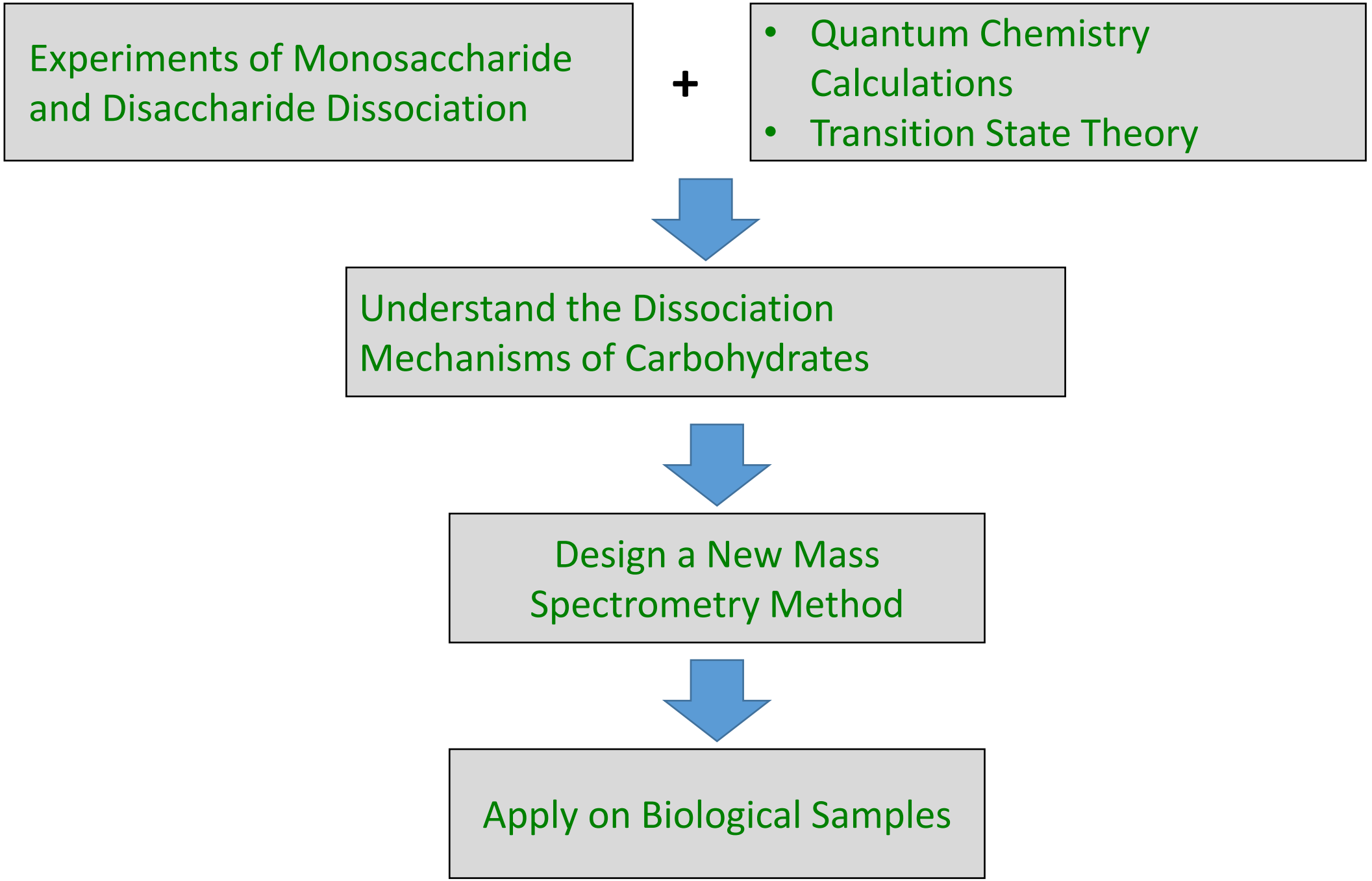
- Multistage tandem mass spectroscopy
- CID, HCD, electron transfer, electron attachment, photodissociation, …
- Sample pretreatment: reduction and permethylation
- H^+, Li^+, Na^+, K^+, Cs^+, Mg^+, Cu^+, Pb^+…, e^-, Cl^-, NO_3^-, …

- Linkage position **Yes**
- Anomer **No**
- Stereoisomer **No**
- Sequence **Yes/No**

Our New Mass Spectrometry

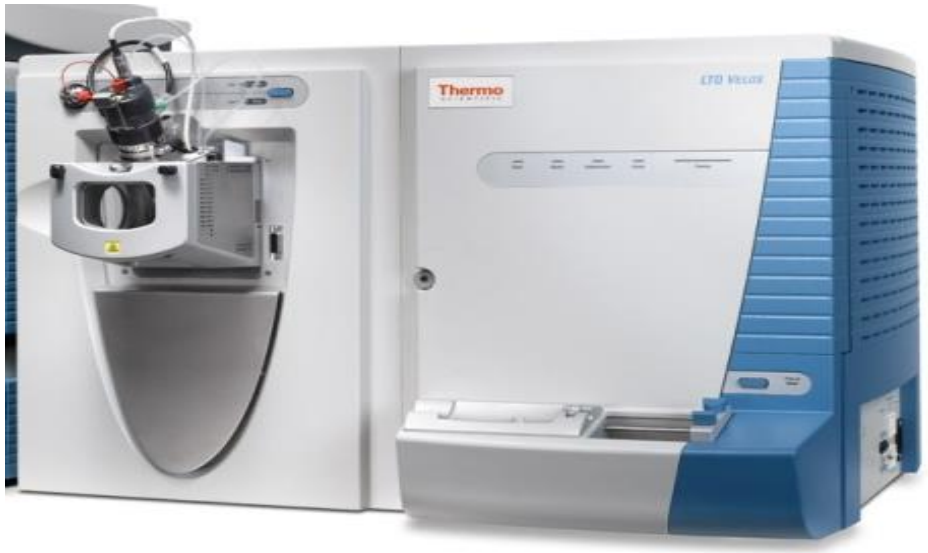
- Multistage tandem mass spectroscopy
- Low energy CID
- No sample pretreatment, Li^+, Na^+,
- In some cases, ^{18}O labelled

- Linkage position **Yes**
- Anomer **Yes**
- Stereoisomer **Yes**
- Sequence **Yes**

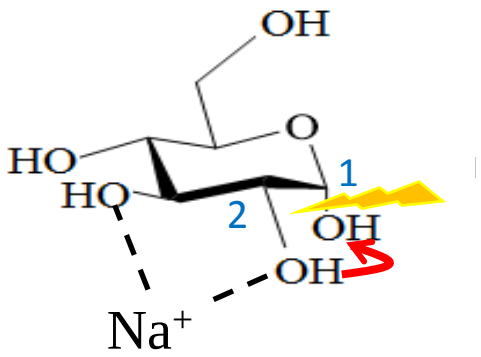

Experiments of Monosaccharide and Disaccharide Dissociation
+
Quantum Chemistry Calculations
Transition State Theory
Understand the Dissociation Mechanisms of Carbohydrates
Design a New Mass Spectrometry Method
Apply on Biological Samples

HPLC-ESI-MS

- HPLC (Thermo Fisher Scientific)
  - Linear ion trap mass spectrometer (LTQ XL, Thermo Fisher Scientific)

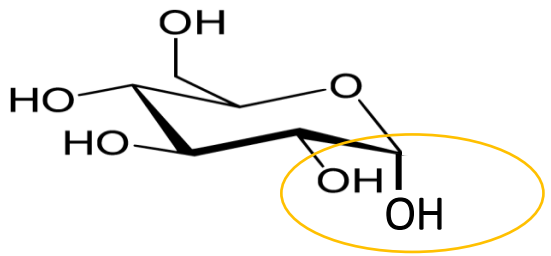


Dissociation mechanism: dehydration

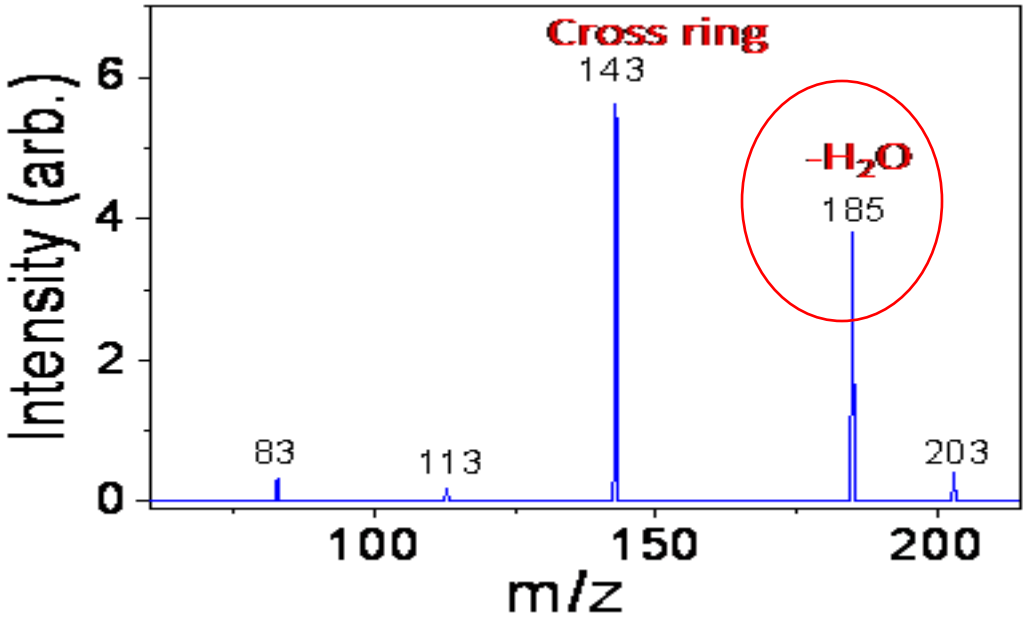


Determination of anomeric configuration at reducing end

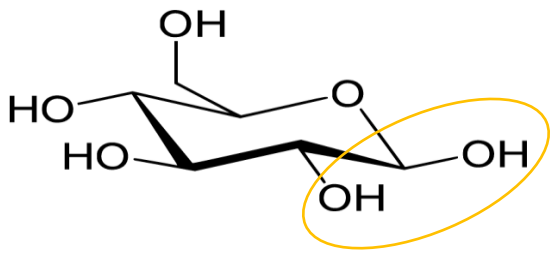
α-glucose



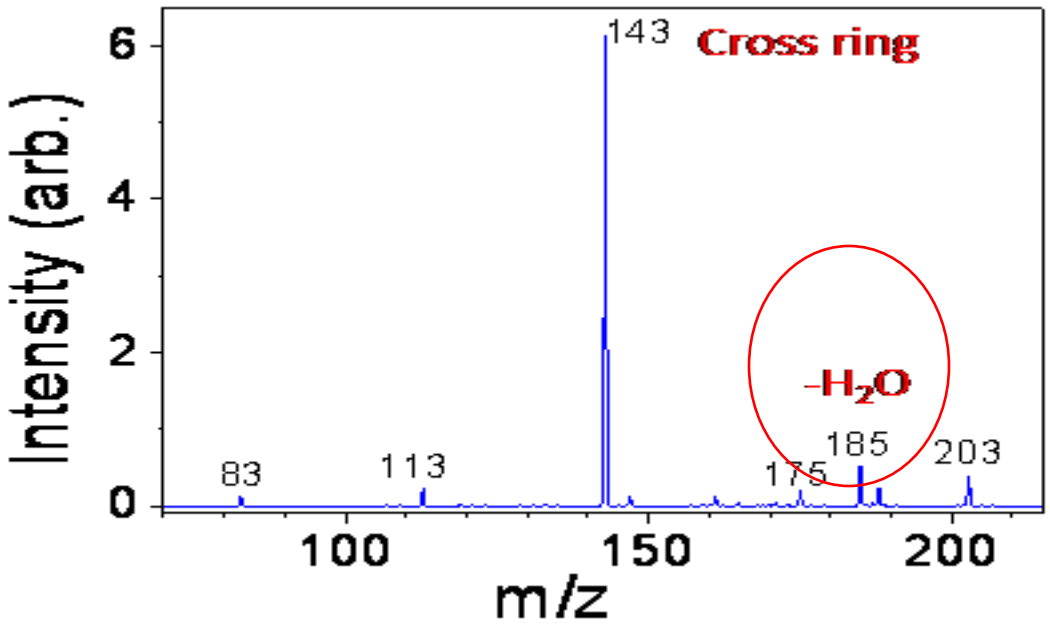
cis



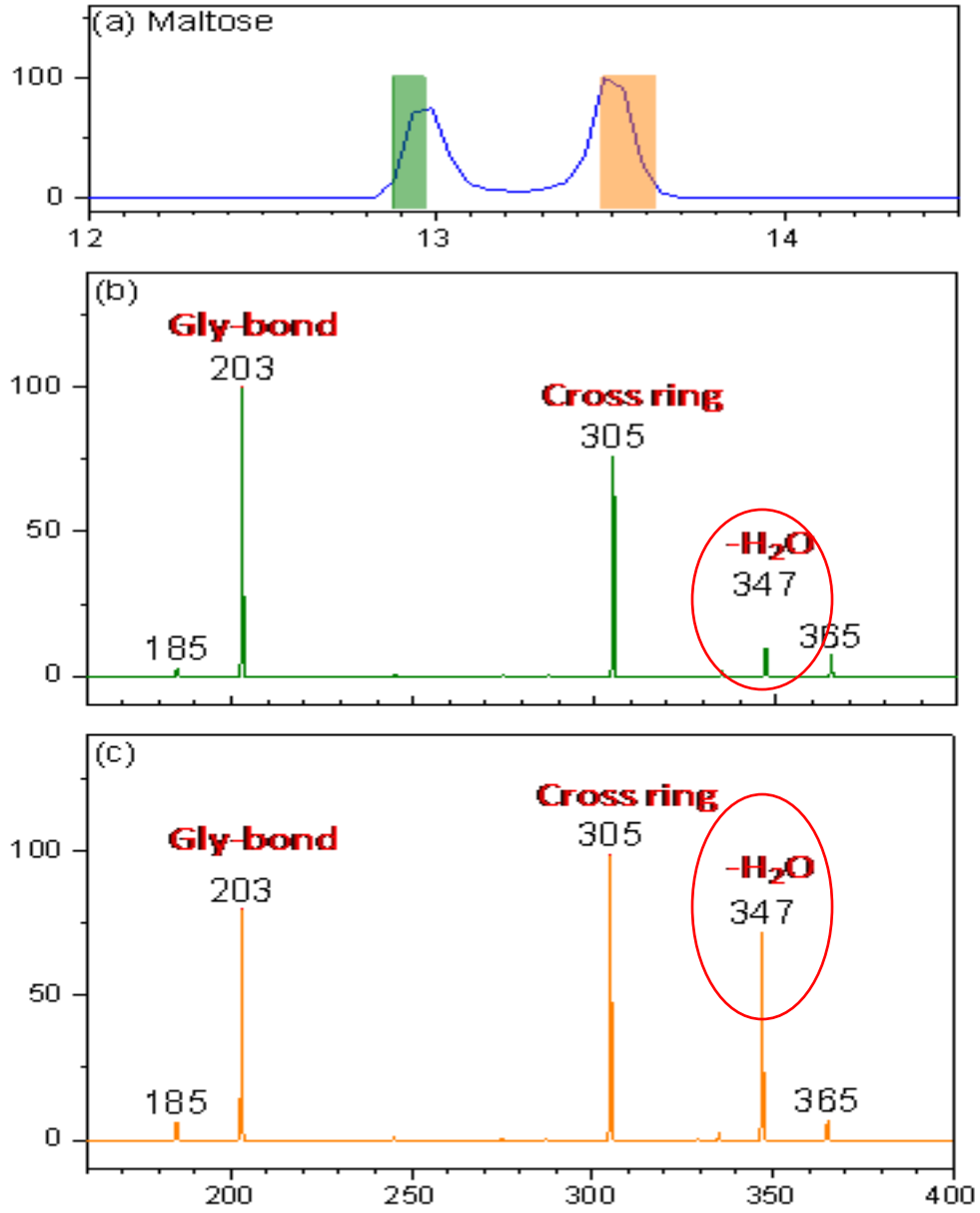
β-glucose



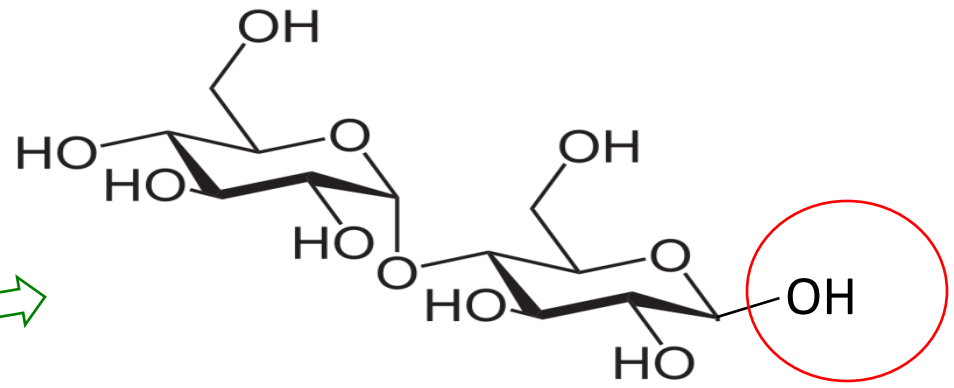
trans



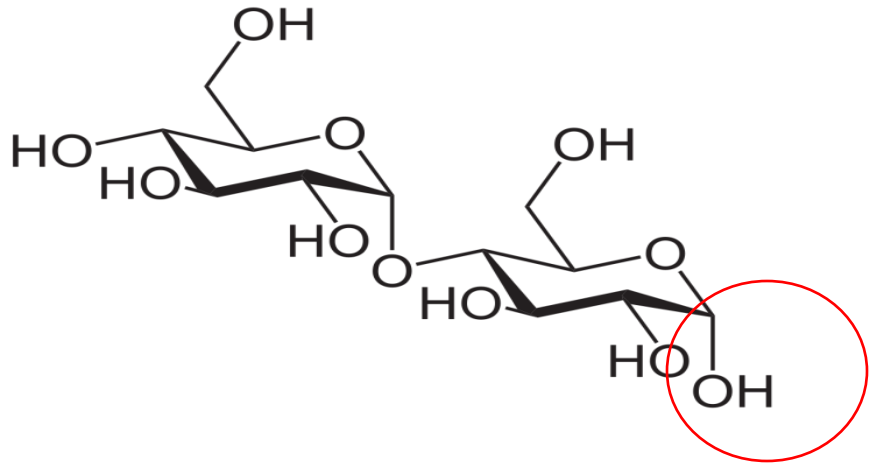
Maltose



β-maltose [α-Glc-(1-4)-β-Glc]

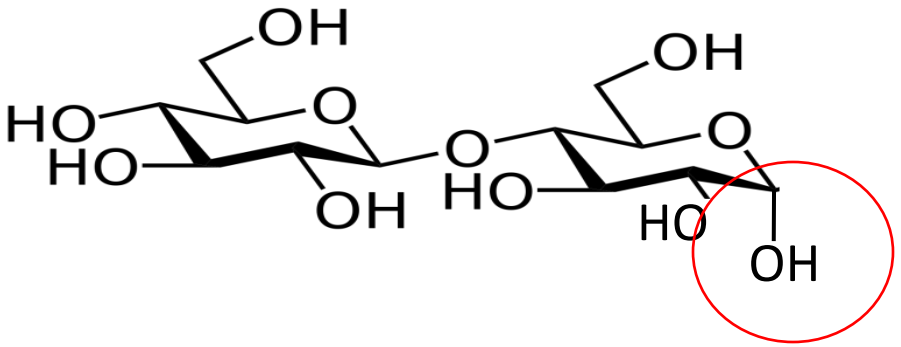


α-maltose [α-Glc-(1-4)-α-Glc]

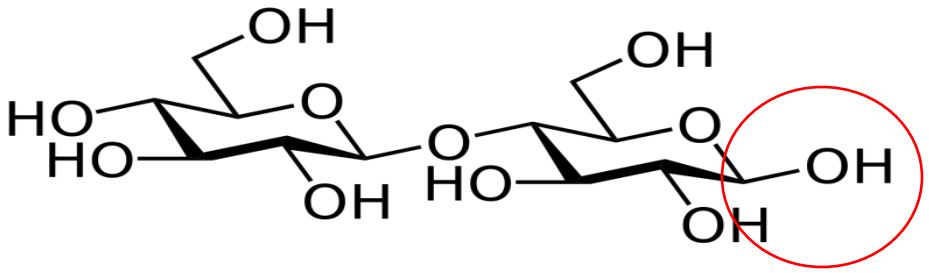


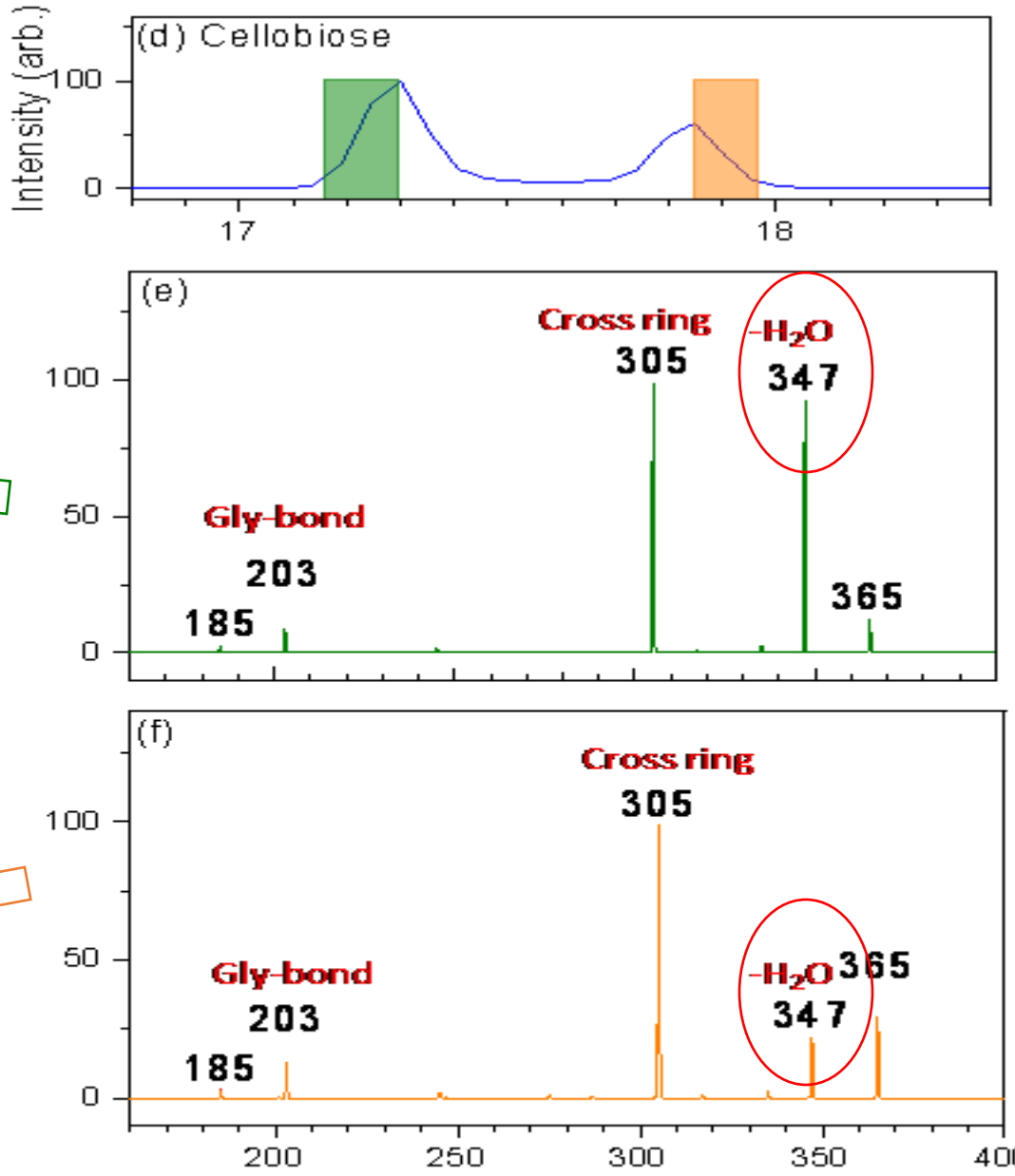
Cellobiose

α-cellobiose [β-Glc-(1-4)-α-Glc]

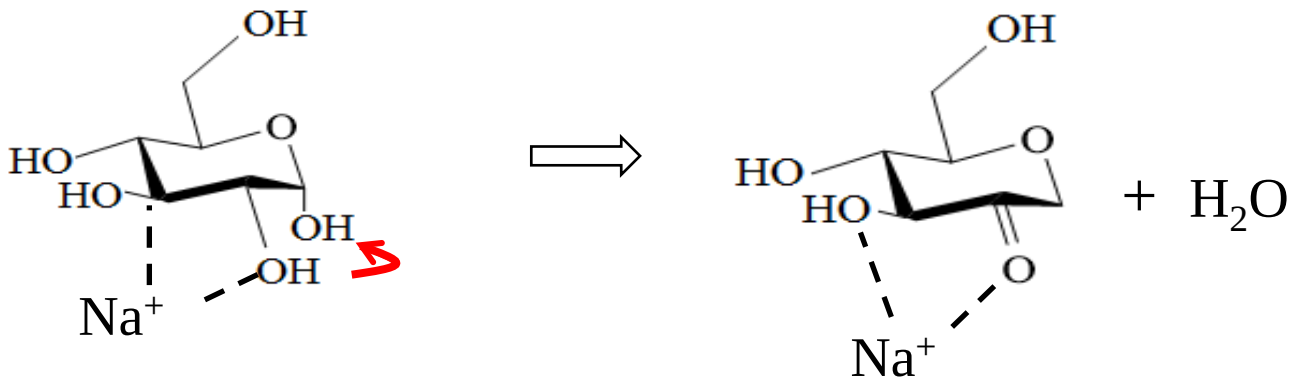


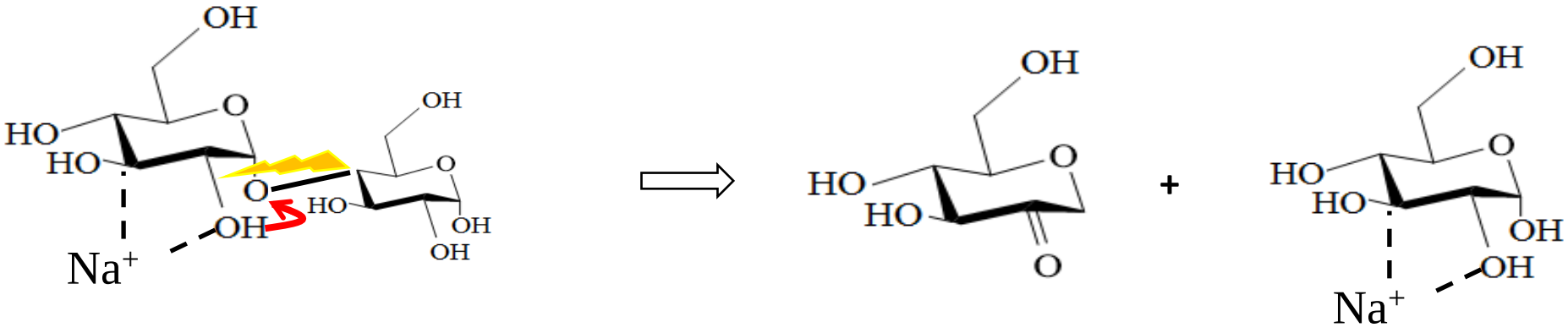
β-cellobiose [β-Glc-(1-4)-β-Glc]





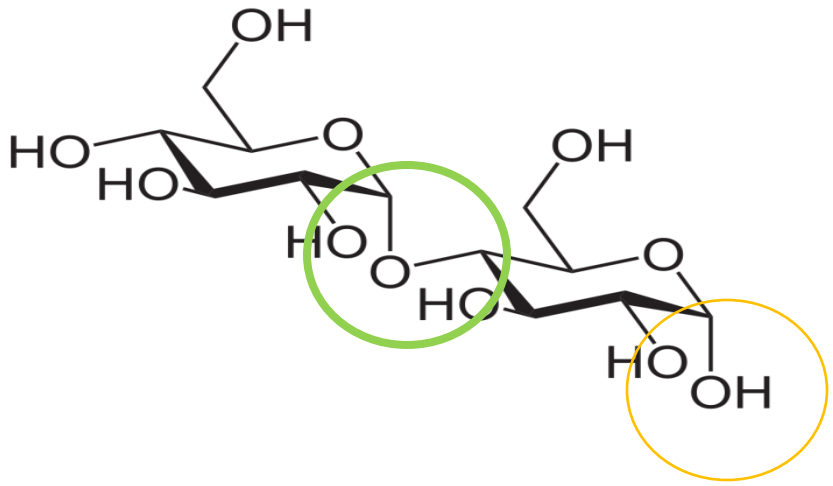
Dissociation mechanism: dehydration





Glycosidic bond cleavage

α-Maltose



α-Cellobiose