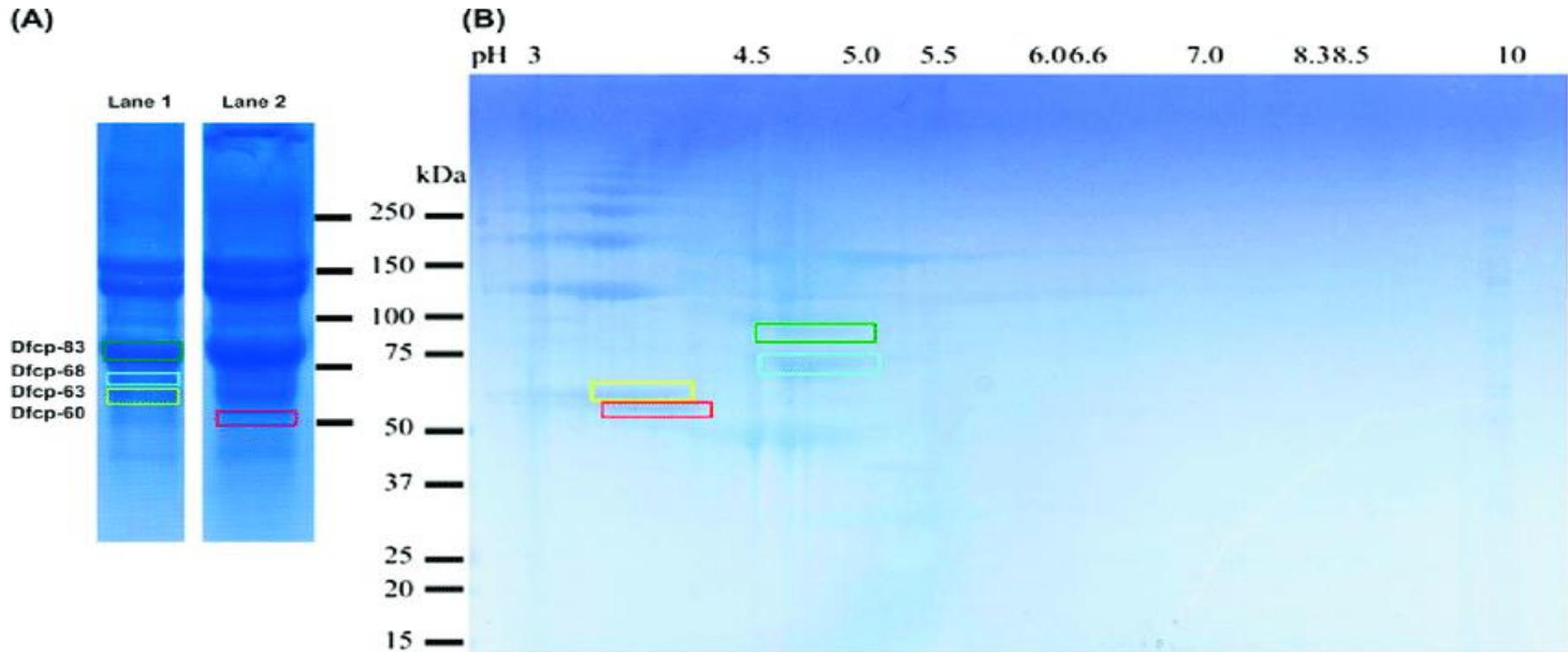
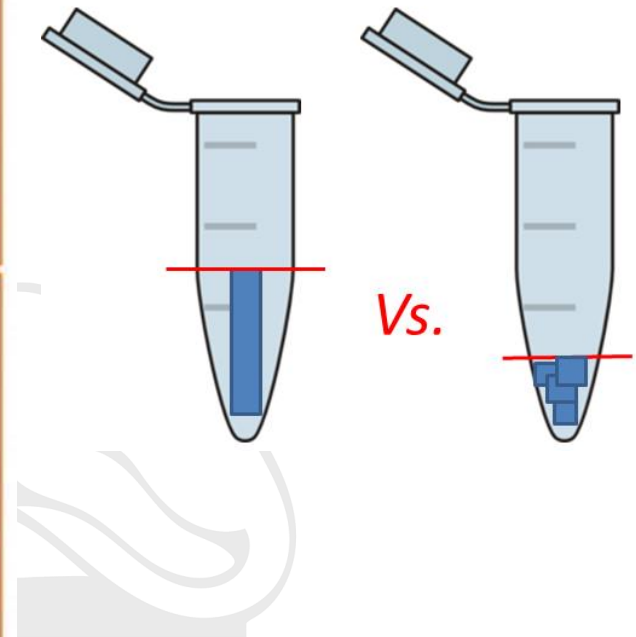


# Select Protein Band and Excise



# Select Protein Band and Excise



Research gate

# Enzymes used for Protein Digestion to Peptides



Enzyme	Site of Cleavage
Trypsin	Lys, Arg (C)
Chymotrypsin	Phe, Trp, Tyr (C)
Asp-N protease	Asp, Glu (C)
Pepsin	Leu, Phe, Trp, Tyr (N)
Elastase	Ala, Gly, Ser (C)
Cyanogen bromide	Met (C)
Endoproteinase Lys C	Lys (C)

# Enzyme Selection and Specification

Protease	Cleavage site	Example of use
<b>Trypsin</b> <i>Specific protease</i>	NNNR NNNK NNN (R is arginine, K is lysine)	Protease of choice for most applications; generates peptides 7–20 amino acids in length with charge characteristics optimal for mass spec analysis
<b>Trypsin/Lys-C Mix, Mass Spec Grade</b> <i>Specific protease</i>	NNNR NNNK NNN (R is arginine, K is lysine)	Reduces missed lysine cleavage sites, increases peptide/protein identification; active under strong denaturing conditions
<b>Lys-C</b> <i>Specific protease</i>	NNNNK NNN (K is lysine)	Digests membrane and other proteolytically resistant proteins; generates larger peptides than tryptic peptides—advantage for certain mass spec methods (for example, electron transfer dissociation)
<b>Arg-C</b> <i>Specific protease</i>	NNNR NNN (R is arginine) Arg-C also can, to a lesser degree, cleave at lysine	Facilitates analysis of histone posttranslational modifications; used in proteome-wide analysis
<b>Glu-C</b> <i>Specific protease</i>	NNNE NNN (E is glutamate) Glu-C also can, to a lesser degree, cleave at aspartate residues	Used as an alternative to trypsin if trypsin produces peptides that are too short or too long or if tryptic cleavage sites are not accessible
<b>Asp-N</b> <i>Specific protease</i>	NNNN DNNN (D is aspartate)	Similar to Glu-C

<b>Chymotrypsin</b> <i>Low Specific protease</i>	NNNN(F/Y/W) NNN (F, Y and W are aromatic residues phenylalanine, tyrosine and tryptophan, respectively)	Digests hydrophobic proteins (for example, membrane proteins)
<b>Pepsin</b> <i>Nonspecific protease</i>	Nonspecific protease (advantage—active at low pH)	Used in structural protein studies and antibody analysis; digests proteolytically resistant, tightly folded proteins
<b>Thermolysin</b> <i>Nonspecific protease</i>	Nonspecific protease (advantage—remains active at high temperature)	Digests proteolytically difficult, tightly folded proteins; used in structural protein studies
<b>Elastase</b> <i>Nonspecific protease</i>	Nonspecific protease	Used to increase protein coverage



# Trypsin Digesting Peptides

## Protein Sequence

RLVEVALGKIGGGANTRGYEVALVNTKFWMCSMVALPGMSWFRH

R=Arginine  
K=Lysine

Trypsin  
digestion  
sites



## Resulting Peptides

LVEVALGK

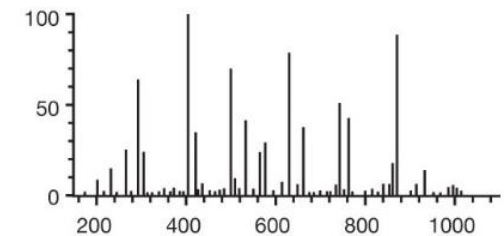
IGGGANTR

GYEVALVNTK

FWMCSMVALPGMSWFR

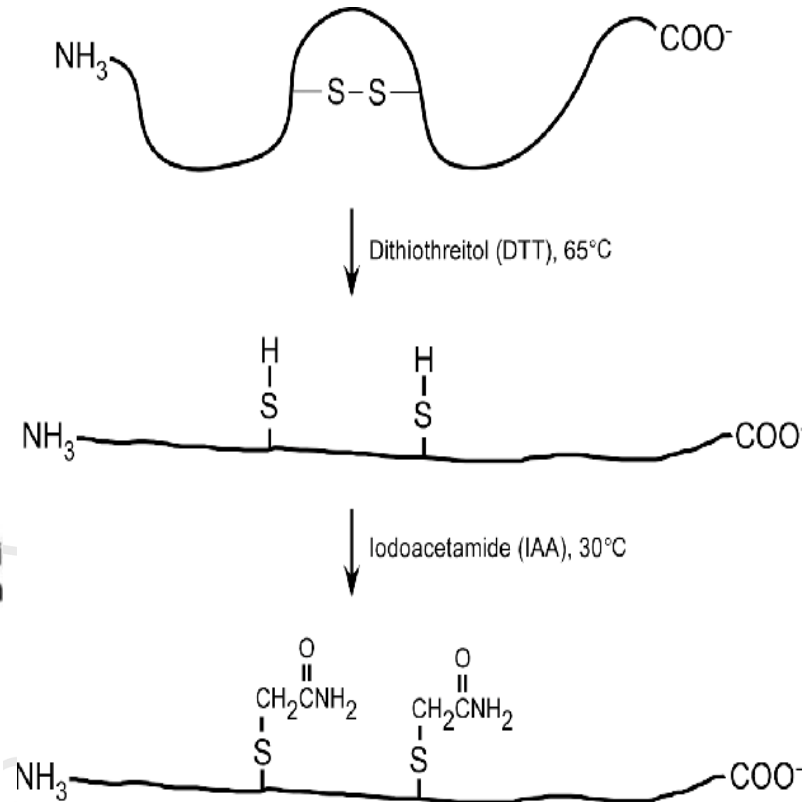
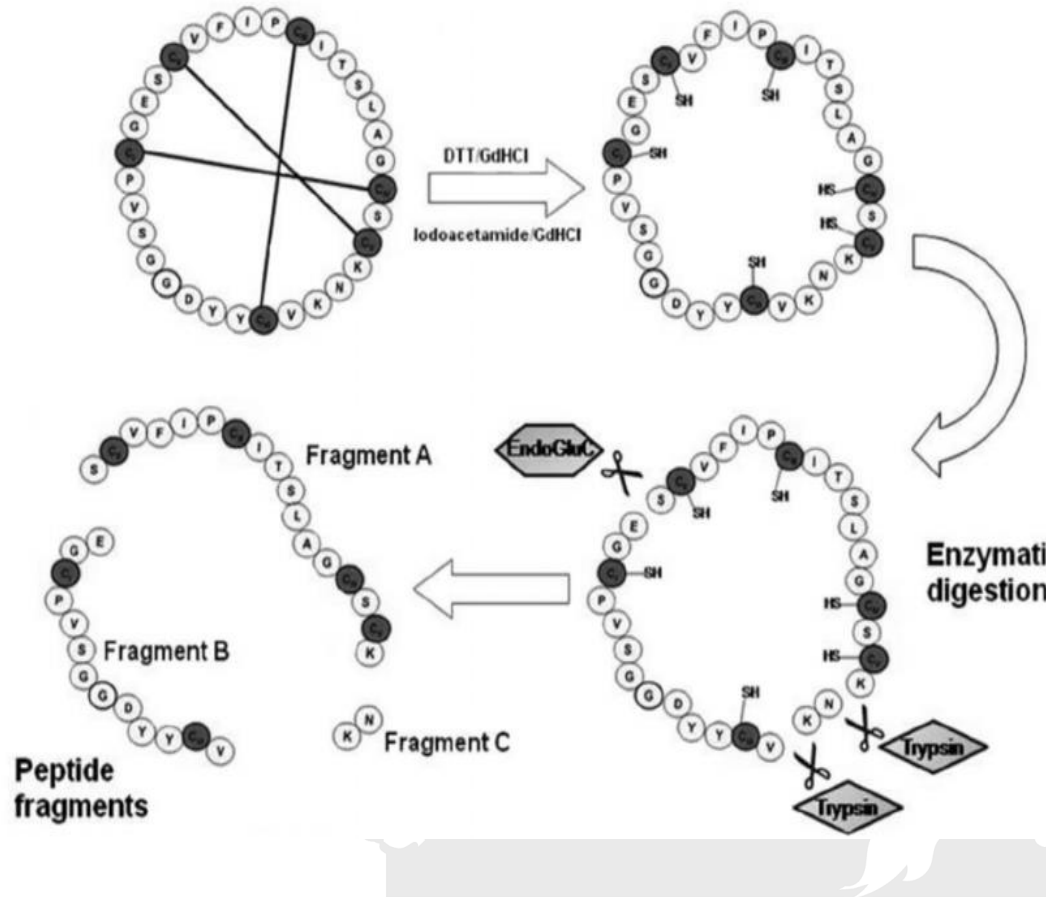


## Mass Spectrometry Analysis

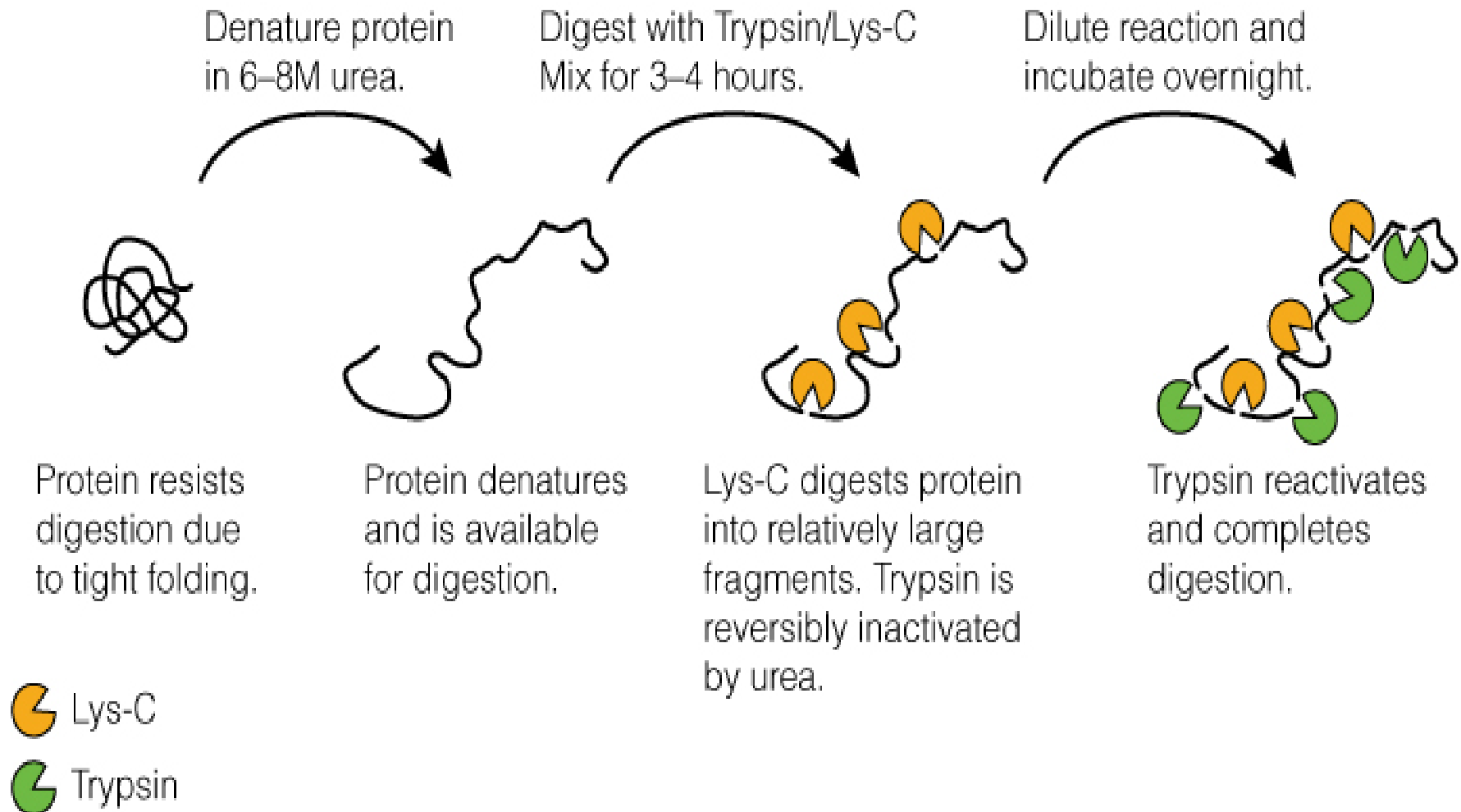




## Addition of DTT and IAA/Acrylamide



# Addition of Enzyme



# Trypsin Digestion Protocol

in Gel

Protein sample

Reduction & Alkylation  
1 hour at 57°C

Optional

Add Rapid Digest Buffer

Add SOLuTrypsin  
1 hour at 60°C

Quench with FA

Analyze

1 to 2 hours



in solution

Protein sample

Add denaturant

Reduction  
1 hour at 57°C

Alkylation  
1 hour at room temperature

Add buffer to dilute denaturant

Add Trypsin  
Overnight at 37°C

Quench with FA

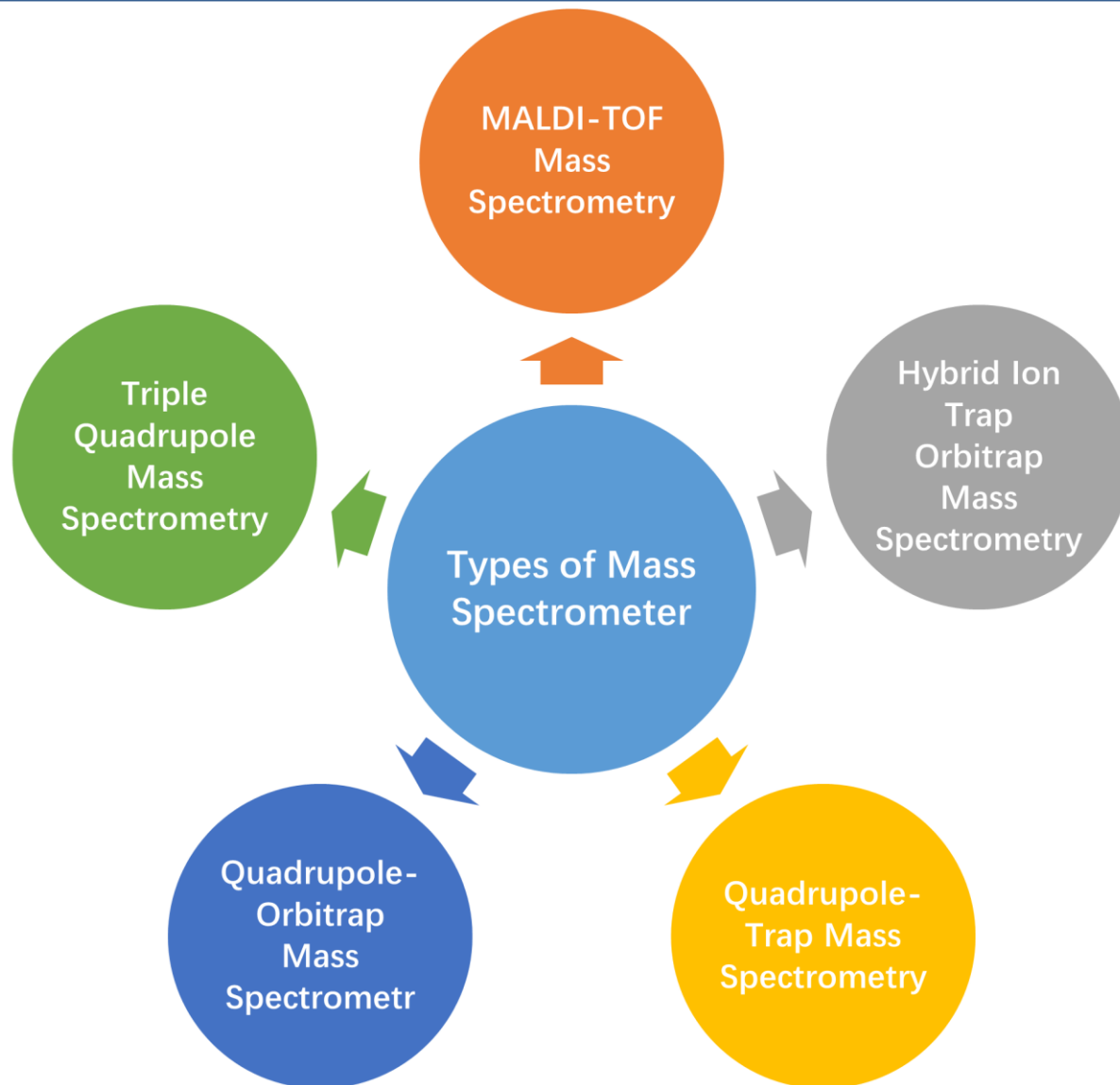
Analyze

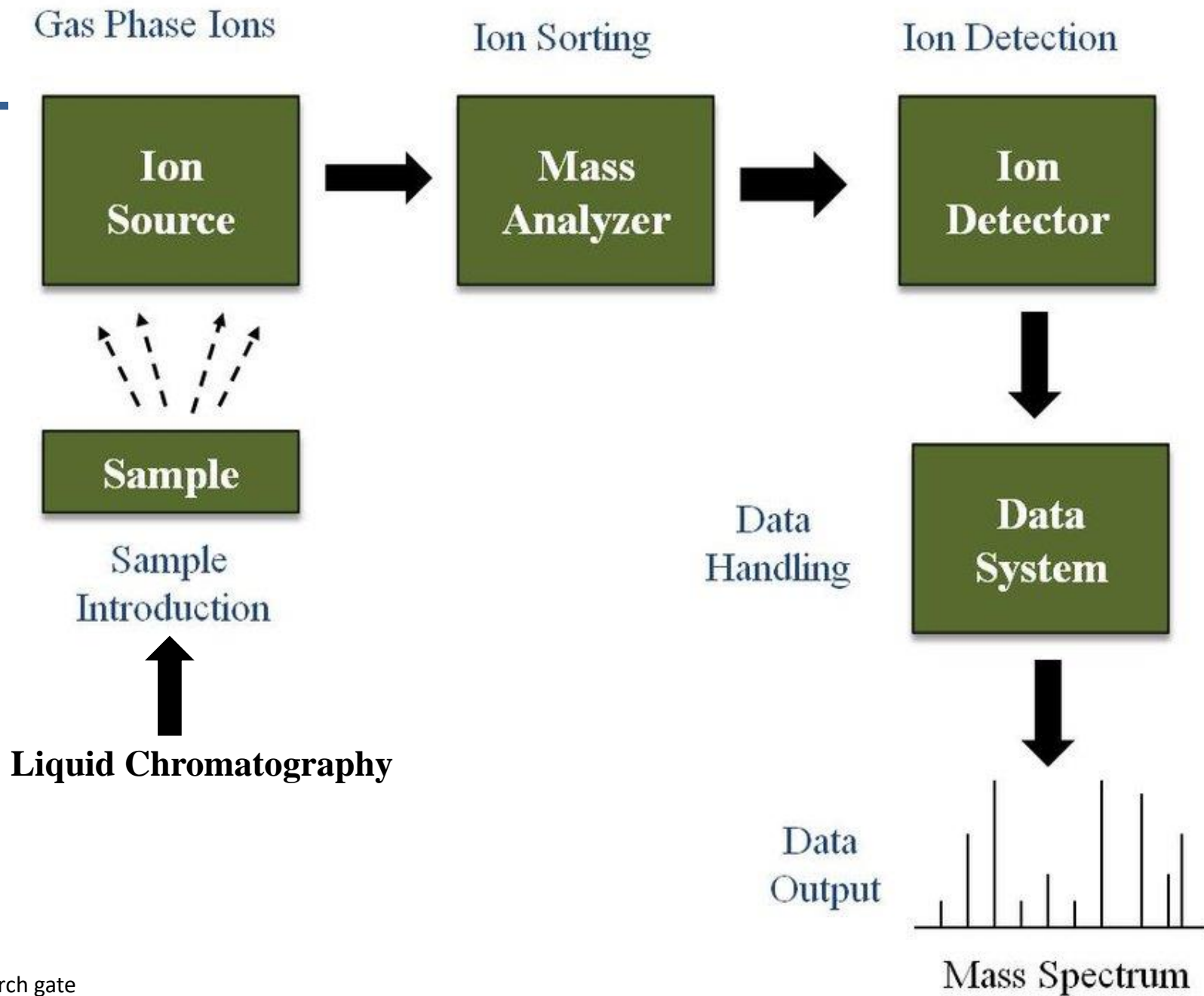
16 to 18 hours





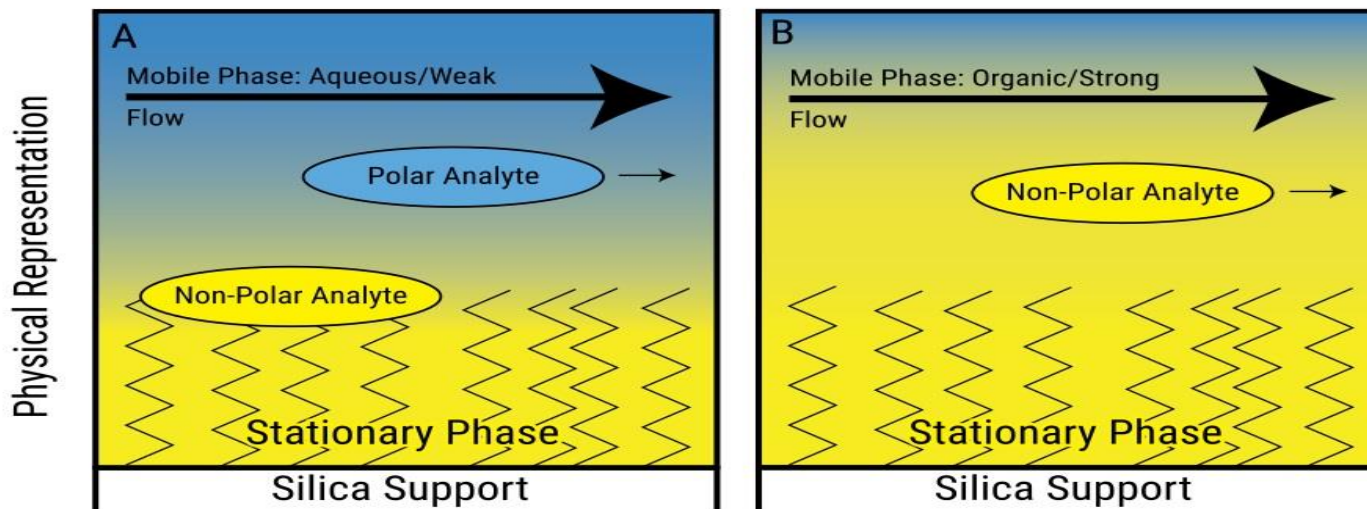
# Mass Spectrometry



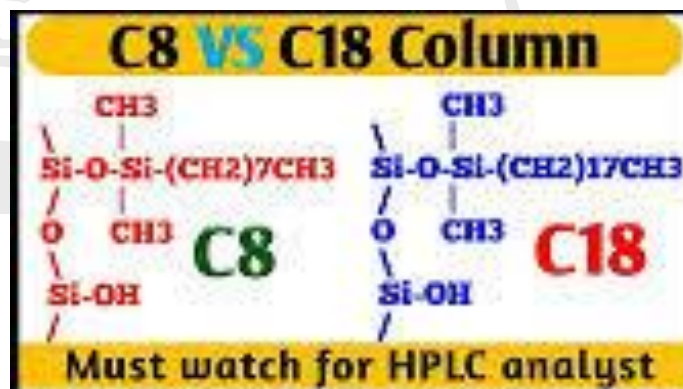


# C18 versus C8

## Reverse Phase Gradient Elution

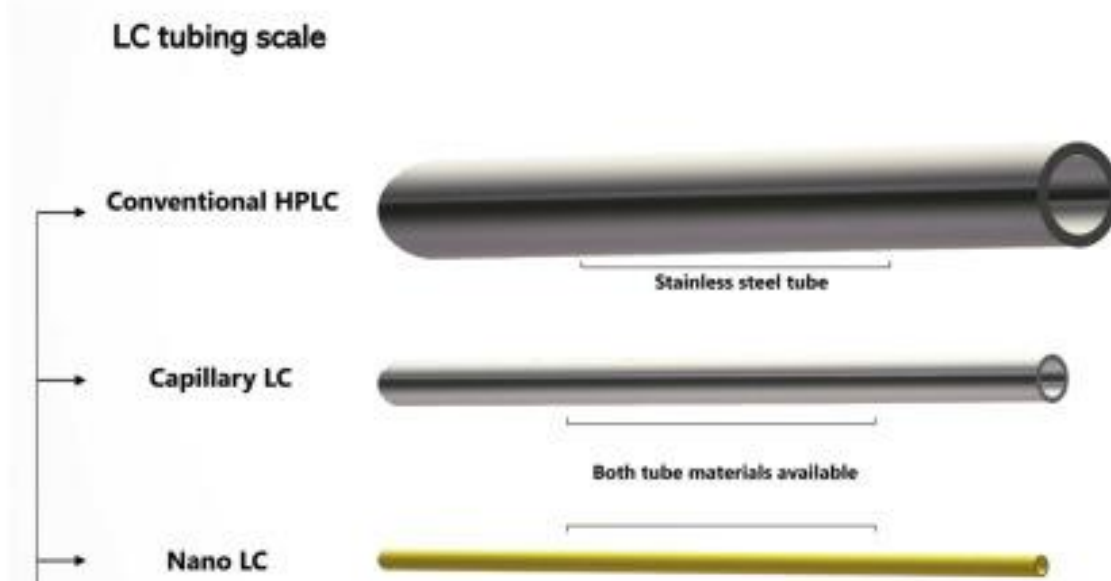


Pediaa.com



Youtube

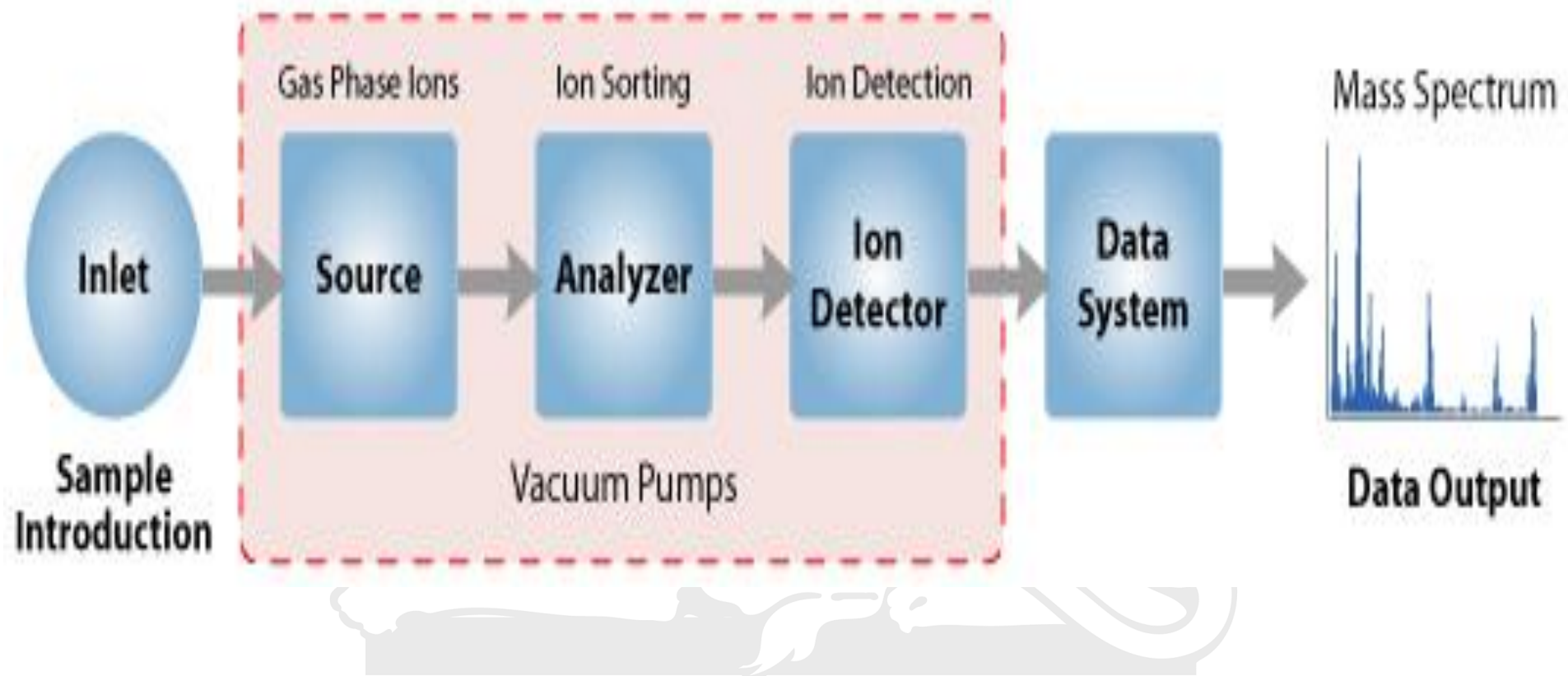
# Columns and Flow Rates



Science Direct

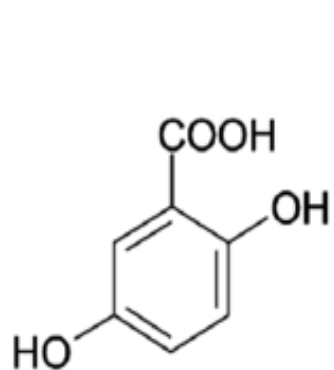
Column Internal Diameter (ID)	Typical Flow Rate Range
75 $\mu\text{m}$ –100 $\mu\text{m}$ (nanoscale)	100 nL/min–1 $\mu\text{L}$ /min
150–300 $\mu\text{m}$ (capillary scale)	3 $\mu\text{L}$ /min–10 $\mu\text{L}$ /min
500 $\mu\text{m}$ –1 mm (microscale)	10 $\mu\text{L}$ /min–100 $\mu\text{L}$ /min
1 mm–2.1 mm (analytical scale)	50 $\mu\text{L}$ /min–2.0 mL/min

# Parts of Mass Spectrometer

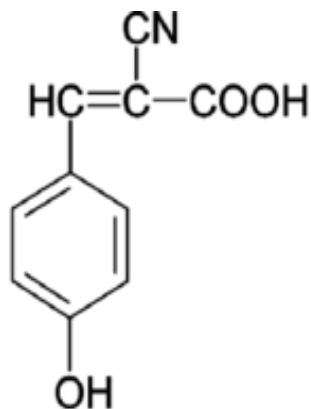




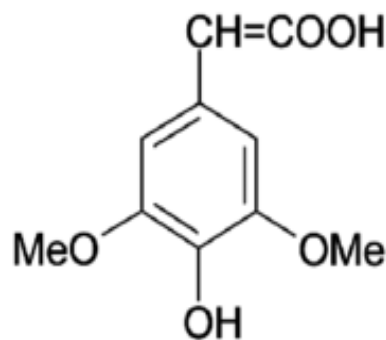
# Matrix



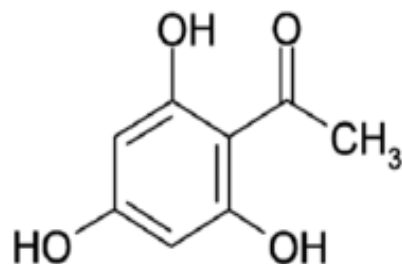
2,5-dihydroxybenzoic Acid  
(2,5-DHB)



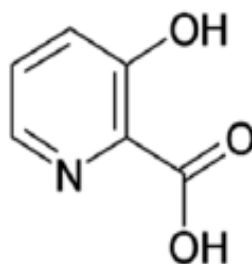
$\alpha$ -cyano-4-hydroxycinnamic Acid  
(CHCA)



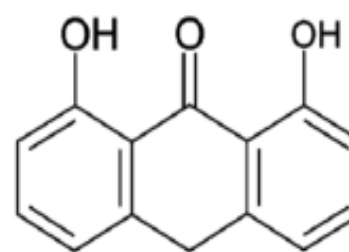
sinapinic Acid (SA)



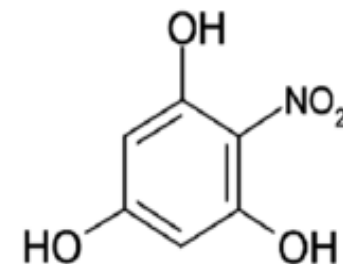
2,4,6-trihydroxyacetophenone  
(THAP)



3-hydroxypicolinic Acid  
(3-HPA)

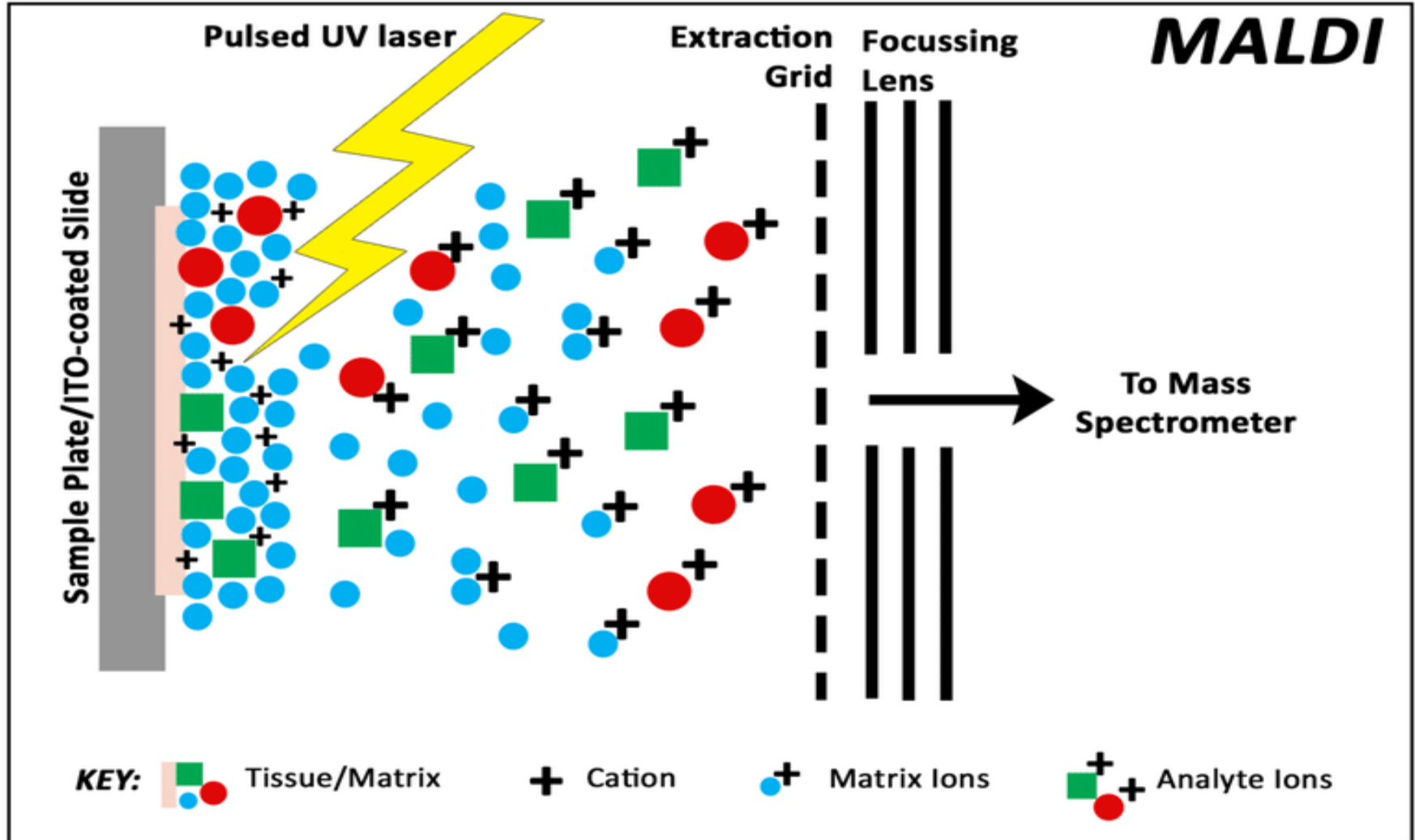


dithranol



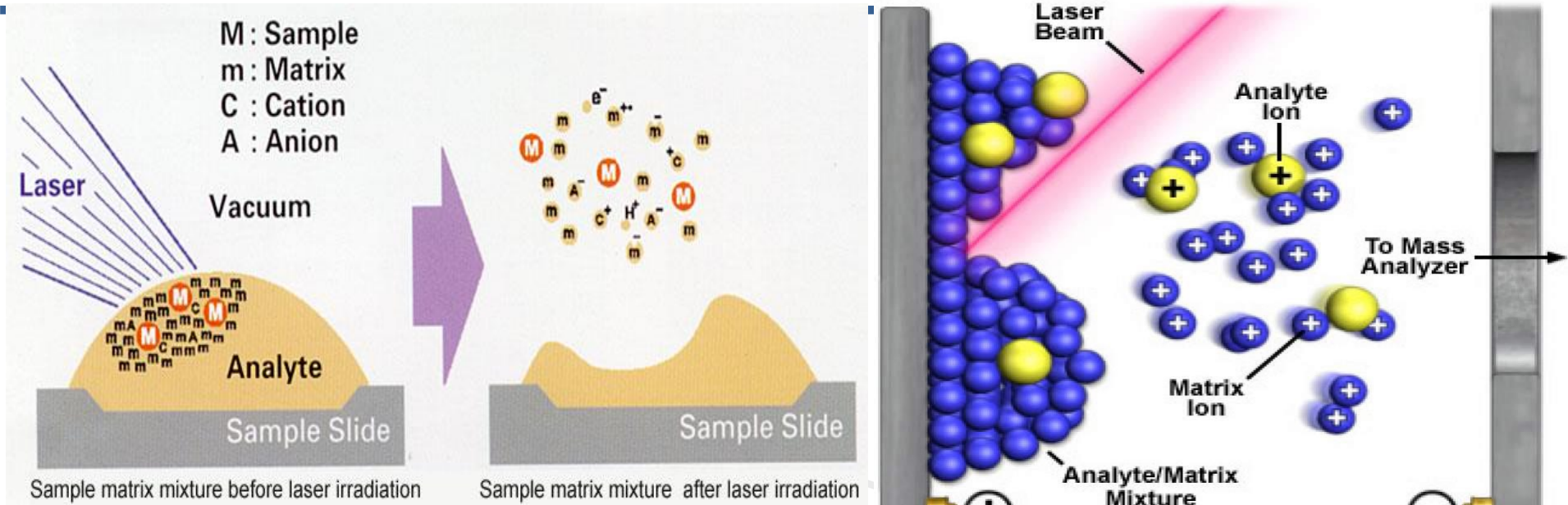
2-nitrophenloroglucinol

# Ionization (generation of ions) in MALDI



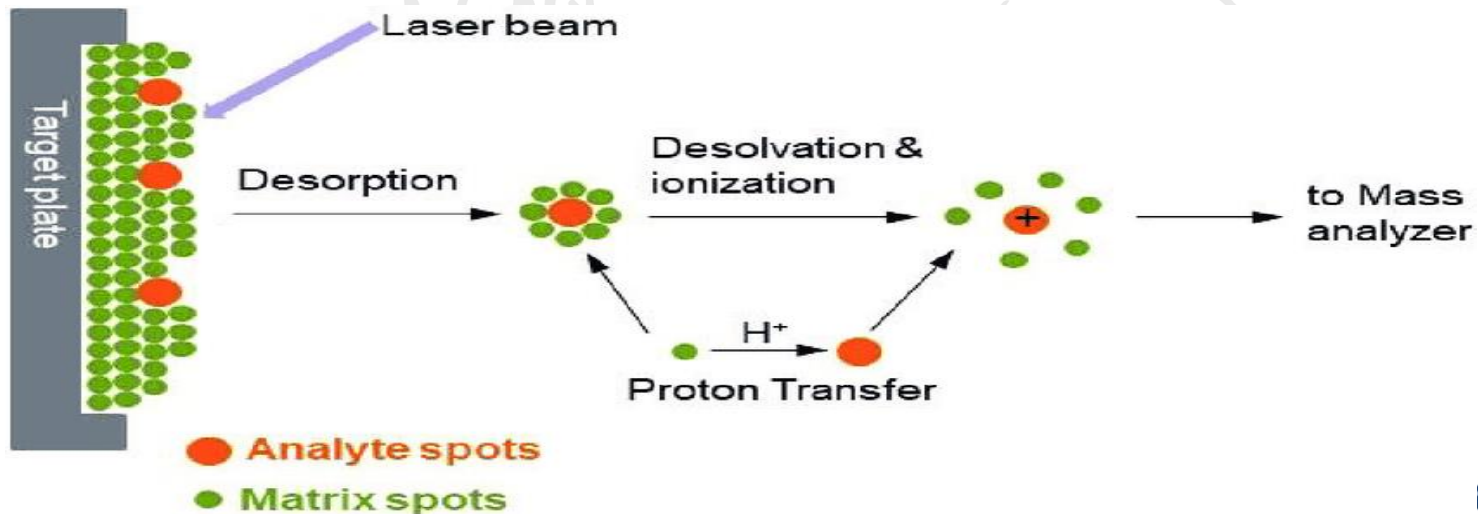
Shimadzu

# Ionization (generation of ions) in MALDI



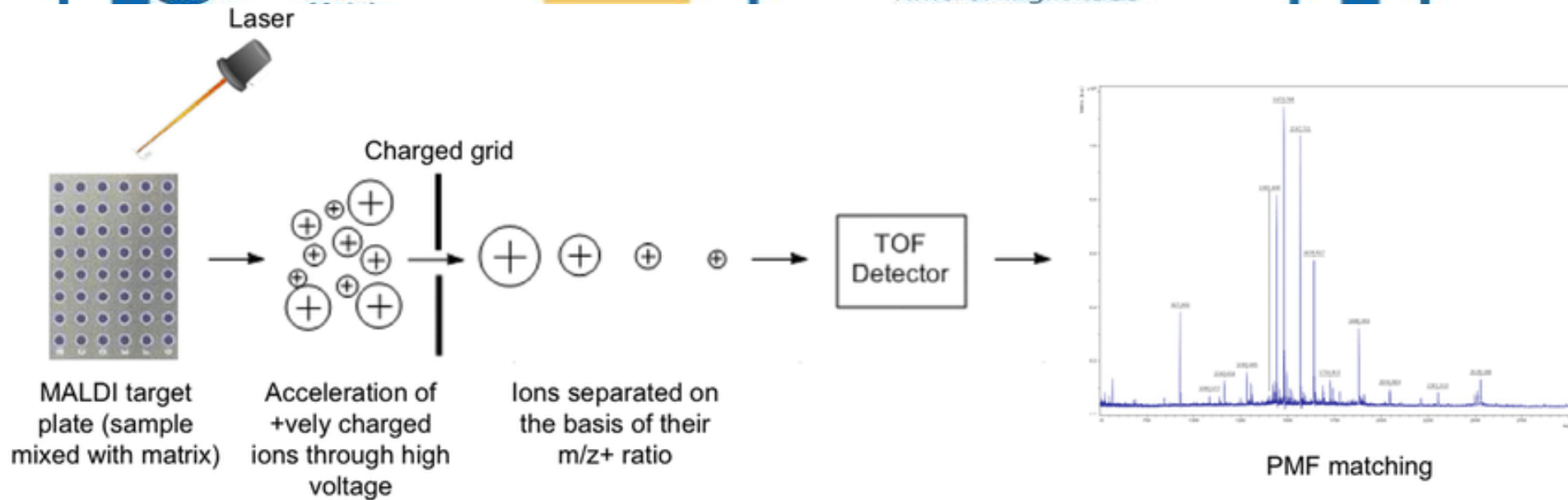
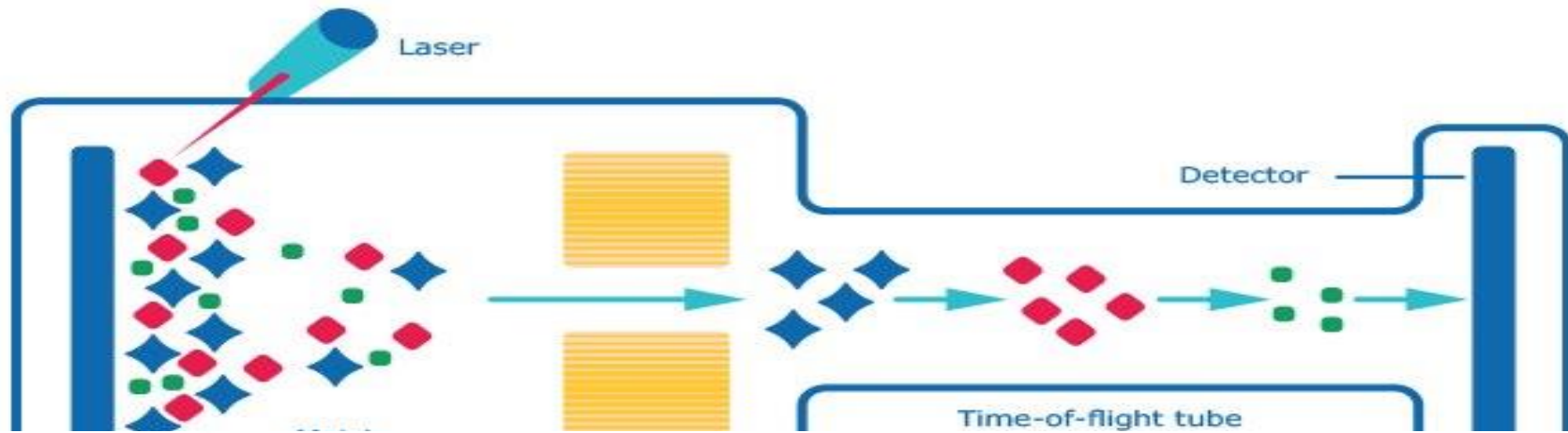
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MagLab



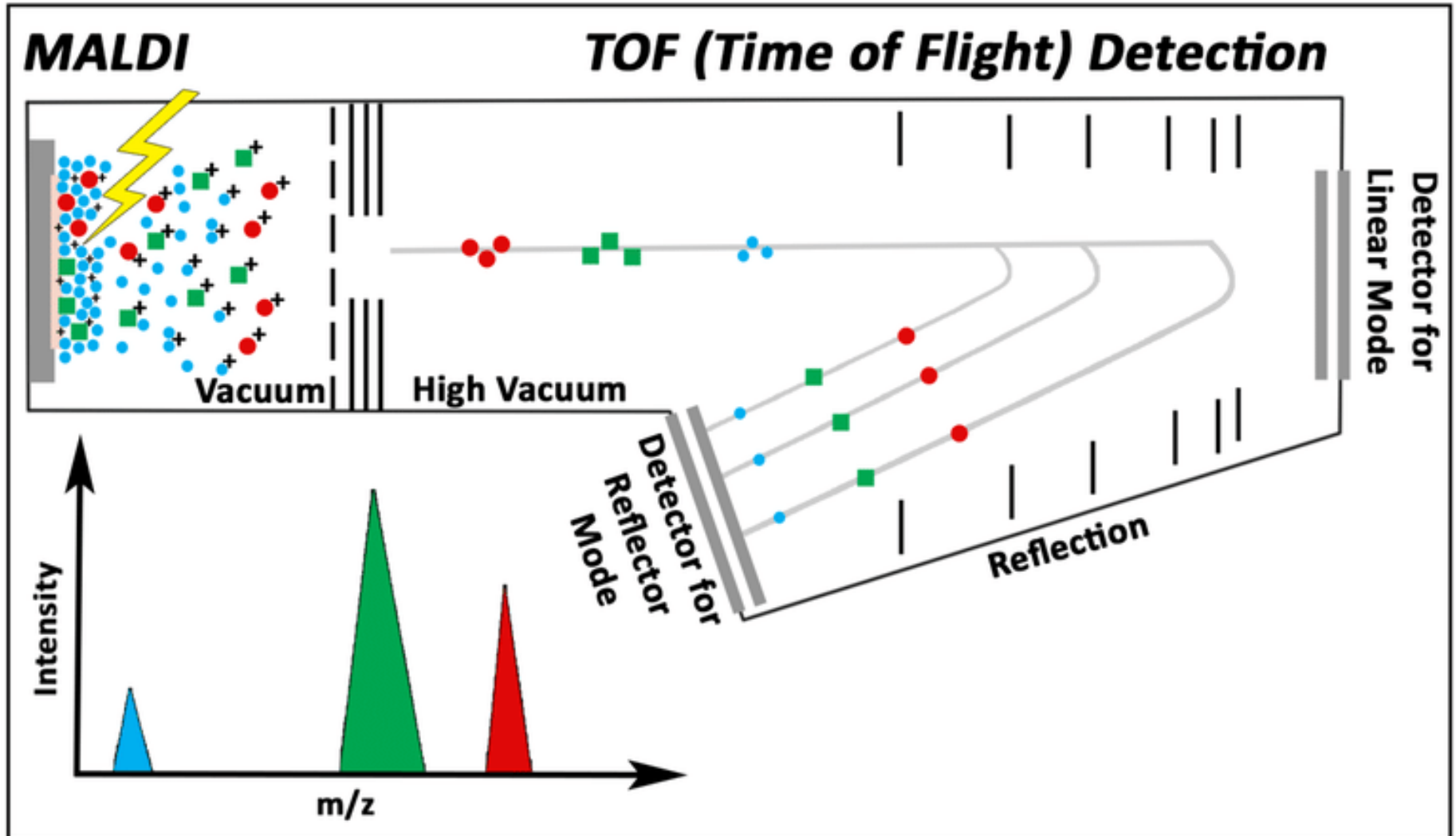
ResearchGate

# Linear Mode in MALDI



Sigma-Aldrich

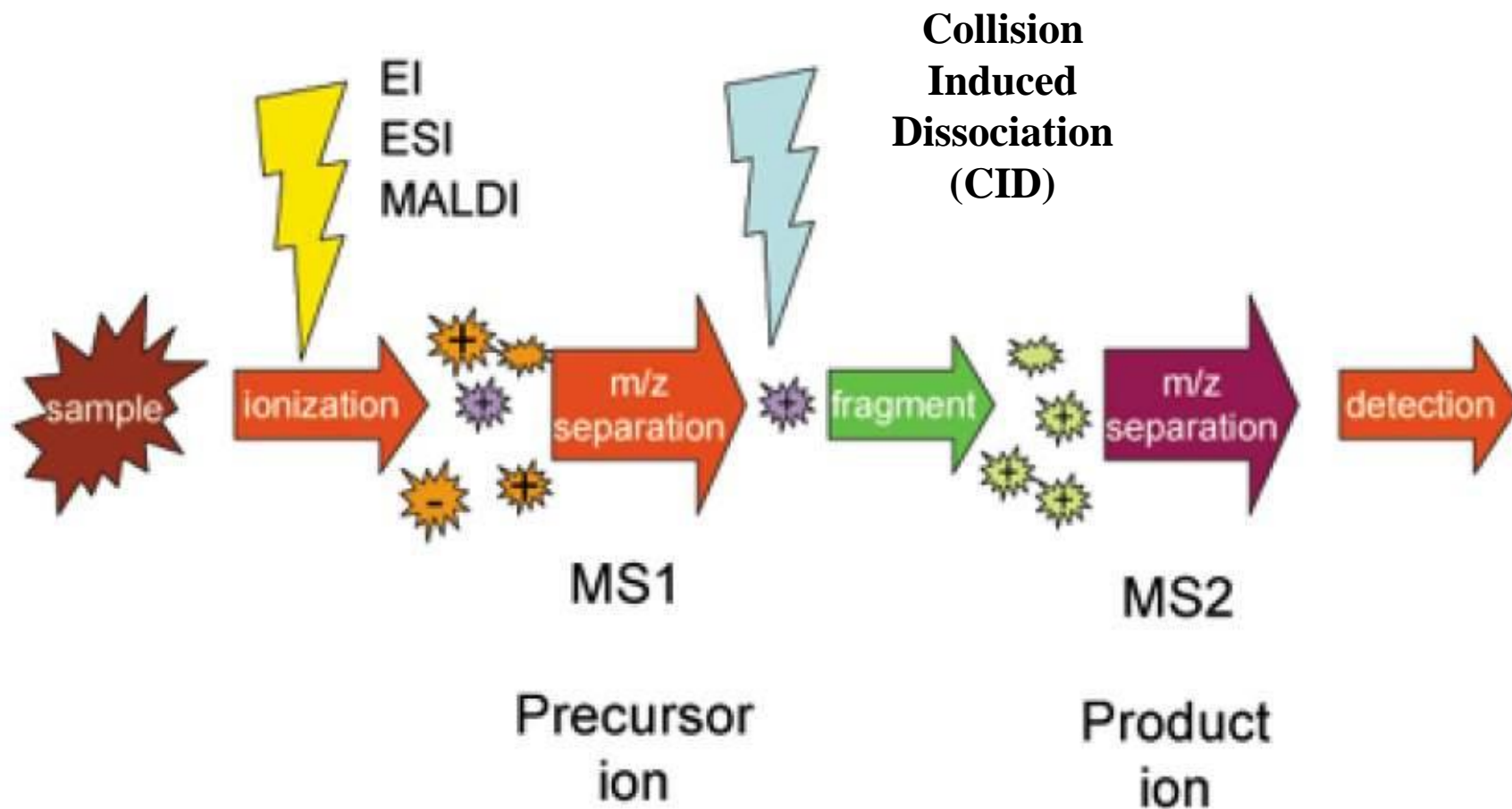
# Reflectron Mode in MALDI



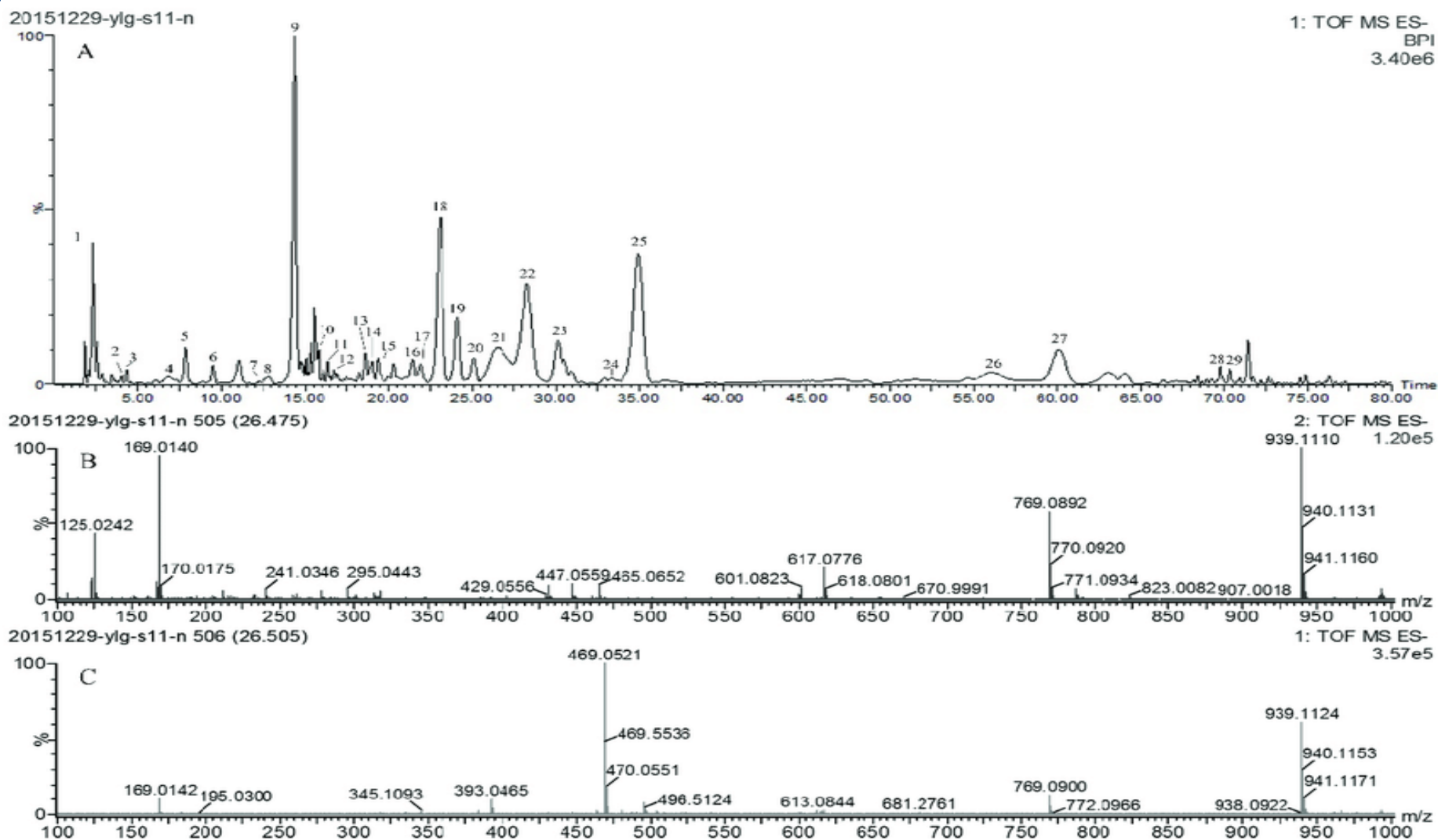
Shimadzu



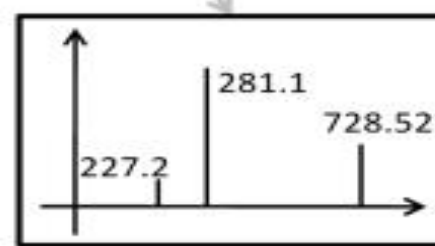
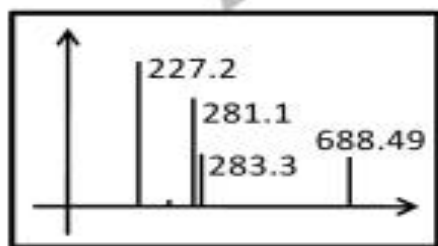
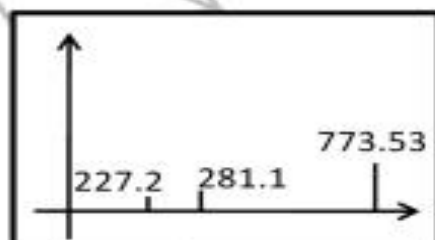
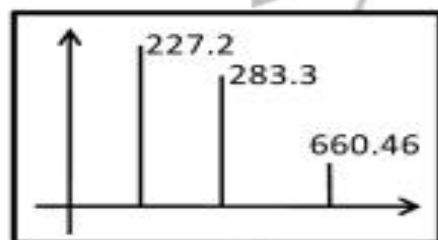
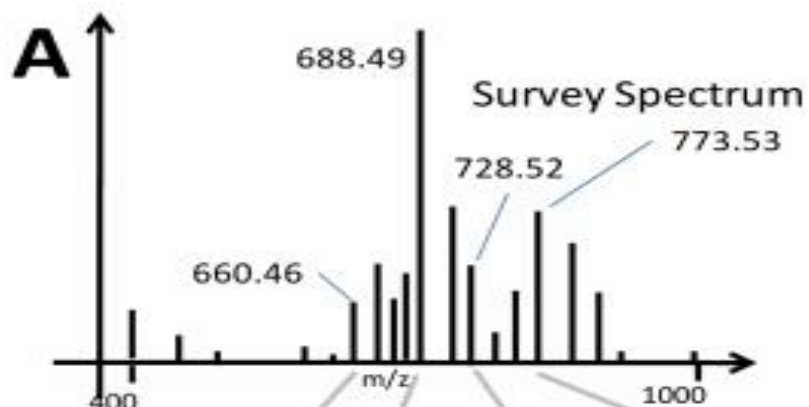
# Parent and Daughter Ion Formation



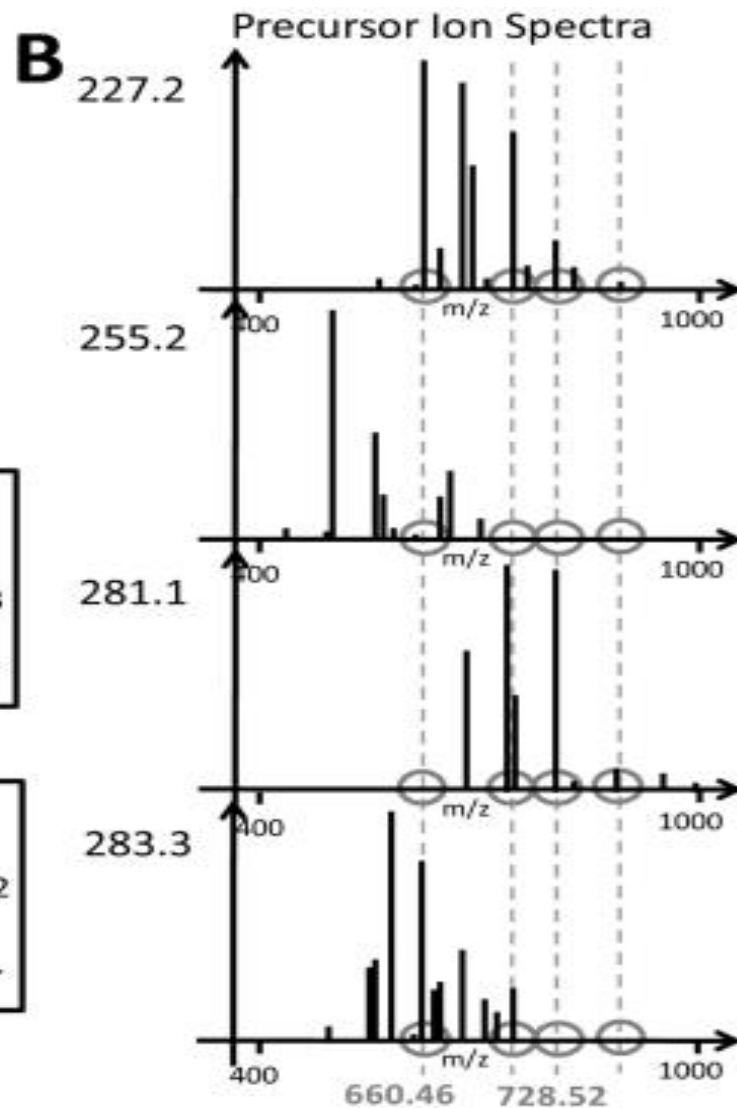
# Data Dependent Acquisition



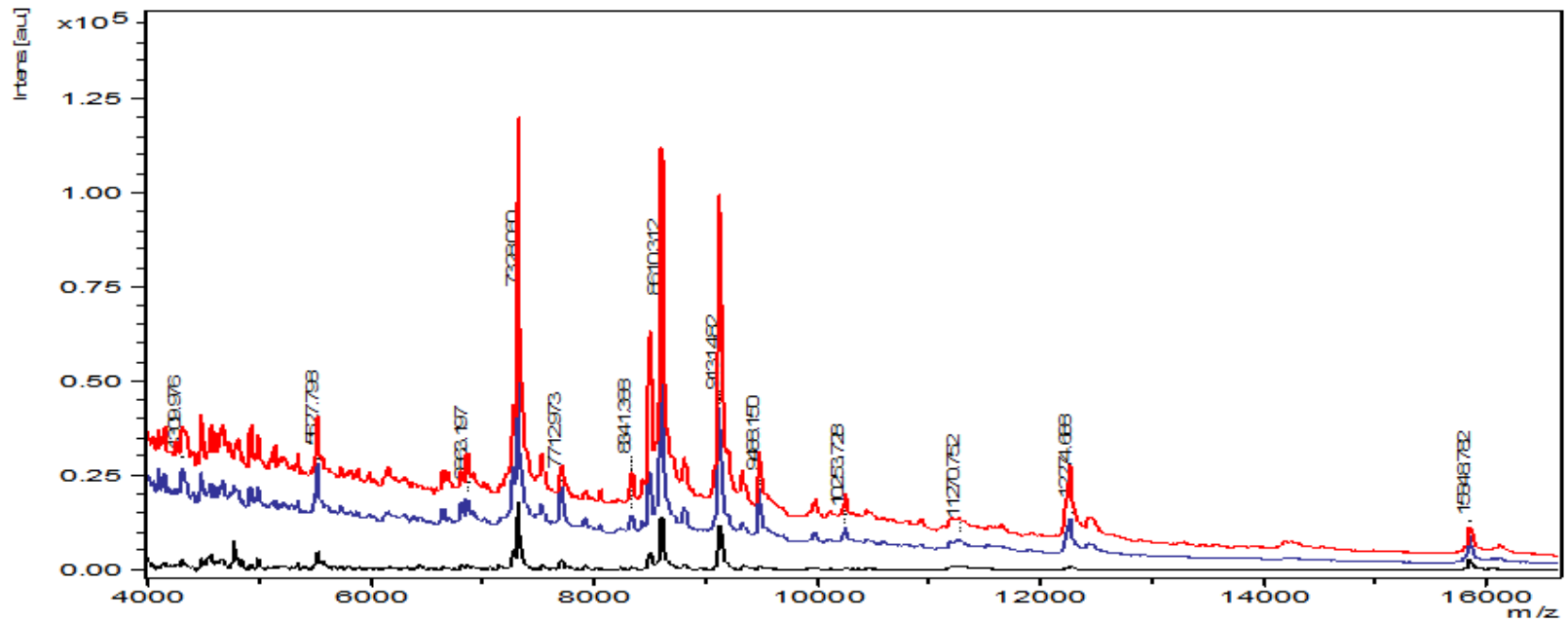
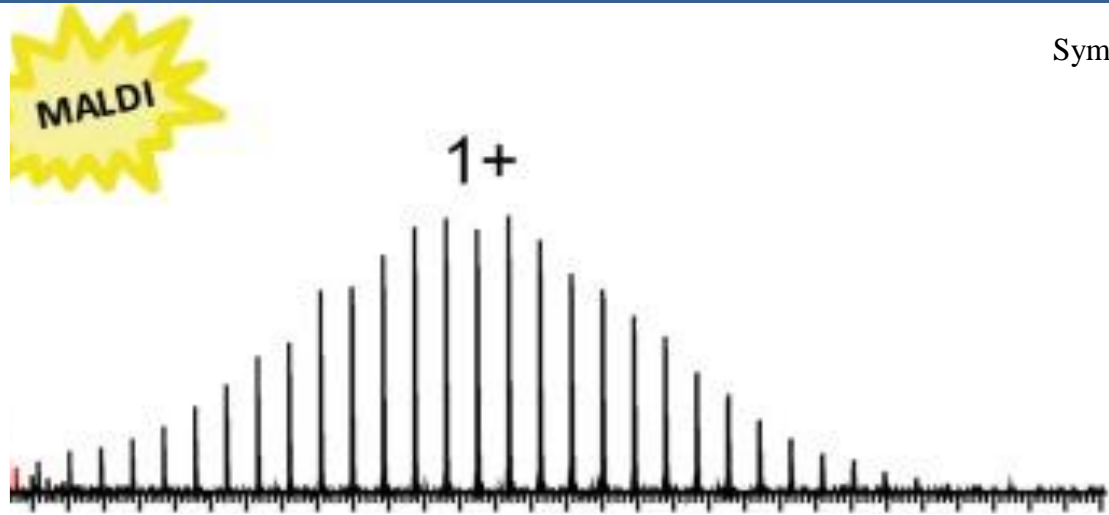
# Data Dependent Acquisition



Fragment Spectra



# MS Spectra



**A**

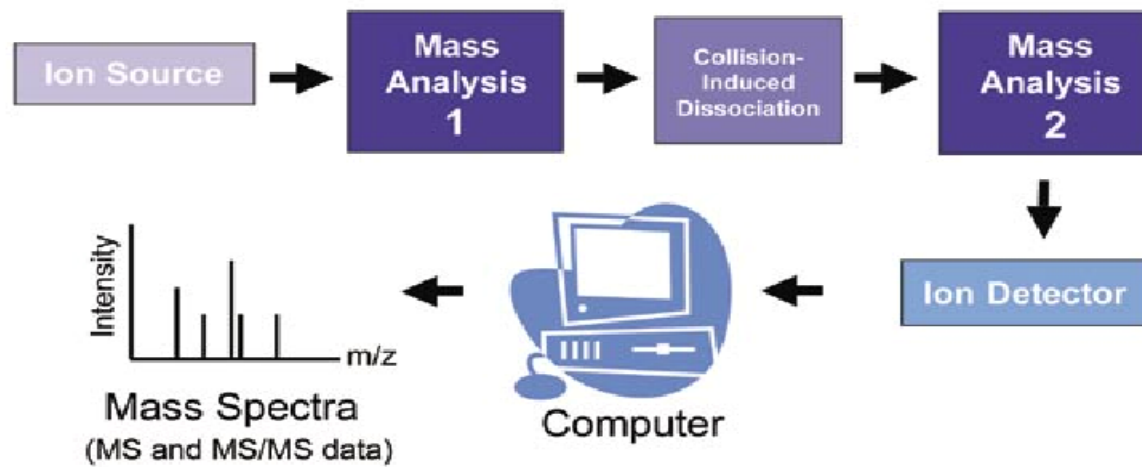
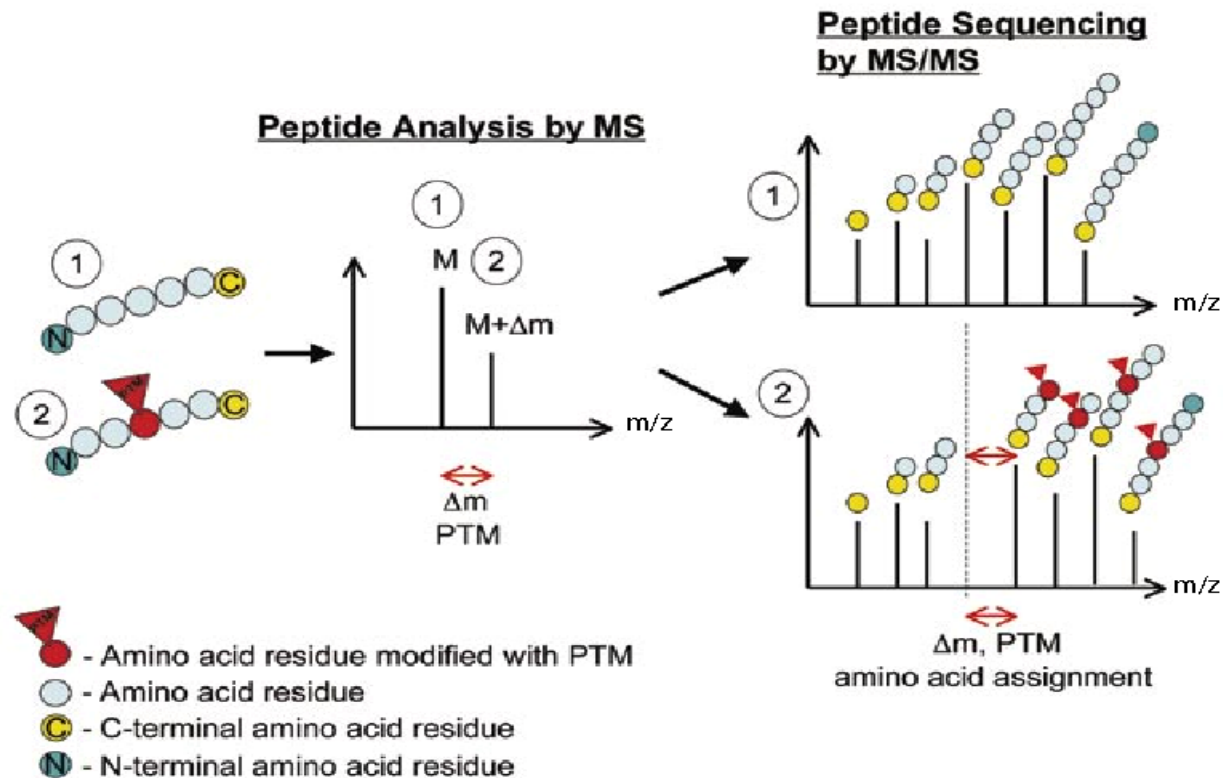
**B**

Figure 1 Tandem mass spectrometry (MS/MS) for mapping posttranslational modifications. Semantic Scholar

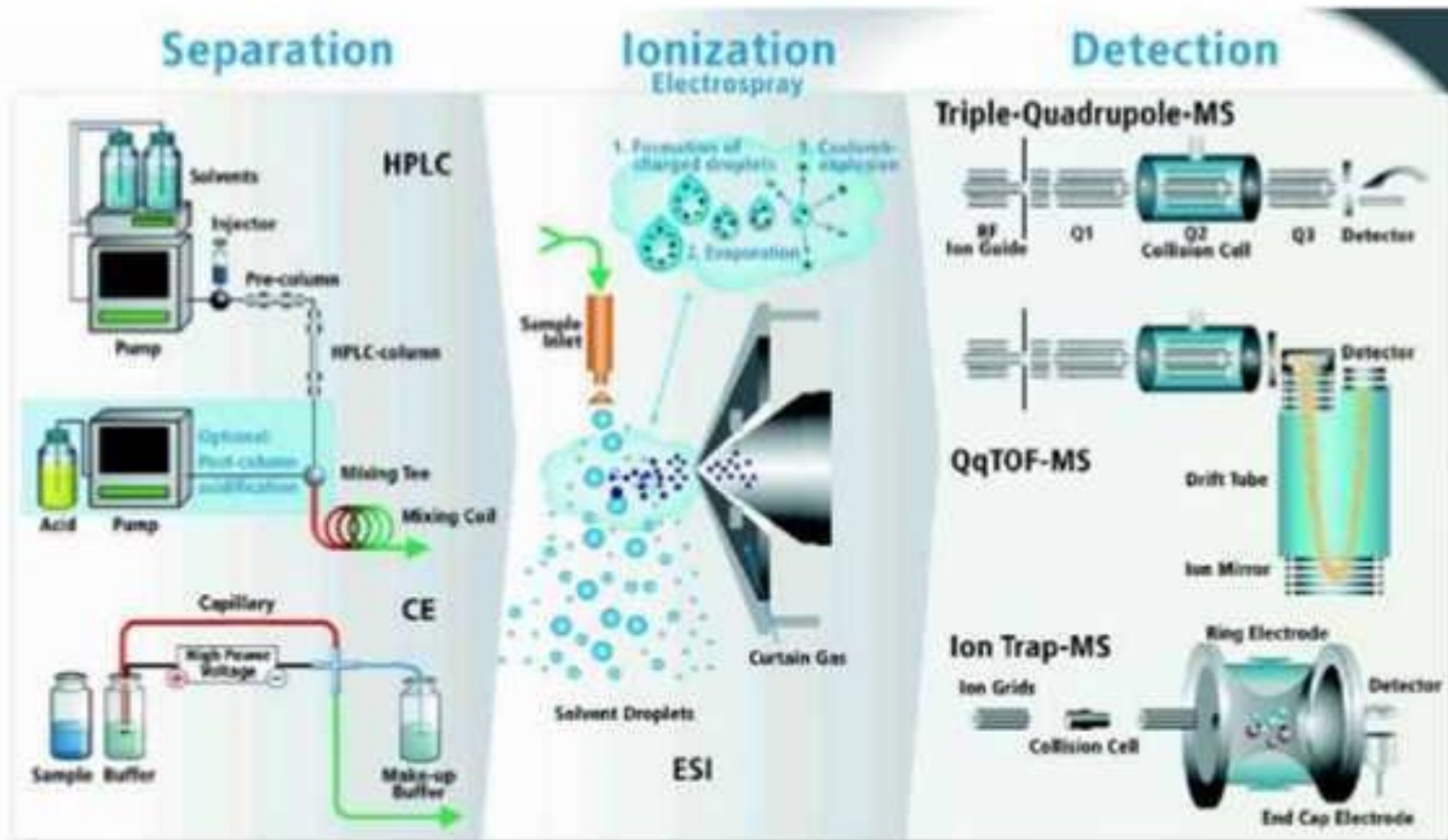


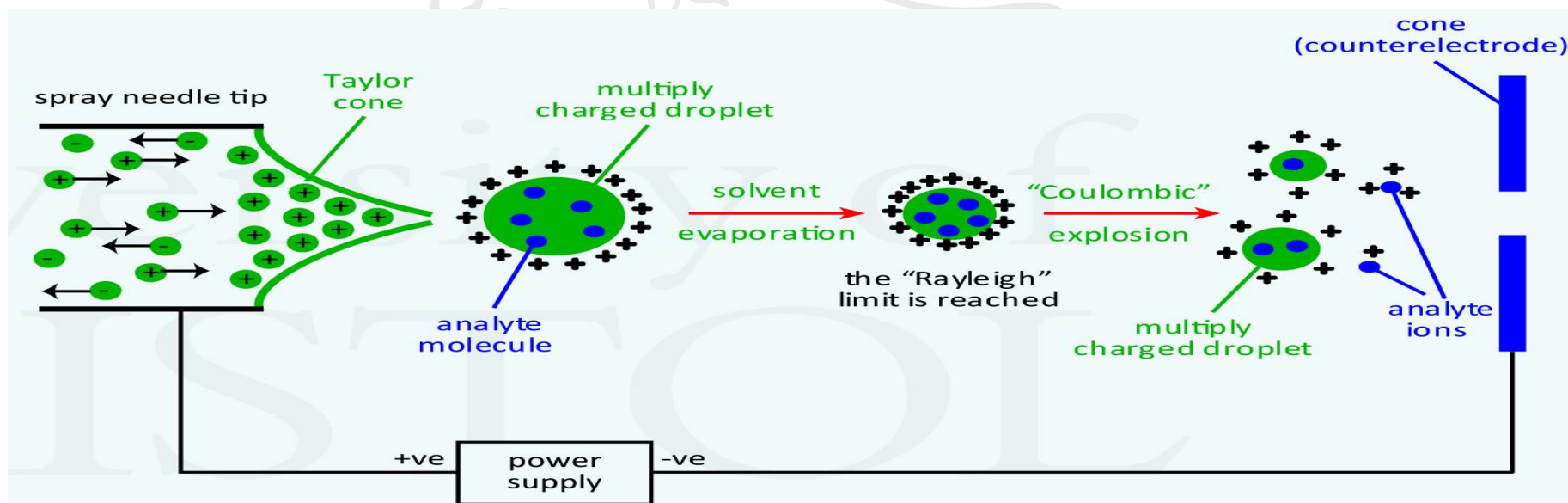
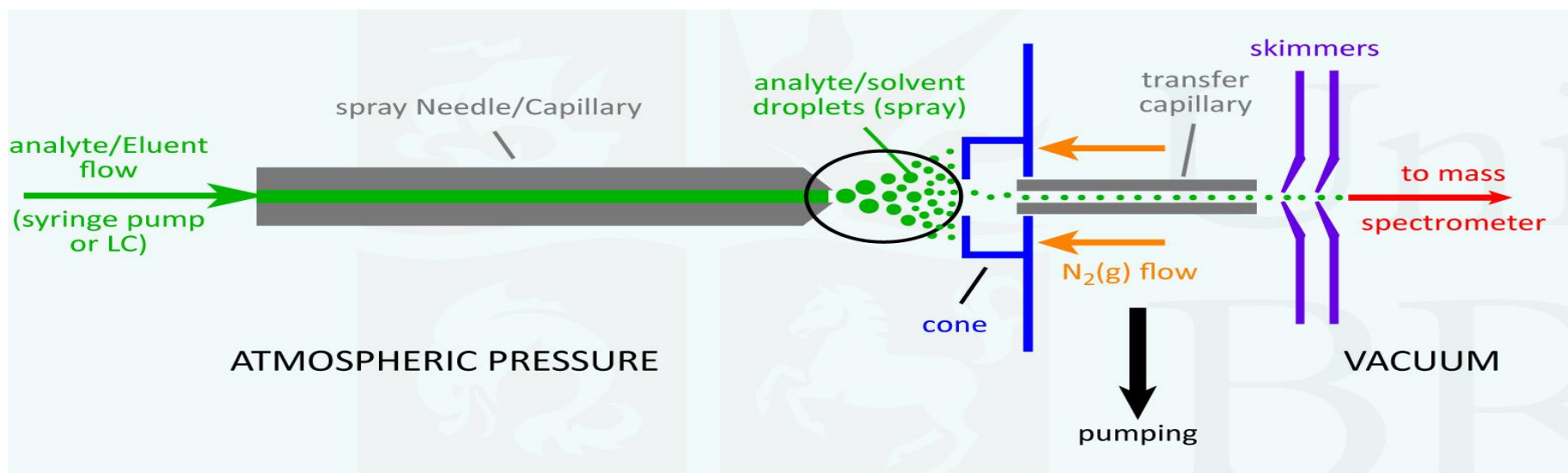
# Advantages and Disadvantages of MALDI

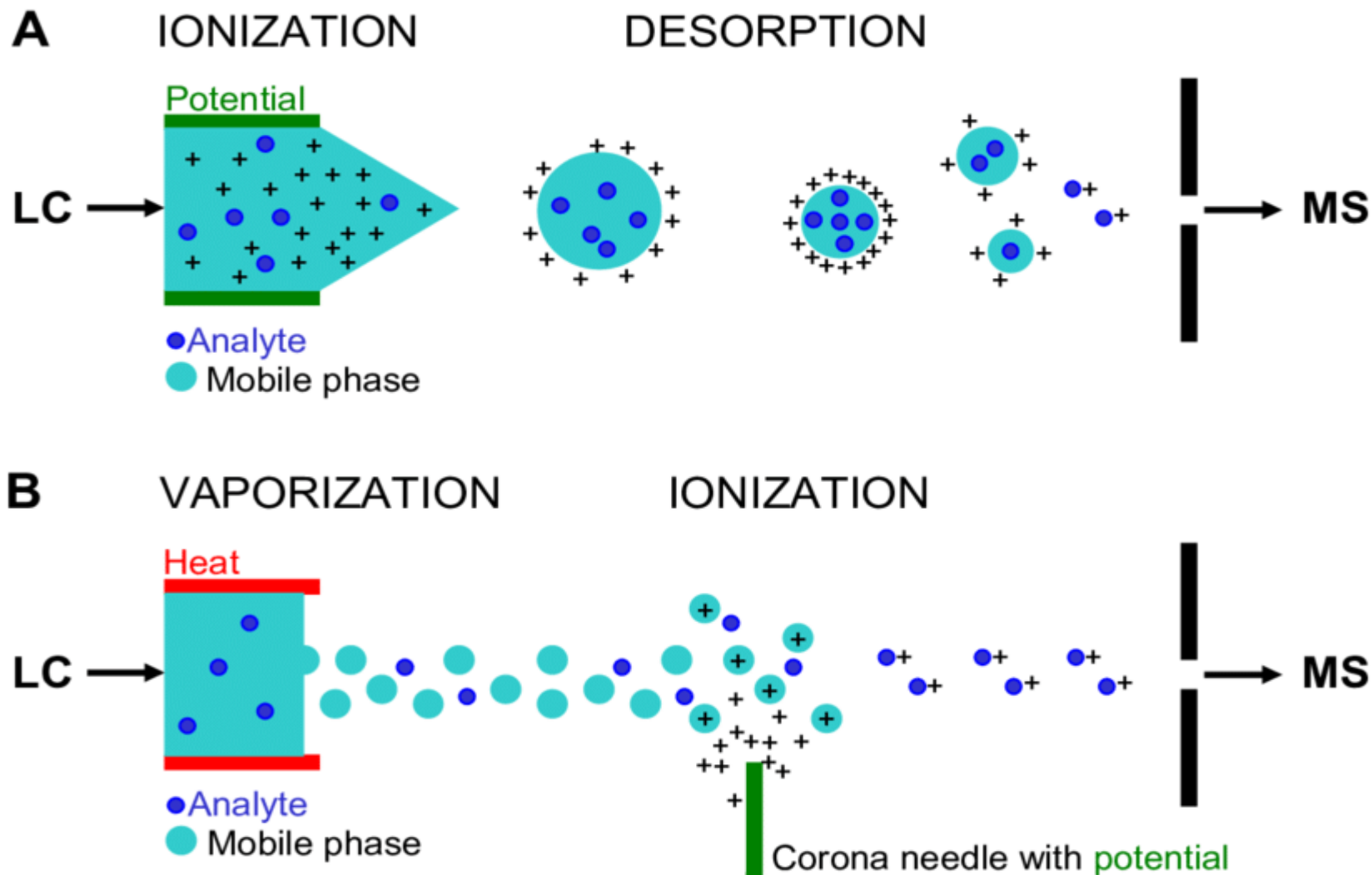


Advantages	Disadvantages
<p>Practical mass range of up to 300,000 Da. Species of much greater mass have been reported.</p> <p>Typical sensitivity on the order of low femtomole to low picomole. Reports have indicated that attomole sensitivity is possible.</p> <p>Soft ionization with little to no fragmentation observed.</p> <p>Tolerance of salts in millimolar concentrations.</p> <p>Suitable for the analysis of complex mixtures.</p>	<p>Low resolution (see Chapter 2). Some MALDI instruments are capable of higher resolution; however, this is only in a relatively low mass range and is accomplished at the expense of sensitivity.</p> <p>Matrix background, which can be problem for compounds below a mass of 1000 Da. This background interference is highly dependent on the matrix material.</p> <p>Possibility of photodegradation by laser desorption/ionization.</p>

# Electrospray Ionisation-Mass Spectrometry (ESI-MS)

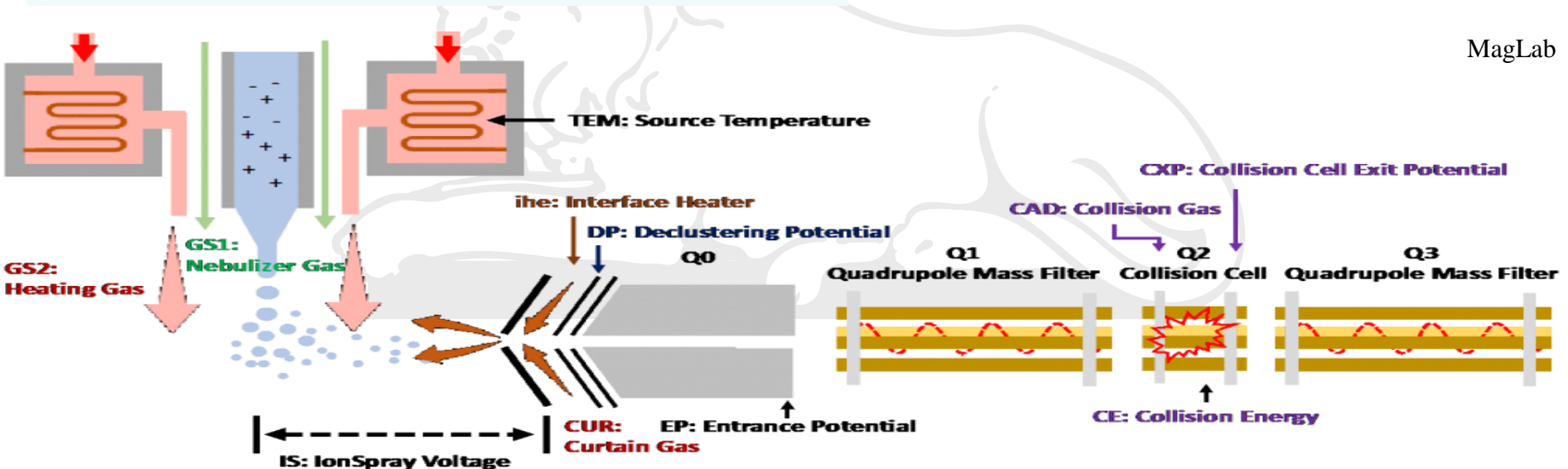
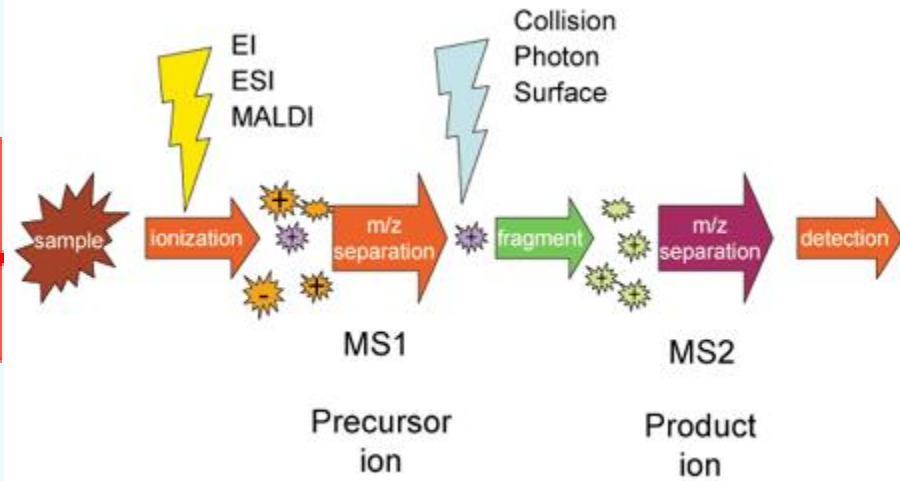
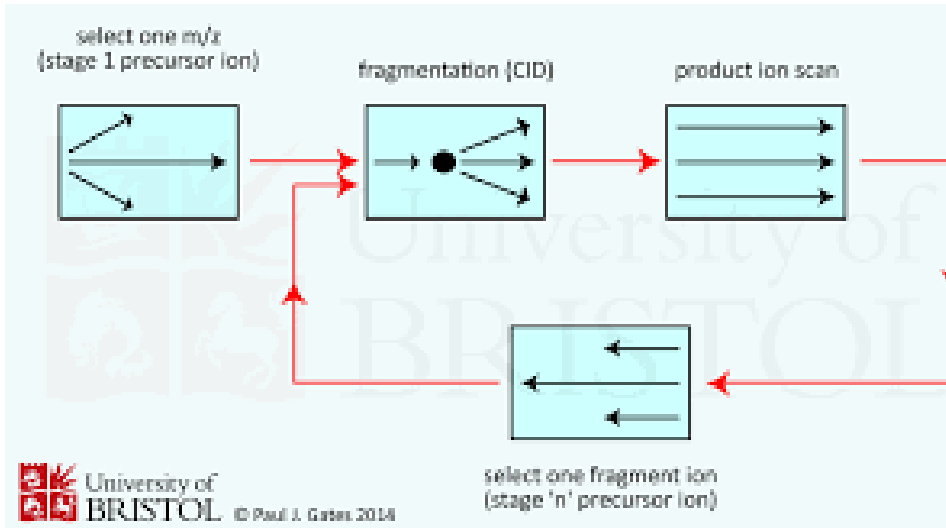






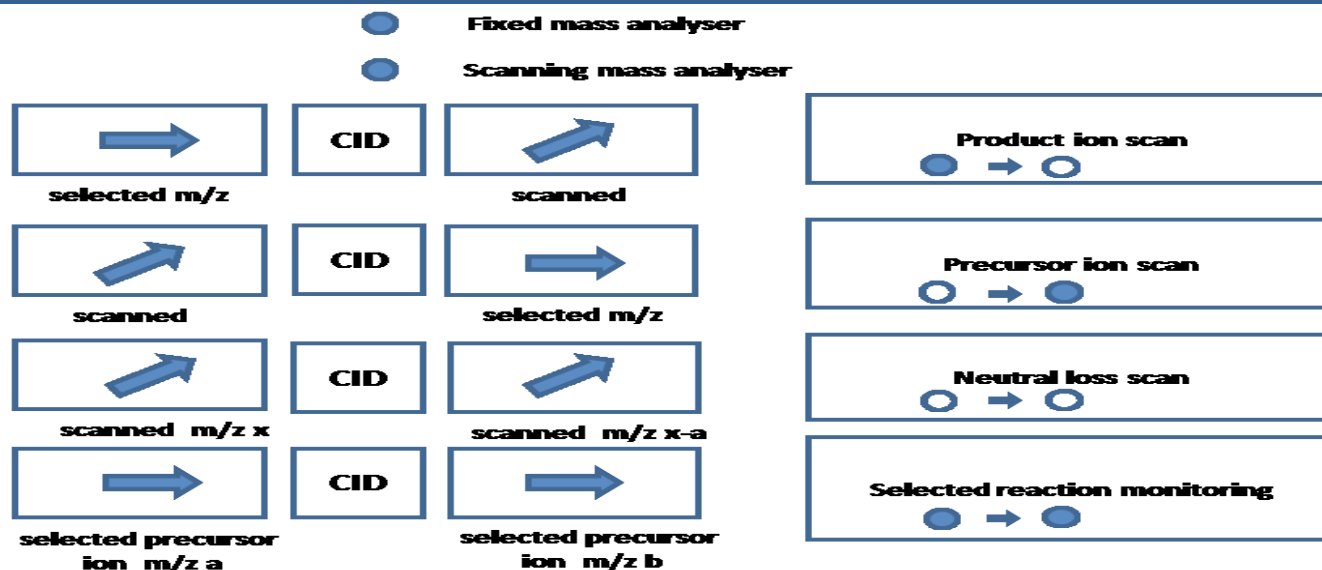


# What Happens inside the Mass Spectrometer





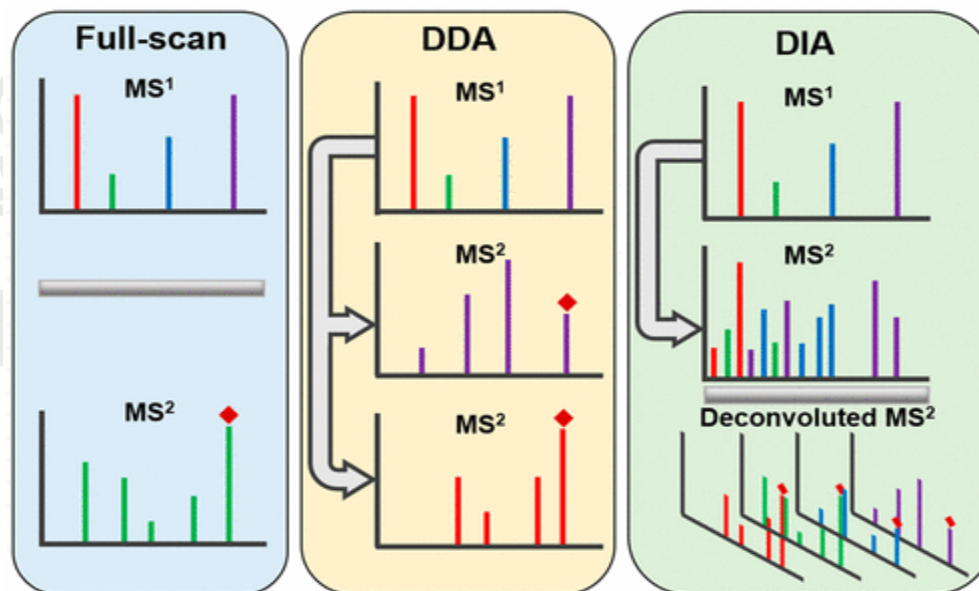
# Quantitation of Proteins based on Intensity



Researchgate

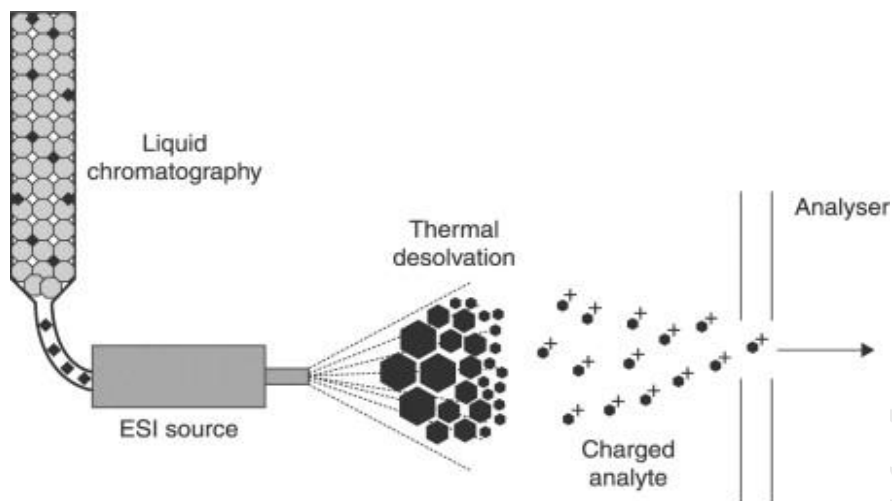
DDA: Data Dependent Acquisition

DIA: Data Independent Acquisition

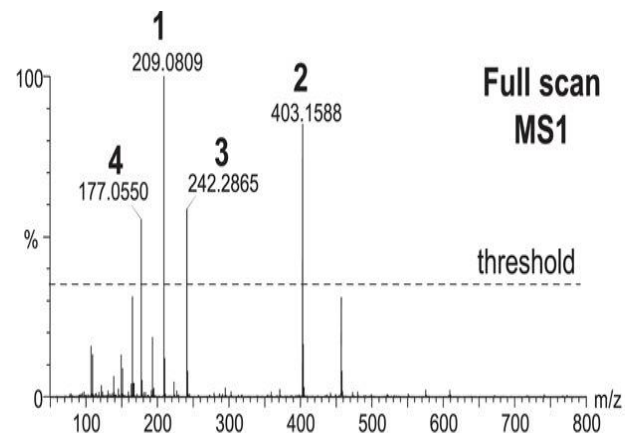
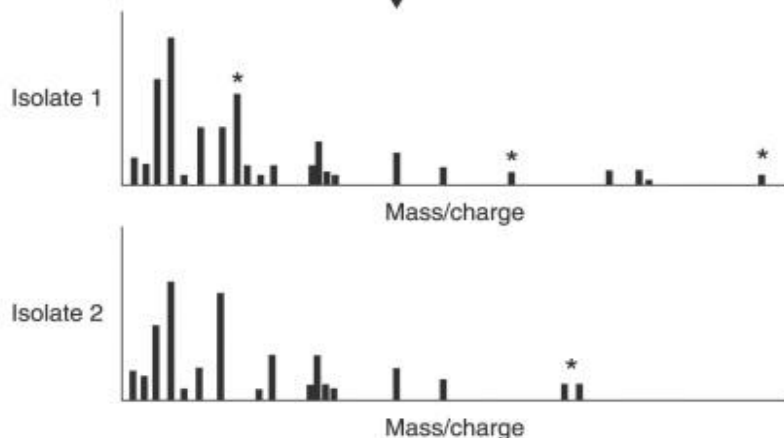


XMol

# What Happens inside the Mass Spectrometer



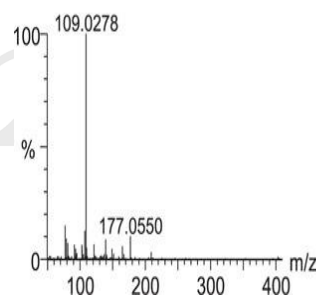
Output for a given retention time



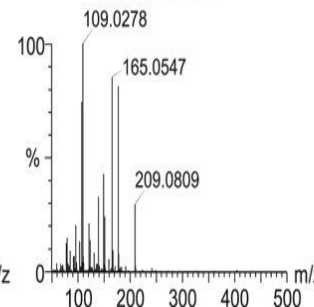
Decision process

- 1: YES
- 2: YES
- 3: NO (noise → excluded)
- 4: YES

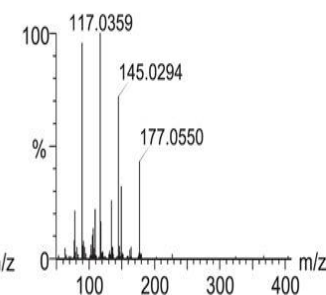
MS/MS-1



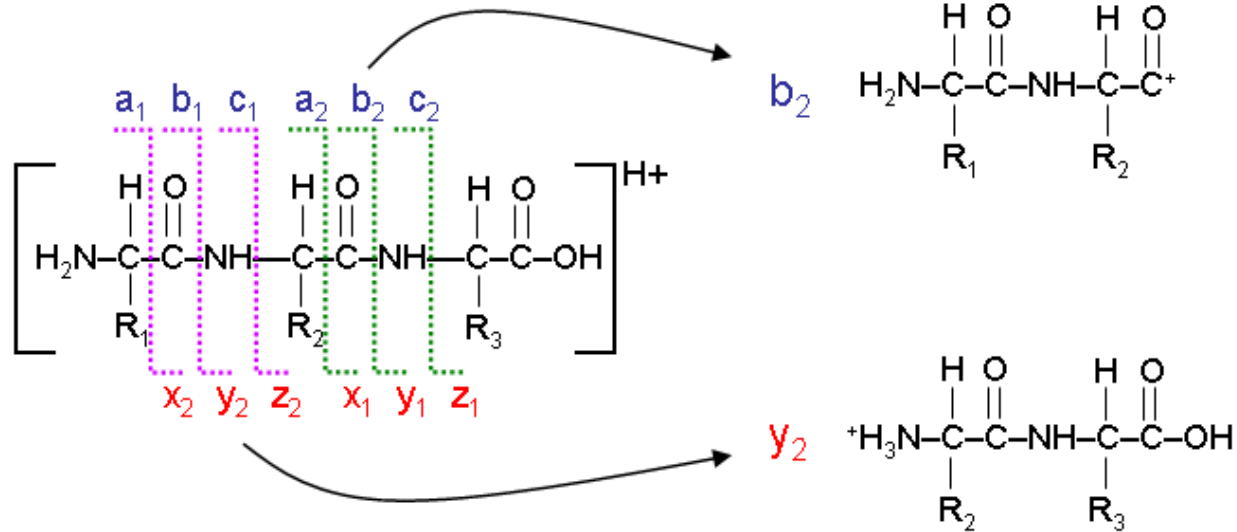
MS/MS-2



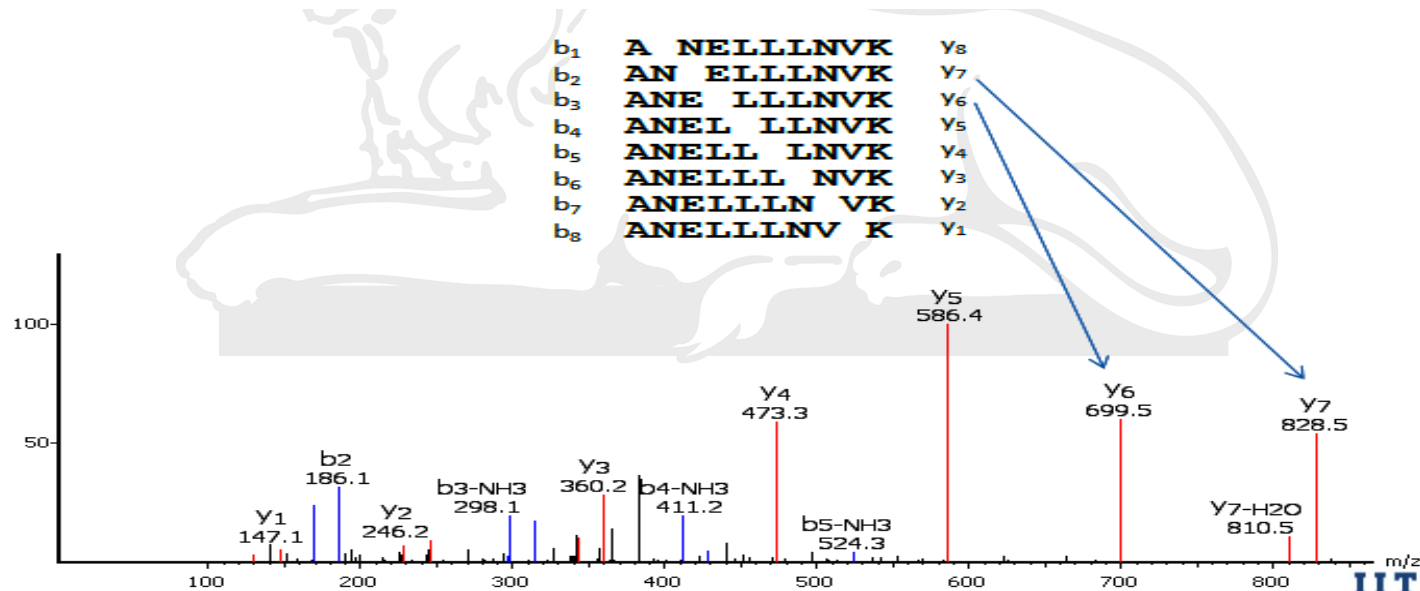
MS/MS-4



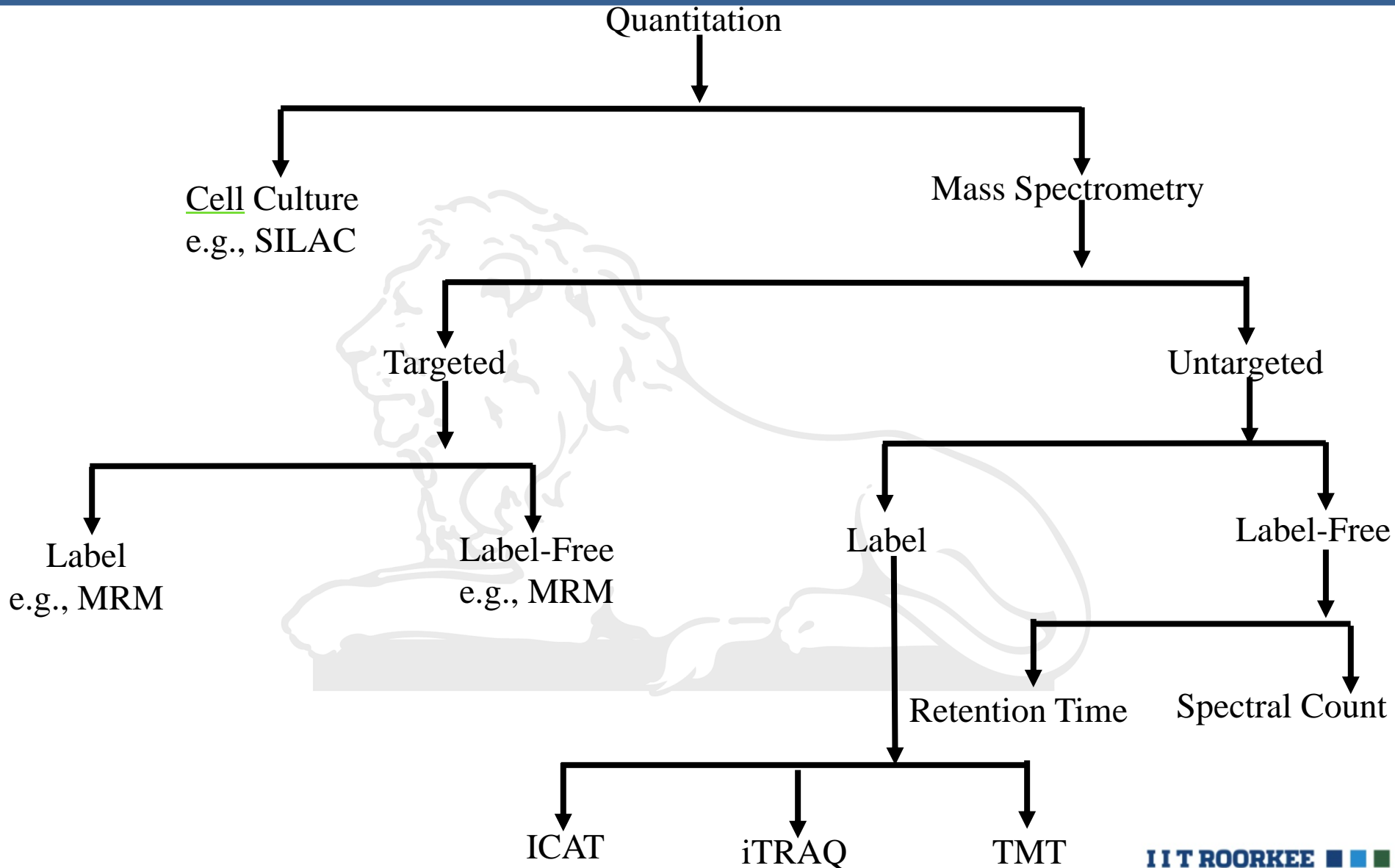
# Fragmentation of Peptides to Form b and Y ions



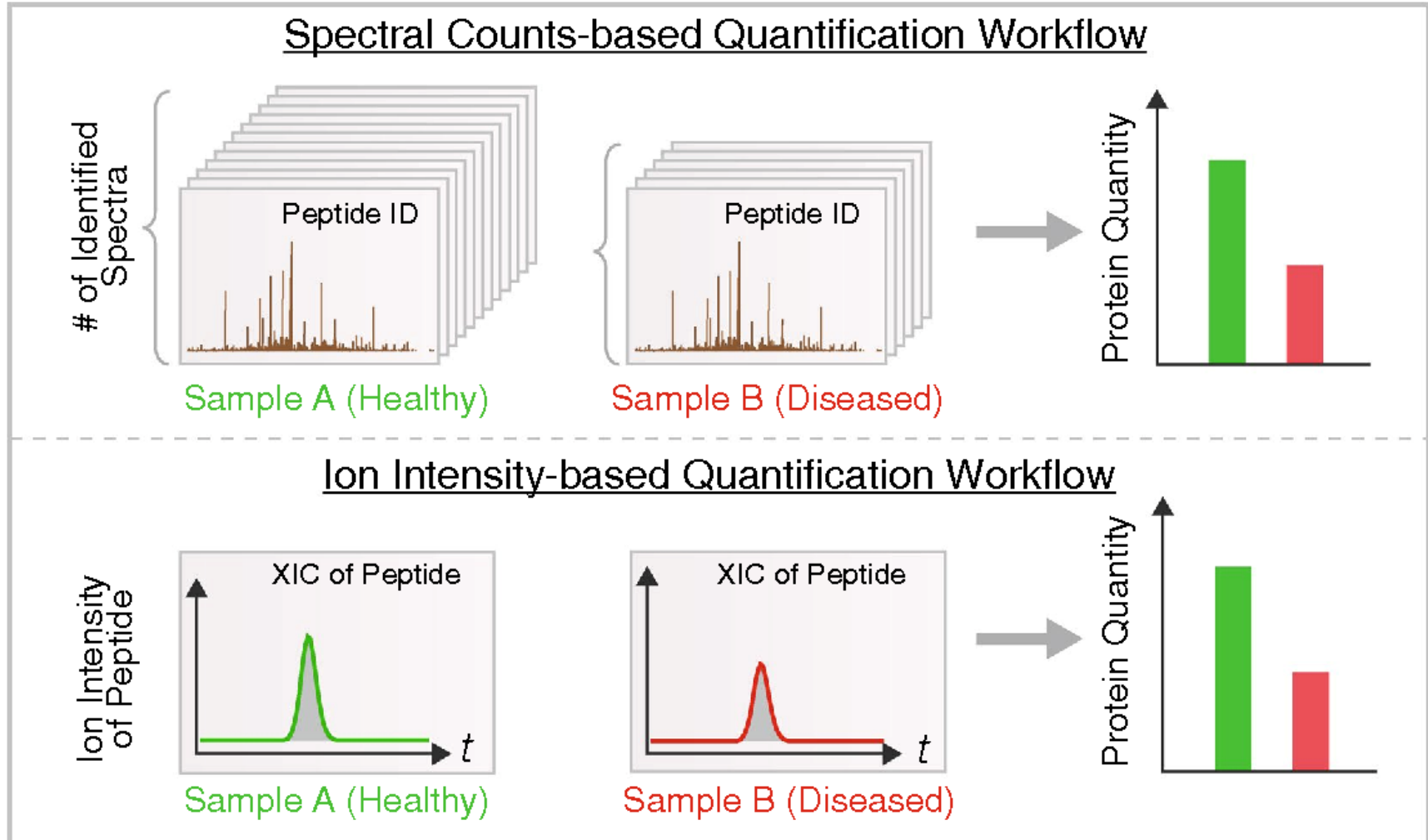
Alchemistmatt.com



# Quantitation of Proteins



# Quantitation of Proteins

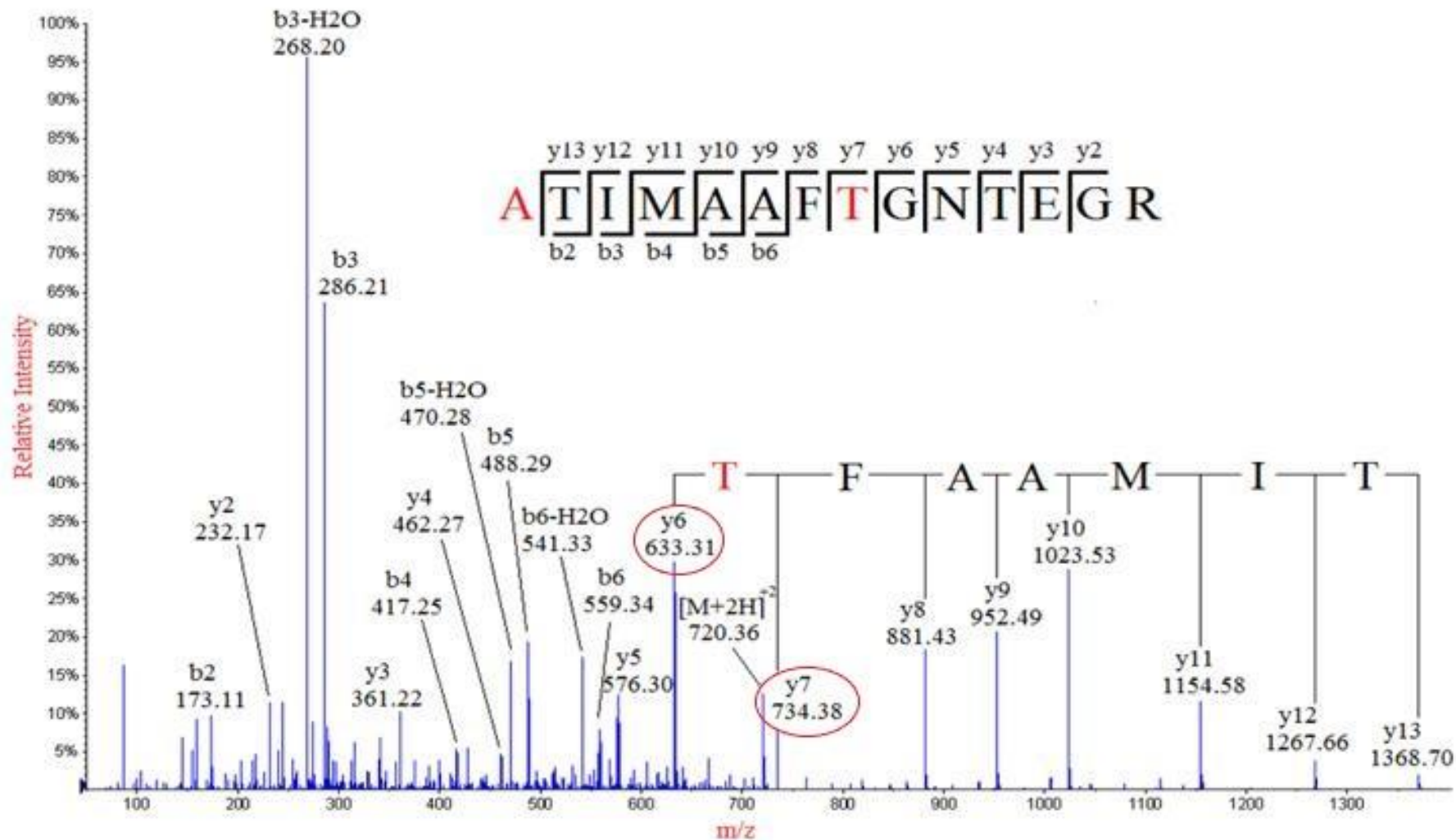


Common label-free quantification in proteomics studies. Two common label-free quantification approaches in use are based on spec

ScienceDirect



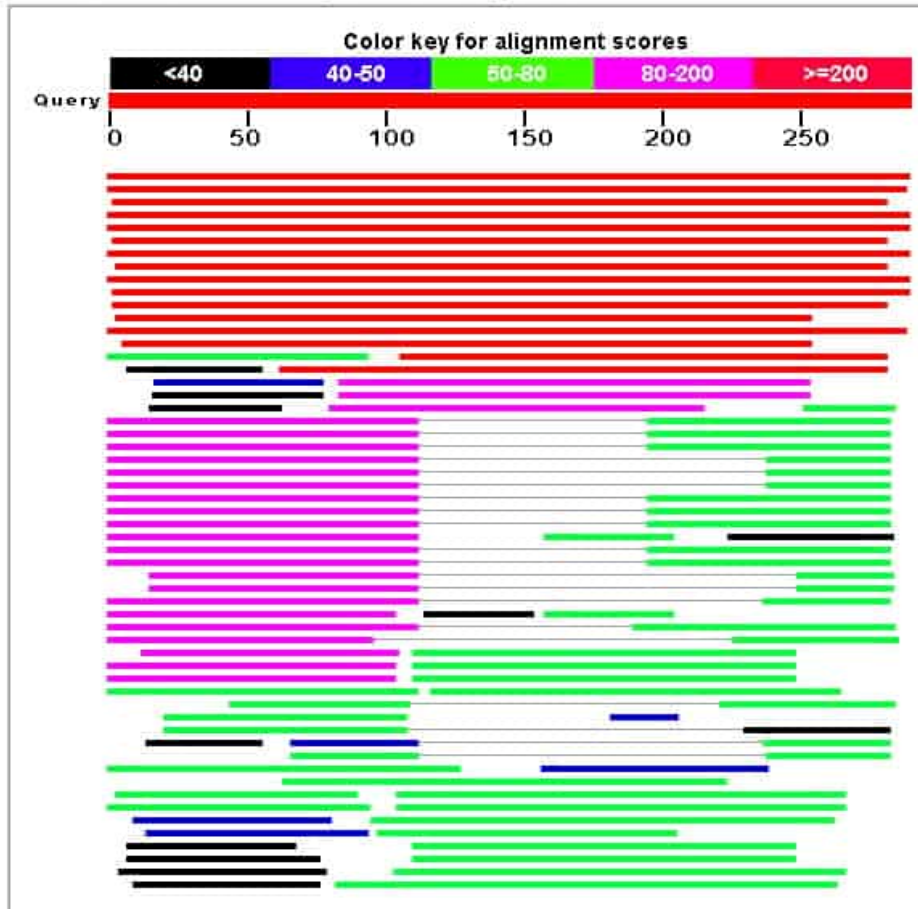
# De nova Sequencing



# BLAST Search

## Distribution of 491 Blast Hits on the Query Sequence

Mouse-over to show define and scores, click to show alignments

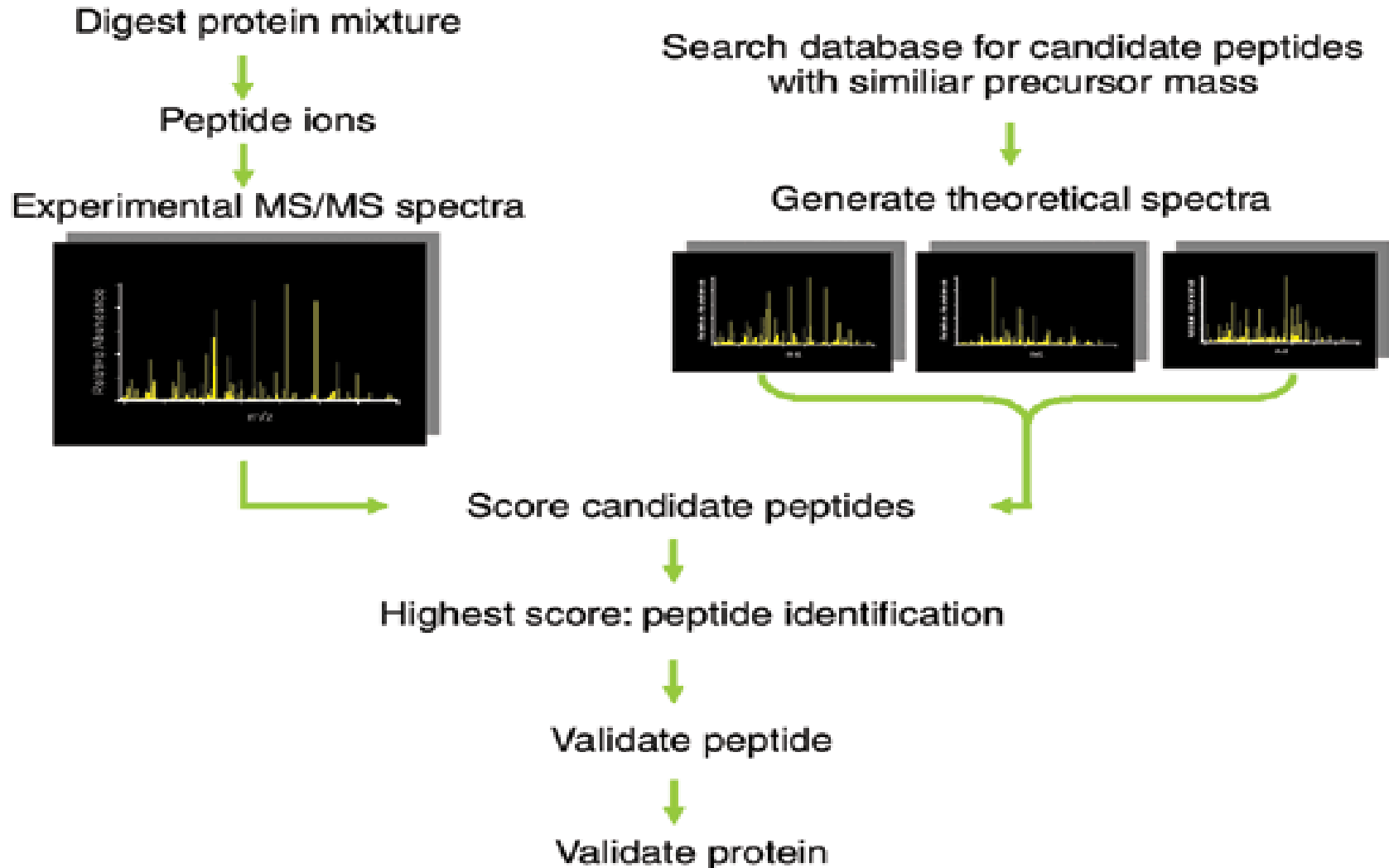


ry=  
gth=588

quences producing significant alignments:

		Score (Bits)	E Value
<a href="#">028577118.1</a>	PREDICTED: Perca flavescens type-2 ice-structurin...	<a href="#">94.9</a>	1e-15
<a href="#">028577120.1</a>	PREDICTED: Perca flavescens type-2 ice-structurin...	<a href="#">89.1</a>	6e-14
<a href="#">028577119.1</a>	PREDICTED: Perca flavescens type-2 ice-structurin...	<a href="#">89.1</a>	6e-14
<a href="#">028577117.1</a>	PREDICTED: Perca flavescens type-2 ice-structurin...	<a href="#">89.1</a>	6e-14
<a href="#">028564253.1</a>	PREDICTED: Perca flavescens type-2 ice-structurin...	<a href="#">85.3</a>	8e-13
<a href="#">028564599.1</a>	PREDICTED: Perca flavescens galactose-specific le...	<a href="#">81.4</a>	1e-11
<a href="#">028564252.1</a>	PREDICTED: Perca flavescens ladderlectin-like (LO...	<a href="#">81.4</a>	1e-11
<a href="#">028564251.1</a>	PREDICTED: Perca flavescens ladderlectin-like (LO...	<a href="#">81.4</a>	1e-11
<a href="#">030429847.1</a>	PREDICTED: Sparus aurata galactose-specific lecti...	<a href="#">77.6</a>	2e-10
<a href="#">030429846.1</a>	PREDICTED: Sparus aurata galactose-specific lecti...	<a href="#">77.6</a>	2e-10
<a href="#">030429845.1</a>	PREDICTED: Sparus aurata galactose-specific lecti...	<a href="#">77.6</a>	2e-10

# Database Searches





**Thank you**