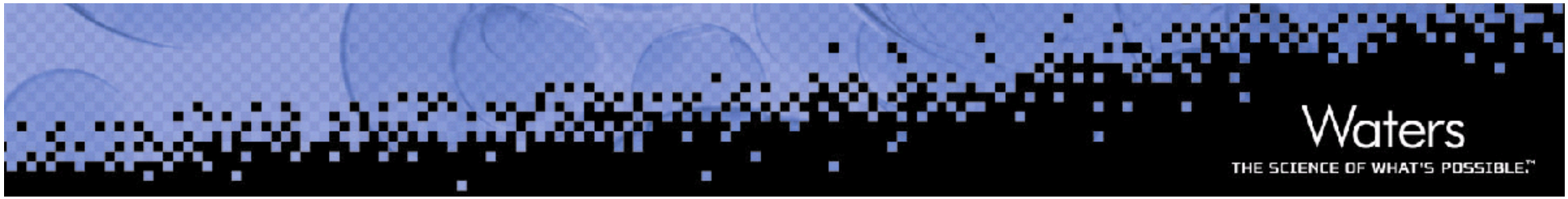

Introduction to Mass Spectrometry and Applications Overview

Denis Calnan
Business Development Manager
(Mass Spectrometry)
Waters Ireland

Overview

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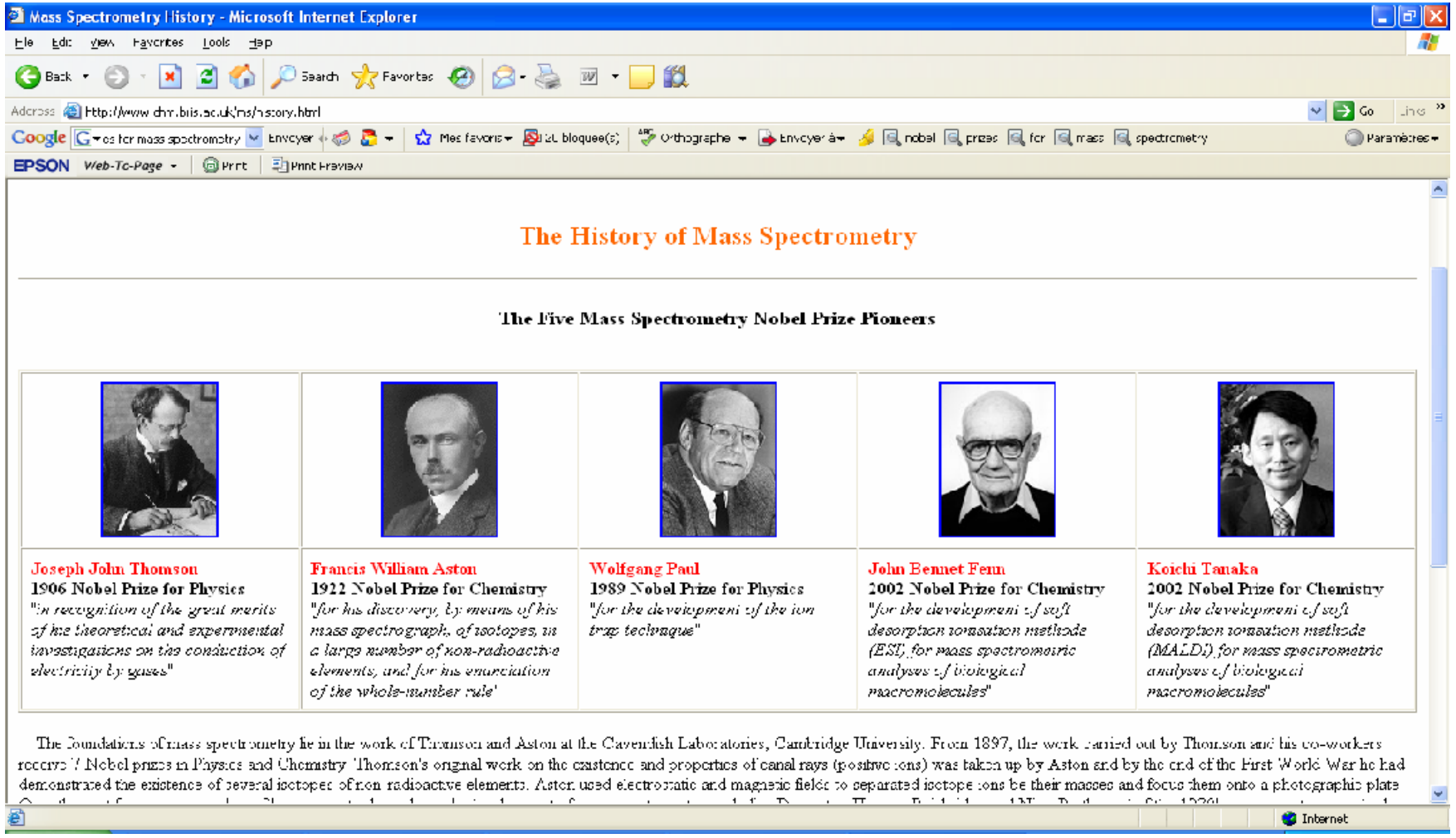
- Mass Spec overview
- Ionisation processes
- Types of instrument
- Areas of application
- Summary


Mass Spectrometry History - Microsoft Internet Explorer
File Edit View Favorites Tools Help
Back Search Favorites
Address http://www.chm.bris.ac.uk/ms/history.html
Go
Google
Envoyer
Mes favoris
Orthographe
Envoyer à
nobel prizes for mass spectrometry
Paramètres
EPSON Web-To-Page
Print
Print Preview
The History of Mass Spectrometry
The Five Mass Spectrometry Nobel Prize Pioneers
Joseph John Thomson
1906 Nobel Prize for Physics
"in recognition of the great merits of his theoretical and experimental investigations on the conduction of electricity by gases"
Francis William Aston
1922 Nobel Prize for Chemistry
"for his discovery, by means of his mass spectrograph, of isotopes, in a large number of non-radioactive elements, and for his enunciation of the whole-number rule"
Wolfgang Paul
1989 Nobel Prize for Physics
"for the development of the ion trap technique"
John Bennet Fenn
2002 Nobel Prize for Chemistry
"for the development of soft desorption ionisation methods (ESI) for mass spectrometric analyses of biological macromolecules"
Koichi Tanaka
2002 Nobel Prize for Chemistry
"for the development of soft desorption ionisation methods (MALDI) for mass spectrometric analyses of biological macromolecules"
The foundations of mass spectrometry lie in the work of Thomson and Aston at the Cavendish Laboratories, Cambridge University. From 1897, the work carried out by Thomson and his co-workers
Internet

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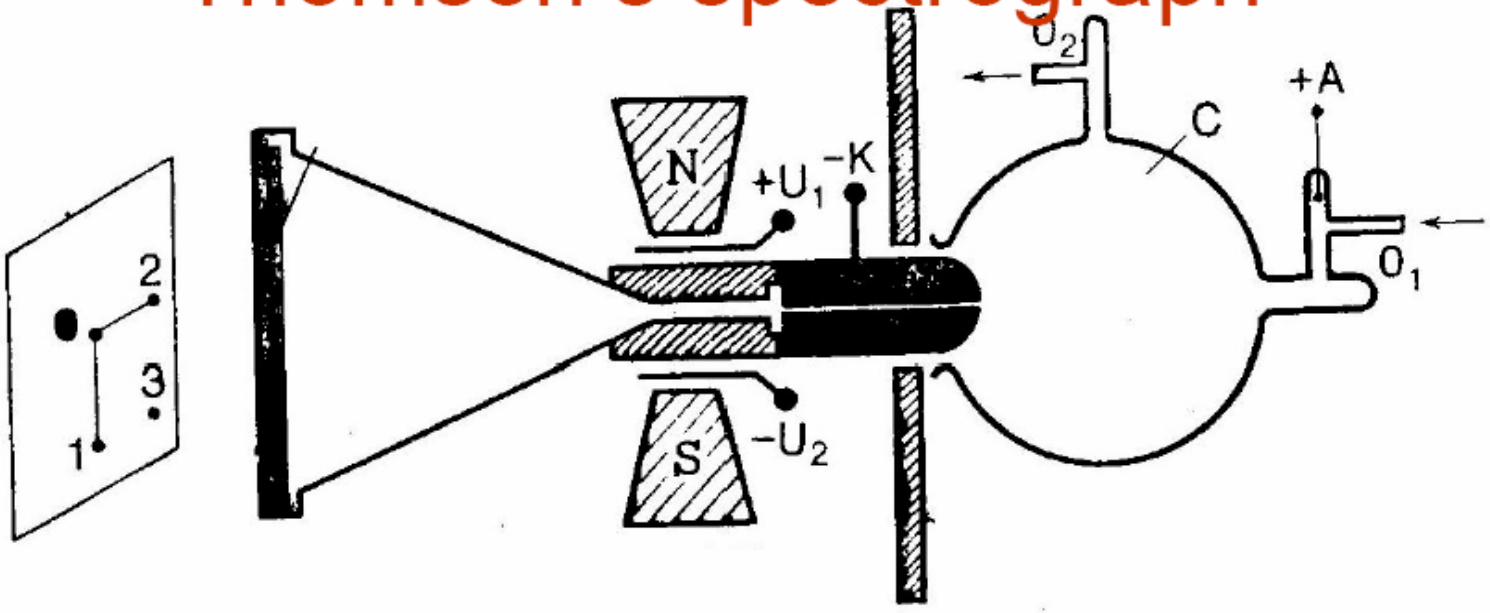
Brief History of MS

- The ability to separate molecules based on mass / charge ratio , m/z was described by J.J. Thomson, 1912 (Nobel Prize 1906)

- 1920's Electron Ionisation (EI)

- 1940's First commercial Mass Spectrometers used in the petrochemical industry

Thomson's spectrograph



"I feel sure that there are many basic problems in chemistry which could be solved with far greater ease with this than with any other method. The method is surprisingly sensitive – more so even than the method of spectrum analysis, requiring an infinitesimal amount of material, and does not require this to be specially purified."

Joseph John Thomson, 1913
The Rays of Positive Electricity and their Applications to Chemical Analysis

Brief History of Mass Spectrometry

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- 1950's first "Time of Flight" (Tof) mass spectrometers
- 1960's Chemical Ionisation introduced
- 1981 Fast Atom Bombardment (FAB) Barber *et al*

Brief History of Mass Spectrometry

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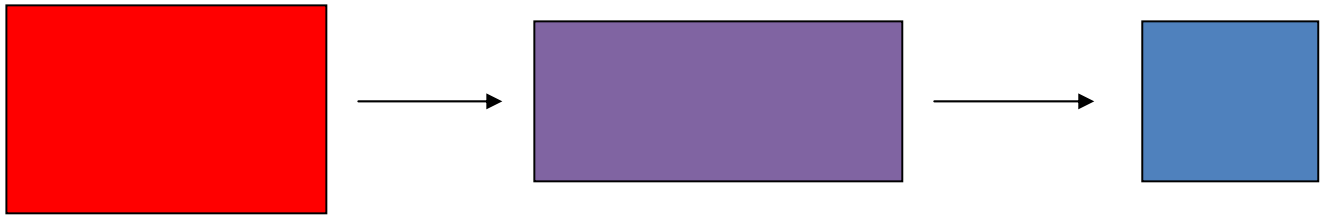
- 1988 "Soft Ionisation" techniques emerged
- Koichi Tanaka –soft laser desorption (leading to MALDI)
- John Fenn – Electrospray Ionisation (ESI)
- This work revolutionised Biological Mass Spectrometry
- Both received the Nobel prize for Chemistry in 2002

"Typical Layout" Mass spectrometer



	Source	Mass analyser	Detector
e.g.	*ESI* *MALDI* *APCI* *EI*	*Tof* *Quadrupole (Q, QqQ)* *Ion Trap* *Magnetic sector*	MCP Photomultiplier

Areas of Application

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- **Biological**

- Proteomics
- Clinical
- Microbes

Areas of Application

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- **Pharmaceutical**

- Drug discovery
- QC in production
- Validation

Areas of Application

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- **Industrial**

- Petrochemicals
- Organic synthesis
- Dyes, perfumes

Areas of Application

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- **Environmental**

- Food contaminants
- Water analysis
- Pesticides
- Dioxins (PCBs)

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The significance of Soft Desorption Ionisation development

A fundamental problem in biological mass spectrometry was how to transfer highly polar, non volatile molecules with a mass of tens of kDa into the gas phase without destroying them

John B. Fenn
Virginia University, USA

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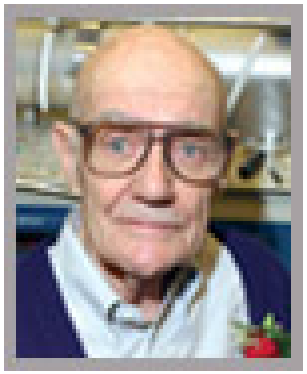
- Fenn and co workers built upon Malcolm Dole's early description of the electrospray principle
- Fenn combined Electrospray Ionisation with quadrupole MS
- This work was presented at the ASMS in 1988 and later published in a landmark paper in 1989 *Science* vol 246
- Multiply charged ions gave rise to spectra identifying proteins >100kDa

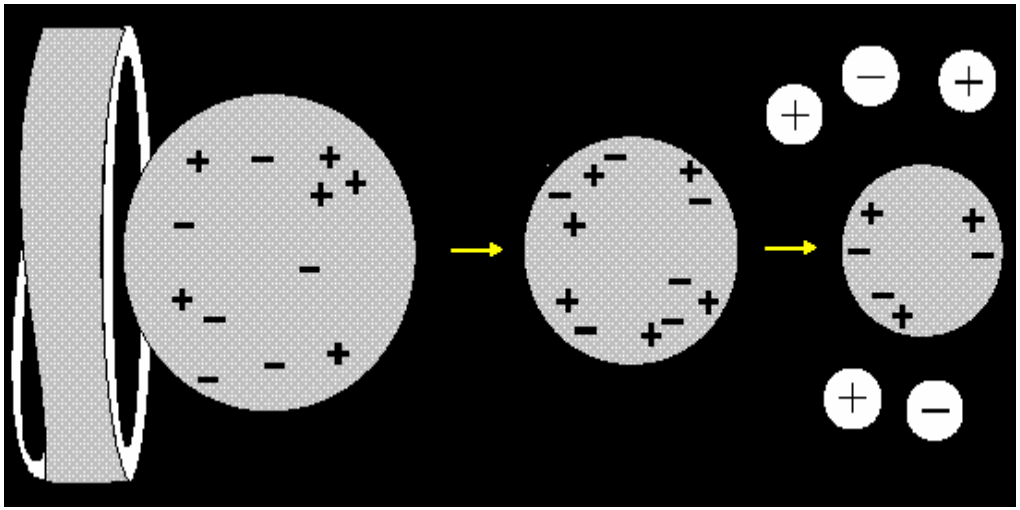


Mechanism (*in simple terms*)

- A solution containing sample molecules is electrostatically spayed through a fine capillary by applying a high electrical potential difference
- Highly charged droplets are produced, the surrounding solvent evaporates and the charge density in the droplet increases
- This leads to the formation of "naked", highly charged molecules (charge state form +1 up to +40 and beyond for large proteins)

Electrospray Ionisation Theory

Capillary
~3kV



Ions evaporate from the surface

As droplet evaporates, the electric field increases and ions move towards the surface.

m/z

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- Molecular ions +ve or -ve
- Mass to charge ratio

“m/z value”

Example Peptide Glufib

Molecular Mass 1569 Da

[M+H]+ = 1570.6 m/z

[M+2H]2+ = 785.8 m/z

Quattro premier XE

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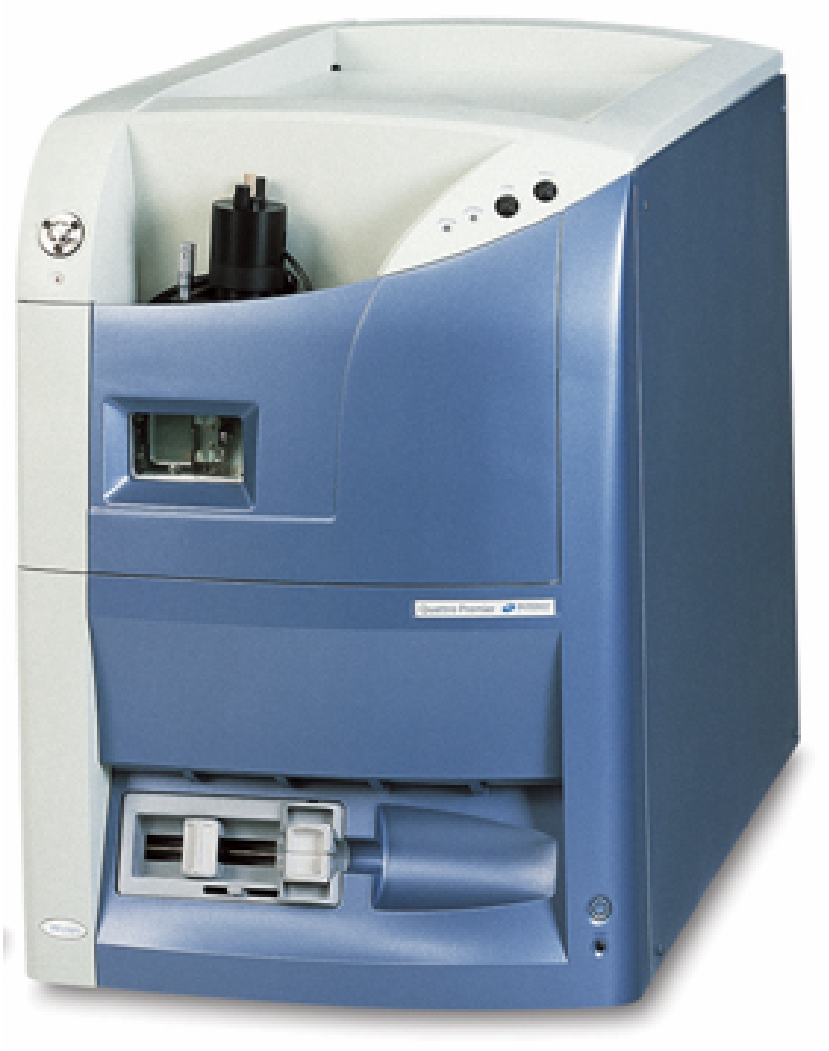

Tandem Quadrupole

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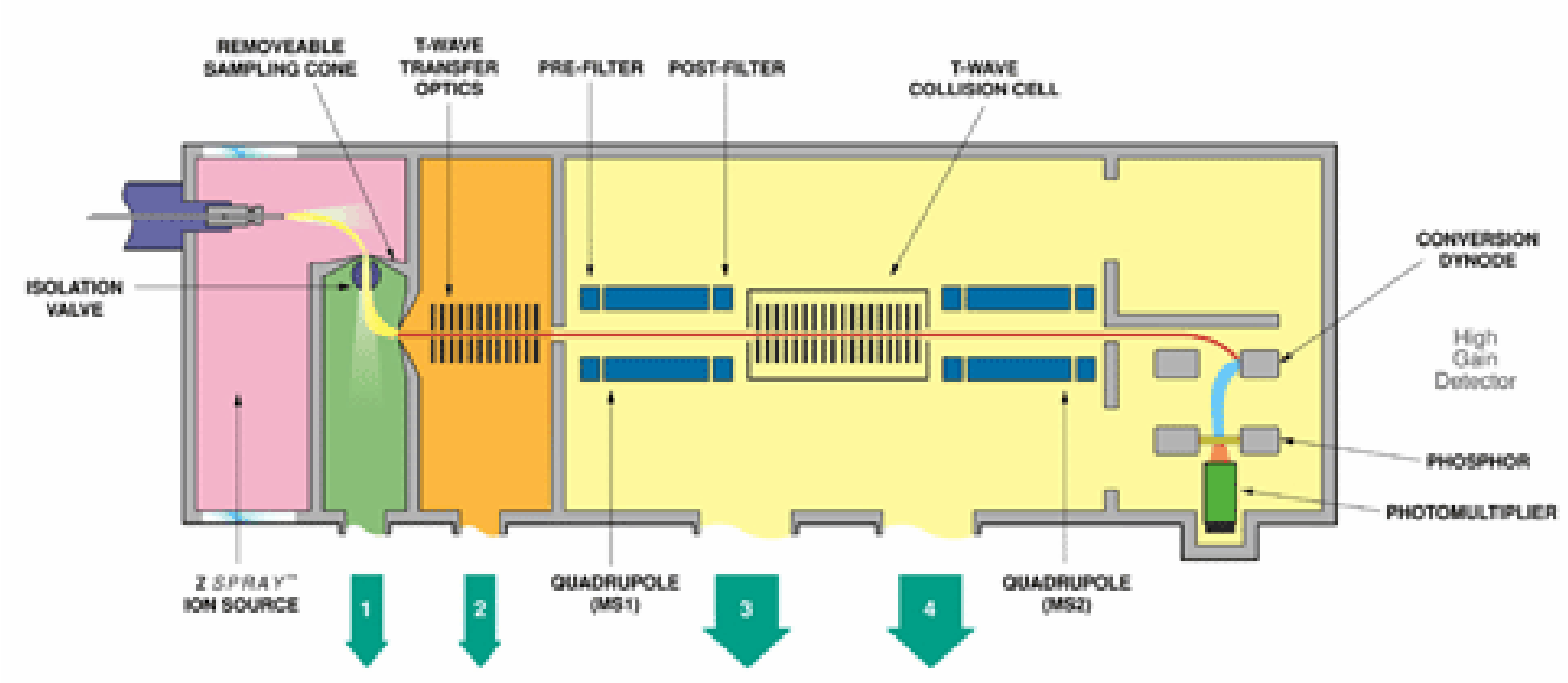

AutoSpec Premier

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MALDI-Tof MS

MALDI micro MX™

MICROMASS
MS TECHNOLOGIES

Maldi Micro MX

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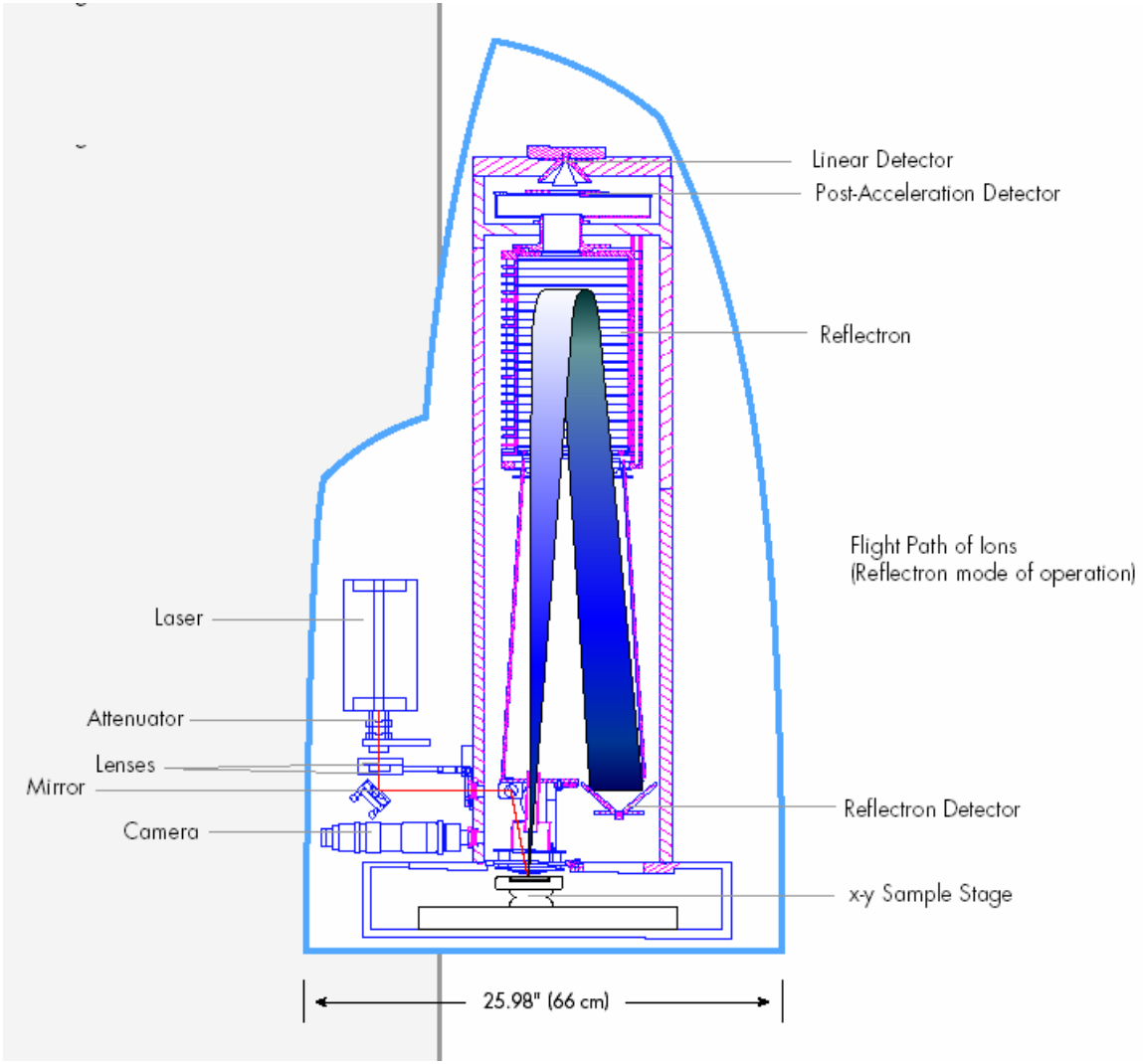

Time of Flight

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- **Time-of-Flight Mass Spectrometry – TOF MS**
  - **The mass of ions is determined by accelerating then and measuring their time-of-flight over a known distance**
  - **Put simply heavy ions fly slowly, light ions fly quickly**

$$neV = \frac{1}{2}mv^2$$

Definitions

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- Nominal mass: The mass of an ion calculated using the integer mass of the most abundant isotope of each element
  - Neglects the mass defect, where H=1, C=12, O=16

- Monoisotopic mass: The mass of an ion calculated using the exact mass of the most abundant isotope of each element
  - Includes the mass defect, where ^{1}H=1.0078, ^{12}C=12.0000, ^{16}O=15.9949

Why Accurate Mass?

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- Confirmation of elemental composition
  - Identification of unknown compounds
  - Patent support and scientific journals
- Additional dimension of specificity
  - Quantitation in accurate mass MS mode rather than MS/MS mode to reduce chemical interferences
  - Differentiation of nominal isobars in combinatorial libraries
  - Improved protein database search results
  - Improved de novo protein sequencing results

Accurate Mass Measurement Theory

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- Accurate mass measurements take advantage of the fact that the combination of elements contained in a molecule have a very specific, non-nominal molecular weight

The Fundamentals of Accurate Mass

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- Carbon has a mass of 12.0000
- Hydrogen has a mass of 1.0078
- Oxygen has a mass of 15.9949
- Nitrogen has a mass of 14.0031

LCT Premier XE

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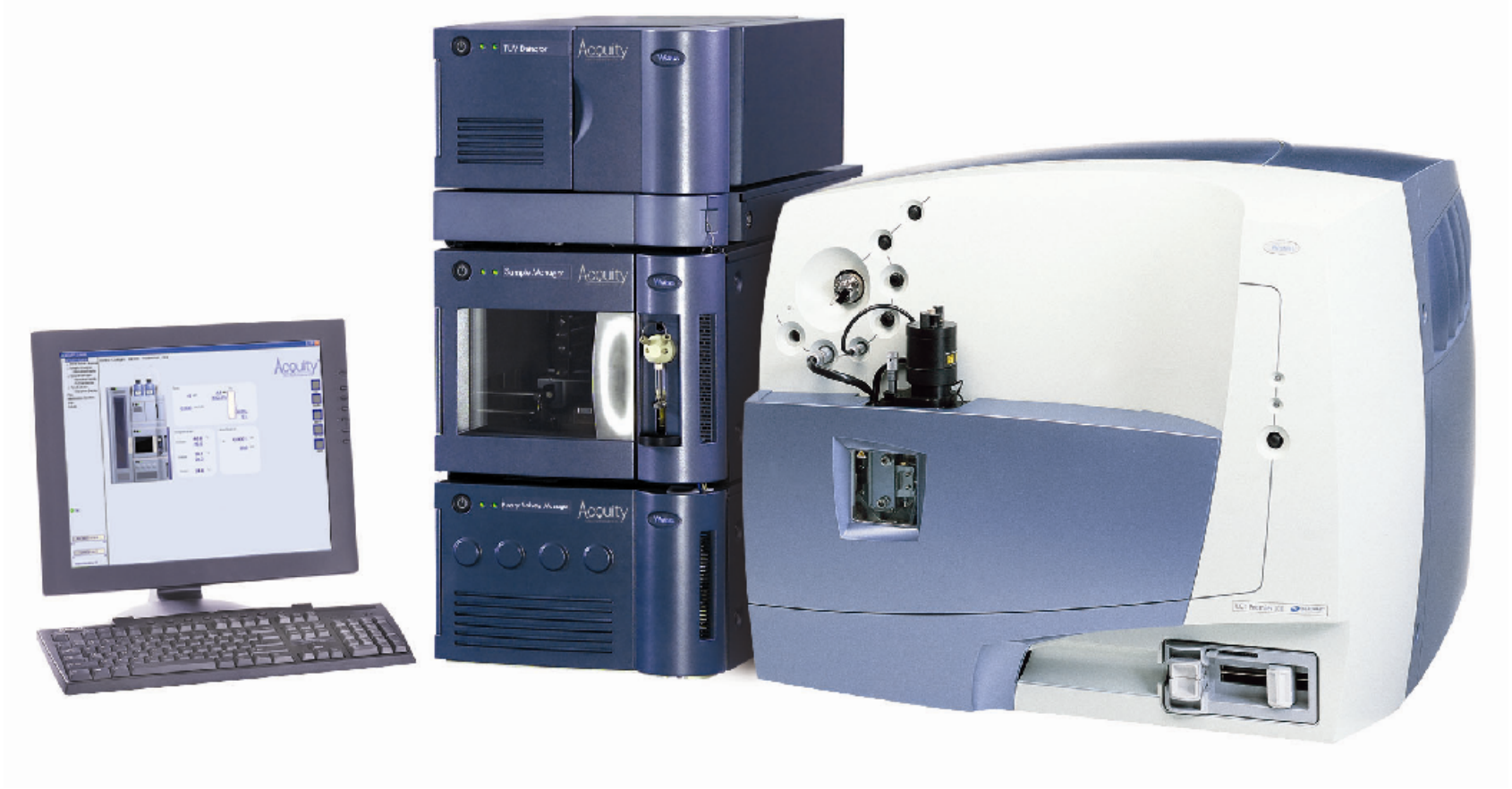

LCT Premier schematics

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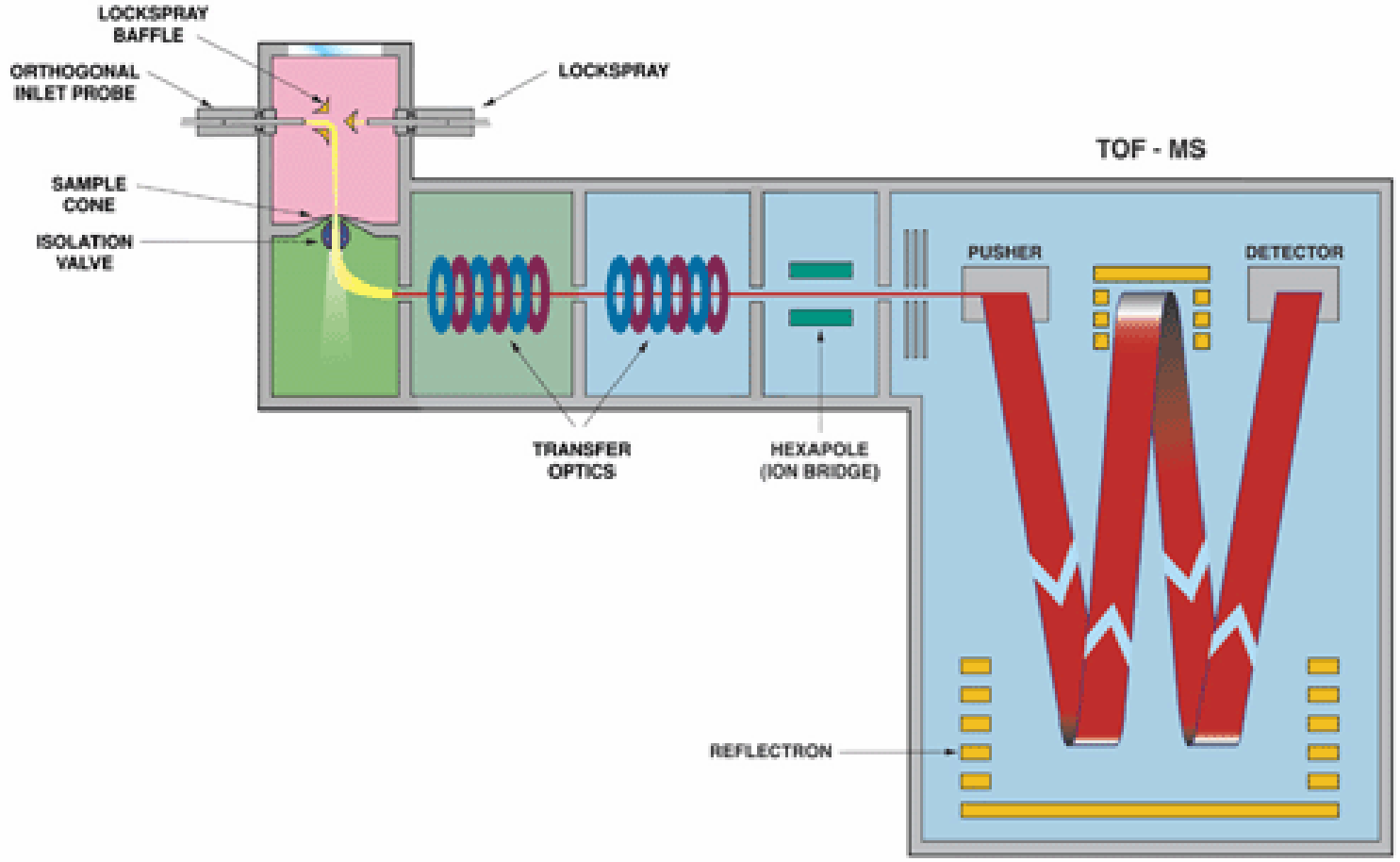

Integrated LockSpray Ion Source

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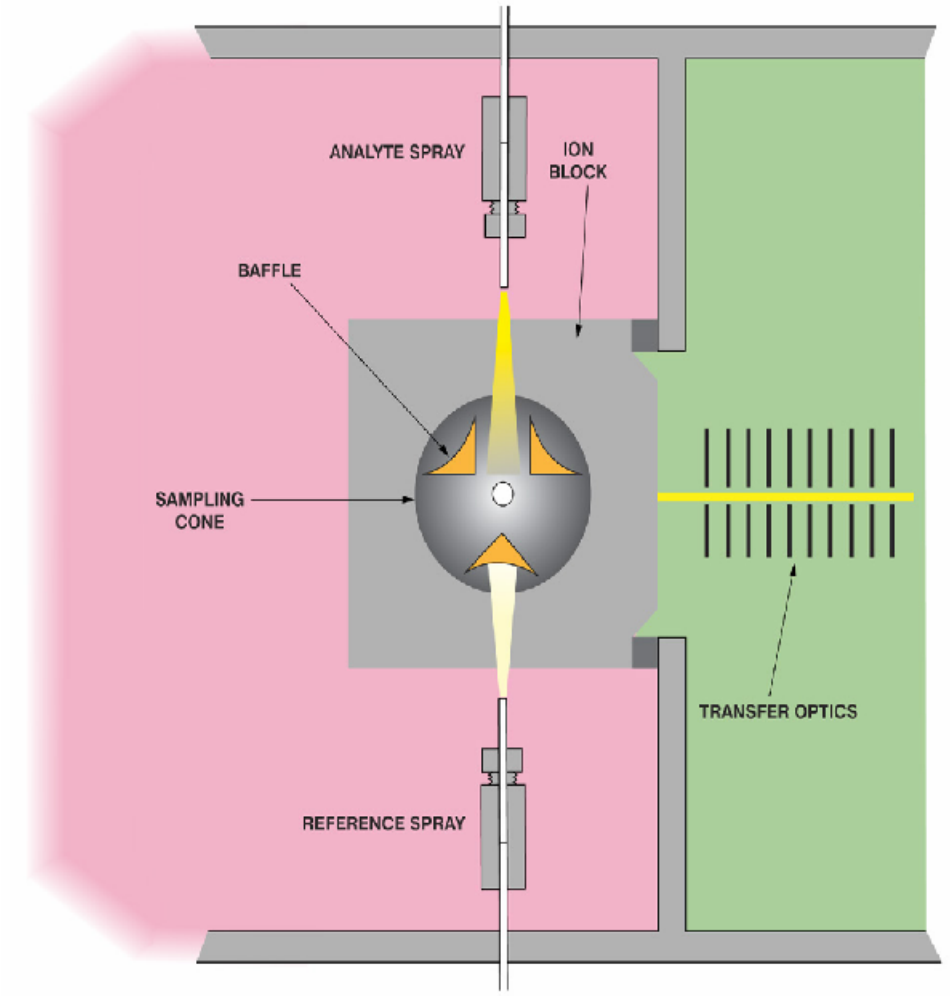

Example 1
Nominal Mass Measurement

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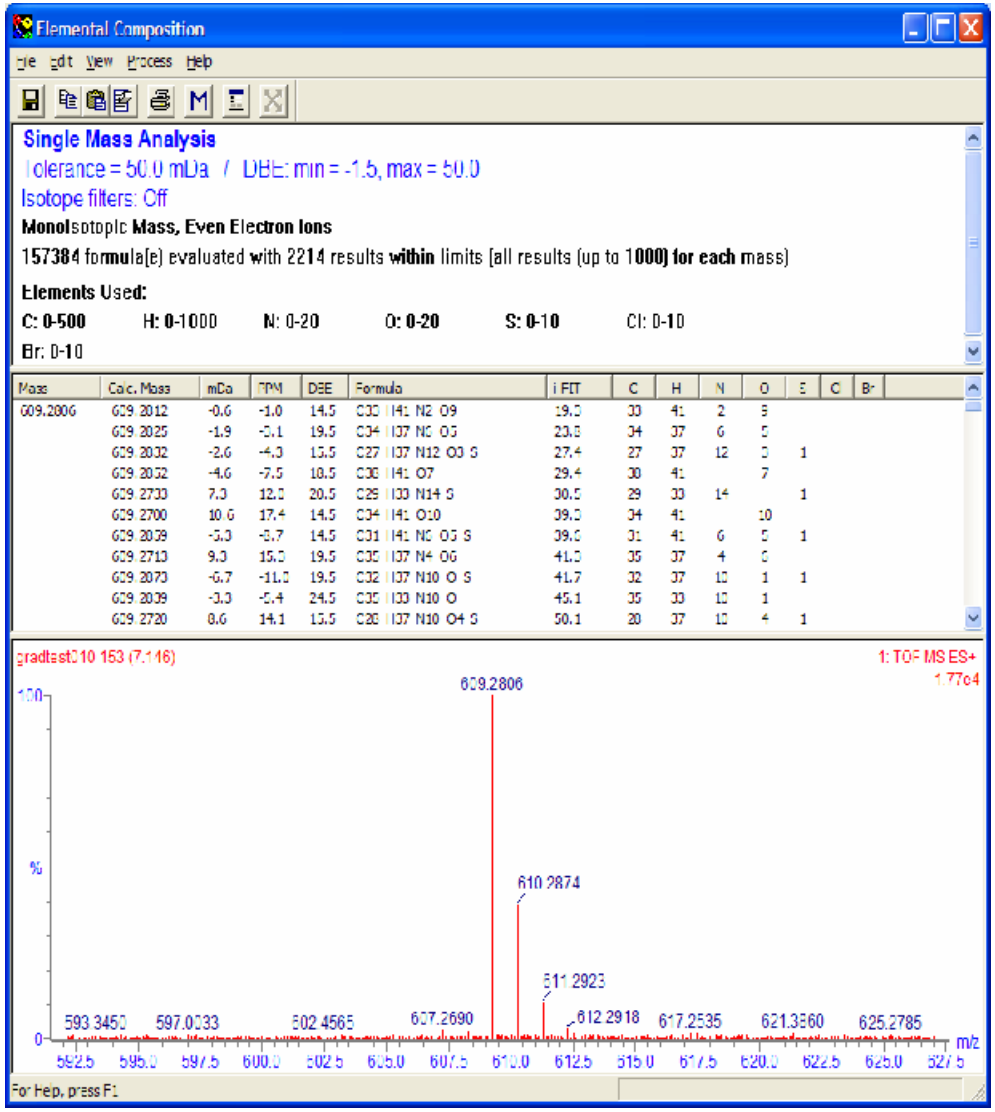

- Nominal mass measurement doesn't provide data specificity
- At m/z609, with 0.1Da error around mass, **2214** possible combinations
- Using wide range of elements:
  - $C_{500}H_{1000}N_{20}O_{20}S_{10}Cl_{10}Br_{10}$

Example 1
i-FIT Filters Off (3ppm)

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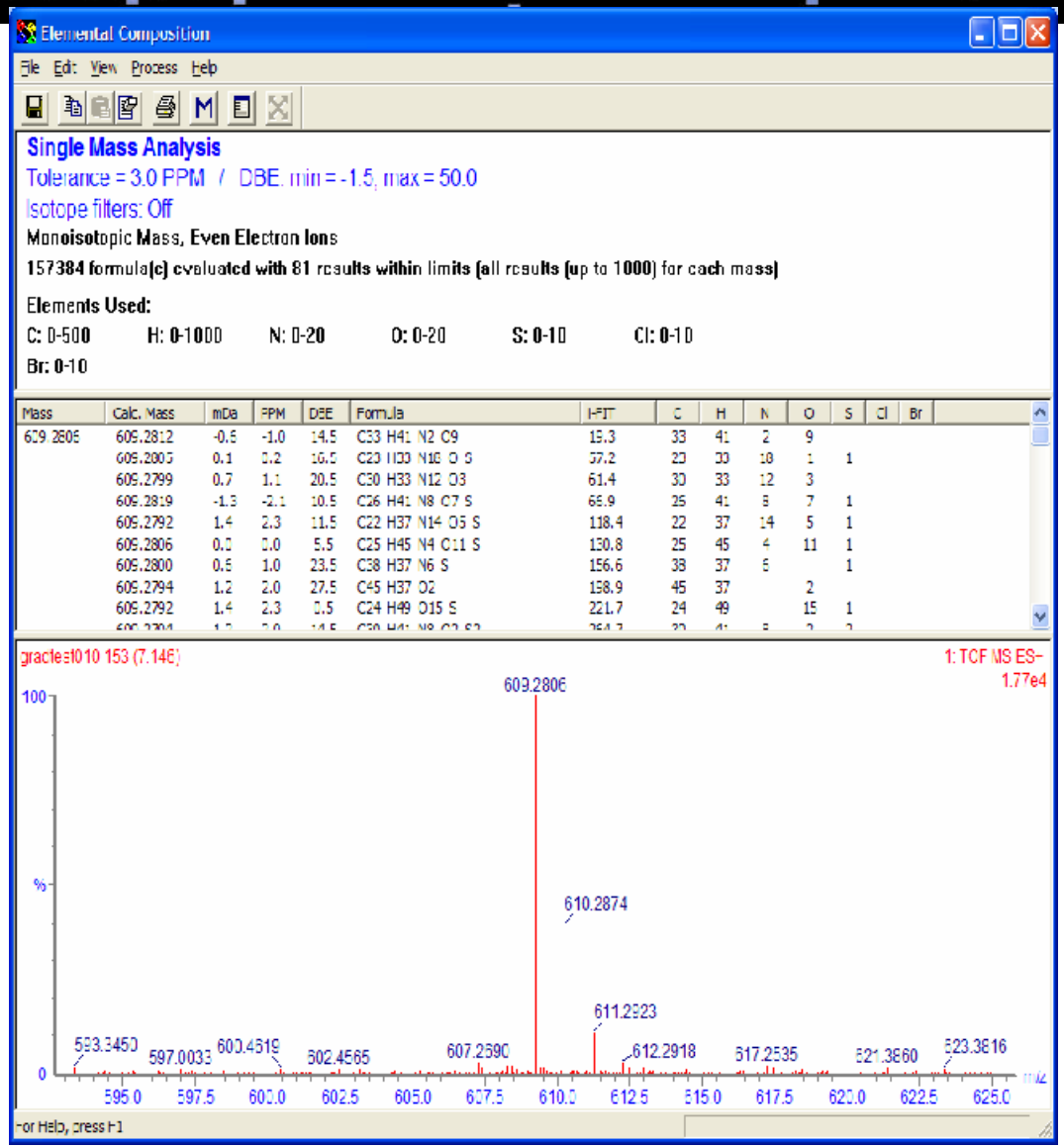

- At m/z609, with 3ppm error around mass, **81** possible combinations
- Using wide range of elements:
  - $C_{500}H_{1000}N_{20}O_{20}S_{10}Cl_{10}Br_{10}$

Example 1
i-FIT Filters On (3ppm)

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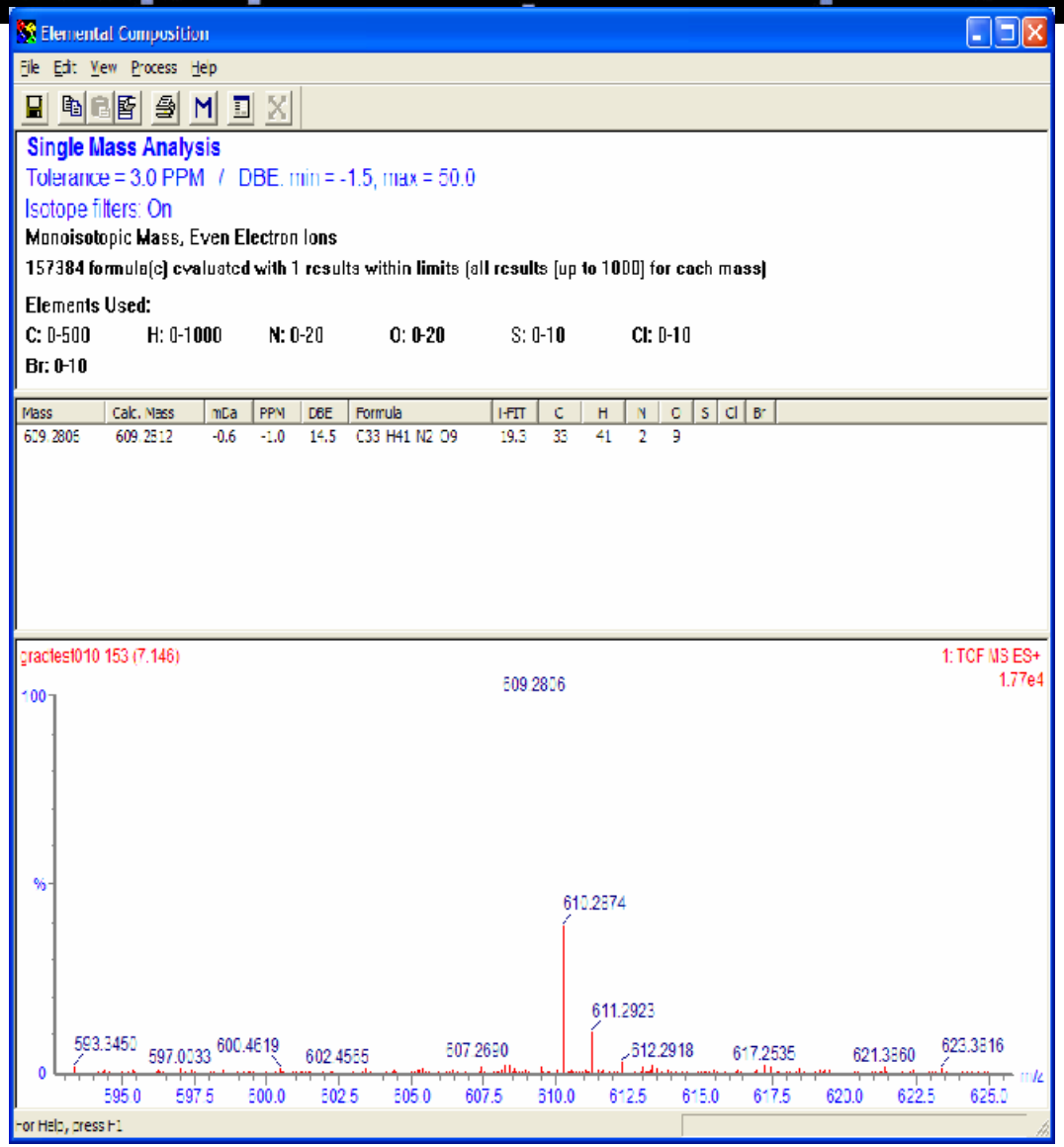

- At m/z609, with 3ppm error around mass, only **1** possible combination
- Using wide range of elements:
  - $C_{500}H_{1000}N_{20}O_{20}S_{10}Cl_{10}Br_{10}$
- Isotope Filters turned on
  - 3% instrument error
  - Carbon range = +/-3

Conclusions

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- LCT Premier XE - simplified exact mass with high sensitivity over a wide dynamic range
  - Ideal for screening applications
- Wizard driven set up procedures for easy operation
- Complete exact mass ionisation coverage with dedicated LockSpray for ESI, ESCi and APcI
- i-FIT – realisation of exact mass measurement
  - Simplification of elemental composition results through isotope interrogation and data interpretation
- System solutions with OpenLynx, ChromaLynx, MarkerLynx and MetaboLynx

GCT Premier

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ACQUITY UPLC™ QTOF Premier™ System

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MALDI Q-Tof Premier

Waters
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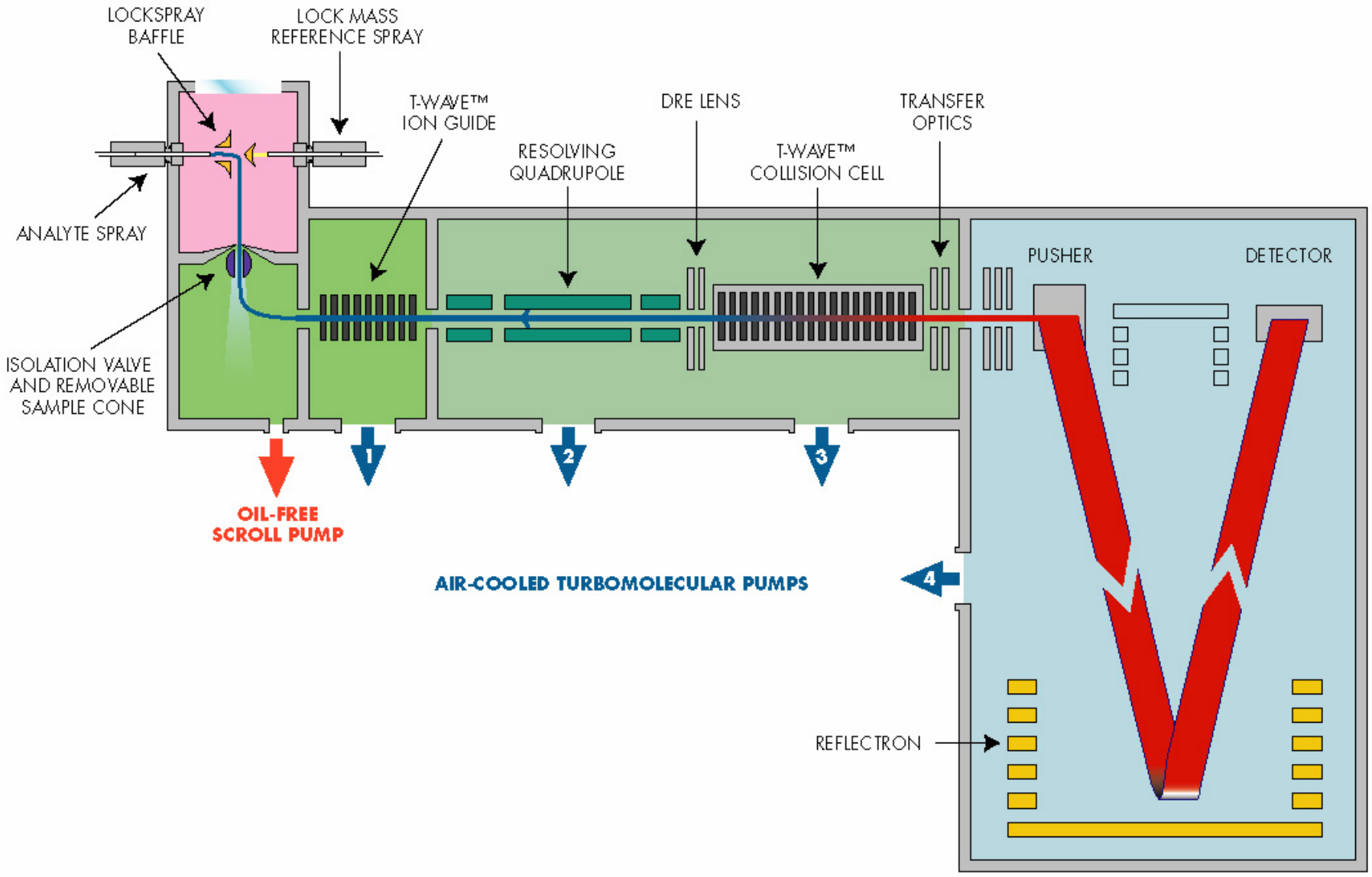
+

MALDI

ESI

ESI-QTof Premier schematic



MALDI-QTof Premier schematic

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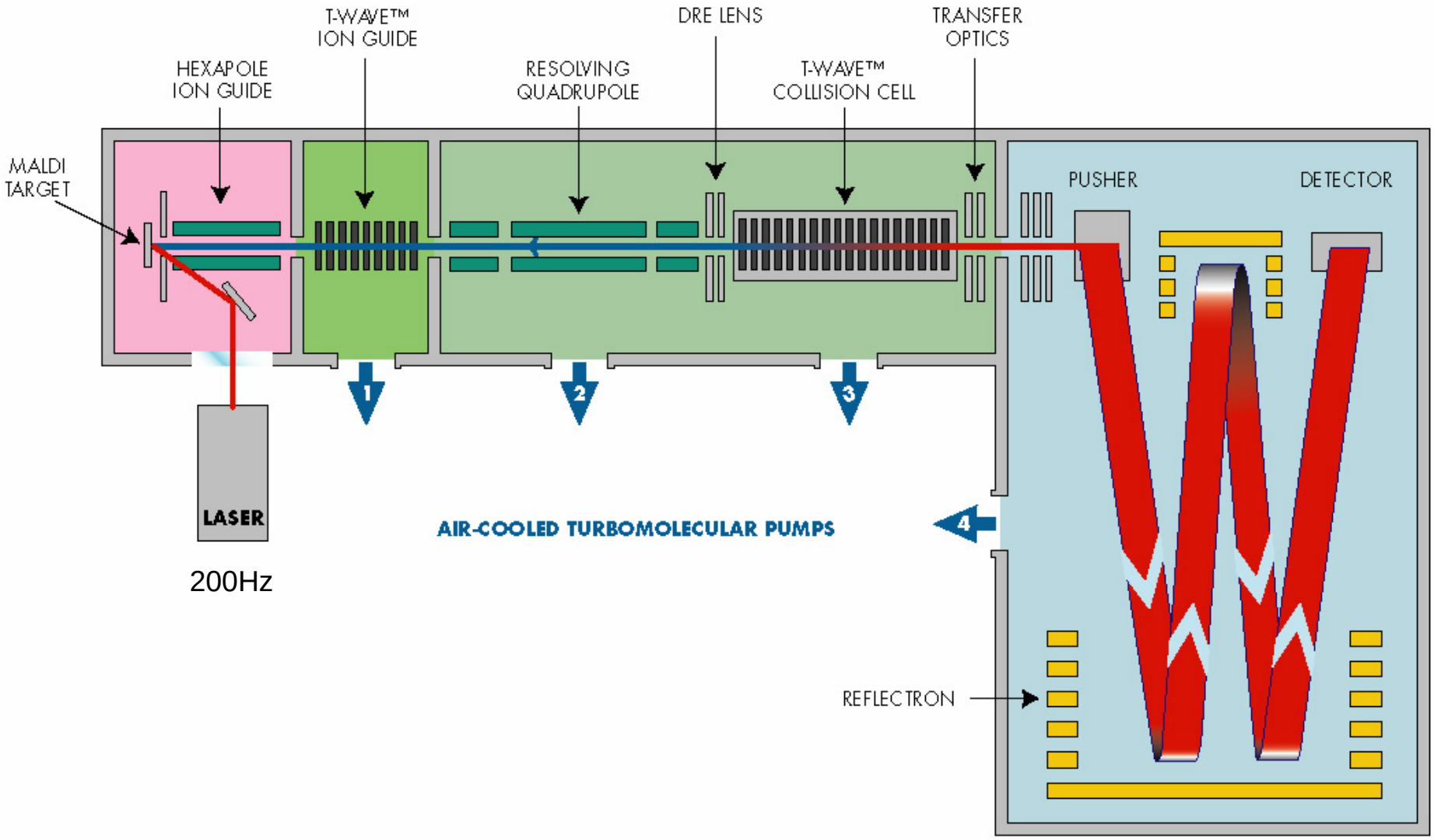

200Hz

Waters phosphopeptide mixture MALDI MS

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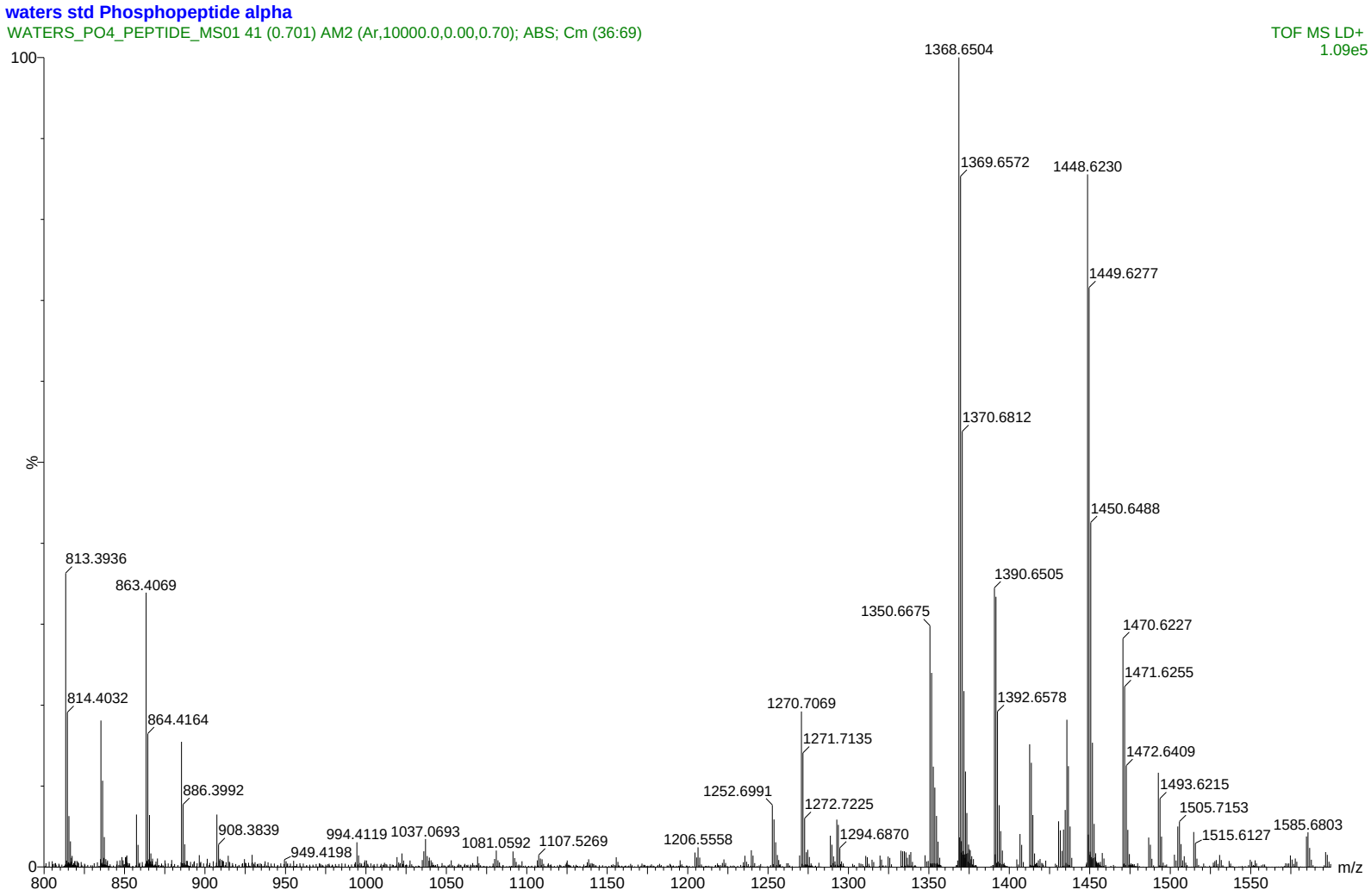
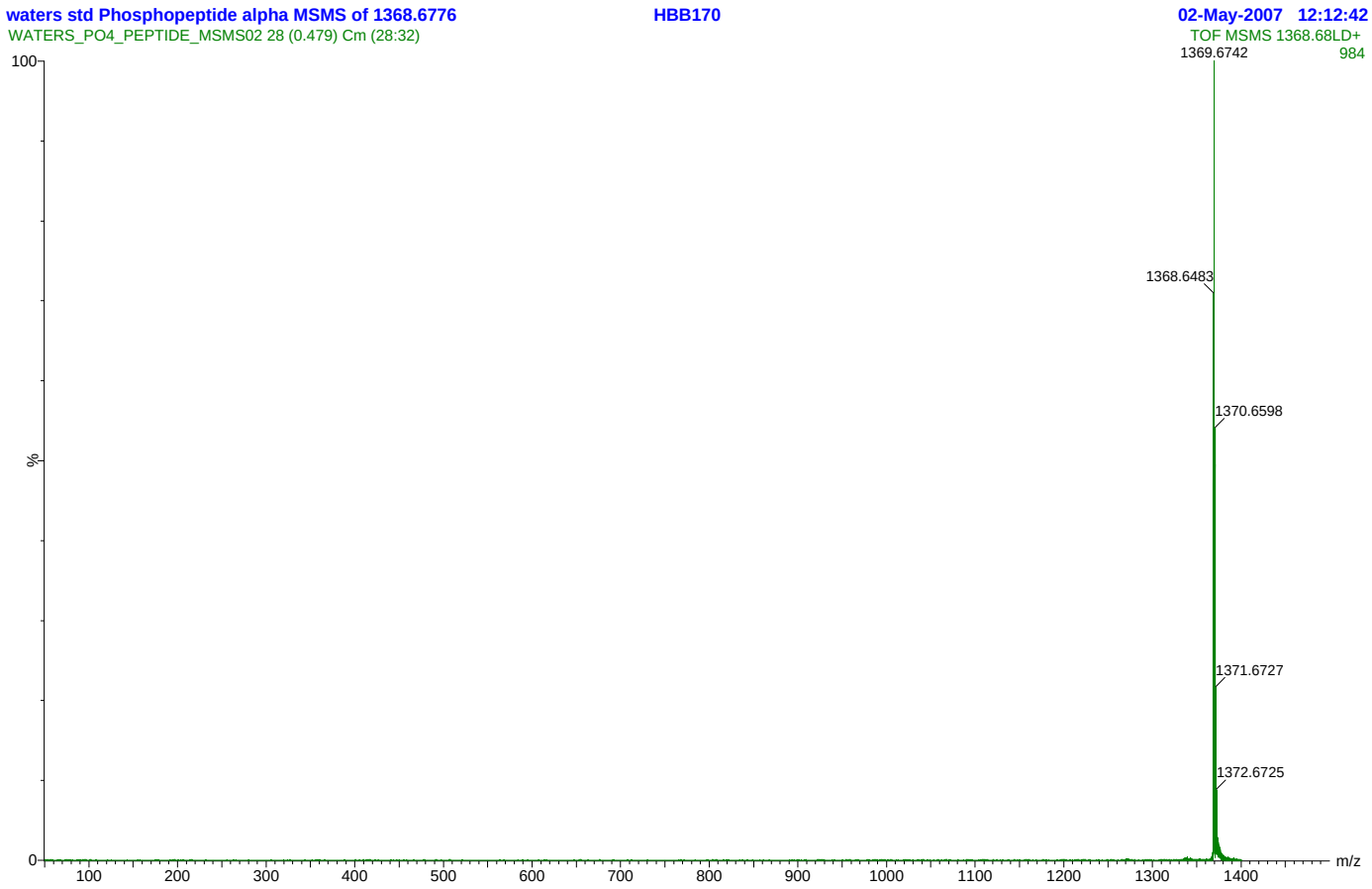


Isolation of phosphopetide ion 1368 m/z



De novo sequencing of phosphopeptide VNQI GpTLSESI K, 1368 m/ z (using MaxEnt3 and BioLynx)

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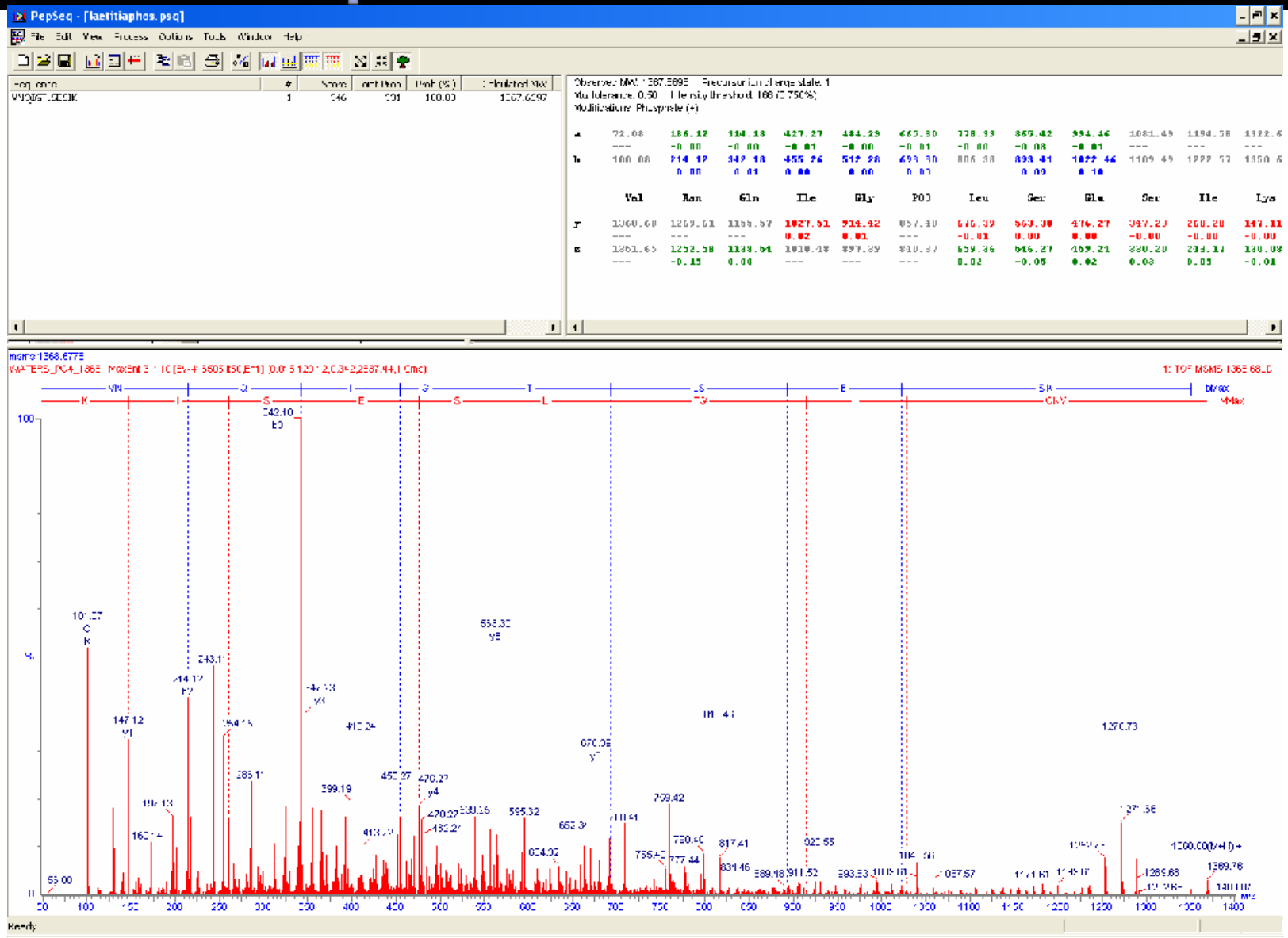
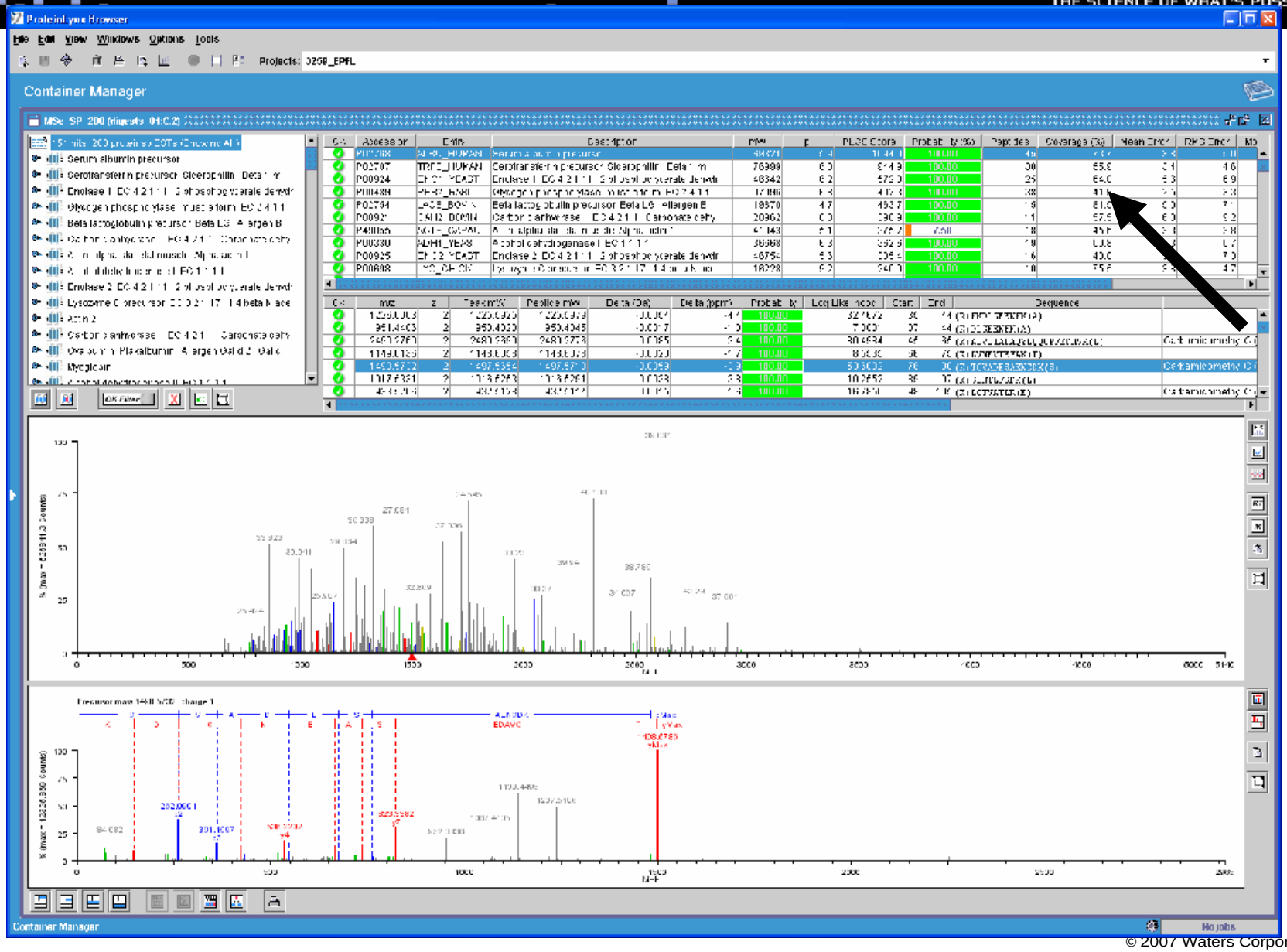


MS^e data analysis (> 70% sequence)



N Glycan MALDI MS

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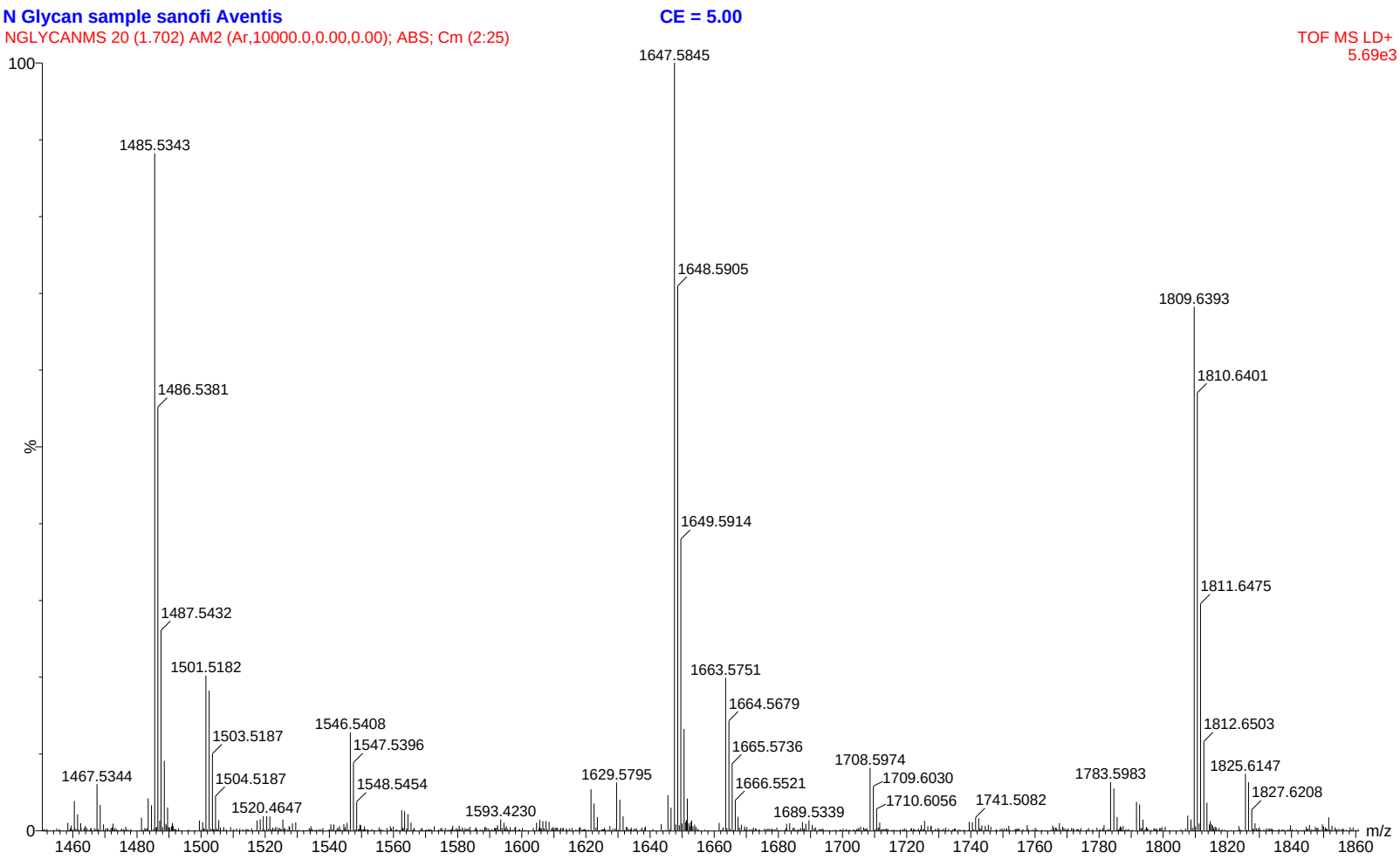

Isolation of glycan m/z 1647

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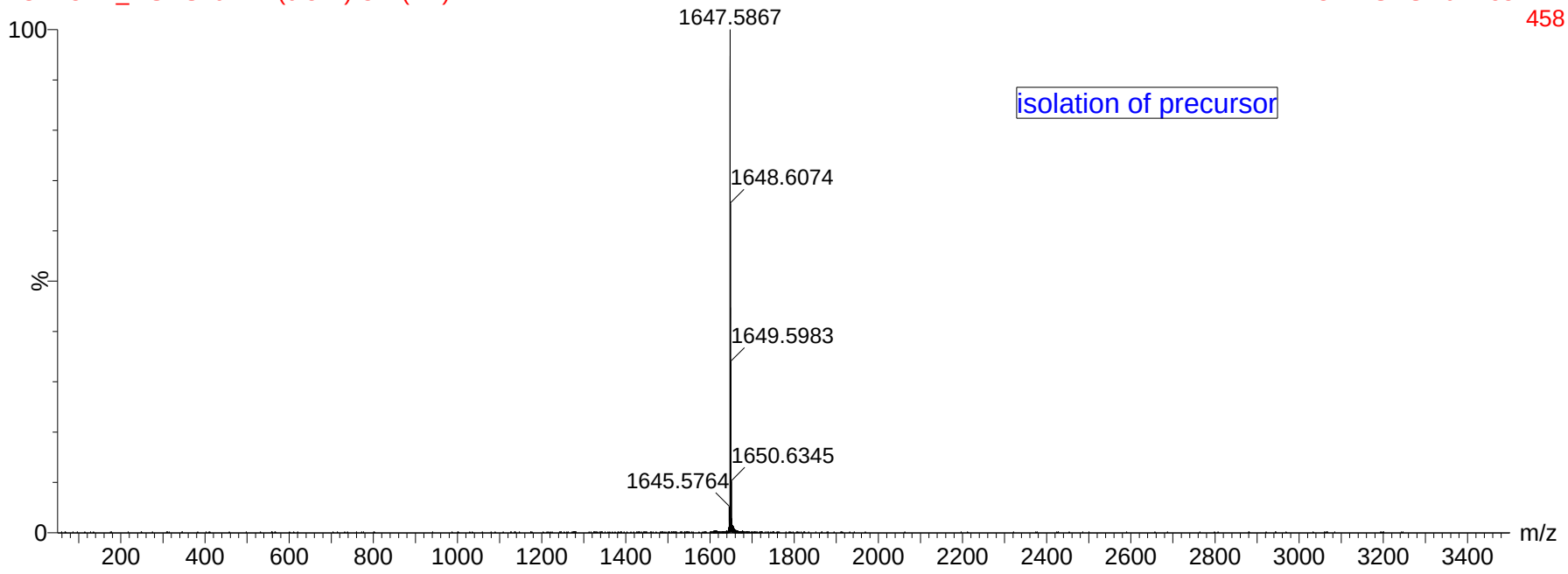
N Glycan sample sanofi Aventis msms m/z 1647.58 **CE = 5.00**

NGLYCAN_MSMS1647 4 (0.341) Cm (2:4)

TOF MSMS 1647.58LD+
458



MALDI MSMS of m/ z 1647

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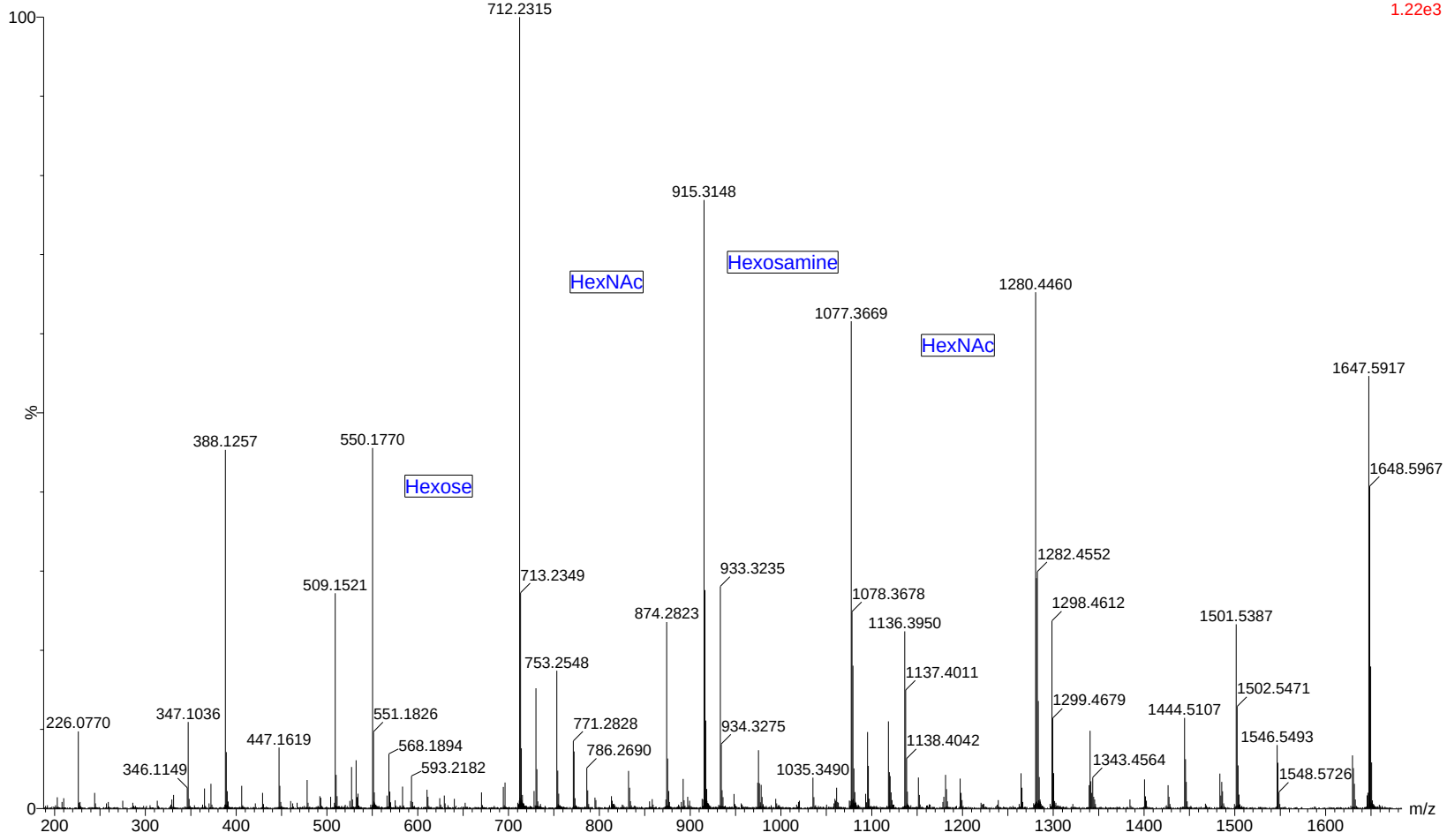
N Glycan sample sanofi Aventis msms m/z 1647.58 **CE = 95.00**

NGLYCAN_MSMS1647 24 (2.045) AM2 (Ar,10000.0,0.00,0.00); ABS; Cm (12:35)

TOF MSMS 1647.58LD+
1.22e3



Biological MS - *Proteomics*

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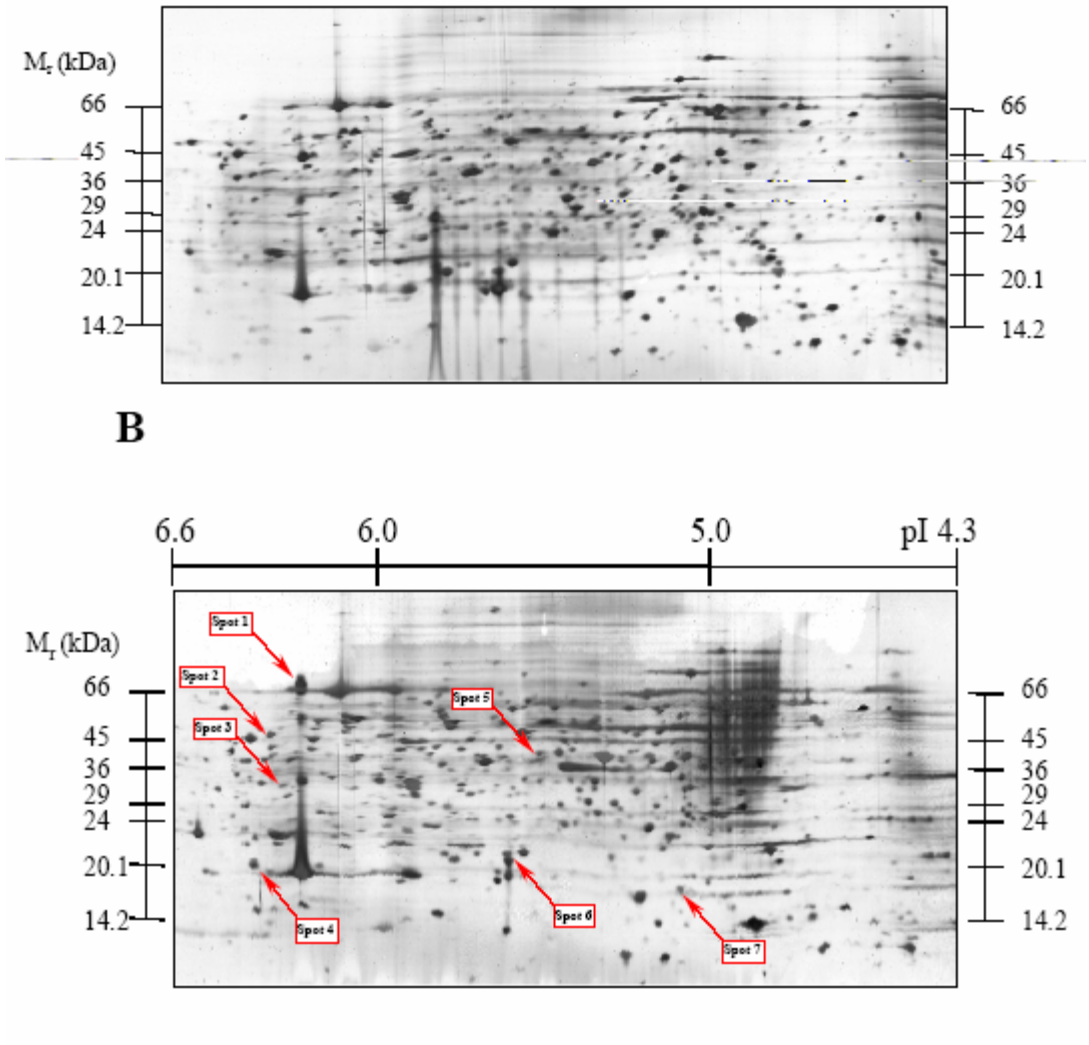
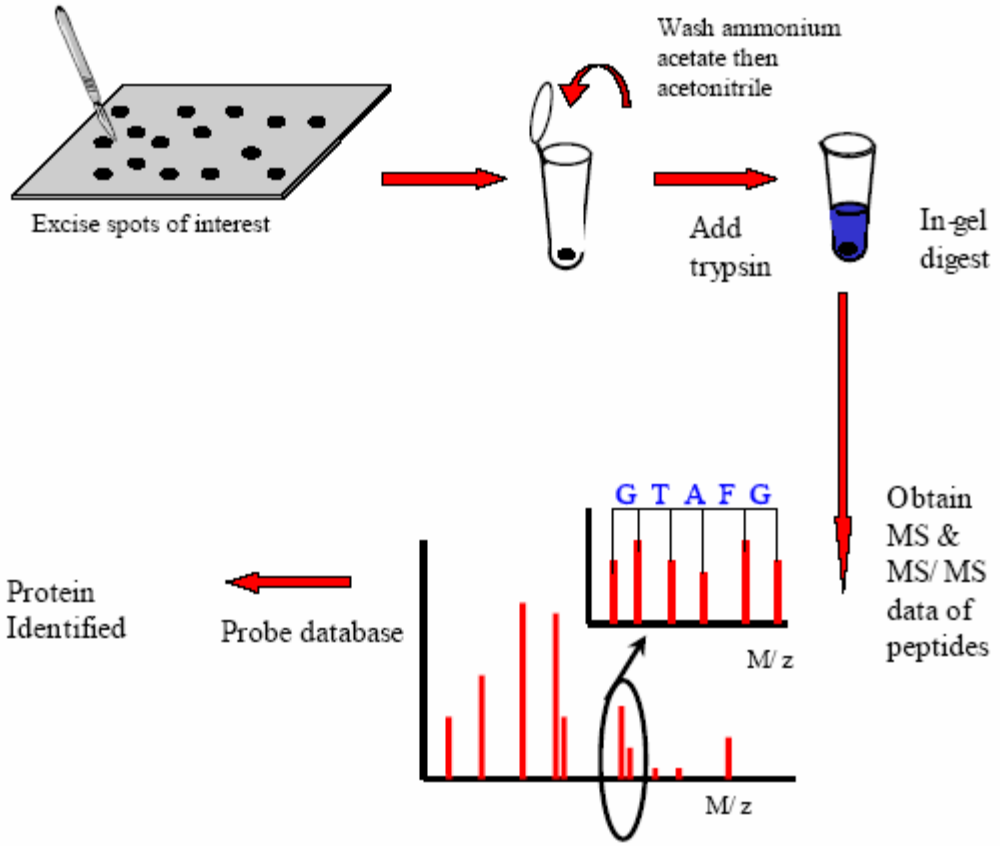
- Example of "Proteomic analysis"
- Protein identification may involve:
- MALDI Tof - Micro mx (peptide mass finger print)
- LC MSMS – QTof Premier with nano ACQUITY UPLC (protein sequencing)

Typical 2-D Gel showing normal and disease state



Identification of proteins from gels



LC MS of Peptides
Q-Tof micro™

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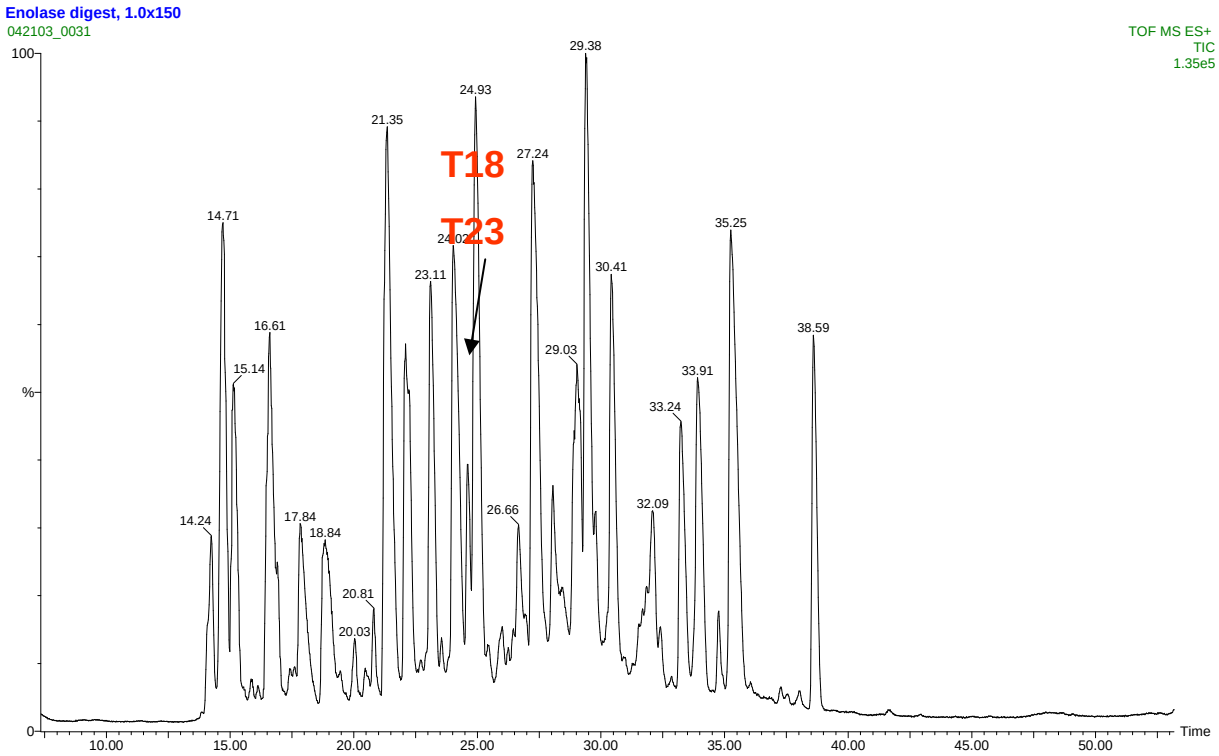

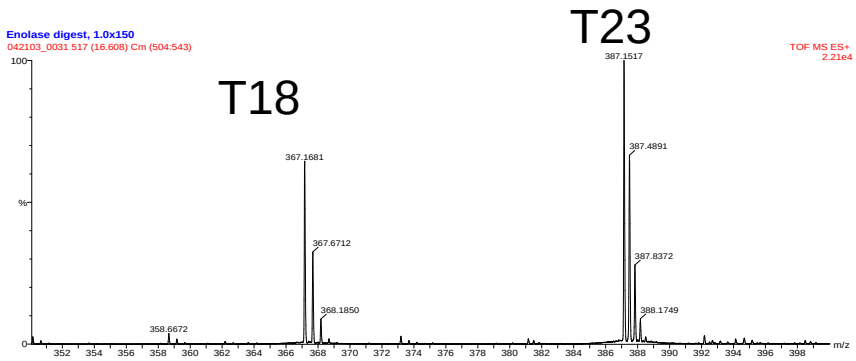

Peptide Sequencing
ESI MSMS data

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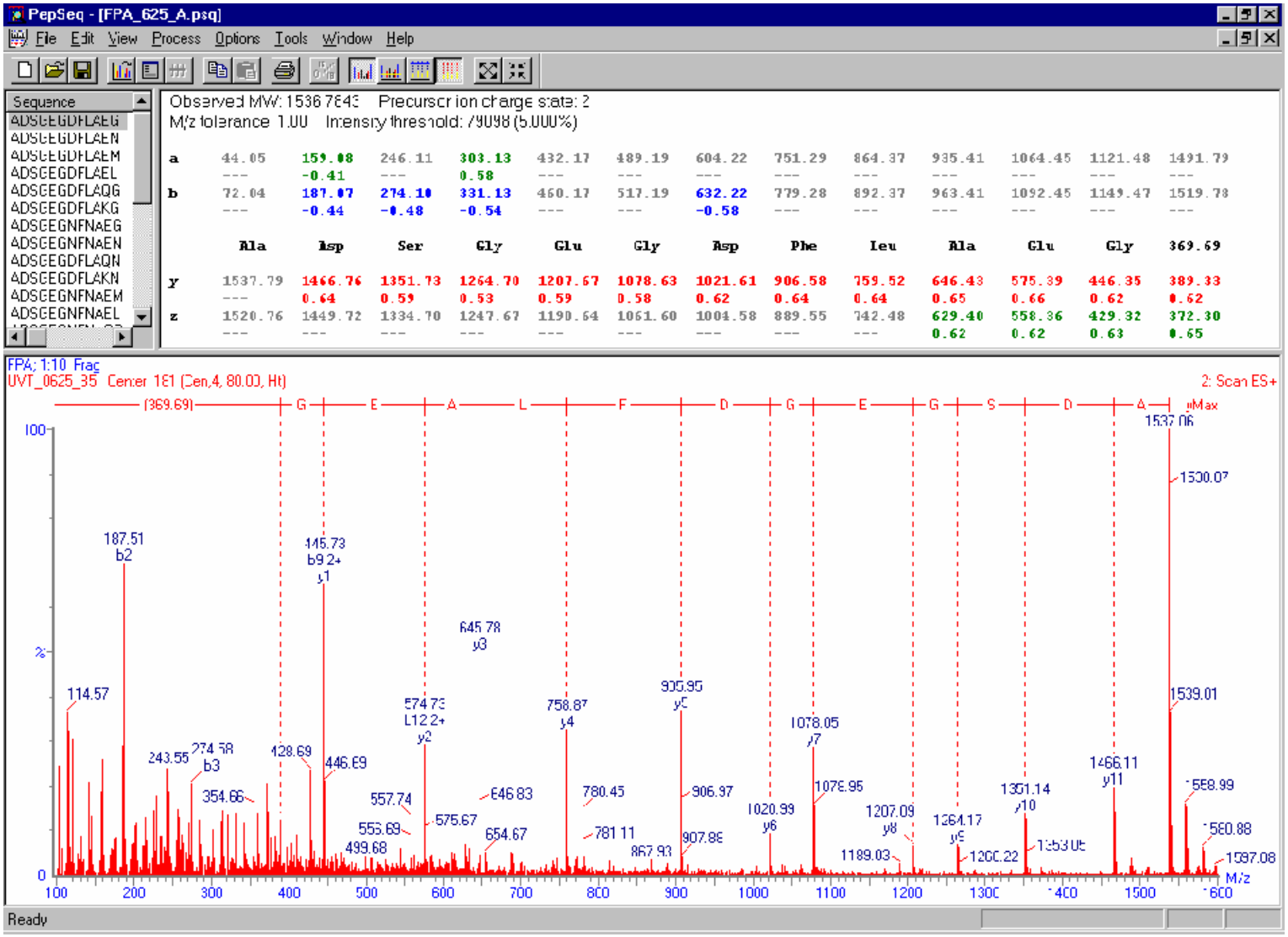

What does it do?

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SYNAPT™
High Definition **Mass Spectrometry**™

This enables the analysis of samples differentiated by size and shape and charge, as well as mass, to deliver increased specificity and sample definition

Enabling technology

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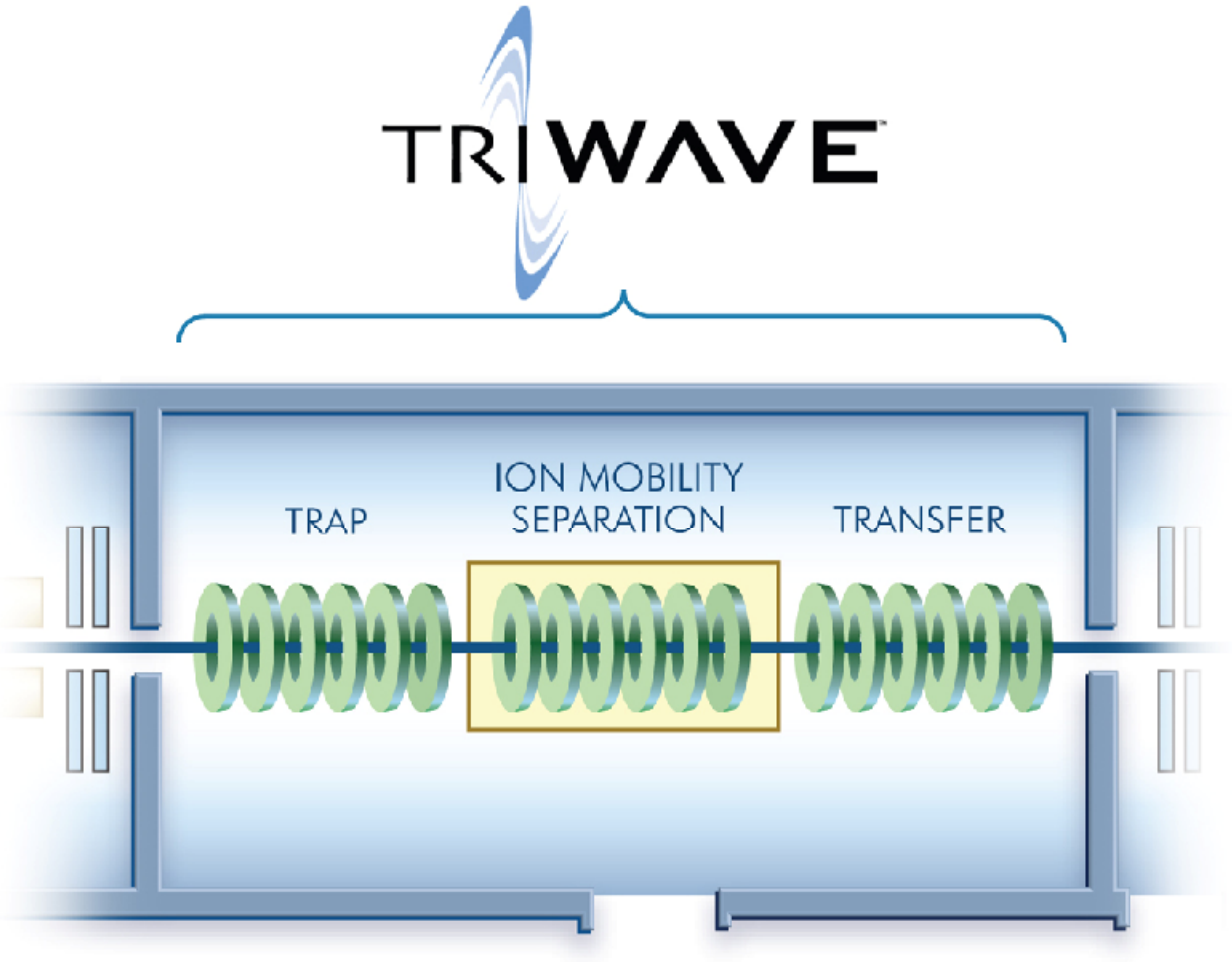

Synapt HDMS system

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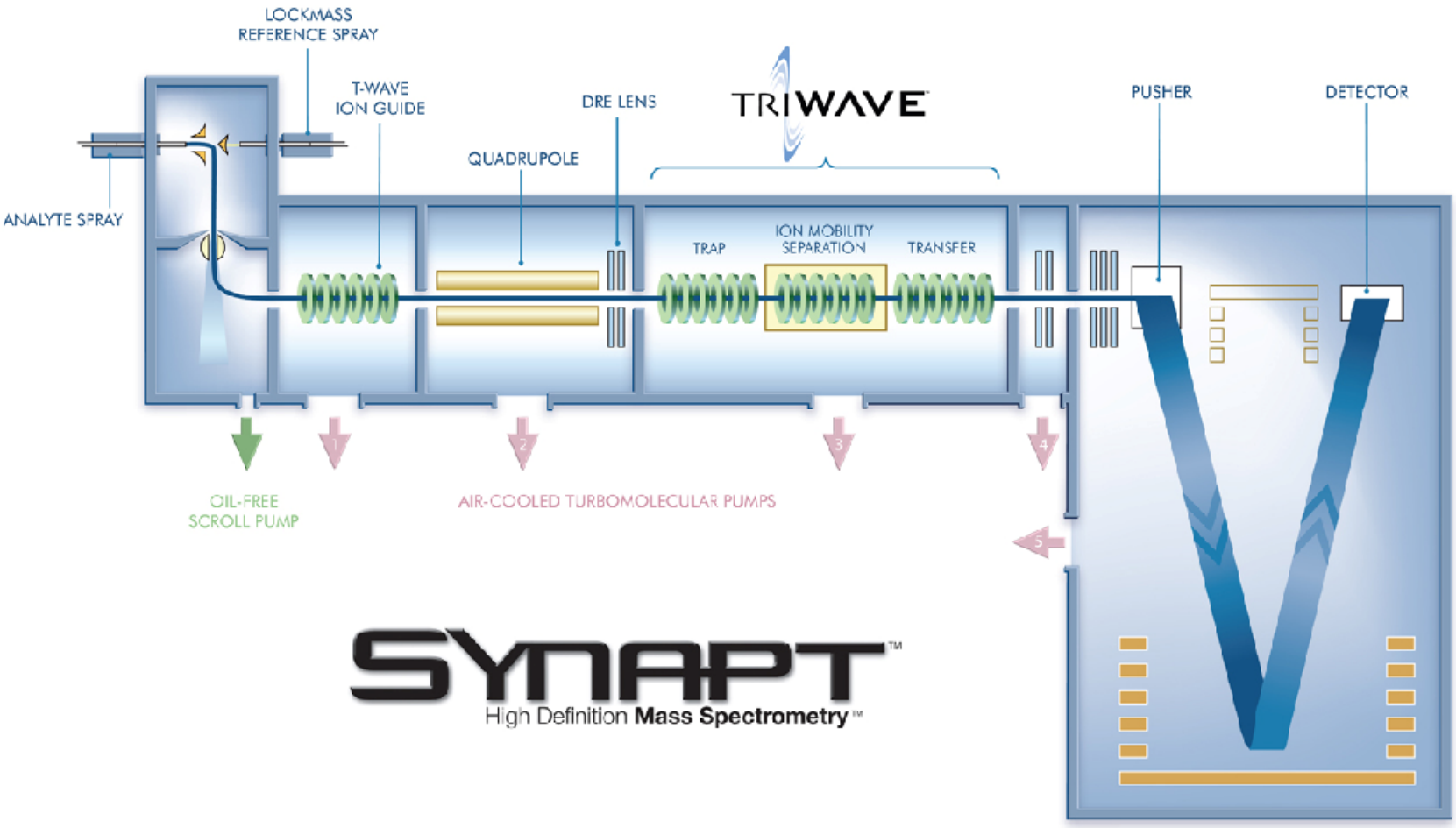

Haemoglobin drift time analysis

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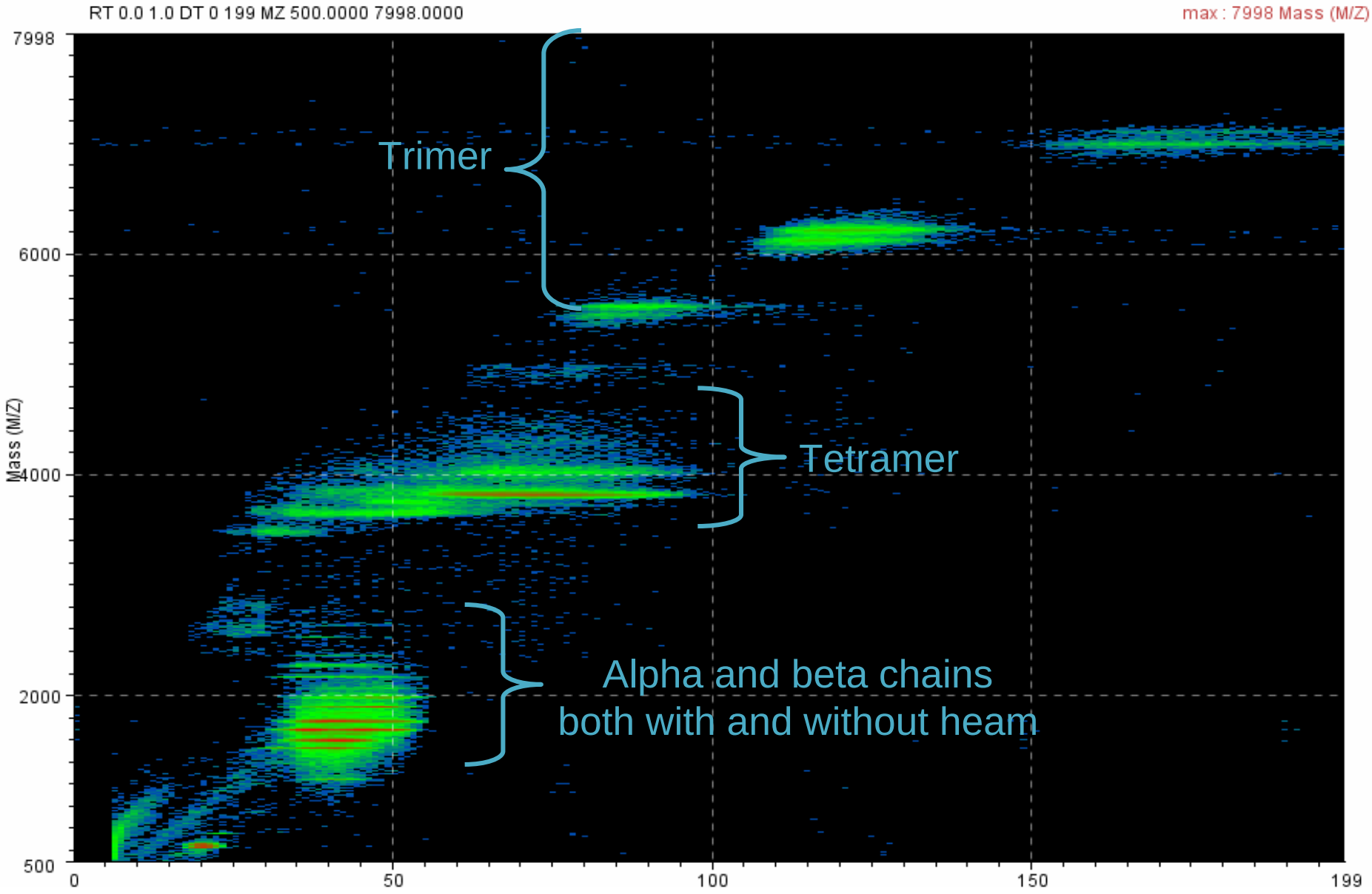

Time Aligned Parallel Fragmentation (TAP)

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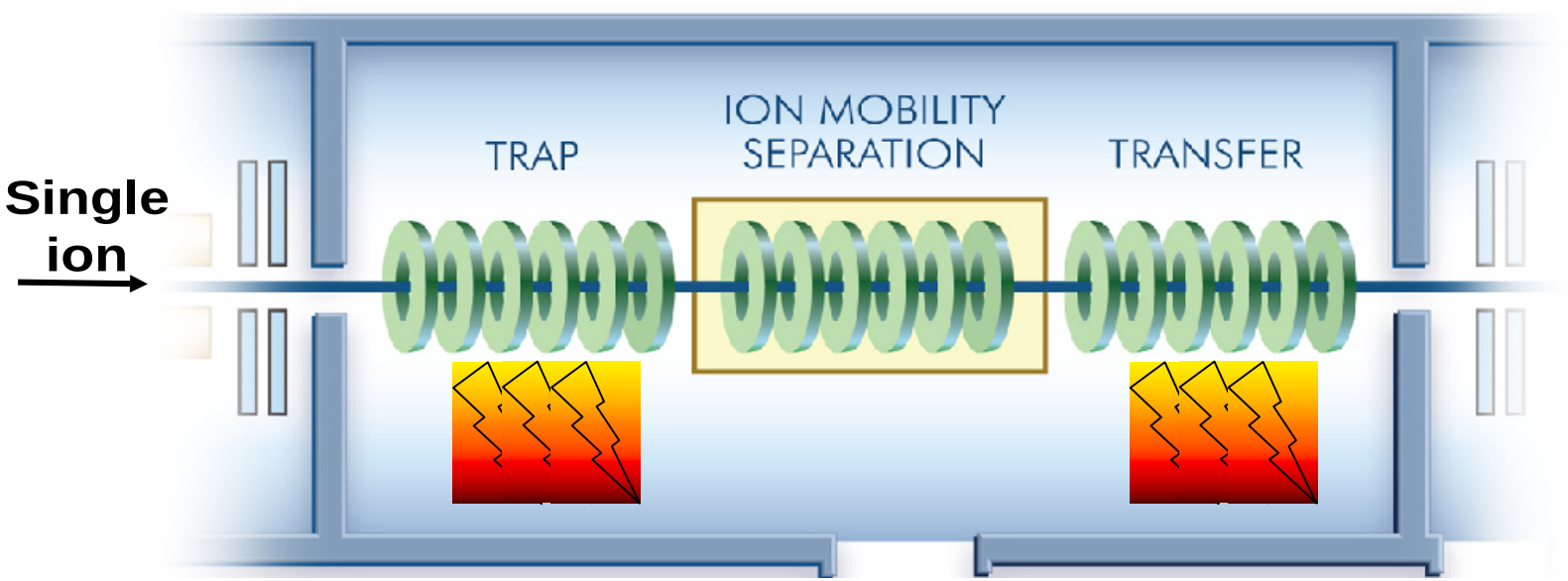

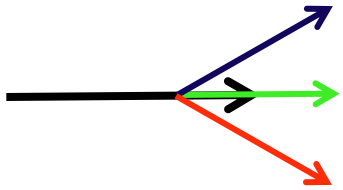

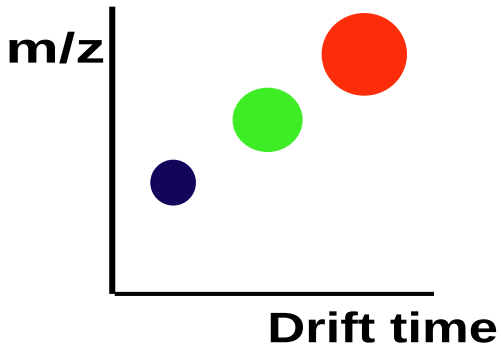

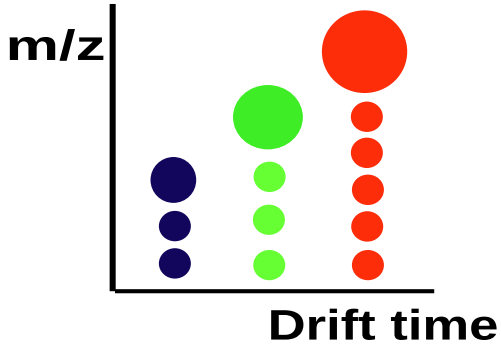

Precursor ion fragmented

Product ions separated by IMS

Precursor and products share same drift time

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

ACQUITY TQD featuring the Waters TQ Detector

SUMMARY

Waters
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- **In Chemistry at Queens**
- LCT P , GCT P , MALDI micro MX
- Variety of ionisation methods
- Flexible sample preparation and sample type
- TOF accurate mass measurement, elemental compostion and structural analysis
- Mass Spectrometry continues to evolve and will become more important in routine analysis

