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Q-Tof Premier

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- Objectives Arose From A Repeated Question At Previous FEBS Meetings (What Can MS Do For Me ?)
  - An Introduction To Mass Spectrometry (MS)
    - What Is MS, Types Of MS, Performance Characteristics
  - An Introduction To The Current Capabilities Of MS
    - Analysing Small Mols, Peptides, Proteins etc.
  - An Introduction To Some Of The Latest Technologies And Applications
    - Biomarker Discovery (Metabonomics And Proteomics), Future Technologies (Combining Shape And Mass ?)

Acknowledgements

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- Professor T.Ozden (Uni. Gazi, Turkey)
- Professor S.Gaskell (Uni. Manchester, UK)
- Professor A.Tishbee (Uni. Israel)
- Likrom (Istanbul, Ankara)
  - MS Course (1-2 Days)
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An Introduction To Mass Spectrometry (MS)

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What Is Mass Spectrometry ?

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- Mass spectrometry is an analytical tool used for measuring the molecular mass of a sample but it is capable of much more, as we shall see !
- For large molecules such as biomolecules**,** molecular masses can be measured to better than 0.01% *i.e.* within a 4 Daltons (Da) for a sample of 40,000 Da.
- This allows minor mass changes to be detected, *e.g.* the substitution of one amino acid for another, or a post-translational modification.

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- For small molecules (< 900 Da) the molecular mass can be measured to within an accuracy of 5 ppm or less,
- This is often sufficient to confirm the molecular formula of a compound, and is also a standard requirement for publication and patent submission.

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- Structural information can be generated using certain types of mass spectrometers, usually those with multiple analysers which are known as tandem mass spectrometers.
- This is achieved by fragmenting the sample inside the instrument and analysing the products generated.
- This procedure is useful for the structural elucidation of organic compounds, peptide or oligonucleotide sequencing.

Summary

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- Mass spectrometers determine the molecular weight of a compound which facilitates the:
  - Determination of modifications and substitutions of intact proteins
  - Determination of protein complex stoichiometry
  - Determination of elemental compositions
  - Determination of peptide and carbohydrate sequences
  - Structural Elucidation
  - Detection of compounds at low levels (femtomole sensitivity)
  - Quantification of target compounds (diagnostics, monitoring)
  - Much much more (biomarkers, purity, clinical) !

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Types Of Mass Spectrometer

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Types Of MS

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- There are many types of mass spectrometer
  - Quadrupole
  - Ion Trap (3D and 2D)
  - Tandem Quadrupole

Low Resolution

  - TOF (axial and orthogonal)
  - TOF/TOF
  - FT ICR/Magetic/Electrostatic Instrument
  - Q-TOF

High Resolution

- Each has its benefits but no one instrument is superior in every way to the rest and so…
- Depending on the application and performance criteria an instrument can be chosen.

Types Of MS

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There are several Classical Performance Criteria

- Sensitivity
- Dynamic Range
- Resolution
- Mass Accuracy

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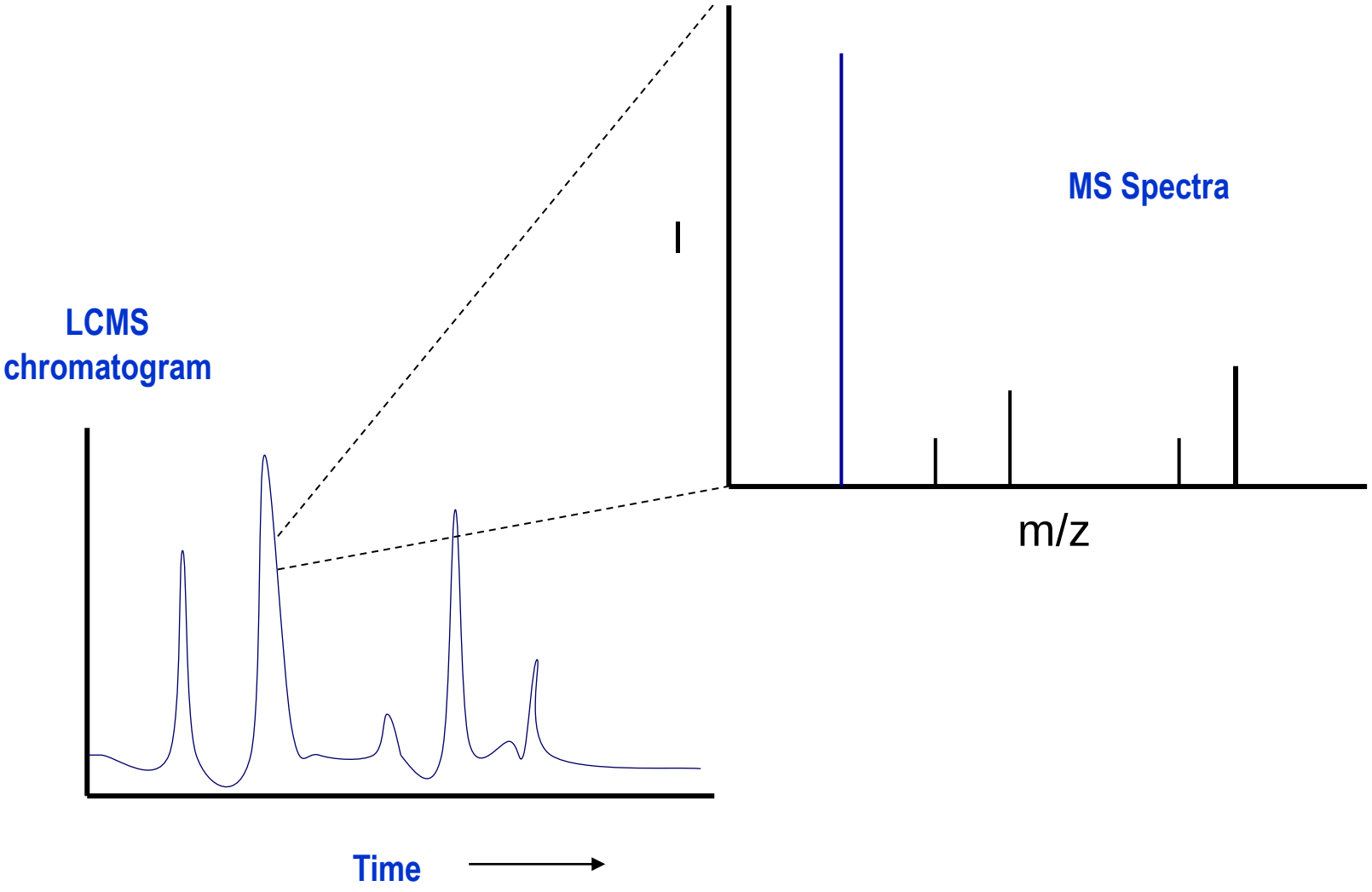

Spectral Resolution

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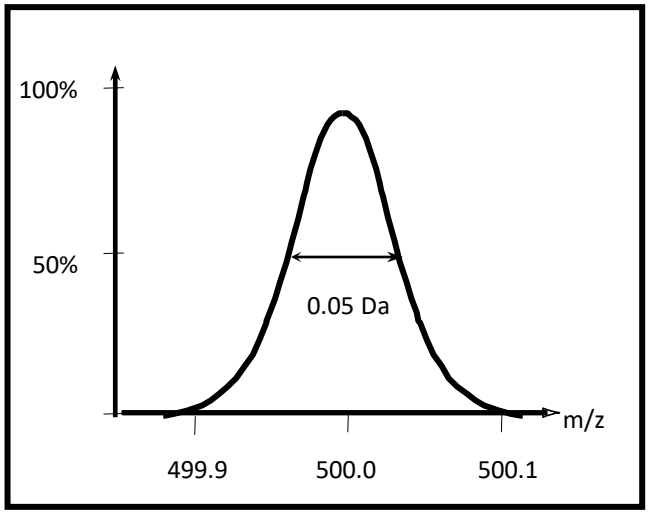

Mass = 500

Peak width (@ 50%) = 0.05Da

Resolution (FWHM) = $\frac{500}{0.05}$ = 10000

FWHM = Full Width Half Maximum

Resolution

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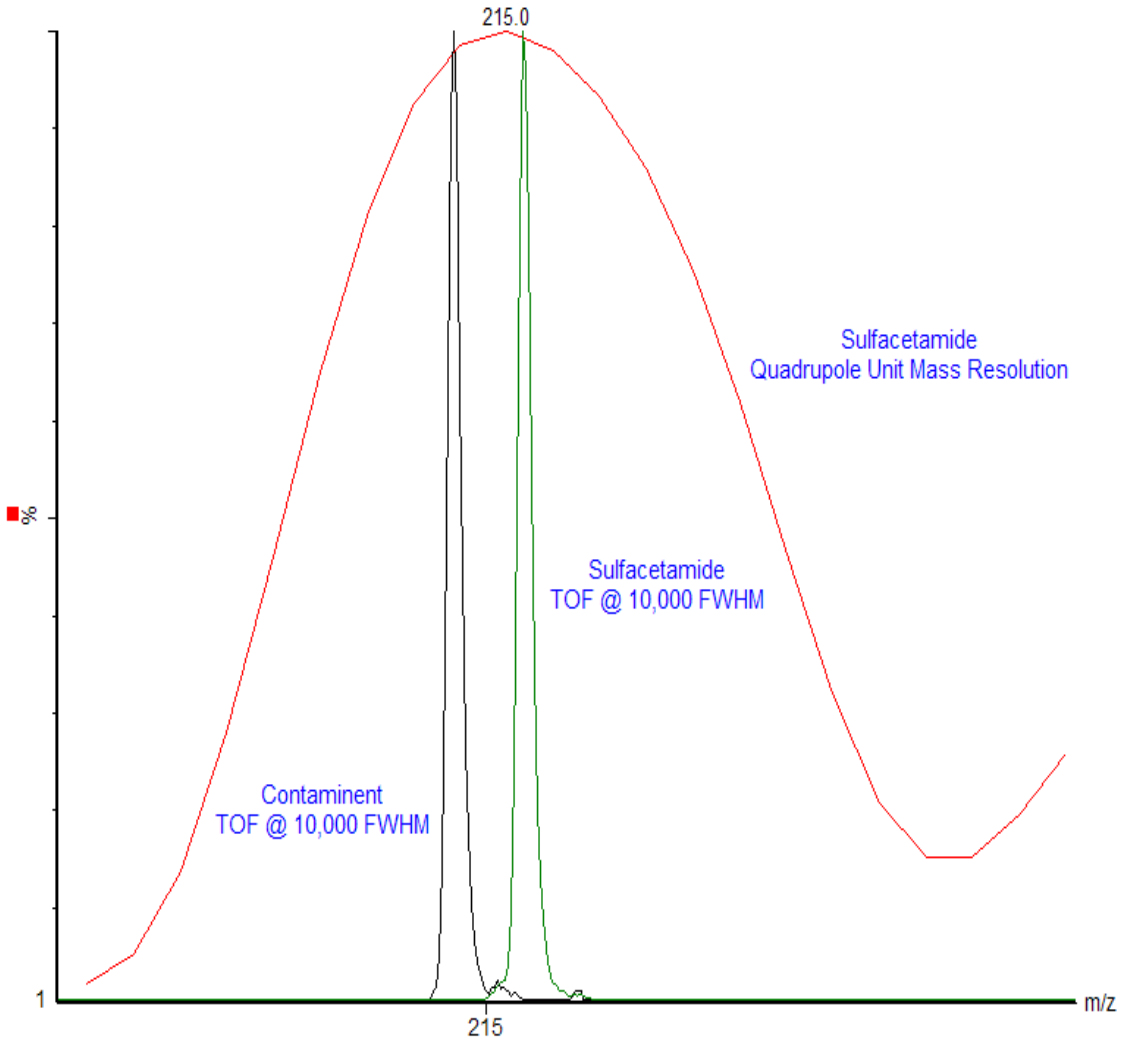

- Quadrupole resolution (quads, traps etc) is not sufficient to differentiate these two compounds
- data with a higher resolving power of (>10,000) clearly shows two distinct peaks.

Mass Accuracy

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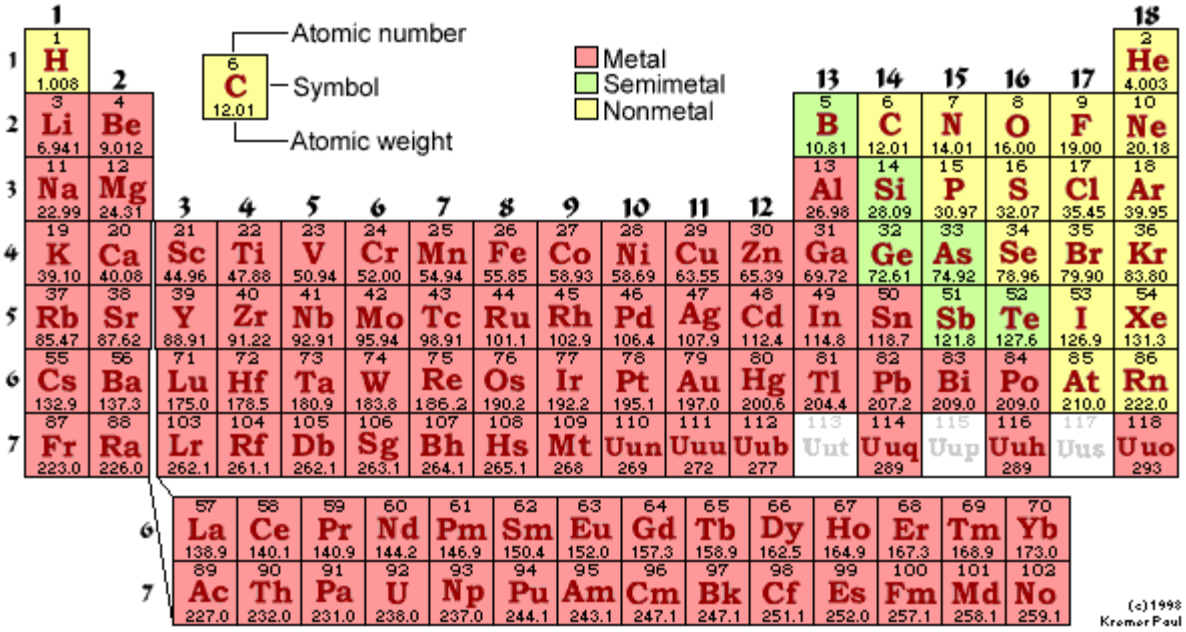

- Every element found in nature has a unique mass
- Elements are combined to produce compounds with distinct masses and physical properties
- Compounds can be detected by mass spectrometry and thus their masses measured
- If a compound mass can be measured with sufficient accuracy, a unique elemental composition can be inferred – the benefit of exact mass

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- carbon has a mass of 12
- hydrogen has a mass of 1
- oxygen has a mass of 16
- nitrogen has a mass of 14

- But this is not strictly true

The Fundamentals of Exact 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

- It is possible to have combinations of atoms which have the same nominal (or integer) mass but different accurate mass
- If such compounds can be mass measured with sufficient accuracy it is possible to determine elemental composition

Simple Examples

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- CO = 27.9949
- N_2 = 28.0061
- C_2H_4 = 28.0313

- These elemental combinations have the same nominal mass but different exact mass
- A nominal mass measurement cannot distinguish these
- If any compounds differ in their elemental compositions by substitution of any of these elements, then the exact mass measurement will show this

Mass Measurement Accuracy

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- The accuracy of the measurement is quoted as the difference (error) between the measured mass and the calculated mass
- The accuracy is measured in
  - milliDaltons (1mDa = 0.001 mass units)
  - ppm = parts per million = $\Delta m/m \times 10^6$

Example:

True' mass = 400.0000

Measured mass = 400.0020

Difference = 0.0020 (2 mDa)

$$\text{ppm error} = \frac{0.002}{400} \times 10^6 = 5 \text{ ppm}$$

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What are the Benefits of Exact Mass?

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- Measurement of mass to 4 decimal places
- High confidence in confirming expected compounds
  - Distinguishes them from compounds of similar mass
- Compound identification
  - Prediction of elemental composition
- Patent submission and publication
  - ACS require better than 5ppm mass accuracy for publication

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Software is key !

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- Mass spectrometric technology advances typically relate to performance characteristics (sensitivity, resolution, mass accuracy etc.)
- These characteristics although indicative of the data quality and therefore the information content that can be acquired by the mass spectrometer are not necessarily guarantees of the data quality and information content that is actually acquired.
- Advanced software has now become a key performance characteristic of MS instrumentation (guaranteeing data quality, simplifying application processes, facilitating the extraction of knowledge)

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An Introduction To The Current Capabilities Of MS

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Current Capabilities

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- Determination of elemental compositions
- Structural Elucidation
- Determination of modifications and substitutions of intact proteins
- Determination of protein complex stoichiometry
- Determination of peptide and carbohydrate sequences
- Quantification of target compounds (diagnostics, monitoring)

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Determination of elemental compositions and Structural Elucidation

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Isotope Filters

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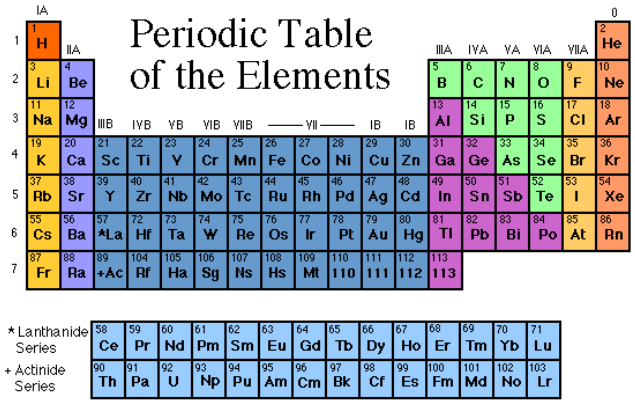
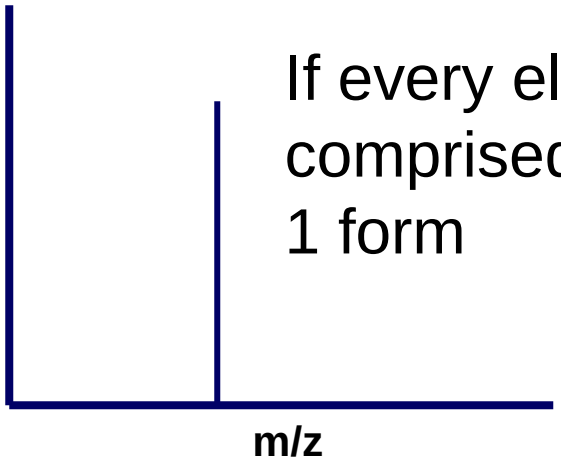
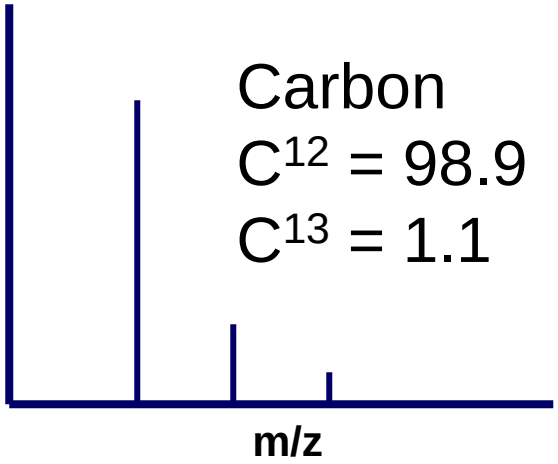




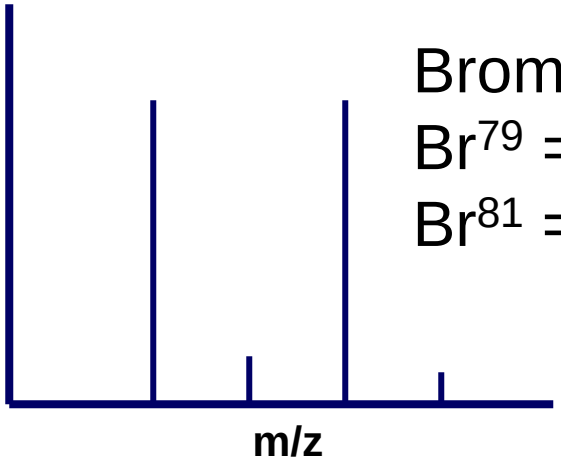
If every element comprised of only 1 form



Carbon

$C^{12} = 98.9$

$C^{13} = 1.1$



Bromine

$Br^{79} = 50.7$

$Br^{81} = 49.3$

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Elemental Composition

File Edit View Process Help

Single Mass Analysis

Tolerance = 3.0 PPM / DBE: min = -1.5, max = 50.0

Element prediction: On

Monoisotopic Mass, Even Electron Ions

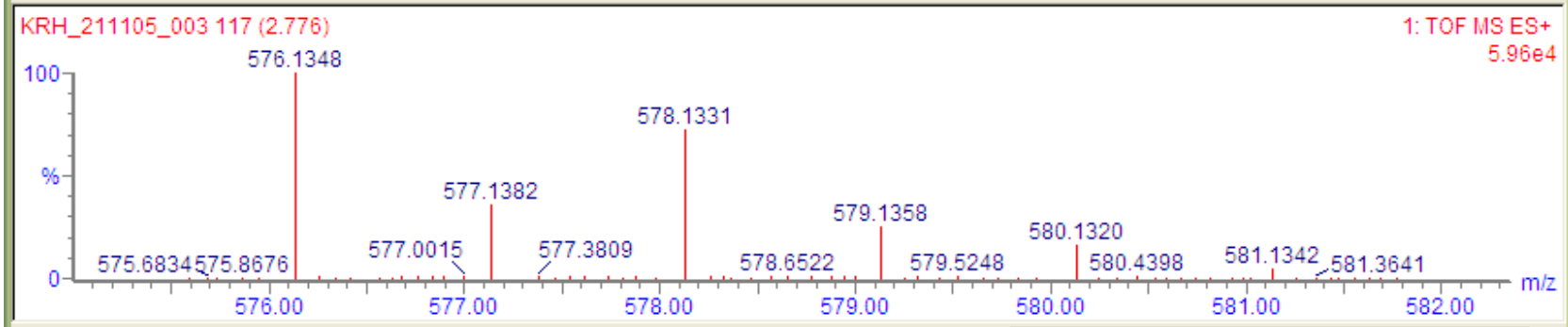
581167 formula(e) evaluated with 3 results within limits (all results (up to 1000) for each mass)

Elements Used:

C: 0-500 H: 0-1000 N: 0-200 O: 0-200 F: 0-100 S: 0-4

Cl: 0-8 Br: 0-8

Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	C	H	N	O	F	S	Cl	Br
576.1348	576.1345	0.3	0.5	18.5	C32 H28 N O5 Cl2	3.7	32	28	1	5			2	
	576.1356	-0.8	-1.4	14.5	C29 H29 N O6 F Cl2	50.0	29	29	1	6	1		2	
	576.1345	0.3	0.5	16.5	C28 H24 N5 F4 Cl2	53.4	28	24	5		4		2	



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Determination of modifications and substitutions of intact proteins

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Overview of Protein Electrospray Ionisation

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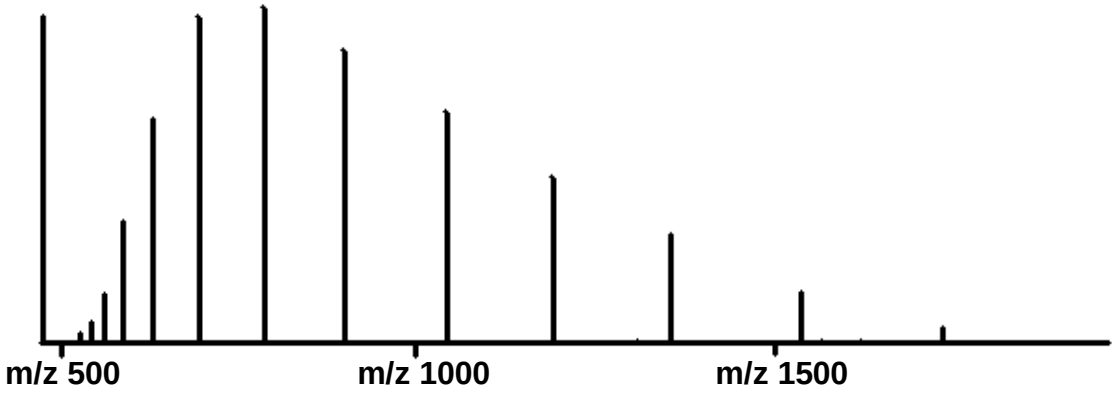
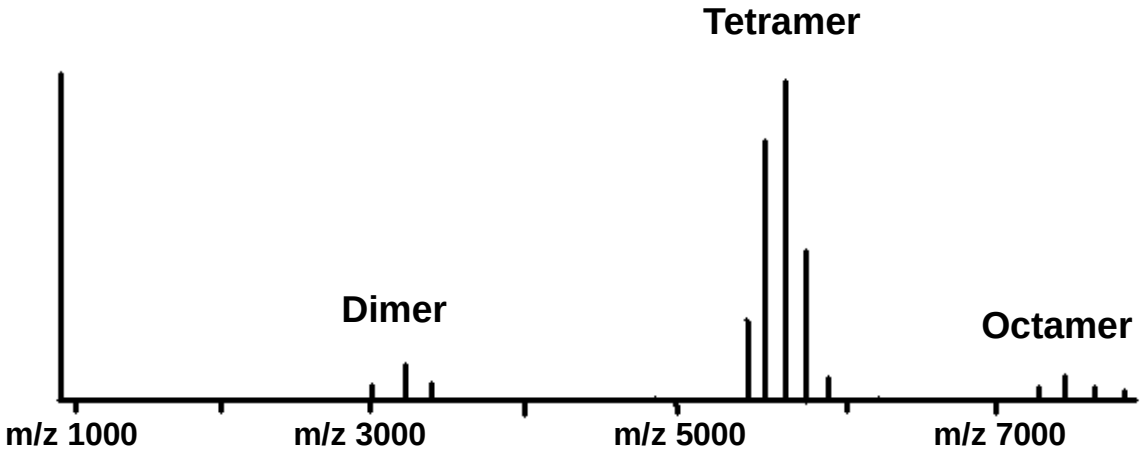


Protein (1pmol/uL) in 50% ACN 0.1%HCOOH, pH ~ 3

Standard source pressures ~1.8 e0mbar



Protein (5-10pmol/uL) in aqueous 10mM ammonium acetate, pH ~ 7

Increased source pressure ~10 e0mbar

Determining the Charge State of a Peak and Mass of the Protein

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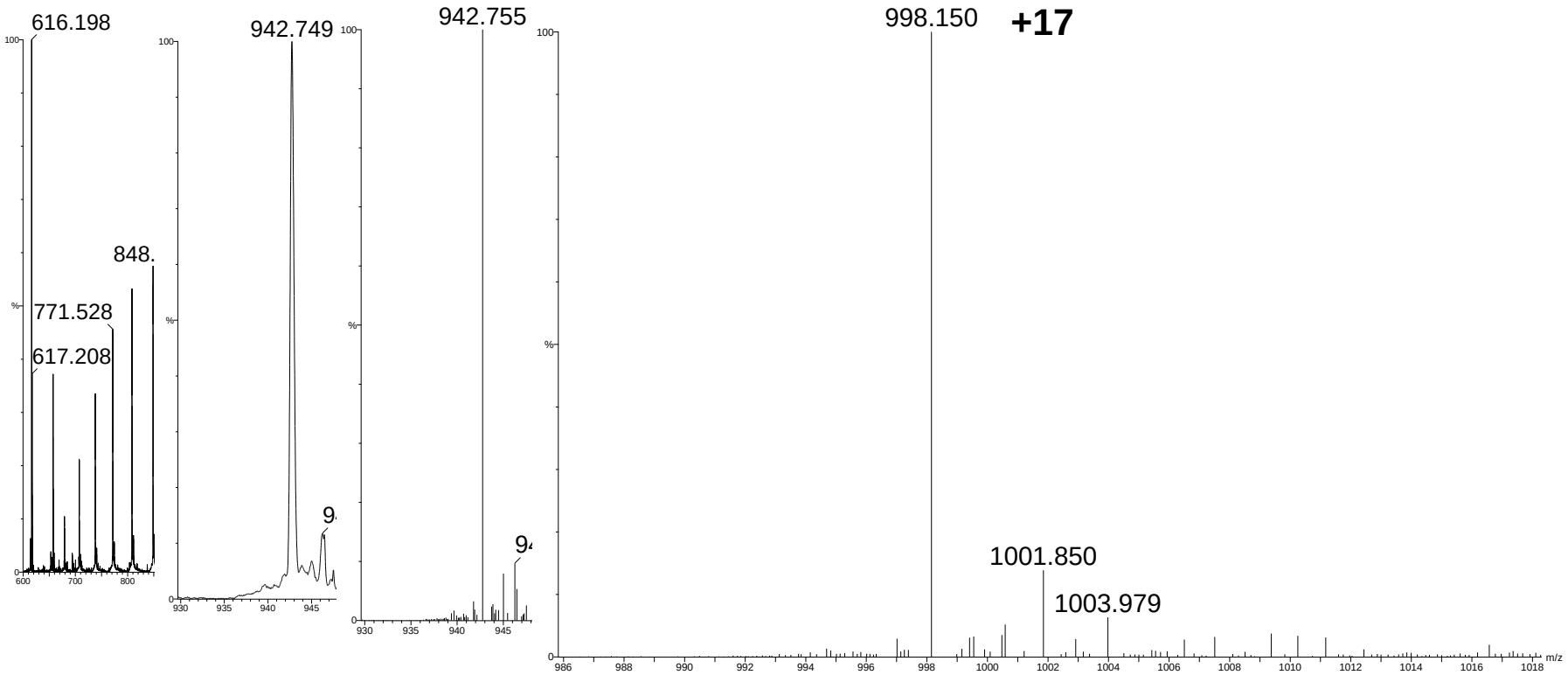

Protein (1pmol/uL)
50% ACN
0.1%HCOOH, pH ~ 3

n = (Mn + 1) – H / Mn – (Mn+1)

n = 942.755 – 1.00794 / 998.150 – 942.755

n = 17.0

998.150 x 17 – (17 x 1.00794) = 16951.415

Maximum Entropy Software

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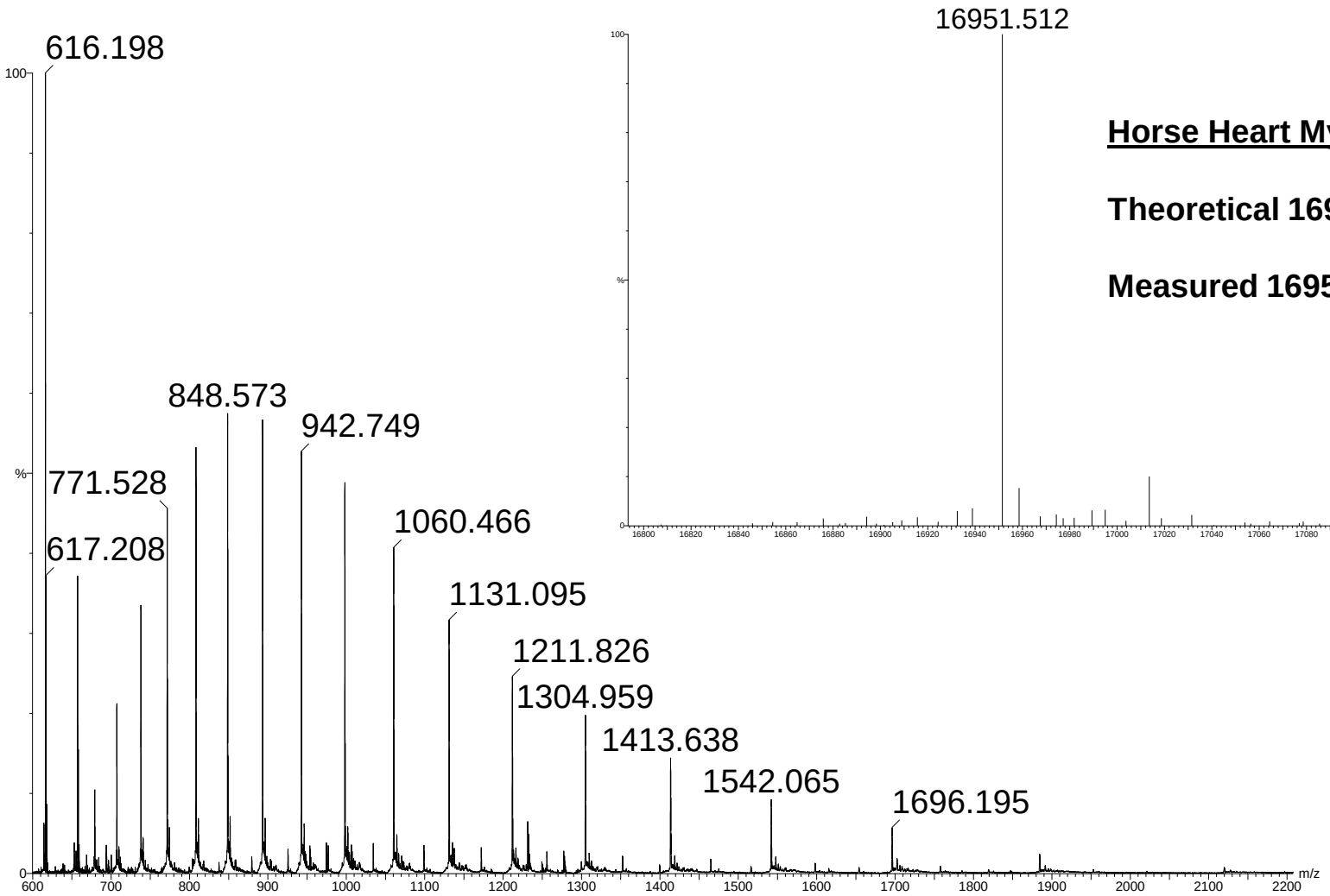

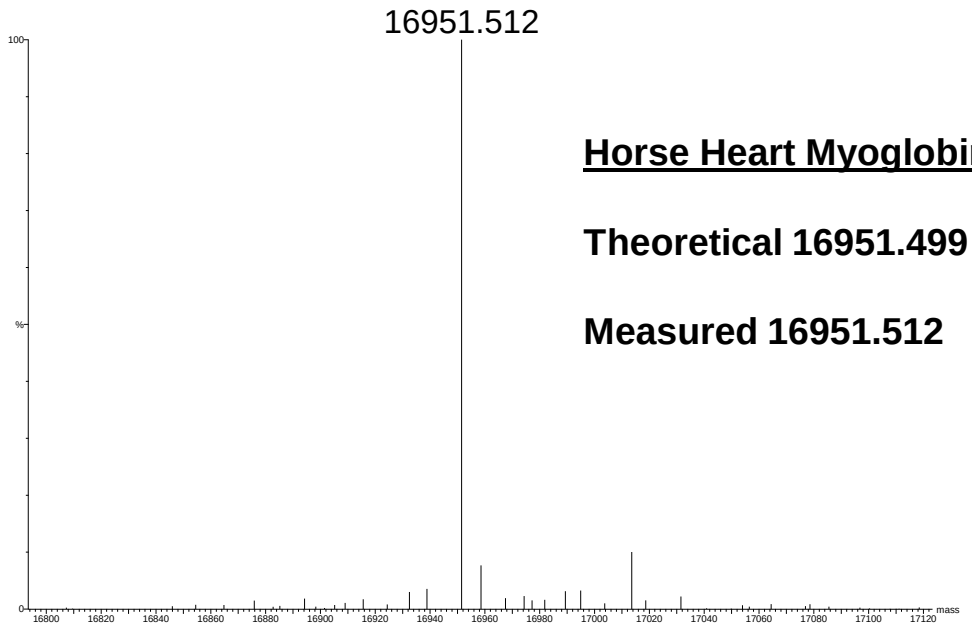

Horse Heart Myoglobin

Theoretical 16951.499

Measured 16951.512

Proteasome 20S

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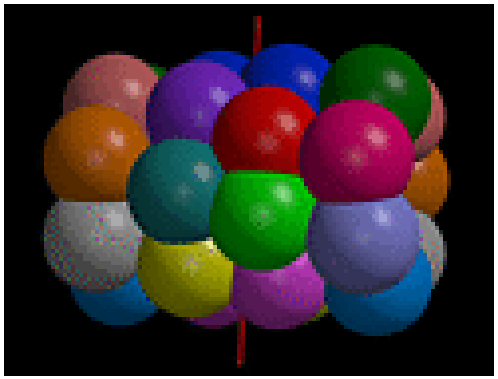
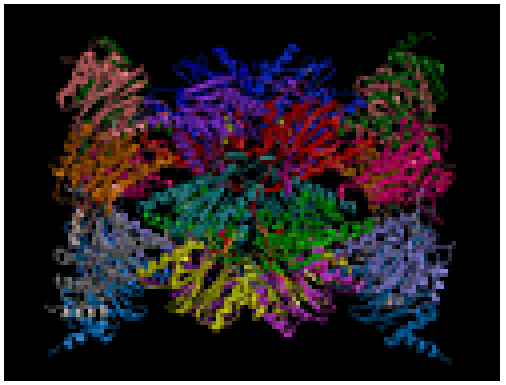
Proteasome 20S

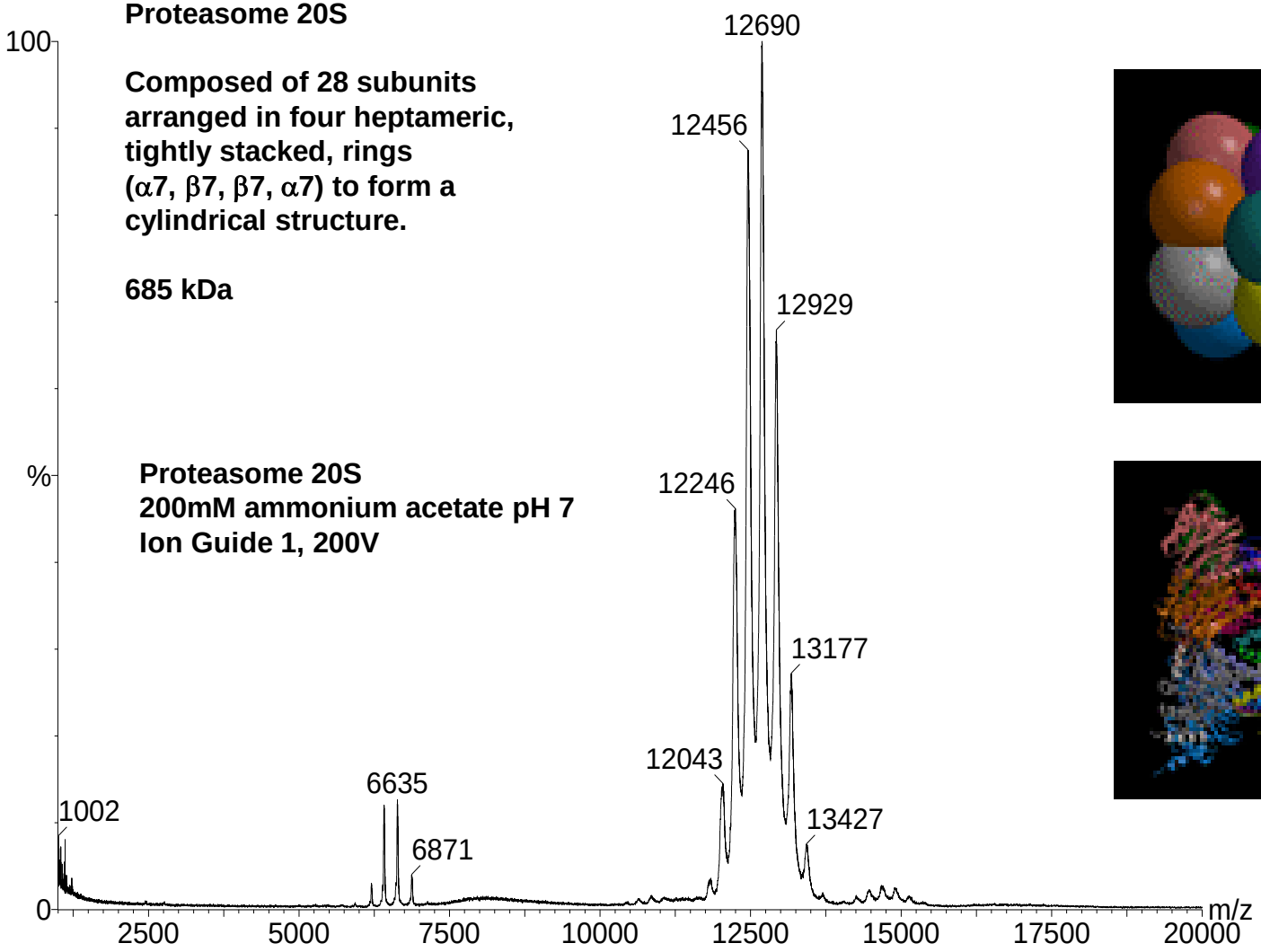
Composed of 28 subunits arranged in four heptameric, tightly stacked, rings (α7, β7, β7, α7) to form a cylindrical structure.

685 kDa



Proteasome 20S
200mM ammonium acetate pH 7
Ion Guide 1, 200V





PttXET16A (Mutant E85A)

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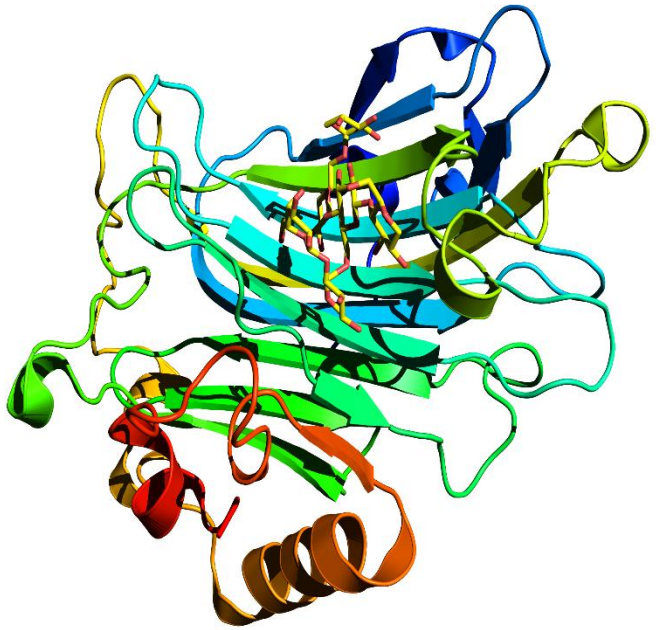
Xyloglucan endotransglycosylases (XET, EC 2.4.1.207) are unique enzymes that perform an endolytic cleavage of a xyloglucan chain. This activity has been proposed to play a major role in the transient cell wall loosening required for cell wall expansion.

Recent evidence suggests that XET activity may also contribute to reinforcing the connections between primary and secondary cell walls in wood-forming tissues.



Populus tremula x tremuloides Xyloglucan Endotransglycosylase 16A (PttXET16A E85A). Glycosylation of this enzyme is heterogeneous

Over expressed in *P. pastoris*

YVAALRKPVDVAFGRNYVPTWAFDHIKYFNGGNEIQLHLDKYTGTGFQSKGSYLFGHFSMQM
KLVPGDSAGTVTAFYLSSQNSEHDAIDFEFLGNRTGQPYILQTNVFTGGKGDREQRIYLWFDP
TKEFHYYSVLWNMYMIVFLVDDVPIRVFKNCKDLGVKFPFNQPMKIYSSLWNADDWATRGGL
EKTDWSKAPFIASYRSFHIDGCEASVEAKFCATQGARWWDQKEFQDLDAFQYRRLSWVRQK
YTIYNYCTDRSRYPSMPPECKRDRDI

YV; signal sequence, A; mutation, NRT; glycosylation site

Average Mr 32088.271

Analysis of PttXET16A (E85A) under non-denaturing conditions

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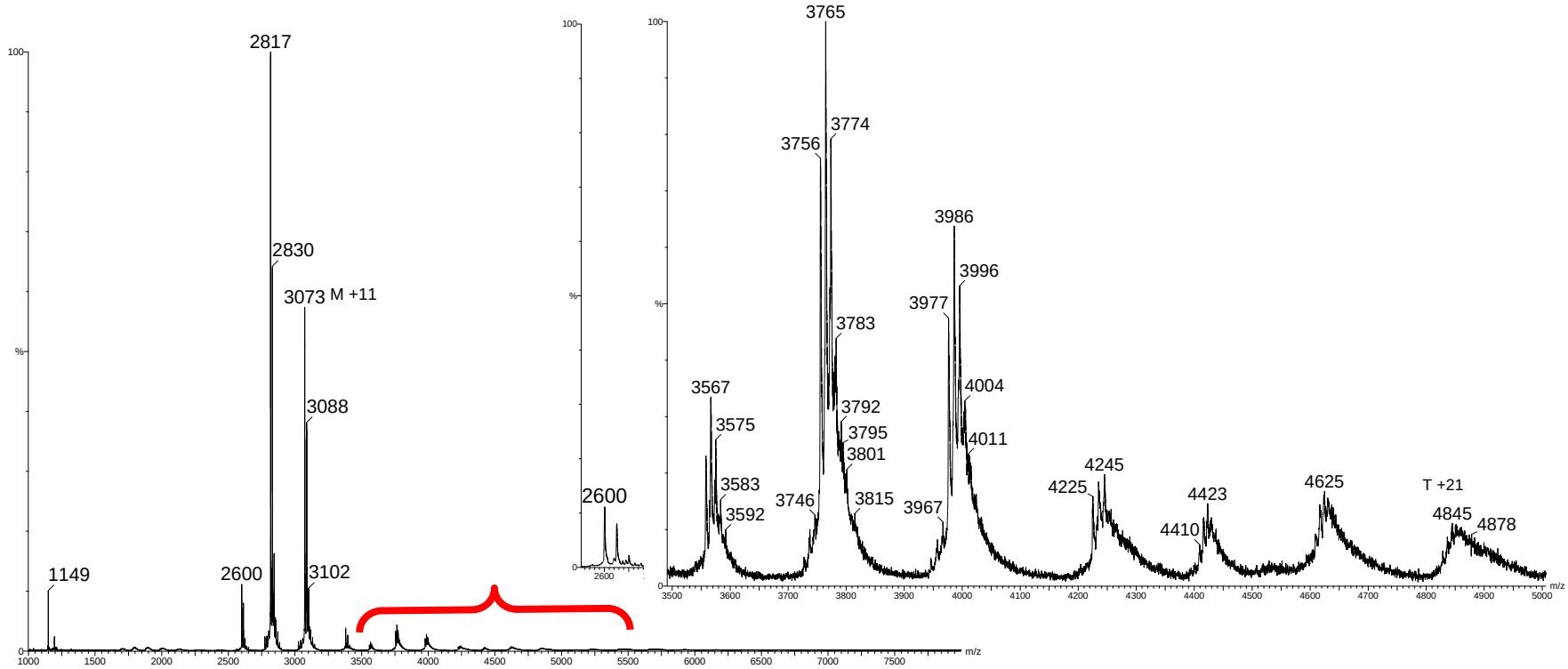

Infusion of PttXET16A (E85A) into LCT Premier at 10pmol/uL
10mM ammonium acetate, pH 5.5, Ion Tunnel 1 150V

Predominant species observed is the heterogeneously glycosylated monomer 33,792 Da

PttXET16A (E85A) Glycosylation

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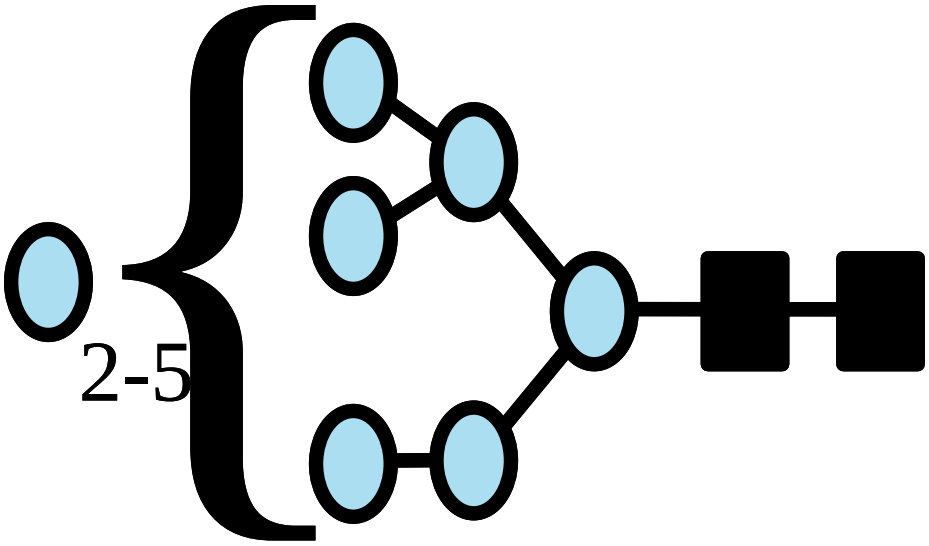

N-Linked high mannose oligosacharide PTM bound to conserved sequence NRT

2NacHex8-11Hex

Mass increments observed above sequence mass 32088.271Da :

1703.529; 2NacHex8Hex

1865.672; 2NacHex9Hex

2027.814; 2NacHex10Hex

2189.956; 2NacHex11Hex

Electrospray oaToF Spectrum of PttXET16A (E85A)

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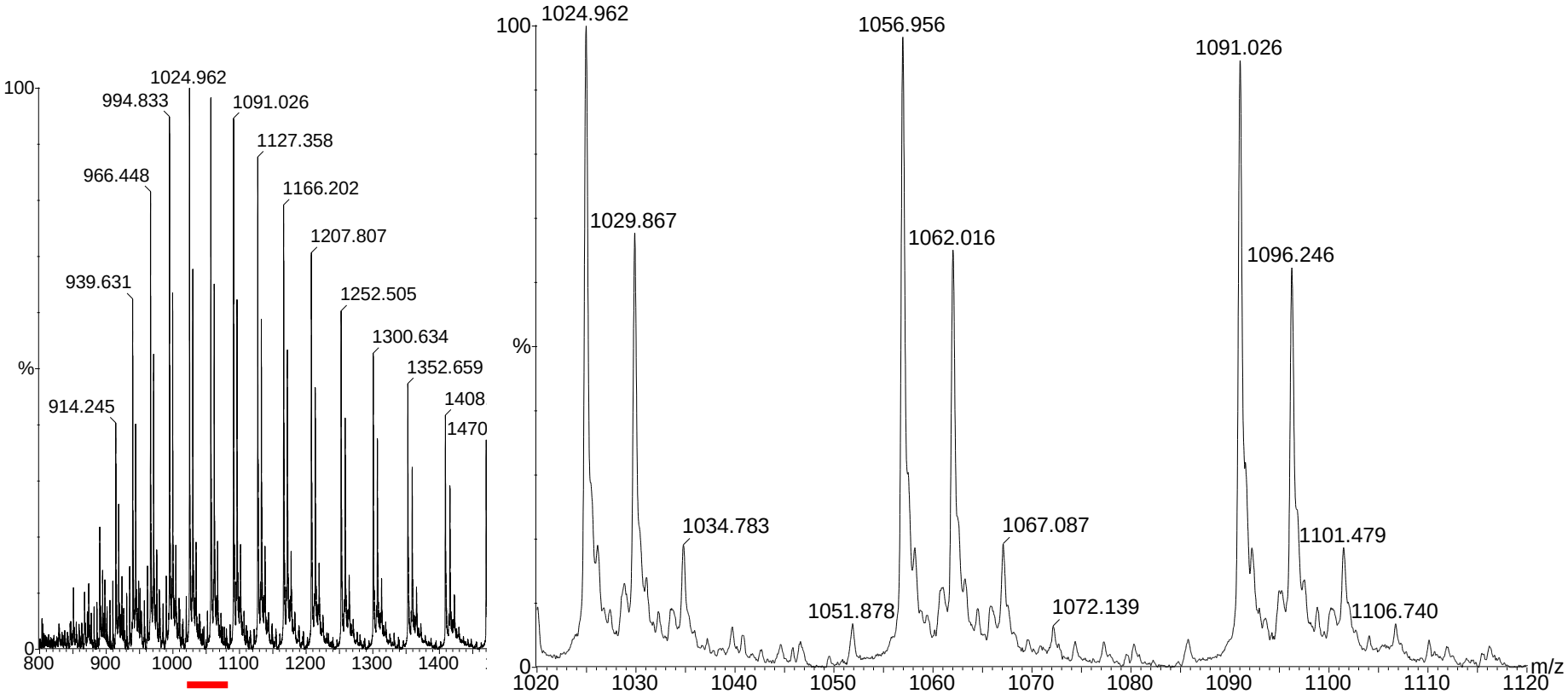

Maximum Entropy Deconvolution

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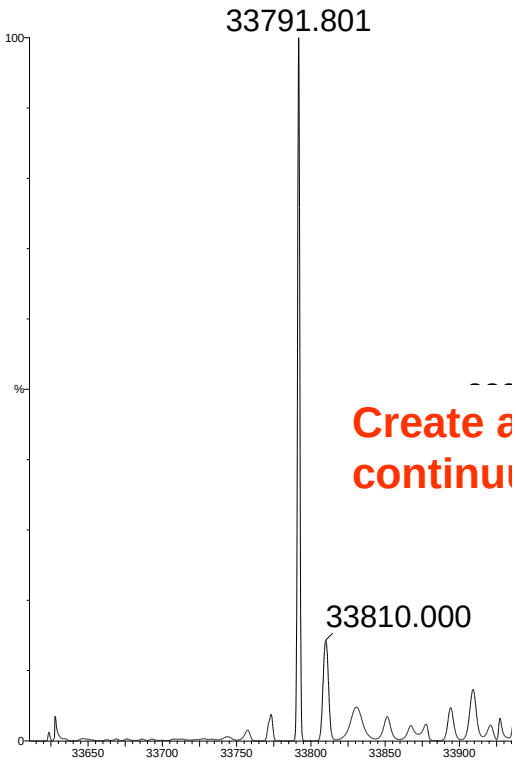

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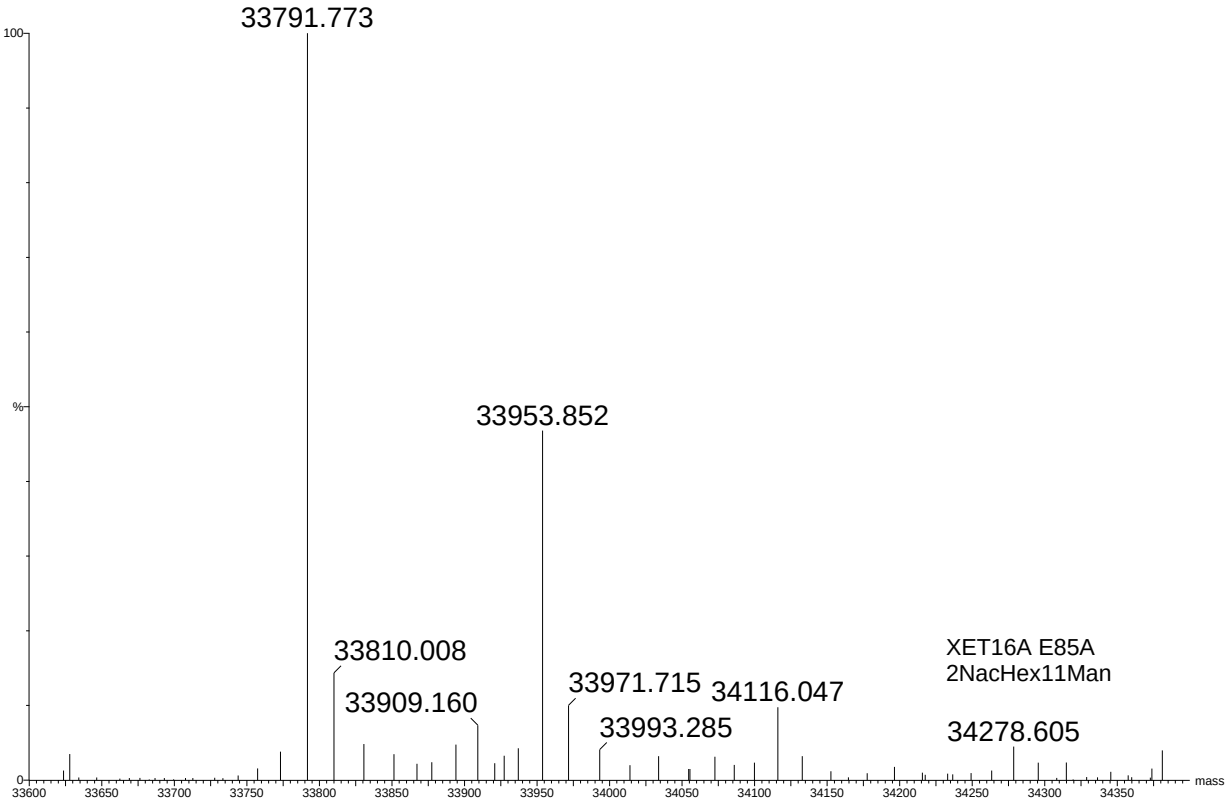

Mass Errors Obtained after Maximum Entropy Deconcolution

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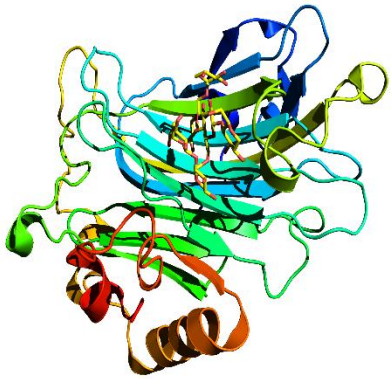
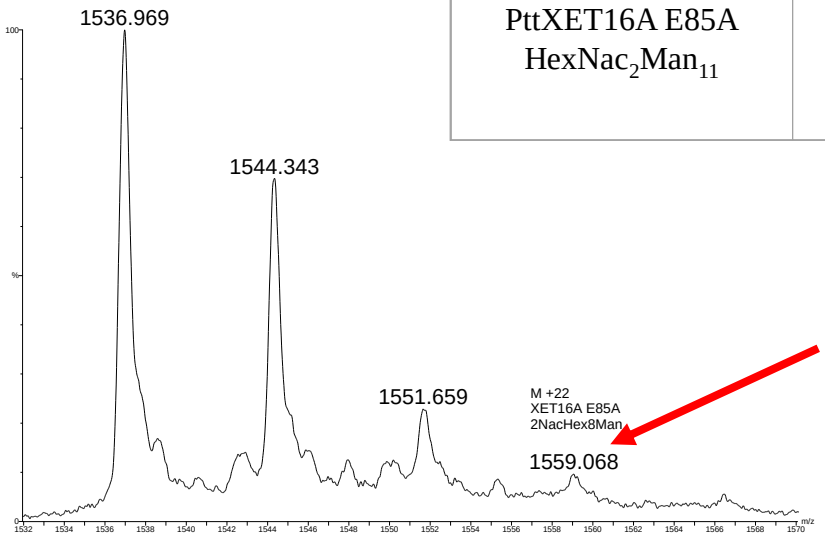


Protein analysed	Expected Molecular Weight (Da)	Mean Measured Molecular Weight ±SD (Da)	Error mDa / ppm
PttXET16A E85A $HexNac_2Man_8$	33791.800	33791.792 ± 0.034 ±1.0 ppm	-8.0 / -0.3
PttXET16A E85A $HexNac_2Man_9$	33953.943	33953.854 ± 0.043 ±1.3 ppm	-89.0 / -2.6
PttXET16A E85A $HexNac_2Man_{10}$	34116.085	34116.081 ± 0.024 ±0.7 ppm	-4.0 / -0.1
PttXET16A E85A $HexNac_2Man_{11}$	34278.228	34278.620 ± 0.137 ±3.9 ppm	392.0 / 11.4



Intensity of this peak is v low, therefore poor mass errors

Acknowledgements

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Marten Snel

Brian Green

Harry Brumer, Kathleen Peins & Martin Baumann

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Determination of protein complex stoichiometry

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Human Hemoglobin

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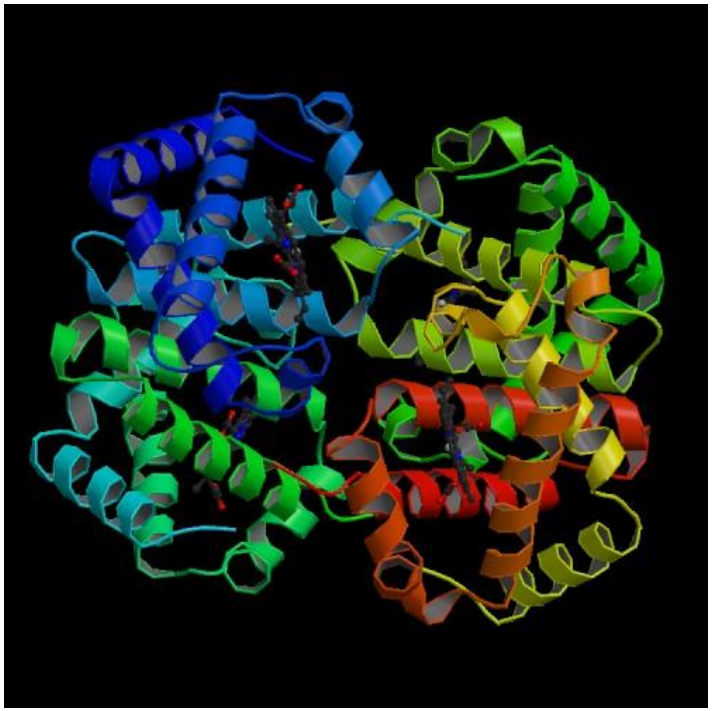
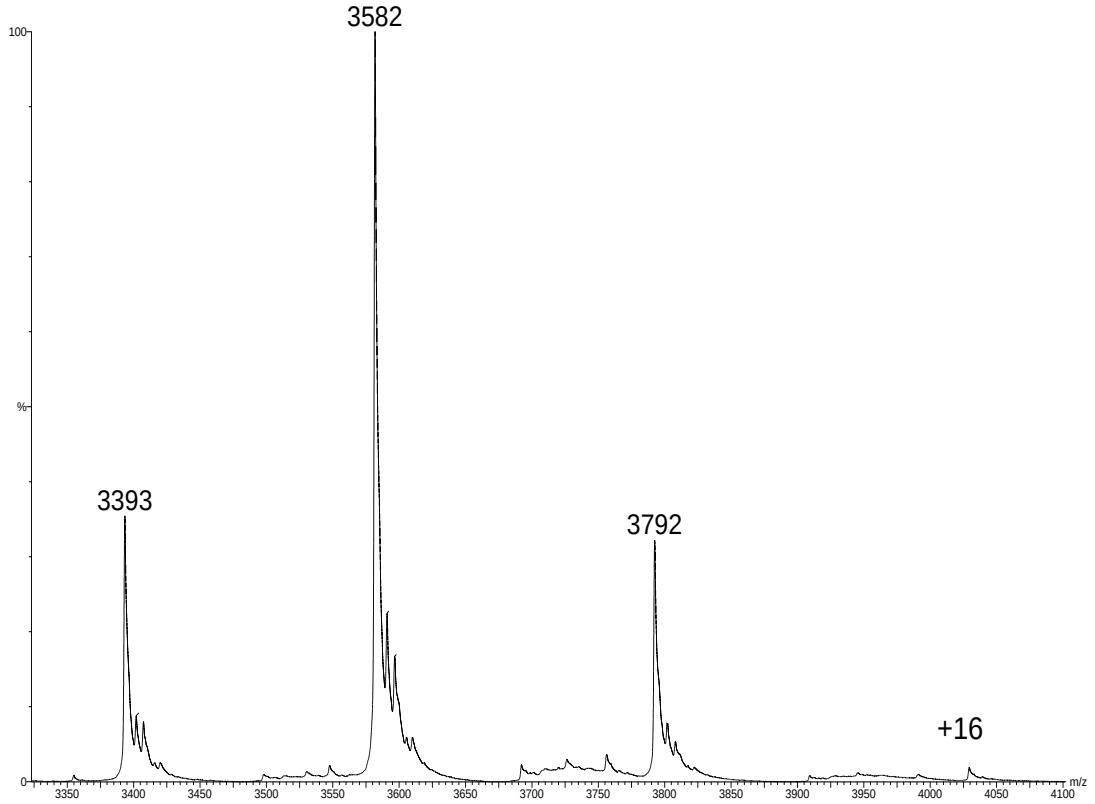


Human Deoxy Hemoglobin

http://www.rcsb.org/pdb



Human blood diluted 200x
10mM ammonium acetate, pH 7.4
Ion Guide 1, 70V

Human Hemoglobin analysed with Ion Guide 1 set to 105V

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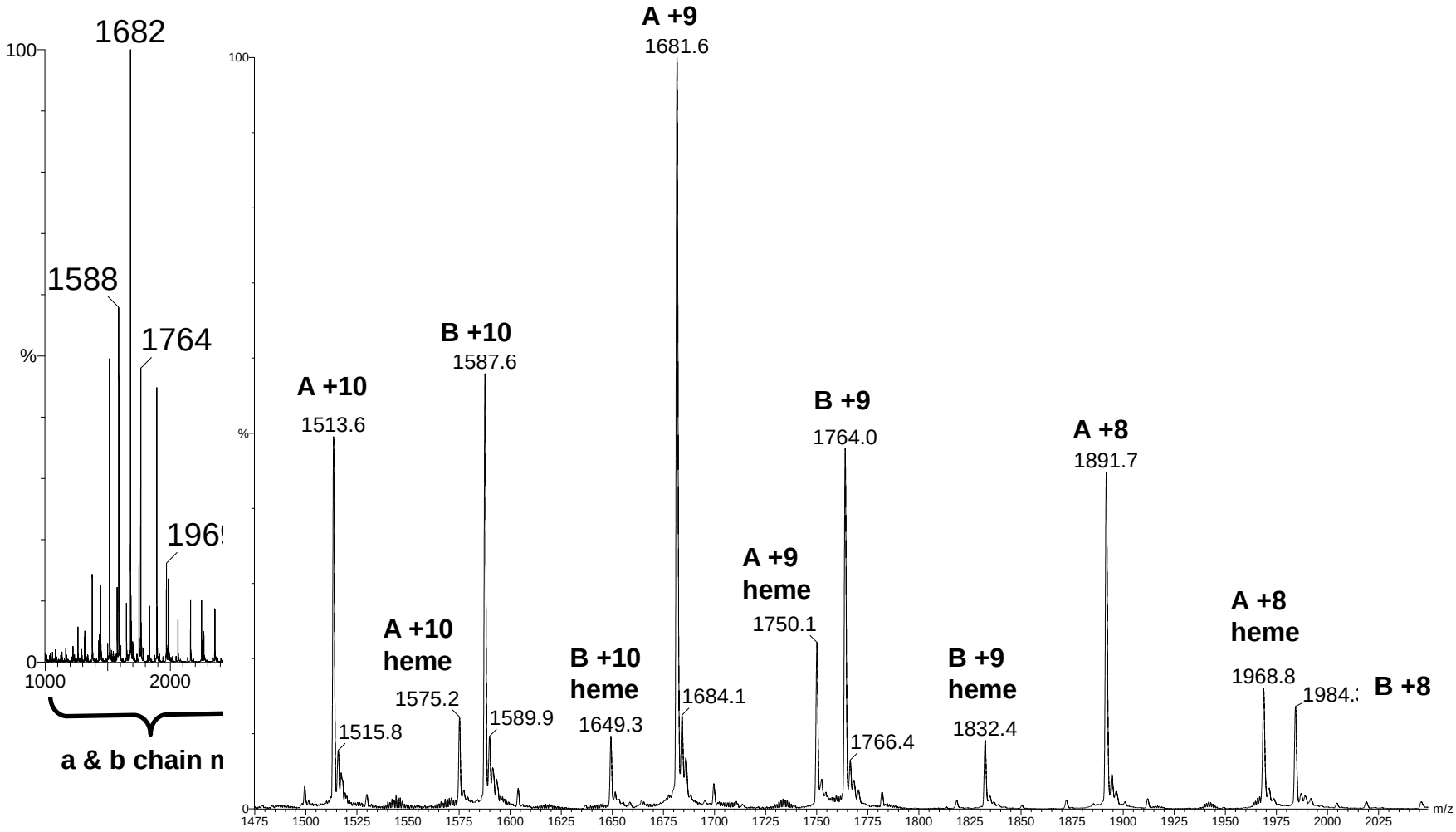

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Determination of peptide and carbohydrate sequences

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Data Dependant Acquisition (DDA)

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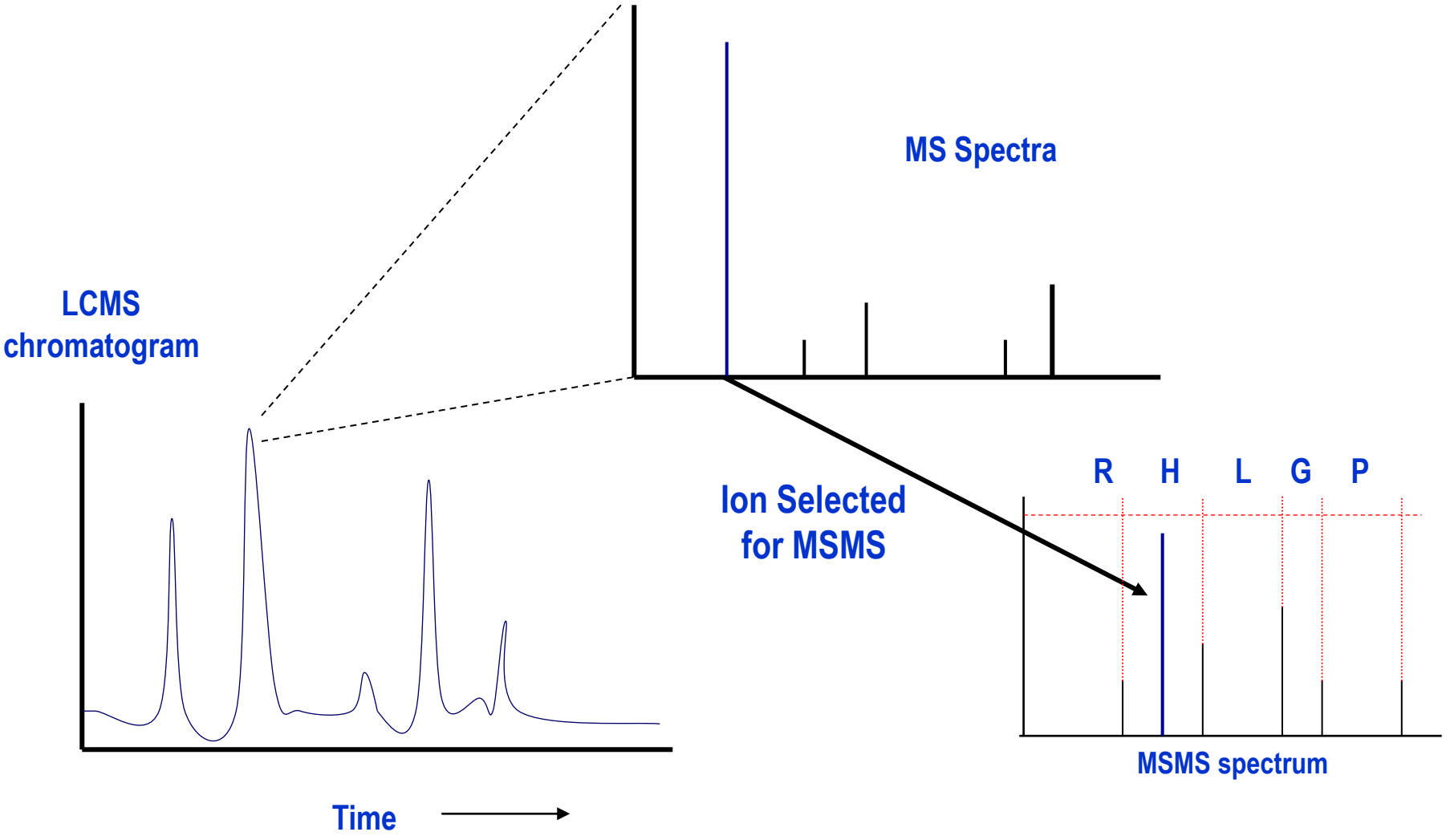

Protein digestion
Databank searching – MS/MS

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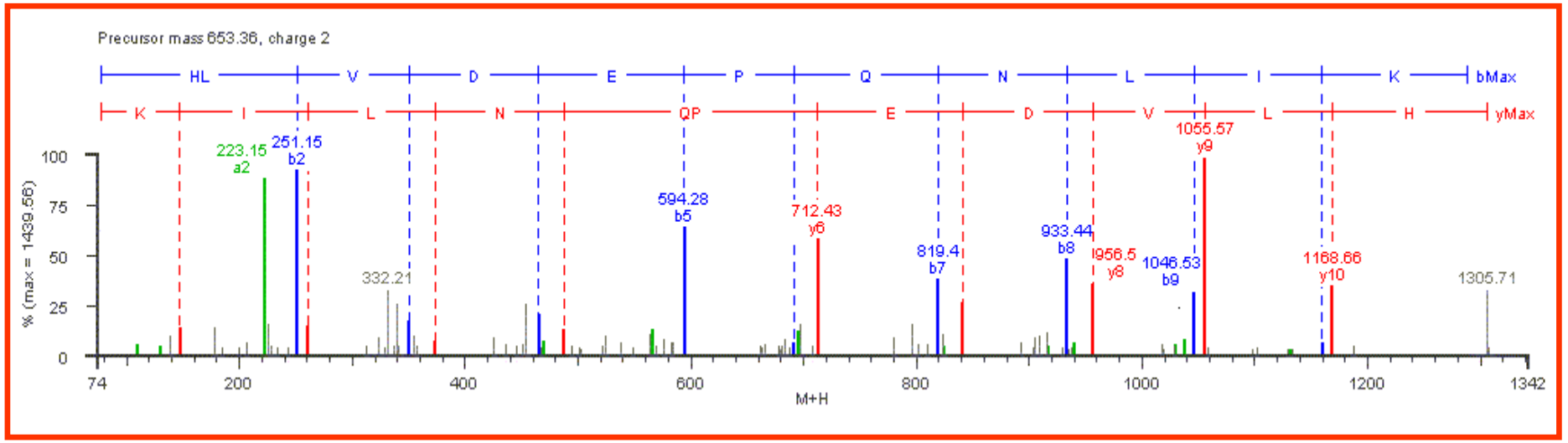

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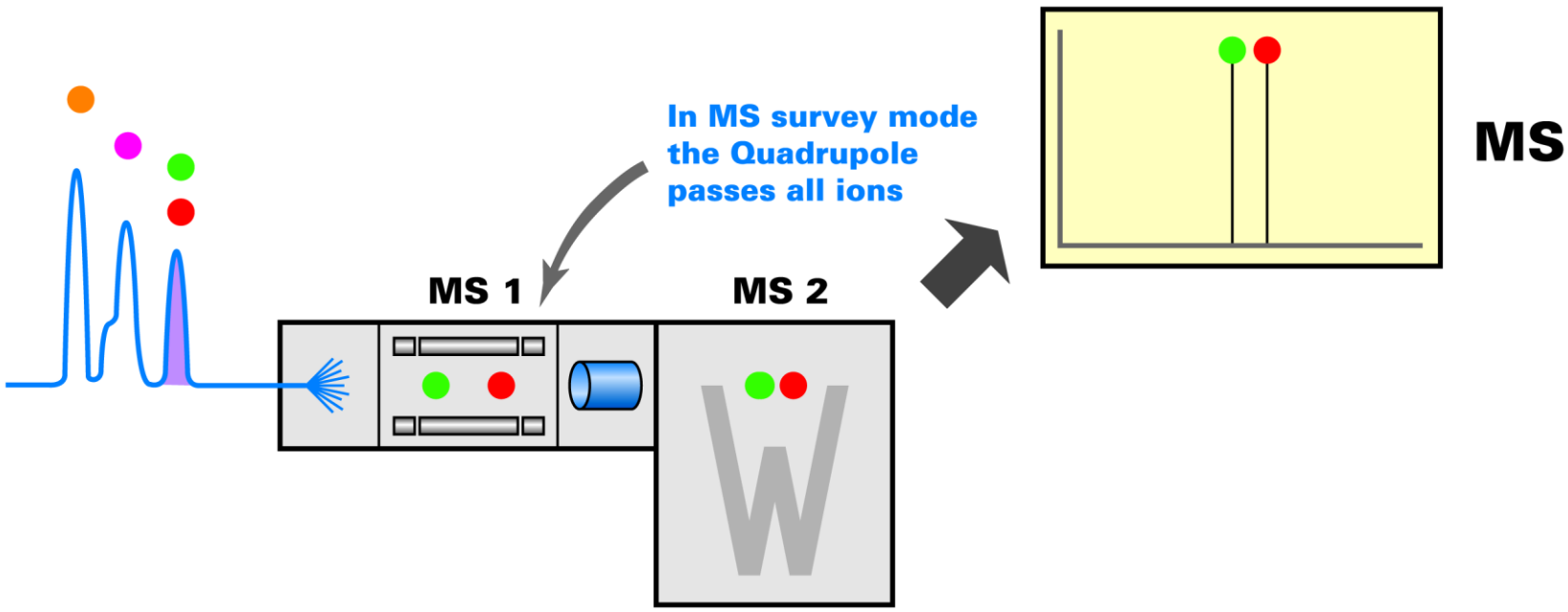
Data Directed Analysis



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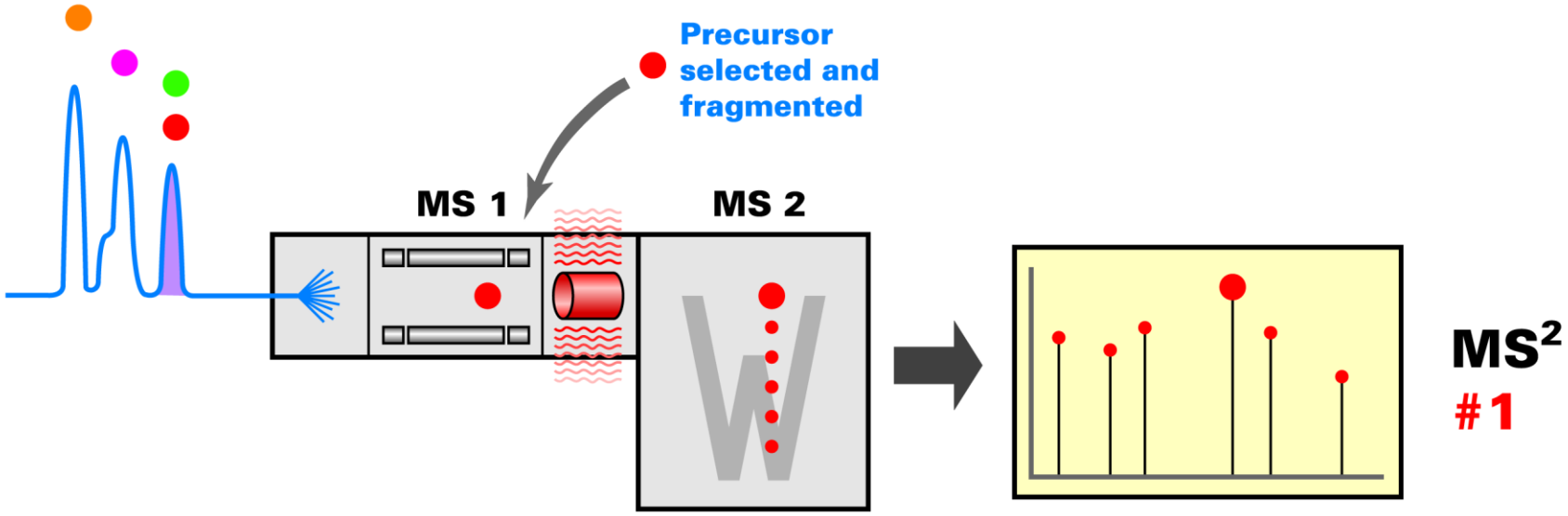
Data Directed Analysis



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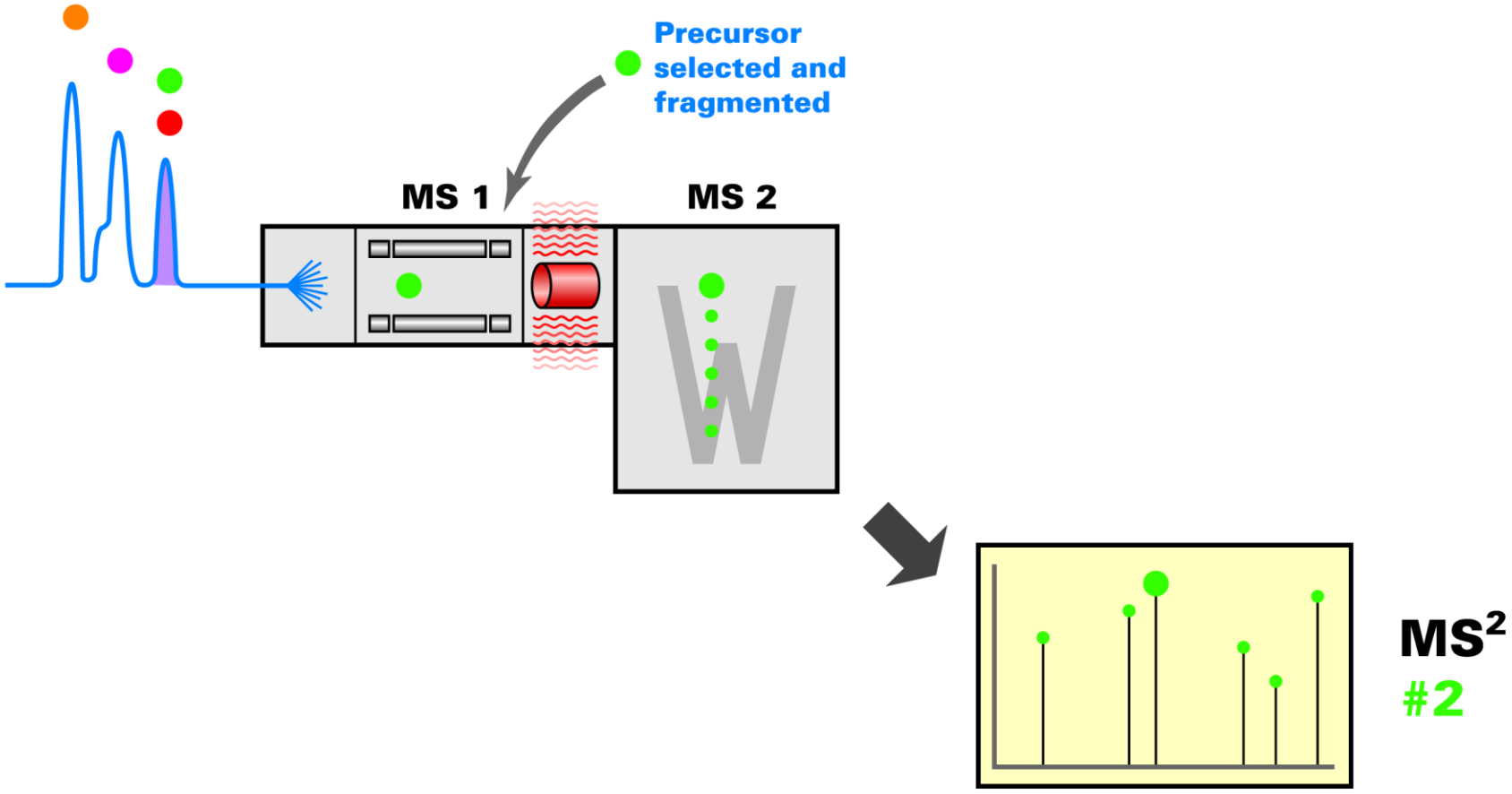
Data Directed Analysis



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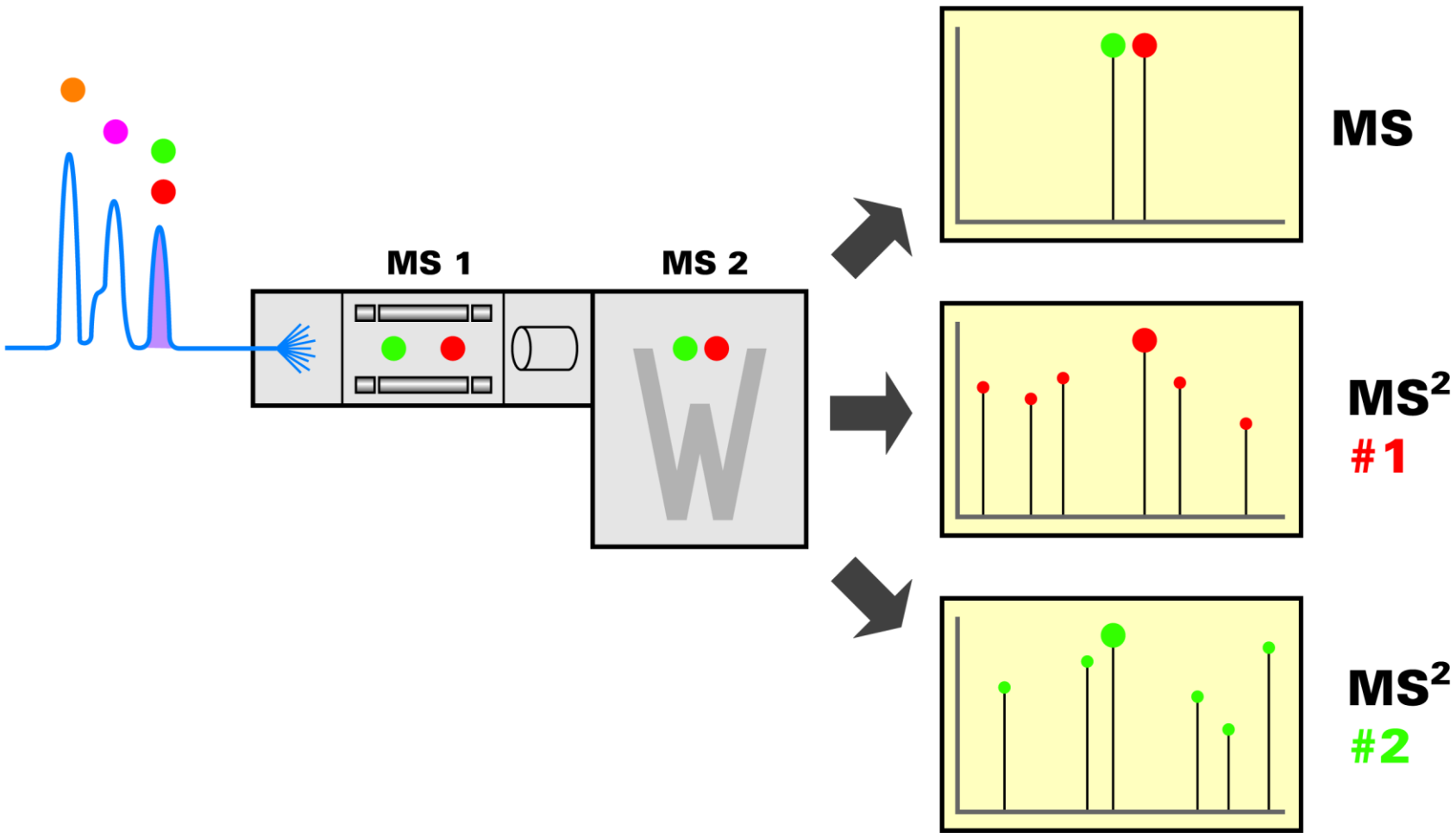
Traditional Data Directed Analysis

…necessitates pre-selection of a preferred analyte type



Summary

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- Mass spectrometers determine the molecular weight of a compound which facilitates the:
  - Determination of modifications and substitutions of intact proteins
  - Determination of protein complex stoichiometry
  - Determination of elemental compositions
  - Determination of peptide and carbohydrate sequences
  - Structural Elucidation
  - Detection of compounds at low levels (femtomole sensitivity)
  - Quantification of target compounds (diagnostics, monitoring)
  - Much much more (Limited only by the researchers imagination) !

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Dr Matt Kennedy

FEBS 2006, Istanbul

An Introduction To Some Of The Latest Technologies And Capabilities

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