

Mass and the Mass Spectrum

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What does a mass spectrometer do?

1. It measures mass better than any other technique.
2. It can give information about chemical structures.

What are mass measurements good for?

To identify, verify, and quantitate: metabolites, recombinant proteins, proteins isolated from natural sources, oligonucleotides, drug candidates, peptides, synthetic organic chemicals, polymers

How is mass defined?

Assigning numerical value to the intrinsic property of "mass" is based on using carbon-12, ^{12}C , as a reference point.

One unit of mass is defined as a Dalton (Da).

One Dalton is defined as 1/12 the mass of a single carbon-12 atom.

Thus, one ^{12}C atom has a mass of 12.0000 Da.

Isotopes

+Most elements have more than one stable isotope.

For example, most carbon atoms have a mass of 12 Da, but in nature, 1.1% of C atoms have an extra neutron, making their mass 13 Da.

+Why do we care?

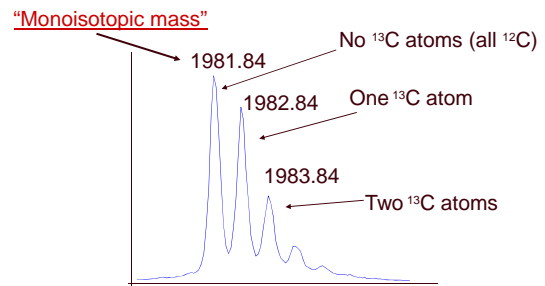
Mass spectrometers can "see" isotope peaks if their resolution is high enough.

If an MS instrument has resolution high enough to resolve these isotopes, better mass accuracy is achieved.

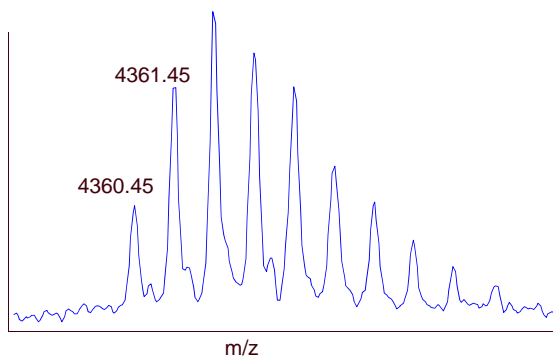
Stable isotopes of most abundant elements of peptides

Element	Mass	Abundance
H	1.0078	99.985%
	2.0141	0.015
C	12.0000	98.89
	13.0034	1.11
N	14.0031	99.64
	15.0001	0.36
O	15.9949	99.76
	16.9991	0.04
	17.9992	0.20

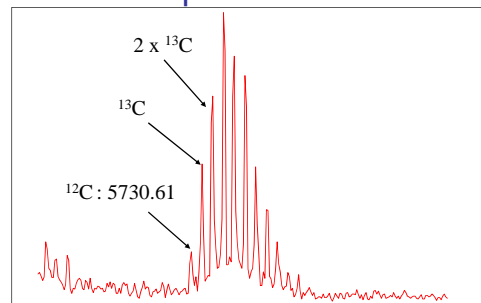
Mass spectrum of peptide with 94 C-atoms (19 amino acid residues)



Isotope pattern for a larger peptide (207 C-atoms)

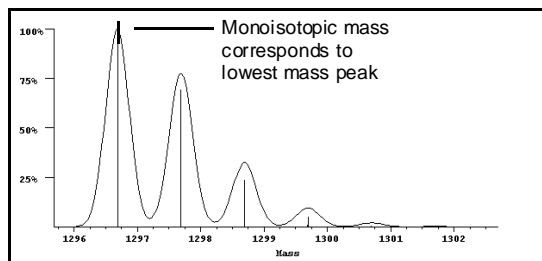


Mass spectrum of insulin



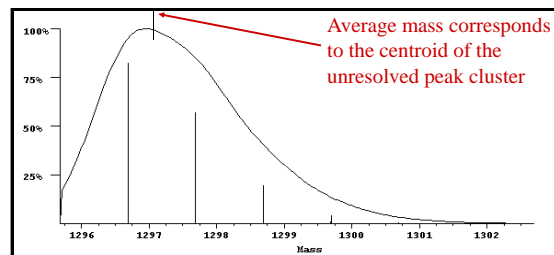
Insulin has 257 C-atoms. Above this mass, the monoisotopic peak is too small to be very useful, and the average mass is usually used.

Monoisotopic mass



When the isotopes are clearly resolved the **monoisotopic mass** is used as it is the most accurate measurement.

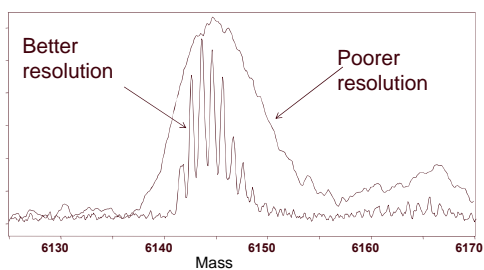
Average mass



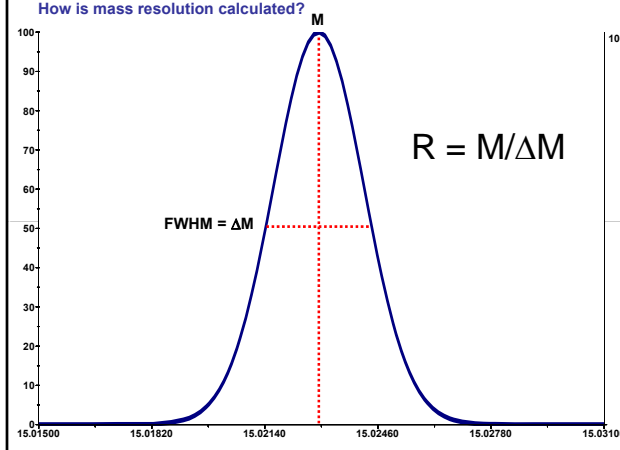
When the isotopes are not resolved, the centroid of the envelope corresponds to the weighted average of all the isotope peaks in the cluster, which is the same as the average or chemical mass.

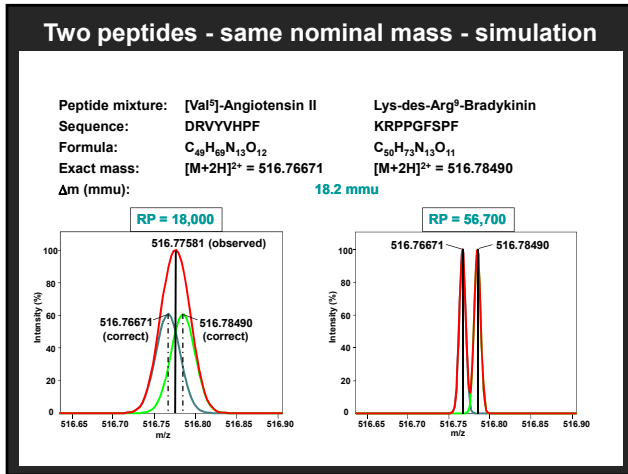
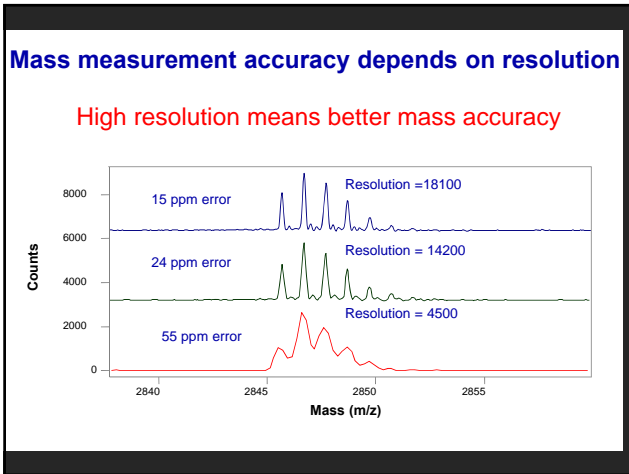
What if the resolution is not so good?

At lower resolution, the mass measured is the average mass.



How is mass resolution calculated?

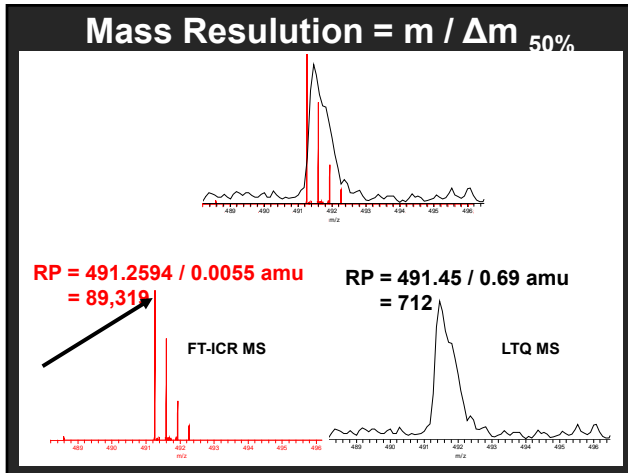




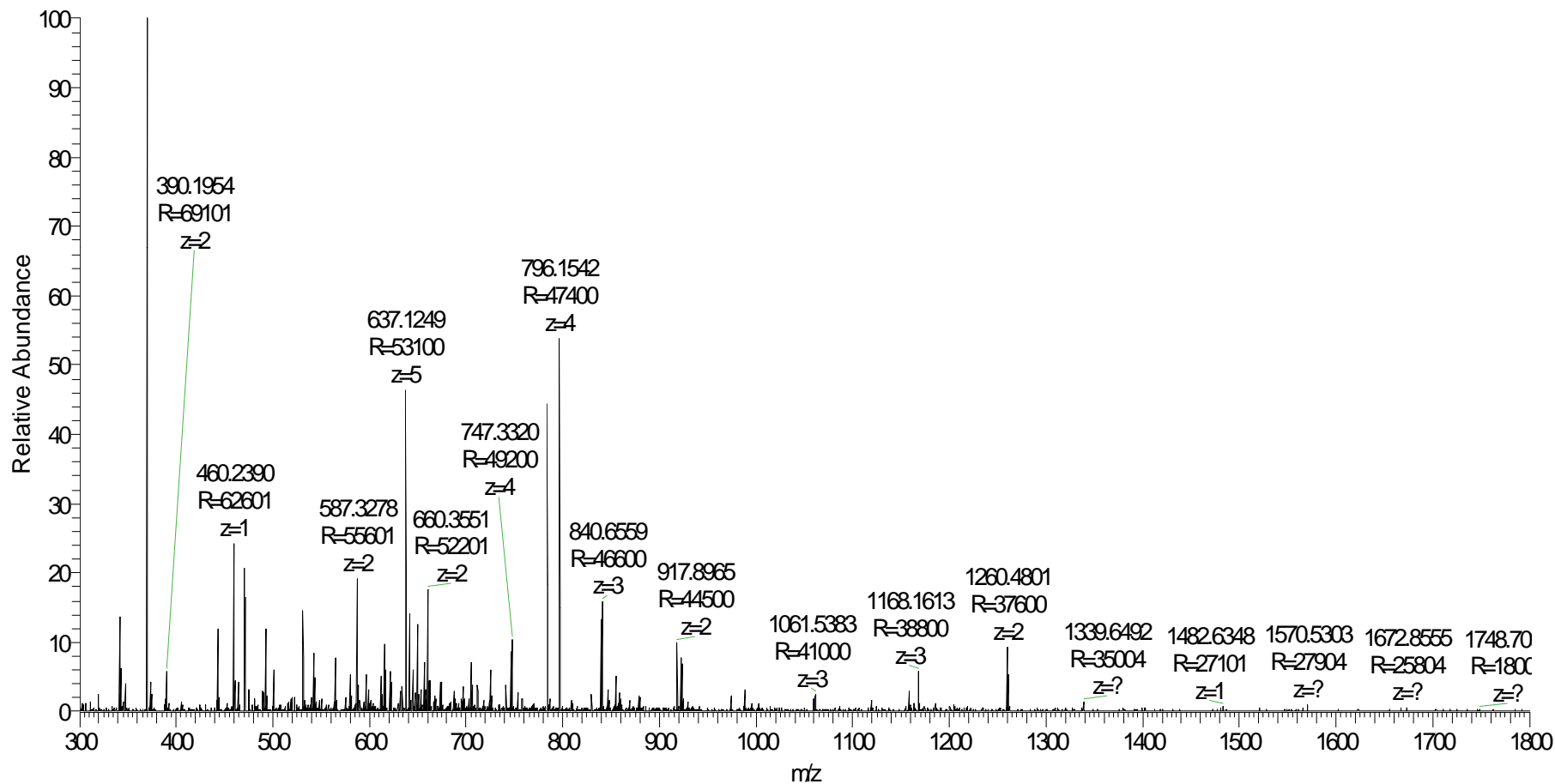
Is Mass Accuracy Important ?

Results for error limit up to 5 ppm

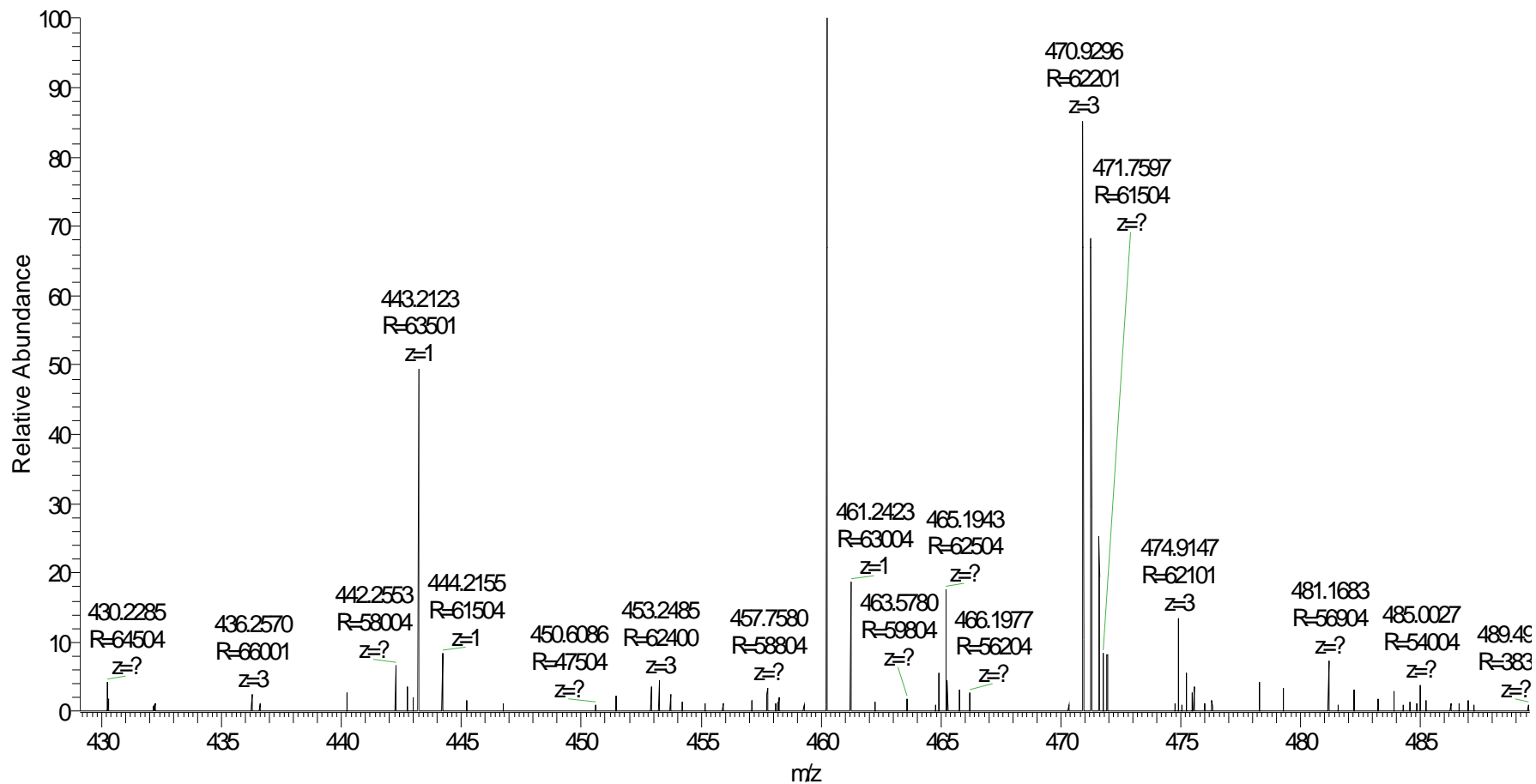
	Theoretical Mass	Delta [ppm]	Delta [mmu]	RDB	Composition
1 ppm (4)	516.76671	0.0	0.0	21.0	C ₂₈ H ₄₉ O ₁₂ N ₁₃
	516.76647	0.5	0.2	15.0	C ₂₈ H ₄₉ O ₁₁ N ₁₃ S ₂
	516.76638	0.6	0.3	12.0	C ₂₁ H ₇₅ O ₁₄ N ₁₃ S ₁
	516.76705	-0.7	-0.3	11.5	C ₁₄ H ₇₇ O ₁₄ N ₁₃ S ₁
2 ppm (10)	516.76738	-1.3	-0.7	18.0	C ₂₈ H ₄₉ O ₁₆ N ₉
	516.76804	1.3	0.7	21.5	C ₂₇ H ₄₉ O ₁₄ N ₁₆
	516.76580	1.8	0.9	15.5	C ₂₇ H ₄₇ O ₁₀ N ₁₃ S ₂
	516.76772	-2.0	-1.0	16.5	C ₂₄ H ₇₃ O ₁₁ N ₁₆ S ₁
	516.76773	-2.0	-1.0	15.0	C ₂₄ H ₇₃ O ₁₁ N ₁₅ S ₁
5 ppm (23)	516.76805	-2.6	-1.3	25.5	C ₂₈ H ₄₉ O ₈ N ₁₄
	516.76537	2.6	1.3	16.5	C ₂₈ H ₇₃ O ₁₅ N ₁₂
	516.76807	-2.6	-1.4	7.0	C ₂₃ H ₇₉ O ₁₄ N ₁₅ S ₂
	516.76513	3.0	1.6	10.5	C ₂₈ H ₈₁ O ₁₄ N ₉ S ₂
	516.76513	3.1	1.6	16.0	C ₂₈ H ₇₉ O ₉ N ₁₅ S ₂
	516.76839	-3.3	-1.7	16.0	C ₂₈ H ₇₆ O ₁₂ N ₁₄ S ₁
	516.76479	3.7	1.9	20.0	C ₂₂ H ₇₅ O ₁₁ N ₉ S ₁
	516.76872	-3.9	-2.0	25.0	C ₂₄ H ₇₁ O ₁₀ N ₁₁
	516.76470	3.9	2.0	17.0	C ₁₄ H ₇₁ O ₁₄ N ₁₆
	516.76874	-3.9	-2.0	6.5	C ₂₈ H ₈₁ O ₁₄ N ₁₀ S ₂
	516.76446	4.3	2.2	11.0	C ₂₈ H ₇₉ O ₁₁ N ₁₁ S ₂
	516.76897	-4.4	-2.3	12.5	C ₂₈ H ₇₃ O ₁₆ N ₁₆
	516.76907	-4.6	-2.4	15.5	C ₂₁ H ₇₇ O ₁₃ N ₁₃ S ₁



ah_20121221_ORBI_ST6Gal2_CID#3136 RT: 12.84 AV: 1 NL: 1.36E7
T: FTMS+pNSI Full ms [300.00-1800.00]



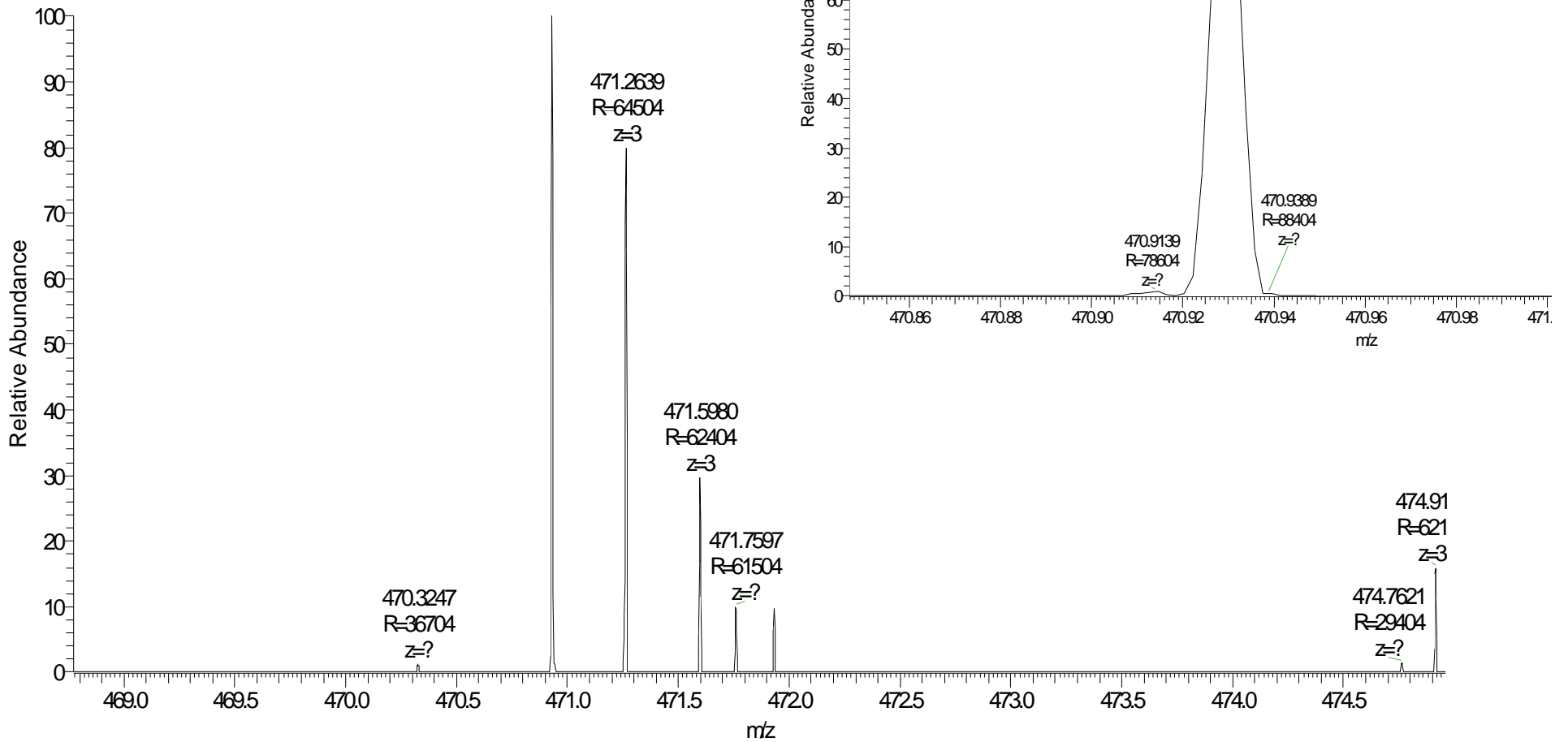
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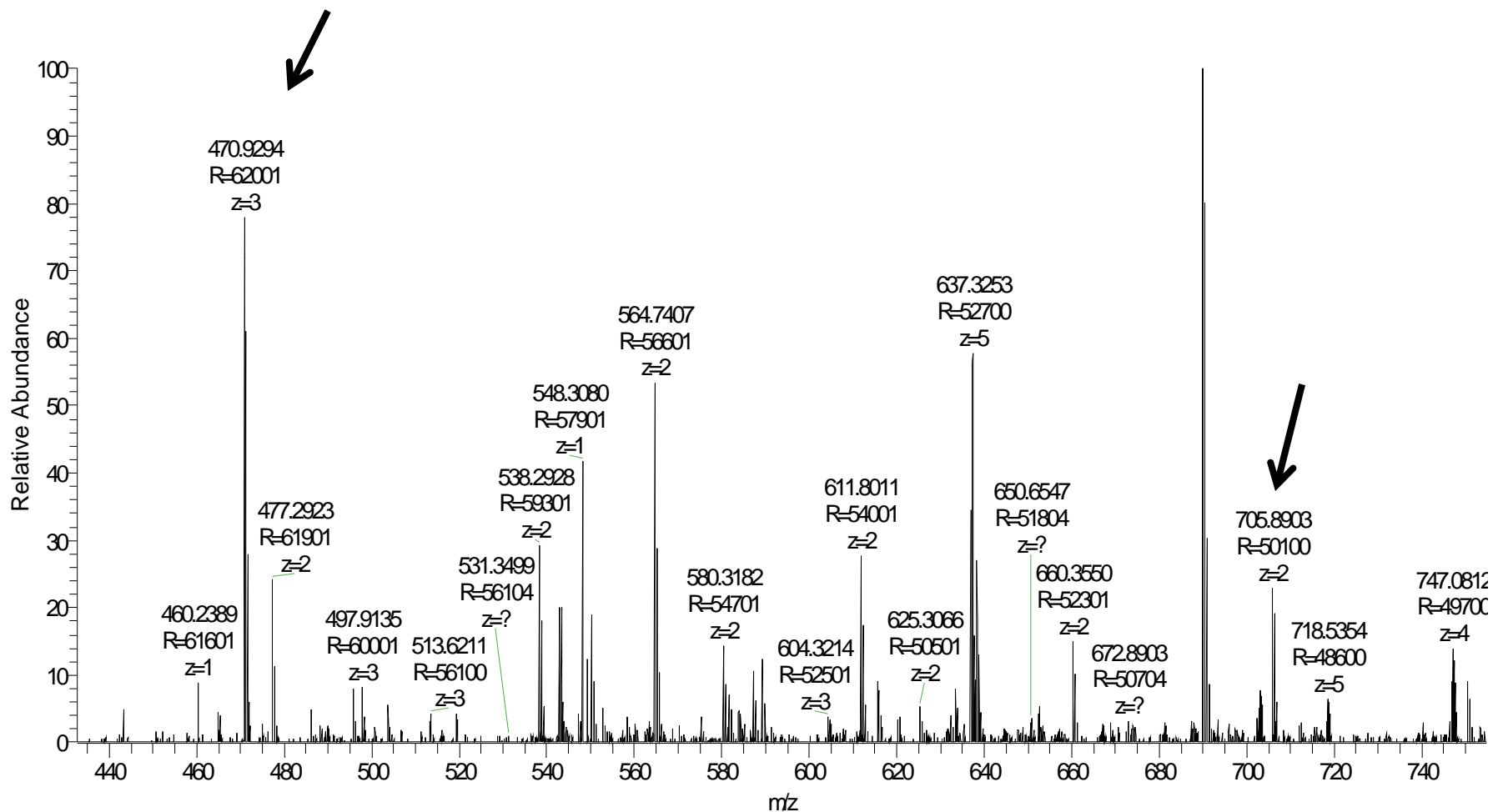
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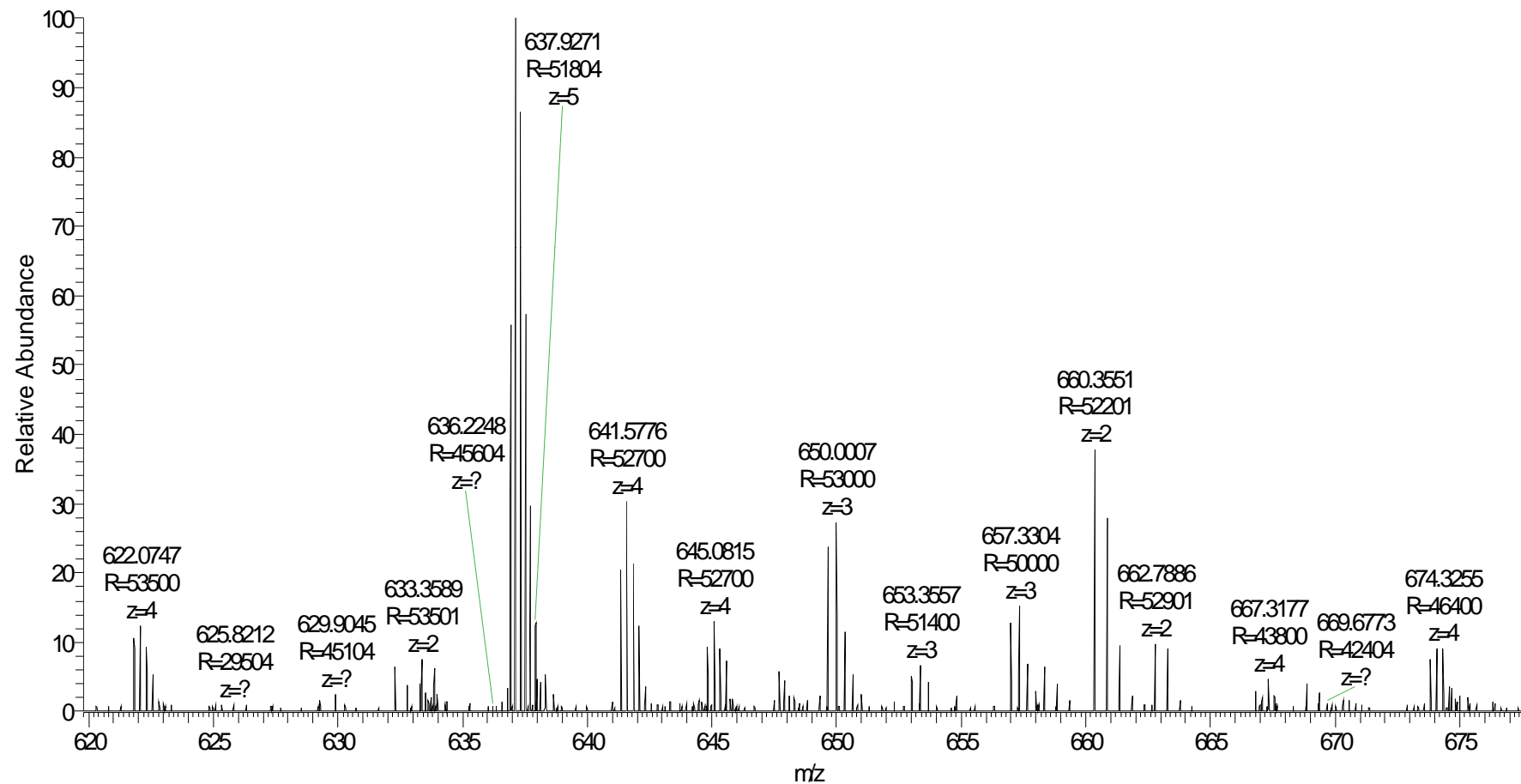
470.9296



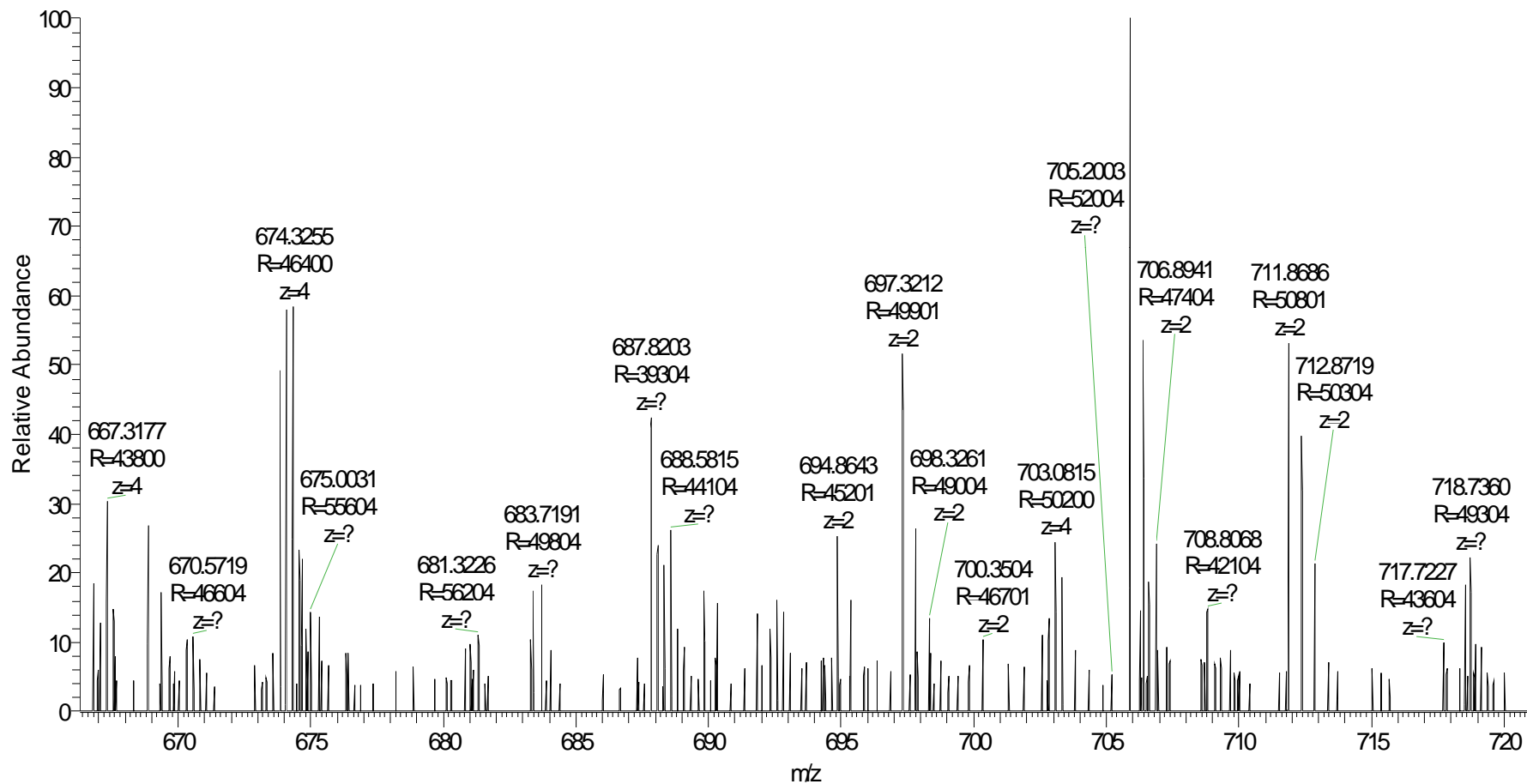
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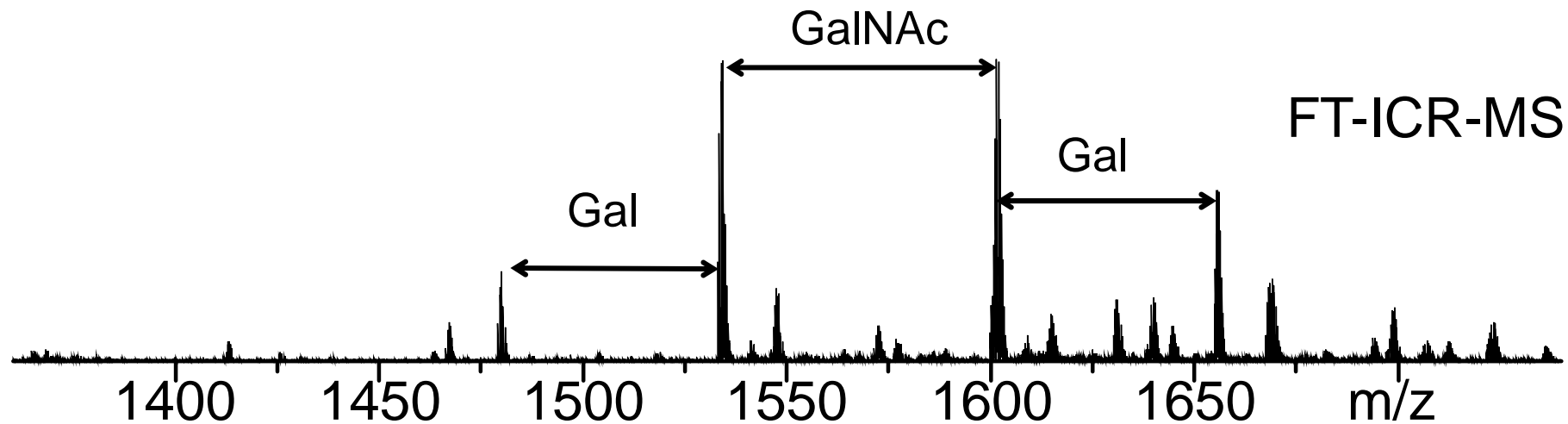


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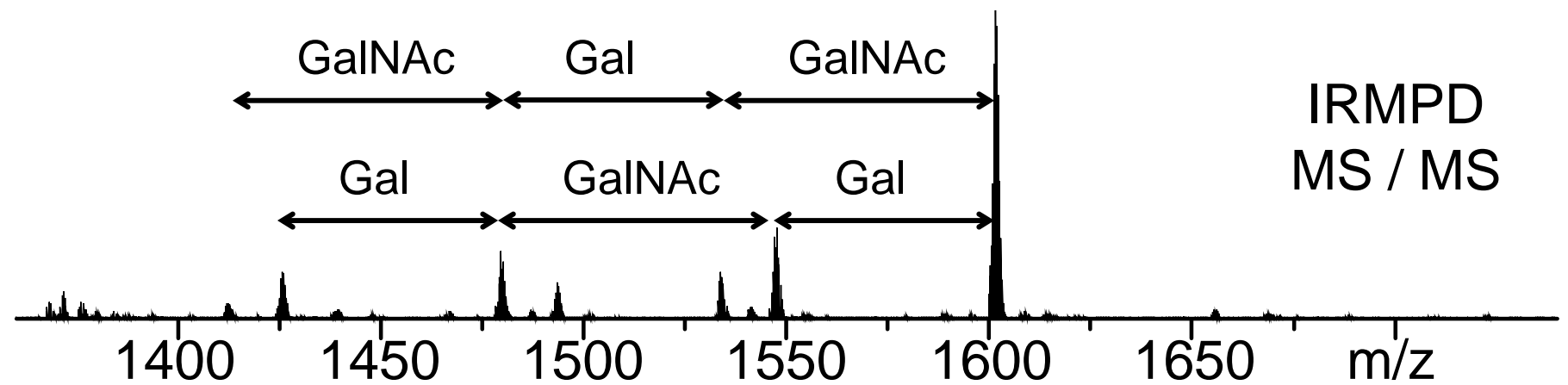
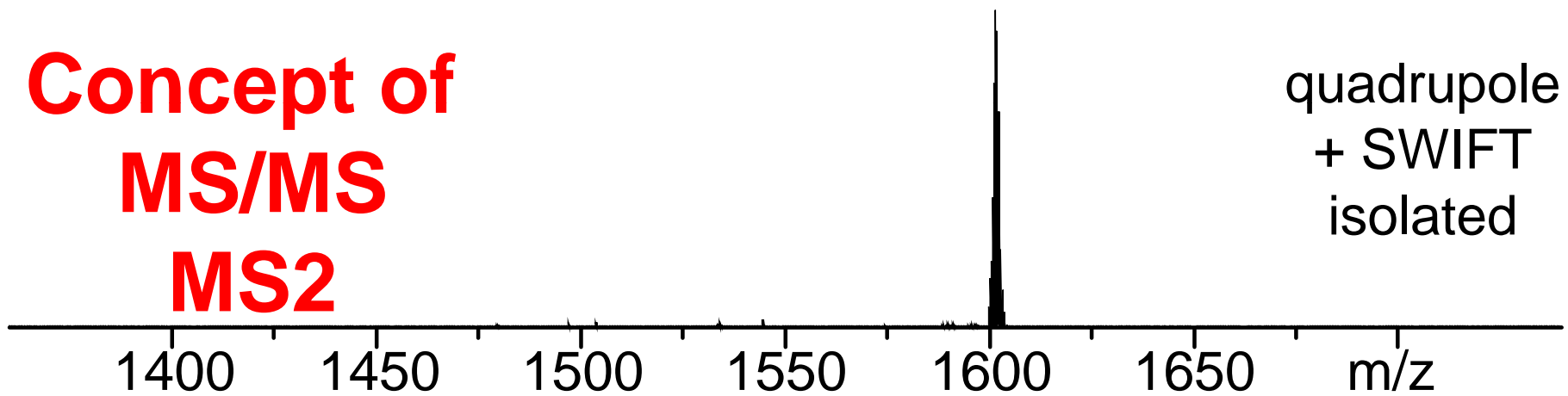
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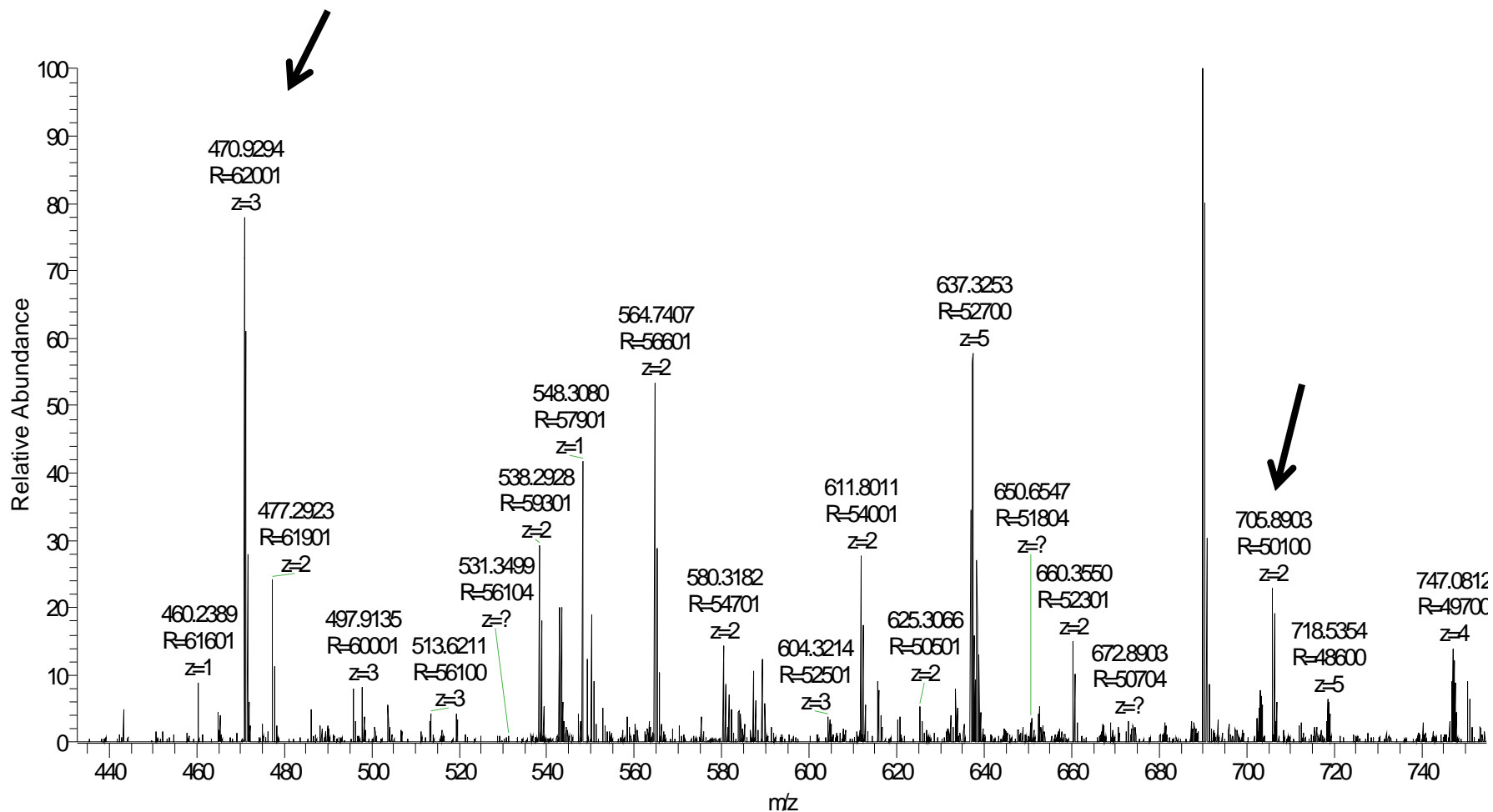


**Concept of
MS/MS
MS2**

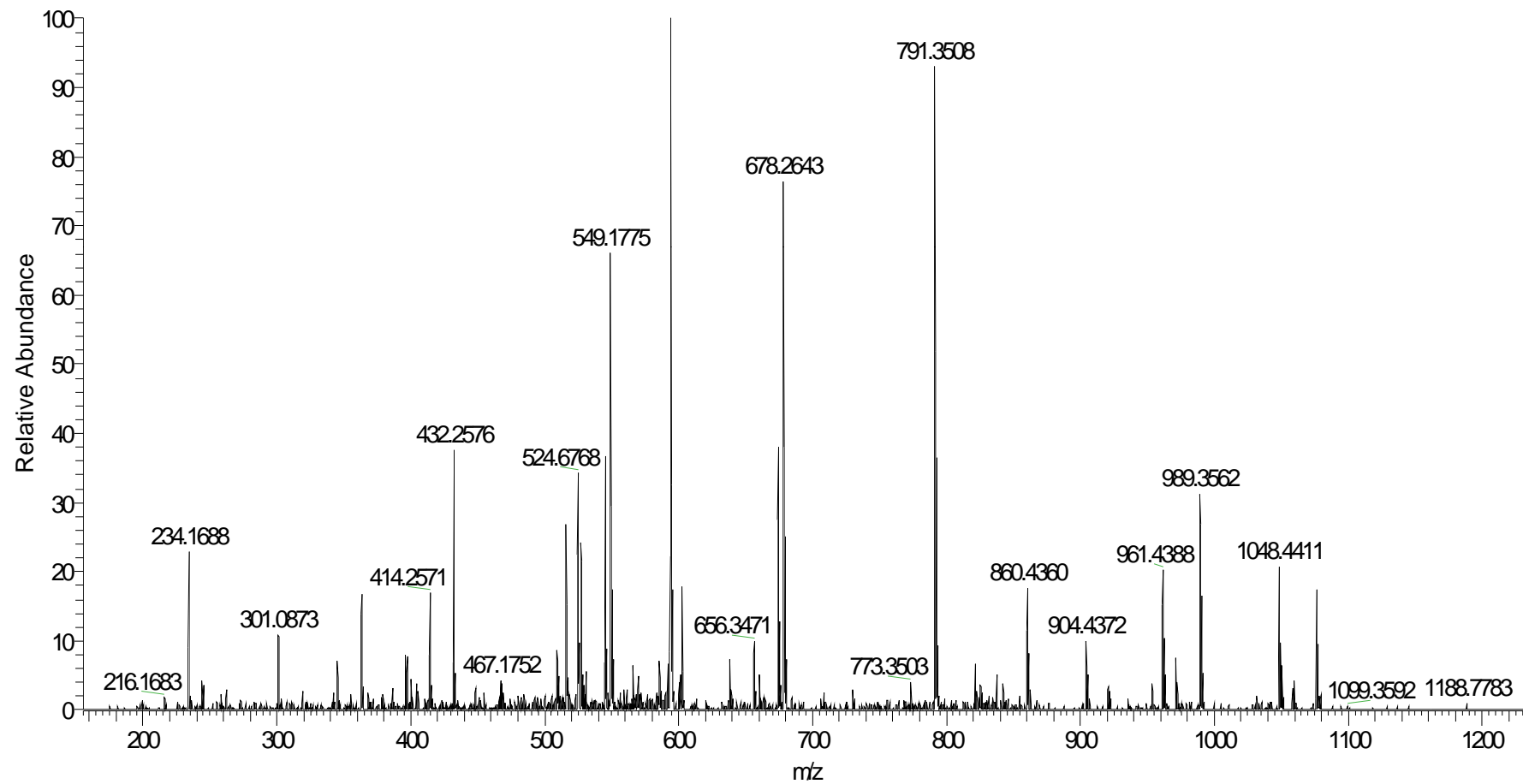
quadrupole
+ SWIFT
isolated



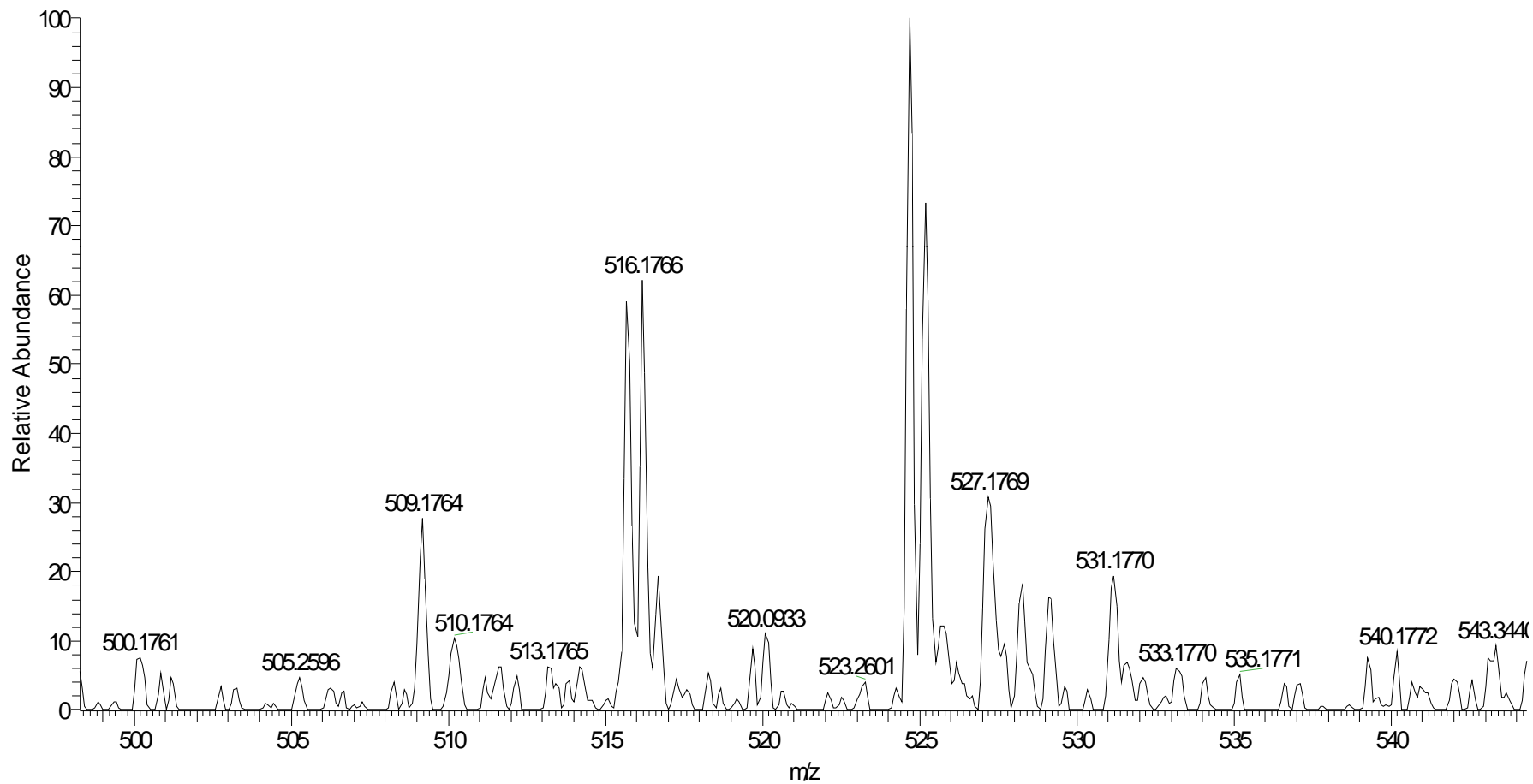
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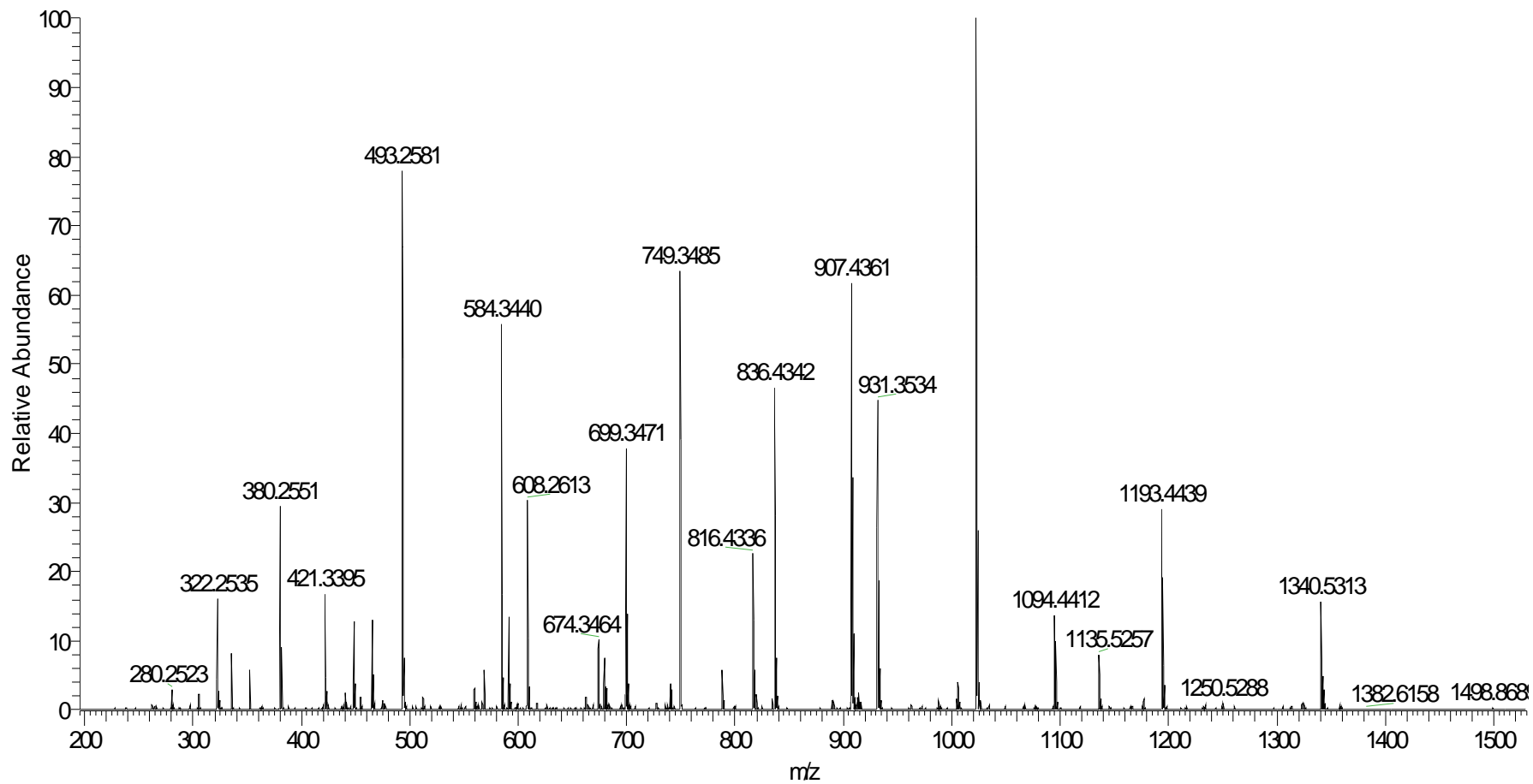
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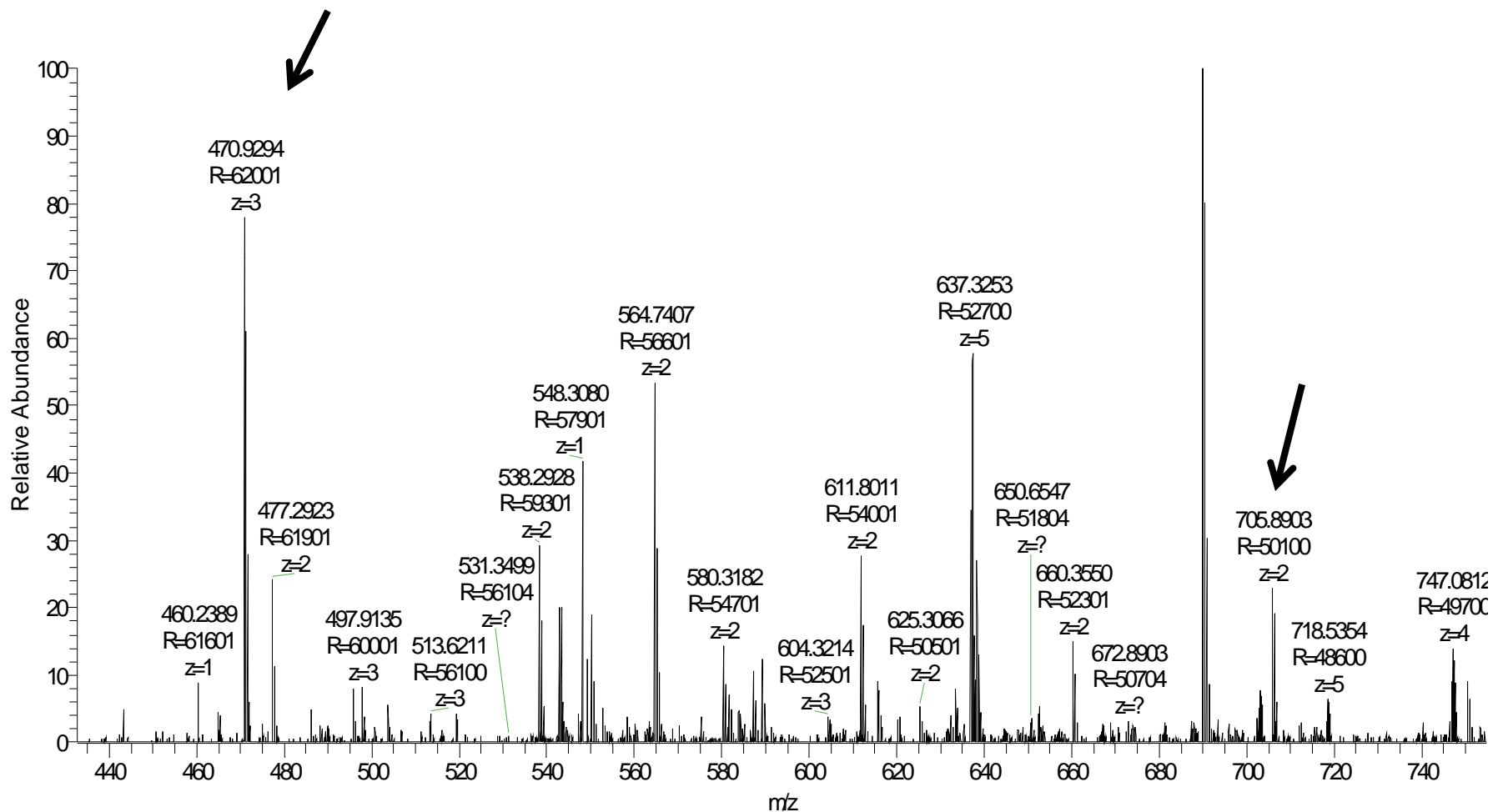
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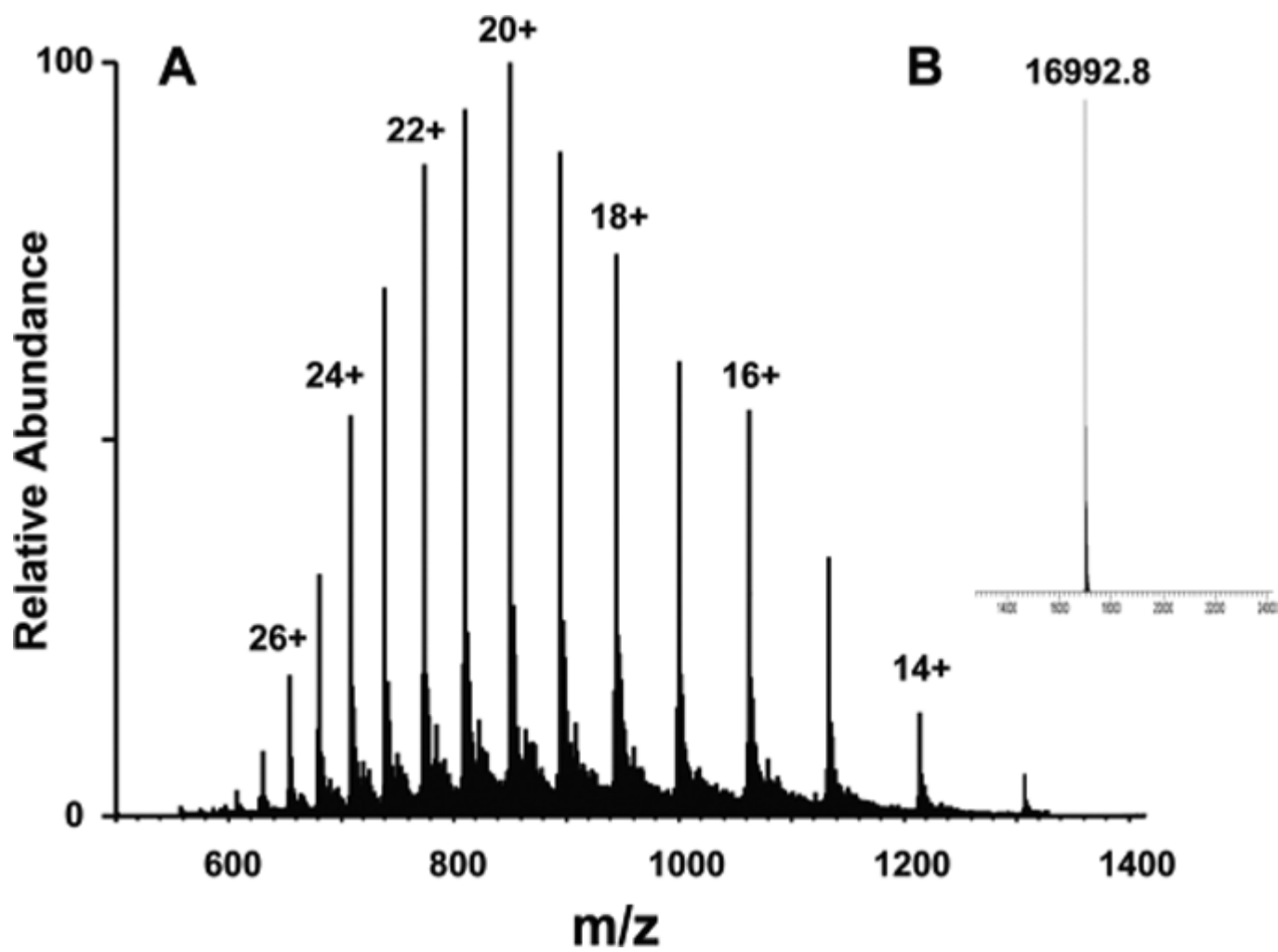
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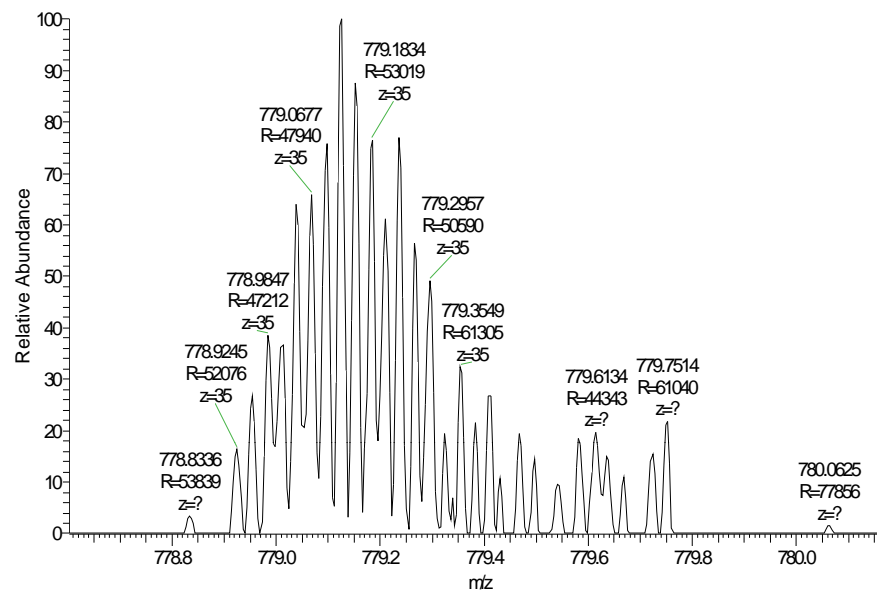
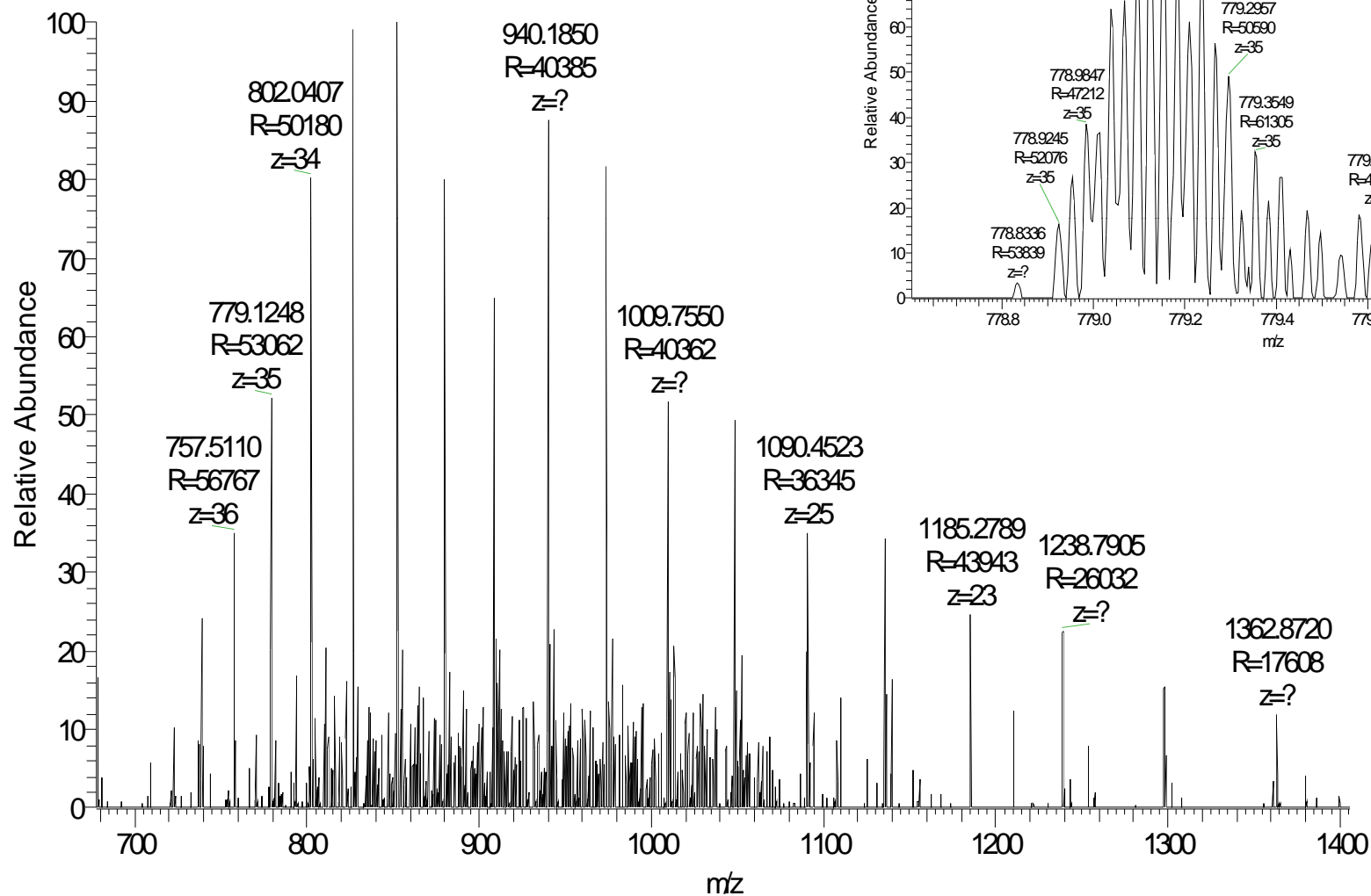
Myoglobin



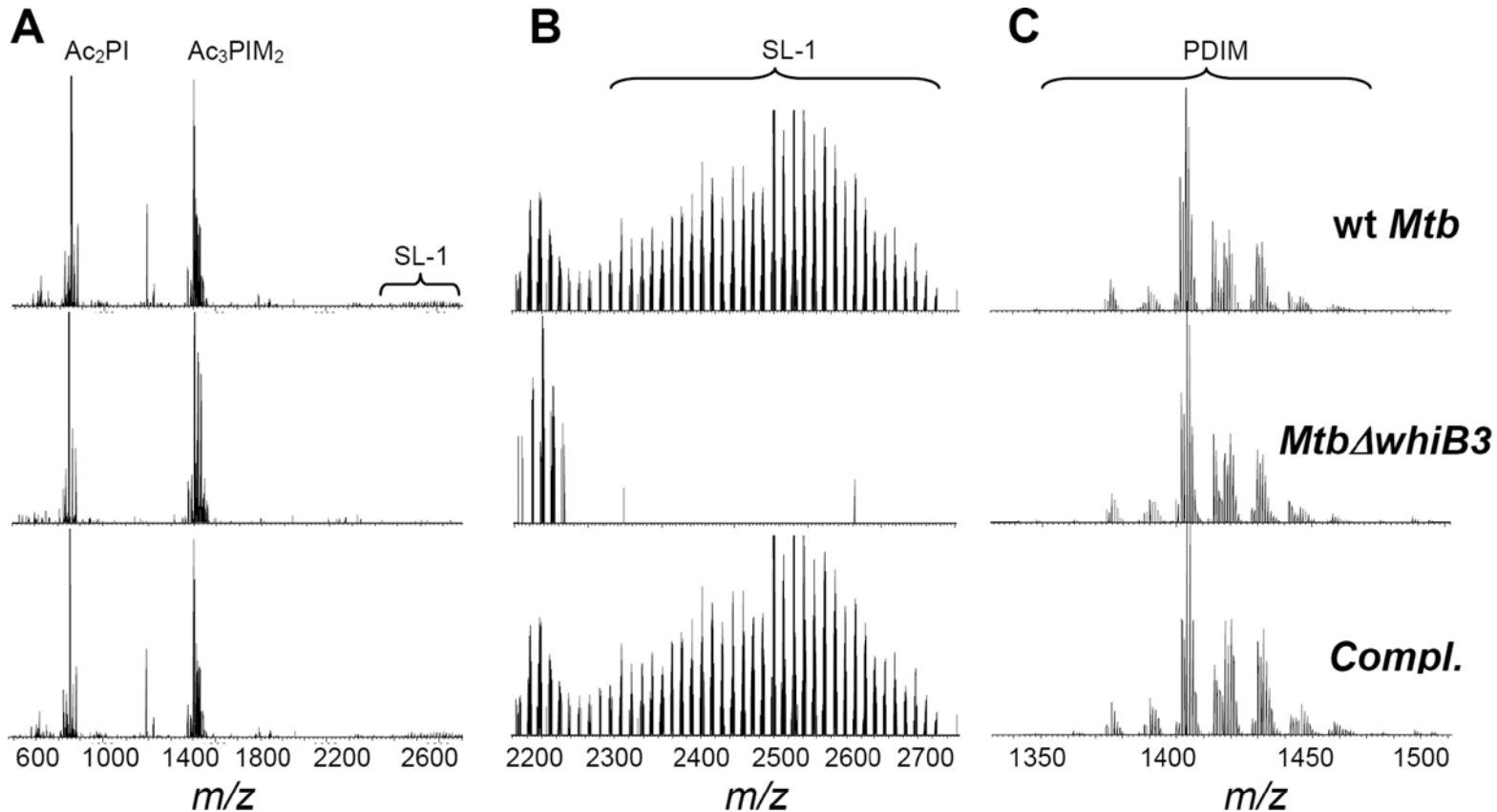
RXR-Ligand binding domain

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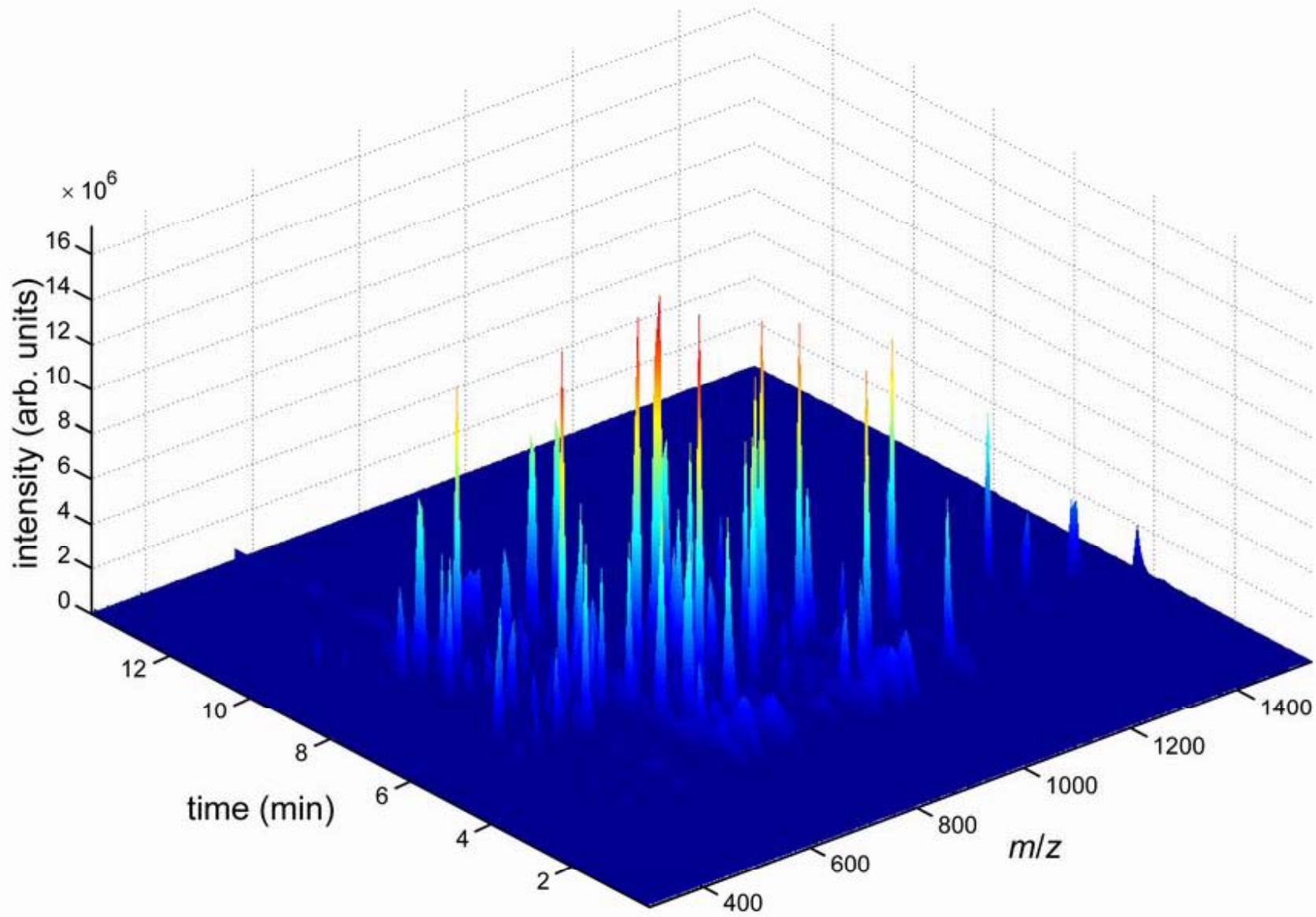
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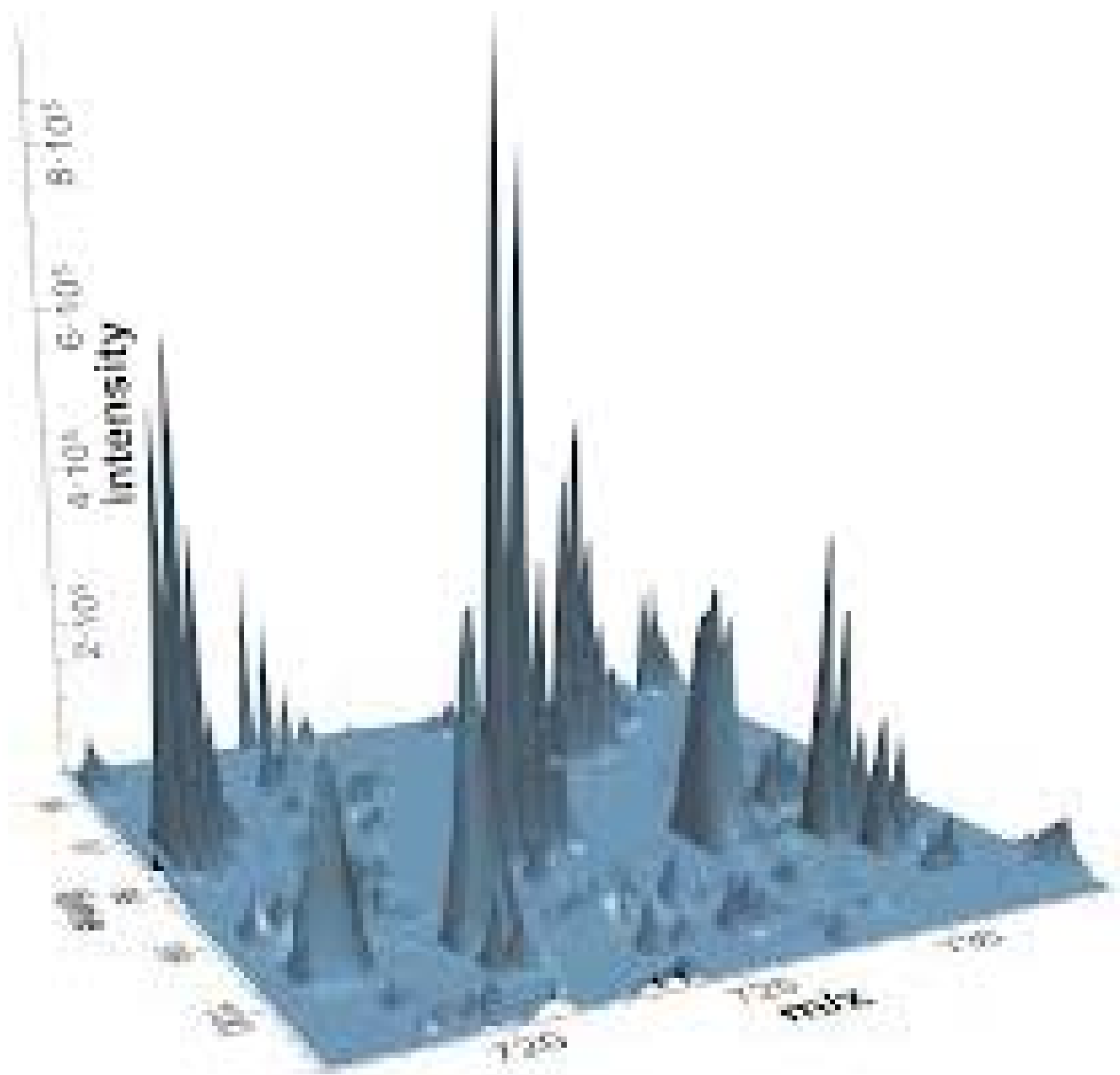


total crude lipids

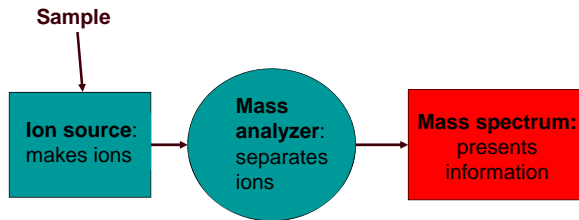


FT-ICR mass spectra of total crude lipids derived from *MtbΔwhiB3* growing in macrophages demonstrate the absence of SL-1. (A) Total crude lipid extracts were prepared from *Mtb* growing inside macrophages and analyzed in the negative ion mode by FT-ICR MS. We observed the presence of Ac_2PI species at m/z 835.5261 and 851.5566, which corresponds to their reported theoretical masses. The dimannose specie esterified to three acyl chains (Ac_3PIM_2) corresponds to mass m/z 1413.8888 was also detected in all the strains tested. Note that the multiple lipofoms of SL-1 were absent in *MtbΔwhiB3*. (B) The SL-1 region of FT-ICR mass spectrum showed a complete absence of this class of lipids ($\sim m/z$ 2300 to 2600) in *MtbΔwhiB3* (C) FT-ICR mass spectra of the PDIM region.

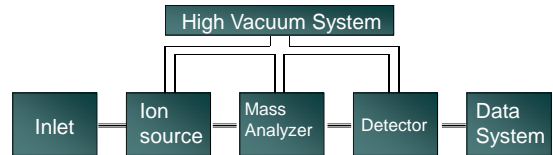




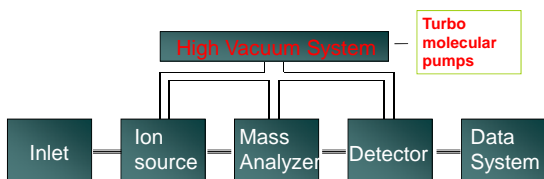
How does a mass spectrometer work?



Mass Spectrometer Block Diagram



Mass Spectrometer Block Diagram



Sample Introduction

