

Life Science and Industrial Applications of the AXIMA Resonance

High Vacuum MALDI-QIT-TOF Mass Spectrometer

AXIMA Resonance



MALDI-QIT-TOF MS

High performance as standard...



Glycomics



Proteomics



Top-Down Proteomics



Non-Enzymatic / Chemical Modifications



Post-Translational Modifications



Small Molecules



Polymer Analysis



Lipidomics

...Results delivered

The AXIMA Resonance

Introduction

The AXIMA Resonance is a versatile MALDI mass spectrometer employed in a broad range of biological and organic analytical applications. Its main advantages are:

- True high-vacuum MALDI ion source
- Quadrupole ion trap with MSⁿ capability for structural elucidation
- High mass resolution and mass accuracy independent of the number of cycles during MSⁿ experiments
- Ability to isolate parent ions with isotopic resolution over a wide mass range - up to 1000 FWHM
- Variable energy CID control during acquisition
- Outstanding sensitivity - uncompromised design, to ensure highly efficient trapping functionality
- Low sample consumption - allowing many more MSⁿ experiments to be performed on the same spot
- Variable repetition rate N₂ laser
- Manual or fully automated operation allowing the seamless analysis of few or many samples as required
- Dedicated software solutions for a wide range of applications

Given these advantages, the AXIMA Resonance is ideally suited for high-end proteomics studies as well as less conventional studies with polymers, lipids and small molecules. The purpose of this document is to give an insight into the use of the AXIMA Resonance in these applications.



Glycomics

Classical glycan and glycopeptide analysis using MS² and MS³

AXIMA Resonance, an ideal solution for carbohydrate research

Glycosylation is one of the major post-translational modifications (PTM) and has significant effects on protein folding and ultimately on protein activity. Identification of the glycan structure forms much of the challenge in this research field. However, of equal importance is the determination of the point of attachment to the protein. The AXIMA Resonance offers the advantage of being able to address both of these challenges.

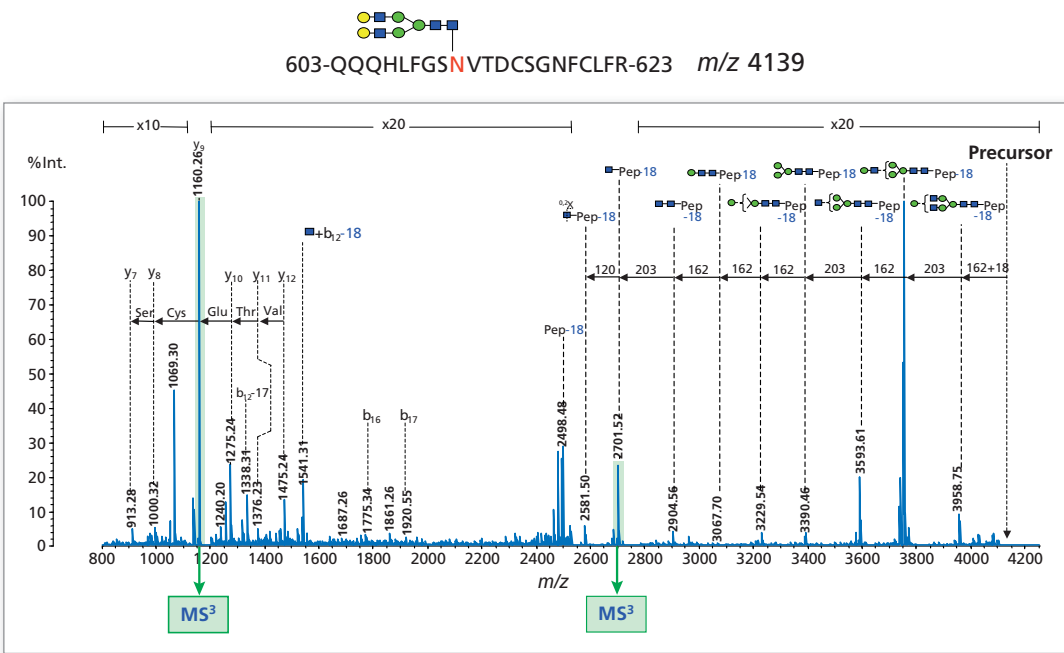


Figure 1: MS² spectrum of a human transferrin glycopeptide [M+H-2Sia]⁺ (m/z 4139) that has already lost two sialic acid moieties. The spectrum exhibits the fragmentation of the glycan part attached to the peptide.

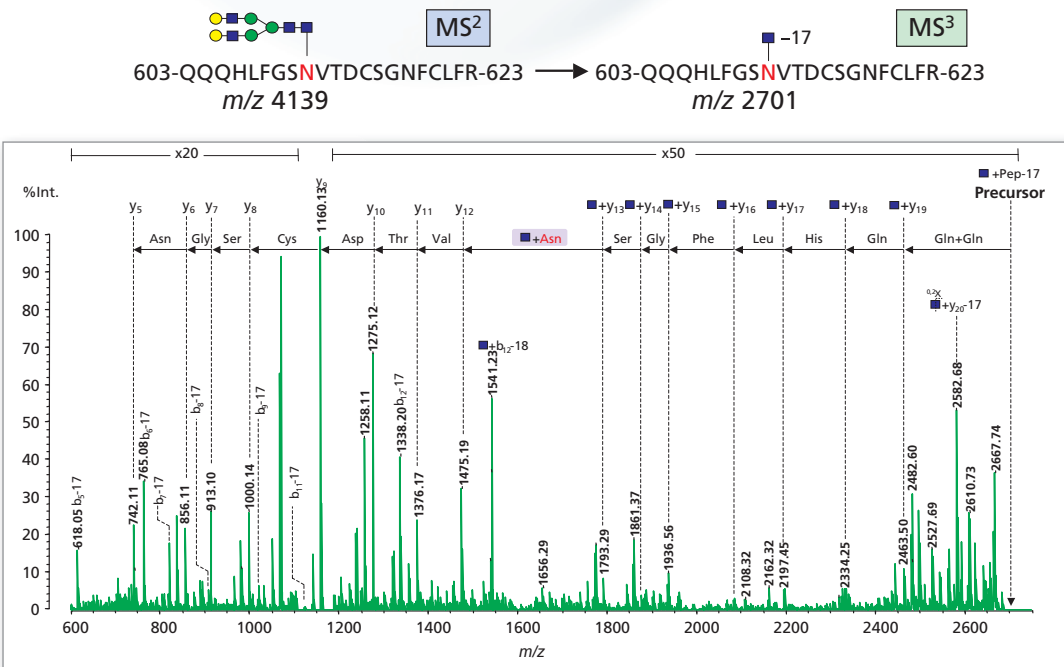


Figure 2: Positive ion MS³ spectrum of the product ion at m/z 2701 (from the MS² analysis of m/z 4139 ion), exhibiting fragmentation of the peptide backbone. The detection of specific fragments allowed the identification of the glycosylation site.

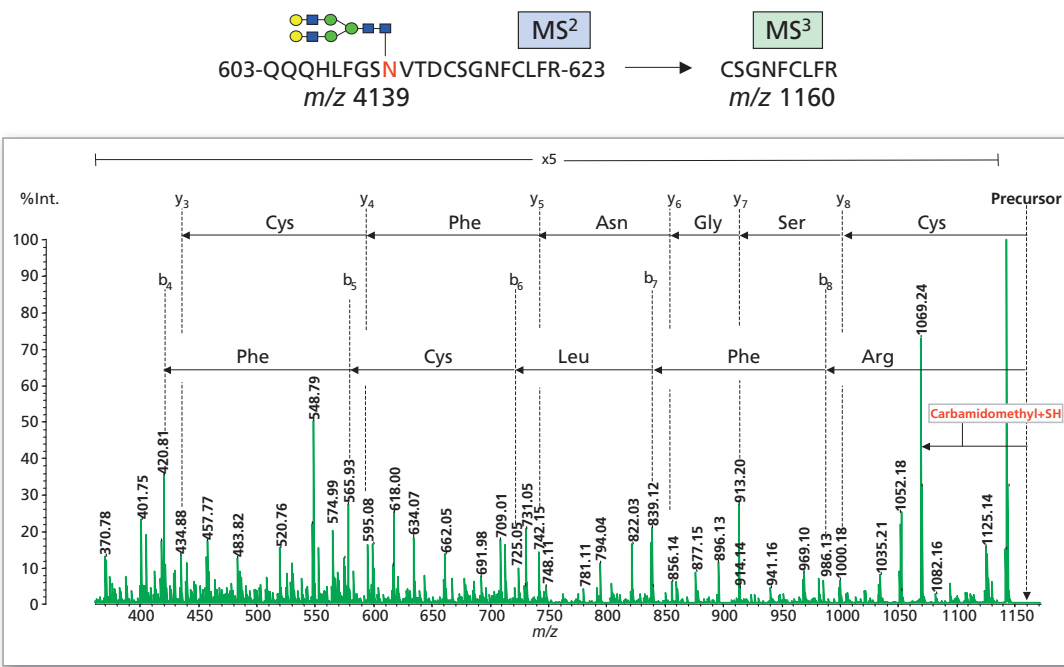


Figure 3: Positive ion MS³ spectrum of the product ion at m/z 1160, exhibiting fragmentation of the peptide backbone. The peptide sequence is completely assigned.

Biosimilars

Accurate Glycan Analyser, a one-stop platform for smarter carbohydrate research

Accurate Glycan Analyser is a software-directed multistage MALDI mass spectrometric approach using the unique MSⁿ capability offered by the AXIMA Resonance and a populated database of real MS and MSⁿ glycan spectra, guiding the user through the process of structural analysis of glycans without requiring an expert level of knowledge.

Unlike others, the Accurate Glycan Analyser database is populated with glycans of high biological relevance, that have been enzymatically generated using the extensive glycogene know-how from the Japanese National Institute of Advanced Industrial Science and Technology. This recombinant technology-based approach is unique in this field and provides the researcher with a database of considerable added value.

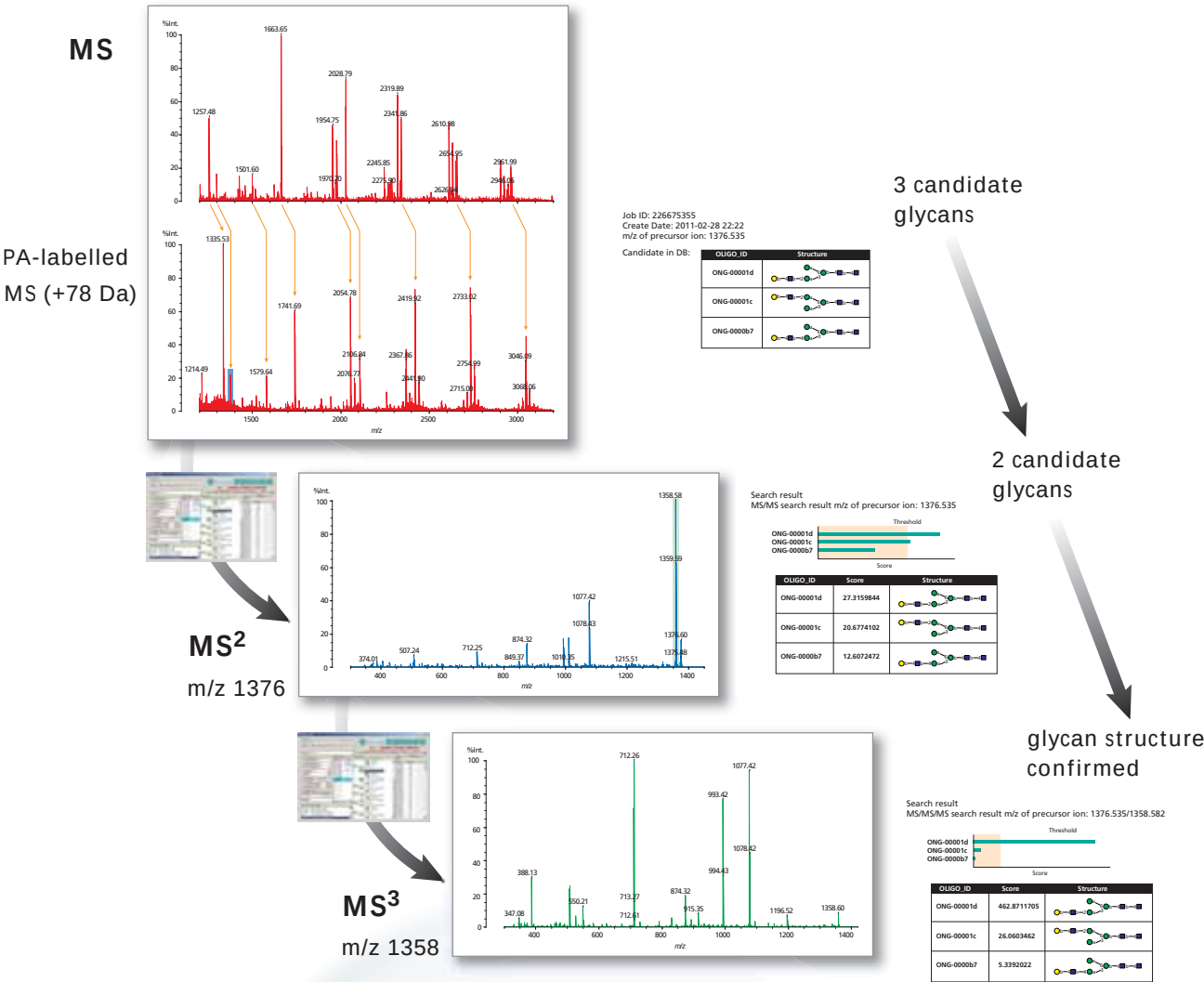


Figure 4: Overview of the Accurate Glycan Analyser - AXIMA Resonance workflow applied to PA-labelled glycans released from fetuin. At the MS level, potential glycan candidates are proposed. The software then suggests MS² precursor masses that can be analysed to confirm the proposed glycan structure. In some cases MS² alone is not sufficient to unequivocally identify a single glycan candidate, and thus the Accurate Glycan Analyser software proposes further MSⁿ precursor candidates (up to MS⁴). For the fetuin example shown, MS³ was necessary to unambiguously identify the glycan structure.

Human Factor IX (FIX), a single-chain glycoprotein, plays an important role in the intrinsic blood coagulation pathway and is used as a treatment for the bleeding disorder haemophilia B. There are two known glycosylation sites (Asn-157 and Asn-167). The production of therapeutically active recombinant and plasma-derived FIX is approved and regulated by US and European agencies. The current regulatory levels of quality control require meticulous characterisation of the glycosylation pattern. However, due to the branched nature of glycans, the unambiguous identification of their structure is not trivial. The Accurate Glycan Analyser - AXIMA Resonance platform facilitates this identification.

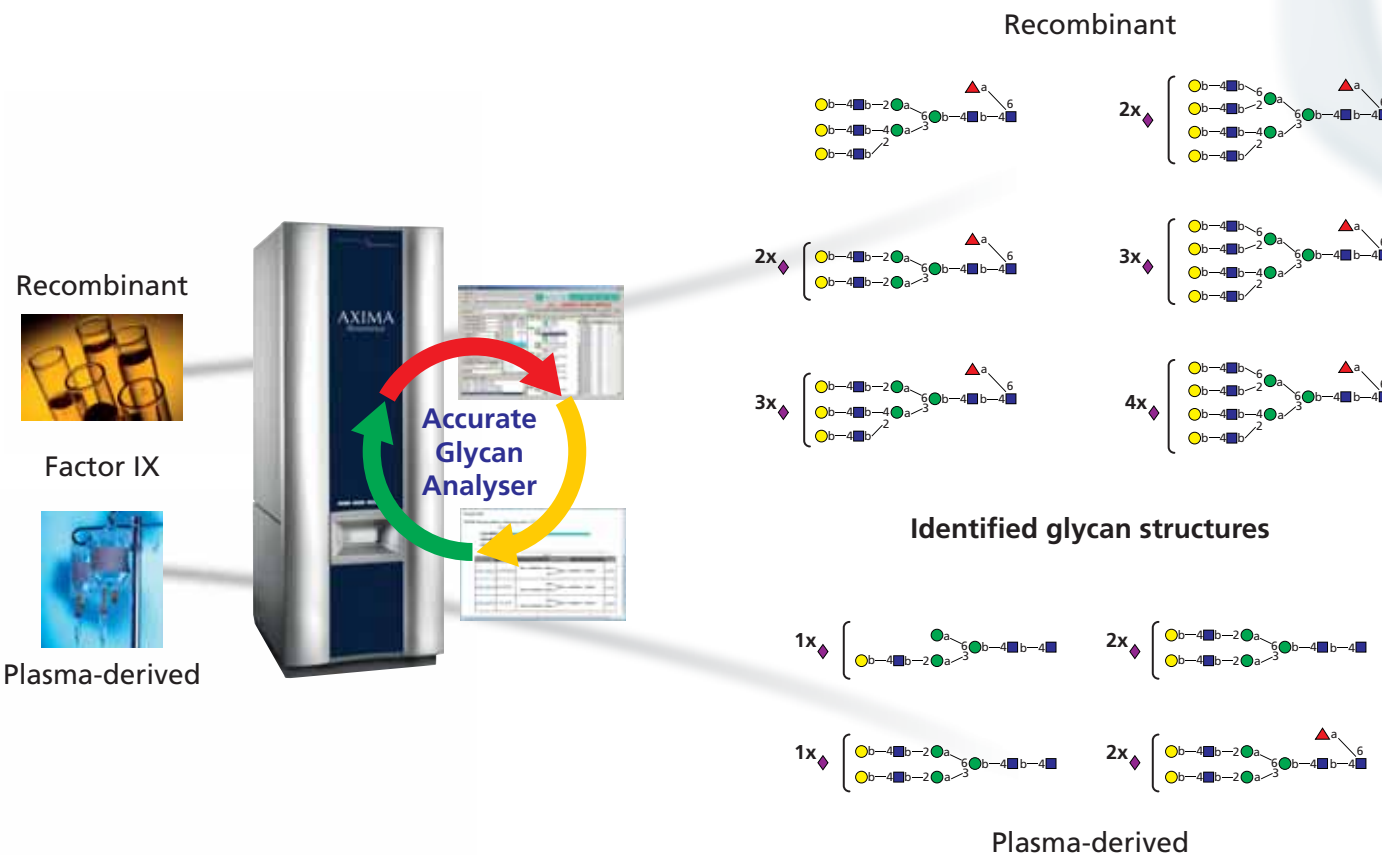


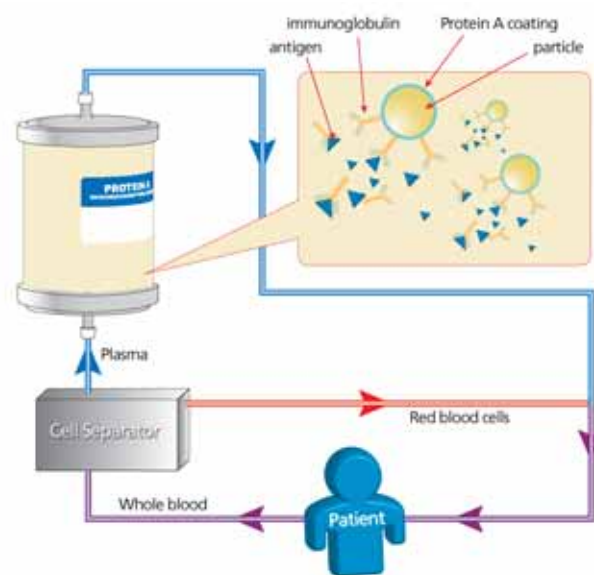
Figure 5: Summary of the identified glycan structures observed in plasma-derived and recombinant FIX using the Accurate Glycan Analyser software in combination with MSⁿ analyses. Significant differences were observed, in particular the extent of fucosylation and the core structures of the identified glycans.

Proteomics

Classical Proteomics

AXIMA Resonance, designed with sequencing and structural characterisation in mind

Peptide mass fingerprint (PMF) and MS² ion searching are widely used to enable high throughput protein identification. The example shown here illustrates the suitability of the AXIMA Resonance for classical proteomics applications. The proteins retained during a novel immunoadsorption therapy for rheumatoid arthritis (schematic 1) were studied in detail, combining 1D gel electrophoresis, PMF and MS² analysis.



Schematic 1: Treatment schematic of Staphylococcal Protein-A based immunoadsorption therapy. The patient plasma is separated from the red blood cells, passed through the immunoadsorption column, recombined with the blood cells and returned to the patient. Immobilised Protein-A binds immunoglobulins and attached proteins (antigens).

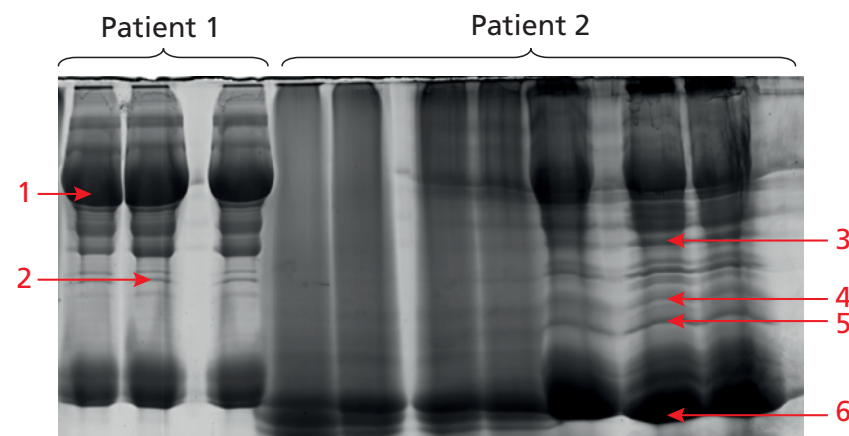


Figure 6: 1D SDS-PAGE of the proteins retained on column during immunoadsorption treatment of rheumatoid arthritis patients 1 and 2. Marked bands were excised and subjected to in-gel digestion with trypsin.

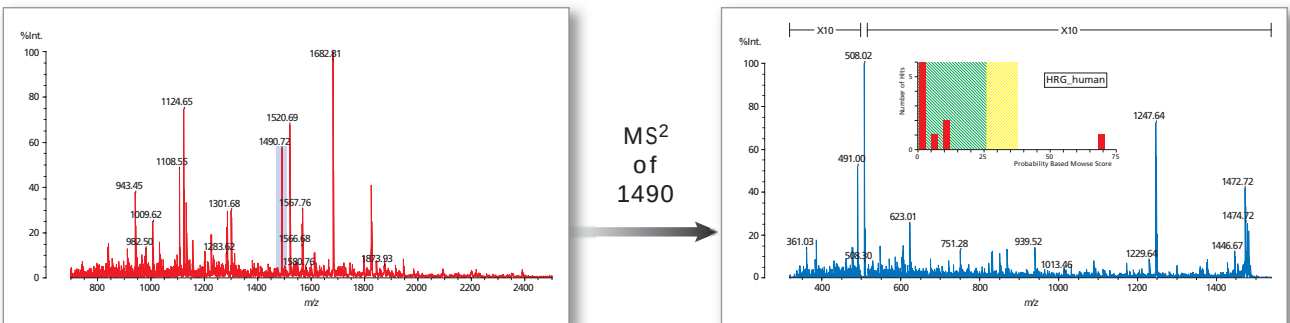


Figure 7: PMF mass spectrum of the tryptic digest of gel band 4 (left) and MS² analysis of m/z 1490 with individual Mascot MS² Ion Search (right) result.

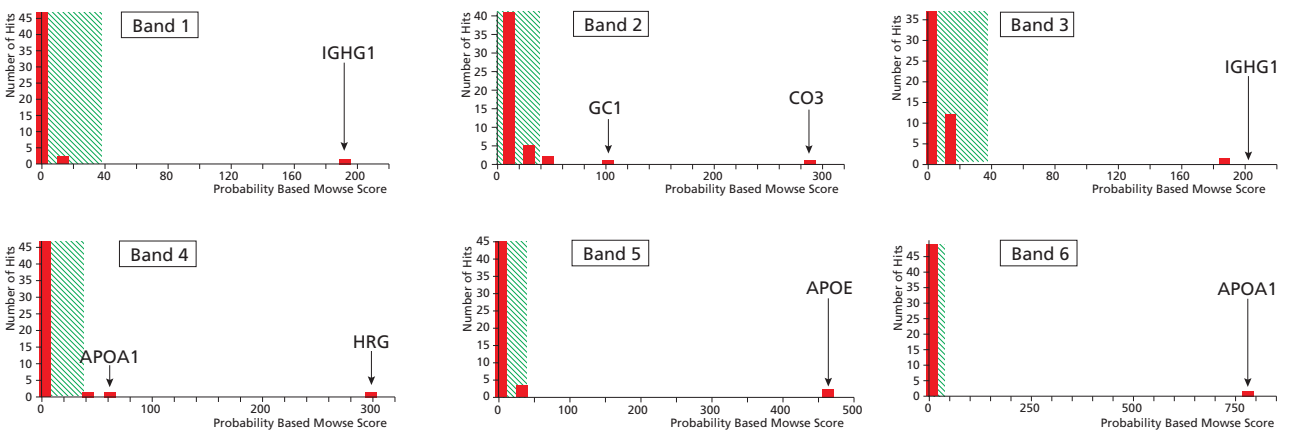


Figure 8: Summary of Mascot Ion Search results for all selected gel bands analysed for each patient. Matches to apolipoprotein A1 (APOA1), apolipoprotein E (APOE) and histidine-rich glycoprotein (HRG) were identified. Lipoprotein A1 has been reported in the literature to be present at an increased level in rheumatoid arthritis patients, however, little is understood of its biological role in inflammatory disease or the biological and therapeutic significance of the partial removal of these proteins from the bloodstream of rheumatoid arthritis sufferers.

Microbial Proteomics

Expanding rapid microbial identification through targeted proteomics

Mass spectrometric microbial identification is generally obtained using straightforward fingerprinting methods (eg: using the Saramis software on the AXIMA IDplus platform). However, the investigation of differences at the fingerprint level using MS² methods can provide more valuable and detailed information regarding the bacterial cells investigated.

The emergence of multi-drug resistant (MDR) enterobacteriaceae that can produce extended-spectrum beta-lactamases (ESBLs) is currently a major worldwide concern. These enzymes confer resistance to a wide spectrum of beta-lactam antibiotics currently used as first-line empirical therapy in the management of gram negative bacterial infections.

Significant differences in the microbial fingerprint were observed between ESBL- and non ESBL-producing *E. coli*. In particular, two peaks at m/z 2341.3 and m/z 3790.0 dominated the fingerprint from ESBL-producing *E. coli*. These potential biomarkers were selected for more in depth analysis by high resolution MS².

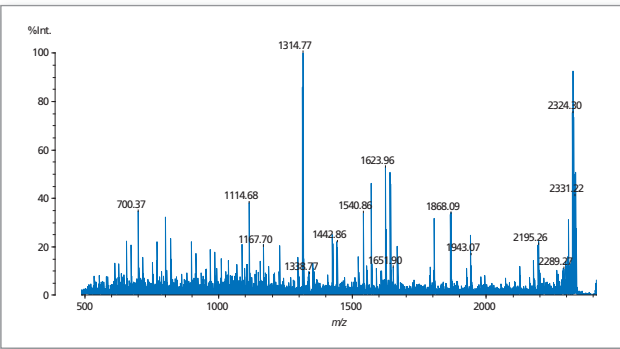


Figure 9: MS² mass spectrum of m/z 2341.

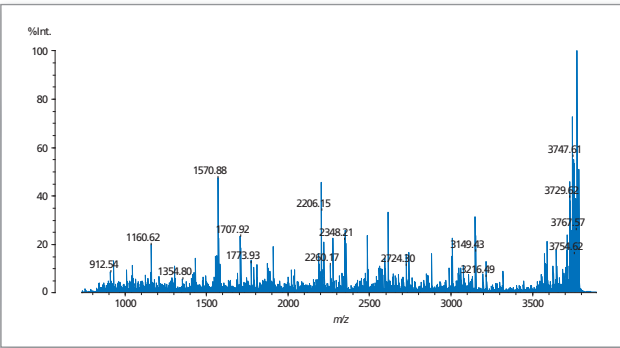


Figure 10: MS² mass spectrum of m/z 3790.

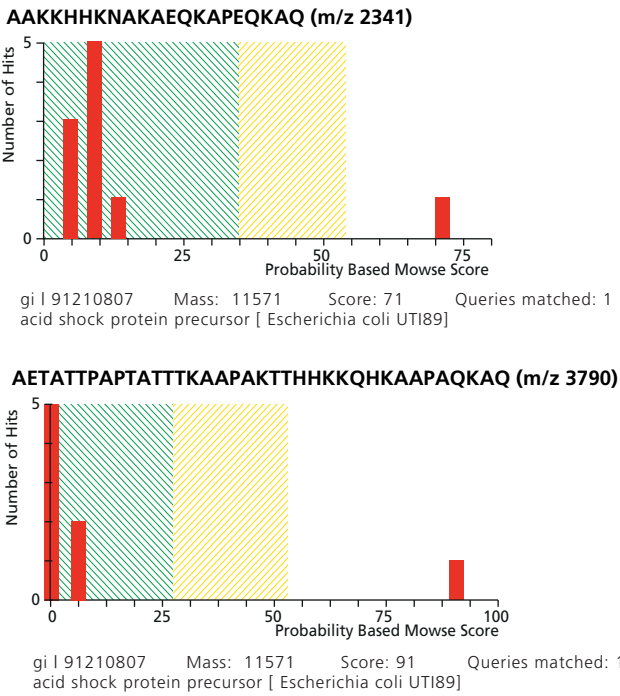
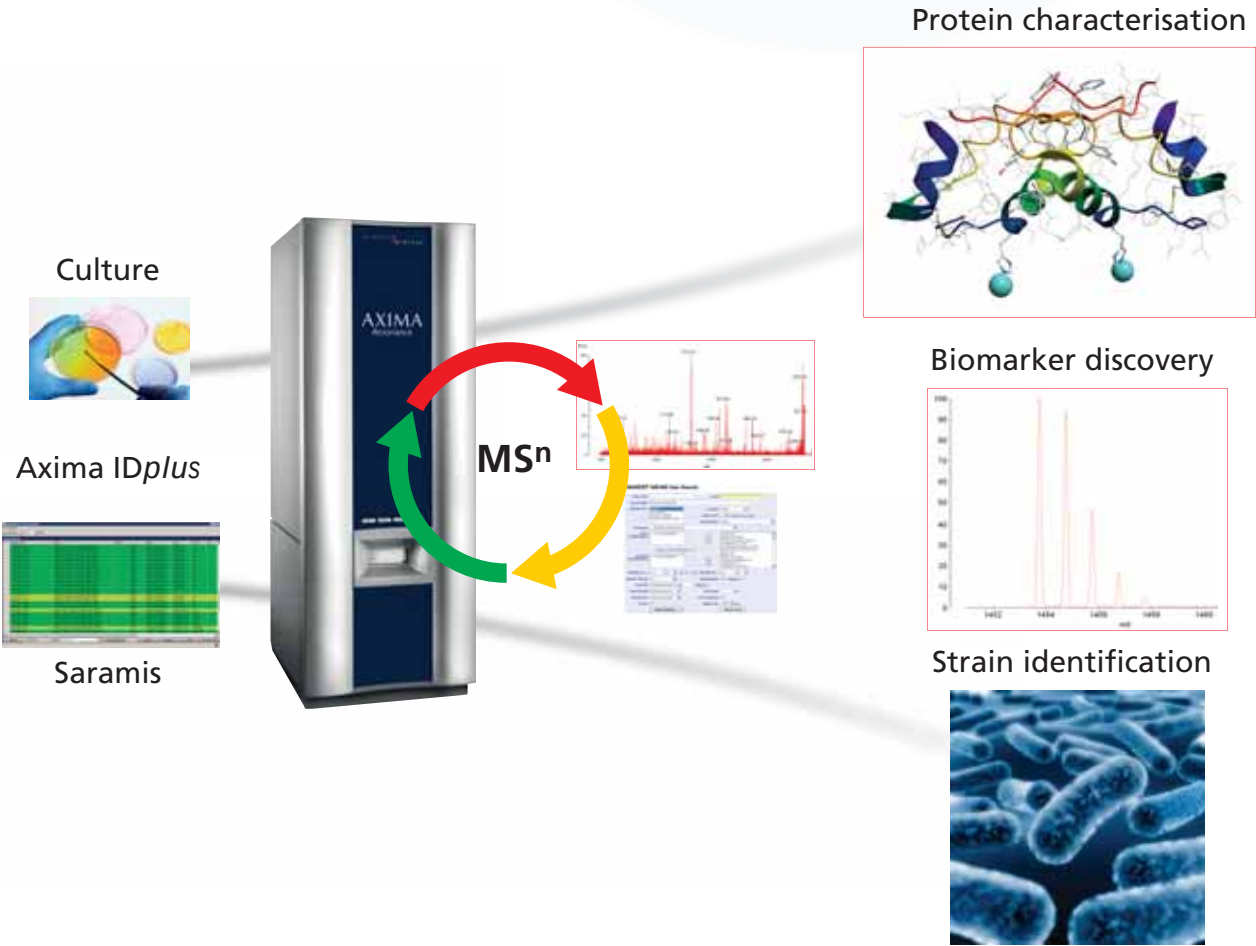


Figure 11: The MS² data were searched using Mascot Ion Search against all *E. coli* proteins in the NCBI database. Both peptides matched with high confidence to the same protein: acid shock protein precursor.



Top-Down Proteomics

Mapping intact proteins by combining In-Source Decay and MSⁿ

In-Source Decay (ISD) is a widely accepted technique in the sequencing of whole proteins by mass spectrometry, commonly referred to as top-down proteomics. Of particular importance is the characterisation of the protein N-terminus. Top-down sequencing using the AXIMA Resonance is achieved by a novel approach combining ISD and pseudo MSⁿ analyses in an ion trap. This has the advantage of desorbing large proteins and maintaining high resolution on the selection and detection of fragment ions.

ISD of human serum albumin provided a significant portion of the N-terminal sequence.

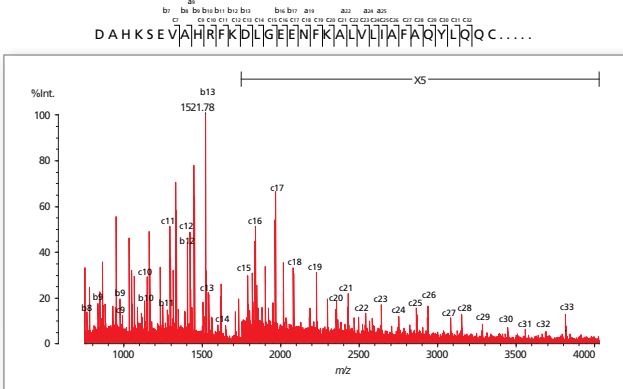


Figure 12: AXIMA Resonance ISD spectrum of HSA protein with its corresponding sequences. HSA protein gave a regular c-ion series from c7 to c33 and some b- and a-ion series. Observed mass resolution was greater than 11500 FWHM for the c33 ion.

Pseudo MS³ and pseudo MS⁴ fully characterised the N-terminus of the protein.

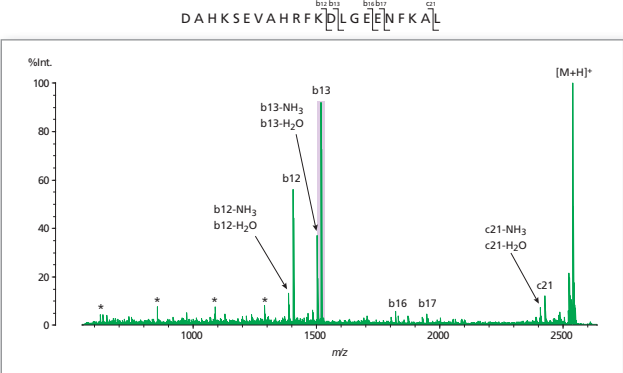
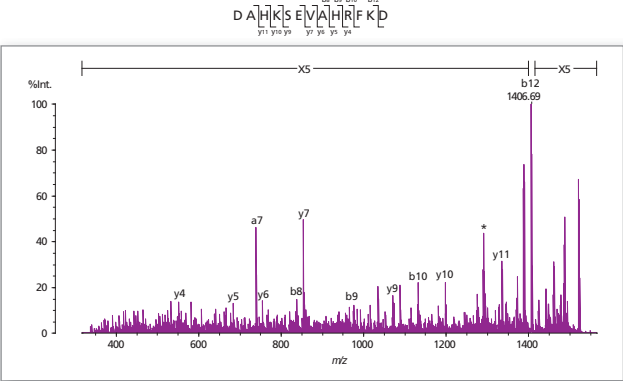


Figure 13: Pseudo MS³ of the c22-ion (m/z 2540.30). The two main species correspond to b12 and b13 ions with their neutral losses of NH₃ and H₂O. Other species are annotated: b16, b17 and c21. The observed mass resolutions for b13 ion, b13- NH₃ and b13- H₂O exceeded 9000 FWHM. The asterisks “*” mark internal fragments.

Figure 14: Pseudo MS⁴ of the b13-ion (product ion of the c22-ion, m/z 1521.75). Many species were identified: b8-b10, b12, y4-y7 and y9-y11. Mass resolution was similar to the pseudo MS³ spectrum.



Non-Enzymatic / Chemical Modifications

Thinking outside the box...

detecting protein modifications that are not in the database

Glycation is the non-enzymatic modification of a protein with a sugar molecule. Known as the Maillard reaction, it is the result of the condensation between the reducing end of a sugar and the reactive amine group of a protein. Unlike the enzyme-controlled glycosylation process, glycation is haphazard and tends to impair the function of the modified protein. Detailed characterisation of glycation products is therefore of high importance in the physiology and pathology of human diseases such as the chronic vascular complications of diabetes. It is also, however, relevant to the field of food processing.

In this study human serum albumin (HSA) glycation was investigated.

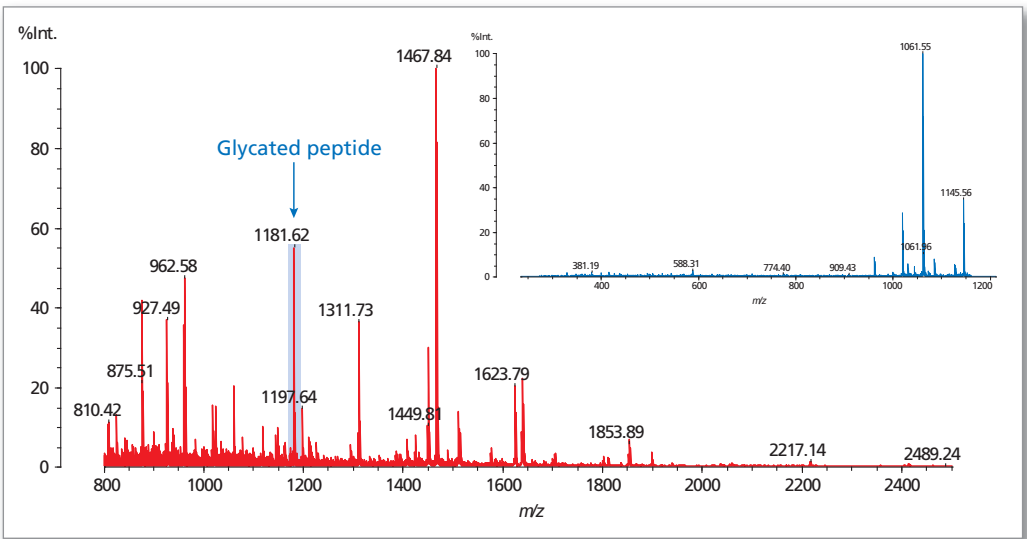


Figure 15: HSA was incubated with glucose for three weeks, digested with trypsin and the PMF was compared to that of unmodified HSA. Several differences between native and glycated HSA PMF were observed. These were analysed with MS², eg: MS² of m/z 1181 (inset).

PTMfinder is Shimadzu proprietary software that uses data mining to investigate protein modifications, including hypothetical and novel modifications. PTMfinder software offers the possibility of screening large MS² datasets for specific peptide modifications.



Figure 16: Glycation is a labile modification under UV MALDI conditions and in MS² produces a distinctive neutral loss pattern indicative of the modification (loss of 36, 120 and 162 Da from the precursor). Using the PTMfinder software, the specific MS² neutral loss signature for glucose was screened against glycated HSA digestion data. The screenshot illustrates the PTMfinder results highlighting the neutral losses on the spectrum.

Post-Translational Modifications

*MSⁿ capability with high mass accuracy -
an ideal alliance for post-translational modification analysis*

Collagen is a highly abundant protein in mammals and constitutes the main protein within connective tissue, tendons, ligaments and skin. Hydroxylation is a common post-translational modification of collagen formed through the reaction of proline amino acid residues with prolyl hydroxylase. Alpha-1 type 1 collagen was investigated through enzymatic degradation and MSⁿ mass spectrometry. Several trypsin-digested peptides were found to contain multiple hydroxylation sites due to the prevalence of proline residues. Mass spectrometric elucidation of the exact location of hydroxylation sites was performed using the AXIMA Resonance mass spectrometer combining MS² and MS³ for hydroxyproline (HyP) identification.

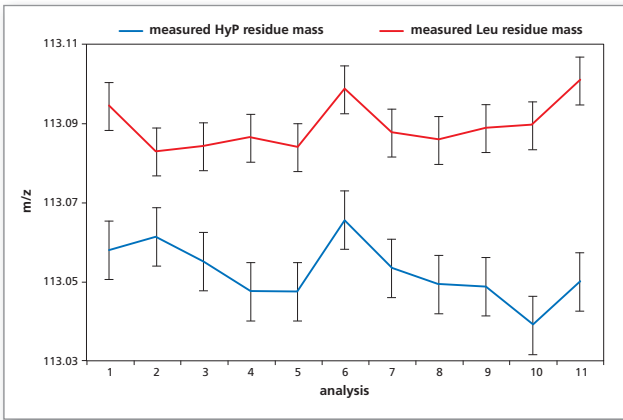
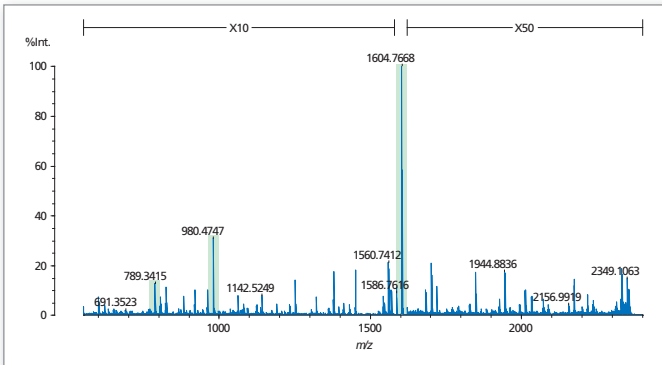


Figure 17: As HyP and Leu/Ile are isobaric in nominal mass (113.04768 and 113.08406 Da respectively), very high accuracy was necessary in order to specifically determine HyP sites. The accuracy of the AXIMA Resonance is sufficient to differentiate HyP from Leu/Ile (0.03638 Da) as illustrated in the statistical graph of multiple measurements of HyP (blue trace) and Leu/Ile (red trace) within HyP-Bradykinin and LHRH peptide sequences respectively.



b	Seq	y
362.2341	G	2031.9843
449.2661	S	1944.9522
546.3189	P	1847.8995
603.3404	G	1790.8780
674.3775	A	1719.8409
789.4044	D	1604.8140
846.4259	G	1547.7925
943.4786	P	1450.7397
1014.5158	A	1379.7026
1071.5372	G	1322.6811
1142.5743	A	1251.6440
1255.6220	HyP	1138.5963
1312.6435	G	1081.5749
1413.6912	T	980.5272
1510.7439	P	883.4744
1567.7654	G	826.4530
1664.8182	P	729.4002
1792.8767	Q	601.3416
1849.8982	G	544.3202
1962.9823	I	431.2361
2034.0194	A	360.1990
2091.0408	G	303.1775
2219.0994	Q	175.1190

Figure 18: MS² spectrum of the proteolytic peptide GSPGADGPAGAPGTPGPQGIAGQR (M+H)⁺ = 2393 Da) from ITRAQ modified collagen alpha-1(I). The localisation of the HyP residue was demonstrated by a shift in the predicted y-ions (highlighted in yellow).

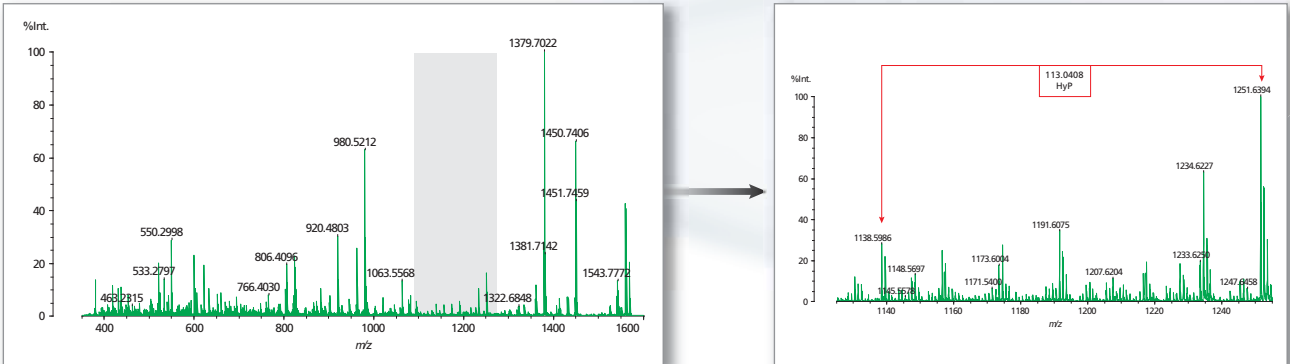


Figure 19: The dominant fragment ion at 1604 Da was selected for MS³ analysis and this confirmed the location of the HyP residue.

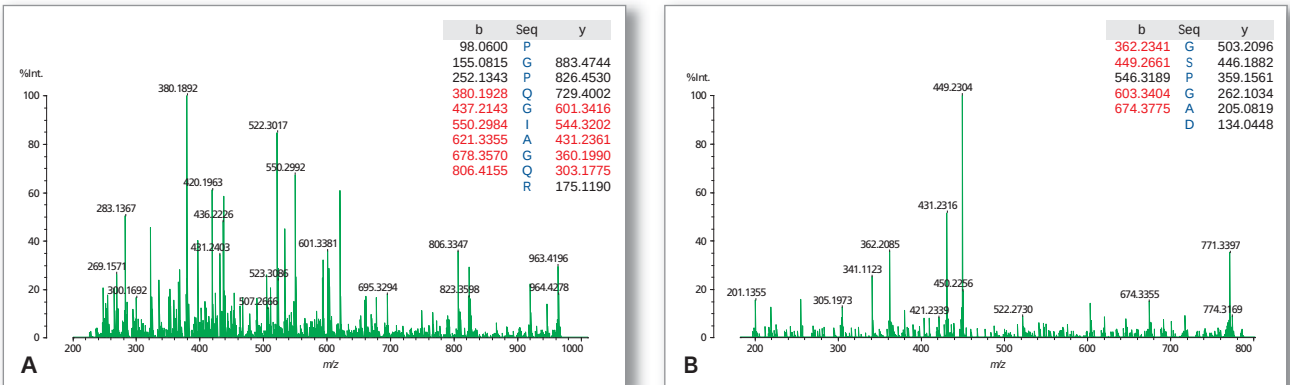


Figure 20: Combination of the MS³ data for the fragment ions 980 Da (A) and 789 Da (B) with that of 1604 Da (Figure 19) provided the complete amino acid sequence for this peptide.

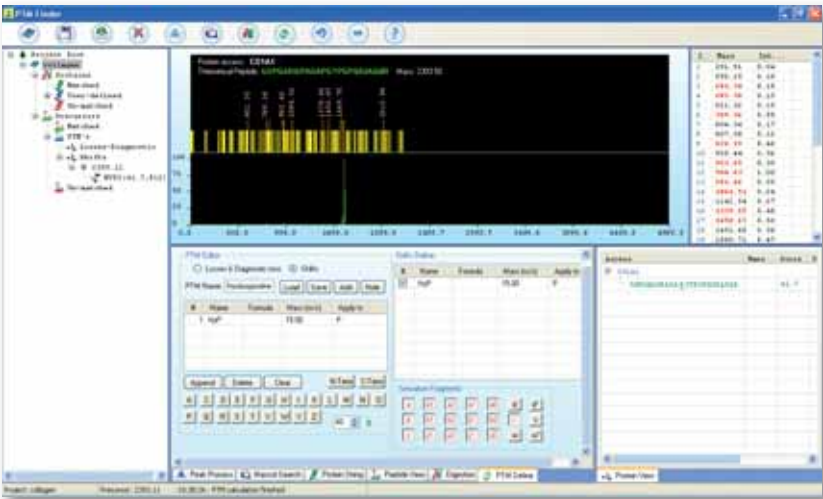


Figure 21: The MS² data were interrogated using PTMfinder software. Here hydroxyproline was defined as a possible modification of proline ($\Delta M = 15$ Da) and the MS² of m/z 2393 was screened. A single HyP modification was correctly identified at amino acid position 12 out of the possible 5 proline sites within the peptide.

Small Molecules

MSⁿ, maximising structural information for small molecules

Anthocyanins belong to the flavonoid group of polyphenolic compounds and have been investigated for multiple health benefits related to their anti-oxidant nature.

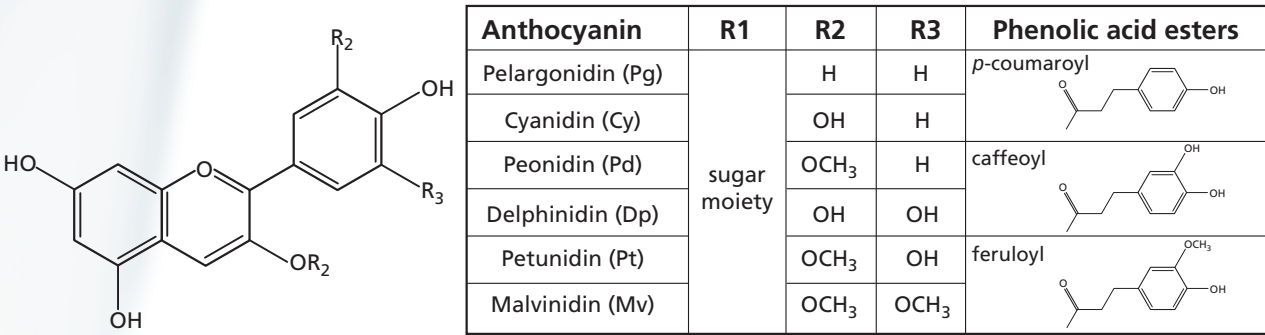


Figure 22: Basic structure of an anthocyanin. Many forms exist, the six most common core structures are shown in the table. The sugar moiety varies between mono-, di- and tri-saccharide units with various phenolic acid ester groups.

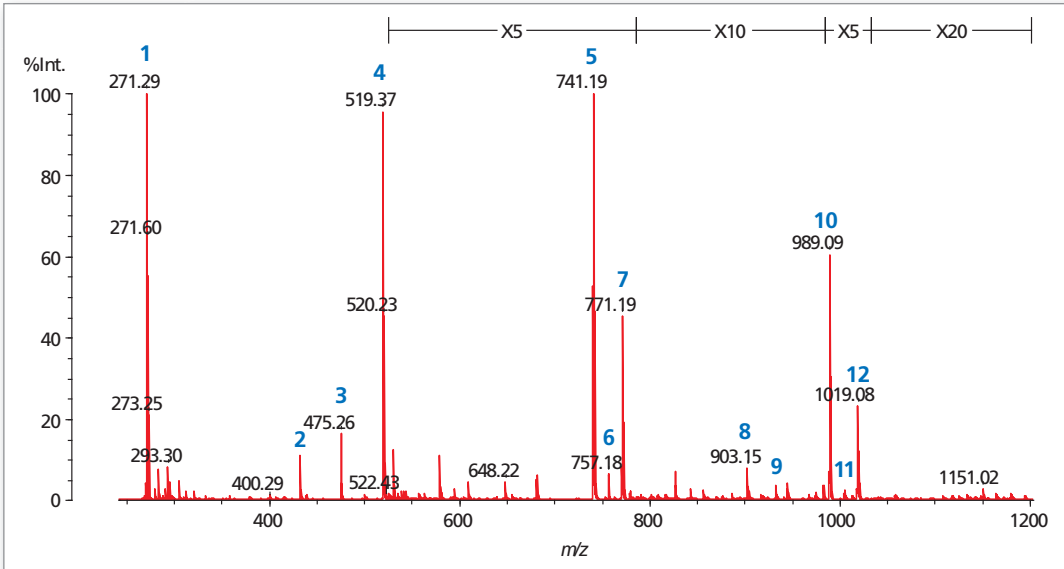


Figure 23: The MS spectrum of red radish extract contains a complex mixture of anthocyanins.

- 1 pelargonidin-aglycone
- 2 pelargonidin-3-glucoside
- 3 pelargonidin-3-glucoside acetylated
- 4 pelargonidin-3-(malonyl) glucoside
- 5 pelargonidin-3-(*p*-coumaroyl) diglucoside
- 6 pelargonidin-3-(caffeoyl) diglucoside
- 7 pelargonidin-3-(feruloyl) diglucoside
- 8 pelargonidin-3-(*p*-coumaroyl) diglucoside-5-glucoside
- 9 pelargonidin-3-(feruloyl) diglucoside-5-glucoside
- 10 pelargonidin-3-(*p*-coumaroyl) diglucoside-5-(malonyl) glucoside
- 11 pelargonidin-3-(caffeoyl) diglucoside-5-(malonyl) glucoside
- 12 pelargonidin-3-(feruloyl) diglucoside-5-(malonyl) glucoside

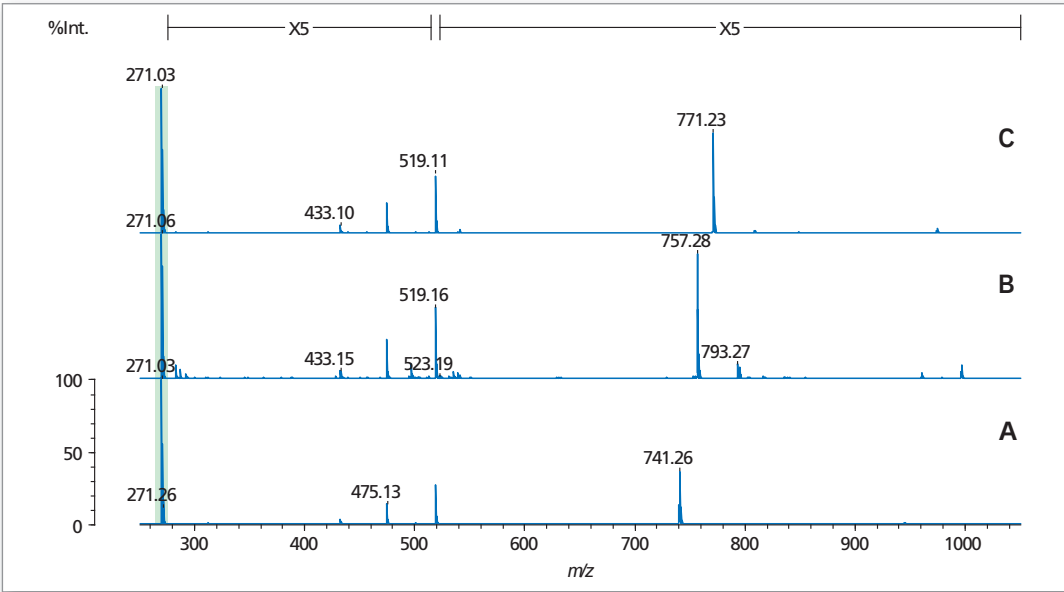


Figure 24: MS² analysis of three of the radish anthocyanins (m/z 989 (A), m/z 1005 (B) and m/z 1019 (C)) indicates that the fragmentation pathway mainly consists of the loss of the sugar moieties: loss of malonyl (86 Da), of 5-(malonyl) glucoside (248 Da), of the modified diglucosides at position 3, of both the modified diglucosides and malonyl and finally complete loss of all the sugar moieties to form the Pg-aglycone.

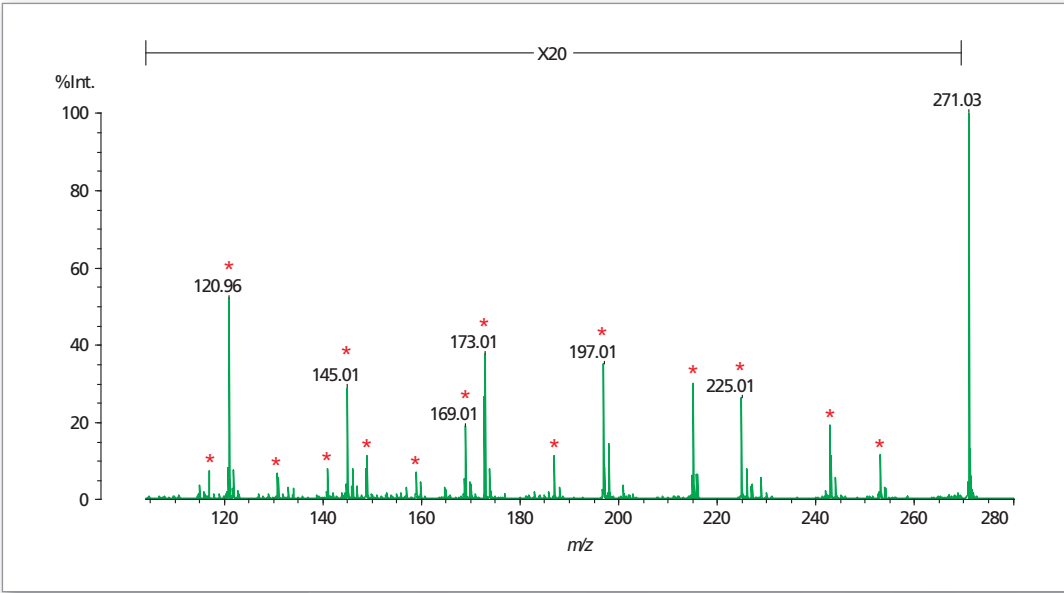


Figure 25: MS³ analysis of the Pg-aglycone fragment ion (m/z 271). Each of the peaks annotated with * corresponds to a known fragment of the Pg-aglycone structure. Fragmentation proceeds through loss of water (18 Da), loss of CO (28 Da) and loss of acetyl (42 Da).

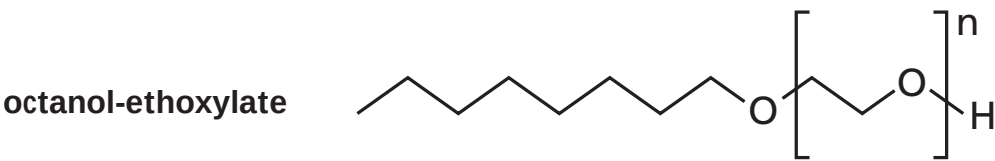
Polymers

Broadening horizons...

compositional analysis of synthetic polymers

Fatty alcohol alkoxylates are used as non-ionic surfactants in home and industrial cleaning agents. Characterised by important properties such as foam suppression, foam control and wetting effects, fatty alcohol alkoxylates are synthesised by reaction of fatty alcohols with alkoxides such as ethylene oxide and propylene oxide amongst others. Alkoxylates are also relevant in a broad range of chemical industrial applications where they are used as dispersal agents and emulsifiers.

MS and MS² were used to characterise the fatty alcohol alkoxylate polymer shown in schematic 2.



Schematic 2: Structure of the fatty alcohol alkoxylate polymers analysed.

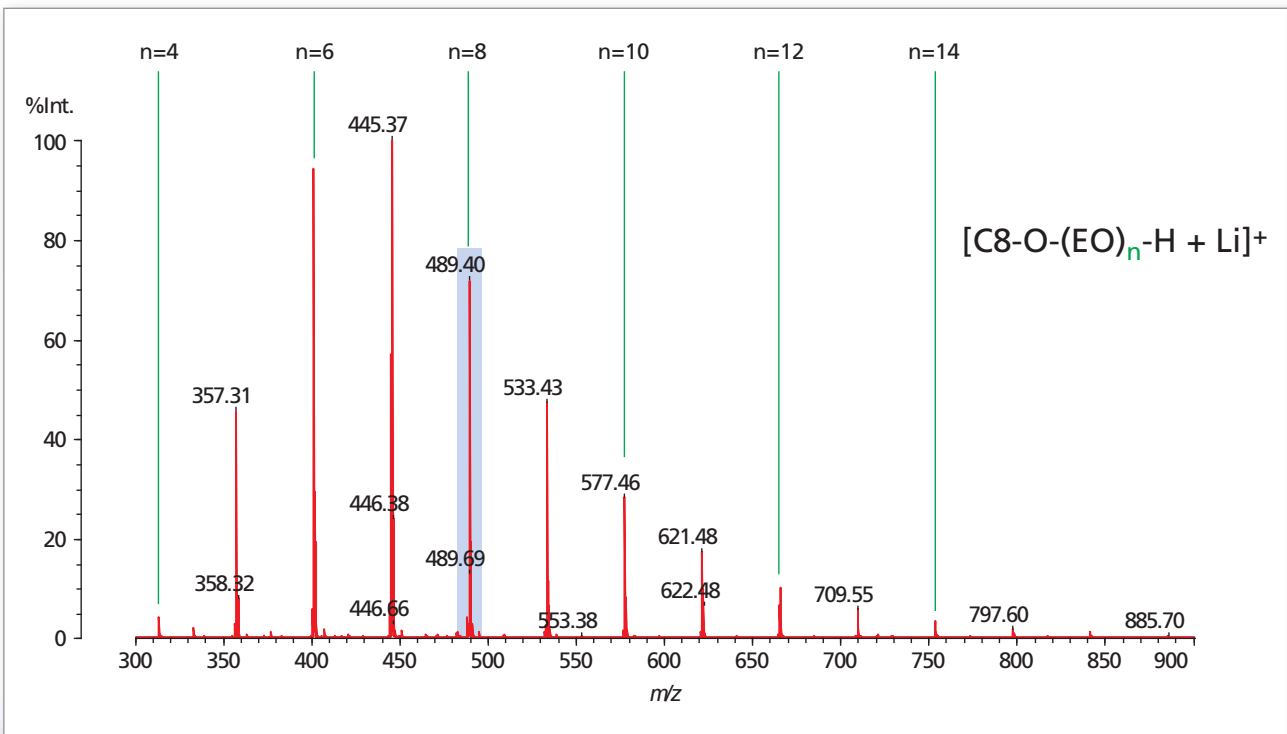


Figure 26: AXIMA Resonance mass spectrum of the ethoxylated octanol. The peaks represent (M+Li)⁺ ions. The mass distribution in the spectrum is asymmetric due to the low degree of ethoxylation in this product.

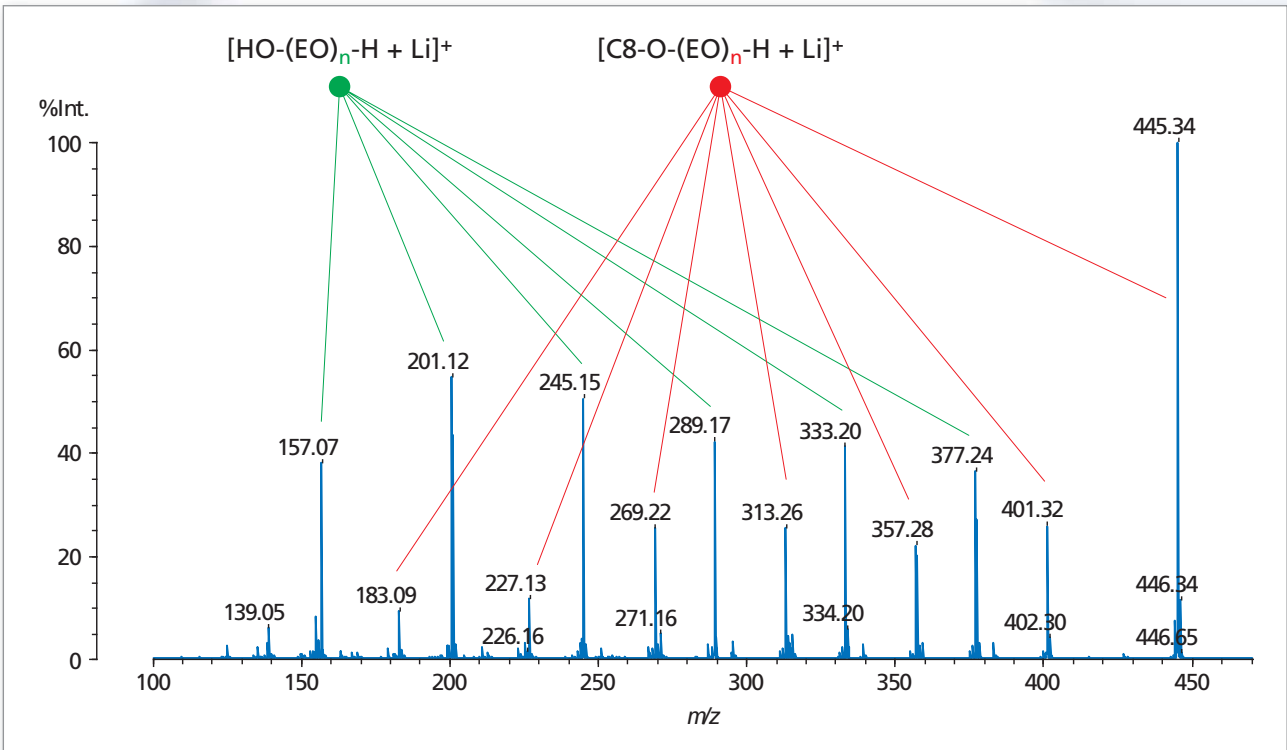
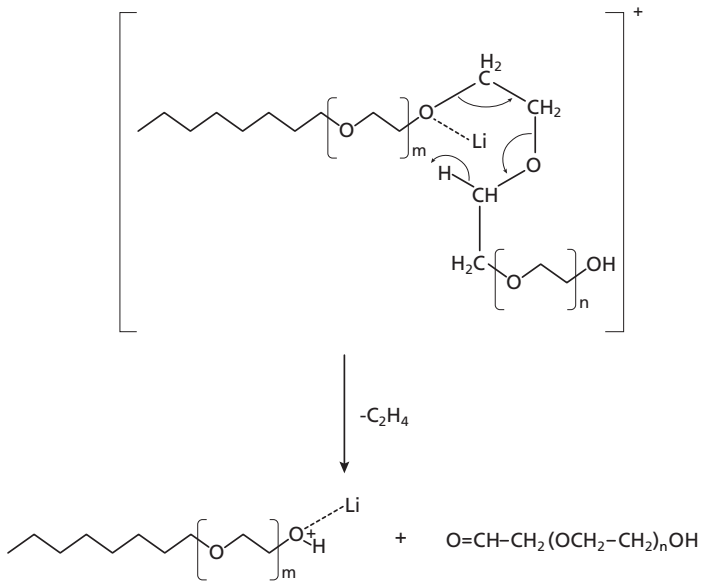


Figure 27: A key advantage of the AXIMA Resonance system is the ability to control the relative collision energy which results in high-quality CID-MS² spectra. This is the MS² spectrum of the lithium adduct precursor ion m/z 489 clearly showing the fragment series [HO-(EO)_n-H+Li]⁺ and [C8-O-(EO)_n-H+Li]⁺ (C8 = C₈H₁₇ and EO = CH₂CH₂O).



Schematic 3: A theory for the fragmentation of the ethoxylated fatty alcohols is based on the rearrangement reaction in which the lithium ion is attached in a transition state to the terminal oxygen. In the next step the elimination of ethylene takes place.

Lipidomics

Revolutionising lipid research with mono-isotopic precursor ion selection and MSⁿ capability

Lipids represent an important class of compound due to their broad range of vital biological functions. Mono-isotopic selection of lipids during MS² analysis is critical due to the natural occurrence of C-C bond unsaturation giving rise to ions that are separated by 2 Da in the mass spectrum.

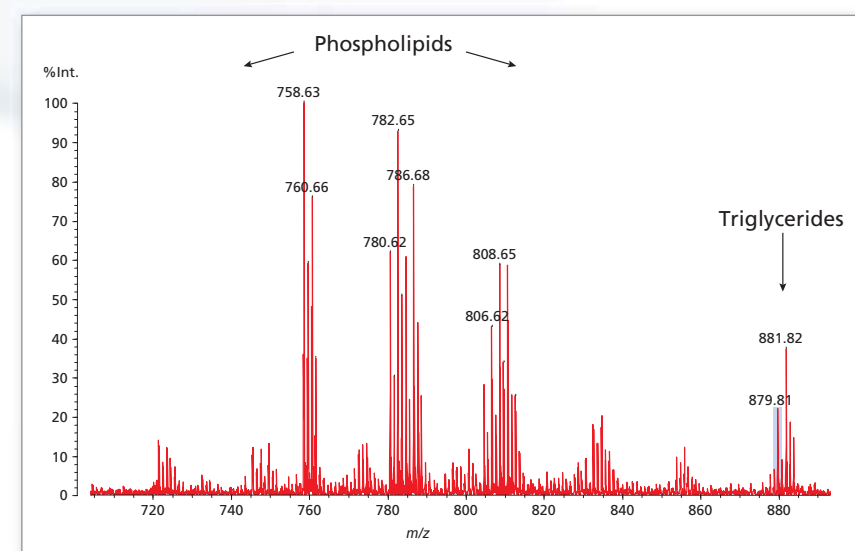


Figure 28: Representation of a typical MALDI mass spectrum of a crude mixture of lipids derived from human plasma. Plasma lipid extracts in MALDI-TOFMS exhibit mostly phospholipids and triacylglycerols (triglycerides or TAG). Depending on the experimental conditions (matrix used, solvent, cations, etc) it is possible to selectively ionise certain classes of lipids individually from a mixture. In this case, the chosen conditions allowed for the detection of mainly the phosphatidylcholine and TAG species.

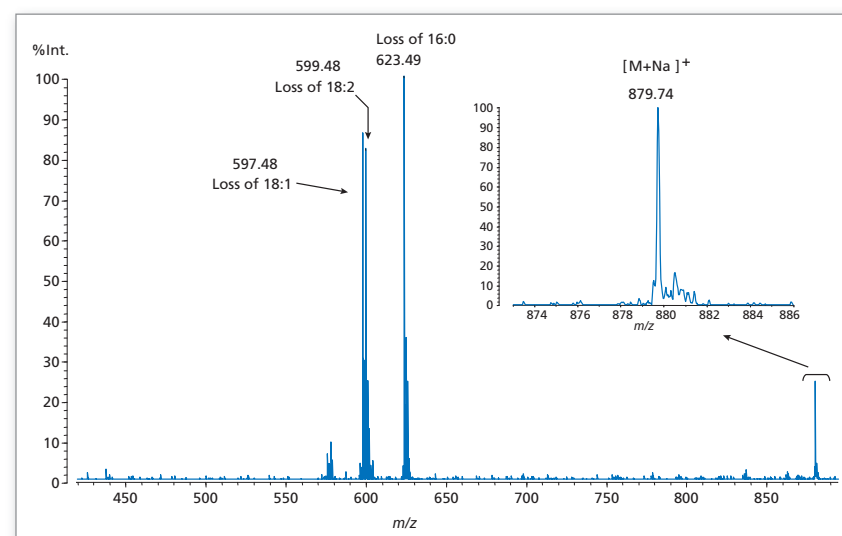


Figure 29: The positive ion MS² spectrum of a TAG (16:0, 18:2, 18:1) is shown. The sodium adduct [M+Na]⁺ (m/z 879.74) was detected and selected for MS² with isotopic resolution in order to perform the CID experiment. The product ion spectrum clearly exhibits the loss of the three different fatty acid chains (loss of RCOOH) where RCOOH can be a 16:0, 18:1 or 18:2 fatty acid.

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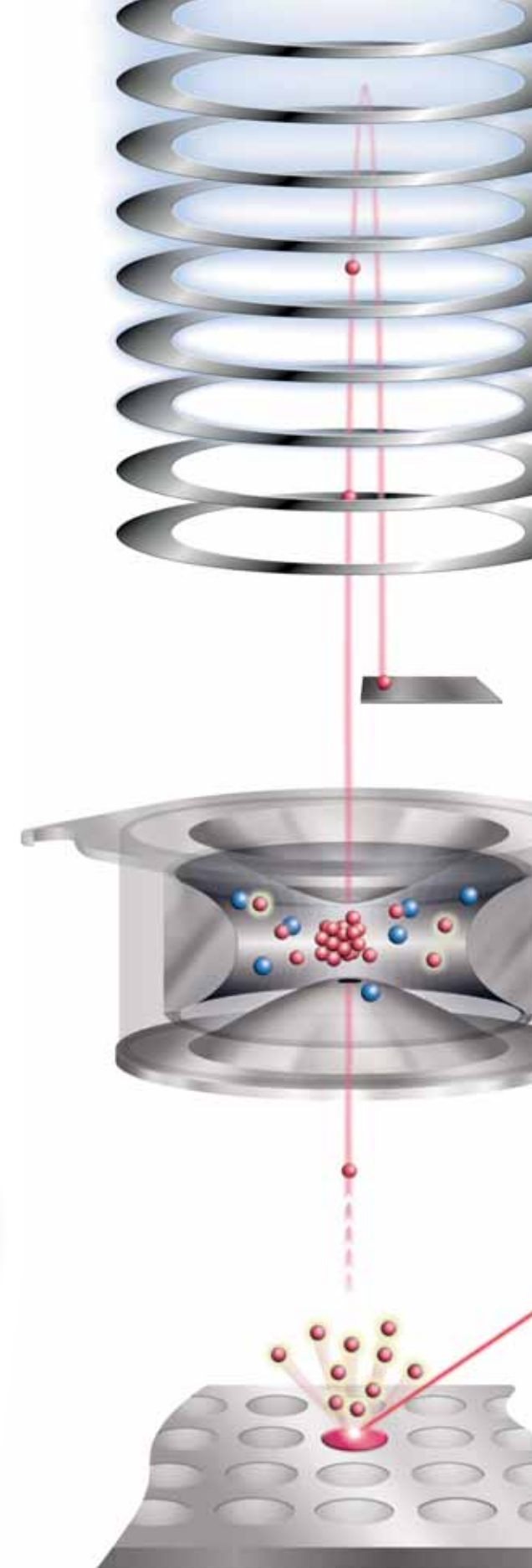
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