Chloroplast proteomics: potentials and challenges

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Abstract

With the available Arabidopsis genome and near-completion of the rice genome sequencing project, large-scale analysis of plant proteins with mass spectrometry has now become possible. Determining the proteome of a cell is a challenging task, which is complicated by proteome dynamics and complexity. The biochemical heterogeneity of proteins constrains the use of standardized analytical procedures and requires demanding techniques for proteome analysis. Several proteome studies of plant cell organelles have been reported, including chloroplasts and mitochondria. Chloroplasts are of particular interest for plant biologists because of their complex biochemical pathways for essential metabolic functions. Information from the chloroplast proteome will therefore provide new insights into pathway compartmentalization and protein sorting. Some approaches for the analysis of the chloroplast proteome and future prospects of plastid proteome research are discussed here.

Key words: Chloroplasts, mass spectrometry, plant cells, plant proteins, proteomics.

Introduction

Proteomics: definition and concepts

Proteomics defines an approach for the systematic identification of all proteins expressed in a cell and is expected to accelerate discoveries in the life sciences. The progress of proteomics and proteomics-related technologies over the last decade is based on two major developments. First, the wealth of genome information paved the way for the large-scale analysis of proteins for which amino acid sequences were deposited into databases (Arabidopsis Genome Initiative, 2000). Second, technological improvements in mass spectrometry, especially the development of soft ionization techniques for peptide analysis, allowed rapid and sensitive protein identification from minute quantities of biological samples (reviewed in Aebersold and Mann, 2003). Although it is generally accepted that the proteome is dynamic and difficult to define, scientists aim at the most complete identification of the protein complement of a cell or a tissue type under certain, well-defined conditions.

In principle, two basic proteomics approaches can be distinguished. ‘Shotgun proteomics’ attempts the identification of all proteins that are present in a sample and results in a list of proteins. Combined with sophisticated protein or peptide fractionation strategies, shotgun proteomics is technically a relatively simple method for high throughput analyses of the proteome of an organelle or a cell type and provides a snapshot of the major protein constituents (Shabanowitz et al., 2000; Washburn et al., 2001; Koller et al., 2002). The biological benefit of the acquired data could be 3-fold. Firstly, proteins in databases that are assigned as hypothetical, putative or unknown proteins, once identified in a proteomics study, are no longer hypothetical and their annotation can be changed to ‘expressed protein’. Secondly, shotgun proteomics performed with a subcellular organelle allows the definition of the organelle proteome and thus for novel insights into intracellular protein trafficking and sorting (reviewed in Taylor et al., 2003). Thirdly, shotgun proteomics can provide limited information about protein amounts, since the number of detected peptides per protein should relate to the abundance of the protein (reviewed in Greenbaum et al., 2003). ‘Functional proteomics’ concentrates on the analysis of specific proteins related to specific biological processes. This could be the identification of proteins that are modified or change their abundance in response to a perturbation. Such changes can be revealed by differential...
display techniques such as 2-dimensional (2-D) gel electrophoresis (O’Farrel, 1975) or isotope-coded affinity tagging (ICAT; Gygi et al., 1999a). Another example of functional proteomics is the identification of proteins isolated by affinity separation methods, such as antibody affinity precipitation, native purification of protein complexes, or affinity ligand binding.

Regardless of the approaches discussed above, only protein fractions of limited complexity can be analysed. This is a consequence of dynamic range limitations that are inherent to all relevant proteomics technologies. The dynamic range represents the ability to detect, analyse, and identify low-abundant proteins in the presence of highly abundant proteins. This limitation is apparent, for example, in 2-D gel electrophoresis, where highly abundant proteins dominate the stained gel while low-abundant proteins are difficult to detect. The dynamic range limitation of a mass spectrometer is a technical parameter that depends on the number of ions that can be detected at any given time point during the analysis and on the sensitivity of the instrument. Since the range of protein concentrations in a cell exceeds the dynamic range of every mass spectrometer, protein fractionation is normally required for proteome analyses aimed at the systematic identification of all cellular proteins. Two basic fractionation designs can be applied. Firstly, proteins or peptides can be fractionated according to their physico-chemical properties such as hydrophobicity or charge. When different chromatographic techniques are combined, this method is referred to as multidimensional fractionation or MudPIT (Washburn et al., 2001; reviewed in Whitelegge, 2002). Secondly, proteins can be fractionated according to their subcellular localization, for example, by enrichment of a specific membrane system or an organelle. This approach provides information about protein localization and allows novel insights into protein sorting. In most of the successful proteome analyses conducted to date these two techniques were combined, with subcellular fractionation preceding multidimensional chromatography.

Chloroplast proteome analyses
In the following sections, the progress that has been made in the analysis of the chloroplast proteome of higher plants will be summarized. Plastids can serve as a paradigm for organelle proteomics because they have several interesting properties. From an evolutionary perspective, it is now accepted that chloroplasts arose from cyanobacteria through endosymbiosis (Goksøyr, 1967). They are semi-autonomous organelles, which depend to a large extent on nucleus-encoded proteins that are imported into the plastid after translation in the cytosol. Plastids perform essential cellular functions and harbour a complex biosynthetic machinery for nitrogen fixation, amino acid biosynthesis, sulphur metabolism, isoprenoid biosynthesis, and gene expression (for review see Staehelin and Newcomb, 2000). Despite their importance for cellular function and development, the current understanding of plastid metabolic functions is far from complete.

In silico approaches to define the plastid proteome
Most of the proteins that constitute a functional chloroplast are nuclear encoded and are imported into the chloroplast after translation in the cytosol. The majority of these proteins have N-terminal amino acid sequences that facilitate their transport into the chloroplast and, therefore, are denoted as transit peptides (reviewed in Bruce, 2001). With the completion of the Arabidopsis genome, the chloroplast proteome has been estimated on the basis of transit peptide prediction by software tools like TargetP or ChloroP (Emanuelsson et al., 1999, 2000). More than 3600 proteins are predicted to be targeted to the chloroplast (http://mips.gsf.de). Recently, this information was combined with a genome-wide search for genes that are potentially of cyanobacterial origin (Abdallah et al., 2000; Martin et al., 2002; Leister, 2003). These genes might have been transferred from the cyanobacterial endosymbiont to the nucleus during evolution and many of the gene products would be routed back to the chloroplast. Martin and colleagues (Martin et al., 2002) have compared 24 990 proteins encoded in the Arabidopsis genome with three cyanobacterial genomes, the yeast genome, and 16 reference genomes from prokaryotes. They identified 9368 proteins to be sufficiently conserved for a primary sequence comparison. From this set of proteins, 866 proteins are shared between Arabidopsis and the cyanobacterial genomes, and 834 proteins branch with cyanobacterial proteins in phylogenetic analyses (Martin et al., 2002). It is conceivable that these 1700 proteins are encoded by genes that were transferred from the plastid to the nucleus. Interestingly, more than half of these proteins are not predicted to localize to the chloroplast by TargetP. On the basis of this observation, it was concluded that cyanobacterial proteins are not generally routed back to their origin, but rather were distributed to different subcellular locations during evolution (Martin et al., 2002; Leister, 2003).

Prediction of subcellular localization could be erroneous, however, because transit peptides are not well conserved (Bruce, 2001), which limits the reliability of all existing prediction programs. Two parameters characterize the performance of prediction programs, the specificity and the sensitivity (Emanuelsson et al., 1999, 2000). On a training set of 778 proteins, the sensitivity (i.e. the prediction of true chloroplast proteins to be chloroplast-localized) of the plant version of TargetP was calculated to be 85% (Emanuelsson et al., 2000). The specificity (i.e. the correct prediction) with this training set was 69%. These values indicate the relatively high degree of uncertainty of computer-based prediction (Emanuelsson et al., 2000).
Besides the lack of primary sequence conservation, import pathways exist that follow different and, to date, unknown import rules, which have not yet been implemented to improve prediction algorithms. A number of proteins have been reported recently that do not follow a canonical import pathway, both for mitochondrial (Pfannner et al., 1987; Folsch et al., 1996) and chloroplast protein import (Miras et al., 2002). For example, dual targeting has been observed for a glutathione reductase that is imported into mitochondria and chloroplasts (Rudhe et al., 2002). Miras and colleagues have demonstrated that a protein without a canonical transit peptide localizes to the inner membrane of the chloroplast envelopes. The data obtained with truncated versions of this protein suggest that neither the N- nor the C-terminus are essential for chloroplast localization (Miras et al., 2002). Other examples of proteins lacking an N-terminal transit peptide have been reported from mitochondria. Two proteins from yeast contain their signal sequence at their C-terminus (Pfannner et al., 1987; Folsch et al., 1996). These findings indicate that the assessment of an organellar proteome on the basis of software-based protein localization prediction will yield incomplete and unreliable data. The systematic identification of proteins from organelles by proteomics is, therefore, an important complementary approach to obtain new information about intracellular protein sorting that will help to define novel rules for protein import into organelles.

**Mass spectrometry-based large-scale approaches to analyse the chloroplast proteome**

The thylakoid lumen: During the last few years, several chloroplast proteomics studies have been published that focused on a suborganellar compartment such as the thylakoid lumen or the chloroplast envelope membrane system (Peltier et al., 2000, 2002; Schubert et al., 2002; Ferro et al., 2002, 2003). Historically, the analysis of the thylakoid lumen proteome preceded other large-scale proteomics efforts, since the lumen proteome is made up of soluble proteins that can easily be analysed by 2-D gel electrophoresis. Furthermore, proteins that are transported into this compartment have a bipartite transit peptide that can be recognized by currently available software prediction tools (Peltier et al., 2002). Therefore, the analysis of the thylakoid lumen proteome was used to fine-tune computer prediction programs and to improve the in silico prediction of the thylakoid lumen proteins from the *Arabidopsis* database (Peltier et al., 2000, 2002). Technically, thylakoid lumen proteins were isolated from intact chloroplasts followed by their separation using 2-D gel electrophoresis. Proteins were then identified by MALDI TOF and LC-ESI-MS/MS (see Glossary) and N-termini were established by Edman degradation. Together, a comprehensive list of more than 100 proteins from the thylakoid lumen is now available (Peltier et al., 2000, 2002; Schubert et al., 2002). Among the proteins are many immunophilines, isomerases, proteases, and several proteins involved in antioxidant metabolism (Peltier et al., 2000, 2002; Schubert et al., 2002).

The combination of 2-D gel electrophoresis and MS/MS analysis allowed the comparison of theoretical isoelectric point (pI) and molecular mass with the experimentally determined values for each of the identified proteins. This comparison revealed several erroneous database annotations, ranging from false N- or C-termini to incorrectly predicted intron/exon boundaries (Peltier et al., 2002). With the help of available EST sequence information, Peltier and colleagues identified one interesting protein produced by alternative splicing (Peltier et al., 2002). This discovery elegantly demonstrates that use of 2-D gel electrophoresis early in the workflow provides valuable additional information for protein identification and correction of database entries. Furthermore, 2-D gel electrophoresis provided an estimate of protein abundance in the thylakoid lumen, which Peltier and colleagues used to assess the dynamic range of the proteins. They found that the concentrations of the most abundant proteins were 10 000-fold higher than the low-abundant proteins that are still visible on the gel. Considering the detection limit of 2-D gels it is likely that the true dynamic range of proteins in the thylakoid lumen is several orders of magnitude higher. Since this dynamic range exceeds the ability of currently available proteomics technology to display and identify low-abundant proteins, the authors conclude that it is impossible experimentally to define the lumen proteome (Peltier et al., 2002). Therefore they developed an approach to predict the thylakoid lumen proteome in silico by using characteristic protein parameters that were derived from the sequenced proteins. Forty-one (41) of the identified proteins from the thylakoid lumen were then used to search for orthologues in all land plants and in *Chlamydomonas reinhardtii* and 201 luminal proteins were retrieved from this search. These proteins constituted the test and training set that was the basis for the prediction of the lumen proteome. Using stringent search criteria in a multistep prediction procedure, 71 proteins were identified as potential candidates to enter the thylakoid lumen via the twin-arginine translocation pathway (TAT-pathway) (Robinson et al., 2001; Peltier et al., 2002). This stringent prediction most likely underestimates the number of proteins that enter the thylakoid lumen and has limitations for the prediction of proteins that are located via the Sec-translocase pathway (Robinson et al., 2001; Schubert et al., 2002).

The constraints discussed above were substantiated by the lumen proteome analysis reported by Schubert and colleagues (Schubert et al., 2002). They reproducibly identified more than 250 proteins from highly purified spinach and *Arabidopsis* thylakoid lumen vesicles by 2-D gel electrophoresis. On the basis of this comparison they
predicted protein localization. It is error-prone and requires careful verification of the proteome of an organelle or a suborganellar compartment, whereas a prediction is a valuable tool to assess the thylakoid lumen proteome reported by the two laboratories are partially complementary. Although software-based prediction is a valuable tool to assess the proteome of an organelle or a suborganellar compartment, it is error-prone and requires careful verification of the predicted protein localization.

The envelope membrane system: In contrast to the soluble thylakoid lumen proteins, it is technically difficult to analyse membrane proteins by 2-D gel electrophoresis. Therefore, a different approach was used for the analysis of the chloroplast envelope membrane proteome. In these studies, a combination of the specific enrichment of envelope membranes combined with an extraction of hydrophobic proteins was followed by protein identification with electrospray ionization mass spectrometry (Ferro et al., 2002, 2003). The specific enrichment of hydrophobic proteins by organic phase extraction (chloroform/methanol), alkaline, or saline treatment was an efficient tool to improve the analysis of transporter proteins localized in the inner and outer envelope membrane (Ferro et al., 2002, 2003). Originally, 54 proteins were identified from highly purified chloroplast envelope preparations after chloroform/methanol extraction of the hydrophobic core. Twenty-one of these proteins contain at least four α-helical trans-membrane domains and are, therefore, most likely transporters in the inner envelope membrane (Ferro et al., 2002). When the hydrophobicity of the identified proteins, defined as amino acid residues per transmembrane domain (Res/TM) was plotted against the isoelectric point (pI), most of the proteins have a high pI value with a Res/TM value smaller than 100. This information was used to search the database for transporters that are potentially localized in the inner envelope membrane and that comply with the criteria pI > 8.8, four trans-membrane domains (TM), Res/TM <100, and the presence of a chloroplast transit peptide. Together, 136 proteins complied with these stringent criteria and are likely transporters in the inner chloroplast envelope membrane (Ferro et al., 2002). By contrast, a purely theory-based in silico search strategy resulted in 541 potential envelope membrane proteins (Koo and Ohlrogge, 2002). This difference suggests that proteomics can provide details about protein composition to narrow predicted proteins to a more accurate and reliable group.

Ferro and colleagues took another step towards a more exhaustive list of experimentally verified envelope membrane proteins (Ferro et al., 2003). The authors extracted proteins with increasing hydrophobicity from envelope membranes (Ferro et al., 2003). The improvement in the dynamic range of the protein fractions resulted in the identification of 112 proteins. Along with the identification of new proteins (including putative transporters), this study provided additional information about N-termini of chloroplast membrane proteins. Several proteins were N-acetylated, which is a frequent post-translational modification in plants (Ferro et al., 2003). Another notable finding was the identification of proteins with homology to mitochondrial protein import components. Although contaminations cannot be ruled out, the high purity of the envelope preparation suggests that these proteins could also be true constituents of the chloroplast envelope membrane. It is possible that the chloroplast protein import machinery is more variable than previously anticipated (Ferro et al., 2003). A comparison of the above-discussed proteins with the proteins extracted from the hydrophobic core (chloroform/methanol extraction) of the envelope membranes of spinach and Arabidopsis supports the view that the composition of the envelope membrane is very dynamic. This is perhaps not surprising, because the envelope system has a critical regulatory function in metabolite and protein exchange between the cytosol and the chloroplast. To establish the complete proteome of the chloroplast envelope membrane system and to elucidate the full dynamics of its protein composition, it will be necessary to investigate several plant model systems under different environmental conditions.

Targeted approaches and functional proteomics

An elegant proteomics approach was applied to define novel thioredoxin targets by affinity chromatography (Balmer et al., 2003). Thioredoxin is known as an important regulator protein that mediates the status of the photosynthetic electron transport to the activity of metabolic and regulatory proteins. An affinity column was constructed with immobilized thioredoxin m and f to search for interacting proteins systematically. To prevent their complete reduction, the immobilized thioredoxin carried a mutation of one cysteine residue to alanine or serine, trapping interacting proteins as mixed disulphides. Several proteins of a chloroplast stroma preparation bound to the column, among them known thioredoxin targets from different metabolic and regulatory pathways including the pentose phosphate and the Calvin cycle, nitrogen and sulphur metabolism, and chloroplast translation.
(Balmer et al., 2003). In addition, 15 potential novel thioredoxin targets were identified from the isoprenoid, tetrapyrrrole, and vitamin biosynthetic pathways (Balmer et al., 2003). This experiment demonstrates that targeted proteomics approaches using affinity chromatography can provide substantially new biological information (Balmer et al., 2003).

Similar to affinity purification, the isolation of native protein complexes can provide biological information about protein/protein interactions. Protein/protein interactions are of major importance for the regulation of cellular processes. While transient interactions cannot be detected with commonly employed proteomics technologies, several analyses of stable chloroplast protein complexes have been reported. One example is the analysis of the chloroplast RNA polymerase which is of major importance for the maintenance of chloroplast functions (Fleischmann et al., 1999). In addition to known RNA polymerase subunits, Fleischmann and colleagues identified a 36 kDa RNA binding protein and an iron superoxo dismutase associated with the highly purified complex. The association of an iron superoxo dismutase with the RNA polymerase complex is unexpected, but most likely reflects the integration of the RNA polymerase in a redox signalling network (Li, 2003). The integration of an RNA binding protein in the complex suggests significant crosstalk between the regulation of gene expression on the transcriptional and post-transcriptional level. The assembly of enzymes from different levels of gene expression into a protein complex is also realized in the 70S ribosome of Chlamydomonas reinhardtii. Recent proteome analyses identified a protein with high homology to the chloroplast endonuclease CSP41 that associates stably with known ribosomal proteins (Yamaguchi et al., 2002, 2003). CSP41 is involved in the regulation of chloroplast mRNA stability and its association with the ribosome suggests a co-ordinated regulation of mRNA stability and translation (Yang et al., 1996). In addition to the examples described above, other targeted analyses of protein complexes established the subunit composition of the chloroplast RNA degradosome (Baginsky et al., 2001), and the CLP protease complex (Peltier et al., 2001). Together, the combination of native protein complex isolation techniques with mass spectrometric identification of its subunits provided valuable new insights into stable protein/protein interaction in the chloroplast and suggested regulatory networks that expand over different levels of gene expression.

The comparison of the protein complement of cells or tissues under different experimental conditions is expected to provide information about the function of proteins that are altered between different states. Several examples are available where large-scale differential display techniques have been employed to study the adaptation of the cellular protein composition to changing conditions. For example, Kubis and colleagues compared the protein complement of purified chloroplasts from the import mutant ppiI with that of wild-type chloroplasts (Kubis et al., 2003). The abundance of photosynthetic proteins is significantly reduced in the mutant while non-photosynthetic proteins are not affected, suggesting that the ppiI mutant is specifically defective in the import of photosynthetic proteins (Kubis et al., 2003). While this conclusion is justified by the data, the proteome comparison between the mutant and wild-type chloroplasts was by no means exhaustive and only the most abundant proteins were analysed. Dynamic range limitations make the identification of low-abundant proteins difficult and are a general problem of large-scale differential display techniques. These problems can be circumvented by the focused analysis of specific sub-proteomes such as, for example, the thylakoid membrane system. The thylakoid membrane system has been studied intensively and several reports are available that investigated its protein composition and the remodelling of the antenna complexes, for example, in response to iron deficiency (Moseley et al., 2002; Stauber et al., 2003).

Since the thylakoid system is composed of proteins that rank among the most abundant chloroplast proteins it has been used as a model to employ novel mass spectrometric concepts. Intact mass measurements have been used to study the state of post-translational modification of thylakoid membrane localized protein complexes (Whitelegge et al., 2002; Gomez et al., 2002). Phosphorylation of D1, D2, CP43, LHCb, and PsbH was confirmed and PsbT was identified as a new phosphoprotein. A second phosphorylation site was identified for PsbH and an interesting correlation of the occurrence of the more highly phosphorylated form with a 32 Da adduct, that presumably derived from oxidation by illumination, was found (Gomez et al., 2002). Intact mass measurements can provide information about post-translational modifications and proteolytic processing of the analysed proteins. This has been demonstrated in a recent study by Zabrouskov and colleagues, who employed Fourier-transform mass spectrometry to identify and characterize intact proteins in a rather complex mixture. This approach corrected the predicted signal peptide cleavage sites for the oxygen-evolving complex protein 3 (Zabrouskov et al., 2003). Clearly, intact mass measurements open an important new field in proteomics that will gain attention in the years to come.

Plastid proteomics: résumé and outlook

After three years of chloroplast proteome research, there is now a realistic estimate of the potential contribution of proteomics approaches to chloroplast research. Probably the most valuable information that has been obtained during this time is new information about subcellular
proteomics with organelles or sub-organellar compartments provides the possibility to obtain insights into protein sorting on a large scale. Limitations for the reliability, however, are contaminations with proteins from other organelles or from the cytosol that can never be ruled out completely. Complementary localization-experiments are necessary to confirm protein localization to an organelle. Currently, existing prediction tools fail to predict a substantial number of the plastid localized proteins and appear to be less reliable than suggested in the literature. Proteomics can help to define novel protein sorting rules and improve existing prediction algorithms for protein localization. It is expected that the importance of this aspect will increase over the years to come as more and more proteome data become available for different plastid types and other cell organelles. Another aspect of importance is the discovery of hypothetical, putative or unknown proteins. Proteomics data demonstrated that a significant number of the proteins that constitute the plastid proteome have one of the above annotations. In combination with high-throughput-mutant screening, the identification of these proteins might reveal novel metabolic or regulatory functions that reside inside the plastid.

Although significant progress has been made to establish the proteome of higher plant plastids, the proteins identified to date represent only a small fraction of the complete chloroplast proteome. The most important difficulties are dynamic range limitation of mass spectrometry-based proteome analyses. This is especially critical for plastid proteome analyses since these organelles are highly specialized for a specific cellular function resulting in a predominance of the enzymes involved. Examples for the latter are the photosynthetic proteins in chloroplasts. At the same time, however, plastids control their gene expression via sophisticated signal transduction chains including proteins and signalling factors of very low abundance (reviewed in Monde et al., 2000; Rodermel, 2001; Link, 2003). The identification of these proteins is difficult in the presence of the highly abundant proteins from dominating metabolic activities. It will be necessary to apply biochemical tools that help to separate the members of abundant metabolic activities and the low-abundant regulatory activities. One possibility to cope with this difficulty is the specific enrichment of signalling proteins by affinity chromatography. One example is the specific enrichment of phosphopeptides by ‘immobilized metal affinity chromatography’ (IMAC; Andersson and Porath, 1986).

What are the next developments that can be expected in the proteomics field and what concepts will increase the benefit of proteomics studies for plastid research? The authors are convinced that proteomics will become a routine technology for the description and the analysis of a biological system in ‘systems biology’ approaches. The combination and correlation of proteomics data with data obtained from transcriptional profiling and metabolomics is important to define regulatory profiling and metabolomics. In several examples, proteomics data were already combined with gene expression data derived from gene chips (Gygi et al., 1999b; Washburn et al., 2003; Corbin et al., 2003; Kubis et al., 2003). These analyses suggested that no general correlation between transcript and protein abundance can be inferred. For some proteins and the amino acid biosynthetic pathways a good correlation has been observed whereas for others no such correlation exists (Gygi et al., 1999b; Washburn et al., 2003). Proteomics is an indispensable tool to define the regulatory layers of gene expression, but an integration of analyses at all levels of gene expression and metabolite concentrations will be necessary for a complete description of a biological system. Controlled perturbations of the systems combined with a careful analysis of the observed changes on the RNA, the protein, and the metabolite level will result in new insights into pathway crosstalk under a variety of experimental conditions.

Glossary

ESI (electrospray ionization): soft ionization technique that is based on the evaporation of highly charged droplets from a fine solvent spray, creating charged analyte molecules in the gaseous phase.

ICAT (isotope-coded affinity tag): allows for a relative quantification of proteins in two different biological samples by mass spectrometry on the basis of stable isotope labeling of cysteine residues.

LC (liquid chromatography): reduces sample complexity prior to mass spectrometry. In most mass spectrometry applications a reversed phase column (C18) is coupled online to a mass spectrometer.

MALDI (matrix-assisted laser desorption ionization): soft ionization technique that uses laser energy to ionize analyte molecules in a crystalline, solid matrix.

Mass spectrometry: the measurement of ion mass to charge ratios (m/z).

MS/MS (tandem mass spectrometry): provides information about an analyte ion. In the first dimension (MS), the molecular mass of an analyte ion (e.g. a peptide) is determined. In the second dimension (MS/MS), the analyte ion is fragmented and the masses of the fragments are determined. This technique provides information about the amino acid sequence of a peptide.

TOF (time of flight): commonly employed mass analyser. Ions of different masses acquire different velocities when accelerated in an electric field.

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