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Introduction to Mass Spectrometer

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1. Introduction

Mass Spectrometer (MS) is a kind of machine which uses an analytical technique to measure the mass-to-charge ratio of ions. This analytical technique is also known as Mass spectrometry. And an ion is an atom or group of atoms which have lost or gained one or more electrons, making them negatively or positively charged. [1]

Mass spectrometry is an important emerging method for the characterization of proteins. The two primary methods for ionization of whole proteins are electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI).

As it is an important tool in proteomics, it is essential to understand not only the results, but also the principles of Mass Spectrometer. This report is devoting to provide a simple but clear explanation to the principles of Mass Spectrometer.

2. General Structure of Mass Spectrometer

Generally, a typical Mass Spectrometer consists of three parts: an ion source, a mass analyzer and a detector. The function of the ion source is to produce ions from the sample. The function of the Mass Analyzer is to separate ions with different mass-to-charge ratios. Then the numbers of different ions are detected by the detector. Finally, the mass spectrum is generated after all the data have been collected. **Fig.1** is a scheme graph of the Mass Spectrometer.

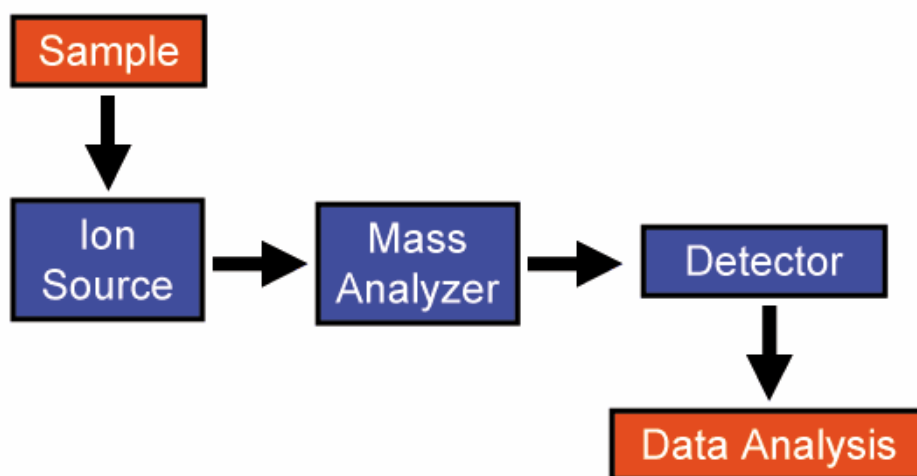


Fig.1 Scheme graph of MS (from wikipedia)

The output, i.e. mass spectrum, is an intensity vs. m/z (mass-to-charge ratio) graph, from which the Chemists are able to draw some clues about the ions.

There are many different kinds of Mass Spectrometers. This paper will discuss about two kinds of Mass Spectrometers, MALDI-TOF MS and ESI Tandem MS, which are most frequently used in proteomics.

3. MALDI-TOF Mass Spectrometer

3.1 Scheme graph of MALDI-TOF MS

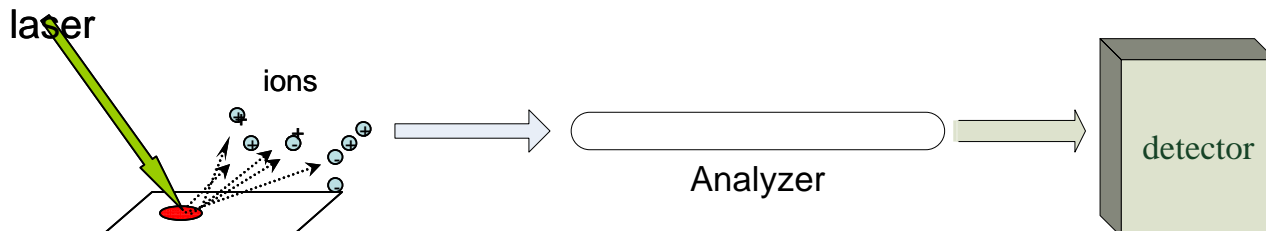


Fig.2 Scheme graph of MALDI-TOF MS

First, let us gain some basic idea about MALDI-TOF Mass Spectrometer. Fig.2 is a scheme graph of MALDI-TOF. It uses laser to ionize the sample proteins and then push the proteins into the analyzer to produce a Mass Spectrum.

Here are the definitions for MALDI-TOF from wikipedia:

Matrix-assisted laser desorption/ionization (MALDI) is a soft ionization technique used in mass spectrometry, allowing the analysis of biomolecules (biopolymers such as proteins, peptides and sugars) and large organic molecules (such as polymers, dendrimers and other macromolecules), which tend to be fragile and fragment when ionized by more conventional ionization methods.[3]

The Time of flight (TOF) method of measuring particle mass-to-charge ratio is done as follows. An ion of known electrical charge and unknown mass enters a mass spectrometer and is accelerated by an electrical field of known strength. This acceleration results in any given ion having the same kinetic energy as any other ion given that they all have the same charge. The velocity of the ion will depend however on the mass-to-charge ratio. [4]

Basically, MALDI is talking about the ionization technique of the sample in the source, while TOF is discussing about the distinguishing technique for the ions in the analyzer.

3.2 Source of MALDI-TOF

The job of the source is to turn the input sample proteins into the output ionized proteins.

In MALDI-TOF MS the specific process is as follows:

First we put the sample proteins into the solvent, i.e. matrix mixed with water. The sample proteins will dissolve in the solvent.

Then we wait until the water in the solvent totally evaporates. When the water evaporates, the sample proteins are surrounded by the matrix, which forms a crystal lattice. We may think like this: the sample proteins are kept in some boxes which are formed by the matrix molecules.

Finally we can put the target, i.e. proteins and 'boxes', into the source, using a beam of laser to fire it. This result in each protein picks up a proton and turns into gas phase.

Please note that both positive and negative ions are obtained, but we always choose to analyze positive ions. Usually the structures of proteins stay intact and the proteins are singly charged after the laser firing.

Fig.3 shows what is going on in the source.

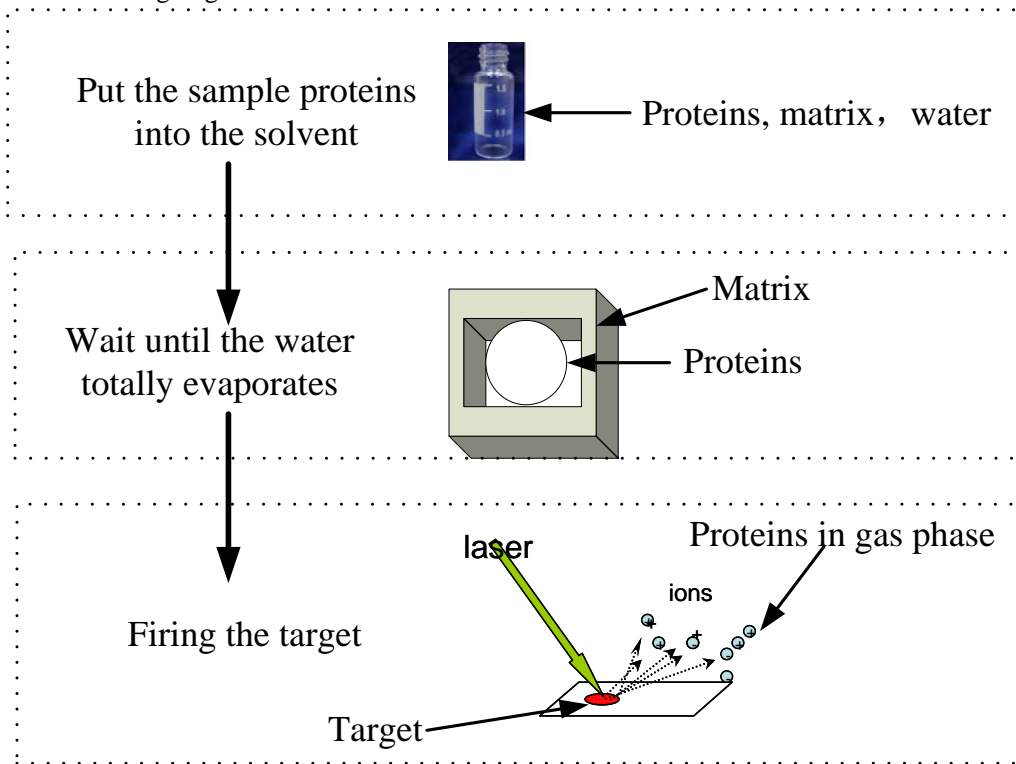


Fig.3 process in the source

3.3 Analyzer of MALDI-TOF

The TOF mass analyzer measures the time it takes for the ions to fly from one end of the analyzer to the other and strike the detector. The flying speeds of ions are proportional to their mass-to-charge ratio.

There are two kinds of mass analyzer, one works in linear mode, and the other works in reflectron mode.

3.3.1 MALDI-TOF Analyzer in linear mode

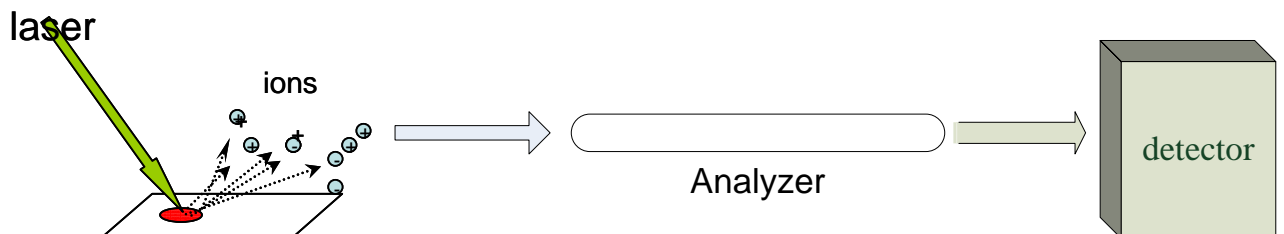


Fig.4 MALDI-TOF Analyzer in linear mode

Fig.4 is the scheme structure of MALDI-TOF MS in linear mode. In linear mode, MALDI-TOF analyzer works in a simple way. It just measure the time for flight for a ion fly from one end to the other.

3.3.2 MALDI-TOF Analyzer in reflectron mode

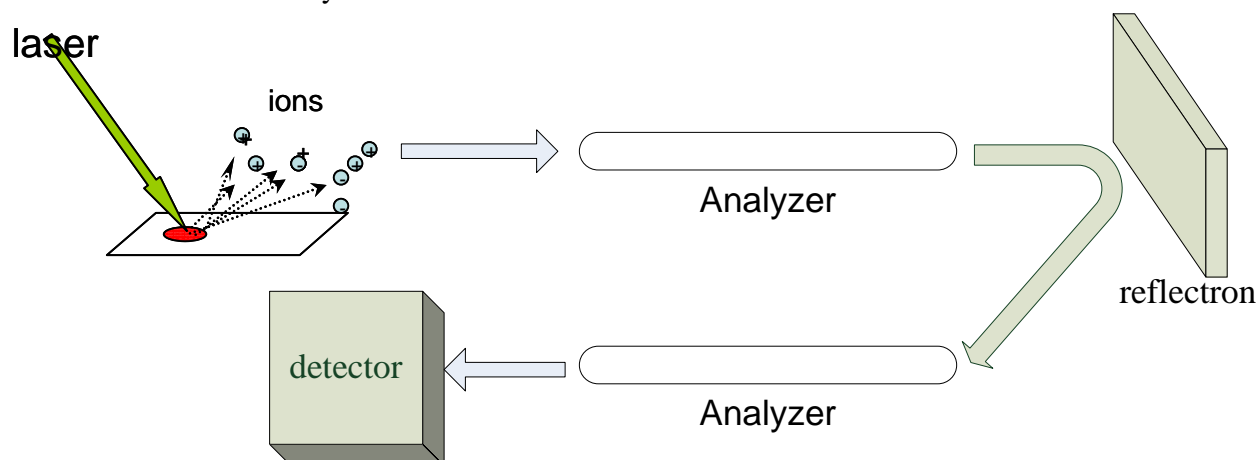


Fig.5 MALDI-TOF Analyzer in reflectron mode

Fig.5 is a scheme graph of MALDI-TOF MS in reflectron mode. Comparing Fig.5 and Fig.4 we can easily find that there is a reflectron in Fig.5, which brings us much advantage.

The workflow in reflectron mode is much the same as that in linear mode. The only difference is that when the ion hit the reflectron, it will reflect and fly towards the detector.

The reflectron focuses ions with the same m/z values, and makes them reach the detector at the same time, which results in more accurate detection.

3.3.3 Comparison of the two modes

The resolution of the linear mode with continuous extraction of ions was poor. The resolution means the ability to distinguish ions with similar m/z values. The resolution of the Mass Spectrometer is like the eyesight of a man. So the MS in linear mode is 'near sighted'.

However, with the help of the reflectron, Mass Spectrometer in reflectron mode enjoys a high resolution. So the reflectron acts like a set of lens which adjust the Mass Spectrometer's 'eyesight'.

Fig.6 gives a comparison of the mass spectrum of linear mode and reflectron mode. This two mass spectrum are derived from the same sample, with Mass Spectrometer working in linear mode and reflectron mode respectively. The x-axle is the m/z value, and the y-axle is the intensity.

In reflectron mode, we can see four peaks clearly from the mass spectrum, while in linear mode, those four peaks merge into one, which make it impossible for us to distinguish them.

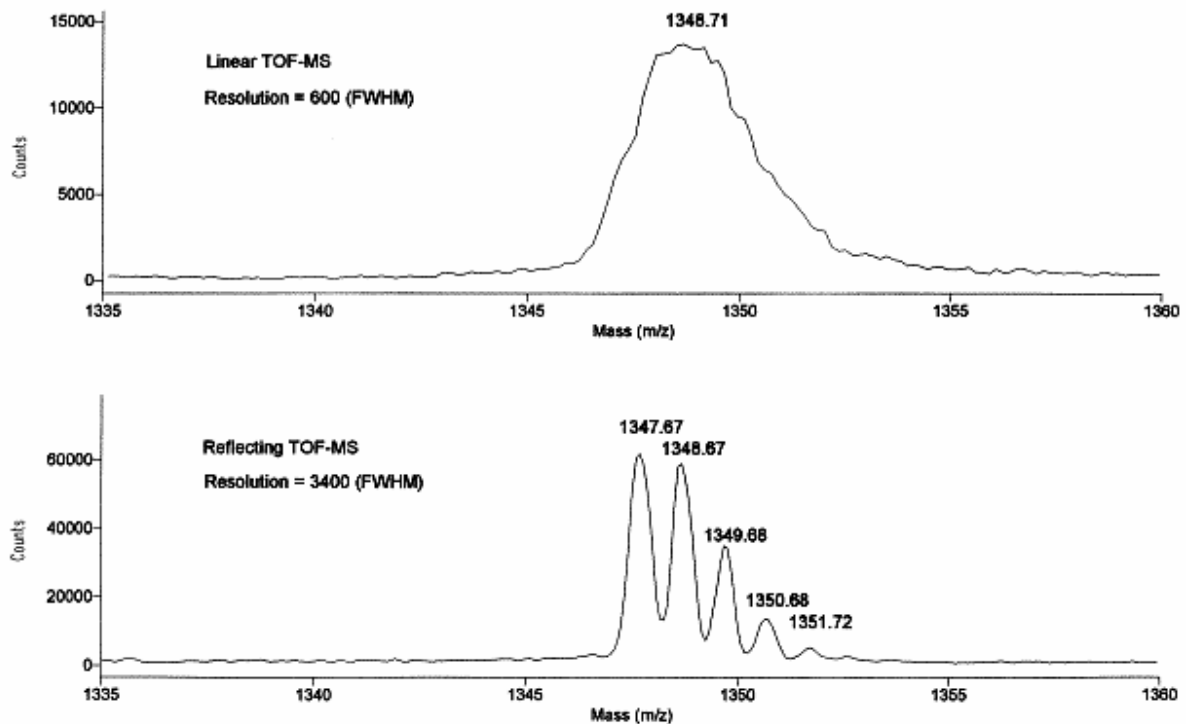


Fig.6 mass spectrum given by both modes for the same material [5]

3.4 Pros and Cons of MALDI-TOF

3.4.1 Pros

- A. User friendly and robust. It was said that a MALDI-TOF instrument in a shared proteomics facility can easily be set up to handle hundreds of analyses per day. [6]
- B. It is compatible with new robotic sample preparation devices designed to aid high-throughput proteomics work. [6]
- C. High resolution
- D. Sensitive, the best instruments are capable of attomole (10^{-18} mole) or better sensitivity under optimum conditions. [6]

3.4.2 Cons

- A. MALDI-TOF instruments are best suited to measuring peptide masses. This type of information is limited.
- B. A successful MALDI-TOF analysis is strongly dependent on the quality of the sample.

4. ESI Tandem MS

4.1 Scheme graph

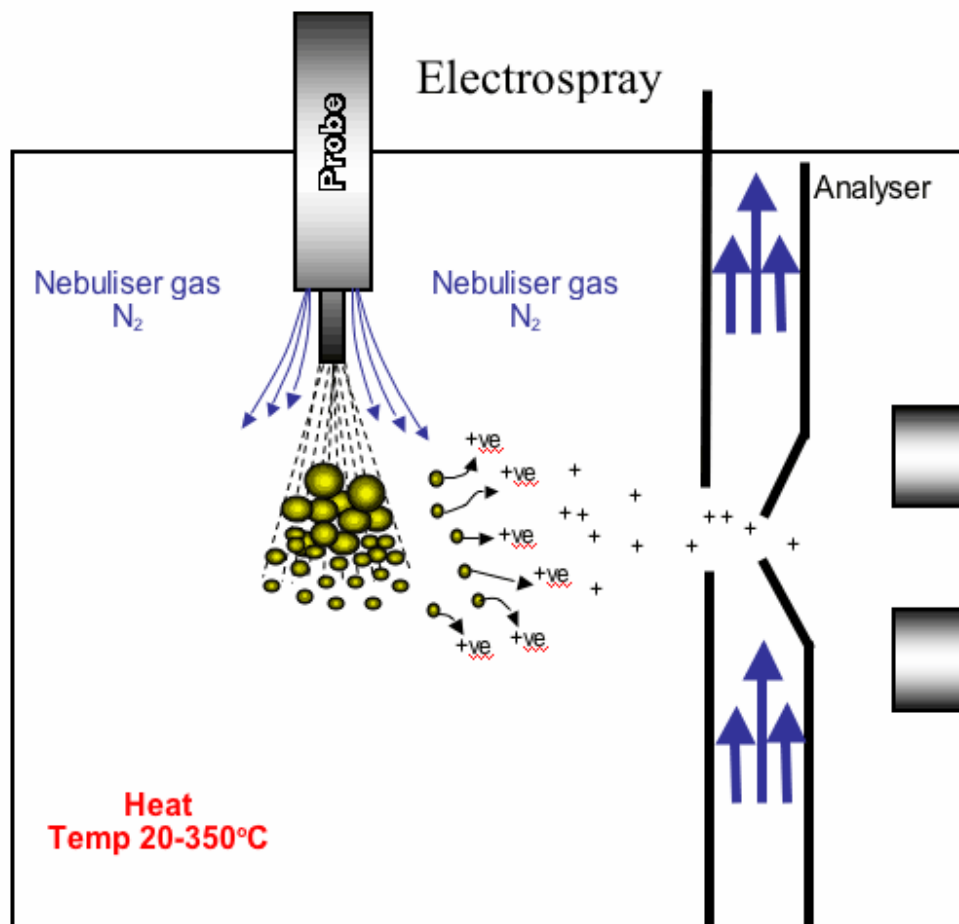


Fig.7 ESI tandem MS

ESI tandem MS is the standard acronym for electrospray ionization tandem mass spectrometry. [6]

ESI is another technique which turns the sample proteins into ions. As can be seen in Fig.7, ESI tandem MS is still composed of three parts: source, analyzer and detector. (Detector is on the right side of analyzer) Tandem mass spectrometry means in this machine, two stages of mass analysis are carried out of the ions.

The basic workflow of ESI tandem MS is as follows: the proteins pass through the source become ions; then the ions pass through the first analyzer and some specific ions are selected; then these selected ions are broken up by a procedure called collision-induced dissociation (CID); finally the second analyzer is used to catch the ions produced after CID.

This section will give some introduction to two kinds of ESI tandem MS, the triple quadrupole mass analyzer and ion-trap mass analyzer.

4.2 Source

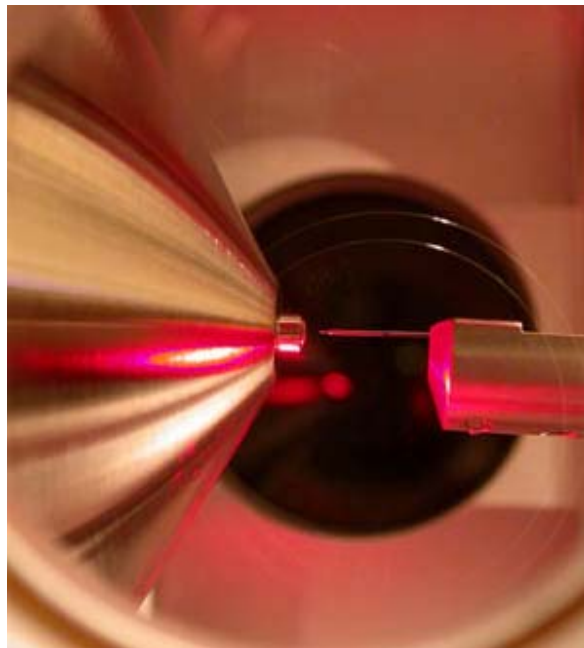


Fig.8 Electro spray (nanoSpray) Ion Source in LTQ-FTICR mass spectrometer. [7]

The purpose of electro spray ionization keeps the same: ionize all the proteins. Fig.8 is a picture of Electro spray Ion Source in LTQ-FTICR mass spectrometer, which may help us gain a sensible impression of ESI.

Here are the main steps of electro spray ionization:

- A. Dissolve the sample proteins in the solvent, which can be easily evaporated.
- B. The liquid (mixture of proteins and solvent) is pushed through a very small, charged and usually metal, capillary.
- C. For some chemical reasons, the proteins become ionized, surrounded by solvent in small droplets.
- D. The droplets are dried so that the ionized proteins are separated from the solvent

Please note that the ionized proteins may be multiply charged, though the structures of proteins stay intact.

Fig.9 shows how the source works.

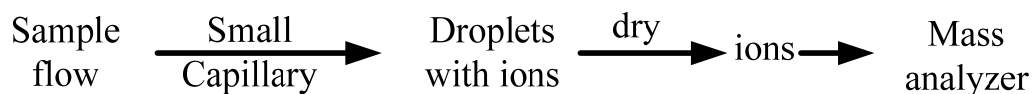


Fig.9 workflow for an ESI source

4.3 Analyzer

4.3.1 The Triple Quadrupole Mass Analyzer

Fig.10 is a scheme graph of Triple Quadrupole Mass Analyzer. As can be seen from Fig.10, the analyzer consists of three single quadrupole mass analyzer. The first Quadrupole acts like a mass filter, only those ions with specific m/z value can pass Q1; in the second Quadrupole Q2, the ions collide with some gas atoms and break into small fragments; given the fragment ions from Q2, Quadrupole Q3 produces the final mass spectrum.

Tandem Mass Spectrometry (MS/MS) with a triple quadrupole

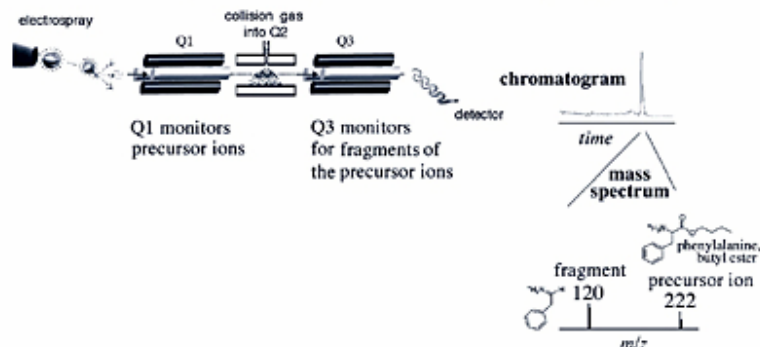


Fig.10 scheme graph of Triple Quadrupole Mass Analyzer [8]

4.3.2 Ion-Trap Mass Analyzer

Fig.11 is the scheme graph of Ion-Trap Mass Analyzer. It might a little bit difficult to read this graph. In fact the principle of Ion-Trap Mass Analyzer is the same as the Triple Quadrupole Mass Analyzer. First, the trap select some specific proteins with certain m/z values, all the other proteins will fly out of the trap; then the voltage is suddenly increased, which leads to the energy increase of the remaining ions, so the ions collide with the gas atoms and produce many fragments; like in Triple Quadrupole Mass Analyzer, the fragments are caught to produce the final mass spectrum.

Tandem Mass Spectrometry (MS/MS) with an ion trap

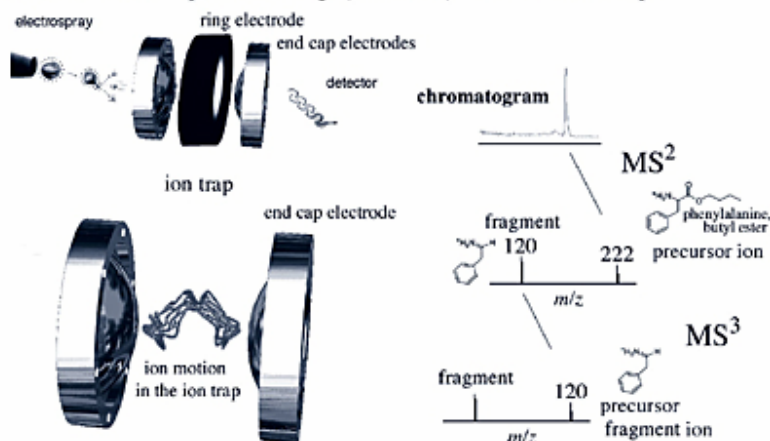


Fig.11 Ion-Trap Mass Analyzer [9]

There is a good analogy mentioned in [6] for Ion-Trap Mass Analyzer. Ion-Trap is “rocks in a can”. A handful of different –sized rocks are scooped up in a can. Then all but one are thrown out. The can is then rattled hard and the remaining rock fragments become pebbles, which are let out one at a time and weighted.

4.3.3 Triple Quadrupole VS Ion-Trap

The similarity between Triple Quadrupole and Ion-Trap is the principle. They both first select some specific ion, then break this specific ion into small pieces and catch the fragments to yield the final mass spectrum.

One of the difference between these two analyzers is that they produce difference fragments for the same protein ion. Trap-ion trends to fragment the ions more thoroughly, i.e. higher efficiency in fragmenting.

4.4 Pros and Cons

Compared with MALDI-TOF, ESI tandem MS have several advantages. One of the advantages is that it gives the information of the identical fragments of a protein, which sometimes are especially helpful in protein identification when we can not draw any clue from MALDI-TOF. Another advantage is the flexibility of combining different tandem MS instruments. We can choose many analyzers to select and fragment ions.

The drawback of tandem MS is its low accuracy and narrow application fields. When the molecule is not so big, no identical fragment is available, then tandem MS is not a good choice any more. It only performs well in limited fields, and can not be widely used.

5. Conclusion

From the previous chapters, we learn two kinds of Mass Spectrometers, MALDI-TOF and ESI tandem MS. A typical mass spectrometer includes three components: source, analyzer and detector. In the source, MALDI-TOF use laser to ionize sample proteins while ESI use a high voltage needle. MALDI-TOF measures the time of ions flying from one end to the other; while tandem MS measure the fragments of the ions. And tandem contains two stages of MS: the first MS is to select specific protein, the second MS is to draw the mass spectrum of the fragments.

6. Reference:

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