LECTURE-11

Hybrid MS Configurations

HANDOUT

PREAMBLE

As discussed in our previous lecture, mass spectrometry is by far the most versatile technique used in proteomics. We had also discussed some of the limitations of Mass spectrometry and how to deal with them, in terms of sample preparation. The two most important features required for a mass spectrometer are sensitivity and resolution. Sensitivity refers to the ability of instrument to detect minute amounts of analyte, whereas resolution refers to the ability of a mass spectrometer to resolve different molecular species with similar but distinct masses. Both of these features need to be addressed properly during the usage of mass spectrometry in clinical proteomics because certain biomarkers are present in sample in the range of femto moles and hidden by the large abundant molecules. Further, during tryptic digestion certain peptides may be generated having extremely close molecular mass. Assigning the correct identity solely on the basis of mass then becomes challenging, which can be mitigated by a highly resolvable instrument. All these problems have been tried to address by configuring the MS instrumentation. In this lecture, we will discuss innovations in the mass spectrometry techniques using hybrid MS configuration.

OUTLINE OF LECTURE

1. Requirement of a hybrid MS configuration
2. Various types of hybrid MS configuration
3. TOF – TOF
4. Triple Quadrapole (TQ)
5. Q – TOF
6. LTQ – Ion trap
7. LTQ – FT-ICR
8. Comparison between various hybrid configurations

BOX-1: TERMINOLOGY

Mass spectrometry - Technique for production of charged molecular species, and their separation by magnetic and electric fields based on mass to charge ratio.

Mass analyzer - The mass analyzer resolves the ions produced by the ionization source on the basis of their mass-to-charge ratios. Various characteristics such as resolving power, accuracy, mass range and speed determine the efficiency of these analyzers.

Mass resolution - Ability of a mass spectrometer to resolve different molecular species with similar but distinct masses.

Mass accuracy - how close a mass measurement is to its true (theoretical or exact) value.

Top-down - An analytical approach of separating and analyzing intact proteins. It involves direct analysis of intact proteins, without previous proteolytic digestion.

Bottom up - analytical approach of separating & analyzing peptides following proteolytic digestion of a sample.

Time-of-Flight (TOF) - A mass analyzer in which the flight time of the ion from the source to the detector is correlated to the m/z of the ion.

Ion traps - An ion trap makes use of a combination of electric and magnetic fields that captures ions in a region of a vacuum system or tube. It traps ions using electrical fields and measures the mass by selectively ejecting them to a detector.

Quadrupole - Quadrupole mass analyzers use oscillating electrical fields to selectively stabilize or destabilize the paths of ions passing through a radio frequency (RF) quadrupole field.

Ion cyclotron resonance (ICR) - A high-frequency mass spectrometer in which the specific ions to be detected are selected by setting a value of the quotient mass/charge, after which they absorb maximum energy through the effect of a high-frequency electric field and a constant magnetic field perpendicular to the electric field.

Orbitrap - An orbitrap is a type of MS analyzer that consists of an outer barrel-like electrode and a coaxial inner spindle-like electrode that form an electrostatic field with quadro-logarithmic potential distribution. Ions that get injected tangentially into the electric field cycle around the central electrode rings and oscillate along the central spindle. The ions are detected by means of the frequency of their harmonic oscillations, which in turn is dependent on the m/z ratio.

Magnetic sector - Double-focusing magnetic sector mass spectrometers make use of static electric or magnetic fields for detection of ions. They provide high sensitivity, high resolution, and reproducibility.

Collision cell - A device that selects a specific ion and further fragments into smaller ions.

Linear Triple Quadrapole (LTQ) - two separate quadrapoles placed at two edges of a collision cell.

1. REQUIREMENT OF A HYBRID MS CONFIGURATION

Before getting into the various hybrid MS configurations, it is essential to know why there was a requirement for combining different mass analyzers to give hybrid configurations. The various mass analyzers discussed in the previous lecture had one or more limitations. For example, the mass resolution of quadrapole was poor while that of TOF was medium, whereas the mass range of ion trap and quadrapole were low. On the other hand, FT-ICR which gave the best resolution and mass coverage was extremely complicated to operate. All these problems surfaced while studying various clinical samples, where the range of analytes may vary upto magnitudes of 10^8 (e.g. Human serum) and discovery of low molecular mass analytes require high sensitivity and mass accuracy.

A complex sample will contribute to various types of analytes. In the area of clinical proteomics, many proteins, having a wide concentration range may be present, and often these proteins act in regulating cellular pathways. On trypsin digestion the number of peptides generated may have overlapping properties, leading to their co-elution. Adding to the challenge is the need to detect very low-abundance species in the presence of highly abundant ones.

The technology of mass spectrometry was too versatile but individual mass analyzers had limitations hence scientist came up with the brilliant idea of making one weakness into the overall strength by combining two different weaklings, whose weakness are independent of each other. Thus was the origin of various combinations of hybrid mass analyzers. Tandem MS makes use of a combination of ion source and

two mass analyzers (hybrid MS configurations), separated by a collision cell, in order to provide improved resolution of the fragment ions. The mass analyzers may either be the same or different. The first mass analyzer usually operates in a scanning mode in order to select only a particular ion, which is further fragmented and resolved in the second analyzer. This can be used for protein sequencing studies.

2. VARIOUS TYPES OF HYBRID MS CONFIGURATIONS

Few hybrid MS configurations were developed to meet the needs of the complex samples.

a) TOF-TOF: Joining two time of flight tubes
b) Triple-Quadrapole: Joining two quadrapole at two ends with a collision cell in between
c) Q–TOF: Joining a quadrapole configuration with time of flight tube
d) LTQ–FTICR: Joining triple quadrapole with Ion cyclotron resonance

A typical MS configuration is shown in animation. Various Hybrid-MS configurations will be described in following section.

Illustration: Typical MS configurations

The ionization source and mass analyzer can be combined in different ways to give varying configurations for the mass spectrometer. Some of the most commonly used MS configurations are MALDI with TOF, ESI with Ion Trap, ESI with Q & TOF and MALDI with Ion Trap.

3. TOF-TOF

TOF or time of flight mass analyzers separate ions by the virtue of their kinetic energy. Trypsin digested peptides having different masses have different kinetic energy. Although the m/z range of TOF was large, the resolution was poor. Combining another TOF tube with the existing tube increased the path length of the ions and hence resulted in better resolution compared to single TOF. However, the addition of another TOF tube resulted into increase in the complexity of the instrument. This problem was mitigated using reflectron, by which the same TOF tube could be used twice for mass analysis. The reflectron uses a constant electrostatic field to reflect the ions. Higher energy, heavier ions penetrate deep into the reflectron and hence are reflected later and become slower, leading to higher resolution. The same path length now becomes twice the path length and hence the time taken becomes twice as it would have been for a single TOF tube. An additional collision cell may be introduced in between to facilitate better resolution.

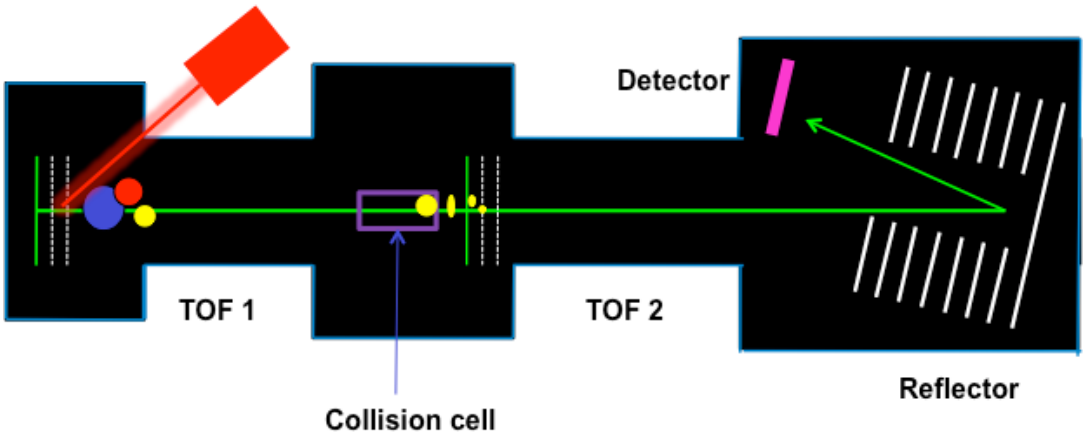

Fig 1. A TOF-TOF hybrid MS with collision cell.

Illustration: Tandem Mass Spectrometry: MALDI-TOF-TOF-MS

A common tandem MS configuration in which the ions are first resolved on the basis of their time of flight in the first TOF analyzer. The selected ions enter the collision cell where they are further fragmented. The fragmented ions are accelerated and further resolved on the basis of their m/z values in the second TOF tube, after which they are detected.

4. TRIPLE QUADRAPOLE (TQ)

The linear quadrapole consists of 4 sets of parallel metallic rods maintained at different potential difference to facilitate sequential elution of ions towards the detector. However, the resolution of quadrapole was very poor as more than two closely related peptides would reach the detector simultaneously. To address the problem, triple quadrapole was designed wherein; a collision cell separated two quadrapoles. The TQ is operated in two modes: radio frequency mode and scanning mode. Radio frequency mode refers to the mode whereby the potential difference is set so that all ions are allowed to move into the next chamber, whereas in the scanning mode only selective ions are allowed to move. In TQ, the first quadrapole is maintained at scanning mode, hence allows selective ions to move into the collision cell where the ion is fragmented into daughter ions. The third quadrapole is maintained at radio frequency mode, allowing all the daughter ions to reach the detector and thus get an idea about the fate of the parent ion. This is particularly useful in a phenomenon known as MRM, multiple reactions monitoring, where the fate of the parent ion is studied. This finds applications in both pharmaceuticals and proteomics, where the metabolism of a particular drug or a protein in question may be studied.

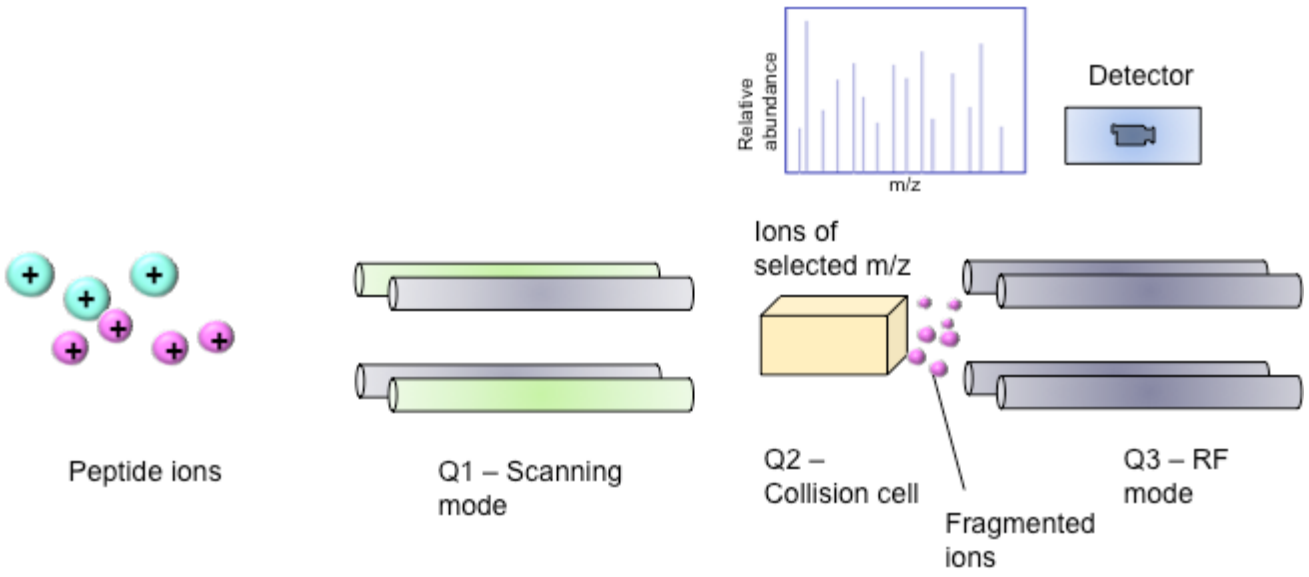

Fig 2. A scheme for Triple Quadrupole (TQ) mass spectrometer.

Illustration: Tandem Mass Spectrometry: Triple quadrupole

The triple quadrupole consists of two sets of parallel metallic rods interspersed by a collision cell. The first quadrupole scans the ions coming from the ionization source and allows only ions of a particular m/z ratio to pass through. These ions enter the collision cell where they are fragmented by collision against an inert gas like argon. The smaller fragments then enter the third quadrupole, which scans all the ions in the radio frequency mode to generate a spectrum based on the varying behavior of ions in an oscillating electrical field.

5. Q – TOF

Q – TOF combines the properties of both triple quadrapole and Time of Flight. The addition of TOF to quadrapole increases the sensitivity and resolution of the instrument. The daughter ions so formed from the third quadrapole now move at different speed as per their mass and hence resolve much better as compared to single TOF or TOF/TOF or TQ.

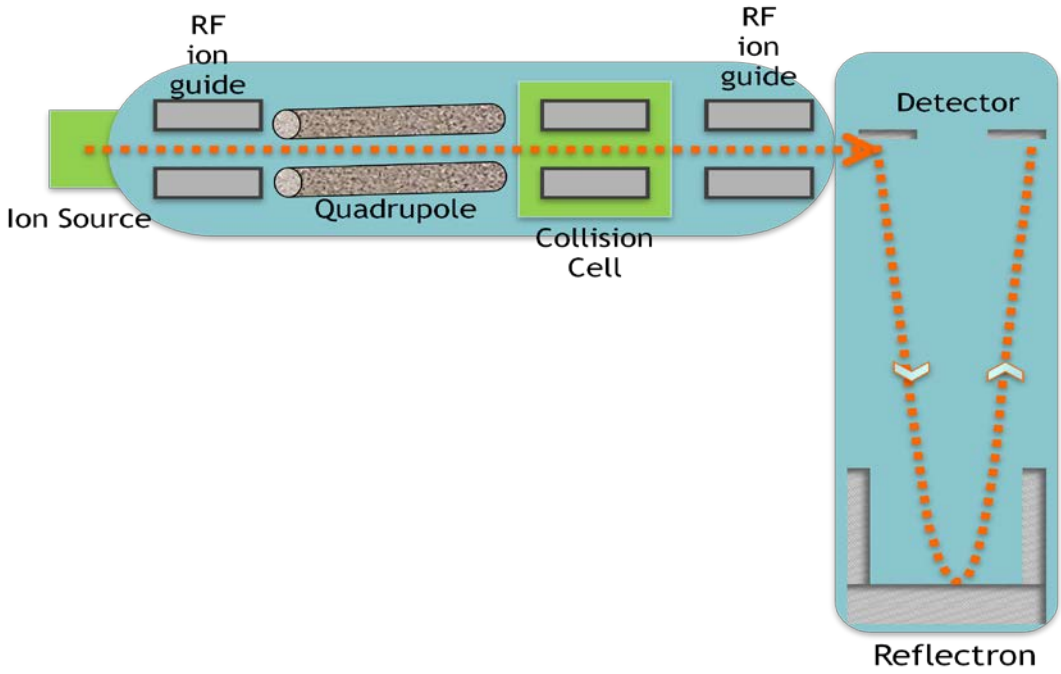

Fig 3: A scheme for Q – TOF mass spectrometer.

6. LTQ – ION TRAP

In LTQ – Ion trap the daughter ions fed from the quadrapole are selectively eluted by the Ion trap, maintained at a threshold potential difference by the ring electrodes. Thus there is an increase in resolution, as the detector now faces fewer ions at a particular time point.

Orbitrap is a high-resolution mass analyzer with high mass accuracy, which is second stage of a hybrid mass spectrometer, whose first stage is typically a linear ion trap. The ions are injected into the Orbitrap tangentially and form oscillating rings around the central electrode, becoming trapped in an electrostatic field.

7. LTQ – FTICR

The best resolution is provided by Linear Triple Quadrapole (LTQ) coupled with Ion Cyclotron Resonance (ICR). The daughter ions released from the third quadrupole are focused towards the detector by the strong magnets placed between the triple quadrupole and the detector. As a result, the daughter ions keep resonating around the magnet before being sequentially eluted. However, ICR being extremely complicated, this configuration finds limited usage for clinical proteomics.

8. COMPARISON BETWEEN VARIOUS HYBRID CONFIGURATIONS

For usage in day-to-day clinical proteomics, a configuration is necessary which gives high-throughput data as well as increased sensitivity and resolution. The reasons for such a criteria are already discussed above. All the configurations discussed have different advantages and limitations; hence to address different biological questions or to analyze different samples, different configurations may be adopted. For example, analyzing complex cerebrospinal fluids, where potential biomarkers may be present at attomolar range, Q–TOF or LTQ-Orbitrap may be beneficial, whereas for multiple reaction monitoring for biomarkers or drug metabolism, TQ may be advantageous. Thus the usage of a particular configuration is subject to the sample being processed, nonetheless all the hybrid MS configurations provide strong analytical platform for highly reproducible and accurate data required for proteomics applications.

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