Matrix-Assisted Laser Desorption Ionization Time of Flight (MALDI TOF)

MALDI is an efficient process for generating gas-phase ions of peptides and proteins for mass spectrometric detection. It is widely used in proteomics research as a high-throughput technique to identify proteins and their post translational modifications.

Learning Objective:
The time-of-flight analyzer resolves ions produced by the ionization source on the basis of their mass-to-charge ratio. The TOF tube can be operated in the linear mode or the reflectron mode depending on the sample to be detected. In case of small molecules, this mode usually provides sufficient resolution. The generated ions are accelerated towards the detector with the lighter ions travelling through the TOF tube faster than the heavier ions. The flight time of the ions is correlated with the m/z ratio.

Fundamentals of MALDI-TOF MS

The time-of-flight analyzer resolves ions produced by the ionization source on the basis of their mass-to-charge ratio. The TOF tube can be operated in the linear mode or the reflectron mode depending on the sample to be detected. In case of small molecules, this mode usually provides sufficient resolution. The generated ions are accelerated towards the detector with the lighter ions travelling through the TOF tube faster than the heavier ions. The flight time of the ions is correlated with the m/z ratio.
The TOF analyzer can also be operated in the reflectron mode, which is more commonly used for proteomics studies. A reflectron, which acts as an ion mirror, is incorporated at one end of the TOF tube. This helps in extending the path length and in turn the flight time of the ion without having to increase the actual size of the instrument. This helps to even out any kinetic energy differences between ions having the same mass and thereby improves the resolution.

Fundamentals of MALDI-TOF MS

The TOF analyzer can also be operated in the reflectron mode, which is more commonly used for proteomics studies. A reflectron, which acts as an ion mirror, is incorporated at one end of the TOF tube. This helps in extending the path length and in turn the flight time of the ion without having to increase the actual size of the instrument. This helps to even out any kinetic energy differences between ions having the same mass and thereby improves the resolution.
The time of flight of a charged ion can be calculated by means of the equation shown. The flight time is directly proportional to the square root of mass of the ion.

\[ t = \left( \frac{m}{2qV_0} \right)^{1/2} \]

**Fundamentals of MALDI-TOF MS**

- \( t \) = time-of-flight (s)
- \( m \) = mass of the ion (kg)
- \( q \) = charge on ion (C)
- \( V_0 \) = accelerating potential (V)
- \( L \) = length of flight tube (m)
Sample preparation and spotting

The protein sample must be prepared suitably before it can be analyzed by MS. The purified protein of interest is excised from the gel on which it has been electrophoresed and dissolved in a suitable buffer. Trypsin is then added to this in order to carry out digestion of the protein. This enzyme cleaves the protein at the C-terminal of the its arginine & lysine residues unless there is a proline present immediately after. The protein is thus digested into smaller fragments of manageable size.
Sample preparation and spotting

Once the protein sample has been digested, all the salt, buffers and any detergents must be removed from this sample. This can be efficiently done with the help of filters (e.g. ZipTip). It offers several advantages such as quick purification, sample enrichment and ensuring there is no contamination. However, it can purify only limited volume of the sample and also adsorbs some amount of the protein sample thereby leading to losses.
Sample preparation and spotting

The purified protein sample is then mixed with an aromatic matrix compound like α-cyano-4-hydroxycinnamic acid, sinapinic acid etc. in the presence of an organic solvent. The components are then mixed thoroughly.
Sample preparation and spotting

The solution containing the organic matrix with the embedded analyte is then spotted on to a metallic MALDI sample plate.
Ionization and detection

The gas phase ions generated are accelerated and travel through the flight tube at different rates. The lighter ions move rapidly and reach the detector first while the heavier ions migrate slowly. The ions are resolved and detected on the basis of their m/z ratios and a mass spectrum is generated. Parameters such as geometric design, power supply quality, calibration method, sample morphology, ion beam velocity etc. all affect the accuracy of mass detection.
Ionization and detection

The target plate containing the spotted matrix and analyte is placed in a vacuum chamber with high voltage and short laser pulses are applied. The laser energy gets absorbed by the matrix and is transferred to the analyte molecules which undergo rapid sublimation resulting in gas phase ions.
Definitions of the components/Keywords

**Fundamentals of MALDI-TOF MS**

1. **Ion source**: One of the major components of any MS instrumentation which fragments the sample into an ionic form for further detection. MALDI and ESI are most commonly used for proteins samples.

2. **Matrix Assisted Laser Desorption Ionization (MALDI)**: MALDI is an efficient ionization source for generating gas-phase ions of peptides and proteins for mass spectrometric detection. Target analyte embedded in dried matrix-sample is exposed to short, intense pulses from a UV laser.

3. **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. Commonly used mass analyzers include Time of Flight (TOF), Quadrupole (Q) and ion trap.

4. **Time-of-Flight (TOF)**: This is 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.

5. **Flight tube**: Connecting tube between the ion source and detector within which the ions of different size and charge migrate to reach the detector.

6. **Refllectron**: The refllectron acts as an ion mirror, and extends the flight length without increasing the instrument size. The refllectron compensates for the initial energy spread of ions having the same mass.

7. **Refllectron detector**: Detects the ions reflected by ion mirror. This over all setup improves the resolution.

8. **Detector**: The ion detector determines the mass of ions that are resolved by the mass analyzer and generates data which is then analyzed. The electron multiplier is the most commonly used detection technique.
Sample Preparation and Spotting

1. **In-gel digestion**: the in-gel digestion is part of the sample preparation process for the mass spectrometric identification of proteins during the course of proteomic analysis. Protein spots or bands excised from the gels are digested using trypsin.

2. **Trypsin**: trypsin is a serine protease found in the digestive system of many vertebrates, where it hydrolyses proteins. It cleaves at the c-terminal of lysine (K) and arginine (R) residues with the exception of k-proline and r-proline sites.

3. **Zip tip**: very small tip like device for removal of salts and other interfering agents from the protein samples before analysis. Zip tips can be incorporated into high throughput robotic devices for automated sample clean up.

4. **Sample/target plate**: multiple well plates on which the samples are spotted.

5. **Anlytes**: the samples that are under study. Analytes may be proteins, peptides or carbohydrates and are ionized prior to mass spectrometric detection.

6. **Matrix**: solution containing high concentration of a uv absorbing molecule deposited on sample plate along with samples. It is essential to select a matrix appropriate for the type of sample to be analysed.

7. **Ion desorption**: A process in which atomic and molecular species residing on the surface of a solid leave the surface and enter the surrounding gas or vacuum.
Ionization and Detection

1. **Laser**: Light amplification by stimulated emission of radiation (LASER or laser) is a mechanism for emitting electromagnetic radiation.

2. **Matrix & analyte**: Solution containing high concentration of UV absorbing molecules embedded with the analyte of interest, deposited on the sample plate. It is essential to select a matrix that is appropriate for the type of sample being analysed. Commonly used matrices are sinapinic acid and a-cyano-4-hydroxycinnamic acid.

3. **Sample plate**: Plate onto which the matrix-analyte solution is spotted.

4. **Matrix Assisted Laser Desorption Ionization (MALDI)**: MALDI is an efficient ionization source for generating gas-phase ion of peptides and proteins for mass spectrometric detection. Target analyte embedded in dried matrix-sample is exposed to short, intense pulses from a UV laser.

5. **Time-of-Flight (TOF)**: This is 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.

6. **Flight tube**: Connector between the ion source and detector within which the ions of different size and charge fly to reach the detector.

7. **Detector**: The ion detector determines the mass of ions that are resolved by the mass analyzer and generates data which is then analyzed. The electron multiplier is the most commonly used detection technique.

8. **Peptide spectrum**: The picks corresponding to individual peptides which are separated on the basis of their m/z ratio.
Which are the two types of ionization sources used for the Mass Spectrometric analysis of biological samples?

- Fast Atom Bombardment and Chemical Ionization
- Electron Transfer Dissociation and Collision Induced Dissociation
- Matrix Associated Laser Desorption Ionization and Electrospray Ionization ✔
- Electron Transfer Dissociation and Matrix Associated Laser Desorption Ionization

Congratulations, you have chosen the correct answer.
Which of the following is not a mass analyzer?

- Time-of-Flight (TOF)
- Quadrupole (Q)
- Ion traps
- MALDI

Congratulations, you have chosen the correct answer.
MALDI is a

Soft ionization technique ✔
Hard ionization technique
Both of them
None of them

Congratulations, you have chosen the correct answer.
Which of the following matrix is most suitable for carbohydrate samples?

- CHCA
- Sinapinic acid
- Dithranol
- DHB

Congratulations, you have chosen the correct answer.
Which of the following statement is right:

- MALDI is more tolerant to salts than ESI **(Correct)**
- MALDI is less tolerant to salts than ESI
- Both of them are equally more tolerant to salts
- There is no effect of salts on MALDI and ESI

Congratulations, you have chosen the correct answer.
Books:

Link, A. J., LaBaer J., Proteomics; A cold spring harbour laboratory manual; cold spring harbour laboratory press.

Research papers:
