Proteomics Course

LECTURE-21
Matrix assisted laser desorption/ionization-Time of Flight (MALDI-TOF)

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Lecture outline

• (I) Basics of MALDI-TOF
• (II) Sample preparation
  • In-gel digestion
  • Zip-tip sample clean-up
  • Matrix and sample plating
• (III) MALDI instrumentation
(I) Basics of MALDI-TOF

Matrix assisted laser desorption/ionization (MALDI)
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MALDI: merits and demerits

- Merits
  - Sample preparation easy
  - More tolerant to salts than ESI
  - Produces mainly singly charged ions

- Demerits
  - Strong dependence on sample preparation methods
TOF mass analyzer consists of ion acceleration and focusing optics and a flight tube.

- Measures m/z ratios of ions based on time it takes for ions to fly in analyzer & strike the detector.

**Time-of-Flight (TOF) equation**

\[ t = \left( \frac{m}{2qV_0} \right)^{\frac{1}{2}} L \]
An overview of MALDI-TOF analysis: Animation
(II) Sample preparation:  
In-gel digestion

In-gel digestion

2D – SDS-PAGE Gel
Spot excision

MALDI-TOF-TOF MS

Protein of interest

Proteins of interest

N-terminus

Trypsin

Digested peptide fragments

TOF 1
TOF 2
Collision Cell

MALDI-TOF-TOF MS

Trypsin digestion
In-gel digestion: reagents

**Coomassie destain**
- 50 mM ammonium bicarbonate (50µL) & 50µL ACN
- incubate (37°C, 10 min) and aspirate the solution

**Dehydration**
- dispense 50µL of ACN and incubate (37°C, 5 min)
- aspirate the solution and re-incubate (37°C, 10 min)

**Reduction**
- dispense 50µL of 10 mM DTT
- incubate (37°C, 20 min)

**In-gel digestion: reagents**

**Alkylation**
- dispense 30µL of 55 mM iodoacetamide
- incubate at (37°C, 20 min) and aspirate the solution

**Dehydration**
- dispense 50µL of acetonitrile
- incubate (37°C, 5 min) and aspirate the solution
- remove residual ACN by incubation (37°C, 5 min)

**Digestion**
- dispense 15µL of trypsin solution
- incubate (RT, 10 min) to allow trypsin to absorb into gel
- add 15 µL of 50 mM ammonium bicarbonate, incubate (37°C, 4 h)
Reduction and alkylation of proteins

Tryptic specificity

Cleaves on the C-terminal of K or R residues
In-gel digestion: Video

Sample clean-up

- In-gel digested protein samples are processed further using ZipTip pipette tips containing C18 or C4 media for enrichment of peptides
- Salts and interfering agents, detergents are washed and finally samples are eluted in a very small volume of solvent
Sample clean-up using ZipTip:

*Video*

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**Sample & Matrix Preparation**

- Matrix selection
- Matrix preparation
- Sample purification
- Sample deposition
- Matrix deposition
- Drying target
- Analysis
Matrix selection

Peptides less than 5000 daltons, lipids and nucleic acids

Peptides and proteins having higher than 5000 daltons and sometimes also use for lipids

$$\text{α-cyano-4-hydroxycinnamic acid (α-cyano)}$$

$$\text{Sinapinic acid}$$

Matrix selection

Small molecules and peptides which are not ionized by other matrices

Used for small nucleotides and phosphorylation studies on proteins

Generally used for nucleotides

$$\text{2,5-Dihydroxybenzoic acid (DHB)}$$

$$\text{Trihydroxyacetophenone (THAP)}$$

$$\text{Picolinic acid}$$
Matrix preparation is done by mixing matrix into a suitable solvent and vortex for few minutes to dissolve it properly.

(III) MALDI-TOF instrumentation: Video
MALDI TOF-TOF

• MALDI can be coupled to tandem TOF-TOF or hybrid Q-TOF analyzers, separated by a collision cell
• Much higher sensitivity than TQ and single TOF

Summary

• (I) Basics of MALDI-TOF
• (II) Sample preparation
• (III) MALDI instrumentation
REFERENCES


• Renata A. Culak, Min Fang, Shurene Bishop Simon, Itaru Dekio, Lakshani K. Rajakaruna and Haroun N. Shah* Changes in the Matrix Markedly Enhance the Resolution and Accurate Identification of Human Pathogens by MALDI-TOF MS. J Anal Bioanal Techniques 2012, S2