Proteomics Course

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

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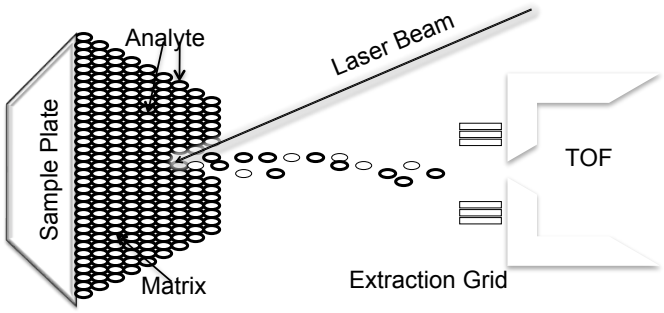
Lecture outline

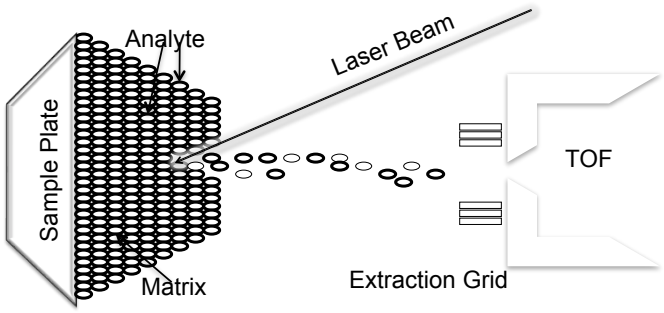
- (I) Basics of MALDI-TOF
- (II) Sample preparation
  - In-gel digestion
  - Zip-tip sample clean-up
  - Matrix and sample plating
- (III) MALDI instrumentation

(I) Basics of MALDI-TOF

Matrix assisted laser desorption/ ionization (MALDI)



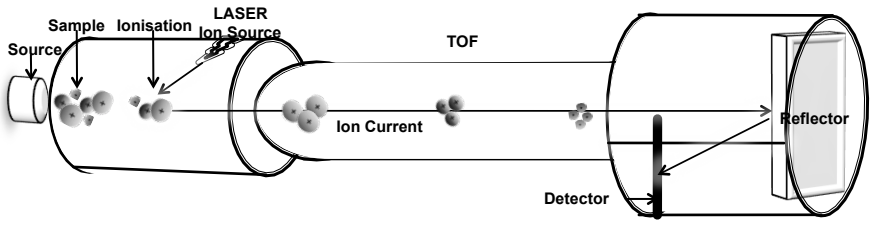
Matrix assisted laser desorption/ ionization (MALDI)



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

Time-of-flight (TOF)

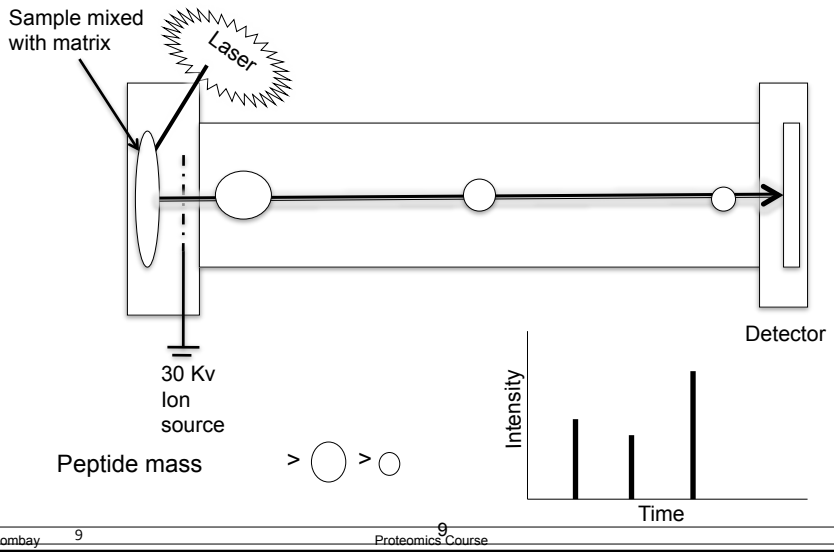


- TOF mass analyzer consists of ion acceleration and focusing optics and a flight tube
- Measures m/z ratios of ions based on time it takes for ions to fly in analyzer & strike the detector

Time of Flight equation

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

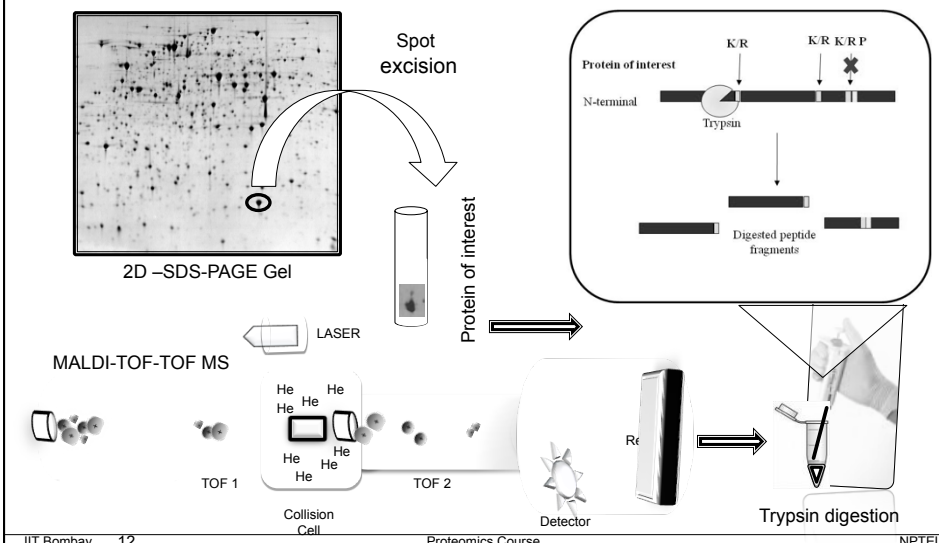
TOF



An overview of MALDI-TOF analysis:
Animation

(II) Sample preparation:
In-gel digestion

In-gel 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

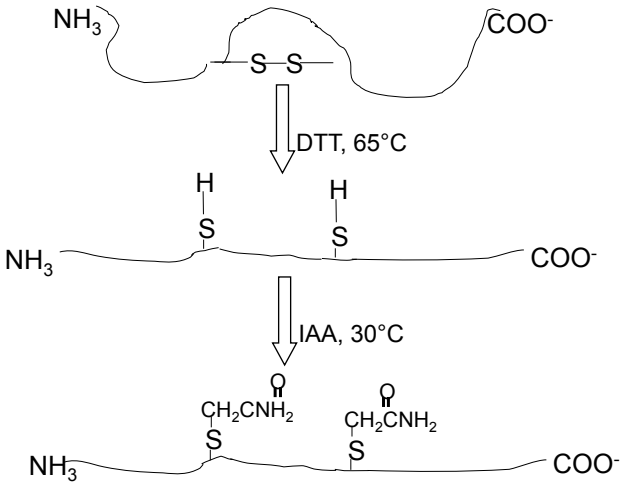
Dehydration

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

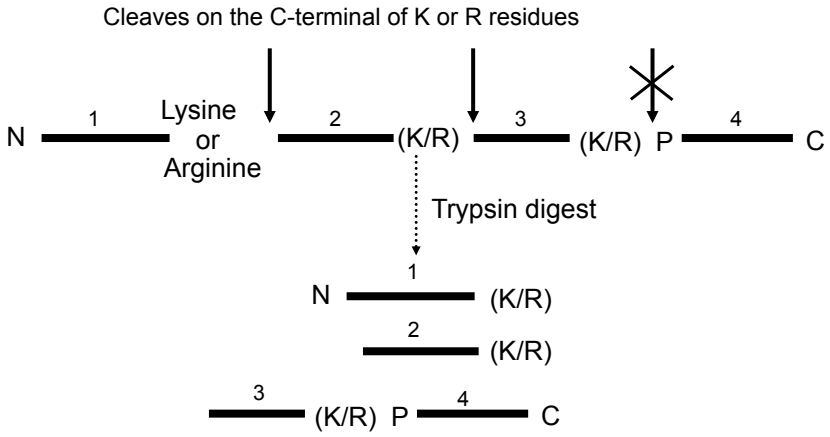
Digestion

- 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



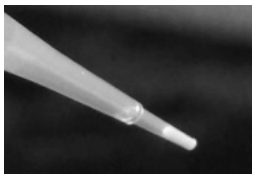
Tryptic specificty



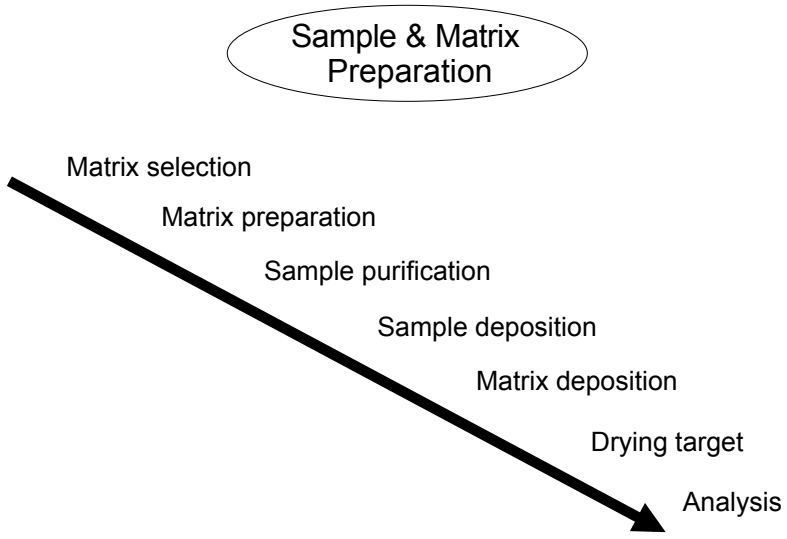
In-gel digestion:
Video

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

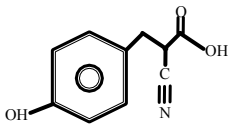


Sample clean-up using ZipTip: *Video*



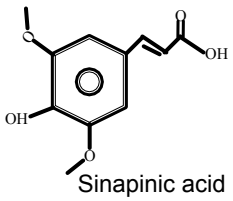
Matrix selection

Peptides less than 5000 daltons, lipids and nucleic acids



α-cyano-4-hydroxycinnamic acid (α-cyano)

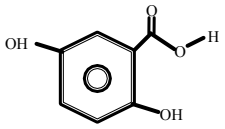
Peptides and proteins having higher than 5000 daltons and sometimes also use for lipids



Sinapinic acid

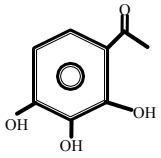
Matrix selection

Small molecules and peptides which are not ionized by other matrices



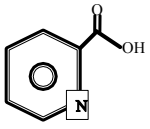
2,5-Dihydroxybenzoic acid (DHB)

Used for small nucleotides and phosphorylation studies on proteins



Trihydroxyacetophenone (THAP)

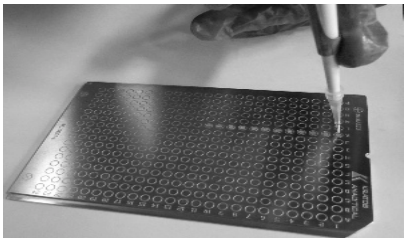
Generally used for nucleotides



Picolinic acid

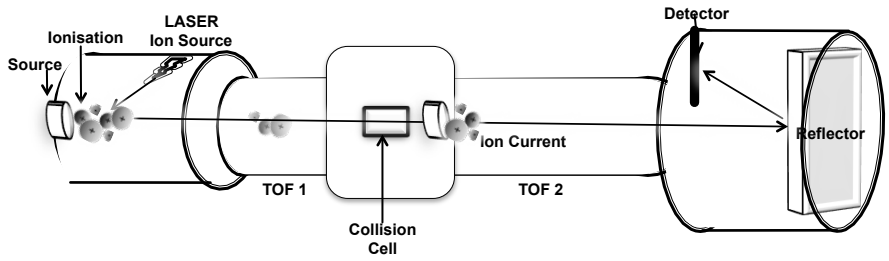
Sample and Matrix Deposition

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

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