Mass spectrometry (MS) - overview

**SAMPLE**: charged/ionized sample (fluid biological samples, proteins, peptides, oligosaccharides, DNA, serum, blood, urine, liquor, tissue extracts, …)

**INSTRUMENT**: mass spectrometer, measurement of mass : charge ratio

**RESULTS**: mass spectrum, mass : charge ratio distribution (molecular weight, molecular formula/composition, structural features)

**OPTIONS**: separation of components on the basis of mass : charge ratio, tandem MS, imaging MS (MSI: mass spectrometry imaging)

**What are the advantages?**
- **HIGH SENSITIVITY**: $10^{-10}$ – $10^{-16}$ g
- **LOW AMOUNT OF SAMPLE**: $10^{-6}$ – $10^{-12}$ g (micro – pico grammm)
- **BROAD MASS RANGE**: 1 – 10$^6$ atomic mass unit (Da)
- **SPECIFICITY AND REPRODUCIBILITY**

**Mass spectrometer**

1. **ION SOURCE**: production of gas phase ions from the sample
   - Positive ion mode: removing an electron (+ charged ion)
   - Negative ion mode: adding an electron (-charged ion)

2. **MASS ANALYZER**: separation “filtering” of ions according to their mass : charge ratio

3. **DETECTOR**: measuring the relative abundance of each mass : charge ratio, electric signal

4. **VACUUM**: minimizing the collisions between ions and air molecules... otherwise ions do not reach the detector

<table>
<thead>
<tr>
<th>ION SOURCE</th>
<th>IONIZATION/EI</th>
<th>CHEMICAL IONIZATION/CI</th>
<th>ATMOSPHERIC PRESSURE CHEMICAL IONIZATION/APCI</th>
<th>ATMOSPHERIC PRESSURE PHOTO IONIZATION/APFI</th>
<th>FAST ATOM BOMBARDMENT/FAI</th>
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**Ionization and mass analyzer**

**ION SOURCE – ionization**
- ELECTRON IMPACT IONIZATION/EI
- CHEMICAL IONIZATION/CI
- ATMOSPHERIC PRESSURE CHEMICAL IONIZATION/APCI
- ATMOSPHERIC PRESSURE PHOTO IONIZATION/APFI
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- ELECTROSPRAY IONIZATION/ESI
- MATRIX-ASSISTED LASER DESORPTION IONIZATION MALDI/

**MASS ANALYZER – separation according to mass and charge**
- SECTOR ANALYSER
- ION TRAP
- FOURIER TRANSFORM ION CYCLOTRON RESONANCE
- QUADRUPOLE
- TIME-OF-FLIGHT TOF/

**Ionization: ELECTROSPRAY IONIZATION(ESI)**

- spray the sample through a capillary into electric field + vacuum → small drops
- the solvent evaporates quickly in the vacuum
- the size of the drop decreases, the charge density in the drop increases
- increasing forces from electrostatic repulsion split the drop into smaller droplets
- multiply charged species for large biomolecules

**Analyzer: QUADRUPOLE**

- the electric field of the quadrupole influences the movement of the ions
- for a given set of electric field ions of one particular mass : charge ratio can pass through the quadrupole and reach the detector, the others collide with the rods
- different mass : charge ratios can be scanned by changing the electric fields
Ionization: MATRIX-ASSISTED LASER DESORPTION (MALDI)

**MATRIX-ASSISTED**: sample is embedded in a matrix on a metal surface

**LASER**: the matrix-sample is irradiated by laser (UV), the laser energy is absorbed

**DESORPTION**: the matrix-sample dissociates (desorped) from the metal surface into vacuum

IALONIZATION: the particles of the sample are ionized by the matrix

Analyzer: TIME-OF-FLIGHT (TOF)

**Analyzer**: TIME-OF-FLIGHT (TOF) analyzer

**Sample on plate**

**Sample in flight tube**

**Detector**: CONVERSION DYNODE, AMPLIFICATION

**Conversion dynode**

**Amplification**

Detector: CONVERSION DYNODE, AMPLIFICATION

**Mass spectrum, mass : charge spectrum**

**Analysis of mass spectra**

**Tandem mass spectrometry: MS/MS, MS²**

**(MS1)** fixed precursor ion with a given m/z ratio

**Fragmentation**

**(MS2)** scan product ion m/z ratio distribution

**eg.**

determine structurally significant fragment ions

**amino acid composition of peptides**

**CID**: collision induced dissociation
Tandem mass spectrometry: MS/MS, MS²

**B** Precursor ion scanning

![Image](59x682 to 285x713)

**C** Neutral loss scanning

![Image](310x679 to 537x713)

**D** Multiple ion monitoring

![Image](59x455 to 285x480)

(Tandem mass spectrometry) 

**MS²:** fix product ion with a given m/z ratio

↑ fragmentation

**MS¹:** scan the m/z ratio distribution of precursor ions

**eg.** group of compounds all give the same fragment ion

functional groups, phosphate ester, carbohydrate modification

Tandem mass spectrometry: MS/MS, MS²

**MS¹:** fix precursor ion with a given m/z ratio and **MS²:** fix product ion with a given m/z ratio

scan the distribution of the corresponding ions

**eg.** identification of components

**Applications**

**MEDICINAL SCREENING FOR CONGENITAL METABOLIC DISORDERS**

- carnitine profile determination, primary/secondary carnitine deficiency, fatty acid oxidation disorders, organic acids
- amino acid profile determination, aminoacylation
- guanidine compounds: disorders of creatine synthesis
- disorders of bile acid synthesis
- taurine, taurine metabolism
- galactosamines
- disorders of ceruloplasmin, ceruloplasmin synthesis
- disorders of ceruloplasmin, ceruloplasmin synthesis
- disorder of carbohydrate metabolism

**THERAPEUTIC DRUG MONITORING**

- anticonvulsant drugs, anticonvulsant drugs, anticonvulsant drugs, anticonvulsant drugs
- immunosuppressive drugs/sirolimus, antimycotic drugs, anticonvulsant drugs, anticonvulsant drugs
- SULFONYLUREA SCREENING
- diagnosis of kidney disease, diagnosis of kidney disease, diagnosis of kidney disease, diagnosis of kidney disease
- METHYLMALONIC ACID IN URINE OR SERUM
- diagnosis of vitamin B12 deficiency

**Applications – medical applications**

**MEDICAL JURISPRUDENCE**

- screening of drugs, doping and poisons
- MICROBIOLOGY
- PHARMACEUTICAL RESEARCH
- MEDICAL SCIENCES
- AGRICULTURE
- SPACE SCIENCES
- FOOD INDUSTRY

**APPLICATIONS**

**PROTEOMICS**

- molecular mass determination
- sequence and structural information
- posttranslational modification (phosphorylation, glycosylation, ...)
- identification of disulfide linkages
- determination of active/binding site
- protein-ligand interaction
- quantitative analysis of protein mixtures

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Mass spectrometry imaging
visualize the spatial distribution of endogenous biomolecules/small molecules, drugs, biomarkers, metabolites, lipids, peptides or proteins/ by their molecular masses within tissues and organs.

GOALS:
• DIAGNOSTIC
diagnostic studies comparing different tissue types (such as tumor versus normal) to aid in pathological diagnosis
• PROGNOSTIC
prognostic studies to categorize patients with long- or short-term survival
• THERAPEUTIC
drug response studies to predict a patient's response to a certain treatment

MSI – detecting cancer specific compounds in tissues
HER2: human epidermal growth factor receptor 2 (HER2) overexpression plays an important role in the development of aggressive breast cancers
Therapy:
• Trastuzumab/Herceptin interferes with HER2 receptors
• It is effective only in HER2 overexpressing cells, side effects in other cells

MSI – detecting anti-cancer drug distribution in tissues
Orally administered anti-cancer drug distribution in whole mouse section.
A) unstained transverse whole mouse section
B) IMS image
The drug is present in high concentrations in the tumour and can also be found in lower concentrations in the digestive system and blood vessels.
Mass spectrometry keywords

- ESI-quadrupole, MALDI-TOF techniques
- Mass-spectrum
- Applications

Fig. 5. MALDI imaging analysis of a bank note: a) and c) are 2D maps of the distribution of dyes with masses 575 Da (red dot) and 785 (red dot). b) and e) display spectra from specific areas on the note, indicating that on these positions different combinations of dyes are present.