Proteomics Course

LECTURE-22
Liquid chromatography-Mass spectrometry (LC-MS/MS)

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Lecture outline

• Mass spectrometry work-flow
• Liquid chromatography
• In-gel digestion
• Ionization source
• Mass analyzers
• Tandem mass spectrometry
(1) In-gel digestion
In-gel digestion:

\textbf{Animation}
(2) Separation technology – Liquid chromatography (LC)

Liquid chromatography (LC)

- Separate mixture components on basis of differences in affinity for stationary & mobile phase
- Removes undesired impurities
- Increased sensitivity, detection of low level proteins
- Separates peptide mixture
Reversed phase (RP) chromatography

- Based upon hydrophobic binding interaction between
  - peptides/proteins (mobile phase)
  - immobilized hydrophobic ligand (stationary phase)

RP-HPLC configuration

Mobile phases

A buffer (0.1% Formic acid, 5% ACN)
B buffer (0.1% Formic acid, 80% ACN)

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B buffer (0.1% Formic acid, 80% ACN)
RP-HPLC with ESI

- RP is used with ESI
  - due to compatibility of RP’s acidic aqueous & polar mobile with ESI
- In-line RP-HPLC is useful
  - desalting peptides before ESI
  - no need for off-line desalting

Strong cation exchange (SCX) resin

- Silica based cation exchange stationary phase
- Sulfonic acid cation-based exchange ligand
- Ligand covalently bound to polymer coated silica
Microcapillary HPLC columns

- Microcapillary HPLC’s low flow rate is more sensitive than standard RP-HPLC
- Microcapillary HPLC columns prepared using fused silica capillary

Multidimensional separations

- Multidimensional separations
  - Size exclusion chromatography (SEC)
  - Ion exchange chromatography (IEX)
  - Capillary electrophoresis (CE)
  - Reversed-phase (RP)
  - Affinity chromatography
Multidimensional approaches coupled with MS

I. SEC $\rightarrow$ RP

II. RP $\rightarrow$ CE
   SEC $\rightarrow$ CE

III. IMAC $\rightarrow$ RP
    Avidin $\rightarrow$ RP

IV. SCX $\rightarrow$ RP

MUltimensional Protein Identification Technology (MudPIT)

SCX – separation by charge

RP (C18) – separation by hydrophobicity
Liquid chromatography: 
*Animation*

(3) Ionization sources
Ionization sources

Gas phase
- Electron ionization
- Chemical ionization (CI)
- Photoionization (PI)

Solution Phase
- Electrospray
- Atmospheric-pressure PI
- Atmospheric-pressure CI

Solid Phase
- Matrix-assisted laser desorption
- Plasma desorption
- Fast Atom Bombardment

Electrospray ionization (ESI)

- ESI requires sample of interest to be in solution
- To ionize samples high voltage is applied to high conductively coated needle
- Distinguishing feature of ESI
  - its ability to produce multiply charged ions
Electrospray ionization (ESI)

- Desolvation of ions occurs at atmospheric pressure and mass analyzer is maintained at lower pressure
- During movement, evaporation reduces droplet size
- Ions when enter into MS, droplets are dried using a stream of inert gas
(4) Mass analyzers
Types of mass analyzers

- Time-of-Flight (TOF)
- Ion Trap
- Quadrupole
- Magnetic Sector
- Orbitrap
- Ion Cyclotron Resonance

Mass analyzers: categories

- Scanning MS
  - TOF
    - MALDI
- Ion-beam MS
  - Quadrupole
- Trapping MS
  - IT, Orbitrap, and FT-ICR
  - ESI
Time of Flight (TOF)
Quadrupole

- Quadrupole (Q) – set of 4 parallel metallic rods
- Radio frequency mode
- Scanning mode
- Neutral loss scan and precursor ion scanning mode
Triple quadrupole mass spectrometer (TQ)

- TQ – 3 arrangements similar to quadrupole

Ion Trap
Ion Trap

- Consist of a chamber surrounded by a ring electrode and two end-cap electrodes
- Voltage applied to ring electrode determines which ion remain in the trap

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Fourier transform ion cyclotron resonance
Fourier transform ion cyclotron resonance

- Uses cyclotron motion (cyclotron frequency) to resolve ions
- Most complex, difficult to operate
- Highest resolution, mass accuracy and sensitivity
- Multiple tandem experiments feasible
- MS/MS of very large ions feasible

(5) Hybrid-MS & MS configuration comparison
MALDI TOF-TOF

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

Q-TOF

- Combines front part of a TQ with a TOF analyzer to measure the mass of the ions
MS: concepts review

**Animation**

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### Performance comparisons of MS instruments

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Resolution</th>
<th>Mass Accuracy</th>
<th>Sensitivity</th>
<th>Scan Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIT/LTQ (Linear Ion Trap)</td>
<td>2000</td>
<td>100 ppm</td>
<td>Femtomole</td>
<td>Fast</td>
</tr>
<tr>
<td>TQ (Triple Quadrupole)</td>
<td>2000</td>
<td>100 ppm</td>
<td>Attomole</td>
<td>Moderate</td>
</tr>
<tr>
<td>LTQ-Orbitrap</td>
<td>100,000</td>
<td>2 ppm</td>
<td>Femtomole</td>
<td>Moderate</td>
</tr>
<tr>
<td>LTQ-FTICR</td>
<td>500,000</td>
<td>&lt; 2 ppm</td>
<td>Femtomole</td>
<td>Slow</td>
</tr>
<tr>
<td>Q-TOF</td>
<td>10,000</td>
<td>2-5 ppm</td>
<td>Attomole</td>
<td>Moderate, Fast</td>
</tr>
</tbody>
</table>

Summary

- Mass Spectrometry work-flow
- In-gel digestion
- Liquid chromatography
- Ionization source
- Mass analyzers
- Tandem mass spectrometry

REFERENCES


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ACKNOWLEDGEMENT

- Agilent Technologies: www.home.agilent.com/agilent/
- Andrew J Link. CSHL Proteomics Course 2008