iBioSeminars: Mass Spectrometry and its Application to Molecular Biology

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Overview of the Lecture

1. What is a mass spectrometer?
2. What can mass spectrometers identify?
3. What are the different types of mass spectrometers?
4. Peptide fragmentation
5. Database searching
6. Practical applications of mass spectrometry

1. What is a mass spectrometer?

A. It measures mass

B. It can give information about chemical structures

1 Sample Preparation

2 Ion source: generates ions

3 Analysis

John B Fenn

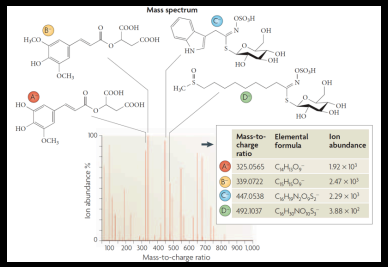
Koichi Tanaka

analyzer: separates ions

The mass-to-charge ratio is often referred to as m/z and is typically unitless

m: the mass number (atomic mass/u)

z: the charge number (Q/e)



2. What can mass spectrometers identify?

A. Small molecules

-Drugs

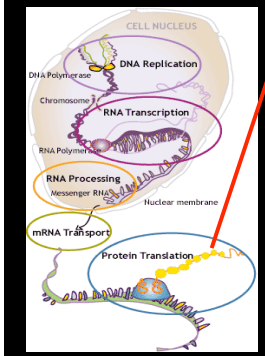
-Residual gases

-Carbon dating

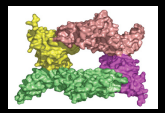
-Trace contaminants or toxins

-Particles from space explorations

B. Identification of biological material (proteins, nucleic acid, lipids)

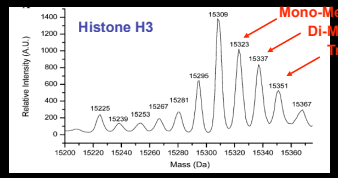


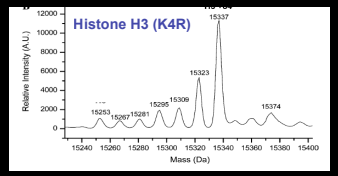
Protein-protein interactions



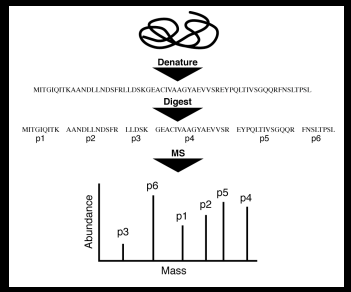
Protein Complexes
"Building Blocks of the Cell"

A. “Top-down” Proteomics: Identification of Intact Proteins

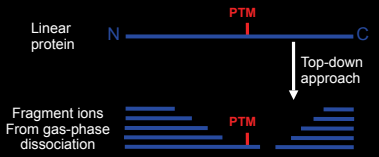




B. “Bottom-Up” Proteomics: Identification at the peptide level to infer proteins



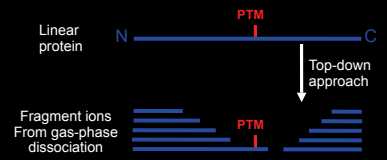
Top-Down vs. Bottom Up



Top-Down (+)

- -access complete protein sequence
- -ability to locate post-translational modifications (PTMs)
- -time-consuming protein digestion is eliminated

Top-Down vs. Bottom Up

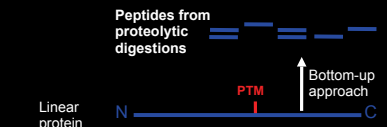


Top-Down (-)

-complex spectra obtained limits approach to single proteins or simple mixtures

-does not work well with proteins > 50 kDa

Top-Down vs. Bottom Up



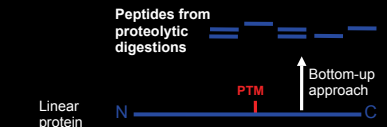
Bottom-Up (+)

-most widely used approach for protein ID

-reverse phase HPLC provides high-resolution separation of peptide digests

-can analyze very complex mixtures

Top-Down vs. Bottom Up



Bottom-Up (-)

-only a fraction of total peptide population of a given protein is identified (less PTMs identified!)

-loss of information about low abundance peptides in mass spectra dominated by high abundance species

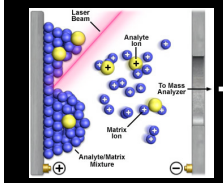
3. What are the different types of mass spectrometers?

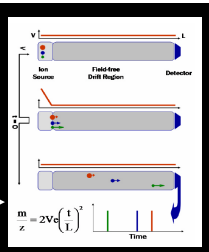
A. Maldi (Matrix-assisted laser desorption/ ionization)-TOF (Time of Flight)

B. LC-MS/MS

A. Maldi-TOF

"Ion Source"





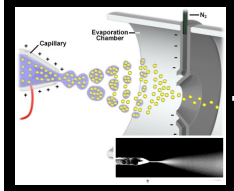
"Mass Analyzer"

Matrix

cinnamic acid

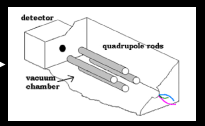
B. LC-MS/MS

"Ion Source"



Electrospray Ionization

"Mass Analyzer"

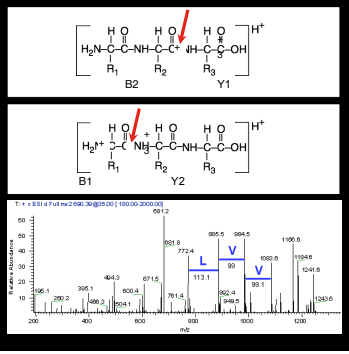


First MS

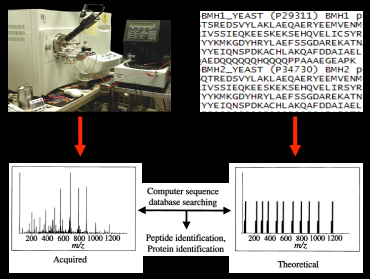
"Peptide Fragmentation"

Second MS

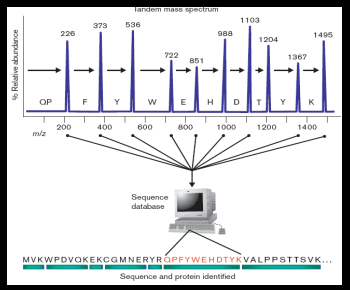
4. Peptide Fragmentation by MS/MS



5. Database searching



The spectra are imperfect….not every predicted (b and y) ion is visible, so computer programs make ideal/theoretical spectra for all the peptides in the sequence databases, and find the best match

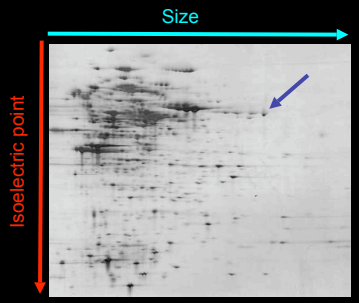


SEQUEST, MASCOT, Prospector

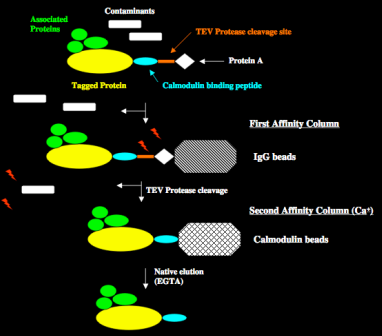
Man programs (SEQUEST, MASCOT, Prospector)….basically same idea as figure above….statistics used to decide on correct and incorrect main thing that differs

6. Practical applications for mass spectrometry

A. Globally identify entire proteomes

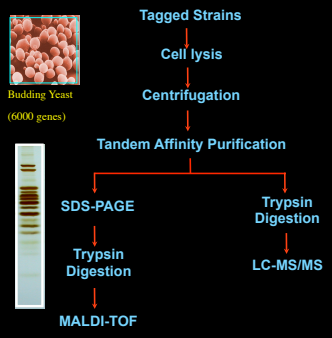


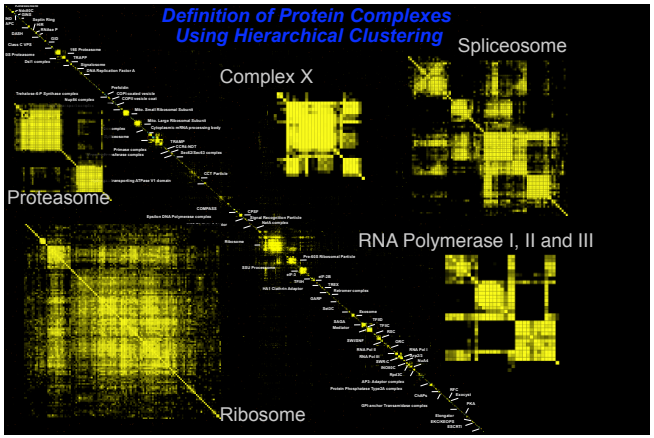
B. Identify purified complexes to generate protein-protein interaction networks



TAP (Tandem Affinity Purification) Tag

Identification of Protein Complexes Using Mass Spectrometry




Definition of Protein Complexes
Using Hierarchical Clustering
Spliceosome
Complex X
Proteasome
RNA Polymerase I, II and III
Ribosome