

SBCNY

NIGMS funded Center

Experimental Methods in Systems Biology

Part of the Coursera Certificate in Systems Biology

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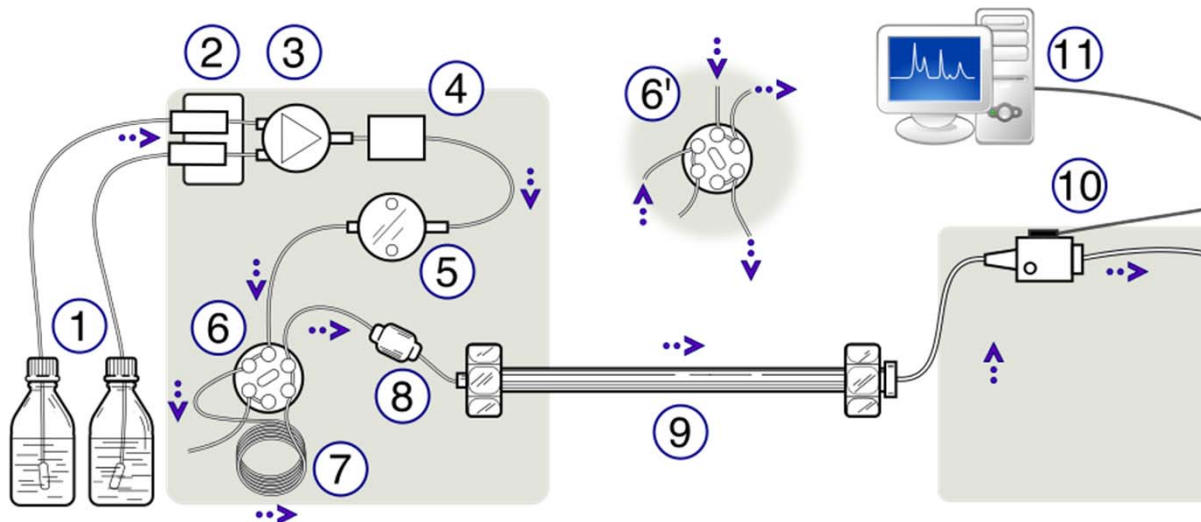
Fall 2014, Week 3 Mass Spectrometry-Based
Proteomics



Icahn School
of Medicine at
**Mount
Sinai**

How Does Tandem Mass Spectrometry Work?

Separation—Liquid Chromatography



http://en.wikipedia.org/wiki/High-performance_liquid_chromatography#mediaviewer/File:HPLC_apparatus.svg

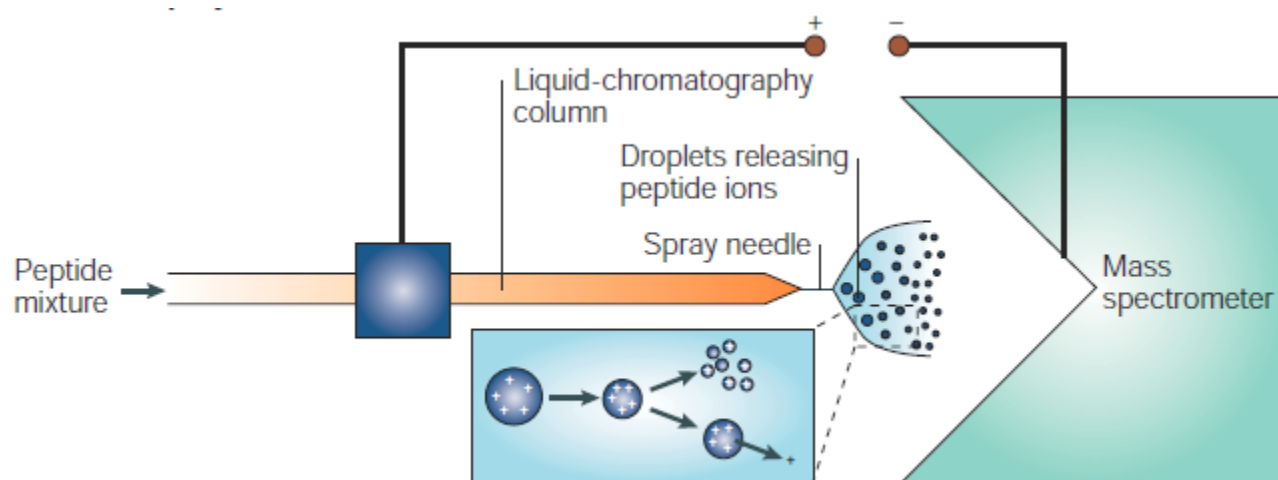
- Most important part is (9) the column
- Peptides/Proteins have different affinity for or migration speeds in the column resin depending on the solvent composition
 - Column resin typically hydrophobic C18 but there are many types
- The solvent is run on a “gradient” across time to elute different peptides from the column
 - Solvent composition changes over time

Ionization Methods: making ions from peptides

- Electron ionization (EI)
- Photo-ionization (PI)
- Field ionization (FI)
- Chemical ionization (CI)
- Thermal ionization (TI)
- Fast Atom Bombardment (FAB)
- **Electrospray (ESI)**
- **Matrix-assisted laser desorption ionization (MALDI)**

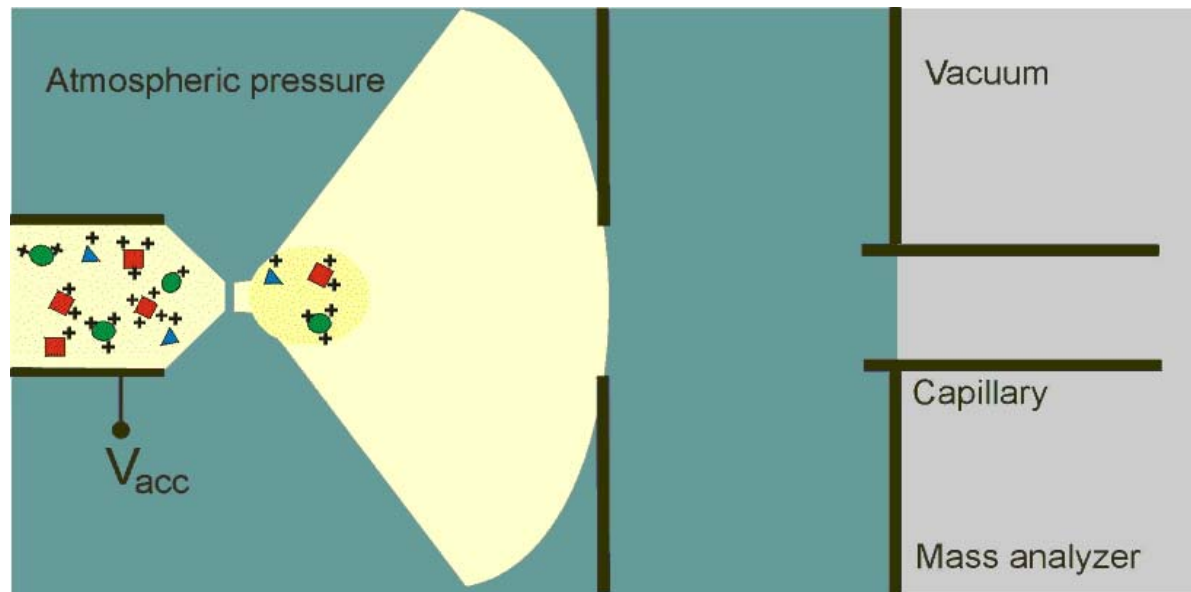
Electrospray Ionization (ESI)

2002 Nobel Prize in
Chemistry to John
Fenn

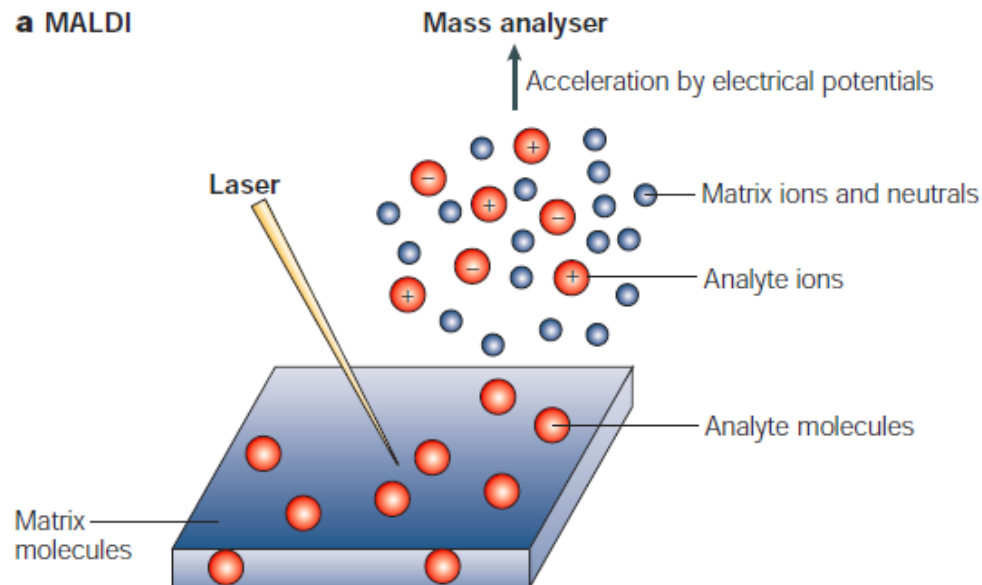


Steen and Mann, Nat Rev Mol Cell Biol, 2004

Electrospray Animation



Matrix-Assisted Laser Desorption Ionization (MALDI)

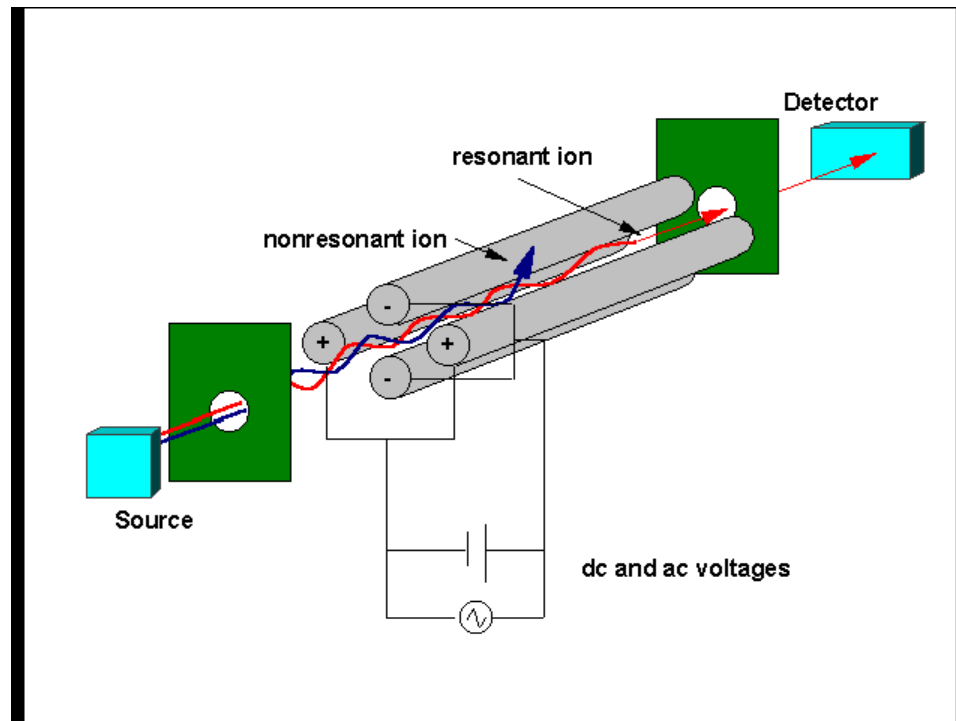


Steen and
Mann, Nat Rev
Mol Cell Biol,
2004

Mass Analyzer: Separation of peptide ions based on
 m/z (\sim size)

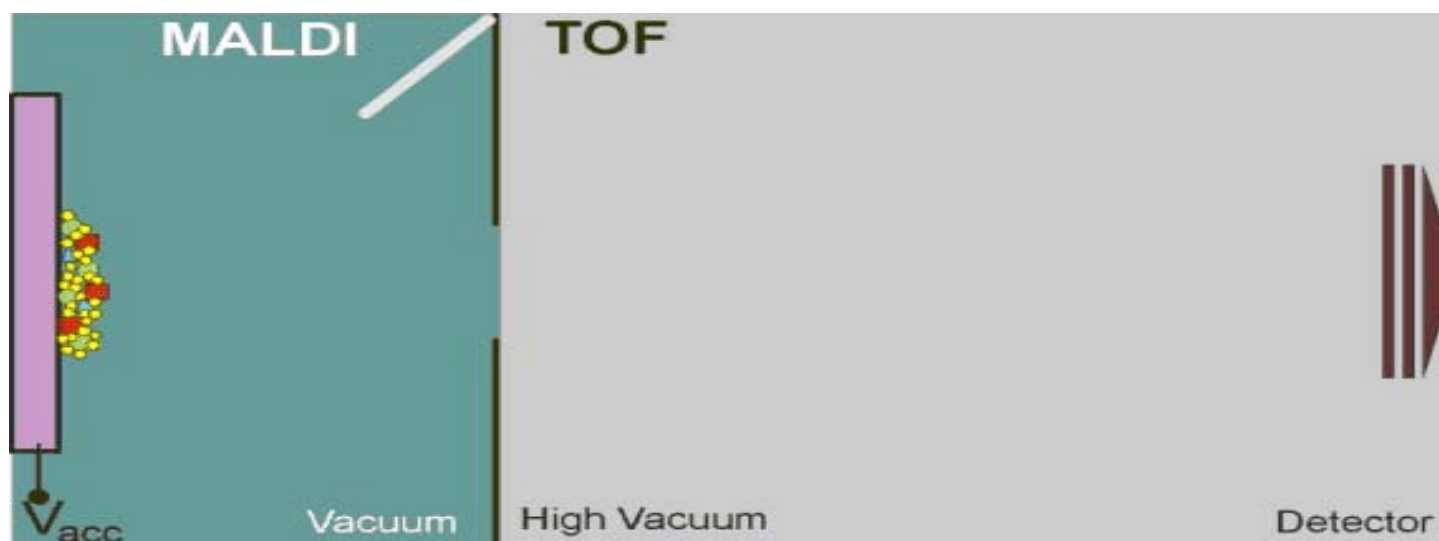
- Electrostatic analyzer
- Magnetic analyzer
- **Quadrupole analyzer (e.g. triple quad)**
- **Time of flight analyzer (TOF)**
- Double-focusing analyzer
- Ion cyclotron resonance analyzer
- **Ion trap analyzer (IT)**

Quadrupole



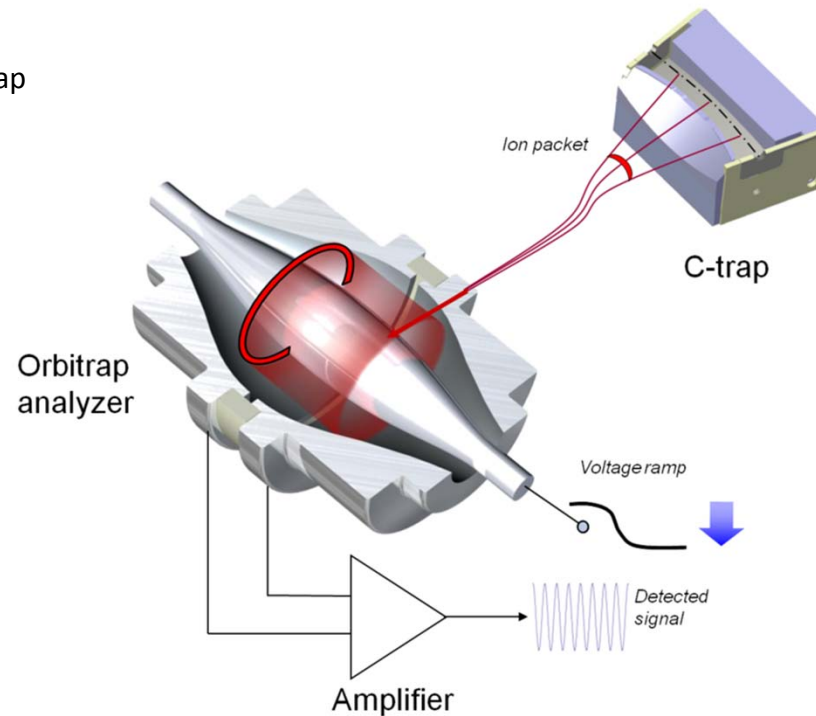
<http://www.tissuegroup.chem.vt.edu/chem-ed/ms/quadrupo.html>

Matrix Assisted Laser Desorption Ionization (MALDI) Time of Flight (TOF) Mass Spectrometry (MS)



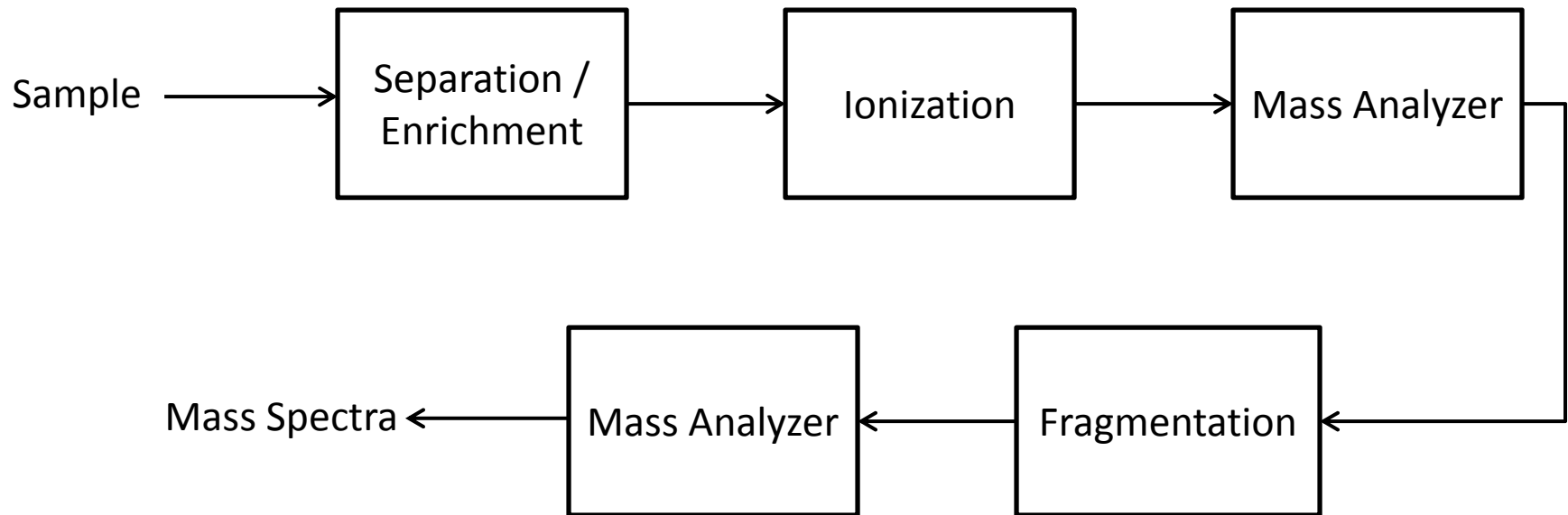
Orbitrap (type of Ion Trap)

<http://en.wikipedia.org/wiki/Orbitrap>



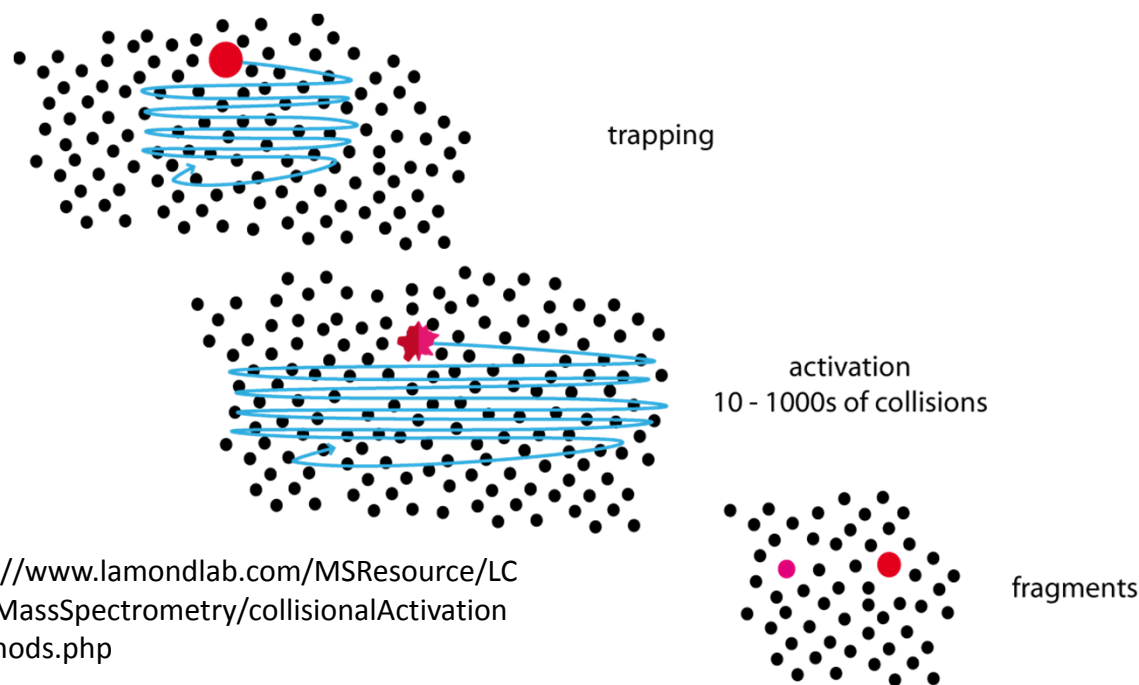
<http://planetorbitrap.com/about-the-technology#.VCDLJhZO3Kc>

General MS/MS Setup for Proteomics



Fragmentation—Fast Atom Bombardment/Collision Induced Dissociation

- Ions are collided with an inert gas (e.g. helium) causing fragmentation



<http://www.lamondlab.com/MSResource/LC/MS/MassSpectrometry/collisionalActivationMethods.php>

Nice Animation of a Q Exactive™ Quadrupole/Orbitrap

<http://planetorbitrap.com/q-exactive#tab:animation>

- This web link takes you to a nice animation of the technology
- We will be showing video of experiments with this machine at the Center for Advanced Proteomics Research at Rutgers