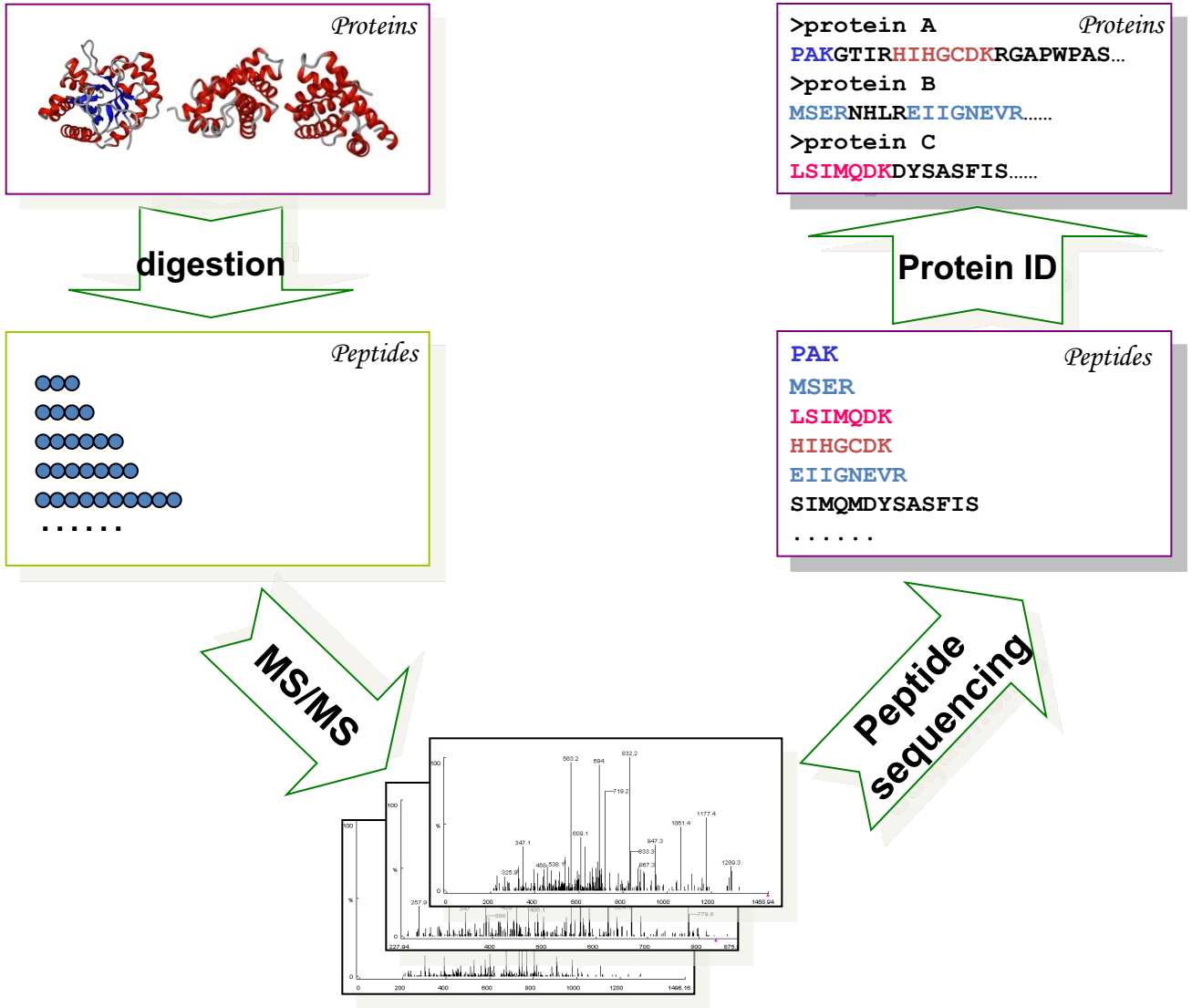
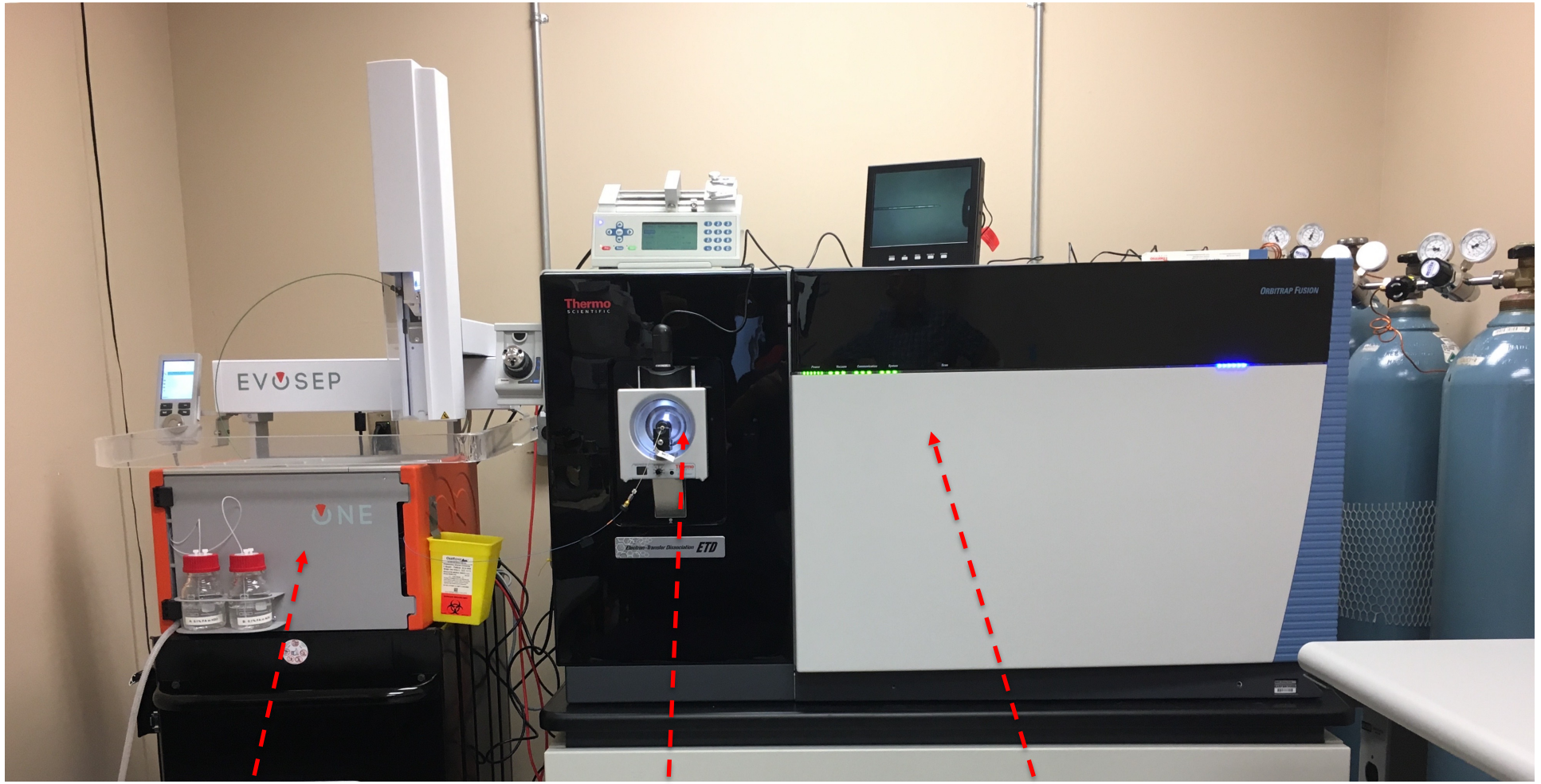
Review:

1. Finished "seeding"
2. proteomics
3. peptide-spectrum match (PSM).

Bottom Up Proteomics



A Typical LC-MS Setup



LC separation

Ionize and inject

measurement

Three Basic Components

- Ionizer
  - provide electric charges to the molecules
  - MALDI, **ESI**
- Mass Analyzer
  - Separates the ions according to m/z
  - Magnetic Sector, Iontrap, **TOF**, Quadrupole, FT, **Orbitrap**
- Detector
  - Detect the separated ions
  - Electron multiplier, Fourier Transform

Ionization

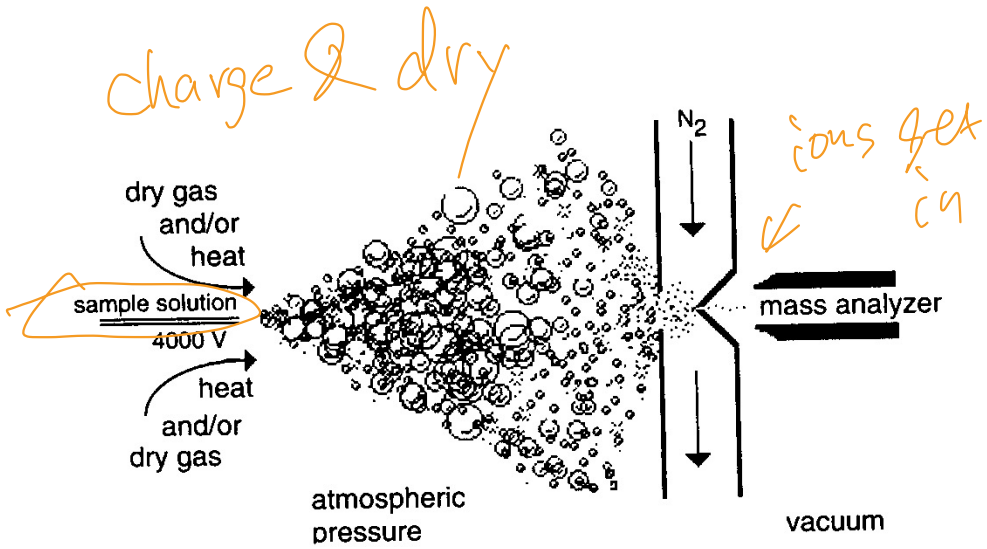
- MALDI (Matrix Assisted Laser Desorption/Ionization)
- ESI (Electrospray Ionization)

Koichi Tanaka

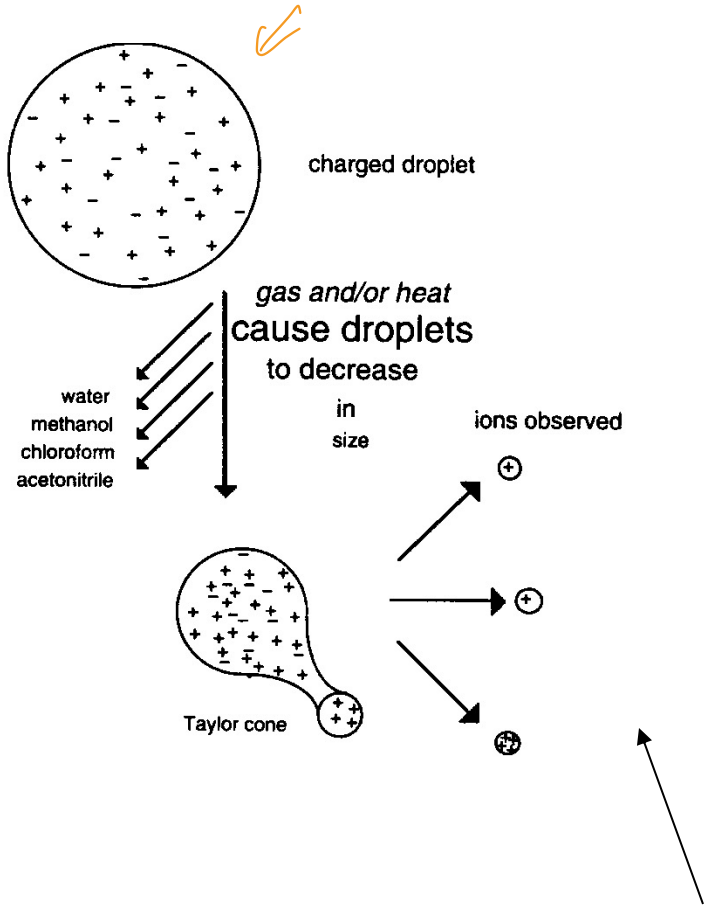
John Fenn

Nobel prize in Chemistry, 2002

Ionization – ESI



Electrospray Ionization: Formation of Charged Droplets



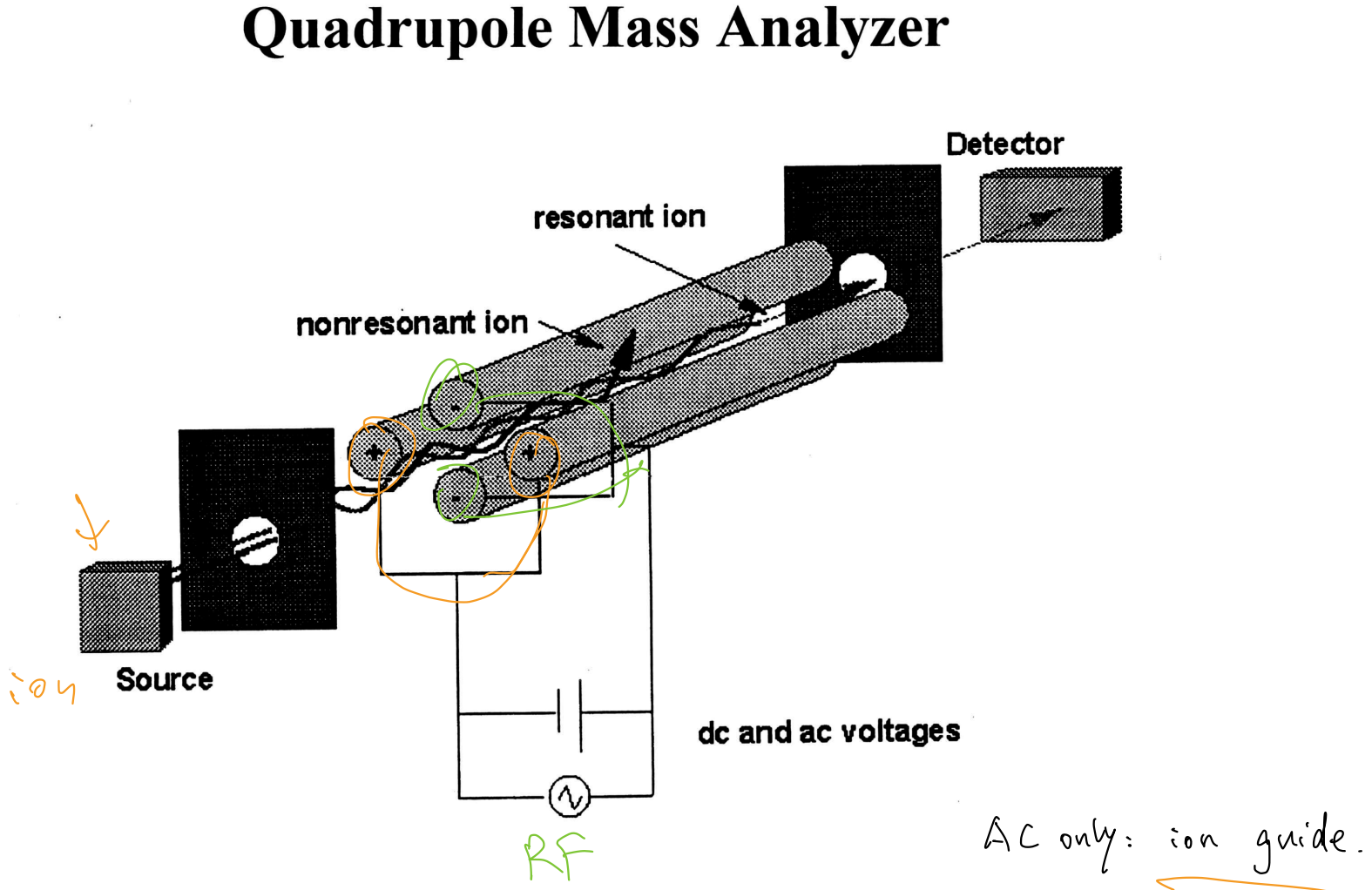
Formation of multiply charged ions

ESI History

- Fenn, J. B.; Mann, M.; Meng, C. K.; Wong, S. F.; Whitehouse, C. M. (1989). "Electrospray ionization for mass spectrometry of large biomolecules". Science 246 (4926): 64–71.
- A.B. from Berea College in his new hometown.
- 1940: Ph.D. from Yale University.
- 1962: He joined the Yale University faculty.
- 1987: he reached the mandatory retirement age (70).
- University-mandated move to smaller laboratory space.
- Started to work on ESI.
- 1994: Fenn joined Virginia Commonwealth University.
- The patent rights to ESI became the subject of a legal case between Yale University and Fenn.
- 2005: Yale was awarded over one million dollars and partial patent rights to the technique.

Fenn's first electrospray ionization source (top) coupled to a single quadrupole mass spectrometer

Mass Analyzer (1) – Quadrupole

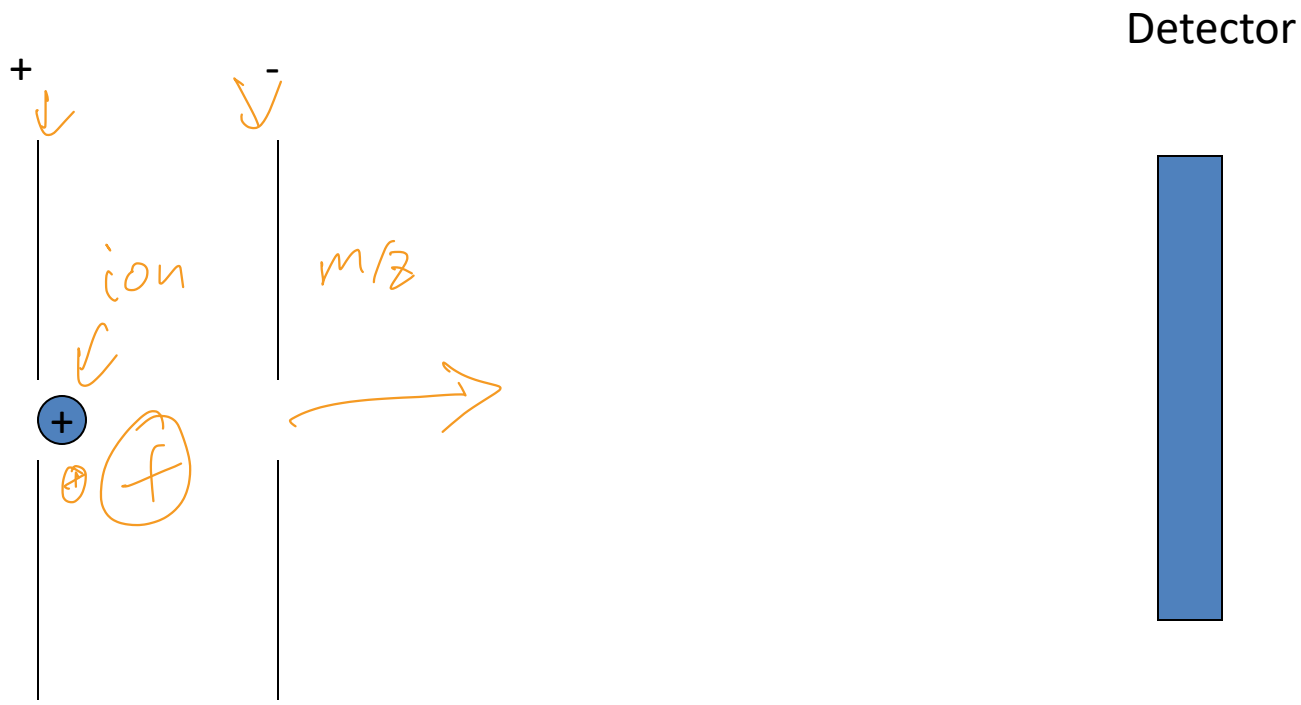


- **Adjusting DC voltage allows different m/z ions to pass. (Mass filter)**
- **The complete spectrum is obtained by scanning whole range.**

Mass range 10- 4,000 Da

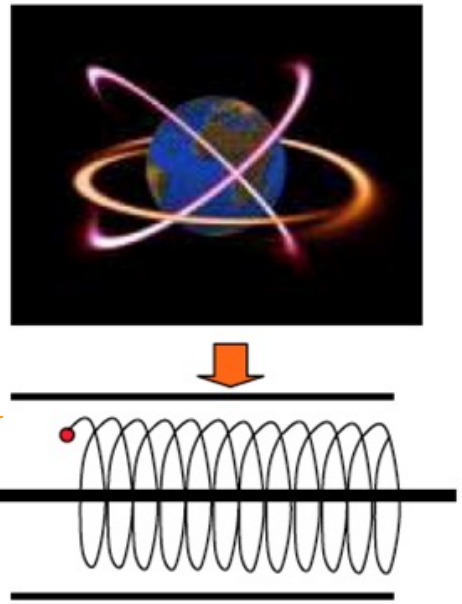
Mass Analyzer (2) – TOF

- Time of Flight.

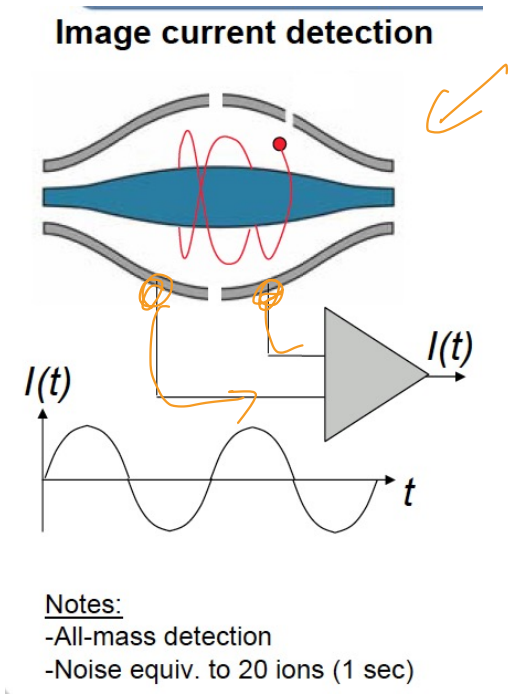


Time of flight is proportional to sqrt(m/z)

Mass Analyzer (3) – Orbitrap



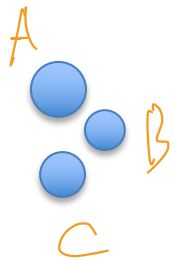
Moving ions are trapped around an electrode.



By shaping the electrode appropriately, ions also move left and right. Left-right frequency proportional to sqrt(m/z).

Fourier transform to convert the time domain signal to frequencies.

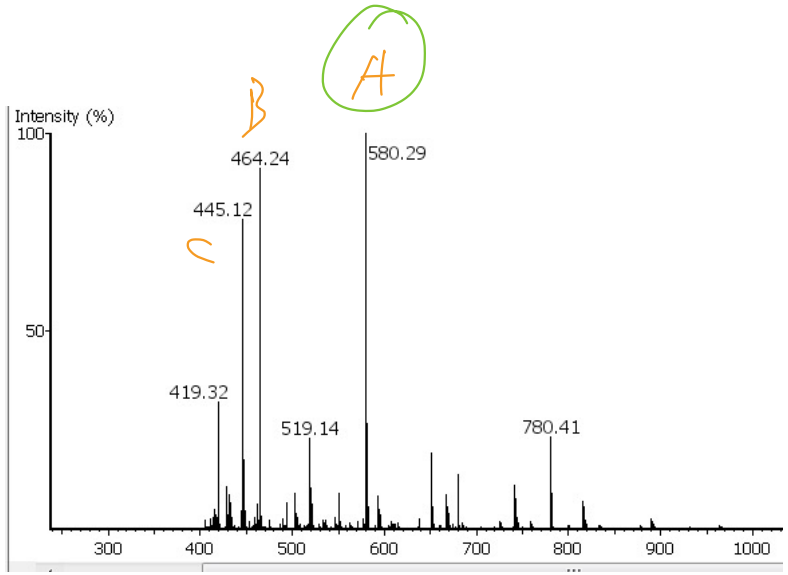
Mass Spectrometer



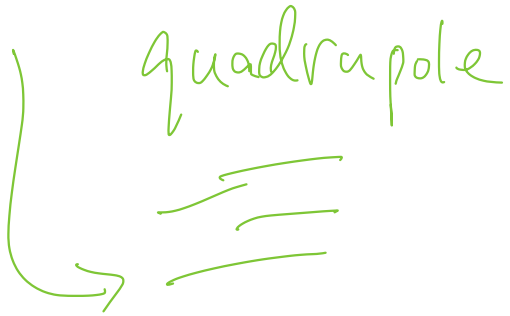
Molecules

Ionizer

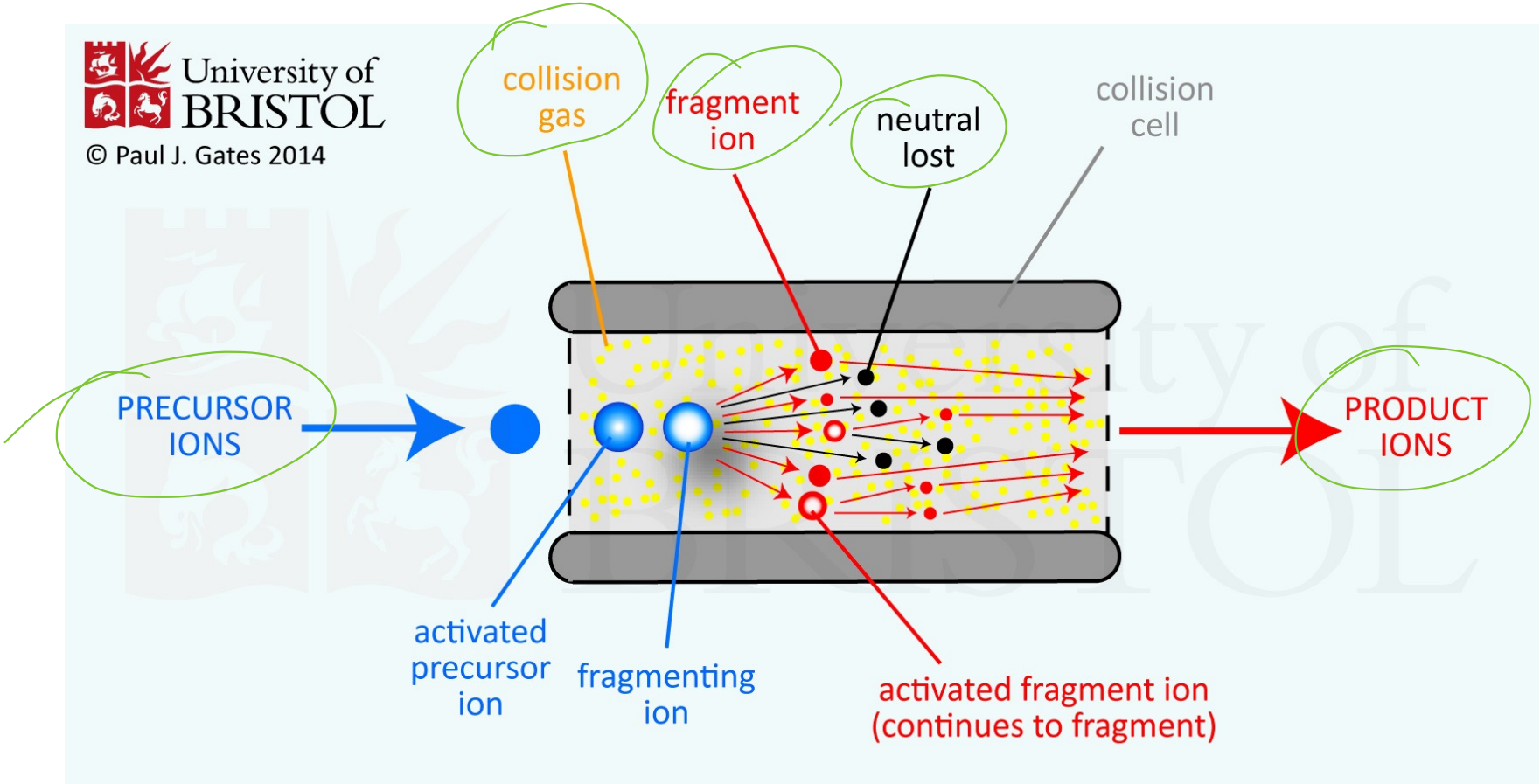
Mass Analyzer



MS Spectrum



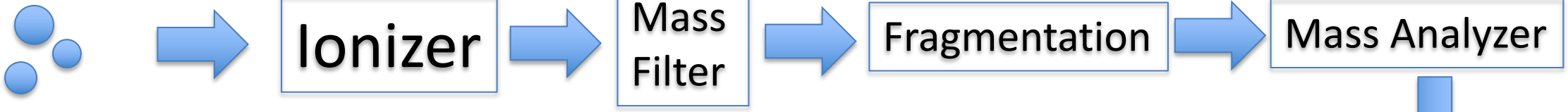
Collision Cell



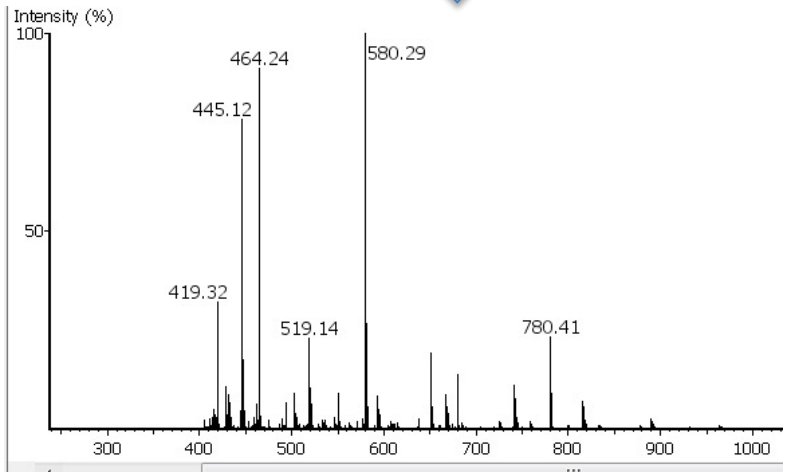
Tandem Mass Spectrometer

parent ion

precursor ion



Molecules



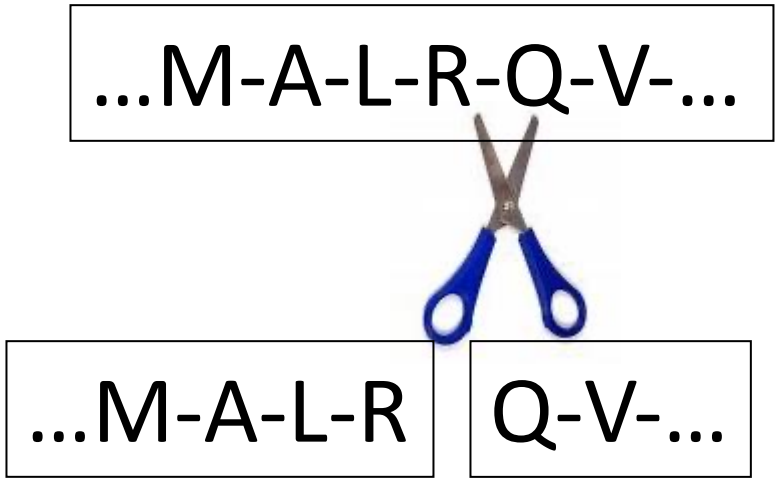
Tandem MS

MS2

MS/MS Spectrum

Protease

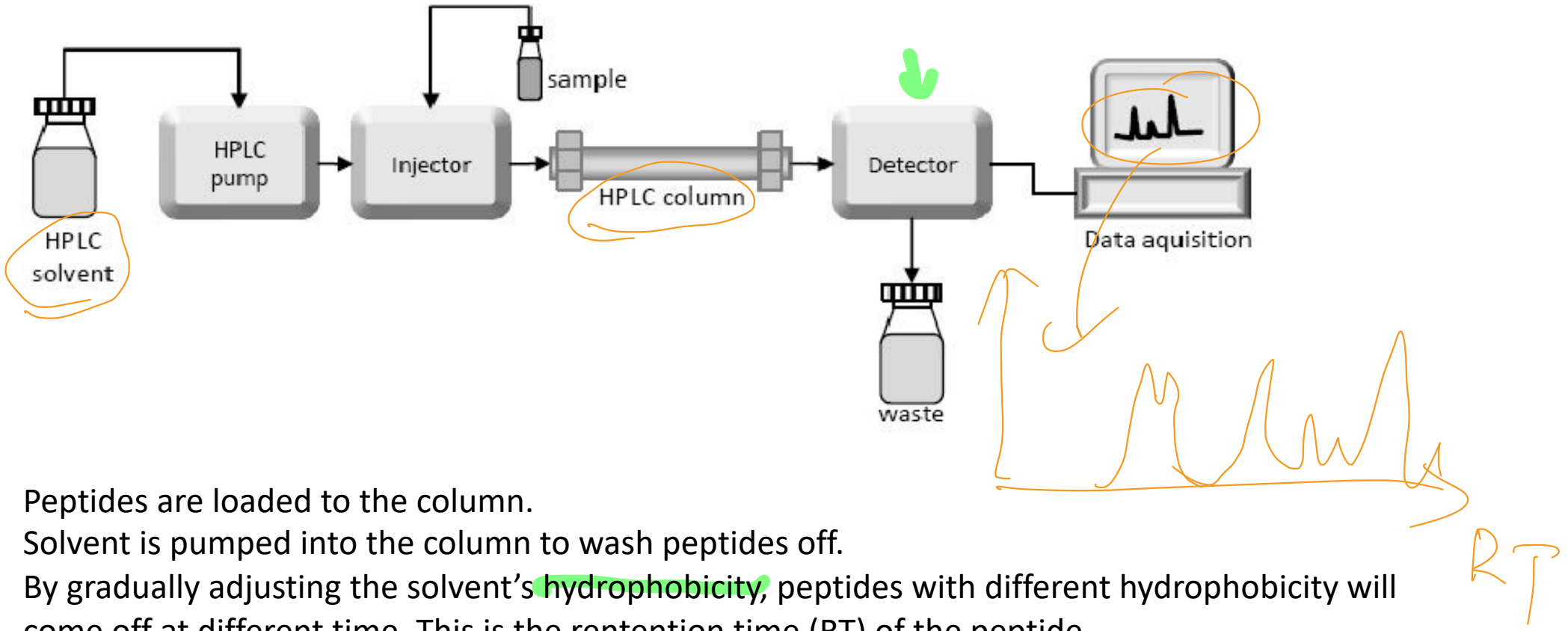
- Proteins are generally too large for mass spec. They need to be cut into short peptides first.
- A **protease** is any enzyme that conducts proteolysis. In another word, a protease breaks protein in water.
- **Trypsin** is the most commonly used enzyme. It digests at site [KR] | [^P]
  - After K or R, but not before P.



Sample Complexity

- Challenge: Suppose there are 10,000 proteins to be analyzed. Each produces 100 peptides. Then there are 1 million peptides.
- If all of them are injected into mass spec simultaneously, we will see a peak everywhere. No useful information at all.
- Solution: separate them and inject a subset of them to mass spec at any given time.

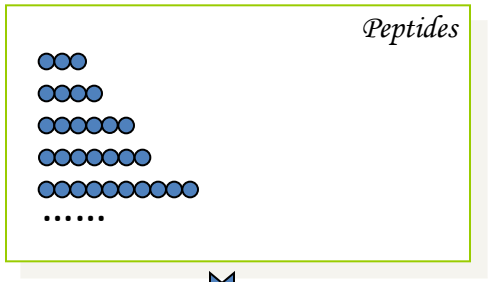
Liquid Chromotography (LC)



1. Peptides are loaded to the column.
2. Solvent is pumped into the column to wash peptides off.
3. By gradually adjusting the solvent's hydrophobicity, peptides with different hydrophobicity will come off at different time. This is the rentention time (RT) of the peptide.
4. Peptides are therefore separated. (But the separation is not perfect..)

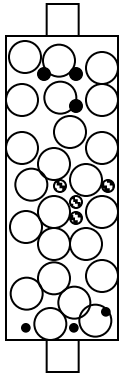
Put All Pieces Together

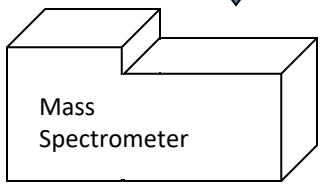
(1) A mixture of protein is digested into peptides with added enzyme (usually trypsin).



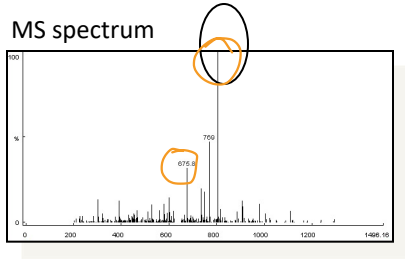
digestion

(2) The resulting peptides are separated with liquid chromatography (LC). Different peptides elute at different time. The separation may not be perfect.



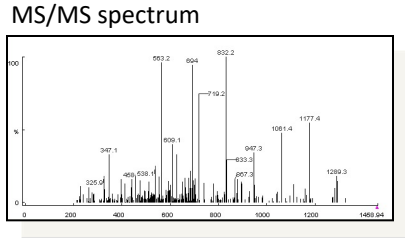


MS



(3) The spectrometer scans the peptide ions at a particular time and obtains a profile MS scan. Each peak in the MS spectrum supposedly corresponds to a peptide.

MS/MS



(4) The spectrometer selects a peak (a peptide ion) with the first mass analyzer, fragments it and produces an MS/MS scan with the second mass analyzer.

Data-dependent Acquisition

DDA

aquire

survey scans