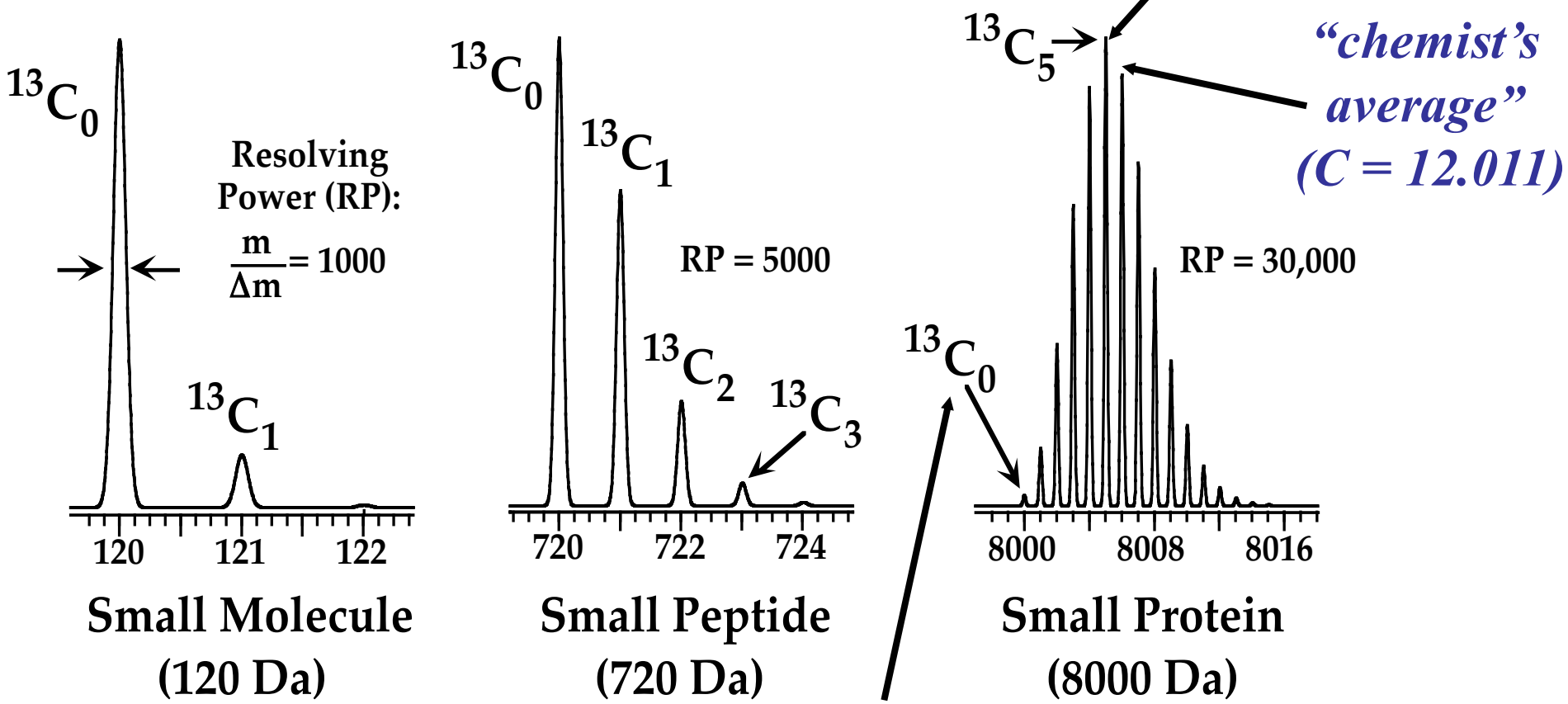
History of Mass Spec
Basics of Mass Spec

History of Mass Spectroscopy

- Developed in early 1900s for study of small molecules
- Extended in early 1990s to study of peptides and proteins
- Nobel Prize awarded in 2002 for development of two main methods:
  - MALDI
  - Electrospray (ESI)

Large Molecule Isotopic Distributions



2002 Nobel Prize in Chemistry

Awarded "for the development of methods for identification and structure analyses of biological macromolecules"

- To **John B. Fenn** and **Koichi Tanaka** "for their development of soft desorption ionisation methods for mass spectrometric analyses of biological macromolecules”
- To **Kurt Wüthrich** "for his development of nuclear magnetic resonance spectroscopy for determining the three-dimensional structure of biological macromolecules in solution” (NMR)

2002 Nobel Prize in Chemistry
Koichi Takeda

- Developed MALDI while working for Shimadzu Corporation in Kyoto, Japan

Matrix-assisted laser desorption ionization (MALDI)

- Sample mixed with carrier substance (“matrix”) and dried on plate
- Laser heats matrix and causes matrix and sample to sublime off surface
- Ions are accelerated and move down tube in vacuum
- “Time of flight” measures mass and charge of substance

MALDI Sample Plate

- Samples are spotted on plate and dried

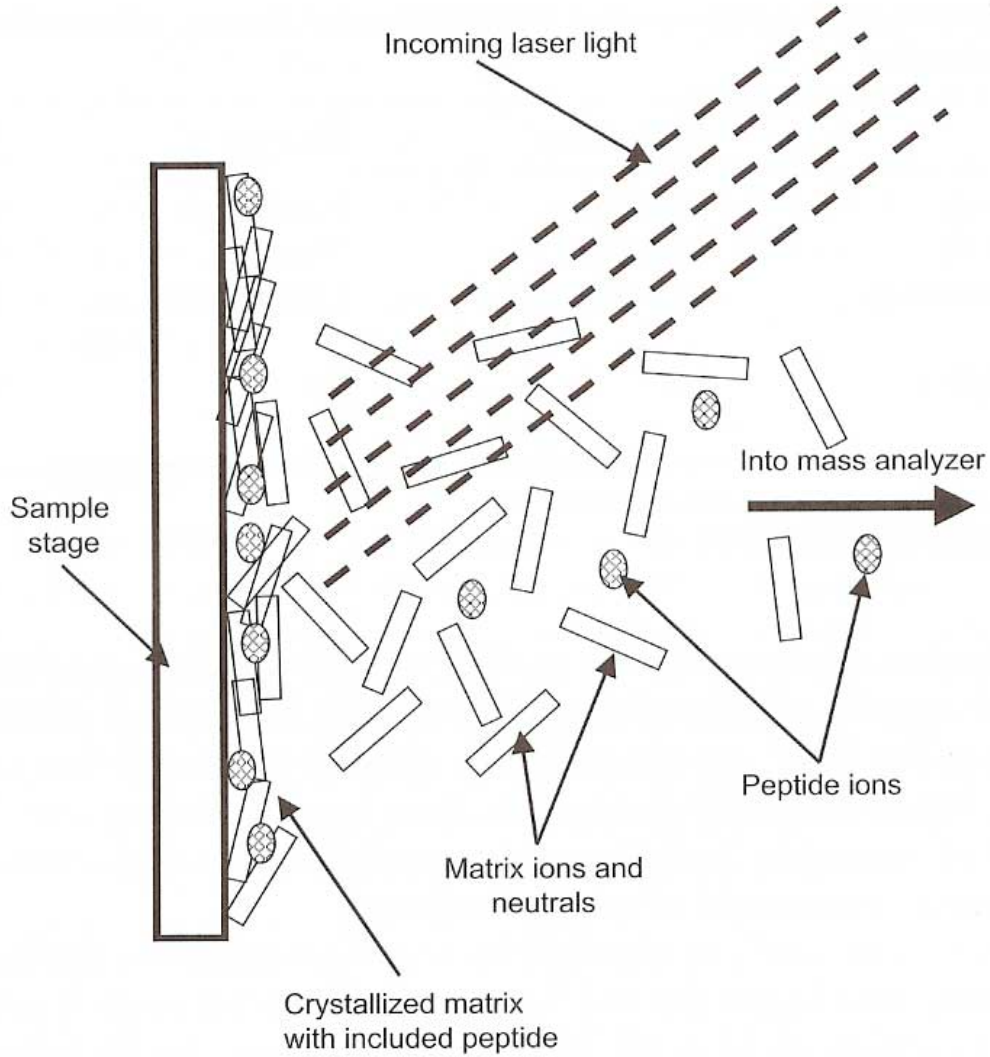
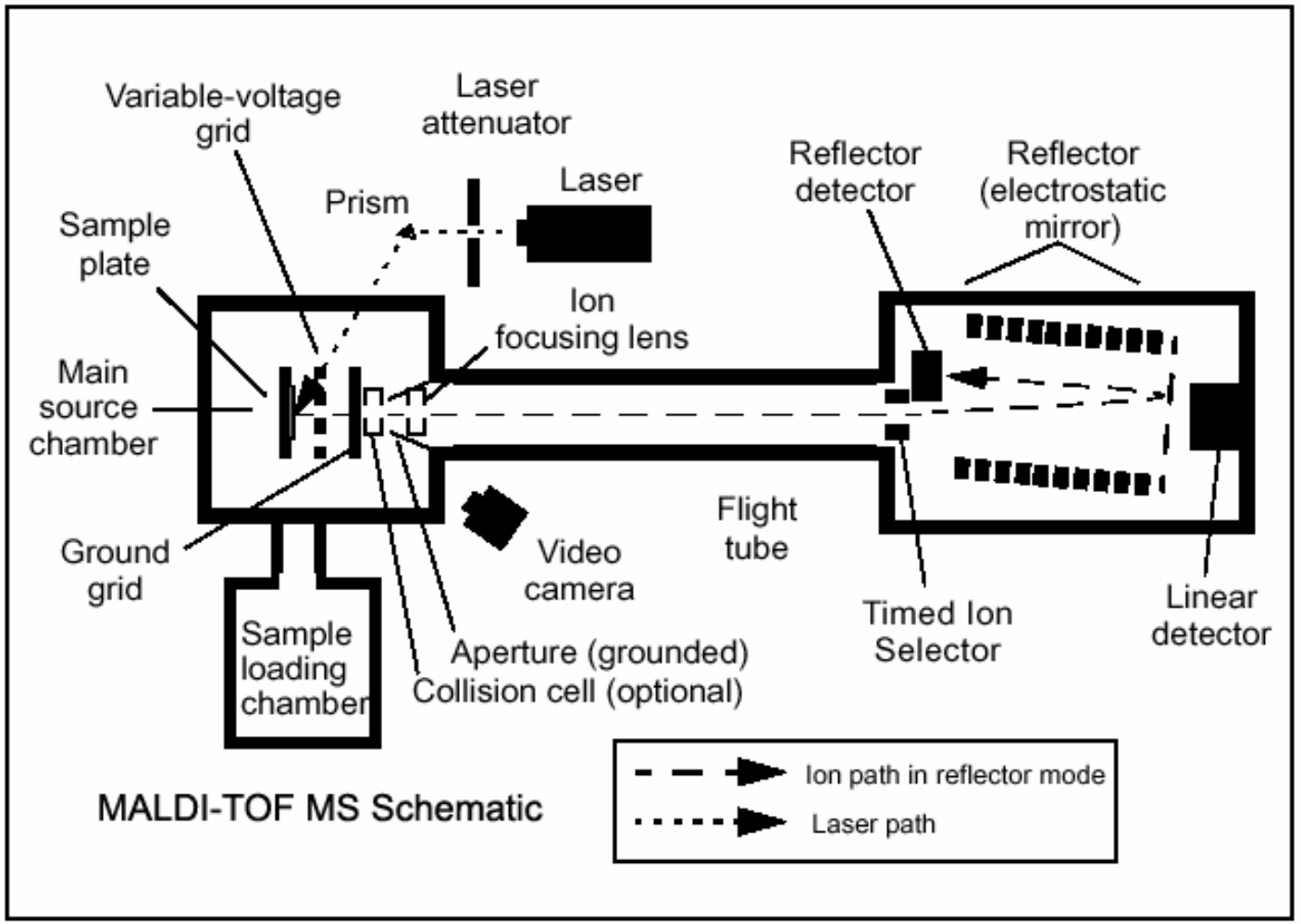


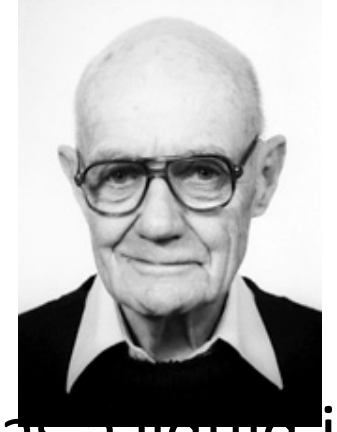
Figure 3.4. A generalized view of the processes associated with matrix-assisted laser desorption/ionization. The protein or peptide analyte are co-crystallized with the matrix compound on the sample stage and are irradiated with UV-laser pulses. The laser pulses vaporize the matrix compound and produce a plume that carries the protonated peptide or protein into the gas phase. The gas-phase ions are directed into the mass analyzer by appropriate electric fields.

From-Kinter & Sherman, "Protein Sequencing and Identification Using Tandem Mass Spectroscopy, 2000

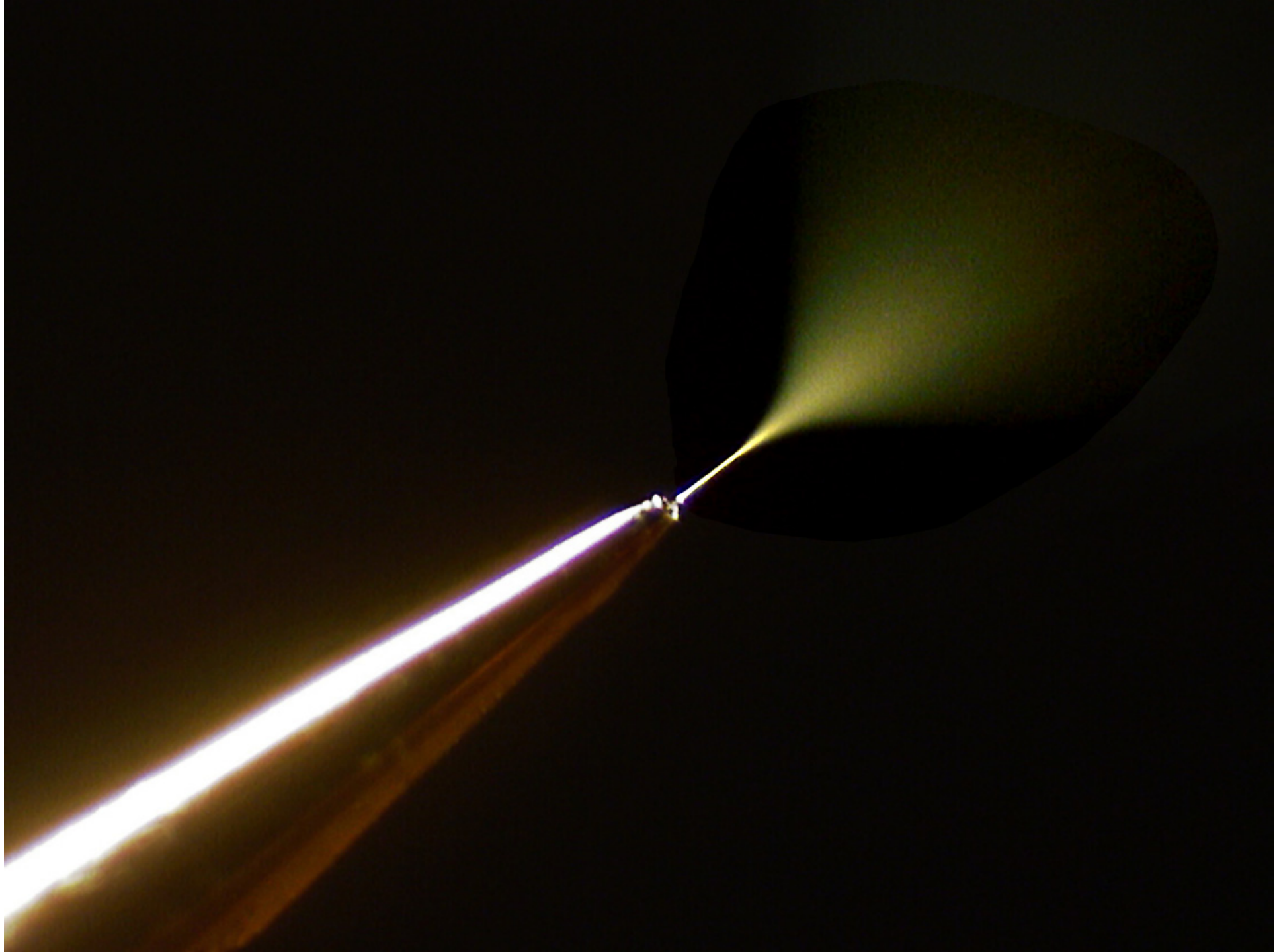
MALDI Time of Flight (TOF)



Nobel Prize - John Fenn

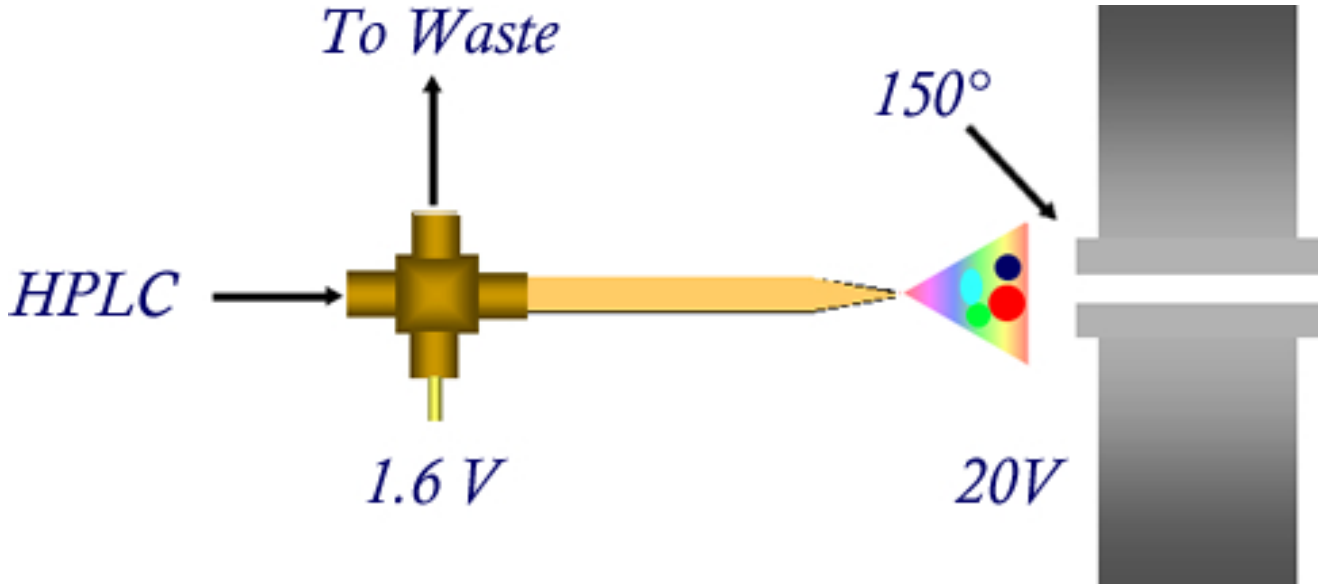


- Electrospray ionization (ESI) involves dispersing sample as a liquid into vacuum so that sample is ionized and solvent sublimes
- Allows samples to be injected into mass spectrometer as they come off column
- Most commonly used method for proteomics



Electrospray allows us to combine MS with chromatography

- Peptides separated on HPLC column
- Elutant from column injected directly into MS using ESI



Routine separation protocol (Gel-based)

- Samples are separated by SDS polyacrylamide gel electrophoresis:
  - Lehninger demo
- Proteins are stained to identify bands
- Band is cut out of gel
- Gel slice is cleaved with trypsin to break protein into peptides
- Peptides extracted from gel slice and injected into MS/MS

Routine Separation (Solution)

- Separate proteins by Isoelectric Focusing (by pI)
- Purify by liquid chromatography
- Immunoaffinity (using antibodies on beads)

➔ Digest with trypsin before MS/MS

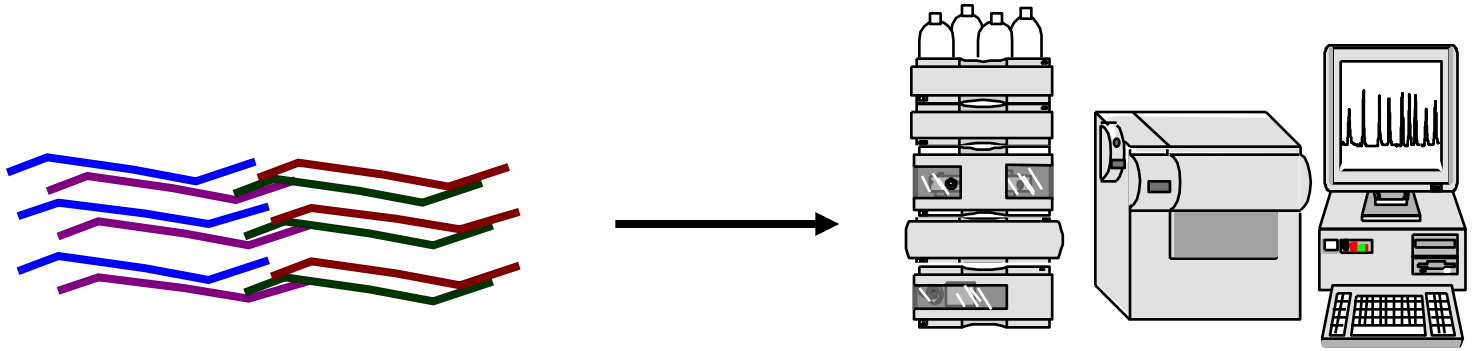
MS Instruments at UIC

- Voyager MALDI
- Thermo LTQ
- Thermo LTQ-FT -
  - Attomole sensitivity (part per billion)
  - Fourier Transform
  - 7 Tesla superconducting magnet (liquid helium)

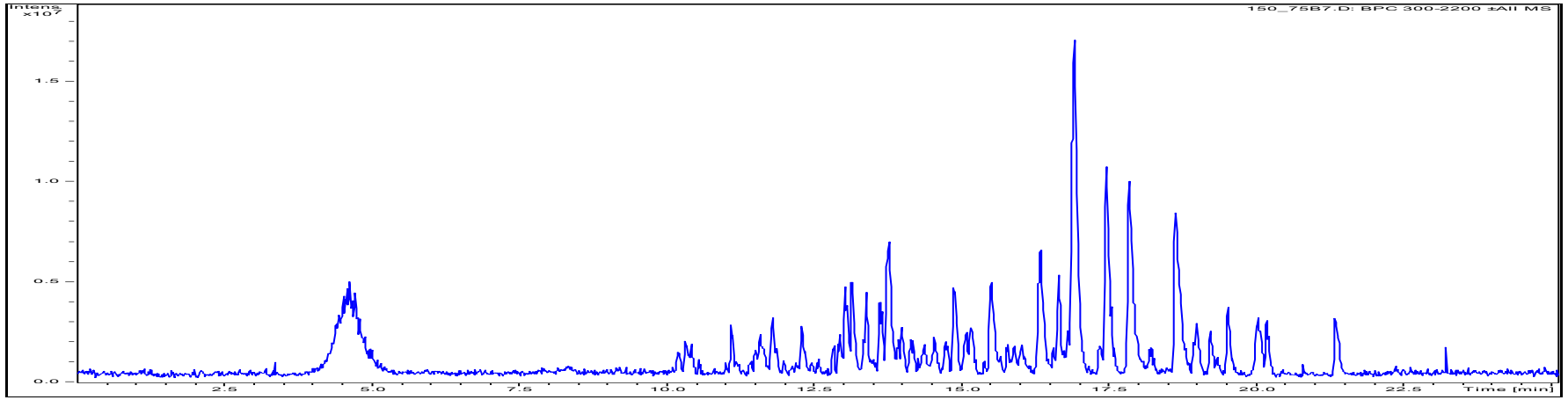
MS/MS fragmentation

- Identifying the sequence in each peptide is the key to protein identification.

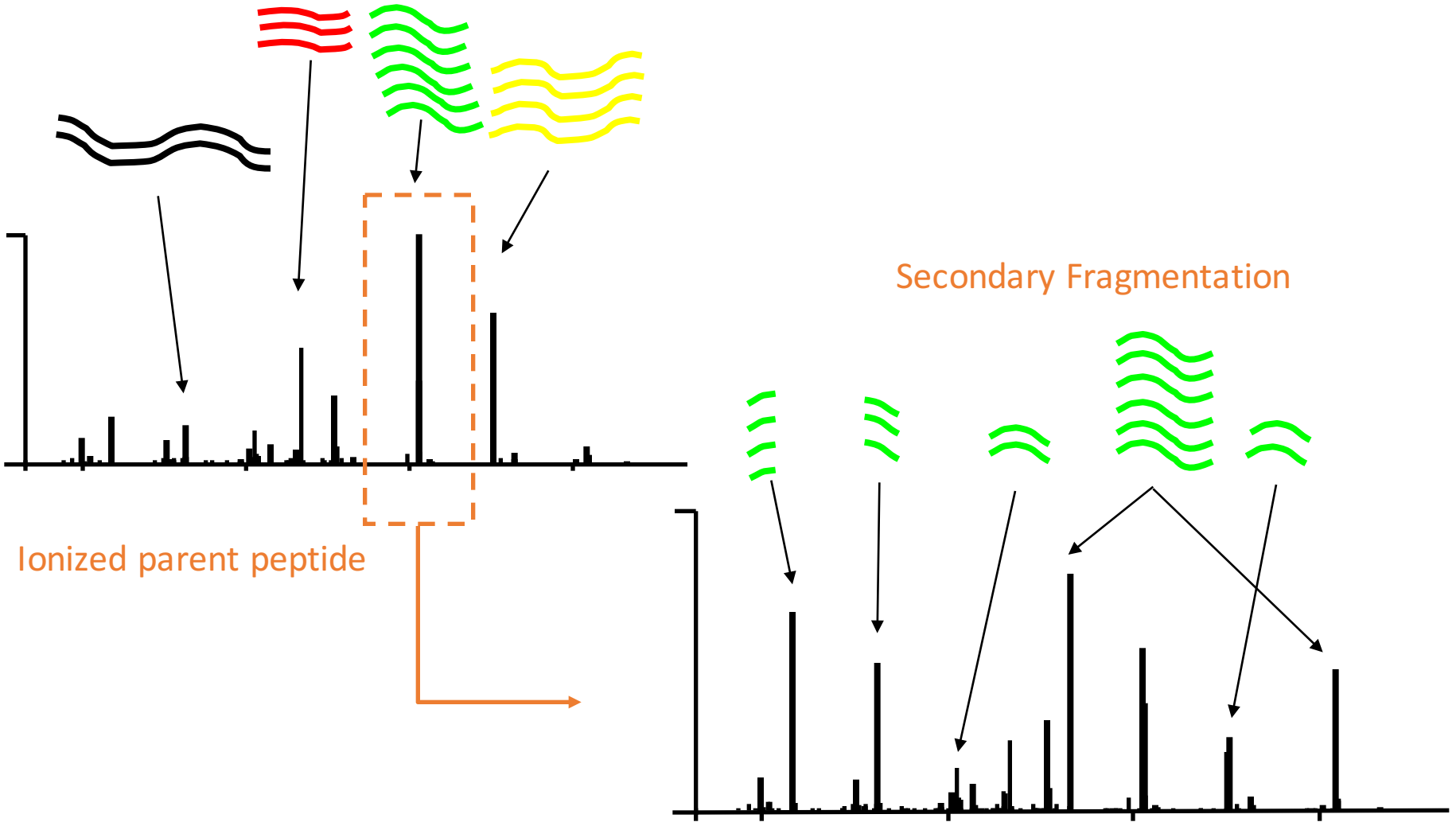
The peptides are injected into a liquid chromatograph/mass spectrometer (LC/MS)….



A chromatogram of peptides is produced

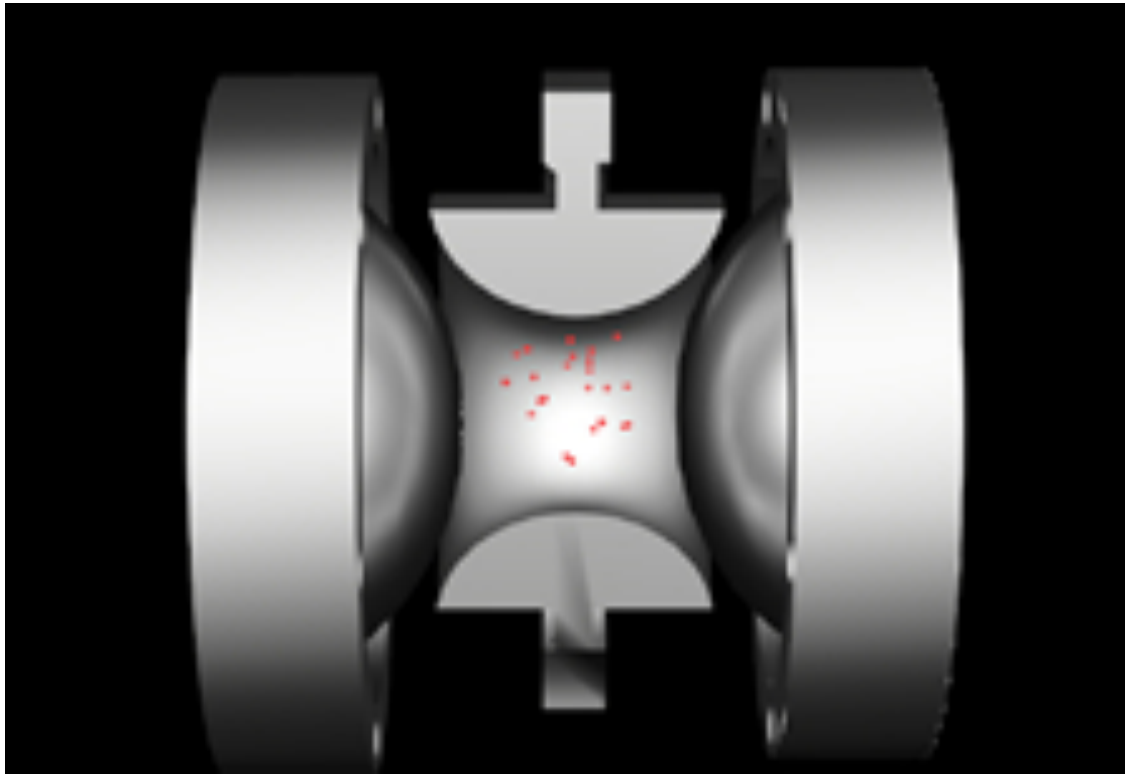


Tandem Mass Spectrum: An Example

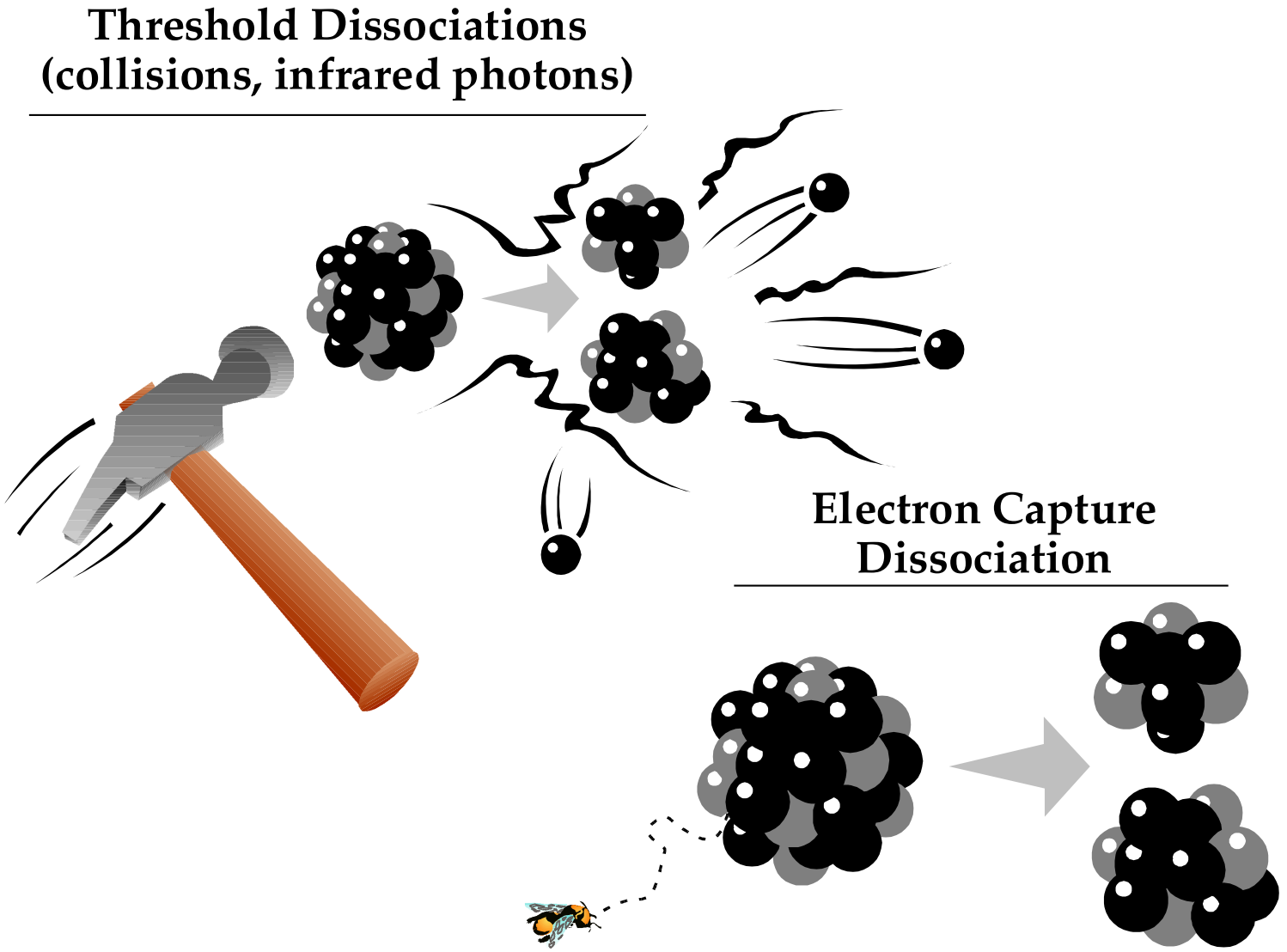


Ion Trap

- Magnets focus and trap the ions before fragmentation by collision with Argon gas

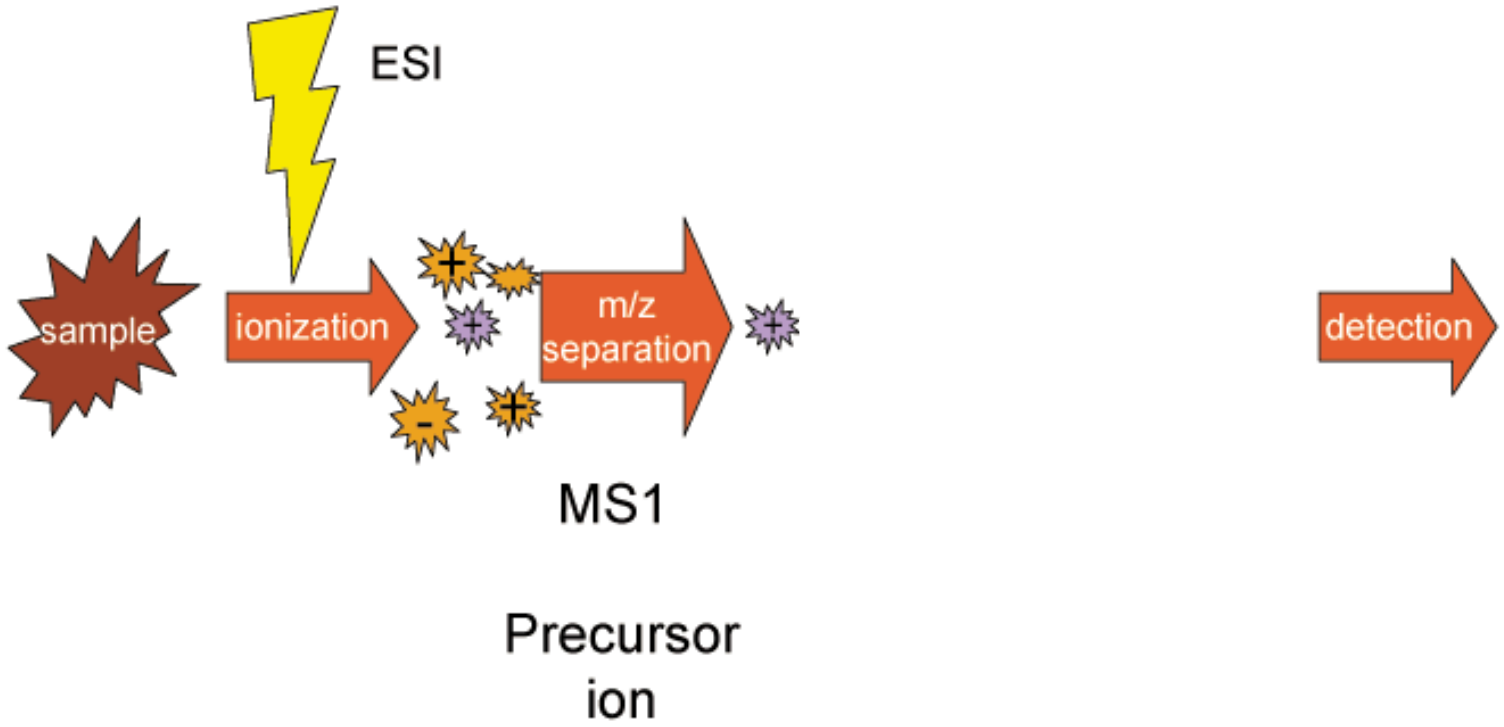


Two General Ways to Fragment Gas Phase Ions

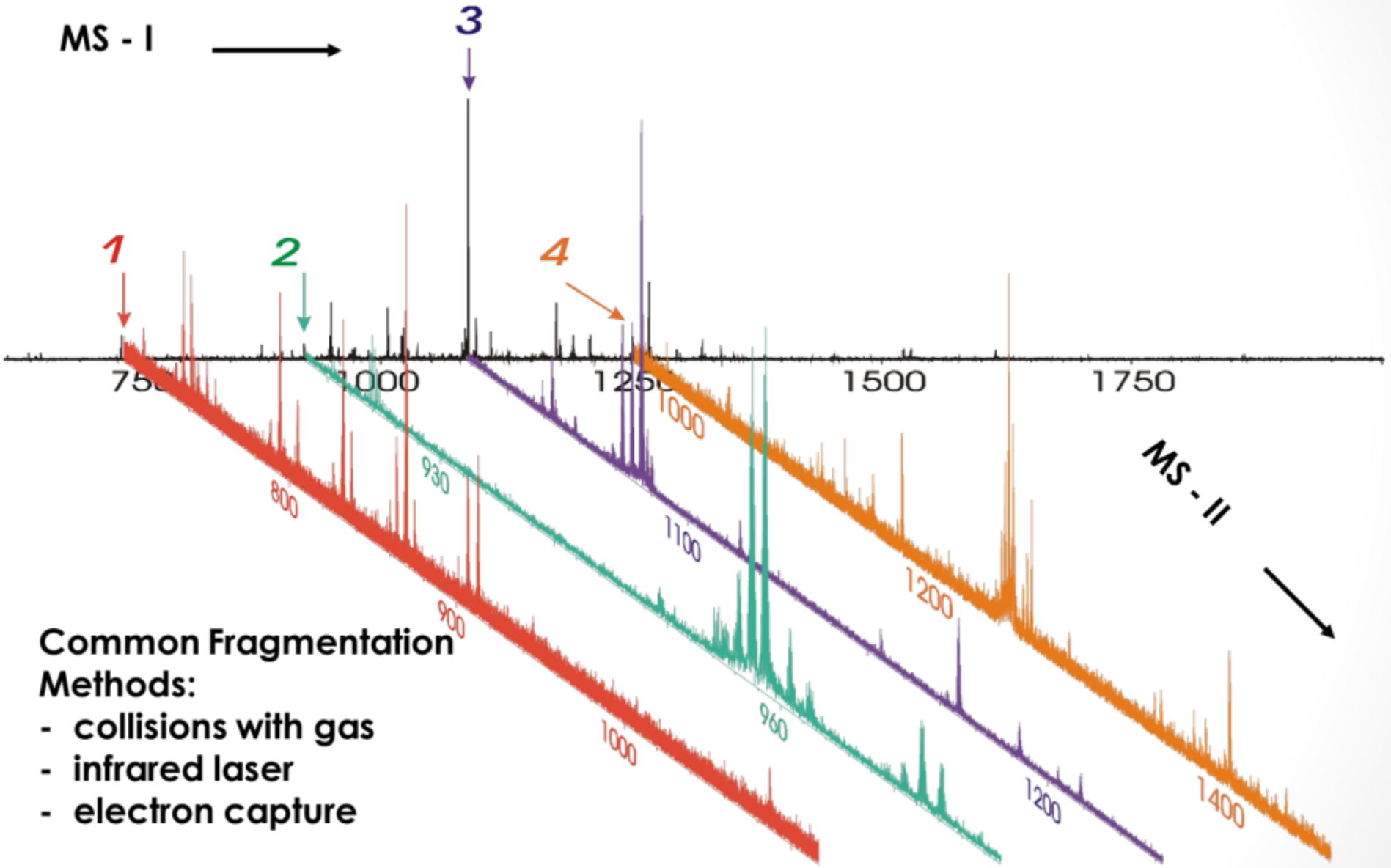


MS/MS Schematic

Breaking Humpty-Dumpty apart.

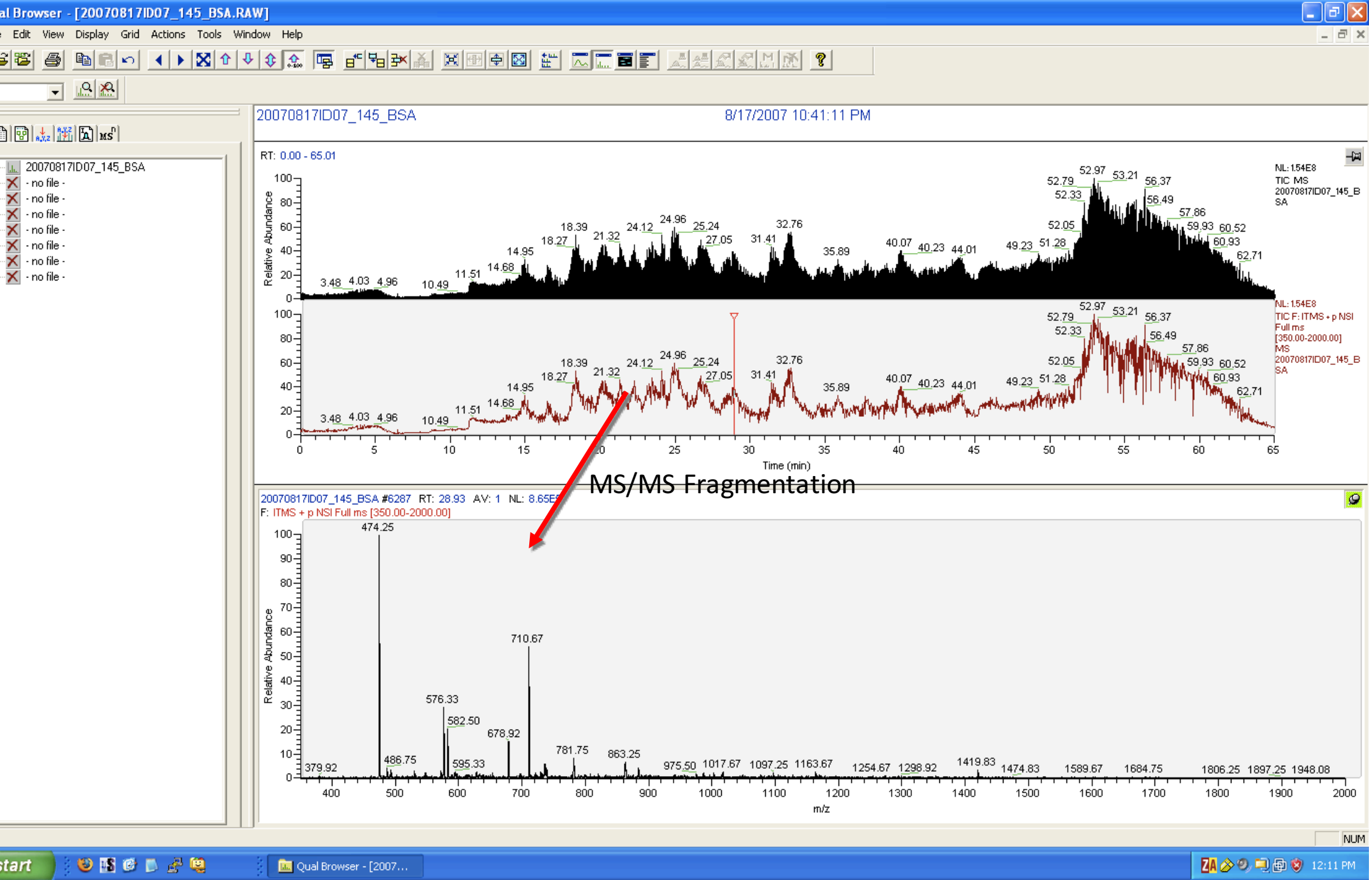


Tandem Mass Spectrometry (MS/MS)



Common Fragmentation Methods:

- collisions with gas
- infrared laser
- electron capture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But…

- Can you tell which peptides were in the BSA peaks?
- You need a computer with sophisticated search algorithms to identify your peptides and the proteins they came from.