Mass Spectrometry

Hyphenated Techniques
GC-MS LC-MS
and
MS-MS

Reasons for Using Chromatography with MS

- Mixture analysis by MS alone is difficult
  - Fragmentation from ionization (EI or CI)
    - Fragments from each component in one spectrum yields intractable interpretation
  - Suppression effects in soft techniques (ESI)
    - Analytes that are less abundant or ionize poorly may not be detected
    - Quantitation of mixtures is less accurate and less sensitive than quantitation of components that have been chromatographically separated

Chromatography -MS Data

- Rather than averaging all data into one spectrum, each full scan is saved separately to create a chromatogram
  - TIC - Total Ion Chromatogram
  - EIC - Extracted Ion Chromatogram
- Scan speed is critical for resolution of close-eluting components
  - peaks could be missed or averaged together
  - spectral tilting
    - Intensities of low/high mass ions skewed

Chromatography -MS Data

- Identification of Components
  - Background subtraction can be useful in cleaning up spectra
    - Can remove background ions
    - Can remove ions from co-eluting tailing compound
  - EIC can support/contradict that two MS peaks are from the same/different compounds
    - Comparing whether time profiles overlap
  - EIC can show if ions come from the peak or are in the background

Offline LCMS

- LC may also be coupled with MS by fraction collection (ion exchange, size exclusion chromatography, TLC, Flash Column)
- Fractions may be analyzed by any compatible MS technique
- Offline Automation
  - LC eluent can be spotted onto a MALDI target using a robot for MS analysis

LC in line with MS

- Liquid chromatography may be directly coupled to mass spectrometry using an APCI or ESI source
- Typical flow rates:
  - 100 µl/min-2.5ml/min: APCI source
  - > 1 ml/min: ESI source, usually need splitter (≥ 4.6 mm column)
  - 1 µl/min - 1 ml/min: ESI source (200 µm- 4.6 mm column)
  - < 1 µl/min: nanospray source (≤ 150 µm column)

Types of LC coupled inline to MS

- Most common= reversed phase (i.e. C18, C8, C4)
- Ion exchange
- Normal phase
- Affinity
- 2D Chromatography (Ion Exchange followed by reversed phase)

Restrictions

- Non-volatile salts should be avoided as signal suppression will result
- Strong ion-pairing agents such as trifluoroacetate should similarly be avoided
- Need polar solvents for ESI
  - Normal phase not compatible with ESI (Use APCI)
- Additives must be compatible with ionization mode (i.e. pH of separation must match desired ions)
  - Or use post-column addition

GCMS

- With the advent of capillary columns, GC columns can be inserted directly into the ionization source
- GC is typically coupled to EI, CI, and FI
- GC-MS is applicable to a narrower range of compounds due to volatility requirements
- When GC/MS is applicable, it is a fairly trouble free technique

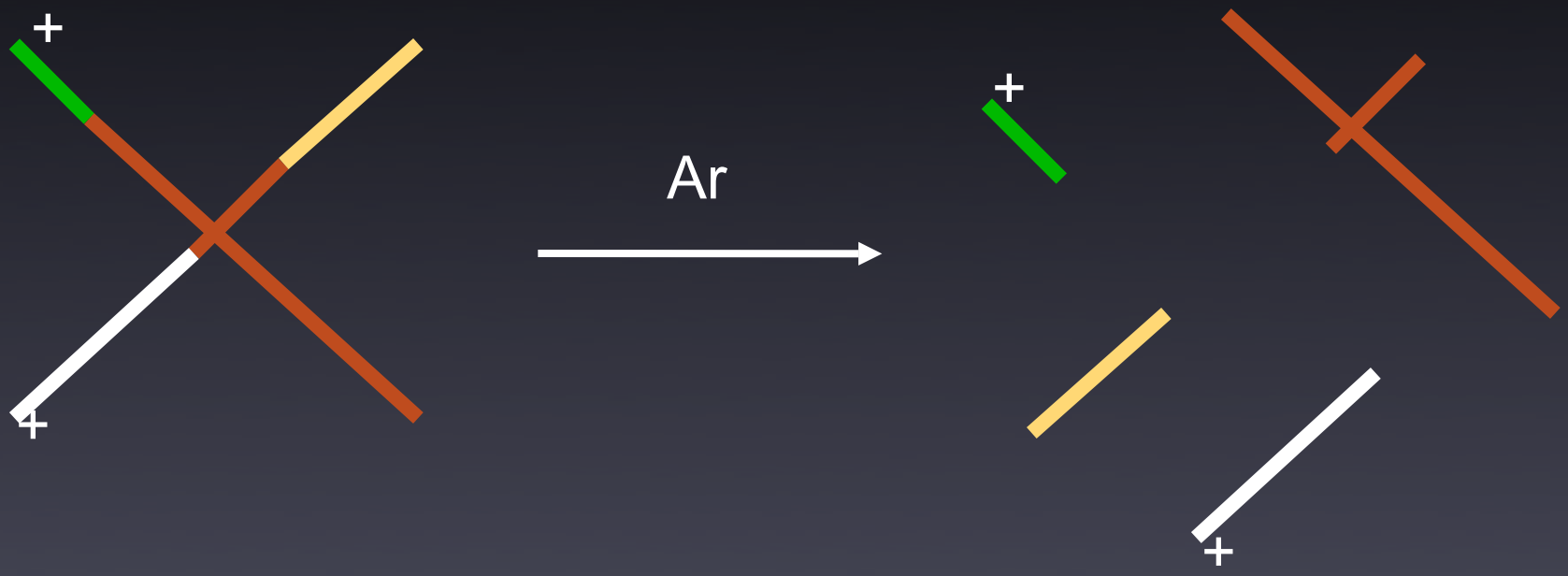
Tandem Mass Spectrometry (MS/MS or MS^n)

- Soft ionization techniques such as ESI only result in parent ions, revealing no structural information
- Hard ionization techniques yield fragments, but specific precursor-product relationships cannot be obtained
- Isolate and fragment precursor ion and measure m/z of product ions to obtain structural information
- One mass analyzer isolates, another scans for products

Common Methods of Fragmentation

- Threshold Dissociation (Weakest bonds are broken)
  - Collision Induced Dissociation (CID)
    - Apply an electric field to accelerate precursor ions and induce bond cleavage via collision with neutral gas molecules (Ar, N_2)
  - Infrared Multiphoton Dissociation (IRMPD)
    - Use infrared laser to break bonds
- Electron Capture Dissociation (ECD) (Random)
  - Multiply-charged cations capture electrons, inducing odd-electron fragmentation

CID



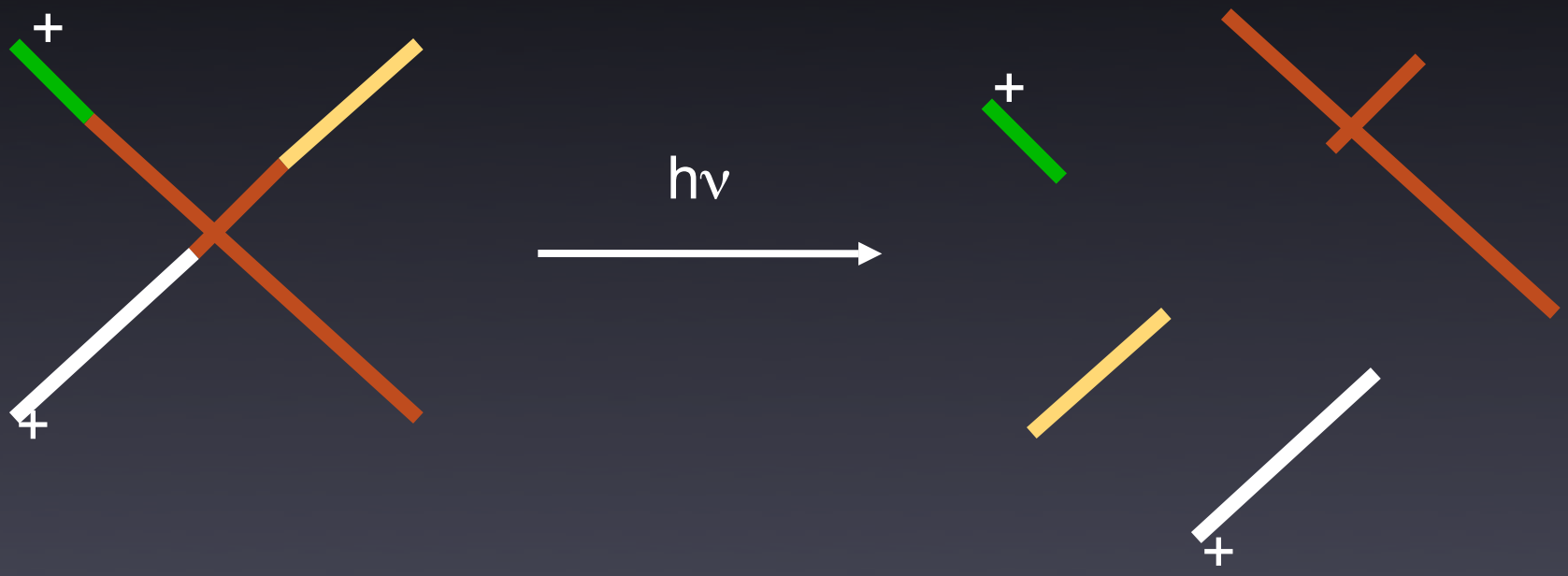
Accelerated Precursor Ion

Fragmentation through one or more collisions

Product Ions

(And Neutrals)

IRMPD



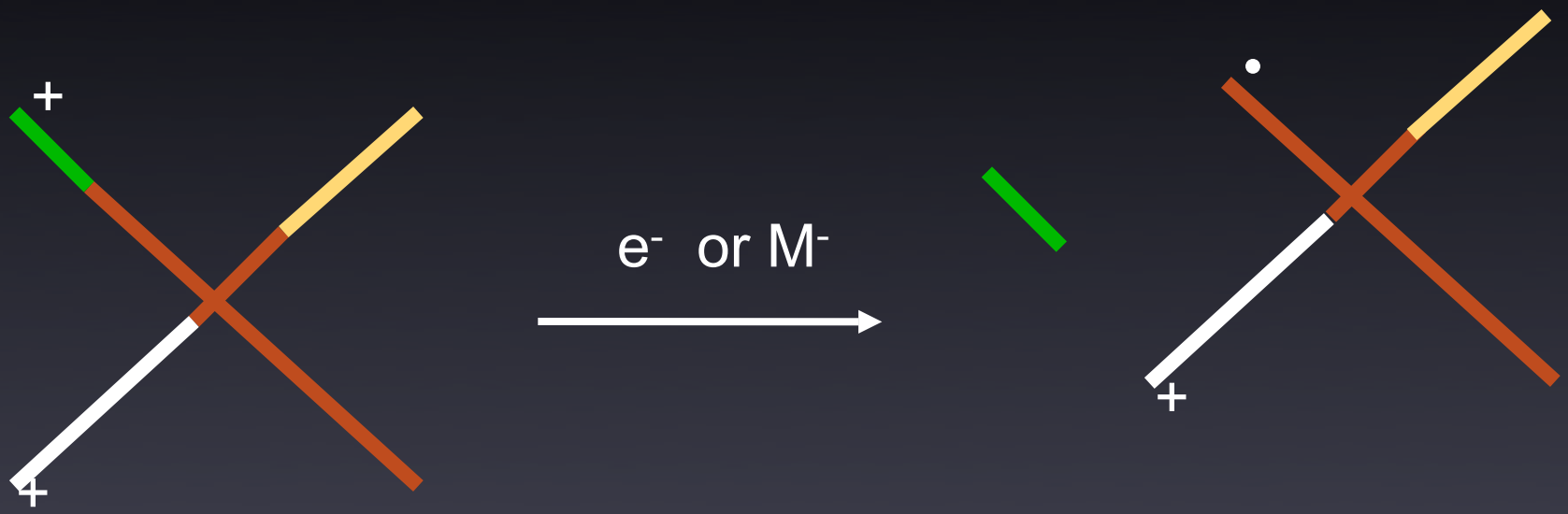
Isolated Precursor Ion

Fragmentation by one or more photons

Product Ions

(And Neutrals)

ECD/ETD



Isolated
Precursor Ion

Capture of one
or more electrons

Product Ions

(And Neutrals)

Instruments for MS/MS

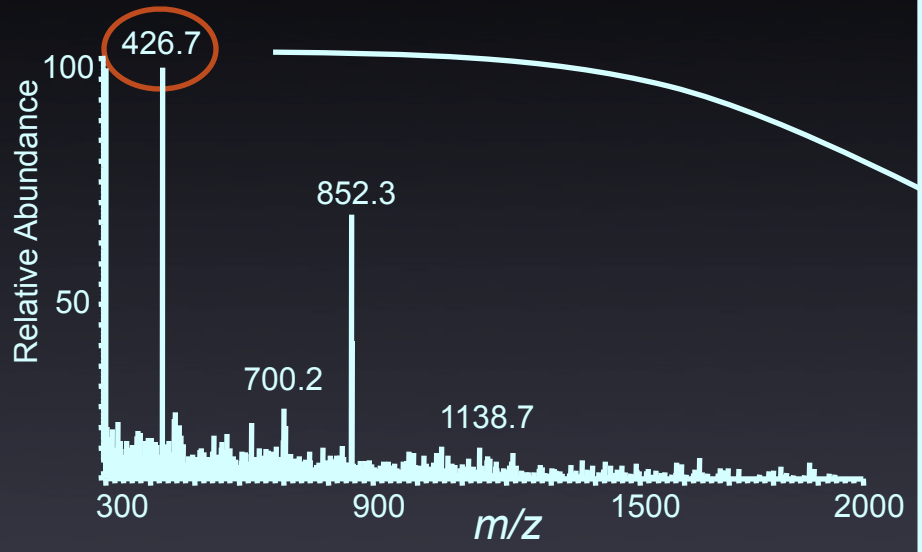
- Instruments where one or more analyzers are placed in series
  - Triple Quadrupole *(CID at eV levels)*
  - Four Sector *(CID at keV levels)*
  - Quadrupole-Time-of-Flight *(Q-TOF) (CID eV)*
  - TOF-TOF
- Trapping Instruments
  - Quadrupole Ion Trap *(CID eV, IRMPD, ECD)*
  - FT-ICR *(CID, SORI-CID, IRMPD, ECD)*

Quadrupole Ion Trap

- MS/MS is done in one analyzer
  - Precursor Ion is Isolated by selective ejection
  - Precursor ion is given kinetic energy
  - Fragments are formed and trapped
  - Trap is scanned to detect products
  - Products can be isolated and fragmented etc.

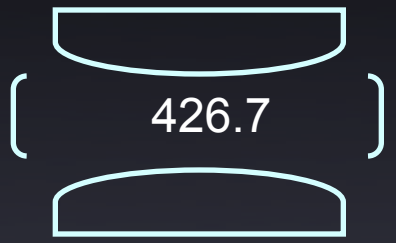
Tandem Mass Spectrometry in an Ion Trap

1) Measure Full Spectrum

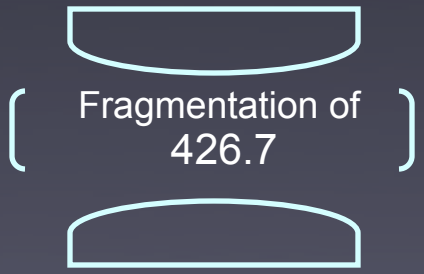


2) Ion Isolation:

e.g., Precursor *m/z* 426.7 ± 1.5



3) Add Collision Energy



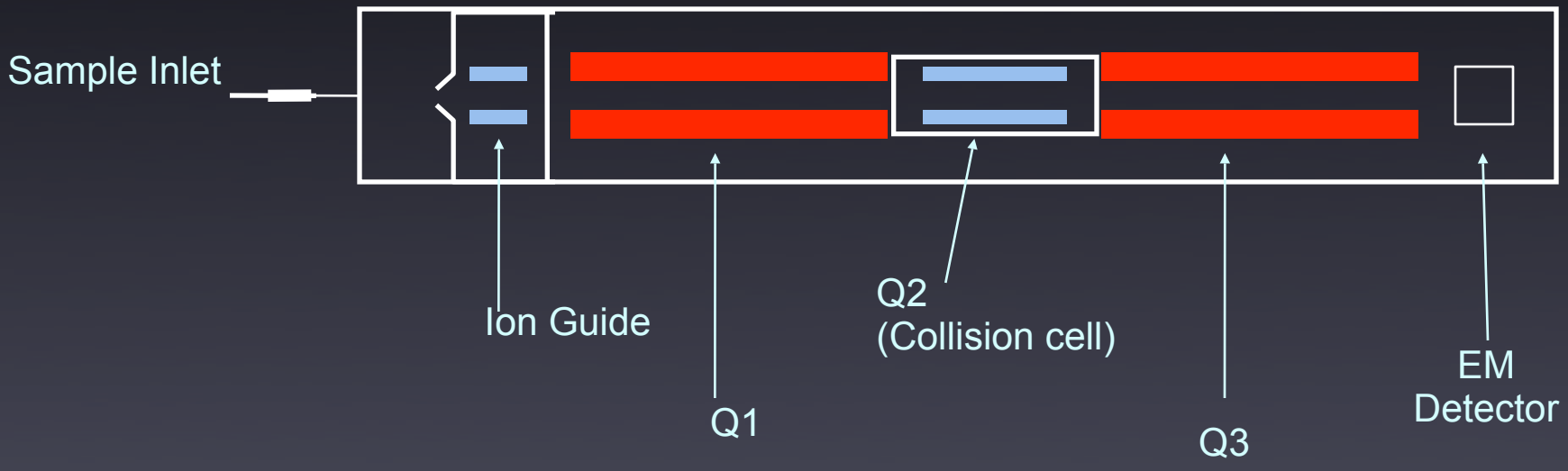
4) Measure *m/z* of Fragment Ions

[For Peptide SLNVALR]

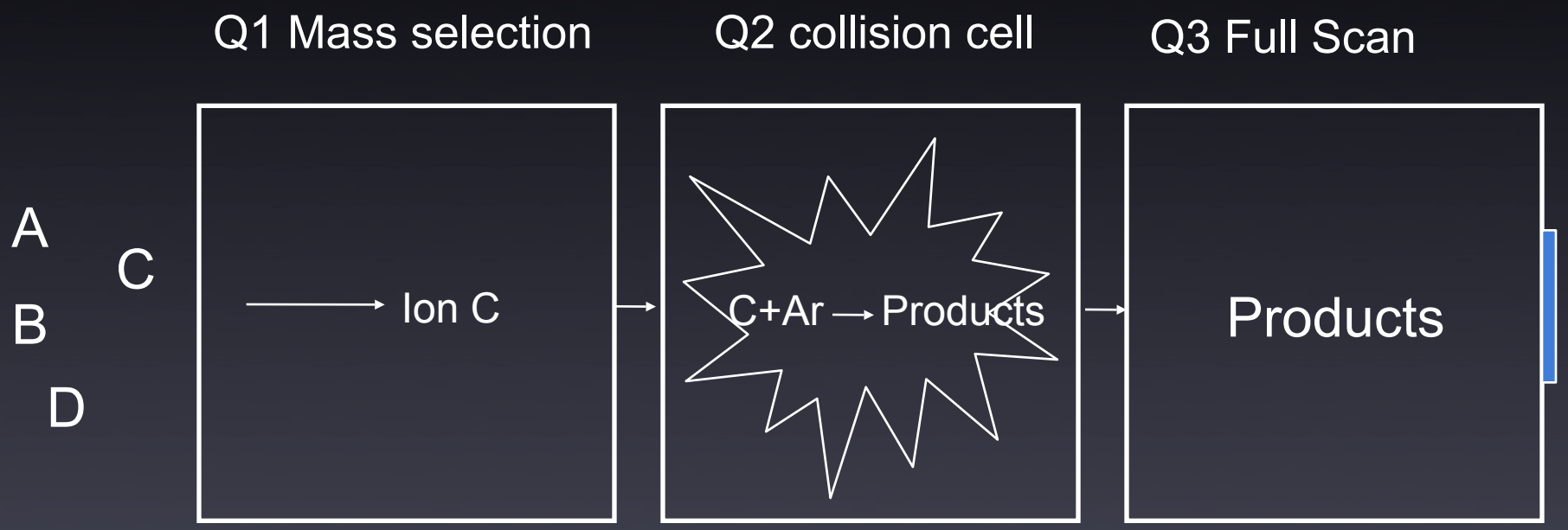
SLNVAL	R
SLNVA	LR
SLNV	ALR
SLN	VALR
SL	NVALR
S	LNVALR

Courtesy of the MSCLS at the Univ. of Minn.

Triple Quadrupole Mass Analyzer



Product Ion Scan Mode in a Triple Quadrupole



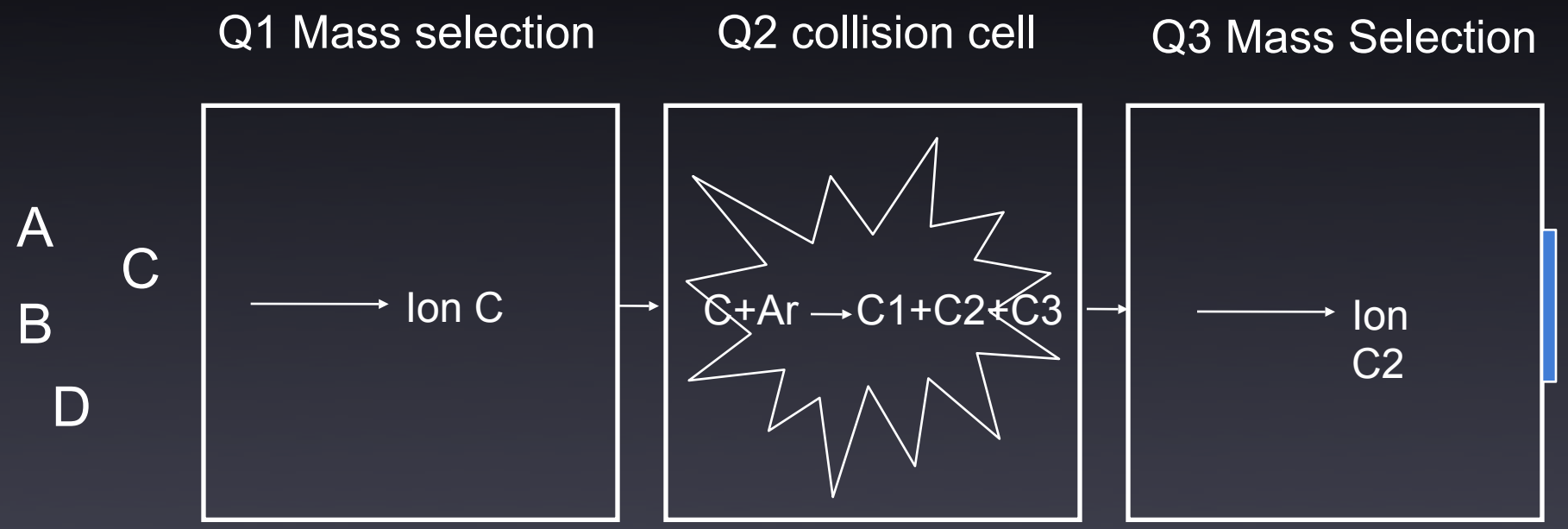
Ions in Source

Q1 only transmits Ion C

Fragment Ion C

Q3 Scans for products

Multiple Reaction Monitoring (MRM) in a Triple Quadrupole



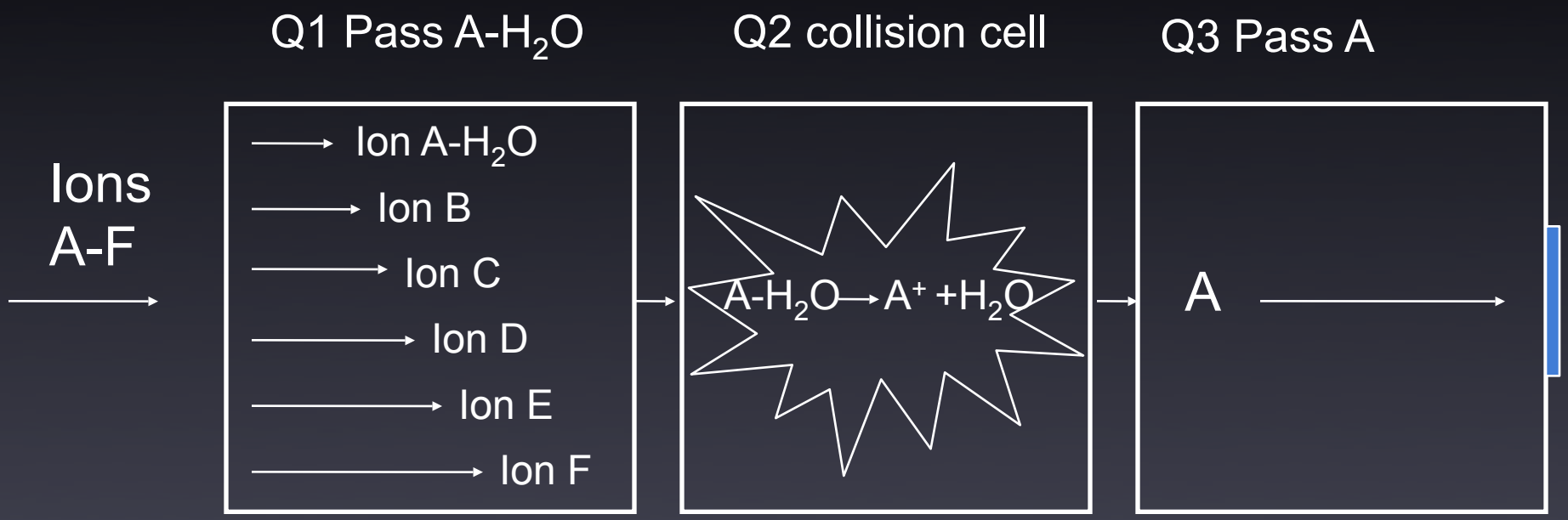
Ions in Source

Q1 only transmits Ion C

Fragment Ion C

Q3 only transmits Ion C2

Neutral Loss Scan Mode in a Triple Quadrupole

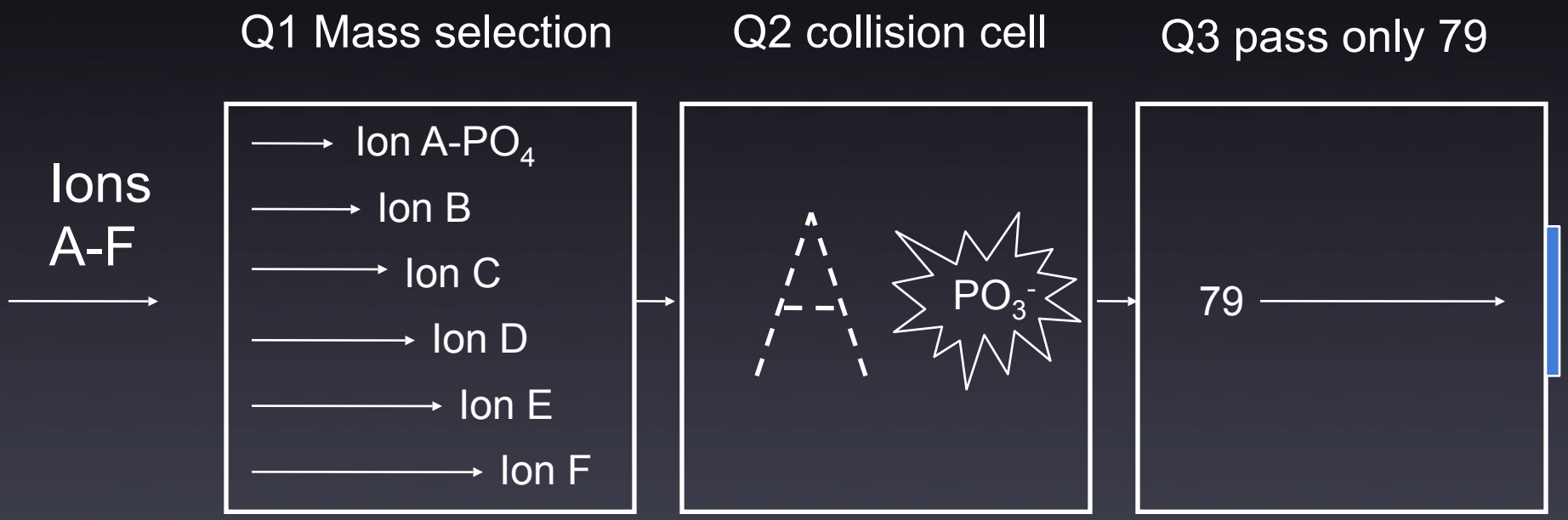


Scans across mass range

Fragment ions one at a time

Scans across mass range at 18 amu lower than Q1

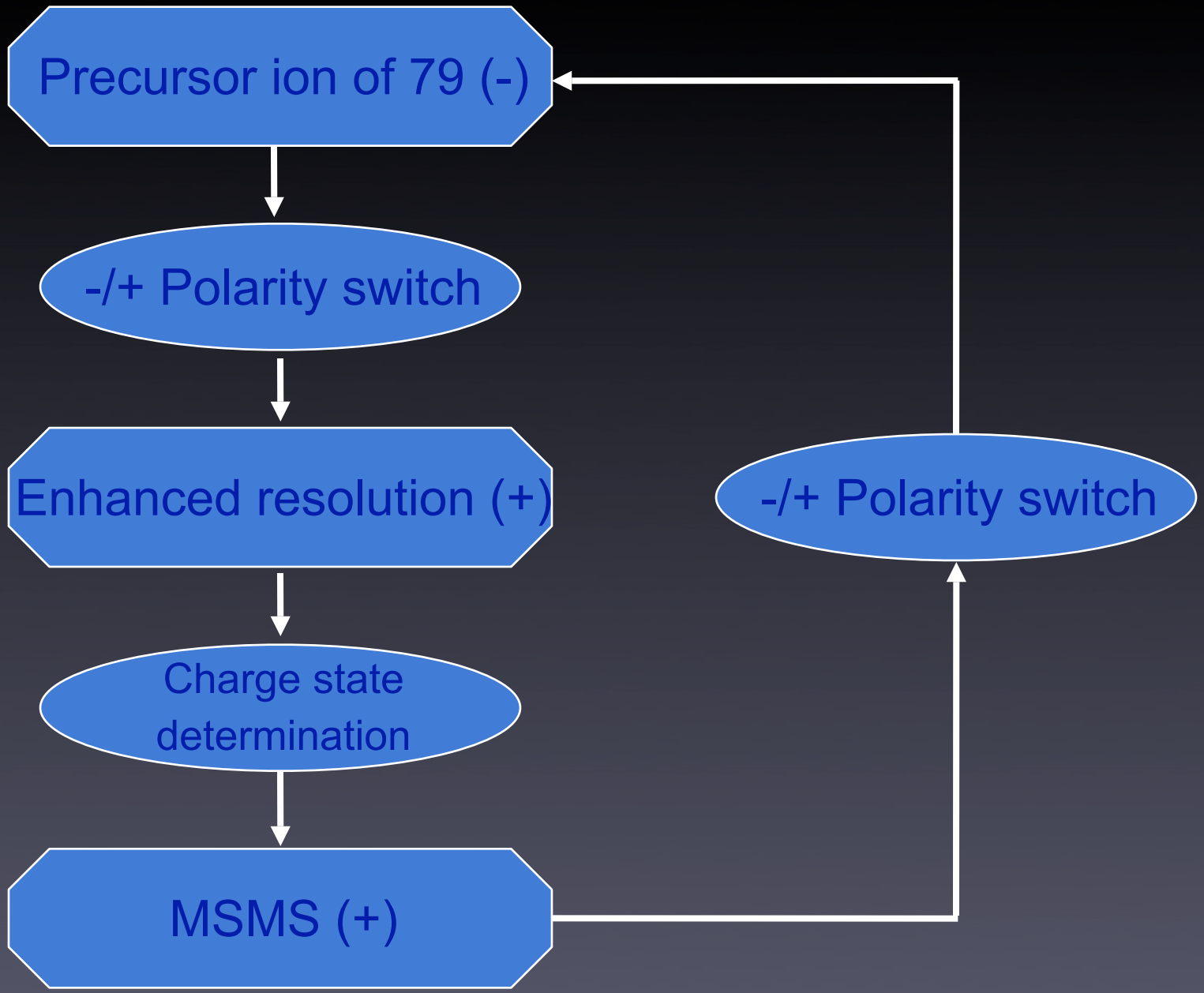
Precursor Ion Scan Mode in a Triple Quadrupole



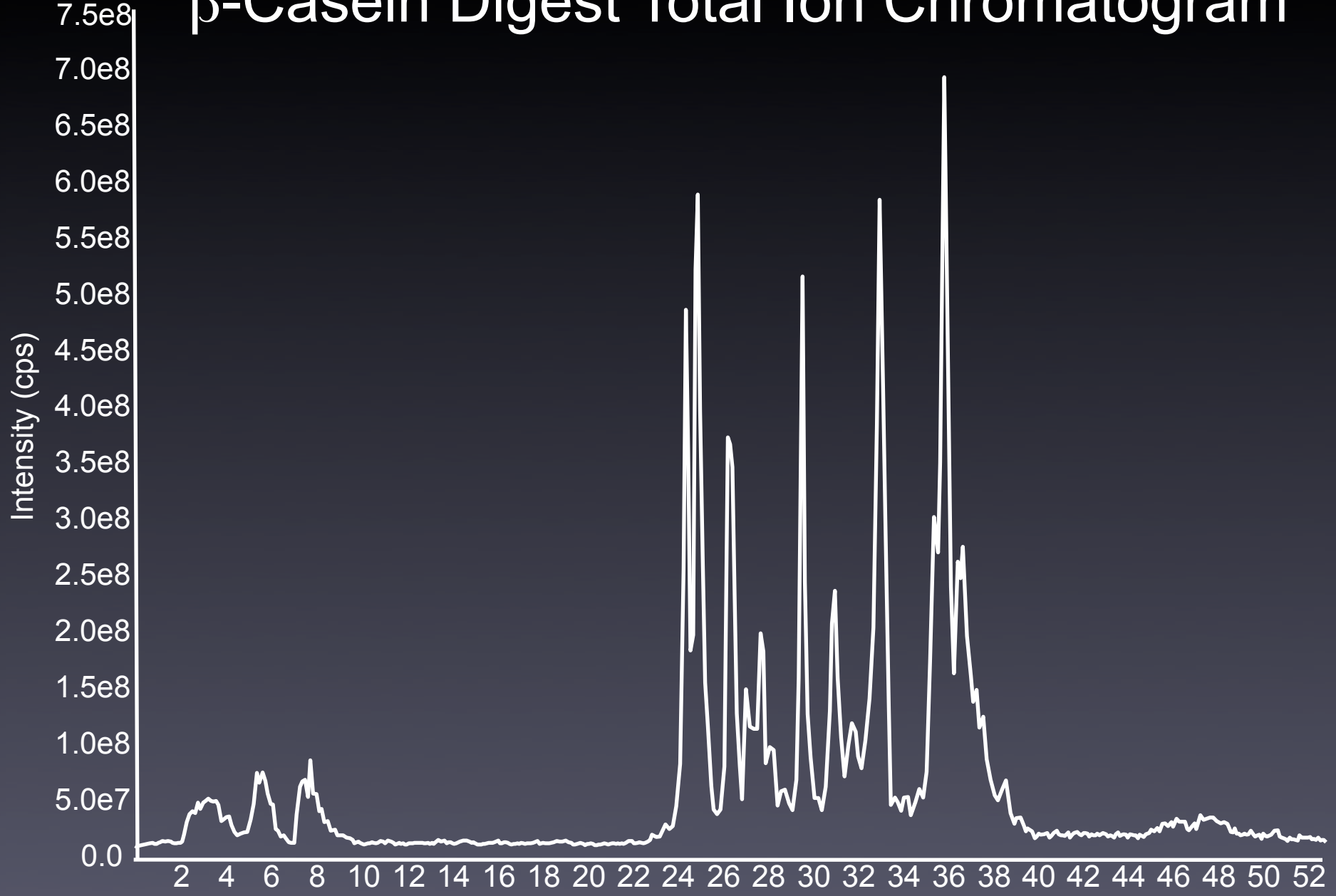
Scans across mass range

Fragment ions one at a time

Transmits only 79

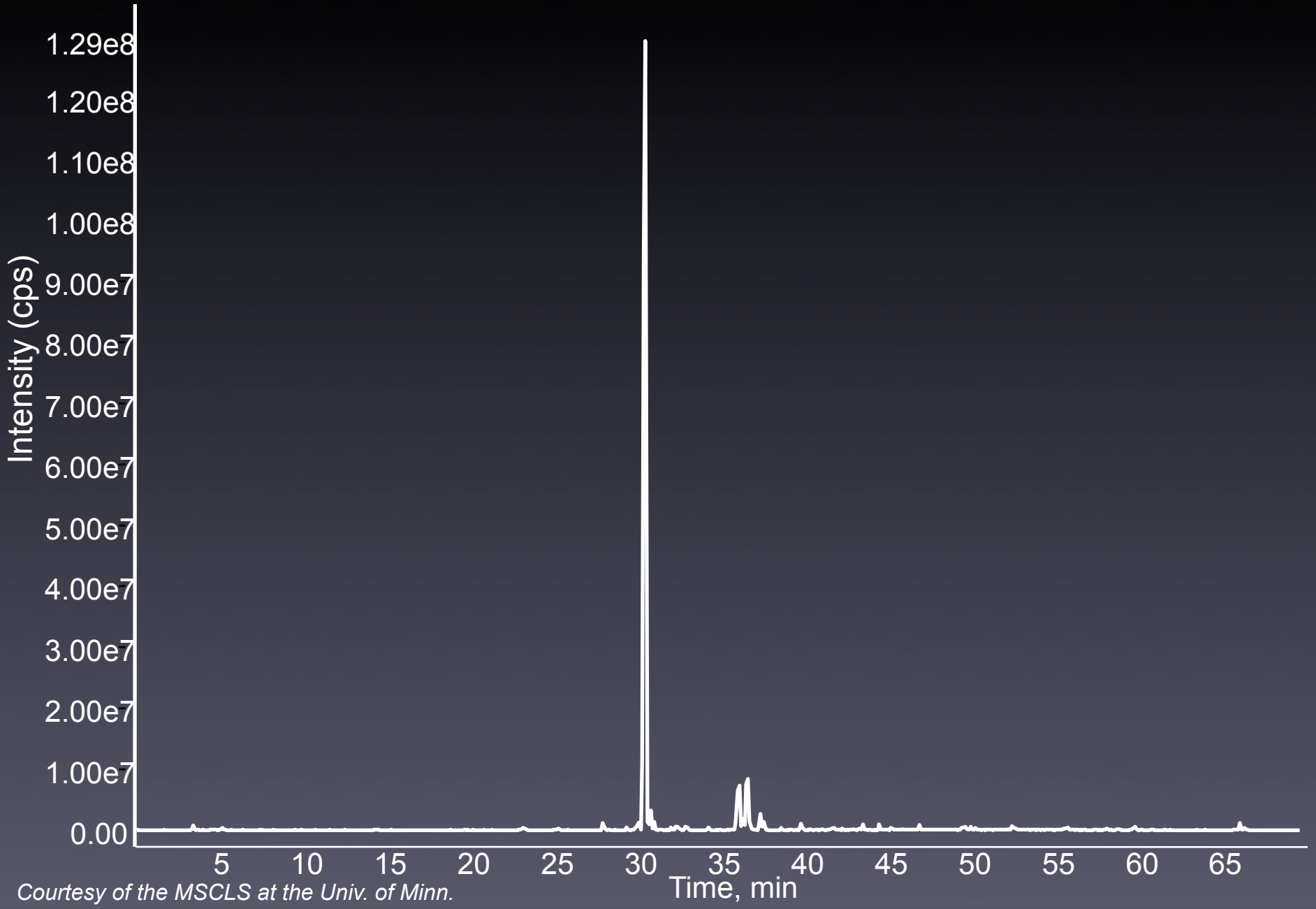

Precursor ion of 79 (-)
-/+ Polarity switch
Enhanced resolution (+)
Charge state determination
MSMS (+)
-/+ Polarity switch

β-Casein Digest Total Ion Chromatogram



Courtesy of the MSCLS at the Univ. of Minn.

Negative Precursor Ion Chromatogram



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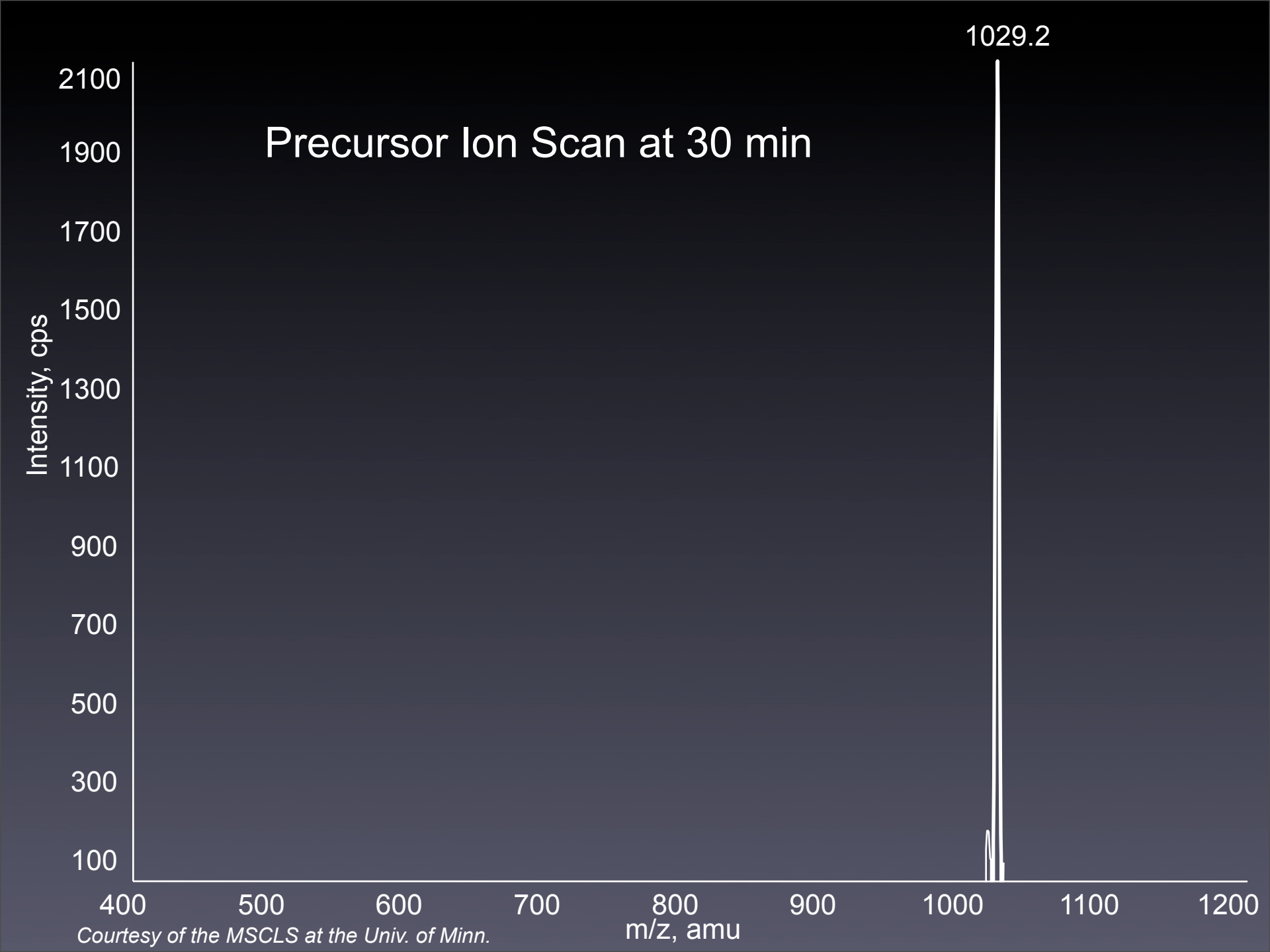
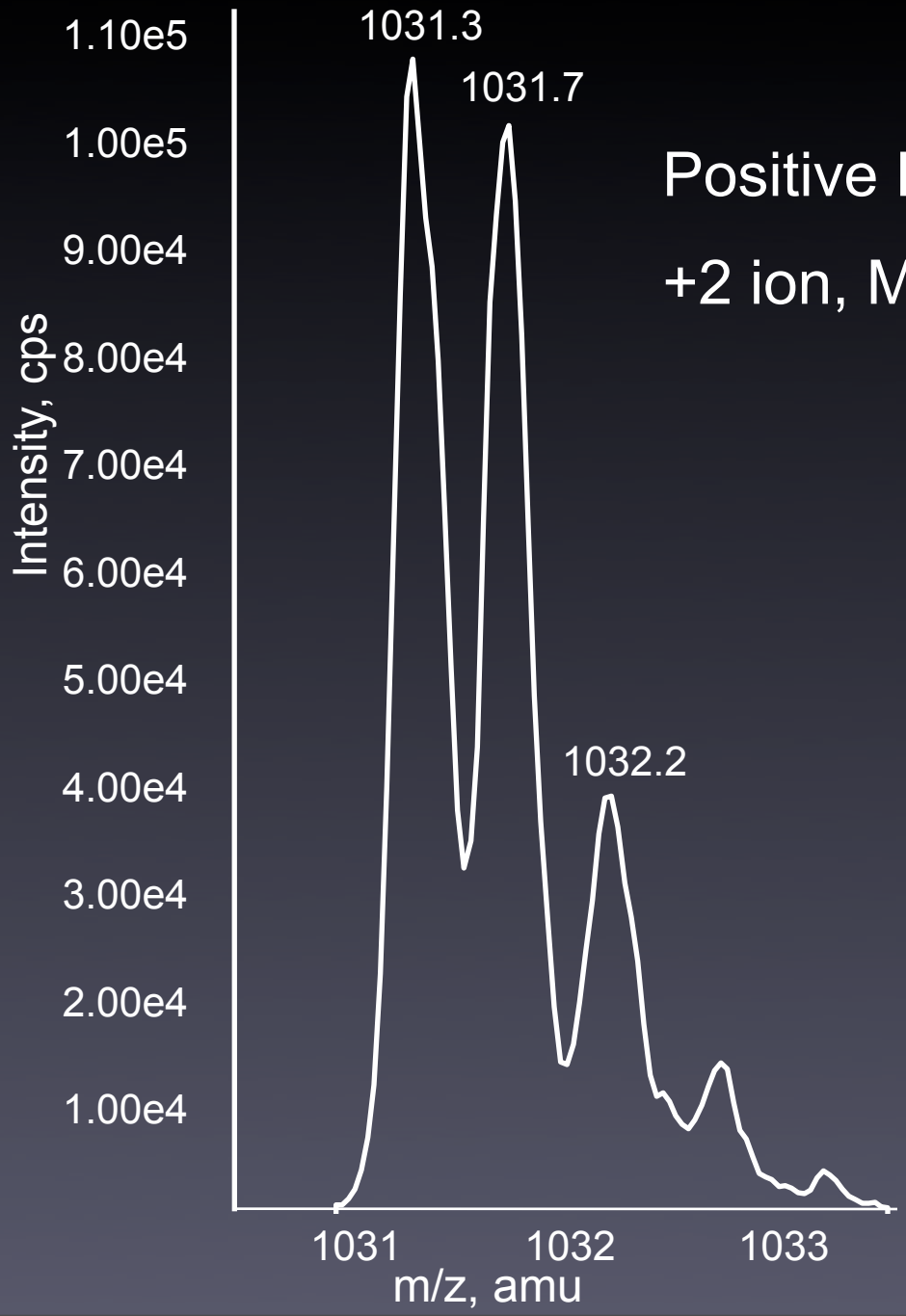

1029.2
Precursor Ion Scan at 30 min
2100
1900
1700
1500
1300
1100
900
700
500
300
100
Intensity, cps
400
500
600
700
800
900
1000
1100
1200
m/z, amu
Courtesy of the MSCLS at the Univ. of Minn.

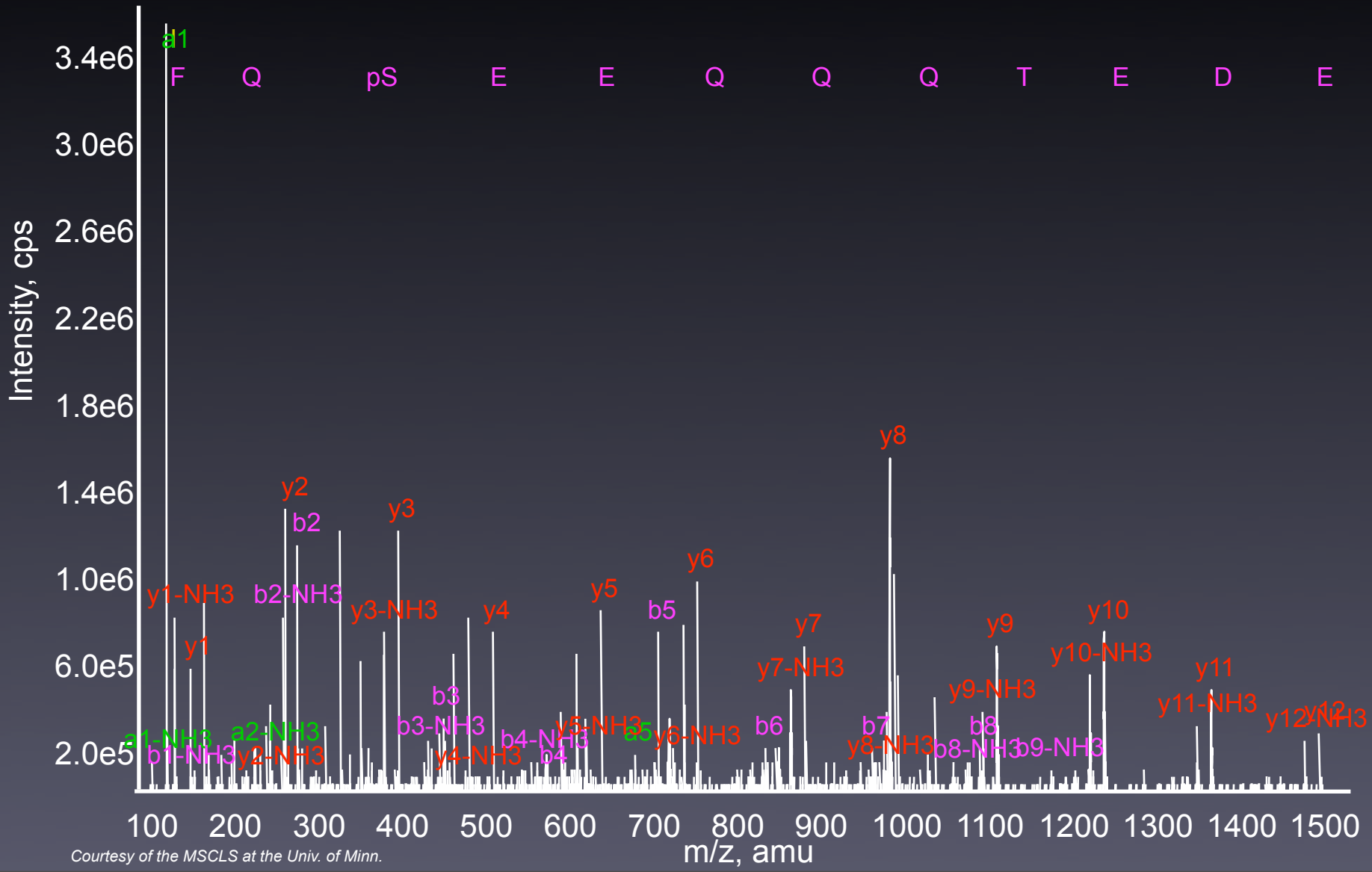


Positive Enhanced Resolution Scan

+2 ion, MW= 2060.6

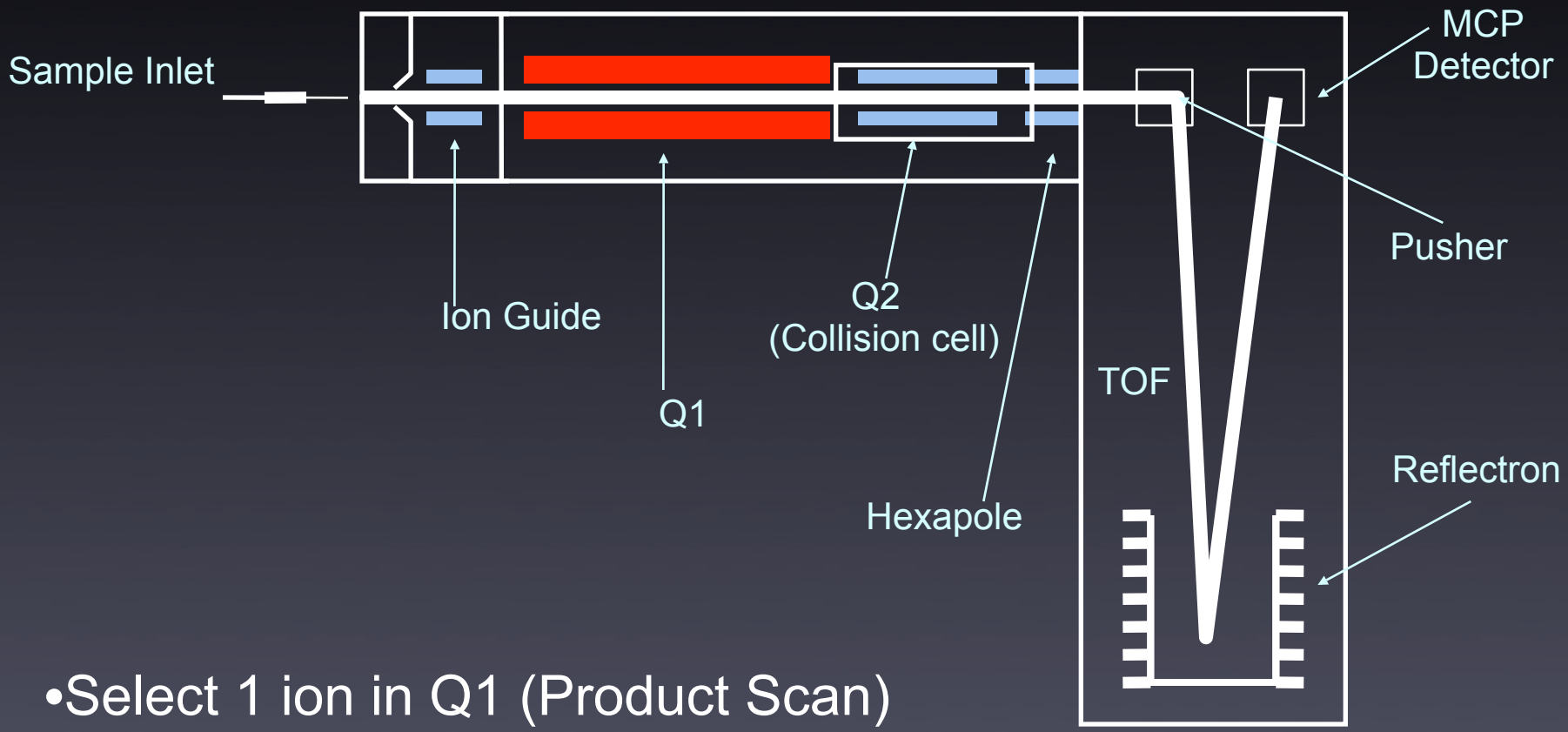
Courtesy of the MSCLS at the Univ. of Minn.

MSMS on ion 1031.3 for sequence determination



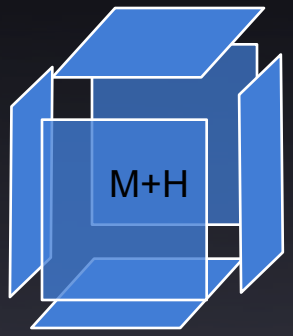
Courtesy of the MSCLS at the Univ. of Minn.

Q-TOF Mass Analyzer



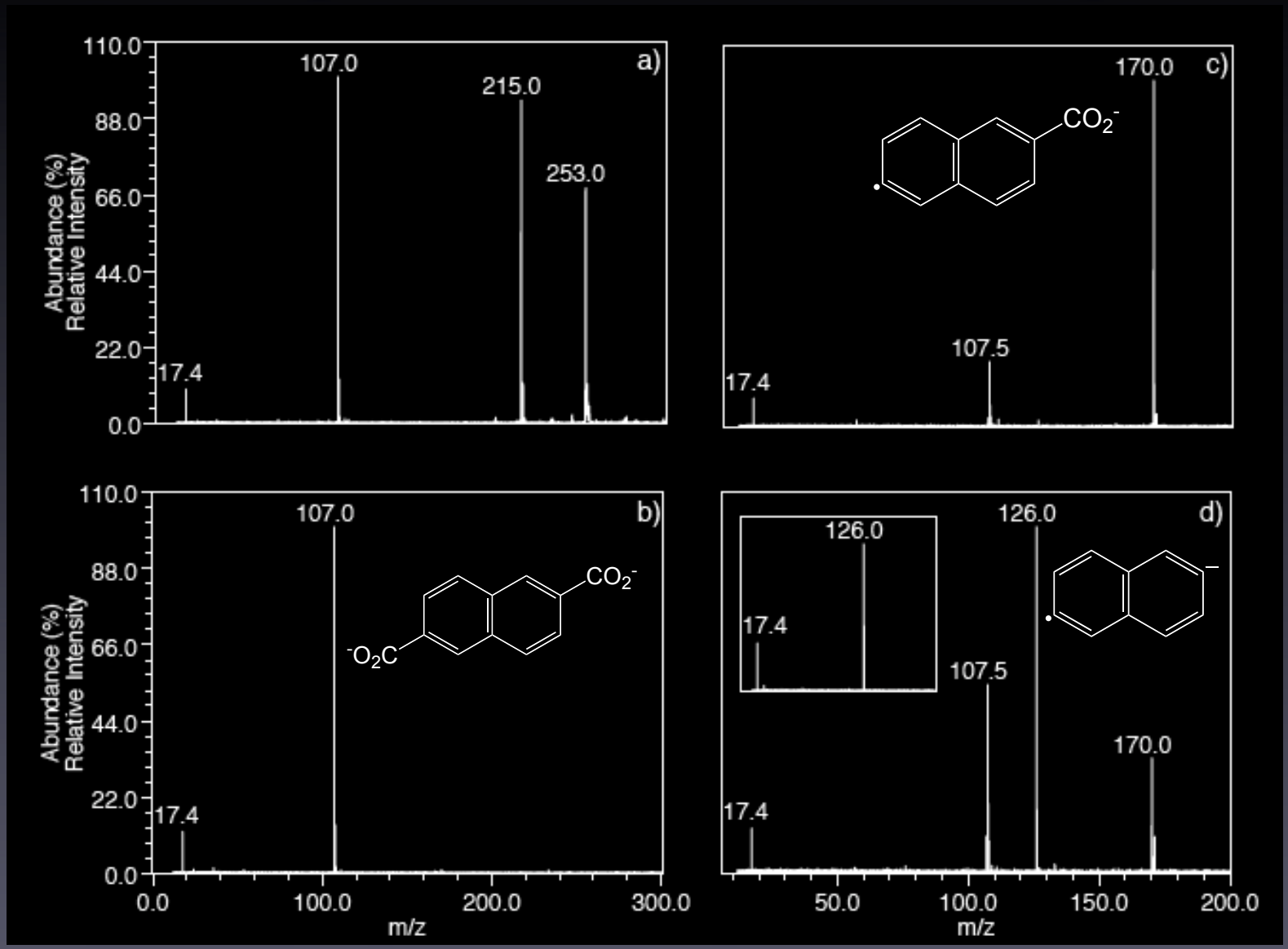
- Select 1 ion in Q1 (Product Scan)
- Scan Q1 (Precursor Scan)
- Cannot perform Neutral Loss Scan

FT-ICR-MS



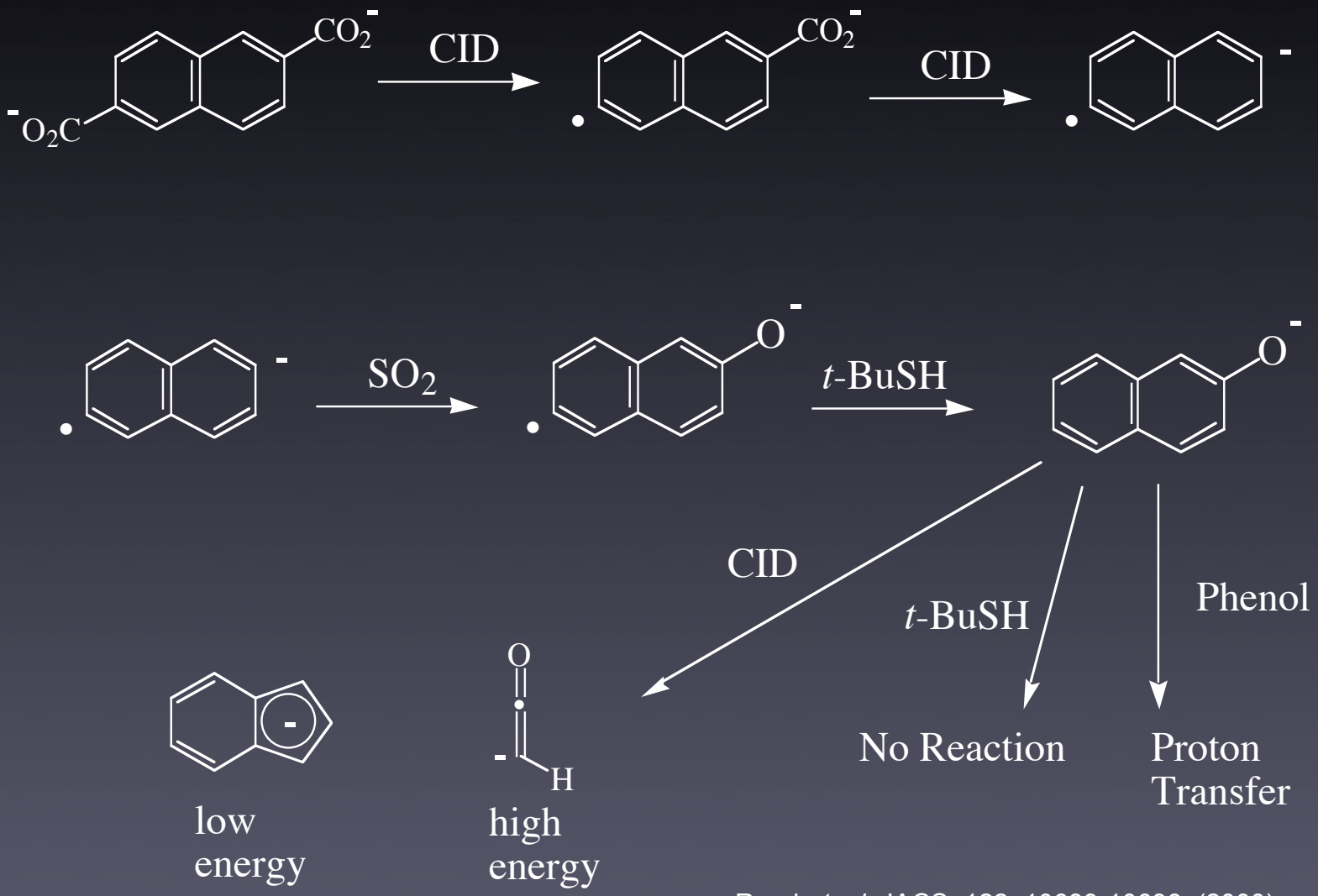
- MS/MS is done in one analyzer
  - RF fields can selectively isolate one ion
  - RF fields (on or off resonance) can accelerate ions followed by collisions and detection of products
  - IR laser or electron source can fragment isolated ions followed by detection of products
  - Processes can be mixed, matched, repeated etc.

MS^4 With FT-ICR

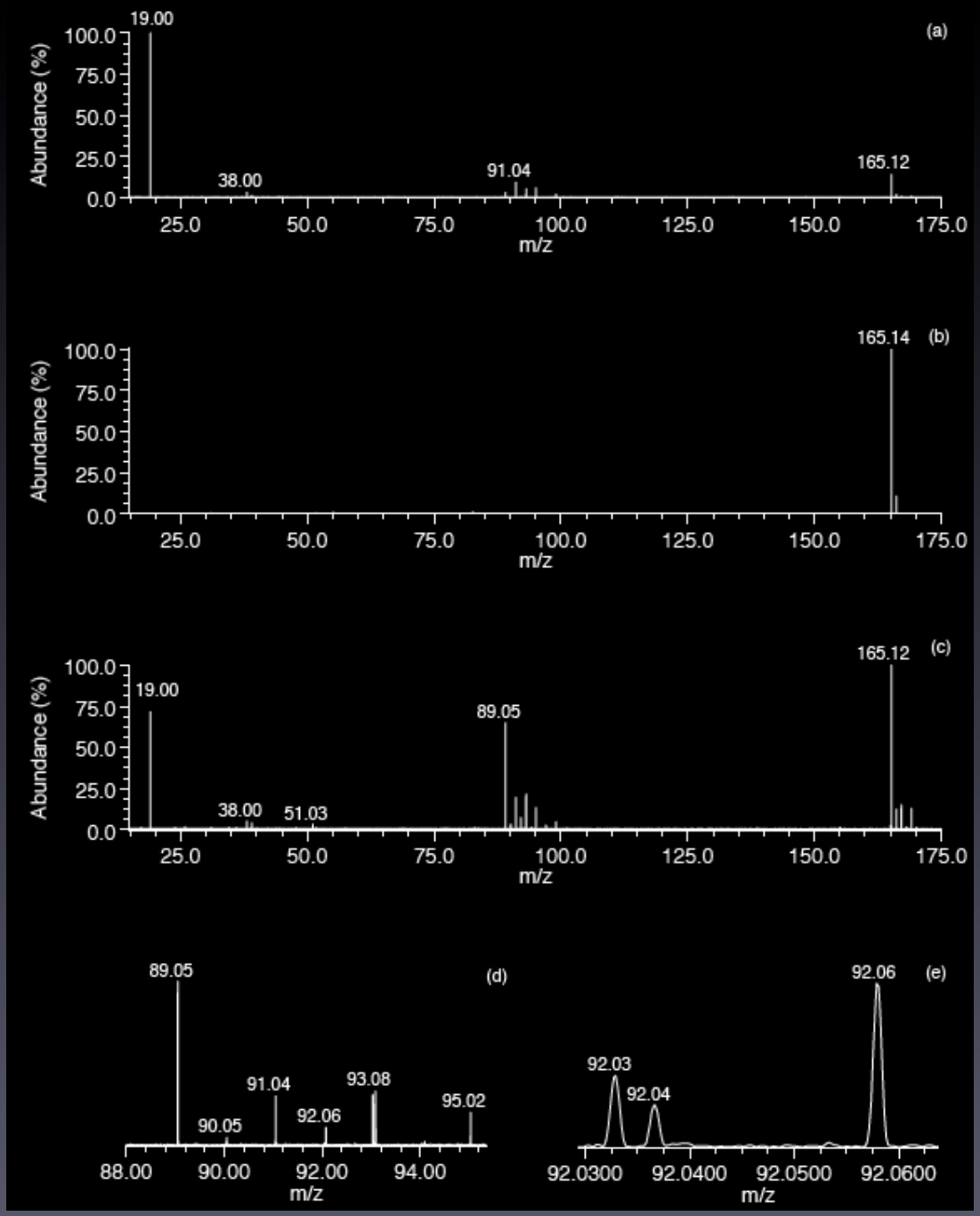


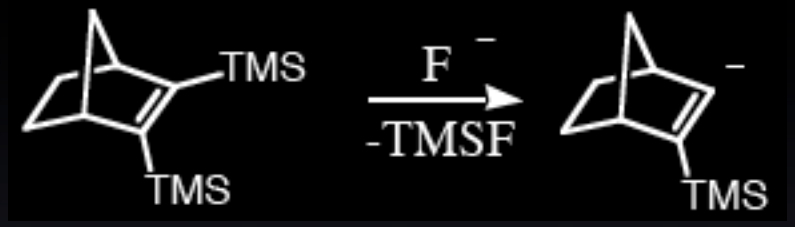
Reed *et. al.* JACS, 122, 10689-10696, (2000)

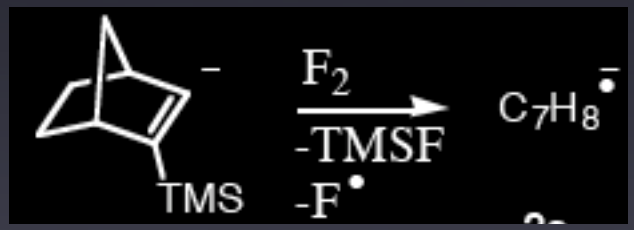
MS^6 With FT-ICR



Reed *et. al.* JACS, 122, 10689-10696, (2000)







Multiple ions at m/z 92

MS/MS Applications

- Structural elucidation aid for unknown compounds
- Functional group confirmation
- Positive Identification of known compounds
  - environmental contaminants
  - biological metabolites
  - controlled substances (illegal drugs, steroids etc.)
- When Combined with Chromatography
  - Screening of mixtures for specific moieties
  - Structure-specific quantitation
- Thermochemical information (BDE etc.)
- Chiral analysis