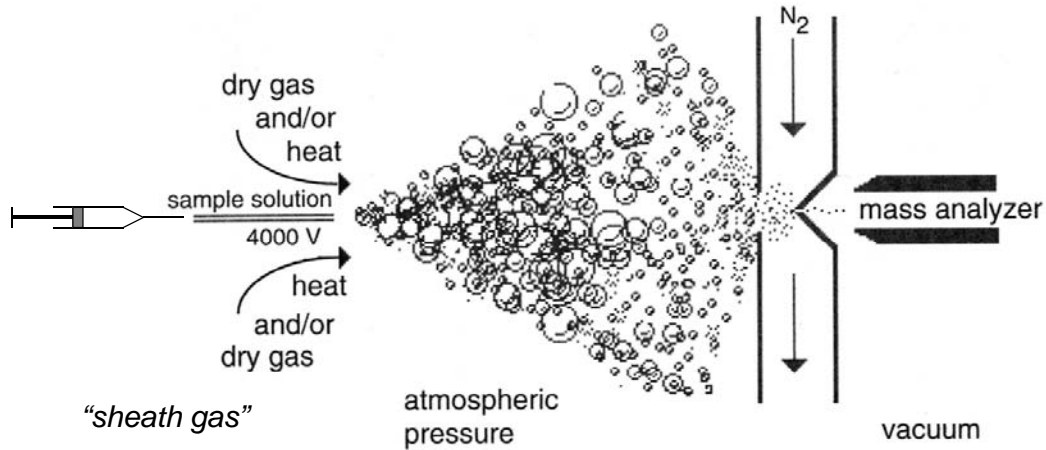
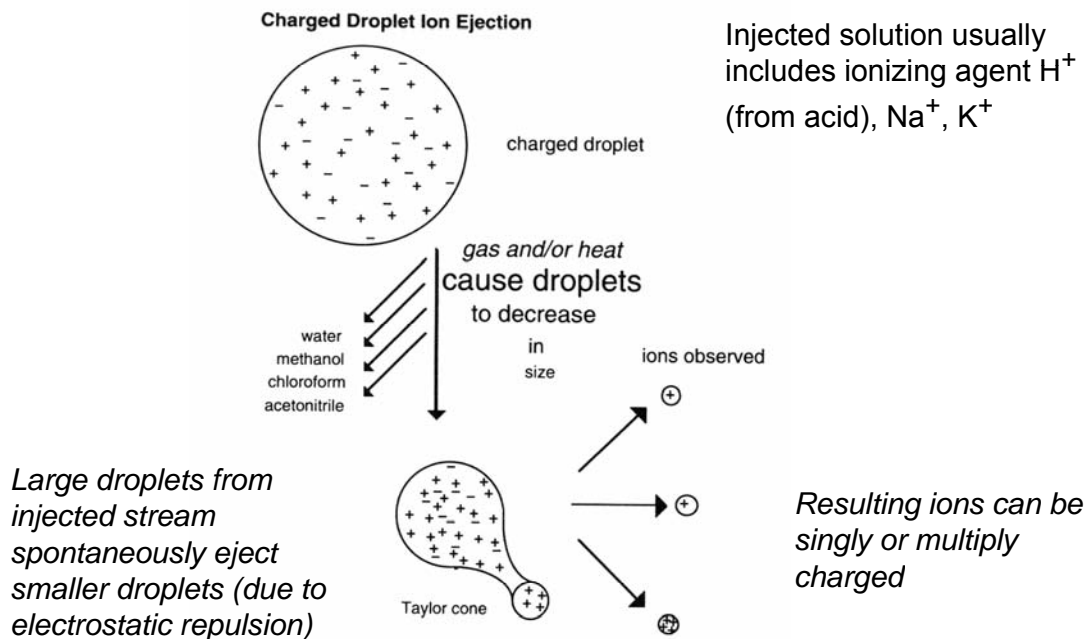


Electrospray Ionization (ESI)

- *sample solution is injected directly into instrument*
- *voltage drop between source, analyzer generates ionized droplets*
- *solvent evaporates away as droplets migrate across gap*

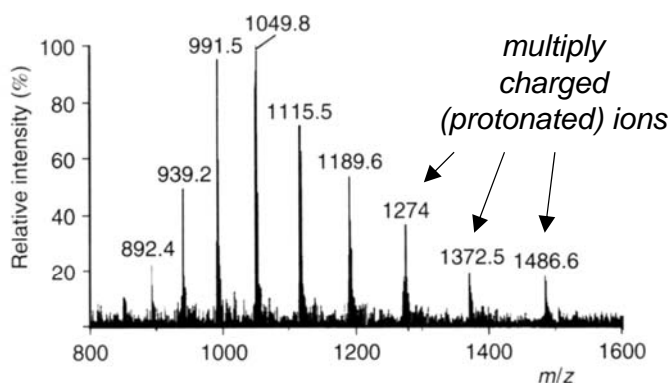
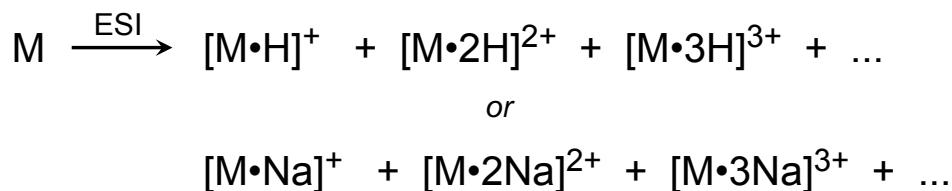


Electrospray Ionization (ESI)



Electrospray Ionization (ESI)

Multiply charged ions frequently observed.



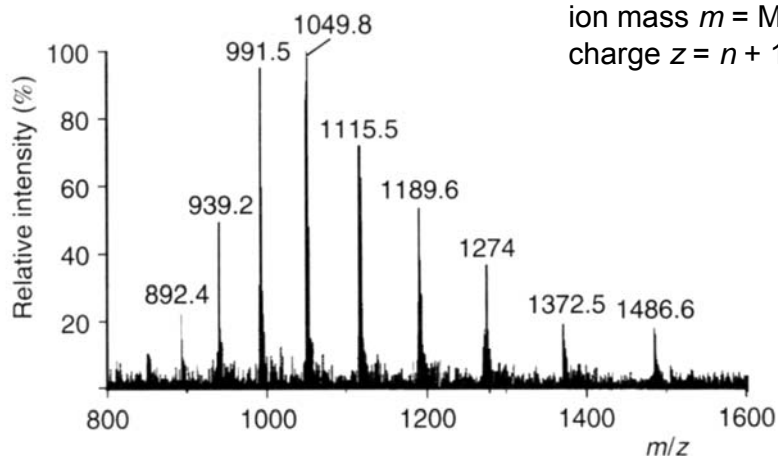
λ lysozyme:
big > 10 kDa protein

*From this series of peaks,
how do we determine mass
of protein?*

Charge Ladders in Electrospray Ionization

We know two things:

1. Any peak corresponds to $[M \cdot nH]^{n+}$;
ion mass $m = M + n$,
charge $z = n$.
2. Peak to that peak's left has one more
proton attached, one more charge;
ion mass $m = M + n + 1$,
charge $z = n + 1$.

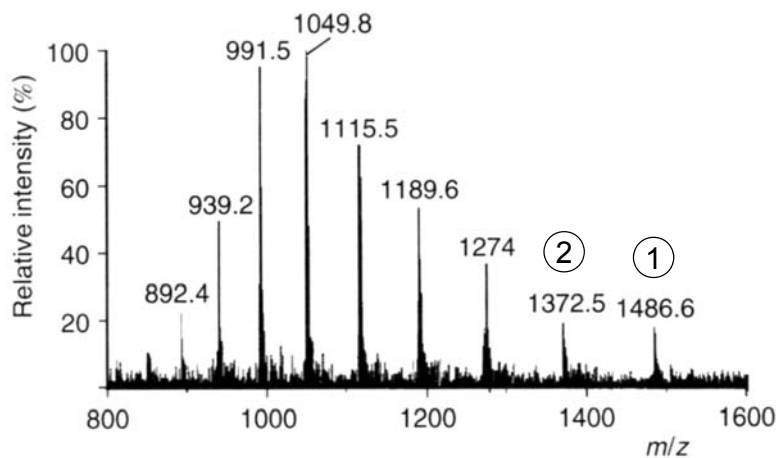


Charge Ladders in Electrospray Ionization

Set of simultaneous equations:

$$(\text{observed } m/z \#1) \cdot z = M + z(H^+)$$

$$(\text{observed } m/z \#2) \cdot (z + 1) = M + z(H^+) + 1$$



Charge Ladders in Electrospray Ionization

Now performed by computer algorithm that searches for ladders, outputs likely mass candidates.

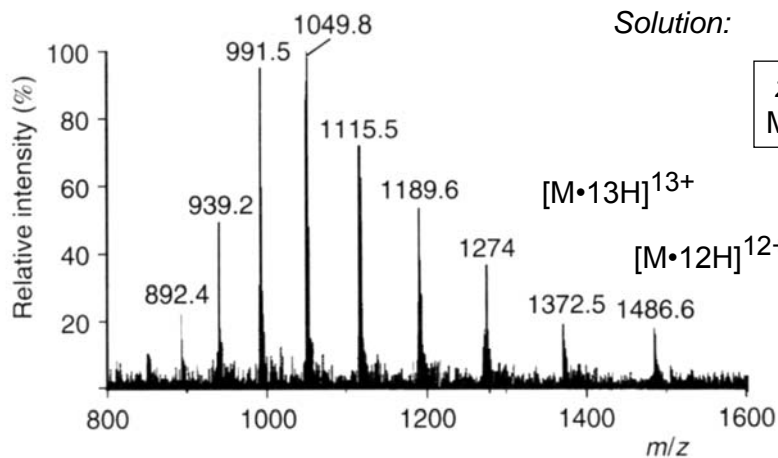
Set of simultaneous equations:

$$1486 \cdot z = M + z$$

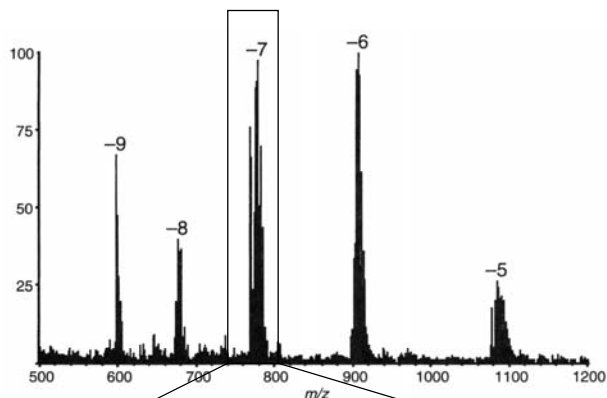
$$1372.5 \cdot (z + 1) = M + z + 1$$

Solution:

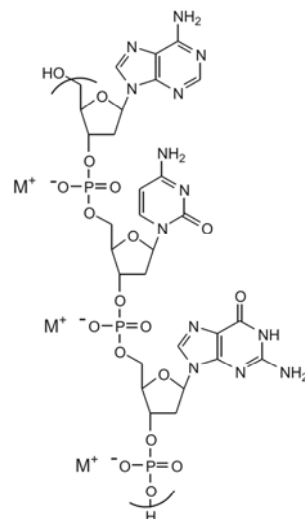
$$\begin{aligned} z &= 12 \\ M &= 17827.2 \end{aligned}$$



actual MW λ lysozyme:
17825.2

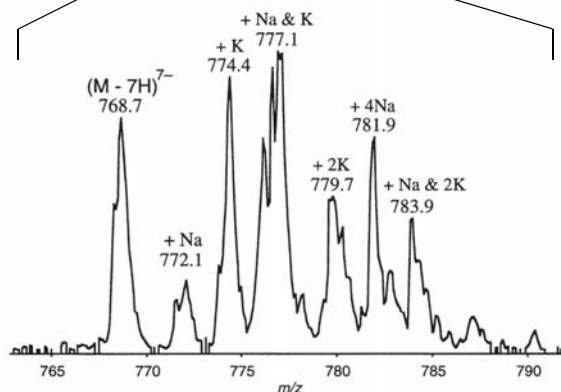


If sample contains a mixture of counterions, can get messy.



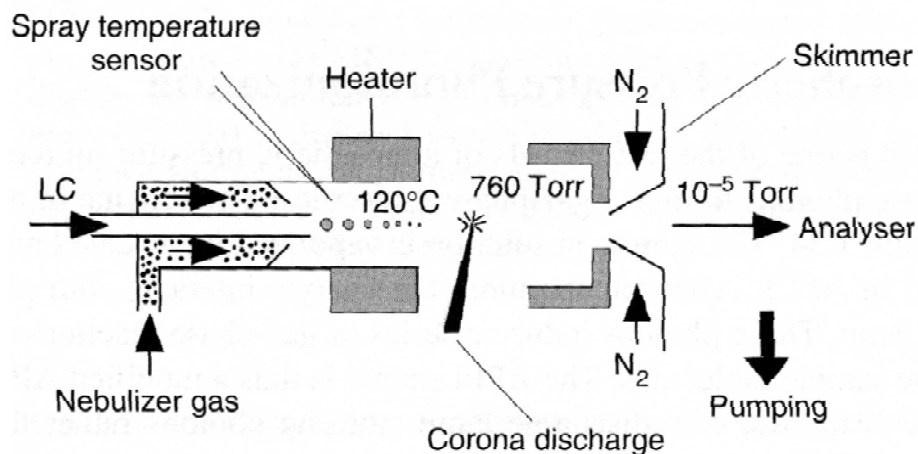
$M^+ = H^+, Na^+, K^+$

ESI-MS
22-mer single-stranded DNA
(difficult to desalt)



Atmospheric Pressure Chemical Ionization (APCI)

Analogous to CI, but uses discharge to generate N_2^{+}/O_2^{+} , which then ionizes evaporated solvent molecules. These then ionize analyte.

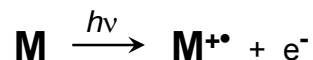


Now common for LC/MS, but appropriate for small molecules only.

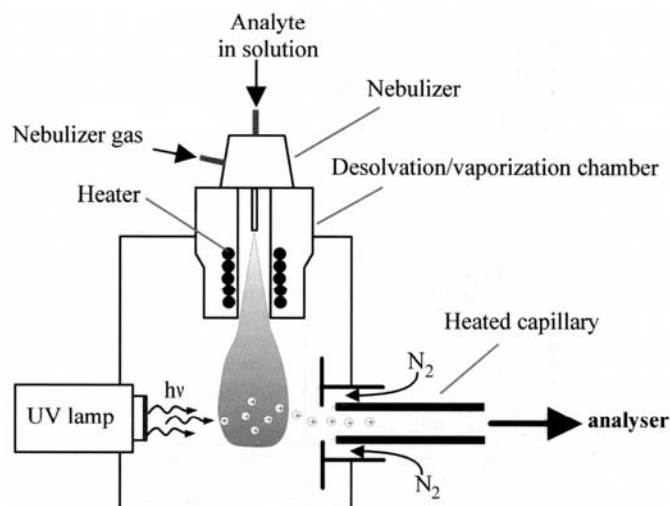
Atmospheric Pressure Photoionization (APPI)

Molecules ionized by UV lamp/laser excitation.

Direct APPI:



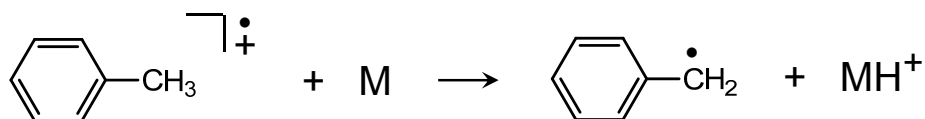
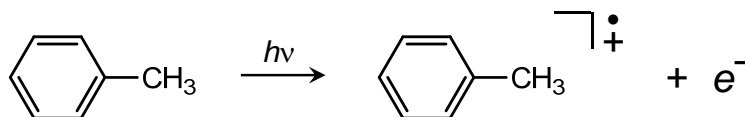
- Requires a chromophore.
- Ionization efficiency is usually low, so yield of radical cations is also low.



Indirect APPI

By using a UV-active dopant, molecules without chromophores can be chemically ionized.

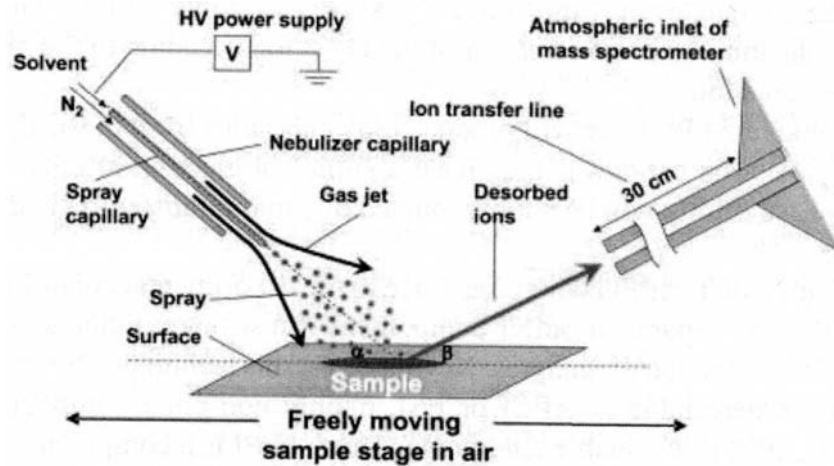
Example: Toluene ($\lambda_{\max} \approx 250 \text{ nm}$)



Result is protonation rather than electron ionization.

Desorption Electrospray Ionization (DESI)

If electrospray source is close enough to counter electrode (sample), intervening space need not be vacuum.



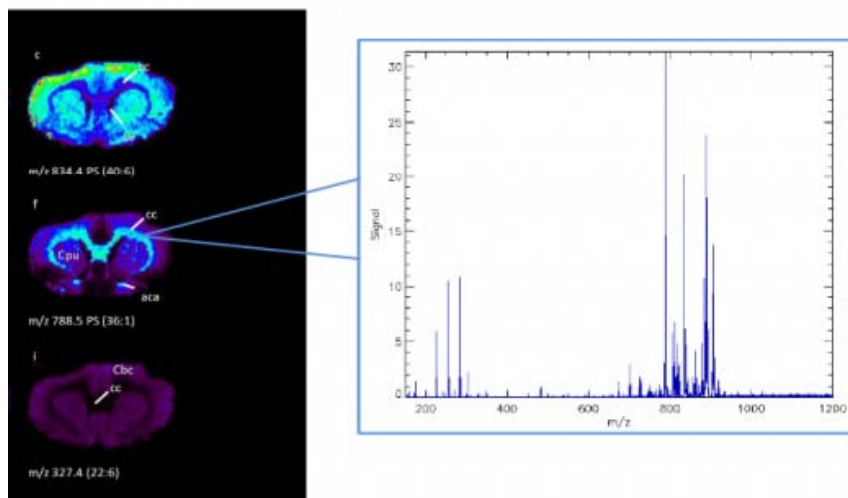
- Instrument works like a “sniffer”.
- Can be used for MS imaging, environmental sampling & analysis of biological tissue.

MS Imaging via DESI

Because source is a movable capillary, can be scanned across sample to yield a mass-based image.

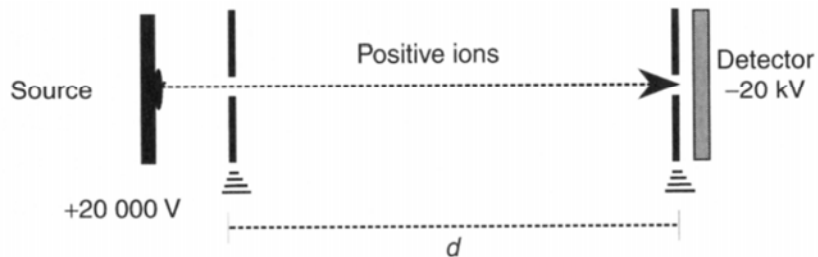
Rat brain tissue slices, imaged by scanning DESI source

Image is mass-selected intensity at each pixel.

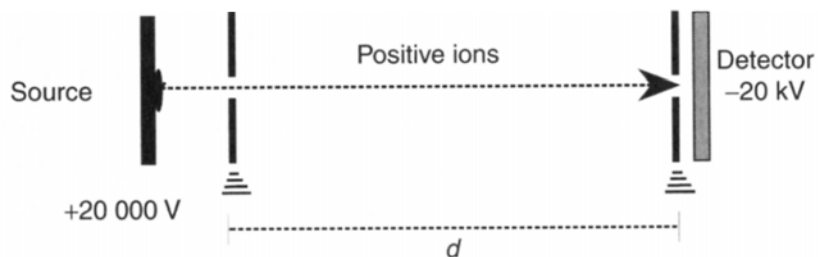


Mass Resolution Based On Time of Flight (TOF)

- Based on simple principle of ions flying through space, accelerating under the influence of an applied electrostatic force.
- Force is the same on all ions, so heavier ions fly slower than light ones.



Mass Resolution Based On Time of Flight (TOF)



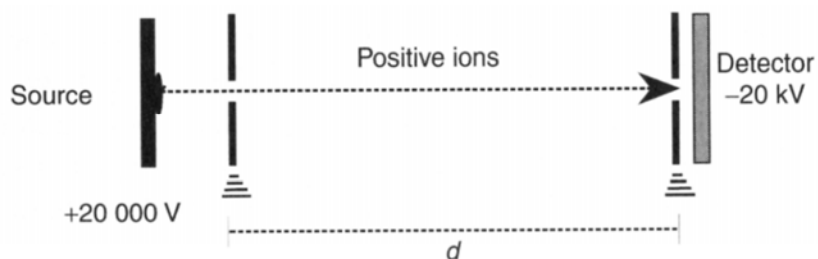
$$E_e (\text{ion}) = qV_S = zeV_S$$

$$E_k (\text{ion}) = \frac{mv^2}{2}$$

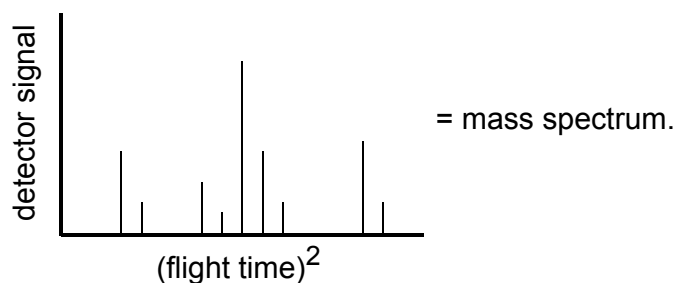
$$E_e (\text{ion}) = E_k (\text{ion}), \text{ so}$$

$$v^2 = \frac{2zeV_S}{m} \quad t^2 = \frac{d^2}{v^2} = \frac{m}{z} \left(\frac{d^2}{2eV_S} \right)$$

Mass Resolution Based On Time of Flight (TOF)

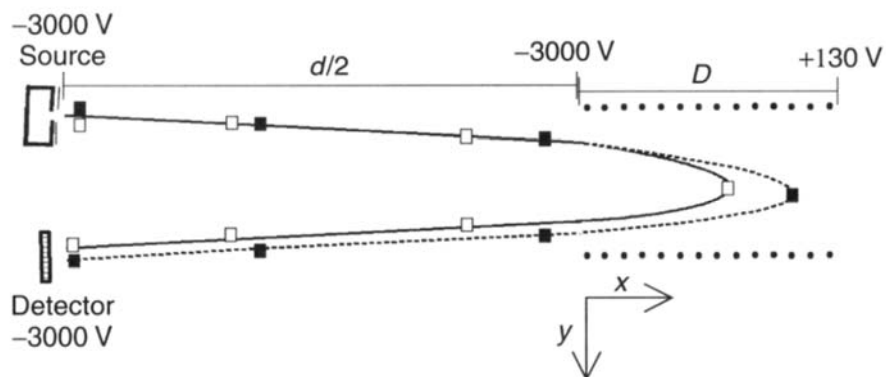


$$t^2 = \frac{m}{z} \left(\frac{d^2}{2eV_s} \right)$$



TOF Focusing by Reflectron

Corrects for kinetic energy dispersion at source.



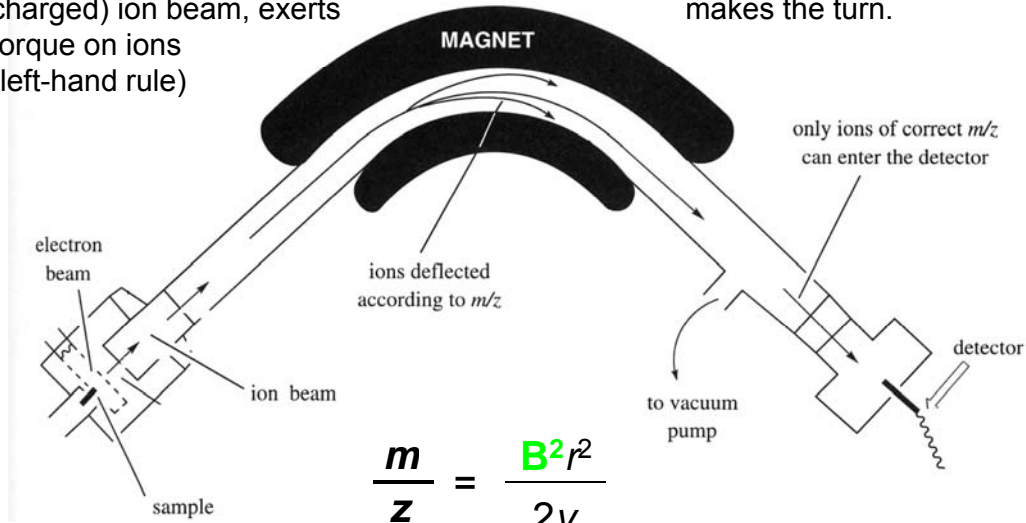
□ & ■ : Same mass, different initial velocity

Mass Resolution: Magnetic Sector Analyzers

Magnetic field, applied perpendicular to (positively charged) ion beam, exerts torque on ions (left-hand rule)



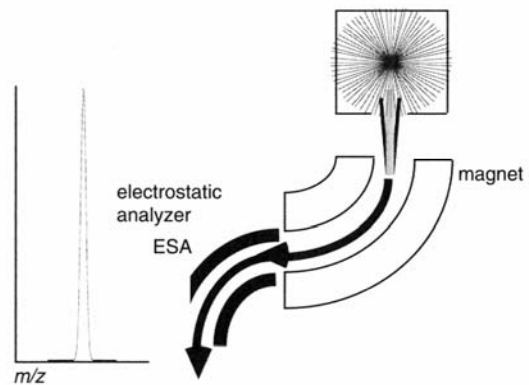
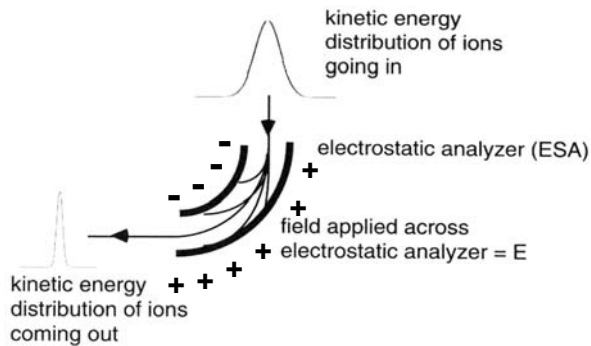
Magnetic field strength set such that only one mass makes the turn.



Scan **B** to obtain mass spectrum.

Mass Resolution: Double-Focusing Analyzers

Resolution improved by adding Electrostatic Analyzer



Double-Focusing Magnetic Sector-Electrostatic Analyzer