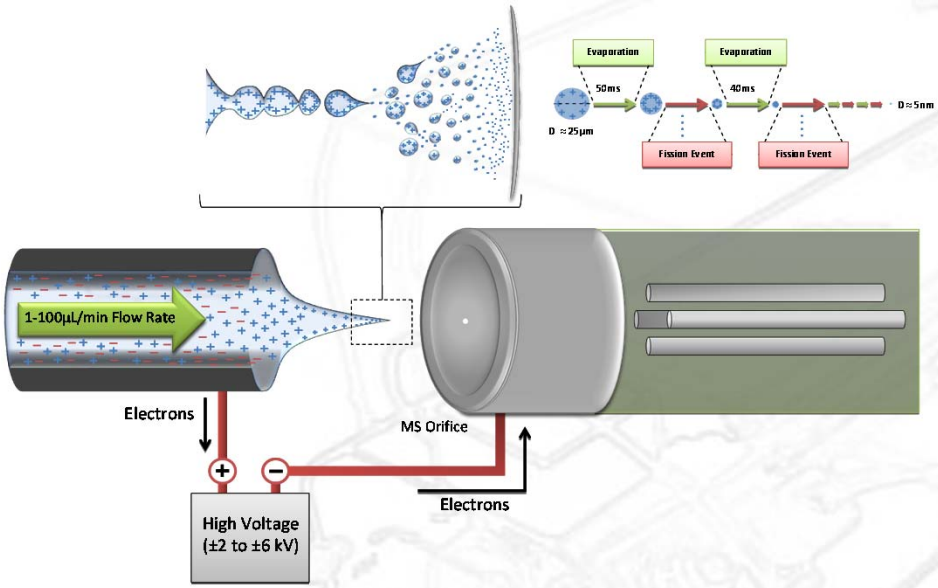
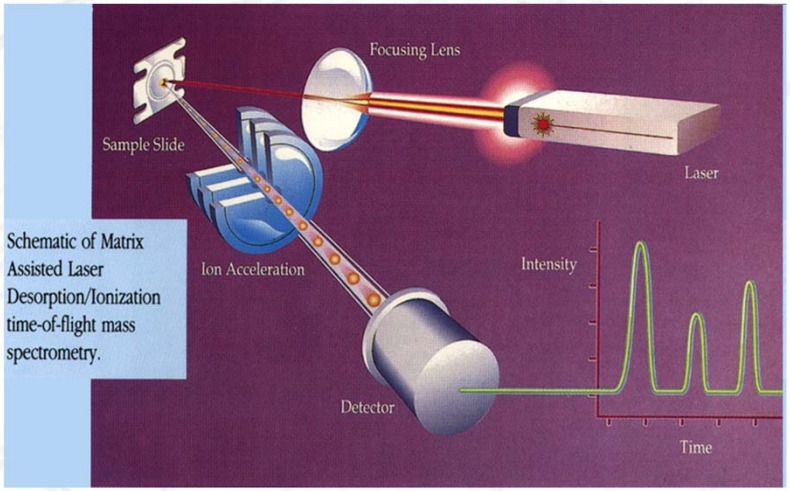
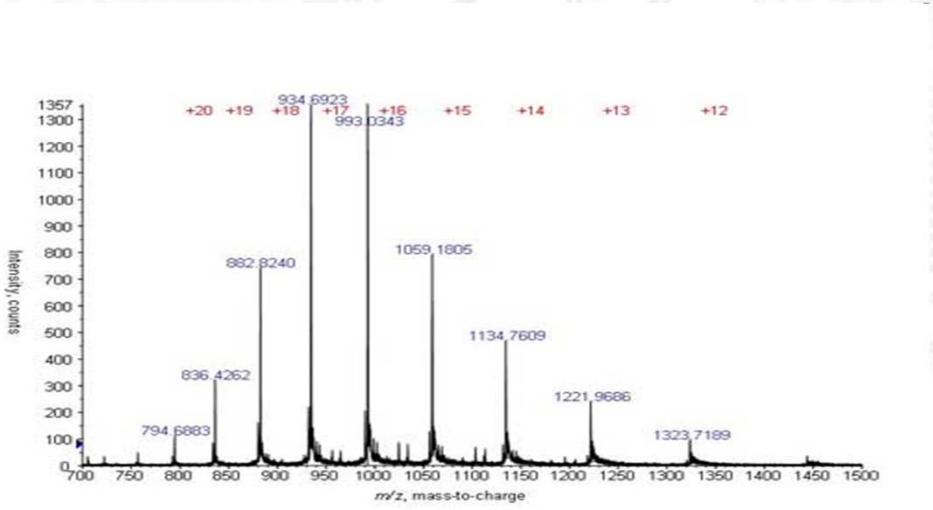
Week 2: Soft Ionization

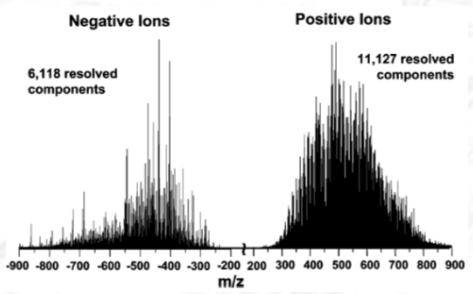


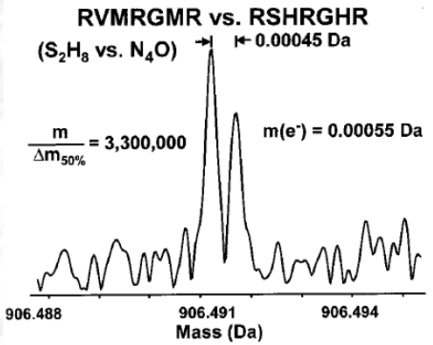




Last Time…

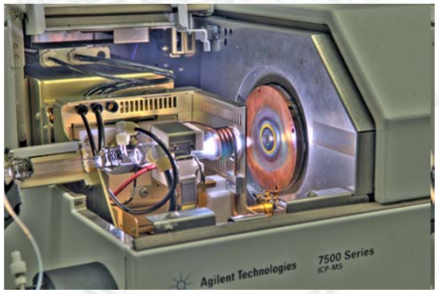
- Mass Spectrometry is super-cool:

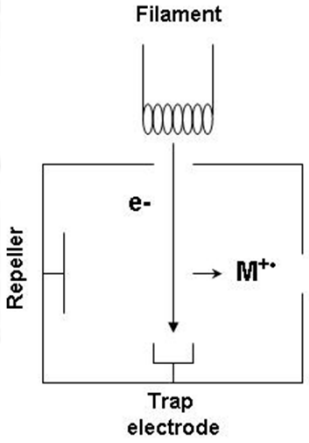




- A Brief History of MS:

- Ionization:





This time…

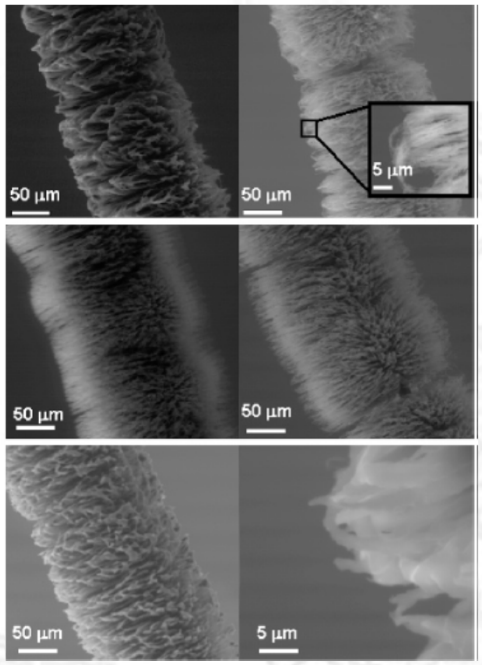
- So far, the ionization techniques we've talked about are:
  - At least somewhat hard; they tend to make ions through fragmentation/dissociation
  - Generally not great at transferring large analytes into the gas phase (at least not intact – see point I above).

- The salient feature of soft ionization is that analytes (even large ones) are transferred intact into the gas phase. This includes:
  - Large Polymers
  - Nucleic Acids and non-covalent complexes thereof
  - Proteins and non-covalent complexes thereof

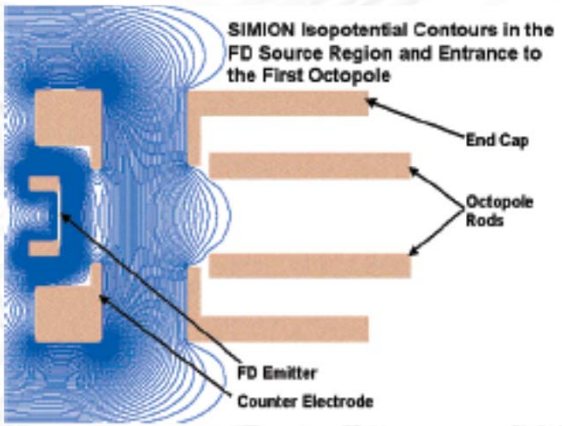
Field Ionization / Field Desorption

- Possibly the first 'soft' ionization method was 'Field Ionization' or 'Field Desorption'

- Involves desorption of ions from a surface in a very high electric field.



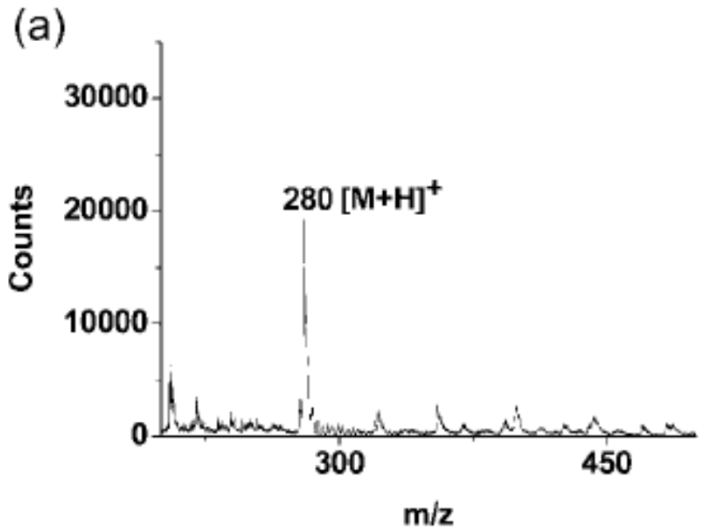
Anal. Chem. **2005,** 77, 1317-1324



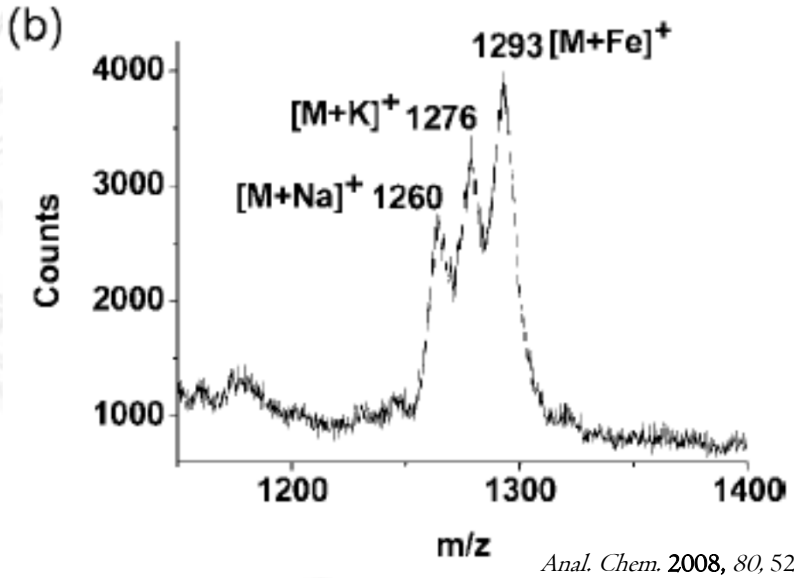
- At tips of metal fibers, exceedingly strong electric field causes electrons to tunnel into metal leaving a positively-charged ion

Field Dissociation Ionization

- Big advantage of FDI: Virtually no fragmentation



- Big disadvantage of FDI: Large molecules are hard to ionize and poorly desolvated



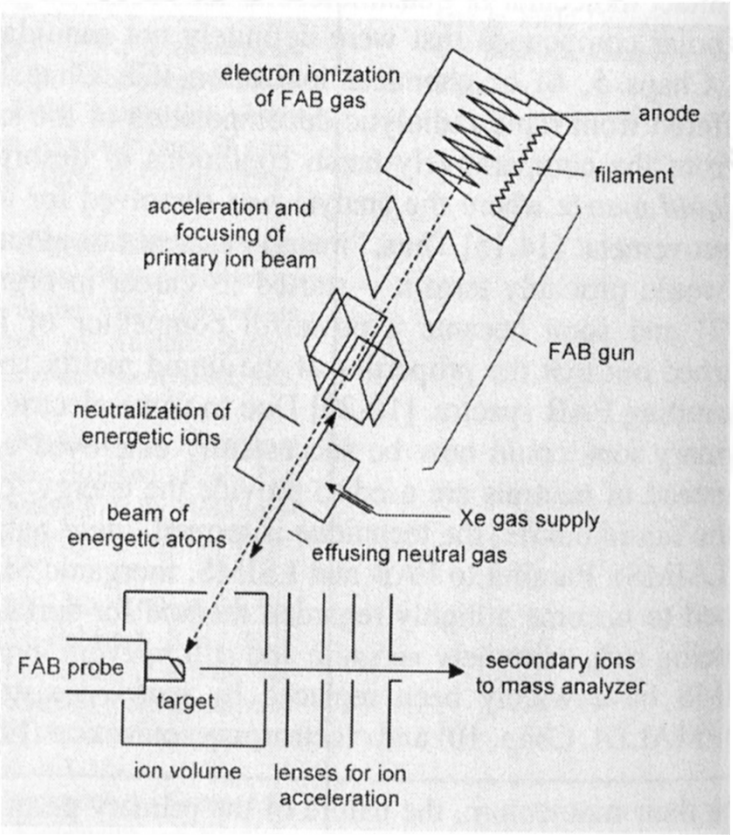
Anal. Chem. 2008, *80,* 5203–5210

Fast Atom Bombardment (FAB)

- FAB is rather like EI, except instead of electrons at tens of eV, we're going to use fast-moving, neutral atoms at tens of keV

- To get our 'fast atoms' (usually Xe) moving fast, we first ionize the gas, then we can accelerate using an electric field

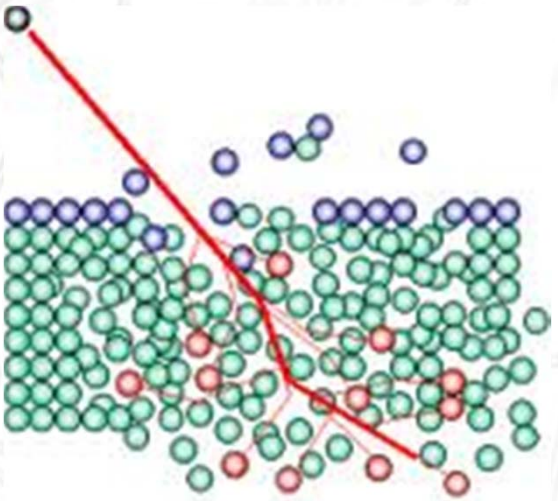
- The ionized gas is made neutral again by charge transfer to a neutral gas (like Xe). For fast particles, charge is lost more efficiently than kinetic energy in these types of collisions.



Fast Atom Bombardment Con't

- Why neutral ions?
  - Because impact of charged atoms (e.g. molecular beam solid analysis) caused electrostatic charging of the surface over time…
- Impaction of high energy neutrals causes ejection of neutrals and secondary ions *via* ablation.

- Impaction of high energy neutrals causes ejection of neutrals and secondary ions *via* ablation.

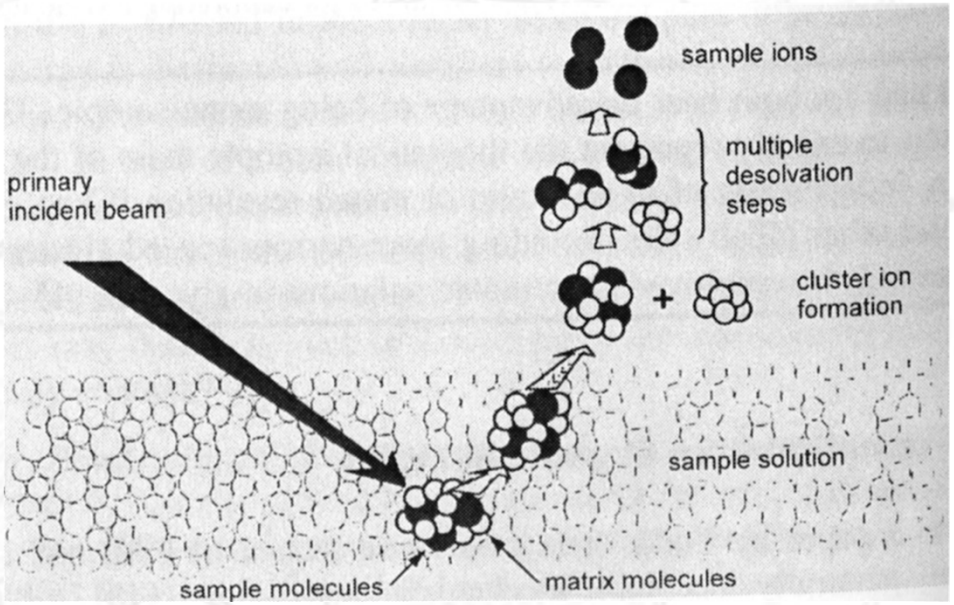


Schematic of the collision cascade

Fast Atom Bombardment Ionization

- Chemical Ionization model:

- Ions are generated via proton transfer reactions with liquid matrix



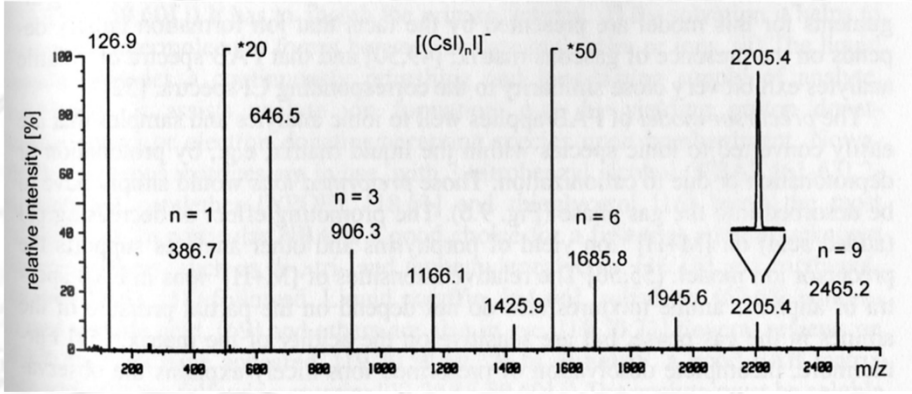
- Preformed ions model: Ions in solution (or solid) are ejected as charged species and desorbed into the gas phase with thermal assisyance.

- Ions of correct charge type are accelerated towards MS, ions of incorrect charge type are pushed away…

Fast Atom Bombardment Matrices

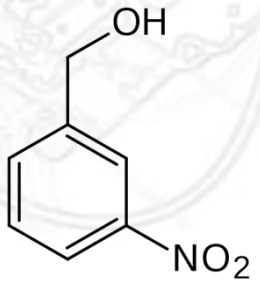
- Salt solids, such as CsI form ionized clusters covering a wide mass range... good for mass calibration.

- FAB spectrum of CsI in negative ion mode.

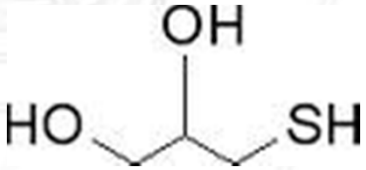


- Matrices for FAB should be: i) good at solvating analyte (alcohol), ii) good at proton transfer (alcohol), iii) good at absorbing primary energy

3-nitrobenzyl alcohol



thioglycerol

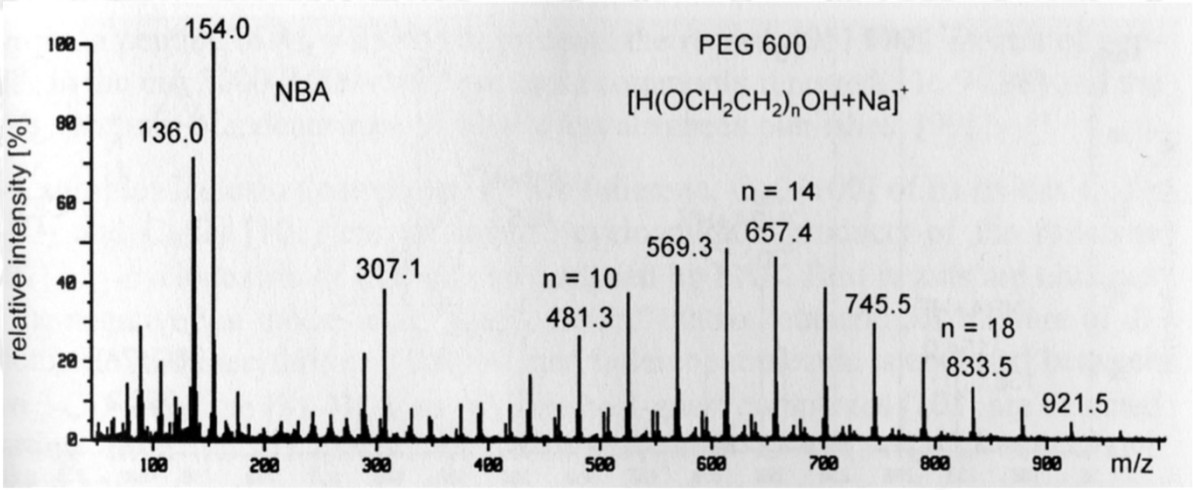


FAB Mass Spectra

- FAB mass spectra are noisy! This is because of: i) unwanted reactions with matrix, ii) radiolytic decay.

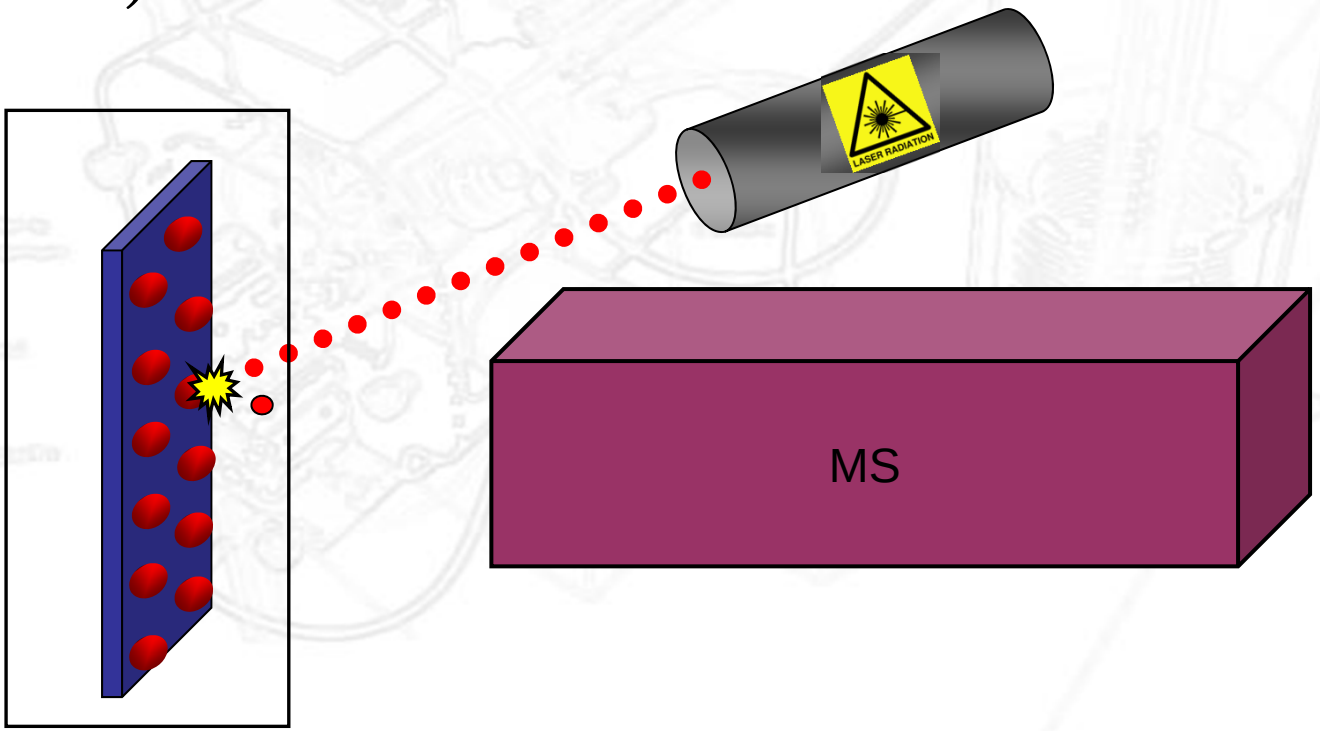
- FAB is quite gentle, generally allowing intact ions up to around 7,000 m/z. Protein ionization (trypsin) at ~23,000 has been demonstrated. Record = CsI cluster at ~ 90,000 m/z.

- Low m/z oftern dominated by matrix clusters; $(Ma_n+H)^+$ or $(Ma_n-H)^-$



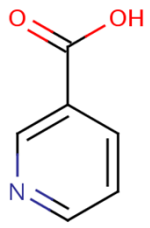
Matrix-Assisted Laser Desorption (MALDI)

- This is the first of the shiny new soft ionization techniques that revolutionized MS as a bioanalytical tool
- Similar to FAB in many respects, except instead of a high energy beam of neutral particles, we use a laser, usually N_2 (λ = 337 nm).
- Two flavors of MALDI: UV (N_2, 3 – 10 ns pulse), IR (TEA-CO_2, 100 ns pulse)

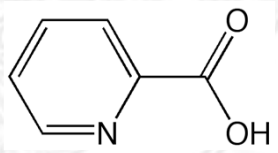


Matrices for MALDI

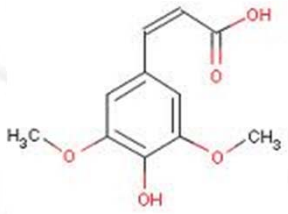
- The effectiveness of MALDI is strongly dependent on the matrix used (Tanaka won nobel for pretty much that alone!)
- Matrices must be:
  - Good at absorbing photons of laser wavelength
  - Efficient at converting absorbed laser energy into heat
  - Potential for charge transfer reactions either from neutral, excited neutral or ionized state
- Result? Matrices for UV-MALDI are often aromatic organic acids:



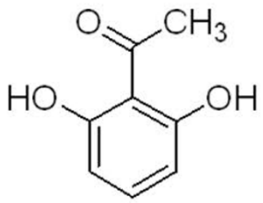
nicotinic (nucleotides)



picolinic (nucleotides)



3,5-dimethoxy-4-hydroxycinnamic (proteins)

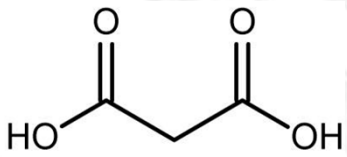


2,6-dihydroxyacetophenone (proteins, phosphopeptides)

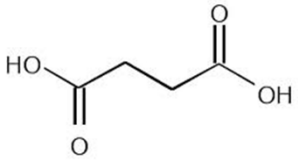
MALDI at Long Wavelengths

- It is also possible (though less common) to use IR lasers (Er:YaG = 2940 nm, TEA:CO_2 = 10,000 nm) for MALDI.

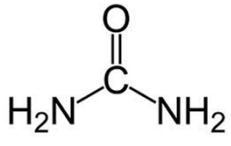
- Matrices for IR MALDI absorb energy via O-H, N-H (~ 3000 nM) and C-O stretch or O-H bend (~10,000 nM).



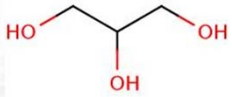
malonic acid



succinic acid



urea



glycerol

MALDI Ion Generation: Primary Ions

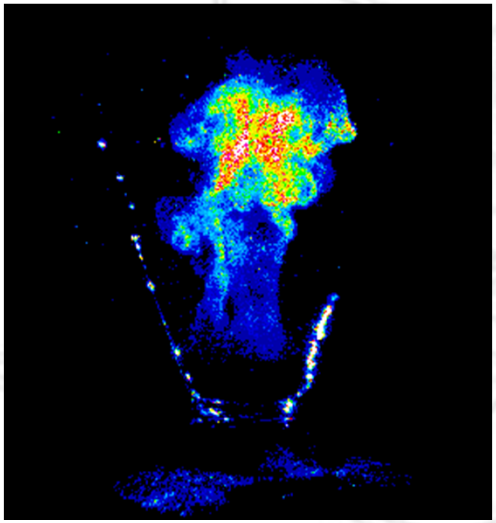
- How precisely primary ions are generated in MALDI is not well understood
- The simplest explanation is photoionization, where excitation of matrix electrons exceeds the ionization potential (IP)
- Based on some pretty rough approximations, matrix IPs are somewhere in the neighbourhood of 8 eV; N_2 lasers carry 3.7 eV per photon, so ionization would require three photons. Unlikely at MALDI laser intensities.
- Direct photoionization might still occur if the matrix IPs are lowered by interactions with analyte.
- Another possibility is 'pooling' where matrix molecules in the excited state (but not ionized) transfer their energy to neighboring excited matrix molecules.

Primary Ions in MALDI: IR Just Don't Cut It

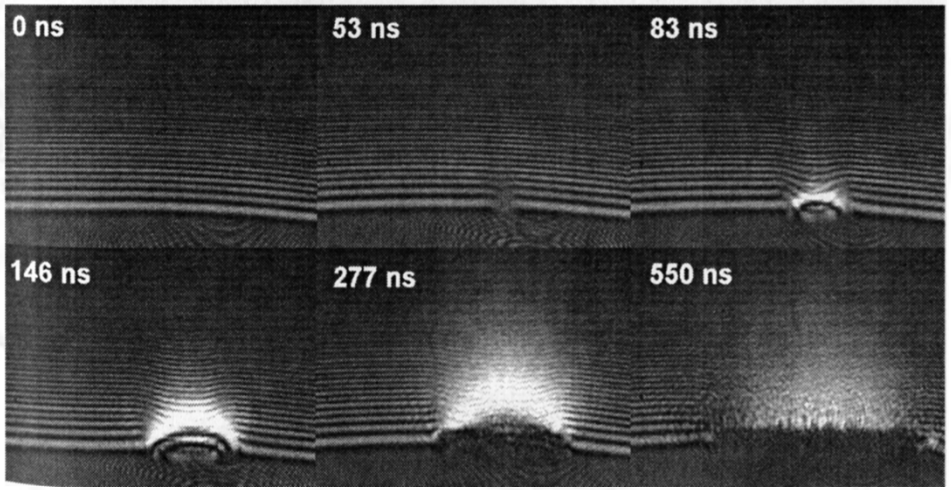
- But these don't explain IR-MALDI where photoionization is extremely unlikely.
- The polar fluid model suggests that the environment of the matrix immediately after laser ablation is like water.
  - This would allow ionization of the matrix analogous what occurs in a polar liquid (*i.e.* $CH_2COOH \longrightarrow CH_2COO^-$)
  - Outside a polar liquid, the amount of energy required to break an OH bond is about the same as a CH bond…
- Alternatively, primary ions might be 'preformed' in solution. An acidic solution will contain preformed ions...

MALDI Ion Generation: The Plume

- When the laser hits the target plate, primary ions are generated (somehow), but also the sample heats up and basically explodes
- The resulting expulsion of 'stuff' is called the 'plume'
- Conditions in the plume are hot (600 – 1000 K) and fast (500 – 1000 m/s)



IR-MALDI Plume
Kermit K. Murray, Website



Maldi plume timeline: Mass Spectrometry, A Textbook;
Jurgen H. Gross

Secondary Ions

- In the plume, primary ions are reactive and there's plenty of heat to push over kinetic barriers.

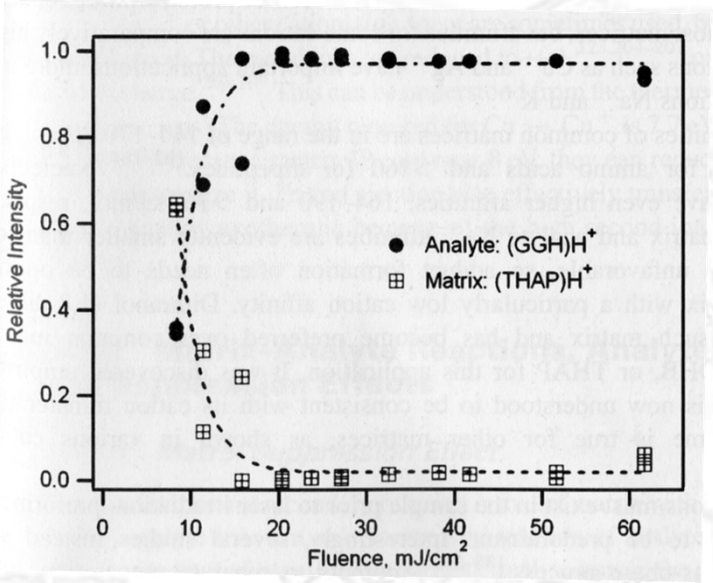
- The result is a lot of charge transfer reactions (like the ones we say in CI), usually via loss or gain of a proton.

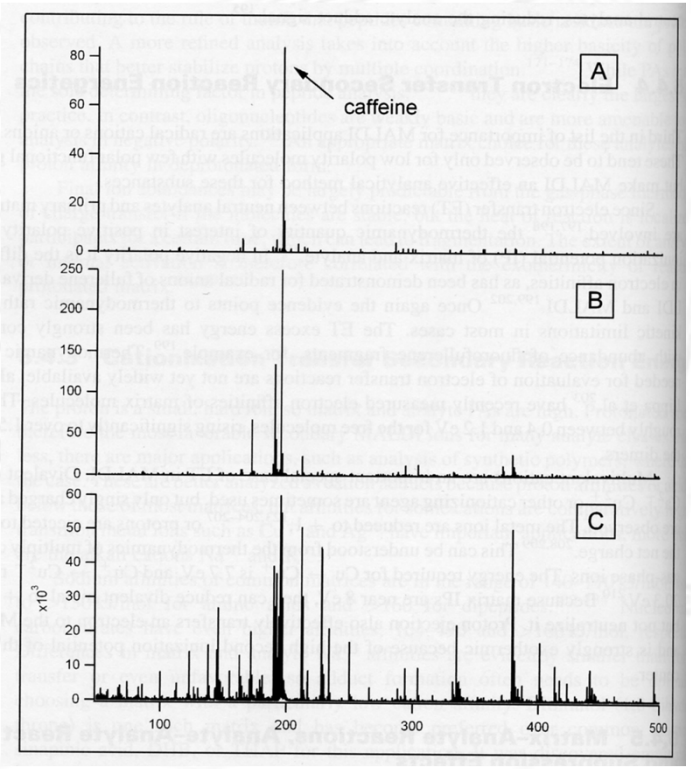
    - Basic residues on proteins and peptides have high proton affinities (PA), thus you want a matrix with low proton affinity when protonated and positive ion mode.

    - Nucleotides have low PAs, so you want a high proton affinity matrix when deprotonated and negative ion mode.

Generating Analyte Ions (Instead of Matrix)

- The main thing to avoid primary matrix ions in the mass spectrum is to strike a balance between efficient charge transfer reactions to analyte (good) and overionization of the matrix (bad)





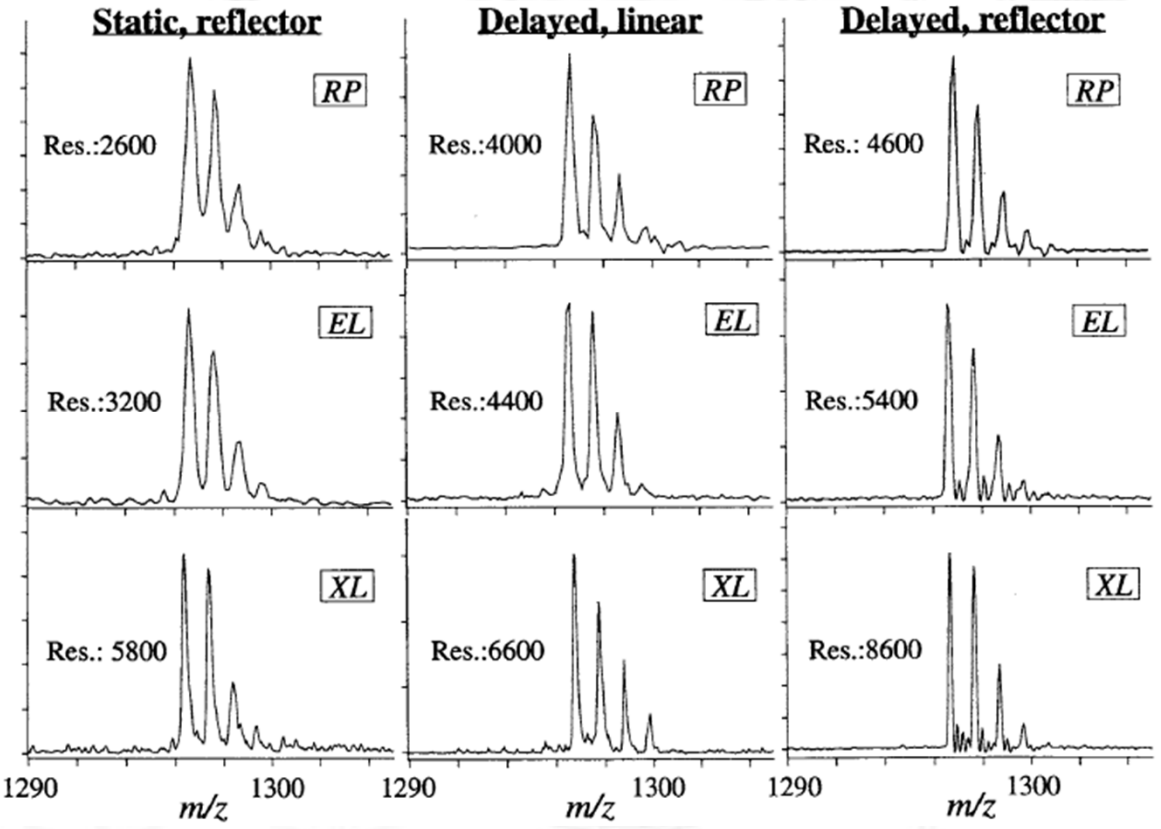
more matrix

higher laser power (overionization)

MALDI Delayed Ion Extraction

- MALDI suffers from the same 'initial kinetic energy' problems as CI, only worse (since much of the laser energy is converted into kinetic)

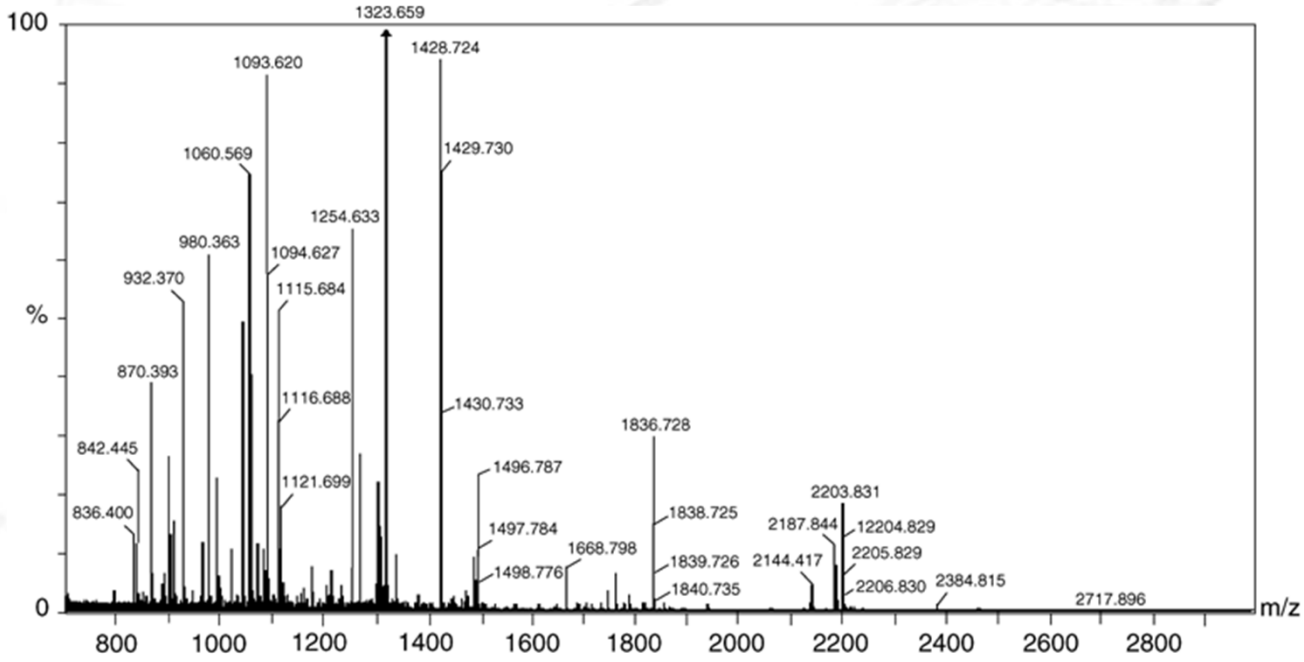
- A solution is to allow the plume to cool for a moment in a field free environment. Cooling occurs due to expansion and is more efficient for hotter molecules, thus after waiting, the distribution of kinetic energies is narrower. This is called delayed extraction:



RAPID COMMUNICATIONS IN MASS SPECTROMETRY, VOL. ***9,*** 1044-1050 (1995)

MALDI Mass Spectra

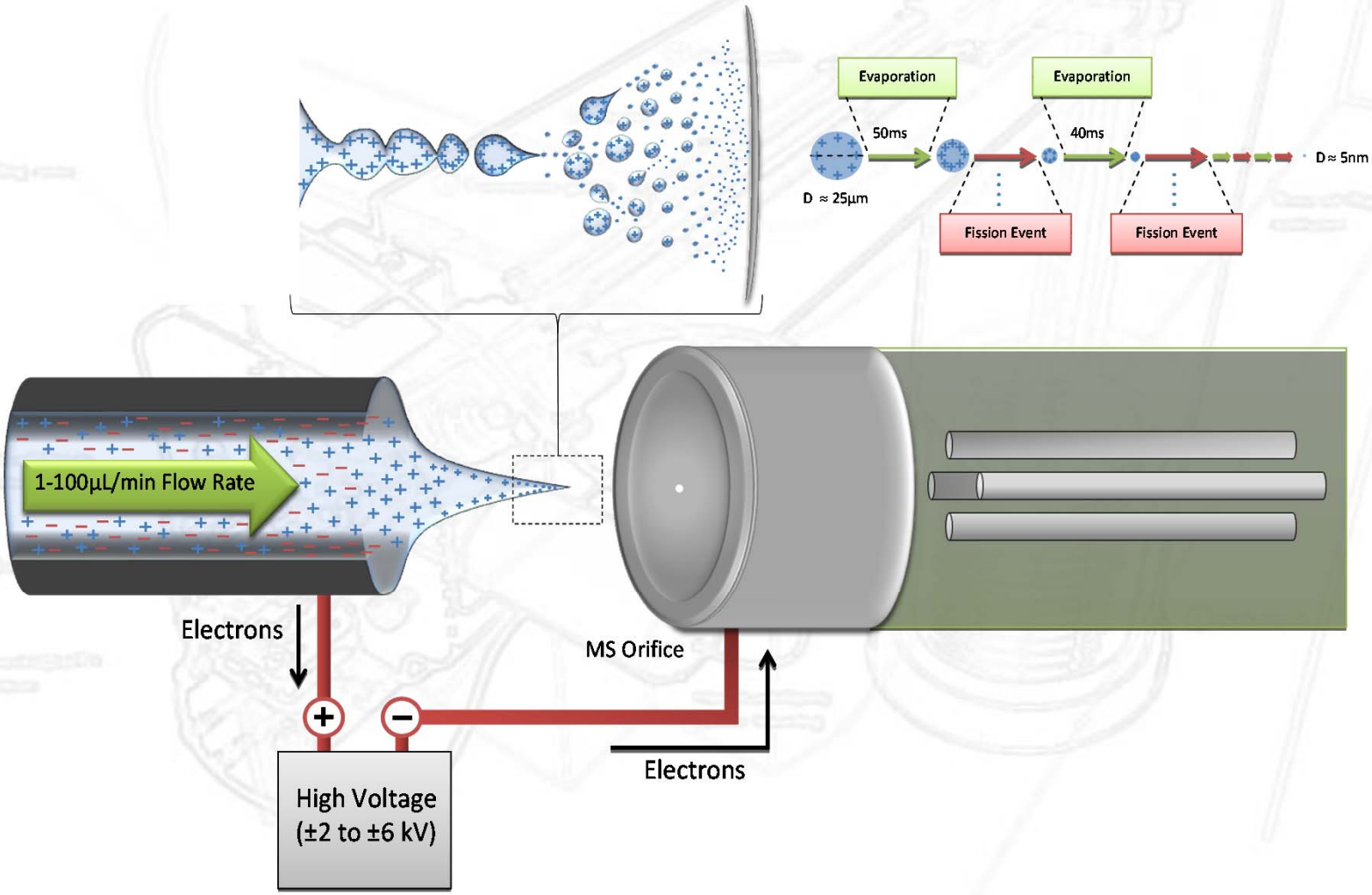
- MALDI Mass Spectra are characterized by matrix ions at low m/z and (usually) singly charged analytes



- Single charging tendency is sometimes an advantage as it simplifies the spectrum (compared to electrospray).

Electrospray Ionization

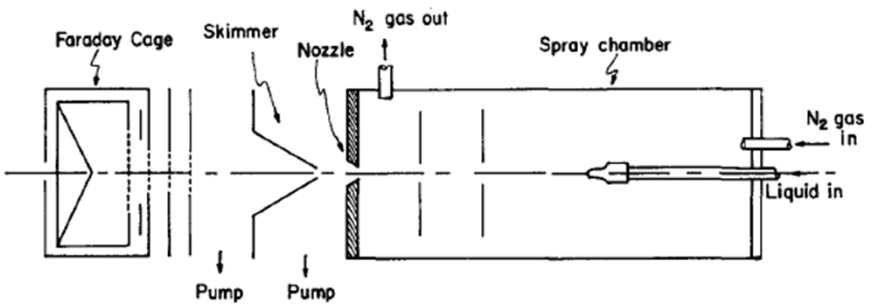
- In electrospray, ions are generated by passing solution through a capillary held at high electric potential (2 – 6 kV)



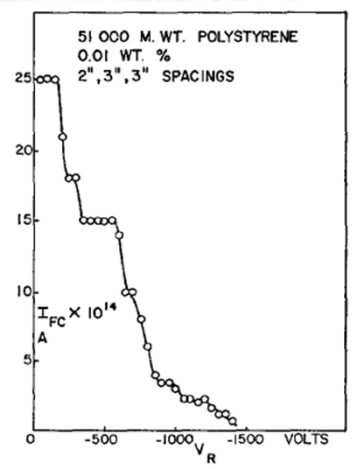
A Little Electrospray History

- The first person to do real science with electrospray was Dole and co-workers in 1968. 'Molecular Beams of Macroions'

- Dole probably should have won the nobel prize, but didn't for two reasons: 1) We wasn't really doing mass spectrometry

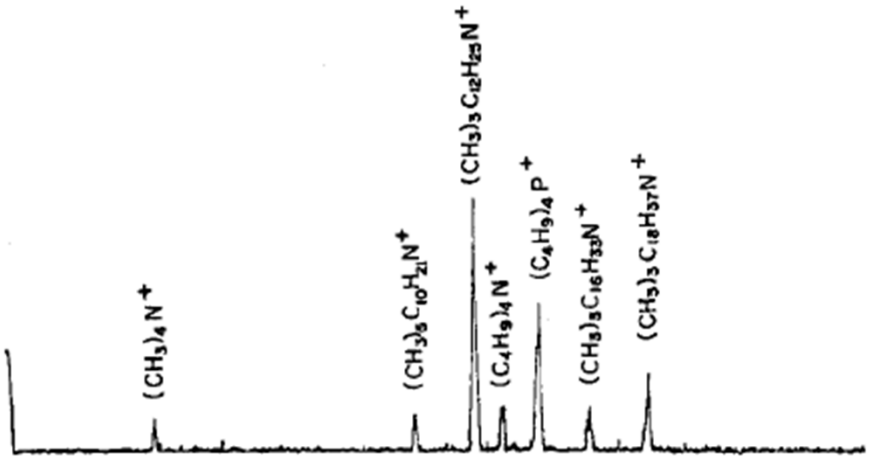


- 2) He was more interested in polystyrene than proteins



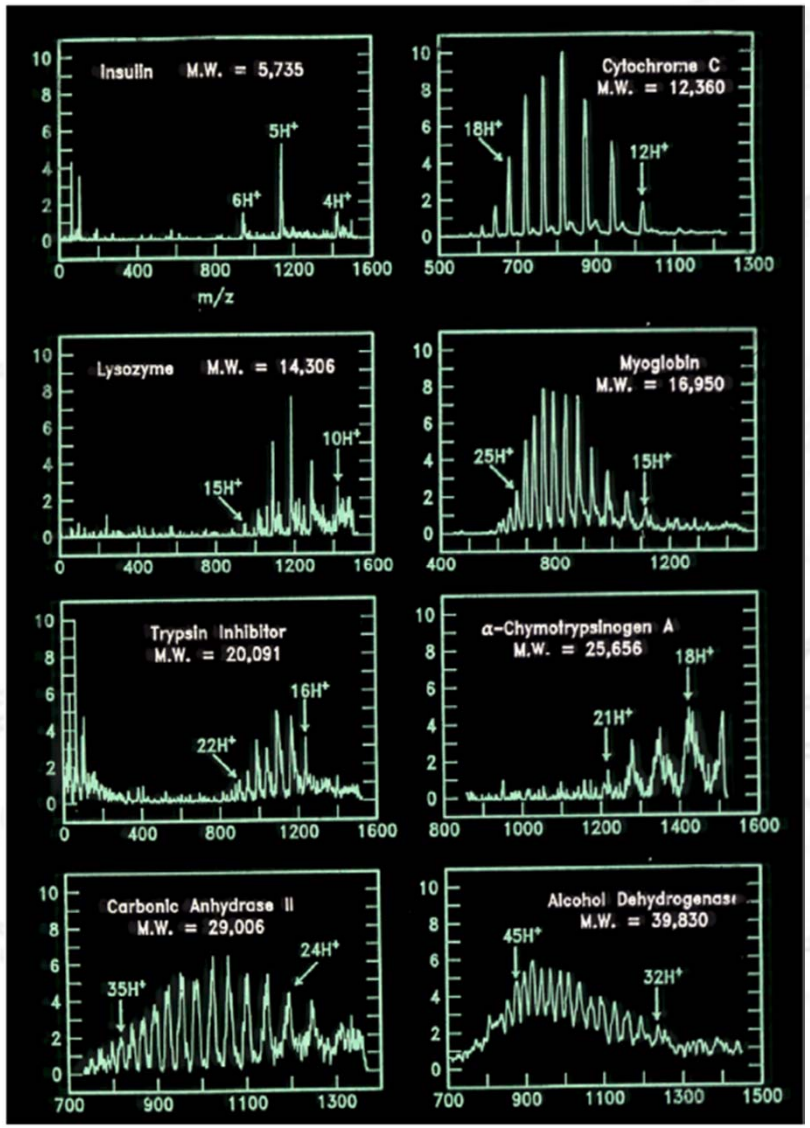
Electrospray History Con't

- Almost 20 years later (1984) Fenn realized the potential of Dole's work and did actual Electrospray mass spectrometry…



- His paper was titled "Electrospray Ion Source – Another Variation on the Free Jet Theme"… can you feel the excitement?
- Then he did this…

Electrospray History Con't

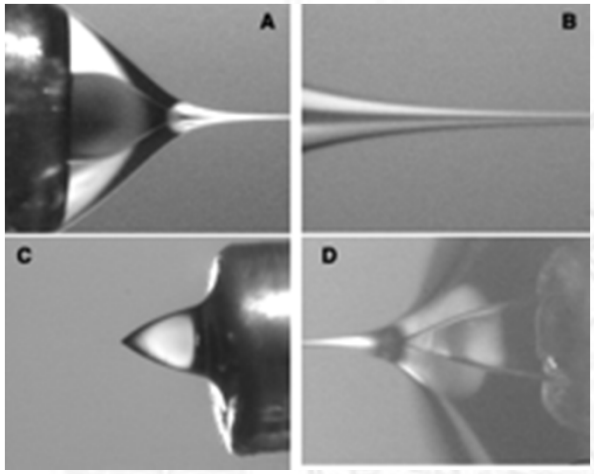


C. K. Meng, M. Mann, J. B. Fenn, *Z. Phys. D* 10, 361 (1988)

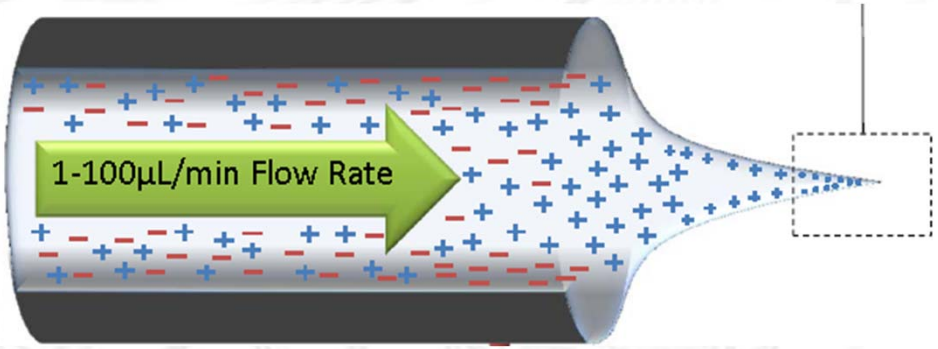
- And the 'gentlest' ionization technique was born!

Electrospray Step 1: The Taylor Cone

- At the tip of the capillary, ions with the same polarity as the capillary try to escape, accumulating at the liquid-air interface.



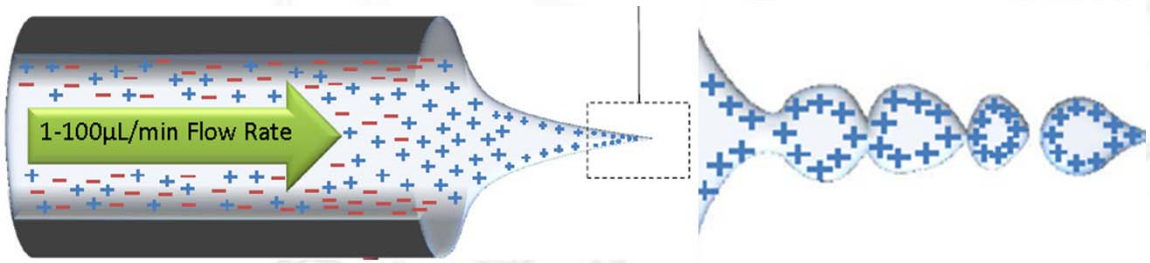
SCIENCE VOL 295, I. G. Loscertales *et al*.



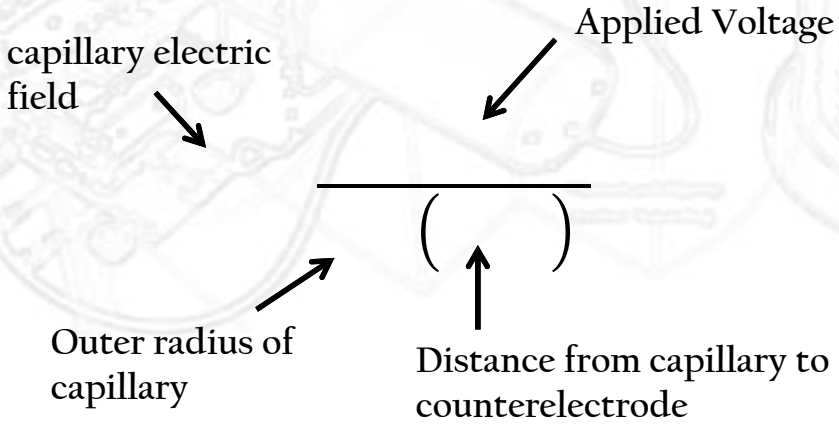
- A combination of surface tension and charge/charge repulsion cause the formation of a 'Taylor cone'

Step 2: Parent Droplet Formation

- At the very narrow tip of the Taylor cone, coulombic repulsion overcomes surface tension, resulting in the emission of μm sized droplets

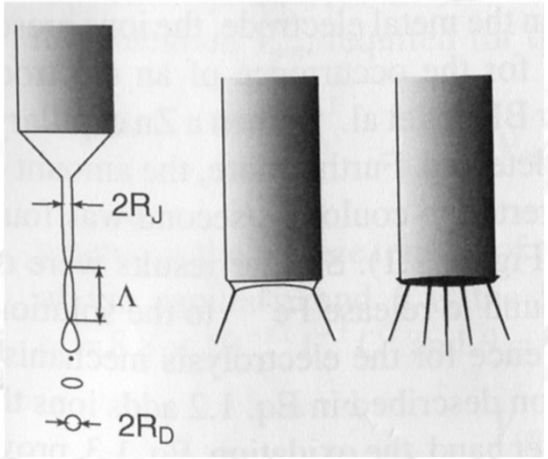


- The initial droplet size is roughly inversely proportional to the electric field at the capillary tip, which is given by:



Stability of the Taylor Cone

- The Taylor cone is stable only within a range of flow rates and capillary voltages.



Increasing Voltage ⟶

- An estimate for the 'onset voltage' of the Taylor cone is given by:

$$V_{onset} \propto \sqrt{\frac{\gamma \cdot d \cdot \left(\frac{1}{2}R_J\right)}{\ln\left(\frac{4d}{R_J}\right)}}$$

distance to counterelectrode

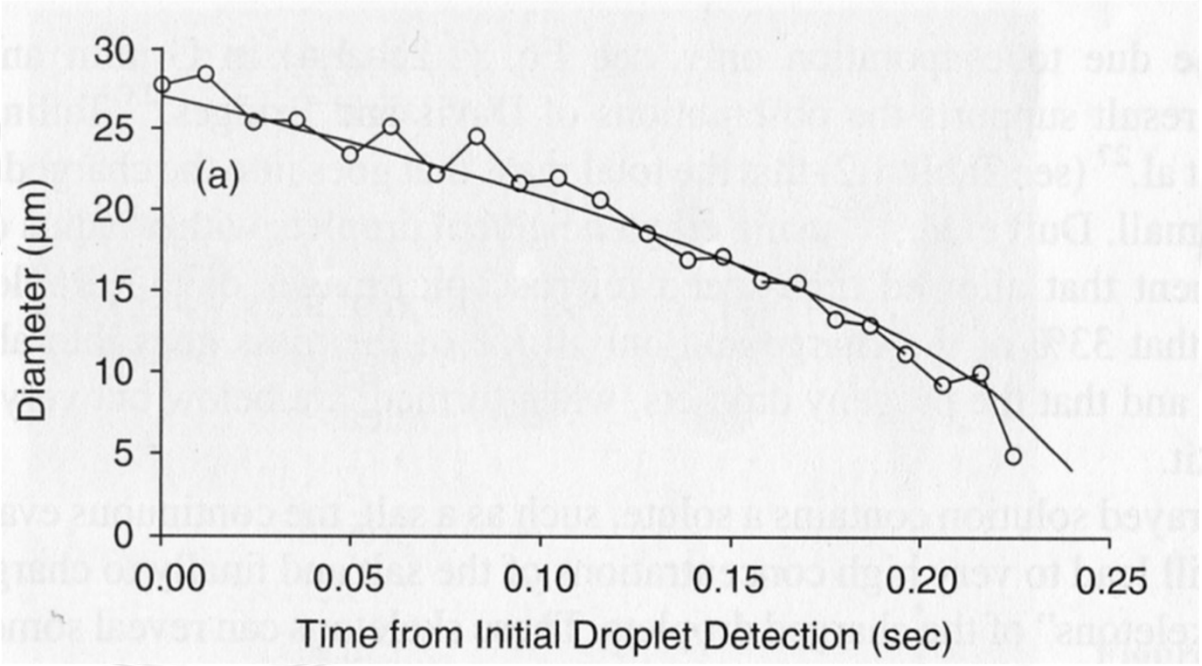
Surface tension of solvent

outer radius of capillary

Step 3: Shrinkage

- Initial droplet sizes vary depending on the flow rate and the electric field, but are typically in the 5 – 30 μm range.

- Droplets shrink over time, mainly via efficient evaporation due to high surface area-to-volume ratio.

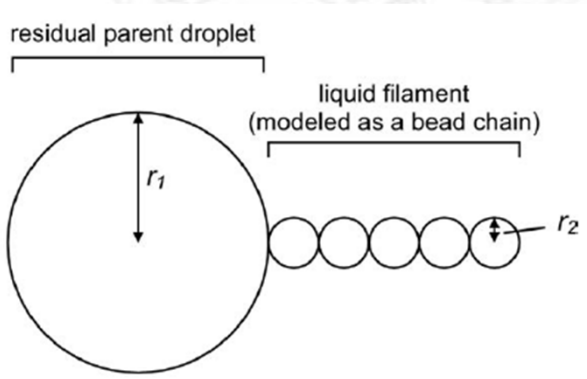


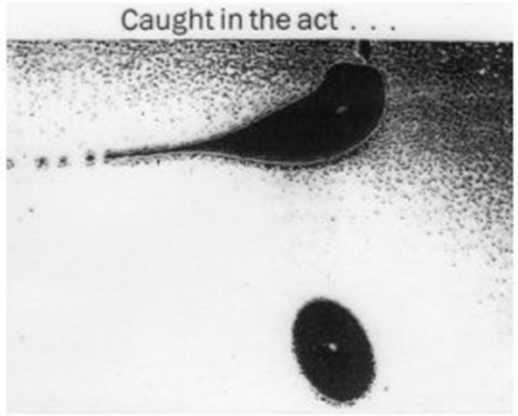
Beauchamp and co-workers, *J. Chem. Phys. B*, 2005

Step 3: Shrinkage, Jet Fission

- As the droplet gets smaller, the coulombic repulsion between charged species on the surface gets stronger until it overcomes surface tension.

- At this point, the droplet undergoes 'jet fission', loosing 2 – 5% of it's mass, but 15 – 20% of it's charge.

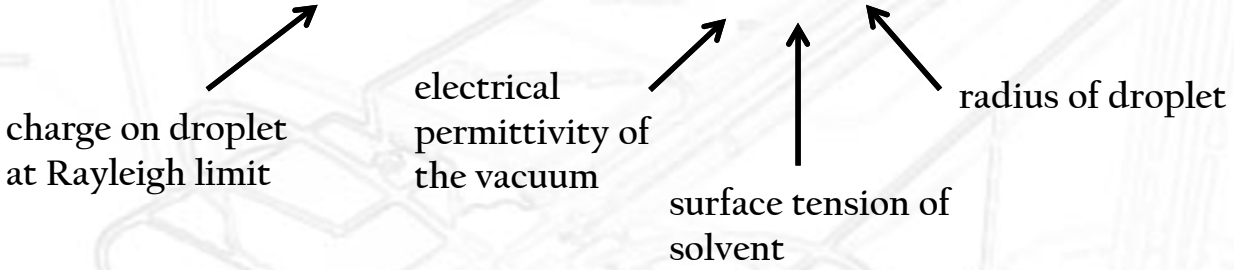




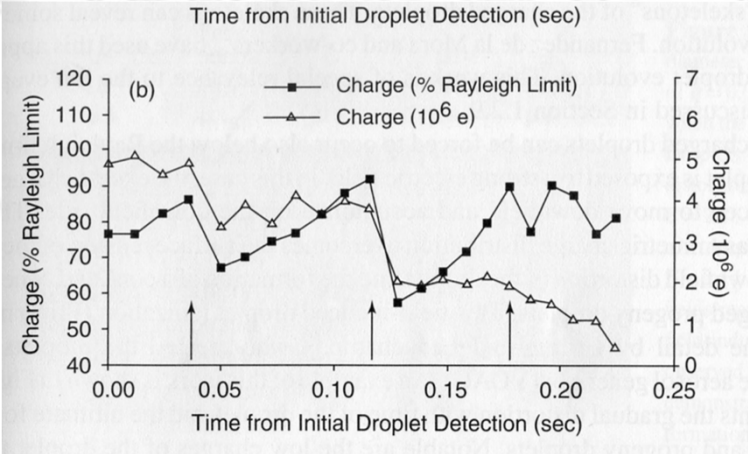
- The result is highly charged progeny droplets that continue to undergo evaporation / jet fission…

Step 3: Jet Fission and the 'Rayleigh Limit'

- The Rayleigh limit is when coulombic repulsion exactly balances surface tension. It is given by:



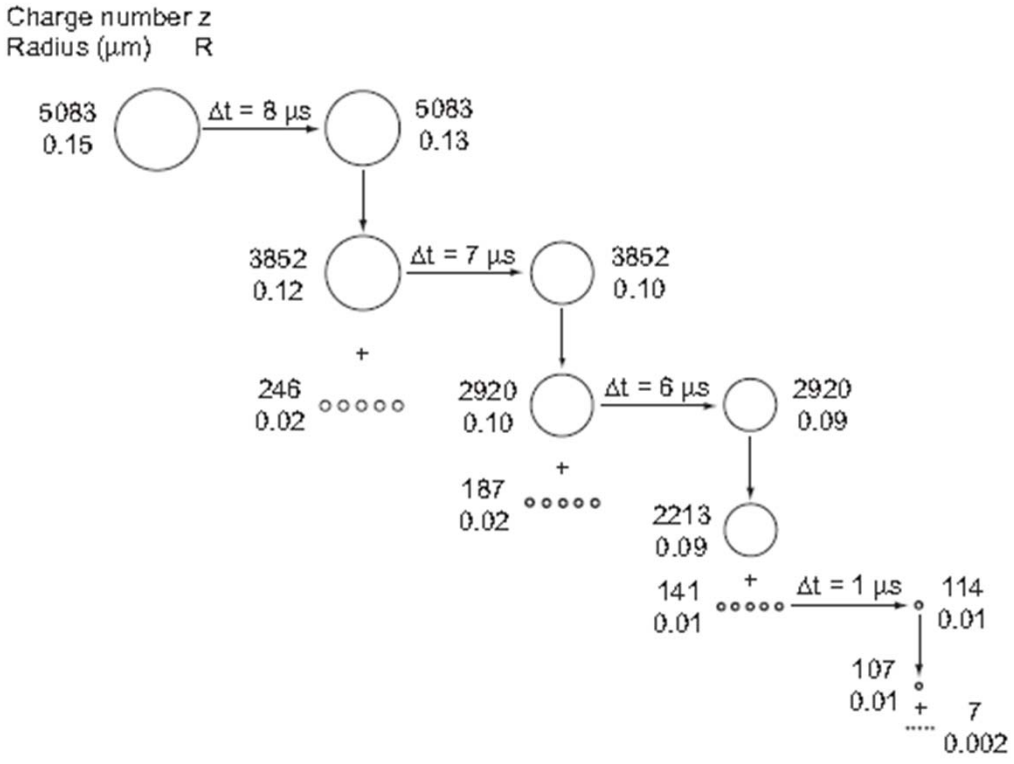
- Jet fission usually occurs between 80% - 95% of the Rayleigh limit:



Beauchamp and co-workers, *J. Chem. Phys. B*, 2005

Step 3: Shrinkage

- Shrinkage of progeny droplets has been well characterized by Paul Kerbarle and co-workers.



- Incidentally, this characterization is also suitable for 'nanospray'

Step 3: Shrinkage – the Final Droplet

- After 10 successive fissions, the parent droplet volume will have decreased 29 – fold, which means a ~ 29-fold increase in analyte concentration

- The frequency of jet fission events in 3rd generation or greater progeny droplets is too high to have been measured experimentally.

- The 'final' droplet radius is usually guestimated at around 10 nm, although the size may vary substantially depending on the analyte. Some large protein complexes, viral capsids *etc.* are › 20 nm in diameter.

Step 4: Ionization

- Ionization in electrospray is not fully understood, but two basic explanations are clearly valid:

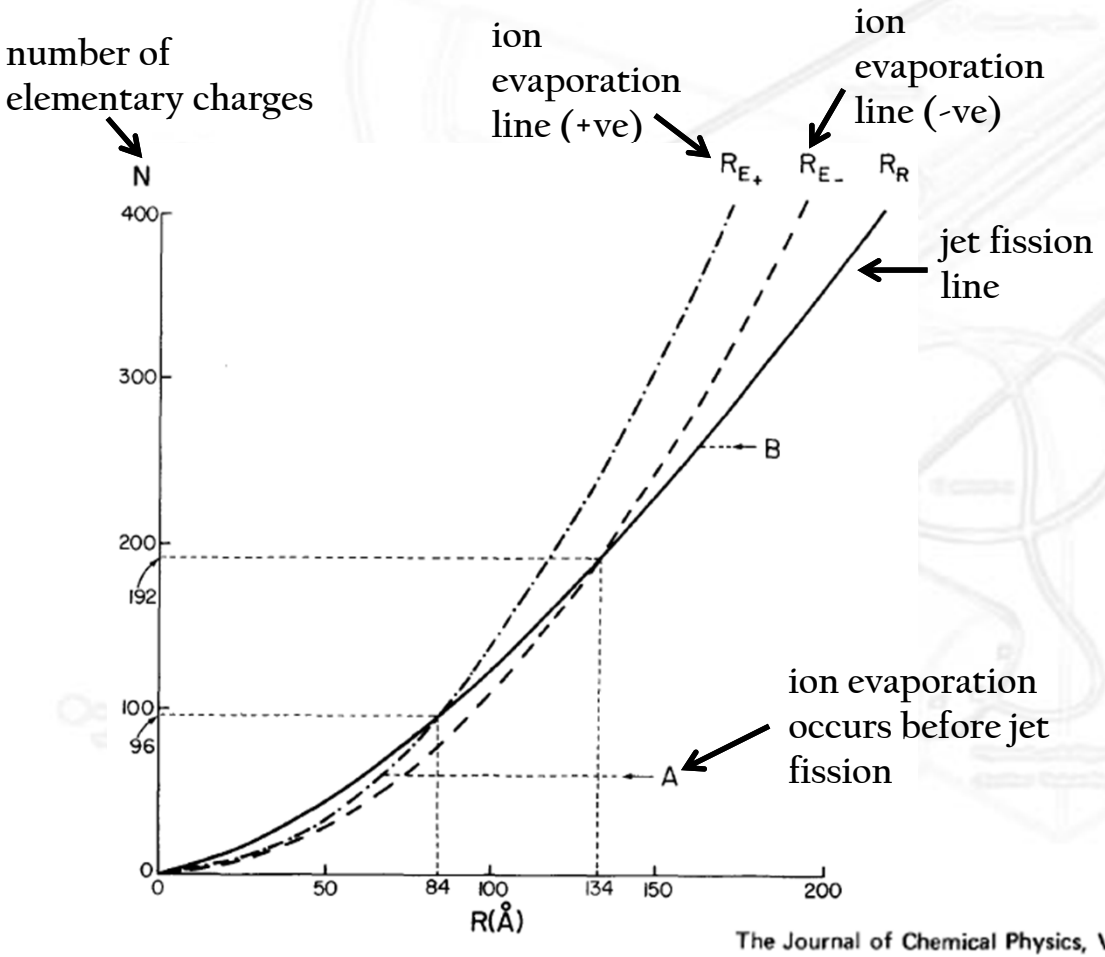
  - The Ion Evaporation Model (IEM), Iribarne and Thomson: Charged species are 'field evaporated' from small droplets

  - The Charged Residue Model (CRM), Dole, Fenn, Others: Solvent evaporates from droplet, leaving a residue of charge on analyte.

- Interesting to note that the proposed models depended on the species under investigation by each group…

Ionization via the IEM

- Initial support for the IEM was from ion mobility measurements of NaCl in water (not done with electrospray!)

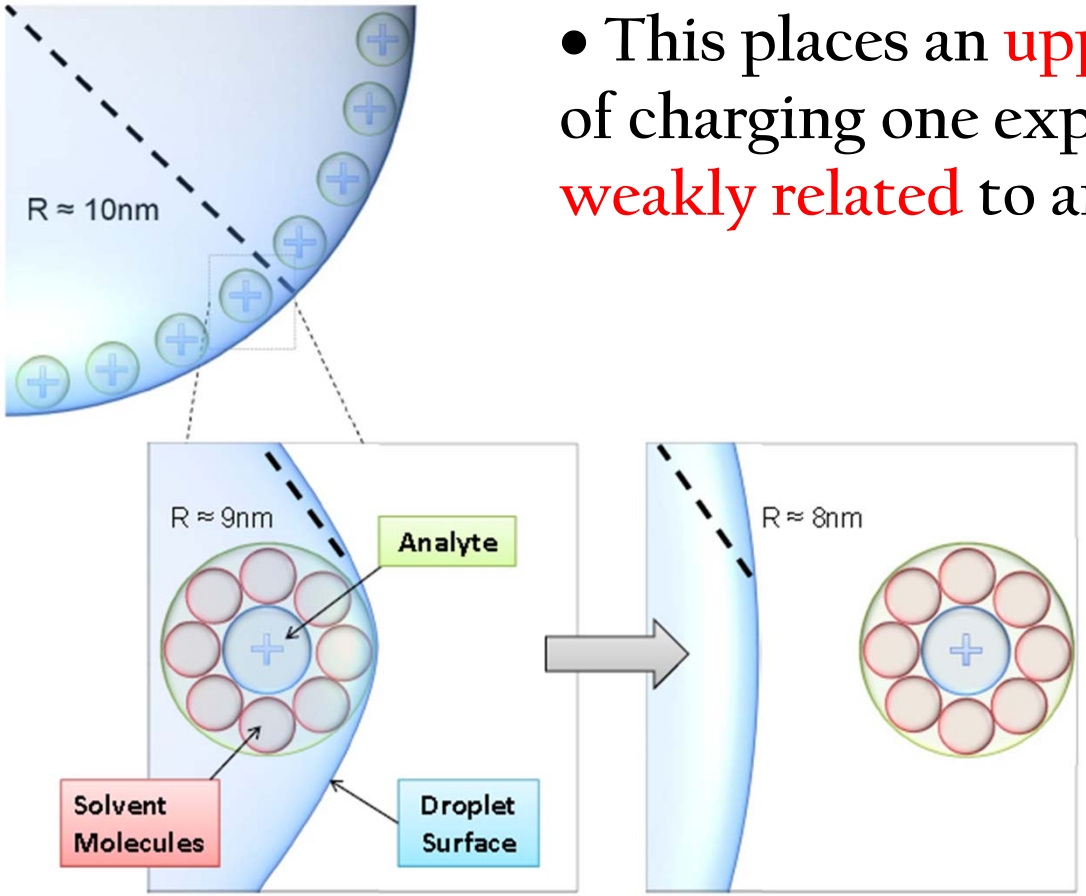


- Bottom line: At small droplet radii, ion evaporation is more energetically favorable than jet fission as a way of relieving coulombic stress.

The Journal of Chemical Physics, Vol. 64, No. 6, 15 March 1976

Consequences of the IEM

- According to the IEM, analytes pick up charge locally as they evaporate from the droplet:

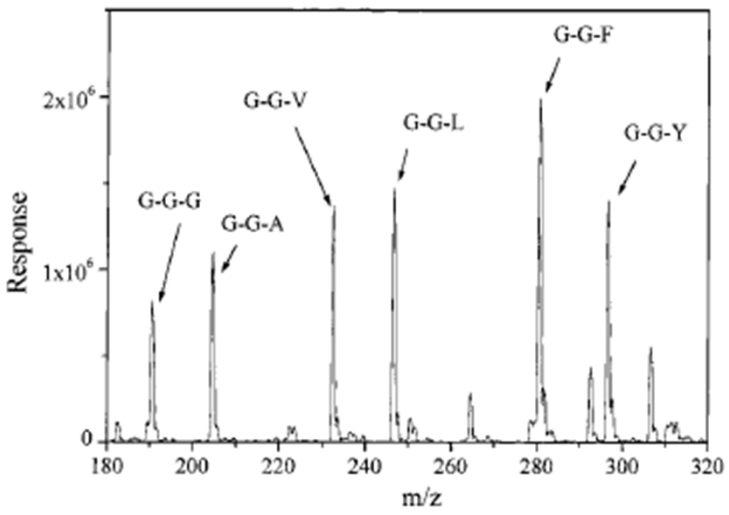


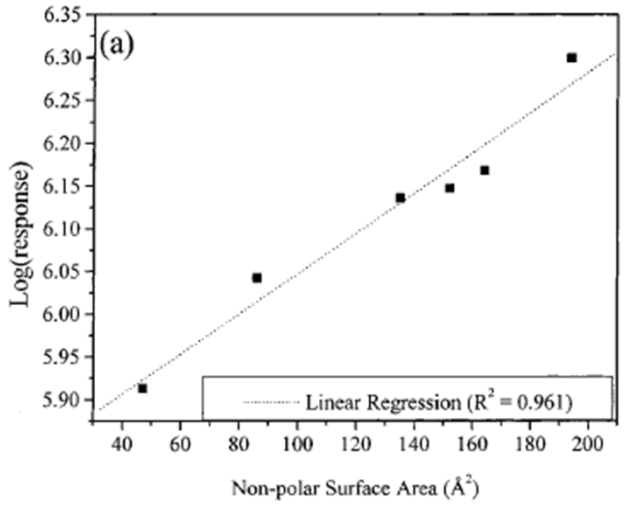
- This places an upper limit on the amount of charging one expects – charging is only weakly related to analyte size

Consequences of IEM Con't

- In the IEM, analytes evaporate as individual species, thus we don't expect to see ions of clusters that do not occur in solution.

- In the IEM, ionization efficiency is directly related to 'surface activity' of the analyte, which is inversely proportional to solvation energy. Thus, hydrophobic species should ionize more efficiently than hydrophilic ones:

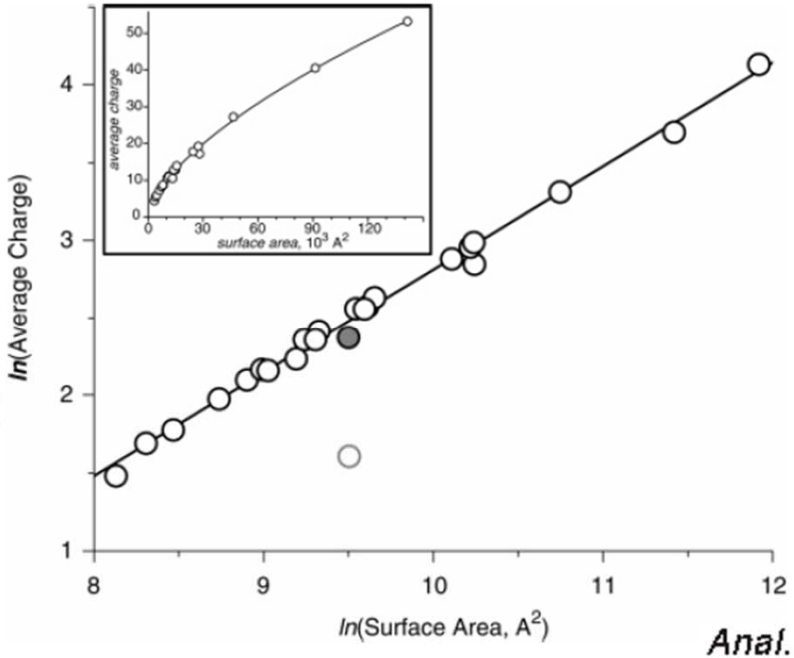




Anal. Chem. **2000**, *72*, 2717−2723

Applicability of IEM

- So, the IEM works quite well for small molecules, but some observations for large analytes are hard to explain…
  - Charging of proteins is well beyond what can be explained by local charge acquisition.
  - Protein charging scales well with protein surface area

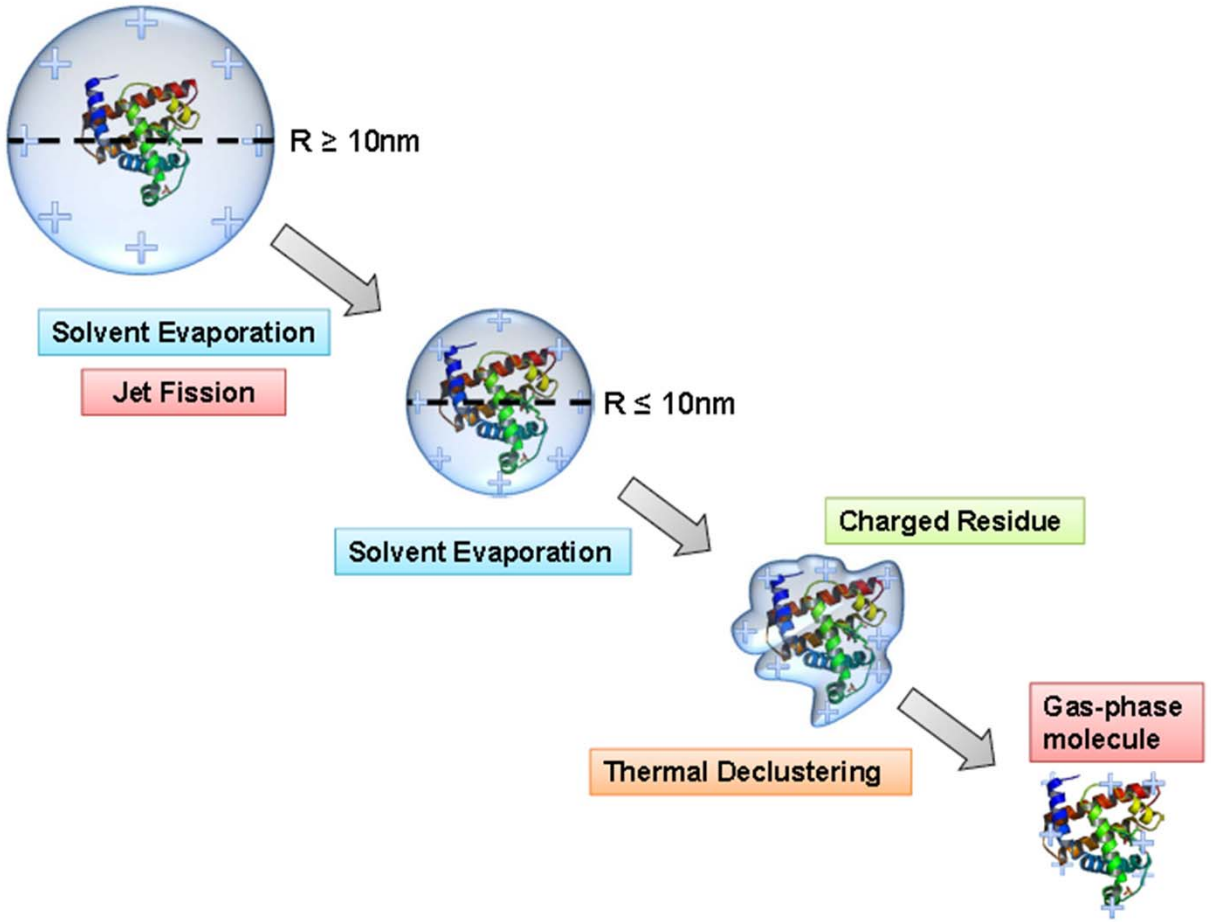


- Non-physiological protein multimers observed at higher protein concentrations.

Anal. Chem. **2005,** *77,* 5370−5379

Ionization via the CRM

- The CRM suggest a simpler ionization process, in which solvent simply continues to evaporate leaving a charged residue on the gas phase analyte



Consequences of the CRM

- This would explain the amount of charge observed on proteins, since virtually all charge carriers in the final droplet (i.e. after the final jet fission event) could contribute to the charge.

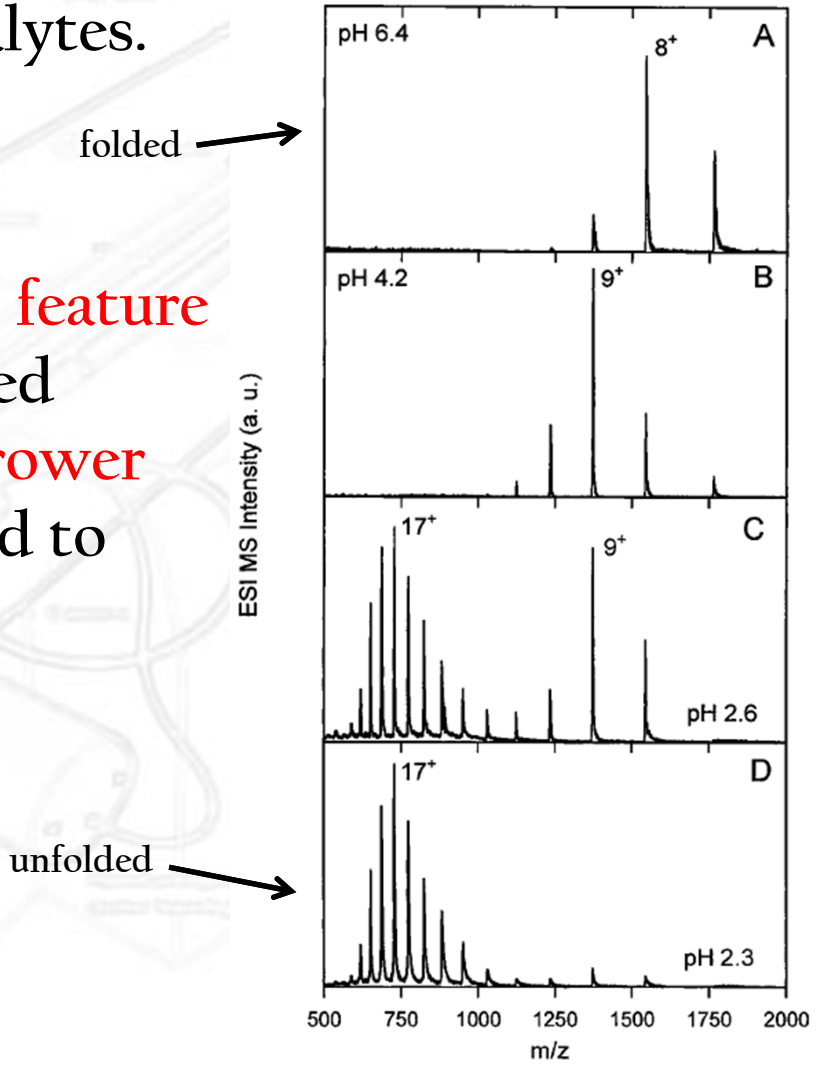
- It would also explain the proportional relationship between surface area and charge on proteins, since greater surface area = more room for charged residue.

- It would also explain the detection of non-physiological multimers, since at high concentration, it is likely that multiple analytes will occupy the same 'final droplet' and ionize as a cluster.

Electrospray Mass Spectra

- Electrospray mass spectra are characterized by multiple charging, especially of large analytes.

- An interesting and important feature of ESI mass spectra is that folded proteins pick up less and a narrower distribution of charge compared to unfolded proteins.



Biochemistry **1997**, *36*, 12296−12302

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