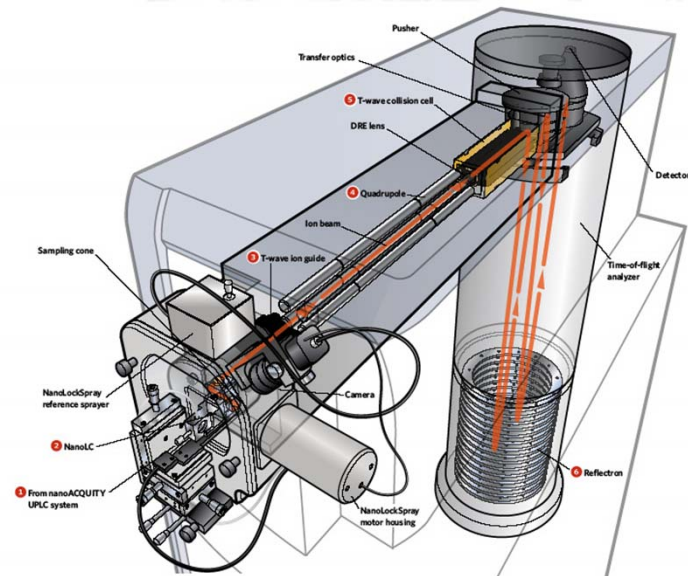
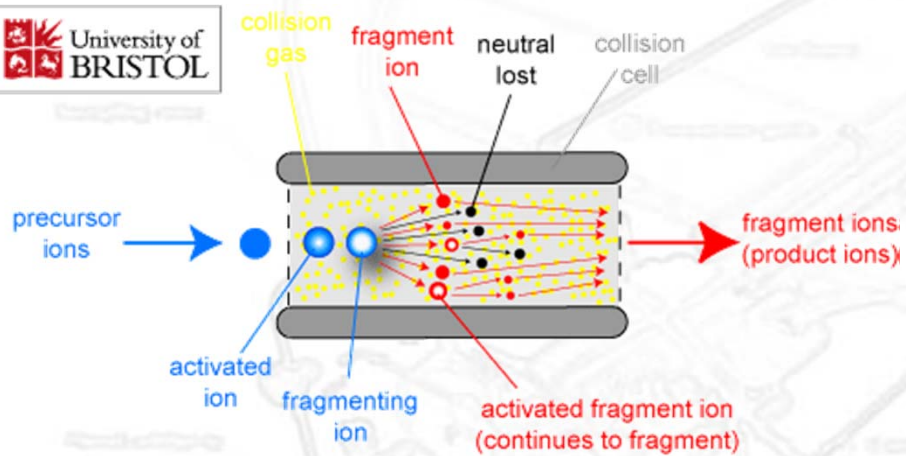
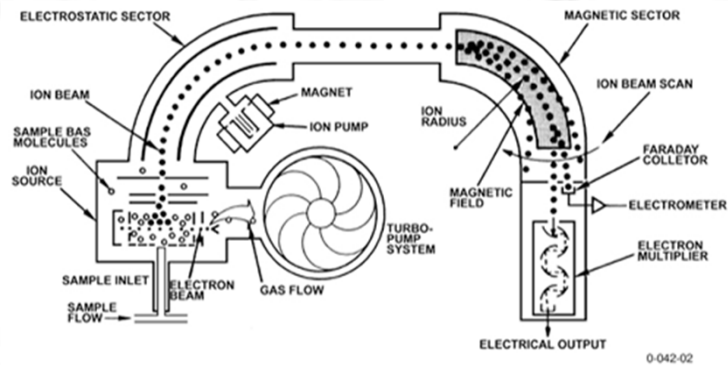


Week 4: Collisional Activation, Mass Analyzers: TOF



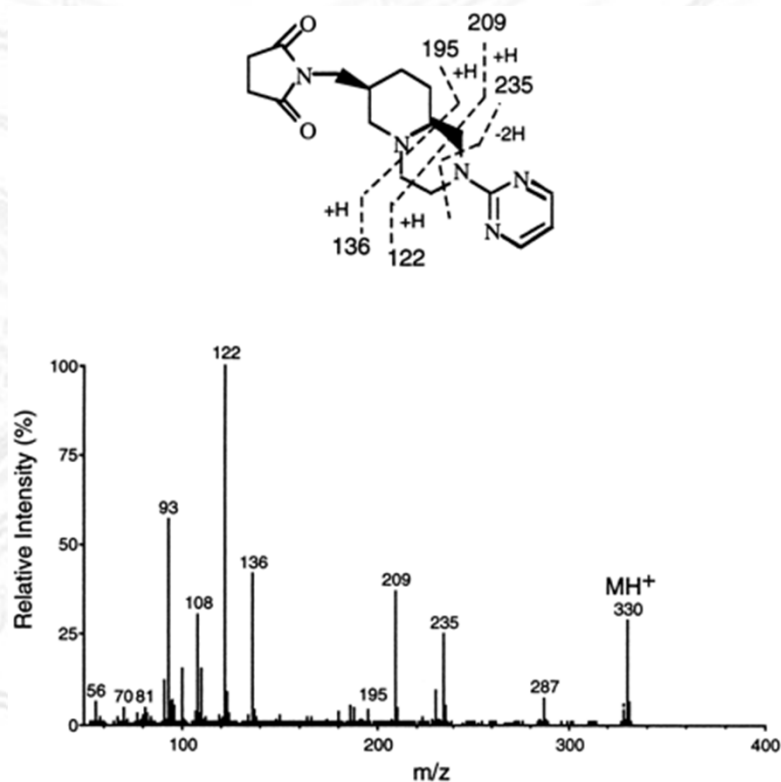
Last Time...

- Mass Analyzers; **Sectors** and **Quadrupoles**:



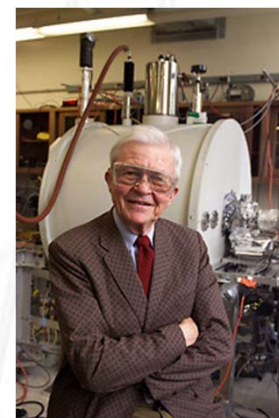
Collisional Activation/Dissociation

- Collisionally Activated Dissociation (CAD) is a method in which we cause an ion to fragment by colliding it with a neutral gas.
- Many of these fragments are ions, which we can analyze by MS to help determine what the original molecule looked like.



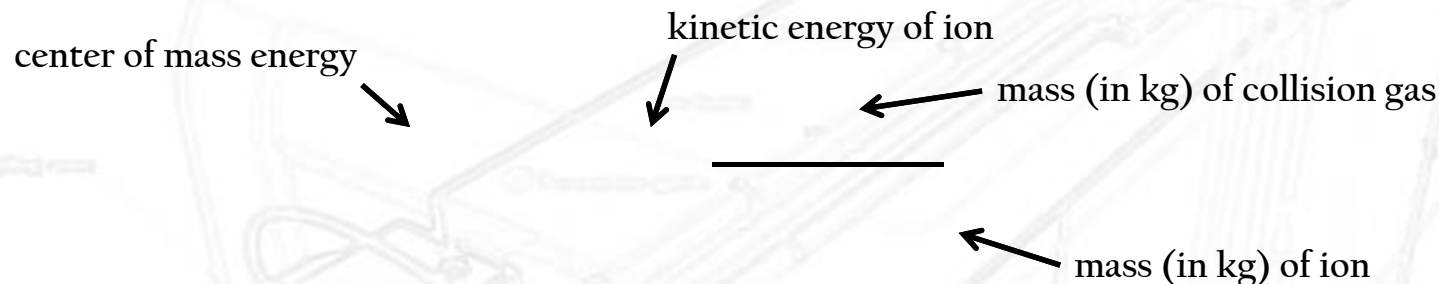
A Brief History of CAD

- ‘Metastable ions’, which were the products of CAD in sector instruments, were identified as early as 1945 (Dibeler and Condon).
- **Fred McLafferty** was the first to look at the chemistry of CAD, he has a reaction named after him – the ‘McLafferty rearrangement’.
- CAD only really started to become popular as a technique with the inclusion of **quadrupoles** in commercial instruments and the resulting ability to do **tandem mass spectrometry**, or MS/MS



CAD – The Fundamentals

- The maximum amount of energy produced by collision of an ion with translational energy E_k with a neutral gas is given by:



- This is the center of mass reference frame for inelastic collisions
- e.g. for our $[\text{DEREK}+\text{H}]^+$ ion accelerated with 10kV, $v = 43,839 \text{ m}\cdot\text{s}^{-1}$, $E_k = 1/2mv^2 = (0.5) * (1.124 * 10^{-24} \text{ kg}) * (4.389 * 10^4 \text{ m}\cdot\text{s}^{-1})^2 = 1.083 * 10^{-15} \text{ J} = 1.083 * 10^{-15} \text{ J} / 1.602 * 10^{-19} \text{ J}\cdot\text{eV}^{-1} = 6757 \text{ eV}$
- This is well above the kind of energy we'd see in a quadrupole instrument, but remember, we were accelerating with 10 kV, which is what **sectors** do.

CAD Fundamentals, Cont.

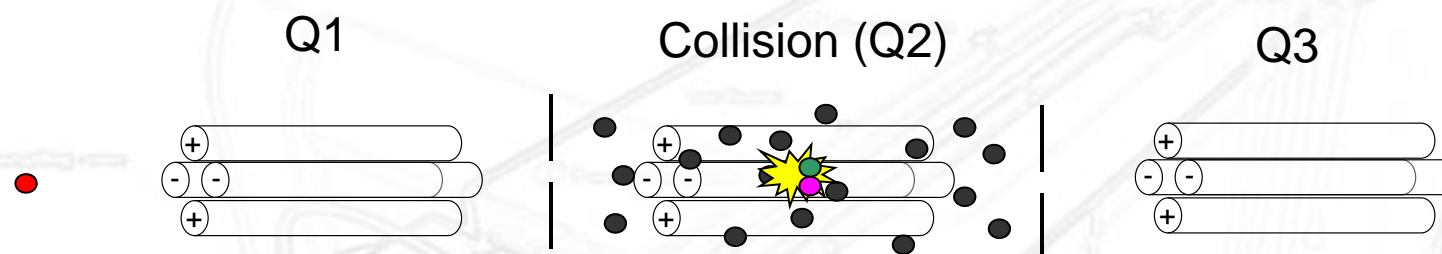
- So, if we're talking center of mass energy, and our collision gas is N_2 ...



- Bond breaking requires tens of eV, so there's enough energy for bond breaking here.
- Of course, in a **quadrupole**, we're accelerating with **2 orders of magnitude less voltage** so we're not going to fragment with a single collision. We need multiple collisions! Heating!

The Triple Quadrupole

- The **triple quadrupole** was the first, and still most widely used instrument specifically for CAD experiments

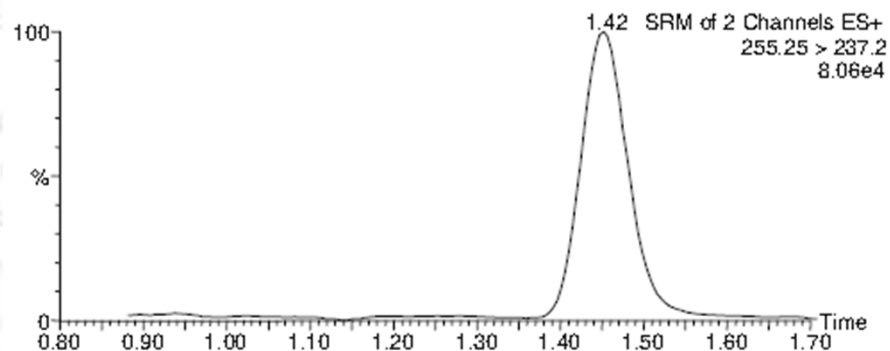
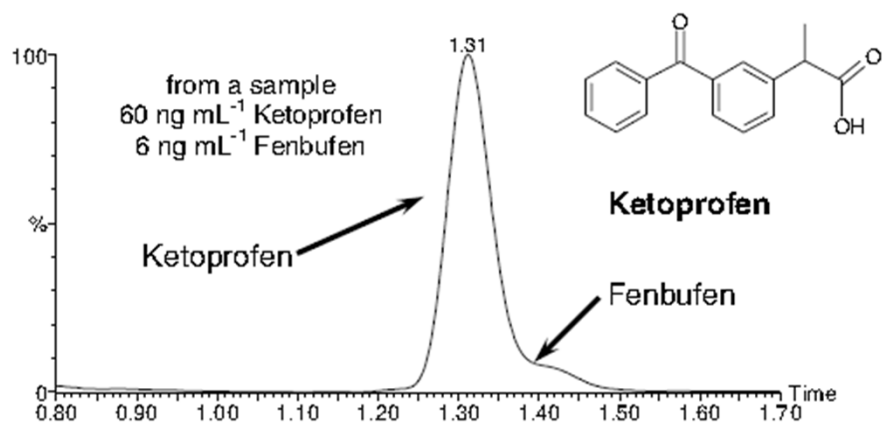
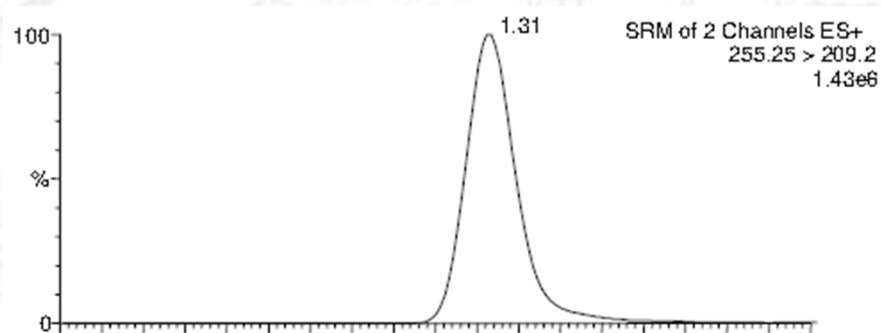
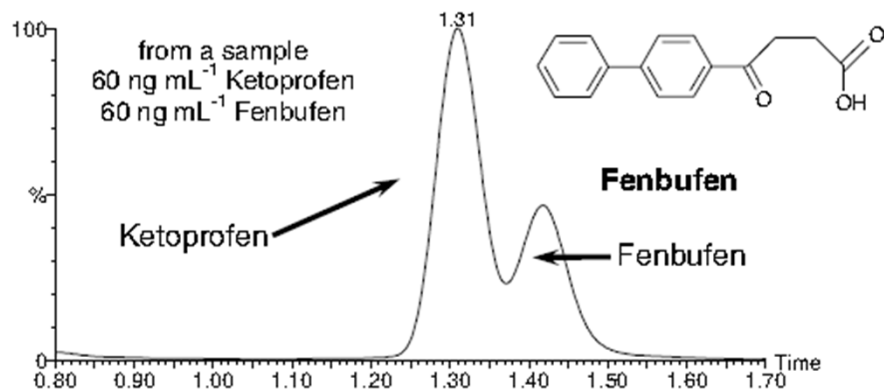


- This allowed (initially) 3 types of scan:
 - **Product ion**: Select in Q1, Scan Q3... characterize molecular structure of known mass ion based on observed fragments.
 - **Precursor ion**: Scan in Q1, Select Q3... look for particular functional group within a set of molecules
 - **Neutral loss**: Scan Q1, Scan Q3 @ $m/z = Q1 - x$... look for a neutral loss with mass x .

Multiple (or Selected) Reaction Monitoring

- Recently, fourth type of scan – Multiple Reaction Monitoring (MRM) has come into use, particularly in proteomics:
 - **MRM or SRM**: Select in Q1, Select in Q3 based on a known ‘MRM transition’
- Note that in order to do this, you already have to know the mass of the precursor and one of its fragments. So why do it?
 1. In proteomics especially, or whenever there is a very complex sample, a particular **precursor mass** may correspond to **more than one analyte**.
 2. Vast improvement in **signal-to noise**. Virtually complete elimination of chemical noise.

Results of MRM/SRM scan



J. THROCK WATSON INTRODUCTION TO
O. DAVID SPARKMAN MASS SPECTROMETRY

CAD in Different Instruments

Instrument Type	Number of Collisions*	eV, Ion Energy	Collision Gas	Collision Time, μ sec	% Efficiency
Single Transmission Quadrupole; In-source CAD	M	1–400	N ₂	5–50	80–95
Triple Quadrupole	S/M	10–100	N ₂ , Ar, Xe, Kr, Ne	5–50	10–40
3D and Linear Quadrupole Ion Trap	M	6–10	He	20,000–40,000	60–80
Double-Focusing Sector-Based	S	2000–8000	He	<5	1–3

CAD Allows Us To Sequence Peptides!

- Probably the most important MS/MS experiment these days is fragmentation of peptides to determine their sequence:

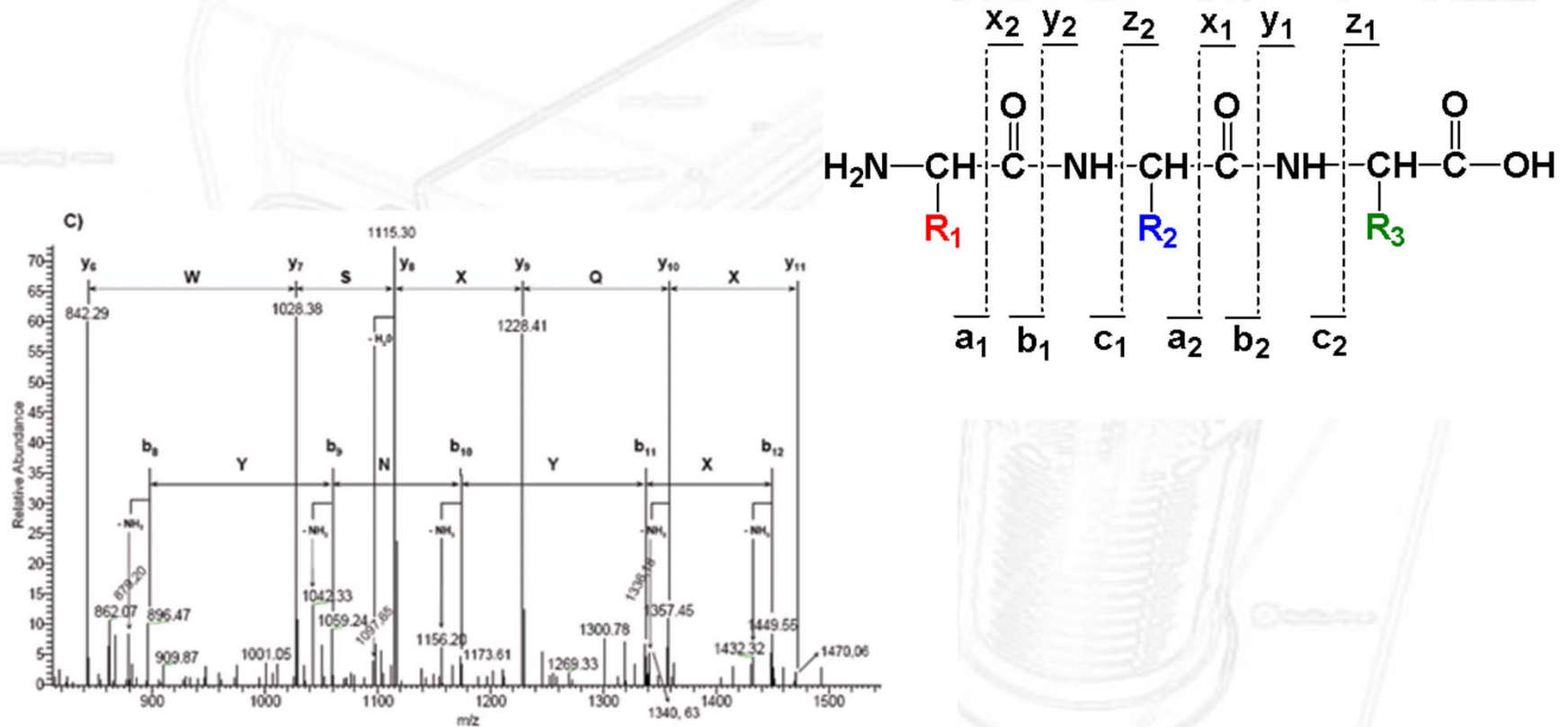
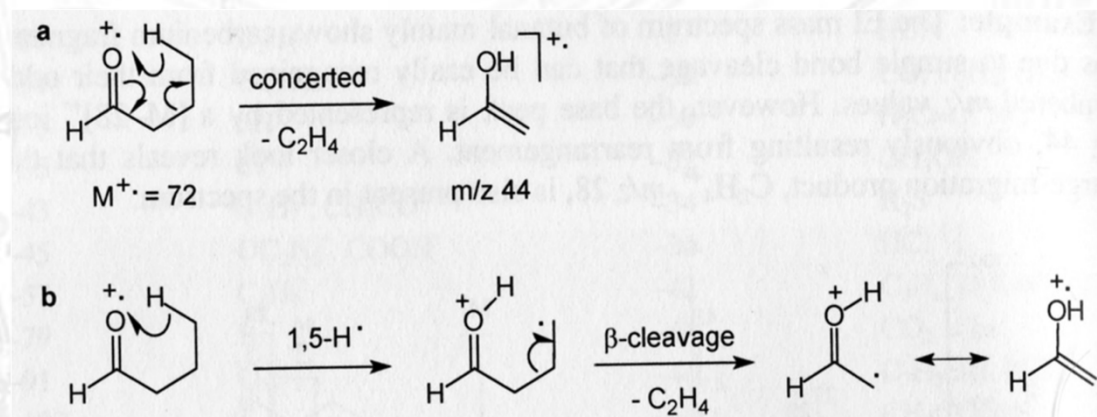


Figura 6S. Interpretação do espectro de massas referente ao peptídeo em estudo, obtido pelo instrumento íon trap. A) Espectro original; B) zoom na região de baixa massa do espectro; C) seqüenciamento do peptídeo (séries -y e -b). * corresponde ao íon precursor (duplamente carregado)

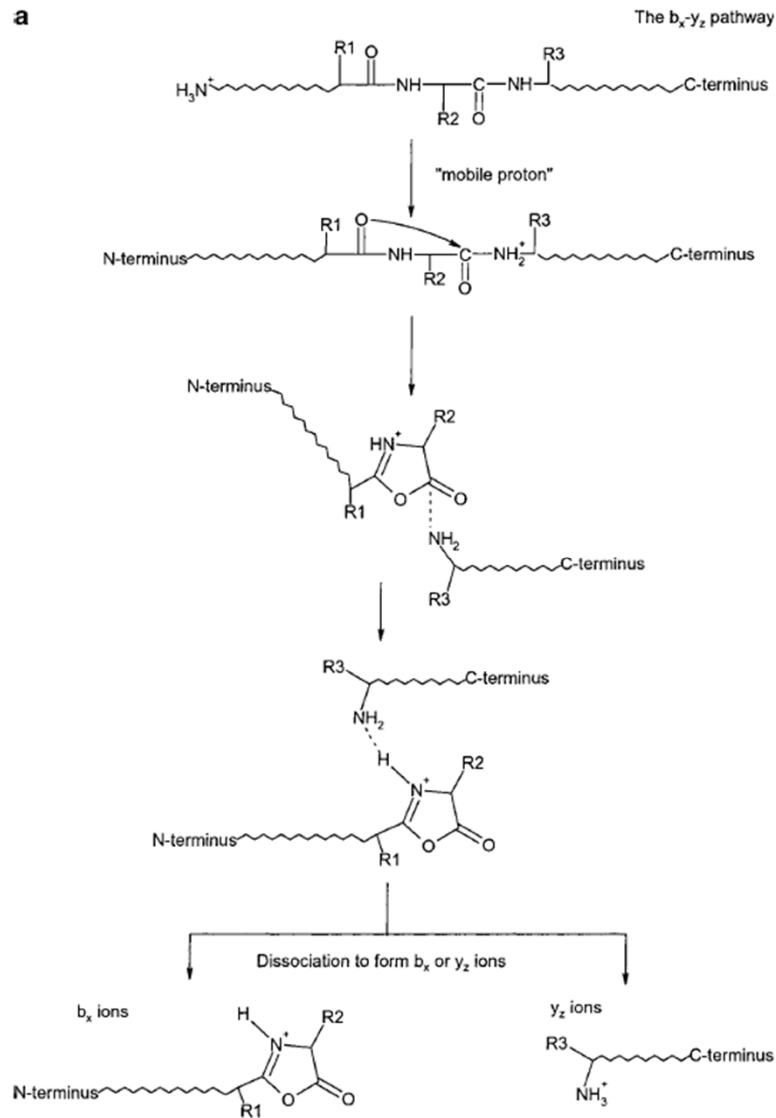
Fragmentation Reactions in CAD

- Fragmentation in CAD occurs not by physical breaking of the bonds, but by (usually) intramolecular **charge rearrangements** that lead to bond breakage.
- The classic example of this is the McLafferty rearrangement:



- This is a γ -H shift followed by β -bond cleavage resulting in the loss of an alkene.

Fragmentation Reactions of Peptides



Mass Spectrometry Reviews, **2005**, 24, 508–548

CAD in Ion traps: MS^n

- Tripple quadrupole instruments allow for a single fragmentation. In a trap, you can in principle do as many as you want using **resonant excitation**...
- To understand resonant excitation, we have to realize that trapped ions have stable orbits with specific frequencies related to their position on the a_u/q_u 3D plot (a_x, a_y, a_z vs. q_x, q_y, q_z).
- If we accumulate all of these terms into a single z direction stability term β_z , we can relate that value to the orbital frequency in the z direction f_z :

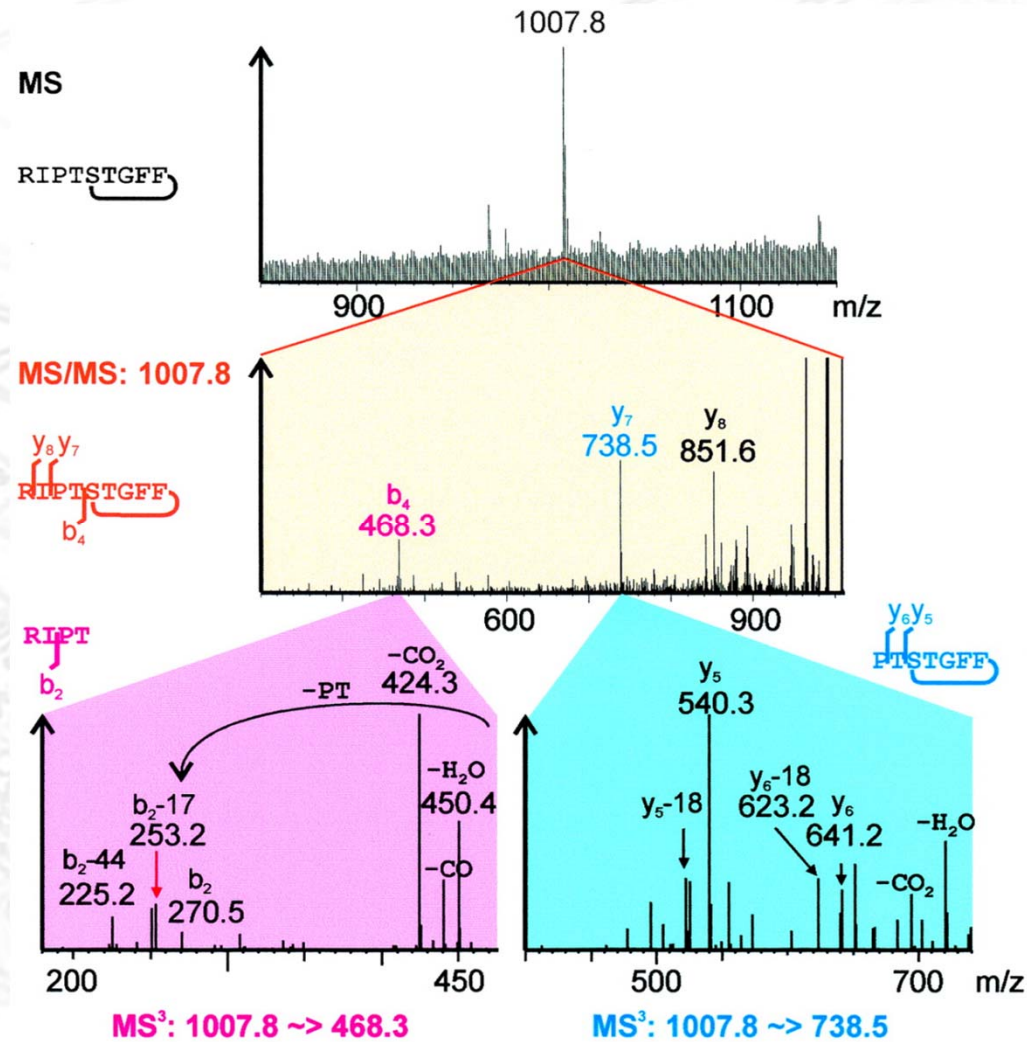
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CAD in Ion traps: MSⁿ, Cont.

- Because β_z increases with q_z , we can calculate f_z for any ion **when a particular V is applied to the end caps.**
- If we match ω to f_z we will excite the orbit of a **single ion**, resulting in a substantial increase in kinetic energy.
- Note that this gives us a tool to eject all ions from the trap except for a **single m/z.**
- Once we have a single ion, we can ‘tickle’ it to add some kinetic energy for fragmentation with the background gas.
- We can then analyze the fragments using a conventional scan. In the next trapping event, we can select, fragment, select fragment, scan... and we’ve now done **MS/MS/MS.**

MSⁿ Example

- Here's some MS³ done on a cyclic lactone-peptide.



Time of Flight (TOF) Mass Analyzers

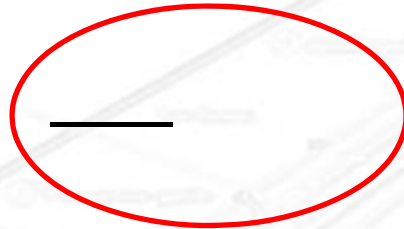
- TOFs use the simplest form of mass analysis: They give the ions a push with an electric field. **Small stuff flies fast, big stuff flies slow... we measure the amount of time it takes to cross a fixed distance.**
- Given the simplicity of the idea, people thought of it pretty early on – as early as 1932.
- Early TOF instruments were actually **quite abundant in the 60's**, however, technological limitations meant that quadrupoles were actually initially better than TOFs (not the case now!) and there were serious declines in sales until the mid-late 90's!
- Resurgence in TOF was driven by MALDI and the collapse of the soviet union.

TOF Mass Analyzers; Innovations Required!

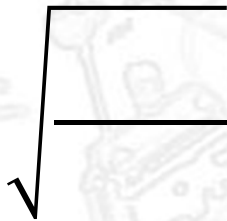
- Early TOF instruments had problems:
 1. Slow
 2. Bad resolution (compared to quads, even)
 3. Non-gaussian peak shape!
 4. High vacuum required for long flight path.
- Solutions?:
 1. Better computers / electronics
 2. Reflectrons, better electronics, MCP
 3. Better electronics – square pulses!
 4. Rotary pump-backed turbo pumps

Basic Principles of TOF

- Lets take a look at our 'kinetic energy after acceleration in an electric field' equation:



- To get the velocity, we can rearrange to:



- The amount of time t for an ion traveling at v to traverse a distance L :

Basic Principles of TOF, Cont.

- Subbing in our equation for v , we get:

$$-\left(\frac{\quad}{\quad}\right)$$

- Note that terms in brackets are **constants!**

- In other words, all else being equal, m/z is proportional to t^2 .

- By rearranging, we can also look at this as **m/z related to flight time:**

$$\sqrt{\frac{\quad}{\quad}} \left(\frac{\sqrt{\quad}}{\quad} \right)$$

- We can accumulate the in-bracket constants into a single term A .

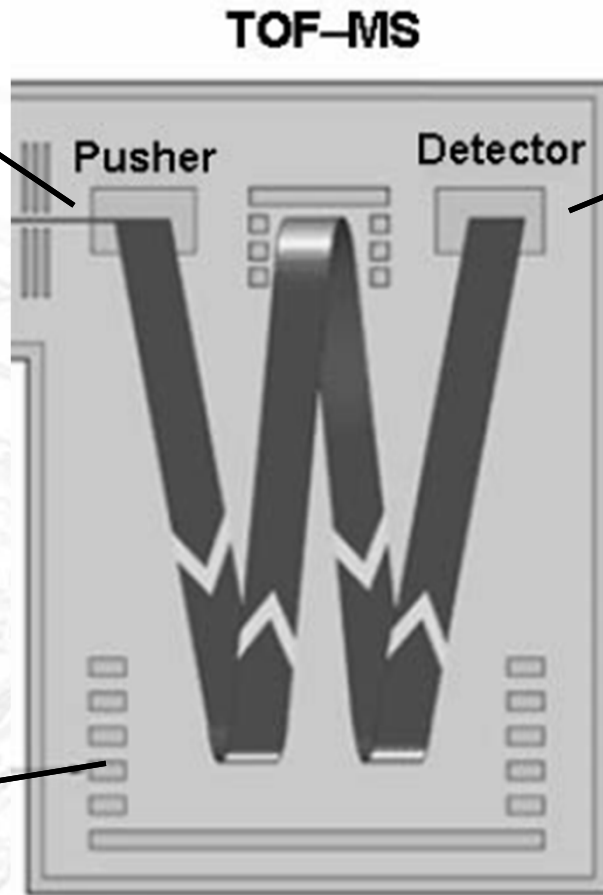
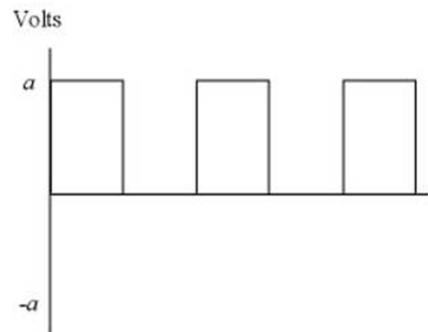
- After adding a second ‘fudge factor’ constant for issues relating the ‘real’ $t = 0$, we get a linear relationship:

$$\sqrt{\frac{\quad}{\quad}}$$

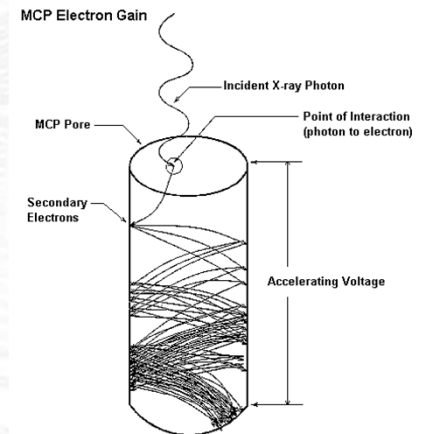
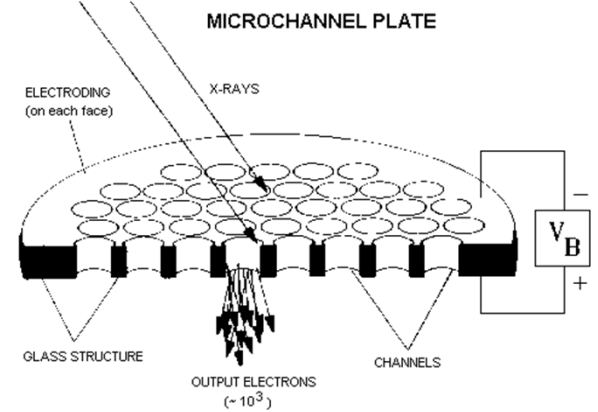
TOF Instruments

- Note that the A term depends on the particulars of the instrument (length of flight tube, ‘pushing’ voltage).
- Also included are any number of other factors that exert a small influence on the flight time and affect both mass accuracy and resolution:
 - ‘Squareness’ of the accelerating pulse
 - Position of the ion with respect to the ‘pusher’
 - Collisions with neutral gas
- Another important factor affecting resolution is **the ability to tell similar flight times apart** – ions with similar m/z will arrive within ns of each other!

Basic Principles of TOF

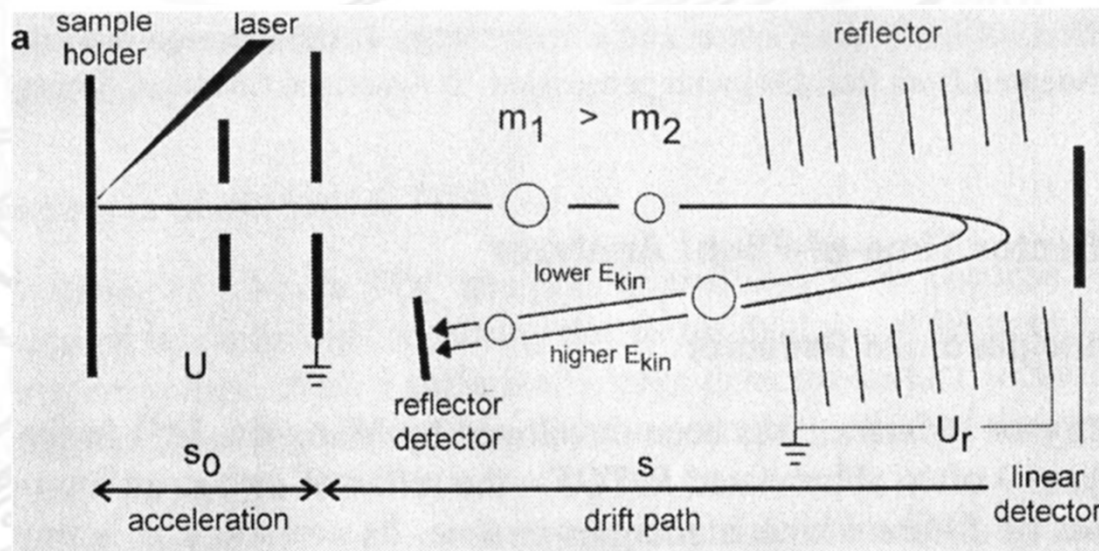


Reflectron



The Reflectron

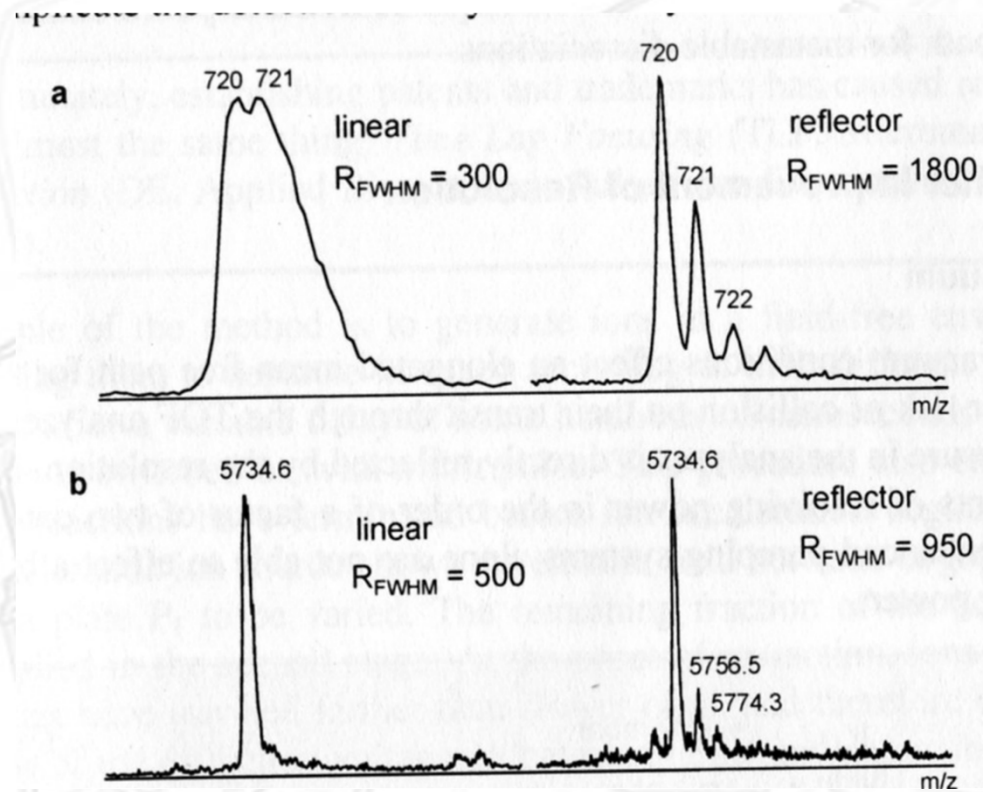
- One of the challenges of getting highly resolved TOF is that ions are located in different positions with respect to the electric field when they are pushed.



- Ions with lower kinetic energy penetrate more shallowly into the reflectron **focusing ions with different kinetic energies** in terms of initial position for their return flight.

Reflectron, Example

- Reflectrons help resolution!



Basic Principles of TOF

- Lets take a look at our 'kinetic energy after acceleration in an electric field' equation:

