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KOŁO NAUKOWE CHEMIKÓW UWr JEŻ

Application of Ion Cyclotron Resonance Mass Spectrometry in Peptides Analysis

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Contents

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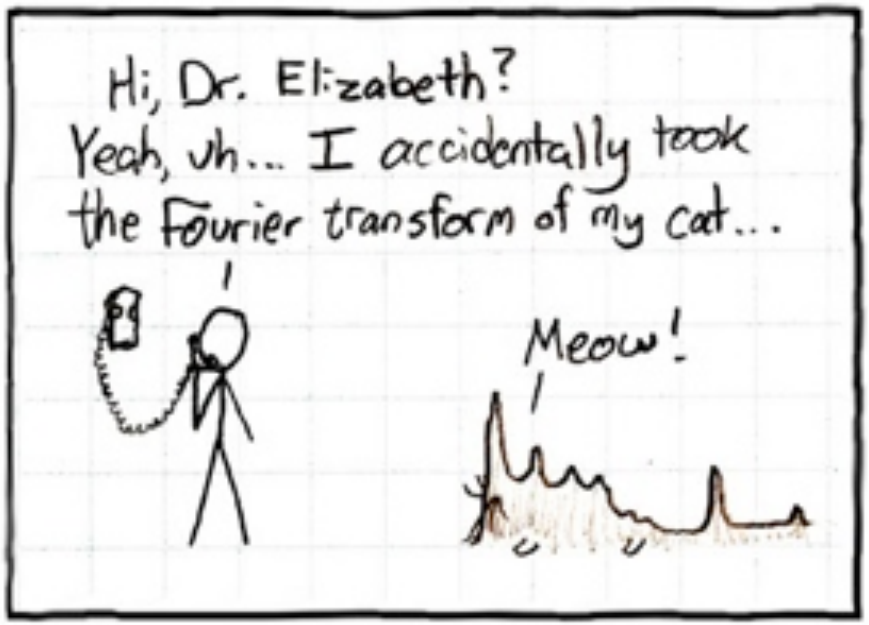
FT-ICR-MS

Fourier Transform Ion Cyclotron Resonance Mass Spectrometer

Bruker Daltonics Apex Ultra 7 T Actively Shielded

FT-ICR-MS

- A kind of mass spectrometer, which analyses the ions by their cyclotron frequencies in a homogeneous magnetic field using the Fourier Transform
- It was developed in 1974 by Comisarov and Marshall

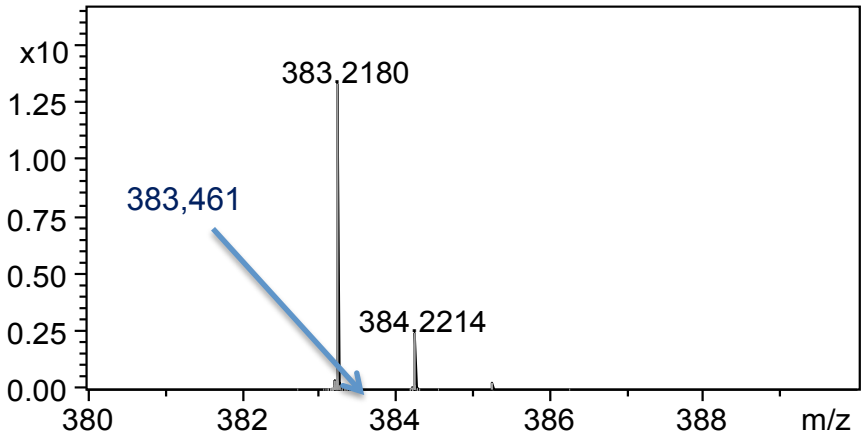


Comisarow M., Marshall A., Fourier transform ion cyclotron resonance spectroscopy, Chem. Phys Lett. 1974, 25, 282-283

https://xkcd.com/26/

Mass spectrometry

- Analytical technique in which the ions mass to charge ratio (*m/z)* is measured by mass spectrometer
- The obtained mass spectrum is a plot of ions abundance as a function of *m/z* values
- The mass spectrum does not show the average mass of ion but its monoisotopic mass thus we can observe the isotopic peaks distribution

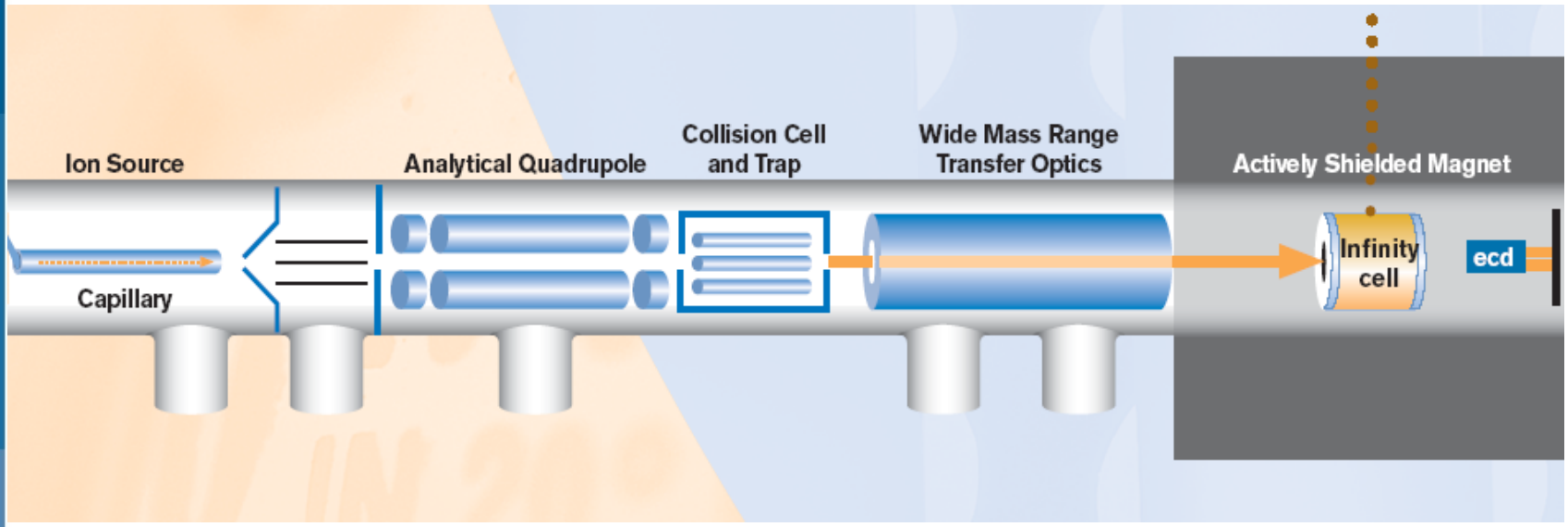


average mass

Magdalena Wierzbicka, New isotopic tags in proteomics research, Bachelor Thesis, Wroclaw 2015

Mass spectrometer

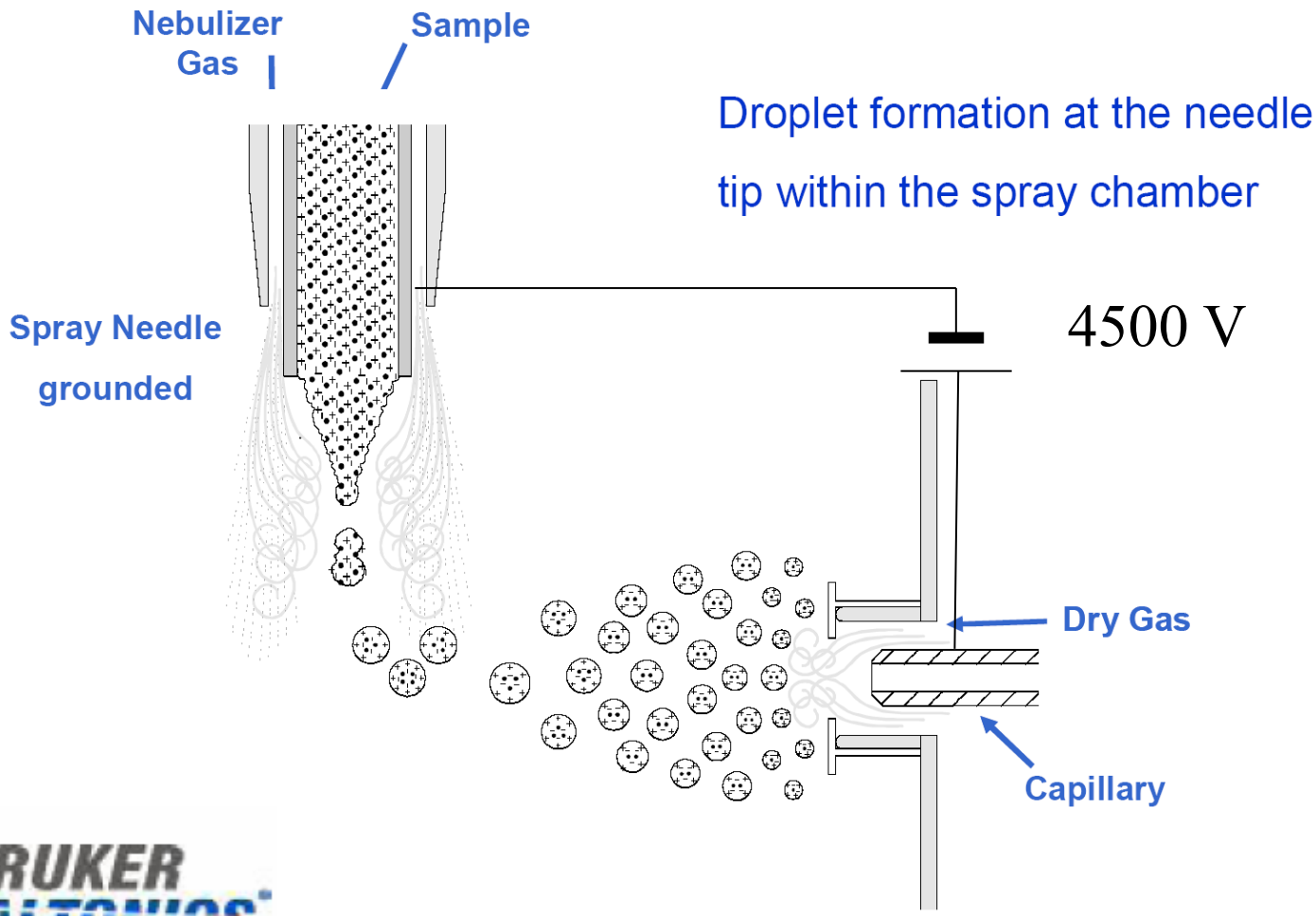
The scheme of FT-ICR Apex Ultra 7 T instrument (Bruker Daltonics, Brema, Germany)



Fourier Transform Mass Spectrometry Apex-Qe, A training course, Bruker Daltonics, 2007

Ion source

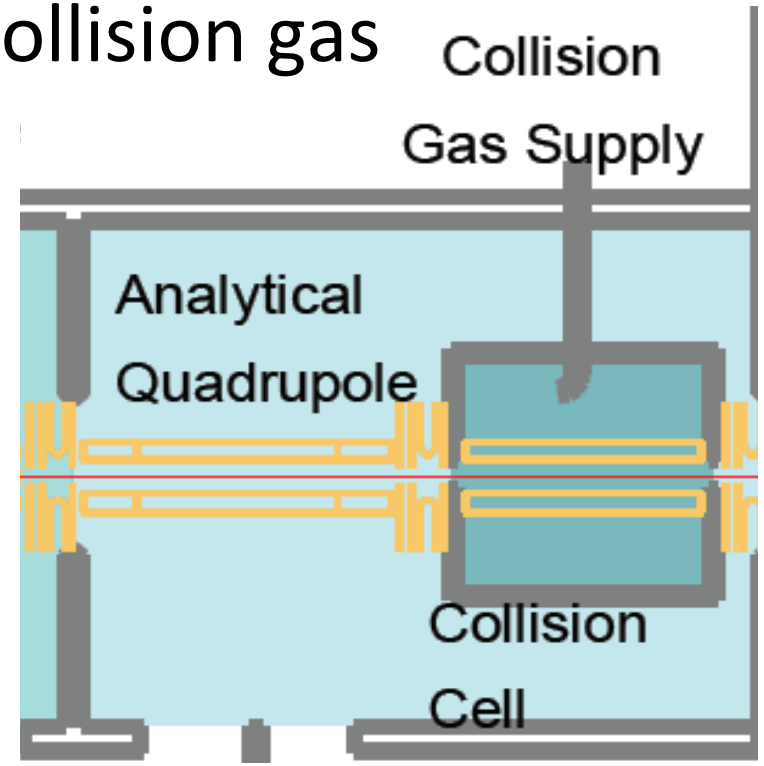
Ionization technique – Electrospray (ESI)



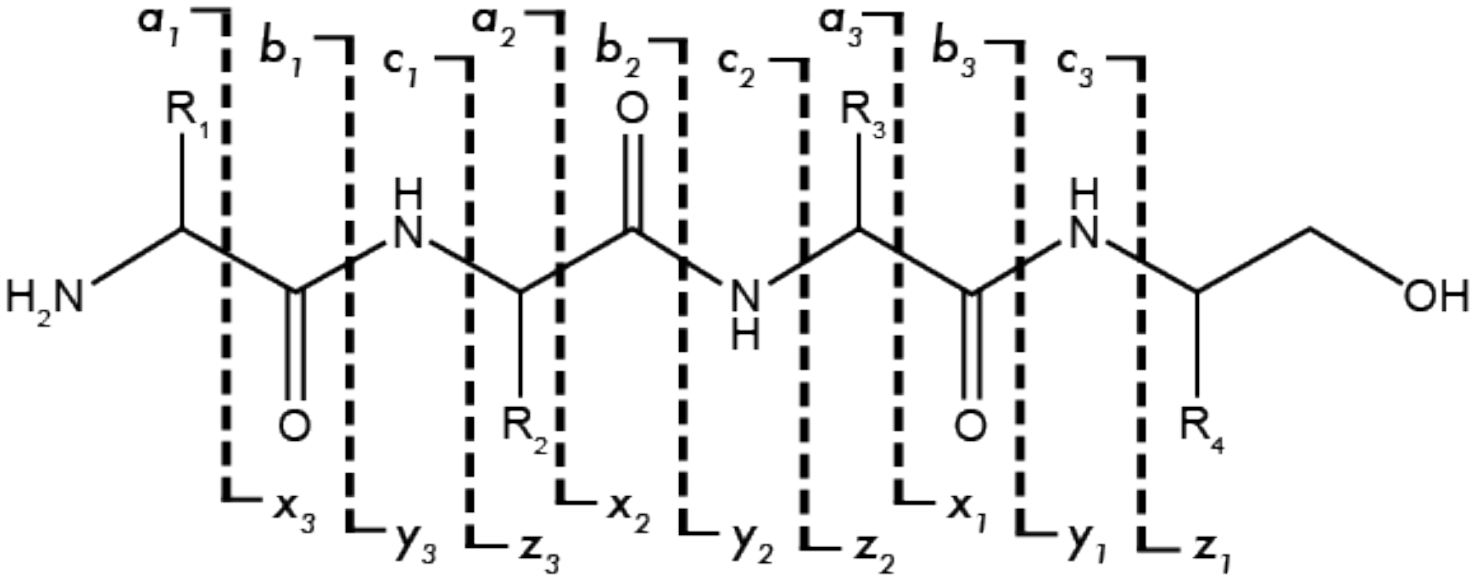
BRUKER DALTONICS

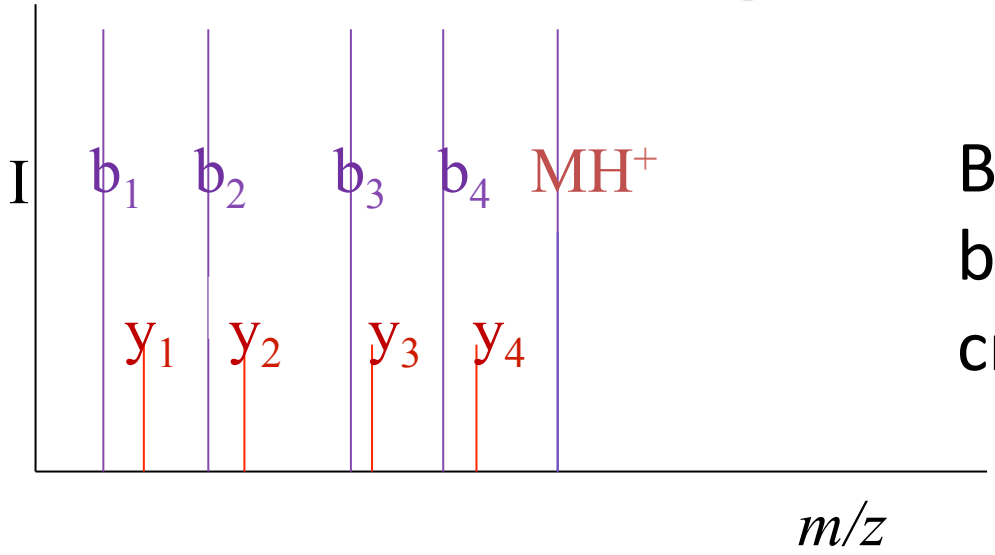
Collision cell

- To facilitate sample analysis, the selected ions can be fragmented into smaller ions (tandem mass spectrometry, MS/MS), using collision induced dissociation CID
- Argon is used as a collision gas



MS/MS



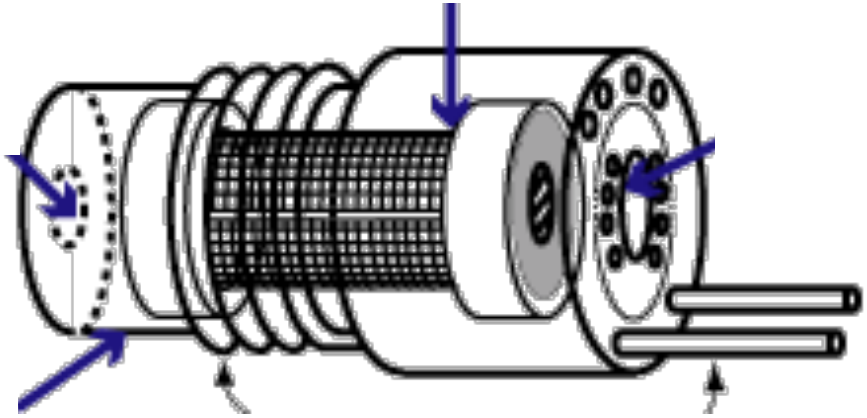


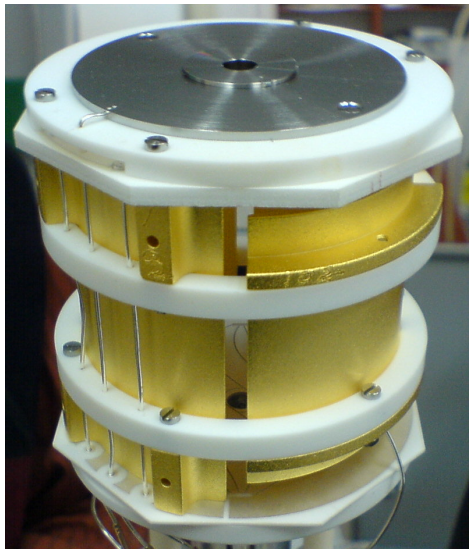
By CID fragmentation b and y type ions are created

Roepstorff P, Fohlman J (1984). "Proposal for a common nomenclature for sequence ions in mass spectra of peptides". Biomed. Mass Spectrom. 11 (11): 601

ICR

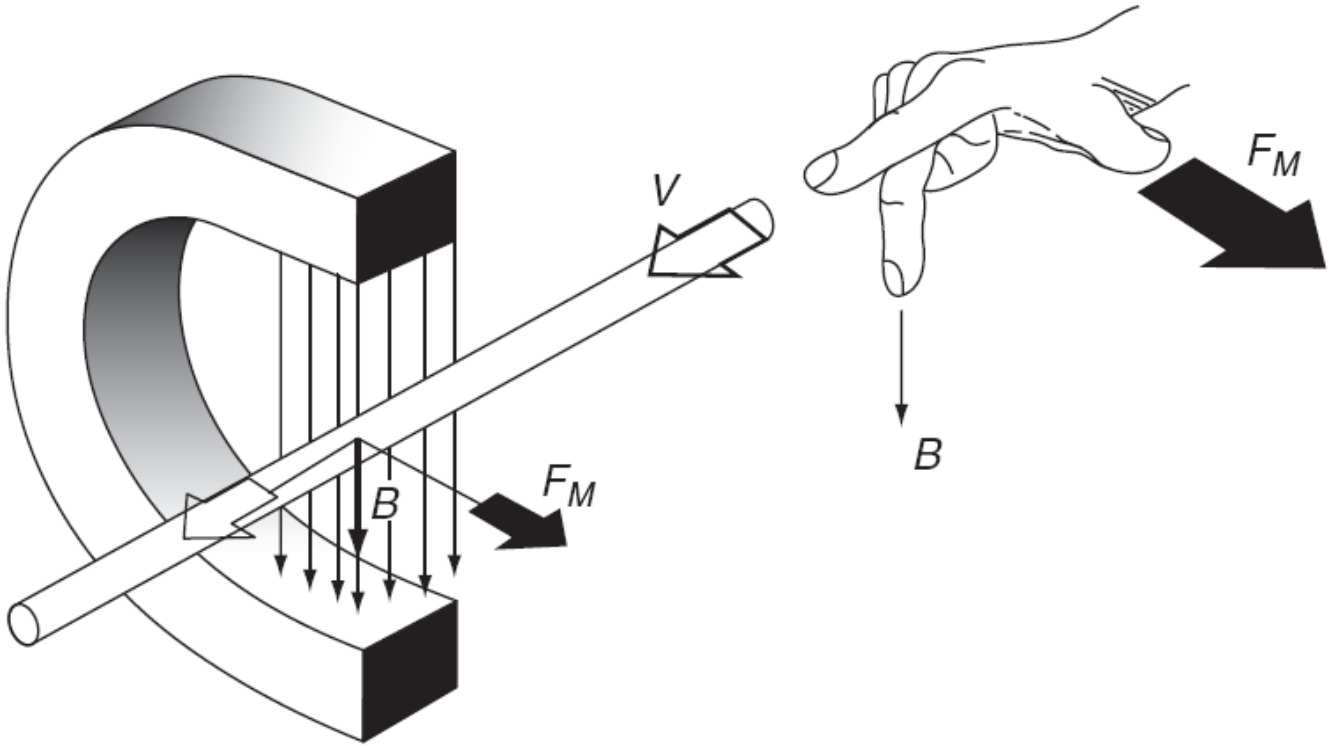
- It works using a superconducting magnet which generates the homogenous magnetic field with the force from 3 to 12 T
- Magnet is cooled by liquid helium
- Inside the magnet there is a FT-ICR trap, which plays the role of ion analyzer and detector
- The entire system is also cooled by liquid nitrogen
- The measurement of *m/z* is based on the ions cyclotron frequencies





Cyclotron frequency

In a magnetic field the charged particle undergoes cyclotron motion and its radius depends on the strength B of magnetic field and the ion velocity v



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Cyclotron frequency

- Moving ion experiences two forces:
- The Lorentz force:
- The centrifugal force:
- If the ion trajectory is stable, these two forces are equal:

$$F = zvB$$

$$F = \frac{mv^2}{r}$$

$$zvB = \frac{mv^2}{r}$$

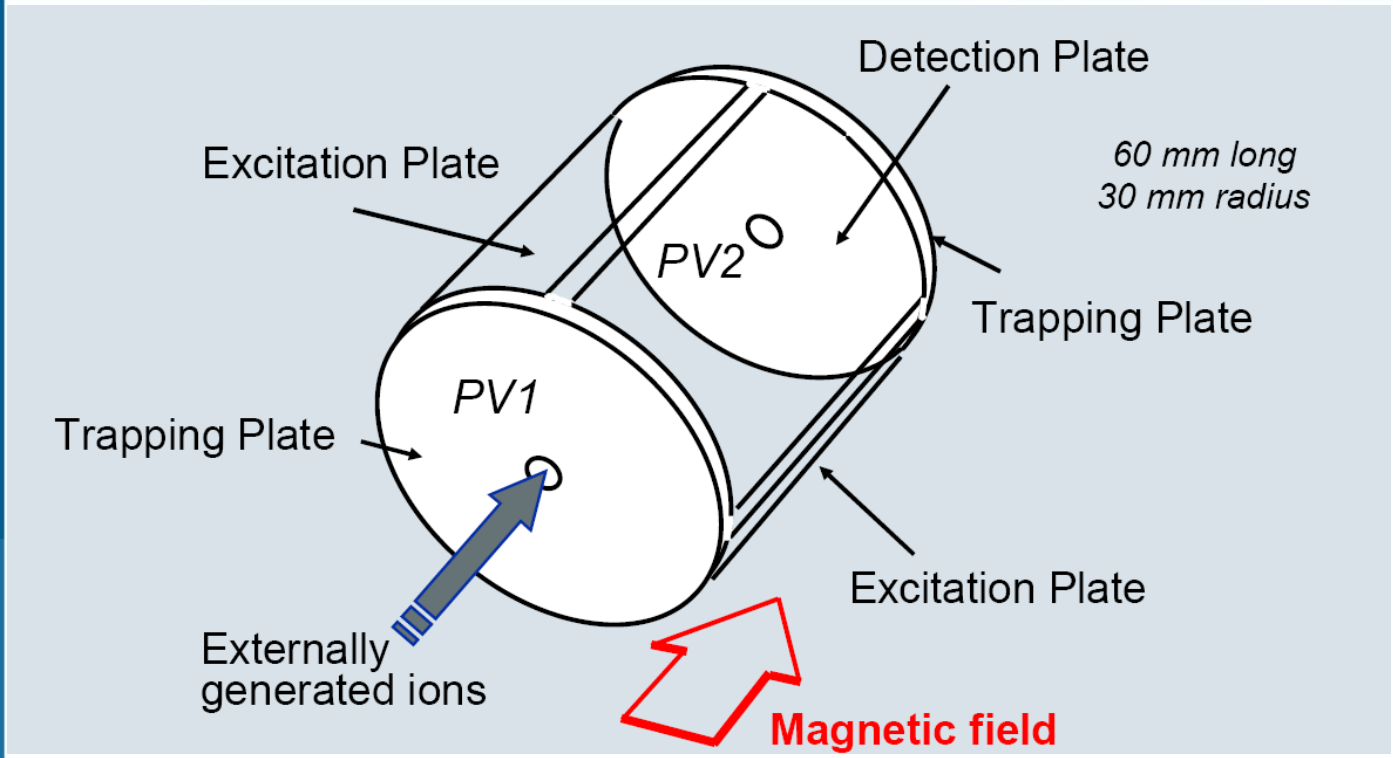
$$f = \frac{v}{2\pi r}$$

- The ion frequency will be:
- Using SI system the *f* will be in Hz, the *B* in T and the *m* in u, so we will obtain the equation of cyclotron frequency

$$f = \frac{z}{m} \cdot \frac{B}{2\pi}$$

$$f = \frac{1{,}535611 \cdot 10^7 B}{m/z}$$

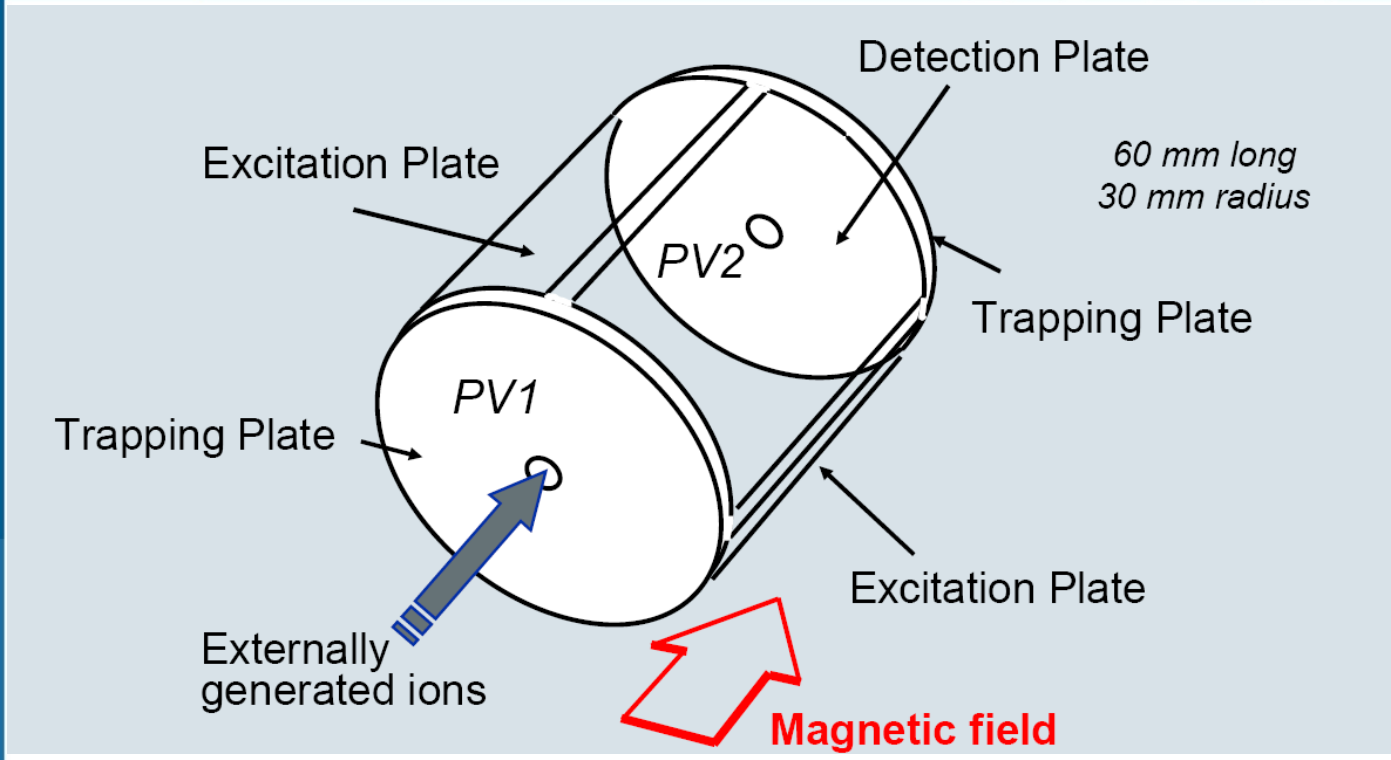
FTICR trap



Fourier Transform Mass Spectrometry Apex-Qe, A training course, Bruker Daltonics, 2007

- Consists of three pairs of metal plates
- Inside the trap, an ultra-high vacuum (10^{-12} Ba) is maintained, allowing the ions collision-free motions
- There could be 10 – 10^6 ions in the trap – the presence of more ions causes the space charge effect which provides to the ion suppression

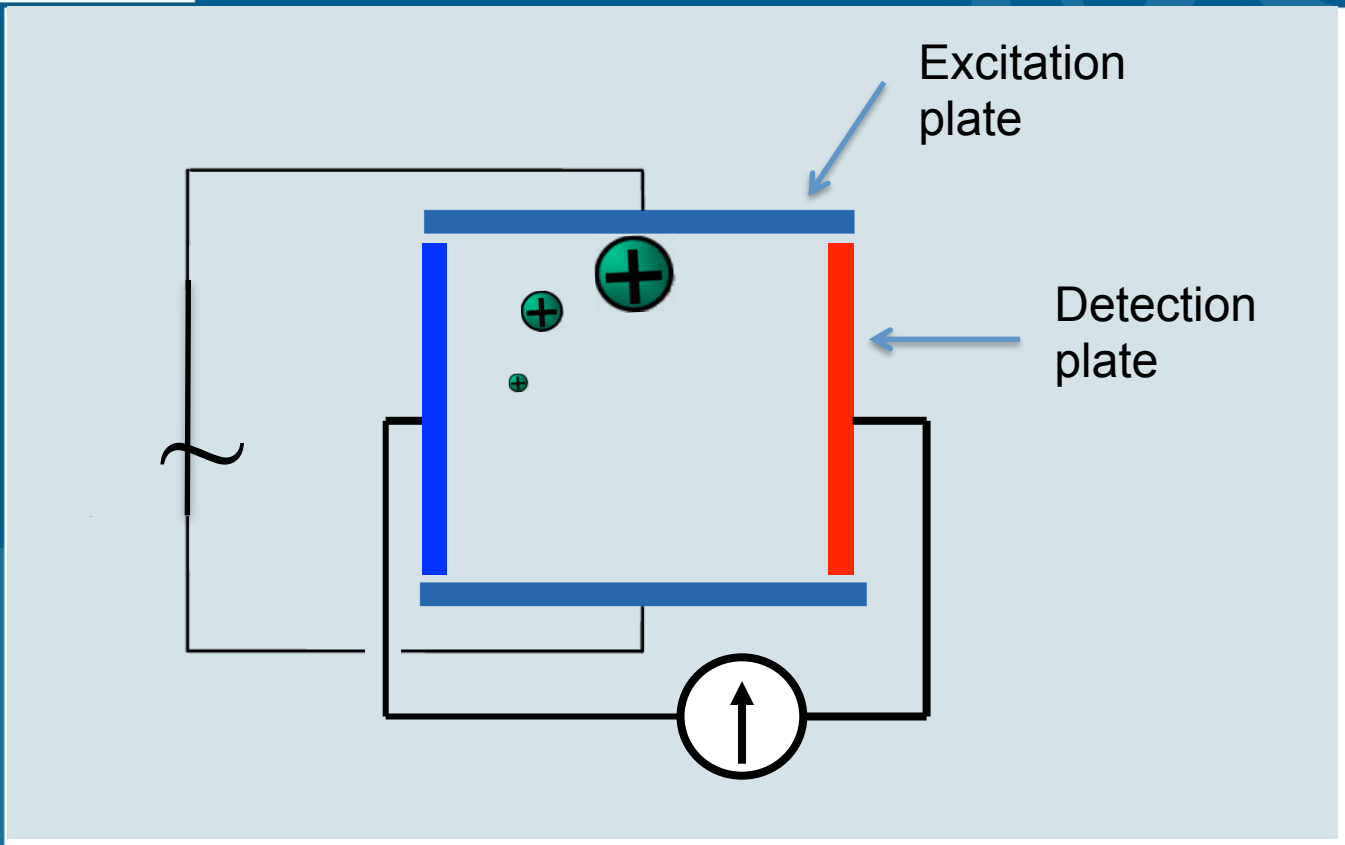
FTICR trap



Fourier Transform Mass Spectrometry Apex-Qe, A training course, Bruker Daltonics, 2007

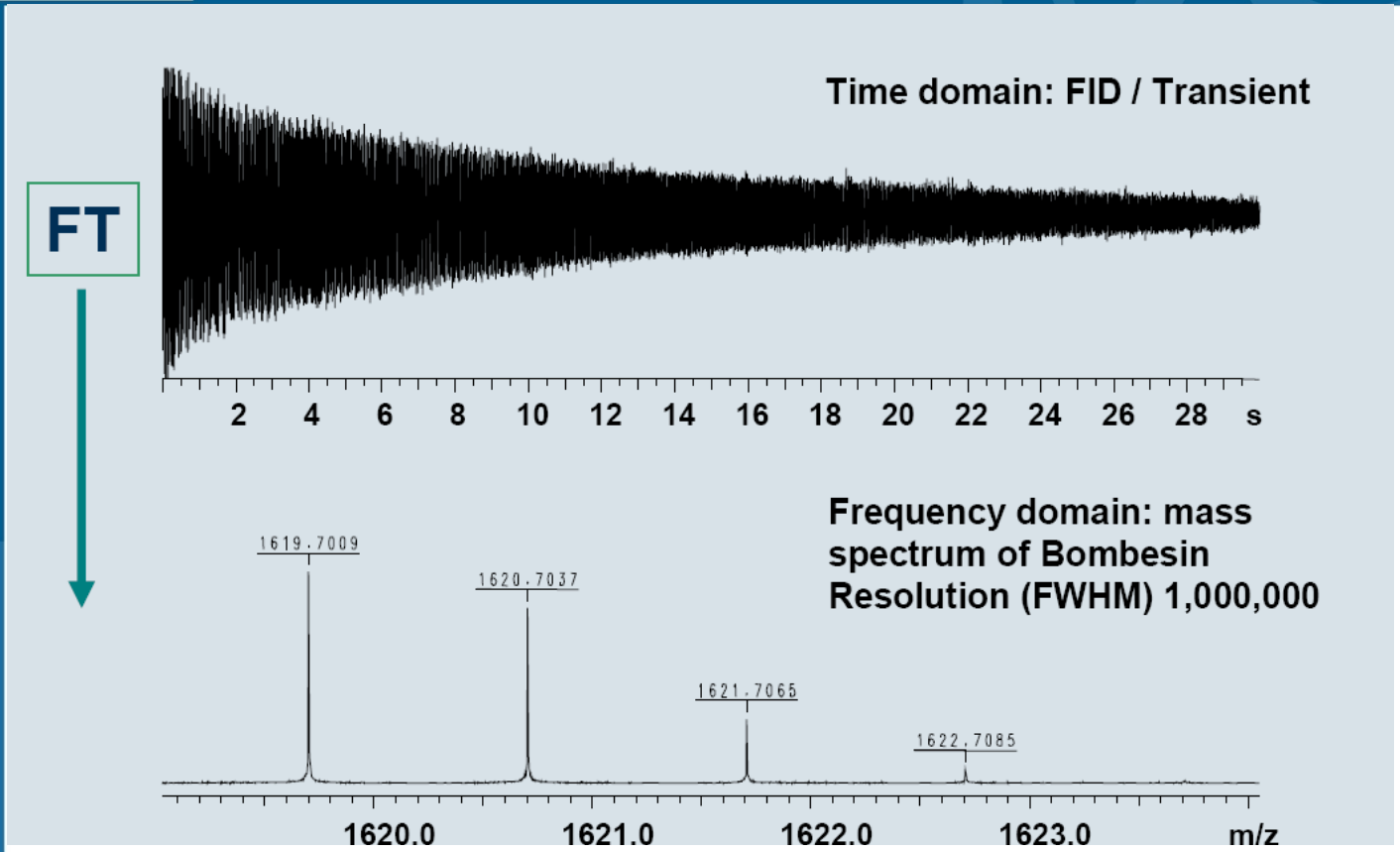
- The ion packet in the trap is excited to a larger orbit by applying an alternating field
- After the excitations, electric field is turned off and then it is possible to detect the ions by detection plates

Detection



- When the cation packet is closing to the detection plate, it attracts electrons from it and when this cation packet is receding from the plate, electrons are coming back to their previous positions
- It causes the small flow of alternating current between two detection plates, which is reinforced and analyzed

Detection and Fourier Transform



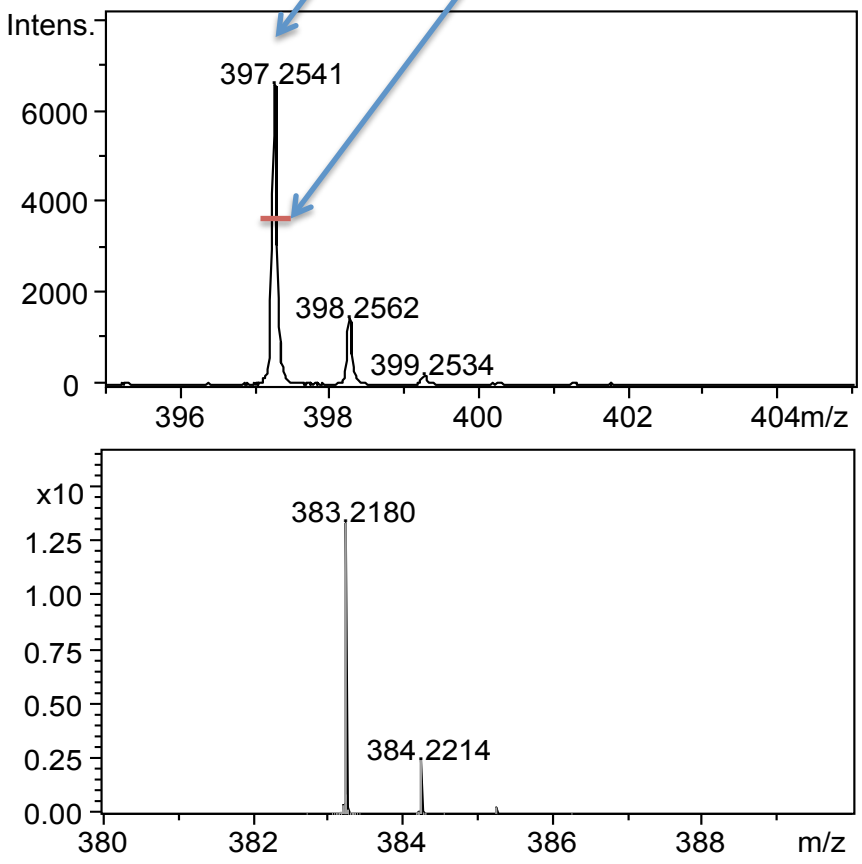
Fourier Transform Mass Spectrometry Apex-Qe, A training course, Bruker Daltonics, 2007

- The signal obtained from detection plates is a superpostion of frequencies with different amplitudes of different ions
- This signal is converted into the mass spectrum by Fourier Transform

Advantages of FT-ICR-MS

- High resolving power
  - ≈ 100 000 FWHM
- High mass accuracy
  - < 1,5 ppm (internal calibration)
  - < 2,0 ppm (external calibration)
- Wide *m/z* measuring range
  - from 20 to 4000 *m/z*
- High sensitivity
  - ≈ 1 fmol

$$RP = \frac{m/z}{\Delta(m/z)_{FWHM}}$$



Magdalena Wierzbicka, New isotopic tags in proteomics research, Bachelor thesis, Wroclaw 2015

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FT-ICR-MS analysis of ASN^{+}-MQIFVKT-OH

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Objectives

I. Synthesis of ASN^{+}-MQIFVKT-OH by Fmoc procedure

II. ESI-MS analysis of the synthesized peptide conjugate

III. The examination of hydrogen-deuterium exchange (HDX) on the α-carbon of derivatized proline residue by ESI-FT-ICR-MS

IV. MS/MS analysis of the isotopically labeled product: ASN^{+}-MQIFVKT-OH (*d*)

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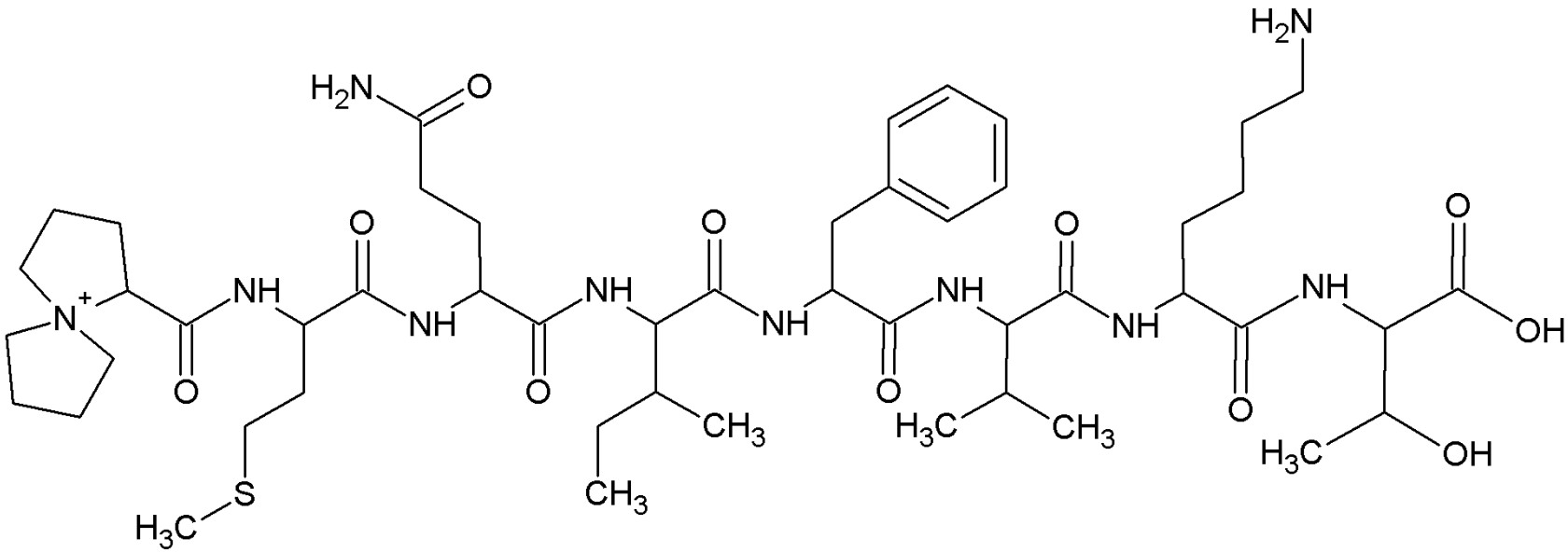
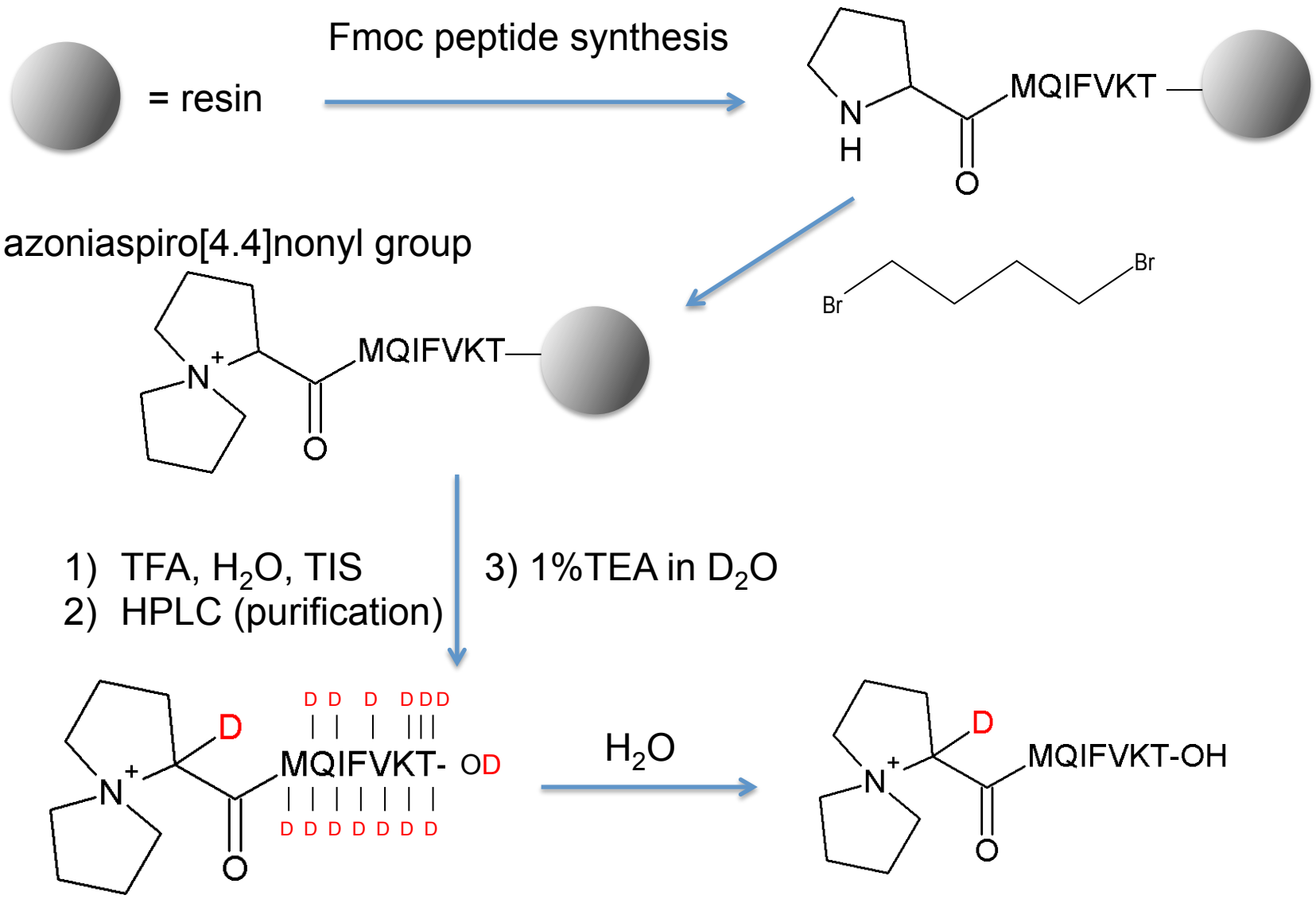
Synthesized peptide conjugate



ASN^{+}-MQIFVKT-OH

Solid phase synthesis



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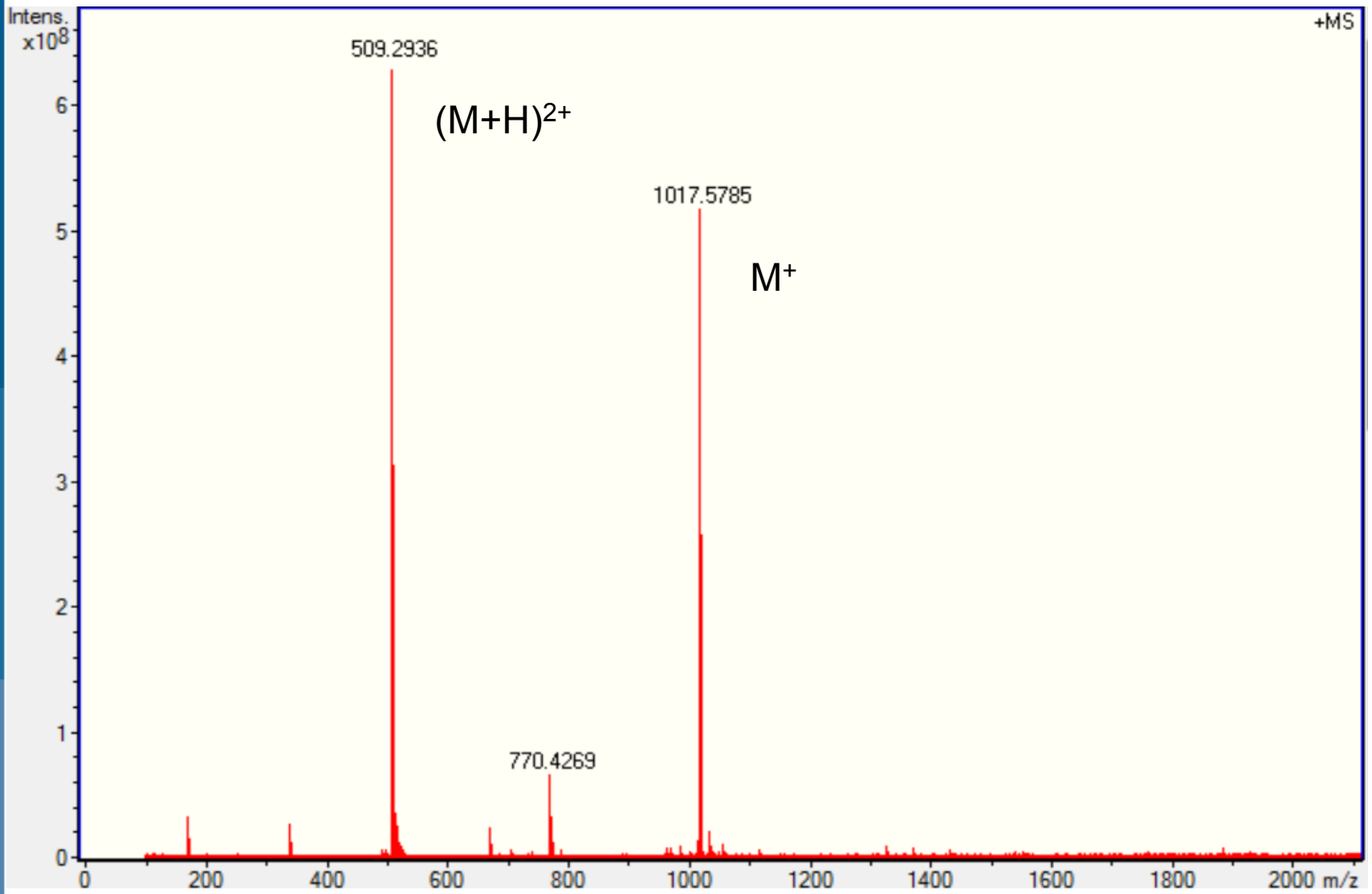
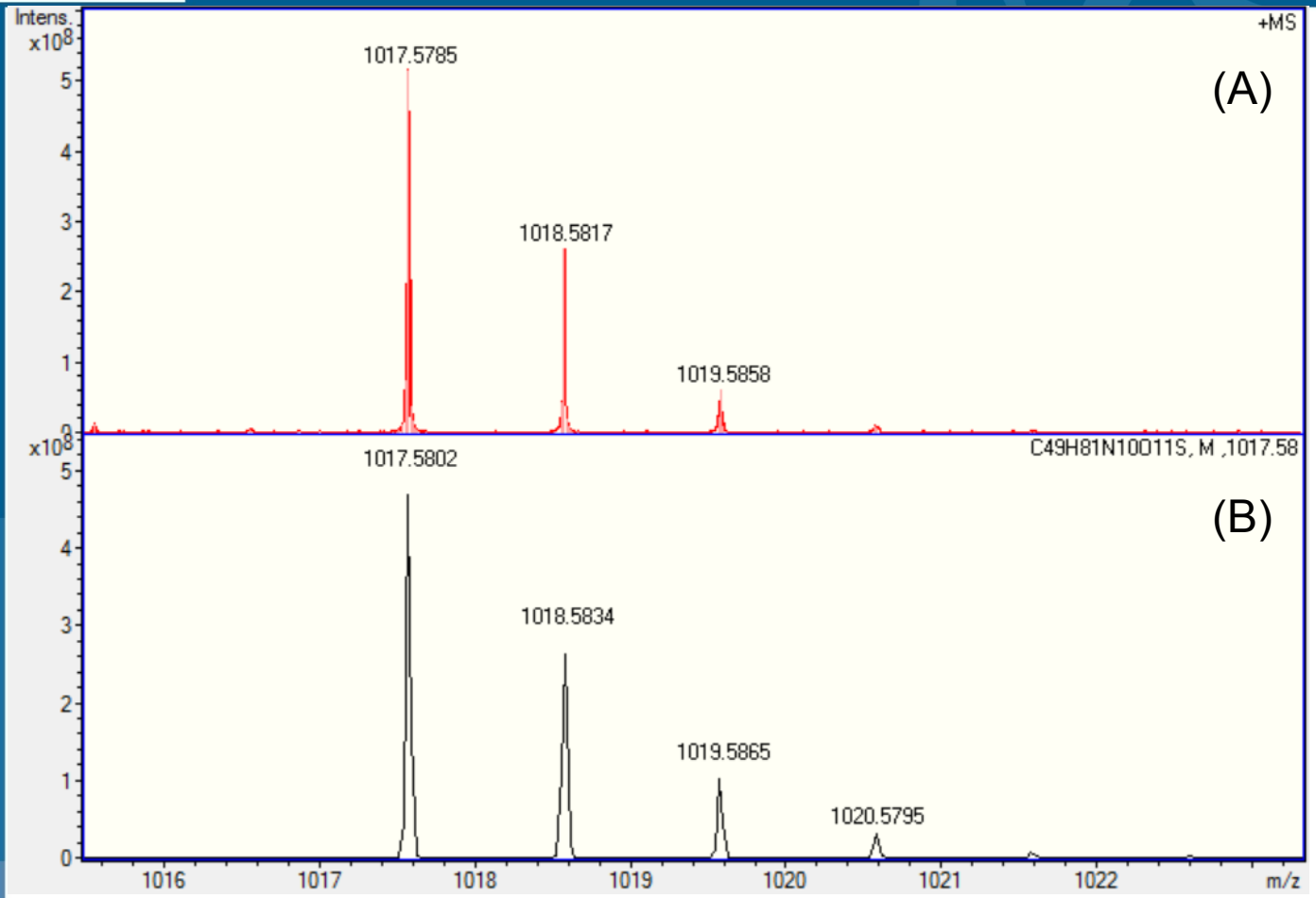
ESI-MS analysis



ESI-MS analysis



- Peptide isotopic envelope: experimental (A) and simulated (B)
- High mass accuracy: 1,67 ppm
- Resolving power: 70 000 FWHM

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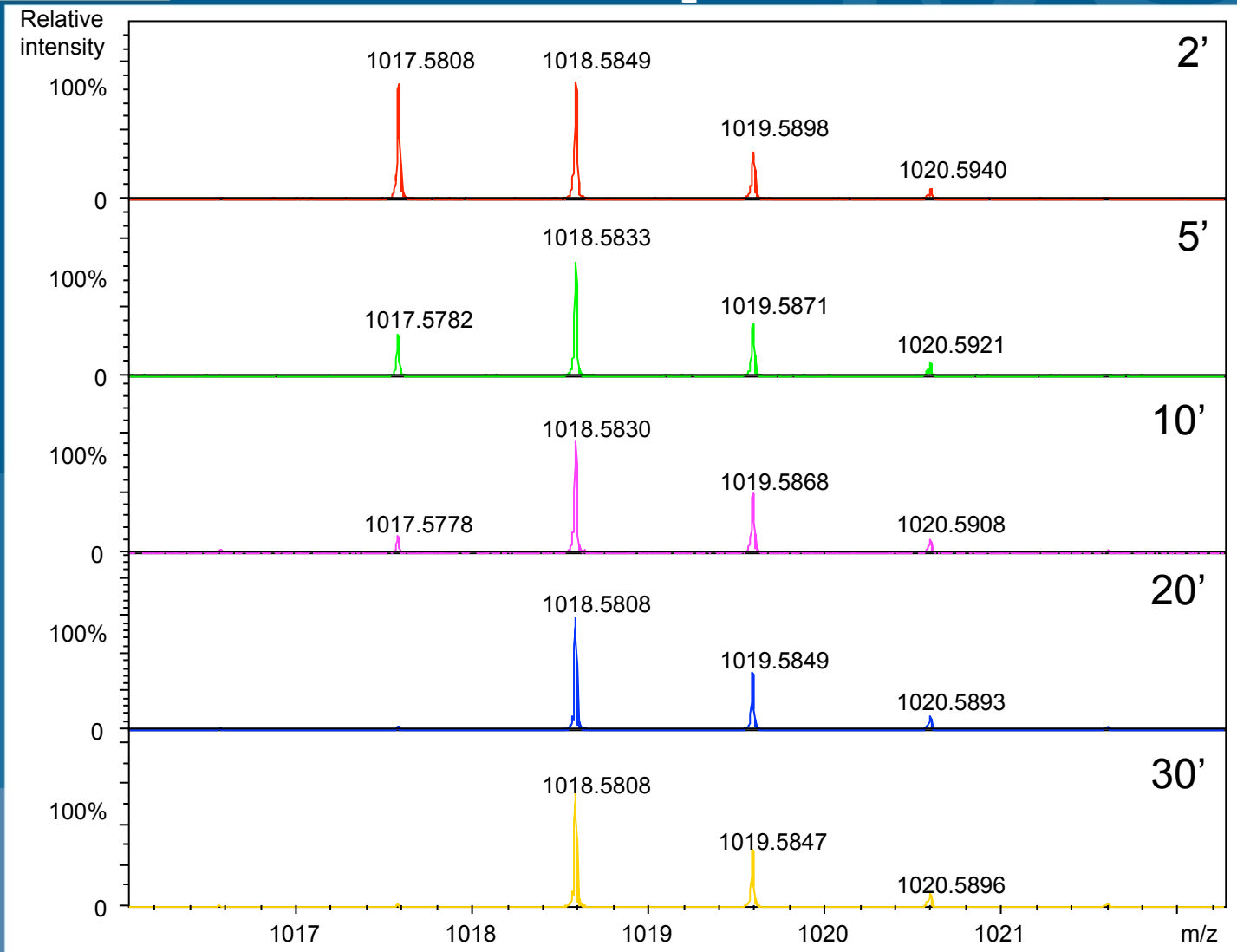
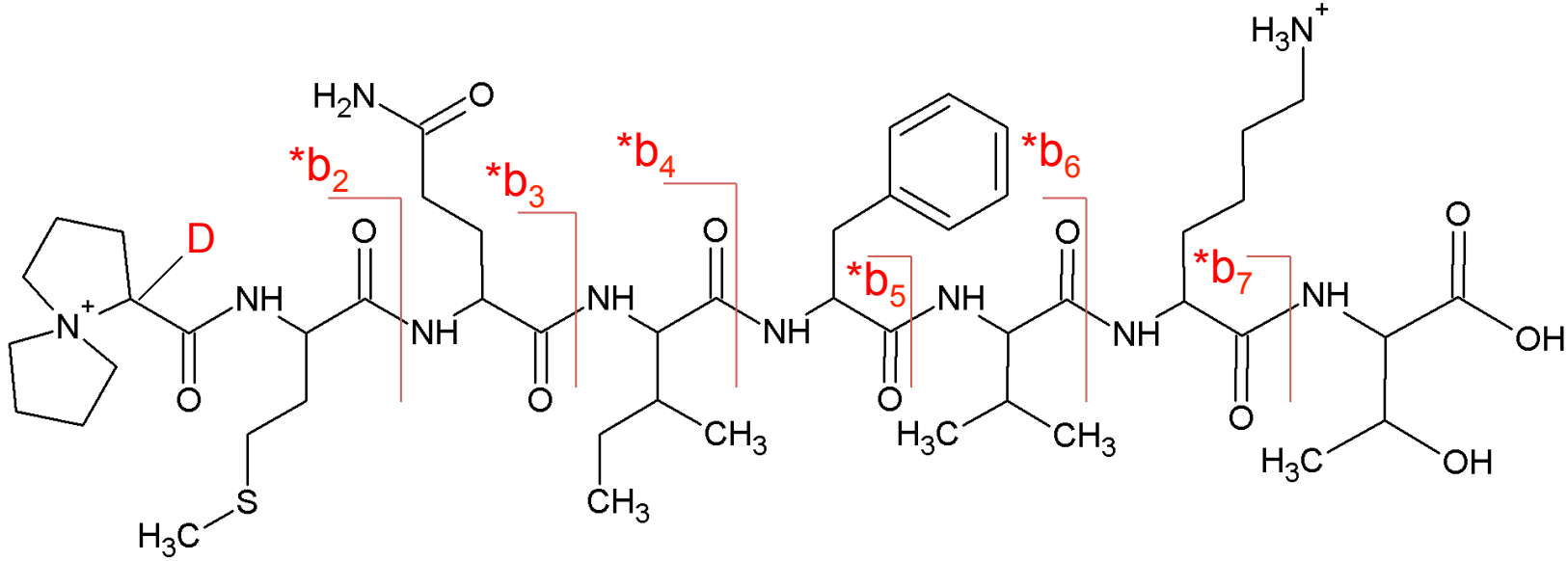
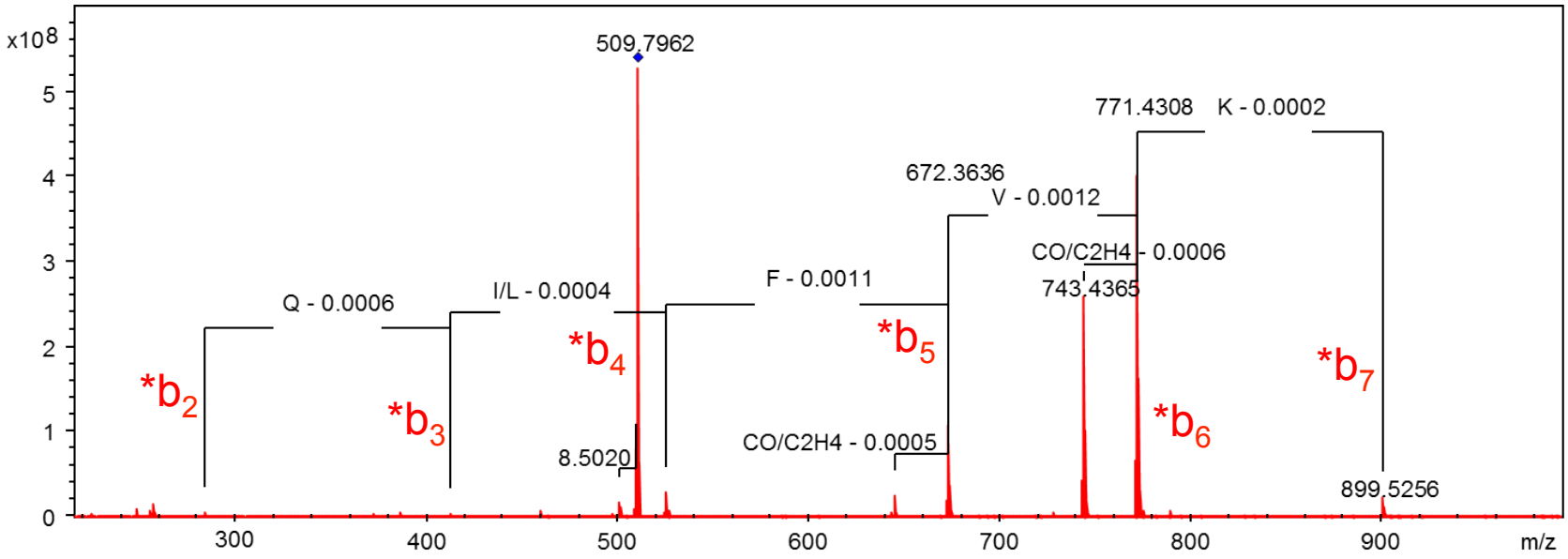
Hydrogen-deuterium exchange in 1% TEA/D_2O, followed by back exchange in H_2O



Full hydrogen-deuterium exchange can be observed after 20 minutes of incubation in 1% TEA/D_2O

MS/MS analysis (parent ion *m/z* 509.8)





On MS/MS spectrum *b type ions are mostly observed

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Conclusions

- Peptide conjugate was synthesized and its structure was confirmed by ESI-MS analysis
- Proline residue was converted into azoniaspiro[4.4]nonyl group, creating the ionization tag
- In the solution of 1% TEA in D_2O, 15 protons undergo hydrogen-deuterium exchange (HDX)
- Labile deuterium atoms like in an amide, a carboxyl and a hydroxyl groups undergo back-exchange in water solution at neutral pH

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Conclusions

- The hydrogen on α-carbon of proline residue undergoes HDX in 1% TEA in D_2O but does not undergo back exchange in water solution
- MS/MS spectrum of peptide shows, that it fragments giving *b – type ions.

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References

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