

Improvements in Peptide Detection for Proteomics Using a Combined Miniaturised Field Asymmetric Ion Mobility-Mass Spectrometry Approach

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Overview

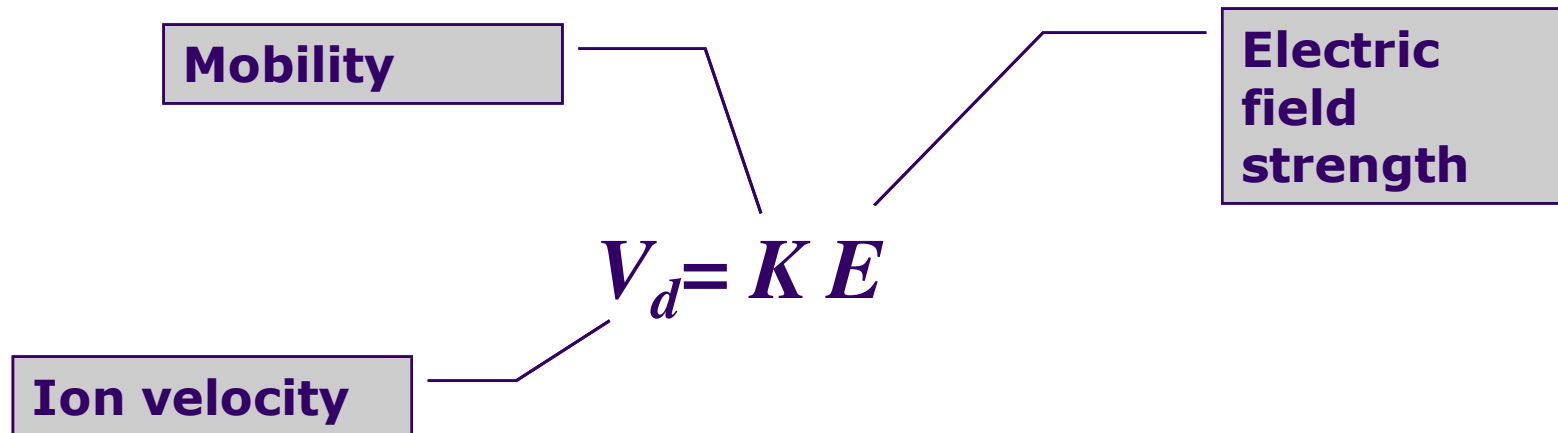
- **Background and aims of experiment**
- **Overview of FAIMS analysis**
- **Miniaturised FAIMS device**
- **Results**
- **Conclusions and further work**

Background and Aims of Experiment

- The identification of proteins is typically unachievable by mass spectrometry alone.
- MS/MS used or mass spectral data compared PMF generated by protein search engines.
- Electrospray ionisation produces multiply charged and isobaric ions which can reduce the confidence in protein identification.
- Pre-separation of gas phase peptide ions using a miniaturised FAIMS device has been evaluated.
 - increase selectivity of the analysis.
 - simplify mass spectral data.
 - improve confidence in rapid protein identification.

Ion Mobility

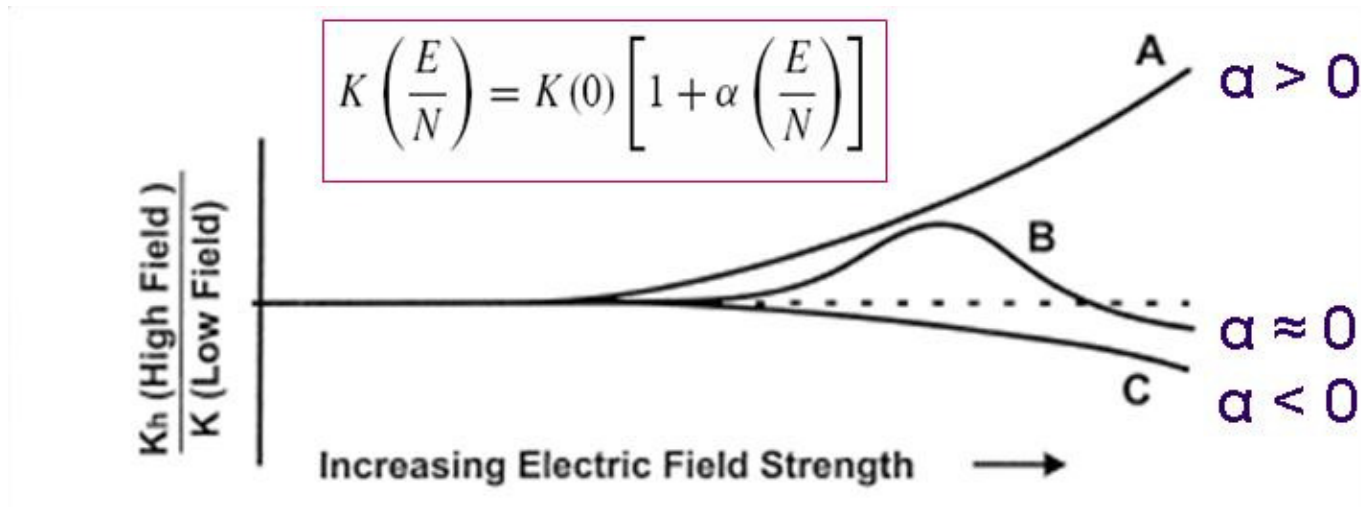
- The mobility of an ion under low field conditions can be calculated mathematically based on the following equation:



- Ions with a higher mobility (K) in an electric field, will move more quickly.

Principle of FAIMS

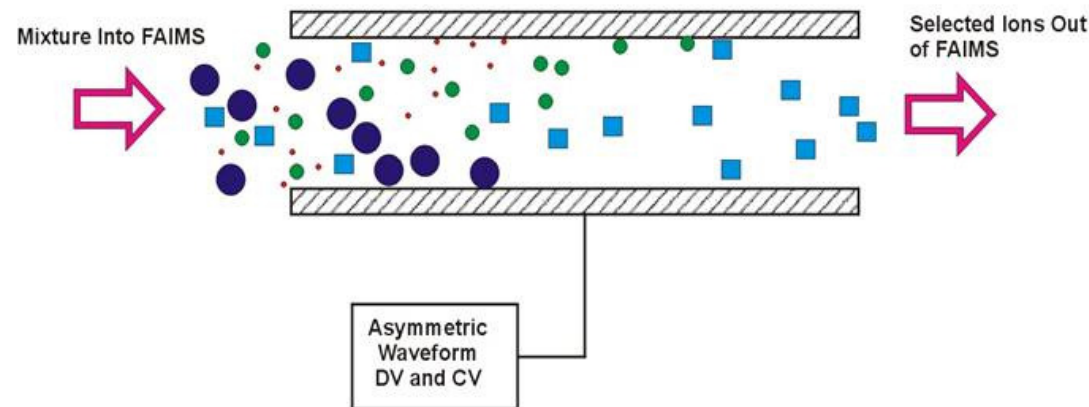
- FAIMS separates gas-phase ions on the basis of their differential mobilities (α) under alternating low and high field conditions.

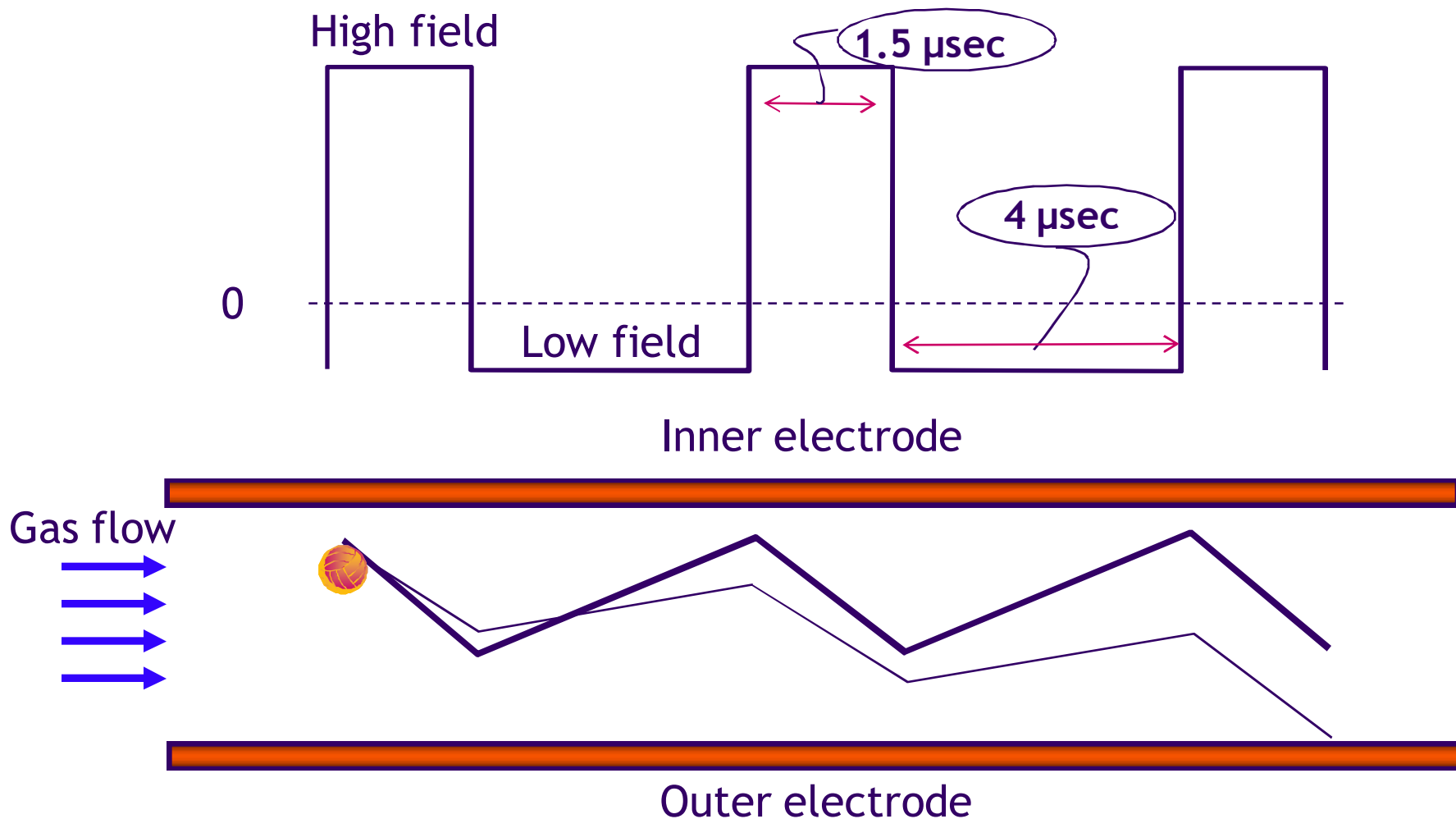


[Purves R W, Guevremont R, Anal. Chem. 1999, 71, 2346-2357]

What is FAIMS Analysis?

- Ions are selectively filtered as a function of the difference in the mobility in low and high electric fields.
- Ions are passed between electrodes, to which an alternating waveform is applied, causing ions to drift towards the electrode.
- A second voltage (CV) is superimposed to selectively allow analytes to pass through the ion filter electrodes.

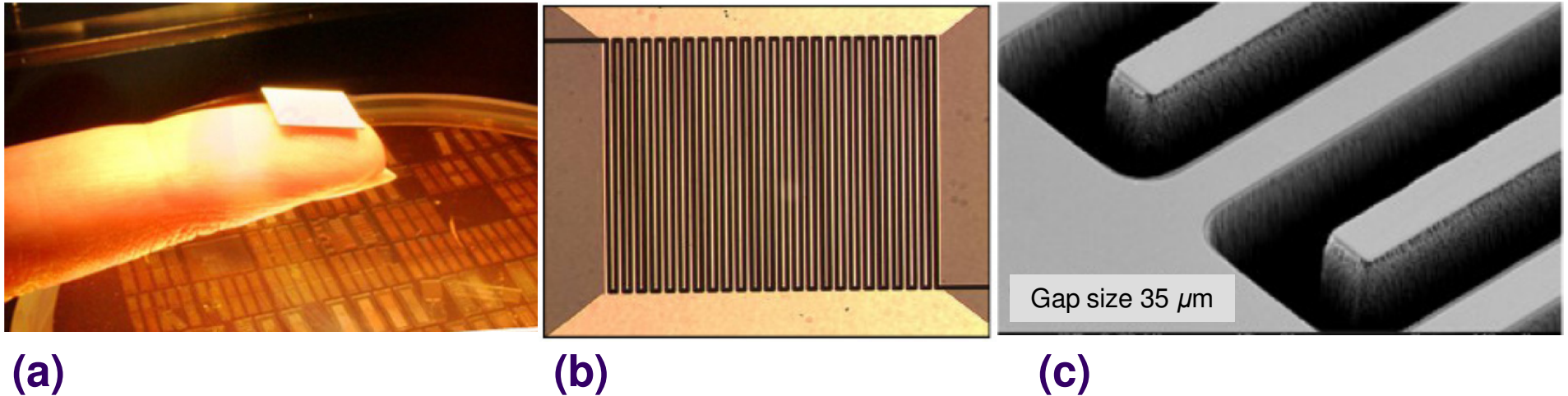




Inappropriate compensation voltage : orange ion is lost

With same compensation voltage superimposed : purple ion transmitted to MS

Owlstone FAIMS Technology

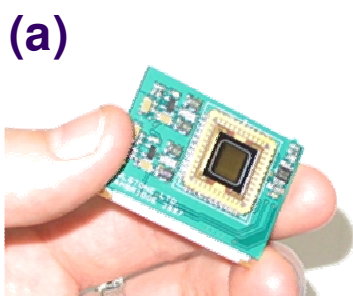


Advantages of miniaturisation include:

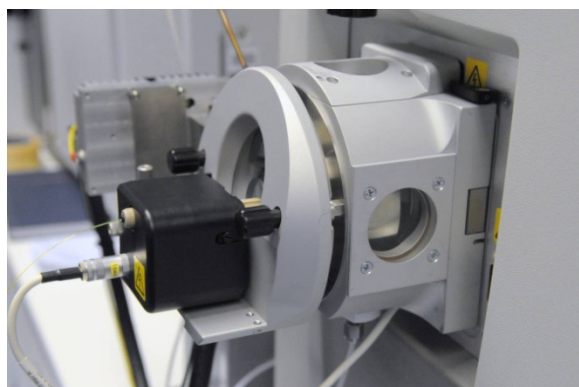
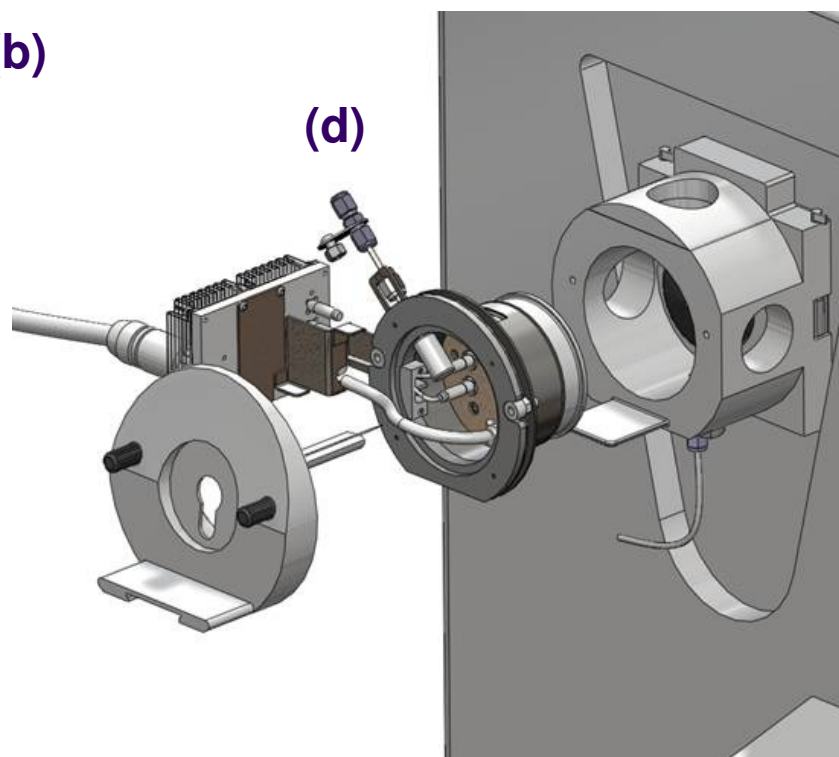
- Higher field strengths capability (75 kV cm^{-1}).
- Shorter ion residence time = higher speed of analysis.
- Cheaper to manufacture than conventional FAIMS devices.

FAIMS chip cartridge

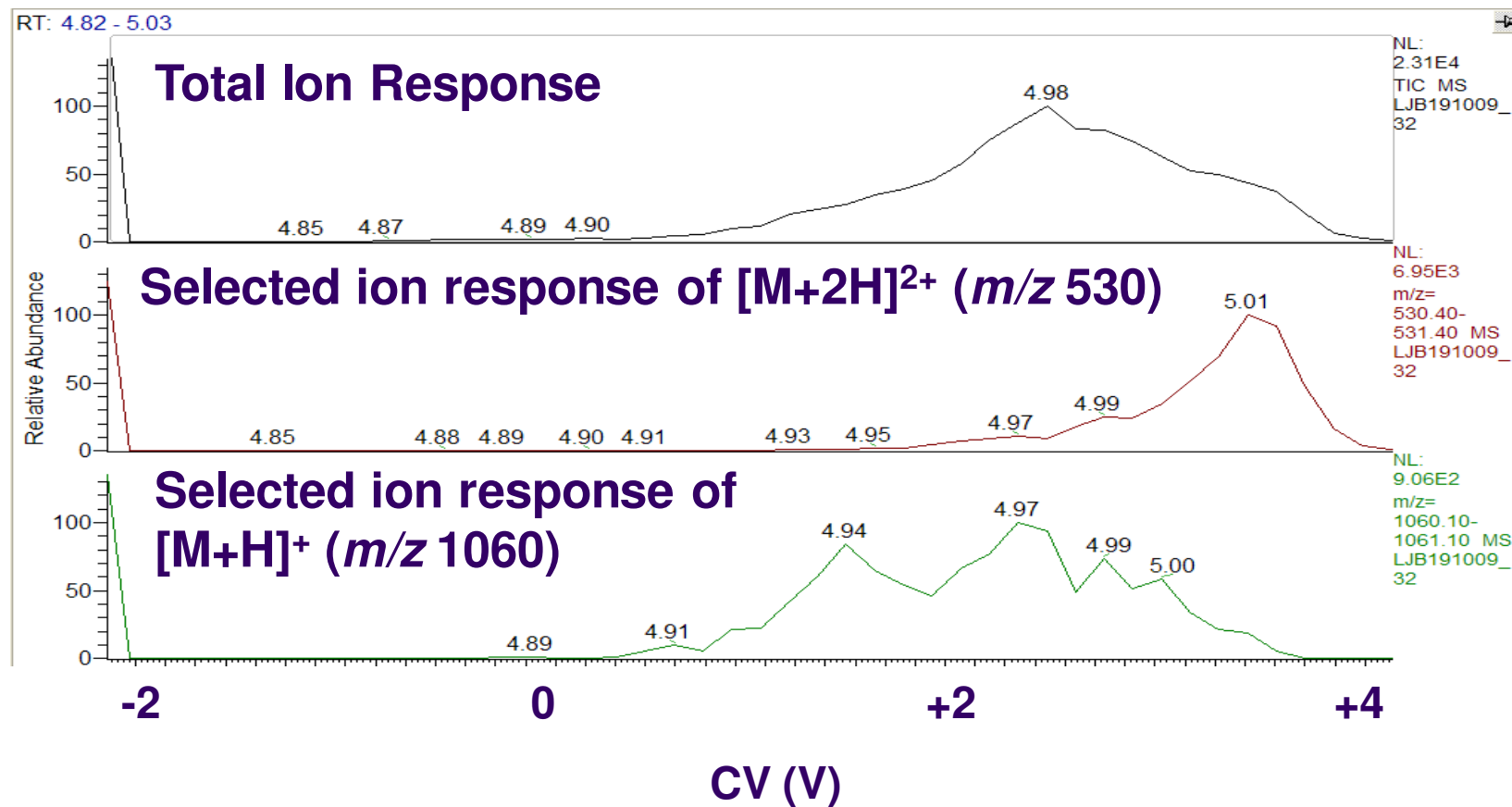
- The FAIMS chip fits inside the Thermo Fisher Scientific LTQ nanospray source housing, between the NSI source and MS.



(b)

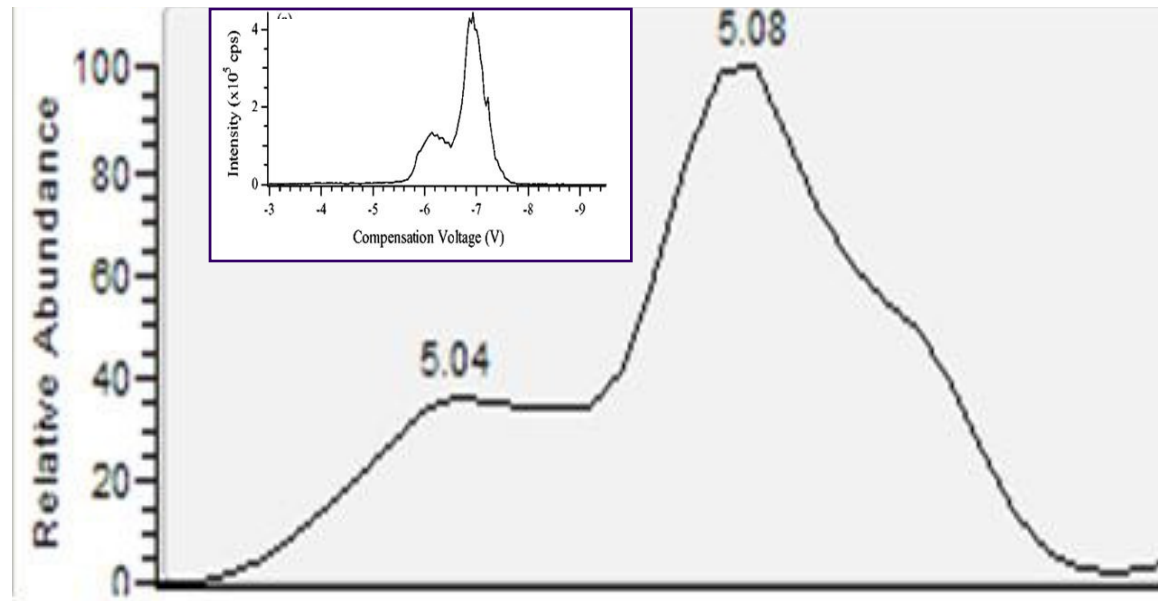


Charge state separation of Bradykinin (RPPGFSPFR) 10 pmol μL^{-1} using miniaturised FAIMS



Sweeping at 0.5v s^{-1} : total analysis time – 12secs.

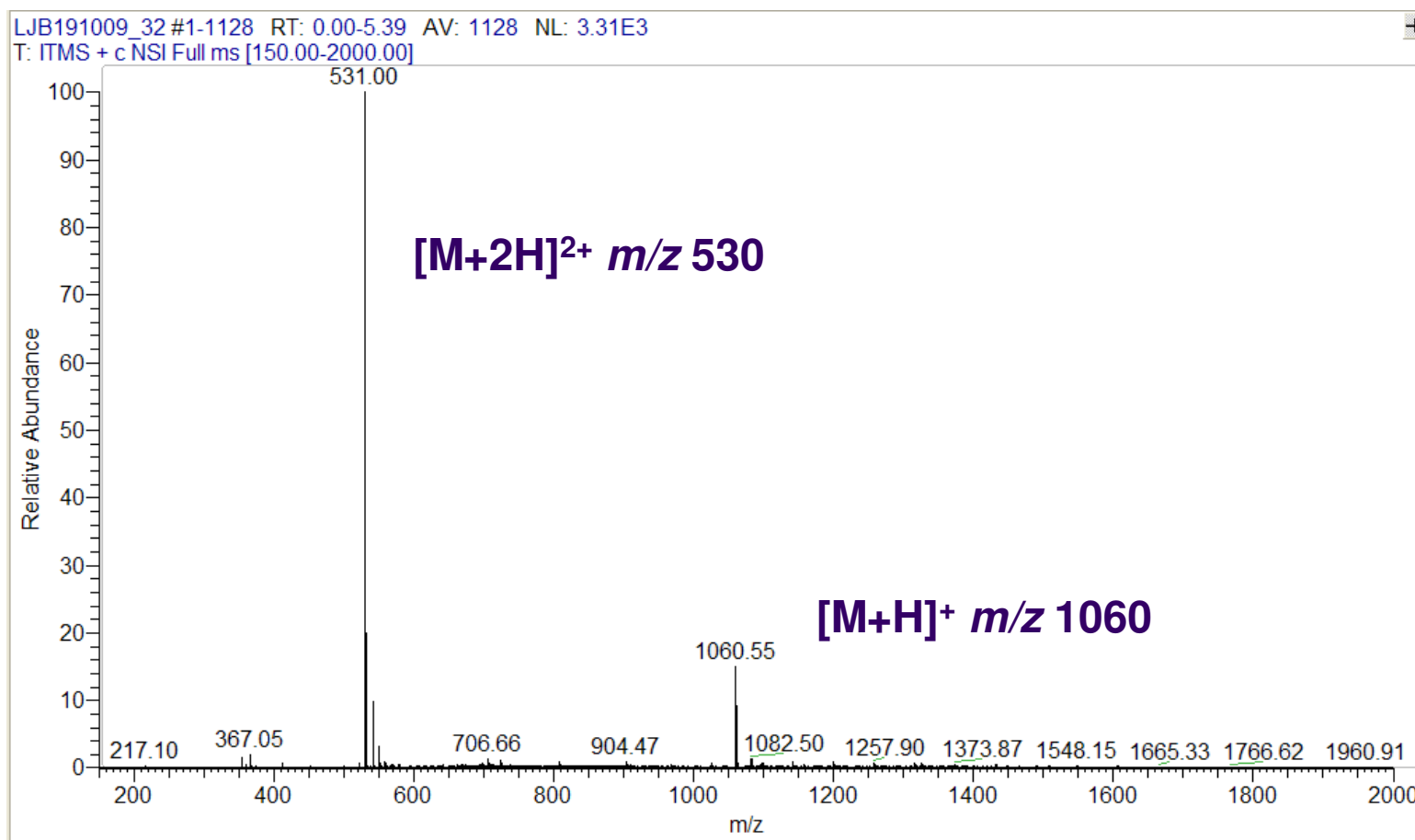
Separation of Conformers of $[M+2H]^{2+}$ ion of bradykinin



- The CV scan is similar to that reported for the FAIMS analysis of the $[M+2H]^{2+}$ ion of bradykinin using cylindrical electrodes.
- Demonstrates capability of miniaturised FAIMS for the identification of peptide conformations.

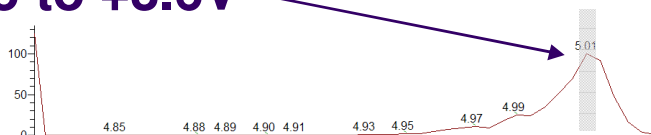
1. Purves, R. W., D. A. Barnett, et al., Rapid Commun. Mass Spectrom., 2001, 15, 1453-1456.

MS spectra without FAIMS analysis

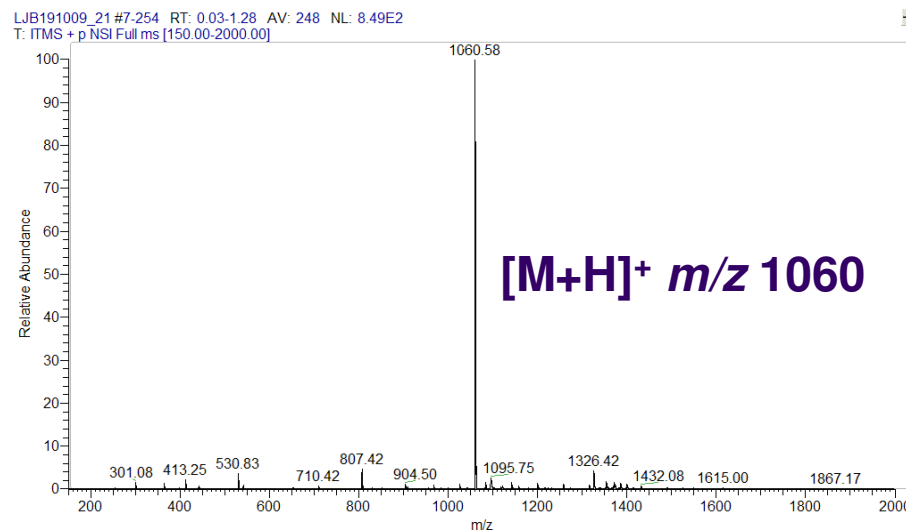
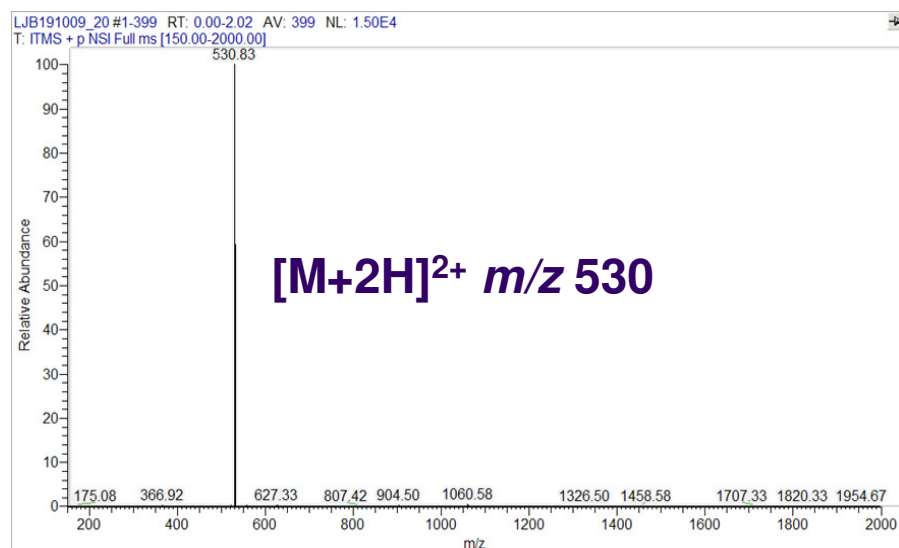
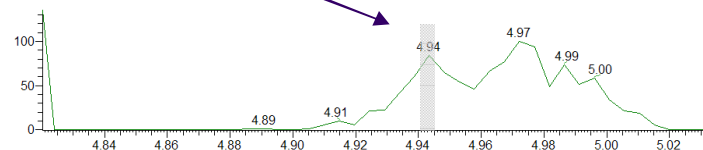


FAIMS-MS spectrum for each charge state

CV: +3.5 to +3.6V



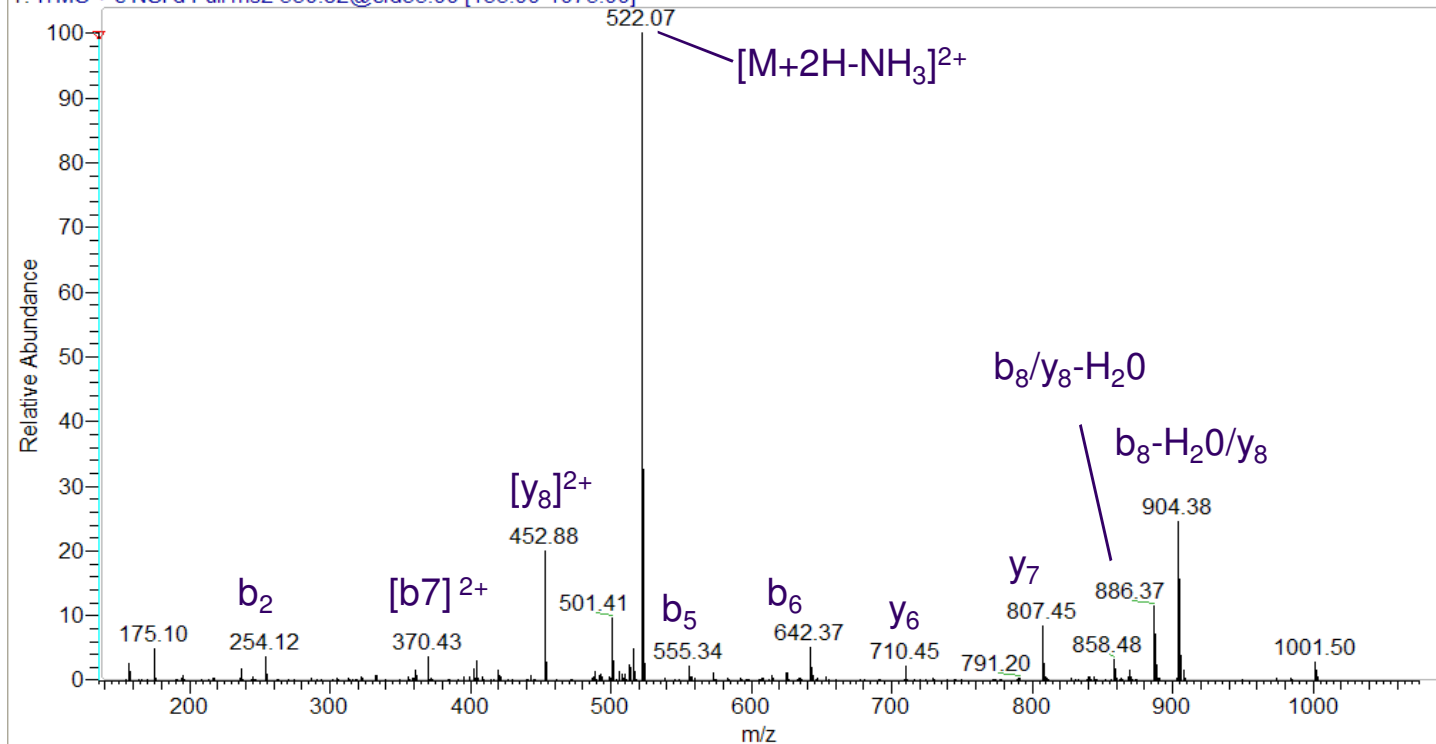
CV: +1.4 to +1.5V



➤ Optimum CV for transmission of each charge state applied (as determined from SIR).

FAIMS-MS² on [M+2H]²⁺ to confirm ion identity

LJB191009_25 #20 RT: 0.13 AV: 1 NL: 4.94E3
T: ITMS + c NSI d Full ms2 530.82@cid35.00 [135.00-1075.00]



MS²

Precursor ions
m/z 530

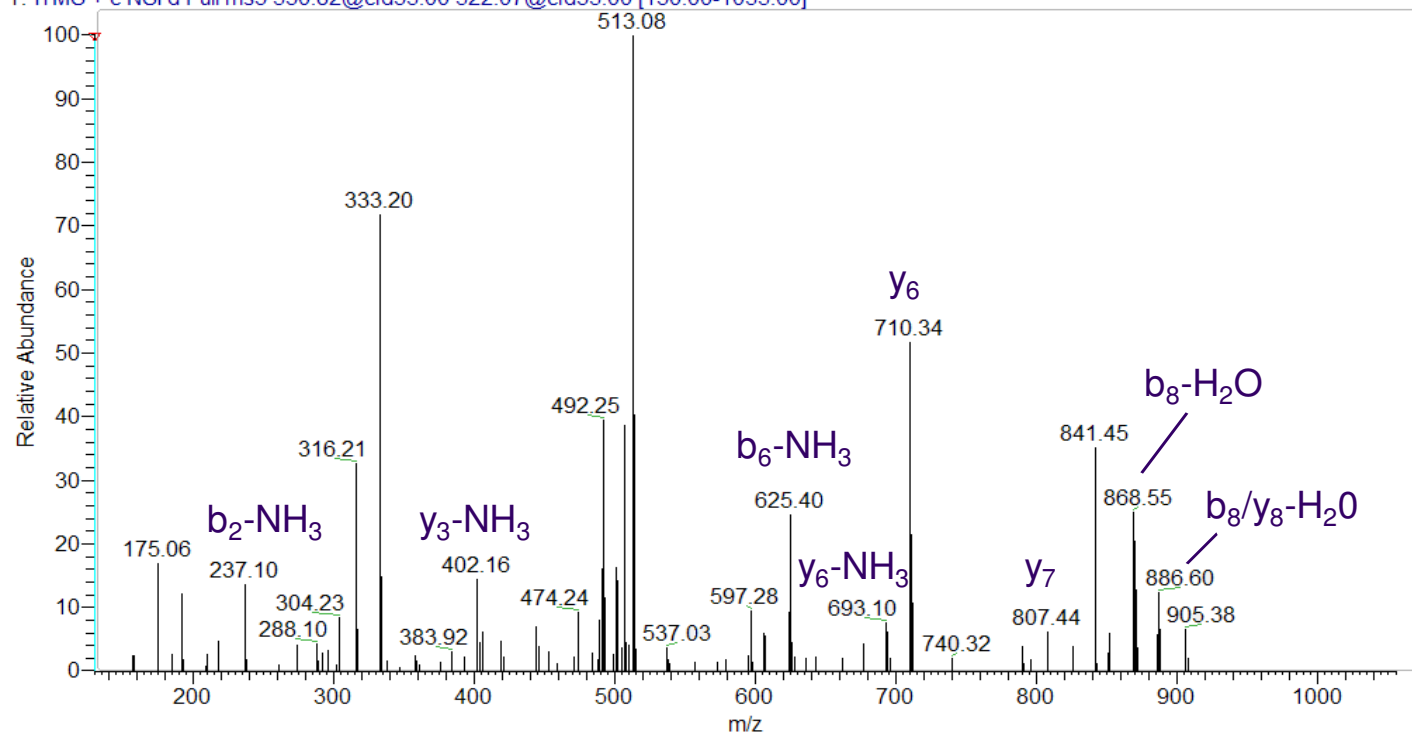
↓ CID 35

spectra

- [M+2H]²⁺ fragments more readily than singly charged species; more b and y fragment ions produced.

FAIMS-MS³ on [M+2H]²⁺ to confirm ion identity

LJB191009_25 #21 RT: 0.13 AV: 1 NL: 2.43E2
T: ITMS + c NSI d Full ms3 530.82@cid35.00 522.07@cid35.00 [130.00-1055.00]



MS³

Precursor ions
m/z 530

↓ CID 35

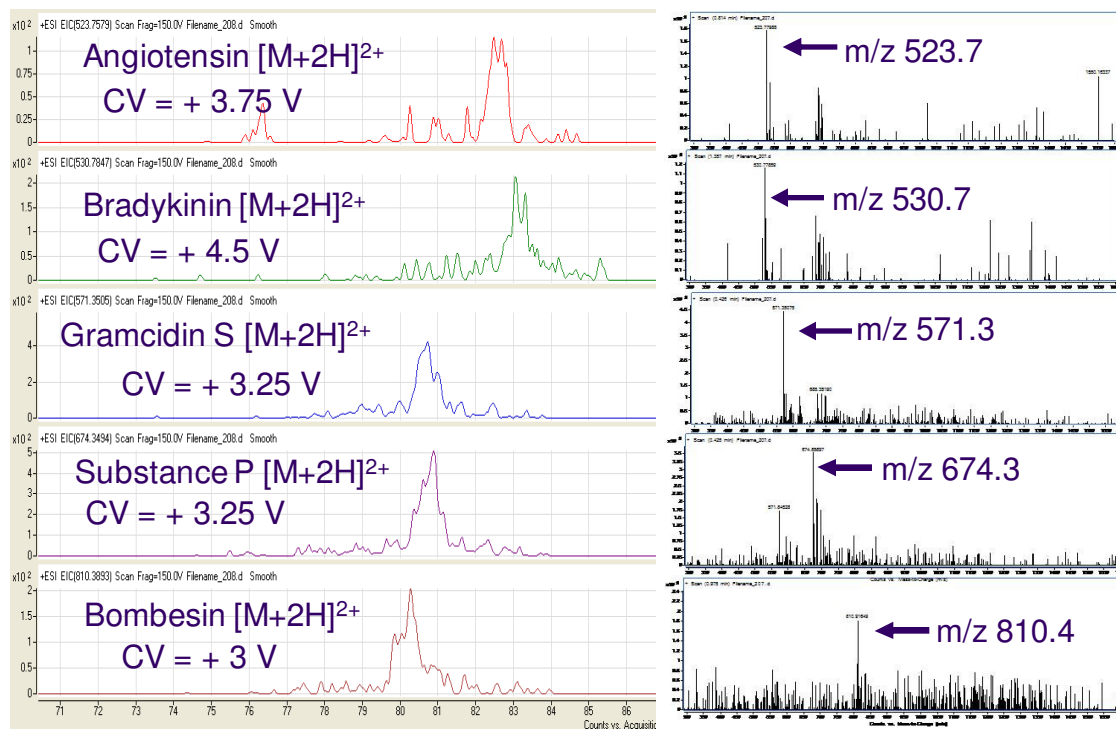
m/z 522

↓ CID 35

spectra

➤ More b and y fragment matches can be used to confirm identity of precursor ions with greater confidence.

Separation of a mixture of $[M+2H]^{2+}$ peptide ions



- **FAIMS-TOF-MS analysis of mixture of peptide $[M+2H]^{2+}$ ions.**
- **Peptides detected between +3 V and + 4.5 V.**
- **Small CV window can be applied, transmit all doubly charged peptide responses.**
- **At 0.5v s⁻¹ this would take 3 seconds; FAIMS analysis compatible with HPLC.**

Conclusions

- Charge state separation of singly and multiply charged peptides has been achieved.
- FAIMS separation of peptide conformers has been demonstrated.
- MS_n fragment ions have been used to confirm the parent ion.
- Separation of peptides of the same charge state can be achieved over a small CV window.

Further Work

- The analysis of protein tryptic digest using FAIMS-MS method to identify individual peptides and fragments for protein identification.
 - Minimise isobaric, non-related interferences
 - Charge state separation of tryptic peptides will reducing complexity of mass spectra.

Acknowledgements

Centre for Analytical Science@Loughborough University

Professor Colin Creaser

Professor Paul Thomas

Dr. Jim Reynolds

Dr. Gushinder Kaur-Atwal

Dr. Victor Bocos-Bintintan Dr. Ran Huo

Gavin Blackburn

Emma Harry

Mark Howdle

Victor Moll

Cristina Guallar

Matthew Turner

Neil Devenport

Victoria Wright

Helen Martin

Aditya Malkar

Owlstone

Danielle Toutoungi

Billy Boyle

Martyn Rush

Funding

Owlstone & Loughborough University

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Centre for Analytical Science



Thank you for listening

Any questions?