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

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Overview

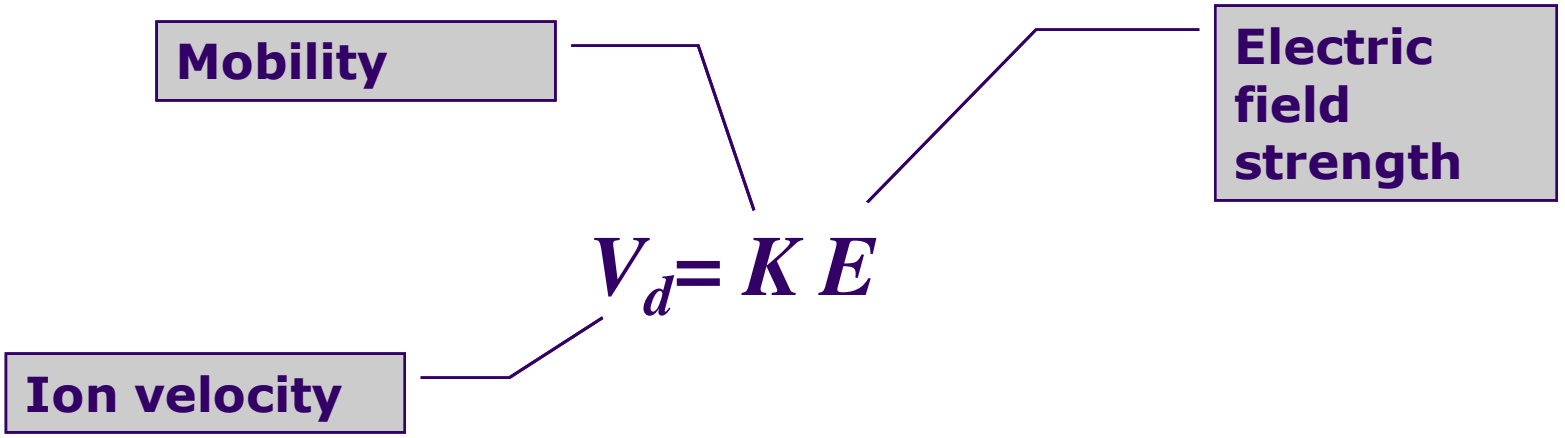
- Background and aims of experiment
- Overview of FAIMS analysis
- Miniaturised FAIMS device
- Results
- Conclusions and further work

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

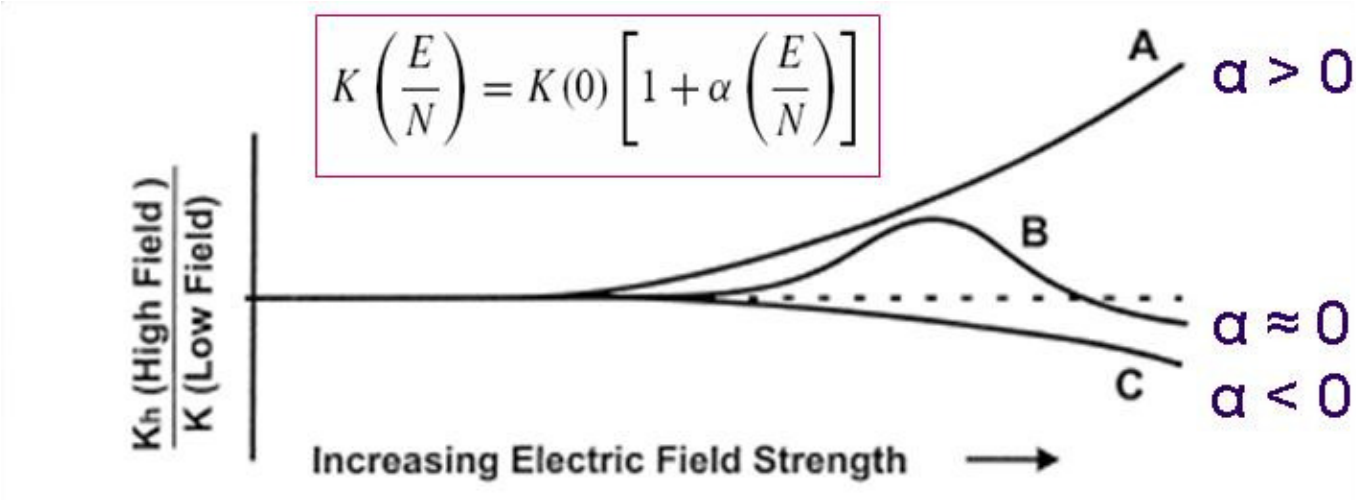
- The mobility of an ion under low field conditions can be calculated mathematically based on the following equation:



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

Principle of FAIMS

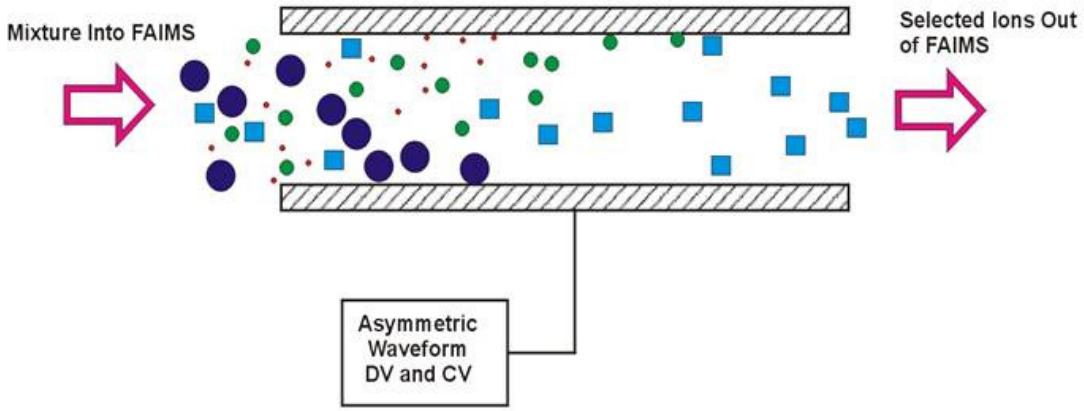
- **FAIMS separates gas-phase ions on the basis of their differential mobilities (α) under alternating low and high field conditions.**

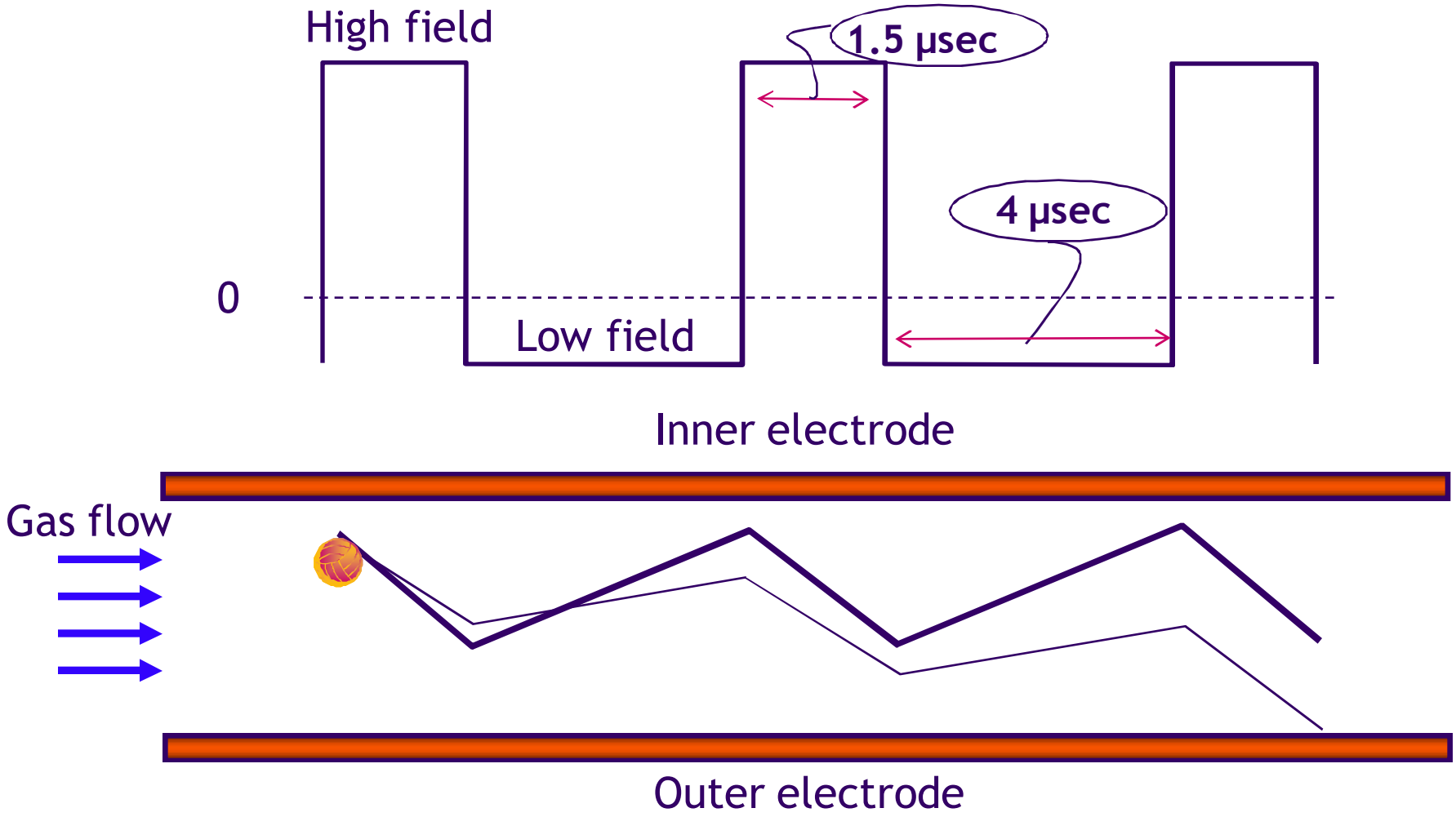


[Purves R W, Guevremont R, Anal. Chem. 1999, 71, 2346-2357]

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.

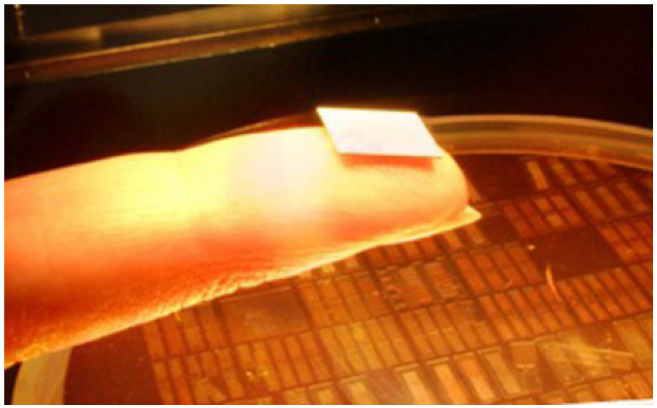




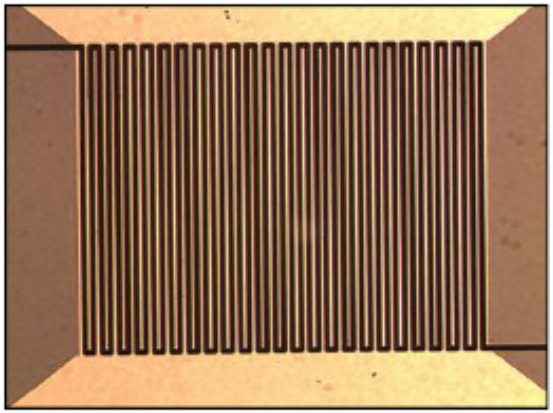
Inappropriate compensation voltage : orange ion is lost

With same compensation voltage superimposed : purple ion transmitted to MS

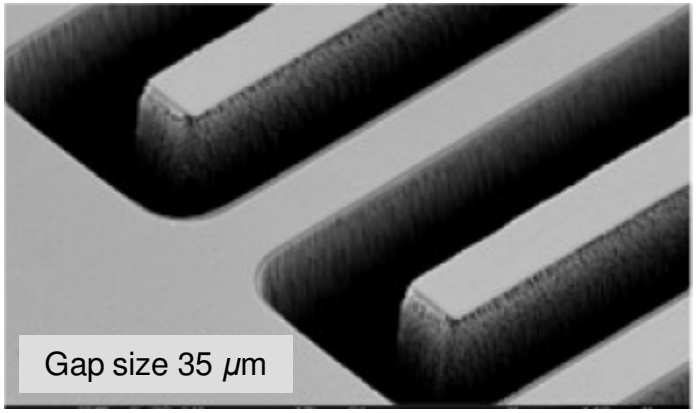
Owlstone FAIMS Technology



(a)



(b)



(c)

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.**

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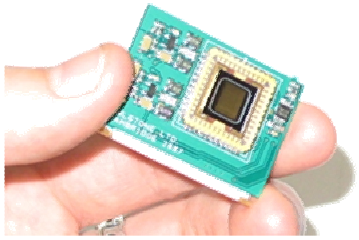
FAIMS chip cartridge

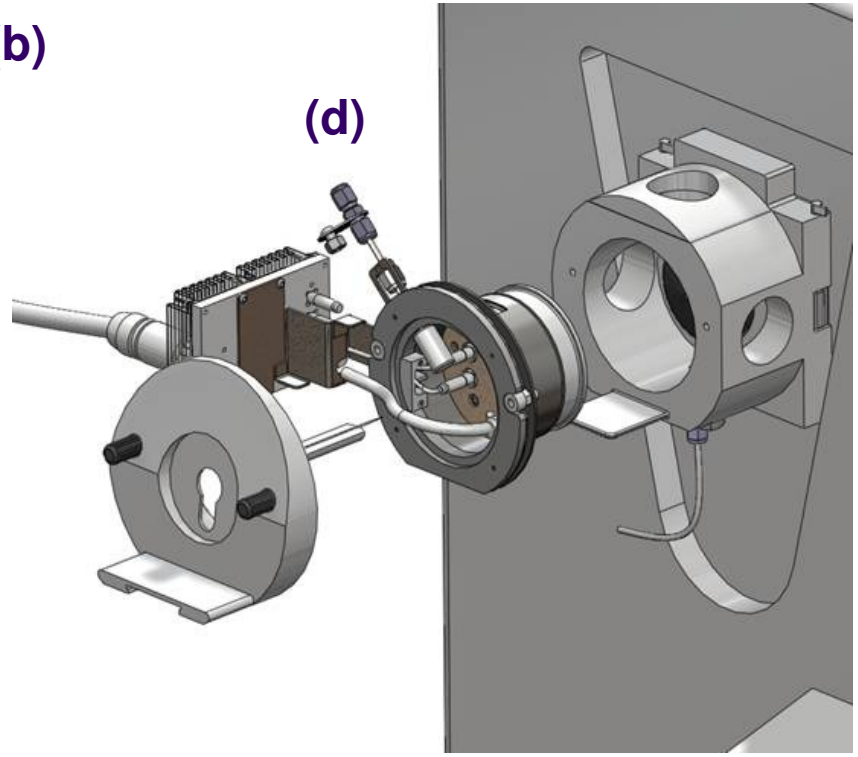
- **The FAIMS chip fits inside the Thermo Fisher Scientific LTQ nanospray source housing, between the NSI source and MS.**

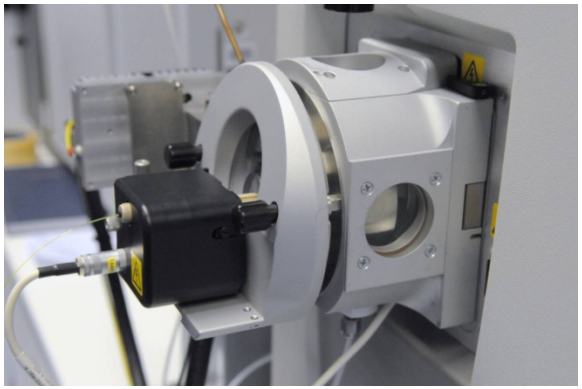
(a)



(b)

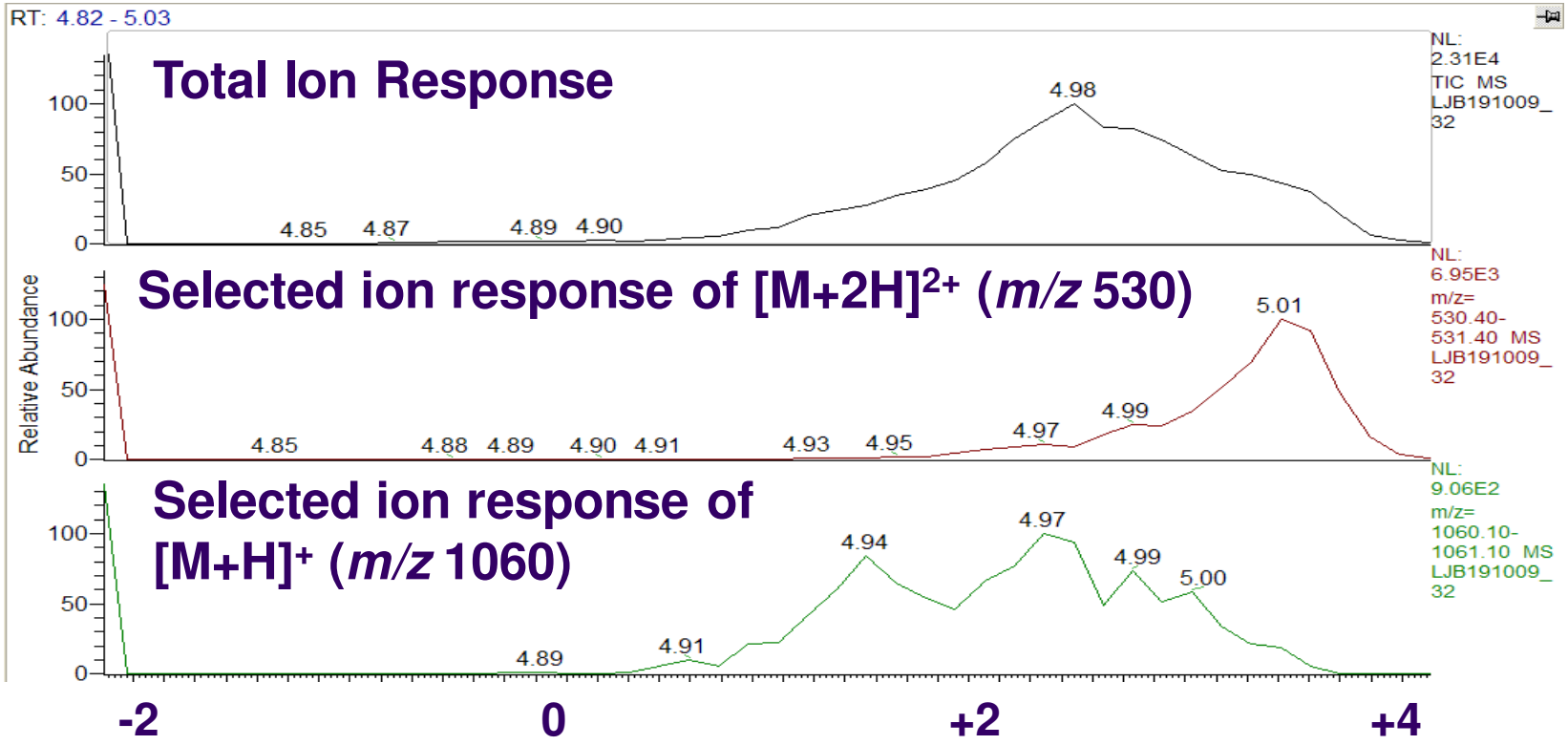
(d)





(c)

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



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

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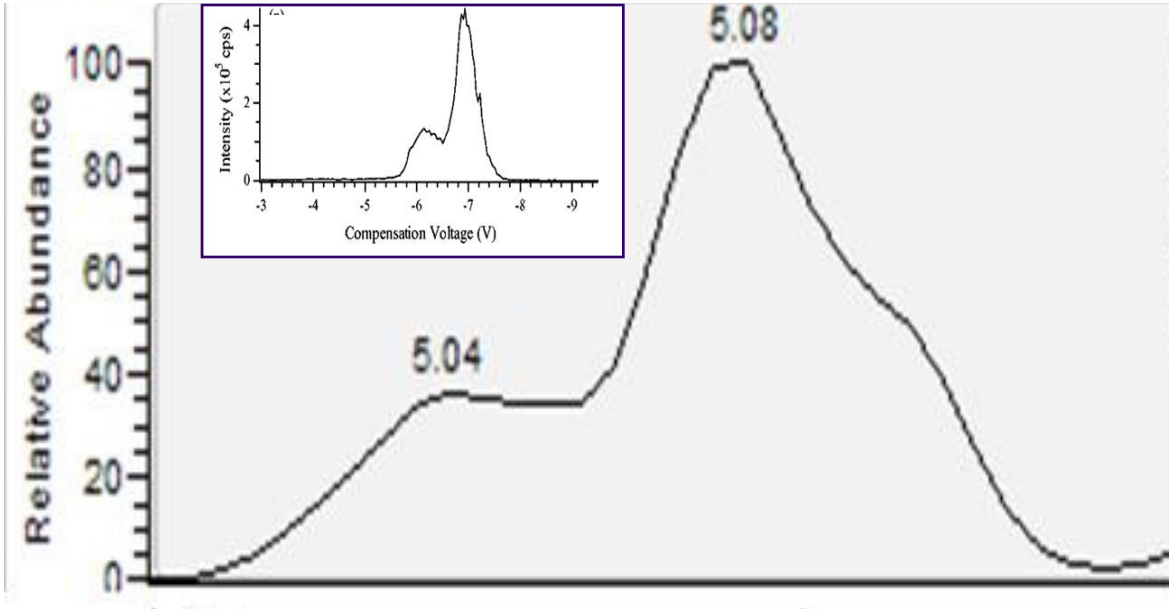
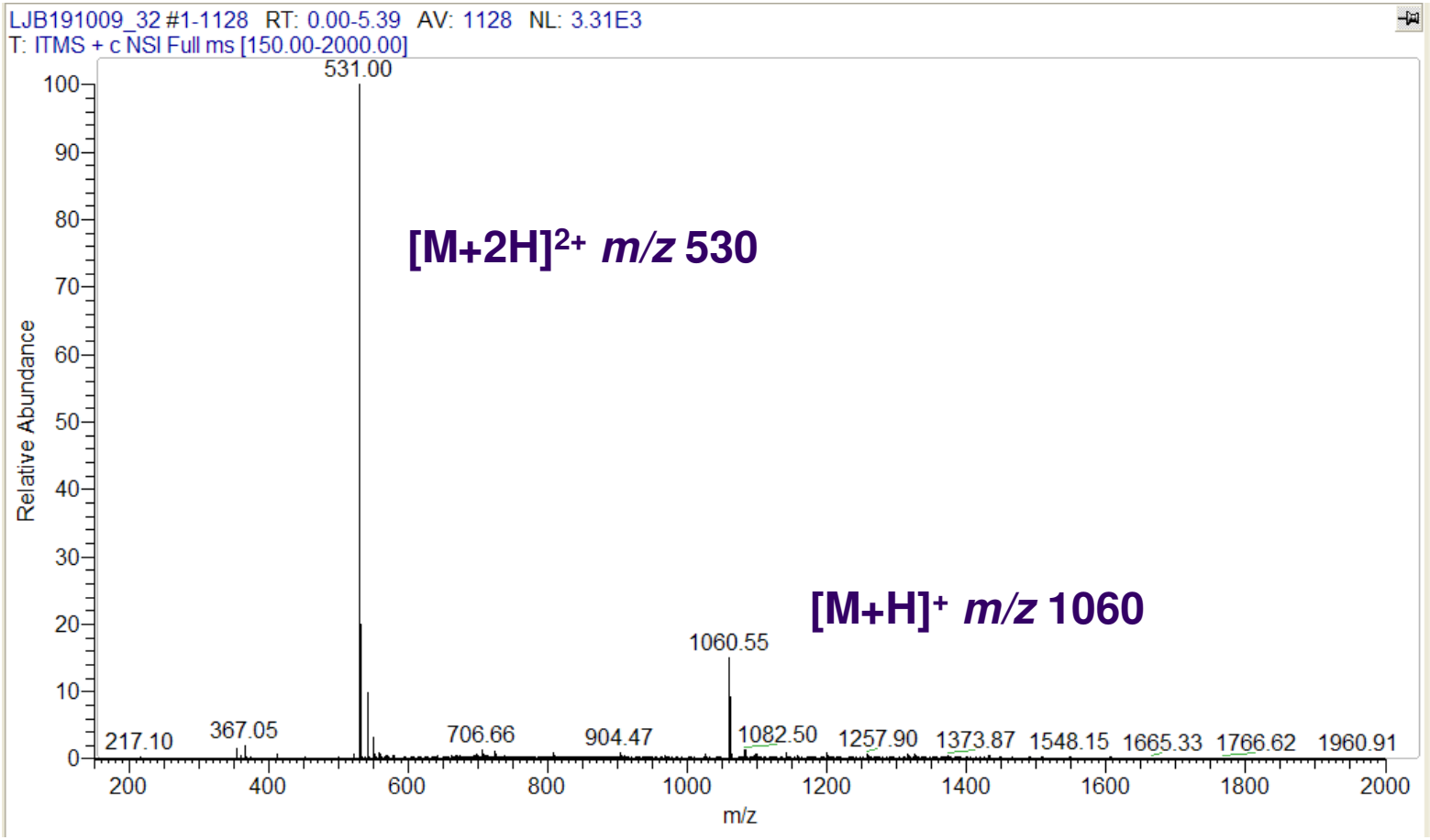
Separation of Conformers of $[M+2H]^{2+}$ ion of bradykinin



- **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.**

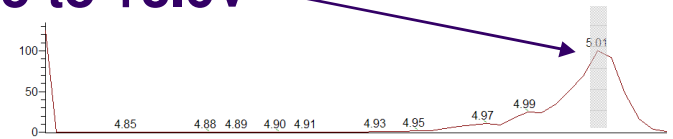
1. Purves, R. W., D. A. Barnett, et al., Rapid Commun. Mass Spectrom., 2001, **15**, 1453-1456.

MS spectra without FAIMS analysis

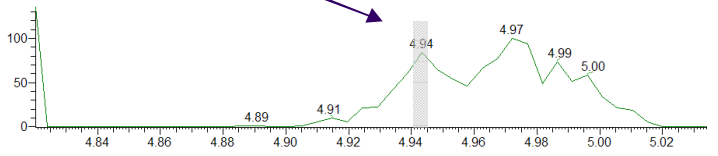


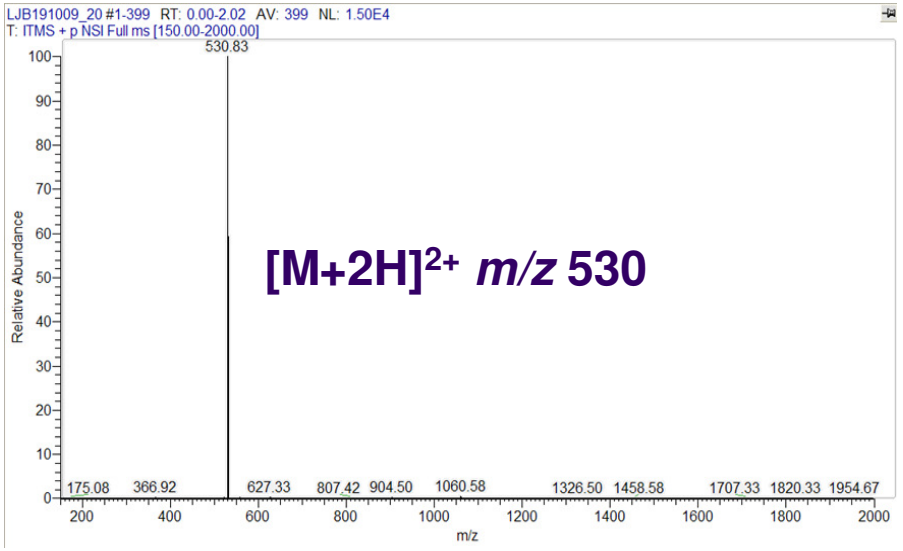
FAIMS-MS spectrum for each charge state

CV: +3.5 to +3.6V

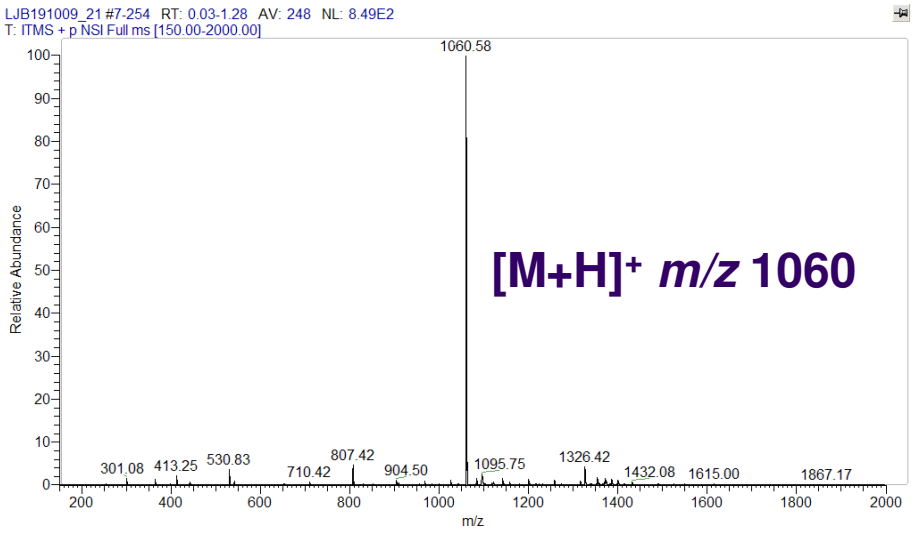


CV: +1.4 to +1.5V





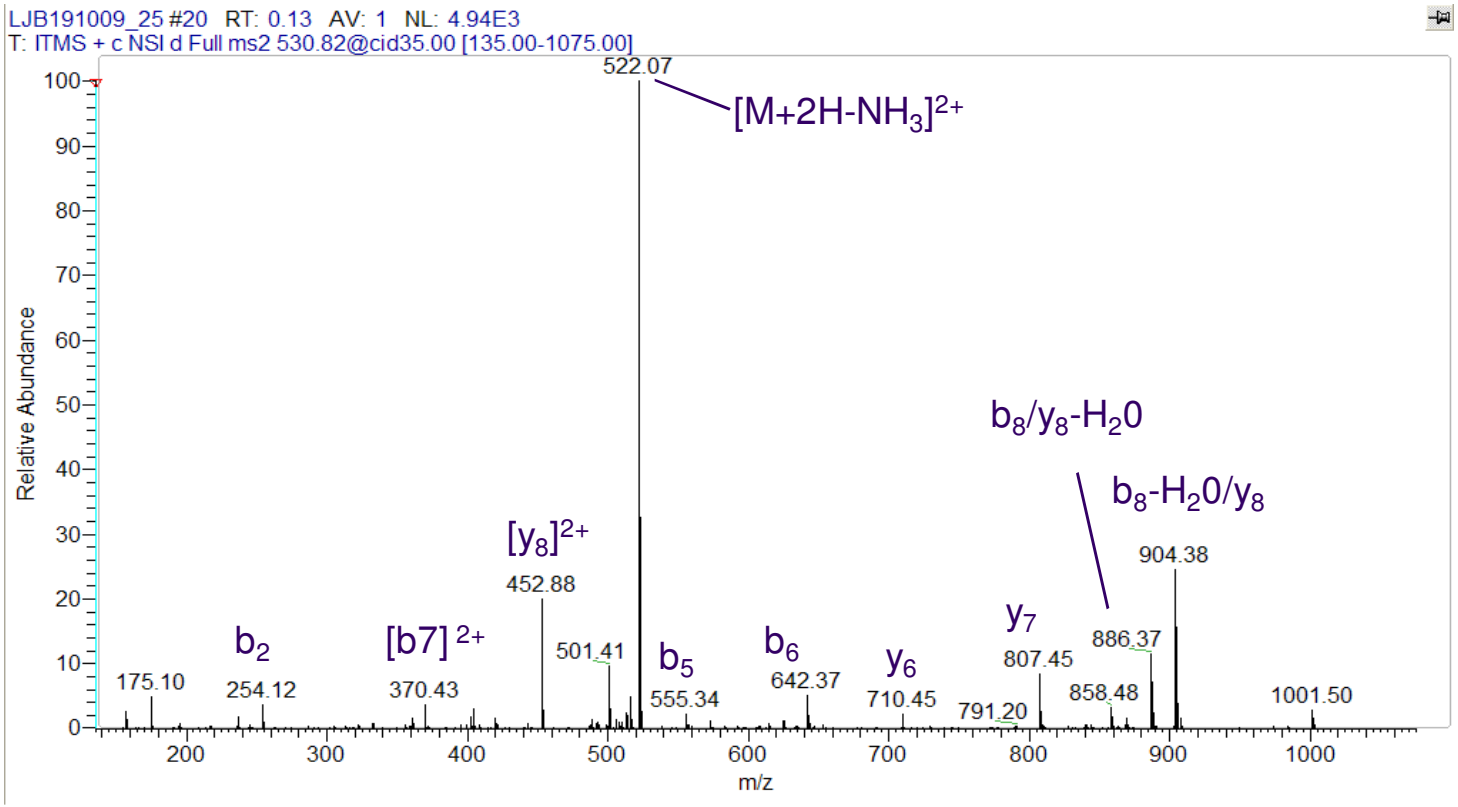
$[M+2H]^{2+}$ *m/z* 530



$[M+H]^{+}$ *m/z* 1060

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

FAIMS-MS^2 on [M+2H]$^{2+}$ to confirm ion identity



MS^2

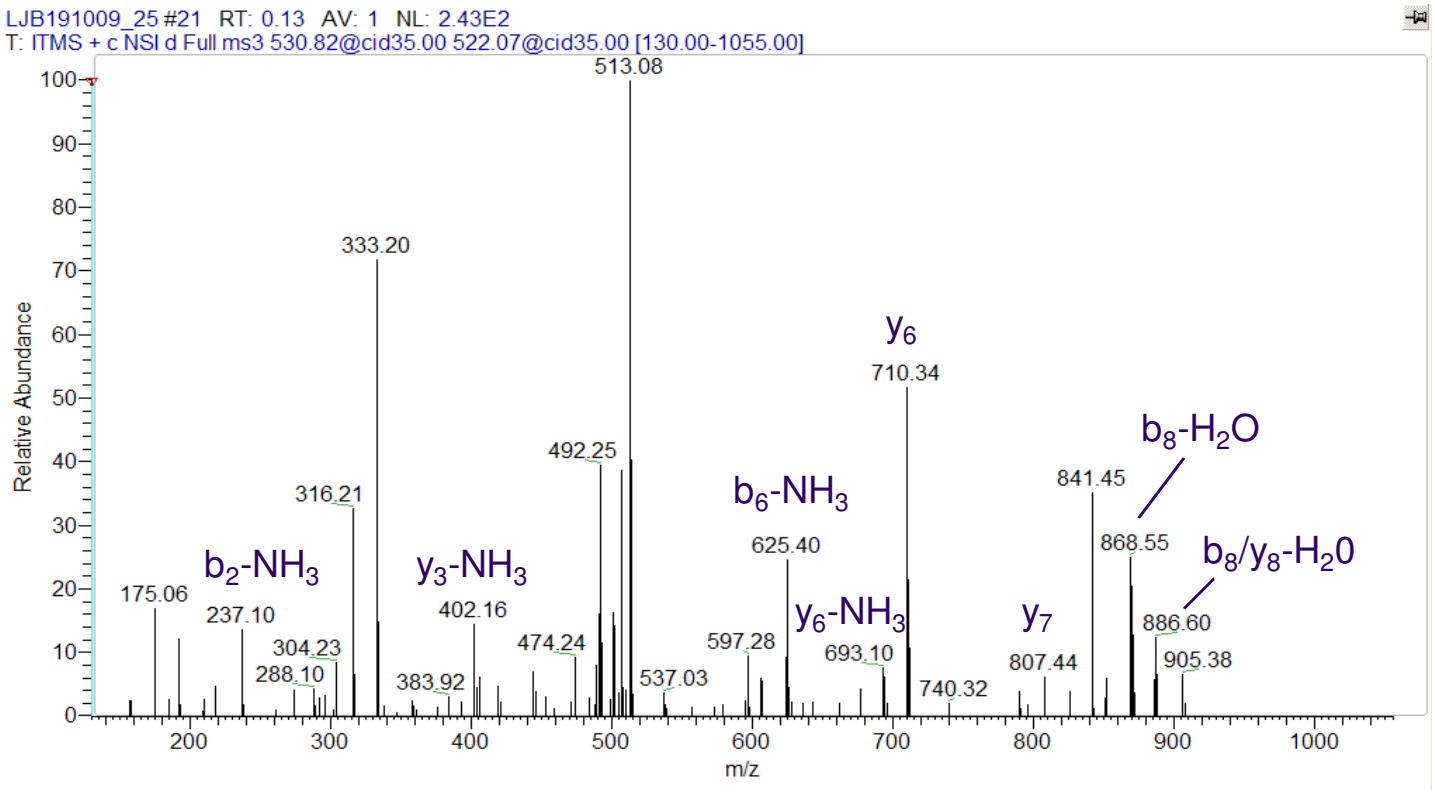
Precursor ions *m/z* 530

CID 35

spectra

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

FAIMS-MS^3 on [M+2H]$^{2+}$ to confirm ion identity



MS^3

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.**

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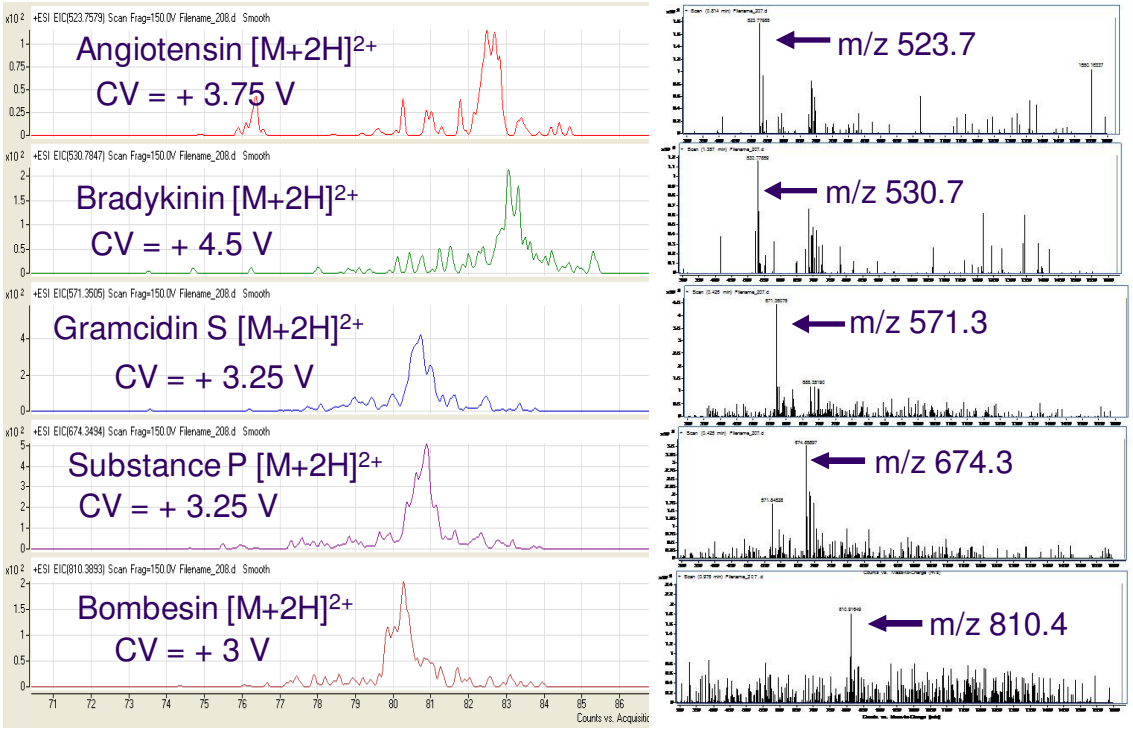
Separation of a mixture of $[M+2H]^{2+}$ peptide ions



- **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.**

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Conclusions

- Charge state separation of singly and multiply charged peptides has been achieved.
- FAIMS separation of peptide conformers has been demonstrated.
- MSn 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.

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Thank you for listening

Any questions?

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