



# Optimization and characterization of the performance of a new ultra-fast FAIMS MS interface



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# Ultra-fast FAIMS fundamentals

*Separation speed* is determined by the ion residence time

- Typically sized FAIMS devices have residence time in **tens to hundreds of milliseconds**
  - This time scale is hardly compatible with **ultra-high speed chromatography** and data dependent FAIMS separation
- 
- How do we achieve **residence time below 1 ms** and still maintain good separation and sensitivity?
- 
- The solution is to **reduce drift length** and **increase dispersion field**
  - Field is increased by going to **narrow (micro-fabricated) channels**

# Ultra-fast FAIMS fundamentals

Peak width ( $w$ ) and mobility “resolution” ( $R$ )

$$w = \frac{4}{K} \sqrt{\frac{D_{\parallel} \ln 2}{t_{RES}}} \quad R = \frac{E_C}{w} = \frac{E_C K}{4} \sqrt{\frac{t_{RES}}{D_{\parallel} \ln 2}}$$

Diffusion at low field  $D_{\parallel} = kTK / q$       high field  $D_{\parallel} \propto E_D^2$

Separation as a function of dispersion voltage

$$\frac{E_C}{N} = -\sum_{n=1}^{\infty} k_n \left( \frac{E_D}{N} \right)^{2n+1} \quad k_1 = -a_1 \langle F_3 \rangle$$

Therefore,  $R \propto E_D^2$  to  $E_D^3$       and       $t \propto E_D^{-4}$  to  $E_D^{-6}$

A. Shvartsburg, Differential ion mobility spectrometry, CRC Press, 2009

# High dispersion field FAIMS

Low field vs. high field

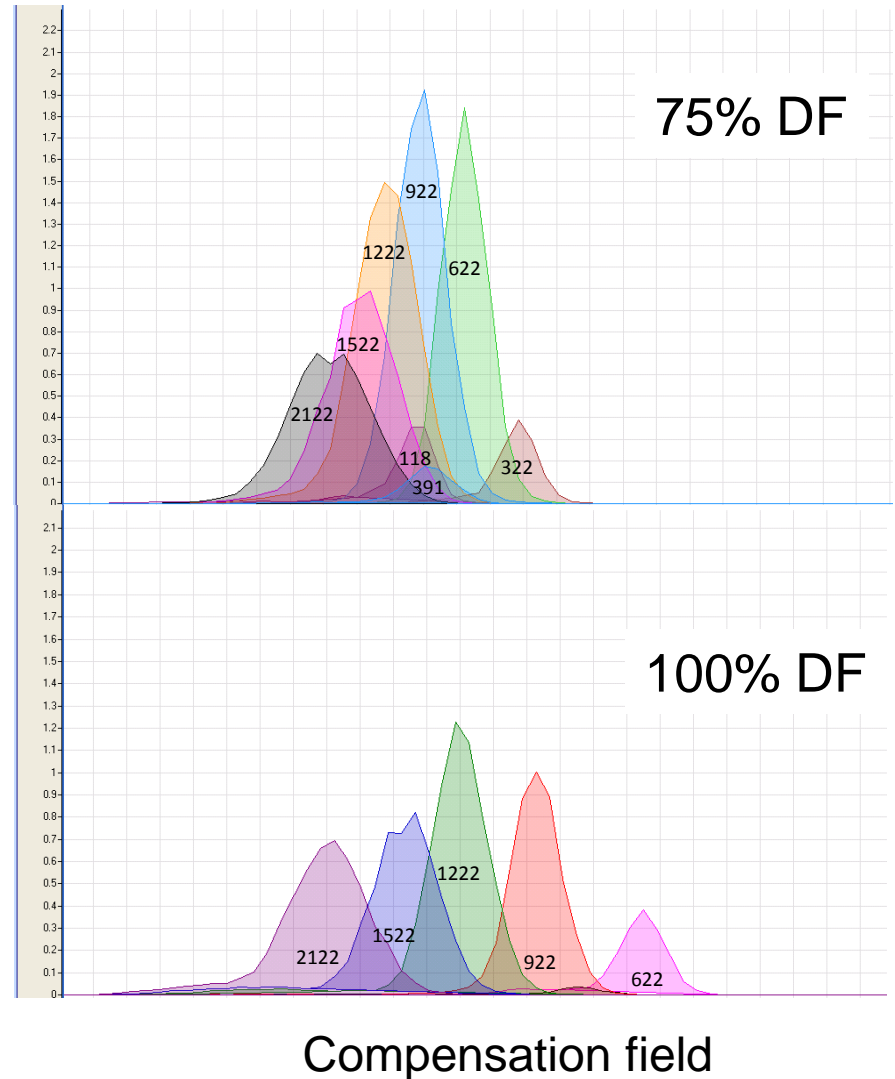
Important point:

Strong transmission discrimination by ion mobility

$$S = \exp\left[-\frac{\pi^2 D_{\parallel} t}{g_e^2}\right]$$

Effective channel width:

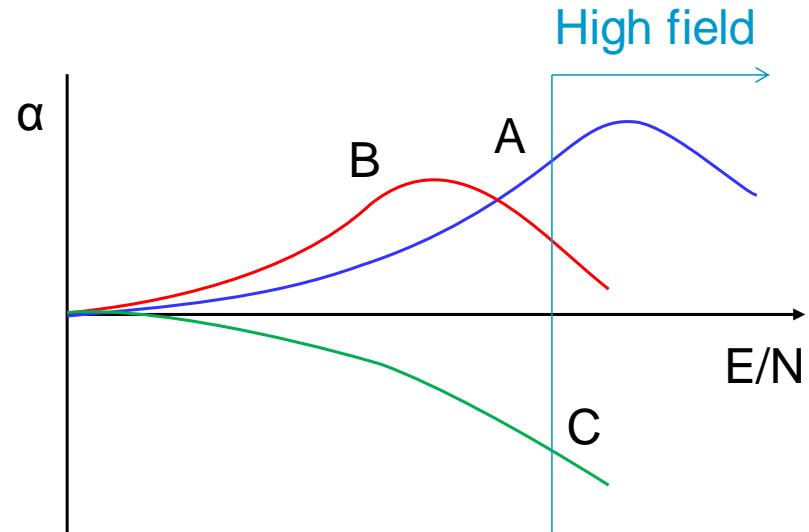
$$g_e \approx g - \frac{K(0)E_D}{3\nu}$$



# High dispersion field FAIMS

Other consequences of using high dispersion fields?

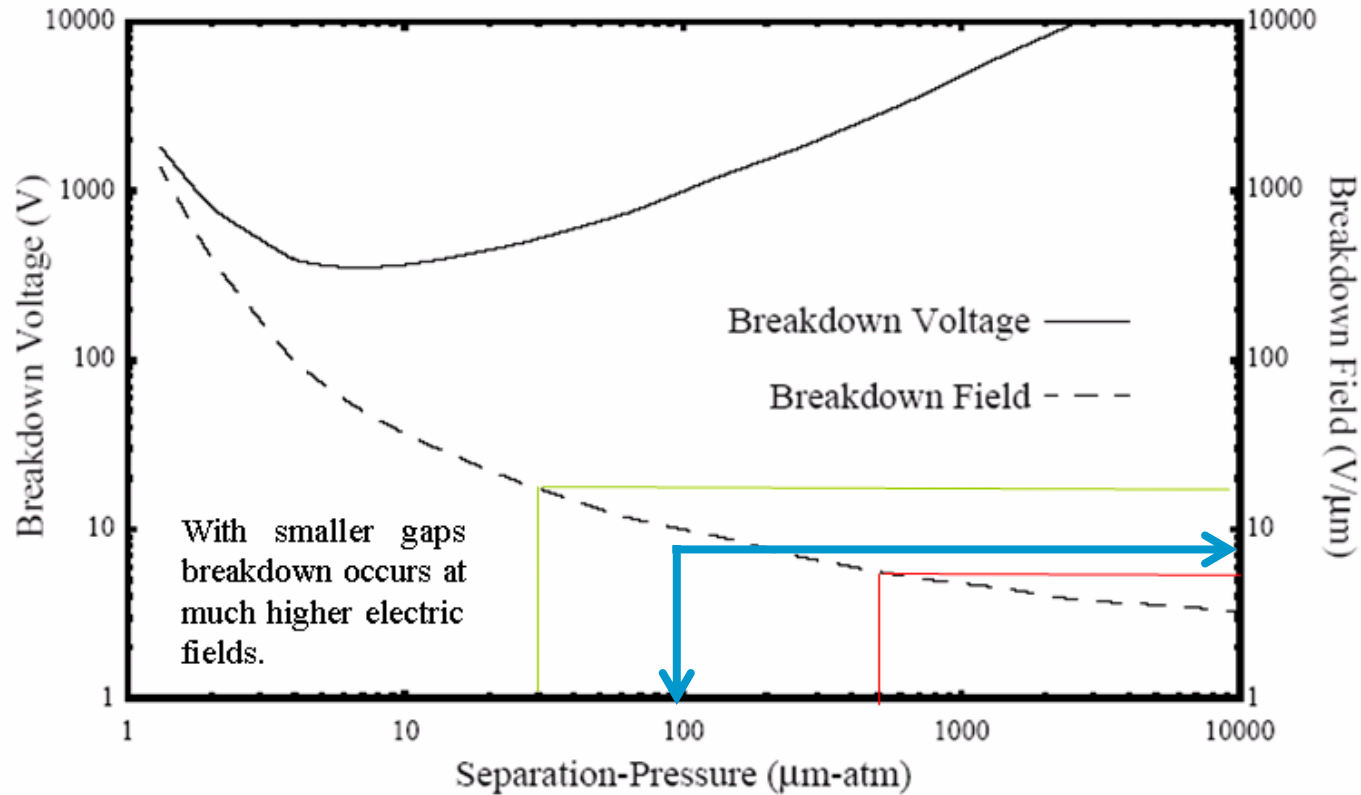
- All ions eventually tend to become C-type at higher field
- Higher order coefficients in ion mobility series become more important



- Temperature of ions grows quickly with dispersion field (as  $E_D^2$ )
  - ✓ One needs to be careful to avoid ion fragmentation
  - ✓ High field can affect ion shape, orientation and collision dynamics which can result in changes in separation

# High dispersion field via smaller electrode gap

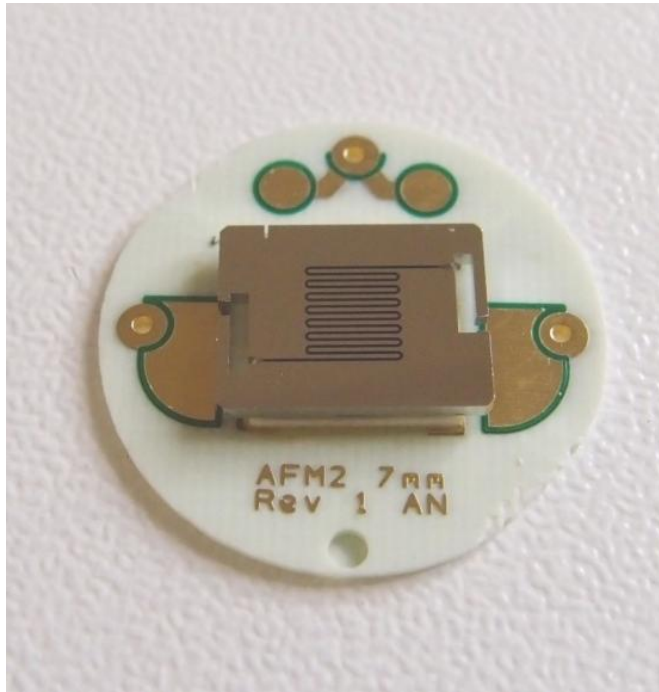
## Paschen curve diagram



Air breakdown field limit rises as gap decreases

# Microscale FAIMS chip made by Owlstone, Ltd.

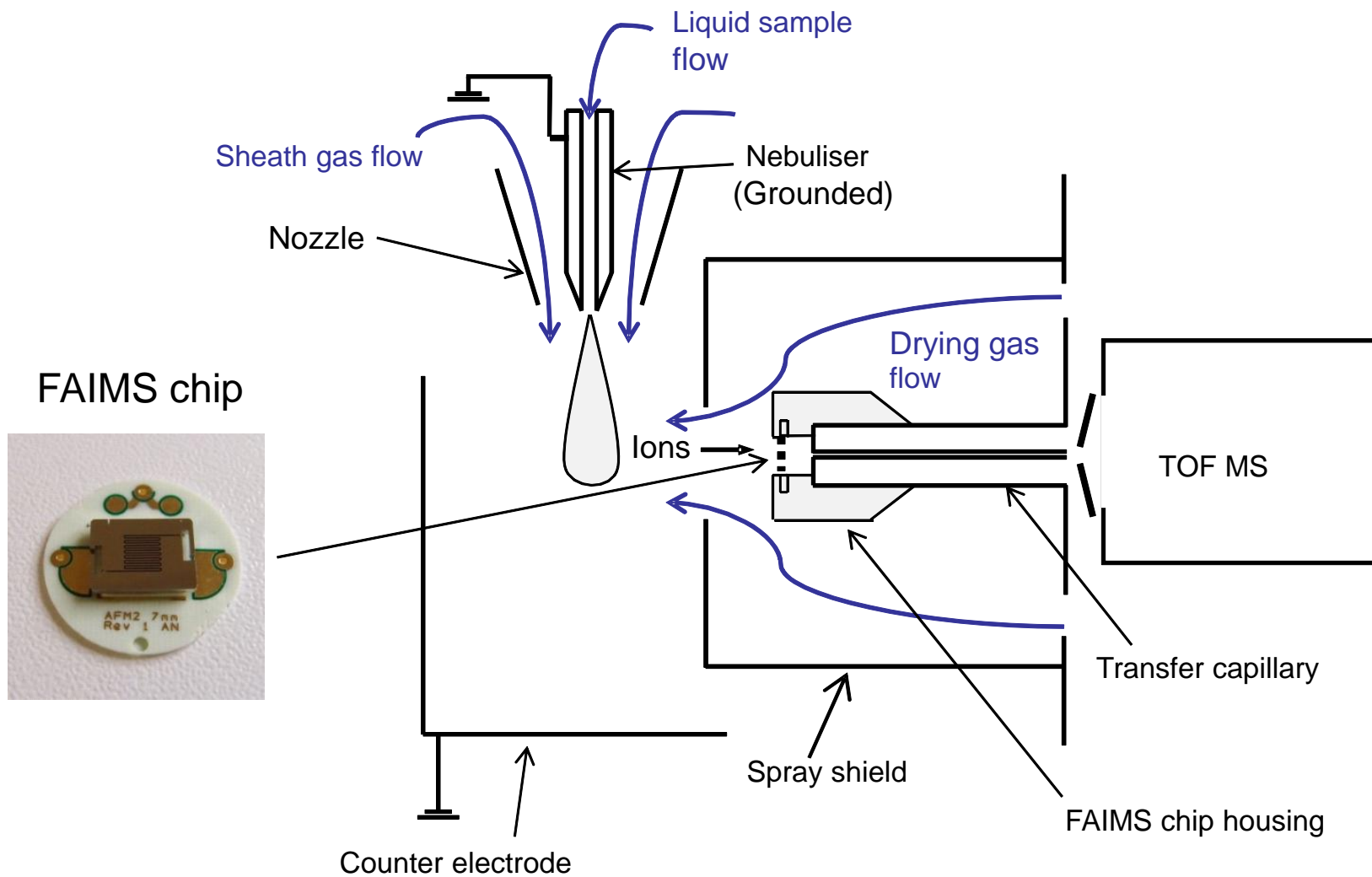
Array of parallel ion channels manufactured in a metal substrate



100 $\mu$ m gap

- Batch micro-fabrication techniques allow extremely precise control over ion channel dimensions on a micron scale.
- Small, stable, mechanically robust device capable of high-performance ion selection.

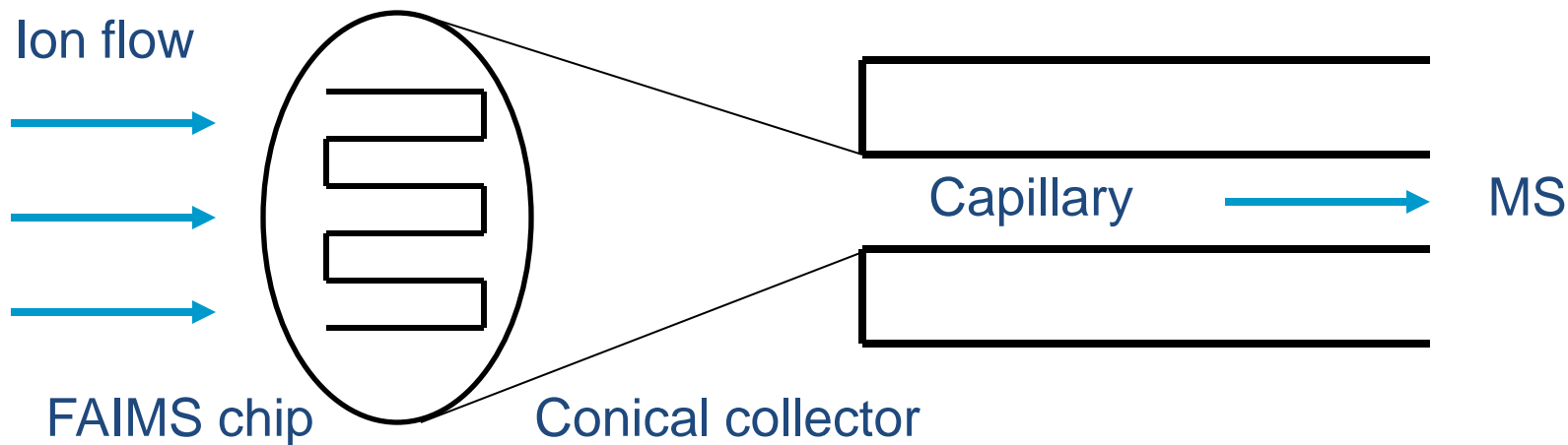
# FAIMS-MS interface schematics



# FAIMS-MS interface schematics

Get better selectivity while maintaining high transmission

1. Get better S/N ratio without impacting S too much
2. Enable operation in FAIMS-off mode
3. Increase sensitivity for high mobility ions

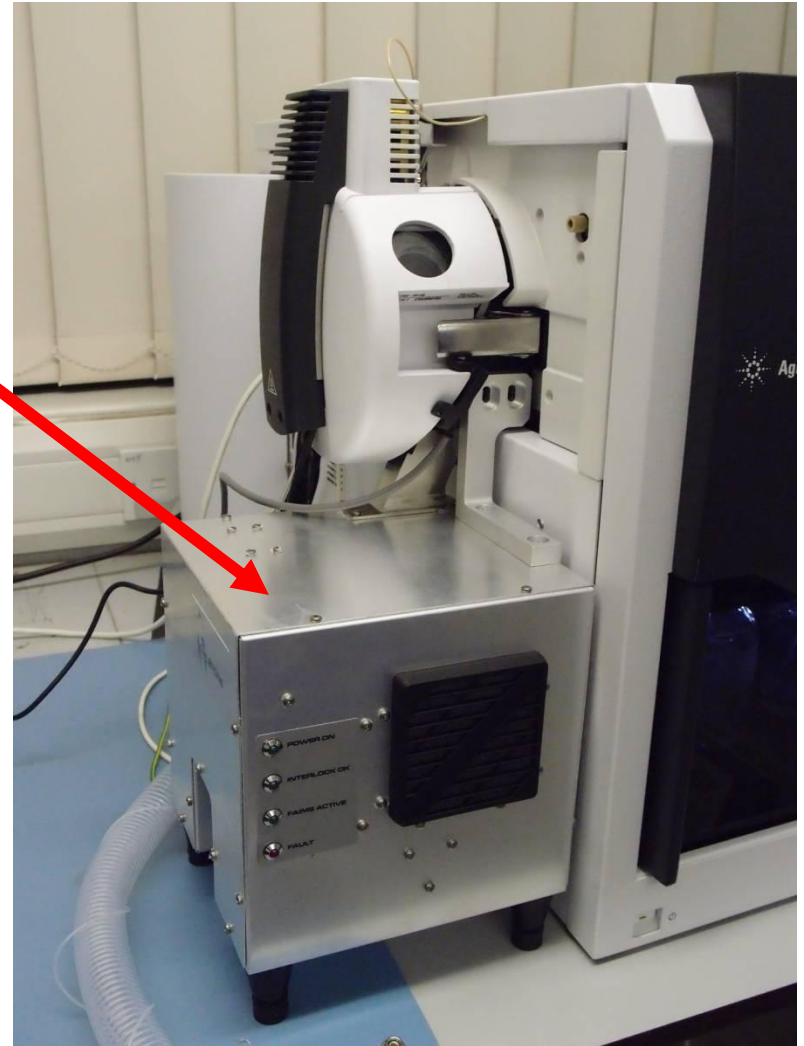
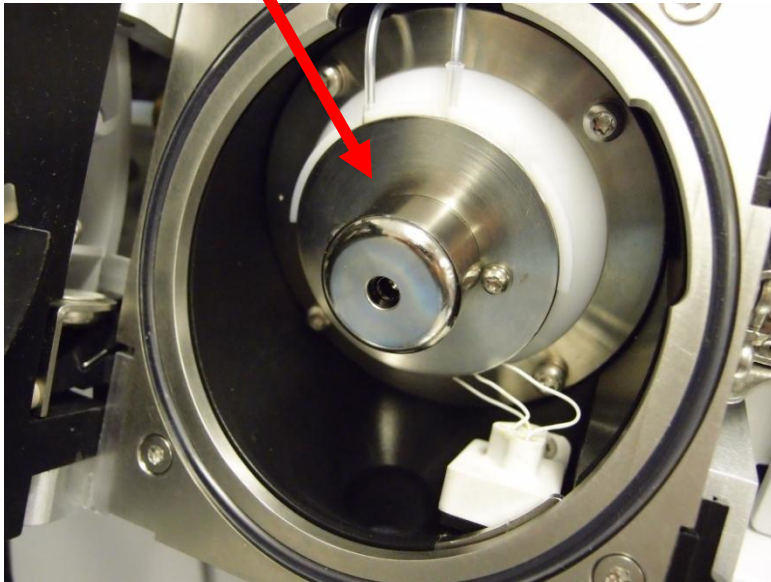


# FAIMS-MS interface

FAIMS-MS system photos

FAIMS control module

Source chamber



# New generation of FAIMS chips

Generation B chip and FAIMS control module are better optimized to achieve desired performance

Generation/parameter	A	B
Channel width	35 $\mu$ m	100 $\mu$ m
Channel length	300 $\mu$ m	700 $\mu$ m
Chip area	15mm <sup>2</sup>	10-20mm <sup>2</sup>
Drive frequency	25MHz	27MHz
DF range	350Td	320Td
CF range	$\pm$ 15Td	$\pm$ 12Td
Residence time	30 $\mu$ s	100-150 $\mu$ s

# Transmission and resolution improvements

Consistent **resolution increase by 50 to 100%** at similar DF settings (up to overall peak capacity of 15-20). Associated transmission gains are summarized below. Blue fields indicated typical operation conditions with FAIMS on and off.

## Typical transmission percentages

m/z	Prototype A zero RF	Prototype B zero RF	Prototype A 70%RF	Prototype B 70%RF	Prototype A 100%RF	Prototype B 100%RF
118	10	35	1.5	7	1	1
322	15	50	4.5	20	1.5	2
622	60	60	35	50	15	15
922	70	70	70	70	45	45
1522	80	80	80	80	60	65

# CF stability and sensitivity to source/sample

CF drift due to solvent/matrix composition known to be a problem for many FAIMS designs, particularly in high flow applications.

## Solution:

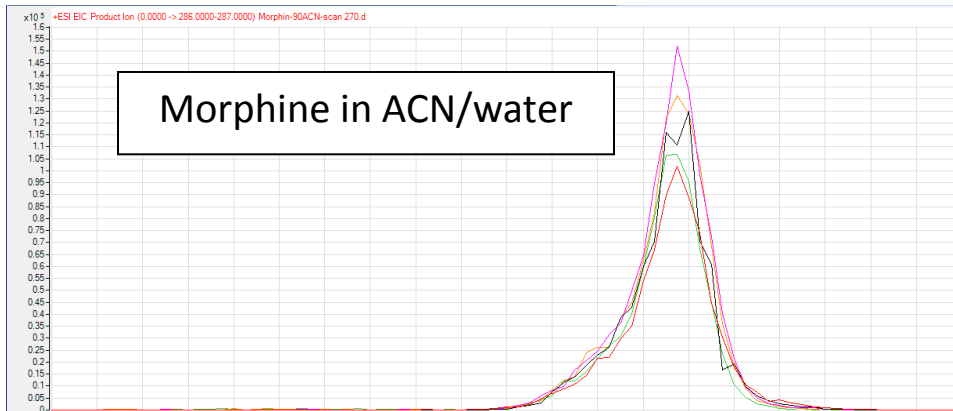
- Upfront desolvation using Agilent Jet Stream source
- High dispersion field may put us in a more favorable place

## Result:

- No appreciable sensitivity to solvent/matrix
- Drying (carrier) gas can be operated in wide range of temperatures

# Sensitivity to solvent composition

Infusion experiment (low flow, high DF)



Compensation field

FAIMS and other settings

DF = 270Td

Flow rate 0.05 ml/min

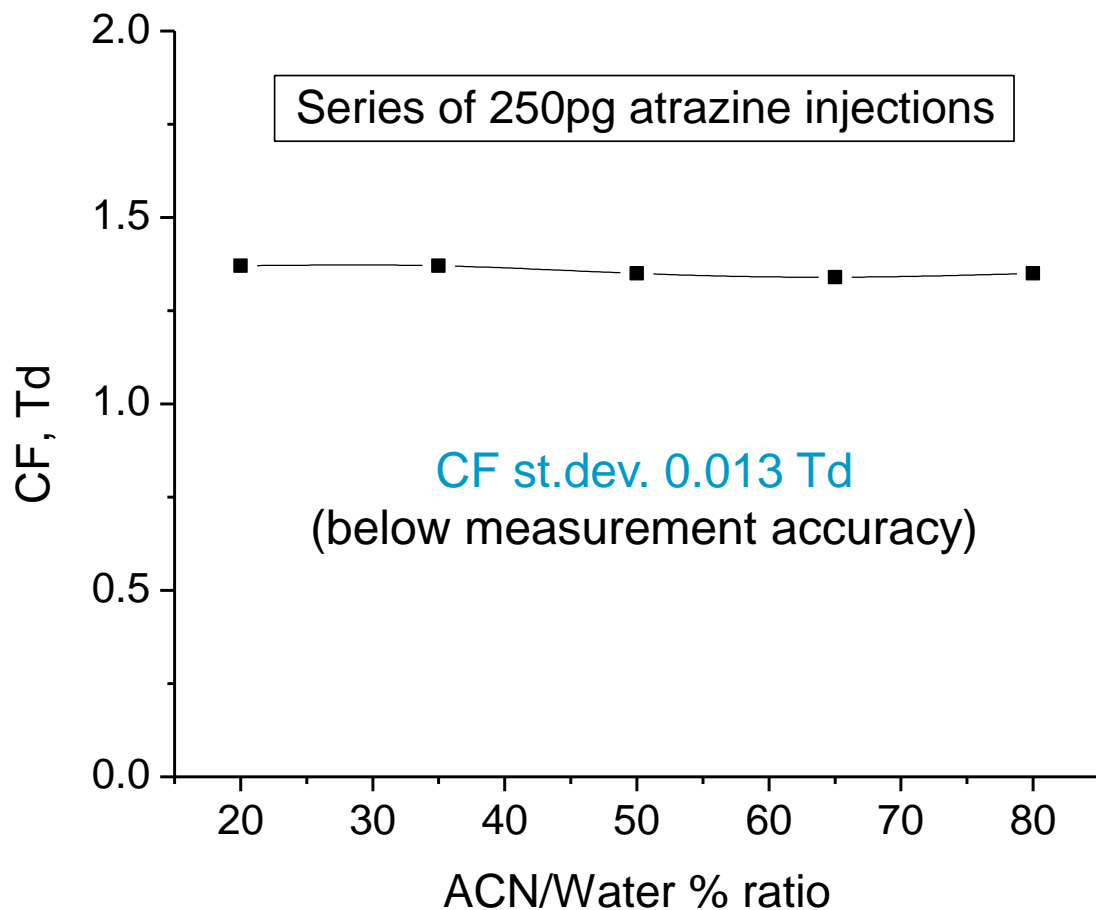
10-90% ACN/water

No shift in peak position detected in a wide range of solvent ratios.

Preliminary experiment with drugs spiked in human plasma suggest similar behavior (data not shown). Optimum CV position is similar to that of the analyte in solvent.

# Low sensitivity to solvent composition

LC injection experiment (high flow, lower DF)

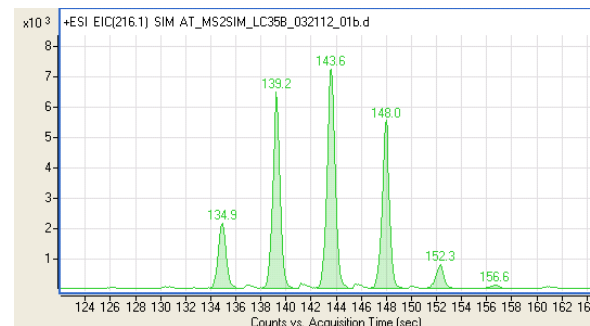


FAIMS and LC settings

DF=220Td

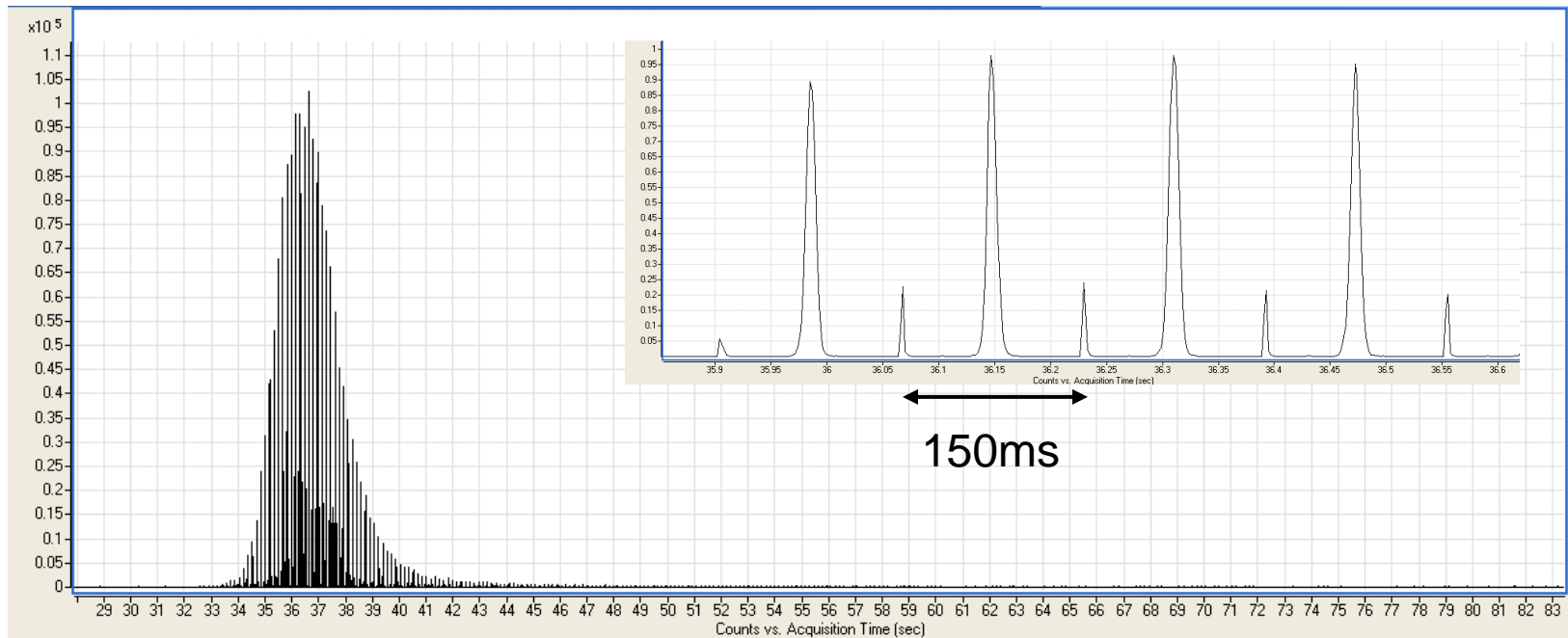
0.25 CV scans/s

Flow 0.4 ml/min



# We have speed now – what do we do with it?

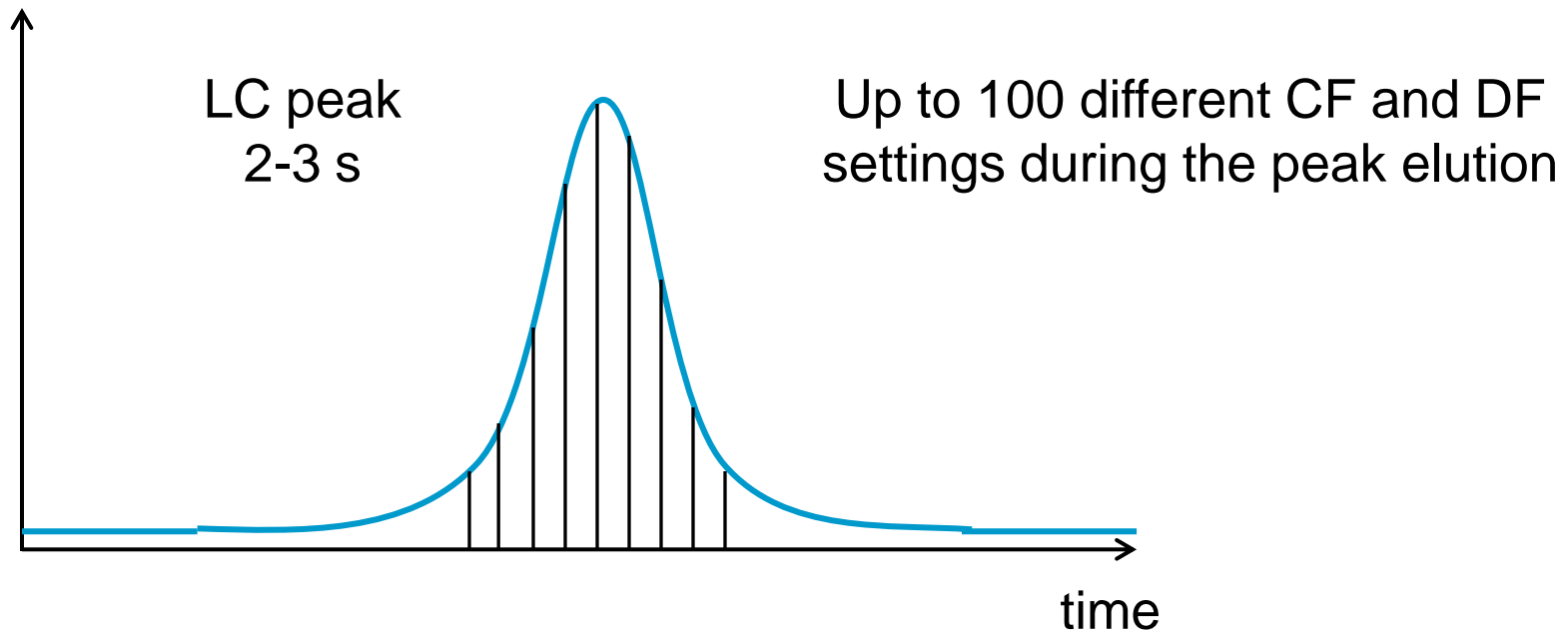
**Benefit #1:** Real-time optimization of CF and DF during chromatographic separation. Under **2 ms** per step in CF scan mode. An MS spectrum is collected at each step.



15 complete CF scans during 2s FWHM of an LC peak

# We have speed now – what do we do with it?

**Benefit #2:** Fast switching of optimized CF/DF values during ultra-high speed chromatography. Under **30 ms** per step in CF/DF “hopping” mode. An MRM transition data or a full TOFMS spectrum is collected at each step.



# Ultra-high field effects on ions

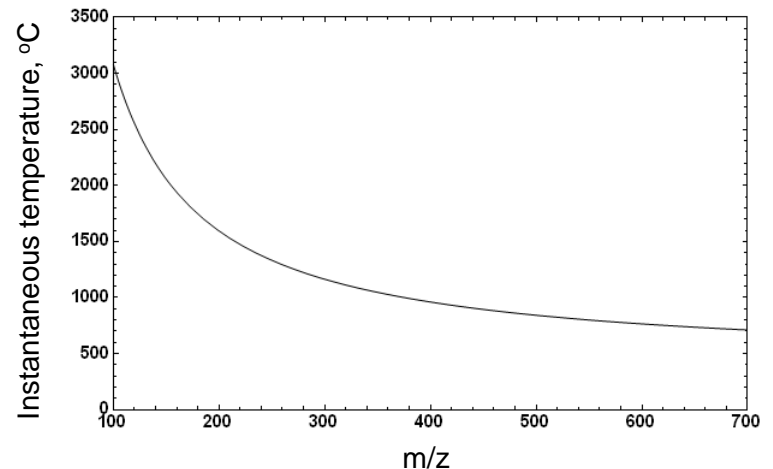
Ion temperature in Ultra-High Field FAIMS environment

$$T_E = T_0 + \Delta T = T_0 + MK^2 E^2 / 3k$$

**Instantaneous** “temperature” can reach over 1000K, particularly for high mobility ions. Interestingly, while one would expect most molecules to fragment readily in these conditions, this is NOT happening in chip-FAIMS.

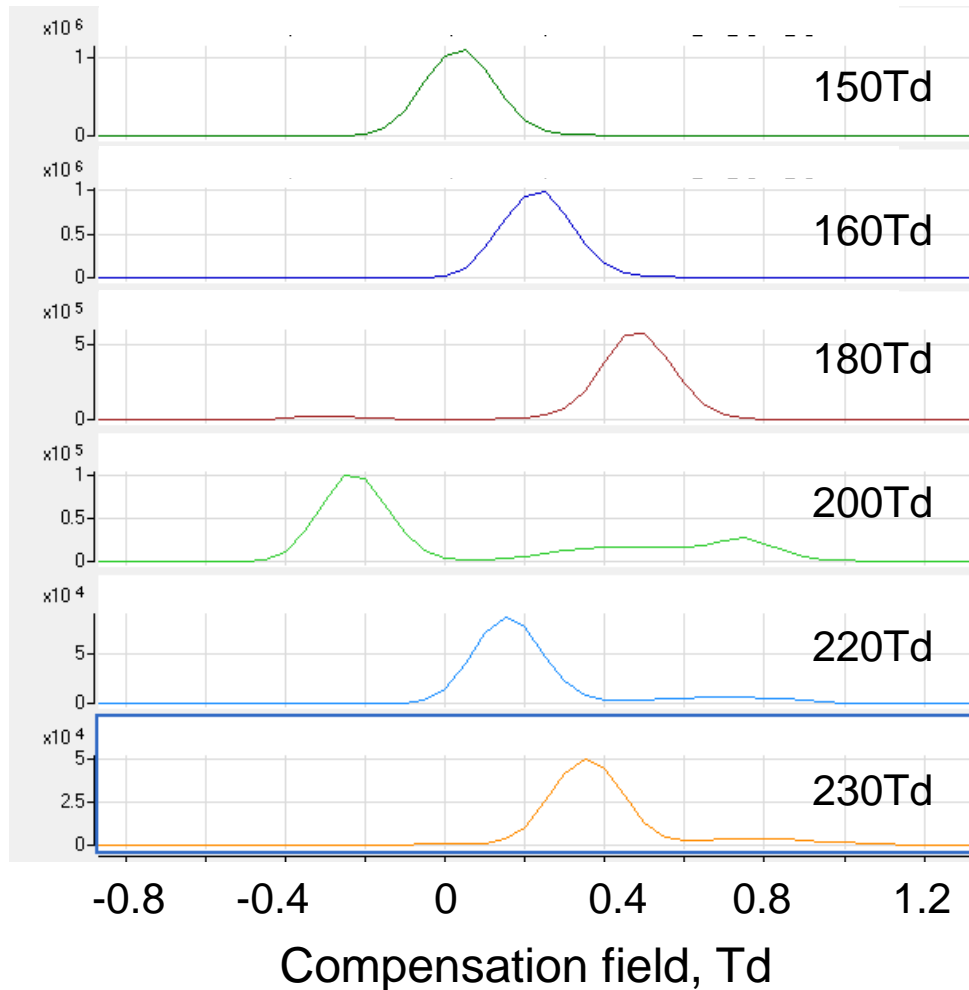
Other effects expected at higher dispersion voltages:

- ✓ Collisional alignment
- ✓ Dipole alignment
- ✓ Isomerization
- ✓ Charge redistribution
- ✓ De-clustering



# Ultra-high field effects on ions

## CF plot for a small m/z ion (110)



## Experimental observation

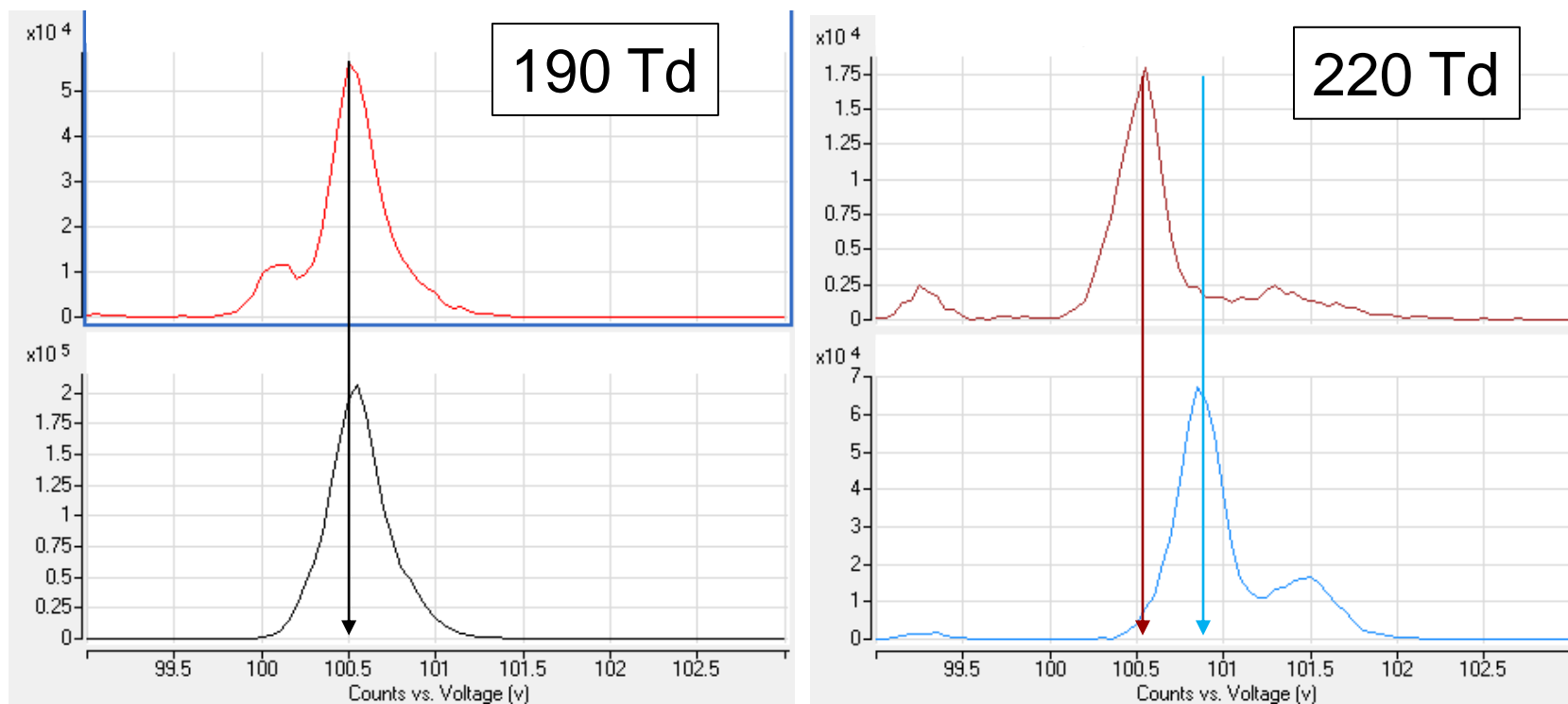
Most of ions show effects of change of peak CF shape and appearance of additional peak at very high field.

This effect can be either detrimental or beneficial for the separation depending on the specific combination of analyte and background.

Ability to perform fast CF/DF 2-dimensional ramping makes method optimization procedure a lot easier.

# Ultra-high field effect for improved separation

Separation of acetaminophen (top) and phenacetin (bottom)

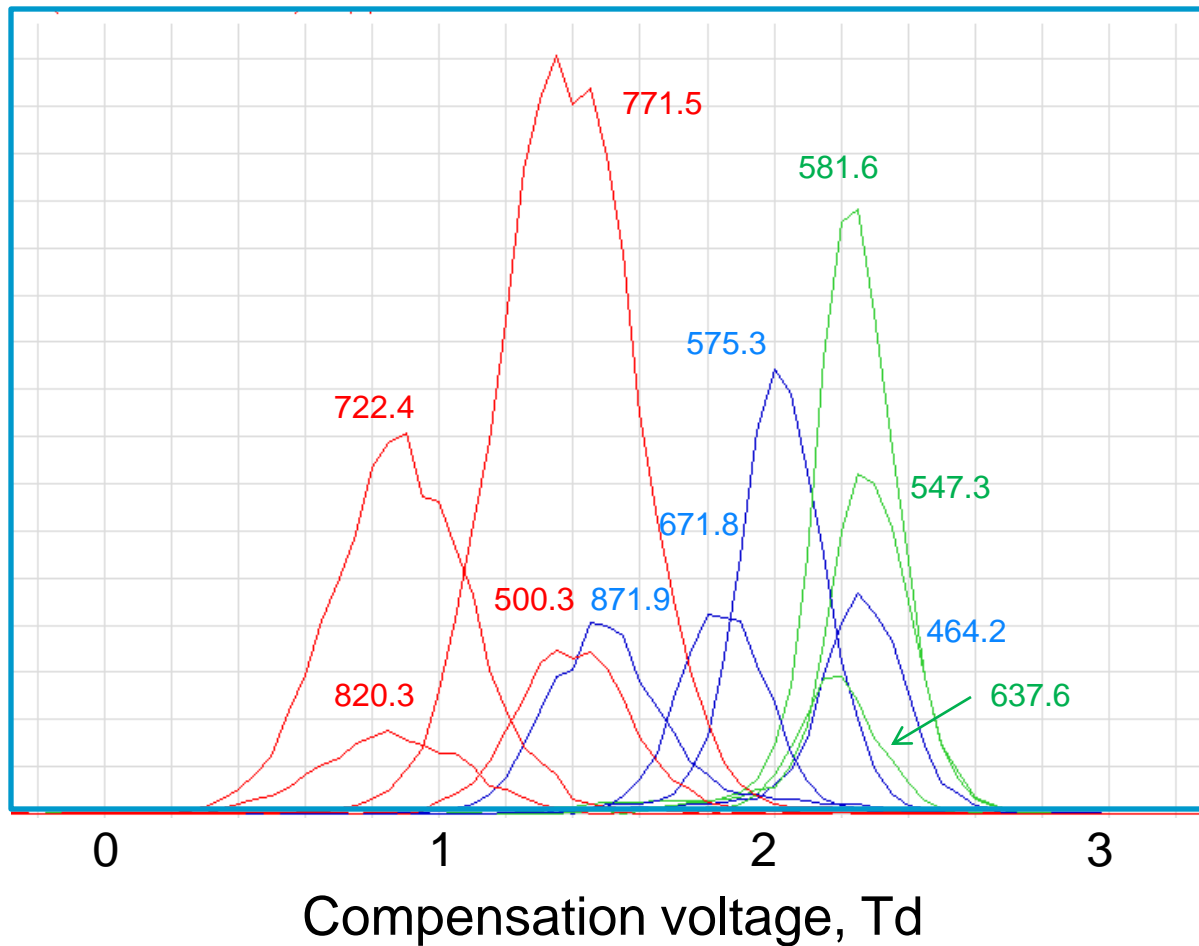


# One more example – charge state discrimination

- FAIMS is capable of efficient separation of **charge state of ions**.
- **Peptide ID routines** require correct identification of ion species which can be complicated in a mixture containing overwhelming number of potential precursors.
- Chip FAIMS is capable of providing significant increase in **confidence of precursor selection** based on charge state discrimination while maintaining very high overall sensitivity.
- High separation speed enables **real-time tuning of FAIMS settings**.
- **Preliminary data** for the separation based on infusion experiment has been collected using AJS ion source.

# Peptide charge state separation

BSA tryptic digest - selection of peptide peaks in CV plot



— 1+  
— 2+  
— 3+

Ion transmission  
50% or better

# Summary

- New chip FAIMS prototypes have been tested. Improvements in selectivity and transmission have been reported.
- Experiments show high degree of compensation field stability and signal linearity.
- Ultra-high field effects on ion mobility have been explored.
- 2 ms per step CF ramping and 30ms hopping for ultra-high speed LC MS applications has been demonstrated.

## Next steps

- Optimization of separation condition for specific applications.
- Better understanding and use of high field effects.
- Evaluation of clustering / de-clustering phenomena at higher DF levels.

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