

# Differential Ion Mobility Separations in Pure Helium and He Mixtures Using Microchips

Alexandre A. Shvartsburg, Yehia Ibrahim, Richard D. Smith

Biological Sciences Division, Pacific Northwest National Laboratory



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## Overview

- FAIMS microchips extremely resistant to electrical breakdown, permit separations in 100% He and all He mixtures with any gas
- We explored the dependences of FAIMS separation parameter (compensation field,  $E_C$ ), resolution, and resolution/sensitivity balance for peptides and metabolites on the He fraction in He/N<sub>2</sub> buffers across the full composition range
- Evaluation involved Owlstone chips of generations I (35  $\mu$ m gap) and current II (100  $\mu$ m gap).
- Evolution of  $E_C$  values between N<sub>2</sub> and He can be rationalized from first principles, showing the path to a *a priori* physical theory for FAIMS separations

## Introduction

- Differential or Field Asymmetric waveform IMS (FAIMS) separations depend on the buffer gas composition much stronger than those using conventional (linear) IMS as the nonlinearity of FAIMS magnifies small effects of specific ion-molecule interactions on ion mobility,  $K$  [1]
- Addition of helium to the buffer (typically nitrogen) broadly raises FAIMS resolution because of:
  - Light gas effect**  
Resolving power scales as  $\sim K^{1/2}$  and thus increases with lighter gases [1, 2]
  - Non-Blanc effect**  
High-field mobilities in mixtures deviate from weighted averages between  $K$  values in pure components. This tends to increase  $E_C$  and thus the resolving power [1].
- Electrical breakdown limits "full-size" FAIMS devices at maximum waveform amplitude (dispersion voltage) to 50% He, so 1:1 He/N<sub>2</sub> has become the buffer of choice.
- Mobilities measured by linear IMS can be related to ion geometries by matching to computed values [3]. That has not been achieved for FAIMS because high-field ion mobilities are much harder to model. Simplest ion-molecule interactions are for He, which has helped interpreting IMS data and would do so for FAIMS.
- Hence enabling FAIMS in helium is desired for both fundamental and analytical reasons

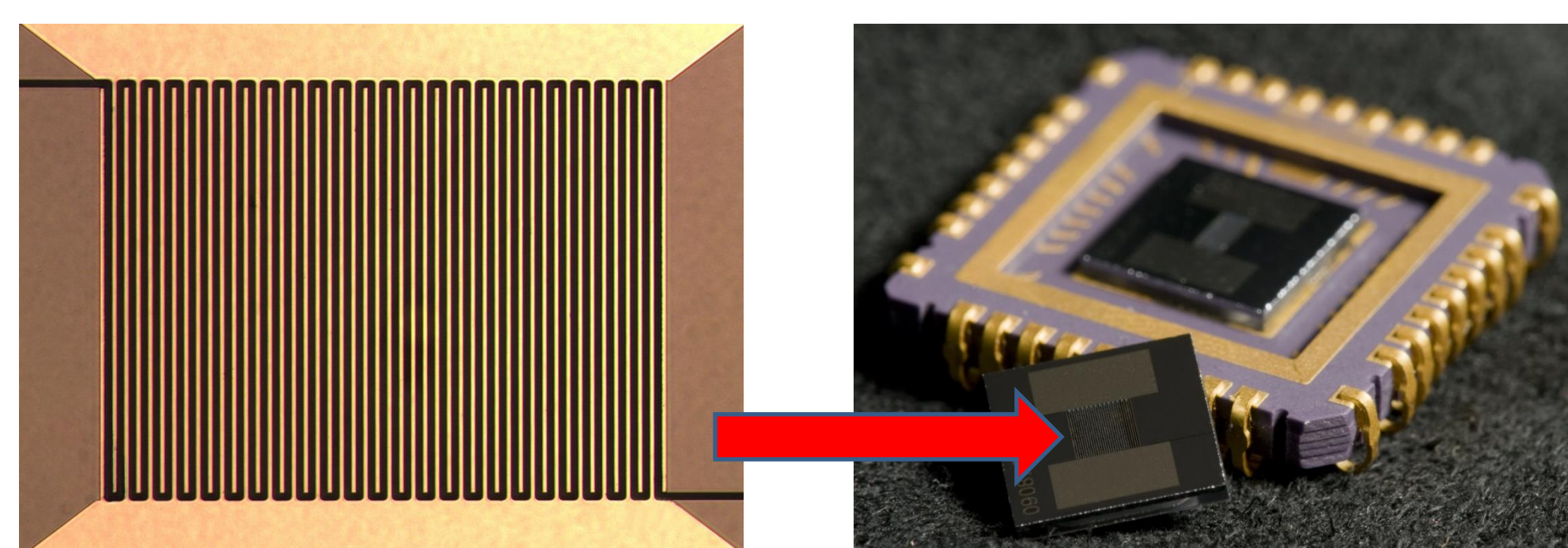
## Methods

### Fundamental premise

By Paschen law, the breakdown field ( $E_{BR}$ ) for any gas goes up with decreasing gap width ( $g$ ): for N<sub>2</sub>, from  $\sim 30$  kV/cm at  $g = 2$  mm to  $\sim 170$  kV/cm at  $g = 0.035$  mm [4]. Hence FAIMS chips with multichannel gaps [5] of  $g = 35 - 100$   $\mu$ m can use much higher dispersion field ( $E_D$ ) than "full-size" devices ( $g = 0.5 - 2.5$  mm). The actual  $E_D \sim 60$  kV/cm (limited by the waveform generator) is just  $\sim 35\%$  of  $E_{BR}$ . Therefore FAIMS microchips should allow high He fractions.

### Implementation

FAIMS microchips (Owlstone Ltd., Cambridge, UK) are etched (with 50% open surface) from silicon wafers, wired via gold vapor deposition, packaged, and mounted on a printed circuit board [4].



Bare chip ( $g = 35$   $\mu$ m)

Chip on PCB

Chip mounts are inserted into the ion path prior to the MS inlet (heated capillary). We explored two systems:

#### System I

With Thermo MS platforms (here, LTQ ion trap [6]):  
Chip ( $g = 35$   $\mu$ m, 0.3 mm thick) spaced from the capillary face;  
Temperature ( $T$ )  $\sim 40 - 60$   $^{\circ}$ C;  
Filtering time ( $t \sim 20 - 80$   $\mu$ s) adjusted by throttling the gas suction behind the chip;  
Dispersion voltage (DV)  $\sim 210$  V;  
Normalized  $E_D \sim 240 - 260$  Td



Front of the LTQ ion trap with FAIMS device in place

#### System II

With Agilent MS platforms (here, 6538 ToF MS):  
Chip ( $g = 100$   $\mu$ m, 0.7 mm thick) affixed to the capillary with  $T = 120 - 150$   $^{\circ}$ C;  
Filtering time ( $t \sim 250$   $\mu$ s) defined by gas flow through the capillary (1.2 L/min);  
DV  $\sim 550$  V;  
Normalized  $E_D \sim 300 - 320$  Td



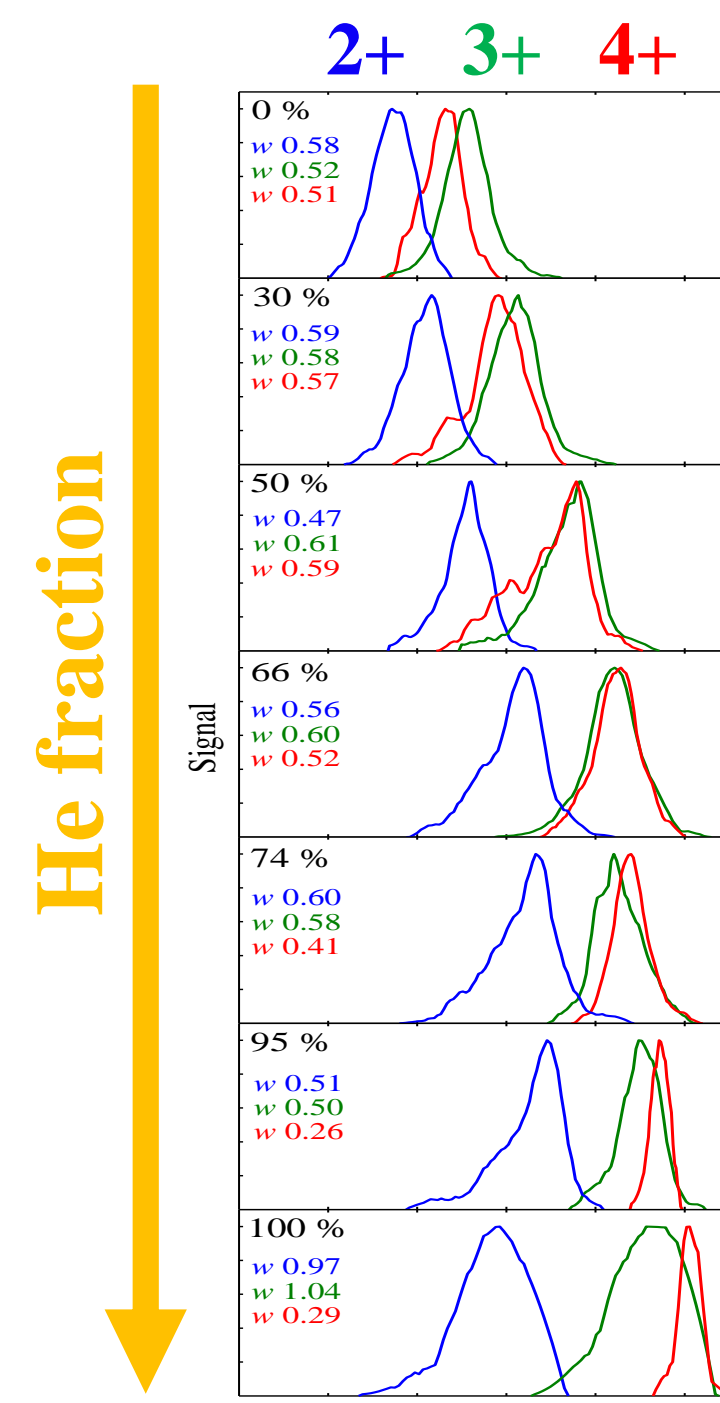
Complete FAIMS instrument with the control module

## Resolving power and resolution for peptides

Evolution of separation properties for model peptides with increasing He fraction in He/N<sub>2</sub> buffers, using System I

Data for Syntide 2 (PLARTLSVAGLPGKK, 1508 Da), charge states  $z = 2 - 4$

Mass-selected FAIMS spectra, peak widths (Td) labeled



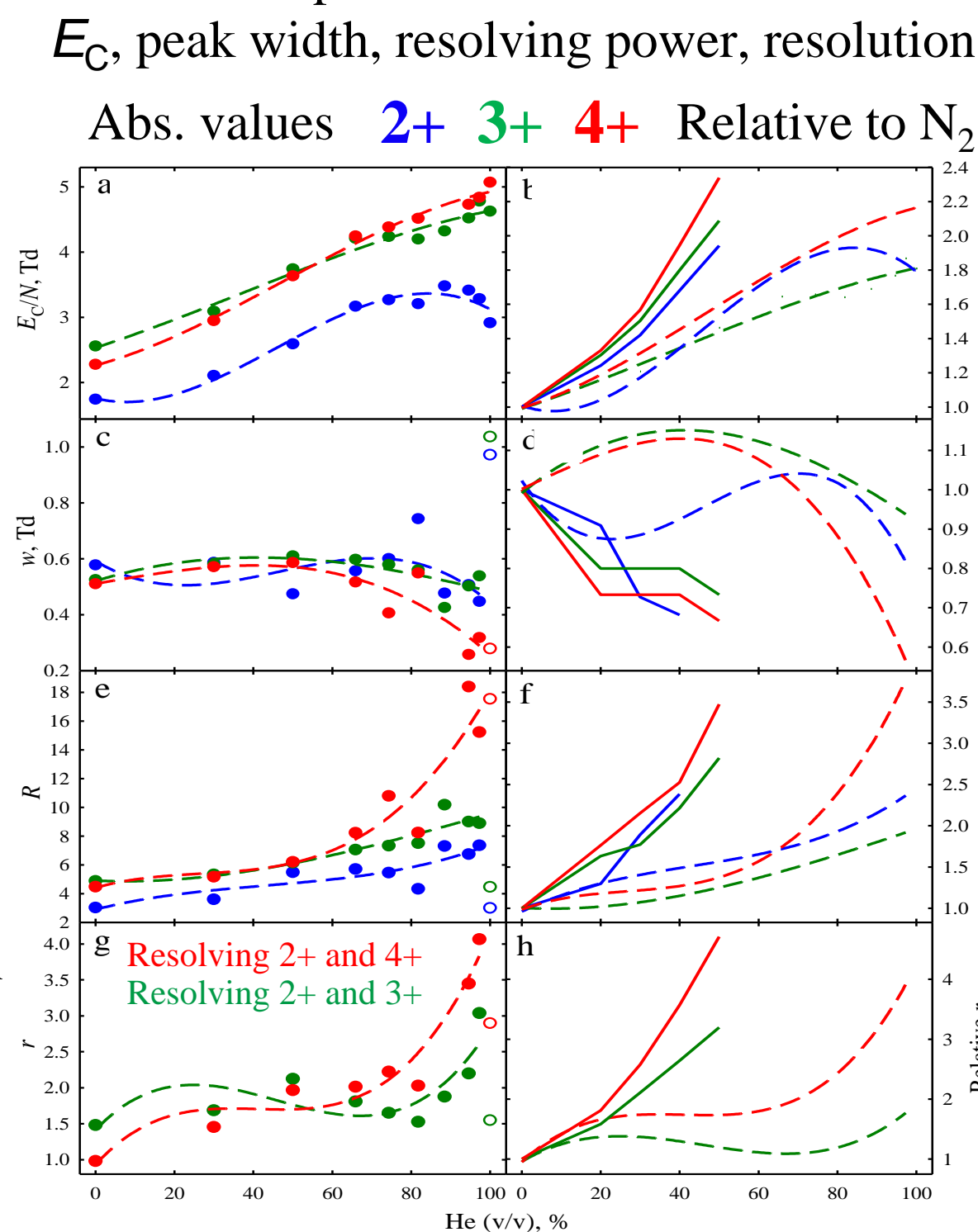
CVs for 2+ (not 3+ or 4+) maximize at  $\sim 85\%$  He

Peak widths decrease only slightly

Resolving power increases by  $\sim 2 - 4$  times

Feature resolution improves by a similar  $\sim 2 - 4$  times

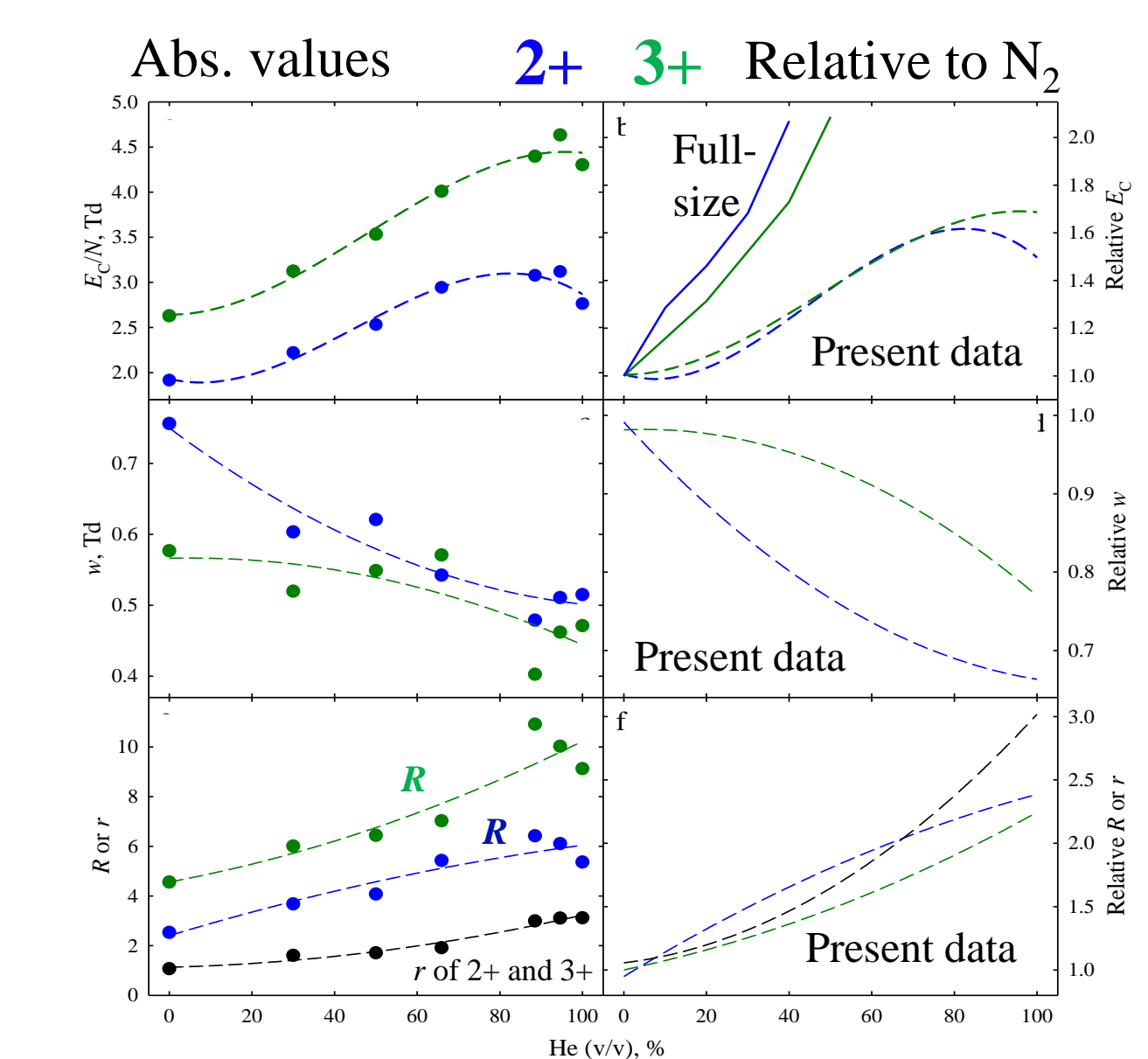
Separation metrics:  $E_C$ , peak width, resolving power, resolution



Dashed lines - regressions through present data (symbols)  
Solid lines - measured for full-size FAIMS up to 50% He

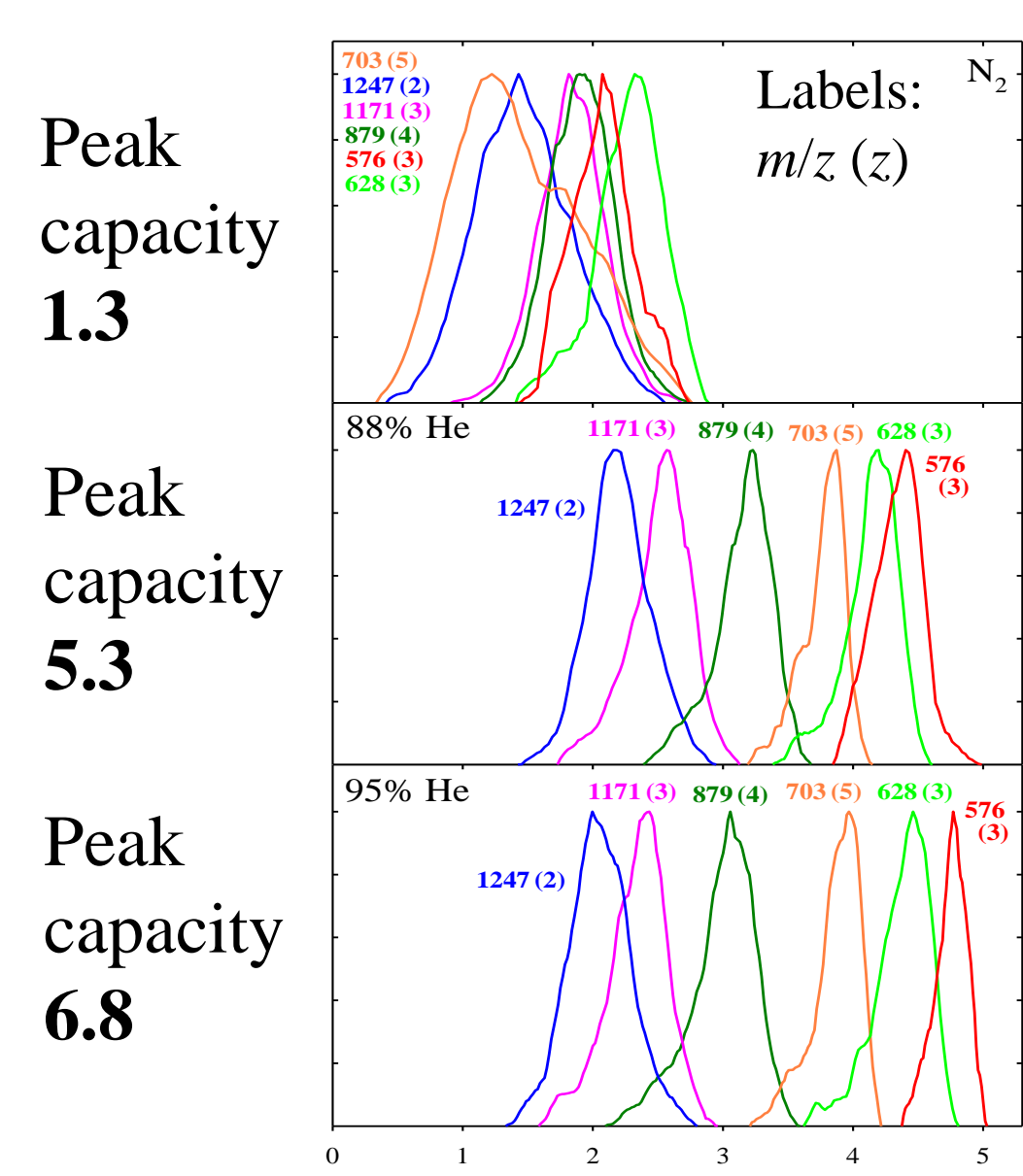
Trends for all separation metrics qualitatively track those with full-size FAIMS devices, but are weaker: relative changes between 0 and 100% He here at most compare to those with full-size units between 0 and 50% He [7]. This is because stronger fields in microchips lead to higher-energy ion-molecule scattering on the repulsive potential wall, which diminishes the distinction between more and less polarizable gas molecules.

Analogous data for phosphopeptide APLpSRGSLPKSYVK (1729 Da),  $z = 2$  and 3



Adding He increases resolving power, narrows peaks, improves resolution; all less so than with full-size devices

Separation of six peptide ions from BSA digest



Peak capacity 1.3

Peak capacity 5.3

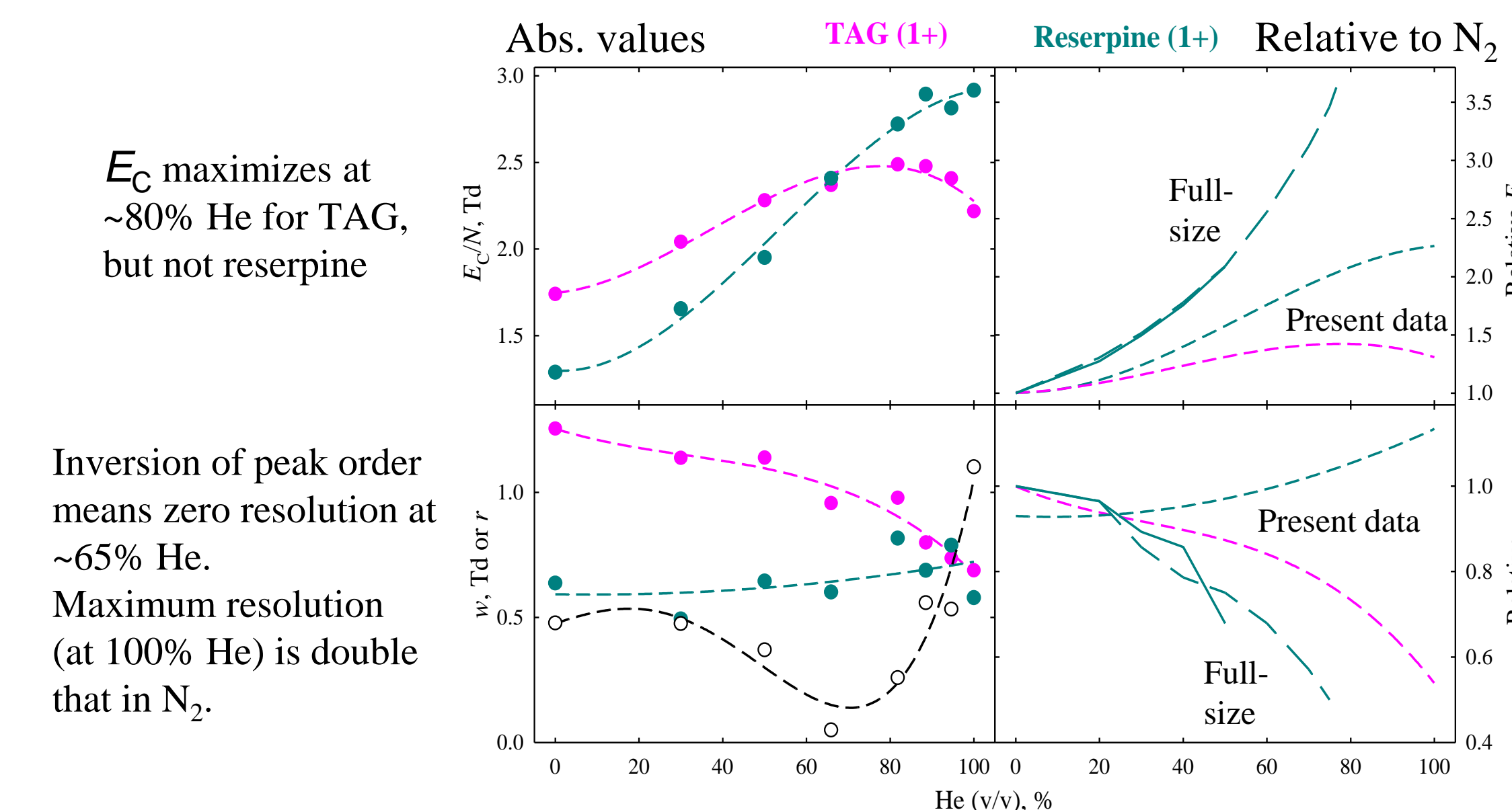
Peak capacity 6.8

Improvement of resolving power critical for peptide separations, even if less than with full-size devices

## Results

### Metabolite analyses

A major emerging FAIMS application is metabolomics. Most metabolite ions generated by ESI are singly charged. We look at the separation of reserpine (609 Da) and lipid triacylglycerol 18:1/18:1/16:0 9Z (TAG, 859 Da)



$E_C$  maximizes at  $\sim 80\%$  He for TAG, but not reserpine

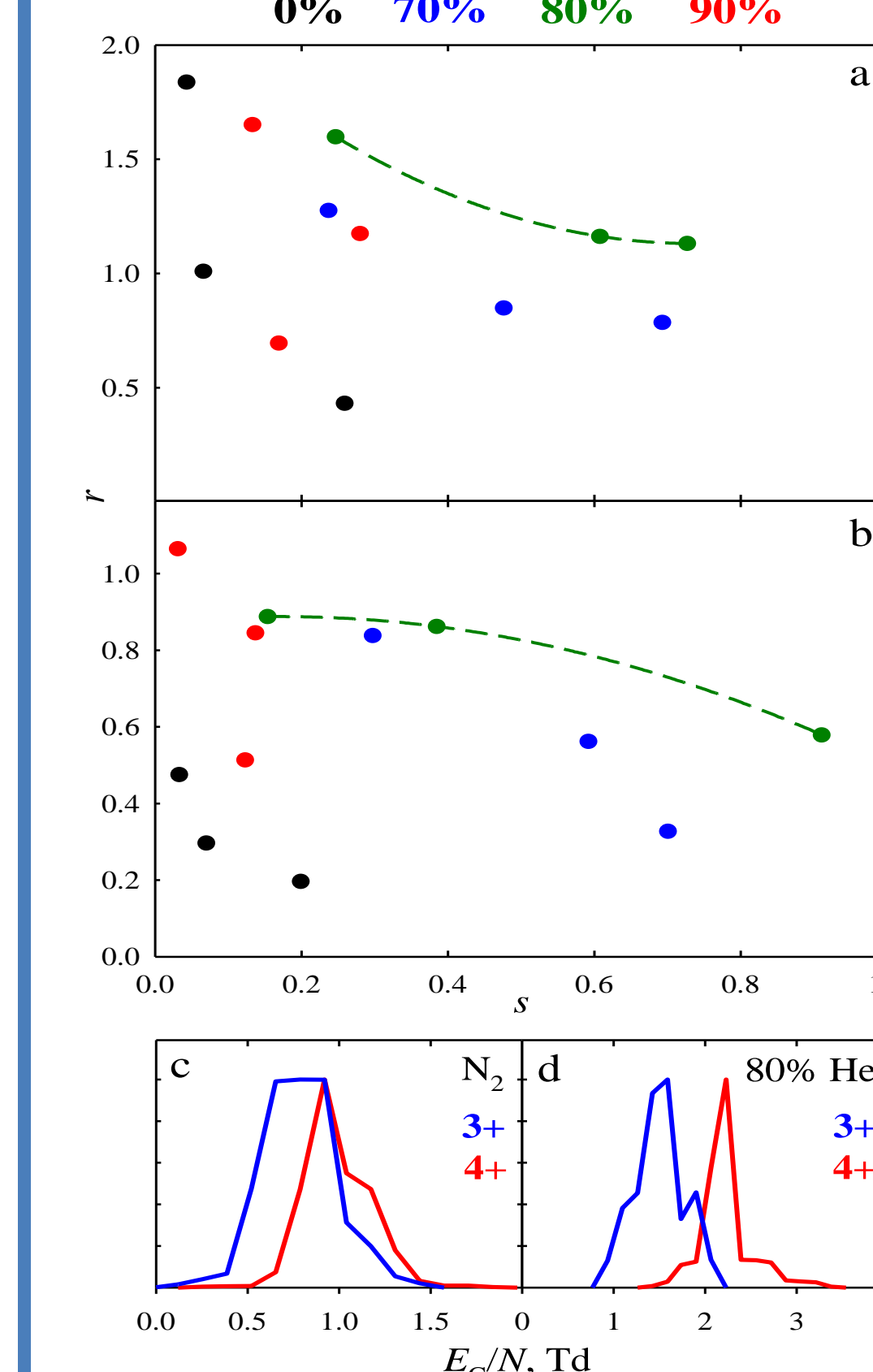
Inversion of peak order means zero resolution at  $\sim 65\%$  He. Maximum resolution (at 100% He) is double that in N<sub>2</sub>.

### Behavior broadly follows that for peptides

Use of He benefits resolution,  $E_C$  for some species maximize at  $\sim 80\%$  He, effects of He weaker than for full-size FAIMS devices.

### Resolution/sensitivity balance

Most important is the balance of resolution and sensitivity. With System II, measured:  
a) melittin (2847 Da) 3+ vs. 4+  
b) melittin 4+ vs. neurotensin (1673 Da) 3+



Best  $r/s$  balance (maximum resolution at equal sensitivity) at  $\sim 80\%$  He

### Toward a priori modeling of FAIMS separations

Absolute  $K$  values that govern linear IMS are accurately calculable from first principles, allowing rational separation design and elucidation of ion geometries. That was impossible for FAIMS because high-field mobilities were a challenge to compute. As with linear IMS, the best hope is analyses in He where ion-molecule potentials are closest to hard-sphere.

For hard-sphere ions,  $E_C$  scales as  $E_D^3$  - must reflect the proportionality of  $\Delta K$  to  $(velocity_{Drift}/velocity_{Brownian})^3$ . Then, for gases A and B and cross-sections  $\Omega$ :

$$E_C(B)/E_C(A) = [\Omega(A)/\Omega(B)]^3 \quad (1)$$

With  $A = N_2$  and  $B = He$ , the  $\Omega$  ratio is  $\sim 1.25$  for small and  $\sim 1.15$  for large peptides. Then the quantity (1) is  $\sim 2$  and  $\sim 1.5$ , respectively, in line with experiment. This agreement is encouraging for the construction of first-principles FAIMS theory for separations in He.

Maxima of  $E_C$  for some ions at  $\sim 80\%$  He are due to non-Blanc effects, not observed for type C ions previously. These can be modeled *a priori* [8].

## Conclusions

- In line with theory [8],  $E_C$  for many species top out at  $\sim 80 - 90\%$  He - the first observation of non-Blanc behavior for type C ions.
- Maximum resolving power at  $80 - 100\%$  He, exceeds that in N<sub>2</sub> by  $\sim 2 - 4$  times. Best resolution/sensitivity balance at  $80\%$  He.
- Effects of He similar to but weaker than those for full-size devices: more energetic collisions shift all gas molecules closer to hard spheres
- Successful estimation of the difference between  $E_C$  in N<sub>2</sub> and He is encouraging for the development of predictive model for FAIMS separations using helium
- Pure and mixed H<sub>2</sub> buffers should also work

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Contact: Dr. Alexandre A. Shvartsburg,  
alexandre.shvartsburg@pnnl.gov

## Career Opportunities

For potential openings with the Omics Separations and Mass Spectrometry Group at PNNL, write to: Dick Smith at [rd@s.pnnl.gov](mailto:rd@s.pnnl.gov); Josh Adkins at [Joshua.Adkins@pnnl.gov](mailto:Joshua.Adkins@pnnl.gov)

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