Differential Ion Mobility Spectrometry

Philippe MAÎTRE – Laboratoire de Chimie Physique
Université Paris-Sud, CNRS, Université Paris-Saclay

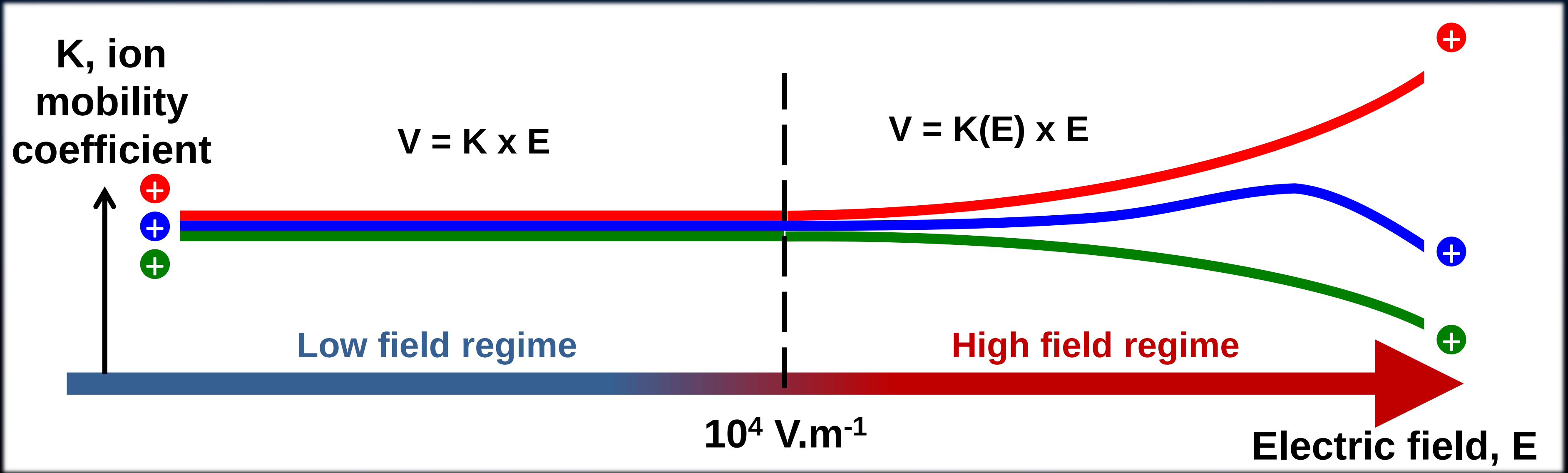
Ecole Thématique CNRS FTMS, Cabourg, 3-6 Avril 2018

Outline

- Introduction
  - FAIMS or DMS/DIMS? Ideas, Origins
  - Separation principle, set-ups, what is spectrum?
- How to improve ion resolution
  - Increasing the separation voltage and/or the residence time and/or adding modifier molecules to the transport gas, and/or …
- Probing DMS/FAIMS selected isomers
  - CID, HDX, MRM, isomer-selective MRM

Ion Mobility versus electric field: non-linear

- **Ion mobility spectrometry (IMS) allows for the (time or space) separation of ion based on their velocity, V, when travelling in a buffer gas under the influence of an electric field, E.**
- **K mobility varies significantly under high electric field**



- **Drift-tube IMS operates with low electric field**
- **Differential ion mobility spectrometry (DIMS or DMS) relies on the difference of ion mobility between low and high electric field**

=> DIMS/DMS uses an asymmetric RF field for separating ions.

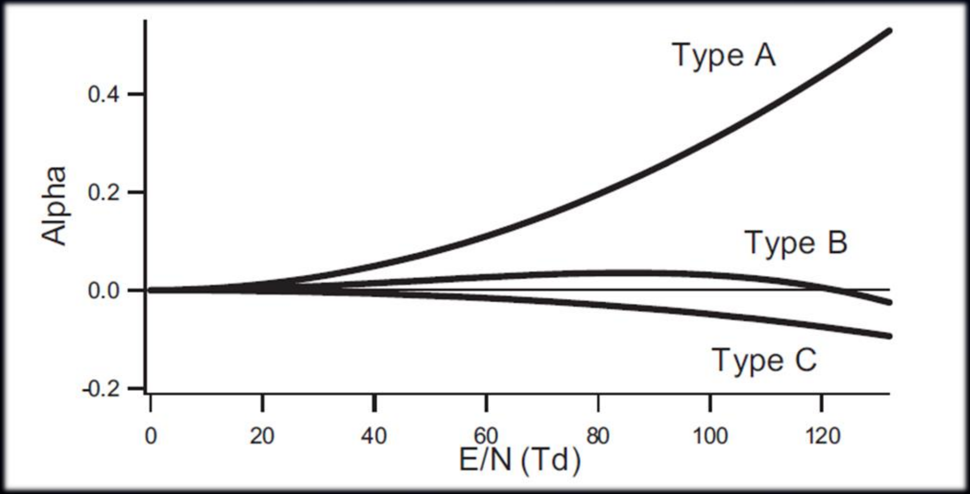
A. A. Shvartsburg, Differential Mobility Spectrometry: Nonlinear Ion Transport and Fundamentals of FAIMS CRC Press, 2008.

Three types of ions

- C: hard sphere, mobility decreases with E strength
- A: ion-molecule clustering/declustering at low/high E
  => Mobility smaller at low electric field
- B: combination of A and B, weakly bound ion-molecule clusters
  => A to C transition

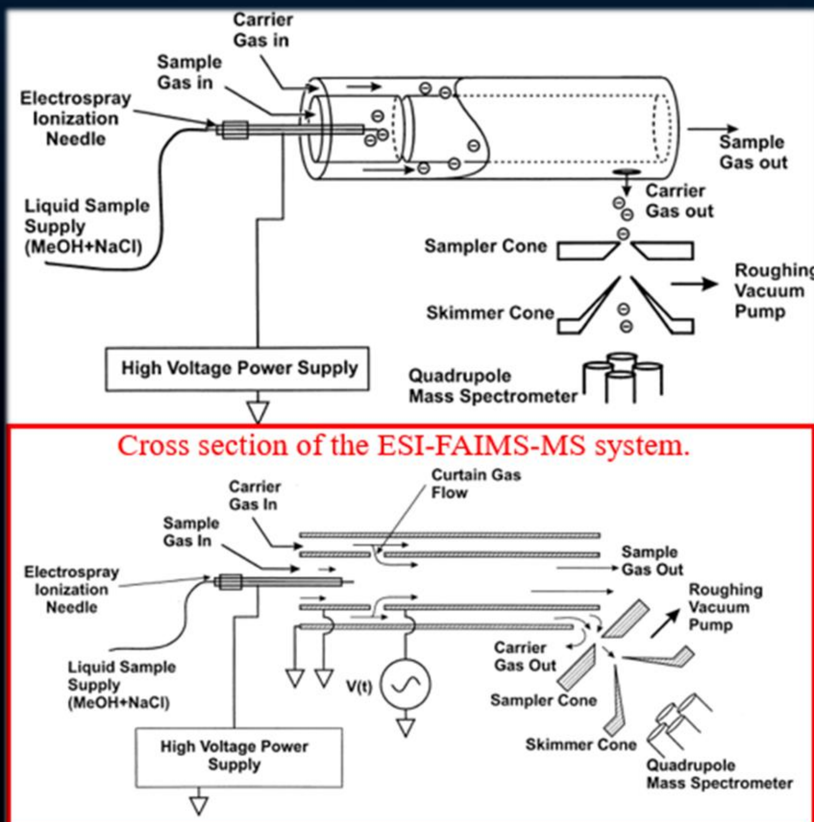
$K(E/N)=K(0)x[1+\alpha(E/N)]$

Alpha=$\alpha(E/N)$], mobility coefficient

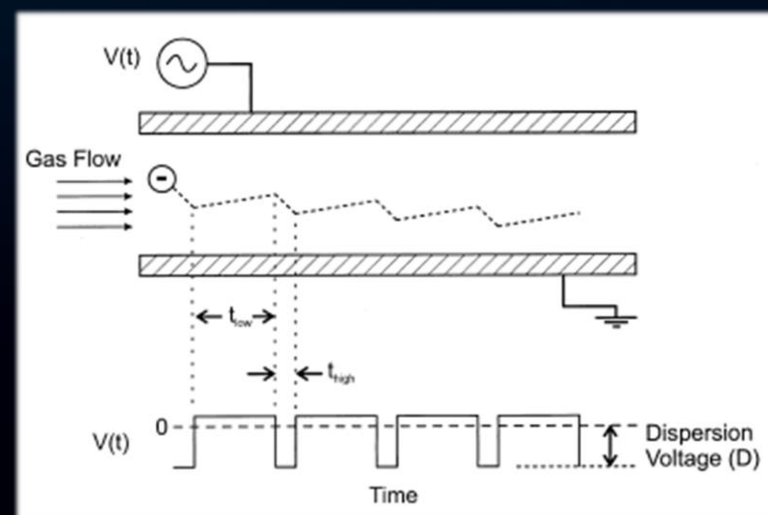


Terminologies/Geometries

- FAIMS = Field Asymmetric Ion Mobility Spectrometry
- 2 co-axial cyclindrical electrodes



- DIMS/DMS= Differential (Ion) Mobility Spectrometry
- 2 planar parallel electrodes



Larry A. Viehlanda, Roger Guevremont, Randy W. Purves, David A. Barnett, Comparison of high-field ion mobility obtained from drift tubes and a FAIMS apparatus, Int. J. Mass. Spectrom. 197 (2000) 123–130

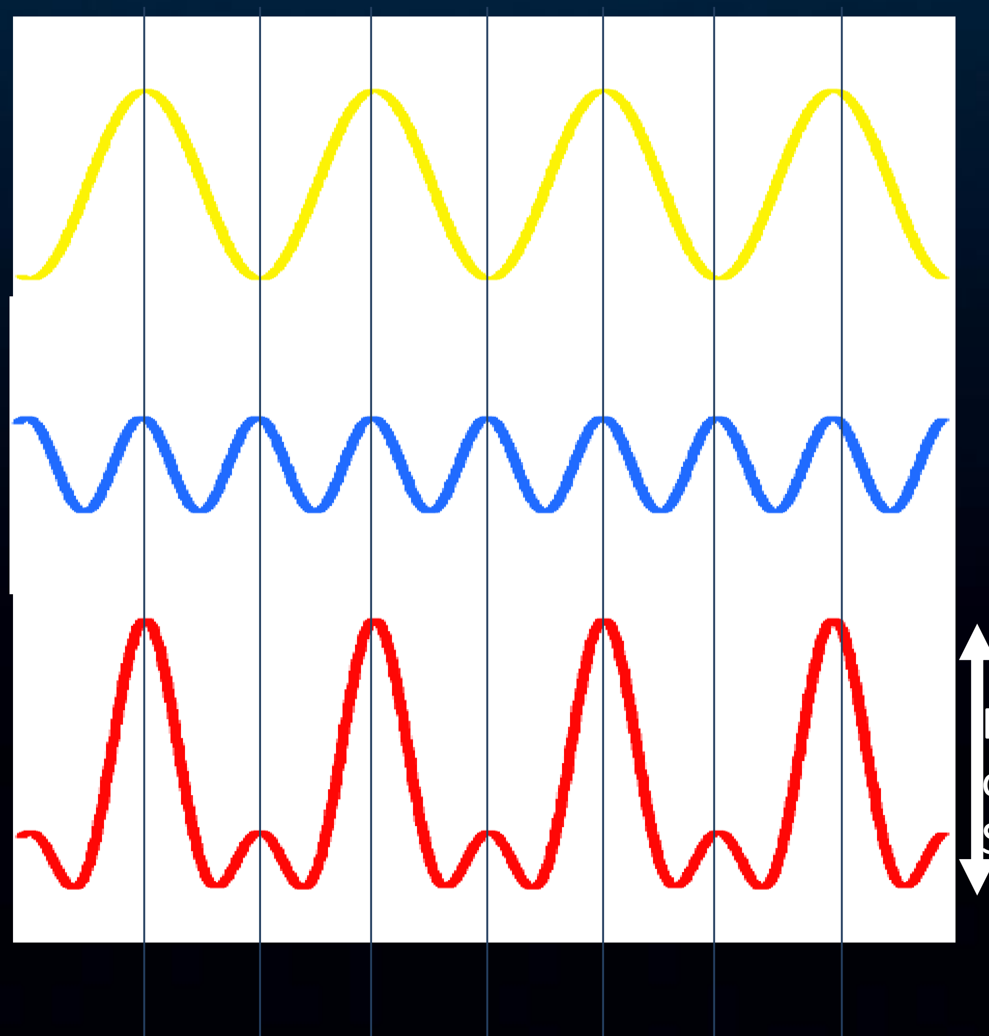
Electric field is periodic and asymetric

The majority of commercial DMS devices use bisinusoidal waveforms

$V_{high} \cos(\omega t)$

$+ V_{low} \cos(2\omega t)$

$= V_{asymetric}$

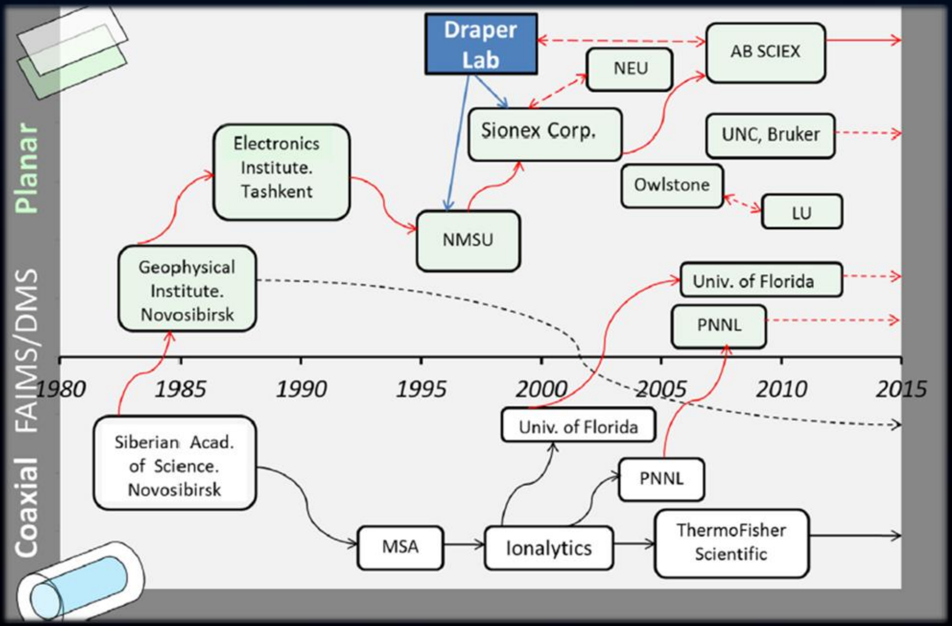


Dispersion Voltage (DV)
or
Separation Voltage (SV)

Alexandre A. Shvartsburg and Richard D. Smith, Optimum Waveforms for Differential Ion Mobility Spectrometry (FAIMS), J Am Soc Mass Spectrom 2008, 19, 1286–1295

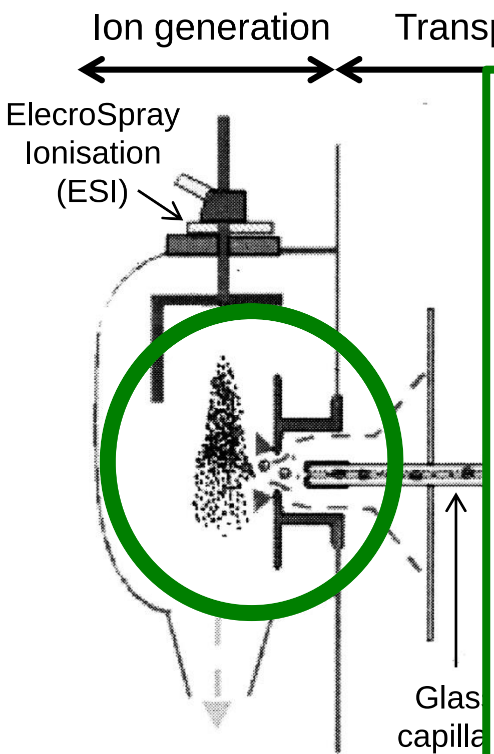
FAIMS and DIMS/DMS: Origins and Evolutions

- The idea of using the specific non-linear electric field dependence of an ion's coefficient of mobility for separation was motivated by the development of field-deployable sensors to detect land mines in the conflict inAfghanistanin the 80 's.
- First report: Gorshkov (1982, USSR patent No. 966583, G01N27/62).
- Development efforts continued in the Soviet Union and then:
  - Cylindrical devices were developed at Mine Safety Associates (MAS, Pittsburgh)
  - Planar devices were developped at New Mexico State University (NMSU) with G. Eiceman
- ~2000: coupling with mass spectrometers and electrospray ion sources

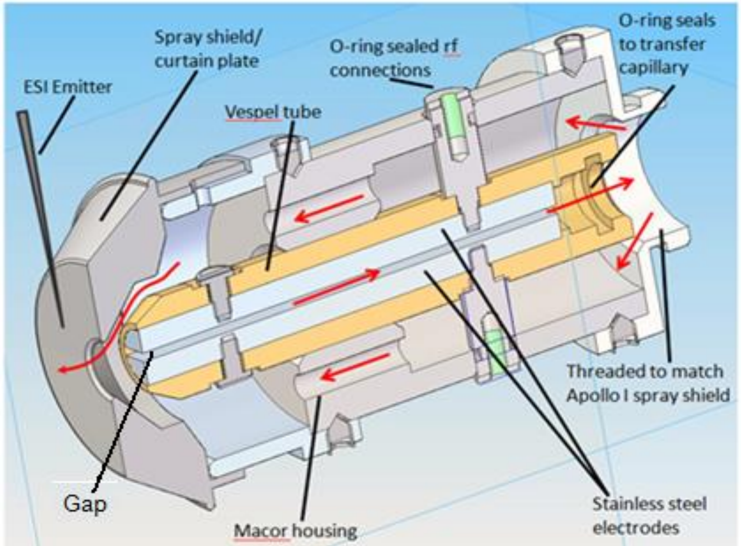


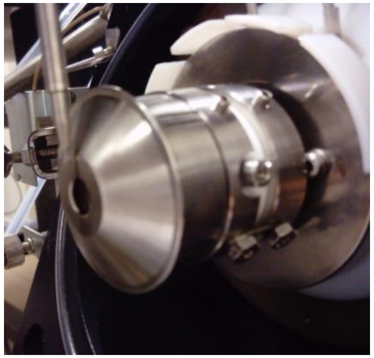
Schneider, B.B., Nazarov, E.G., Londry, F., Vouros, P., Covey, T.R.: DIFFERENTIAL MOBILITY SPECTROMETRY/MASS SPECTROMETRY HISTORY, THEORY, DESIGN OPTIMIZATION, SIMULATIONS, AND APPLICATIONS. Mass Spectrometry Reviews. ***35****, 687-737 (2016)*

Our DMS-Ion-Trap Experimental Set-up



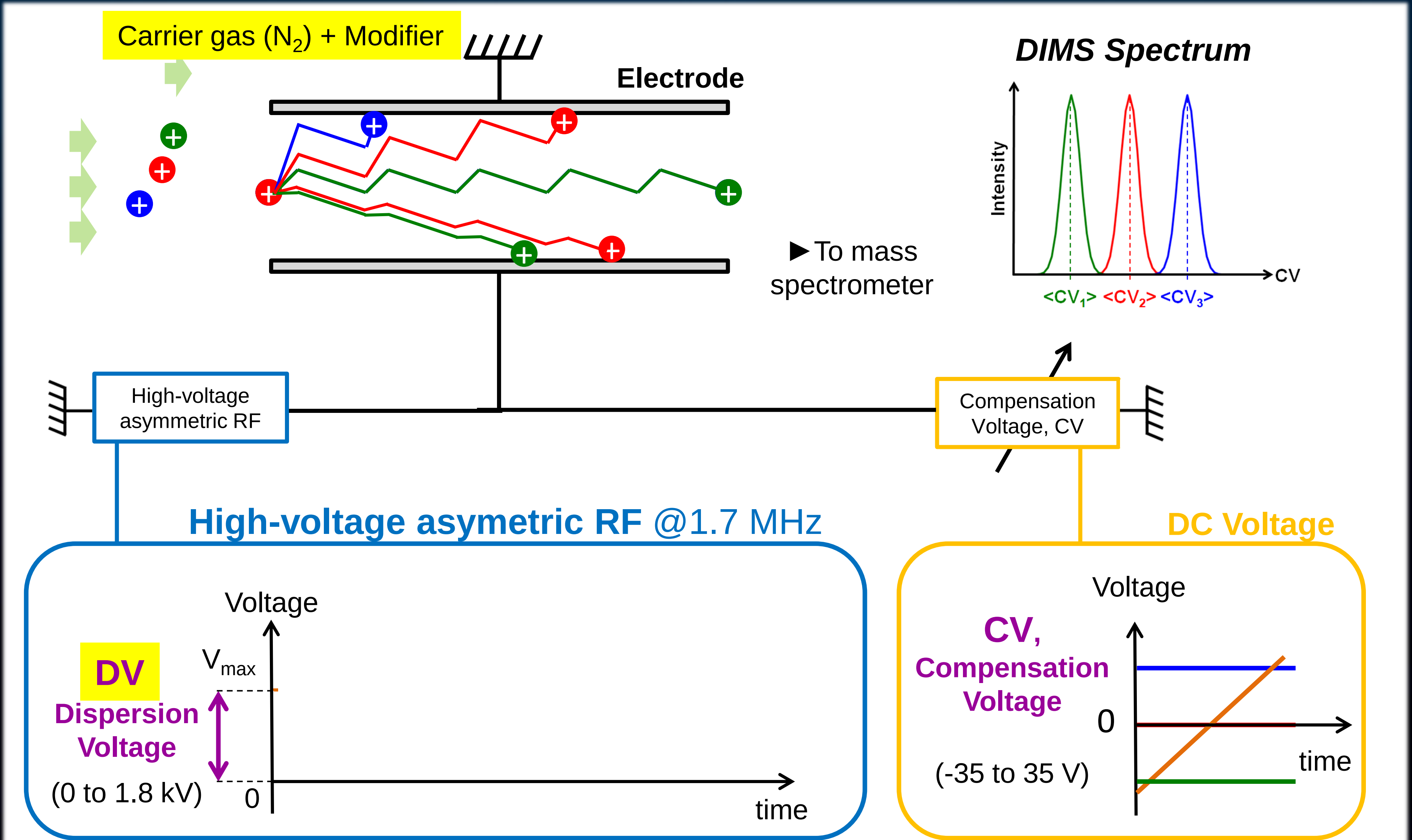
Differential Ion Mobility Spectrometry (DIMS)





- ✓ **Ion separation much faster with DIMS (ms) than with LC (min.)**
- ✓ **DIMS acts as an ion filter => ion trap filled with DIMS-selected ions**
- ✓ **Isomer/isobar separation, and also reduction of background signal**
- ○ **No clear understanding of the ion mobility under high electric field**

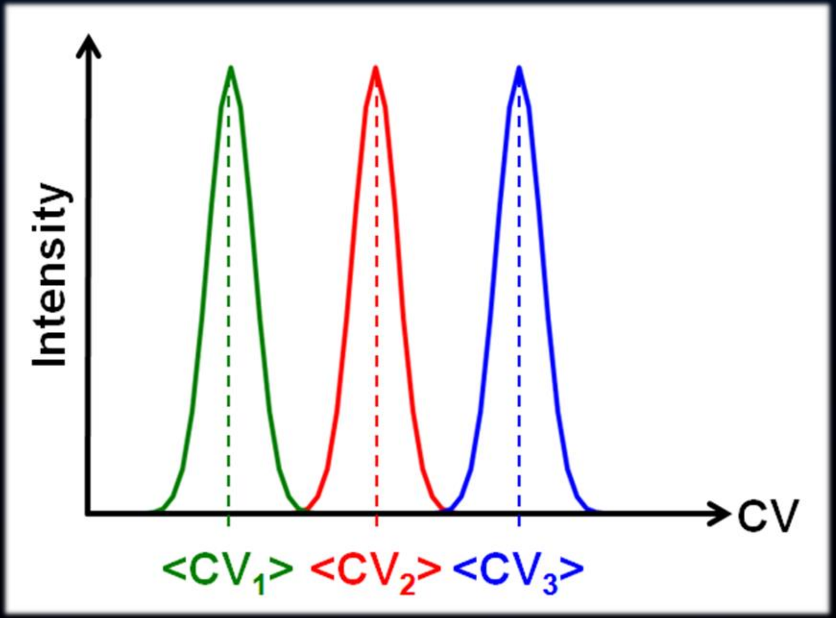
Separation principle and DMS spectrum



Berthias, F.; Maatoug, B.; Glish, G. L.; Moussa, F.; Maitre, P., Resolution and assignment of differential ion mobility spectra of sarcosine and isomers. J. Am. Soc. Mass Spectrom. 2018

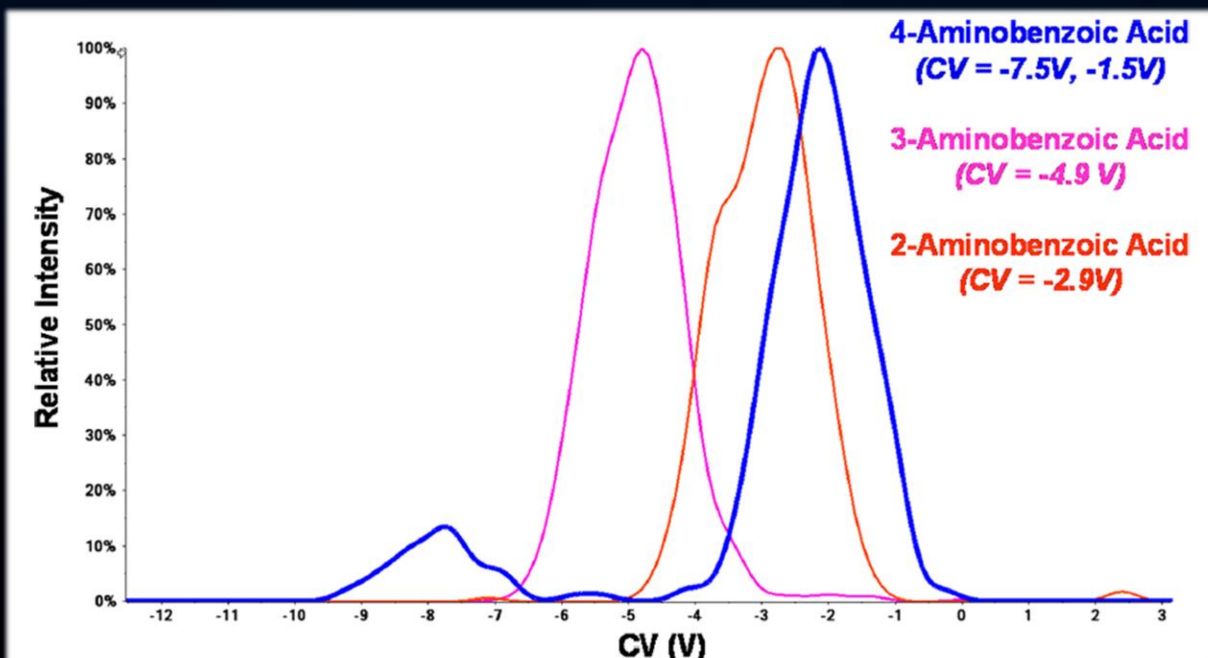
DIMS Spectrum

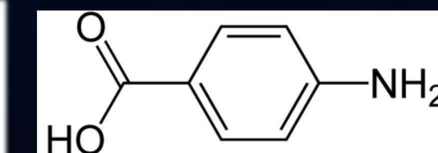
- Ion intensity versus:
  - Compensation Voltage (CV) in Volt
  - Electric field E (CV/Gap between electrodes)
  - E/N (where N is the buffer gas density)

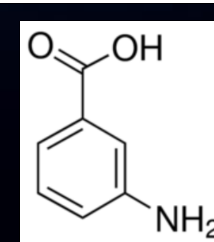


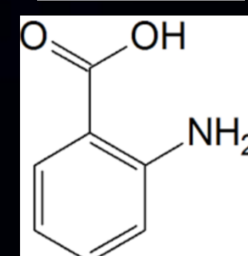
Separation of isomers of small molecules

- Separation of three isomers of aminobenzoic acid
- In the case of 4-aminobenzoic acid (para), two baseline resolved peaks were observed





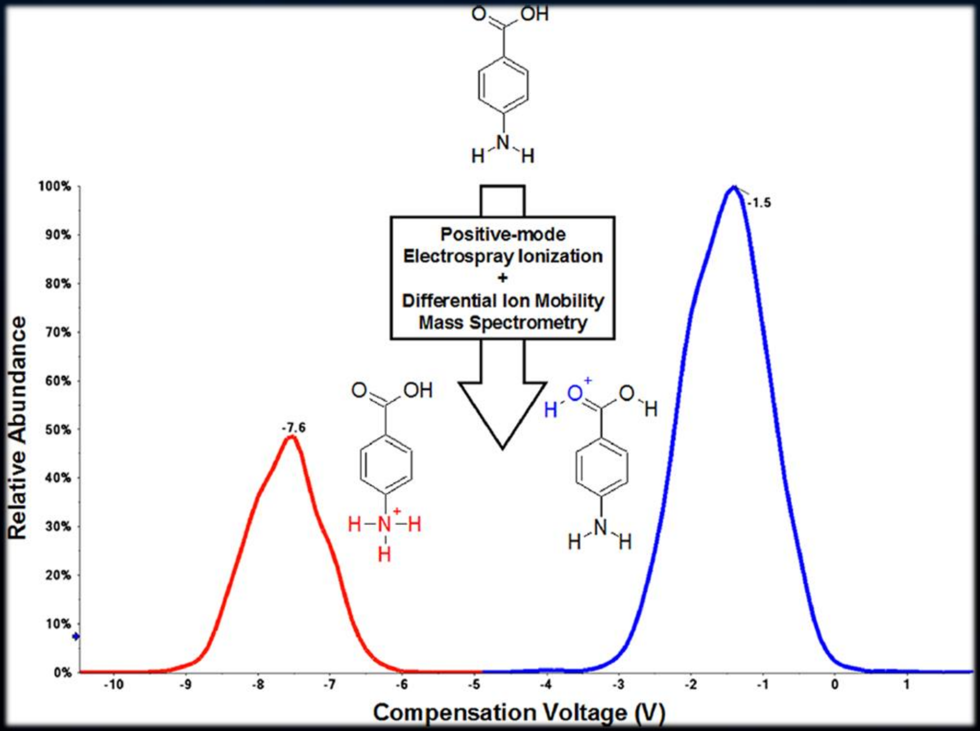




J. L. Campbell, J. C. Y. Le Blanc, and B. B. Schneider, *Probing Electrospray Ionization Dynamics Using Differential Mobility Spectrometry: The Curious Case of 4-Aminobenzoic Acid, Anal. Chem. 2012, 84, 7857−7864*

Separation of protomers of 4-aminobenzoic acid

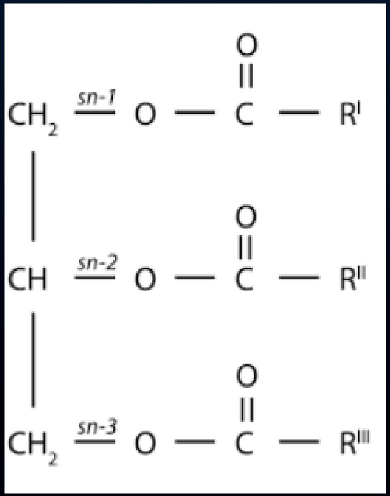
- Two competitive protonation sites (amine or acid)



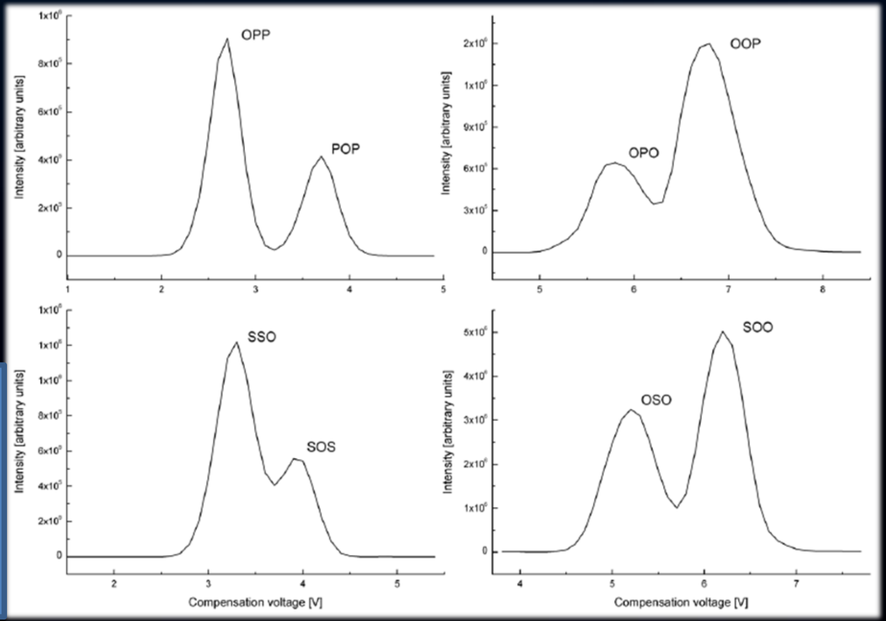
J. L. Campbell, J. C. Y. Le Blanc, and B. B. Schneider, *Probing Electrospray Ionization Dynamics Using Differential Mobility Spectrometry: The Curious Case of 4-Aminobenzoic Acid, Anal. Chem. 2012, 84, 7857−7864*

Separation of regioisomers of triacylglyceride (TAG)

- Quantification of TAG regioisomer ratios using FAIMS; In OPP/POP, fatty acyl chains in positions *sn-2* and *sn-3* are exchanged



Fatty acids:
O = Oleic, $CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$ (18:1)
P = Palmitic, $(CH_3)(CH_2)_{14}COOH$ (16:0)
S = Stearic, $(CH_3)(CH_2)_{16}COOH$ (18:0)

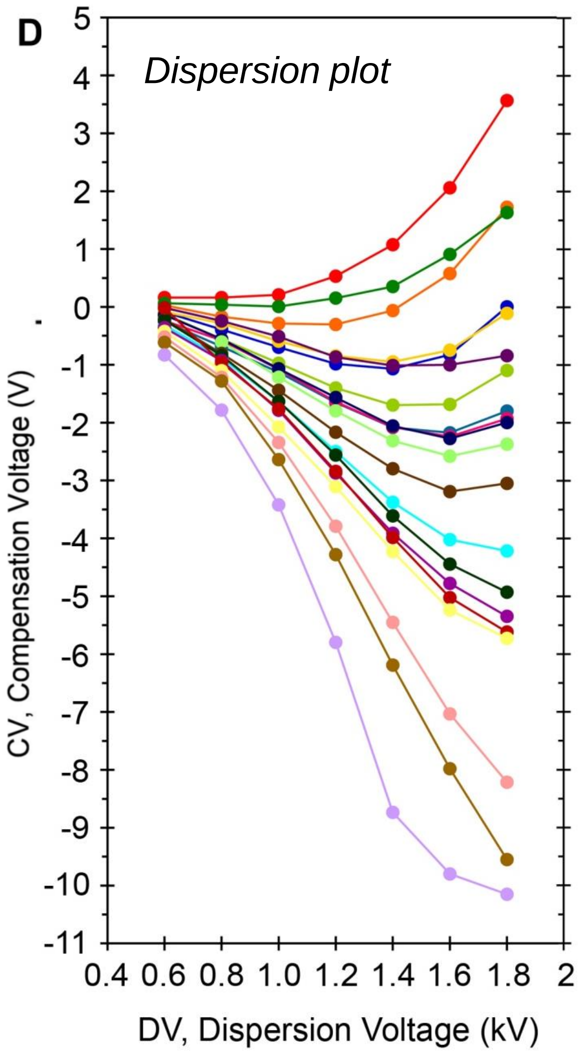


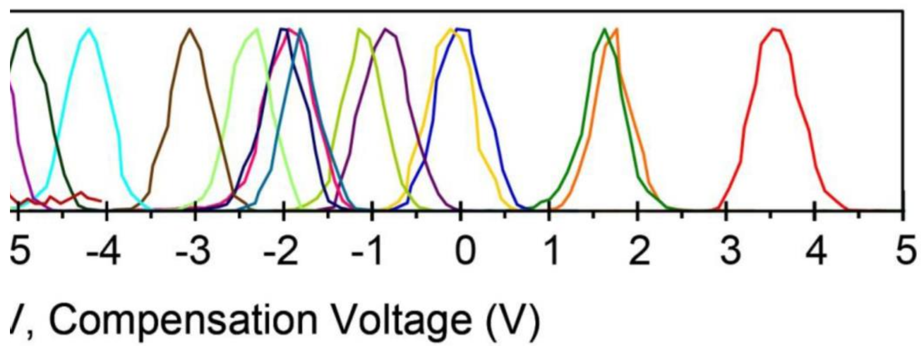
Martin Šala, Miroslav Lísa, J. Larry Campbell and Michal Holčapek, Determination of triacylglycerol regioisomers using differential mobility spectrometry, Rapid Commun. Mass Spectrom. 2016, 30, 256–264

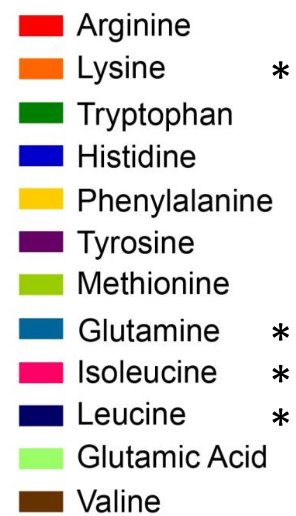
How to improve ion resolution

- Increasing the separation/dispersion voltage
- Adding modifier molecules to the transport gas
- Incresing the residence time

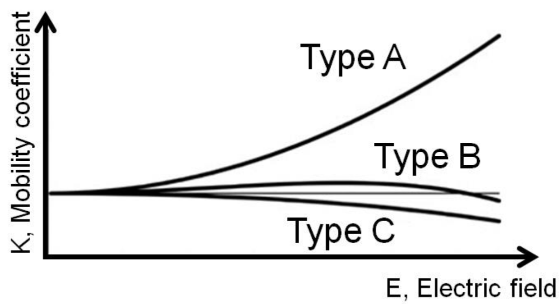
Separation of the 20 common amino acids







- **The higher the DV, the better the resolution.**
- **Three different behaviors are observed:**

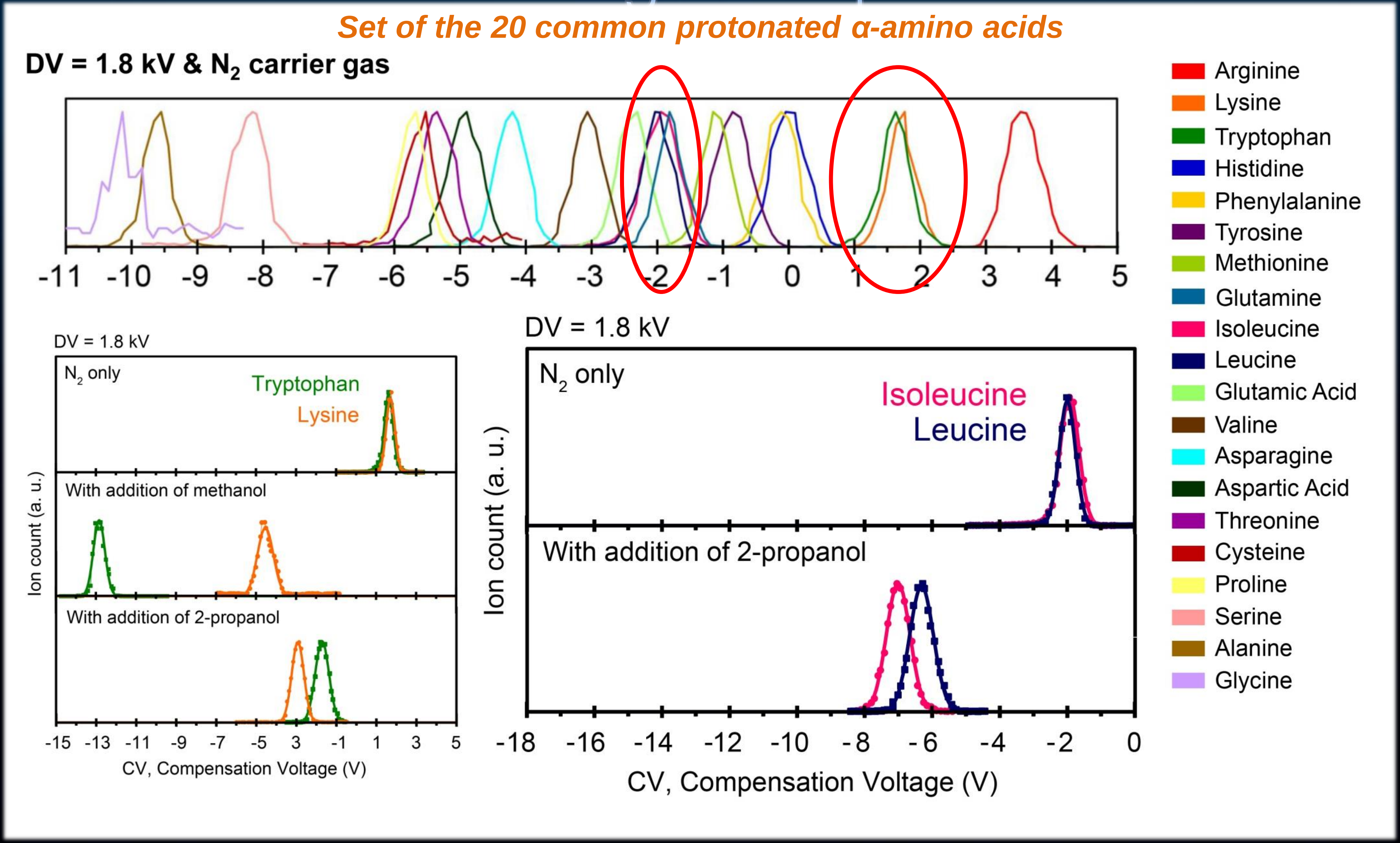


Three classes of ions:

Type A: clustering/de-clustering behavior is supposed to occur
Type C: hard sphere type collisions with the carrier gas.
Type B: mixed type A and C behaviour with the electric field strength.

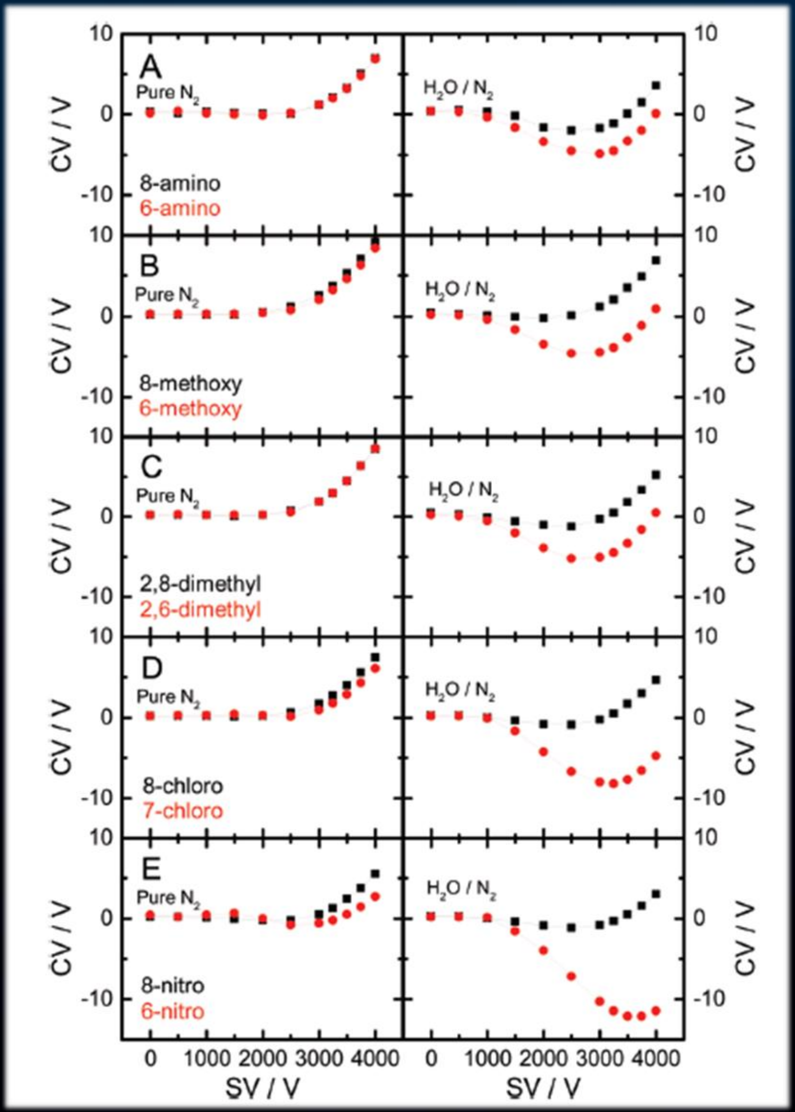
Berthias, F.; Moussa, F.; Maitre, P., To be submitted 2018

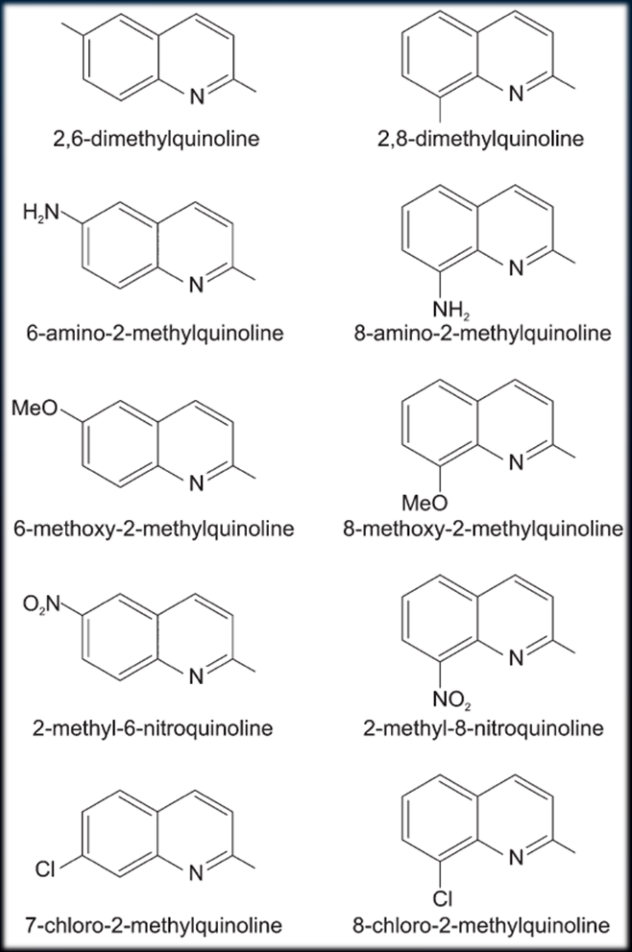
Addition of modifier gas in the N_2 carrier gas: improved resolution



Berthias, F.; Moussa, F.; Maitre, P., To be submitted 2018

Modifier gas effect on separation of quinoline-based drugs

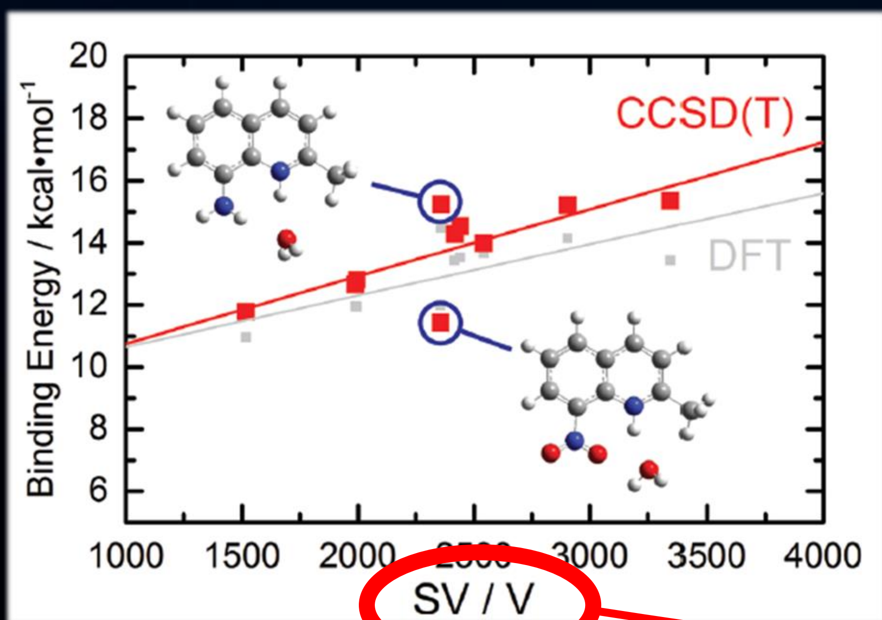


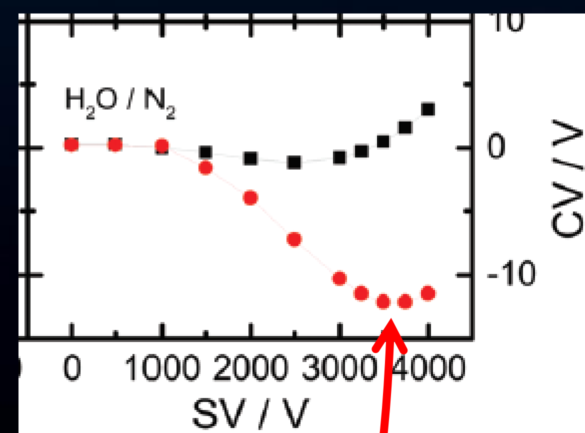


Hopkins, W. S.; Campbell, J. L. and coworkers; Using differential mobility spectrometry to measure ion solvation: an examination of the roles of solvents and ionic structures in separating quinoline-based drugs. *Analyst* 2015, *14* (20), 6897-6903.

Interpretation of the CV shifts / Modifier gas

- Correlation between:
  - Ion-Molecule (modifier) binding energy
  - SV at the DMS dispersion plot extrema for various methylquinolinium derivatives in an N_2 environment seeded with 1.5% H_2O (v/v).



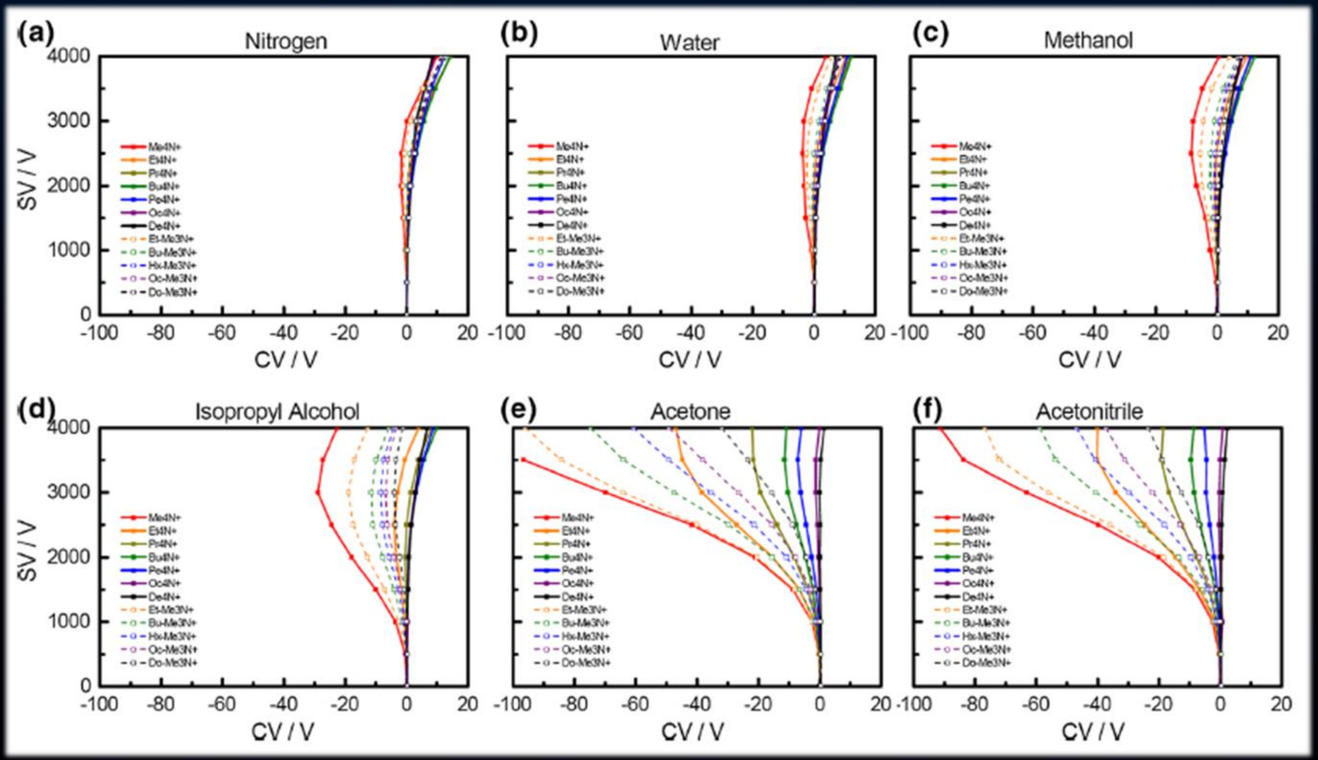


Hopkins, W. S.; Campbell, J. L. and coworkers; Using differential mobility spectrometry to measure ion solvation: an examination of the roles of solvents and ionic structures in separating quinoline-based drugs. *Analyst* 2015, *14* (20), 6897-6903.

Modifier gas effect on CV of tetraalkylammoniums

- A (significant) negative CV shift is observed upon addition of modifier gas

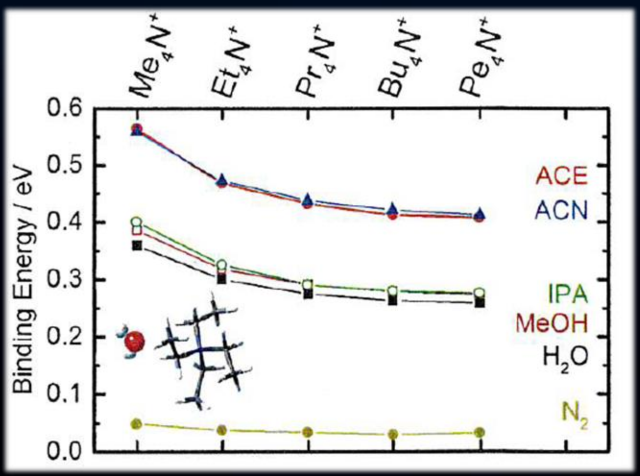
  => RP and PC increase

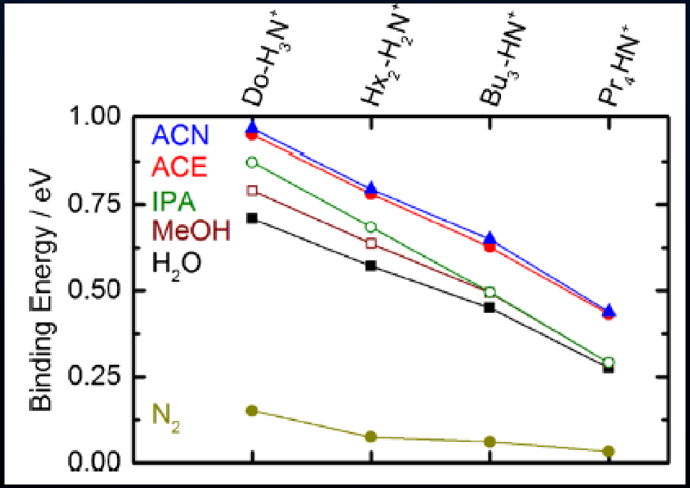


Campbell, J.L., Zhu, M., Hopkins, W.S.: Ion-Molecule Clustering in Differential Mobility Spectrometry: Lessons Learned from Tetraalkylammonium Cations and their Isomers. Journal of the American Society for Mass Spectrometry. ***25****, 1583-1591 (2014)*

Interpretation of the CV shifts / Modifier gas

- The larger is the ion-modifier binding energy, the larger is the negative CV shift





Calculated BEs of $C_{12}H_{28}N^+$ isomers protonated dodecylamine ($Do\text{-}H_3N^+$) protonated dihexylamine ($Hx_2H_2N^+$) , tributylamine ($Bu_3\text{-}HN^+$) , and tetrapropylammonium (Pr_4N^+)

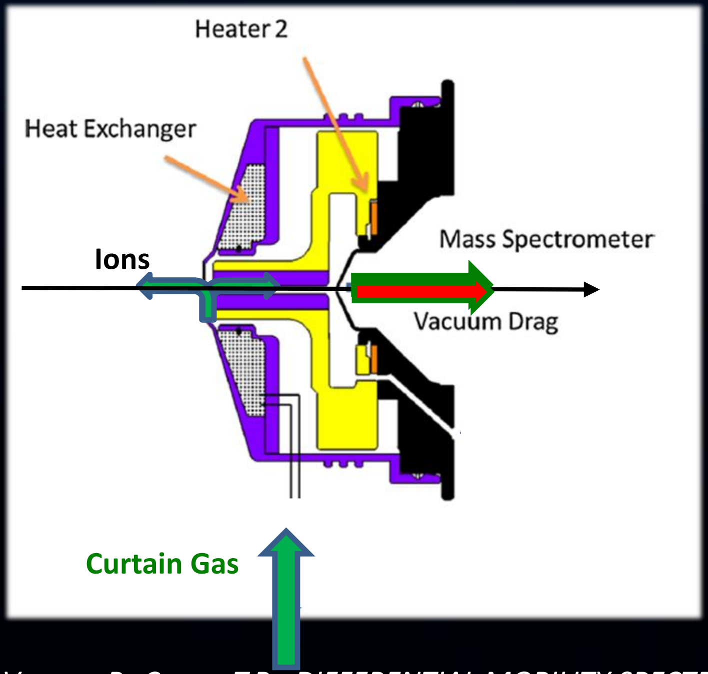
Campbell, J.L., Zhu, M., Hopkins, W.S.: Ion-Molecule Clustering in Differential Mobility Spectrometry: Lessons Learned from Tetraalkylammonium Cations and their Isomers. Journal of the American Society for Mass Spectrometry. ***25****, 1583-1591 (2014)*

How to improve the resolution? Throttle gas

1) The longer is the residence time (RT) of the ions in DMS device, the better is the resolution

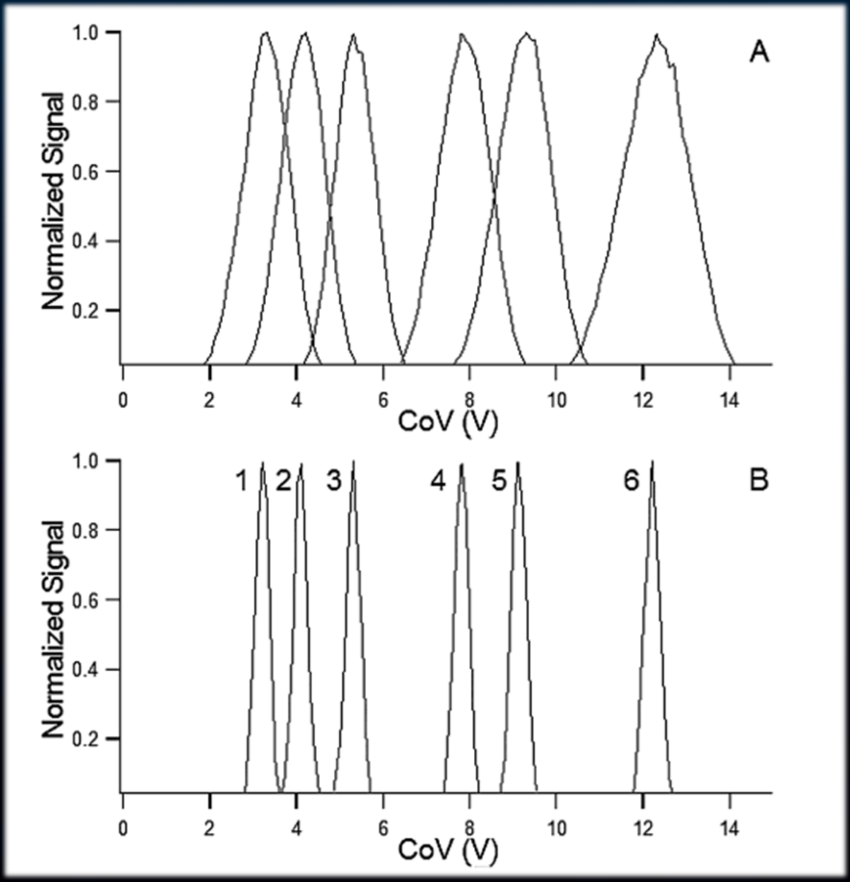
2) RT is controlled by the flow of the gas going through the DMS device, and the maximum transport gas-flow rate is determined by the vacuum drag of the MS

3) By adding a throttle gas flow, one can reduce the is essentially controlled by the speed of the gas flow, which depends on the curtain gas flow (in) and vacuum drag (out)



Schneider, B.B., Nazarov, E.G., Londry, F., Vouros, P., Covey, T.R.: DIFFERENTIAL MOBILITY SPECTROMETRY/MASS SPECTROMETRY HISTORY, THEORY, DESIGN OPTIMIZATION, SIMULATIONS, AND APPLICATIONS. Mass Spectrometry Reviews. ***35****, 687-737 (2016)*

Effect of the throttle gas on the DMS resolution



- Sample= six-component mixture of durgs: (1) Phenylalanine (m/z 166.1), (2) Histidine (m/z 156.1), (3) Methylhistamine (m/z 126.0), (4) Minoxidil (m/z 210.1), (5) Cimetidine (m/z 253.1), and (6) Perphenazine (m/z 404.2).

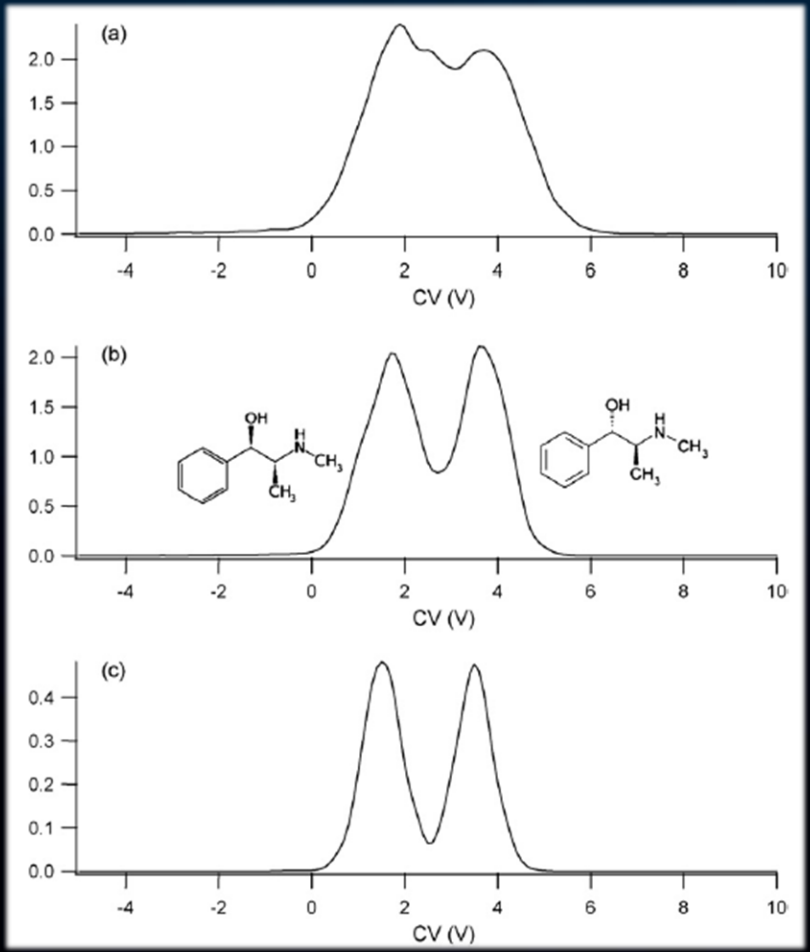
- (A) Throttle gas off => residence time of the ions = 6.5 ms
  - Peak capacity (PC) = 6.5
  - Resolving power (RP) = 2.6-6.7
- (B) Throttle gas on => residence time = 20 ms
  - Peal capacity = 22.5
  - Resolving Power = 11.3-28.4

$$RP = \frac{CoV}{FWHM}$$

$$PC = \frac{CoV_{max} - CoV_{min}}{FWHM}$$

Schneider, B.B., Nazarov, E.G., Londry, F., Vouros, P., Covey, T.R.: DIFFERENTIAL MOBILITY SPECTROMETRY/MASS SPECTROMETRY HISTORY, THEORY, DESIGN OPTIMIZATION, SIMULATIONS, AND APPLICATIONS. Mass Spectrometry Reviews. ***35****, 687-737 (2016)*

Throttle gas improves separation of diastereoisomers



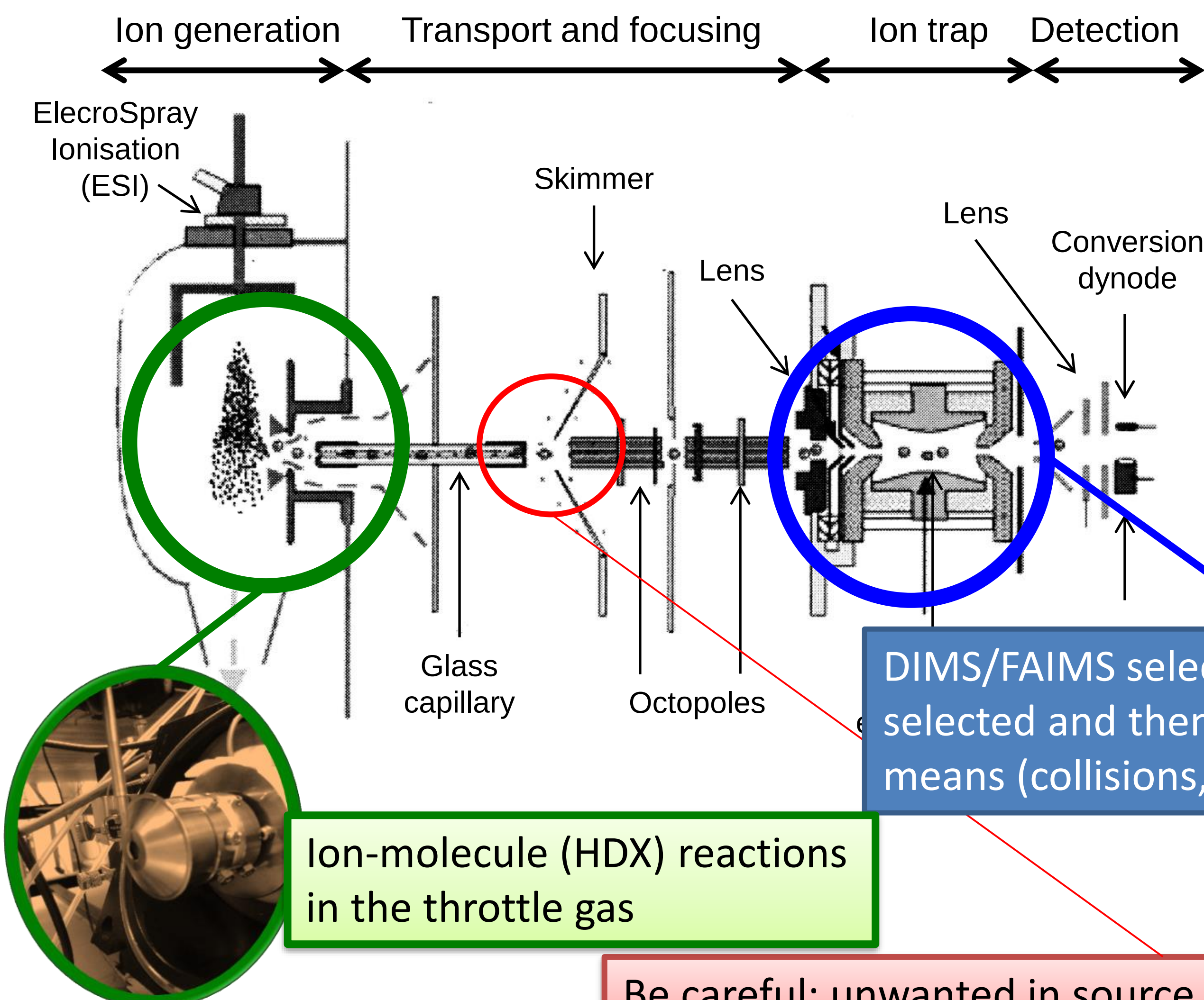
- Nearly baseline resolution when increasing adding the throttle gas resulting in decreasing DMS transport gas:
  - (a) 2.5, (b) 2, and (c) 1 L/min.
- Relative ion counts vs CV
  - Ion loss when RT increases

B. B. Schneidera, T. R. Coveya, S. L. Coyb, E. V. Krylovb, E. G. Nazarov, *Planar differential mobility spectrometer as a pre-filter for atmospheric pressure ionization mass spectrometry, Int. J. Mass. Spectrom. 298 (2010) 45–54*

Probing DMS/FAIMS selected isomers

CID, HDX, MRM, isomer-selective MRM

MS/MS on DIMS/FAIMS selected ions



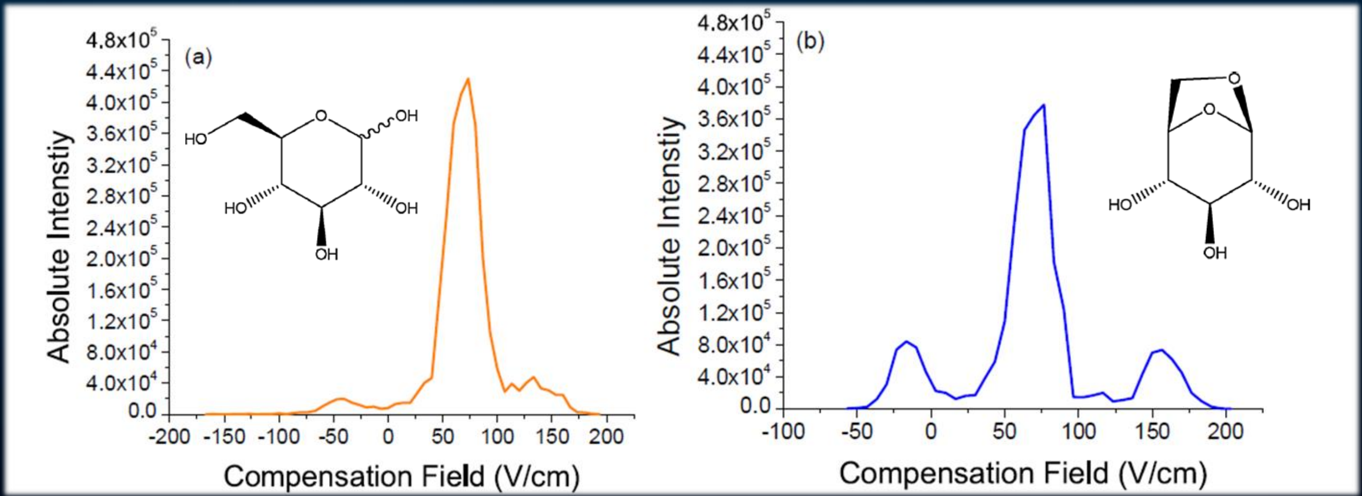
DIMS/FAIMS selected ions can be mass-selected and then activated by different means (collisions, electrons, photons)

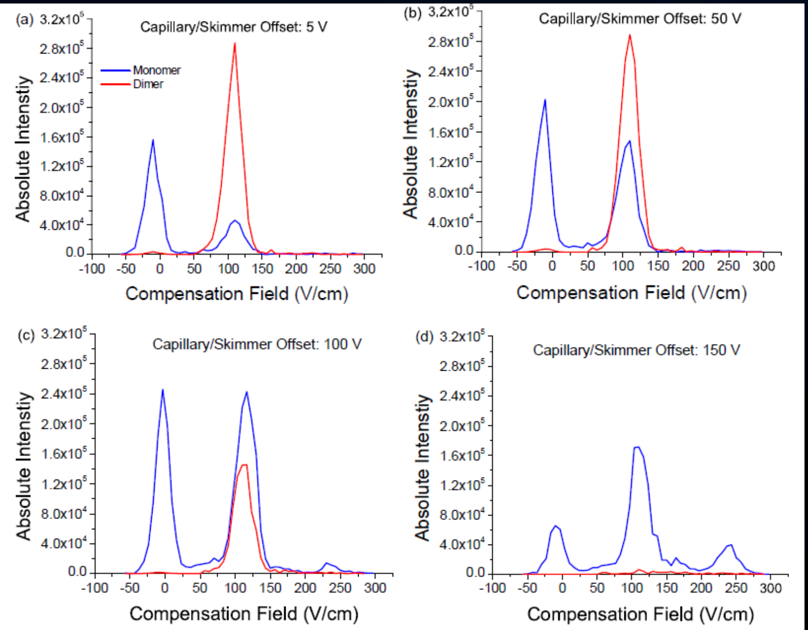
Ion-molecule (HDX) reactions in the throttle gas

Be careful: unwanted in source fragmentations of adducts

Post-DIMS fragmentation of adducts (dimer)

Three DIMS peaks were observed for both lithium cationized glucose and levoglucosan.





Only one corresponds to the monomers, the two others to dimers.

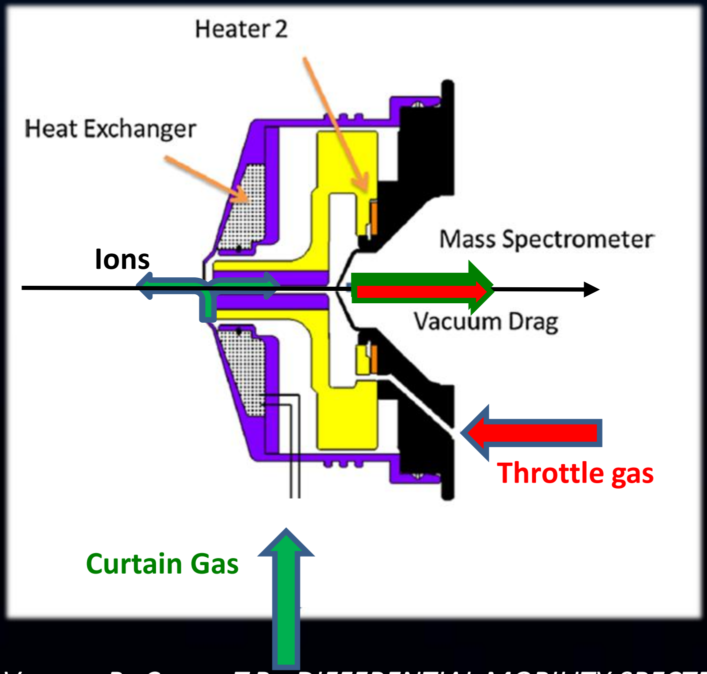
As the voltage is increased the dimer peak begins to fragment, resulting in decreased intensity for the dimer peaks and increased intensity for monomer peak

M. T. Campbell, and G. L. Glish, Fragmentation in the ion transfer optics after differential ion mobility spectrometry produces multiple artifact monomer peaks, Int. J. Mass Spectrom.425 (2018) 47–54

Probing DMS selected isomers through ion-molecule reactions

Adding reagent in the throttle gas:

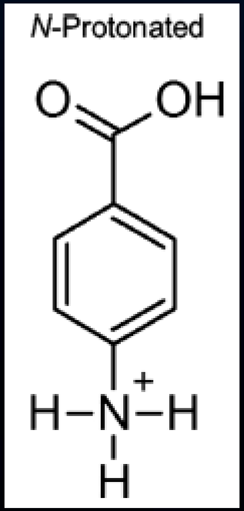
- DMS-selected ion-molecule reactions
- Hydrogen exchange reactions (HDX)

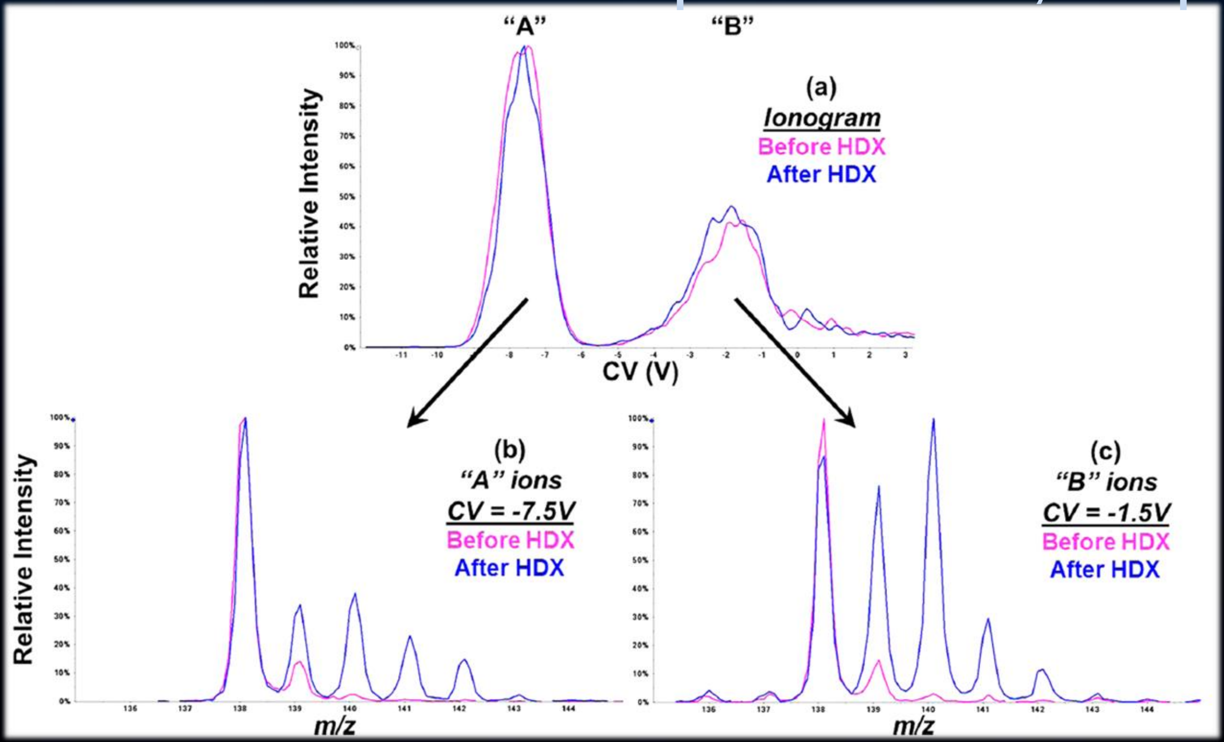


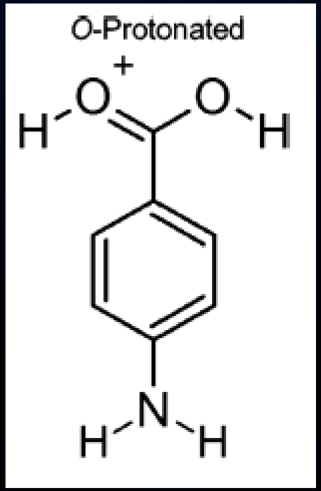
Schneider, B.B., Nazarov, E.G., Londry, F., Vouros, P., Covey, T.R.: DIFFERENTIAL MOBILITY SPECTROMETRY/MASS SPECTROMETRY HISTORY, THEORY, DESIGN OPTIMIZATION, SIMULATIONS, AND APPLICATIONS. Mass Spectrometry Reviews. ***35****, 687-737 (2016)*

Probing protomers: Hydrogen−Deuterium exchange

- D_2O was introduced in the throttle gas (N_2)
  ⇒A and B ions separated by DMS, and then HD
- Slow (CV=-7.5 V) versus fast (CV=-1.5 V) was observed for N- versus O-protonated, respectively



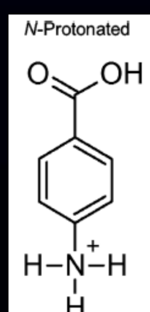


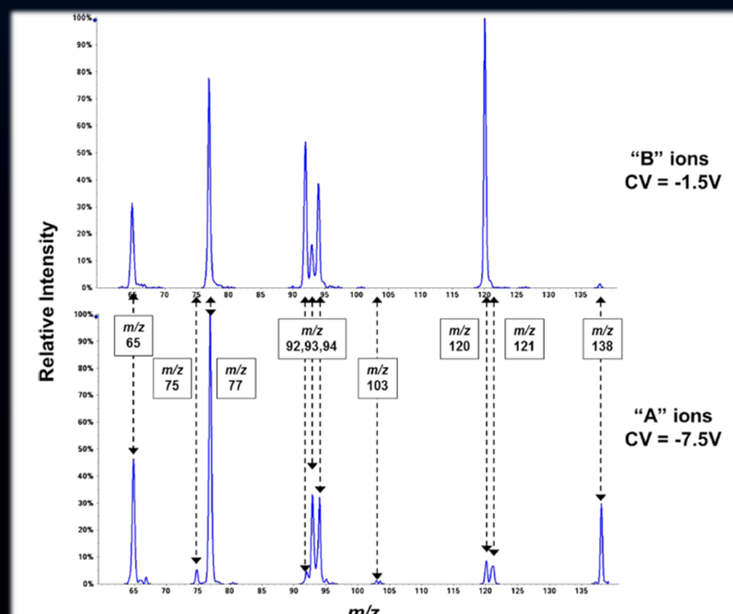


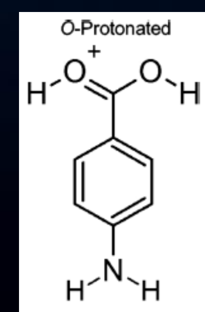
J. L. Campbell, J. C. Yves Le Blanc, and B. B. Schneider, Probing Electrospray Ionization Dynamics Using Differential Mobility Spectrometry: The Curious Case of 4-Aminobenzoic Acid, Anal. Chem. 2012, 84, 7857−7864

CID spectra of DMS selected isomers

- A (CV=-7.5 V) and B (CV=-1.5 V) protomers were selected and submitted to CID.
- Distinct CID mass spectra were oberved

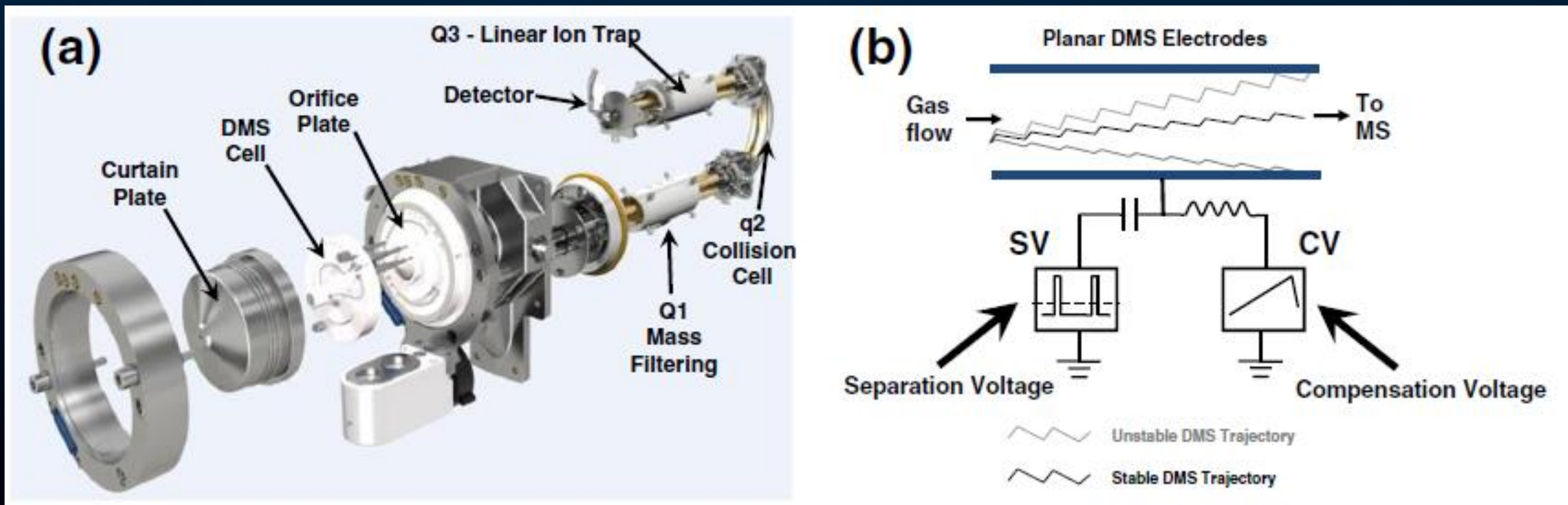


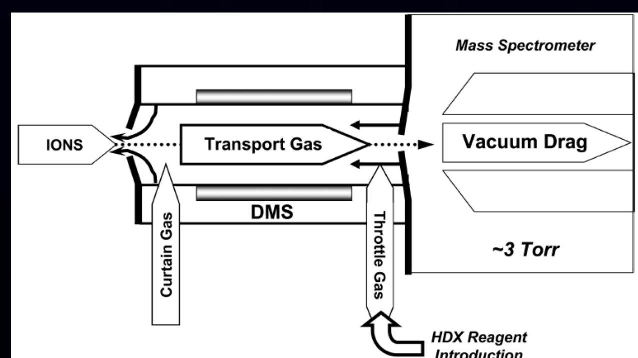




J. L. Campbell, J. C. Yves Le Blanc, and B. B. Schneider, Probing Electrospray Ionization Dynamics Using Differential Mobility Spectrometry: The Curious Case of 4-Aminobenzoic Acid, Anal. Chem. 2012, 84, 7857−7864

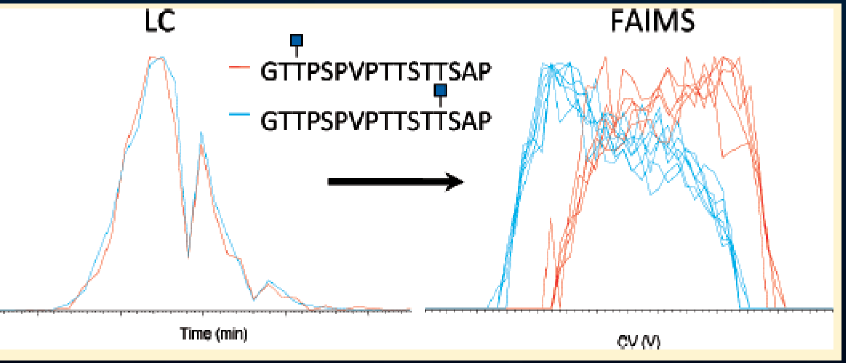
Sciex DIMS-triple quadrupole





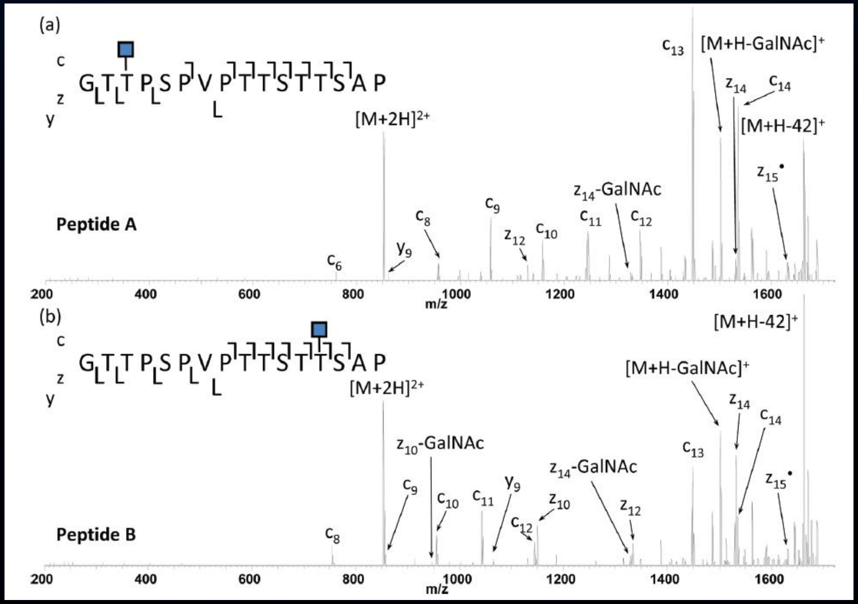
Separation and Identification of Isomeric Glycopeptides

Analysis of intact glycopeptides by MS/MS is challenging: multiple isomers both within the attached glycan and the location of the modification on the peptide backbone.



2 isomers (different location of the modification):
- coelute following reversed-phase LC
- Can be partially separated by FAIMS

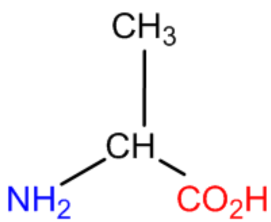
ETD mass spectrum of $[M + 2H]^{2+}$ ions recorded at a CV of −23.7 V (a) and −25.5 (b)



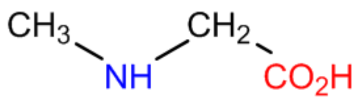
A. J. Creese and H. J. Cooper, Separation and Identification of Isomeric Glycopeptides by High Field Asymmetric Waveform Ion Mobility Spectrometry, Anal. Chem. 2012, 84, 2597−2601

Separation and identification of isomers: the sarcosine and α-alanine case

α-alanine



sarcosine

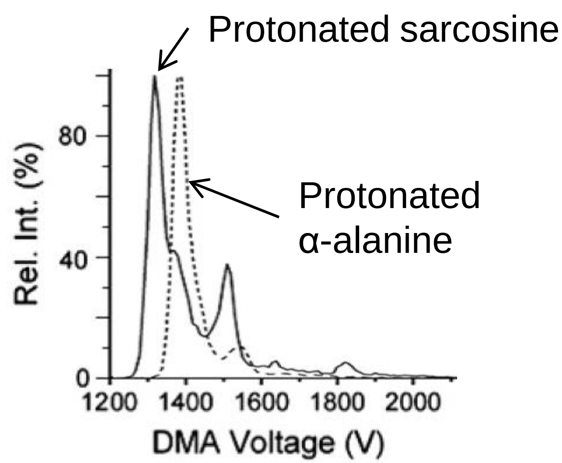


Sarcosine has recently been identified as a potential metabolite; it can up-regulate the expression of some genes, involved in cell cycle progression of metastatic models of prostate cancer. LC-MS/MS (MRM) was used.

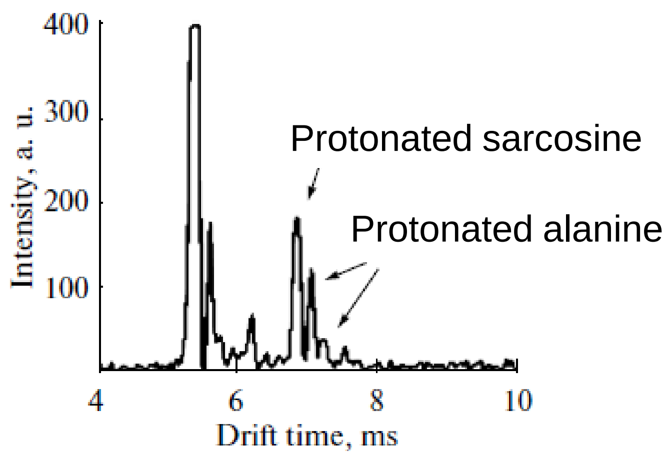
Sreekumar *et al. Nature* **2009,** *457* (7231), 910-914.

Heger *et al. PLoS One* **2016**, 11 (11).

Using time-separated based IMS technique, few attempts have been made to separate sarcosine from isomers:



Martinez-Lozano *et al. Journal of the American Society for Mass Spectrometry* **2010,** *21* (7), 1129-1132.

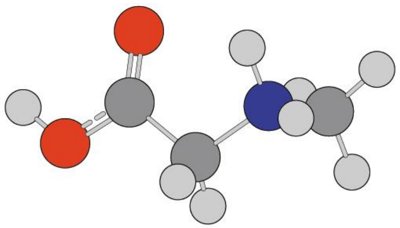


Mirmahdieh *et al. Journal of Analytical Chemistry* **2014,** *69* (6), 513-518.

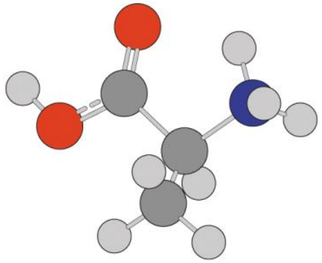
Berthias, F.; Maatoug, B.; Glish, G. L.; Moussa, F.; Maitre, P., Resolution and assignment of differential ion mobility spectra of sarcosine and isomers. J. Am. Soc. Mass Spectrom. 2018

Separation and identification of isomers: the sarcosine and α-alanine case

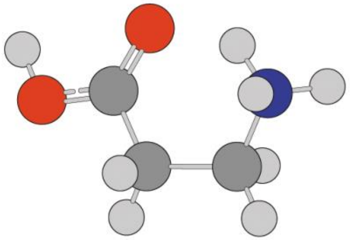
- **Protonated sarcosine (Sar):**



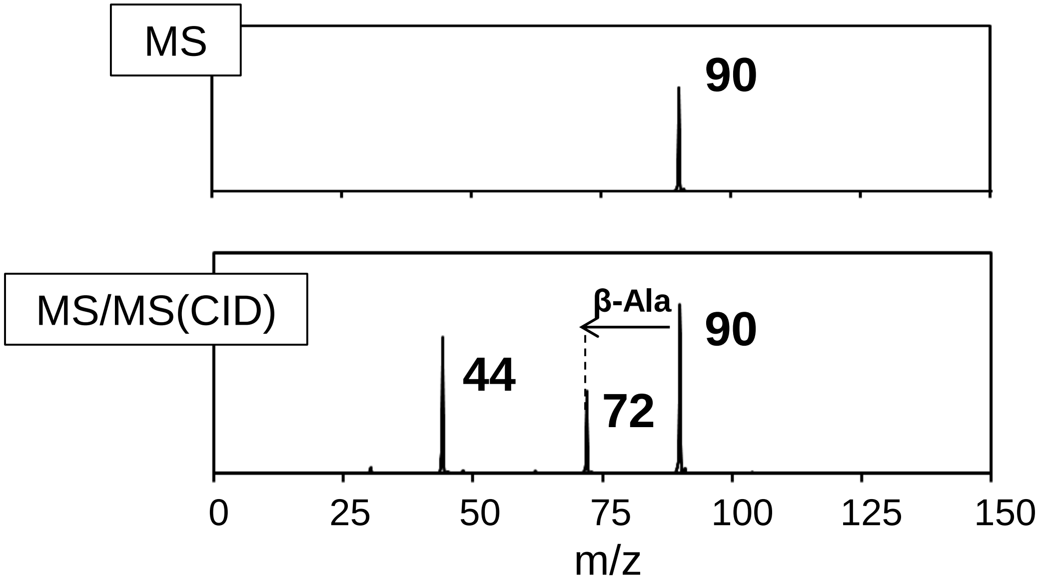
- **Protonated α-alanine (α-Ala):**



- **Protonated β-alanine (β-Ala):**



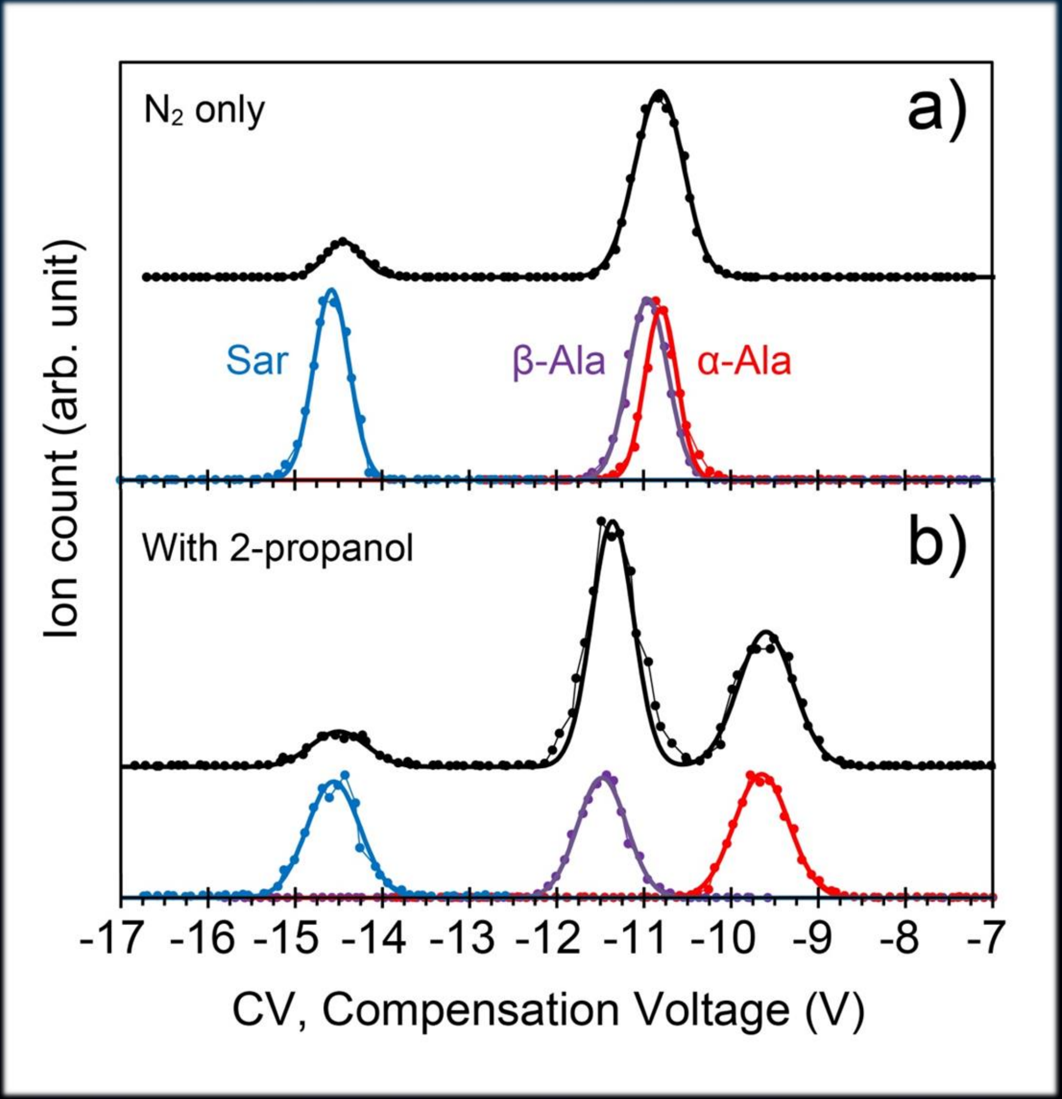
Equimolar mixture of sarcosine, α-alanine and β-alanine:



Distinguishing sarcosine and α-alanine is difficult (same characteristic *m/z* 44 fragment)

Berthias, F.; Maatoug, B.; Glish, G. L.; Moussa, F.; Maitre, P., Resolution and assignment of differential ion mobility spectra of sarcosine and isomers. J. Am. Soc. Mass Spectrom. 2018

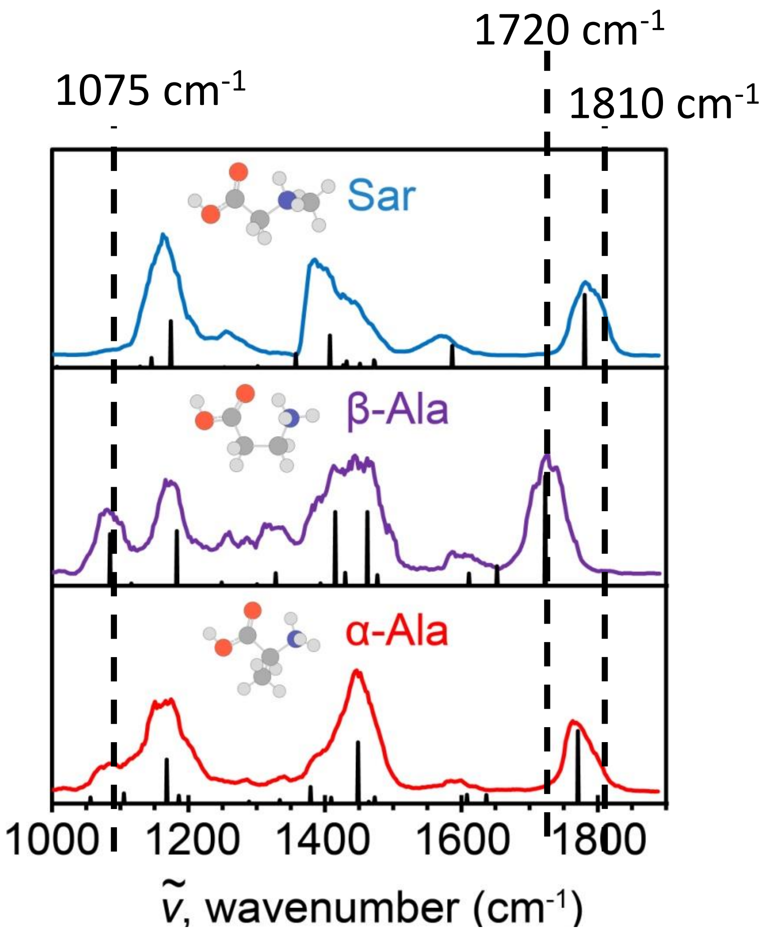
DIMS separation of the 3 isomers

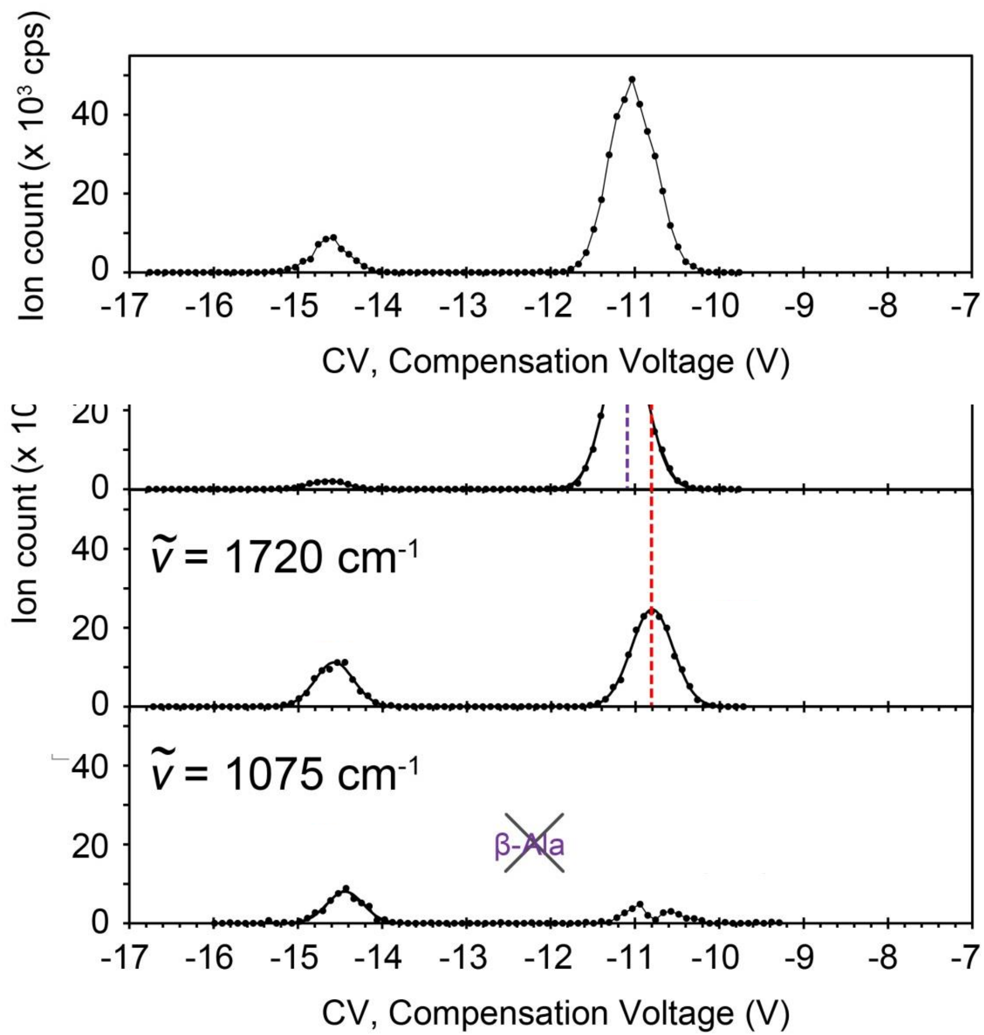


Berthias, F.; Maatoug, B.; Glish, G. L.; Moussa, F.; Maitre, P., Resolution and assignment of differential ion mobility spectra of sarcosine and isomers. J. Am. Soc. Mass Spectrom. 2018

IR identification of DIMS peaks: DIMS-MS/MS (IRMPD) spectra

DIMS spectra of equimolar mixture of protonated



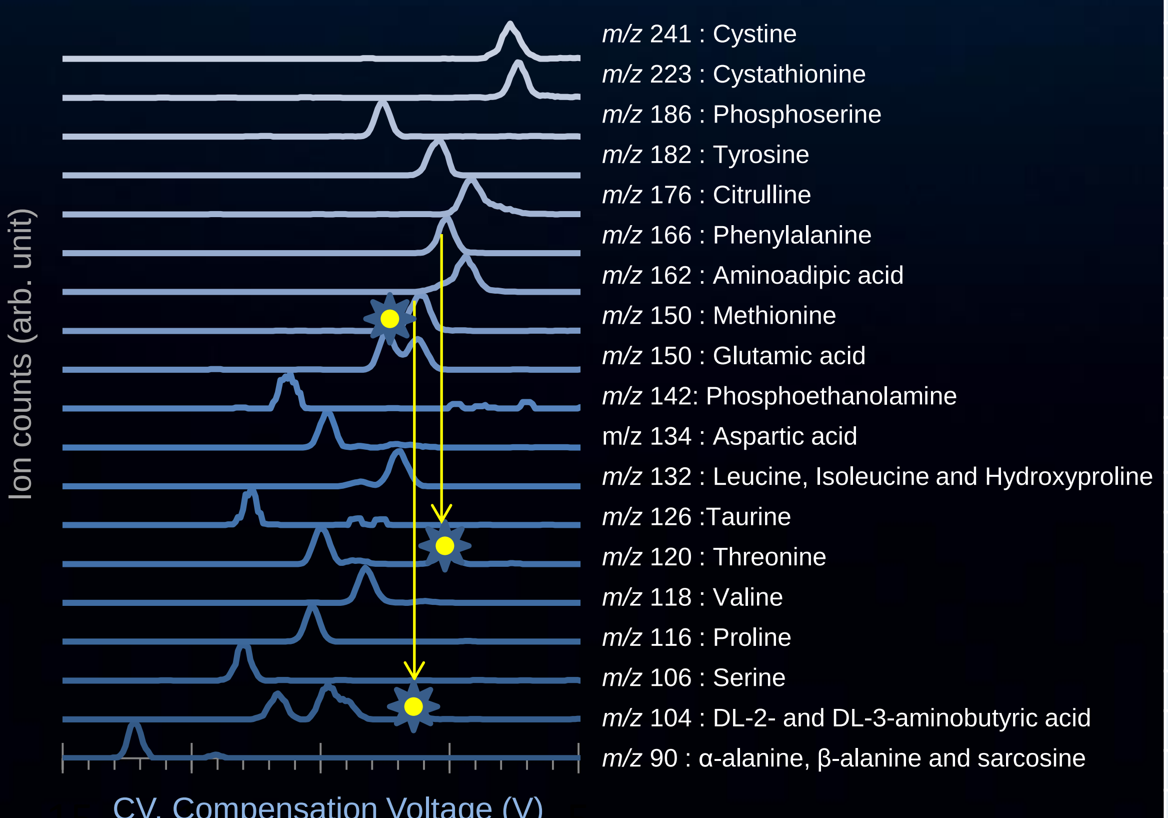


IR absorption spectra of protonated sarcosine, α- and β-alanine.

Berthias, F.; Maatoug, B.; Glish, G. L.; Moussa, F.; Maitre, P., Resolution and assignment of differential ion mobility spectra of sarcosine and isomers. J. Am. Soc. Mass Spectrom. 2018

Towards complex biological (urine, …) sample: DIMS analysis of Standard Mixtures

DIMS-MS/MS analysis of diluted urine samples is under study

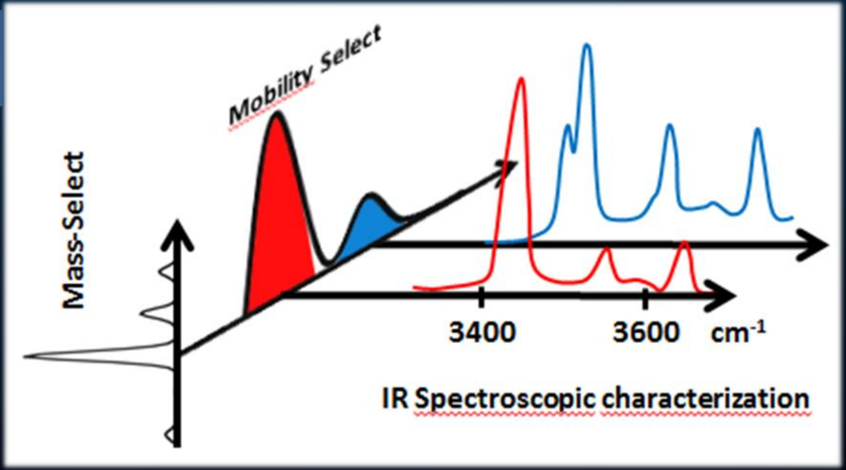


m/z (MH+)	Compound	Concentration (µmol/ml)
241	Cystine	2.5
223	Cystathionine	1.25
186	Phosphoserine	1.25
182	Tyrosine	2.5
176	Citrulline	2.5
166	Phenylalanine	2.5
162	Aminoadipic acid	1.25
150	Methionine	2.5
150	Glutamic acid	2.5
142	Phosphoethanolamine	1.25
142	Phosphoethanolamine	1.25
134	Aspartic acid	2.5
132	Leucine	2.5
132	Isoleucine	2.5
132	Hydroxyproline	2.5
126	Taurine	1.25
120	Threonine	2.5
118	Valine	2.5
116	Proline	2.5
106	Serine	2.5
104	DL-2-aminobutyric acid	1.25
104	DL-3-aminobutyric acid	2.5
90	α-alanine	2.5
90	β-alanine	2.5
90	Sarcosine	6.25

Two IMS-MS/MS(IR) modes

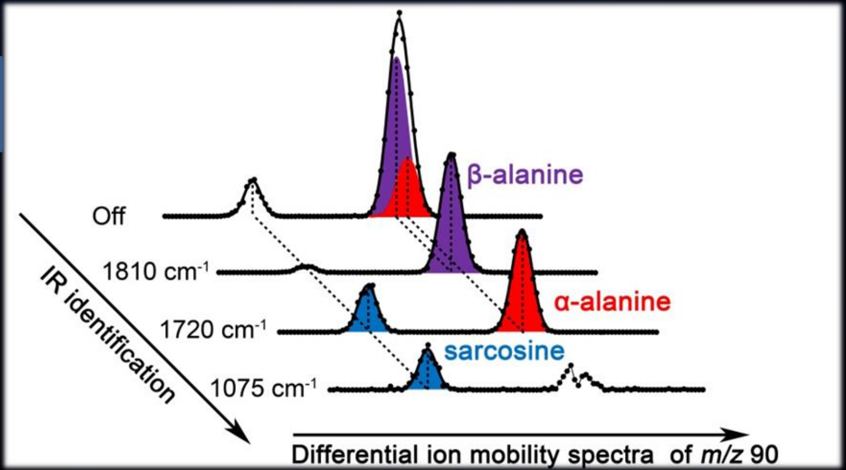
DIMS (fixed CV) → MS/MS(scanned v)

- IR spectroscopy of DIMS-filtered isomers
- Structural characterization of DIMS-selected isomers => better understanding of high voltage mobility?



DIMS (scanned CV) → MS/MS(fixed v)

- Identification of DIMS peaks
- Resolution of overlapping DIMS peaks
- Increasing the resolution



Berthias, F.; Maatoug, B.; Glish, G. L.; Moussa, F.; Maitre, P., Resolution and assignment of differential ion mobility spectra of sarcosine and isomers. J. Am. Soc. Mass Spectrom. 2018

Summary

- Differential Ion Mobiity (DIMS/FAIMS) : an efficient separation method, especially for small ions
- Two modes:
  - CV scanned: Ion chromatograms
  - CV fixed: Ion filter
- High ion count which facilitates subsequent MS/MS
- While structural information (Collision Cross Section, CCS) can be derived with drift tube IMS, no structural info can be derived with DIMS/FAIMS

An open DIMS-MS/MS-IRMPD(CLIO) Platform