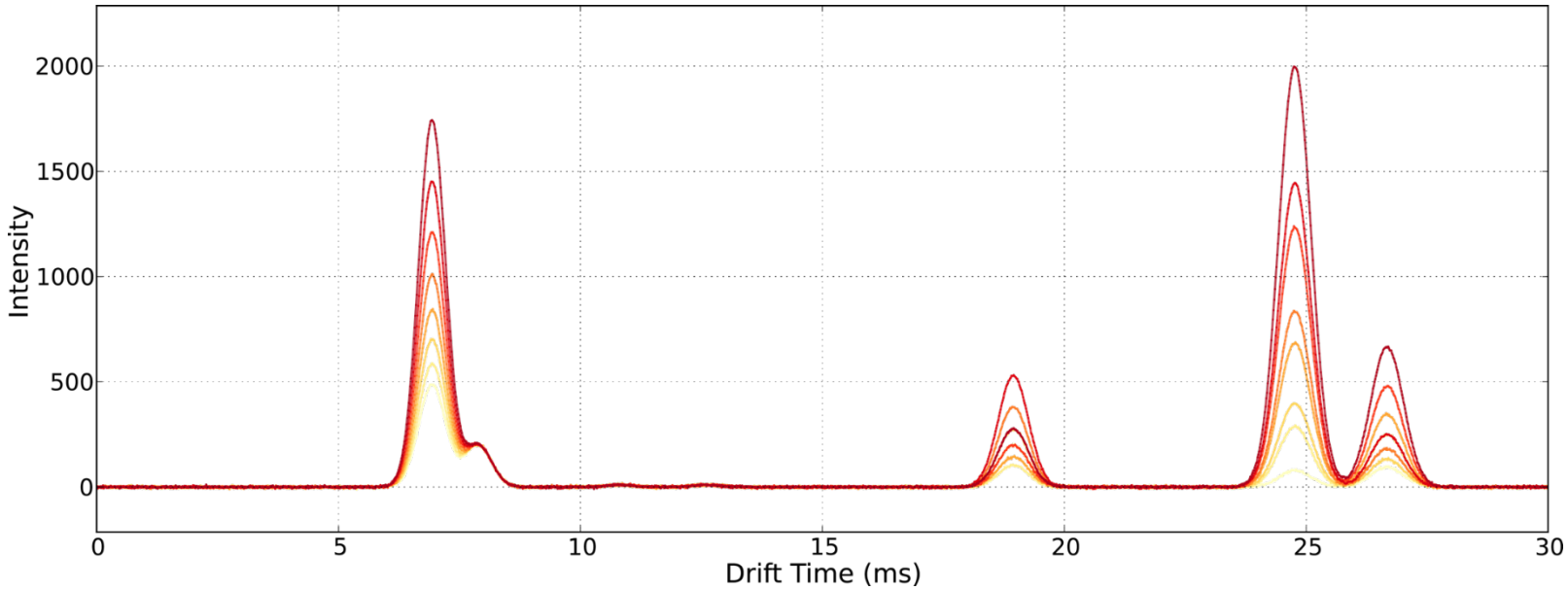
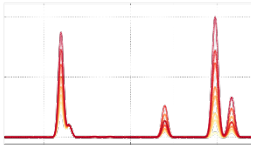
Slide Set #1, 09:00am, Sunday, June 5, 2016

ASMS SHORT COURSE ON ION MOBILITY MASS SPECTROMETRY (IMMS)

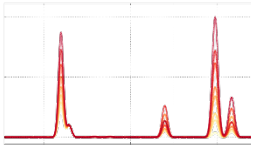
Brian Clowers, John McLean and Erin Baker





GOALS OF THIS COURSE

- **To gain an introductory *understanding* of the fundamentals of ion mobility spectrometry and its analytical capabilities.**
- **To describe the various types of IMMS instrument that are commercially available.**
- **To appreciate the "Value Added" aspect of IMS when coupled to MS.**
- **To provide the tools to *evaluate* different instruments and determine which will best suit your analytical needs.**
- **To demonstrate the variety of applications of IMMS.**



Outline of Course

Sunday

9:00	Introduction
9:45	IMS Theory
10:15	Break
10:45	Drift Tube IMS
12:00	Lunch
1:15	Traveling Wave IMS
2:30	Break
3:00	FAIMS
3:45	Future Directions & Discussion
4:30	Adjourn

Explosives and Narcotics Detection

ITEMISER

VaporTracer2

Morpho Detection
Wilmington, MA

IONSCAN

Smiths Detection
Mississauga, Ont.
Canada

SABRE 4000

Hand-Held Ion Mobility Spectrometers

Products in Development or Off-the-Shelf

Lightweight Chemical Detector

Gated-Grid IMS

Smiths Detection
Watford, UK

ChemPro-100

Open-Loop IMS

Environics, Oy
Mikkeli, Finland

microDMx

AC-DC IMS

Sionex Corp.
Waltham, MA

μ-IMS

Gated-Grid IMS

G.A.S.
Gesellschaft fur Analytische Sensorsysteme
Dortmund, Germany

In-Service and Merging Military Detectors (approximate relative sizes)

ACADA

MCAD

LCD

JUNO

RAID-1

RAID-M

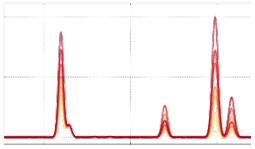
M43-A1

CAM

M-90

ChemPro

Ion Mobility coupled with Mass Spectrometry



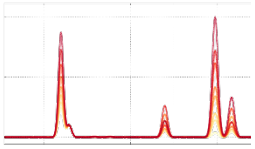
Synapt HDMS

Traveling Wave IMS-TOF

Drift Tube IMS-Quad

Drift Tube IMS-TOF

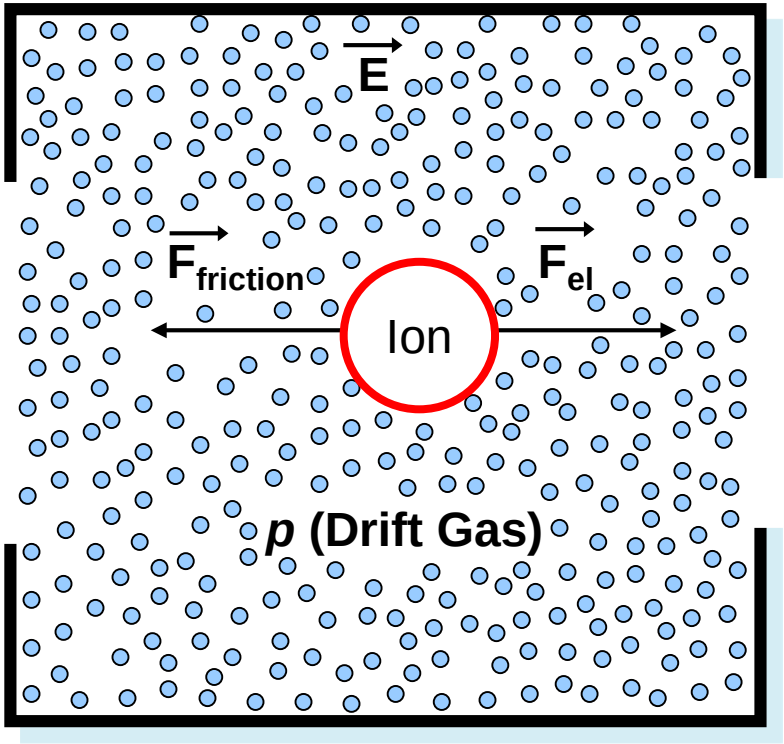
DMS-MS



Overview

- IMS Concept & History
- Ion Mobility Spectrometers
- Ion Mobility-Mass Spectrometers

Ion Mobility Concept

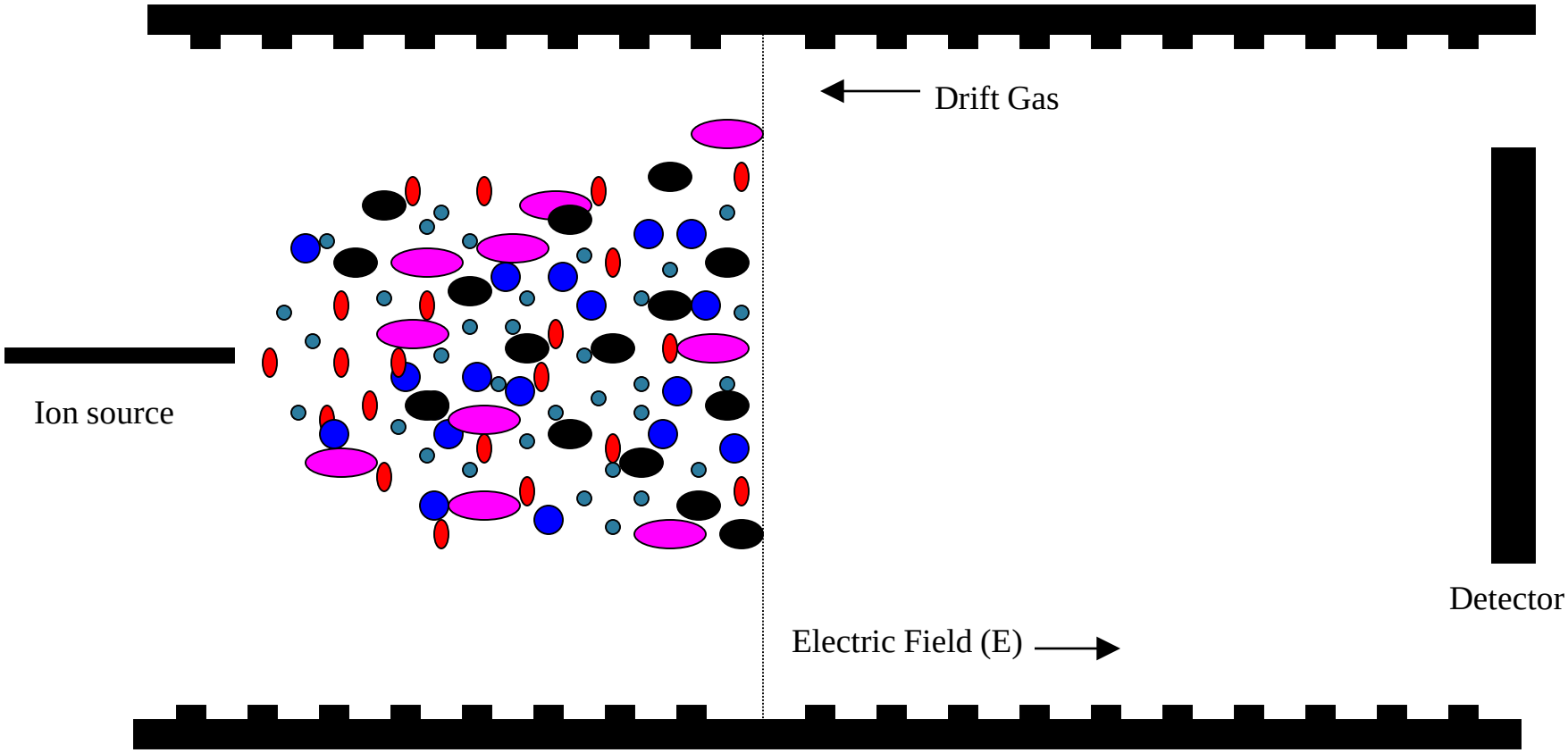


Drift Cell

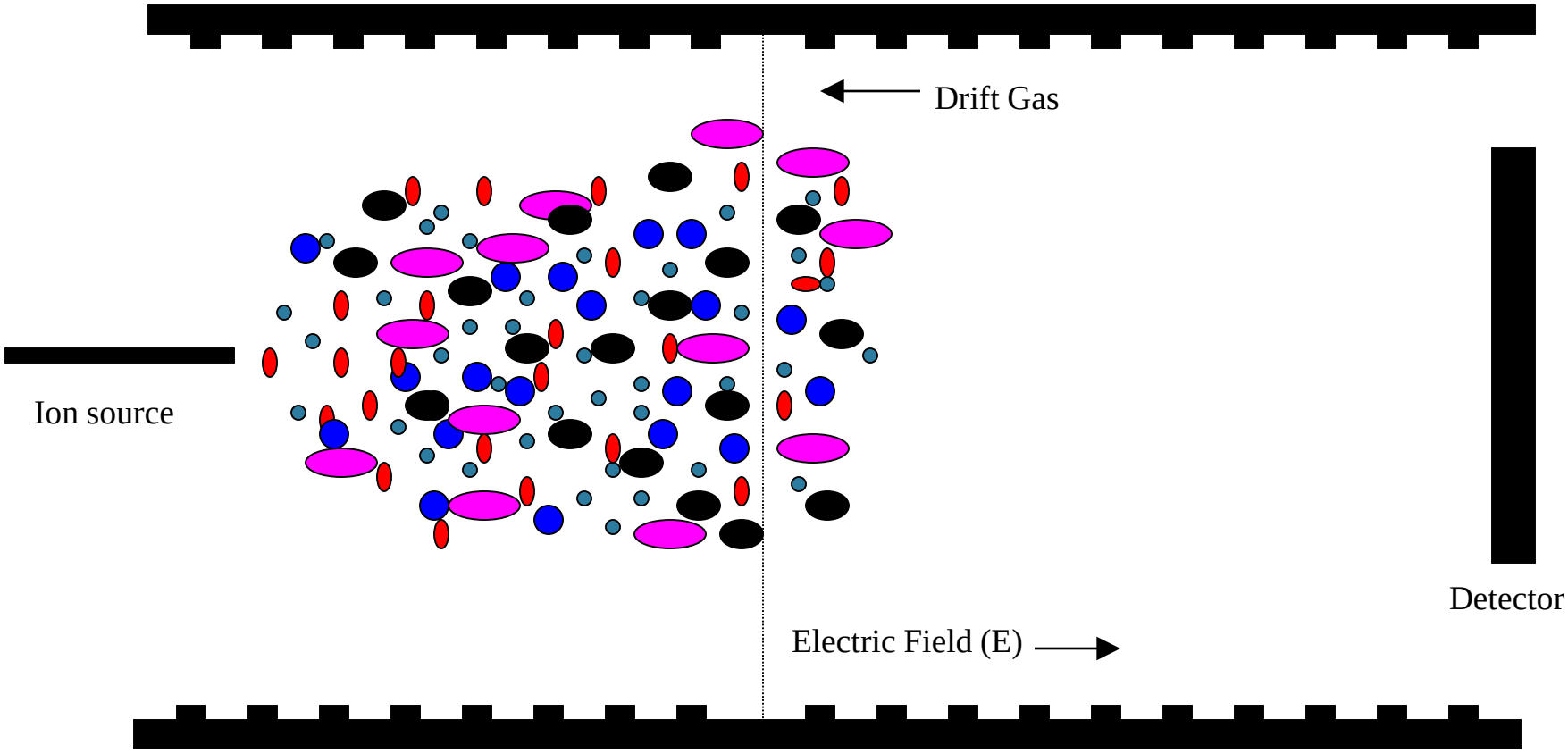
velocity is constant

$v = K \vec{E}$
K = ion mobility

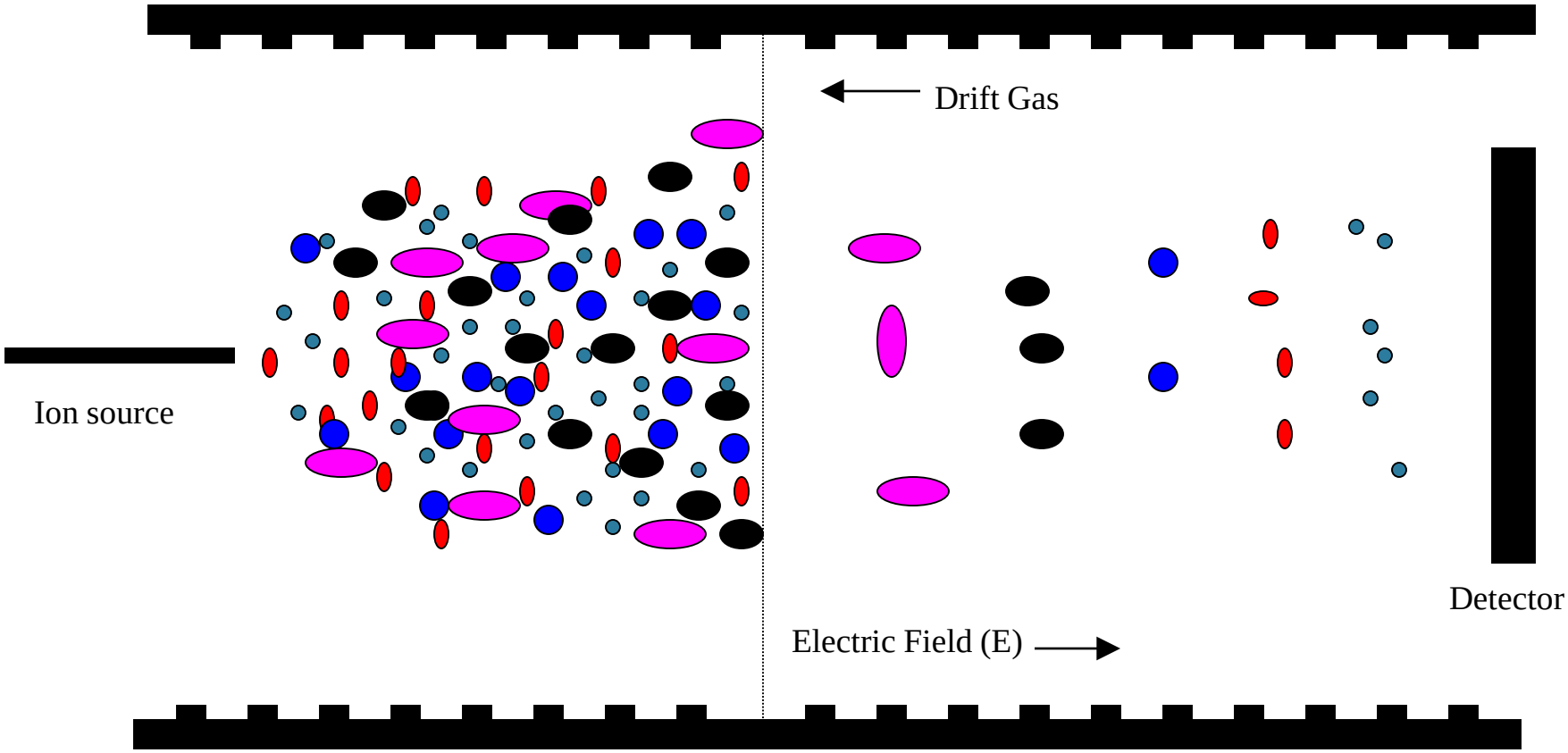
Ion Mobility Experiment



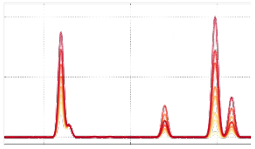
Ion Mobility Experiment

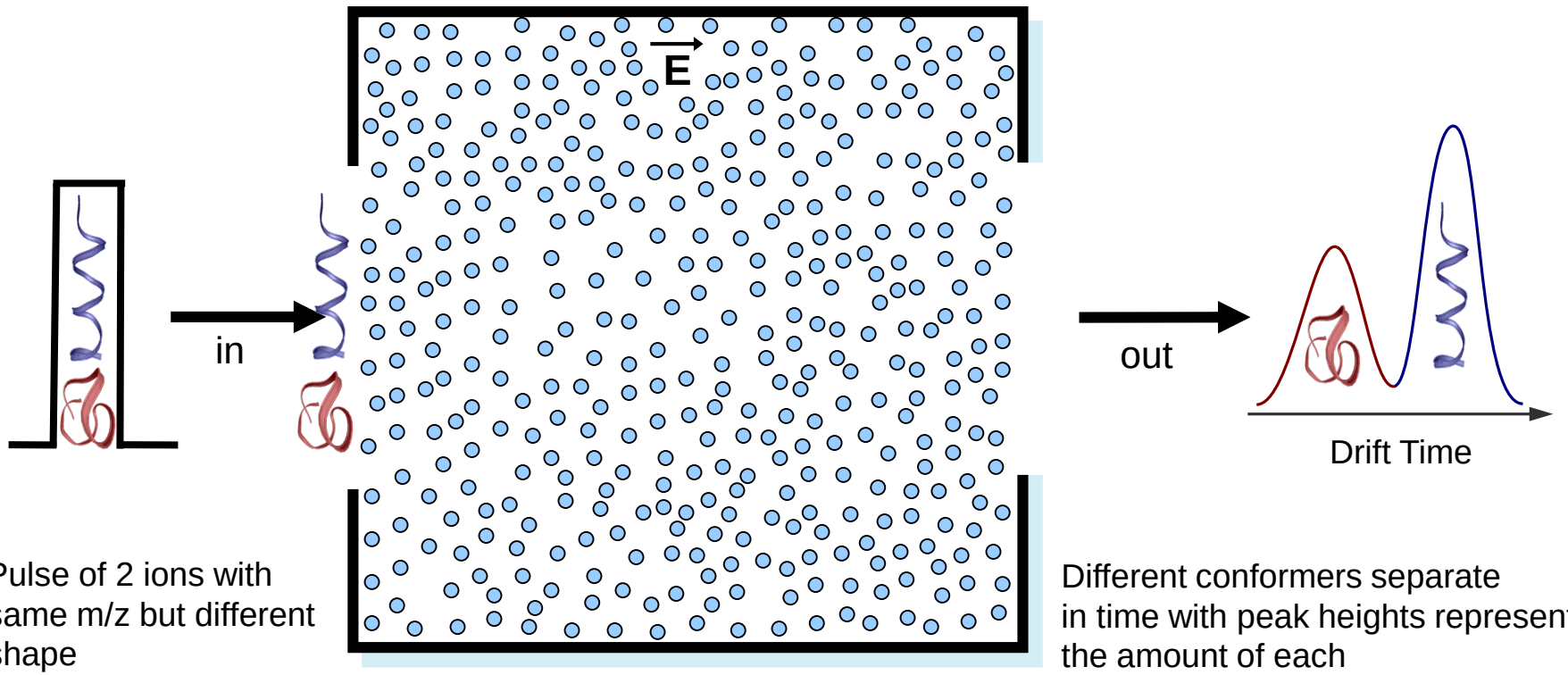


Ion Mobility Experiment



Ion Mobility Concept





Pulse of 2 ions with same m/z but different shape

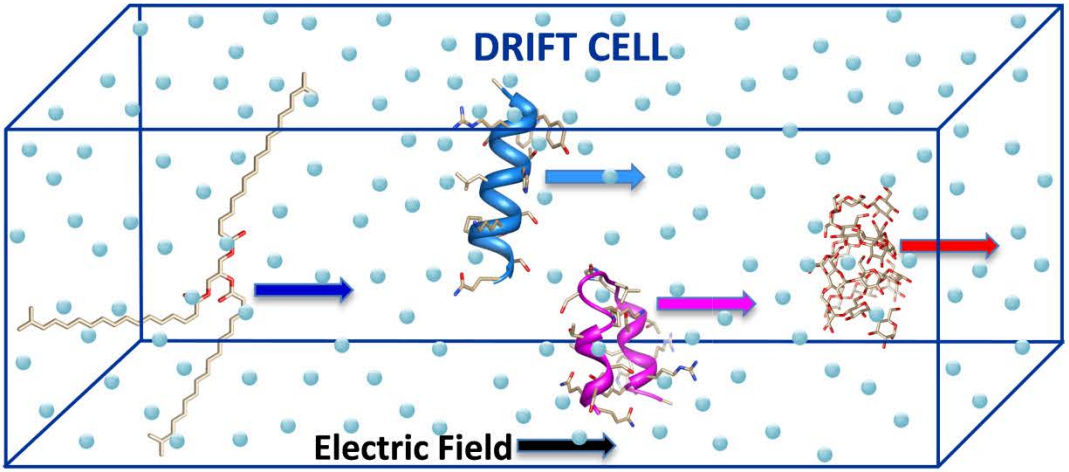
Different conformers separate in time with peak heights representing the amount of each

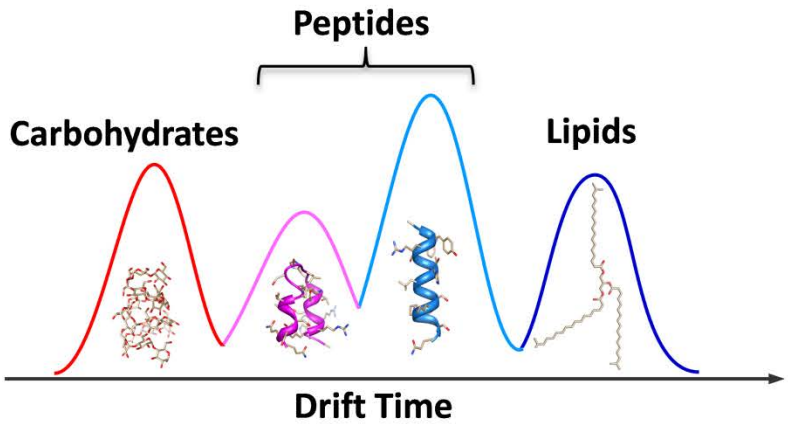
Drift Cell

velocity is constant

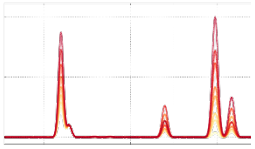
$v = K \vec{E}$
K = ion mobility

Ion Mobility Concept

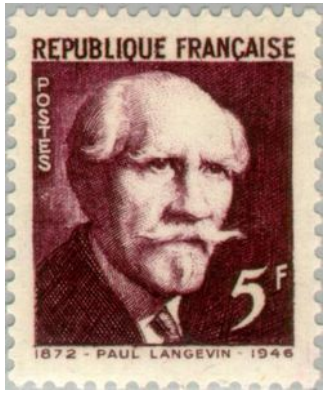




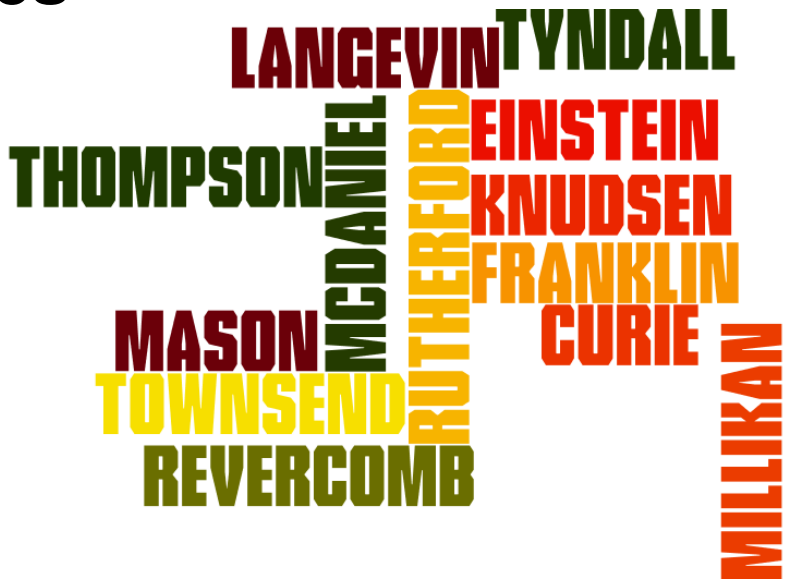
Genesis of Ion Mobility

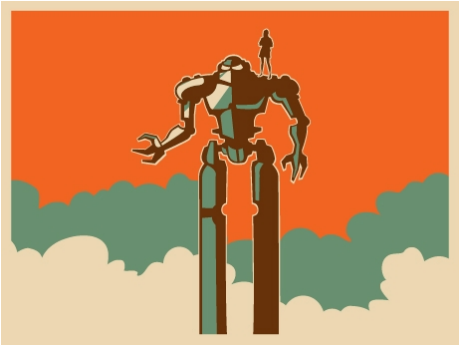


- Pioneers measuring electrical current
  - Faraday
  - Gauss

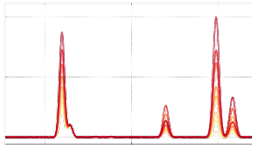


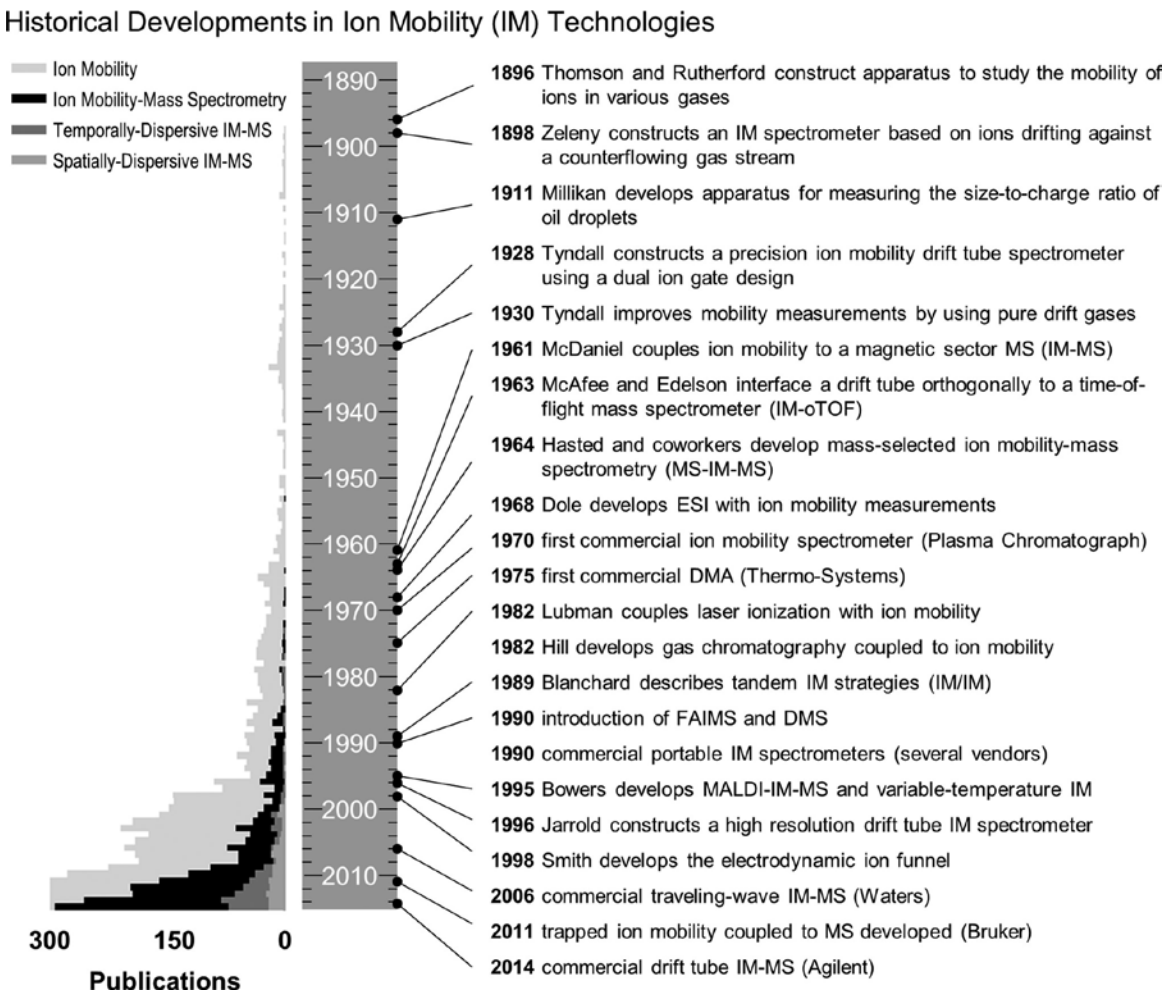
- Gas-Phase Kinetics
  - Diffusion
  - Ion Generation

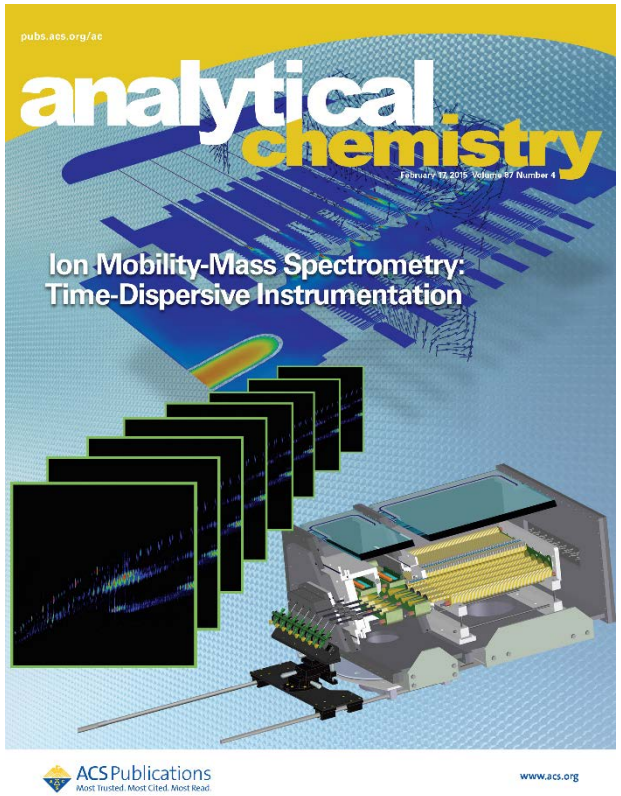




Evolution of IMS and IMMS

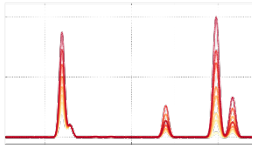


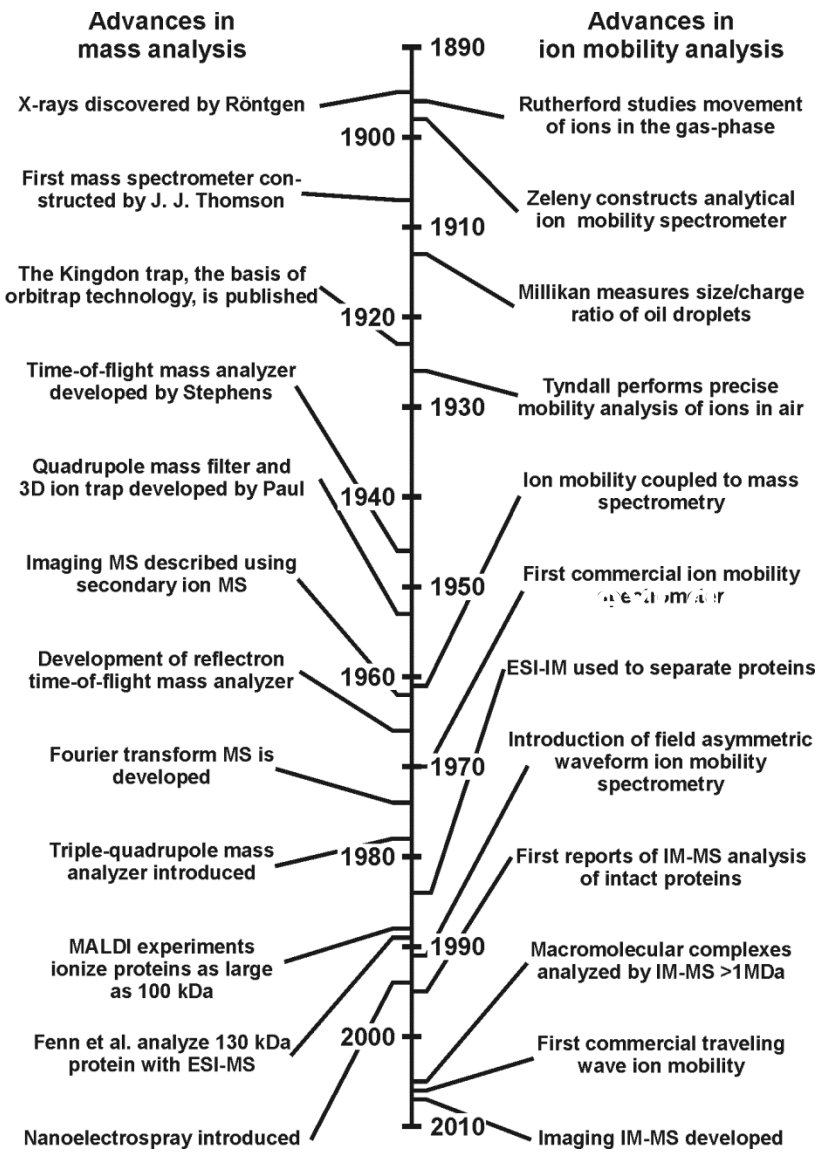




J. C. May and J. A. McLean, *Analytical Chemistry* 87, 1422-1436 (2015).

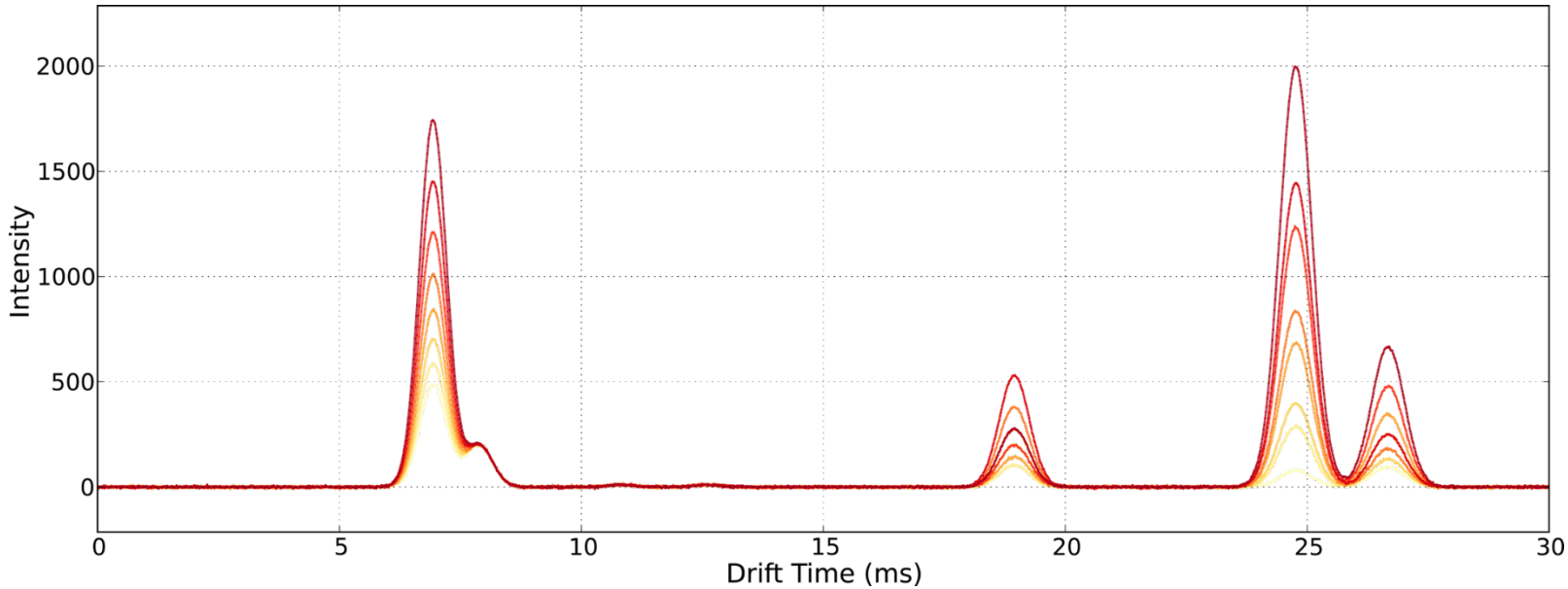
Evolution of IMS with MS





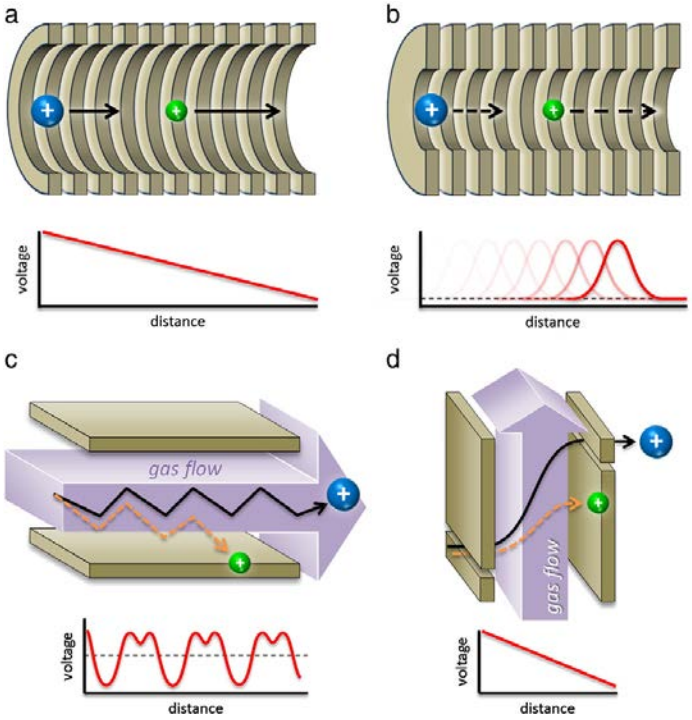
K. M. Hines, J. R. Enders, and J. A. McLean, *Encyclopedia of Analytical Chemistry*, R. Myers and D. C. Muddiman, Eds., John Wiley & Sons (2012).

Ion Mobility Spectrometers



Types of Ion Mobility Spectrometers

- ***Static Fields***
  - Drift Tube IMS (DTIMS)
  - Differential Mobility Analyzer (DMA)
- ***Dynamic Fields***
  - Field Asymmetric IMS (FAIMS)
  - Trapped IMS (TIMS)
  - Traveling Wave IMS (TWIMS)



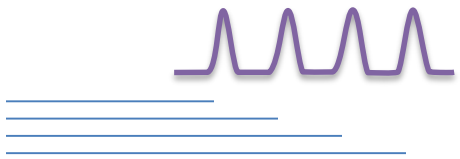
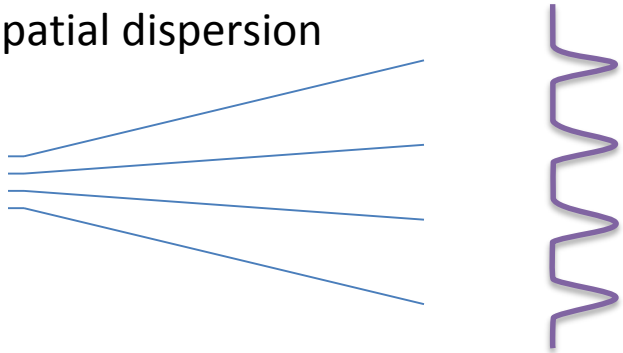
Biochimica et Biophysica Acta 1811 (2011) 935–945

All the flavors of ion mobility spectrometry…

Name	Type	Ion Migration	Electric Field	Gas Flow	MS Analogue
Drift Tube (IMS) Plasma Chromatography Gas-Phase Electrophoresis	Temporal Dispersive (time-of-flight)	→	→	0	Time-of-flight MS
Traveling Wave (T-Wave)		→	→→→	0	Wave MS "Solitron"*
High-Field Asymmetric IMS (FAIMS) Differential Mobility Spectrometry (DMS) RF Ion Mobility Spectrometry	Spatial Dispersive (Filter)	→	↑	→	Quadrupole MS
Differential Mobility Analyzer (DMA) Electrical Aerosol Analyzer (EAA) Gas-Phase Electrophoretic Molecular Mobility Analyzer (GEMMA)		→	→	↑	Magnetic Sector MS
Counterflow Techniques (CMA)	Either	→	→	←	Ion Trap
Counterfield Techniques (Pulsed Reverse Field IMS)		→	←	→	Ion Trap

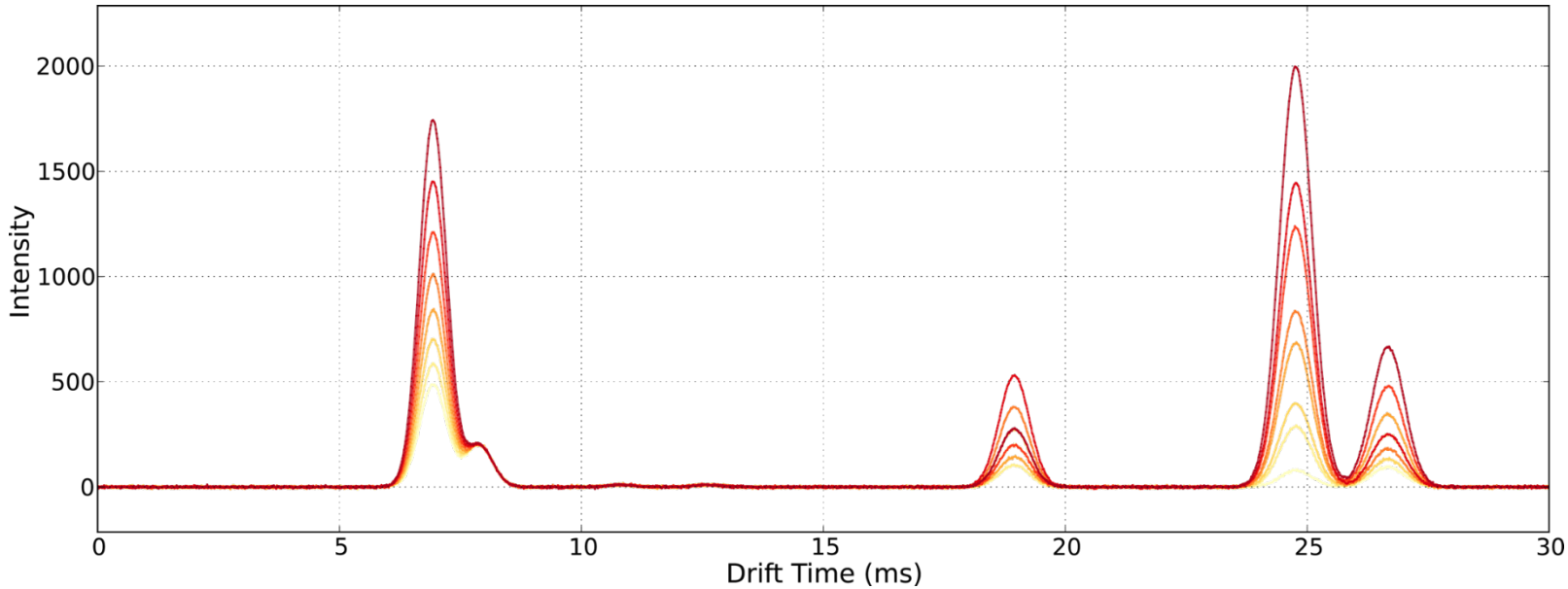
*L.S. Tung, W.L. Barr, R.S. Lowder, R.F. Post "Mass, Charge, and Energy Separation by Selective Acceleration with a Traveling Potential Hill" J. Appl. Phys. 80(7) 1996

All the flavors of ion mobility spectrometry…

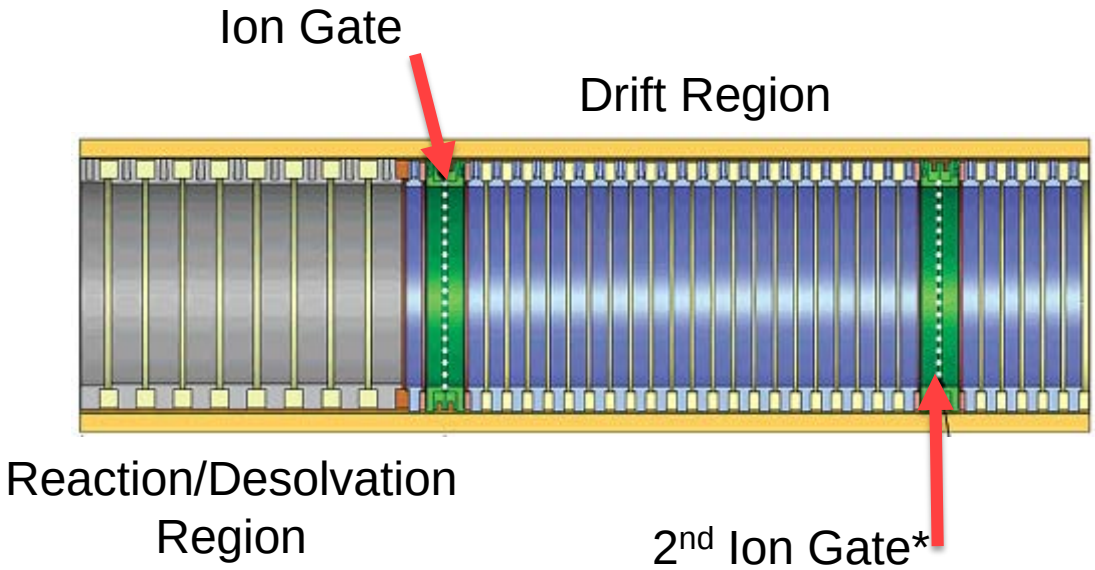
Name	Type	Ion Migration	Electric Field	Gas Flow	MS Analogue
Drift Tube (IMS) Plasma Chromatography Gas-Phase Electrophoresis	Temporal Dispersive (time-of-flight)	Temporal dispersion 			
Traveling Wave (T-Wave)					
High-Field Asymmetric IMS (FAIMS) Differential Mobility Spectrometry (DMS) RF Ion Mobility Spectrometry	Spatial Dispersive (Filter)	Spatial dispersion 			
Differential Mobility Analyzer (DMA) Electrical Aerosol Analyzer (EAA) Gas-Phase Electrophoretic Molecular Mobility Analyzer (GEMMA)					
Counterflow Techniques (CMA)	Either	→	→	←	Ion Trap
Counterfield Techniques (Pulsed Reverse Field IMS)		→	←	→	Ion Trap

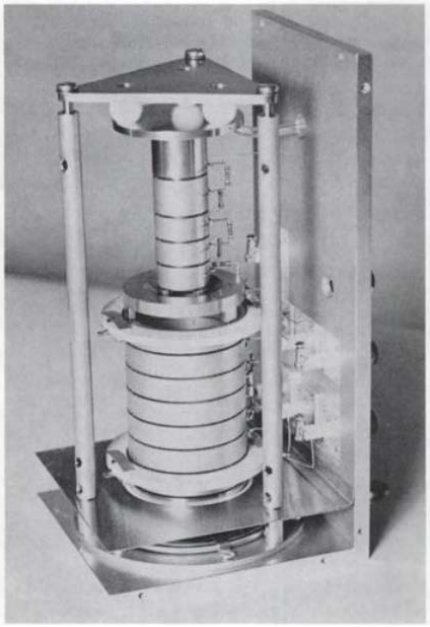
*L.S. Tung, W.L. Barr, R.S. Lowder, R.F. Post "Mass, Charge, and Energy Separation by Selective Acceleration with a Traveling Potential Hill" J. Appl. Phys. 80(7) 1996

Drift Tube IMS



Anatomy of Drift Cell





Analytical Chemistry, Vol. 77, No. 18, September 15, 2005

ANALYTICAL CHEMISTRY, VOL. 43, NO. 11, SEPTEMBER 1971

Explosives and Narcotics Detection

ITEMISER

VaporTracer2

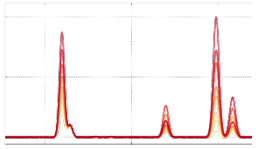
GE Ion Track
Wilmington, MA

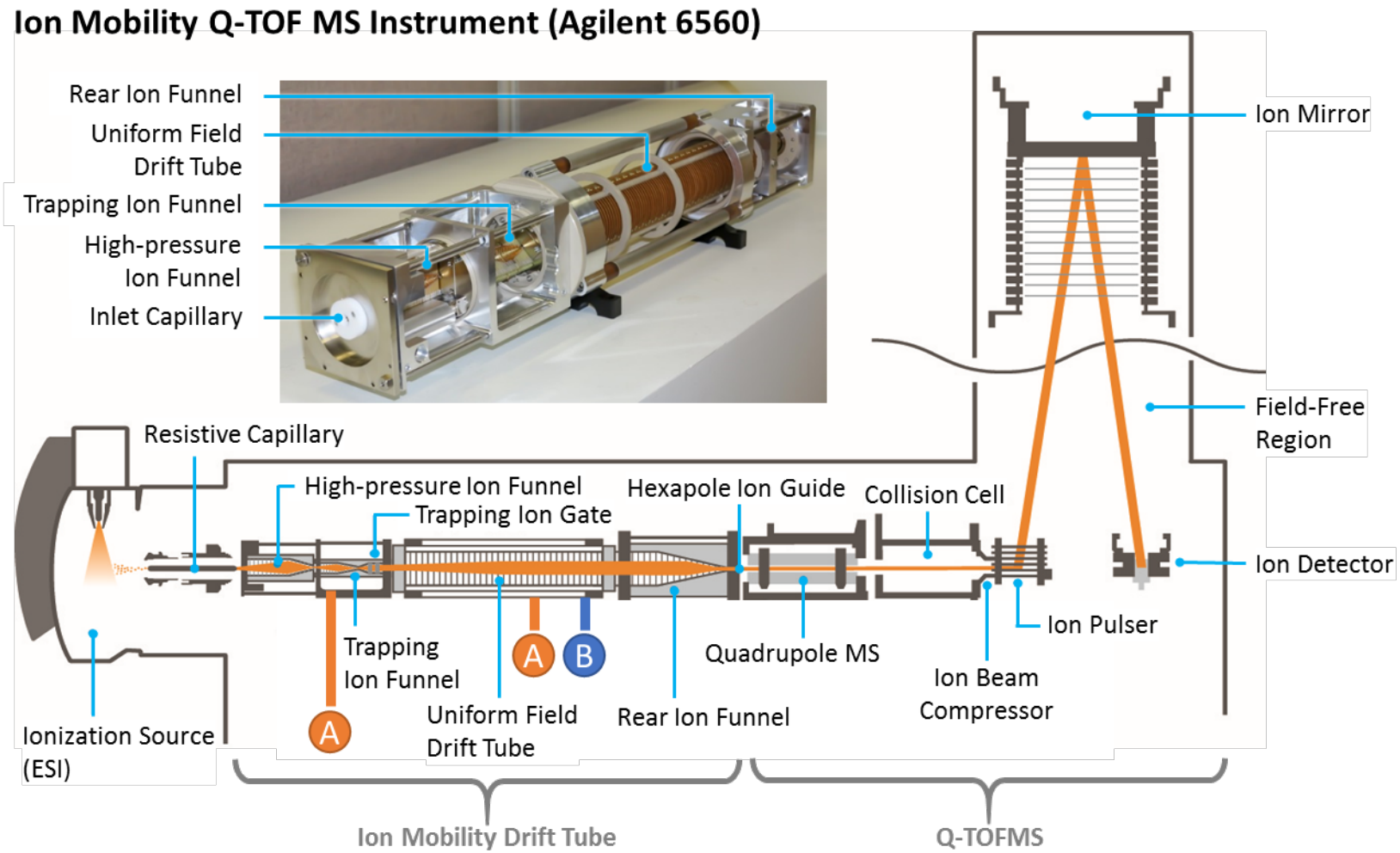
IONSCAN

Smiths Detection
Mississauga, Ont.
Canada

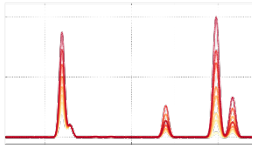
SABRE 4000

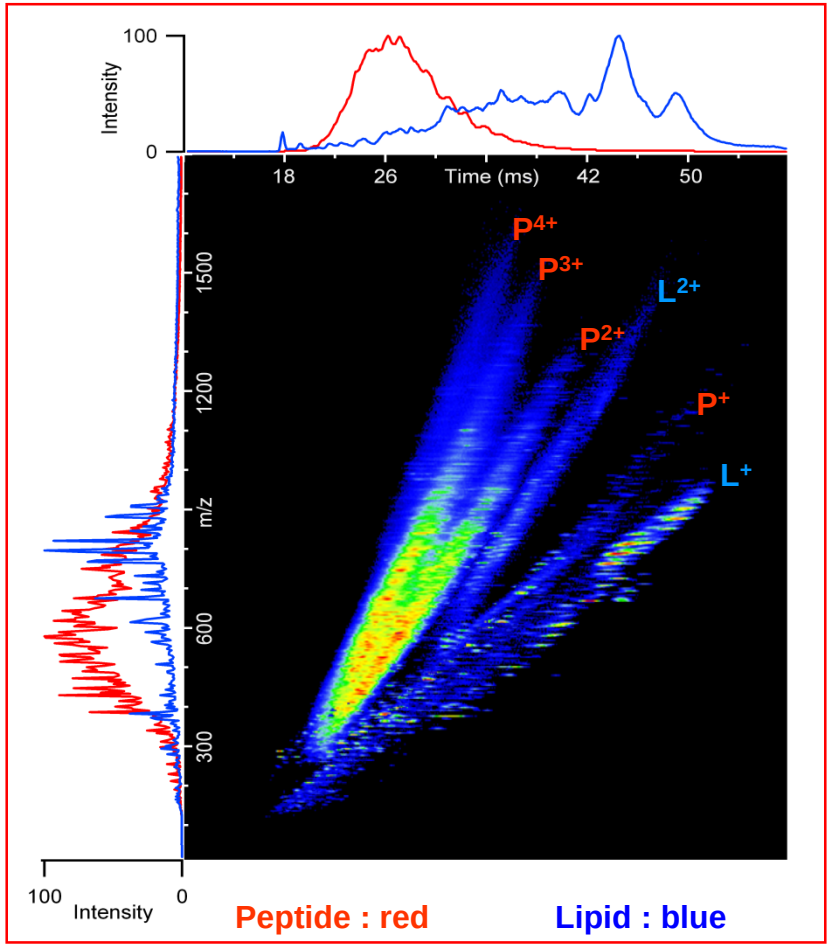
Recent Drift Tube Instrument

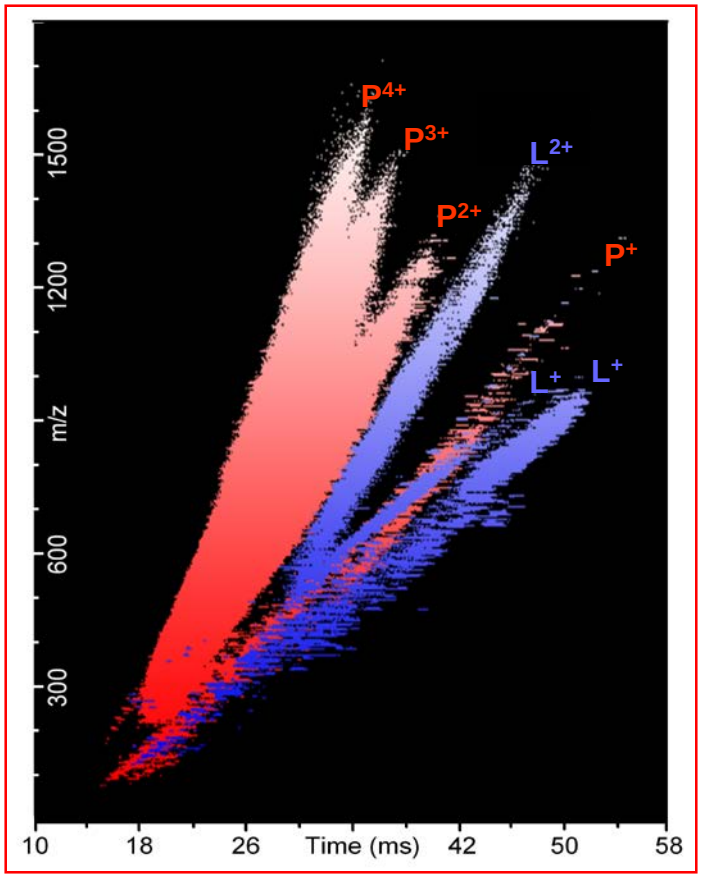




Distinguish isomers & different classes of ions

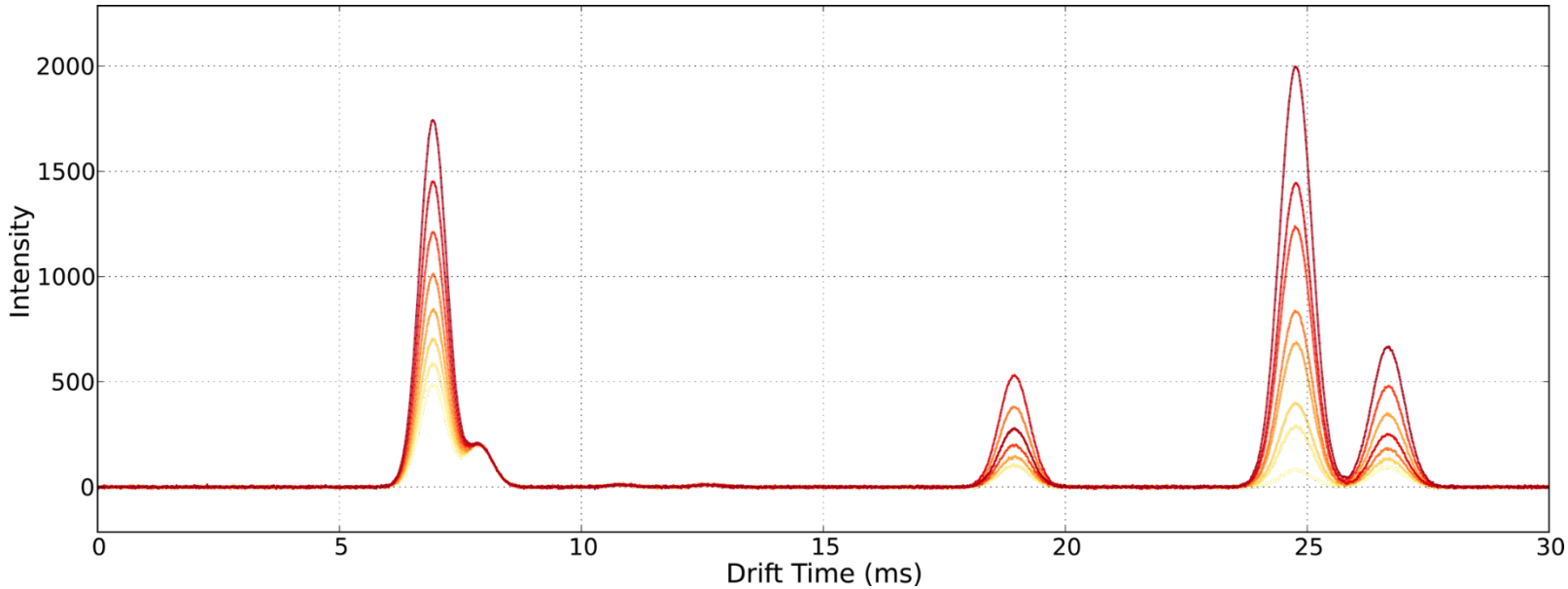




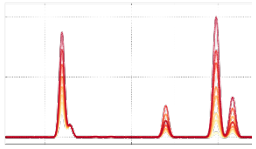


Peptides and lipids are easily distinguished

Dynamic Field IMS

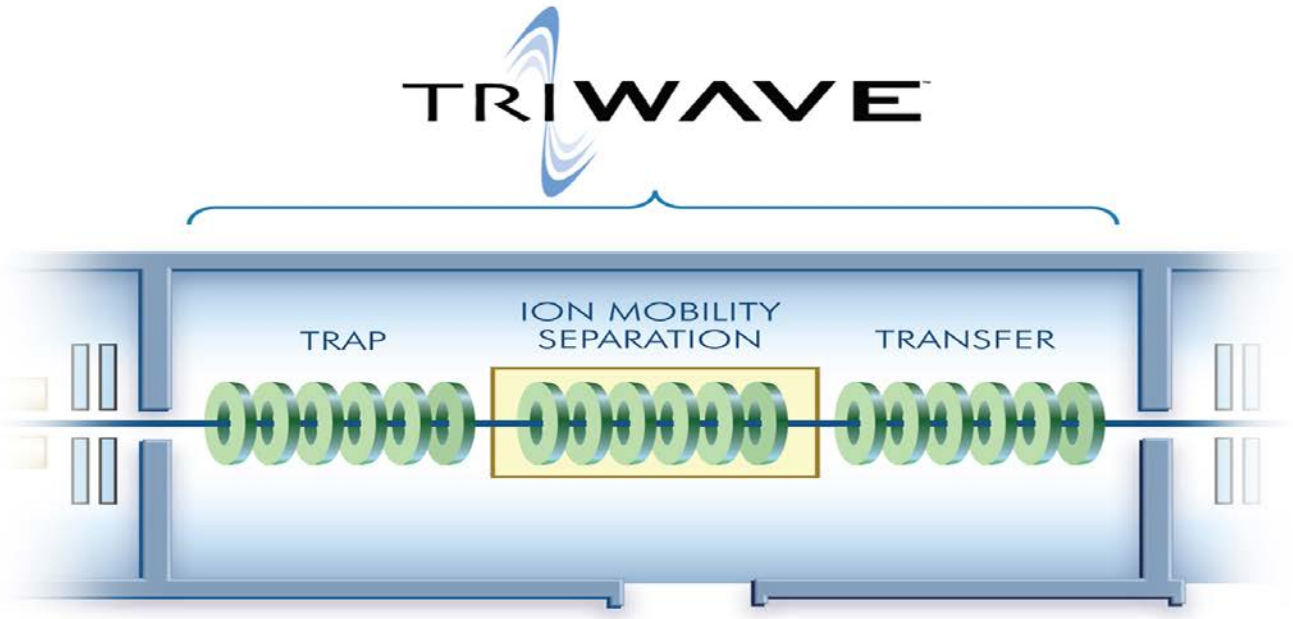


Traveling Wave IMS

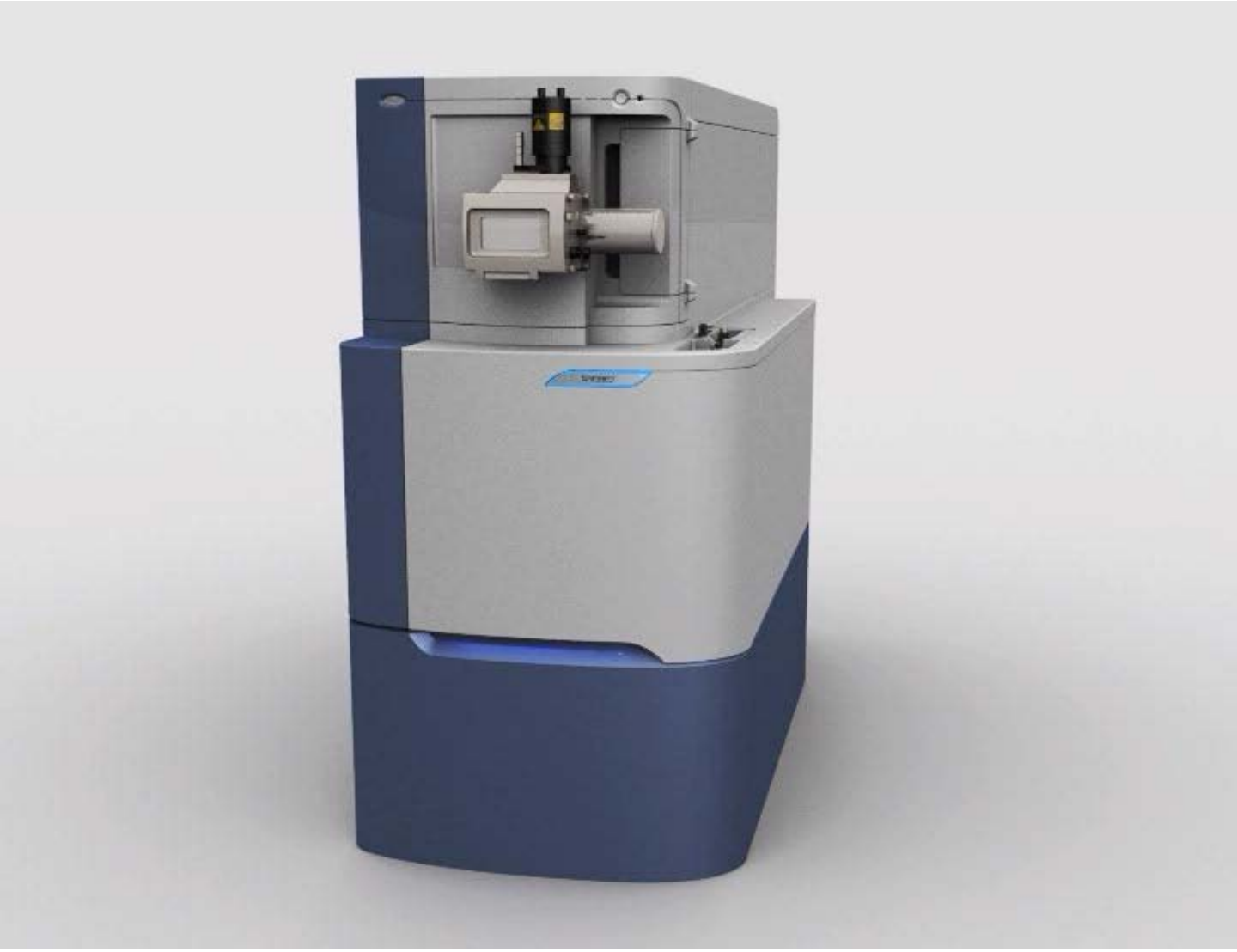


For IMS Analysis Nitrogen Is Admitted Into The Ion Mobility Separator (~0.5 mbar)

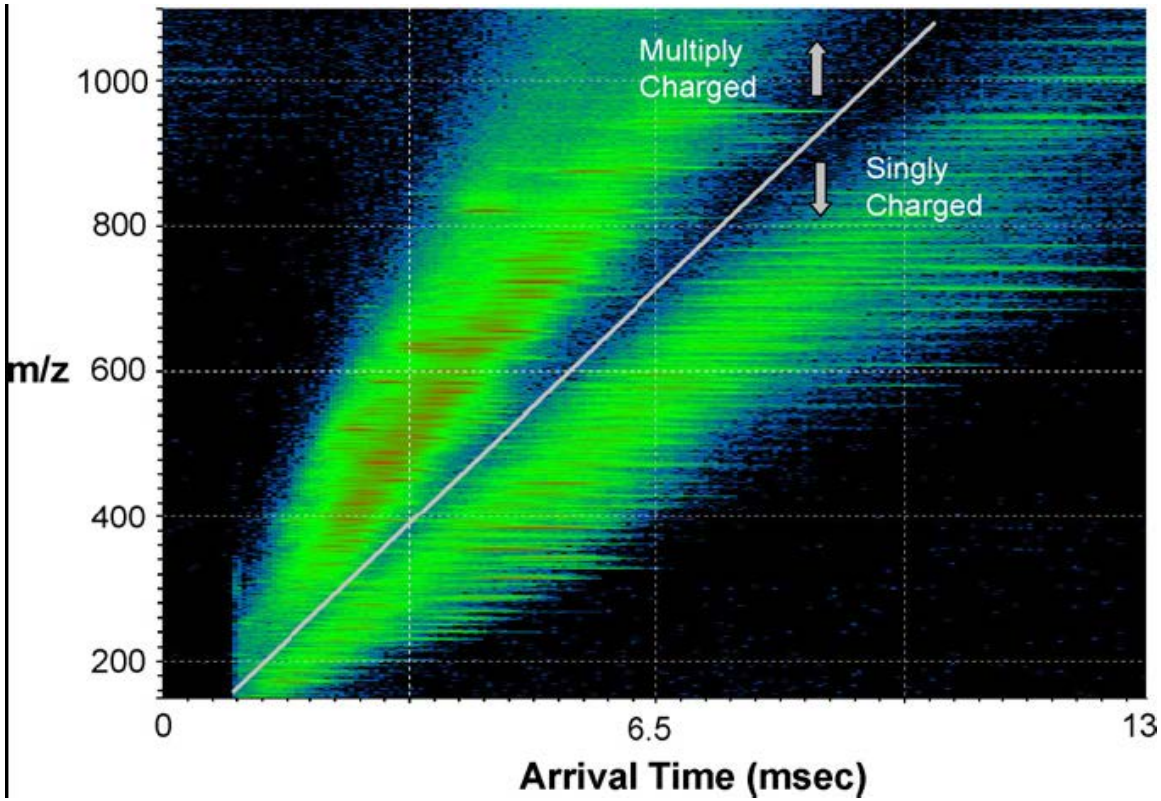
Fragmentation (CID) can be induced in both the TRAP & TRANSFER Regions (~10^{-3} mbar)



Traveling Wave IMS

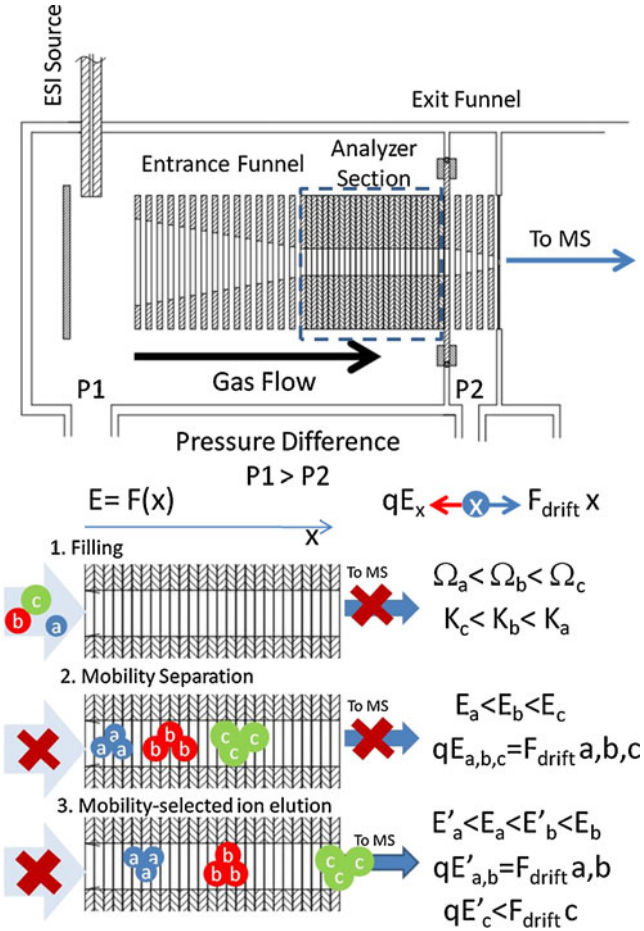


Charge Separation of a Protein Digest

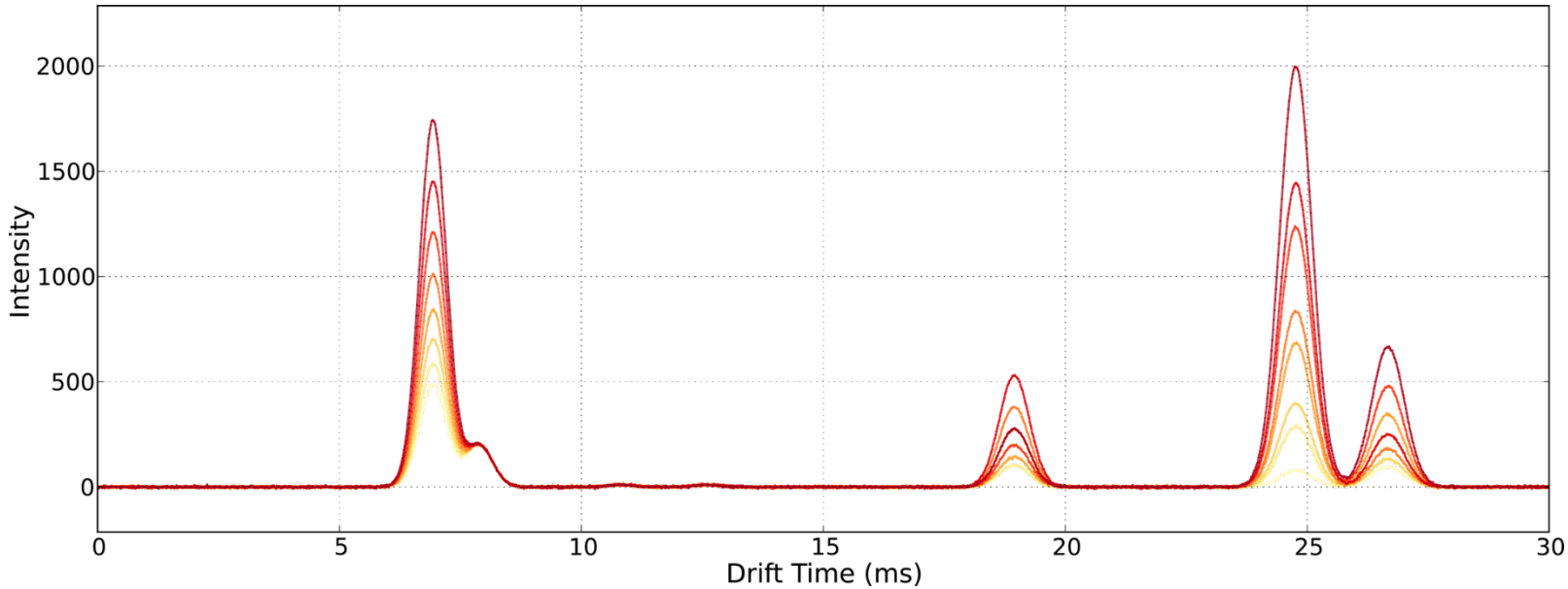


A 2D plot of ion arrival time vs. m/z for a protein digest mixture obtained using TWIMS. Reprinted from International Journal of Mass Spectrometry vol. 261, S. D. Pringle, K. Giles, J. L. Wildgoose, J. P. Williams, S. E. Slade, K. Thalassinos, R. H. Bateman, M. T. Bowers, and J. H. Scrivens, “An investigation of the mobility separation of some peptide and protein ions using a new hybrid quadrupole/traveling wave IMS/TOF instrument”, 1-12. Copyright (2007) with permission from Elsevier.

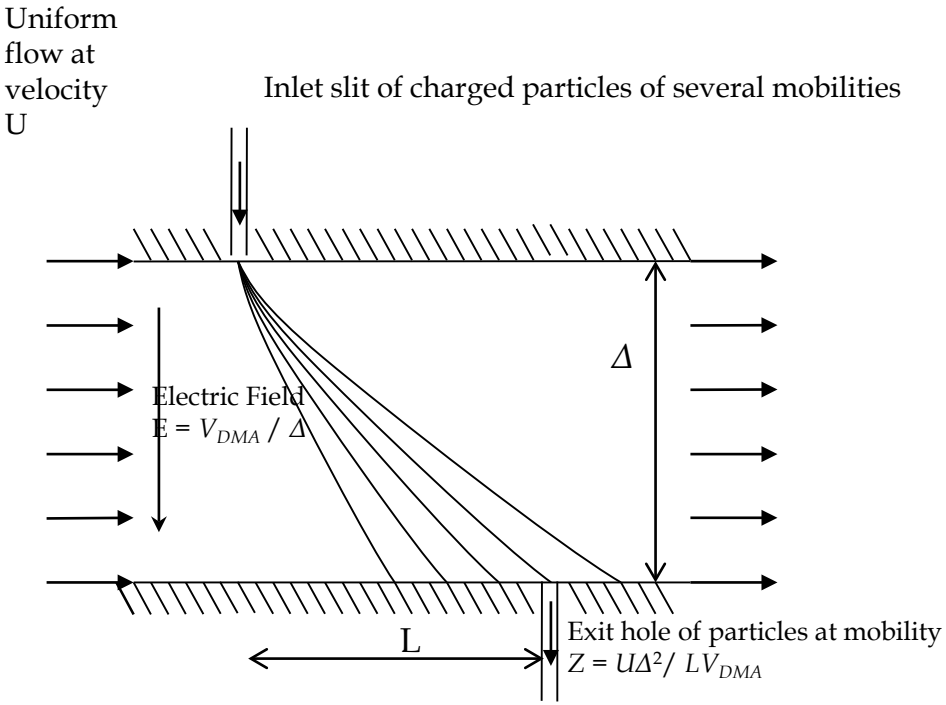
TRAPPED ION MOBILITY SPECTROMETRY

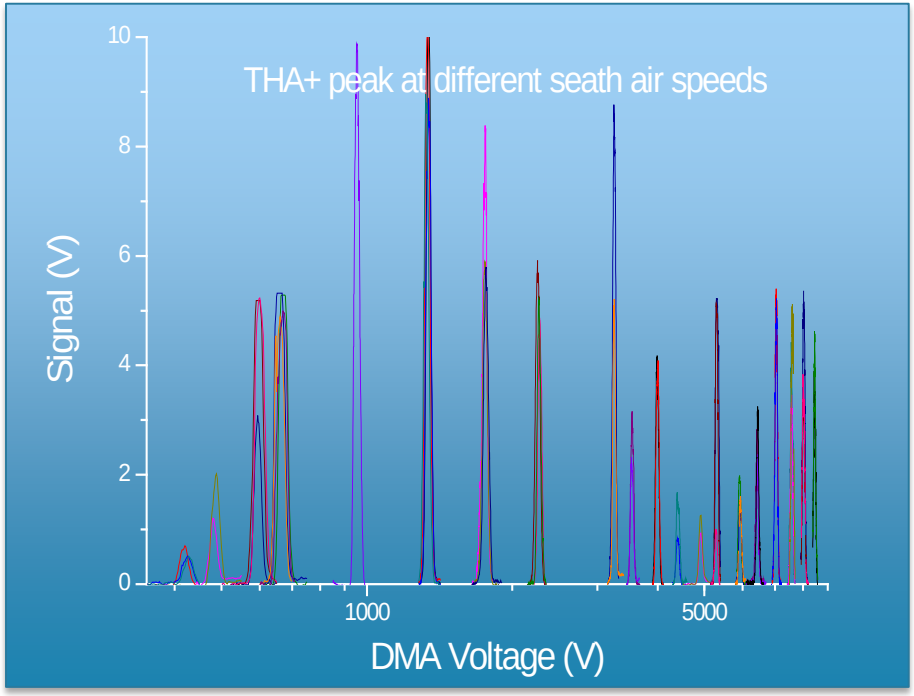


Orthogonal Field IMS

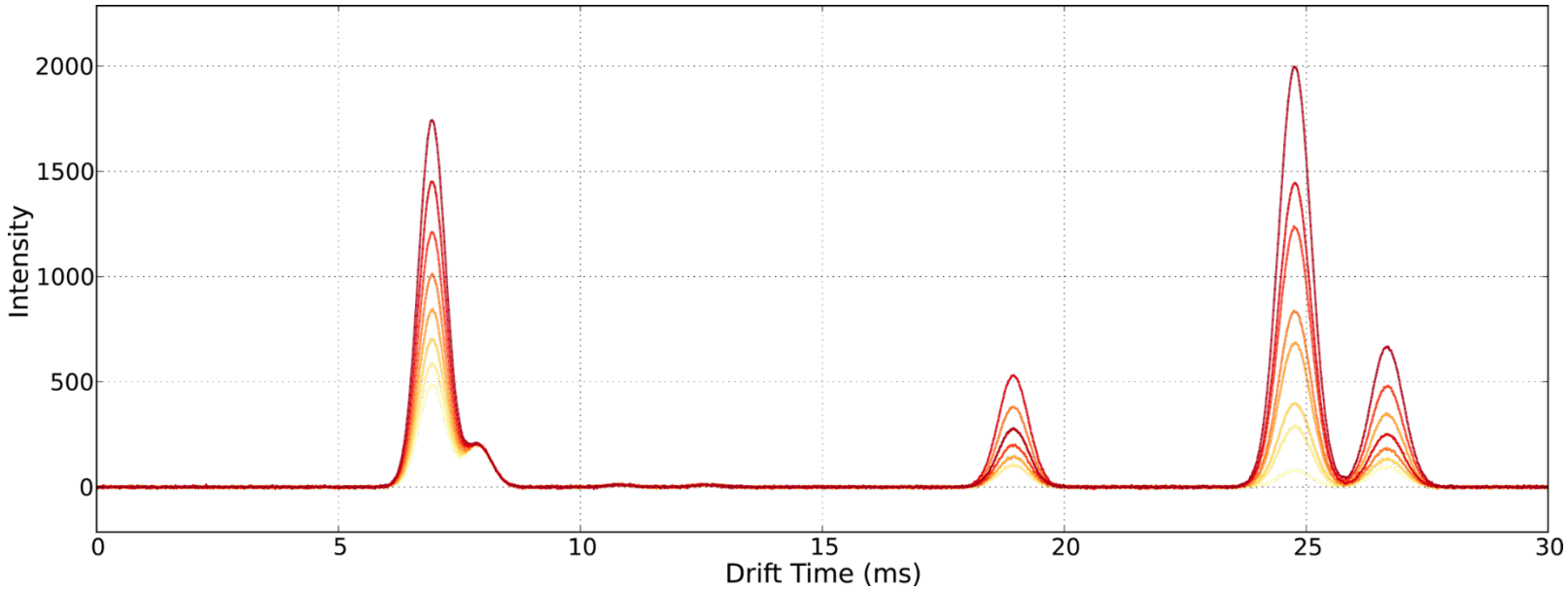


Differential Mobility Analyzer



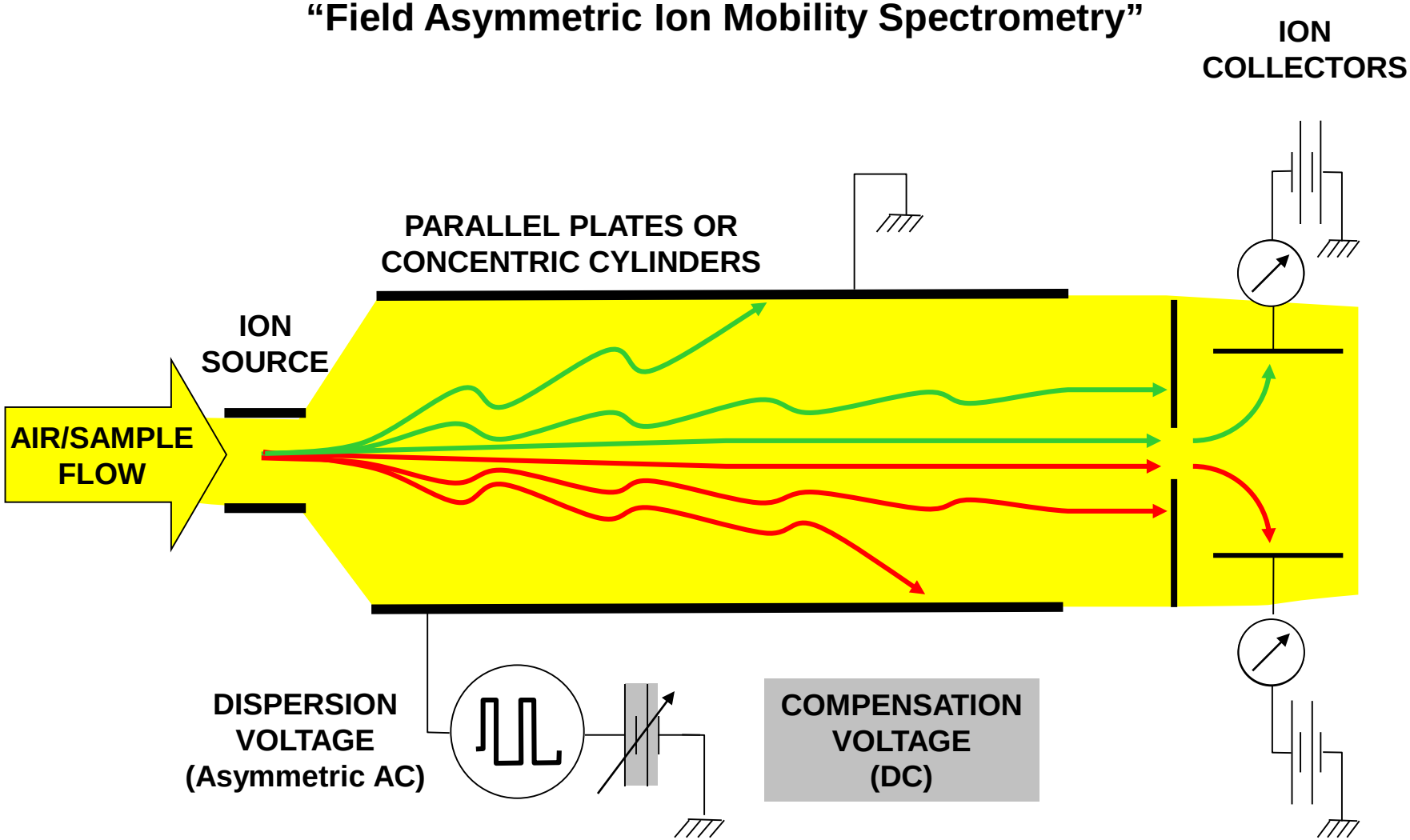


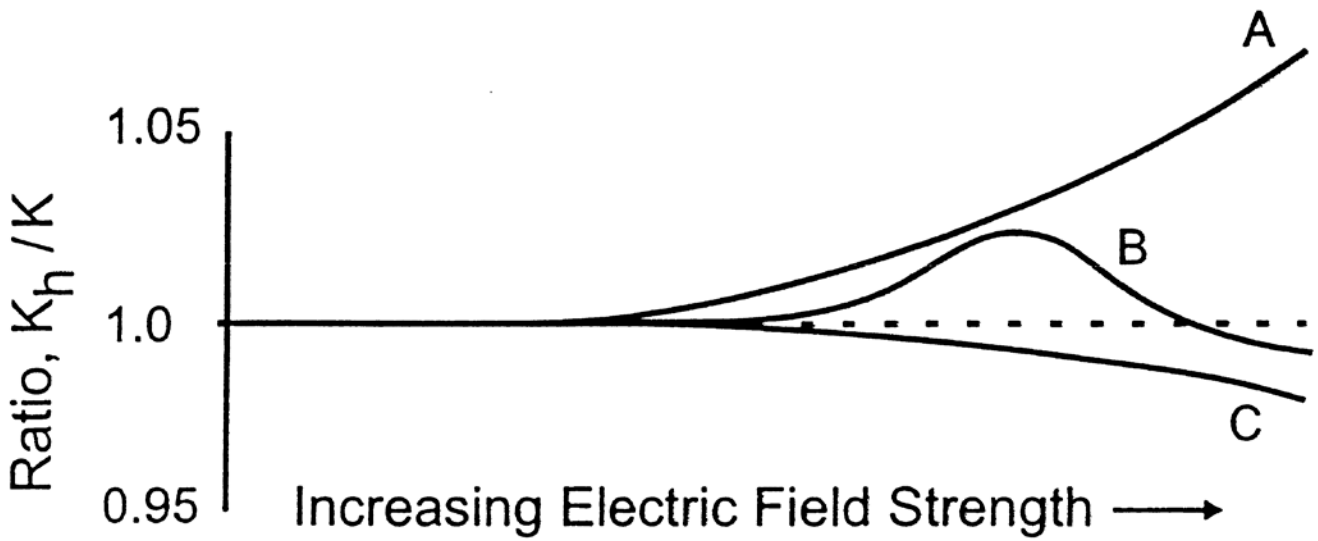
FIELD ASYMMETRIC WAVEFORM ION MOBILITY SPECTROMETRY (FAIMS)



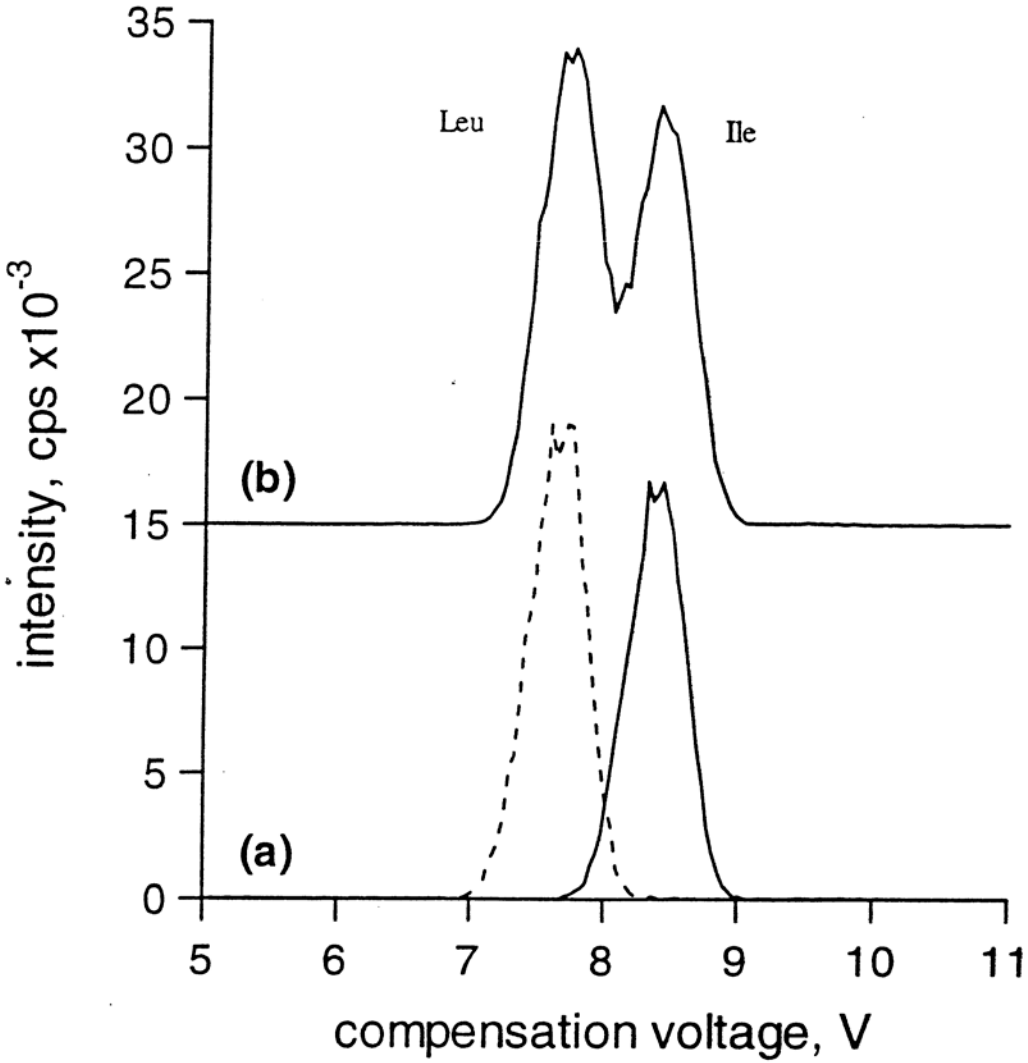
RF-DC Ion Mobility Spectrometry

"Field Ion Spectrometry" "Differential Mobility Spectrometry"
"Field Asymmetric Ion Mobility Spectrometry"



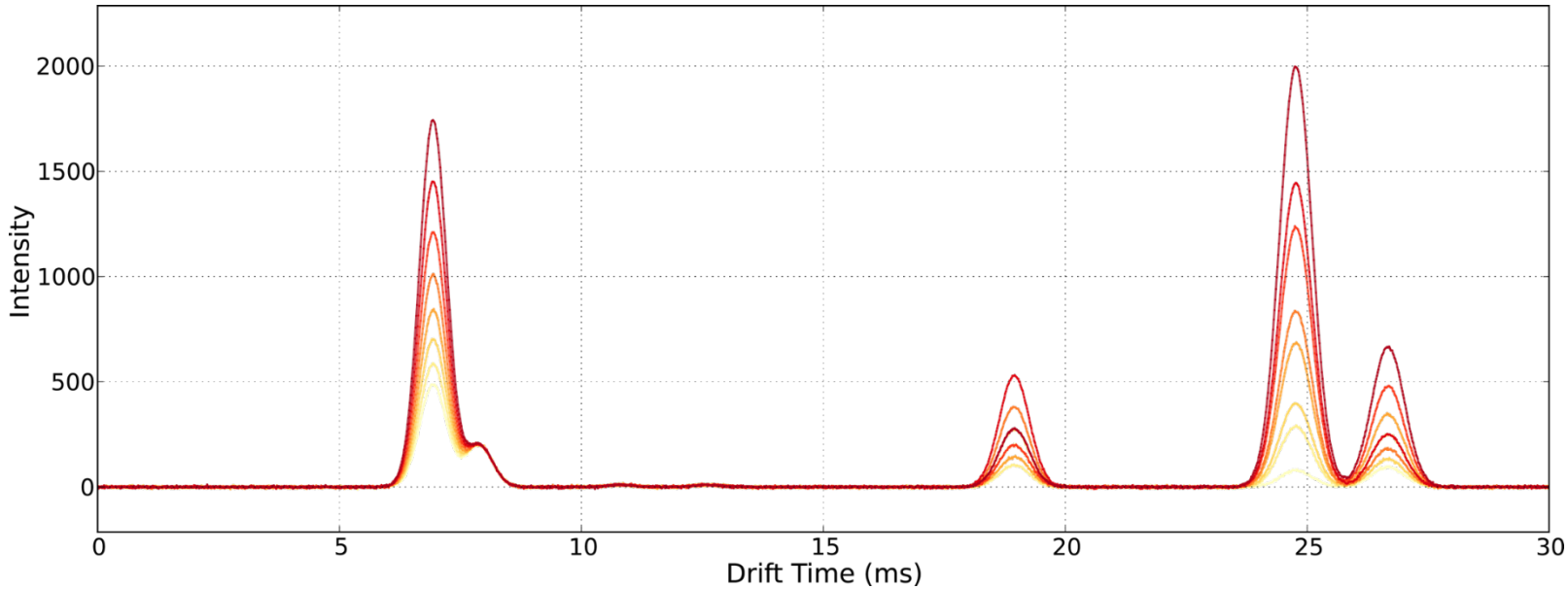


D. Barnett, R. Guevremont, and R. W. Purves "*Determination of Part-per-trillion Levels of Chlorate, Bromate, and Iodate by Electrospray Ionization - High Field Asymmetric Waveform Ion Mobility Spectrometry - Mass Spectrometry*", submitted.

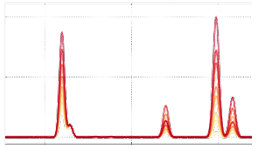


D. Barnett, B. Ells, R. Guevremont, and R. W. Purves "Separation of Leucine and Isoleucine by Electrospray Ionization – High Field Asymmetric Waveform Ion Mobility Spectrometry – Mass Spectrometry", 21st Capillary Chromatography and Electrophoresis Conference, Park City, Utah, July 1999.

ION MOBILITY MASS SPECTROMETERS



Value Added Information to Mass and Mass Fragmentation



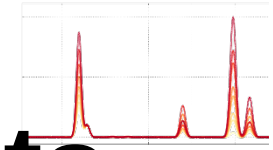
- Pre-separation step prior to mass spectrometry
- Increase in peak capacity
- Separation of isomers and isobars
- Separation of conformers
- Chiral Separations
- Reduction of chemical and random noise
- Charge state separation
- Identification of charge location
- Measurement of ion size
- Class identification by mobility-mass correlation lines
- Isotope measurements in complex mixtures

TYPES OF IMMS INSTRUMENTS

Ion Mobility Spectrometers

Mass Spectrometers

Types of IMMS Instruments



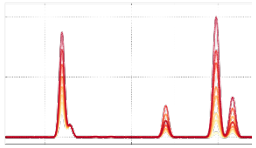
Ion Mobility Spectrometers

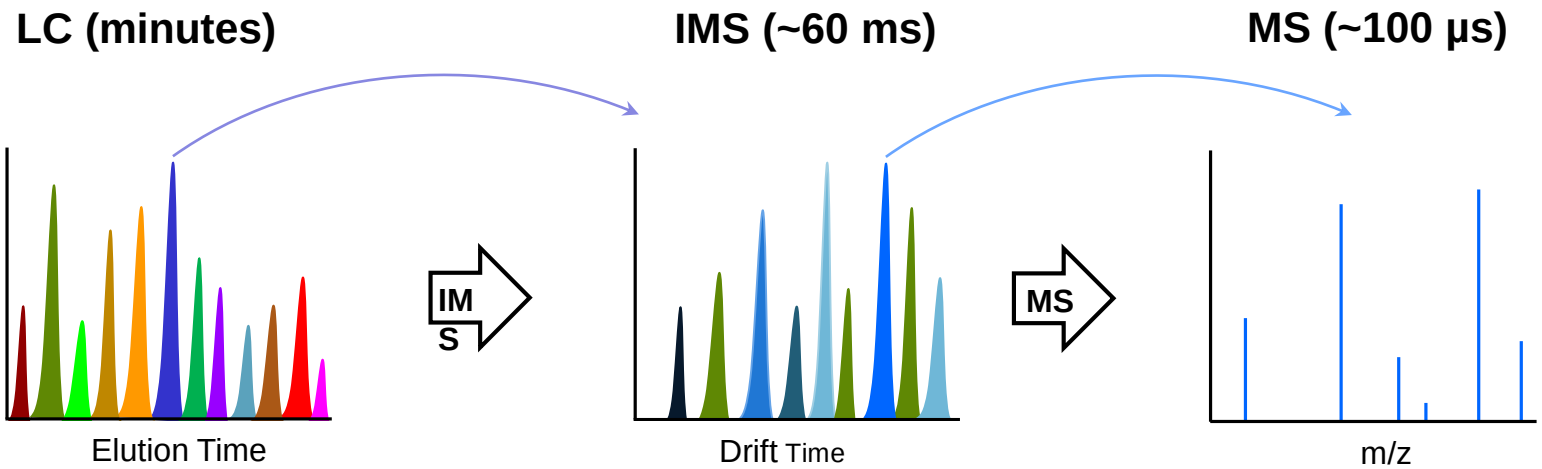
- Drift Time IMS
  - Low Pressure
  - Atmospheric Pressure
  - High Pressure
- Orthogonal Field IMS
  - FAIMS
  - DMS
  - DMA
- Dynamic Field IMS
  - Traveling Wave
  - Trapped Ion

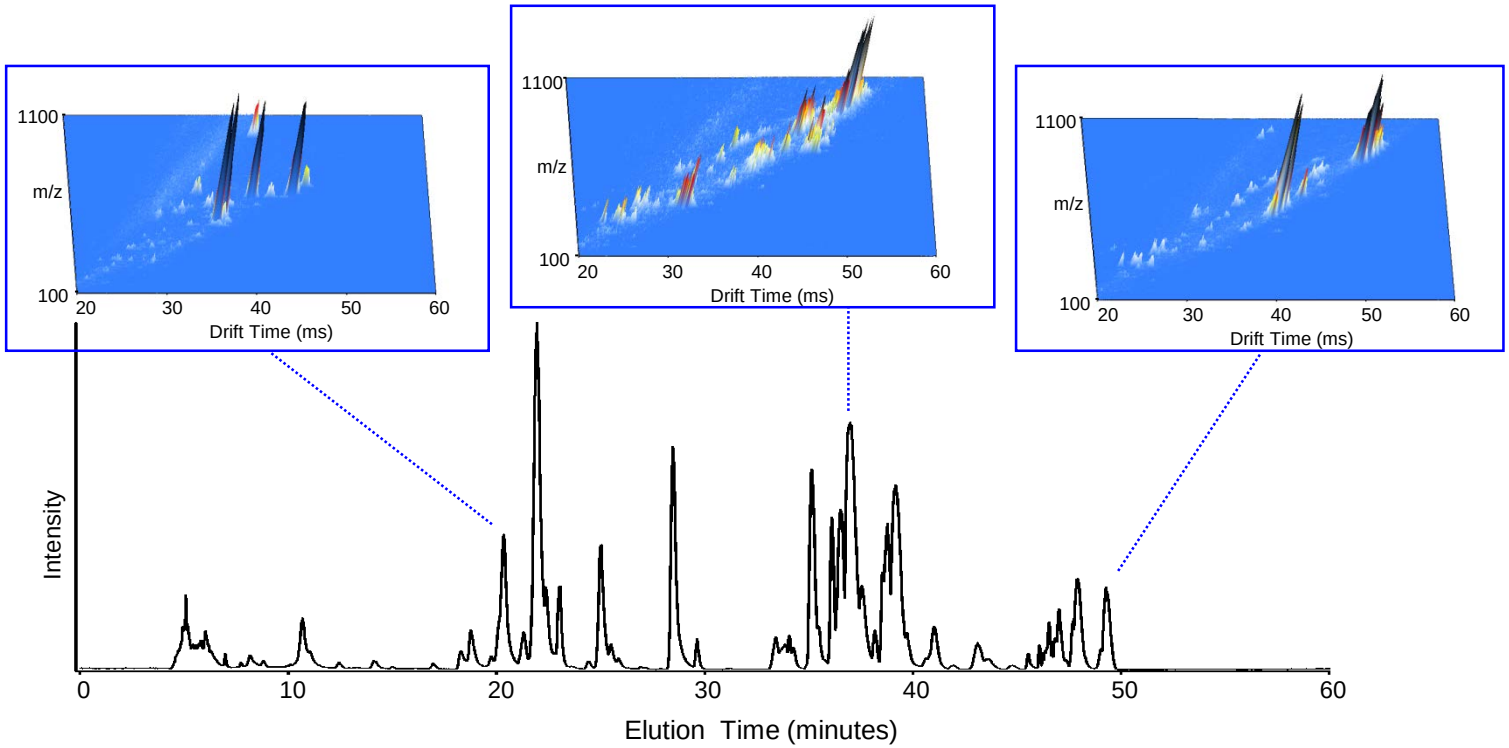
Mass Spectrometers

- Quadrupole
- Time-of-flight
- Ion trap
- Fourier Transform Ion Cyclotron
- Magnetic Sector

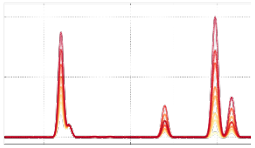
Multidimensional Analyses

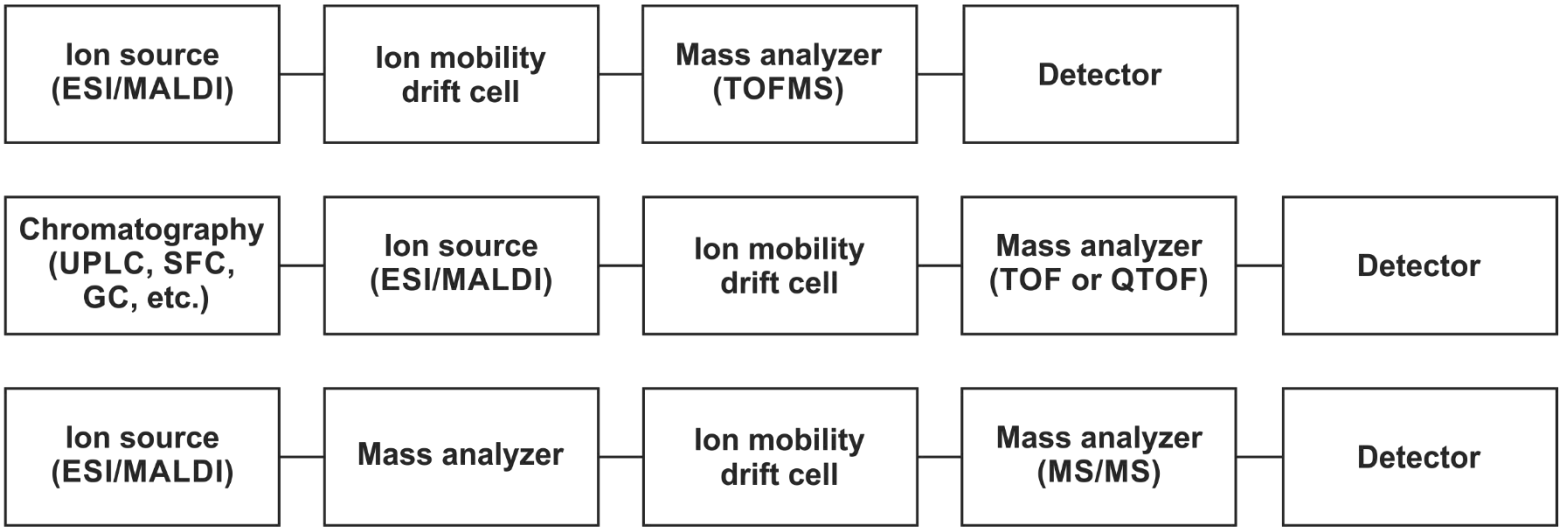


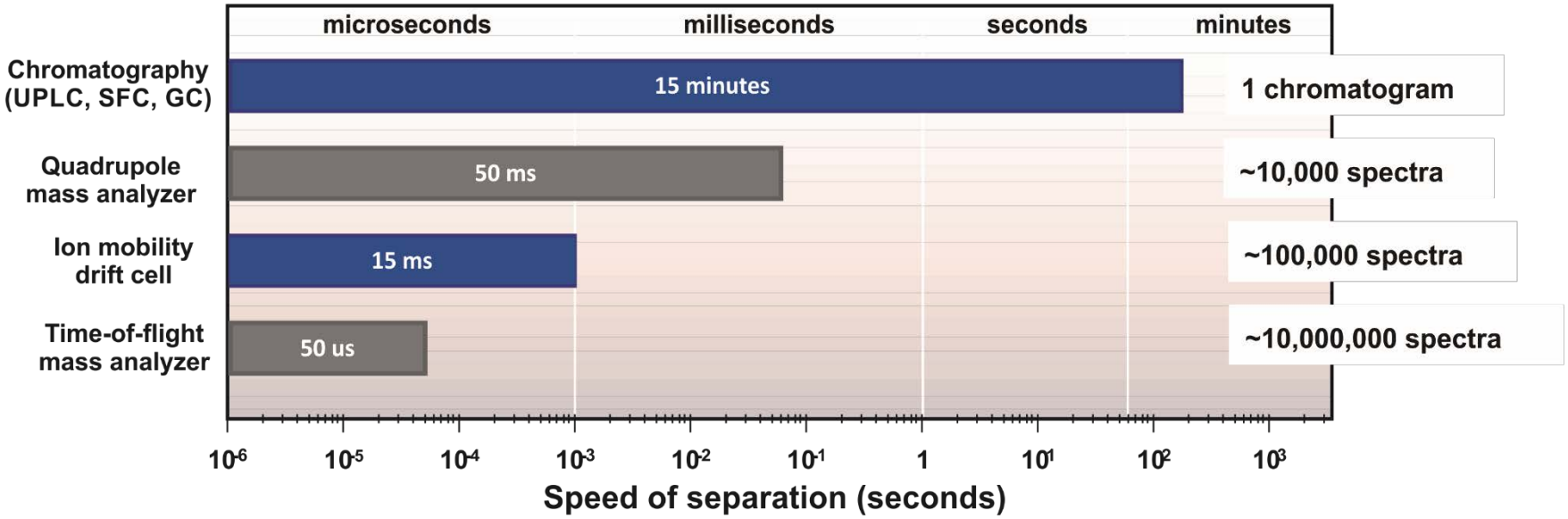




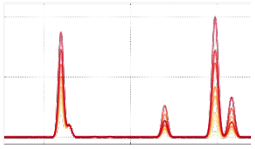
Multidimensional analyses & timescale

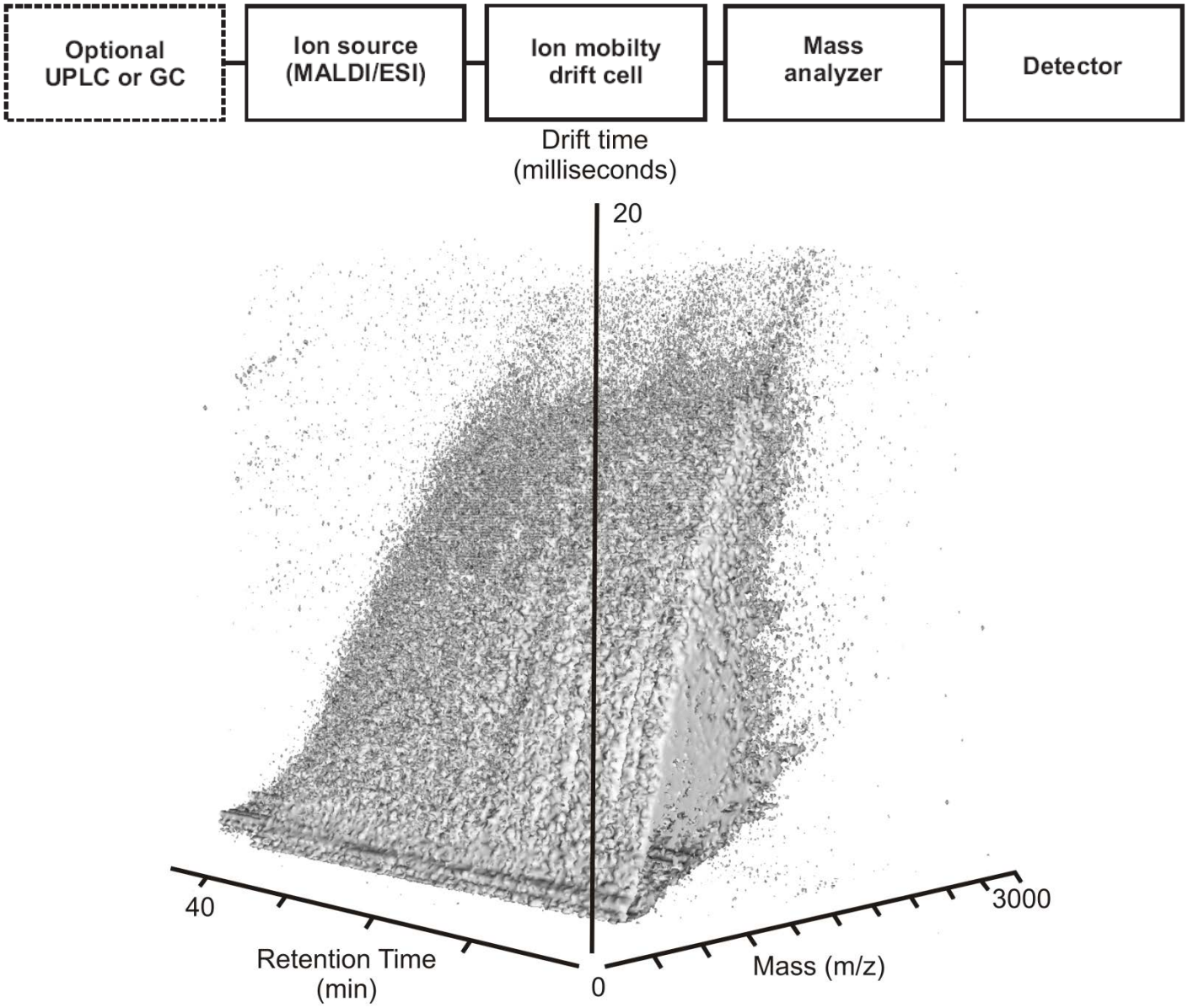


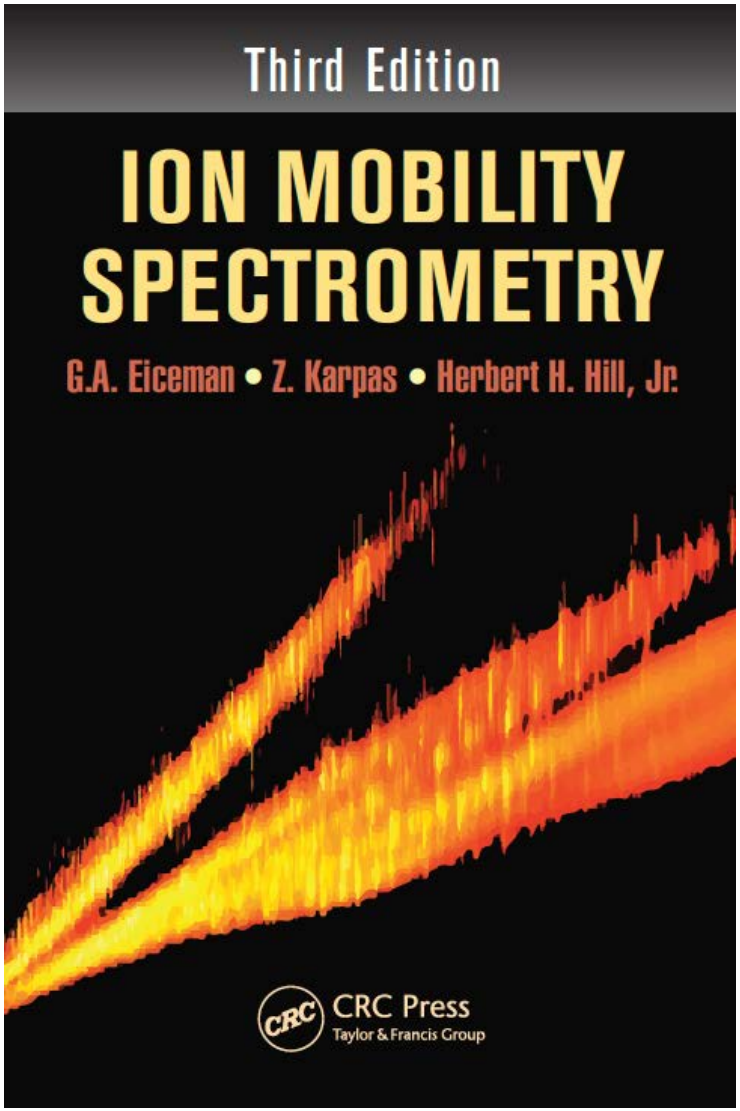




High dimensional molecular characterization





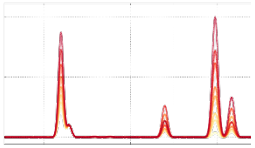


http://www.springer.com/chemistry/analytical+chemistry/journal/12127

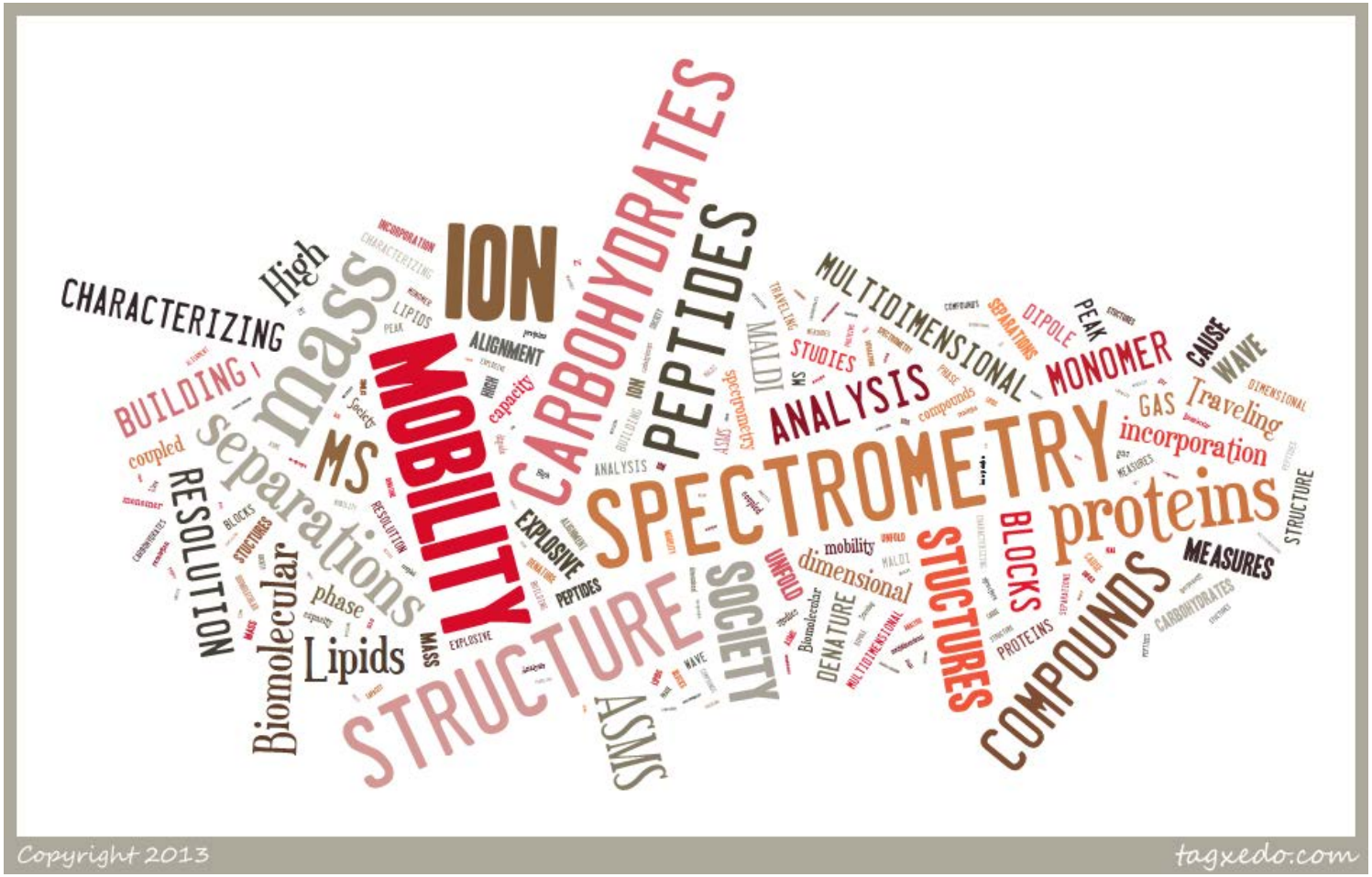
ISIMS Conference 2015
Boston, MA
July 24 – July 28, 2016

IMS
Short Course

July 23-24, 2016



QUESTIONS?



CHARACTERIZING
High
mass
ION
CARBOHYDRATES
PEPTIDES
MULTIDIMENSIONAL
MONOMER
CAUSE
WAVE
BUILDING
MOBILITY
ALIGNMENT
MALDI
STUDIES
ANALYSIS
GAS
Traveling
incorporation
coupled
separations
MS
SPECTROMETRY
proteins
RESOLUTION
EXPLOSIVE
STRUCTURE
SOCIETY
STUCTURES
BLOCKS
MEASURES
dimensional
mobility
Biomolecular
phase
Lipids
ASMS
COMPOUNDS
DENATURE
PROTEINS
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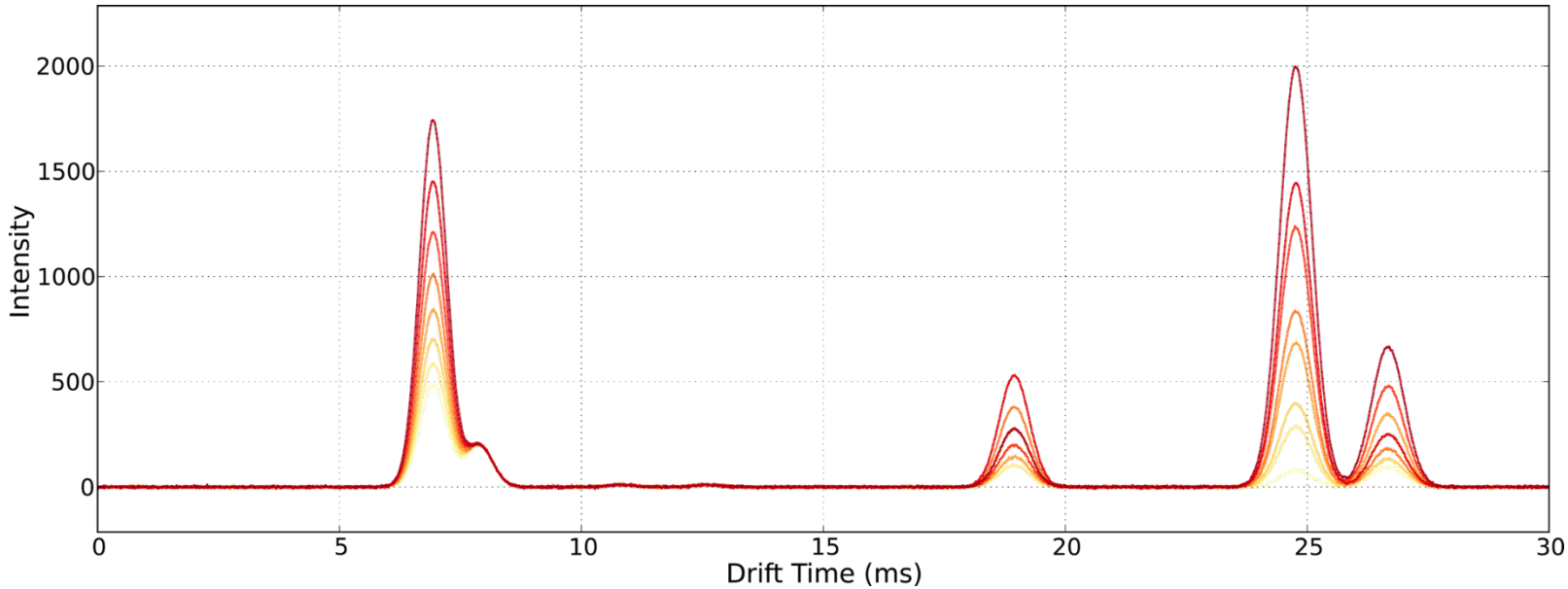
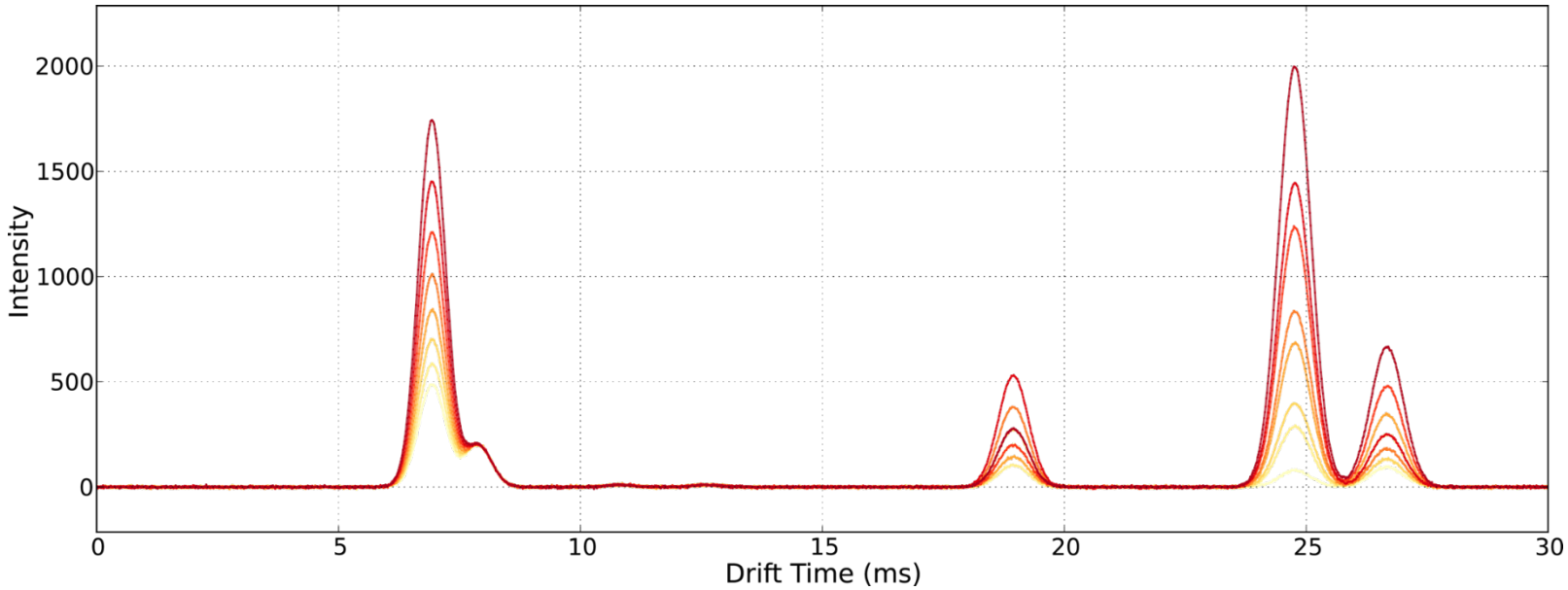
COFFEE BREAK



Drift Tube Ion Mobility Mass Spectrometry (DTIMMS)



Types of IMMS Instruments

Ion Mobility Spectrometers

- Drift Tube IMS
  - Pressure > 760 Torr
  - Pressure = 760 Torr
  - Pressure < 760 Torr
- Orthogonal Field IMS
  - FAIMS
  - DMS
  - DMA
- Traveling Wave IMS

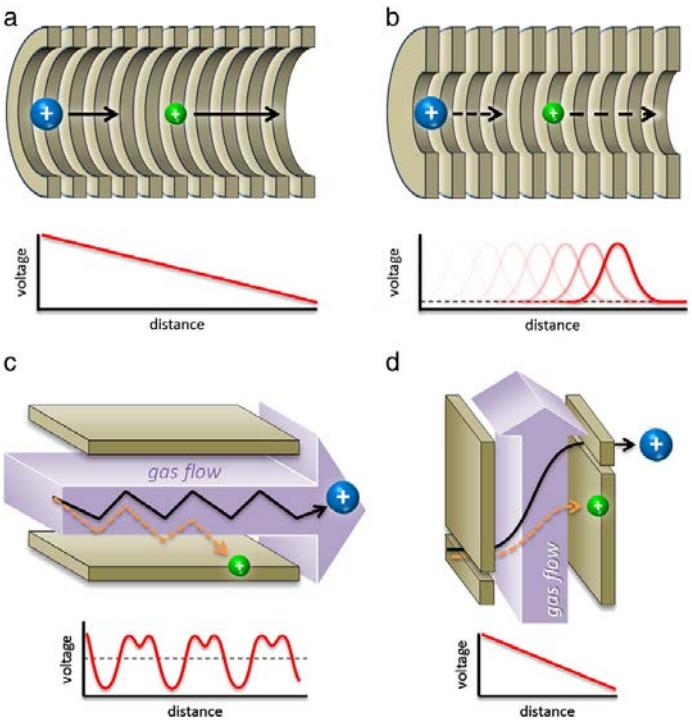
Mass Spectrometers

- Quadrupole
- Time-of-flight
- Ion trap
- Fourier Transform Ion Cyclotron Resonance
- Orbitrap

Commercially available
Research

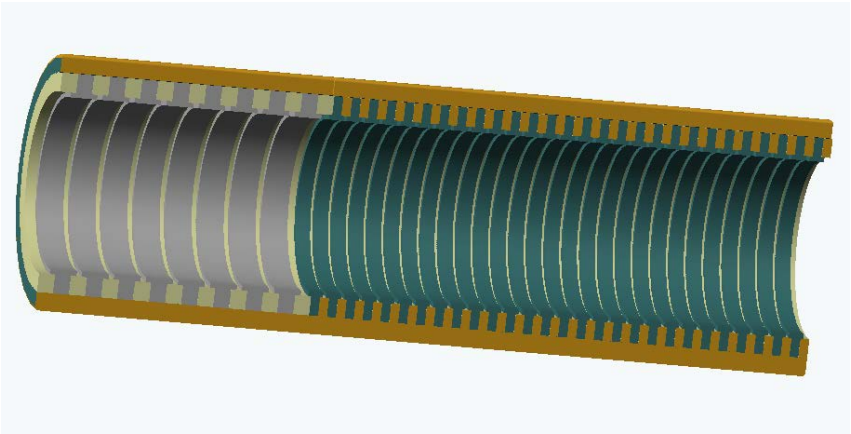
DTIMS-MS

- Commercially Available Instruments
  - IMS$_{(atm)}$-Q MS
  - IMS$_{(atm,\ 2\text{-}4\ torr)}$-TOF
- Research Instruments
  - IMS$_{(atm,\ \sim 10\ torr)}$-IT
  - IMS$_{(atm)}$-FTICR
  - IMS$_{(atm)}$-QqQ MS



Biochimica et Biophysica Acta 1811 (2011) 935–945

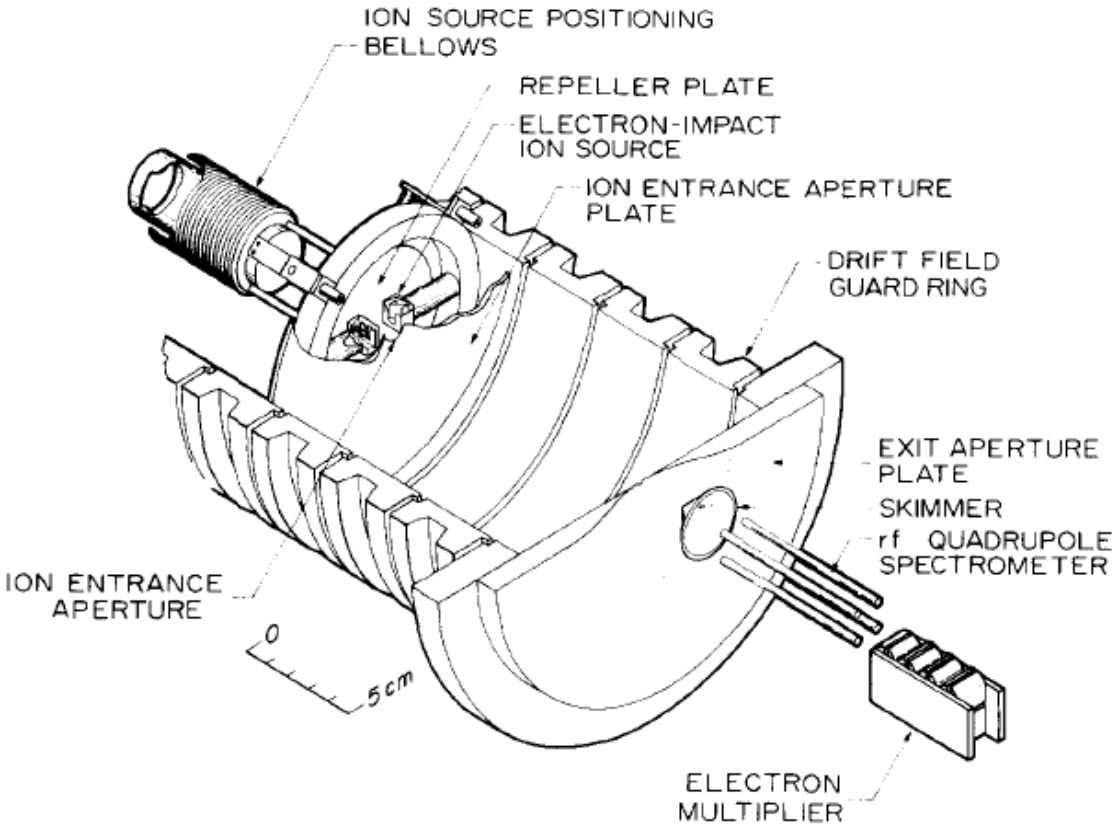
Typical AP Ion Mobility Technology



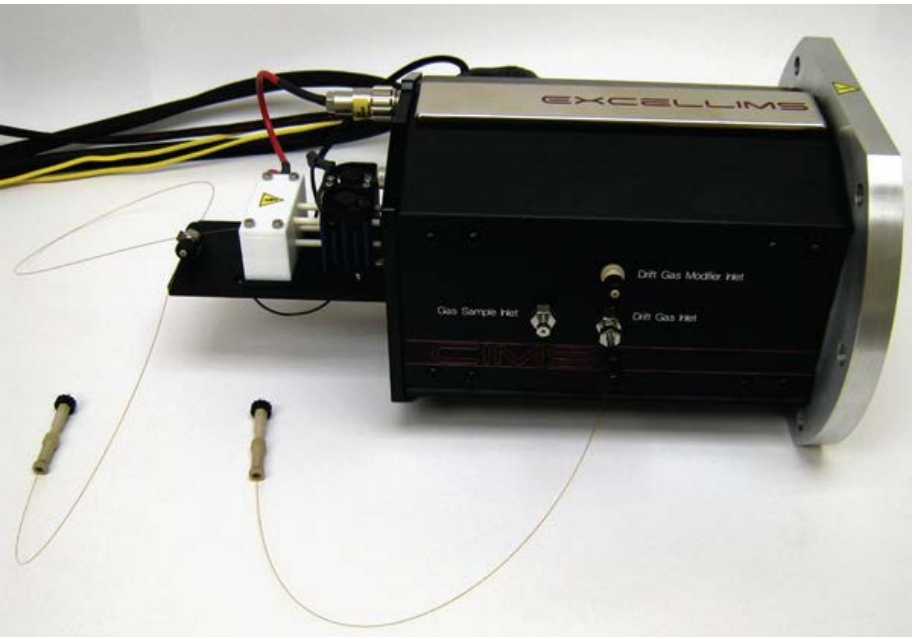
•*L* 10-30 cm
•*T* 100-250 ^{0}C
•*P* ambient
•*E*/*N* 1-10 Td

- Stacked-ring drift tube, external resistor series voltage divider, Bradbury-Nielsen or Tyndall gate.
- AP, especially with ESI sources, requires higher temperatures (100-250 ^{0}C) for declustering.
- “Reaction region” drift zone for ion desolvation prior to gating, especially with ESI.
- Temperature is a significant parameter and control issue

IMS-QUAD



E.W. McDaniel and L.A. Viehland, The transport of slow ions in gases: experiment, theory, and applications



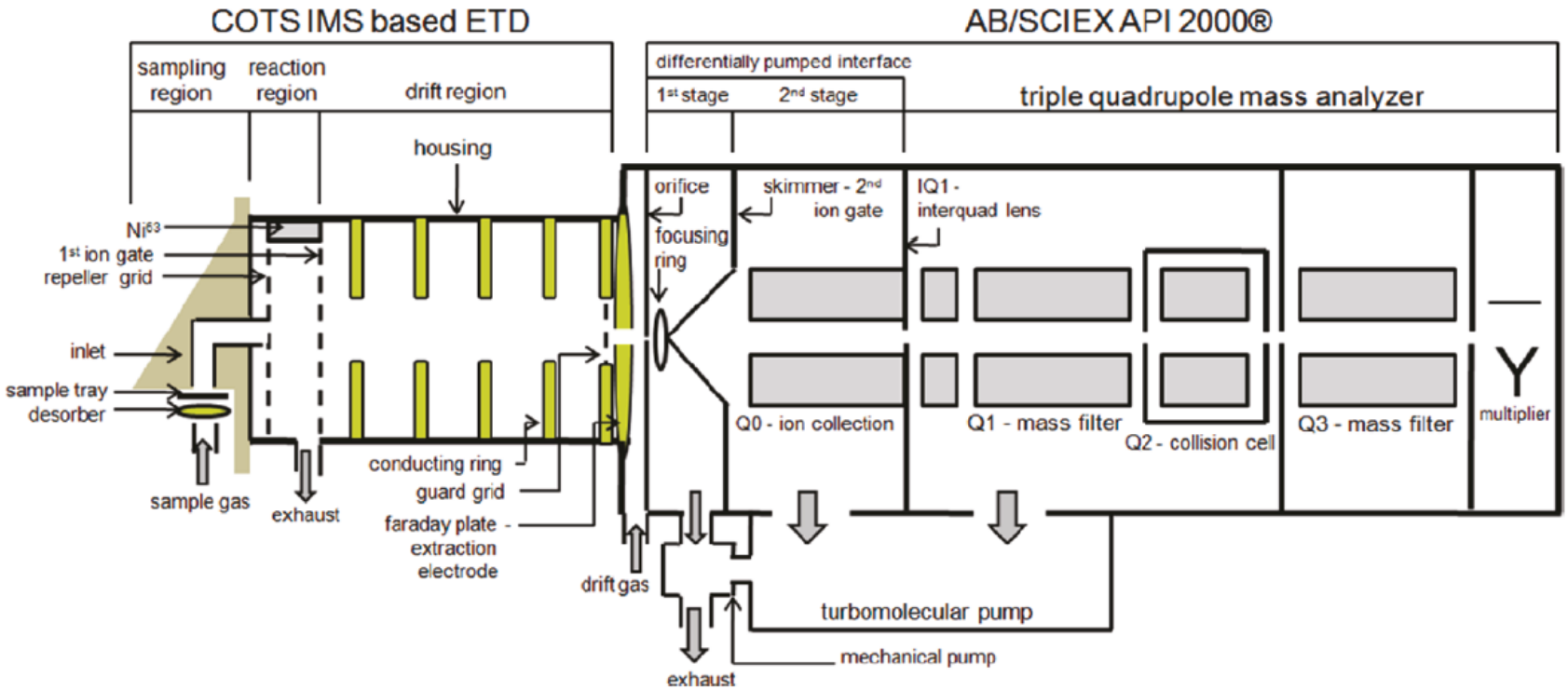
IMS-QUAD

ESI – IMS (RP ~ 60-80)

Differentially Pumped Interface

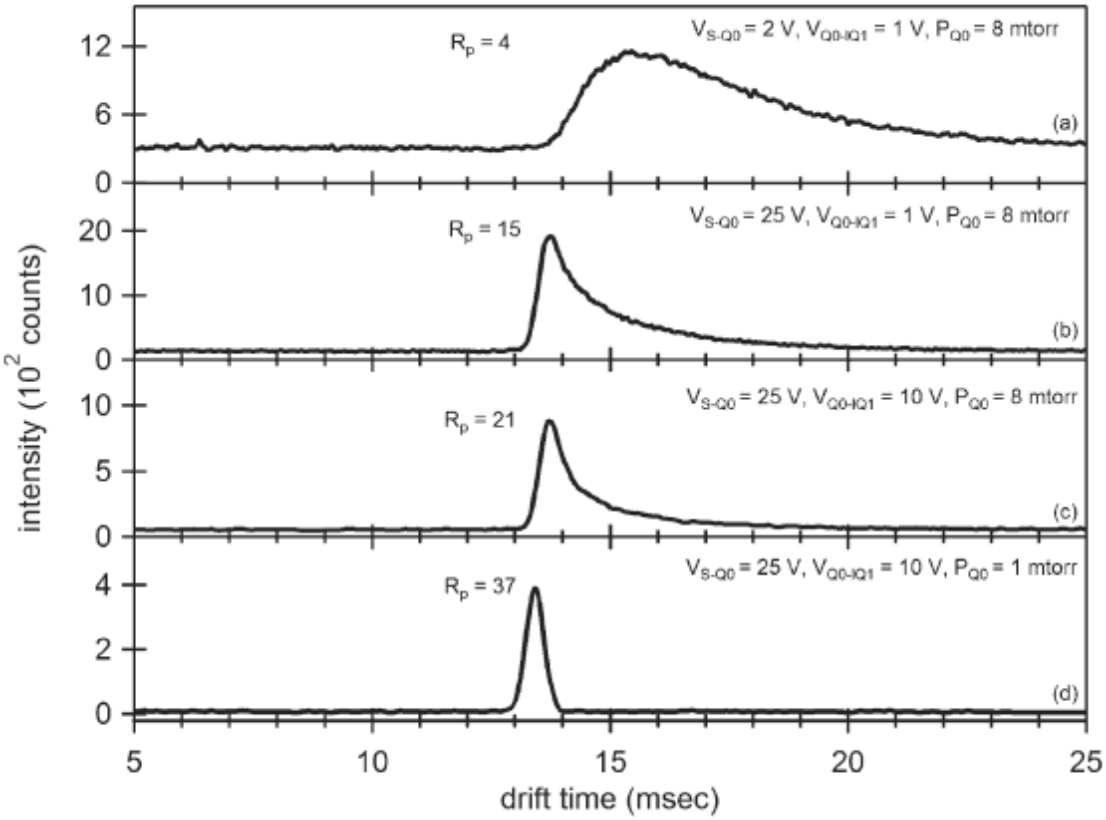
www.excellims.com

IMS – TRIPLE QUAD



dx.doi.org/10.1021/ac201999a |Anal. Chem. 2011, 83, 8596–8603

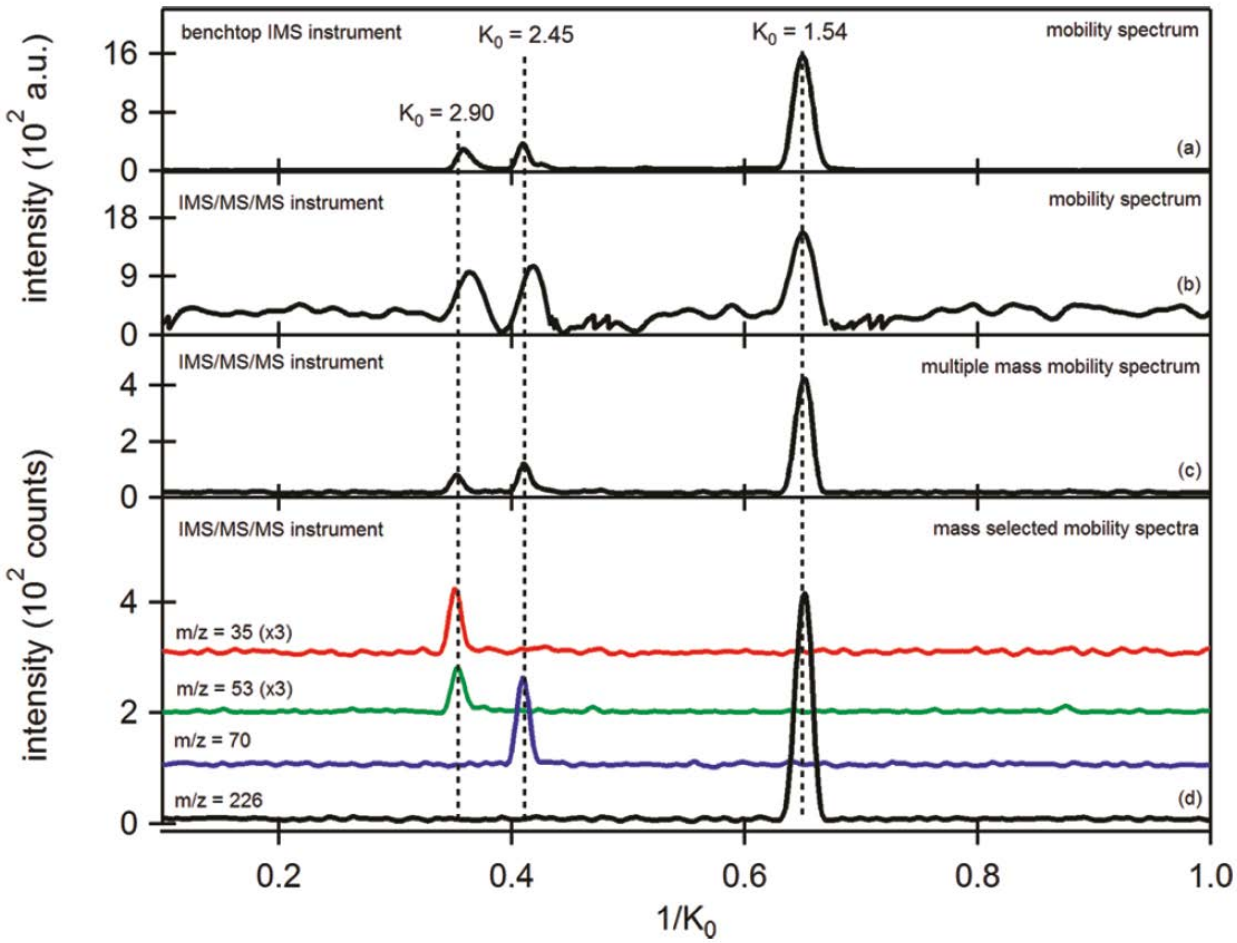
IMS-QQQ



- Peak Broadening
  - No axial field in multipoles

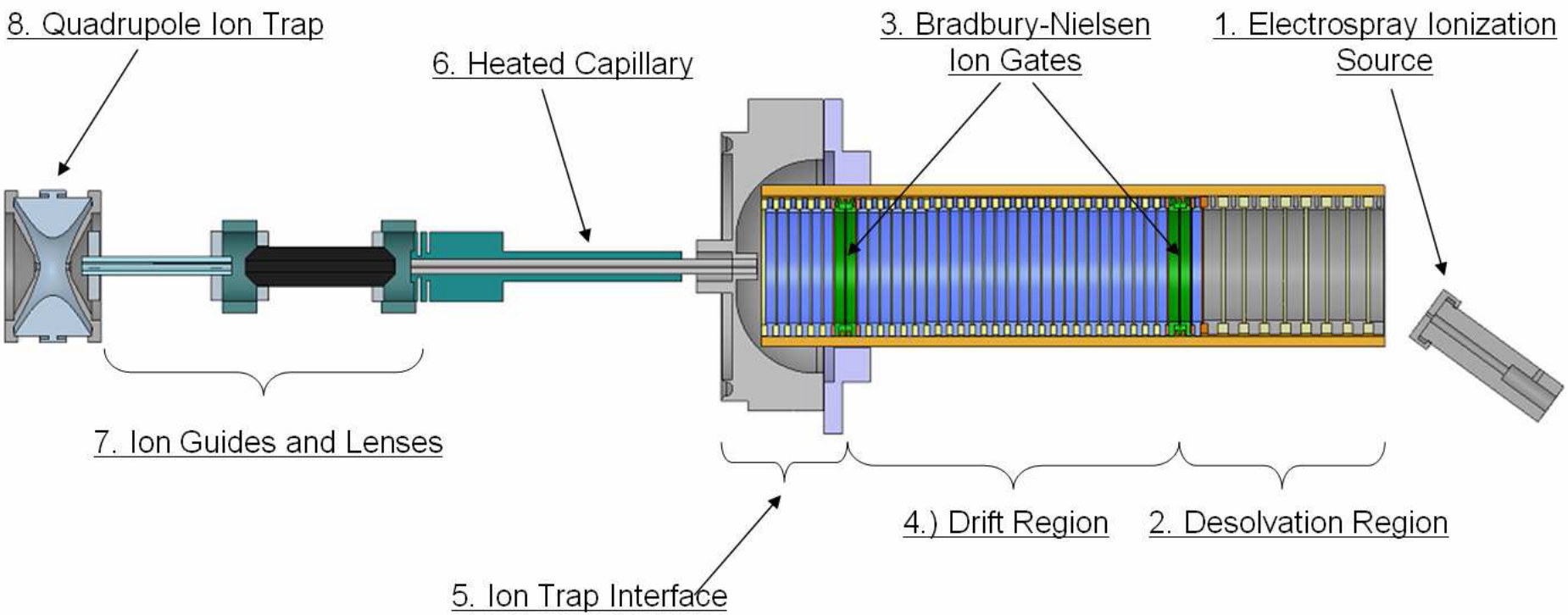
dx.doi.org/10.1021/ac201999a |Anal. Chem. 2011, 83, 8596–8603

IMS-QQQ



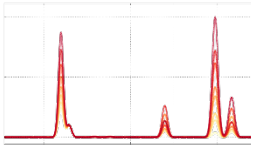
dx.doi.org/10.1021/ac201999a |Anal. Chem. 2011, 83, 8596–8603

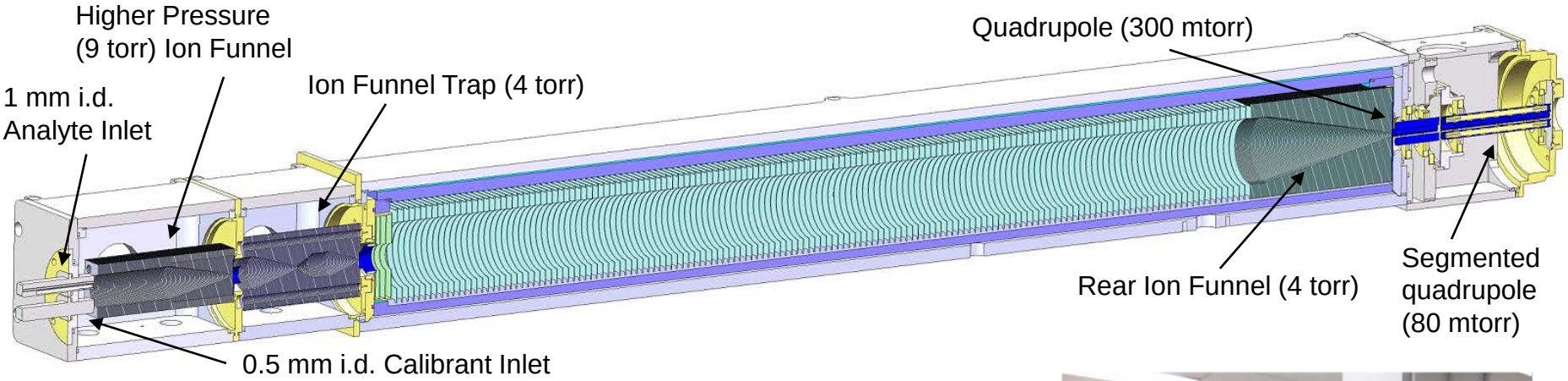
IMS - IT



Mass Analysis of Mobility-Selected Ion Populations Using Dual Gate Ion Mobility Quadrupole Ion Trap Mass Spectrometry, B. H. Clowers, H. H. Hill, Jr., Anal. Chem. 2005, 77, 5877-5885

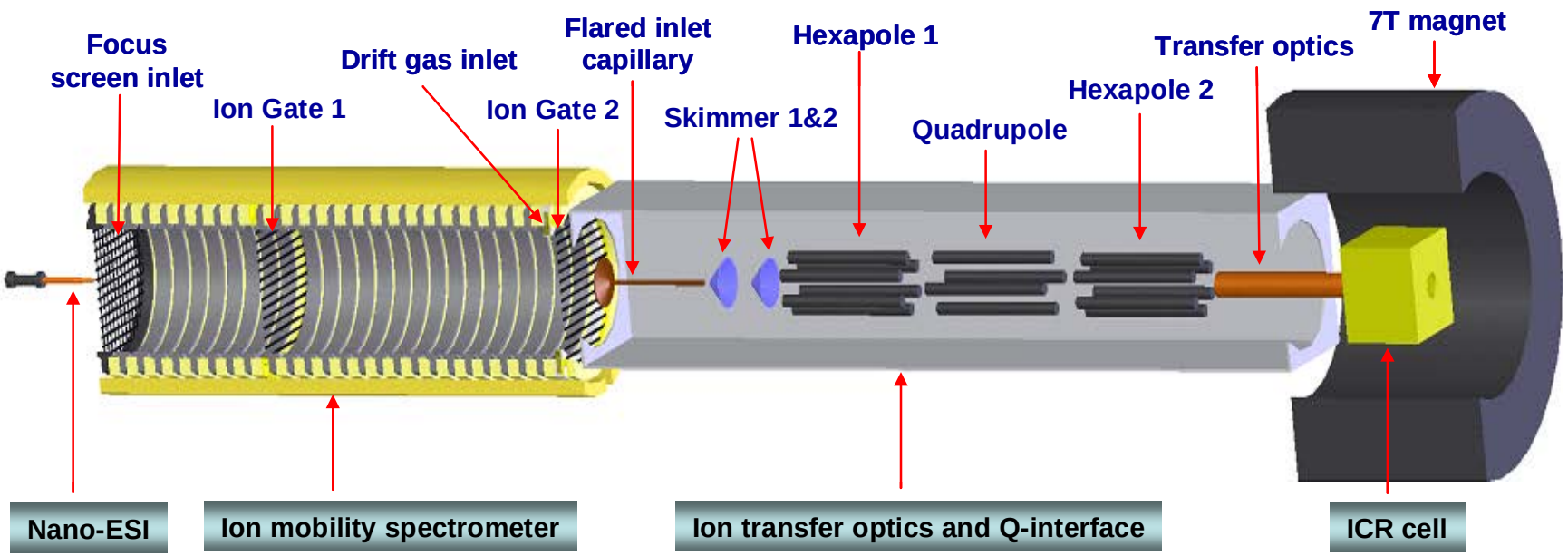
IMS-QExactive (Orbitrap)





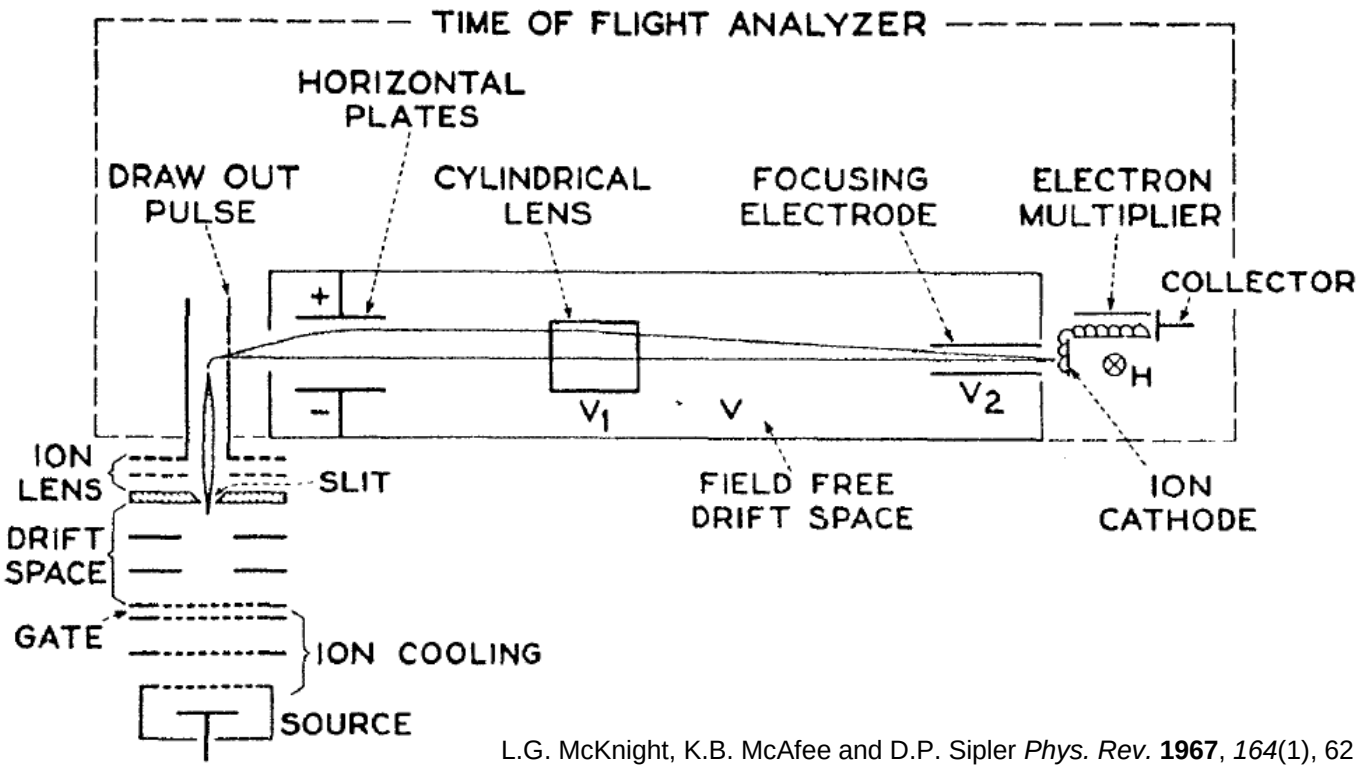
Belov, ASMS 2011, IMS-Orbitrap Multiplexing

IMS - FTICR



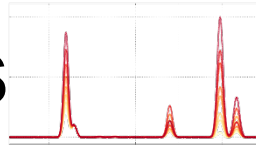
Design and performance of an atmospheric pressure ion mobility Fourier transform ion cyclotron resonance mass spectrometer, X. Tang, J. E. Bruce, H. H. Hill Jr., Rapid Communications Mass Spectrom. 21 (2007) 1115-1122.

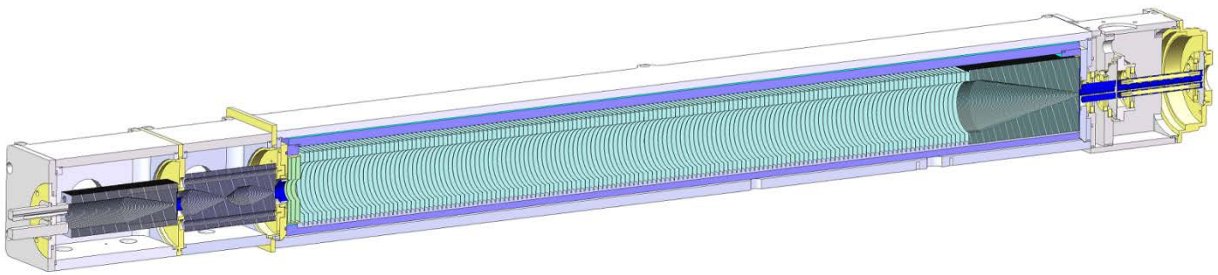
IMS - TOF



L.G. McKnight, K.B. McAfee and D.P. Sipler *Phys. Rev.* **1967**, *164*(1), 62

Low Pressure Ion Mobility Systems

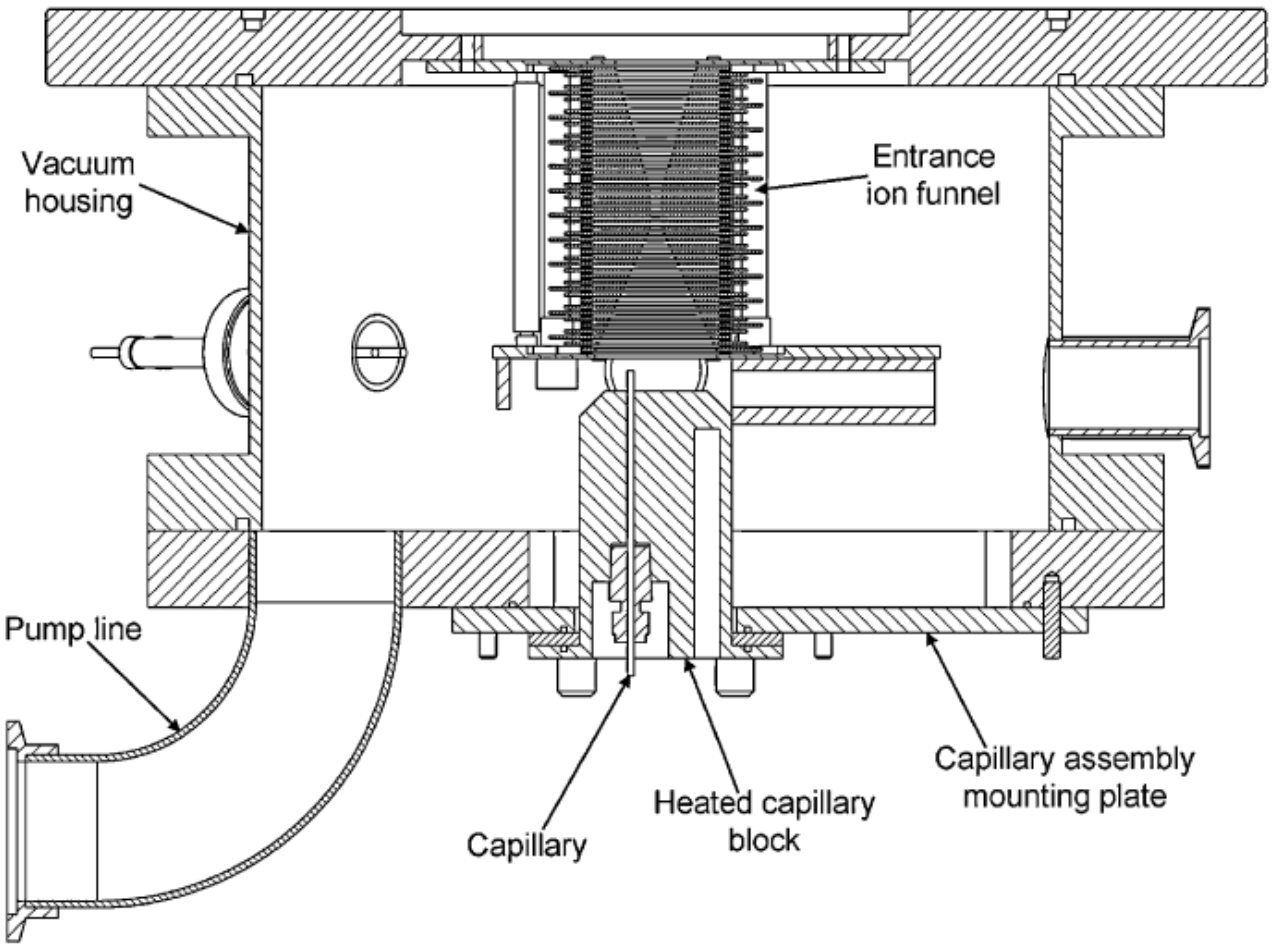




- Stacked-ring drift tube with external voltage divider, single-mesh or gridless gating by voltage pulse
- Low pressure tubes usually operate at ambient or sub-ambient temperature
- No reaction region in drift tube, but often uses apertures and/or He buffer regions to define pressure zones
- Pressure is main measurement and control issue

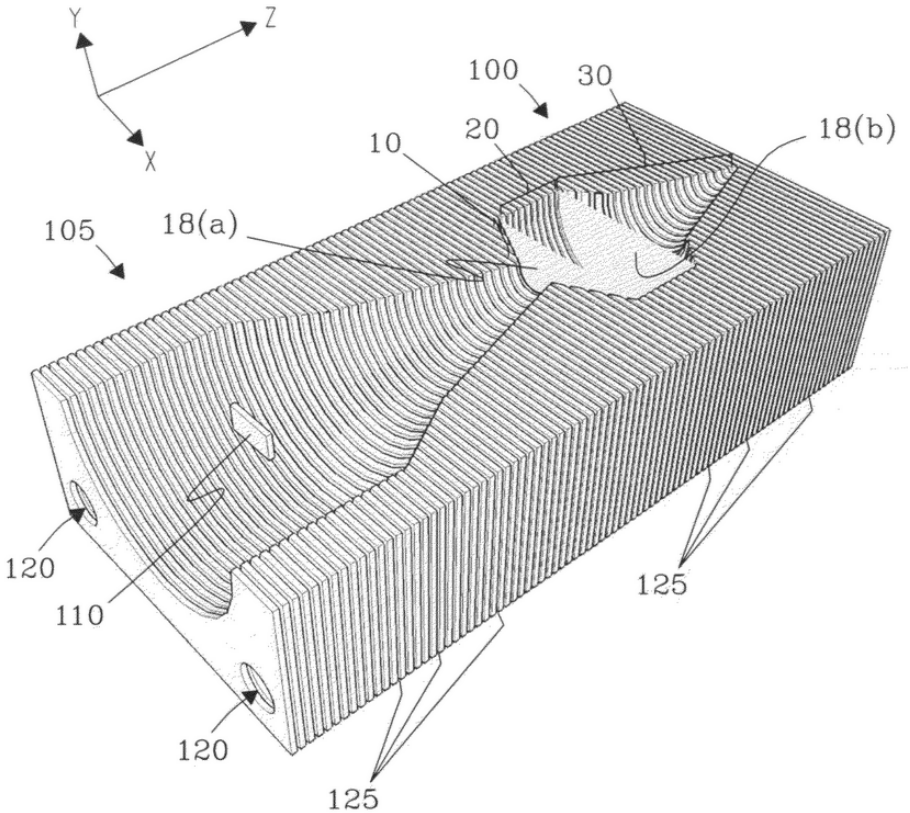
- *L* 30-200 cm
- *T* ambient
- *P* 1-10 torr
- *E*/*N* 4-40 Td

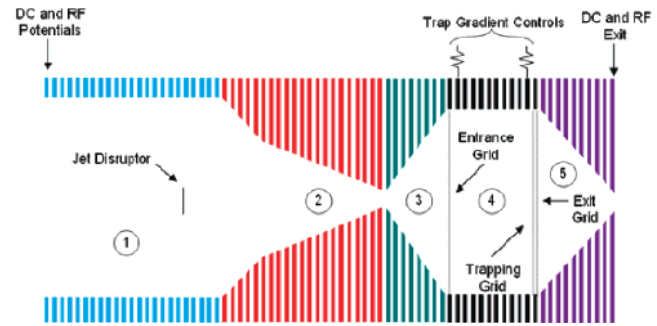
Low Pressure Ion Funnel Interface



P.R. Kemper et al. / International Journal of Mass Spectrometry 287 (2009) 46–57

Ion Funnel Trap

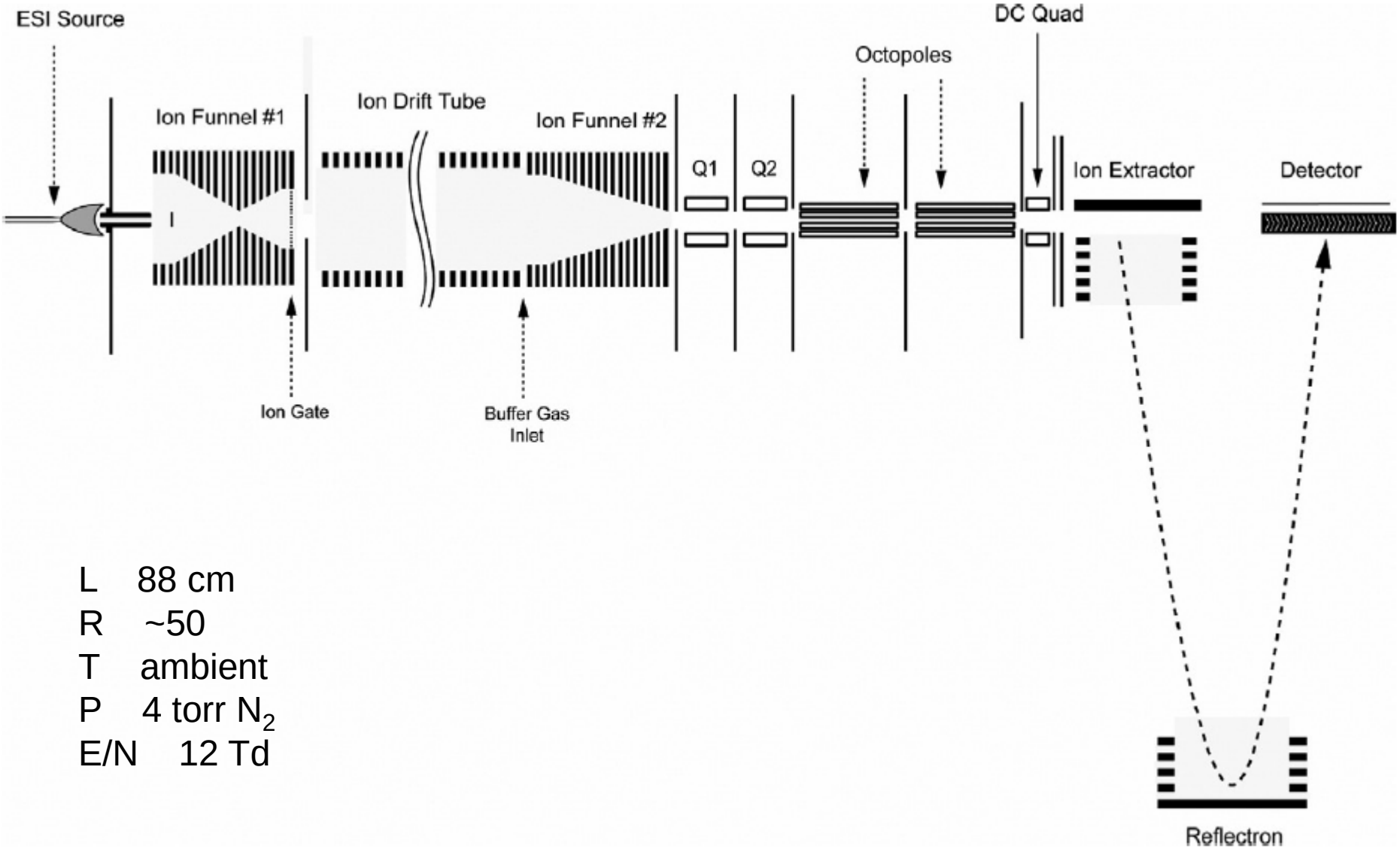




Analytical Chemistry, Vol. 80, No. 3, February 1, 2008

- Larger trapping volume
- Ejection is mobility dependent
  - Trapped Ion Mobility Spectrometry (TIMS)

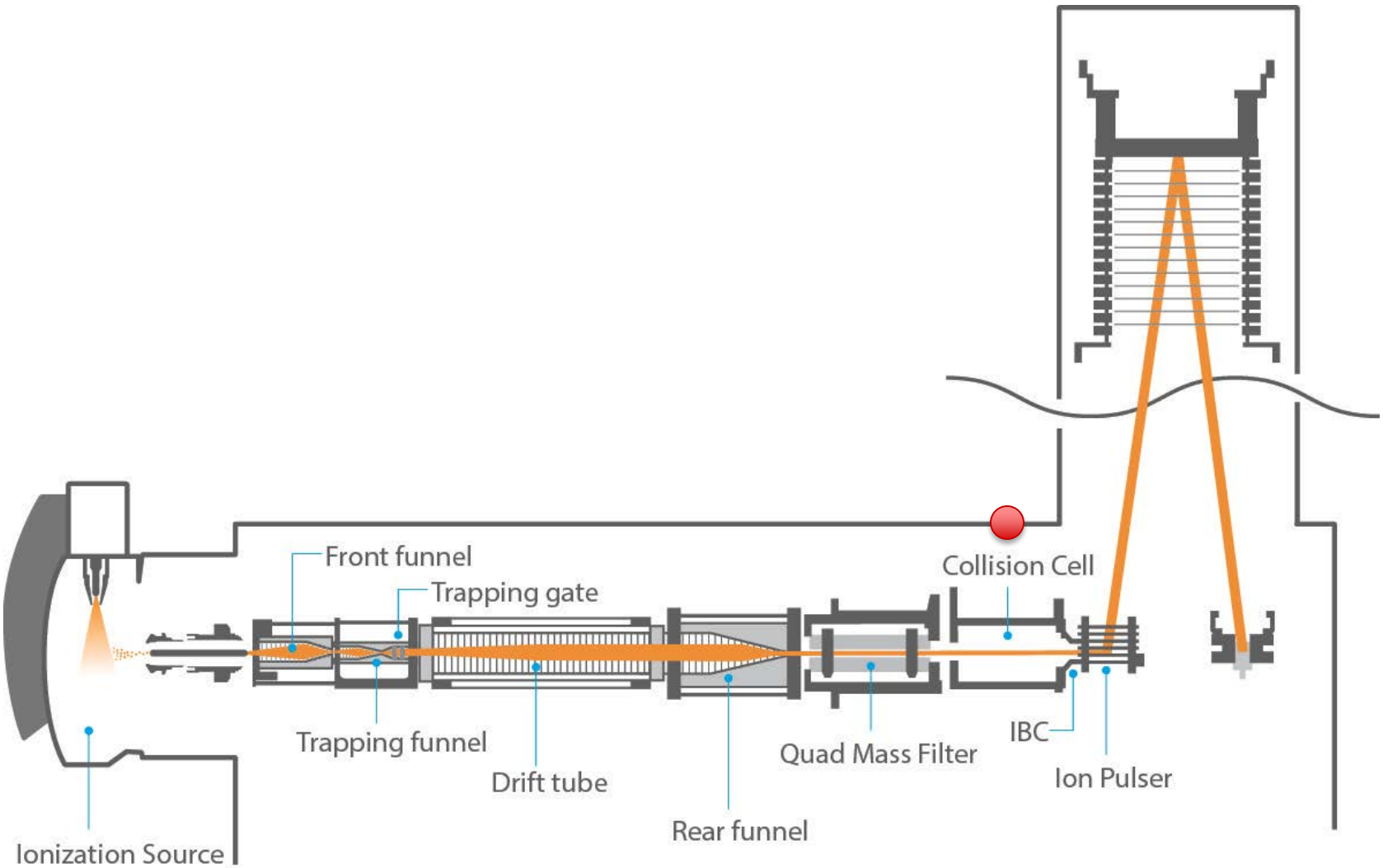
IMS-TOF Systems



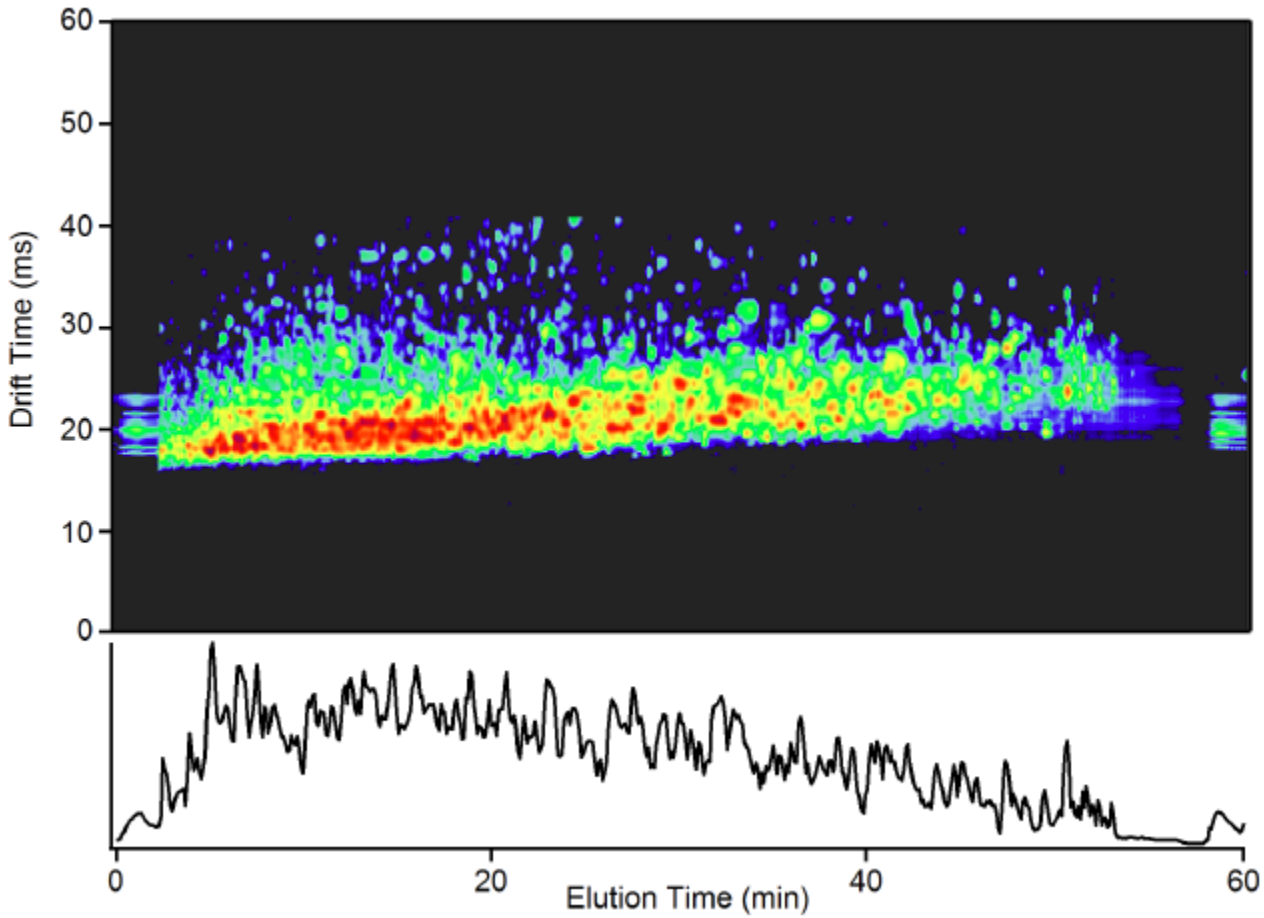
L 88 cm
R ~50
T ambient
P 4 torr N_2
E/N 12 Td

J Am Soc Mass Spectrom 2007, 18, 1176–1187

IMS-TOF Systems (2012)

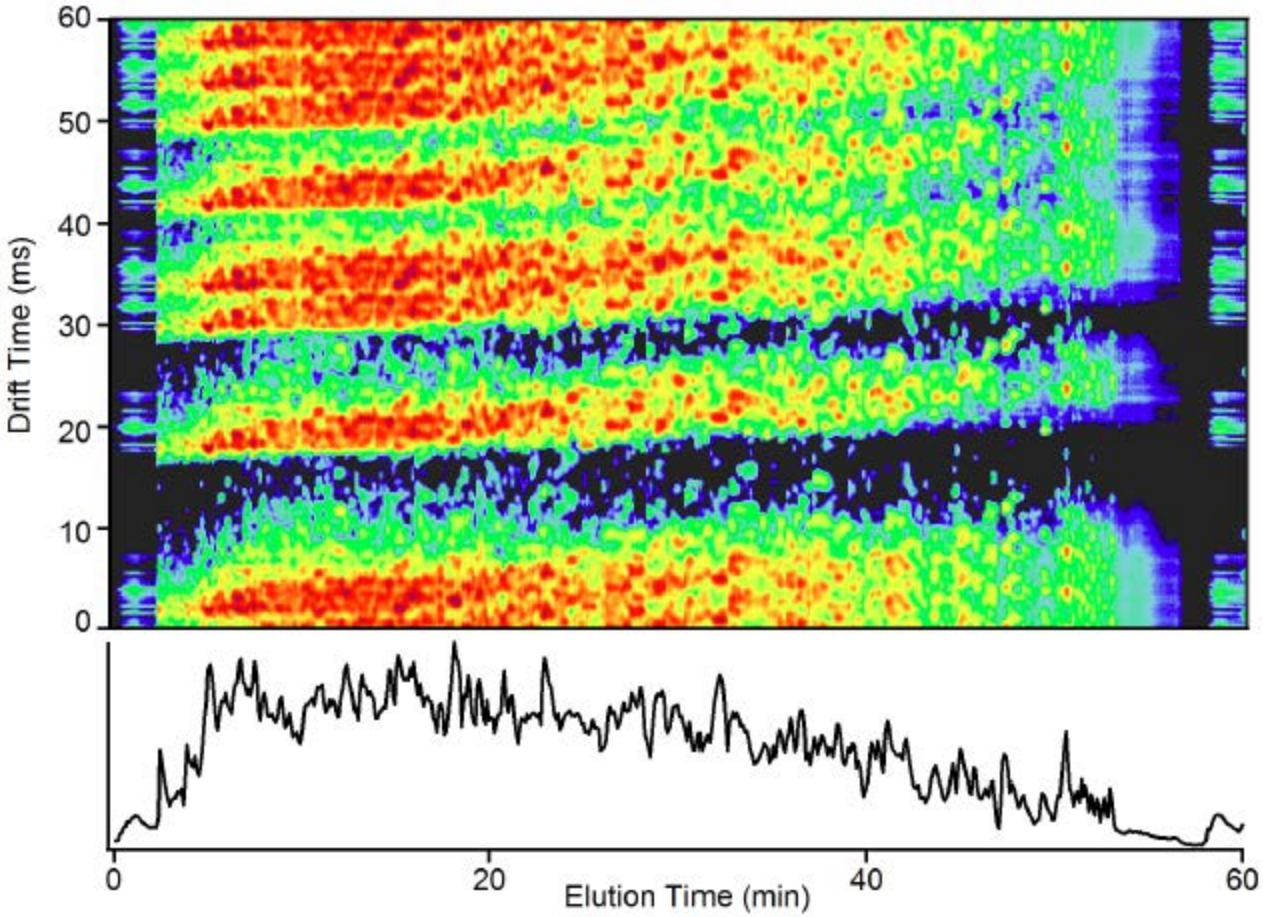


Traditional Pulsing Scheme



Baker et. al, ASMS 2011, High-Throughput Proteomics Platform Demonstrated for Liver Disease Progression Biomarker Verification

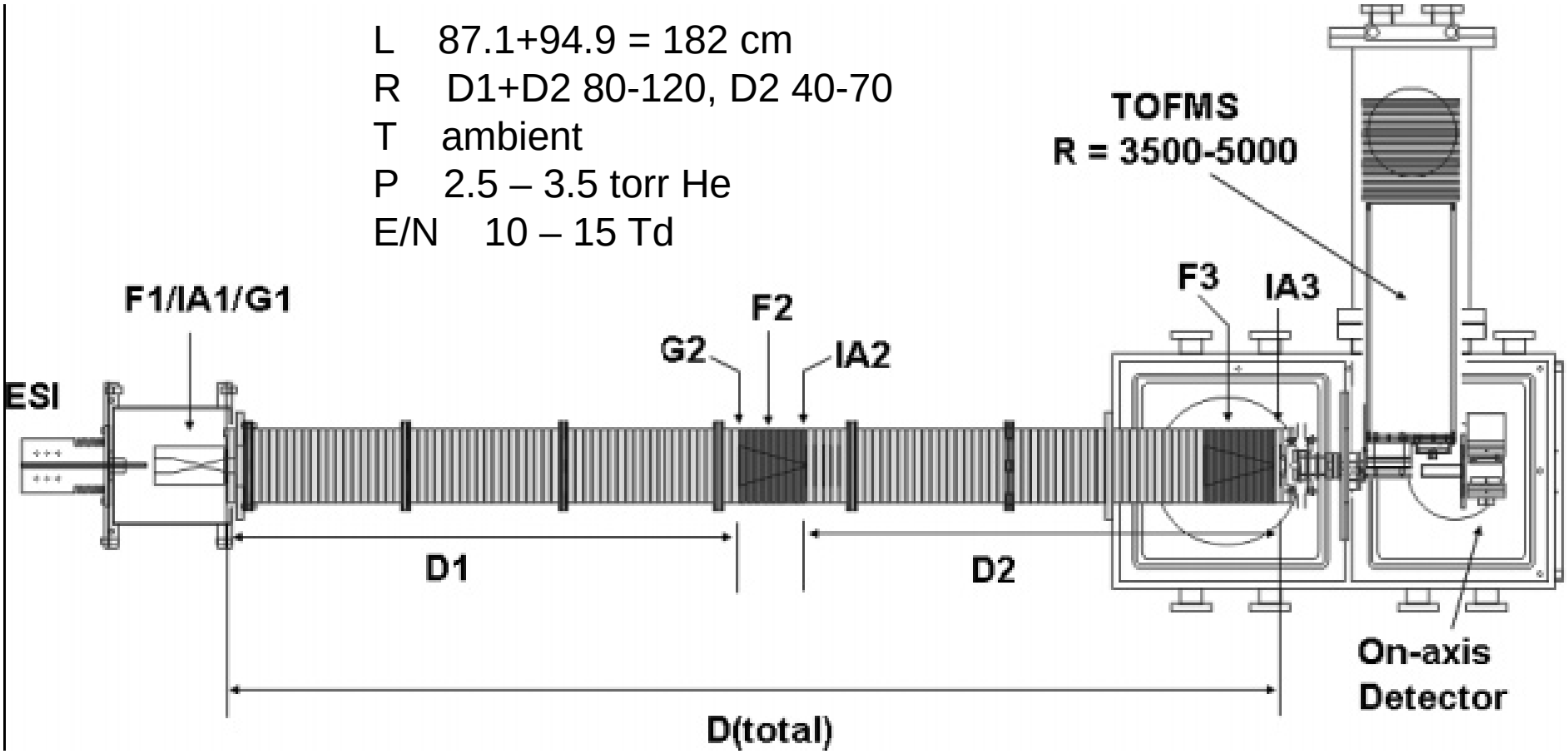
Hadamard Pulsing Scheme



Baker et. al, ASMS 2011, High-Throughput Proteomics Platform Demonstrated for Liver Disease Progression Biomarker Verification

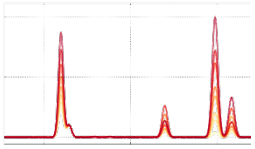
IMS-IMS-TOF

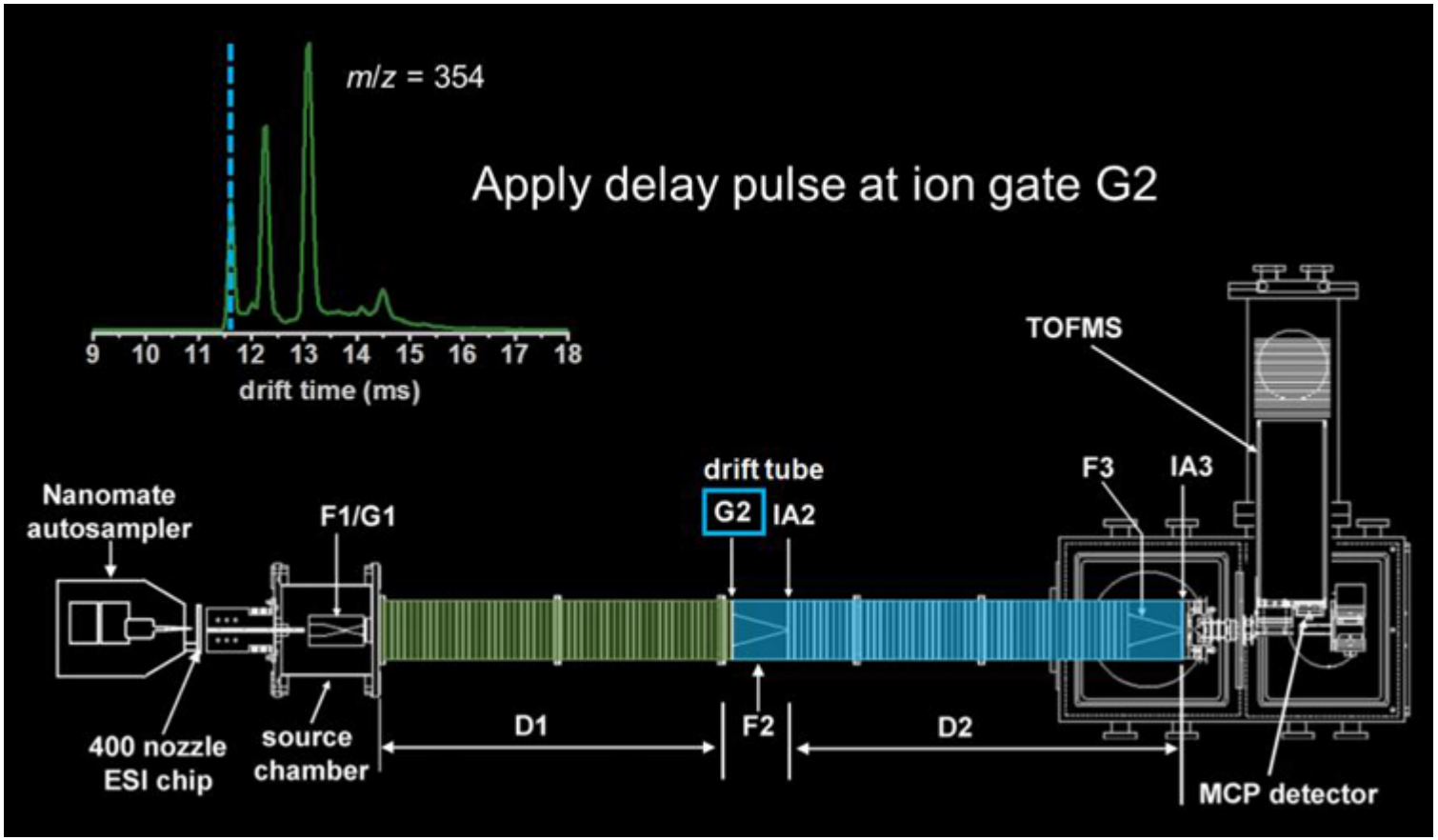
L 87.1+94.9 = 182 cm
R D1+D2 80-120, D2 40-70
T ambient
P 2.5 – 3.5 torr He
E/N 10 – 15 Td



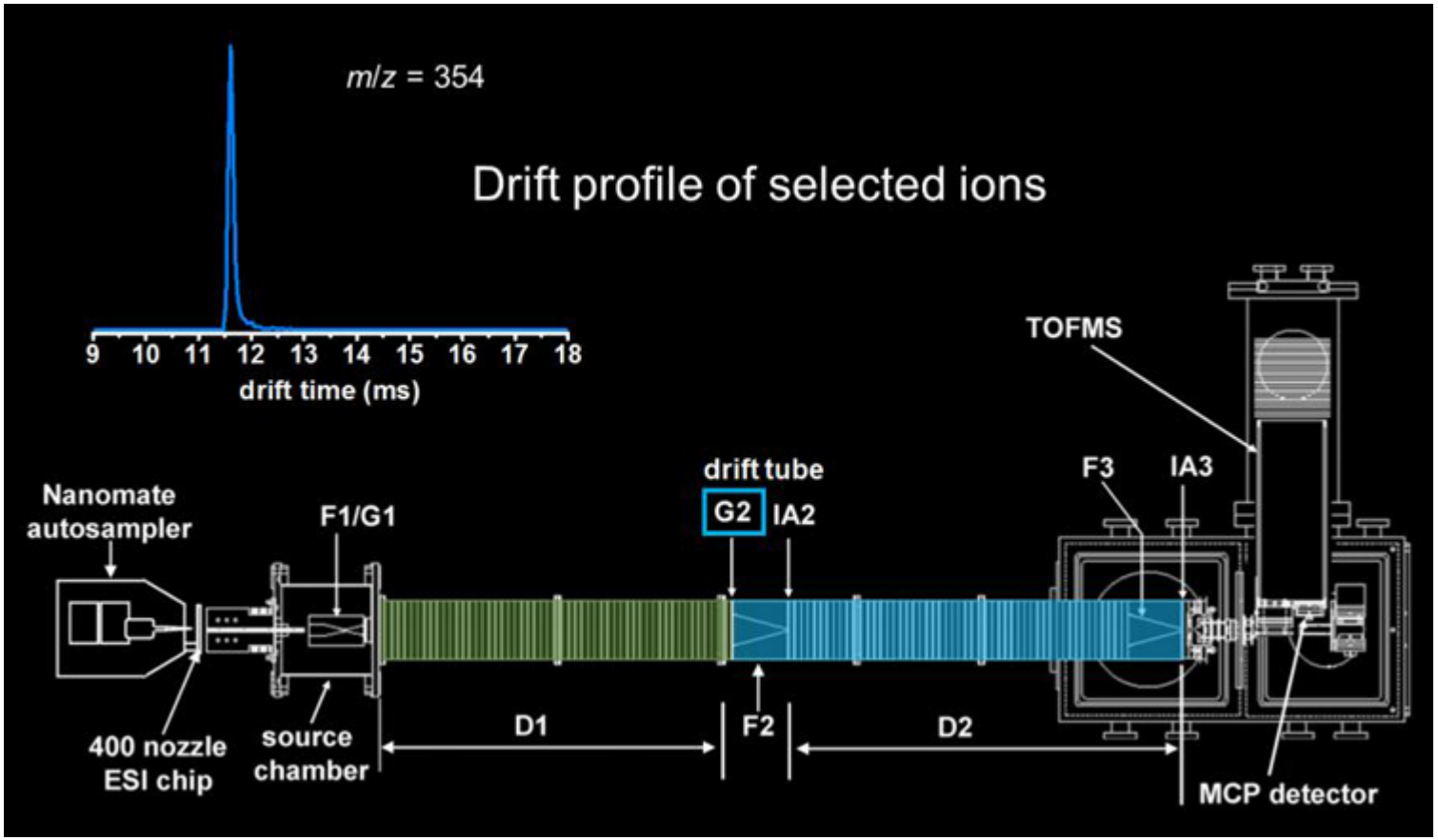
An IMS-IMS Analogue of MS-MS, S. L. Koeniger, S. I. Merenbloom, S. J. Valentine, M. F. Jarrold, H. R. Udseth, R. D. Smith, and D. E. Clemmer, Anal. Chem. 78 (2006) 4161-4174

IMS-IMS-TOF

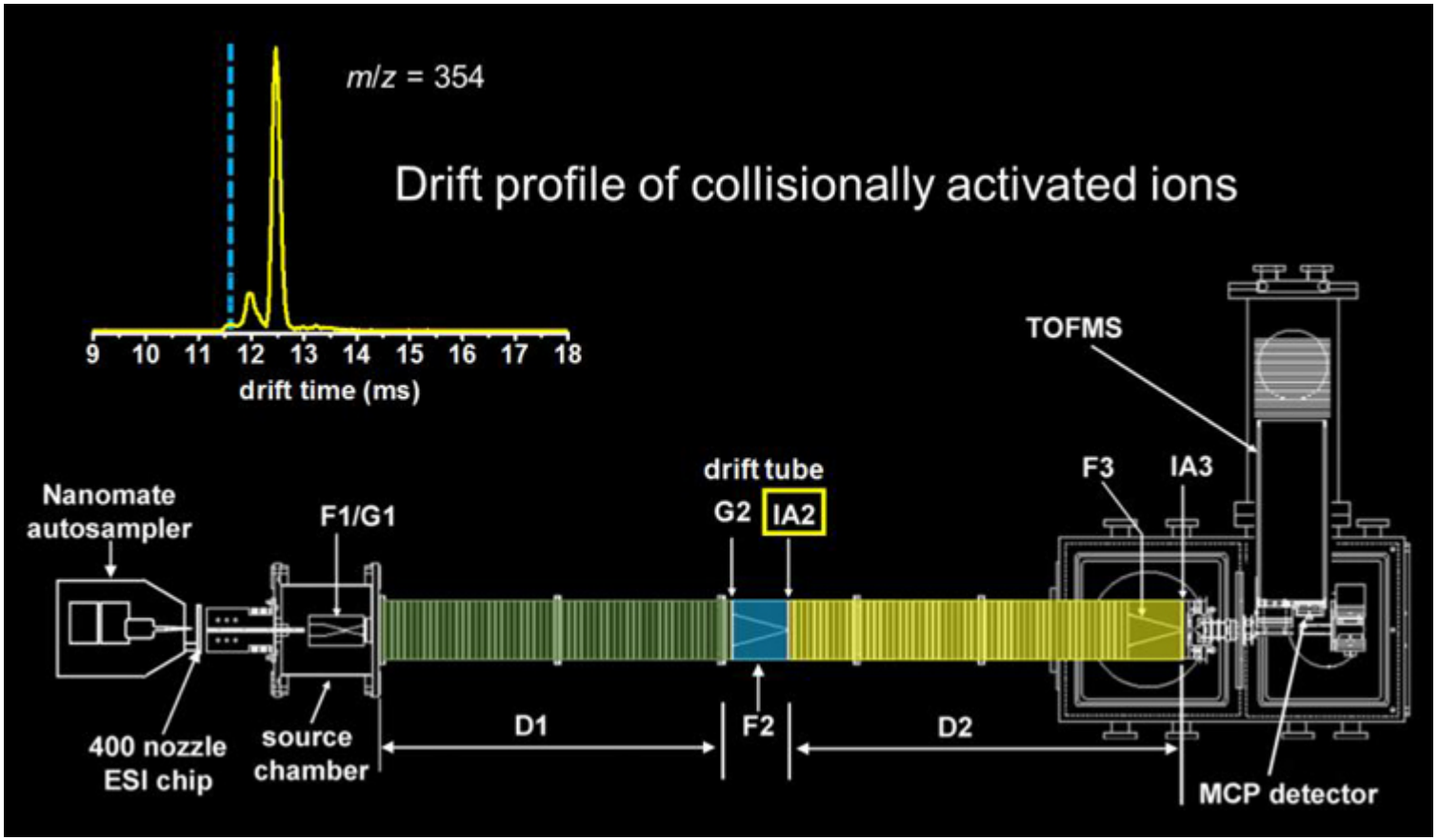



m/z = 354
Apply delay pulse at ion gate G2
9 10 11 12 13 14 15 16 17 18
drift time (ms)
TOFMS
drift tube
G2
IA2
F3
IA3
Nanomate autosampler
F1/G1
400 nozzle ESI chip
source chamber
D1
F2
D2
MCP detector

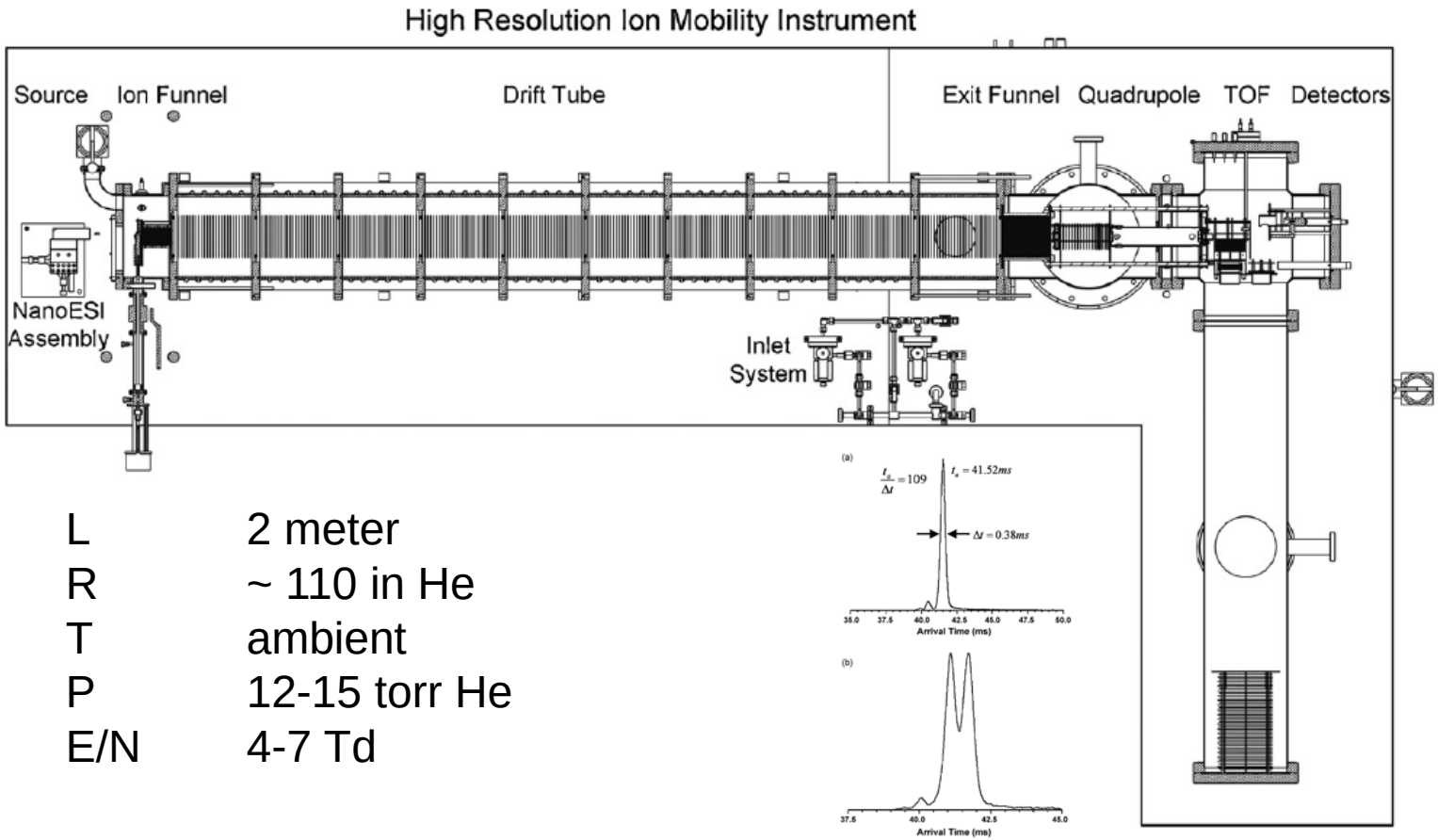
IMS-IMS-TOF


m/z = 354
Drift profile of selected ions
9 10 11 12 13 14 15 16 17 18
drift time (ms)
TOFMS
Nanomate autosampler
F1/G1
drift tube
G2
IA2
F3
IA3
400 nozzle ESI chip
source chamber
D1
F2
D2
MCP detector

IMS-IMS-TOF


m/z = 354
Drift profile of collisionally activated ions
9 10 11 12 13 14 15 16 17 18
drift time (ms)
TOFMS
Nanomate autosampler
F1/G1
drift tube
G2
IA2
F3
IA3
400 nozzle ESI chip
source chamber
D1
F2
D2
MCP detector

Two Meter IMS

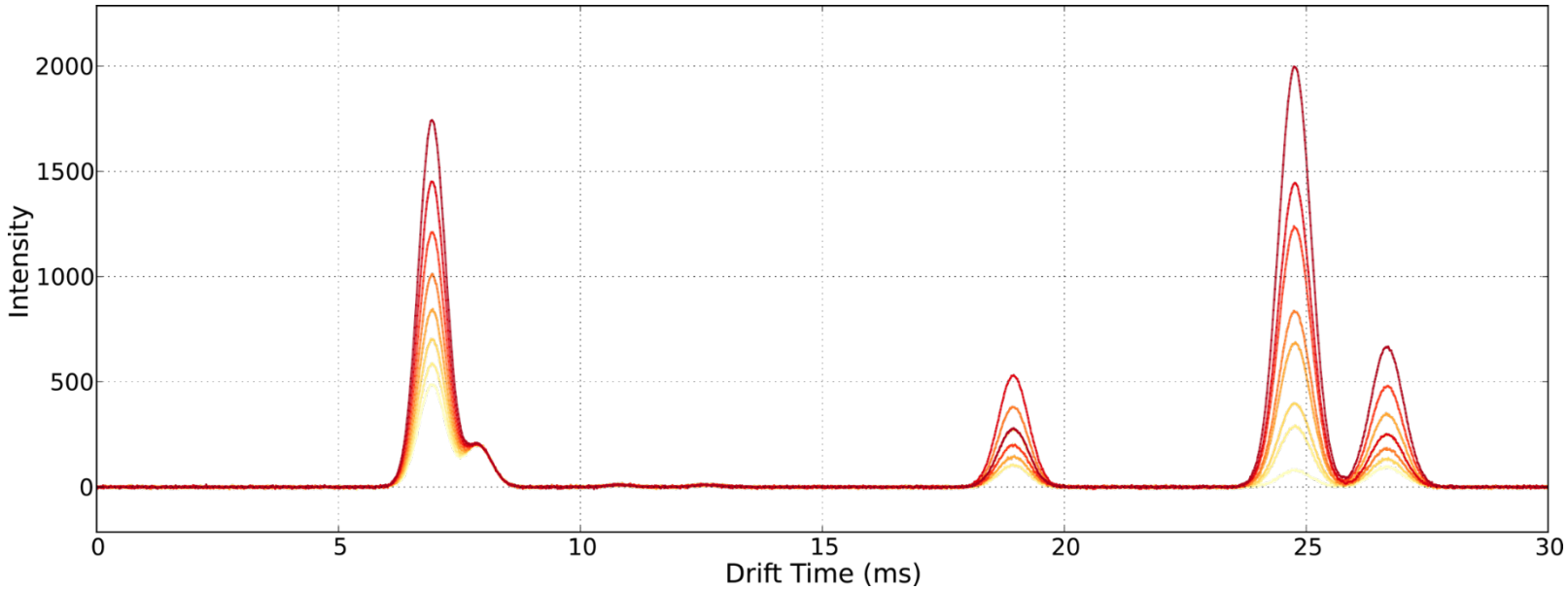


L	2 meter
R	~ 110 in He
T	ambient
P	12-15 torr He
E/N	4-7 Td

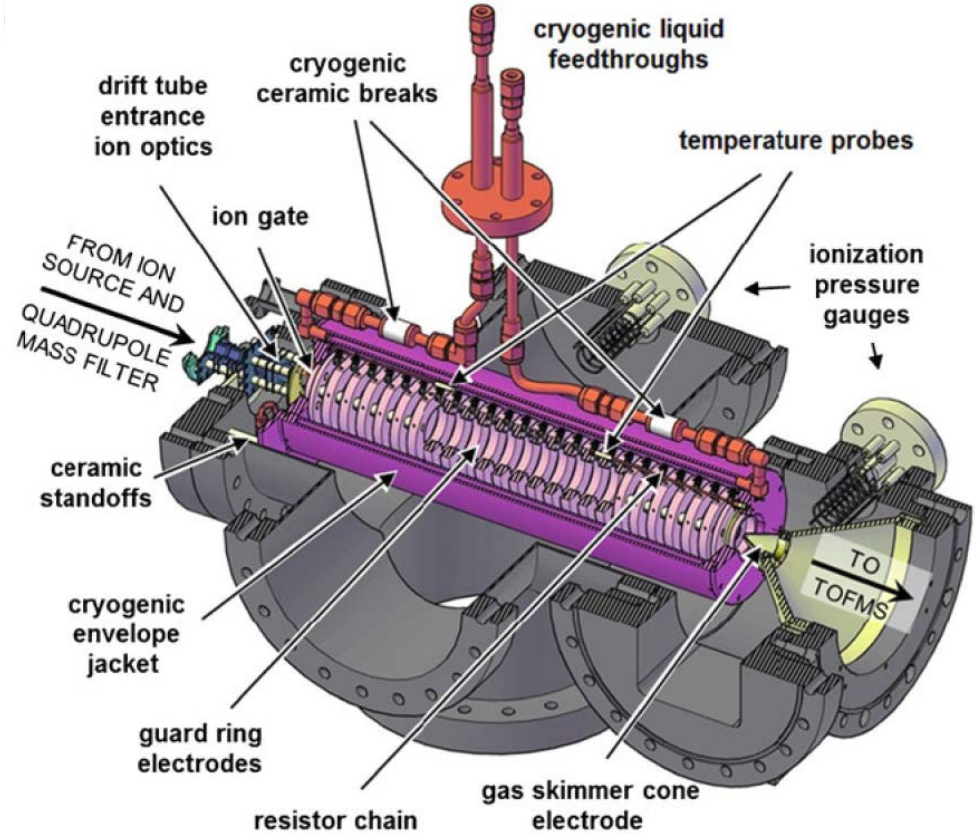
Fig. 1. Overview of the instrument. The length of the drift tube is 2.00 m. The drawing is to scale.

A new, higher resolution, ion mobility spectrometer, P. R. Kemper, N. F. Dupuis, M. T. Bowers, Int. J. Mass Spectrom. 287 (2009) 46-57.

ALTERNATIVE AND HYBRID INSTRUMENTATION



Variable Temperature System



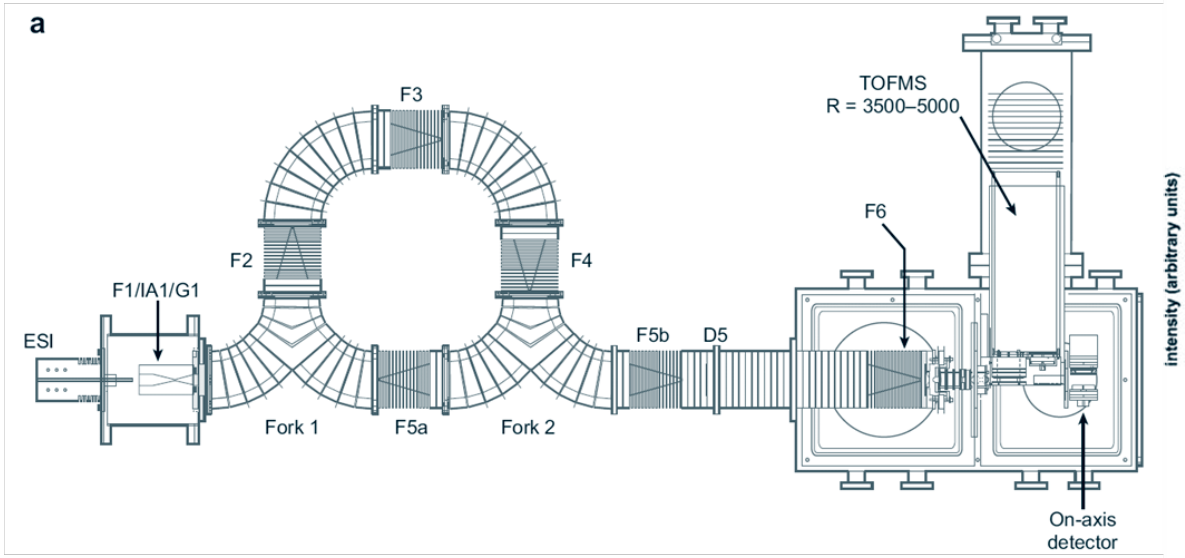
J. Am. Soc. Mass Spectrom. (2011) 22:1134–1145
DOI: 10.1007/s13361-011-0148-2

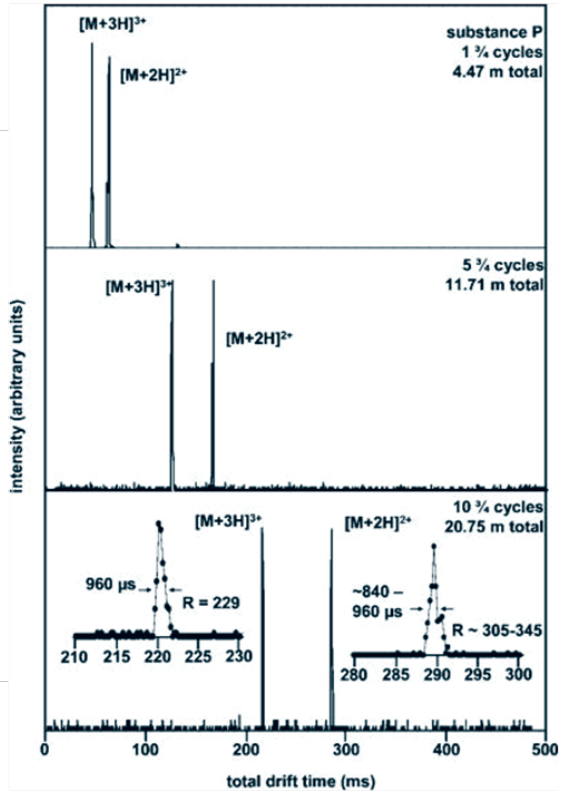
Fundamental Ion Studies

- Kinetics
- Clustering Equilibria

Cyclotrons

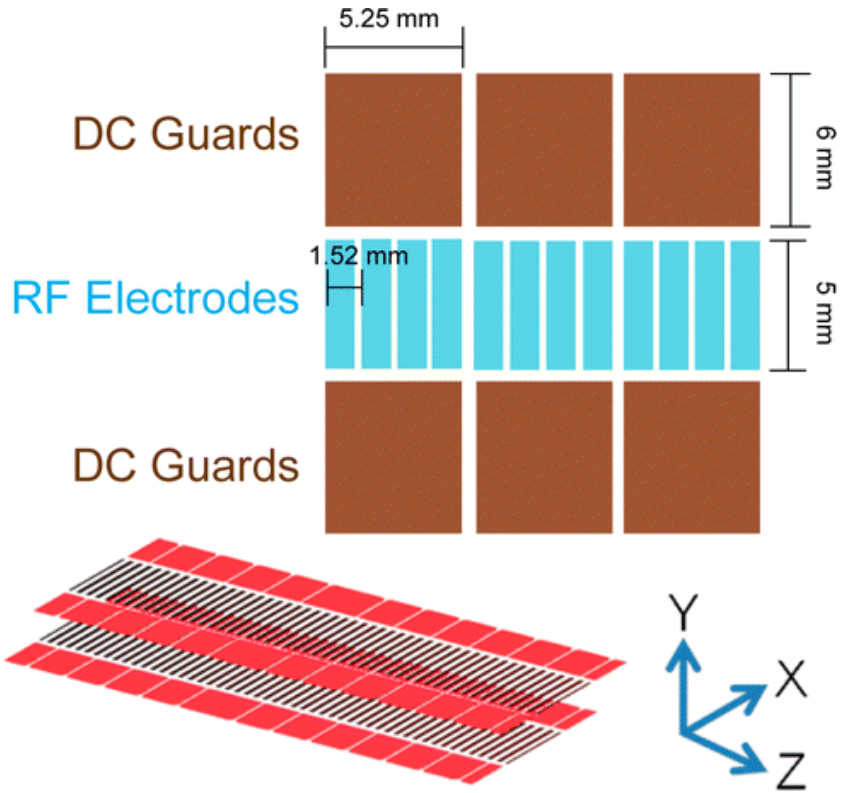
- **Mobility filtering device**
- **Filtering resolving power of ~300**

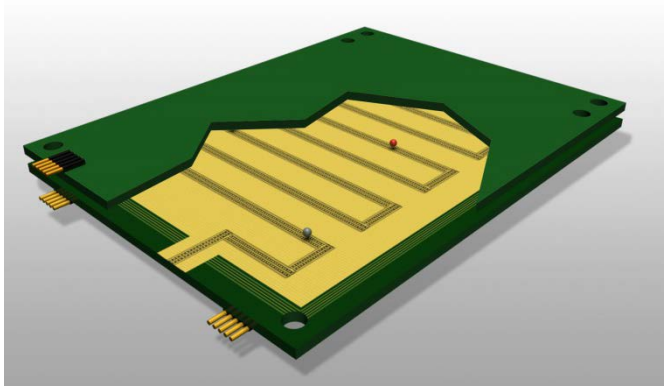




High-Resolution Ion Cyclotron Mobility Spectrometry, S. I. Merenbloom, R. S. Glaskin, Z. B. Henson, and D. E. Clemmer, Anal. Chem. 81 (2009) 1482-1487.

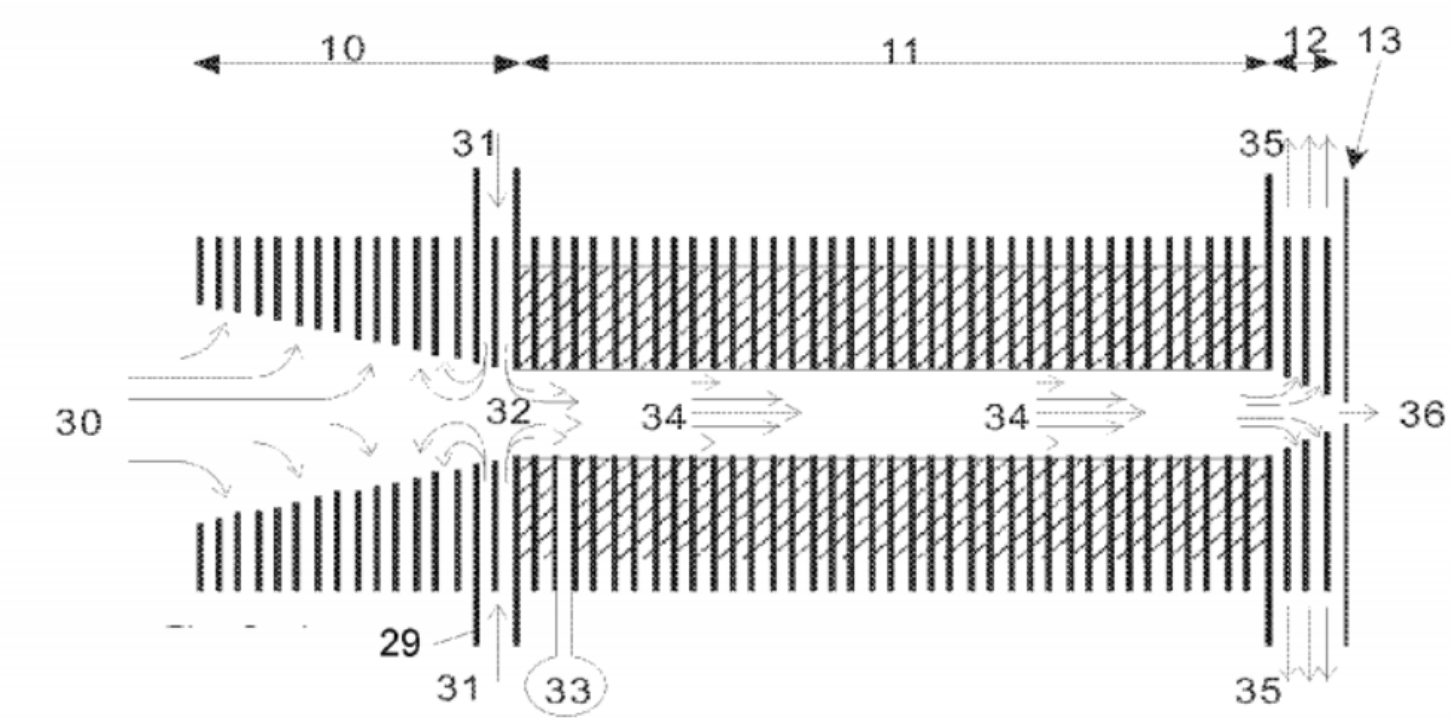
Printed Circuit Boards





Experimental Evaluation and Optimization of Structures for Lossless Ion Manipulations for Ion Mobility Spectrometry with Time-of-Flight Mass Spectrometry, I. K. Webb , S. V. B. Garimella, A. V. Tolmachev , T-C. Chen , X. Zhang, R. V. Norheim, S. A. Prost , B. LaMarche, G. A. Anderson, Y. M. Ibrahim, and R. D. Smith, Anal. Chem. 86 (2014) 9169-9176.

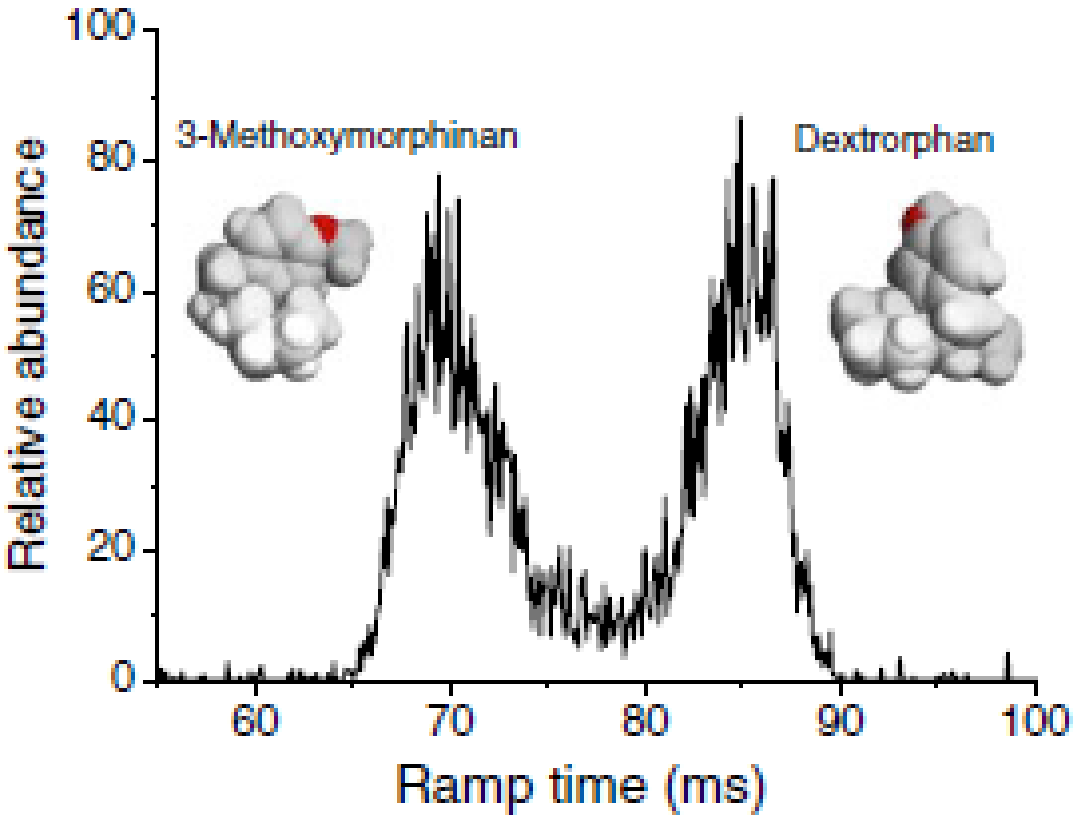
Trapped Ion Mobility Spectrometry



- Currently under development -- Bruker

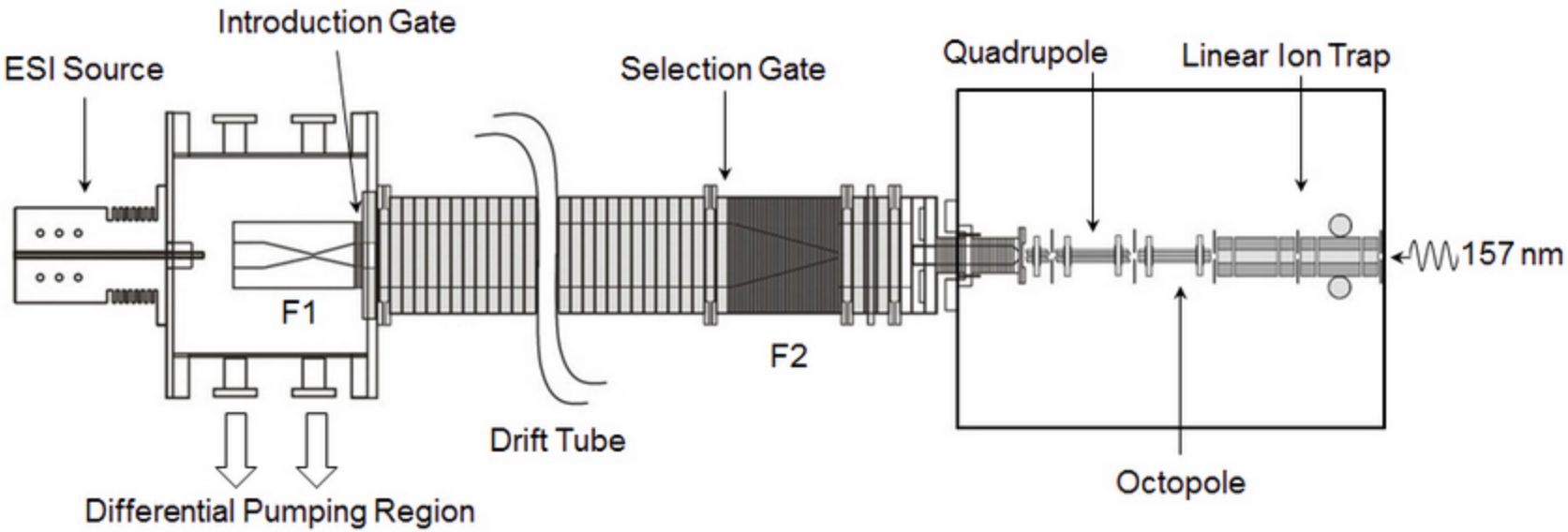
US 20120273670A1

Trapped Ion Mobility Spectrometry



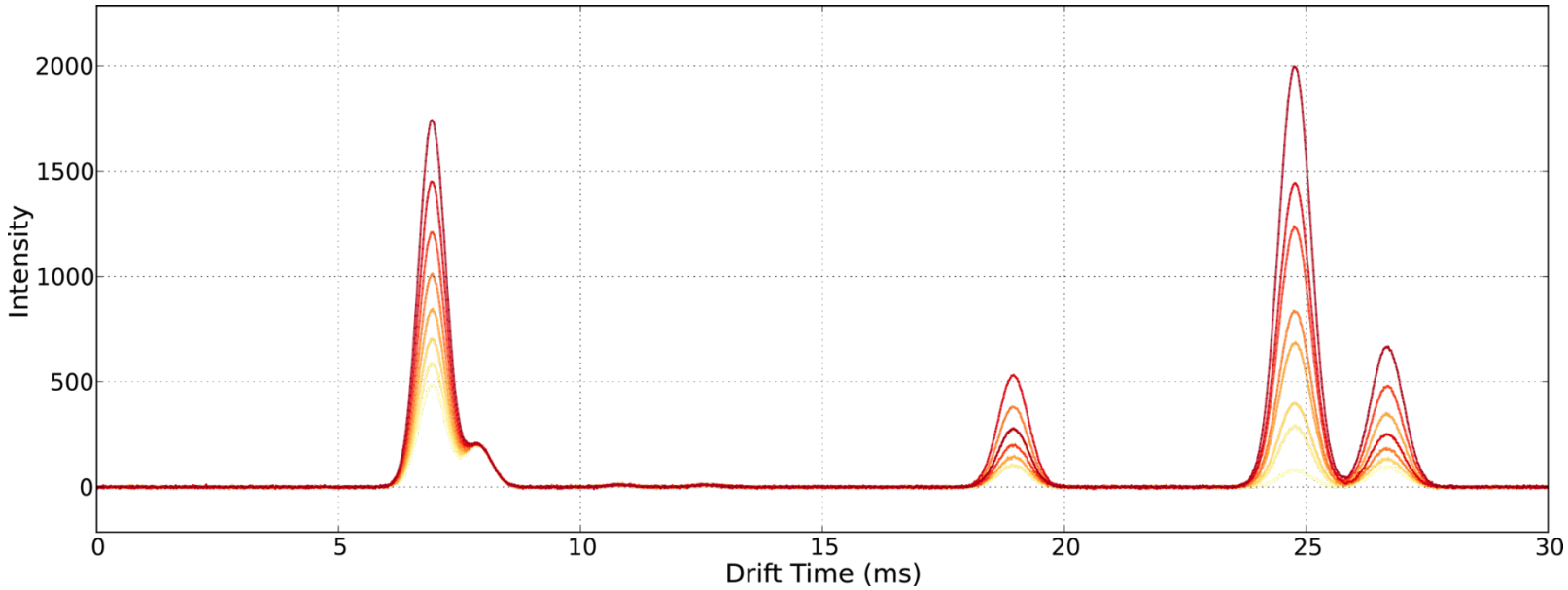
Int. J. Ion Mobil. Spec. (2011) 14:93-98

Photofragmentation

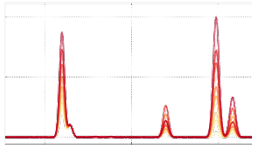


Zucker, S. M.; Lee, S.; Webber, N.; Valentine, S. J.; Reilly, J. P. and Clemmer, D. E. An Ion Mobility/Ion Trap/Photodissociation Instrument for Characterization of Ion Structure. *J. Am. Soc. Mass Spectrom.*, **2011**, *22*, 1477–1485.

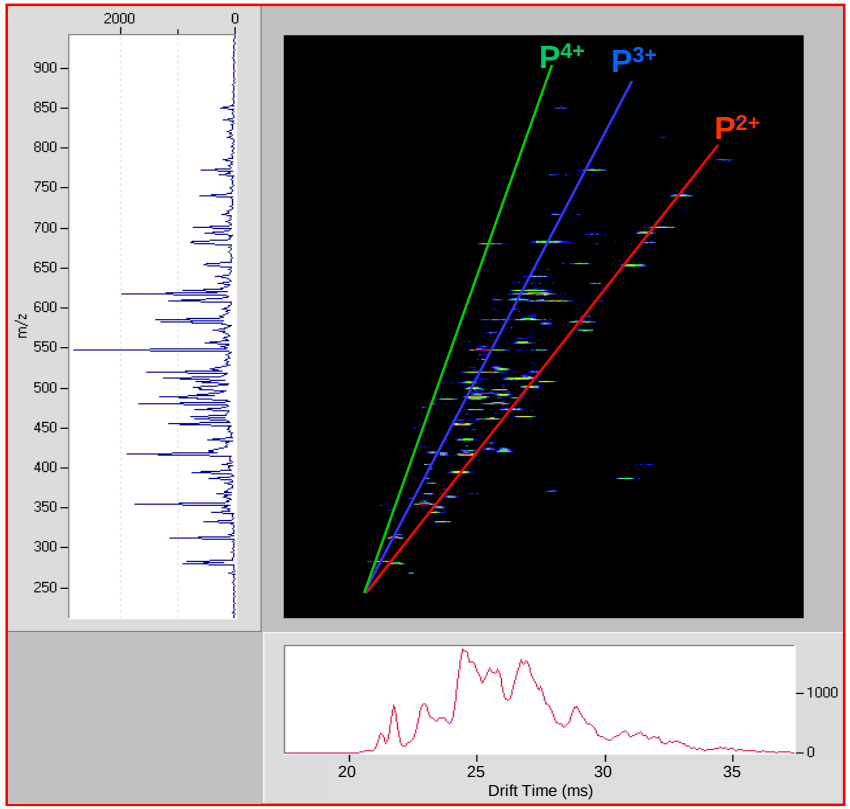
BENEFITS OF
IMS DRIFT TIME SEPARATION



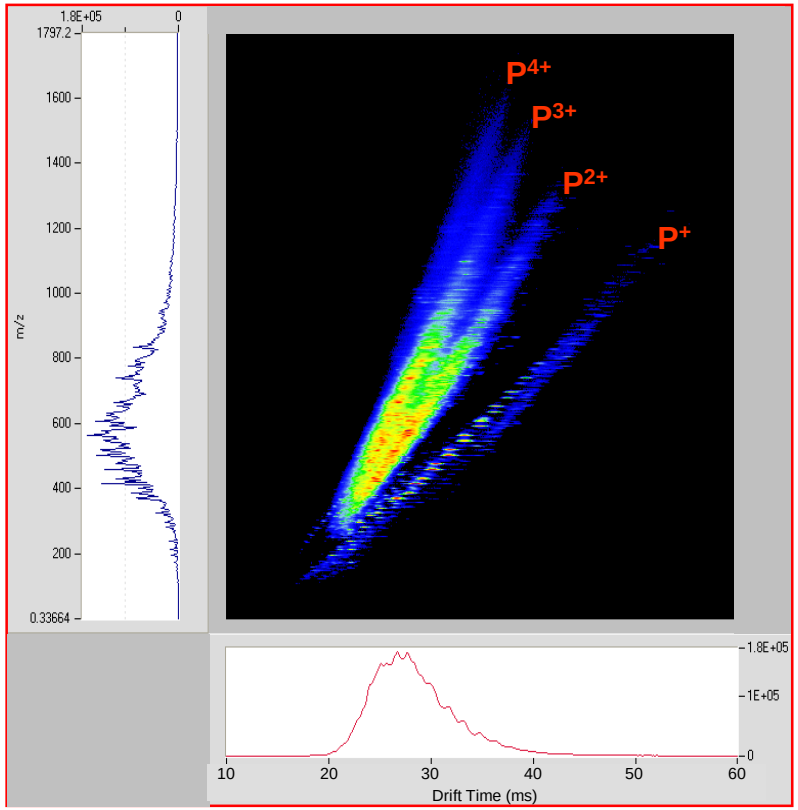
Benefits of IMS drift time separation



1. Separates different charge states, isomers and classes of compounds

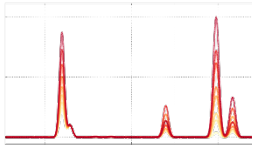


BSA tryptic digest (25 µg/mL)
(5 sec acquisition)

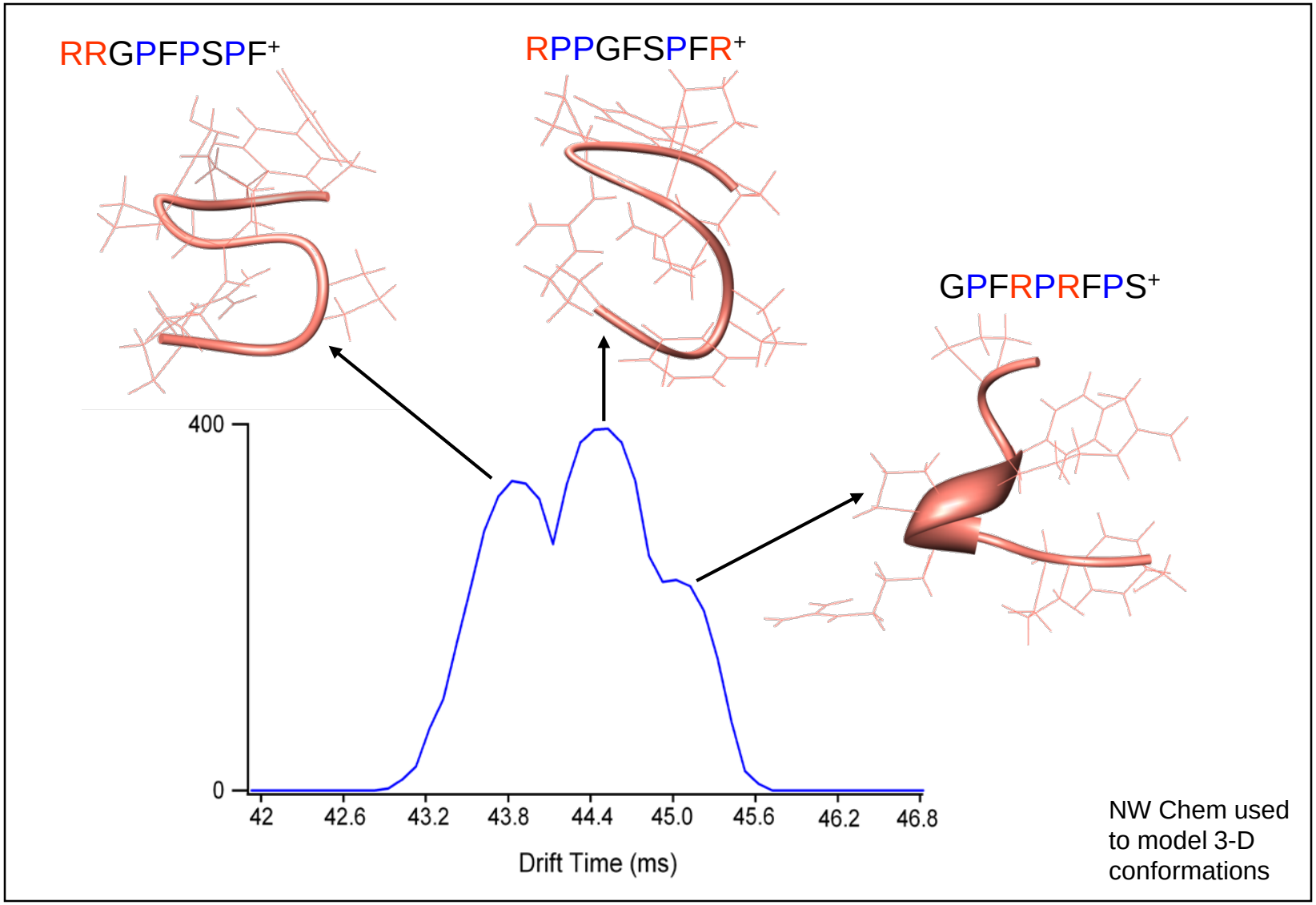


Human Plasma tryptic digest (0.5 mg/mL)
(summed LC run for 50 minutes)

Benefits of IMS drift time separation

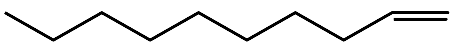


1. Separates different charge states, isomers and classes of compounds

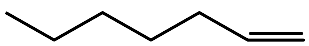


Benefits of IMS drift time separation

1. Separates different charge states, isomers and classes of compounds

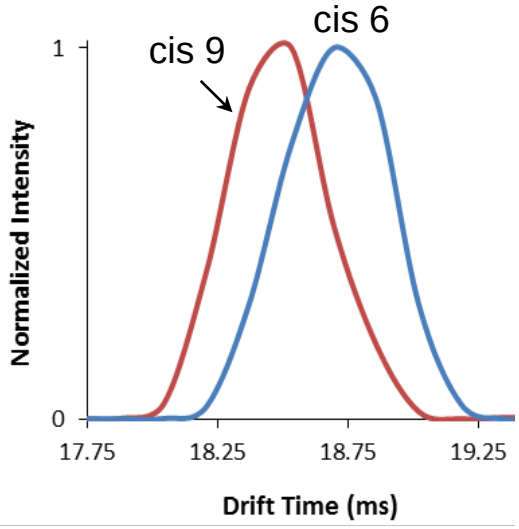


cis 9



cis 6

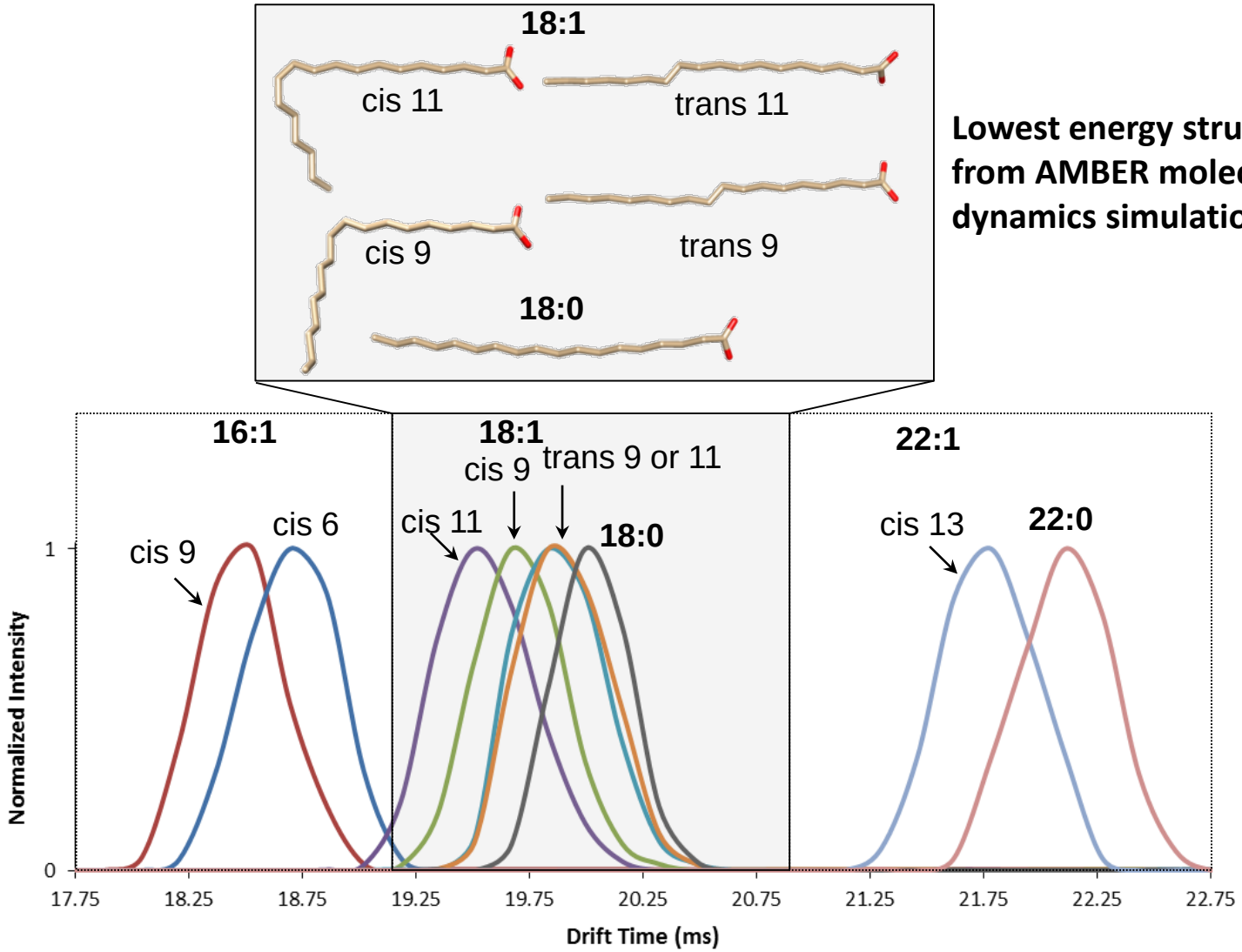
16:1



Fatty acid analysis with IMS

Benefits of IMS drift time separation

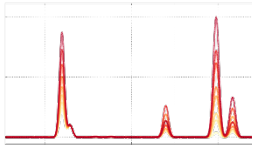
1. Separates different charge states, isomers and classes of compounds



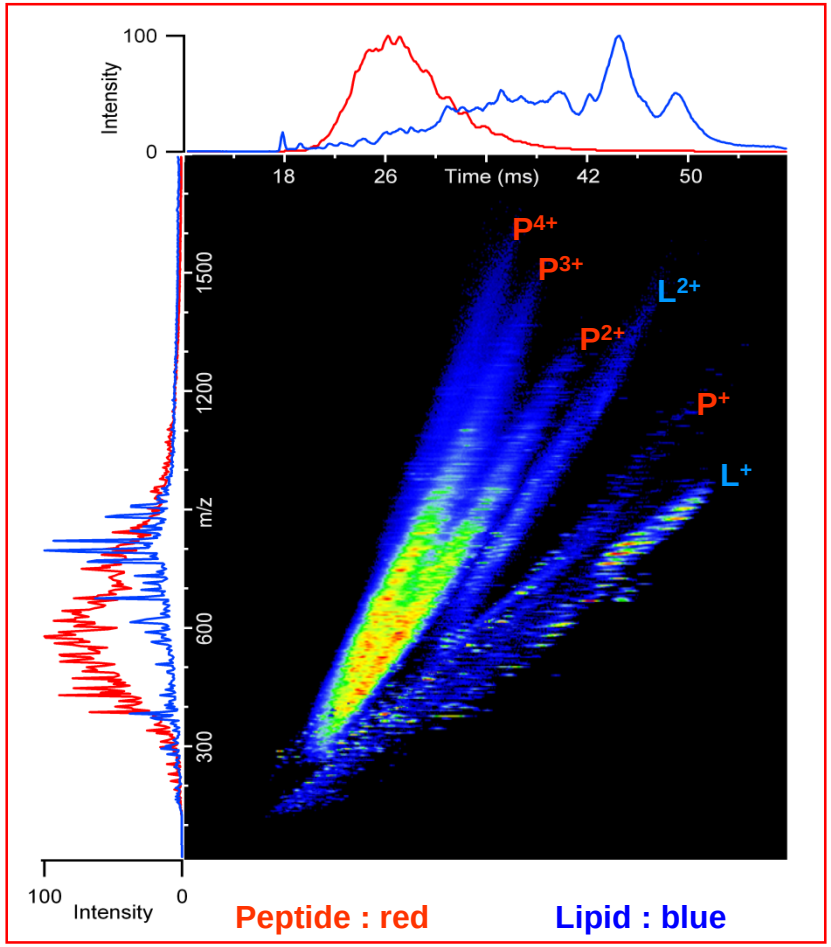
Lowest energy structures from AMBER molecular dynamics simulations

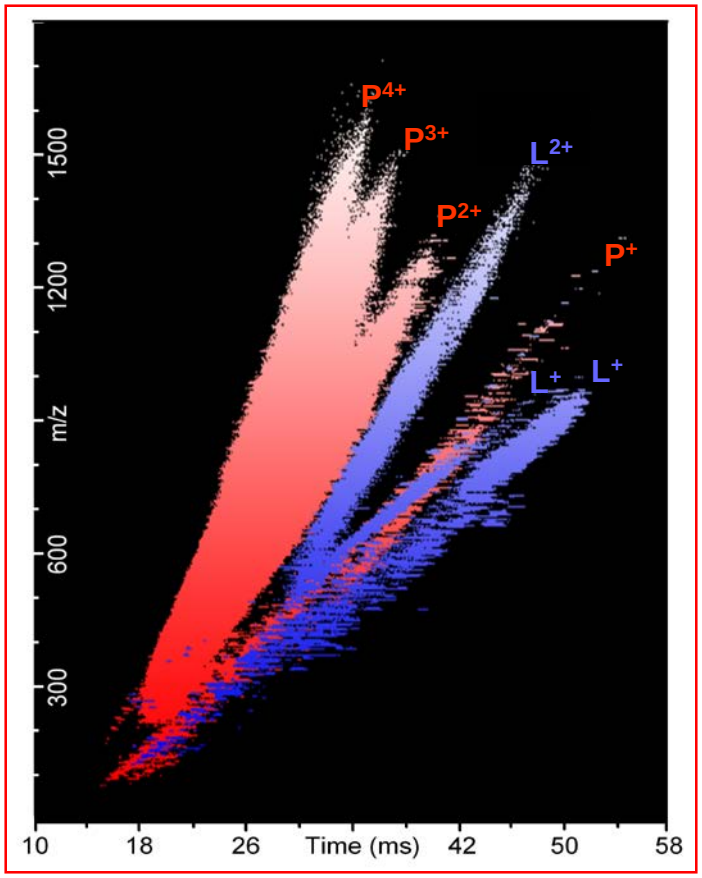
Fatty acid analysis with IMS

Benefits of IMS drift time separation



1. Separates different charge states, isomers and classes of compounds

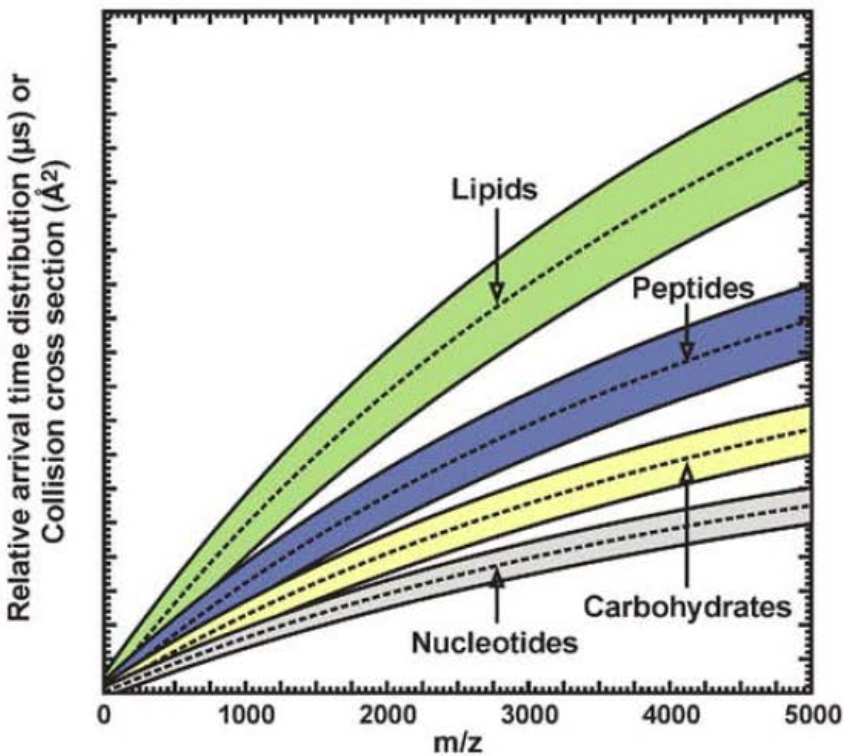


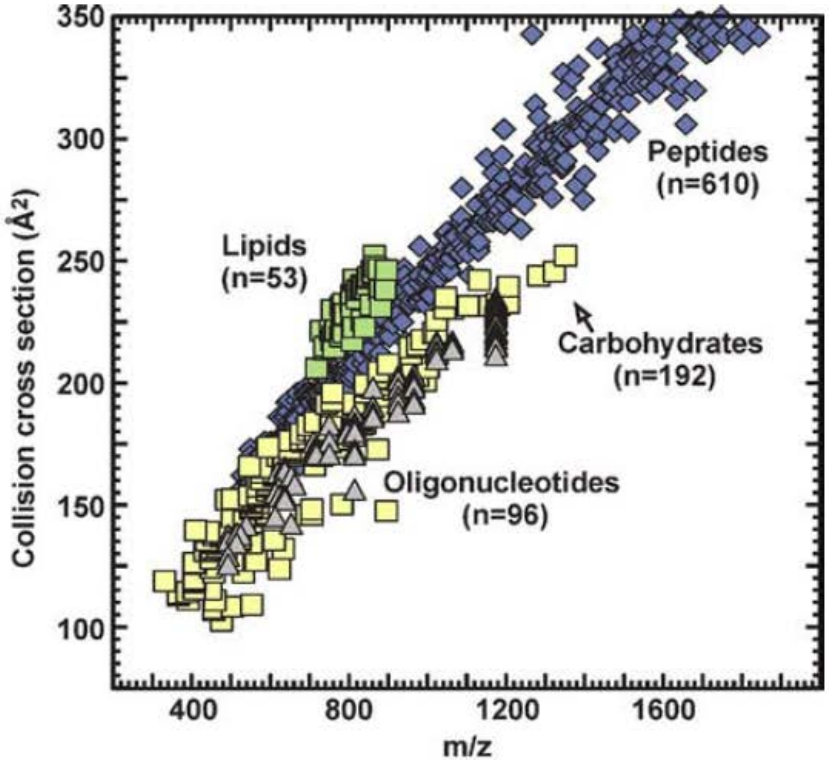


Peptides and lipids are easily distinguished

Benefits of IMS drift time separation

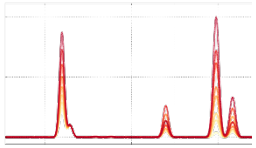
1. Separates different charge states, isomers and classes of compounds



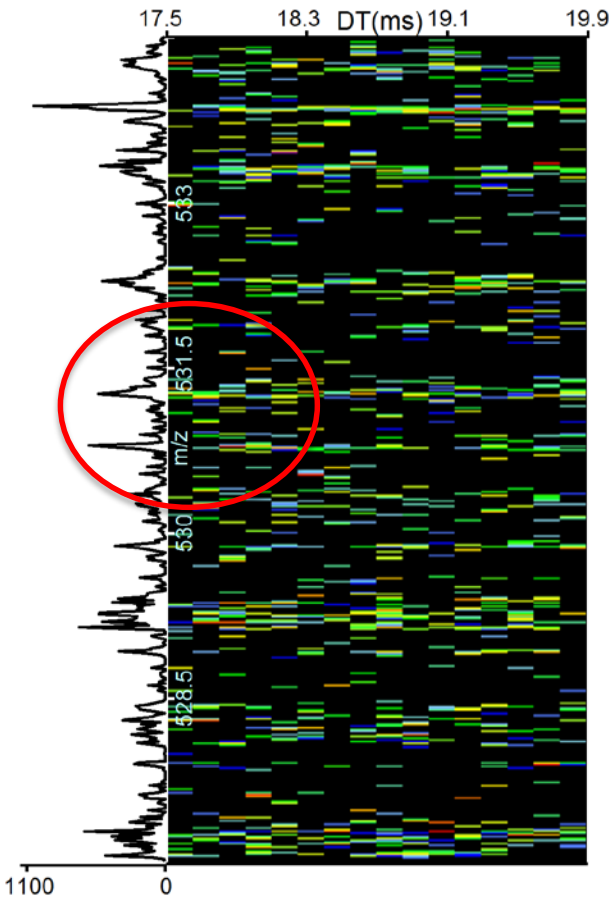


J Am Soc Mass Spectrom 2009, 20, 1775–1781

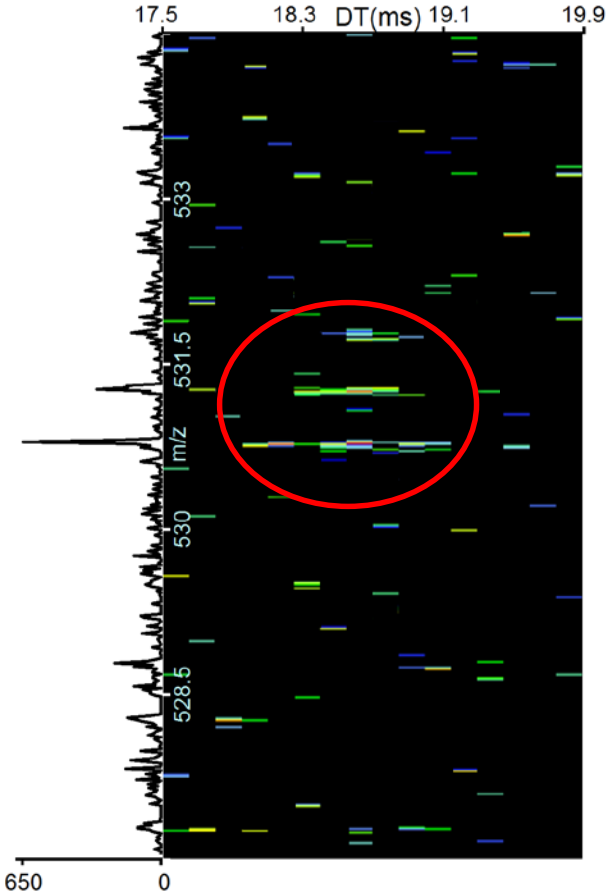
Benefits of IMS drift time separation



2. Improved Sensitivity & Increase Feature Detection & Confidence

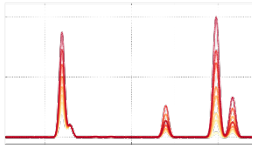


QTOF MS of Bradykinin (100 pM)

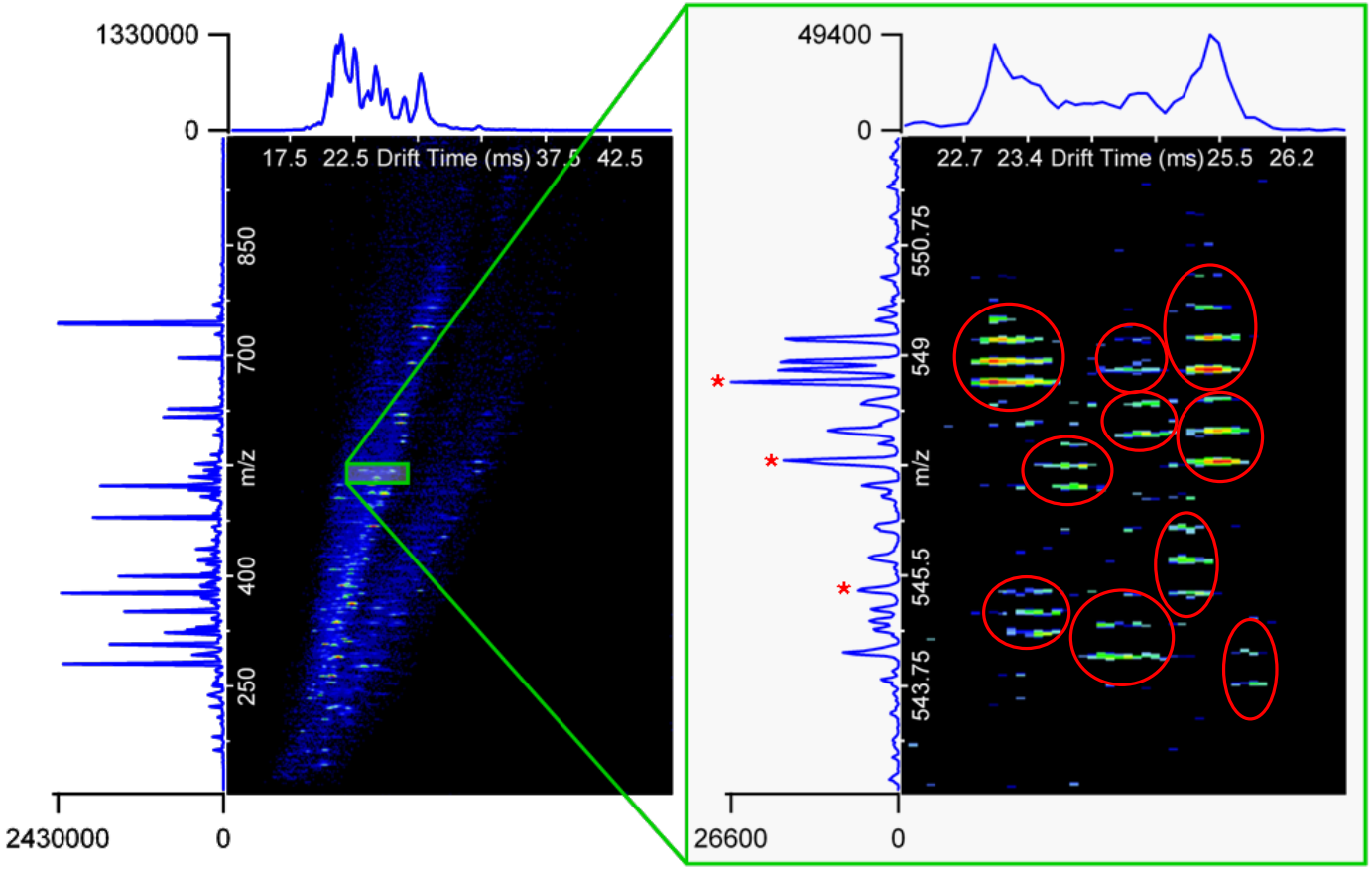


IMS-QTOF MS of Bradykinin (100 pM)

Benefits of IMS drift time separation



2. Improved Sensitivity & Increase Feature Detection & Confidence



Only 3 features discerned without drift time dimension (*)

Benefits of IMS drift time separation

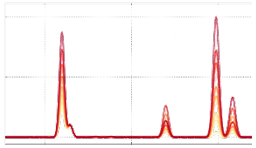
2. Improved Sensitivity & Increase Feature Detection & Confidence

Spiking Level	Non-Serum Peptide	60-min LC-IMS-TOF MS	60-min LC-TOF MS	100-min LC-Velos-Orbitrap
100 pg/mL	Melittin	ND	ND	ND
100 pg/mL	Dynorphin A Porcine Fragment 1-13	✓	ND	ND
1 ng/mL	Des Pro Ala Bradykinin	✓	ND	ND
1 ng/mL	Leucine Enkephalin	✓	ND	ND
10 ng/mL	3X FLAG Peptide	✓	✓	ND
10 ng/mL	Substance P	✓	✓	✓
100 ng/mL	Methionine Enkephalin	✓	✓	✓
100 ng/mL	[Ala92]-Peptide 6	✓	✓	✓

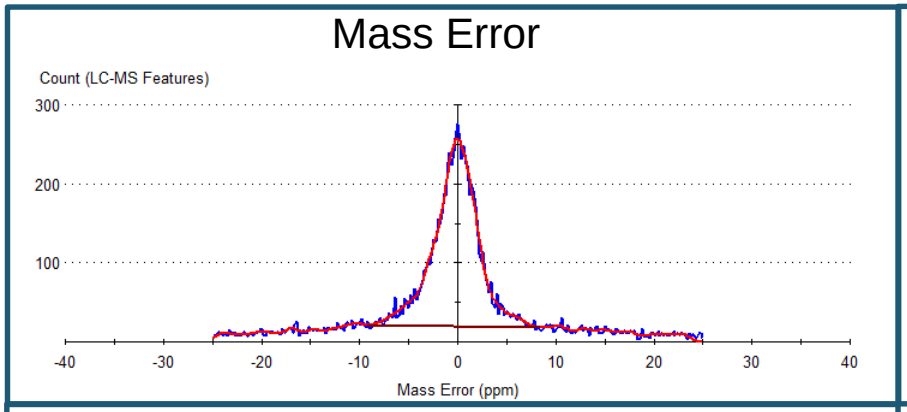
8 peptides spiked in human serum

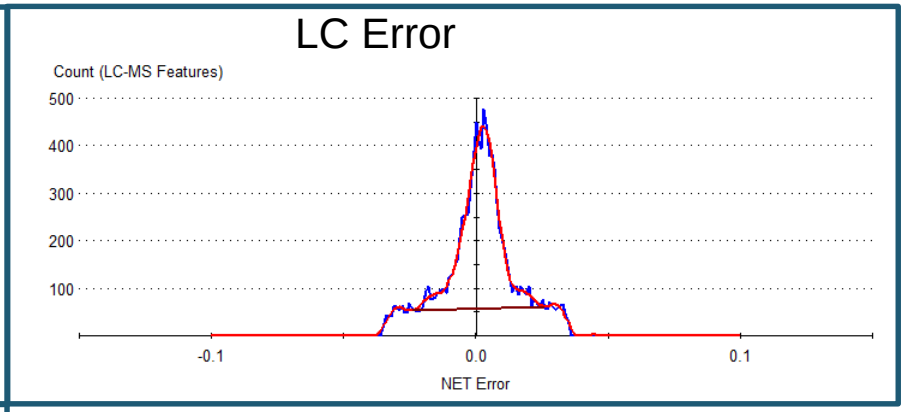
Sample analyzed using Velos-Orbitrap, TOF MS and IMS-TOF MS instruments

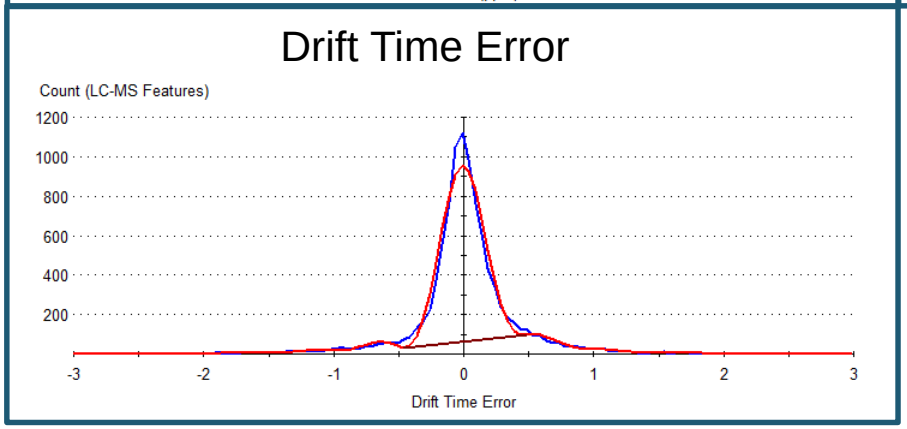
Benefits of IMS drift time separation



3. Utilizing IMS in databases reduces FDR due to extra dimension







Human Serum Peptide Analysis

60-min LC-IMS-MS

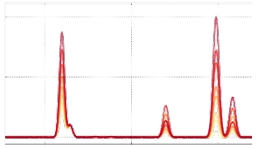
40519 Features with FDR <5%

If you only use LC and MS

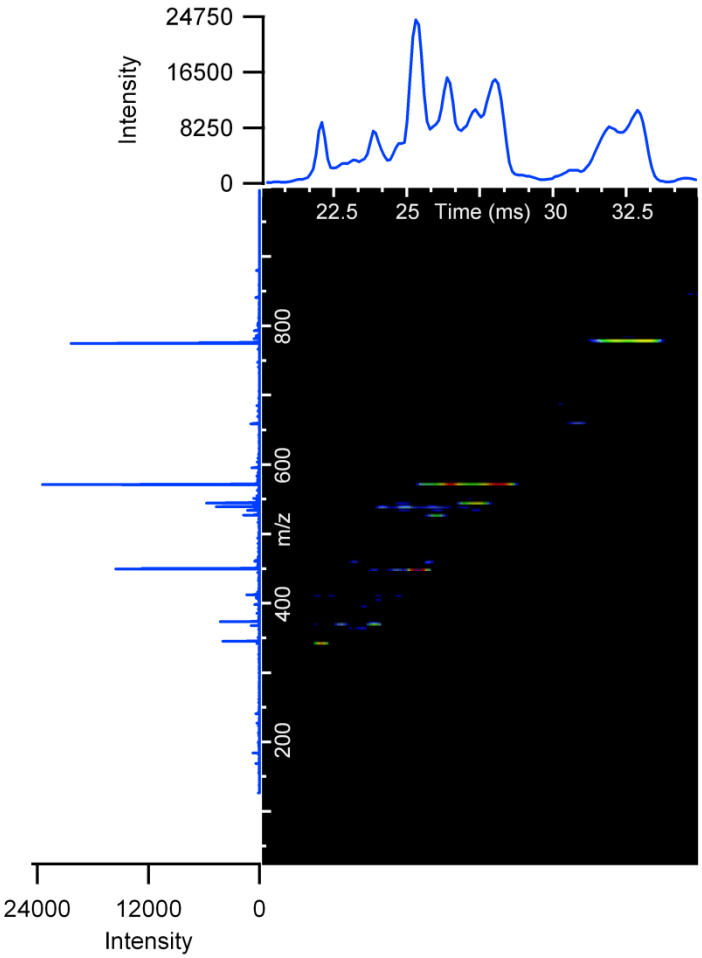
29007 Features with FDR <5%

Extra dimension adds confidence to LC-IMS-MS Features Matches to AMT Tag DB

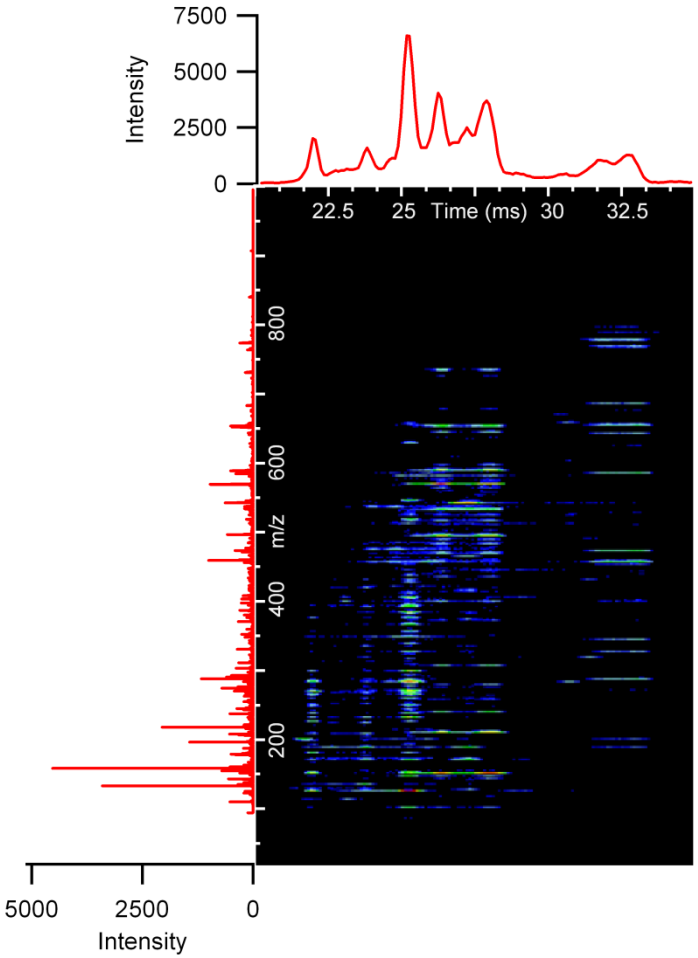
Benefits of IMS drift time separation



4. IMS-(CID)-MS Multiplexed Fragmentation



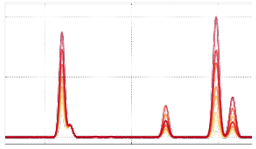
Parent Spectrum



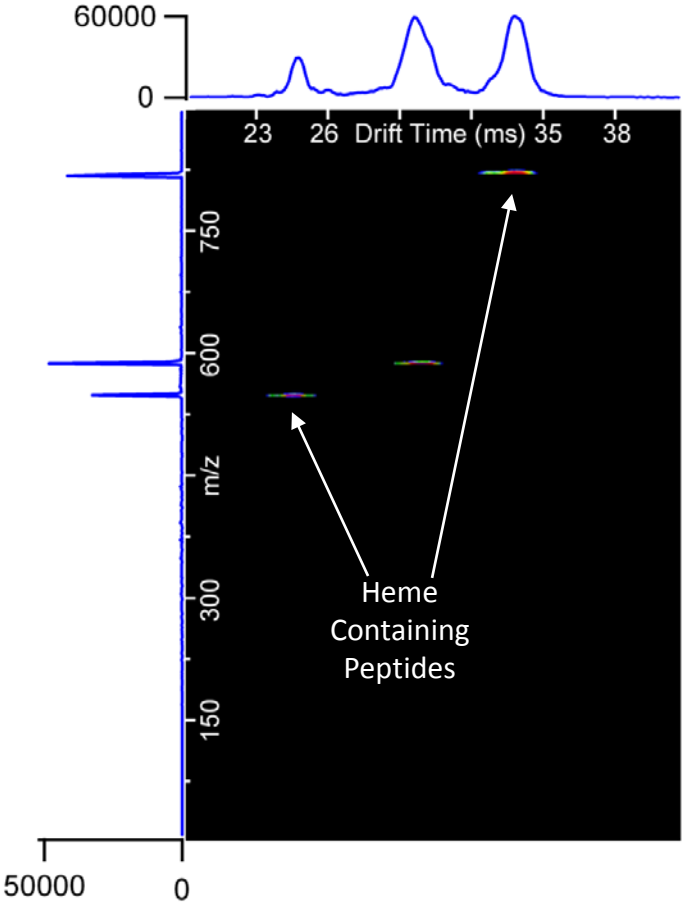
Fragmentation Spectrum

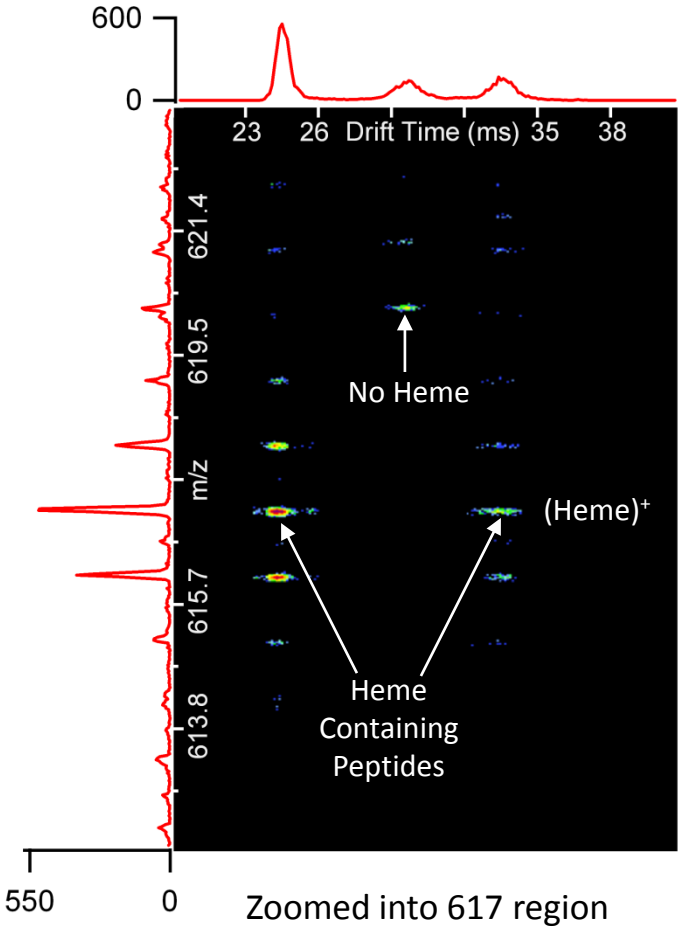
Fragments have the same drift time as precursors

Benefits of IMS drift time separation



4. IMS-(CID)-MS Multiplexed Fragmentation





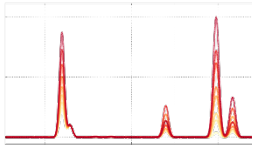
Zoomed into 617 region

Parent Spectrum (3 Peptides Observed in 1 sec LC acquisition)
2 hemepeptides identified

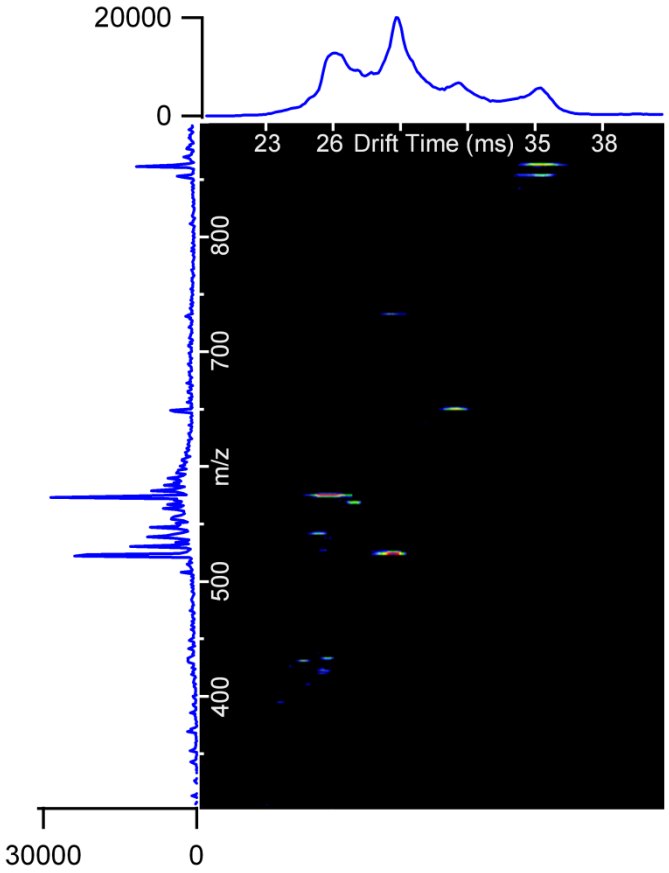
Fragmentation Spectrum

Find multiple hemepeptides simultaneously by looking for heme$^+$ ion at 617

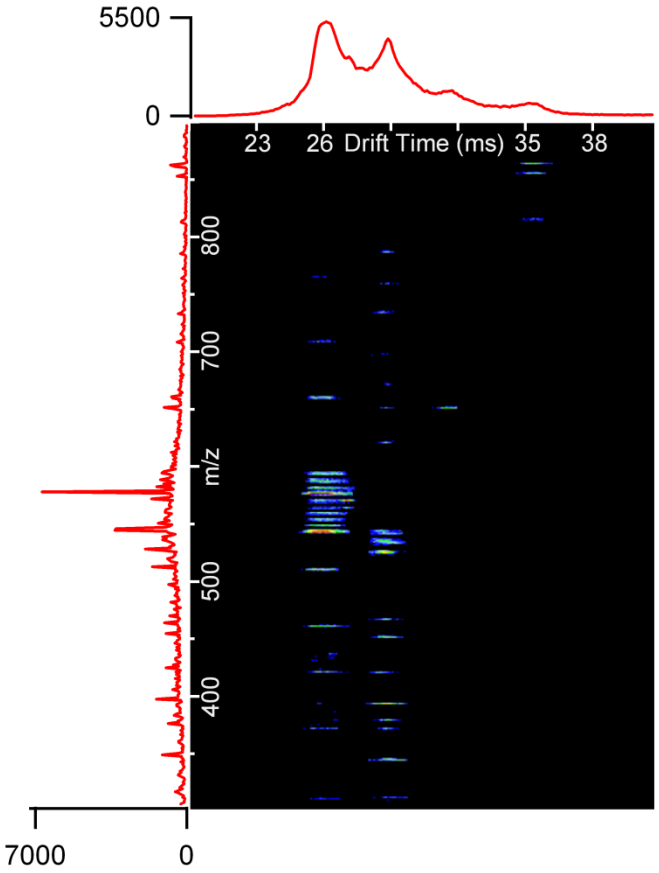
Benefits of IMS drift time separation



4. IMS-(CID)-MS Multiplexed Fragmentation



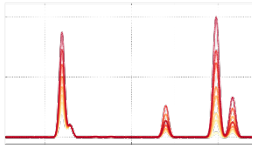
Parent Spectrum (11 Peptides Observed in 1 sec LC acquisition)



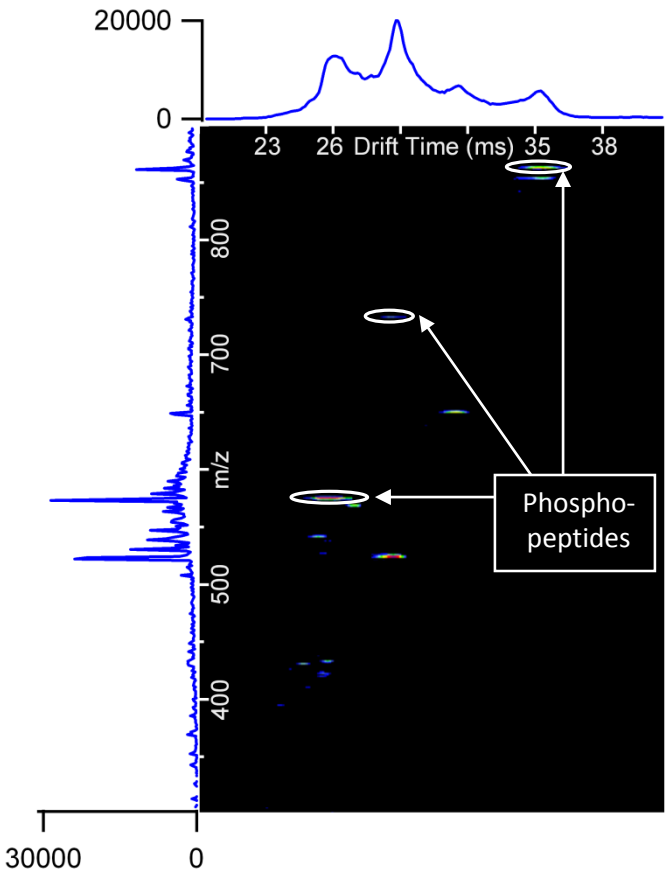
Fragmentation Spectrum

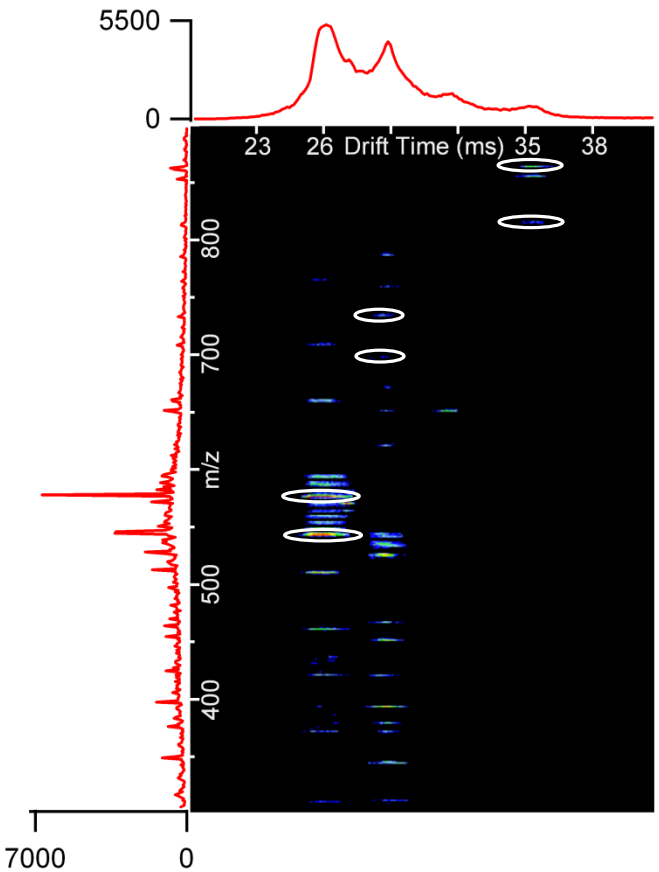
Find multiple phosphopeptides by looking for PO_3 or PO_4 mass difference

Benefits of IMS drift time separation



4. IMS-(CID)-MS Multiplexed Fragmentation



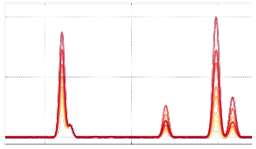


Parent Spectrum (11 Peptides Observed in 1 sec LC acquisition)
3 Phosphopeptides Identified

Fragmentation Spectrum

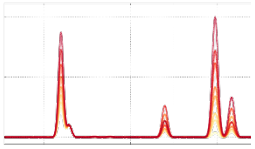
Find multiple phosphopeptides by looking for PO_3 or PO_4 mass difference

The Complementary Union of IMS and MS



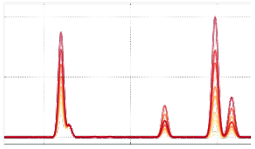
- Reduces chemical and electronic noise, increasing the signal to noise ratio
  - Aids in deisotoping and charge state identification
- Separates based on CCS/charge ratio rather than mass/charge ratio, providing a second dimension of separation for a mass spectrometer
- Separate isomers and conformers
- Provides structural information when compared with molecular models

The Complementary Union of IMS and MS

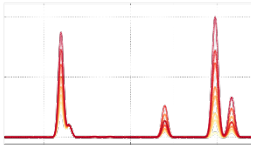


- Rapid separations prior to MS (10s of ms)
- Higher resolving powers than approach or exceed those from gas or liquid chromatography
  - Increases peak capacity
- Separation selectivity can be adjusted by altering drift gas
- Rapid isomeric separations are possible when chiral modifiers are used as drift gases
- Compatible with all MS based ionization sources
  - ESI, SESI, MALDI, ^{63}Ni, Corona, Photoionization, etc.

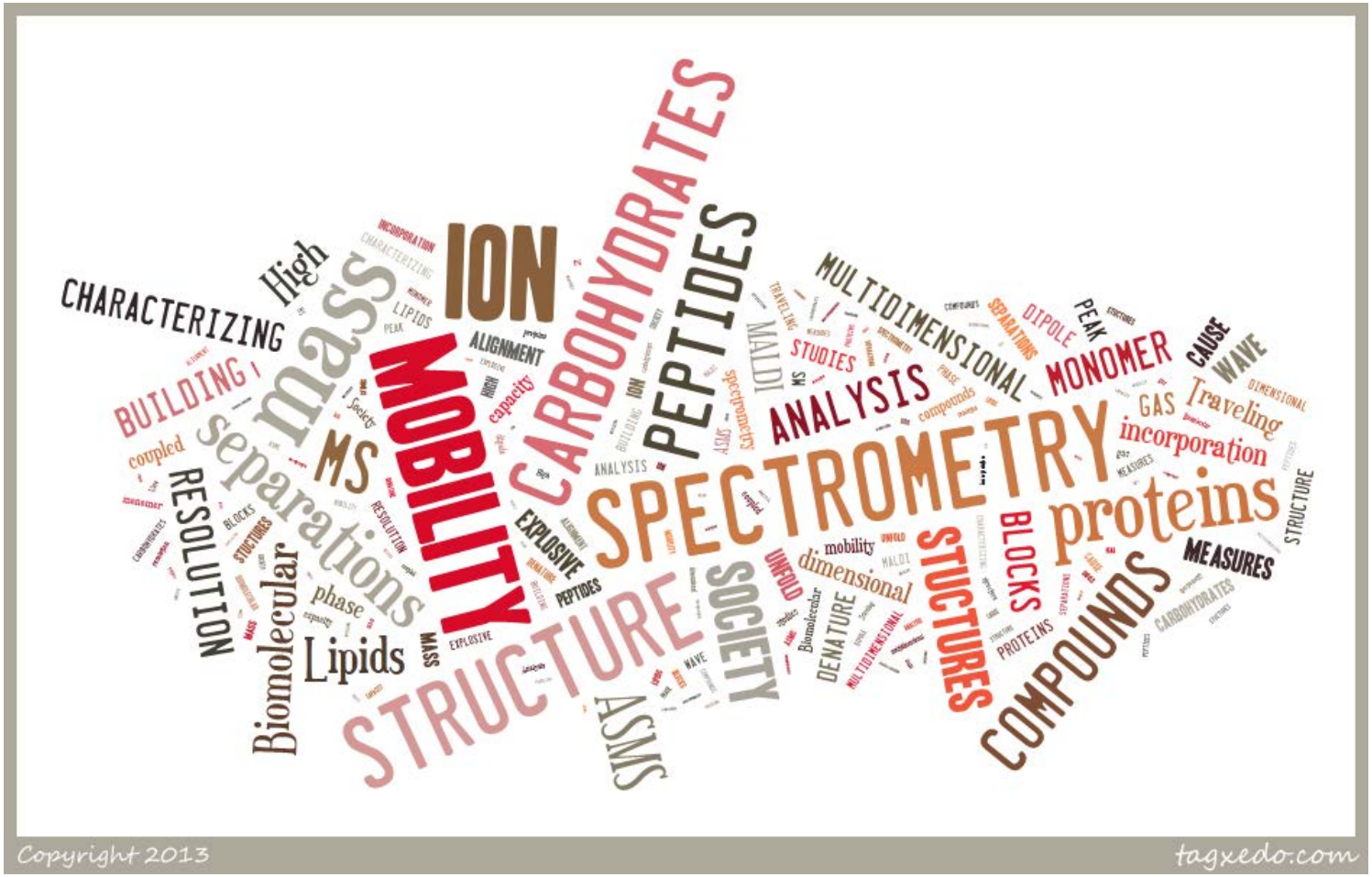
The Complementary Union of IMS and MS



- Produces mobility-mass correlation (ion-density) curves that can be used to identify classes of compounds
- At the IMS-MS interface, collision induced dissociation of the mobility separated ion can provide fragmentation pattern information
- Interfaces easily with both gas and liquid chromatography
  - Additional dimension of separation (Peak Capacity)
- Facilitates the measurement of isomers



QUESTIONS?



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BUILDING
MS
separations
RESOLUTION
coupled
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