Energetic characterization using thermometer ions of a trapped ion mobility mass spectrometer (TIMS)

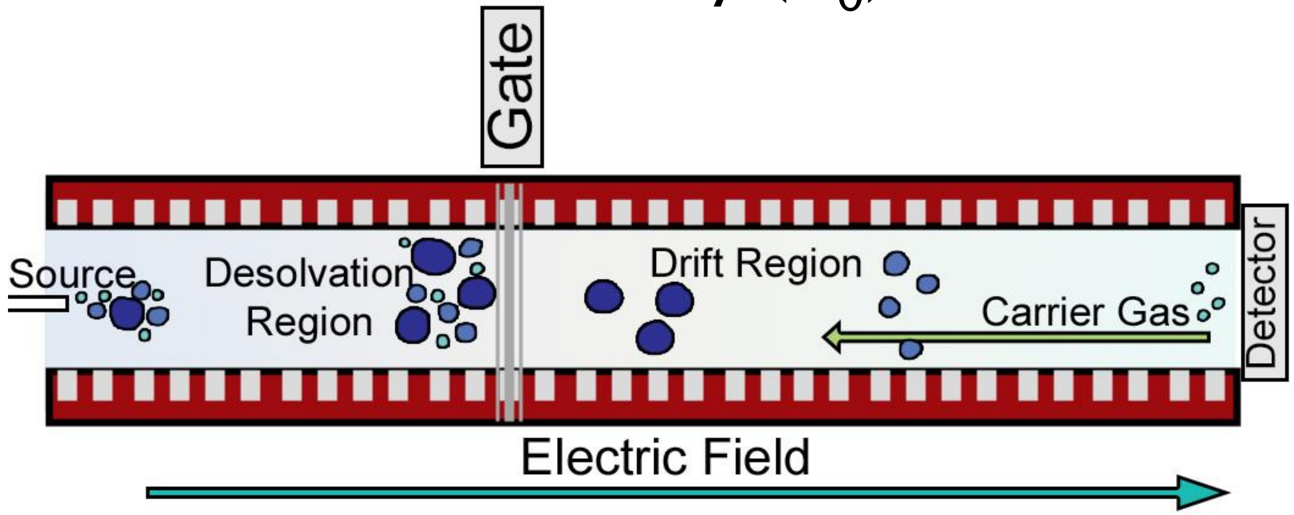
Cameron N. Naylor, Mark E. Ridgeway, Melvin A. Park, Brian H. Clowers

Drift Tube IMS

- Most wide-spread analytical technique in the world
- Ions pass through a stationary gas in an electric field
- Mobilities/ Collision-cross sections reported
- Mobility (K) normalized to reduced mobility (K_0)

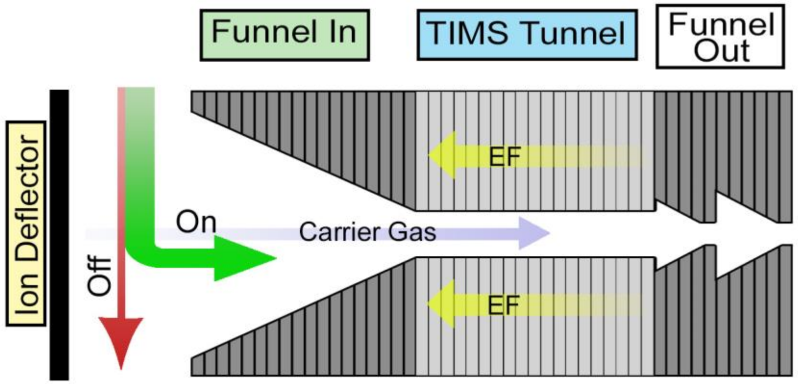
$$K_0 = K \frac{P}{P_0}\frac{T_0}{T} = K\frac{N}{N_0} = \frac{l^2}{Vt_d} * \frac{P}{P_0}\frac{T_0}{T}$$

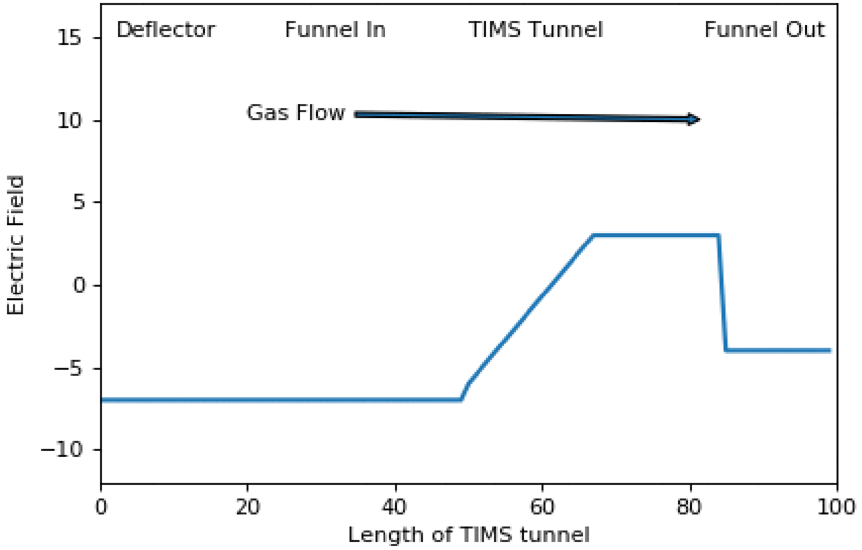
$$\Omega_D = \frac{3}{16}\frac{q}{N}\sqrt{\frac{1}{m}+\frac{1}{M}}\sqrt{\frac{2\pi}{kT}}\frac{1}{K}$$



Trapped Ion Mobility-Mass Spectrometry

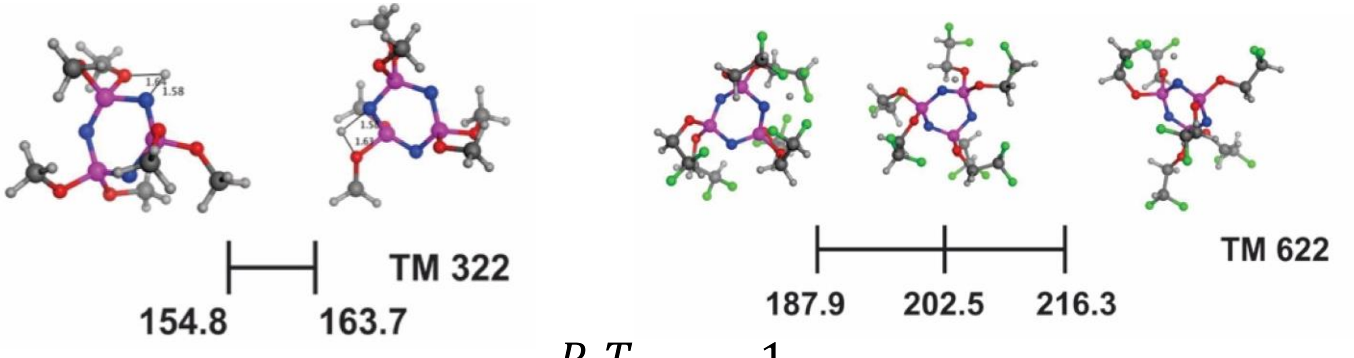
- Non-static electric field
- Collisions with buffer gas provide separation
- Gas flow determined by pressure
- Deflector acts as gate



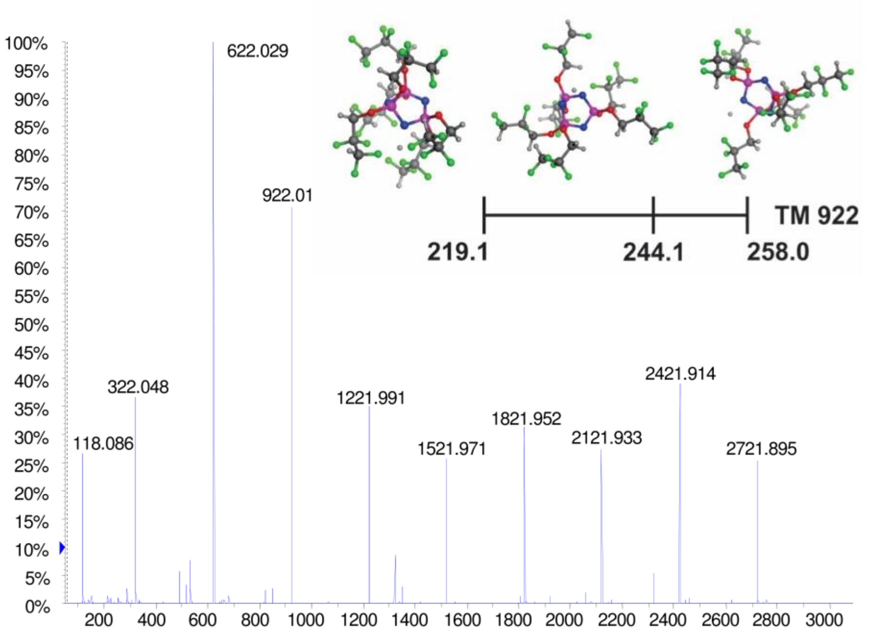


Challenges with TIMS

- Exact temperature unknown
- No direct calculation for mobility/collision cross sections
  - Relies on literature values for calibration
  - Only calibrant used is Agilent Tune Mix
  - Mobilities reported as an elution voltage



$$K_0 = K\frac{P}{P_0}\frac{T_0}{T}\ \alpha\ \frac{1}{V_{elute}}$$



Stow, S. M. *et al. Anal. Chem.* **2017**, *89* (17), 9048–9055.
Agilent. *https://www.agilent.com/cs/library/.../G1969-85000cofa872022-U-LB86189.pdf*
Accessed June 22,2018.

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$$K_0 = K \frac{P}{P_0} \frac{T_0}{T} \alpha \frac{1}{V_{elute}}$$

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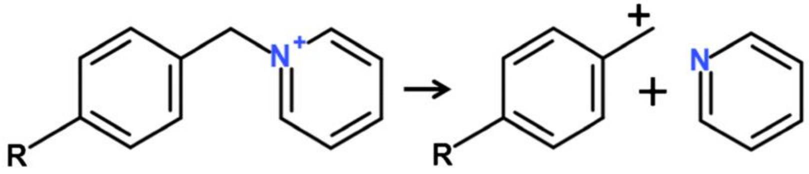
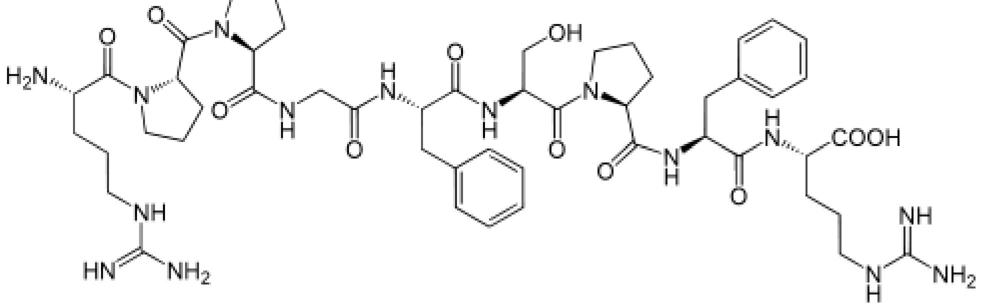
Agilent. *https://www.agilent.com/cs/library/.../G1969-85000cofa872022-U-LB86189.pdf*
Accessed June 22,2018.

Thermometer Ions

- Class of compound with known bond dissociation energy (BDE) for one fragmentation pathway
- BDE found computationally or experimentally
- Also class of proteins depending on conformations present in mobility domain
- Ex. benzylamines, benzylpyridiniums, bradykinin, ubiquitin, leucine enkephlin



RPPGFSPFR



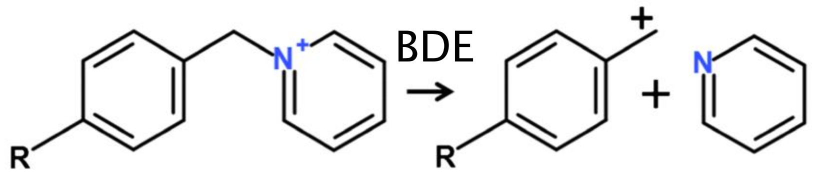
Pierson, N. A.; Valentine, S. J.; Clemmer, D. E. *J. Phys. Chem. B* **2010**, *114* (23), 7777–7783.
Wells, J. M.; McLuckey, S. A. *Methods Enzymol.* **2005**, *402* (1993), 148–185

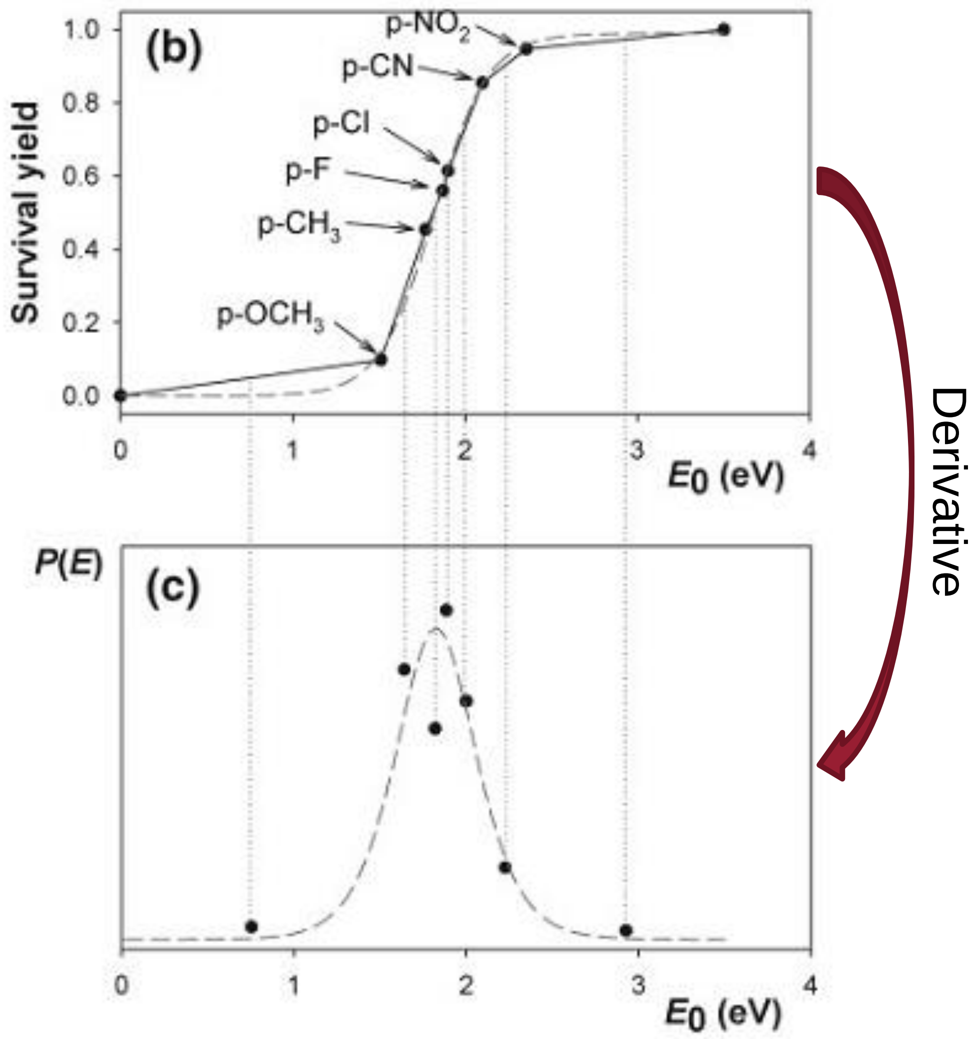
Survival Theory

- Homologous series of thermometer ions with differing bond dissociation energies compare fragmentation ratios

$$Survival\ Yield = \frac{I_{Parent}}{I_{Parent} + I_{Fragment}}$$

- Internal energy described with a Boltzmann distribution

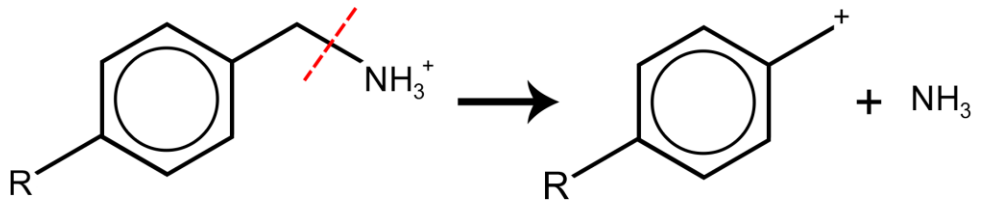




Gabelica, Valerie. De Pauw, Edwin. Intrernal Energy and Fragmentation of Ions Produced in Electrospray Sources. Mass Spectrometry Reviews, 2005, 24,566-587.

4-substituted benzylammonium

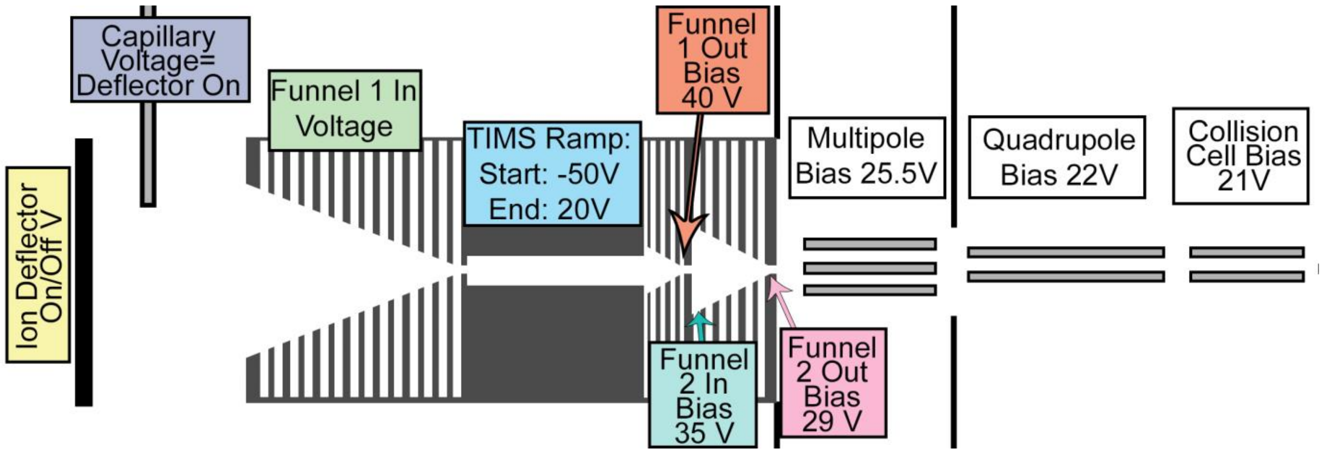
4-sub. Benzylamine	BDE[1] (eV)	Parent *m/z*	Fragment *m/z*
Nitro	1.96	153	136
Trifluoromethyl	1.845	176	159
Chloro	1.52	142	125
T-butyl	1.35	164	147
Methoxy	1.05	138	121

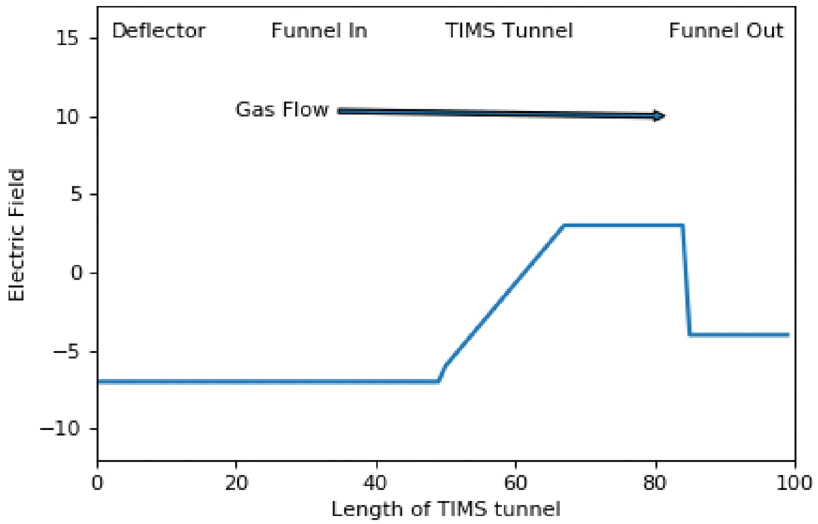


Sample Prep: 5 μM each benzylamine in 50:50 MeOH/H_2O

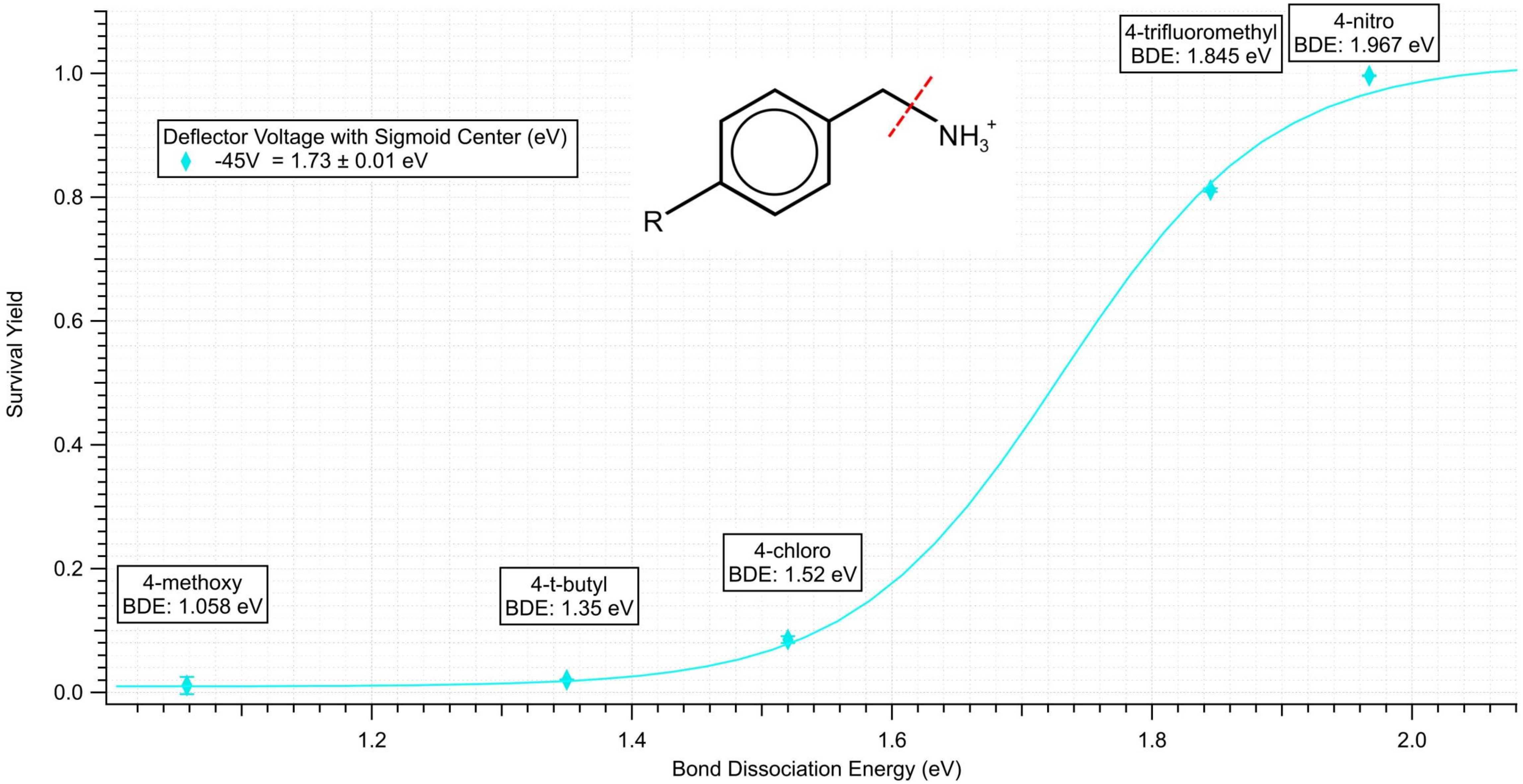
1.Stephens, E. R.; Dumlao, M.; Xiao, D.; Zhang, D.; Donald, W. A. *J. Am. Soc. Mass Spectrom.* **2015**, *26* (12), 2081–2084.

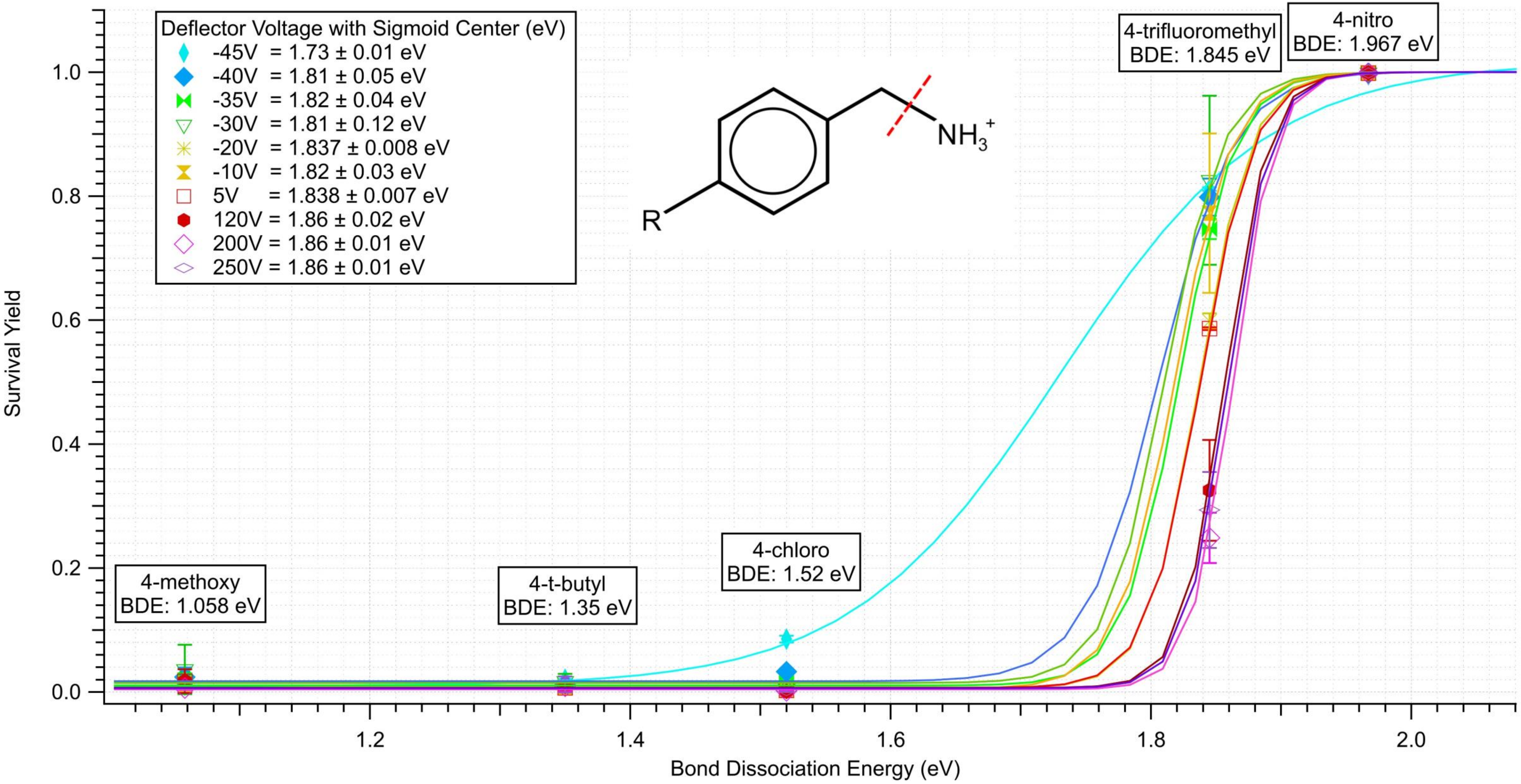
TIMS Settings





Capillary (V)	Deflector On (V)	Deflector Off (V)	Funnel 1 In (V)
-45	-45	-150	-50
-40	-40	-150	-50
-35	-35	-150	-50
-30	-30	-150	-50
-20	-20	-150	-50
-10	-10	-150	-30
5	5	-150	-50
120	120	0	100
200	200	0	150
250	250	0	200


Deflector Voltage with Sigmoid Center (eV)
-45V = 1.73 ± 0.01 eV
R
NH_3^+
4-methoxy
BDE: 1.058 eV
4-t-butyl
BDE: 1.35 eV
4-chloro
BDE: 1.52 eV
4-trifluoromethyl
BDE: 1.845 eV
4-nitro
BDE: 1.967 eV
Survival Yield
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
Bond Dissociation Energy (eV)


Deflector Voltage with Sigmoid Center (eV)
-45V = 1.73 ± 0.01 eV
-40V = 1.81 ± 0.05 eV
-35V = 1.82 ± 0.04 eV
-30V = 1.81 ± 0.12 eV
-20V = 1.837 ± 0.008 eV
-10V = 1.82 ± 0.03 eV
5V = 1.838 ± 0.007 eV
120V = 1.86 ± 0.02 eV
200V = 1.86 ± 0.01 eV
250V = 1.86 ± 0.01 eV
R
NH3+
4-methoxy
BDE: 1.058 eV
4-t-butyl
BDE: 1.35 eV
4-chloro
BDE: 1.52 eV
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Survival Yield
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0.0
0.2
0.4
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1.0
1.2
1.4
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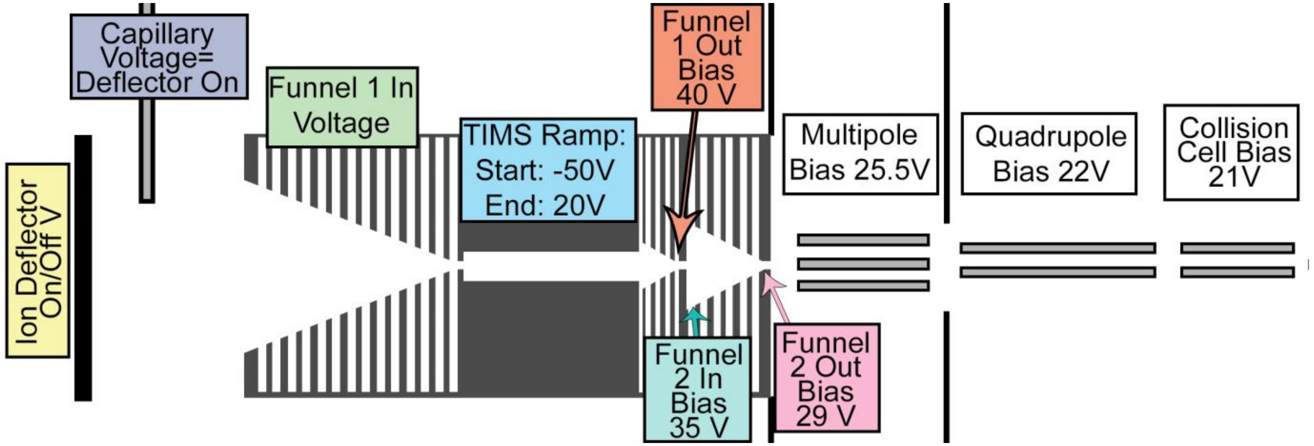
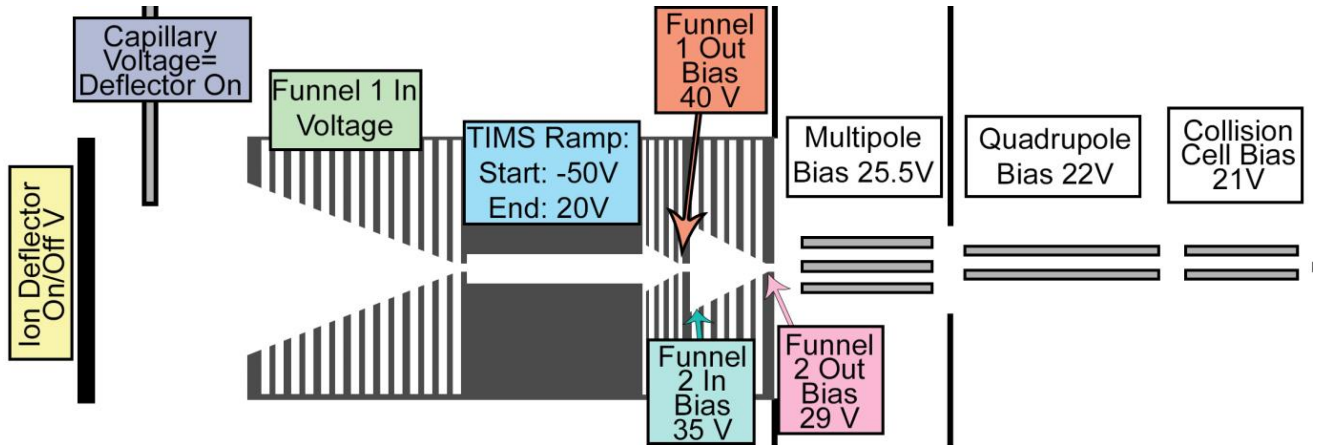
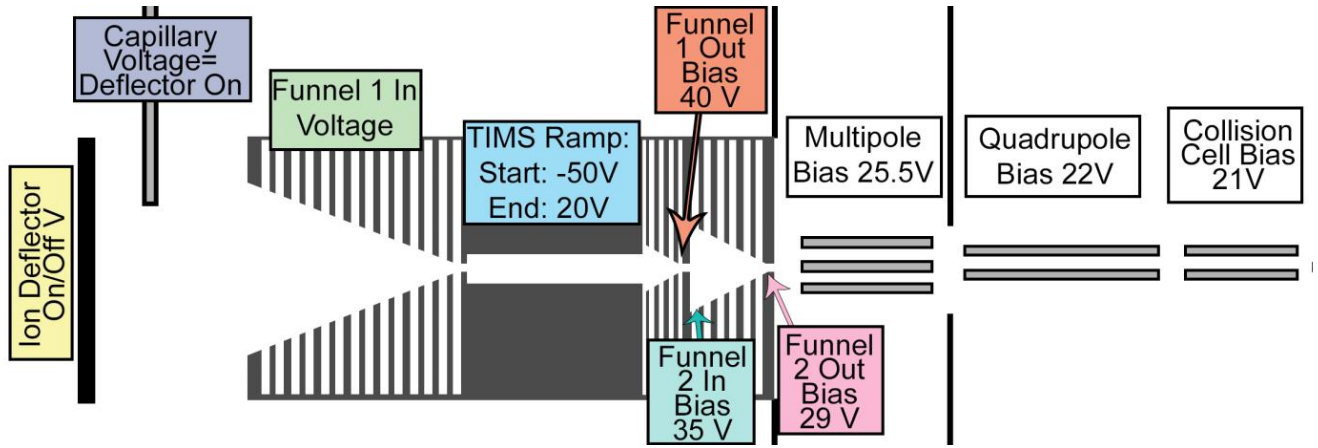
2.0



	Capillary (V)	Deflector On (V)	Deflector Off (V)	Funnel 1 In (V)
Softest possible (1.73 eV)	-45	-45	-150	-50



	Capillary (V)	Deflector On (V)	Deflector Off (V)	Funnel 1 In (V)
Softest possible (1.73 eV)	-45	-45	-150	-50
Softer (1.82 eV)	-40	-40	-150	-50
	-35	-35	-150	-50
	-30	-30	-150	-50
	-20	-20	-150	-50
	-10	-10	-150	-30
	5	5	-150	-50



	Capillary (V)	Deflector On (V)	Deflector Off (V)	Funnel 1 In (V)
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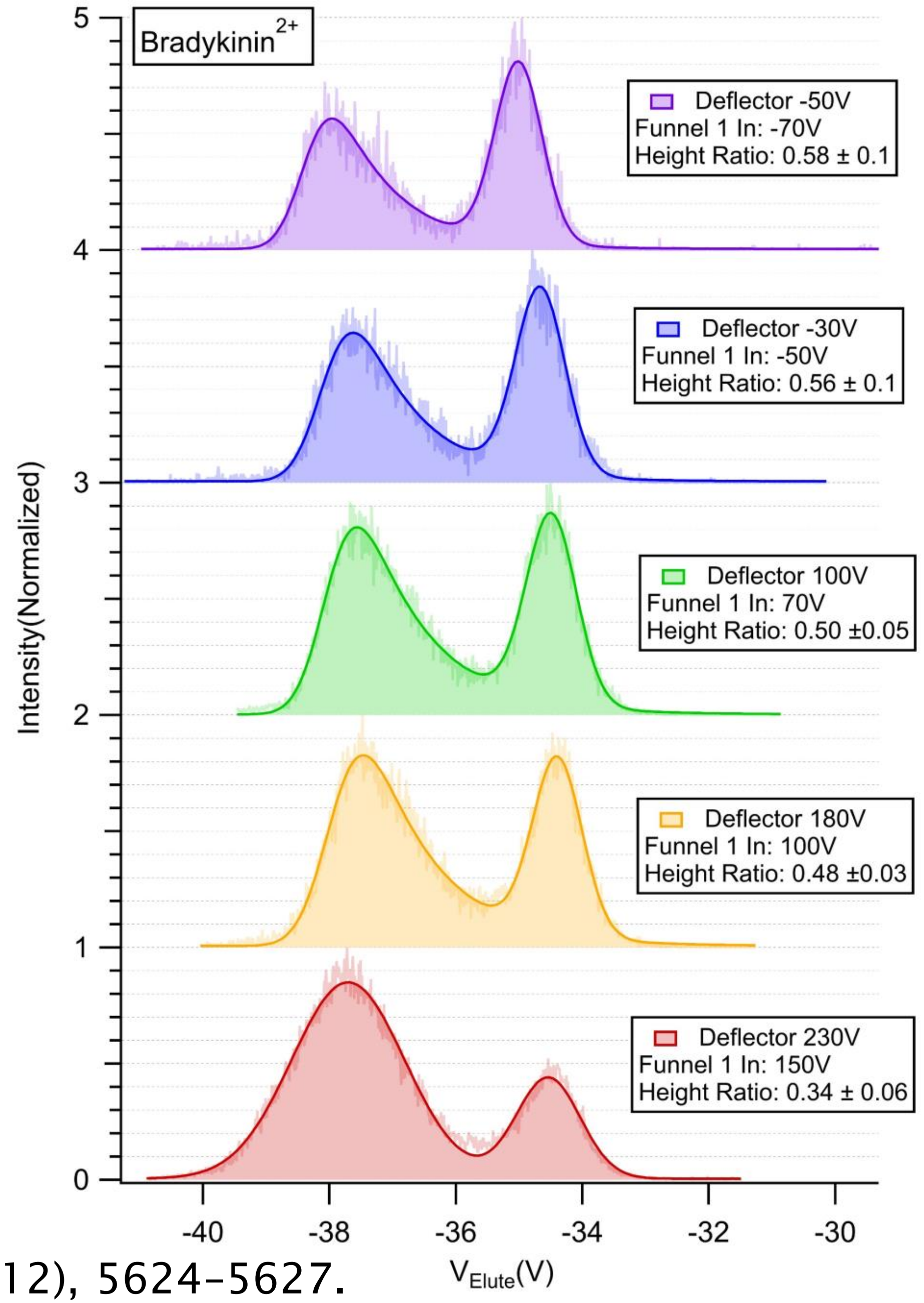
	-30	-30	-150	-50
	-20	-20	-150	-50
	-10	-10	-150	-30
	5	5	-150	-50
Harshest possible (1.86 eV)	120	120	0	100
	200	200	0	150
	250	250	0	200

Bradykinin

- Bradykinin^{2+} has two mobilities that shift in abundance depending on energy felt
- Smaller CCS (right) higher abundance at low energy
- Larger CCS (left) higher abundance at high energy

$$Height\ Ratio = \frac{I_{Right\ Peak}}{I_{Right\ Peak} + I_{Left\ Peak}}$$



Silveira, J. A.; Ridgeway, M. E.; Park, M. A. Anal. Chem. 2014, 86 (12), 5624–5627.

So the average energy is 1.73-1.86eV?

- Carpenter et al. report their final survival yield as 1.68 ± 0.10 eV for their mass spectrometer
- T_{char} =725 ± 23K

1 eV = 96kJ/mol

- What about mobilities?

Carpenter, J. E.; McNary, C. P.; Furin, A.; Sweeney, A. F.; Armentrout, P. B. J. Am. Soc. Mass Spectrom. 2017, 28 (9), 1876–1888.

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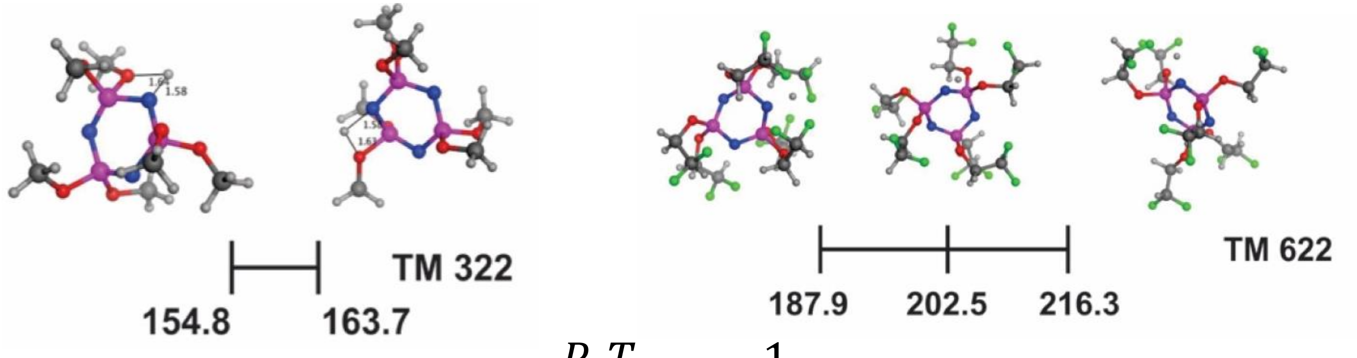
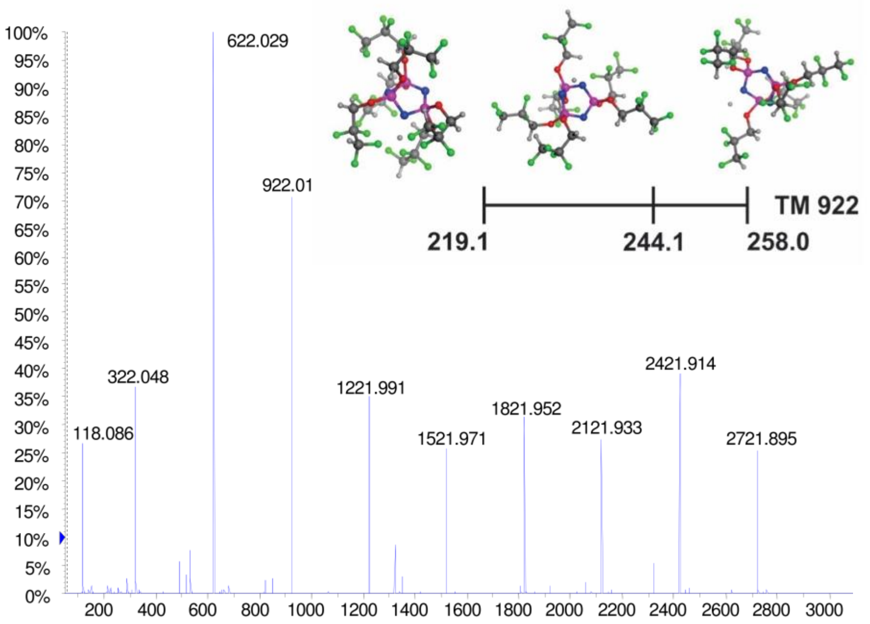
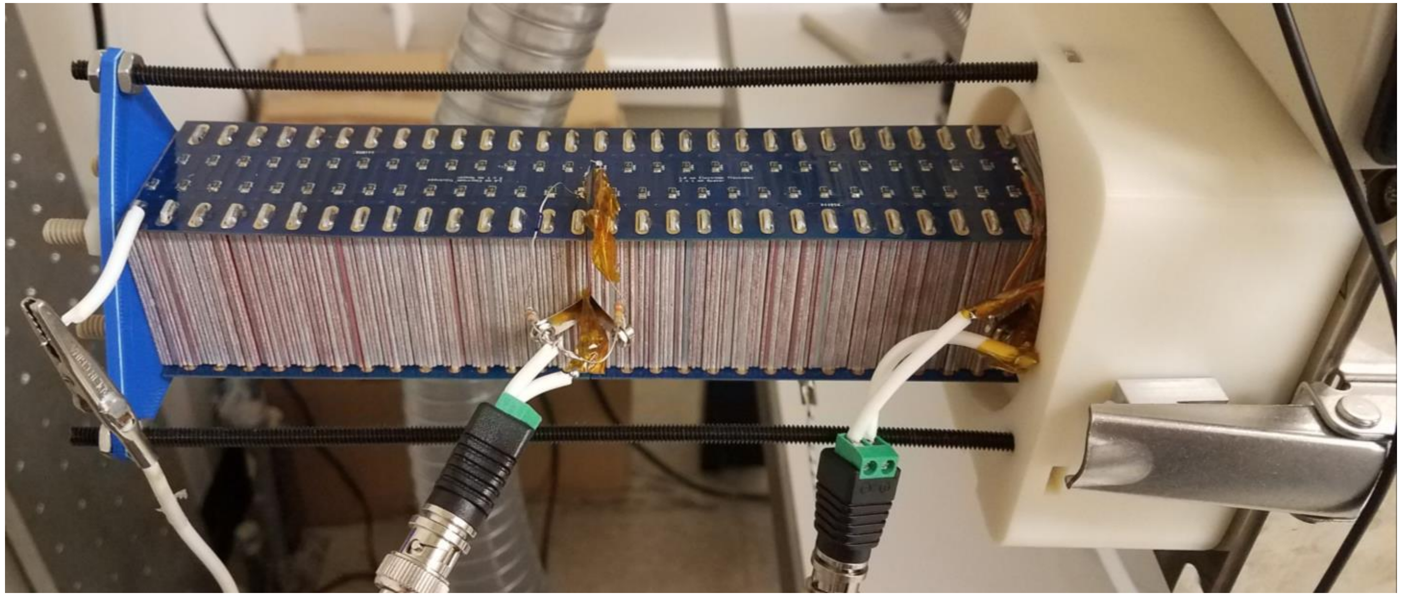


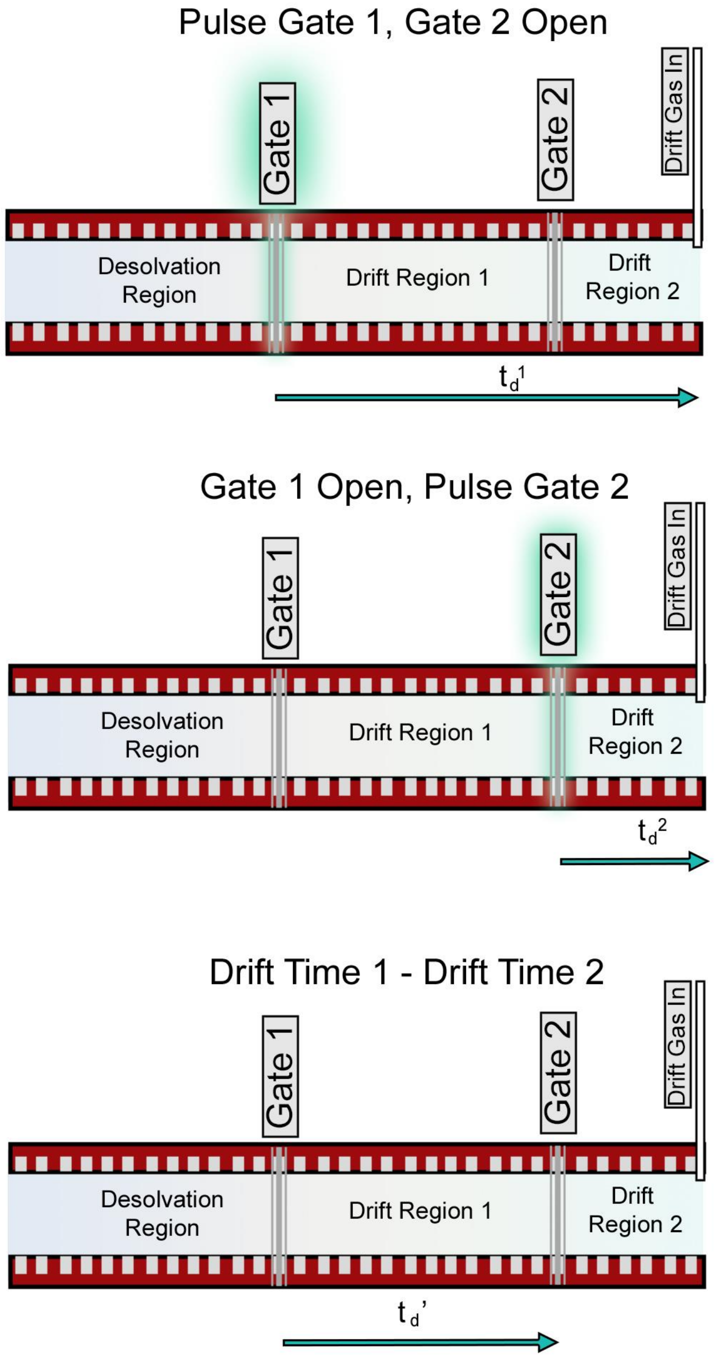


$$K_0 = K \frac{P}{P_0}\frac{T_0}{T} \; \alpha \; \frac{1}{V_{elute}}$$

Stow, S. M. *et al. Anal. Chem.* **2017**, *89* (17), 9048–9055.
Agilent. *https://www.agilent.com/cs/library/.../G1969-85000cofa872022-U-LB86189.pdf* Accessed June 22,2018.

PCBIMS-TIMS





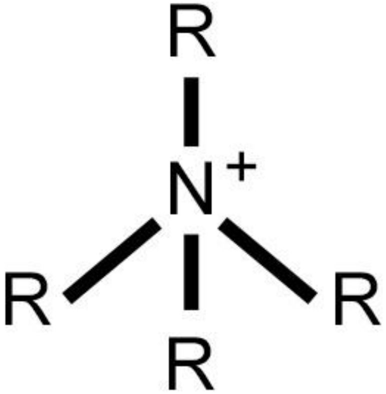
Reinecke, T.; Clowers, B. H. *HardwareX* **2018**, *4*.

3 Standard Classes

Agilent Tune Mix

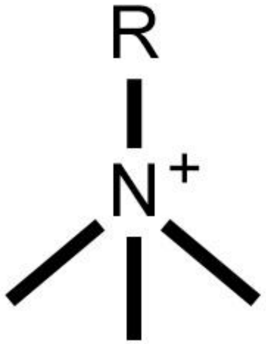
m/z range: 322-1522

Tetraalkylammonium Salts
(TXA salts)

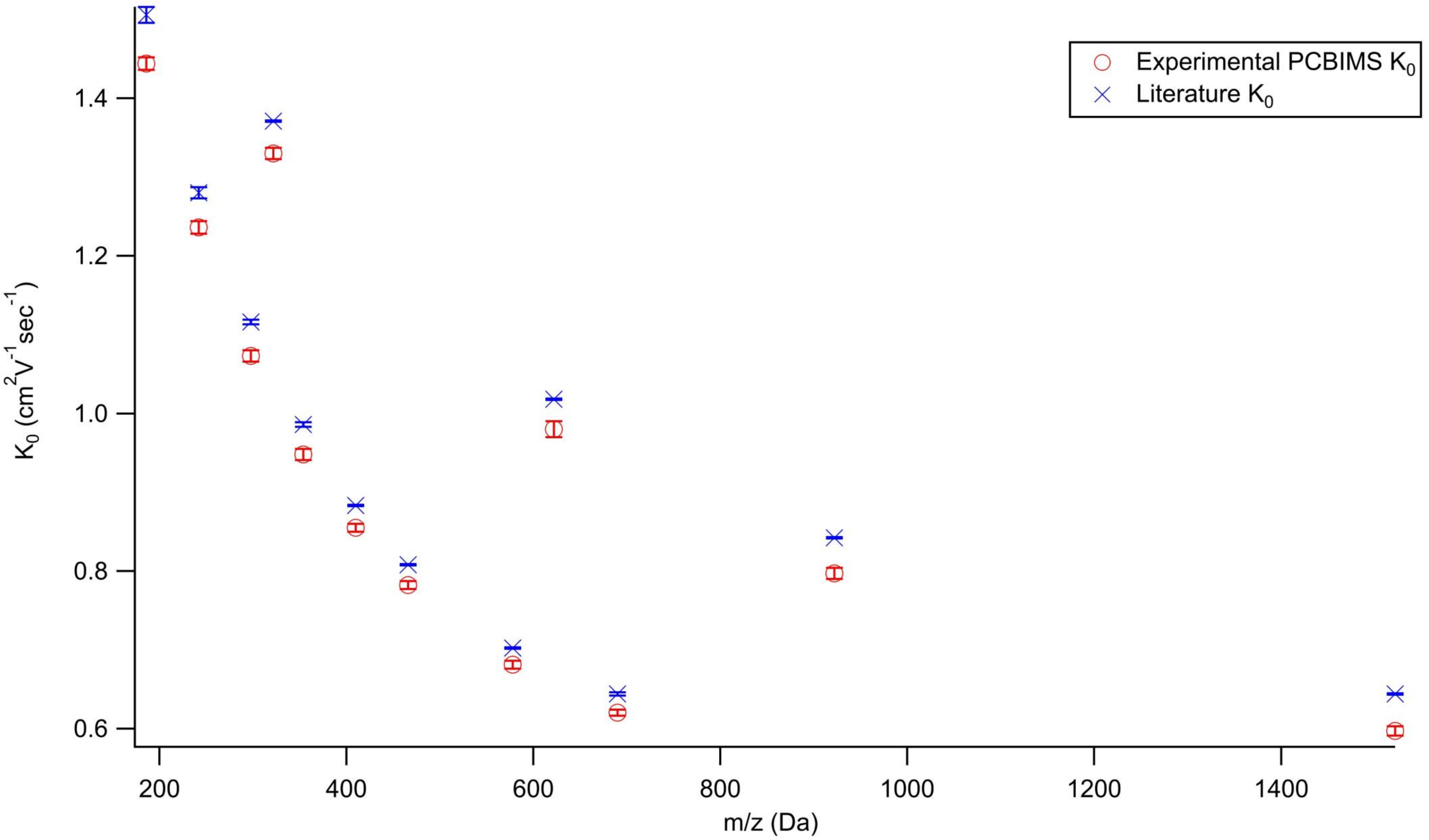


m/z range: 186-690

Alkyl-trimethylammonium Salts
(XTMA salts)

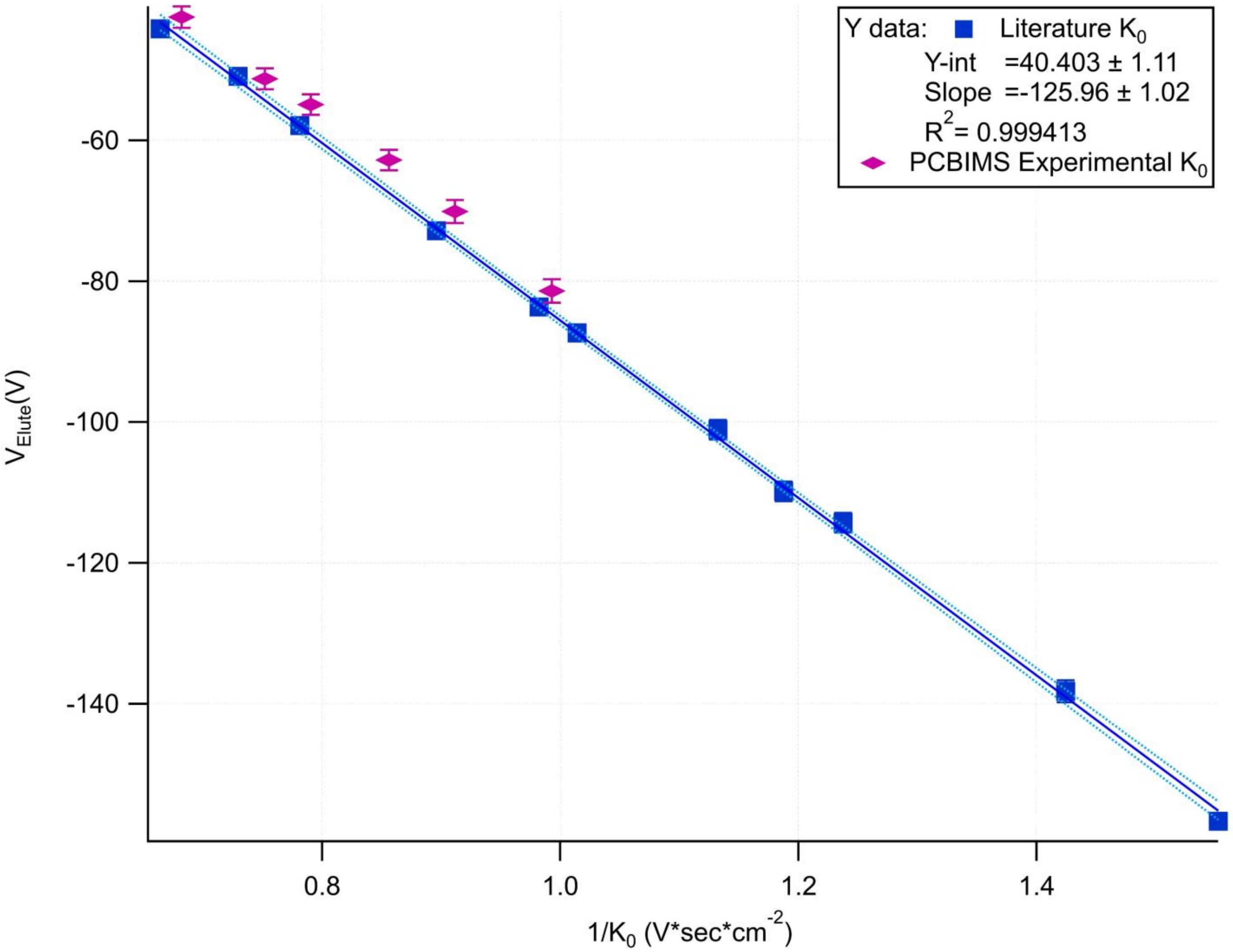


m/z range: 144-312

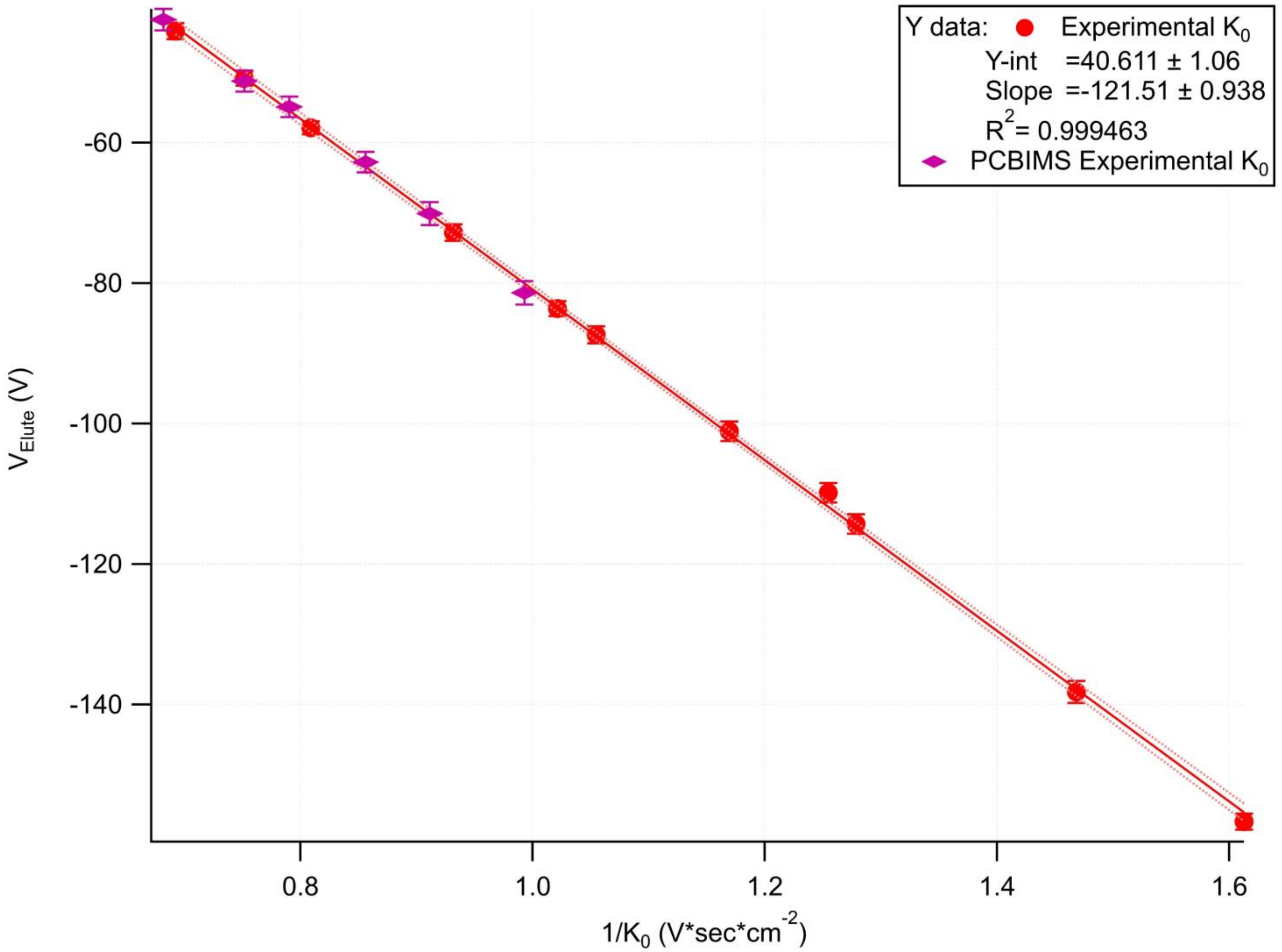


Experimental PCBIMS K0
Literature K0
K0 (cm2V-1sec-1)
m/z (Da)
1.4
1.2
1.0
0.8
0.6
200
400
600
800
1000
1200
1400

TIMS Calibration- Literature Values

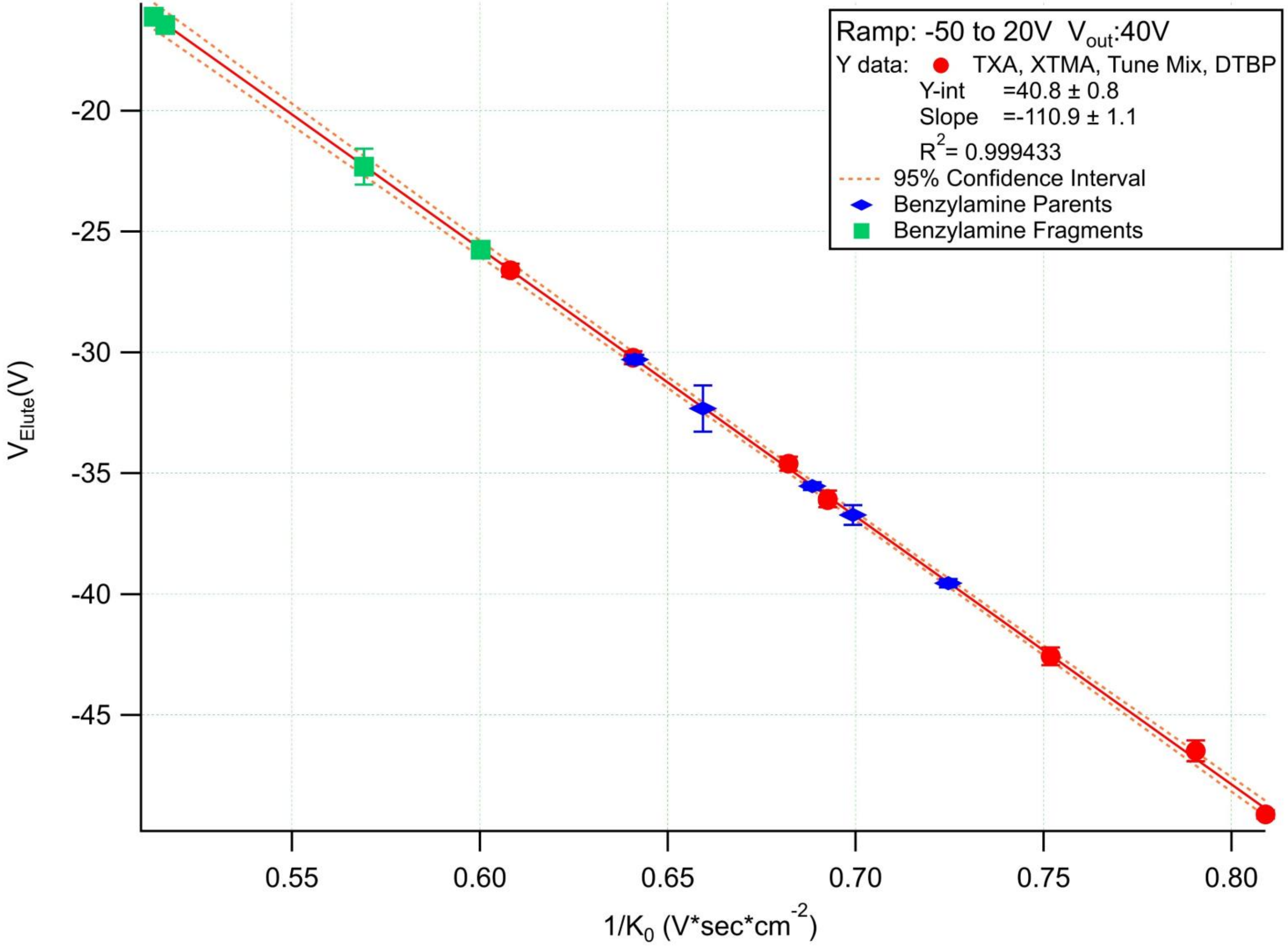


TIMS Calibration- PCBIMS Values



Mobilities of Benzylamines

Thermometer Ion	Parent K_0	Fragment K_0
Nitro	1.453 ± 0.003	--------------
Trifluoromethyl	1.430 ± 0.007	1.76 ± 0.02
Chloro	1.559 ± 0.005	1.937 ± 0.006
T-butyl	1.380 ± 0.003	1.666 ± 0.004
Methoxy	1.52 ± 0.02	1.95 ± 0.05



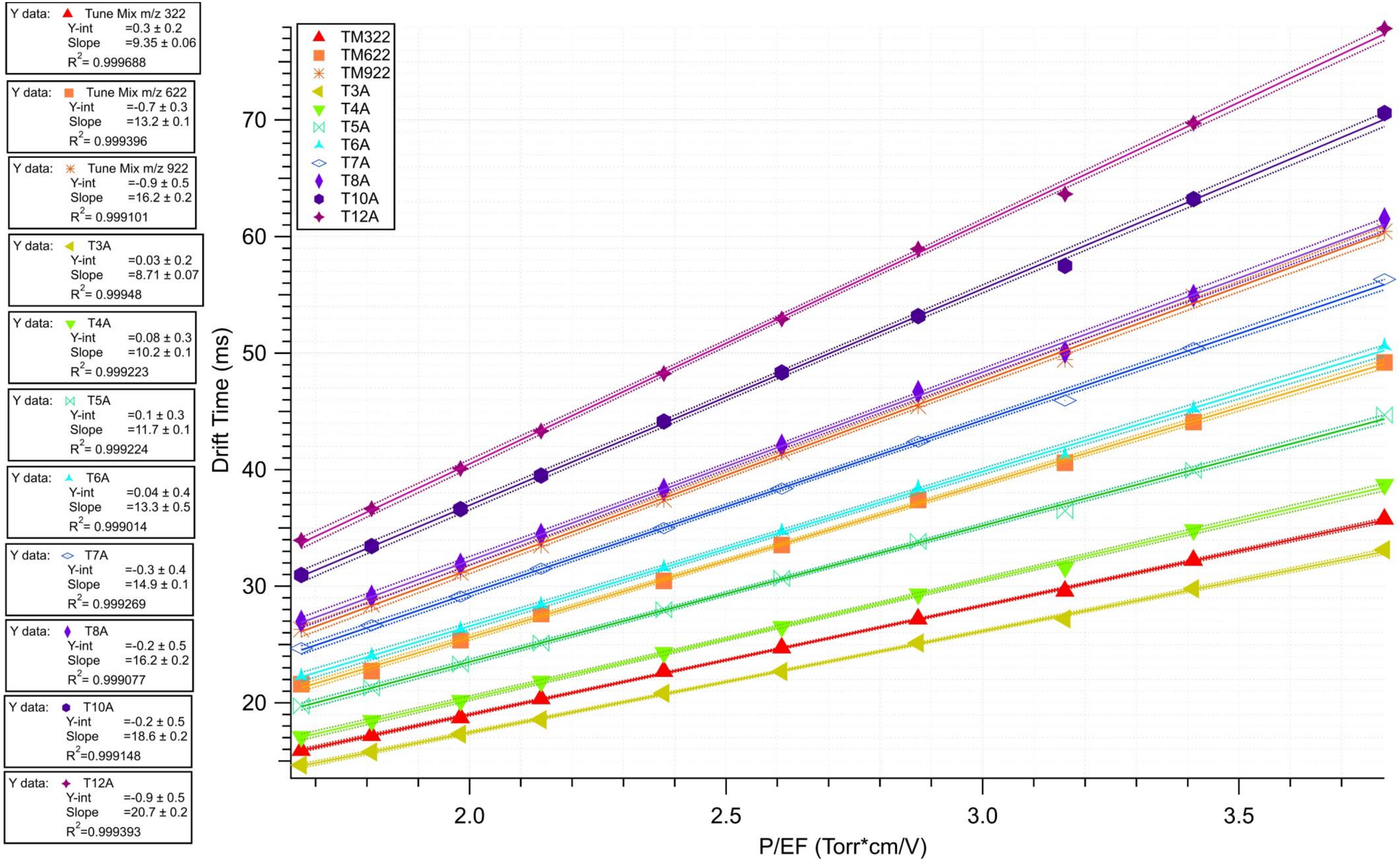
Conclusions

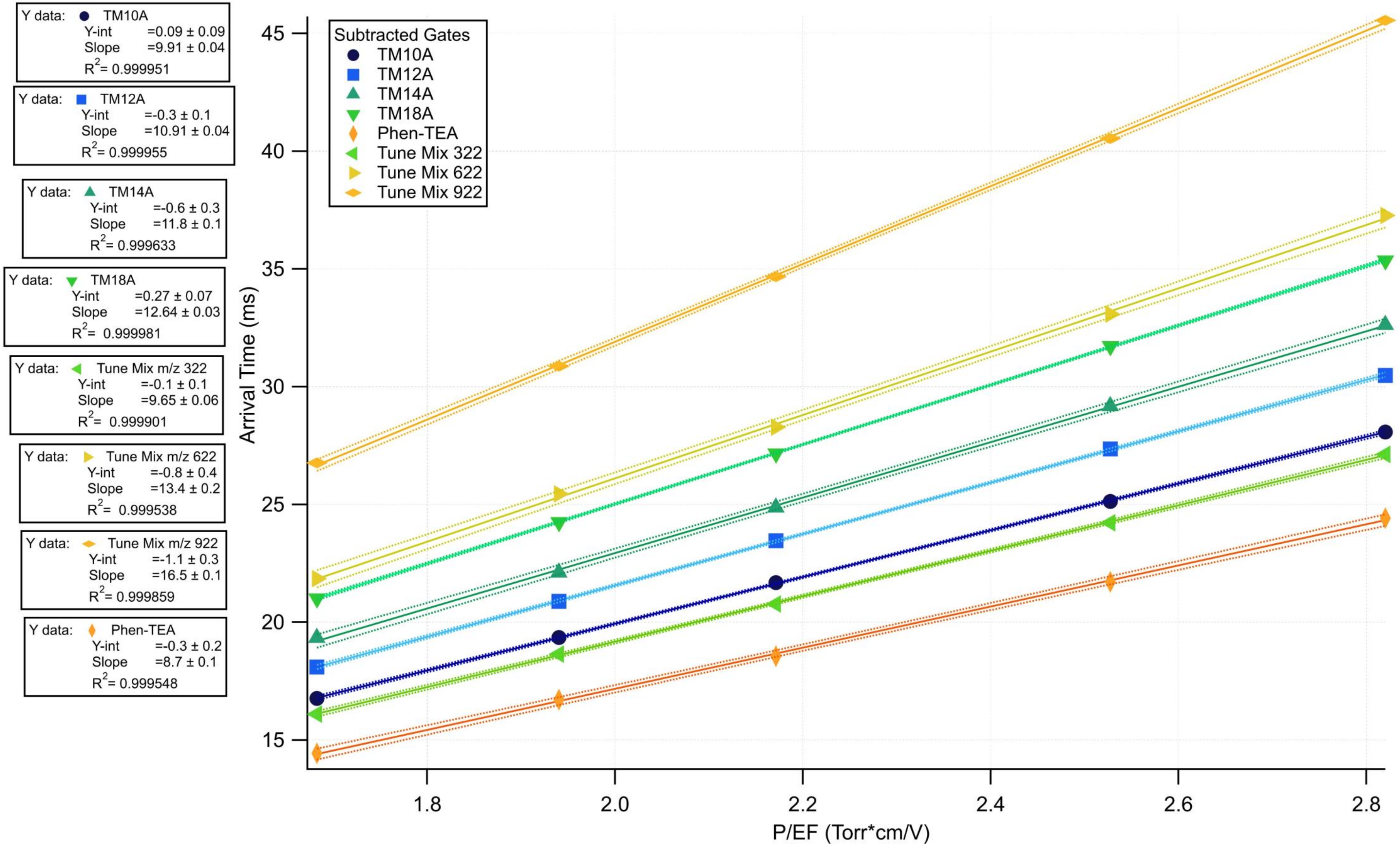
- TIMS voltages can be modified to induce or reduce fragmentation
- PCBIMS has been coupled to a TIMS for accurate mobility values
- Mobilities of para-substituted benzylammonium ions have been reported

Acknowledgements

- Clowers Research Group:
  - Zhihao (Joe) Yu
  - Kelsey Morrison
  - Pearl Kwantwi-Barima
  - Andrew Pemberton
  - Nathan Buzitis
- Dr. Ing. Tobias Reinecke
- Alumni Members:
  - Austen Davis
  - Peyton Nosbusch
- Department of Chemistry WSU
- Bruker Daltonics Inc.

Questions?


Y data: Tune Mix m/z 322
Y-int =0.3 ± 0.2
Slope =9.35 ± 0.06
R2= 0.999688
Y data: Tune Mix m/z 622
Y-int =-0.7 ± 0.3
Slope =13.2 ± 0.1
R2= 0.999396
Y data: Tune Mix m/z 922
Y-int =-0.9 ± 0.5
Slope =16.2 ± 0.2
R2= 0.999101
Y data: T3A
Y-int =0.03 ± 0.2
Slope =8.71 ± 0.07
R2= 0.99948
Y data: T4A
Y-int =0.08 ± 0.3
Slope =10.2 ± 0.1
R2= 0.999223
Y data: T5A
Y-int =0.1 ± 0.3
Slope =11.7 ± 0.1
R2= 0.999224
Y data: T6A
Y-int =0.04 ± 0.4
Slope =13.3 ± 0.5
R2= 0.999014
Y data: T7A
Y-int =-0.3 ± 0.4
Slope =14.9 ± 0.1
R2= 0.999269
Y data: T8A
Y-int =-0.2 ± 0.5
Slope =16.2 ± 0.2
R2= 0.999077
Y data: T10A
Y-int =-0.2 ± 0.5
Slope =18.6 ± 0.2
R2=0.999148
Y data: T12A
Y-int =-0.9 ± 0.5
Slope =20.7 ± 0.2
R2=0.999393
TM322
TM622
TM922
T3A
T4A
T5A
T6A
T7A
T8A
T10A
T12A
Drift Time (ms)
20
30
40
50
60
70
2.0
2.5
3.0
3.5
P/EF (Torr*cm/V)


Y data: TM10A
Y-int =0.09 ± 0.09
Slope =9.91 ± 0.04
R2= 0.999951
Y data: TM12A
Y-int =-0.3 ± 0.1
Slope =10.91 ± 0.04
R2= 0.999955
Y data: TM14A
Y-int =-0.6 ± 0.3
Slope =11.8 ± 0.1
R2= 0.999633
Y data: TM18A
Y-int =0.27 ± 0.07
Slope =12.64 ± 0.03
R2= 0.999981
Y data: Tune Mix m/z 322
Y-int =-0.1 ± 0.1
Slope =9.65 ± 0.06
R2= 0.999901
Y data: Tune Mix m/z 622
Y-int =-0.8 ± 0.4
Slope =13.4 ± 0.2
R2= 0.999538
Y data: Tune Mix m/z 922
Y-int =-1.1 ± 0.3
Slope =16.5 ± 0.1
R2= 0.999859
Y data: Phen-TEA
Y-int =-0.3 ± 0.2
Slope =8.7 ± 0.1
R2= 0.999548
Subtracted Gates
TM10A
TM12A
TM14A
TM18A
Phen-TEA
Tune Mix 322
Tune Mix 622
Tune Mix 922
Arrival Time (ms)
P/EF (Torr*cm/V)
15
20
25
30
35
40
45
1.8
2.0
2.2
2.4
2.6
2.8

	Abbreviation	Direct Calculation K_0 ($cm^2V^{-1}sec^{-1}$)	Literature K_0 ($cm^2V^{-1}sec^{-1}$)
tetrapropyl ammonium	T3A	1.444 ± 0.008	1.506
tetrabutyl ammonium	T4A	1.236 ± 0.008	1.28
tetrapentyl ammonium	T5A	1.073 ± 0.007	1.116
tetrahexyl ammonium	T6A	0.948 ± 0.007	0.986
tetraheptyl ammonium	T7A	0.855 ± 0.005	0.883
tetraoctyl ammonium	T8A	0.782 ± 0.005	0.808
tetradecyl ammonium	T10A	0.681 ± 0.005	0.702
tetradodecyl ammonium	T12A	0.620 ± 0.004	0.644
decyltrimethylammonium	10TMA	1.265 ± 0.002	
dodecyltrimethylammonium	12TMA	1.168 ± 0.004	
trimethyl-tetradecylammonium	14TMA	1.097 ± 0.007	
trimethyloctadecylammonium	18TMA	1.007 ± 0.004	
benzyltriethylammonium	Phen-TEA	1.466 ± 0.007	
Agilent Tune Mix *m/z* 322	TM322	1.330 ± 0.007	1.371
Agilent Tune Mix *m/z* 622	TM622	0.98 ± 0.01	1.018
Agilent Tune Mix *m/z* 922	TM922	0.797 ± 0.007	0.842
Agilent Tune Mix *m/z* 1522	TM1522	0.597 ± 0.006	0.644