Estimating Collision Cross Sections from Travelling Wave IM-MS Data: How it is done and what are the Problems?

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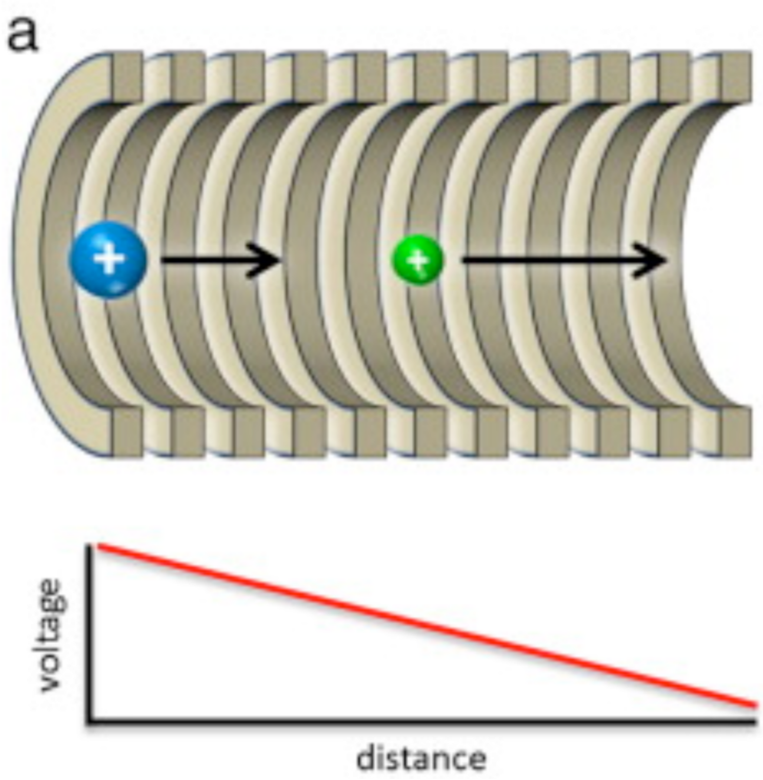
www.fhi-berlin.mpg.de/mp/pagel

Drift Tube IMS

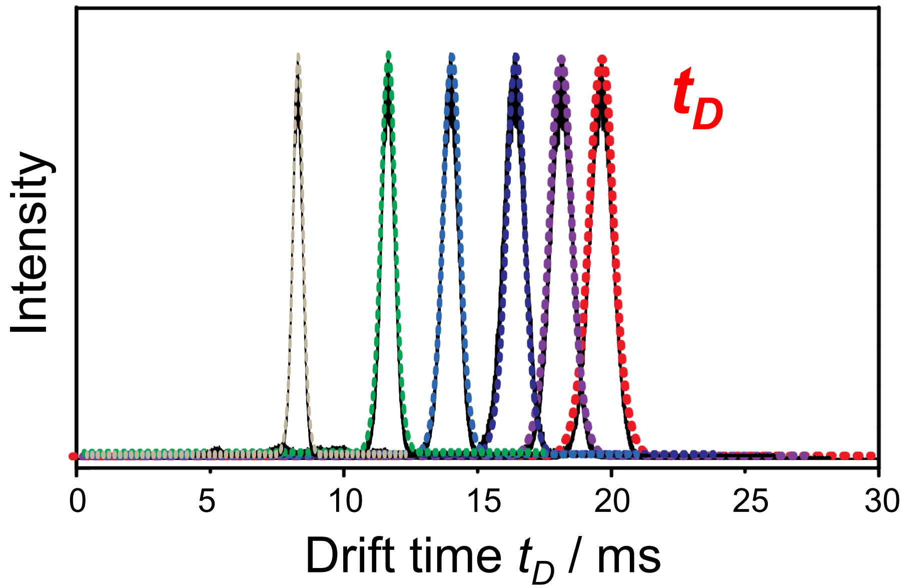
DT IMS
Drift tube IMS

TW IMS
Travelling wave IMS

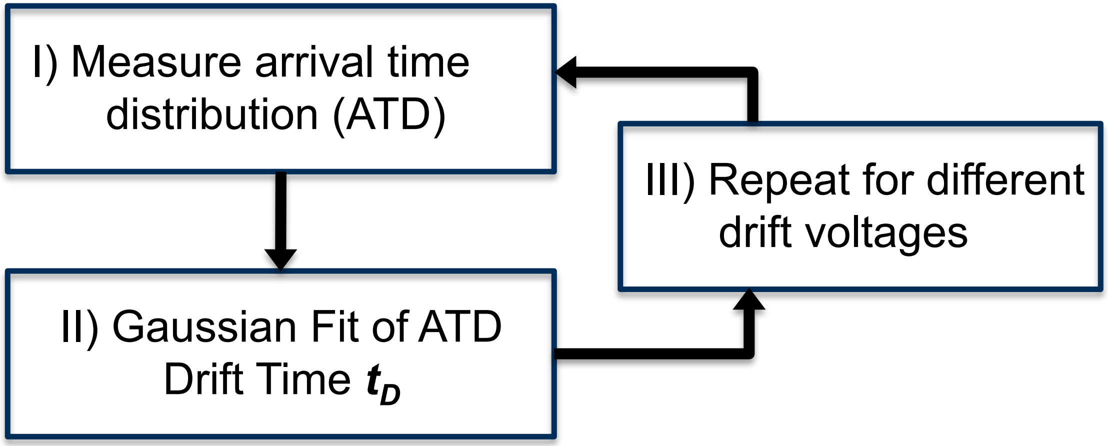


Kliman, M.; May, J.C.; McLean, J.A. *Biochim. Biophys. Acta* **2011**, *1811*, 935.

Collision Cross Section Determination



Drift Voltage	t_D
50 V	19 ms
55 V	18 ms
60 V	16 ms
70 V	14 ms
100 V	11 ms
150 V	8 ms

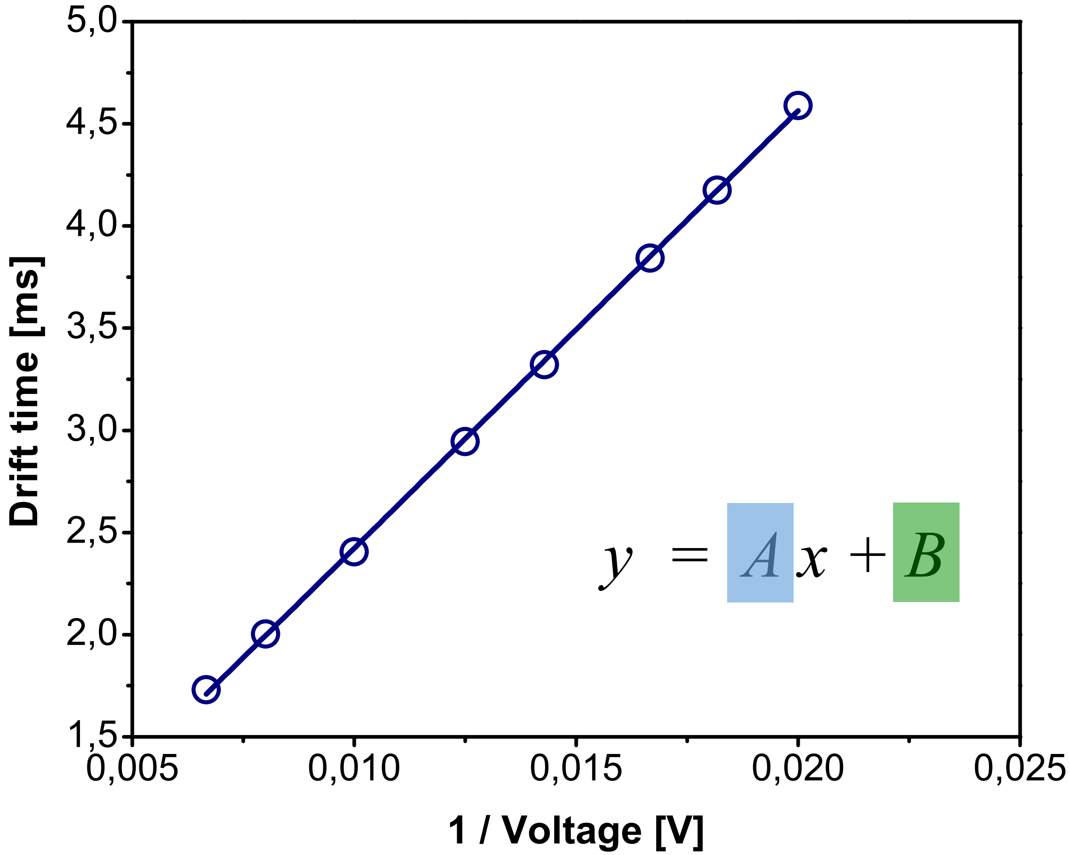


Bush, M. F.; Hall, Z.; Giles, K.; Hoyes, J.; Robinson, C. V.; Ruotolo, B. T. *Anal. Chem.* **2010**, *82*, 9557.
Pagel, K.; Natan, E.; Hall, Z.; Fersht, A. R.; Robinson, C. V. *Angew. Chem. Intl. Ed.* **2013**, *125*, 379.

Direct CCS Measurements on DT IMS Instruments

$$v = KE$$

v: Velocity of the ion
E: Electric field
K: Mobility



$$t_D = \frac{\text{length}}{KE} + t_0$$

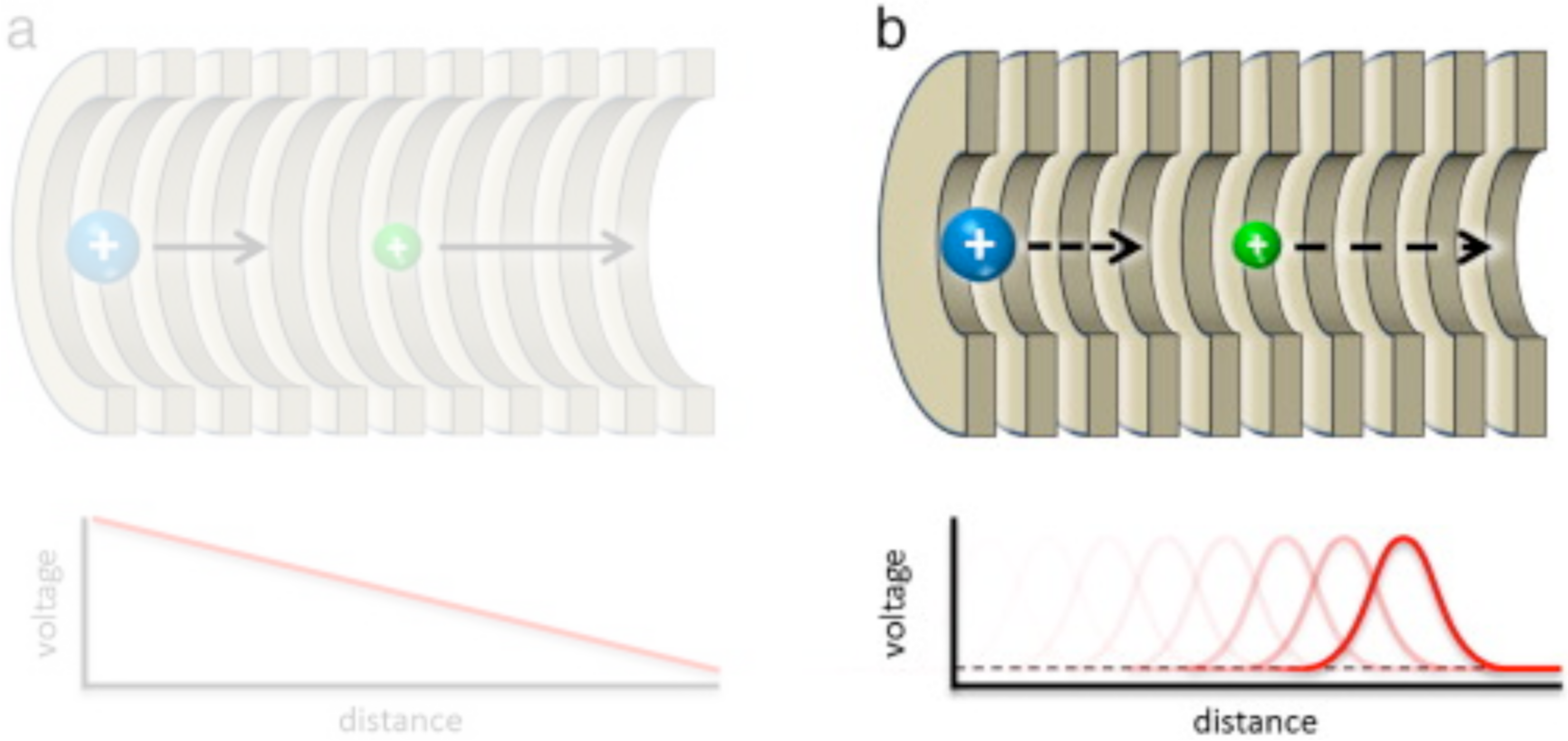
$$\Omega = \frac{3e}{16N}\sqrt{\frac{2\pi}{\mu k_B T}}\frac{1}{K}$$

Bush, M. F.; Hall, Z.; Giles, K.; Hoyes, J.; Robinson, C. V.; Ruotolo, B. T. *Anal. Chem.* **2010**, *82*, 9557.
Pagel, K.; Natan, E.; Hall, Z.; Fersht, A. R.; Robinson, C. V. *Angew. Chem. Intl. Ed.* **2013**, *125*, 379.

Travelling Wave IMS

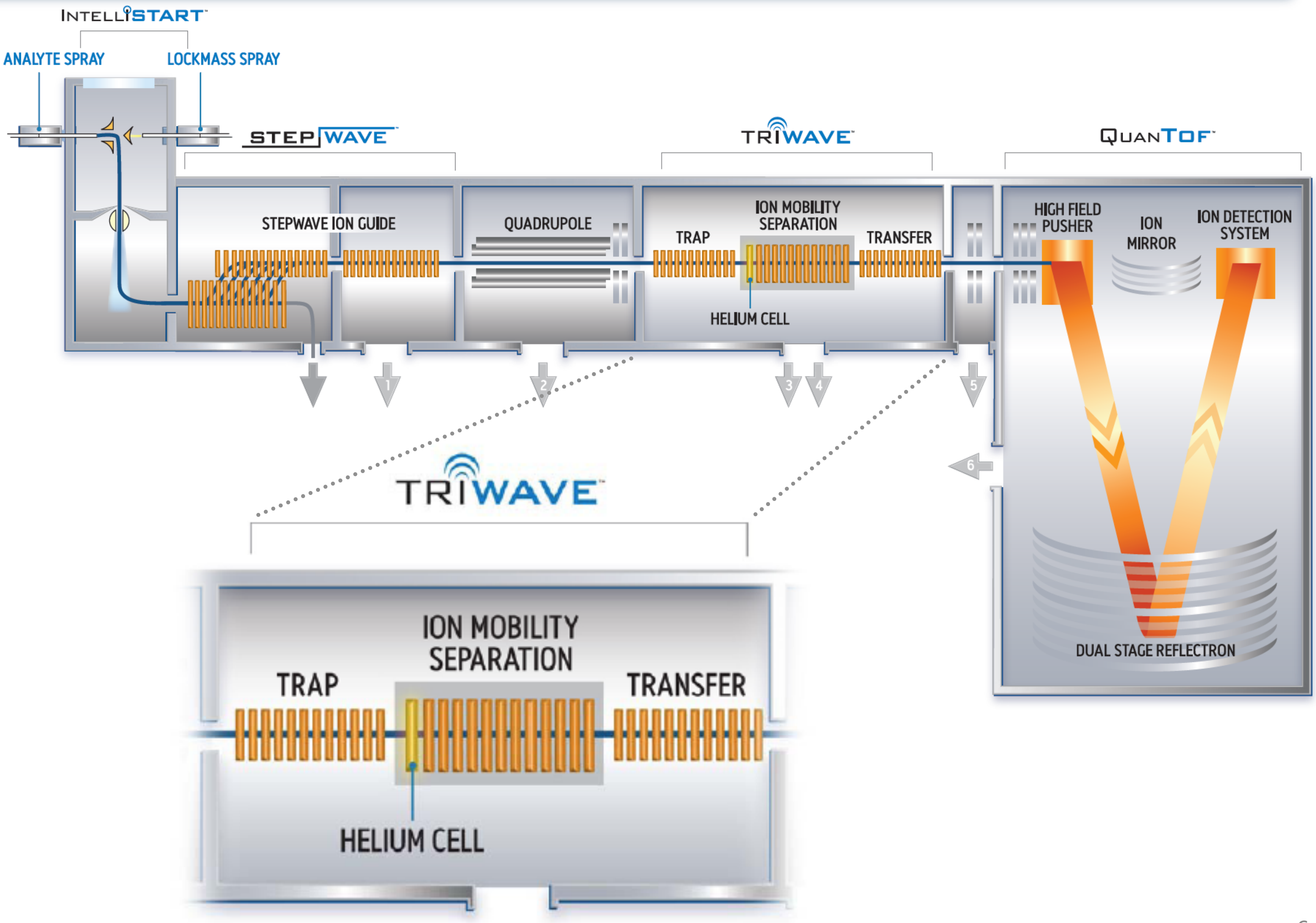
DT IMS
Drift tube IMS

TW IMS
Travelling wave IMS

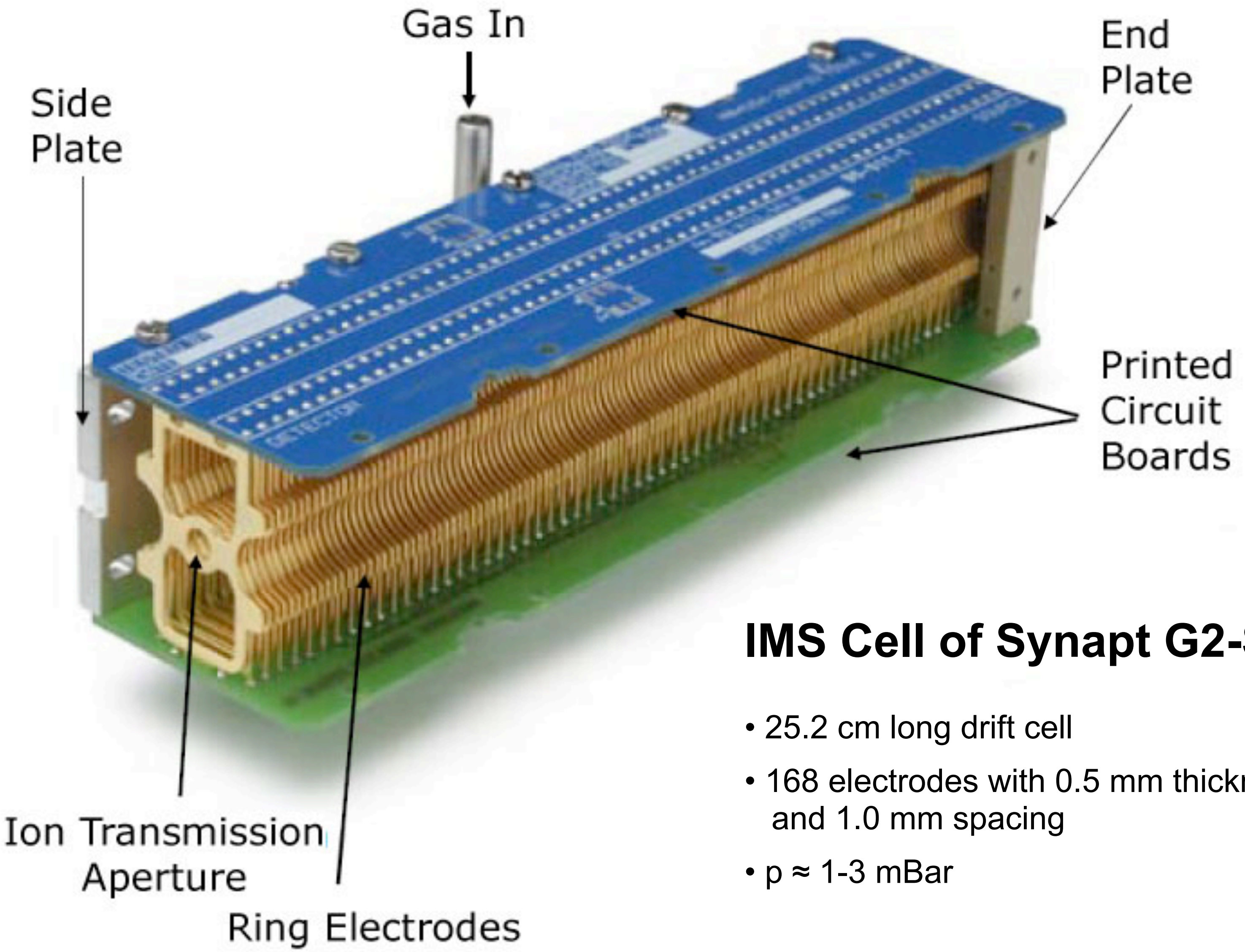


Kliman, M.; May, J.C.; McLean, J.A. *Biochim. Biophys. Acta* **2011**, *1811*, 935.

Instrumentation - Synapt G2-S



Synapt IMS Cell

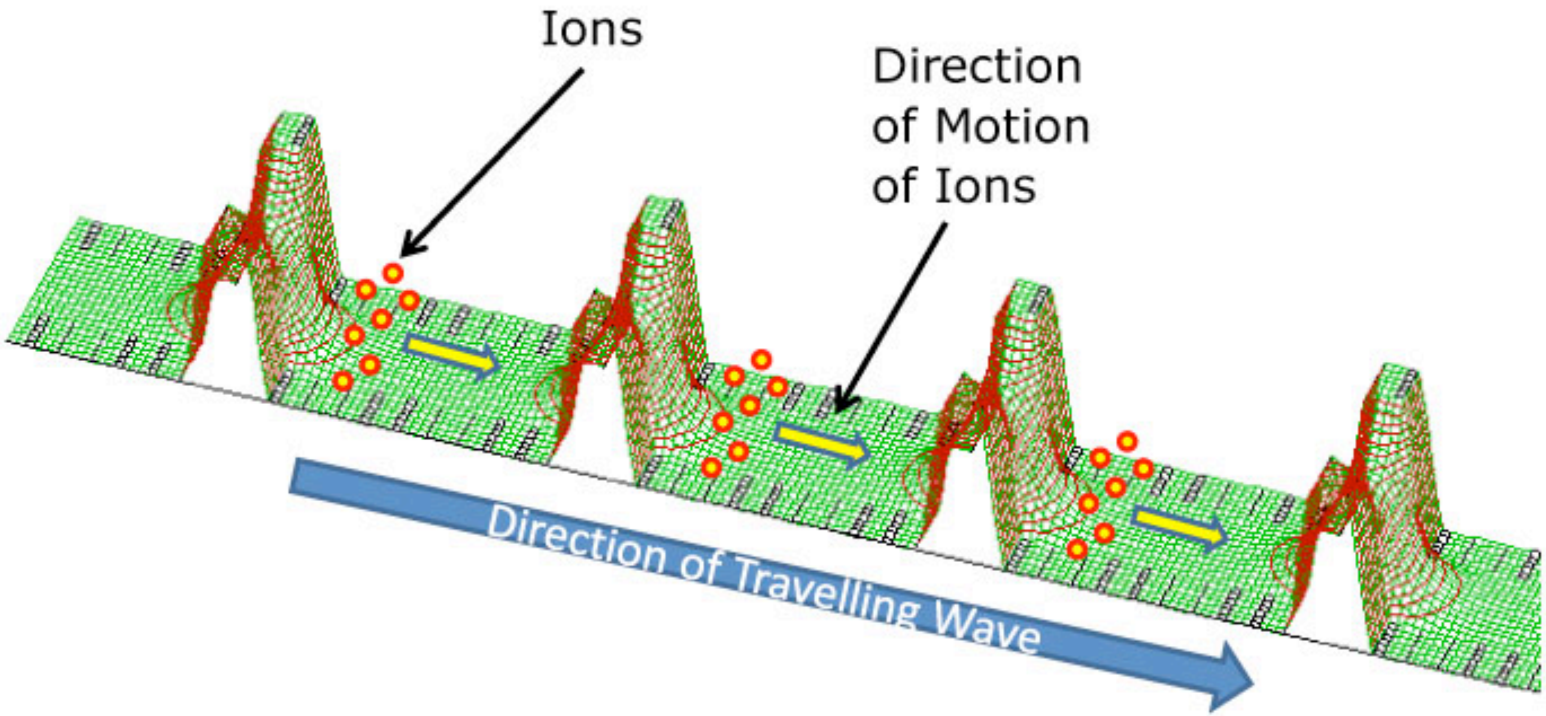


IMS Cell of Synapt G2-S

- 25.2 cm long drift cell
- 168 electrodes with 0.5 mm thickness and 1.0 mm spacing
- p ≈ 1-3 mBar

→ IMS Resolution **t/Δt ≈ 40**

Why can CCSs not be determined directly?



I) Non-uniform electric field

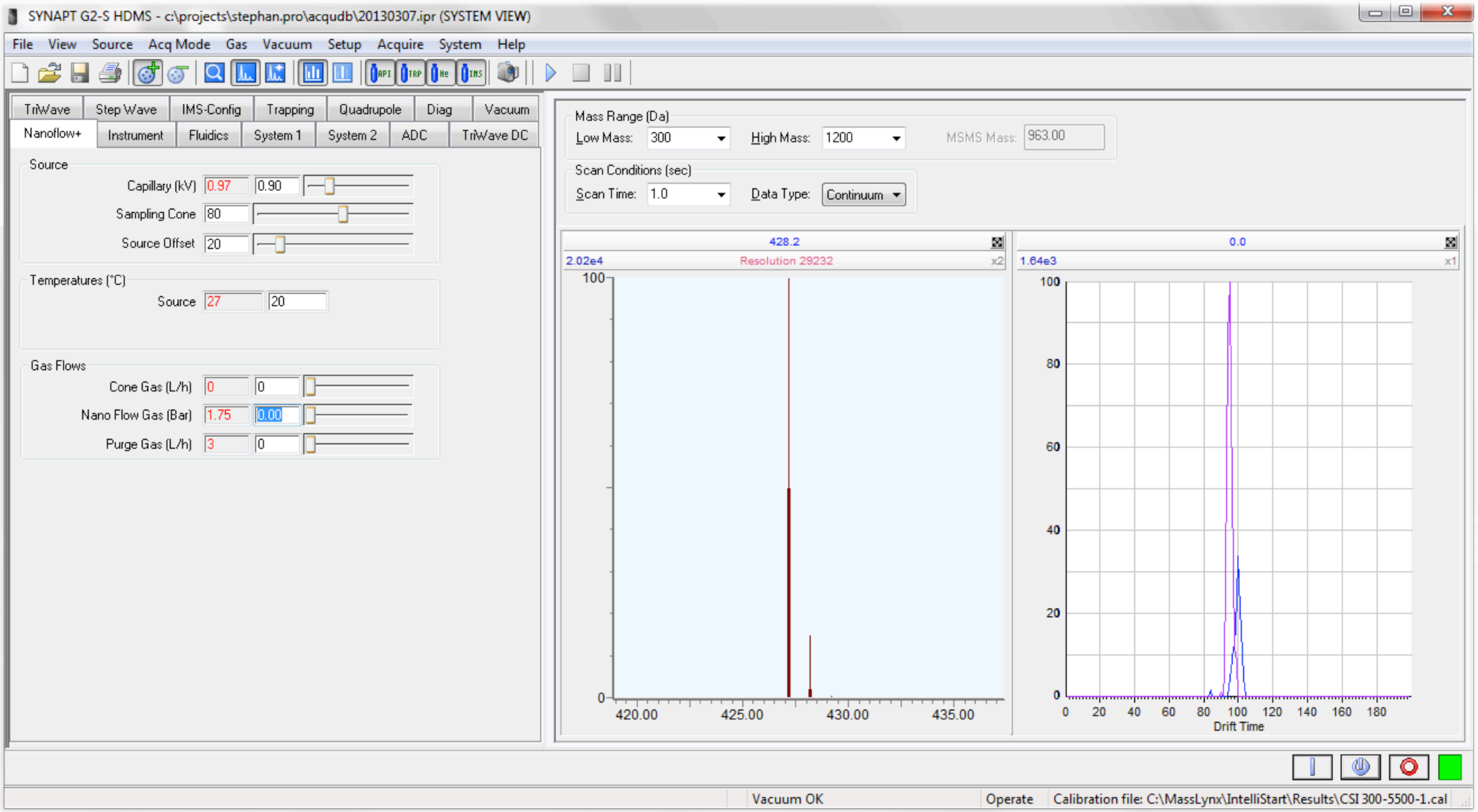
CCSs cannot be calculated directly from TW IMS data

II) Pressure reading

Pirani gauges do not read pressure accurate enough

Pringle, S. D.; Giles, K.; Wildgoose, J. L.; Williams, J. P.; Slade, S. E.; Thalassinos, K.; Bateman, R. H.; Bowers, M. T.; Scrivens, J. H. *Int. J. Mass Spectrom.* ***2007****, 261*, 1.

How to Calibrate?



I) Optimize Instrument Conditions for Separation

I) Optimize Instrument Conditions

I a) Make it Spray Properly

- Adjust needle position, capillary voltage, source conditions etc.

I b) Set Mass Range

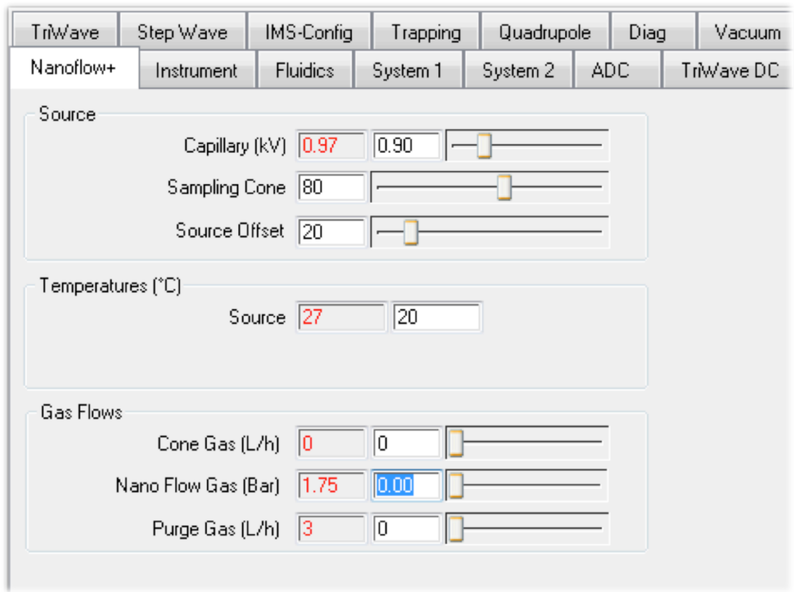
- Pusher frequency is set according to mass window

I c) Set Gas Flow

- Adjust gas flow in Trap, He Cell, IMS Cell to achieve best separation at maximum transmission

I d) Wave Height (WH) and Wave Velocity (WV)

- Adjust WH and WV to achieve maximum separation
- Do NOT use variable WV/WV
- Avoid roll-over and harmonic interferences with pusher



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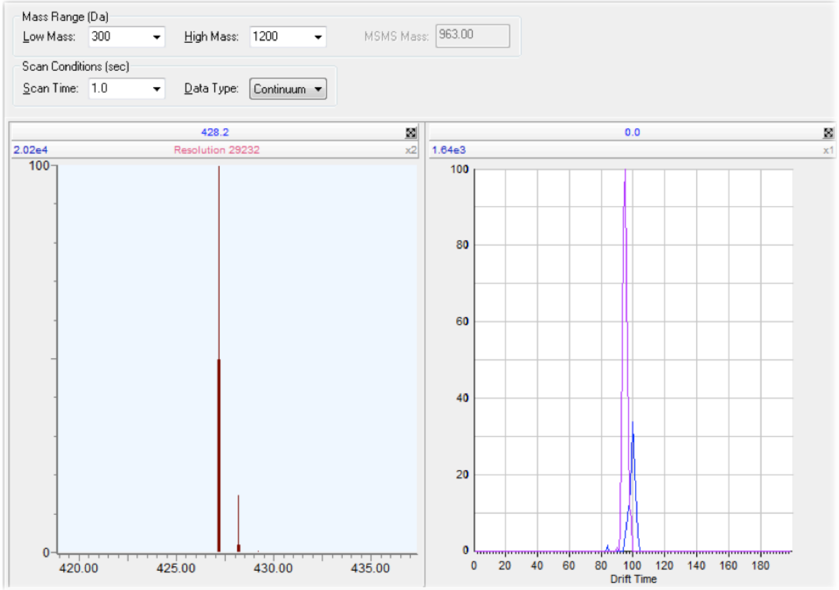

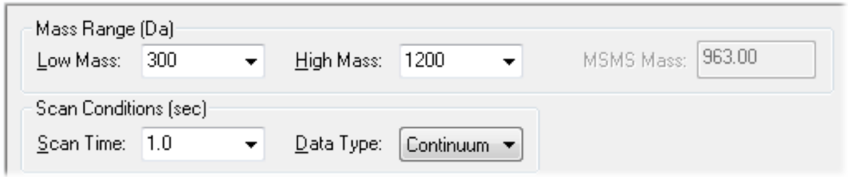

→ Mass range determines number of bins per ATD peak

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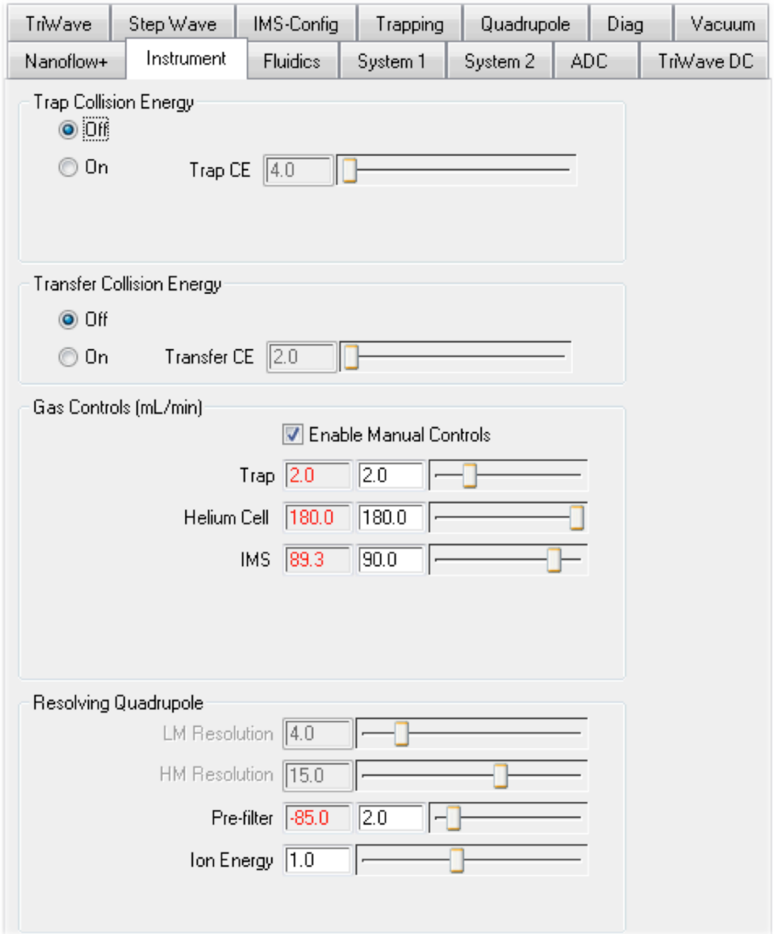

Readings of the vacuum gauges may be inaccurate

→ flow readings are not

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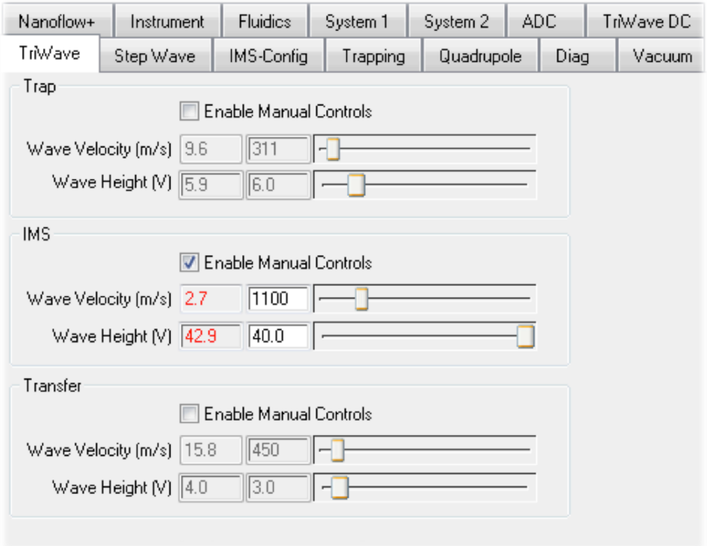
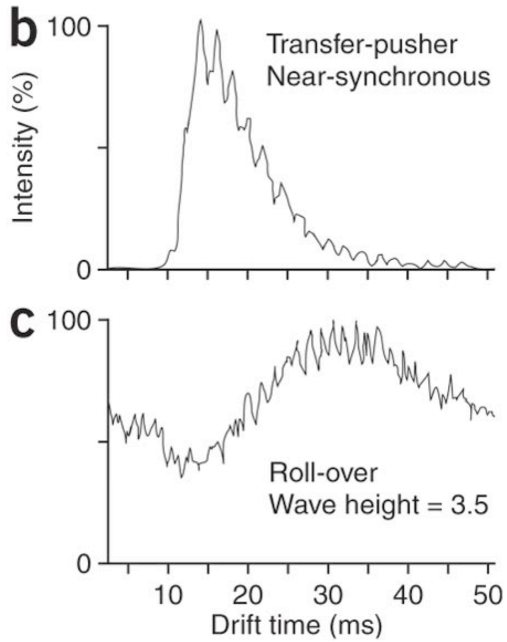




Ruotolo, B. T.; Benesch, J. L. P.; Sandercock, A. M.; Hyung, S.-J.; Robinson, C. V. *Nat. Protoc.* **2008**, *3*, 1139.

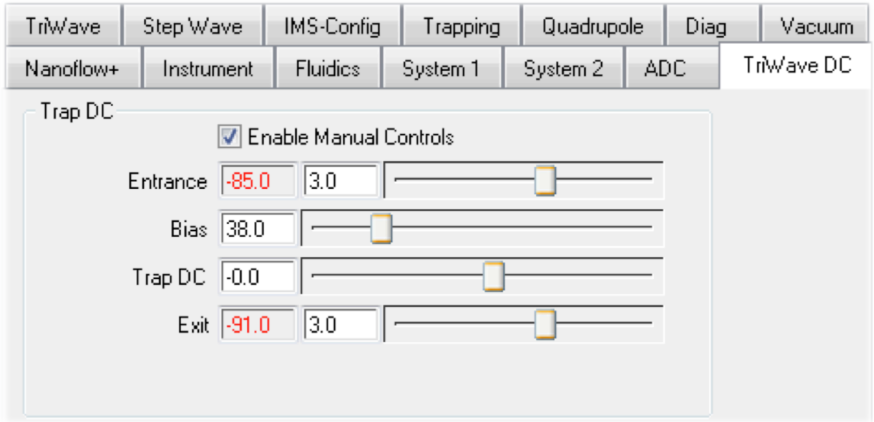
I) Optimize Instrument Conditions

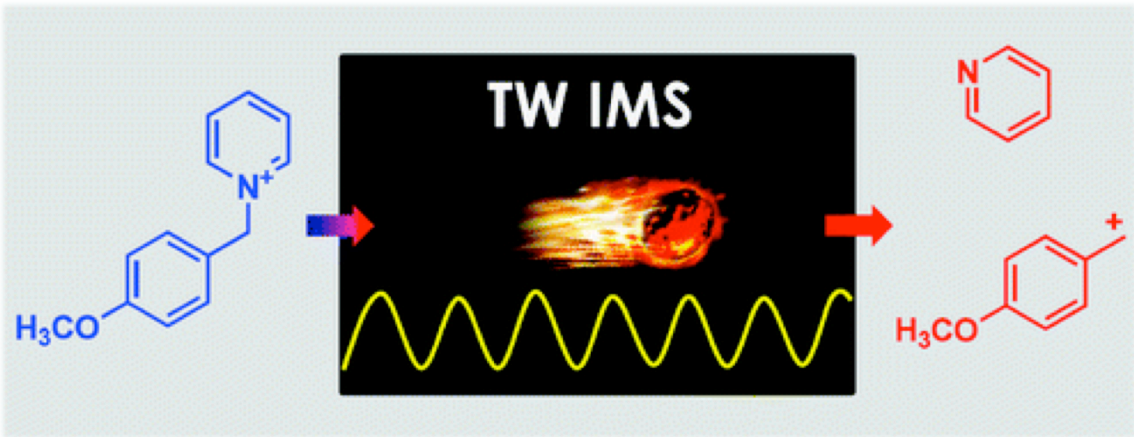
I e) **Set Trap DC bias**

- Injection into the He and IMS cell increases the temperature of the ions and can result in fragmentation

I f) **Save *ipr* File**

- All instrumental parameters are stored in *'extern.inf'*





Default value of 45 V is often too high for low *m/z* ions

→ reduce if necessary, even if transmission decreases

→ if needed adjust gas flow, WH and WV

Morsa, D.; Gabelica, V.; De Pauw, E. *Anal. Chem.* **2011**, *83 (14)*, 5775.
Merenbloom, S.I.; Flick, T.G.; Williams E.R. *J. Am. Soc. Mass Spectrom.* **2012**, *23 (3)*, 553.

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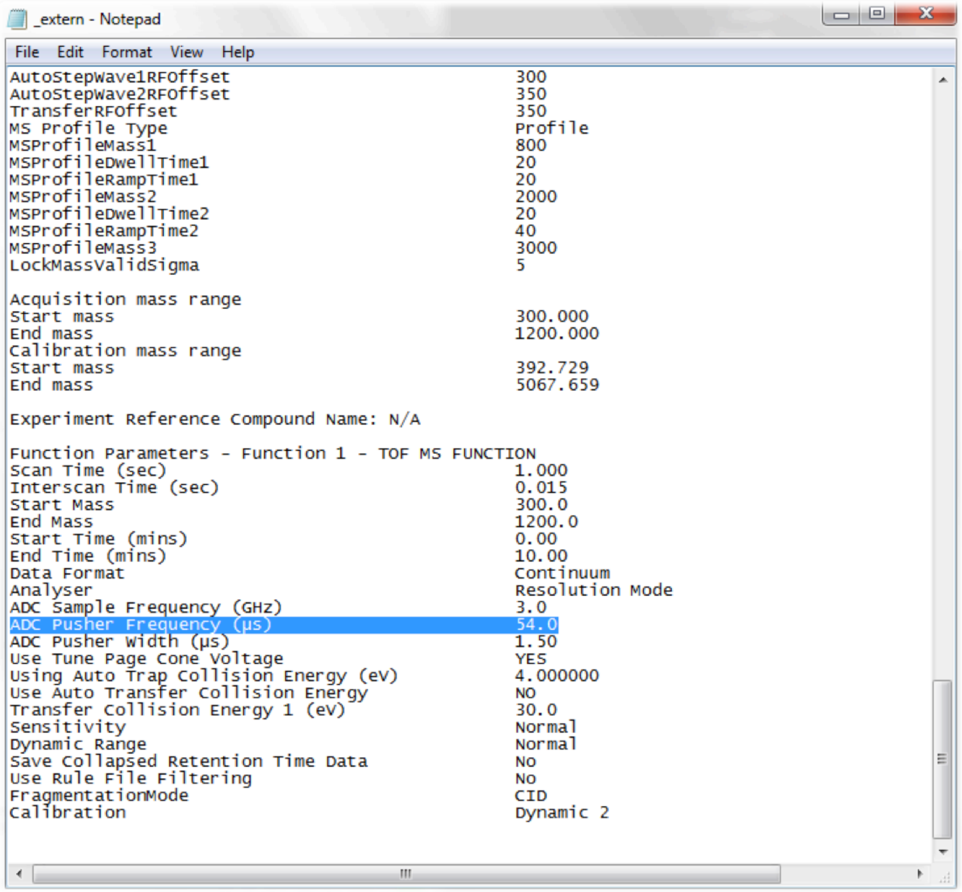


_extern - Notepad

File Edit Format View Help

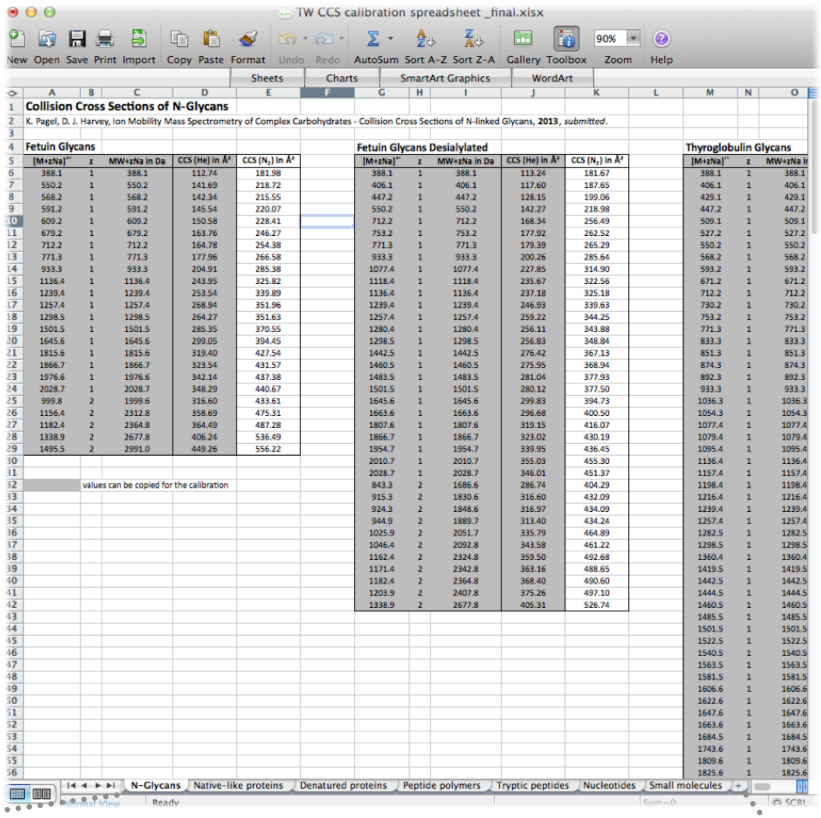
AutoStepWave1RFOffset	300
AutoStepWave2RFOffset	350
TransferRFOffset	350
MS Profile Type	Profile
MSProfileMass1	800
MSProfileDwellTime1	20
MSProfileRampTime1	20
MSProfileMass2	2000
MSProfileDwellTime2	20
MSProfileRampTime2	40
MSProfileMass3	3000
LockMassValidSigma	5
Acquisition mass range	
Start mass	300.000
End mass	1200.000
Calibration mass range	
Start mass	392.729
End mass	5067.659
Experiment Reference Compound Name: N/A	
Function Parameters - Function 1 - TOF MS FUNCTION	
Scan Time (sec)	1.000
Interscan Time (sec)	0.015
Start Mass	300.0
End Mass	1200.0
Start Time (mins)	0.00
End Time (mins)	10.00
Data Format	Continuum
Analyser	Resolution Mode
ADC Sample Frequency (GHz)	3.0
ADC Pusher Frequency (μs)	54.0
ADC Pusher Width (μs)	1.50
Use Tune Page Cone Voltage	YES
Using Auto Trap Collision Energy (eV)	4.000000
Use Auto Transfer Collision Energy	NO
Transfer Collision Energy 1 (eV)	30.0
Sensitivity	Normal
Dynamic Range	Normal
Save Collapsed Retention Time Data	NO
Use Rule File Filtering	NO
FragmentationMode	CID
Calibration	Dynamic 2

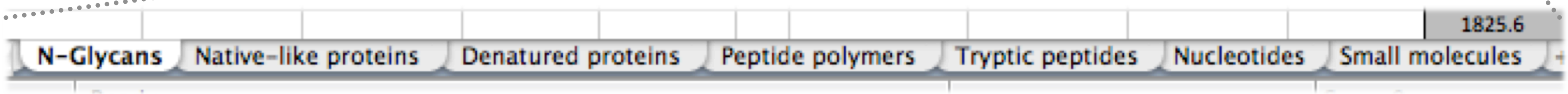
If *ipr* file is not saved prior to analysis, the wrong settings may be stored during data acquisition!

II) Measure Calibrants

II) Measure Calibrants

- Use calibrants of the same molecular type as analyte
- Typical calibrants and calibrant mixtures can be found in the attached xls file
- Use previously saved ipr file
- Do not change Gas flow, WH, WV, and mass range
- repeat for at least three different WHs or WVs





III) Measure Analyte

II) Measure Calibrants

- Use calibrants of the same molecular type as analyte
- Typical calibrants and calibrant mixtures can be found in the attached xls file
- Use previously saved ipr file
- Do not change Gas flow, WH, WV, and mass range
- repeat for at least three different WHs or WVs

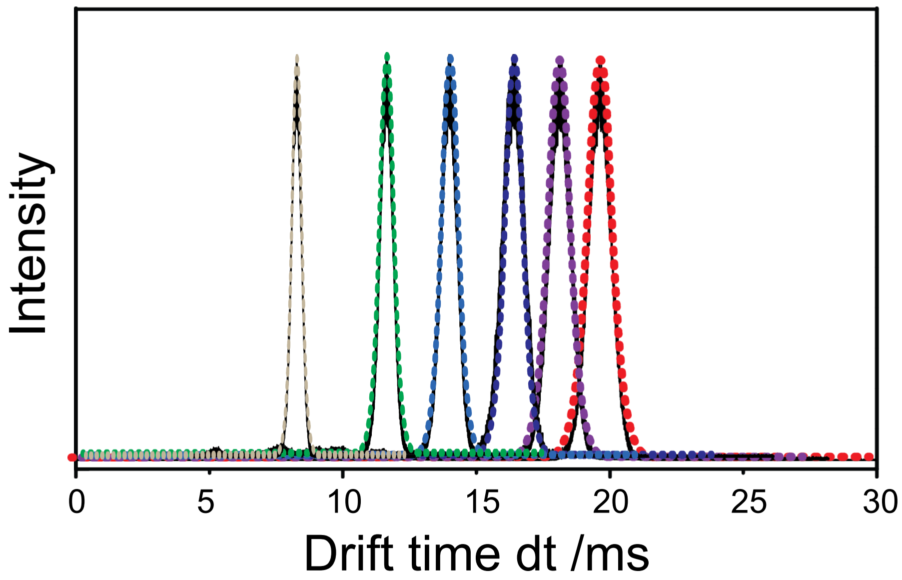
III) Measure Analyte

- Procedure similar to II)

IV) Determine Drift Time

IV) Determine Drift Time for Calibrants and Analyte

- Extract ATDs via DriftScope and MassLynx
- If required convert bins into dt by multiplying with ADC pusher frequency from '*extern.inf*'
- Fit with Gaussian to determine dt



Calibrant	dt
A	19 ms
B	18 ms
C	16 ms
D	14 ms
E	11 ms
F	8 ms

V) Create Calibration

V a) Correct dt for *m/z* dependent flight time

$$dt' = dt - C\sqrt{\frac{m}{z}} \quad (1)$$

- *C* is an empirically determined constant that depends on the instruments Enhanced Duty Cycle (EDC) delay coefficient (typically C = 0.001 x EDC delay)

  → Value can be found under *System – Acquisition Setup*

V b) Correct Ω for charge and reduced mass

$$\Omega' = \frac{\Omega}{z\sqrt{1/\mu}} \quad (2)$$

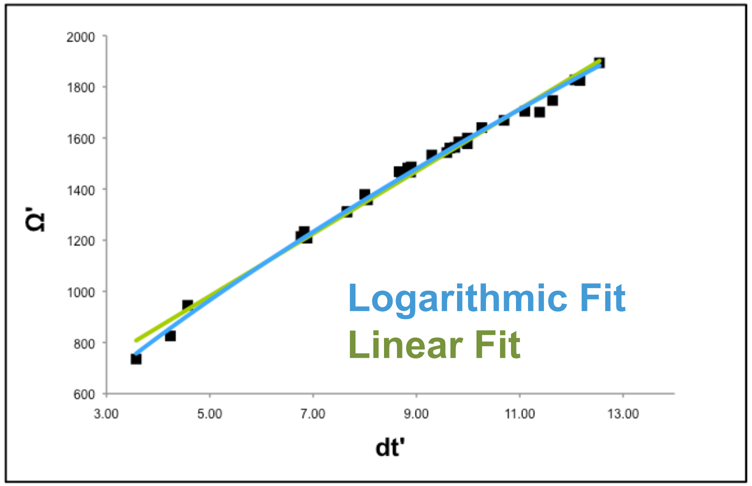
z	charge
$1/\mu$	reduced mass
Ω	literature Collision Cross Section

$$\frac{1}{\mu} = \frac{1}{M_{Ion}} + \frac{1}{M_{Gas}} \quad (3)$$

Ruotolo, B. T.; Benesch, J. L. P.; Sandercock, A. M.; Hyung, S.-J.; Robinson, C. V. *Nat. Protoc.* **2008**, *3*, 1139.
Bush, M. F.; Hall, Z.; Giles, K.; Hoyes, J.; Robinson, C. V.; Ruotolo, B. T. *Anal. Chem.* **2010**, *82*, 9557.

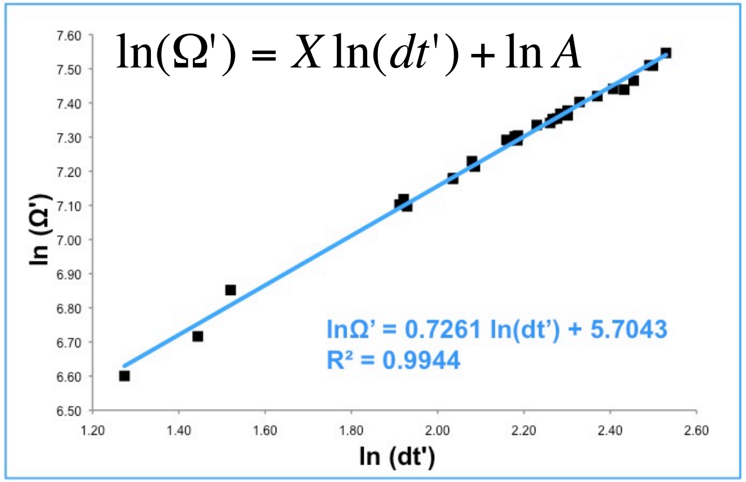
V) Create Calibration

V c) Create Calibration Plot

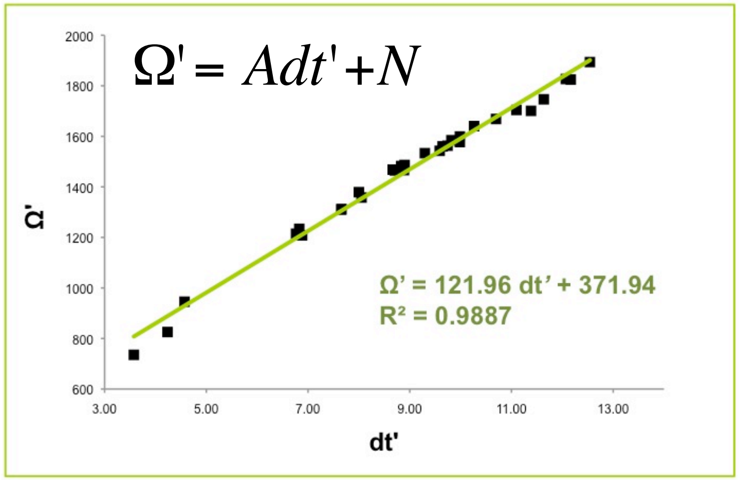


- Calibration plot can be generated as logarithmic fit or linear fit.
- R^2 value of the linearized fit provides a good indication which method is better
- R^2 should not be lower than 0.98

Logarithmic Fit



Linear Fit



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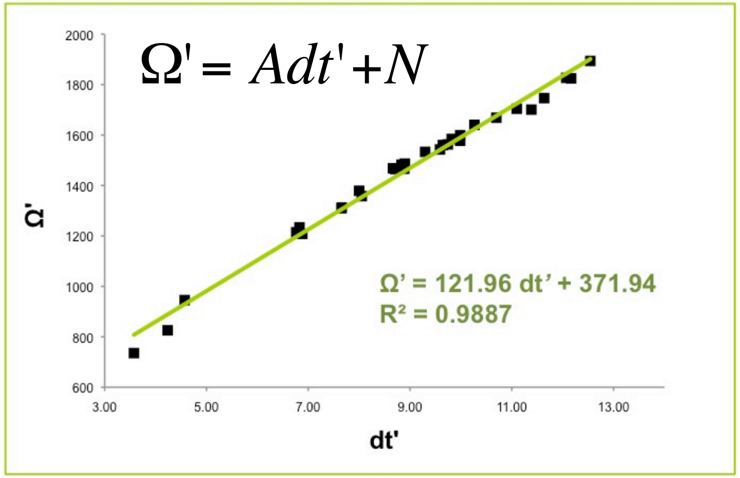
Thalassinos, K. et al. Anal. Chem. **2009,** *81*, 248.

VI) Estimate CCS

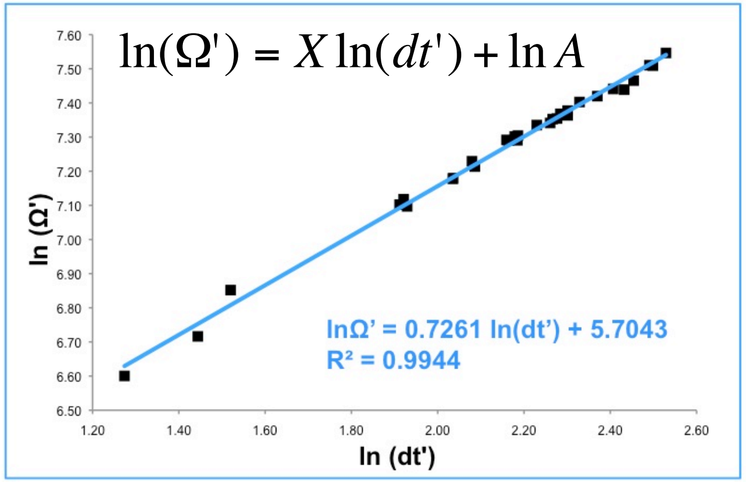
Linear Fit



- correct analyte dt using eq. *(1)*
- Calculate analyte Ω’ using dt’ and regression parameters A and N
- Calculate analyte Ω from Ω’ using eq. *(2)*

$$\Omega' = Adt' + N$$

Logarithmic Fit



$$\ln(\Omega') = X \ln(dt') + \ln A$$

$$\rightarrow \quad \Omega' = Adt'^{X}$$

$$\rightarrow \quad \Omega = A \boxed{z\sqrt{\frac{1}{\mu}}(dt')^{X}}$$

$$\Omega = A\,(dt'')$$

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Thalassinos, K. et al. Anal. Chem. **2009,** *81*, 248.

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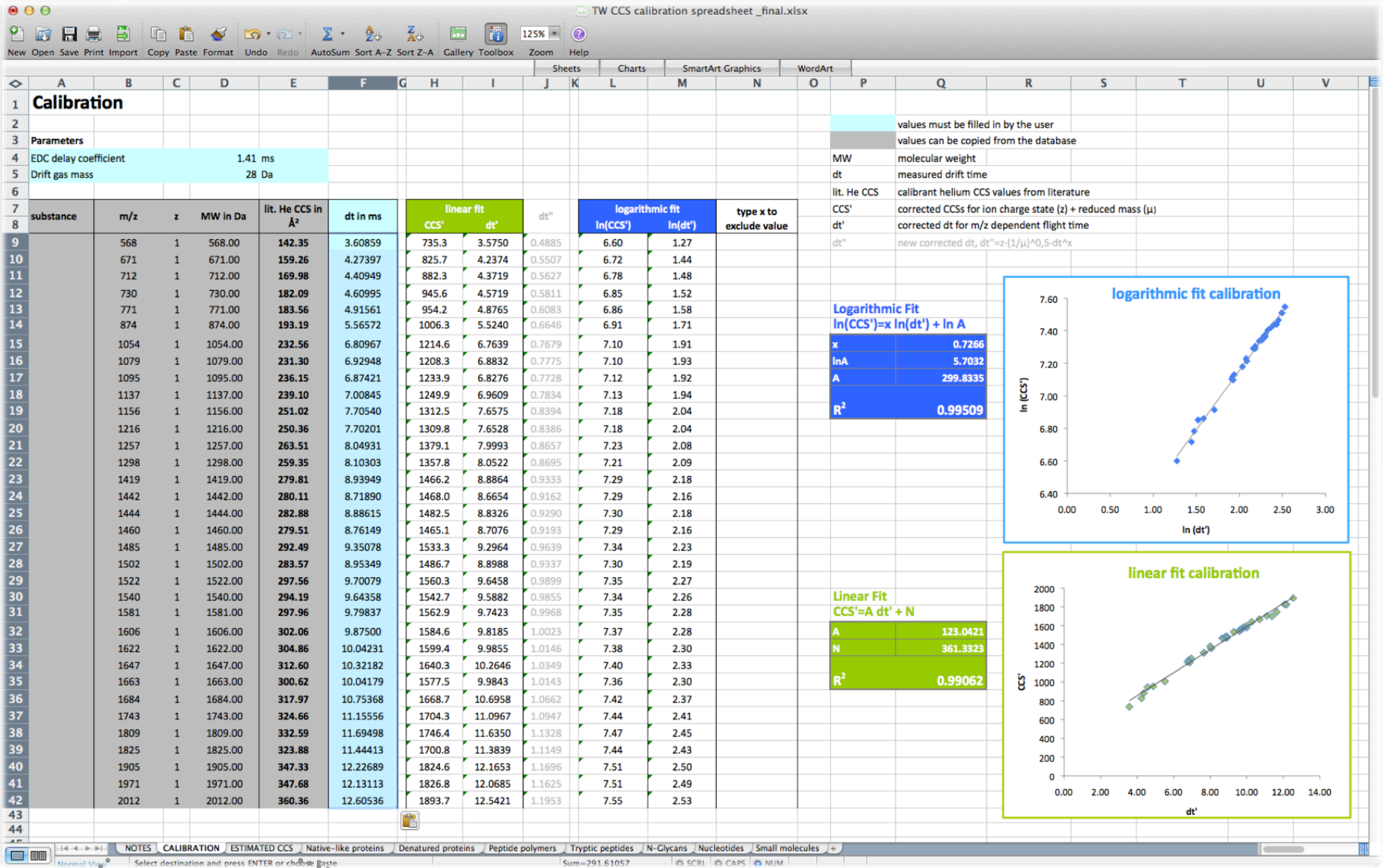


Calibration

Parameters

EDC delay coefficient	1.41	ms
Drift gas mass	28	Da

substance	m/z	z	MW in Da	lit. He CCS in Å²	dt in ms	linear fit CCS'	linear fit dt'	dt''	logarithmic fit ln(CCS')	logarithmic fit ln(dt')	type x to exclude value
	568	1	568.00	**142.35**	3.60859	735.3	3.5750	0.4885	6.60	1.27	
	671	1	671.00	**159.26**	4.27397	825.7	4.2374	0.5507	6.72	1.44	
	712	1	712.00	**169.98**	4.40949	882.3	4.3719	0.5627	6.78	1.48	
	730	1	730.00	**182.09**	4.60995	945.6	4.5719	0.5811	6.85	1.52	
	771	1	771.00	**183.56**	4.91561	954.2	4.8765	0.6083	6.86	1.58	
	874	1	874.00	**193.19**	5.56572	1006.3	5.5240	0.6646	6.91	1.71	
	1054	1	1054.00	**232.56**	6.80967	1214.6	6.7639	0.7679	7.10	1.91	
	1079	1	1079.00	**231.30**	6.92948	1208.3	6.8832	0.7775	7.10	1.93	
	1095	1	1095.00	**236.15**	6.87421	1233.9	6.8276	0.7728	7.12	1.92	
	1137	1	1137.00	**239.10**	7.00845	1249.9	6.9609	0.7834	7.13	1.94	
	1156	1	1156.00	**251.02**	7.70540	1312.5	7.6575	0.8394	7.18	2.04	
	1216	1	1216.00	**250.36**	7.70201	1309.8	7.6528	0.8386	7.18	2.04	
	1257	1	1257.00	**263.51**	8.04931	1379.1	7.9993	0.8657	7.23	2.08	
	1298	1	1298.00	**259.35**	8.10303	1357.8	8.0522	0.8695	7.21	2.09	
	1419	1	1419.00	**279.81**	8.93949	1466.2	8.8864	0.9333	7.29	2.18	
	1442	1	1442.00	**280.11**	8.71890	1468.0	8.6654	0.9162	7.29	2.16	
	1444	1	1444.00	**282.88**	8.88615	1482.5	8.8326	0.9290	7.30	2.18	
	1460	1	1460.00	**279.51**	8.76149	1465.1	8.7076	0.9193	7.29	2.16	
	1485	1	1485.00	**292.49**	9.35078	1533.3	9.2964	0.9639	7.34	2.23	
	1502	1	1502.00	**283.57**	8.95349	1486.7	8.8988	0.9337	7.30	2.19	
	1522	1	1522.00	**297.56**	9.70079	1560.3	9.6458	0.9899	7.35	2.27	
	1540	1	1540.00	**294.19**	9.64358	1542.7	9.5882	0.9855	7.34	2.26	
	1581	1	1581.00	**297.96**	9.79837	1562.9	9.7423	0.9968	7.35	2.28	
	1606	1	1606.00	**302.06**	9.87500	1584.6	9.8185	1.0023	7.37	2.28	
	1622	1	1622.00	**304.86**	10.04231	1599.4	9.9855	1.0146	7.38	2.30	
	1647	1	1647.00	**312.60**	10.32182	1640.3	10.2646	1.0349	7.40	2.33	
	1663	1	1663.00	**300.62**	10.04179	1577.5	9.9843	1.0143	7.36	2.30	
	1684	1	1684.00	**317.97**	10.75368	1668.7	10.6958	1.0662	7.42	2.37	
	1743	1	1743.00	**324.66**	11.15556	1704.3	11.0967	1.0947	7.44	2.41	
	1809	1	1809.00	**332.59**	11.69498	1746.4	11.6350	1.1328	7.47	2.45	
	1825	1	1825.00	**323.88**	11.44413	1700.8	11.3839	1.1149	7.44	2.43	
	1905	1	1905.00	**347.33**	12.22689	1824.6	12.1653	1.1696	7.51	2.50	
	1971	1	1971.00	**347.68**	12.13113	1826.8	12.0685	1.1625	7.51	2.49	
	2012	1	2012.00	**360.36**	12.60536	1893.7	12.5421	1.1953	7.55	2.53	

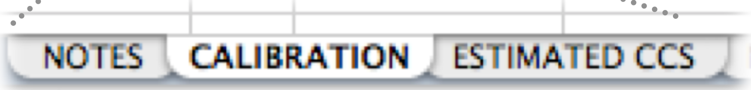
	values must be filled in by the user
	values can be copied from the database
MW	molecular weight
dt	measured drift time
lit. He CCS	calibrant helium CCS values from literature
CCS'	corrected CCSs for ion charge state (z) + reduced mass (μ)
dt'	corrected dt for m/z dependent flight time
dt''	new corrected dt, dt''=z·(1/μ)^0,5·dt'^x

Logarithmic Fit
ln(CCS')=x ln(dt') + ln A

x	0.7266
lnA	5.7032
A	299.8335
R^2	0.99509

Linear Fit
CCS'=A dt' + N

A	123.0421
N	361.3323
R^2	0.99062



VII) Repeat procedure for different WH or WV

Estimated CCSs can be averaged when no systematic changes occur