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

Johanna Hofmann and Kevin Pagel

Fritz Haber Institute of the Max Planck Society, Department of Molecular Physics, Berlin, Germany

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



Drift Tube IMS





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

Collision Cross Section Determination



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





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





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

Instrumentation - Synapt G2-S





Synapt IMS Cell





Why can CCSs not be determined directly?





I) Non-uniform electric field

II) Pressure reading

CCSs cannot be calculated directly from TW IMS data

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 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



TriWave	Step Wave	IMS-Config	Trapping	Quadrupo	ole Diag	g Vacuum
Nanoflow+	Instrument	Fluidics	System 1	System 2	ADC	TriWave DC
Source	Capillary Sampling ((kV) <mark>0.97</mark> Cone 80	0.90 -	-]	Ξ	
	Source O	ffset 20				
─ Temperatu	res (°C) So	urce 27	20			
Gas Flows						
	Cone Gas (l	./h) 0	0		_	
N	lano Flow Gas (Bar) 1.75	0.00		_	
	Purge Gas (I	./h) <mark>3</mark>	0		Ξ	



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– Mass Range Low Mass:	(Da) 300	•	<u>H</u> igh Mass:	1200 🔻	MSMS Mass:	963.00
- Scan Conditi	ions (sec)					
<u>S</u> can Time:	1.0	•	<u>D</u> ata Type:	Continuum 🔻		

→ Mass range determines number of bins per ATD peak



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TriWave	Step Wave	IMS-Config	Trapping	Quadrupo	ole Dia	g Vacuum
Nanoflow+	Instrument	Fluidics	System 1	System 2	ADC	TriWave DC
Trap Collisi	on Energy					
🔘 On	Trap CE	4.0]		-	
- Transfer Co	ollision Energy					
💿 Off						
🔘 On	Transfer CE	2.0]		-	
- Gas Contro	Is (mL/min) T Helium C IM	 ✓ Enab rap (2.0 ell (180.0 4S (89.3) 	le Manual Cor 2.0 180.0 90.0	ntrols 		
- Resolving (Quadrupole				_	
	LM Resolut	ion 4.0			_	
	HM Resolut	ion 15.0]		_	
	Pre-fi	lter -85.0	2.0 -]	_	
	lon Ene	rgy 1.0]	-	

Readings of the vacuum gauges may be inaccurate

 \rightarrow flow readings are not



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Nanoflow+	Instrument	Fluidics	System 1	System 2	ADC	TriWave DC
TriWave	Step Wave	IMS-Config	Trapping	Quadrupo	ole Dia	g Vacuum
Trap	E	nable Manual	Controls			
Wave Vel	ocity (m/s) 9.6	311	-]		_	
Wave	Height (V) 5.9	6.0	—]—		=	
IMS						
	📝 E	nable Manual	Controls			
Wave Vel	ocity (m/s) 2.7	1100	[]		_	
Wave	Height (V) 42.9	40.0			-	
Transfer						
	E	nable Manual	Controls			
Wave Vel	ocity (m/s) [15.8	450	-]		_	
Wave	Height (V) 4.0	3.0			_	









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.



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'

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

extern - Notepad		
File Edit Format View Help		
AutoStepWave1RFOffset AutoStepWave2RFOffset TransferRFOffset MS Profile Type MSProfileMass1 MSProfileRampTime1 MSProfileMass2 MSProfileRampTime2 MSProfileRampTime2 MSProfileRass3 LockMassValidSigma	300 350 350 Profile 800 20 20 200 200 200 20 40 3000 5	•
Acquisition mass range Start mass End mass Calibration mass range Start mass End mass Experiment Reference Compound Name: N/A Function Parameters - Function 1 - TOF MS FUNCT: Scan Time (sec) Interscan Time (sec) Start Mass End Mass Start Time (mins) Data Format Analyser ADC Pusher Frequency (GH2) ADC Pusher Width (µs) Use Tune Page Cone Voltage Using Auto Trap Collision Energy (eV) Use Auto Transfer Collision Energy 1 (eV) Sensitivity	300.000 1200.000 392.729 5067.659 ION 1.000 0.015 300.0 1200.0 0.00 10.00 Continuum Resolution Mode 3.0 54.0 1.50 YES 4.000000 No S0.0 Normal	
Save Collapsed Retention Time Data Use Rule File Filtering FragmentationMode Calibration	No No CID Dynamic 2	

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

	☆ 1 Col	A lision Cr	B C ross Sections o	D f N-Glycans	Sheets E	Charts F	G H	rtArt Graphics	Word/ J	K	L M	N
♦ Use calibrants of the same molecular	2 K. Pa 3 4 Fetu	in Glyca	ns	Mass Spectrome	try of Complex Ca	rbohydrates -	Fetuin Glycan	s Desialylated	d Glycans, 2013,	submitted.	Thyroglobuli	in Glycans
type as analyte	6 7 7 8 9 10 6 11 12 13	*2Naj 188.1 150.2 168.2 191.2 109.2 179.2 112.2 112.2 113	I 388.1 1 388.1 1 550.2 1 568.2 1 591.2 1 609.2 1 712.2 1 771.3	112.74 112.74 141.69 142.34 145.54 150.58 163.76 164.78 177.96	2181.98 218.72 215.55 220.07 228.41 246.27 254.38 266.58		[M+2Na] 2 388.1 1 406.1 1 447.2 1 550.2 1 712.2 1 753.2 1 771.3 1 933.3 1	MW+2Na in Da 388.1 406.1 447.2 550.2 712.2 753.2 771.3 933.3	113.24 117.60 128.15 142.27 168.34 177.92 179.39 200.26	181.67 187.65 199.06 218.98 256.49 262.52 265.29 285.64	(M+2X4) 388.1 406.1 429.1 447.2 509.1 527.2 550.2 558.2	1 38 1 40 1 42 1 44 1 50 1 52 1 55 1 56
 Typical calibrants and calibrant mixtures app be found in the attached via file 	14 15 16 17 18 19 19	133.3 136.4 239.4 257.4 298.5 501.5	1 933.3 1 1136.4 1 1239.4 1 1257.4 1 1298.5 1 1501.5 1 1606.6	204.91 243.95 253.54 268.94 264.27 285.35	285.38 325.82 339.89 351.96 351.63 370.55		1077.4 1 1118.4 1 1136.4 1 1239.4 1 1257.4 1 1280.4 1	1077.4 1118.4 1136.4 1239.4 1257.4 1280.4	227.85 235.67 237.18 246.93 259.22 256.11	314.90 322.56 325.18 339.63 344.25 343.88 240.04	593.2 671.2 712.2 730.2 753.2 771.3	1 59 1 67 1 71 1 73 1 75 1 77
can be found in the attached xis file	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	645.6 815.6 866.7 976.6 028.7 199.8 156.4	1 1645.6 1 1815.6 1 1866.7 1 1976.6 1 2028.7 2 1999.6 2 2312.8	299.05 319.40 323.54 342.14 348.29 316.60 358.69	394,45 427,54 431,57 437,38 440,67 433,61 475,31		1298.5 1 1442.5 1 1460.5 1 1483.5 1 1501.5 1 1645.6 1 1663.6 1	1298.5 1442.5 1460.5 1483.5 1501.5 1645.6 1663.6	256.83 276.42 275.95 281.04 280.12 299.83 296.68	348.84 367.13 368.94 377.93 377.50 394.73 400.50	833.3 851.3 874.3 93.3 1036.3 1054.3	1 83 1 85 1 87 1 89 1 93 1 10 1 10
 Use previously saved ipr file 	27 1 28 1 29 1 30 31 32	182.4 338.9 495.5	2 2364.8 2 2677.8 2 2991.0	364,49 406.24 449.26 or the calibration	487.28 536.49 556.22		1807.6 1 1866.7 1 1954.7 1 2010.7 1 2028.7 1 843.3 2	1807.6 1866.7 1954.7 2010.7 2028.7 1686.6	319.15 323.02 339.95 355.03 346.01 286.74	416.07 430.19 436.45 455.30 451.37 404.29	1077.4 1079.4 1095.4 1136.4 1157.4 1198.4	1 107 1 107 1 109 1 113 1 115 1 115
 Do not change Gas flow, WH, WV, and mass range 	33 34 35 36 37 38 39 40 41 42 43						915.3 2 924.3 2 944.9 2 1025.9 2 1046.4 2 1162.4 2 1162.4 2 1182.4 2 1203.9 2 1338.9 2	1830.6 1848.6 1848.7 2051.7 2051.7 2092.8 2324.8 2324.8 2342.8 2342.8 2342.8 2342.8 2342.8 2342.8 2342.8	316.60 316.97 313.40 335.79 343.58 359.50 363.16 368.40 375.26 405.31	432.09 434.09 434.24 464.89 461.22 492.68 488.65 490.60 497.10 526.74	1216.4 1239.4 1257.4 1282.5 1298.5 1360.4 1419.5 1442.5 1444.5 1460.5 1485.5	1 121 1 123 1 125 1 125 1 128 1 129 1 136 1 141 1 144 1 144 1 144 1 144
 repeat for at least three different 	45 46 47 48										1522.5 1540.5 1563.5 1581.5	1 152 1 154 1 156 1 159
WHs or WVs	50 51 52 53 54 55										1602.6 1622.6 1647.6 1663.6 1684.5 1743.6 1809.6	1 160 1 162 1 164 1 166 1 168 1 174
			N-Glyc	ans Native-li ady	ike proteins 📜 🛛	Denatured pr	roteins Pept	tide polymers 🚽	Tryptic peptid	s Nucleotides	Small molecules +	- G SC
	6 a a a .											



II) Measure Calibrants

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- Use previously saved ipr file
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- repeat for at least three different WHs or WVs

III) Measure Analyte

• Procedure similar to II)



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
А	19 ms
В	18 ms
С	16 ms
D	14 ms
Е	11 ms
F	8 ms

V) Create Calibration



Va) Correct dt for *m/z* dependent flight time

$$dt' = dt - C_{\sqrt{\frac{m}{z}}} \qquad (1)$$

• C is an empirically determined constant that depends on the instruments Enhanced Duty Cycle (EDC) delay coefficient (typically $C = 0.001 \times EDC$ delay)

 \rightarrow Value can be found under System – Acquisition Setup

Vb) Correct Ω for charge and reduced mass

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

Ζ charge 1/μ reduced mass

Ω literature Collision Cross Section

 $\frac{1}{\mu} = \frac{1}{M_{Ior}} + \frac{1}{M_{Cor}}$

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.

(3)

V) Create Calibration



V c) Create Calibration Plot



- Calibration plot can be generated as logarithmic fit or linear fit.
- R² value of the linearized fit provides a good indication which method is better
- R² should not be lower than 0.98

Logarithmic Fit



Linear Fit



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



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

$$\rightarrow \Omega' = Adt'^X$$

$$\Rightarrow \quad \Omega = A \left[z \sqrt{\frac{1}{\mu}} (dt')^X \right]$$

$$\Omega = A\left(dt^{\prime\prime}\right)$$

Ruotolo, B. T.; Benesch, J. L. P.; Sandercock, A. M.; Hyung, S.-J.; Robinson, C. V. *Nat. Protoc.* **2008**, *3*, 1139. Thalassinos, K. et al. Anal. Chem. **2009**, *81*, 248.

VI) Estimate CCS



						-		Shee	ts Chart	s Smart	Art Graphics	WordA	rt	1			1		
A	В	С	D	E	F	GH		J	K L	M	N	0	Р	Q	R	S	Т	U	
Calibrat	on																		
														values must be filled i	n by the user				
Parameters														values can be copied	from the database	e			
EDC delay coeff	icient		1.41	. ms									MW	molecular weight					
Drift gas mass			20	o Da									lit. He CCS	calibrant belium CCS	values from literat	ture			
				lit. He CCS in		lin	ear fit	1.0	logari	thmic fit	type x to	1	CCS'	corrected CCSs for ior	n charge state (z) +	+ reduced ma	iss (µ)		
substance	m/z	z	MW in Da	Ų	dt in ms	CCS'		dt"	In(CCS')	ln(dt')	exclude value		dt'	corrected dt for m/z d	lependent flight t	ime			
	568	1	568.00	142.35	3.60859	735.3	3.5750	0.4885	6.60	1.27			dt"	new corrected dt, dt"	=z·(1/μ)^0,5·dt^x				
	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			Localtha		7.60	log	arithmic fit c	alibration	
	7/1 874	1	771.00	183.56	4.91561	954.2	4.8765	0.6083	6.85	1.58			In(CCS')=	$\ln(dt') + \ln A$	- 1				
	1054	1	1054.00	232.56	6 80967	1214.6	6 7639	0.7679	7.10	1.71				0 7266	7.40				
	1079	1	1079.00	231.30	6.92948	1208.3	6,8832	0.7775	7.10	1.93			InA	5.7032	7.00			2	
	1095	1	1095.00	236.15	6.87421	1233.9	6.8276	0.7728	7.12	1.92			A	299.8335	7.20			£	
	1137	1	1137.00	239.10	7.00845	1249.9	6.9609	0.7834	7.13	1.94					<u> </u>			/	
	1156	1	1156.00	251.02	7.70540	1312.5	7.6575	0.8394	7.18	2.04			R ²	0.99509	5				
	1216	1	1216.00	250.36	7.70201	1309.8	7.6528	0.8386	7.18	2.04					6.80		7		
	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					6.60		•		
	1419	1	1419.00	279.81	8.93949	1466.2	8.8864	0.9333	7.29	2.18		-			6.40				
	1442	1	1442.00	280.11	8.71890	1468.0	8.6654	0.9162	7.29	2.16					0.00	0 0.50	1.00 1.50	2.00 2.50	
	1444	1	1444.00	282.88	8 76149	1462.5	8 7076	0.9290	7.30	2.16							In (dt')		
	1485	1	1485.00	292.49	9.35078	1533.3	9,2964	0.9639	7.34	2.23					-L		()		
	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					2000	I	inear fit calib	ration	
	1540	1	1540.00	294.19	9.64358	1542.7	9.5882	0.9855	7.34	2.26			Linear Fit		2000				•
	1581	1	1581.00	297.96	9.79837	1562.9	9.7423	0.9968	7.35	2.28			CCS'=A dt	' + N	1800				
	1606	1	1606.00	302.06	9.87500	1584.6	9.8185	1.0023	7.37	2.28			A	123.0421	1600			A Sector	
	1622	1	1622.00	304.86	10.04231	1599.4	9.9855	1.0146	7.38	2.30			N	361.3323	1400				
	1647	1	1647.00	312.60	10.32182	1640.3	10.2646	1.0349	7.40	2.33			D ²	0.99062	1200				
	1684	1	1684.00	317.97	10.75368	1668 7	10.6958	1.0562	7.30	2.50				0.33002	8 1000		AN .		
	1743	1	1743.00	324.66	11.15556	1704.3	11.0967	1.0947	7.44	2.41					600	4			
	1809	1	1809.00	332.59	11.69498	1746.4	11.6350	1.1328	7.47	2.45					400				
	1825	1	1825.00	323.88	11.44413	1700.8	11.3839	1.1149	7.44	2.43					400				
	1905	1	1905.00	347.33	12.22689	1824.6	12.1653	1.1696	7.51	2.50					200				
	1971	1	1971.00	347.68	12.13113	1826.8	12.0685	1.1625	7.51	2.49					0.00	2.00 4	.00 6.00 8.00	10.00 12.00	14
	2012	1	2012.00	360.36	12.60536	1893.7	12.5421	1.1953	7.55	2.53							dt'		
						*													_
	NOTES	CALIBR	ATION JESTIM	ATED CCS Native	-like proteins	Denatured prot	eins Peptide	polymers J	Tryptic peptides	N-Glycans Nu	cleotides Small mol	ecules	+ /						
Normal Vie	Select of	lestinati	on and press E	NTER or choose gas	te			Su	m=291.61057	O SCRL	O CAPS O NUM								

						*****					_						_	_	
						~ 0	****							nnant	nro	00	dura	for	

Estimated CCSs can be averaged when no systematic changes occur