## Creating a calibration

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7	A 140.28	560.55	28.0134	0.09		0.92	sec (m/	(z) indeper	ident			0.085	sec (m/	z) dependent	)	-								=
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10	Charge State	m/z	Published Cross Sections	Corrected Cross Sections	Arrival T	imes		Cross Se	ction (Po	wer)	Cross Se	ction (Line	ar) ti	d)		t(d)		ti	(d)'			sqoot(1/F	Reduce	
12				2	4						-		a a											1
13	2	507.30	252.18	658.320945	19			241.214	0		253.926	214.72733	0	0.72946 0		1.71	0		0.79	0		0.19153	0.1915	
14	2	536.32	253.92	663.347492	20			249.9	0		258.482	214.57058	0	0.81775 0		1.8	0	1	0.88	0		0.19139	0.1913	1
15	2	568.78	259.73	679.020895	21			257.927	0		263.023	214.4141	C	.90589 0		1.89	0	100	0.97	0		0.19125	0.1912	1
16	2	656.84	282.95	740.924181	25			285.888	0		281.627	214.06728		.26111 0		2.25	0		1.33	0		0.19094	0.1909	1
17	2	626.33	278.59	729.135429	24			279.541	0		277.033	214.17643		.17273 0		2.16	0		1.24	0		0.19104	0.1910	1
18	2	724.40	310.43	813.677412	30			314.32	0		305.25	213.85814		.70766 0		2.7	0		1.78	0		0.19076	0.1907	1
19	2	693.87	302.28	791.983815	28			303.673	0		295.823	213.94762		1.5292 0		2.52	0		1.6	0		0.19084	0.1908	6
20	2	809.92	354.9	931.174513	42			366.427	0		362.464	213.6433		2.7835 0		3.78	0		2.86	0		0.19057	0.1905	1
21	2	1156.57	415.18	1092.12689	55			407.862	0		423.136	213.09717	3	1.93859 0		4.95	0		4.03	0		0.19008	0.1900	1
22	2	593.34	263.79	689.979494	22			265.516	0		267.645	214.30707	9	0.99453 0		1.98	0		1.06	0		0.19116	0.1911	
23	2	640.36	287.78	753.368911	26			292.322	0		286.572	214.12495		.35198 0		2.34	0		1.42	0		0.191	0.191	
24	2	664.84	286.28	749.738502	26			292.119	0		286.389	214.0403		.35069 0		2.34	0		1.42	0		0.19092	0.1909	1
25	2	766.35	299	784.126239	29			308.702	0		300.166	213.74677		.61559 0		2.61	0		1.69	0		0.19066	0.1906	1
26	2	977.45	363.5	955.13459	42			365.578	0		361.532	213.33111	2	.77596 0		3.78	0		2.86	0		0.19029	0.1902	1
27	2	857.45	375.98	986.94981	46			380.52	0		381.413	213.54236	3	.14129 0		4.14	0		3.22	0		0.19048	0.1904	1
28	2	1134.0853	422.37	1110.90811	56			410.862	0		428.034	213.12249	4	.02948 0		5.04	0	2	4.12	0		0.1901	0.190	1

Step 1: Fill experimental parameters (purple-coloured cells)

Step 2: Enter published cross section values and charge state, m/z, arrival times as measured on the Synapt  $\Lambda$ 

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9 10	Protein Analysed	digests	*																					
11	Charge State	m/z	Published Cross Sections	Corrected Cross Sections	Arriv	ral Times		Cross See	tion (Po	wer)	Cross Se	ction (Line	ar)	t(d)''			t(d)			t(d)'			sqoot(1/F	leduce
13	2	507.30	252.18	658.320945		19		241.214	0		253.926	214.72733		0.72946	0		1.71	0		0.79	0		0.19153	0.1915
14	2	536.32	253.92	663.347492		20		249.9	0		258.482	214.57058		0.81775	0		1.8	0		0.88	0		0.19139	0.1913
15	2	568.78	259.73	679.020895		21	-	257.927	0		263.023	214.4141		0.90589			1.89	0		0.97	0		0.19125	0.1912
16	2	656.84	282.95	740.924181		25	-	285.888	0		281.62/	214.06/28		1.26111	0		2.25	0		1.33	0		0.19094	0.1909
17	2	724.40	210.39	729.135429		20		214.22	0		205 25	214.17043		1.1/2/3			2.10	0		1.24	0		0.19104	0.1910
19	2	693.87	302.28	791.983815		28		303.673	0		295.823	213.94762		1.5292	0		2.52	0		1.6	0		0.19084	0.1908
20	2	809.92	354.9	931,174513		42		366.427	0		367.464	213.6433		2.7835	ő		3.78	0		2.86	0		0.19057	0.1905
21	2	1156.57	415.18	1092,12689		55		407,862	0		423,136	213.09717		3,93859	ő		4.95	0		4.03	ő		0.19008	0,1900
22	2	593.34	263.79	689.979494		22		265.516	0		267.645	214.30707		0.99453	0		1.98	0		1.06	0		0.19116	0.1911
23	2	640.36	287.78	753.368911		26		292.322	0		286.572	214.12495		1.35198	0		2.34	0		1.42	0		0.191	0.191
24	2	664.84	286.28	749.738502		26		292.119	0		286.389	214.0403		1.35069	0		2.34	0		1.42	0		0.19092	0.1909
25	2	766.35	299	784.126239		29		308.702	0		300.166	213.74677		1.61559	0		2.61	0		1.69	0		0.19066	0.1906
26	2	977.45	363.5	955.13459		42		365.578	0		361.532	213.33111		2.77596	0		3.78	0		2.86	0		0.19029	0.1902
27	2	857.45	375.98	986.94981		46		380.52	0		381.413	213.54236		3.14129	0		4.14	0		3.22	0		0.19048	0.1904
28	2	1134.0853	422.37	1110.90811		56		410.862	0		428.034	213.12249		4.02948	0		5.04	0	5	4.12	0		0.1901	0.1901

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6	A	В	28.0134	0.09			0.92	sec (m/	z) indeper	dent			0.085	sec (m	n/z) depen	ndent										
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11	Charge State	m/z	Published Cross Sections	Corrected Cross Sections	An	ival Time	15		Cross Se	tion (Pc	ower)	Cross Se	ction (Line	ar)	t(d)''			t(d)			t(d)'			sqoot(1/R	Reduce	Ì
12					4				6																	
13	2	507.30	252.18	658.320945		19			241.214	0		253.926	214.72733		0.72946	0		1.71	0		0.79	0		0.19153	0.1915	
14	2	536.32	253.92	663.34/492		20			249.9	0		258.482	214.5/050		0.81775	0		1.8	0		0.88	0		0.19139	0.1913	
15	2	656.84	239.73	740 924181		21			257.927			281.627	214.4141		1.26111	8		2.25	0		1.33	0		0.19125	0.1912	
17	2	626.33	278.59	729,135429		24			279,541	0		277.033	214,17643		1,17273	ő		2.16	0		1.24	ő		0.19104	0,1910	
18	2	724.40	310.43	813.677412		30			314.32	0		305.25	213.85814		1.70766	0		2.7	0		1.78	0		0.19076	0.1907	
19	2	693.87	302.28	791.983815		28			303.673	0		295.823	213.94763		1.5292	0		2.52	0		1.6	0		0.19084	0.1908	
20	2	809.92	354.9	931.174513		42			366.427	0		362.464	213.6433		2.7835	0		3.78	0		2.86	0		0.19057	0.1905	
21	2	1156.57	415.18	1092.12689		55			407.862	0		423.136	213.09717	7	3.93859	0		4.95	0		4.03	0		0.19008	0.1900	
22	2	593.34	263.79	689.979494		22			265.516	0		267.645	214.30707	7	0.99453	0		1.98	0		1.06	0		0.19116	0.1911	
23	2	640.36	287.78	753.368911		26			292.322	0		286.572	214.12495	5	1.35198	0		2.34	0		1.42	0		0.191	0.191	
24	2	664.84	286.28	749.738502		26			292.119	0		286.389	214.0403		1.35069	0		2.34	0		1.42	0		0.19092	0.1909	
25	2	766.35	299	784.126239		29			308.702	0		300.166	213.74677	1	1.61559	0		2.61	0		1.69	0		0.19066	0.1906	
26	2	977.45	363.5	955.13459		42			365.578	0		361.532	213.33111		2.77596	0		3.78	0		2.86	0		0.19029	0.1902	
27	2	857.45	375.98	986.94981		46			380.52	0		381.413	213.54236		3.14129	0		4.14	0		3.22	0		0.19048	0.1904	
28	2	1134.0853	422.37	1110.90811		56	_		410.862	0	. a	428.034	213.12249		4.02948	0	<b>)</b>	5.04	0	ŝ.	4.12	0	. 2	0.1901	0.1901	

Step 3: Plot t(d)" on the x axis versus Corrected Cross sections

Step 4: Either fit a linear or power trend. We have found that a linear trend is more suitable when analysing peptides while a power trend when analysing proteins (unpublished results). The excel sheet can handle both cases (linear calibration and results colour-coded green while power calibration and results colour -coded orange).



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8	and the second second		_			/														_
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11	Charge State	m/z	Sections	Sections	Arrival Time	5	cross se	ction (Power	) Cross Se	ction (Linea	r) t(d)	_	t(	a)		t(d)	_	_	sqoot(1/R	teduce
13	2	507.30	252.18	658.20945	19		241,214	0	253,976	214,72733	0.7294	0		1.71	0	0.79	0	100	0.19153	0.1915
14	2	536.32	253.92	653.347492	20		249.9	0	258.482	214.57058	0.8177	ō		1.8	0	0.88	0		0.19139	0.1913
15	2	568.78	259.73	679.020895	21		257.927	0	263.023	214.4141	0.9058	0		1.89	0	0.97	0		0.19125	0.1912
16	2	656.84	282.95	740.924181	25		285.888	0	281.627	214.06728	1.2611	0		2.25	0	1.33	0		0.19094	0.1909
17	2	626.33	278.59	729.135429	24		279.541	0	277.033	214.17643	1.17273	0		2.16	0	1.24	0		0.19104	0.1910
18	2	724.40	310.43	813.677412	30		314.32	0	305.25	213.85814	1.7076	6 0		2.7	0	1.78	0		0.19076	0.1907
19	2	6 2.87	302.28	791.983815	28		303.673	0	295.823	213.94762	1.5292	0		2.52	0	1.6	0		0.19084	0.1908
20	2	809.92	354.9	931.174513	42		366.427	0	362.464	213.6433	2.7835	0		3.78	0	2.86	0		0.19057	0.1905
21	2	1156.57		0	55		407.862	0	423.136	213.09717	3.9385	0		4.95	0	4.03	0		0.19008	0.1900
22	2	593.34		0	22		265.516	0	267.645	214.30707	0.99453	0		1.98	0	1.06	0		0.19116	0.1911
23	2	640.36		0	26		292.322	0	286.572	214.12495	1.3519	0		2.34	0	1.42	0		0.191	0.191
24	2	664.84		0	26		292.119	0	286.389	214.0403	1.3506	0		Z.34	0	1.42	0		0.19092	0.1909
25	2	/66.35		0	29		308.702	0	300.166	213./4677	1.6155	0		2.61	0	1.69	0		0.19066	0.1906
20	2	977.45		0	42		365.5/8		361.532	213.33111	2.7759	0		3.78	U	2.86	0		0.19029	0.1902
28	2	1134 0953		0	46		410 862	0	428.034	213.34236	4 0794			5.04	0	4.12	0		0.19048	0.1904
20	4	1104-0000		0	50		410.002		420.034	210112249	411/94		6	3.04	· ·	4.12		100	0.1901	0.170

Step 5: Insert fit parameters and charge state, m/z, and arrival times for the ions of interest

The resulting estimated cross sections (for each type of fit) are displayed here

## What calculations are performed by the spreadsheet

- Measure arrival time in scans (scan number (n))
- Convert to time (multiply by pusher time)
- t<sub>d</sub> = n\* pusher time (msecs)
- Subtract T-wave offset
- T-wave offset related to wave velocity, the time indicated on readout after wave velocity in mobility region  $(t_m)$  and transfer region  $(t_t)$  is time per pair of plates. The mobility cell has 61 pairs of plates so  $61^*t_m$  is subtracted. The transfer region has 31 pairs of plates so  $31^*t_t$  is subtracted.
- At 300 m/sec  $t_m$  and  $t_t$  are equal to 10  $\mu sec$
- Subtraction would therefore be [(61\*10)+(31\*10)]µsec = 920µsec
- This is not *m*/*z* dependent!
- For these values  $t_d' = t_d 920 \mu sec$
- Then correct for m/z dependent time of flight.
- From Bob Bateman calculations.
- For m/z 1000 the TOF flight time is 44  $\mu$ s and the transit time from the exit of the TriWave to the TOF is 41  $\mu$ s, giving a total time of 85  $\mu$ s (micro-sec) to be subtracted from the drift time. The correction time to the drift time is proportional to the square root of the m/z value.

• 
$$td'' = td' - \sqrt{\left(\frac{m/z}{1000}\right)} \times 0.085$$
 msec

- This is corrected effective drift time.
- To obtain calibration coefficients use published cross section data. (Ω)
- Correct published cross sections by taking into account reduced mass and charge state.

• 
$$\Omega' = \frac{\Omega}{e \times \left(\frac{1}{M_L} + \frac{1}{M_N}\right)^{\frac{1}{2}}}$$

- e = Charge, Mi = mass of ion, MN = mass of mobility gas.
- Plot  $\Omega'$  against  $t_d''$
- Fit curve to equation type  $y = Ax^N$
- Calculate A and N from fit.
- Convert experimental measurements to estimated cross sections by calculating.

1

• 
$$\Omega = td''^N \times A \times e \times \left(\frac{1}{M_I} + \frac{1}{M_N}\right)^{\frac{1}{2}}$$