Supplemental Material

Collisional Cross Sections with T-Wave Ion Mobility Spectrometry without Experimental Calibration

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Table S-1. Unadjusted drift times (in ms) of the ions formed from denaturing solutions (excluding polyalanine) obtained with a 20 V wave height and the various wave velocities. The pressure in the TWIMS cell was 3.2 mbar in all experiments. Ion mobility collision cross sections (IMS CCS) are taken from reference [1].

	m		IMS CCS	Wave Velocity (m/s)					
Species	(kDa)	z	(nm^2)	100	250	500	1000	1500	2000
Angiotensin II	1.0	2	3.35	2.73	4.37	9.11	18.41	28.67	41.85
Apo-myoglobin	17.0	18	44.4	2.73	9.11	18.10	35.72	57.35	100.79
Apo-myoglobin	17.0	19	45.7	2.73	8.57	17.37	34.51	54.74	94.41
Apo-myoglobin	17.0	20	47.0	2.73	8.20	16.71	32.87	52.06	87.91
Apo-myoglobin	17.0	21	48.2	2.73	7.90	15.92	31.65	49.39	81.16
Apo-myoglobin	17.0	22	49.2	2.73	7.53	15.25	30.25	47.51	77.15
Apo-myoglobin	17.0	23	50.1	2.73	7.17	14.64	29.10	45.32	72.99
Apo-myoglobin	17.0	24	50.9	2.73	6.87	13.91	27.82	43.07	67.92
Bradykinin	1.1	2	3.44	2.73	4.19	8.75	17.62	27.28	40.22
Cytochrome *c*	12.4	14	32.0	2.73	7.65	15.49	31.28	49.30	82.04
Cytochrome *c*	12.4	15	33.3	2.73	7.23	14.70	29.34	46.15	76.24
Cytochrome *c*	12.4	16	34.5	2.73	6.74	13.97	28.01	43.65	70.59
Cytochrome *c*	12.4	17	36.0	2.73	6.38	13.24	26.43	41.13	65.67
Cytochrome *c*	12.4	18	36.7	2.73	6.01	12.58	25.09	38.71	60.45
Cytochrome *c*	12.4	19	37.9	2.73	5.83	11.97	23.75	36.69	55.52
Ubiquitin	8.6	9	20.9	2.73	7.90	15.92	31.95	51.33	99.88
Ubiquitin	8.6	10	22.0	2.73	7.23	14.70	29.22	45.74	80.31
Ubiquitin	8.6	11	23.4	2.73	6.72	13.55	27.70	42.89	69.62
Ubiquitin	8.6	12	24.8	2.73	6.20	12.98	25.79	40.15	64.15
Ubiquitin	8.6	13	26.0	2.73	5.83	12.09	24.18	37.30	58.68

Table S-2. Unadjusted drift times (in ms) of the ions formed from denaturing solutions (excluding polyalanine) obtained with a 40 V wave height and the various wave velocities. The pressure in the TWIMS cell was 3.2 mbar in all experiments. Ion mobility collision cross sections (IMS CCS) are taken from reference [1].

	m		IMS CCS	Wave Velocity (m/s)					
Species	(kDa)	z	(nm2)	100	250	500	1000	1500	2000
Angiotensin II	1.0	2	3.35	2.73	1.28	2.37	5.04	7.78	11.48
Apo-myoglobin	17.0	18	44.4	2.73	2.00	4.80	9.96	15.67	26.73
Apo-myoglobin	17.0	19	45.7	2.73	1.82	4.56	9.48	14.94	24.97
Apo-myoglobin	17.0	20	47.0	2.73	1.64	4.37	9.11	14.22	23.57
Apo-myoglobin	17.0	21	48.2	2.73	1.64	4.19	8.75	13.67	22.17
Apo-myoglobin	17.0	22	49.2	2.73	1.46	4.01	8.38	13.06	20.84
Apo-myoglobin	17.0	23	50.1	2.73	1.28	3.83	8.02	12.39	19.56
Apo-myoglobin	17.0	24	50.9	2.73	1.28	3.64	7.65	11.85	18.41
Bradykinin	1.1	2	3.44	2.73	1.28	2.37	4.92	7.47	11.06
Cytochrome *c*	12.4	14	32.0	2.73	1.40	4.19	8.57	13.49	22.54
Cytochrome *c*	12.4	15	33.3	2.73	1.28	3.83	8.08	12.70	20.65
Cytochrome *c*	12.4	16	34.5	2.73	1.28	3.64	7.71	12.03	19.20
Cytochrome *c*	12.4	17	36.0	2.73	1.28	3.46	7.29	11.36	17.80
Cytochrome *c*	12.4	18	36.7	2.73	1.28	3.28	6.93	10.81	16.64
Cytochrome *c*	12.4	19	37.9	2.73	1.28	3.10	6.56	10.15	15.61
Ubiquitin	8.6	9	20.9	2.73	1.58	4.19	8.81	13.97	23.45
Ubiquitin	8.6	10	22.0	2.73	1.28	3.95	8.08	12.76	20.59
Ubiquitin	8.6	11	23.4	2.73	1.28	3.70	7.78	11.97	19.14
Ubiquitin	8.6	12	24.8	2.73	1.28	3.46	7.23	11.12	17.25
Ubiquitin	8.6	13	26.0	2.73	1.28	3.28	6.80	10.57	16.22

Table S-3. Unadjusted drift times (in ms) of the polyalanine ions obtained with a 20 V wave height and the various wave velocities. The pressure in the TWIMS cell was 3.2 mbar in all experiments. Ion mobility collision cross sections (IMS CCS) are taken from reference [2].

Alanine	m		IMS CCS	Wave Velocity (m/s)				
Residues	(Da)	z	(nm^2)	250	500	1000	1500	2000
3	231	1	1.51	2.73	6.01	12.03	18.71	26.97
4	302	1	1.66	3.46	7.35	14.76	22.78	32.44
5	373	1	1.81	4.19	8.81	17.74	27.46	39.42
6	445	1	1.95	5.10	10.39	20.72	32.02	46.01
7	516	1	2.11	6.01	12.21	24.18	37.69	54.76
8	587	1	2.28	7.11	14.34	28.37	43.77	66.06
9	658	1	2.43	8.02	16.04	32.26	50.14	76.35
10	729	1	2.56	8.99	17.86	35.40	55.67	85.95
11	800	1	2.71	10.02	19.87	39.88	62.35	96.31
12	871	1	2.82	11.12	21.75	43.58	67.46	-
13	942	1	2.94	12.15	23.87	47.84	75.20	-
14	1013	1	3.06	13.00	25.76	51.05	80.75	-
11	800	2	2.96	2.92	6.38	12.52	19.50	29.40
12	871	2	3.09	3.10	6.87	13.85	21.51	31.22
13	942	2	3.20	3.46	7.47	14.94	23.51	34.02
14	1013	2	3.33	3.83	8.08	16.10	25.39	37.51
15	1084	2	3.44	4.07	8.63	17.19	27.34	40.49
16	1155	2	3.57	4.37	9.29	18.65	28.92	43.34
17	1227	2	3.69	4.74	9.84	19.93	31.53	46.80
18	1298	2	3.80	5.10	10.57	21.20	33.35	50.75
19	1369	2	3.93	5.53	11.24	22.54	35.48	54.58
20	1440	2	4.04	5.71	11.91	23.63	37.75	61.50
21	1511	2	4.16	6.07	12.58	25.33	39.69	62.11
22	1582	2	4.28	6.50	13.12	26.18	42.12	66.36
23	1653	2	4.37	6.74	13.91	28.19	44.37	69.43
24	1724	2	4.48	7.29	14.82	29.28	46.92	76.53
25	1795	2	4.58	7.65	15.61	30.74	49.29	80.13
26	1866	2	4.70	8.32	16.34	32.86	51.63	85.47
19	1369	3	4.82	3.28	7.11	14.64	23.06	32.90
20	1440	3	4.91	3.70	7.84	15.61	25.15	35.22
21	1511	3	5.01	3.77	8.48	16.22	25.88	39.27
22	1582	3	5.18	4.13	8.93	17.31	27.98	40.41
23	1653	3	5.32	4.37	9.29	18.71	29.71	42.82
24	1724	3	4.58	4.74	9.84	19.62	30.80	45.15

Table S-3. (cont.)

Alanine Residues	m (Da)	z	IMS CCS (nm^2)	Wave Velocity (m/s)				
				250	500	1000	1500	2000
25	1795	3	5.61	4.92	10.30	20.59	32.81	48.48
26	1866	3	5.76	5.29	10.94	21.87	34.26	51.84
27	1937	3	5.92	5.47	11.48	23.33	35.81	54.23
28	2009	3	6.06	5.83	12.03	24.05	38.54	58.32
29	2080	3	6.21	6.20	12.67	25.63	40.18	62.03
30	2151	3	6.34	6.44	13.12	26.85	41.46	65.51
31	2222	3	6.49	6.74	13.85	27.88	43.19	69.43
32	2293	3	6.66	7.11	14.40	28.80	45.83	71.89
33	2364	3	6.74	7.41	15.04	30.01	47.74	76.45

Table S-4. Unadjusted drift times (in ms) of the polyalanine ions obtained with a 40 V wave height and the various wave velocities. The pressure in the TWIMS cell was 3.2 mbar in all experiments. Ion mobility collision cross sections (IMS CCS) are taken from reference [2].

Alanine	m		IMS CCS	Wave Velocity (m/s)				
Residues	(Da)	z	(nm^2)	250	500	1000	1500	2000
3	231	1	1.51	1.28	1.46	3.22	4.92	6.87
4	302	1	1.66	1.28	1.82	3.83	6.01	8.57
5	373	1	1.81	1.28	2.16	4.74	7.23	10.51
6	445	1	1.95	1.28	2.67	5.47	8.51	12.58
7	516	1	2.11	1.28	3.10	6.50	10.02	15.31
8	587	1	2.28	1.28	3.64	7.59	11.85	18.29
9	658	1	2.43	1.64	4.19	8.51	13.30	21.57
10	729	1	2.56	2.00	4.68	9.60	14.82	24.42
11	800	1	2.71	2.19	5.10	10.57	16.58	28.13
12	871	1	2.82	2.55	5.65	11.60	18.47	32.38
13	942	1	2.94	2.92	6.26	12.88	20.53	38.17
14	1013	1	3.06	3.10	6.74	13.73	22.24	42.61
11	800	2	2.96	1.28	1.64	3.46	5.38	7.71
12	871	2	3.09	1.28	1.82	3.83	5.83	8.32
13	942	2	3.20	1.28	1.94	4.01	6.32	9.17
14	1013	2	3.33	1.28	2.00	4.37	6.87	9.96
15	1084	2	3.44	1.28	2.25	4.74	7.41	10.88
16	1155	2	3.57	1.28	2.43	5.10	7.96	11.79
17	1227	2	3.69	1.28	2.55	5.47	8.51	12.82
18	1298	2	3.80	1.28	2.73	5.83	8.99	13.79
19	1369	2	3.93	1.28	2.92	6.14	9.60	14.76
20	1440	2	4.04	1.28	3.10	6.50	10.21	15.80
21	1511	2	4.16	1.28	3.28	6.87	10.63	16.89
22	1582	2	4.28	1.28	3.46	7.17	11.30	18.16
23	1653	2	4.37	1.28	3.64	7.65	12.03	19.56
24	1724	2	4.48	1.34	3.83	7.96	12.58	20.78
25	1795	2	4.58	1.46	4.01	8.35	13.43	22.23
26	1866	2	4.70	1.64	4.25	8.87	14.22	23.93
19	1369	3	4.82	1.28	1.82	4.01	6.20	8.66
20	1440	3	4.91	1.28	2.10	4.43	6.93	10.08
21	1511	3	5.01	1.28	2.10	4.62	7.29	10.63
22	1582	3	5.18	1.28	2.19	4.74	7.47	11.06
23	1653	3	5.32	1.28	2.37	5.10	8.02	11.79
24	1724	3	4.58	1.28	2.55	5.47	8.29	12.58

Table S-4. (cont.)

Alanine Residues	m (Da)	z	IMS CCS (nm^2)	Wave Velocity (m/s)				
				250	500	1000	1500	2000
25	1795	3	5.61	1.28	2.61	5.65	8.84	13.24
26	1866	3	5.76	1.28	2.73	6.01	9.20	14.28
27	1937	3	5.92	1.28	2.92	6.20	9.75	14.88
28	2009	3	6.06	1.28	3.10	6.56	10.21	15.98
29	2080	3	6.21	1.28	3.28	6.87	10.81	17.01
30	2151	3	6.34	1.28	3.46	7.23	11.30	17.93
31	2222	3	6.49	1.28	3.64	7.53	11.72	18.89
32	2293	3	6.66	1.28	3.83	7.84	12.45	19.50
33	2364	3	6.74	1.34	3.92	8.20	12.67	20.65

Table S-5. Unadjusted drift times (in ms) of the ions formed from buffered aqueous solutions obtained with a 20 V wave height and the various wave velocities. The pressure in the TWIMS cell was 3.2 mbar in all experiments. Ion mobility collision cross sections (IMS CCS) are taken from reference [1].

Species	m (kDa)	z	IMS CCS (nm^2)	Wave Velocity (m/s) 100	250	500	1000
Bovine Serum Albumin	66.5	14	44.9	2.73	13.24	28.71	84.38
Bovine Serum Albumin	66.5	15	44.9	2.73	12.82	26.72	77.09
Bovine Serum Albumin	66.5	16	44.7	2.73	11.60	24.93	64.76
Bovine Serum Albumin	66.5	17	44.9	-	11.41	24.24	63.12
Concanavalin A	103.4	20	60.8	2.86	13.85	30.32	96.35
Concanavalin A	103.4	21	60.9	2.83	12.40	27.76	82.68
Concanavalin A	103.4	22	60.5	2.73	11.48	25.76	75.33

Table S-6. Unadjusted drift times (in ms) of the ions formed from buffered aqueous solutions obtained with a 40 V wave height and the various wave velocities. The pressure in the TWIMS cell was 3.2 mbar in all experiments. Ion mobility collision cross sections (IMS CCS) are taken from reference [1].

Species	m (kDa)	z	IMS CCS (nm^2)	Wave Velocity (m/s) 100	250	500	1000	1500
Bovine Serum Albumin	66.5	14	44.9	-	3.10	7.47	18.96	-
Bovine Serum Albumin	66.5	15	44.9	-	2.98	7.71	18.63	43.01
Bovine Serum Albumin	66.5	16	44.7	-	2.81	6.62	18.25	42.58
Bovine Serum Albumin	66.5	17	44.9	-	2.67	6.32	16.22	43.22
Concanavalin A	103.4	20	60.8	2.86	3.28	7.96	23.51	-
Concanavalin A	103.4	21	60.9	2.83	2.98	7.29	21.32	-
Concanavalin A	103.4	22	60.5	2.73	2.73	6.80	19.50	-

Table S-7. Collision cross sections (in nm^2) of the ions formed from denaturing solutions (excluding polyalanine) obtained with a 20 V wave height and the various wave velocities. The pressure in the TWIMS cell was 3.2 mbar in all experiments. Ion mobility collision cross sections (IMS CCS) are taken from reference [1].

	m		IMS CCS	Wave Velocity (m/s)				
Species	(kDa)	z	(nm^2)	250	500	1000	1500	2000
Angiotensin II	1.0	2	3.35	3.54	3.46	3.46	3.52	3.67
Apo-myoglobin	1.1	18	44.4	44.4	43.4	43.0	44.5	51.1
Apo-myoglobin	12.4	19	45.7	45.5	44.9	44.6	45.8	52.3
Apo-myoglobin	12.4	20	47.0	46.9	46.3	45.7	47.0	53.1
Apo-myoglobin	12.4	21	48.2	48.3	47.4	47.1	48.0	53.5
Apo-myoglobin	12.4	22	49.2	49.5	48.6	48.2	49.3	54.6
Apo-myoglobin	12.4	23	50.1	50.5	49.8	49.4	50.3	55.5
Apo-myoglobin	12.4	24	50.9	51.6	50.6	50.3	51.1	55.8
Bradykinin	17.0	2	3.44	3.48	3.40	3.39	3.43	3.60
Cytochrome *c*	17.0	14	32.0	31.7	31.2	31.2	32.0	35.9
Cytochrome *c*	17.0	15	33.3	33.1	32.5	32.3	33.1	37.0
Cytochrome *c*	17.0	16	34.5	34.1	33.8	33.7	34.3	38.0
Cytochrome *c*	17.0	17	36.0	35.3	34.9	34.7	35.4	38.8
Cytochrome *c*	17.0	18	36.7	36.3	36.0	35.8	36.3	39.4
Cytochrome *c*	17.0	19	37.9	37.8	37.1	36.8	37.3	39.8
Ubiquitin	8.6	9	20.9	20.7	20.3	20.3	21.0	25.5
Ubiquitin	8.6	10	22.0	22.1	21.7	21.5	22.0	25.4
Ubiquitin	8.6	11	23.4	23.4	22.9	23.0	23.4	25.9
Ubiquitin	8.6	12	24.8	24.6	24.4	24.2	24.7	27.1
Ubiquitin	8.6	13	26.0	25.9	25.5	25.4	25.7	28.0

Table S-8. Collision cross sections (in nm^2) of the ions formed from denaturing solutions (excluding polyalanine) obtained with a 40 V wave height and the various wave velocities. The pressure in the TWIMS cell was 3.2 mbar in all experiments. Ion mobility collision cross sections (IMS CCS) are taken from reference [1].

	m		IMS CCS	Wave Velocity (m/s)				
Species	(kDa)	z	(nm^2)	250	500	1000	1500	2000
Angiotensin II	1.0	2	3.35	-	3.62	3.61	3.65	3.84
Apo-myoglobin	1.1	18	44.4	46.2	45.3	45.5	46.5	52.5
Apo-myoglobin	12.4	19	45.7	47.4	46.6	46.8	47.9	53.7
Apo-myoglobin	12.4	20	47.0	48.5	48.0	48.2	49.2	54.9
Apo-myoglobin	12.4	21	48.2	50.9	49.3	49.6	50.6	55.9
Apo-myoglobin	12.4	22	49.2	51.7	50.6	50.8	51.7	56.8
Apo-myoglobin	12.4	23	50.1	-	51.7	51.9	52.6	57.5
Apo-myoglobin	12.4	24	50.9	-	52.6	52.9	53.6	58.1
Bradykinin	17.0	2	3.44	-	3.62	3.57	3.58	3.77
Cytochrome *c*	17.0	14	32.0	32.6	32.9	32.7	33.5	37.6
Cytochrome *c*	17.0	15	33.3	-	33.7	34.0	34.8	38.5
Cytochrome *c*	17.0	16	34.5	-	35.1	35.4	36.1	39.6
Cytochrome *c*	17.0	17	36.0	-	36.3	36.5	37.2	40.4
Cytochrome *c*	17.0	18	36.7	-	37.5	37.7	38.4	41.3
Cytochrome *c*	17.0	19	37.9	-	38.5	38.7	39.2	42.2
Ubiquitin	8.6	9	20.9	21.6	21.2	21.3	21.9	24.7
Ubiquitin	8.6	10	22.0	-	22.8	22.7	23.3	25.7
Ubiquitin	8.6	11	23.4	-	24.3	24.5	24.7	27.2
Ubiquitin	8.6	12	24.8	-	25.7	25.7	26.0	28.1
Ubiquitin	8.6	13	26.0	-	27.1	27.0	27.4	29.5

Table S-9. Collision cross sections (in nm^2) of the polyalanine ions obtained with a 20 V wave height and the various wave velocities. The pressure in the TWIMS cell was 3.2 mbar in all experiments. Ion mobility collision cross sections (IMS CCS) are taken from reference [2].

Alanine	m		IMS CCS	Wave Velocity (m/s)				
Residues	(Da)	z	(nm^2)	250	500	1000	1500	2000
3	231	1	1.51	1.54	1.51	1.50	1.52	1.57
4	302	1	1.66	1.66	1.62	1.61	1.63	1.68
5	373	1	1.81	1.78	1.75	1.74	1.76	1.83
6	445	1	1.95	1.93	1.88	1.86	1.89	1.96
7	516	1	2.11	2.07	2.02	2.00	2.04	2.13
8	587	1	2.28	2.24	2.19	2.17	2.20	2.34
9	658	1	2.43	2.37	2.31	2.31	2.35	2.52
10	729	1	2.56	2.50	2.44	2.42	2.48	2.67
11	800	1	2.71	2.63	2.57	2.57	2.62	2.82
12	871	1	2.82	2.76	2.69	2.68	2.73	-
13	942	1	2.94	2.88	2.81	2.81	2.87	-
14	1013	1	3.06	2.96	2.91	2.90	2.97	-
11	800	2	2.96	3.03	2.98	2.93	2.97	3.13
12	871	2	3.09	3.09	3.06	3.05	3.09	3.21
13	942	2	3.20	3.22	3.17	3.15	3.21	3.33
14	1013	2	3.33	3.35	3.28	3.25	3.32	3.49
15	1084	2	3.44	3.43	3.38	3.35	3.44	3.61
16	1155	2	3.57	3.54	3.49	3.47	3.53	3.73
17	1227	2	3.69	3.66	3.59	3.58	3.68	3.88
18	1298	2	3.80	3.78	3.71	3.69	3.78	4.04
19	1369	2	3.93	3.92	3.82	3.80	3.89	4.19
20	1440	2	4.04	3.98	3.93	3.89	4.02	4.45
21	1511	2	4.16	4.09	4.04	4.03	4.12	4.47
22	1582	2	4.28	4.22	4.12	4.10	4.25	4.63
23	1653	2	4.37	4.29	4.25	4.25	4.36	4.74
24	1724	2	4.48	4.46	4.39	4.34	4.49	4.98
25	1795	2	4.58	4.56	4.50	4.45	4.60	5.10
26	1866	2	4.70	4.76	4.61	4.60	4.71	5.27
19	1369	3	4.82	4.71	4.64	4.66	4.75	4.90
20	1440	3	4.91	4.94	4.84	4.79	4.94	5.06
21	1511	3	5.01	4.97	5.01	4.87	5.01	5.32
22	1582	3	5.18	5.16	5.13	5.02	5.19	5.39
23	1653	3	5.32	5.29	5.22	5.20	5.34	5.55
24	1724	3	5.45	5.47	5.36	5.32	5.43	5.69
25	1795	3	5.61	5.56	5.48	5.44	5.60	5.90
26	1866	3	5.76	5.74	5.64	5.60	5.72	6.10
27	1937	3	5.92	5.83	5.77	5.79	5.85	6.24
28	2009	3	6.06	6.00	5.91	5.87	6.07	6.48

Table S-9. (cont.)

Alanine Residues	m (Da)	z	IMS CCS (nm^2)	Wave Velocity (m/s)				
				250	500	1000	1500	2000
29	2080	3	6.21	6.18	6.06	6.07	6.20	6.69
30	2151	3	6.34	6.29	6.17	6.21	6.30	6.88
31	2222	3	6.49	6.43	6.34	6.33	6.43	7.09
32	2293	3	6.66	6.60	6.47	6.44	6.63	7.22
33	2364	3	6.74	6.73	6.61	6.57	6.78	7.45

Table S-10. Collision cross sections (in nm^2) of the polyalanine ions obtained with a 40 V wave height and the various wave velocities. The pressure in the TWIMS cell was 3.2 mbar in all experiments. Ion mobility collision cross sections (IMS CCS) are taken from reference [2].

Alanine	m		IMS CCS	Wave Velocity (m/s)				
Residues	(Da)	z	(nm^2)	250	500	1000	1500	2000
3	231	1	1.51	-	1.55	1.54	1.55	1.58
4	302	1	1.66	-	1.67	1.64	1.67	1.72
5	373	1	1.81	-	1.78	1.79	1.80	1.88
6	445	1	1.95	-	1.94	1.91	1.94	2.05
7	516	1	2.11	-	2.08	2.08	2.10	2.26
8	587	1	2.28	2.33	2.24	2.24	2.29	2.47
9	658	1	2.43	2.47	2.40	2.37	2.42	2.68
10	729	1	2.56	2.61	2.53	2.52	2.56	2.84
11	800	1	2.71	2.69	2.63	2.65	2.70	3.04
12	871	1	2.82	2.83	2.77	2.77	2.85	3.22
13	942	1	2.94	2.99	2.90	2.91	2.99	3.41
14	1013	1	3.06	3.06	3.00	3.00	3.10	3.51
11	800	2	2.96	-	3.11	3.05	3.09	3.19
12	871	2	3.09	-	3.24	3.19	3.19	3.29
13	942	2	3.20	-	3.32	3.25	3.31	3.44
14	1013	2	3.33	-	3.36	3.37	3.44	3.58
15	1084	2	3.44	-	3.53	3.50	3.56	3.73
16	1155	2	3.57	-	3.65	3.63	3.69	3.88
17	1227	2	3.69	-	3.73	3.75	3.81	4.05
18	1298	2	3.80	-	3.85	3.87	3.91	4.20
19	1369	2	3.93	-	3.97	3.97	4.05	4.35
20	1440	2	4.04	-	4.09	4.08	4.17	4.51
21	1511	2	4.16	-	4.20	4.20	4.26	4.67
22	1582	2	4.28	-	4.31	4.29	4.40	4.85
23	1653	2	4.37	-	4.42	4.44	4.54	5.03
24	1724	2	4.48	4.64	4.53	4.53	4.65	5.19
25	1795	2	4.58	4.73	4.63	4.64	4.81	5.37
26	1866	2	4.70	4.88	4.76	4.79	4.95	5.57
19	1369	3	4.82	-	4.83	4.85	4.90	5.01
20	1440	3	4.91	-	5.12	5.08	5.16	5.38
21	1511	3	5.01	-	5.12	5.17	5.28	5.52
22	1582	3	5.18	-	5.22	5.23	5.34	5.63
23	1653	3	5.32	-	5.40	5.42	5.53	5.81
24	1724	3	5.45	-	5.58	5.61	5.62	6.00
25	1795	3	5.61	-	5.64	5.70	5.80	6.16
26	1866	3	5.76	-	5.76	5.87	5.92	6.40
27	1937	3	5.92	-	5.94	5.97	6.10	6.54
28	2009	3	6.06	-	6.11	6.14	6.24	6.78

Table S-10. (cont.)

Alanine Residues	m (Da)	z	IMS CCS (nm^2)	Wave Velocity (m/s)				
				250	500	1000	1500	2000
29	2080	3	6.21	-	6.28	6.28	6.43	7.01
30	2151	3	6.34	-	6.45	6.45	6.58	7.20
31	2222	3	6.49	-	6.61	6.59	6.70	7.40
32	2293	3	6.66	-	6.78	6.73	6.92	7.52
33	2364	3	6.74	6.95	6.85	6.88	6.98	7.75

Table S-11. Collision cross sections (in nm^2) of the ions formed from buffered aqueous solutions obtained with a 20 V wave height and the various wave velocities. The pressure in the TWIMS cell was 3.2 mbar in all experiments. Ion mobility collision cross sections (IMS CCS) are taken from reference [1].

Species	m (kDa)	z	IMS CCS (nm^2)	Wave Velocity (m/s) 250	500	1000
Bovine Serum Albumin	66.5	14	44.9	41.3	42.3	48.4
Bovine Serum Albumin	66.5	15	44.9	43.6	43.9	50.6
Bovine Serum Albumin	66.5	16	44.7	44.4	45.3	50.8
Bovine Serum Albumin	66.5	17	44.9	46.8	47.5	53.4
Concanavalin A	103.5	20	60.8	60.1	61.9	70.5
Concanavalin A	103.5	21	60.9	60.1	62.5	72.2
Concanavalin A	103.5	22	60.5	60.7	63.3	73.7

Table S-12. Collision cross sections (in nm^2) of the ions formed from buffered aqueous solutions obtained with a 40 V wave height and the various wave velocities. The pressure in the TWIMS cell was 3.2 mbar in all experiments. Ion mobility collision cross sections (IMS CCS) are taken from reference [1].

	m		IMS CCS	Wave Velocity (m/s)			
Species	(kDa)	z	(nm^2)	250	500	1000	1500
Bovine Serum Albumin	66.5	14	44.9	42.3	43.3	49.0	-
Bovine Serum Albumin	66.5	15	44.9	44.5	47.0	51.6	52.3
Bovine Serum Albumin	66.5	16	44.7	46.3	46.9	53.0	55.9
Bovine Serum Albumin	66.5	17	44.9	48.3	48.8	54.0	59.2
Concanavalin A	103.5	20	60.8	61.9	63.5	70.3	-
Concanavalin A	103.5	21	60.9	62.3	64.3	72.7	-
Concanavalin A	103.5	22	60.5	63.0	65.3	74.4	-

Polynomial Fits Obtained with Different Adjusted Cross Sections

The drift times of the reference ions calculated using DTIMS cross sectional values, a 20 V wave height, and a 1000 m/s wave velocity are shown as a function of adjusted cross section in Figure S-1a. These reference ions have adjusted cross sections of between 7.5 and 17.0 nm^2 $Da^{1/2}$. The drift times of purely hypothetical ions with adjusted cross sections ranging by half-integer values from 7.5 to 17.0 nm^2 $Da^{1/2}$ are also calculated using the same wave parameters (Figure S-1b). The data in Figure S-1a and S-1b are fit with second-order polynomials (shown as black lines), and these fits both have root-mean-square (rms) deviations of 0.3%. The none-zero rms deviations obtained with these polynomial fits likely result from the uncertainties in the calculated drift times (<0.4% with 10,000 or more wave steps, Figure 3a). The drift times predicted by these two polynomial fits are the same to within less than 0.1% for all of the adjusted cross sections within the range of adjusted cross sections used (Figure S-1c). The difference between the two polynomial fits is greatest for adjusted cross sections below ~12 nm^2 $Da^{1/2}$ because most of the reference ions (70%) have adjusted cross sectional values that are below 12 nm^2 $Da^{1/2}$. In contrast, the adjusted cross sections of the hypothetical ions are evenly distributed across the range of values used. Thus, the polynomial fit obtained with the reference ions is slightly more well defined for the lower adjusted cross sectional values, whereas the polynomial fit obtained with the hypothetical ions is not biased towards either high or low values. The results in Figure S-1 indicate that nominally identical polynomial fits can be obtained using adjusted cross sections obtained with DTIMS cross sectional values and using purely hypothetical adjusted cross sections as long as the same range of adjusted cross sections is used.

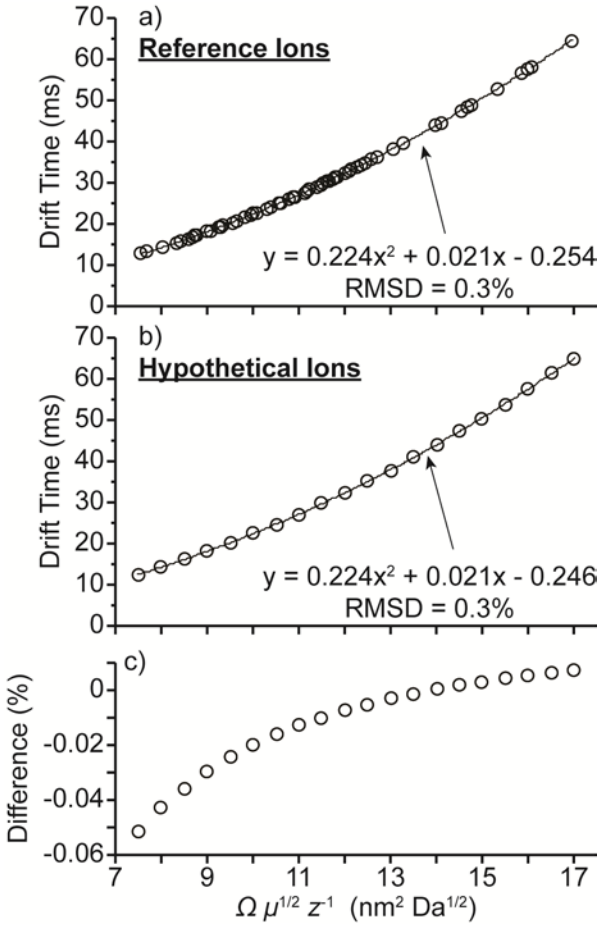

Figure S-1. Calculated drift times as a function of adjusted cross section obtained (a) for the reference ions using DTIMS cross sectional values and (b) for purely hypothetical ions. These results are obtained with a 20 V wave height and a 1000 m/s wave velocity. Black lines are second-order polynomial fits to the data. “RMSD” denotes root-mean-square deviation. (c) The difference between the polynomial fit obtained for the reference ions and that obtained for the hypothetical ions as a function of adjusted cross section.

References

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