Electronic Supplementary Material (ESI) for Analyst.

Modular calibrant sets for the structural analysis of nucleic acids by ion mobility spectrometry mass spectrometry

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Supporting Material

Table 1S. Name, sequence, and mass of single-stranded (ss) and double-stranded (ds) species employed to generate calibration sets. Species marked with ◆ were used exclusively as ss calibrants, whereas those marked with * were combined with the respective complementary strand (not shown) to form a duplex construct of corresponding length. Average masses are reported.

Name	Sequence (5' → 3')	ss mass (u)	ds mass (u)
6mer◆*	CCCGGG	1793.4	3586.4
14b◆*	GTTAAGTCGTATTA	4292.9	8523.7
18b*	AAGAAGGTAACGAGTAGG	5645.7	10997.2
22b*	TCAGAAGAAGGTAACGAGTAGG	6881.5	13468.8
24b◆*	TATCAGAAGAAGGTAACGAGTAGG	7498.9	14703.6
32b◆*	TTAAACAGTATCAGAAGAAGGTAACGAGTAGG	9978.6	19644.9
48b◆*	TTAAACAGTAGGAAGAAAGAGGGAAGTTATAATAATGTAACGAGTAGG	15117.9	29531.3
64b◆*	TTAAACAGGAGAACACAATTCAGATAGGAAGAAAGAGGGAAGTCAGAAGAAGGTAACGAGT-AGG	20087.1	39415.6
80b◆	TTAAACAGGAGAACACAATTATAGTAAGACCACAGGCAGATAGGAAGAAAGAGGGAAGTCA-GAAGAAGGTAACGAGTAGG	25072.4	N/A
96b◆	TTAAACAGGAGAACACAATTATAGTAAGACCACAGGCAGATAGGAAGAAAGAGGGAAGTCA-GAAATCAAACATGAAGTAGGAAGGTAACGAGTAGG	30056.6	N/A

Table 2S. Comparison of CCS values obtained from the EHSS and TJM algorithms for five ds constructs in the study. Each value is the average and relative standard deviation (RSD%) obtained from 20 configurations along the simulation trajectory. A relative deviation (Δ%) was calculated between corresponding EHSS and TJM values. An average RMSD of ±3.9% was calculated from the various Δ%, which decreased to ±0.7% upon exclusion of the 14a:b species. Uncertainty regarding the conformation of this species in the gas phase may be the source of its high Δ% (see ***Results and Discussion***).

Duplex	**z**	**CCS_{EHSS} (Å^2) ±RSD%**	**CCS_{TJM} (Å^2) ±RSD%**	**Δ%**	**RMSD**
$(6mer)_2$	3	496.9 ± 1.0%	491.3 ± 1.0%	**-1.1%**	
10a:b	5	775.8 ± 1.9%	767.7 ± 1.7%	**-1.1%**	
14a:b	5	967.0 ± 1.4%	1162.5 ± 1.3%	**+16.8%**	**±3.9%**
18a:b	6	1405.1 ± 0.8%	1398.8 ± 1.3%	**-0.4%**	
22a:b	7	1791.8 ± 0.6%	1790.2 ± 0.9%	**-0.1%**	

Table 3S. Comparison of CCS values obtained by the EHSS method in either He or N_2 ($EHSS_{He}$ and $EHSS_{N2}$, respectively) for the calibrant sets in this study. An average RMSD of ±3.4% was obtained from corresponding values, which indicated a negligible difference between the two datasets. The fact that the ss series displayed a larger RMSD than the ds counterpart (i.e., ±4.9% versus ±1.5%) could be explained by intrinsic errors associated with modeling structures devoid of well-defined secondary structure.

Species	z	CCS $EHSS_{He}$ ($Å^2$) ±RSD%	CCS $EHSS_{N2}$ ($Å^2$) ±RSD%	Δ%	RMSD
6mer	3	321.6 ± 1.1%	322.9 ± 1.1%	-0.4%	
14b	4	685.2 ± 1.9%	743.7 ± 5.7%	-7.9%	
24b	4	991.5 ± 1.6%	992.1 ± 1.9%	-0.1%	
	5	1127.1 ± 1.0%	1000.6 ± 0.9%	+12.6%	
32b	5	1221.3 ± 1.8%	1217.8 ± 1.6%	+0.3%	±4.9%
	6	1411.5 ± 1.2%	1367.9 ± 0.5%	+3.2%	
48b	7	1919.4 ± 0.5%	1869.7 ± 1.3%	+2.7%	
64b	8	2528.1 ± 1.1%	2507.9 ± 0.6%	+0.8%	
80b	9	2865.0 ± 0.6%	2868.3 ± 0.6%	-0.1%	
96b	10	3524.2 ± 0.7%	3505.1 ± 0.6%	+0.5%	
$(6mer)_2$	3	497.2 ± 1.0%	496.9 ± 1.0%	+0.1%	
14a:b	5	913.7 ± 0.9%	967.0 ± 1.4%	-5.5%	
18a:b	6	1402.7 ± 0.7%	1405.1 ± 0.8%	-0.2%	
22a:b	7	1796.5 ± 0.7%	1791.8 ± 0.6%	+0.3%	
	8	1750.6 ± 1.7%	1746.5 ± 1.4%	+0.2%	
24a:b	7	1872.4 ± 0.8%	1868.4 ± 0.5%	+0.2%	
32a:b	8	2445.1 ± 1.1%	2446.2 ± 1.0%	<0.1%	±1.5%
	9	2430.1 ± 0.4%	2432.5 ± 0.4%	-0.1%	
48a:b	10	3625.4 ± 0.3%	3623.7 ± 0.4%	<0.1%	
	11	3660.6 ± 0.5%	3668.8 ± 0.4%	-0.2%	
64a:b	12	4651.0 ± 0.5%	4654.8 ± 0.7%	-0.1%	
	13	4804.2 ± 0.7%	4810.8 ± 0.7%	-0.1%	
	14	4909.4 ± 0.4%	4908.0 ± 0.7%	<0.1%	

Table 4S. Comparison of EHSS CCS values obtained in this study and reported in previous publications. The individual Δ% values were greater for the larger ds construct and the unconstrained ss species, which again point towards possible deficiencies in their gas-phase models.

Species	z	CCS_{EHSS} (Å^2) ±RSD%	Literature CCS (Å^2)	Δ%	RMSD
gc14 (dsDNA)	7	1049.5 ± 0.7%	1016	+3.3%	
gc18 (dsDNA)	9	1245.8 ± 0.5%	1254	-0.7%	
mix26 (dsDNA)	13	2145.0 ± 0.9%	2035	+5.4%	±4.5%
10dT (ssDNA)	3	486.1 ± 2.2%	452	+7.5%	
16nt hairpin	4	630.0 ± 0.4%	620	+1.6%	

Table 5S. Name, sequence, and mass of test samples employed to evaluate the various calibration curves. Species marked with * were combined with the respective complementary strand (not shown) to form a duplex construct of corresponding length. Species marked with ◆ were used exclusively as ss samples. Average masses are reported.

Name	Sequence (5' → 3')	ss mass (u)	ds mass (u)
10b*	CGCGCGCGCG	N/A	6060.0
16b◆	GAAGGTAACGAGTAGG	5019.3	N/A
30a*◆	GTATTCCGTAGTTCAAATTGCATACTGGAG	9326.0	18472.0
36a*	CCTACTCGTTACCTTCTTCTGACTTCCCTCTTTCTT	N/A	21572.0
56a*◆	GAGAACACAATTCAGATAGGAAGAAAGAGGGAAGTCAGAAGAAGGTAACGAGTAGG	16866.9	33733.8
70b◆	TTAAACAGGAGAACACAATTATAGTAAGACCACAGGCAGATAGGAAGAAAGAGGGAAGTCAGAAGAAGGT	21909.3	N/A
16hp	TGCGATACTCATCGCA	4841.2	N/A
28hp	GCGTTCATCAGAGTCATCTGATGAACGC	8588.6	
36hp	GCTATCCAAGTCTTGCTCGAGCAAGACTTGGATAGC	11060.2	
48hp	GGTTCTCTGGTTAGCCAGAGAGCTGACTCTCTGGCTAACTAGAGAACC	14783.6	

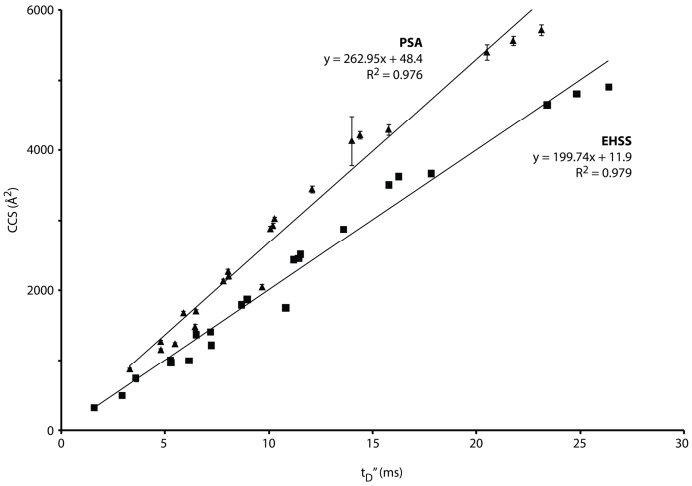

Figure 1S. Calibration curves obtained by including all detected charge states. The curves correlate experimental t_D" data with the corresponding reference values provided by either the EHSS (■) or PSA (◆) algorithm.