Structural Analysis of Activated SgrAI/DNA Oligomers using Ion Mobility Mass Spectrometry — Supporting Material

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Peak qualities in Figure S4C and Figure S5C

Although the peaks in Figures S4-S5 have a relatively low signal/noise ratio, these peaks are reproducible (Fig. S6-S8) and exhibit the expected characteristics of peaks corresponding to the assigned oligomers of SgrAI and DNA. For example, they follow the pattern of electrospray peaks in that a series of continuous whole numbers for the three peaks can be found, and the masses calculated using their *m/z* and assigned charge state are similar. In addition, the mass calculated from the peaks divided by the basic building block unit mass (ie. the DBD or SgrAI dimer+2PC DNA) is close to a whole number (ie. 10 and 19, Fig. S4-S5, respectively). Further, after the collision cross section is calculated, based on the drift time and the charge state, the CCSs of these large species fall on the same straight line found for the smaller species (species with one to six basic building blocks, Fig. 3). This would be very unlikely if the peaks were only noise. In short, both the masses and CCS, which are measured independently from each other, support the assignment of these peaks to the 10×DBD and 19×DBD oligomers.

Region selection was necessary to observe the peaks for the 10×DBD and 19×DBD complexes (Fig. S4-S5) leading to differences in *m/z* peak widths when compared to peaks for complexes that are observable without region selection (*e.g.* 1×DBD, 2×DBD, 3×DBD, and 6×DBD complexes, Fig. 1C). If many species fall in the same *m/z* range, as we suspect is the case for the larger oligomers of DBD, then region selection of the mobiligram is necessary to distinguish among them. The more complicated the mixture is, the narrower the selection must be. The narrow region selection leads to narrower *m/z* peak widths because of exclusion of different "editions" of the complexes containing different degrees of solvent attachment (ions and/or neutral species). These other "editions" have similar, but not exact masses, which leads to *m/z* peak broadening in general (see Figs. S2-3, where CID is used to reduce peak broadening). Figures S9-S10 show the effect of region selection on the widths of peaks identified for the 10×DBD and 2xDBD complexes. Hence, the *m/z* peaks for complexes identified without region selection (*e.g.* 1×DBD, 2×DBD, 3×DBD, and 6×DBD complexes, Fig. 1C) are broader than those identified only after region selection (10×DBD and 19×DBD complexes, Fig. S4-S5).

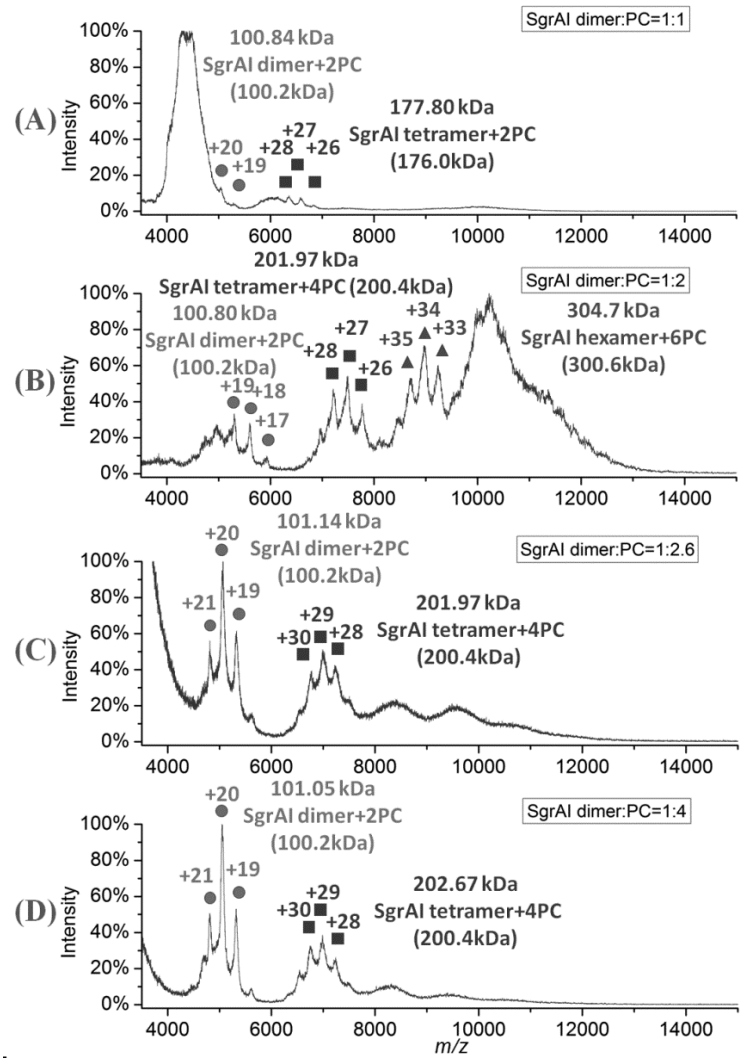


Figure S1. Spectra of different molar ratios of SgrAI dimer to PC DNA. (A) The spectrum of SgrAI:PC=1:1. (B) The spectrum of SgrAI:PC=1:2. (C) The spectra of SgrAI:PC=1:2.6. (D) The spectrum of SgrAI:PC=1:4.

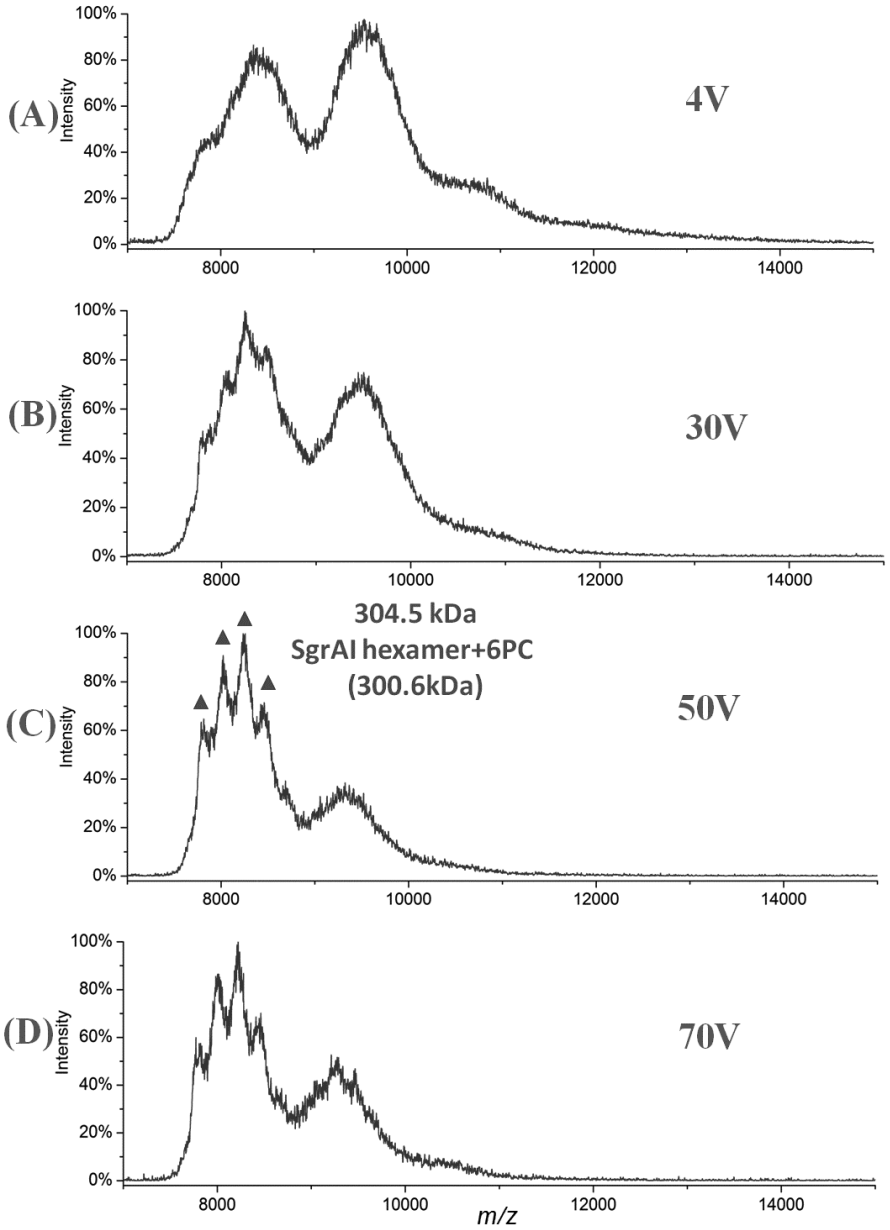


Figure S2. CID of MS/MS quadrupole-selected high *m/z* range in radio frequency profile mode, *m/z* 8,000-30,000 selected, of SgrAI dimer:PC DNA=1:2.6. When the collision voltage is increasing from 4V (A) to 50V (C), SgrAI hexamer+6PC DNA complex (peaks with triangles) appear at 30 V and become clearer at 50 V. When the collision voltage was above 50V (D), the spectrum does not change significantly.

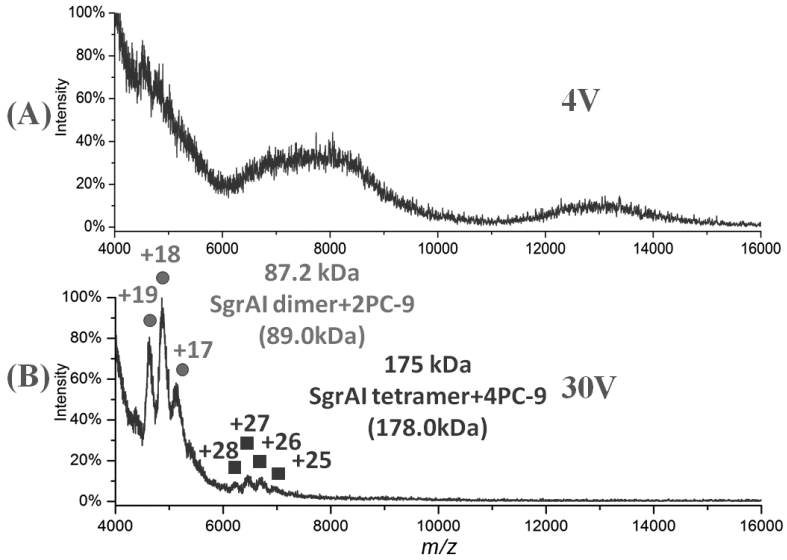


Figure S3. CID of SgrAI dimer:PC-9 DNA=1:2. When the collision voltage was 4 V (A), there are no distinct peaks. A small amount of large *m/z* (12,000-14,000) species was observed. By applying 30V collision voltage, SgrAI dimer+2PC-9 DNA (peaks with circles) and SgrAI tetramer+4PC-9 DNA (peaks with squares) complexes were observed.

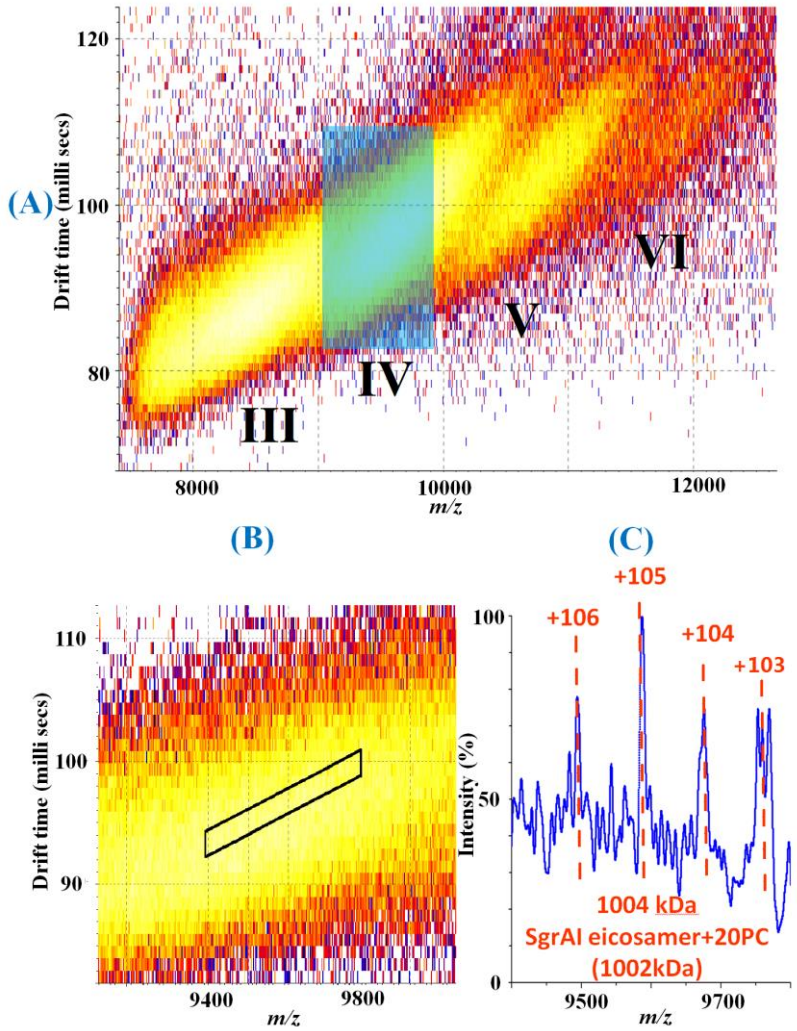


Figure S4. Mobiligram of SgrAI dimer:PC DNA=1:2.6. (B) is a blow up of the blue region in (A). (C) is the mass spectrum extracted from the boxed region in (B) which is the highest intensity region in the cluster. Based on (B) and (C), the peaks of SgrAI eicosamer+20PC DNA complex were observed.

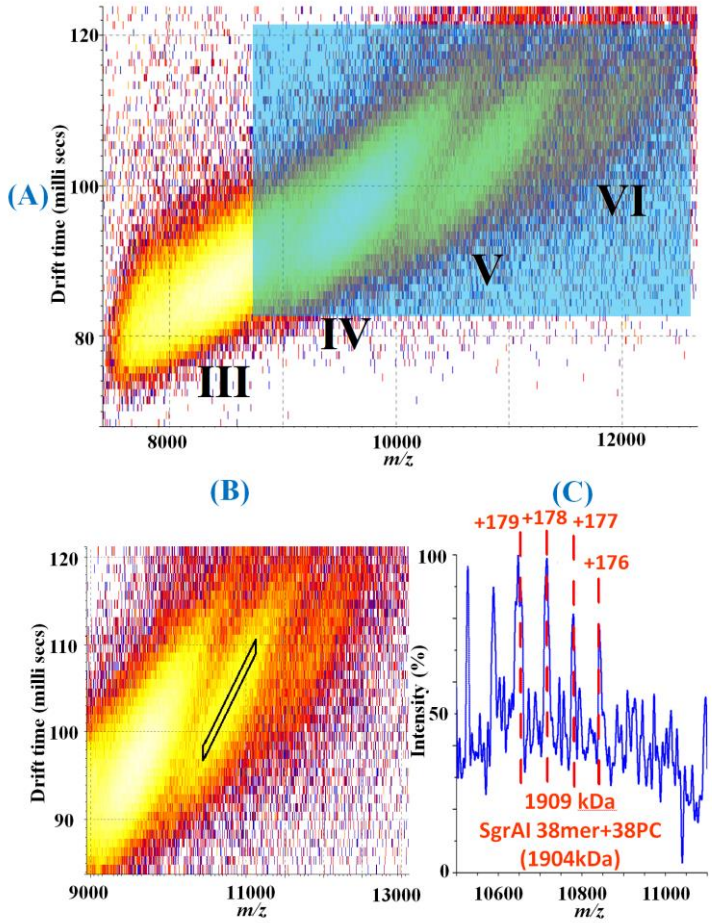


Figure S5. Mobiligram of SgrAI dimer:PC DNA=1:2.6. (B) is blow up of the blue region in (A). (C) is the mass spectrum extracted from the boxed region in (B) which is the highest intensity region in the cluster. Based on (B) and (C), the peaks of SgrAI 38mer+38PC DNA complex were observed.

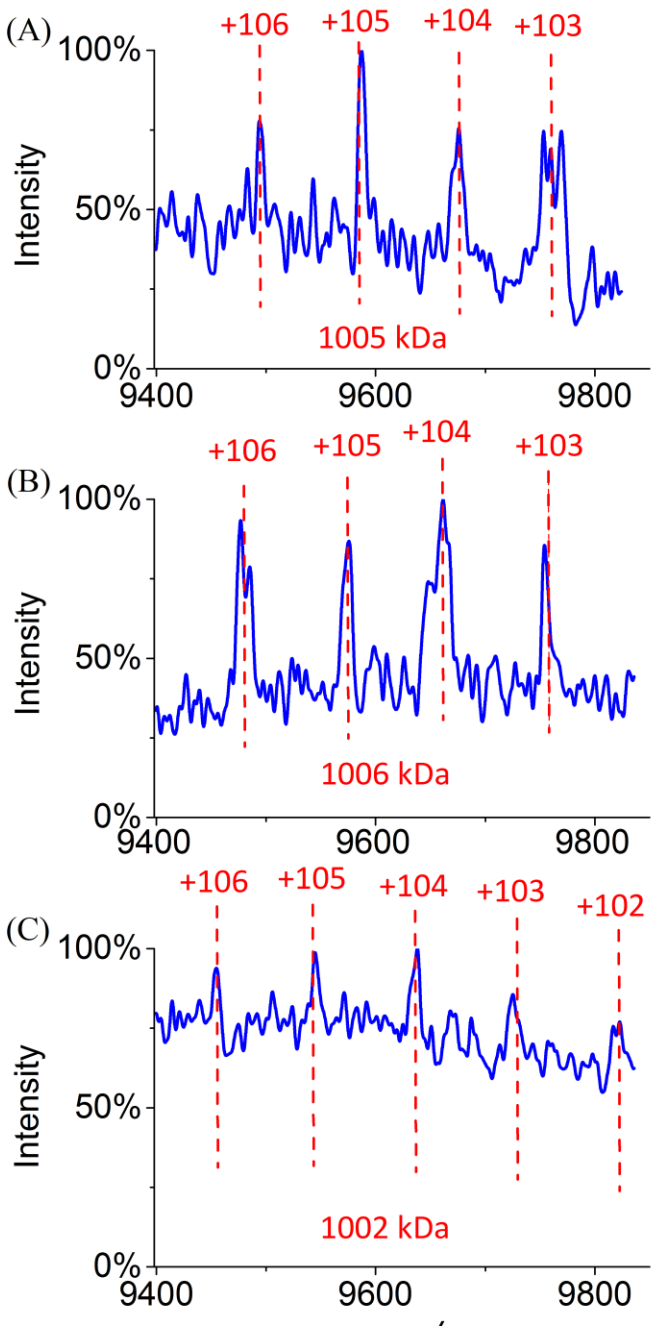


Figure S6. Three independent measurements of the 10xDBD. The masses calculated from the peaks are listed in each spectrum. The theoretical *m/z* of the peaks are labeled with dashed lines.

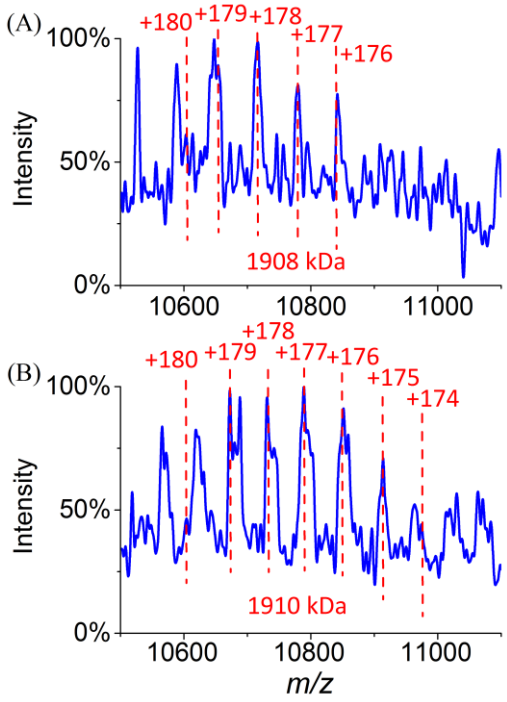


Figure S7. Two independent measurements of the 19xDBD. The masses calculated from the peaks are listed in each spectrum. The theoretical *m/z* of the peaks are labeled with dashed lines.

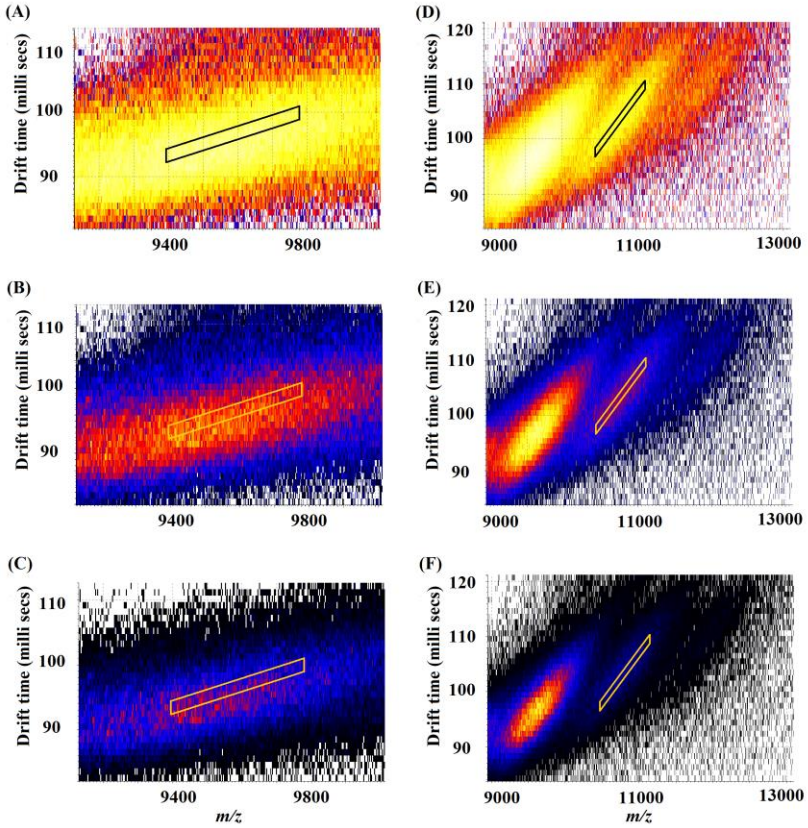


Figure S8. Mobiligrams in different color scales. (A) to (C) show the selected region (boxes), from which the 10×DBD was observed in cluster IV (Fig. S4), drawn with log, square root and

linear scales respectively. (D) to (E) show the selected region (boxes) from, which the 19×DBDwas observed in cluster V, drawn with log, square root and linear scales respectively.

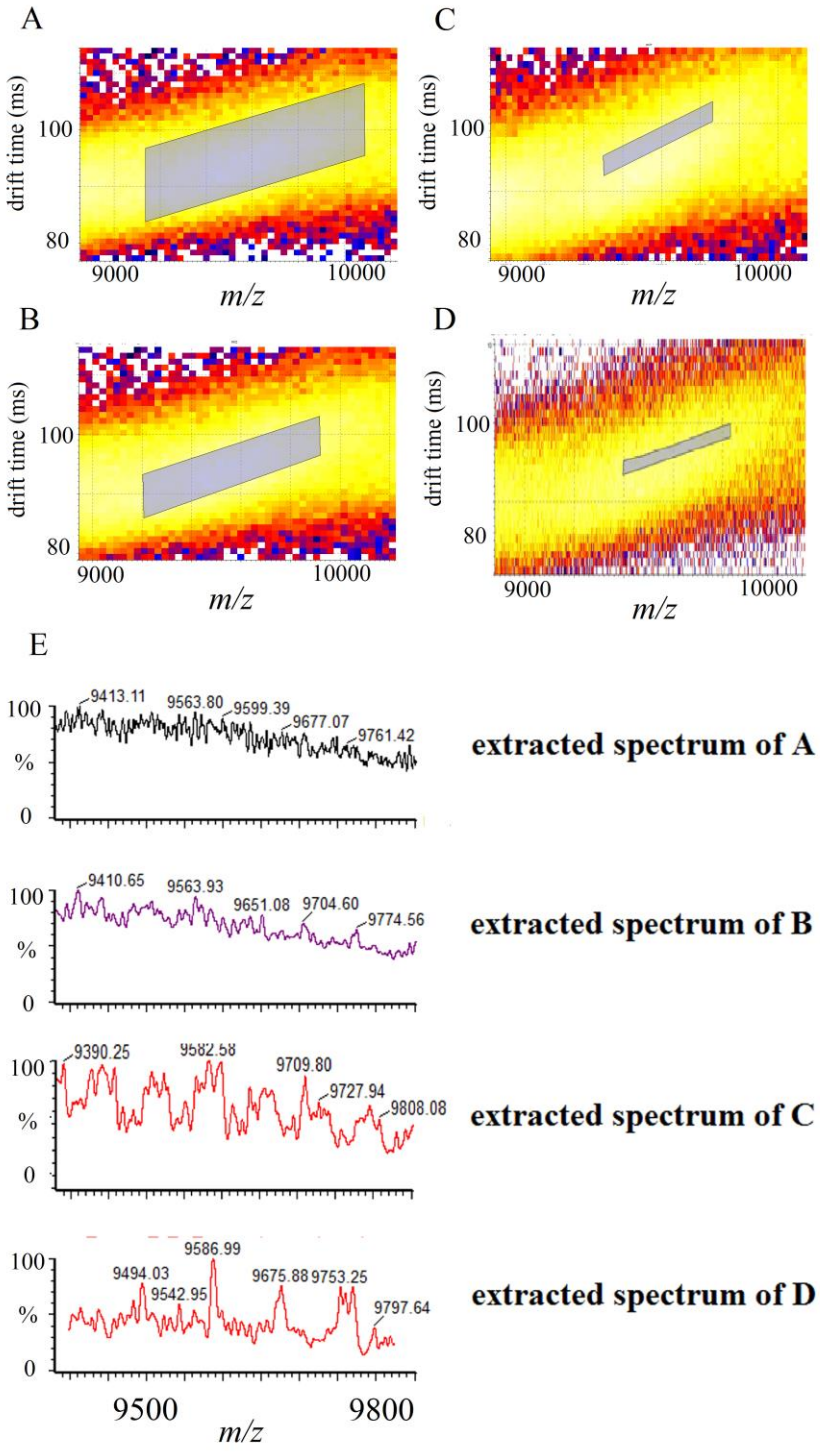
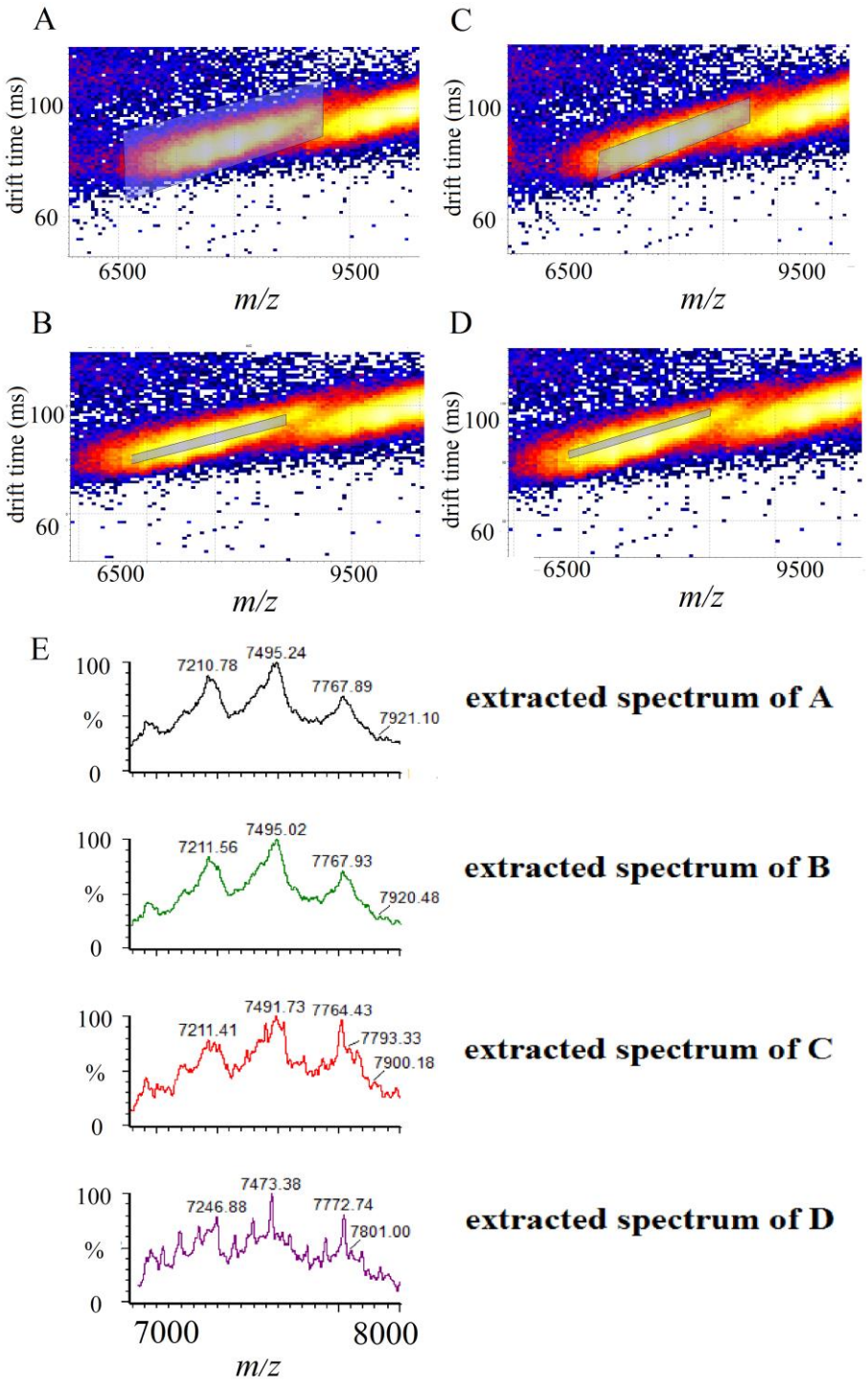


Figure S9. Influence of different widths of selected regions. The mobiligram is the zoom in region from Figure S4 in which 10×DBD was identified. (A)-(D) The width of the selected region varies, becoming narrower from A to D. The spectra are shown in (E). The narrower the region, the narrower the selected peaks are because of less interference from neighboring species with similar

but slightly different drift times.



FigureS10. Influence of different widths of selected regions from region identified as containing 2xDBD in the 1:2.6 SgrAI:PC DNA sample. (A)-(D) The width of the selected region varies from wider to narrower. The spectra are shown in (E). The narrower the region is, the narrower the

selected peaks are because of less interference from neighboring species with similar but slightly different drift times.

Table S1. Masses of SgrAI/DNA complexes

Species		Experimental mass (kDa)	Standard deviation (kDa)	Theoretical mass (kDa)
SgrAI without DNA	dimer	76.0	0.2	75.8
	tetramer	153.5	0.4	151.6
SgrAI dimer:40-2 =1:1	dimer+40-2	101.9	0.1	100.3
	tetramer+2*40-2	198.0	0.2	200.6
	hexamer+3*40-2	299.4	0.5	300.9
SgrAI dimer:PC =1:1	dimer+2PC	100.84	0.07	100.2
	tetramer+2PC	177.8	0.02	176
SgrAI dimer:PC =1:2	dimer+2PC	100.8	0.09	100.2
	tetramer+4PC	201.97	0.05	200.4
	hexamer+6PC	304.7	0.2	300.6
	dodecamer+12PC	605	2	601.2
SgrAI dimer:PC =1:2.6	dimer+2PC	101.14	0.08	100.2
	tetramer+4PC	202.4	0.3	200.4
	eicosamer+20PC	1004	2	1002
	38mer+38PC	1909	1	1904
SgrAI dimer:PC =1:4	dimer+2PC	101.05	0.05	100.2
	tetramer+4PC	202.67	0.06	200.4

Table S2. CID data (collision voltage 50V) of SgrAI dimer:PC DNA=1:2.6

	Experimental mass (kDa)	Standard deviation (kDa)	Theoretical mass (kDa)
Hexamer+6PC	304.5	0.2	300.6

Table S3. CID data (collision voltage 30V) of SgrAI dimer:PC-9 DNA=1:2

	Experimental mass (kDa)	Standard deviation (kDa)	Theoretical mass (kDa)
Dimer+2PC-9	87.2	0.7	89

Tetramer+4PC-9	175	1	178

Table S4. IM-MS CCSs of SgrAI PC DNA complexes

	Z	CCS (nm^2)	Standard deviation (nm^2)
Dimer+2PC	19	54.9	0.1
	18	53.4	0.6
	17	53.3	0.2
Tetramer+4PC	29	88.2	0.4
	28	87.5	0.3
	27	87.3	0.4
	26	87.1	0.2
Hexamer+6PC	36	123.5	0.4
	35	122.4	0.3
	34	122.4	0.3
	33	122.1	1.3
Dodecamer+12PC	51	202	3
	50	203	4
	49	205	4
Eicosamer+20PC	106	372	2
	105	372	3
	104	370	2
38mer+38PC	178	667	3
	177	668	3
	176	669	3

Table S5. IM-MS CCSs of SgrAI dimer with different DNA complexes

	Z	CCS (nm^2)	Standard deviation (nm^2)
Dimer without DNA	18	48.3	0.1
	17	46.1	0.1
	16	45.35	0.03
Dimer+2PC-9	18	49.5	0.4
	17	48.0	0.7

Dimer+40-2	19	54.24	0.06
	18	53.6	0.2
	17	53.5	0.3

Table S6. Calculated CCSs of SgrAI dimer with different DNA complexes

	PA (nm^2)	**EHSS (nm^2)**	**1.14PA (nm^2)**
3MQY+4bp	44.41	58.27	50.63
Conformation 1	55.11	71.33	62.82
Conformation 2	53.99	6987	61.53
Conformation 3	50.31	66.14	57.35