Supplementary information for

Crystal structures of B-DNA dodecamer containing the epigenetic modifications 5-hydroxymethylcytosine or 5-methylcytosine

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Table S1:

Thermodynamic parameters of studied oligonucleotides determined from thermal melting experiment detected by UV absorption spectroscopy at 260 nm in 0.1 cm cells in 10 mM sodium phosphate buffer, pH 7.2 with 50 mM sodium chloride. Values were calculated according to Mergny and Lacroix (60). DD0 represents unmodified Drew-Dickerson dodecamer.

	DD0	**DDm3**	**DDm9**	**DDh3**	**DDh9**
T_m [°C]	58.9	58.9	60.2	59.5	56.4
ΔH_{VH} [kJ.mol^{-1}]	-240	-219	-206	-244	-184
ΔS [kJ.mol^{-1}.K^{-1}]	-0.639	-0.579	-0.536	-0.651	-0.475
$T\Delta S$ at 37°C [kJ.mol^{-1}]	-198	-179	-166	-202	-147
ΔG^0 at 37°C [kJ.mol^{-1}]	-42	-40	-40	-42	-37

Figure S1: RMSD values for individual nucleotides between reference model 1BNA and other selected PDB models of Drew-Dickerson dodecamer. A) A strand, B) B strand.

Figure S2: RMSD values for individual nucleotides between reference model 1BNA and our models of Drew-Dickerson dodecamer with modified bases at position either 3 or 9. A) A strand, B) B strand.

Figure S3: RMSD values for individual nucleotides for each combination of two of our presented Drew-Dickerson dodecamer models. A) A strand, B) B strand.

Figure S4: RMSD values for individual nucleotides for each combination of two sequences from DDm3, DDh3 and DDh3b, which is similar to DDh3 but crystallized in Mg containing conditions. A) A strand, B) B strand.

Figure S5: Intra base and inter base pair parameters of all base pairs in all studied sequences together with original Drew model (1BNA) and initial model of our molecular replacement (1DPN).

Figure S6: Structures and electron density maps of individual 5-methylcytosines. Upper left: DDm3 / A strand. Upper right: DDm3 / B strand; Lower left: DDm9 / A strand. Lower right: DDm9 / B strand. Electron density maps were calculated with the program CCP4mg (61) at sigma = 1.

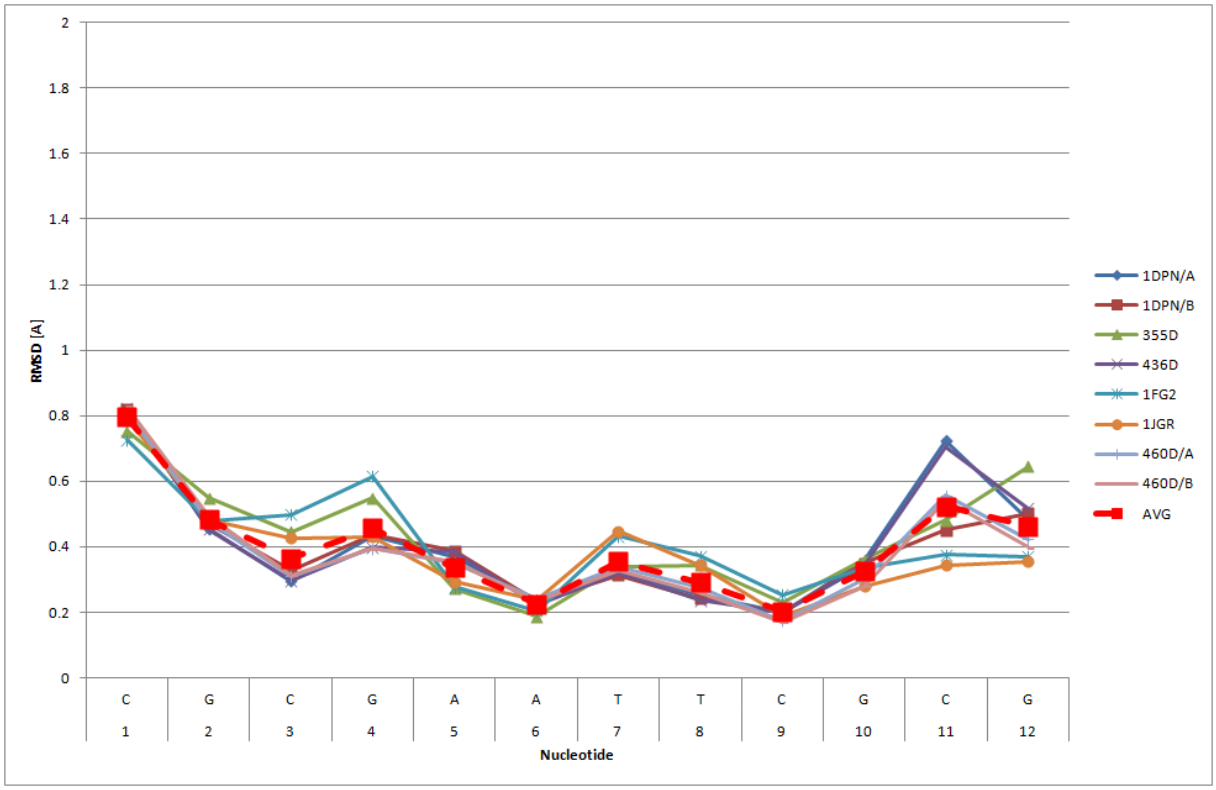
Figure S7: Analysis of the binding of water molecules and cations to DDm3, DDm9, DDh3, DDh3b and DDh9 with the program Nucplot (59).

Figure S8: Raw UV melting curves (A) and folded fraction plots (B) of the oligonucleotides, recorded at 260 nm, in 10 mM sodium phosphate buffer, pH 7.2, with 0.1 mM EDTA and 50 mM sodium chloride. All curves are taken from renaturation experiments.

61. McNicholas, S., Potterton, E., Wilson, K.S. and Noble, M.E.M. (2011) Presenting your structures: the CCP4mg molecular-graphics software. *Acta Cryst.*, **D67**, 386-394.

Figure S1:

A)


RMSD [A]
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
C G C G A A T T C G C G
1 2 3 4 5 6 7 8 9 10 11 12
Nucleotide
1DPN/A
1DPN/B
355D
436D
1FG2
1JGR
460D/A
460D/B
AVG

B)

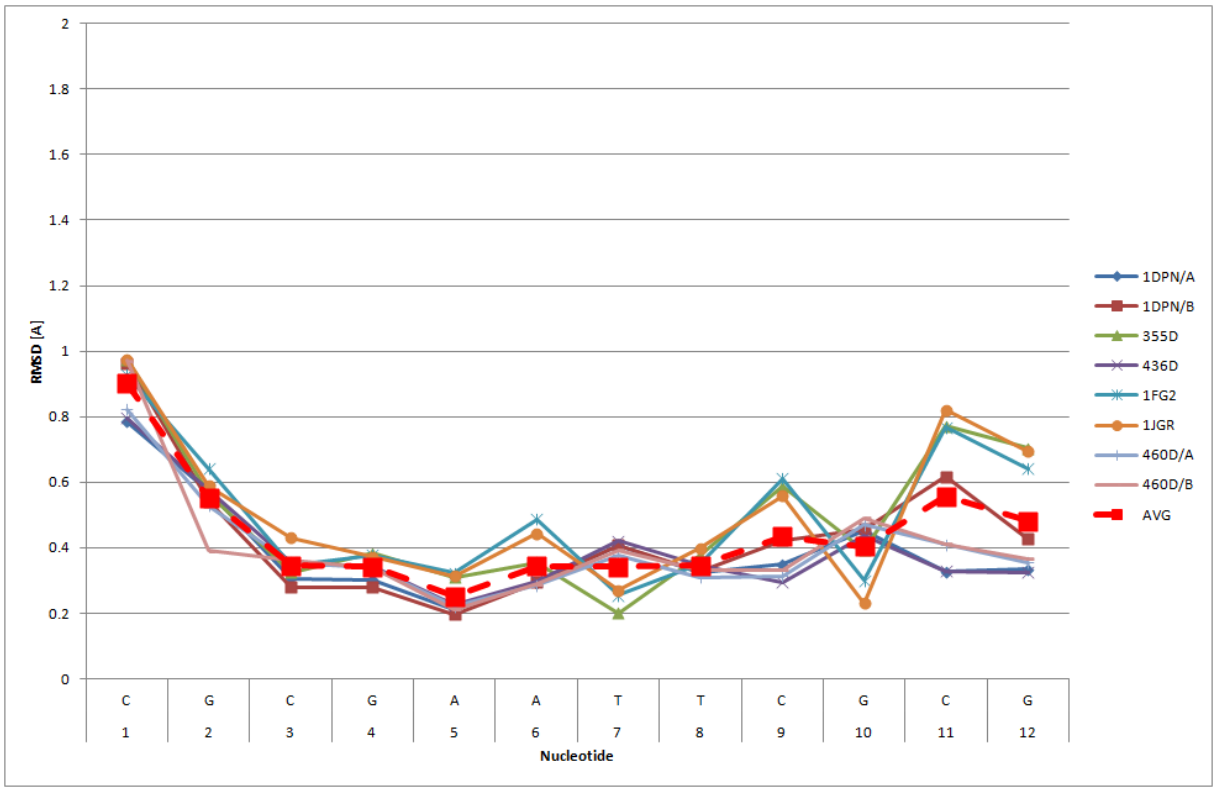
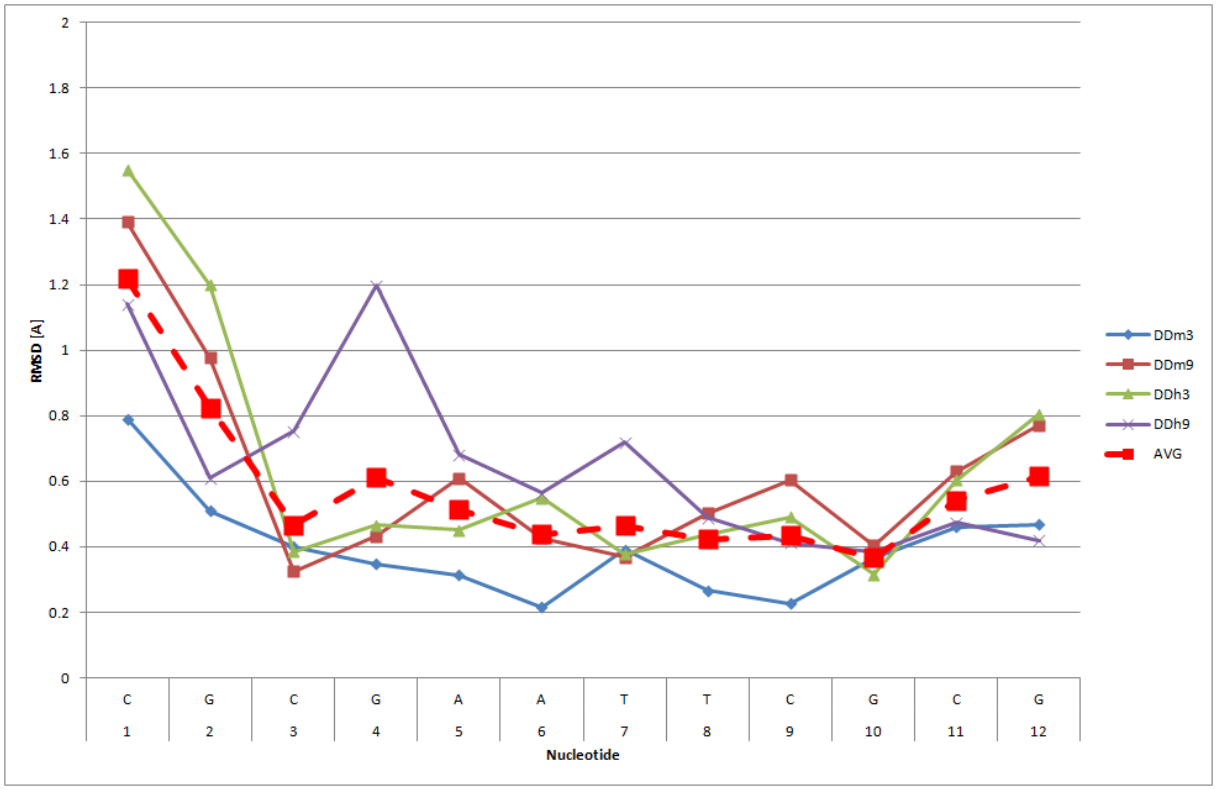

RMSD [A]
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
C G C G A A T T C G C G
1 2 3 4 5 6 7 8 9 10 11 12
Nucleotide
1DPN/A
1DPN/B
355D
436D
1FG2
1JGR
460D/A
460D/B
AVG

Figure S2:

A)


RMSD [A]
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
C G C G A A T T C G C G
1 2 3 4 5 6 7 8 9 10 11 12
Nucleotide
DDm3
DDm9
DDh3
DDh9
AVG

B)

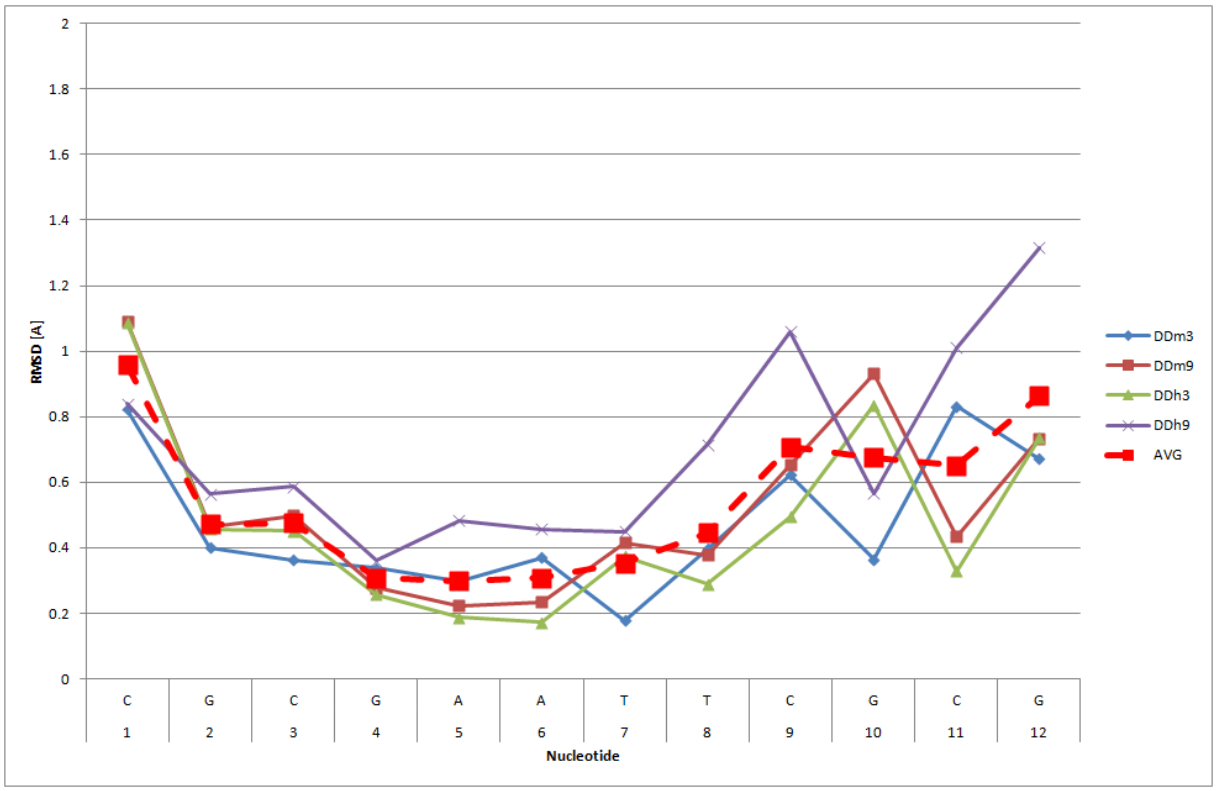
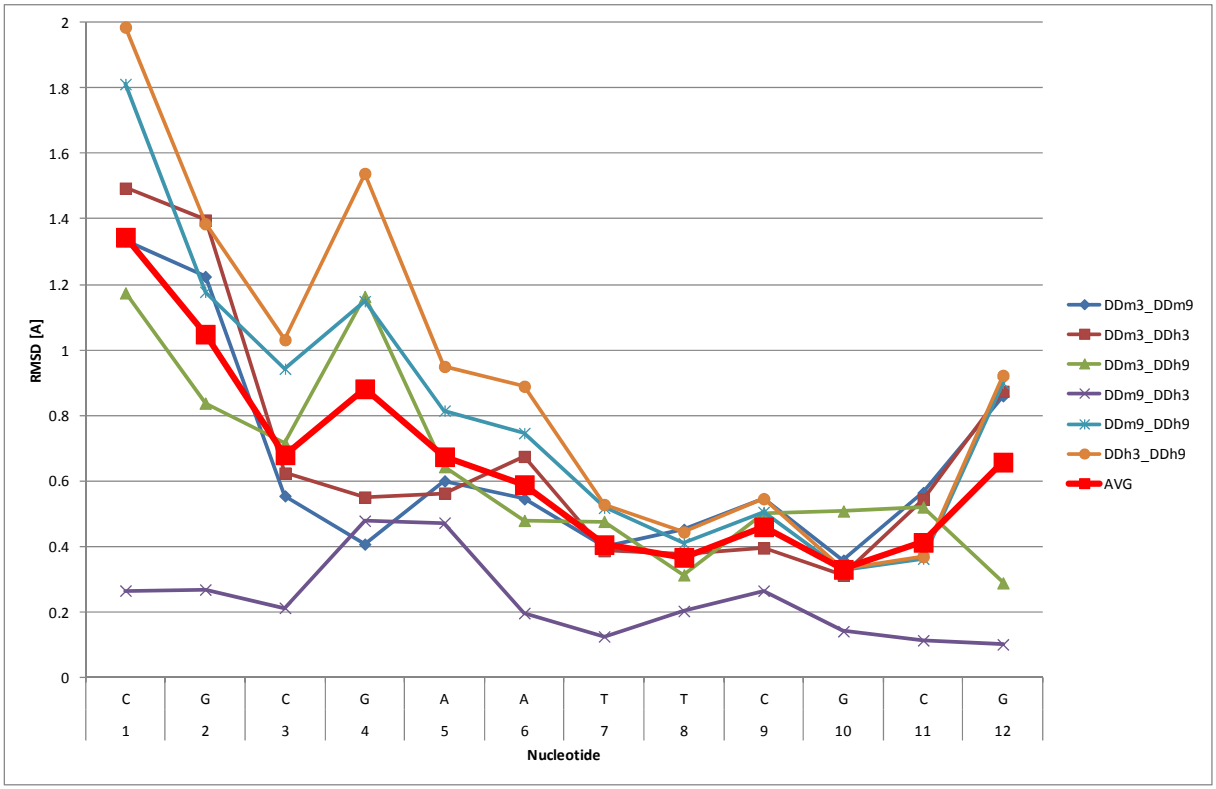

RMSD [A]
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
C G C G A A T T C G C G
1 2 3 4 5 6 7 8 9 10 11 12
Nucleotide
DDm3
DDm9
DDh3
DDh9
AVG

Figure S3:

A)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1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
C 1
G 2
C 3
G 4
A 5
A 6
T 7
T 8
C 9
G 10
C 11
G 12
Nucleotide
DDm3_DDm9
DDm3_DDh3
DDm3_DDh9
DDm9_DDh3
DDm9_DDh9
DDh3_DDh9
AVG

B)

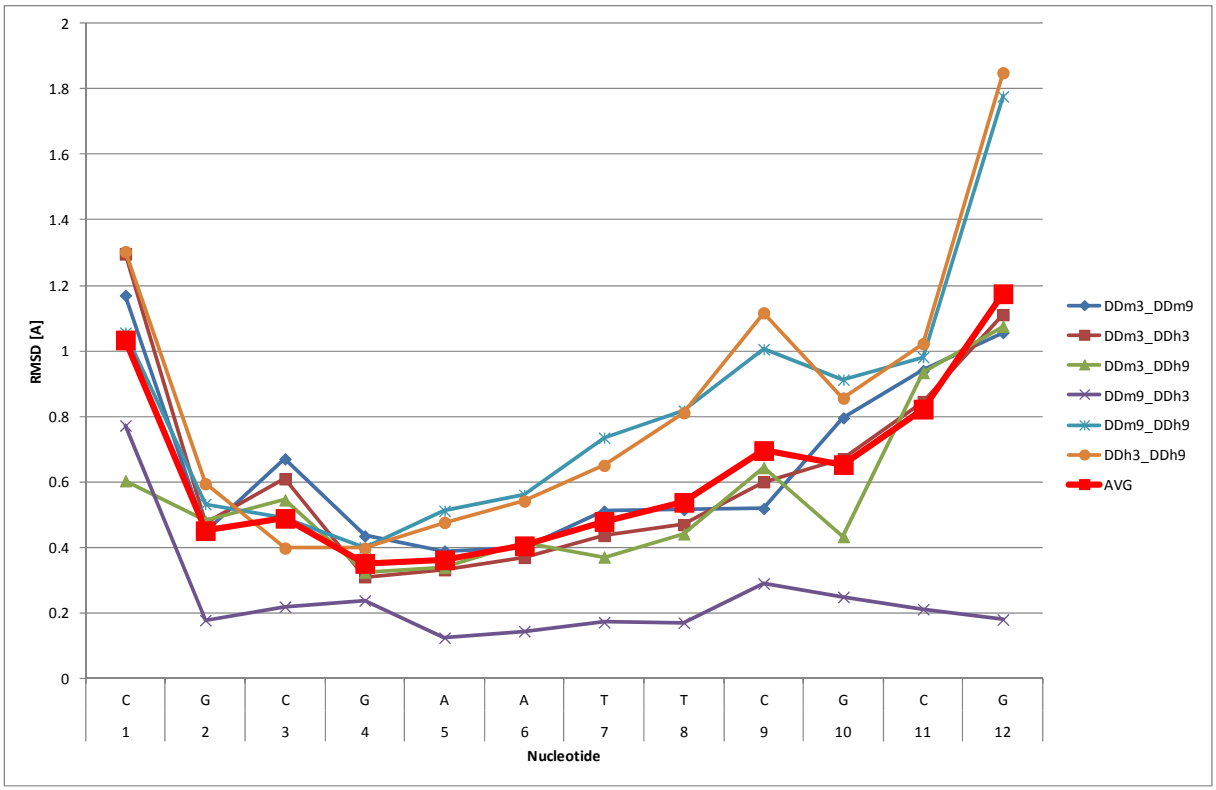
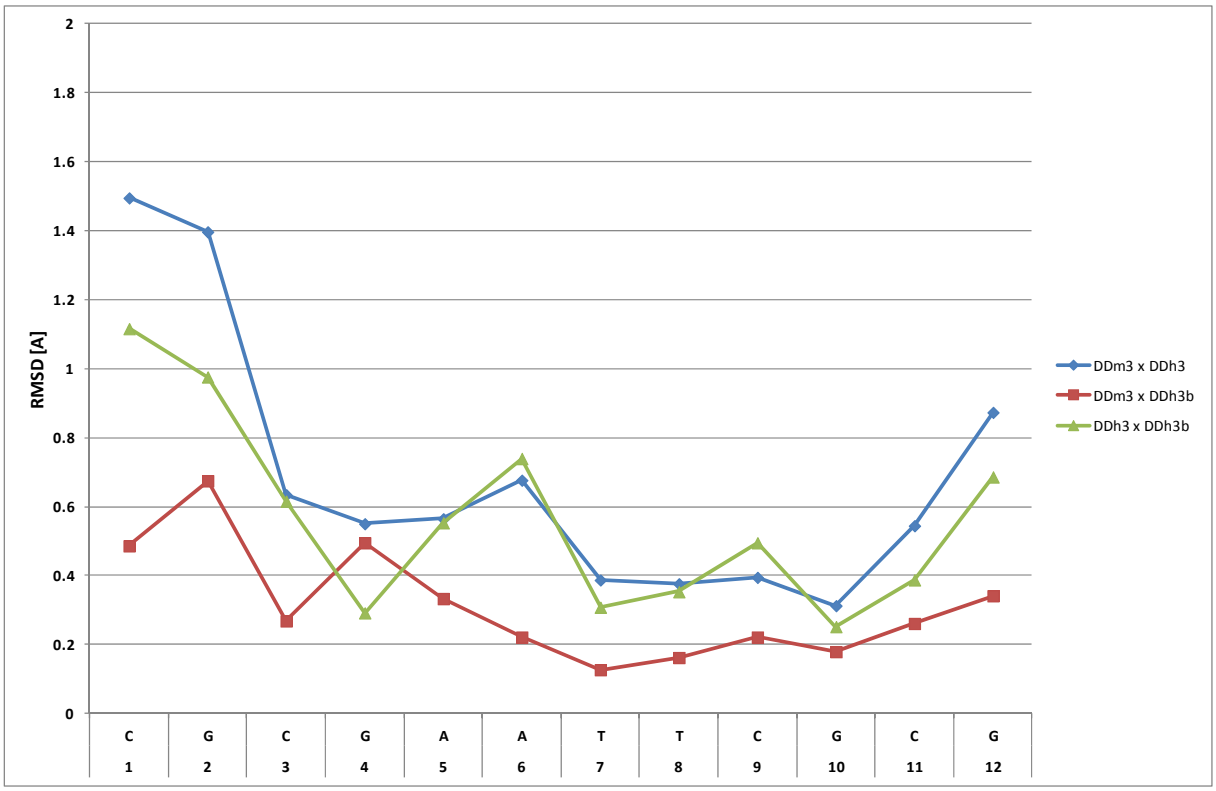

RMSD [A]
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
C 1
G 2
C 3
G 4
A 5
A 6
T 7
T 8
C 9
G 10
C 11
G 12
Nucleotide
DDm3_DDm9
DDm3_DDh3
DDm3_DDh9
DDm9_DDh3
DDm9_DDh9
DDh3_DDh9
AVG

Figure S4:

A)


RMSD [A]
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
C 1
G 2
C 3
G 4
A 5
A 6
T 7
T 8
C 9
G 10
C 11
G 12
DDm3 x DDh3
DDm3 x DDh3b
DDh3 x DDh3b

B)

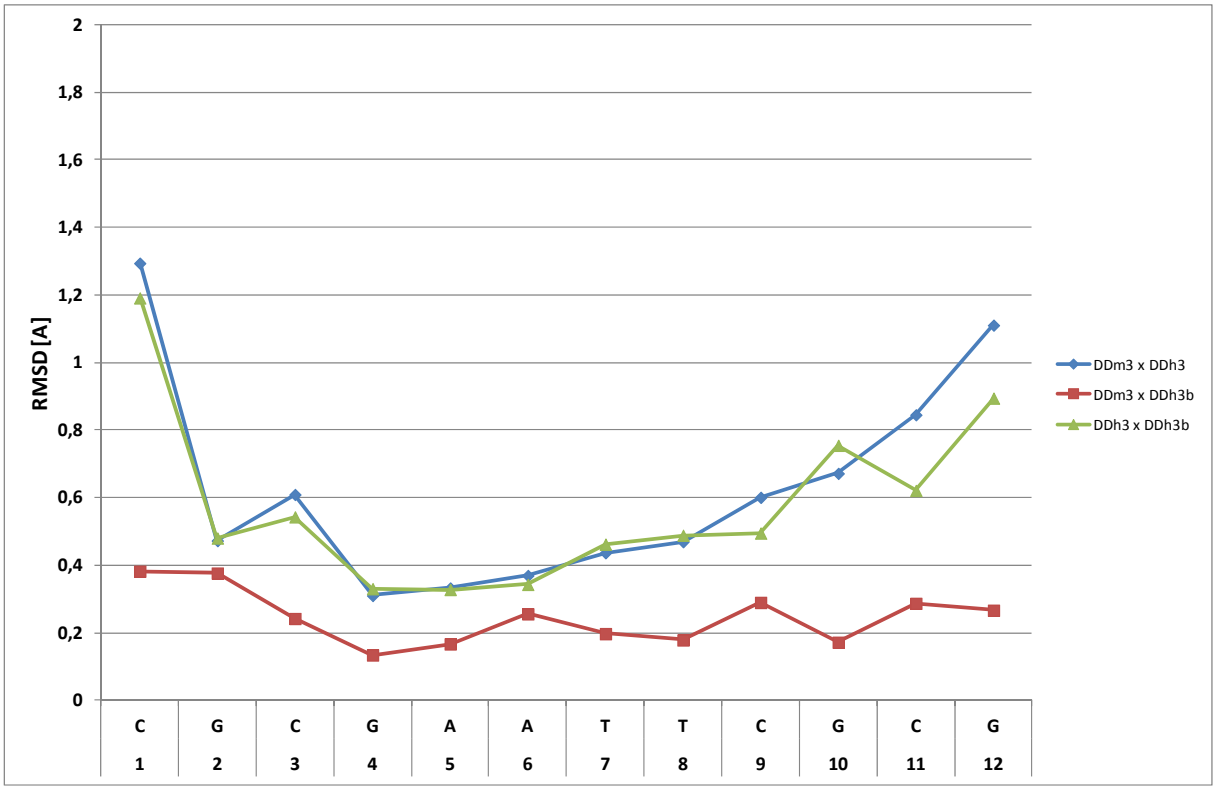
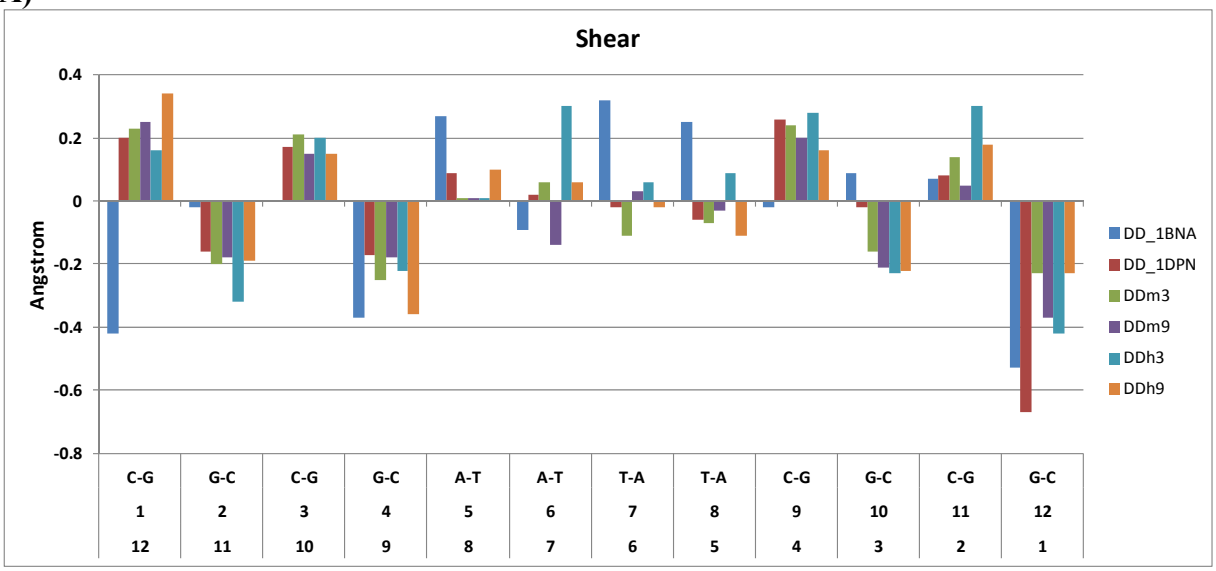
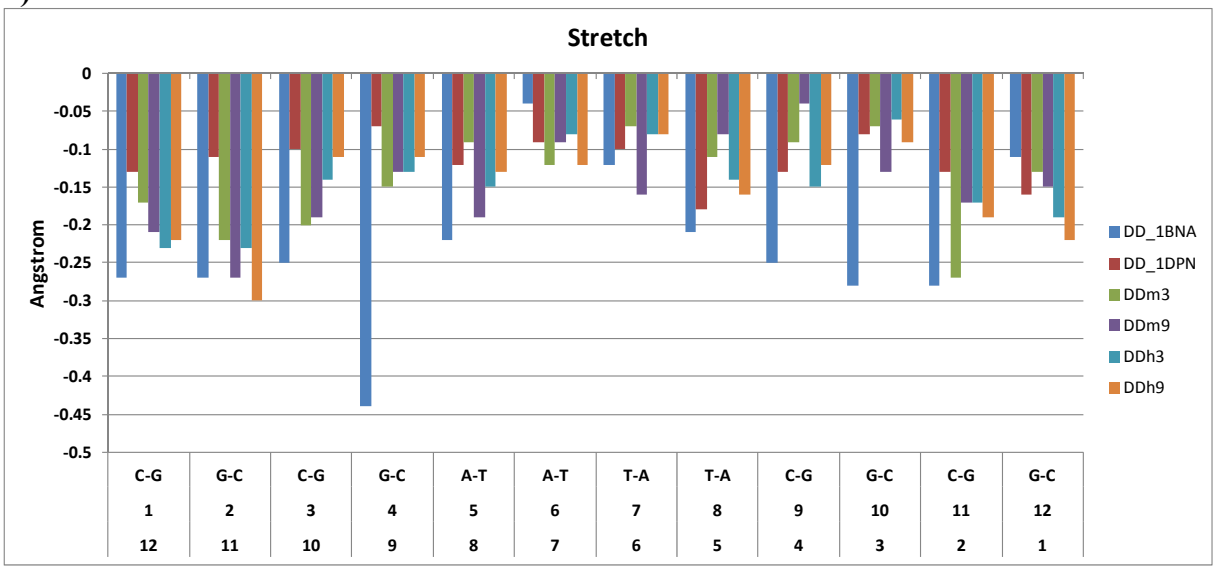

RMSD [A]
2
1,8
1,6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
C 1
G 2
C 3
G 4
A 5
A 6
T 7
T 8
C 9
G 10
C 11
G 12
DDm3 x DDh3
DDm3 x DDh3b
DDh3 x DDh3b

Figure S5:

A)



B)



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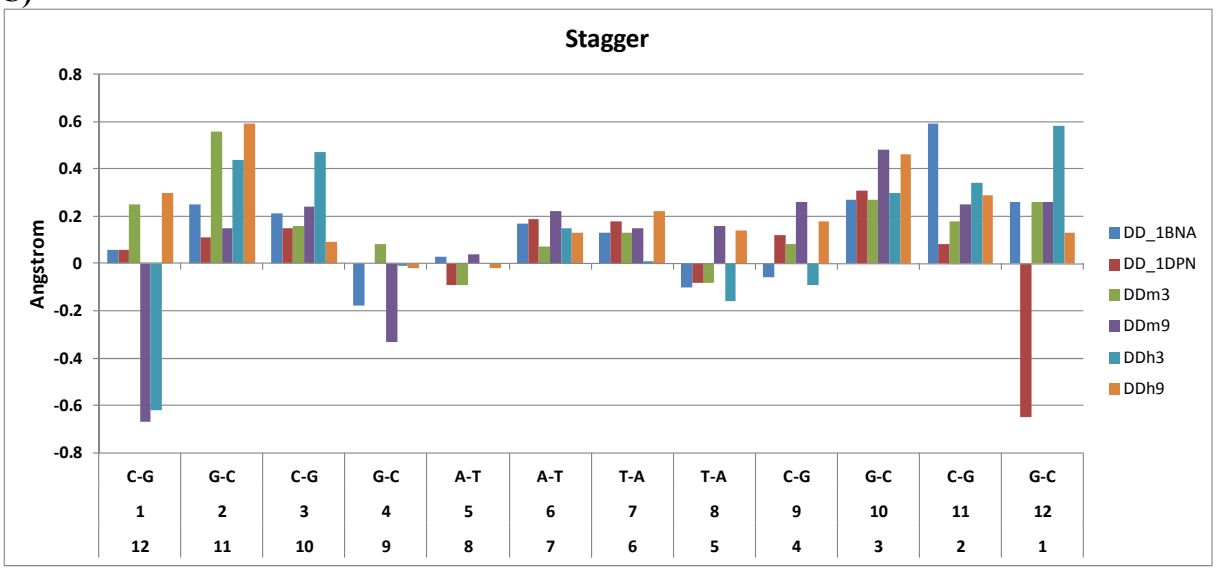

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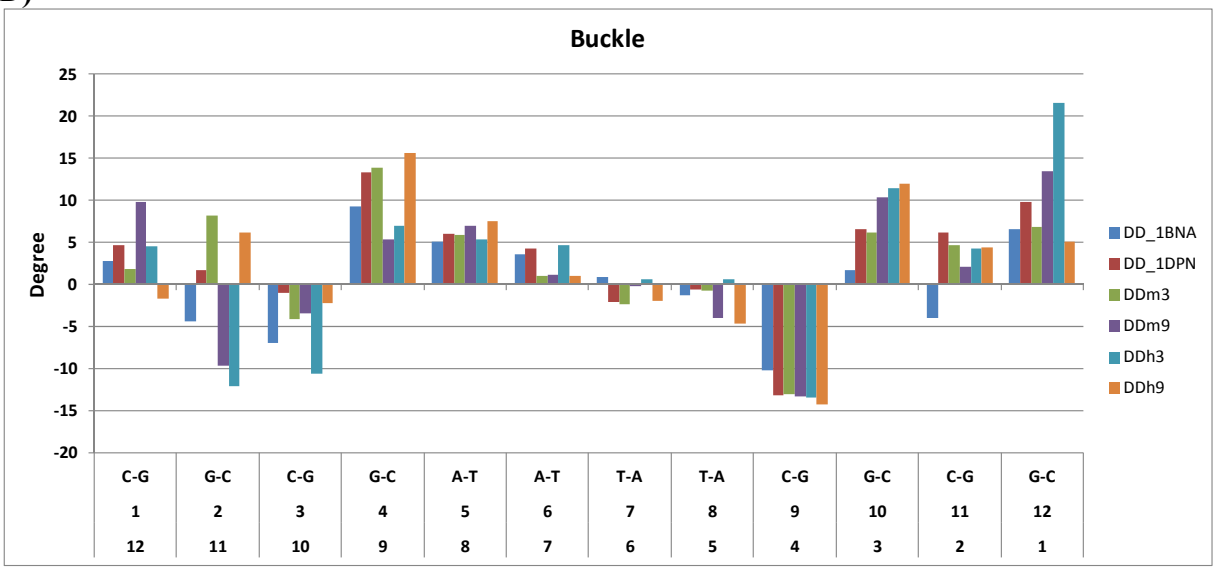
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Degree
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15
10
5
0
-5
-10
-15
-20
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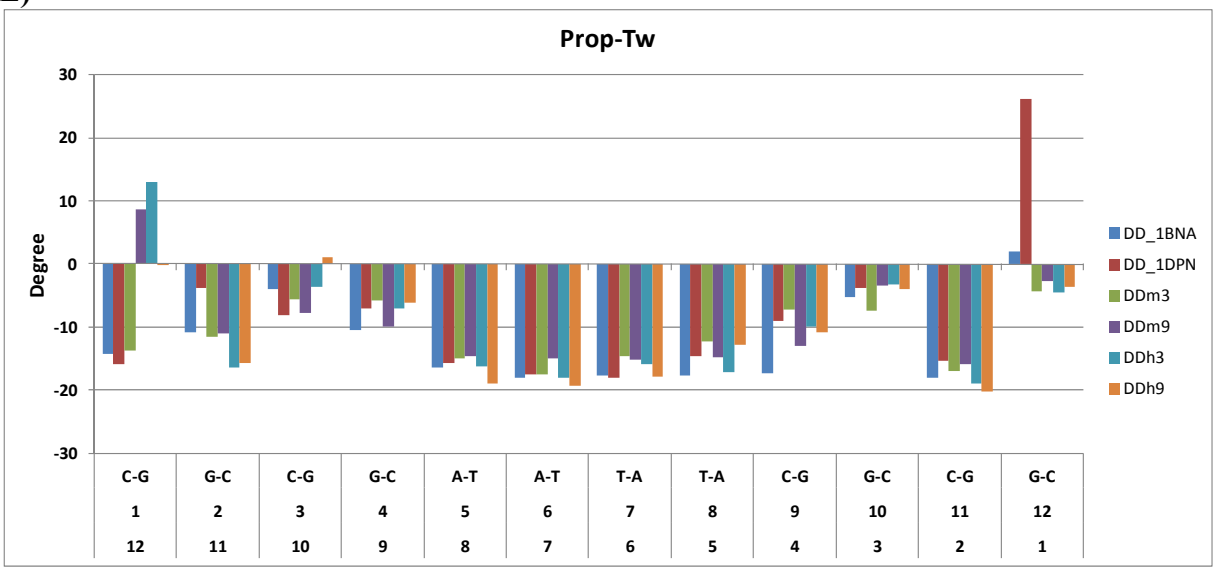
G-C 2 11
C-G 3 10
G-C 4 9
A-T 5 8
A-T 6 7
T-A 7 6
T-A 8 5
C-G 9 4
G-C 10 3
C-G 11 2
G-C 12 1
DD_1BNA
DD_1DPN
DDm3
DDm9
DDh3
DDh9

E)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Degree
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0
-10
-20
-30
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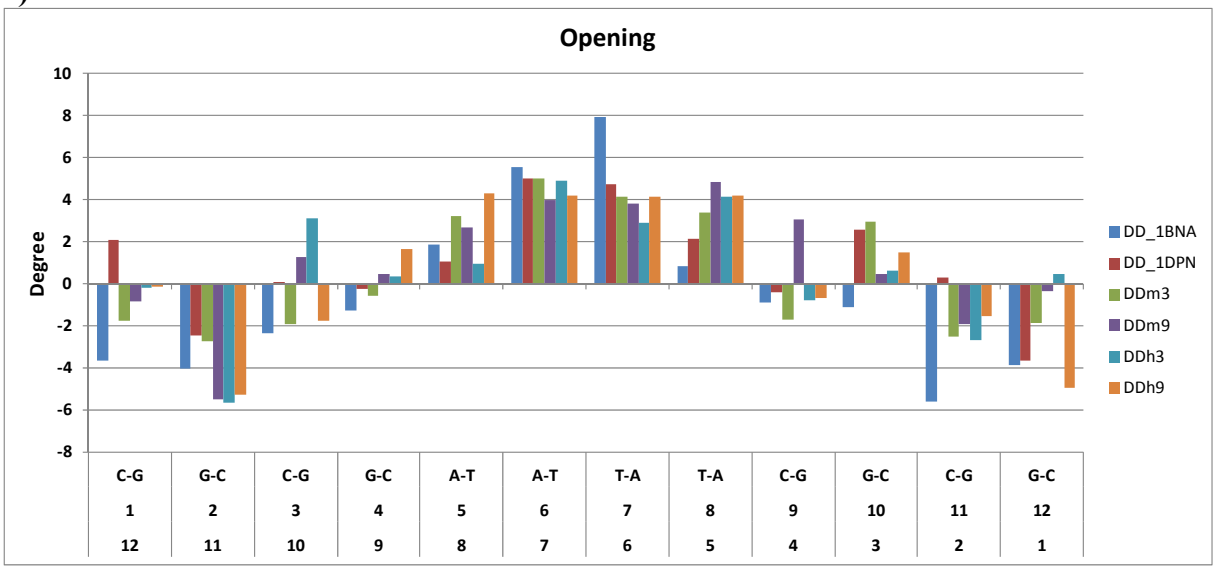
G-C 2 11
C-G 3 10
G-C 4 9
A-T 5 8
A-T 6 7
T-A 7 6
T-A 8 5
C-G 9 4
G-C 10 3
C-G 11 2
G-C 12 1
DD_1BNA
DD_1DPN
DDm3
DDm9
DDh3
DDh9

F)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Degree
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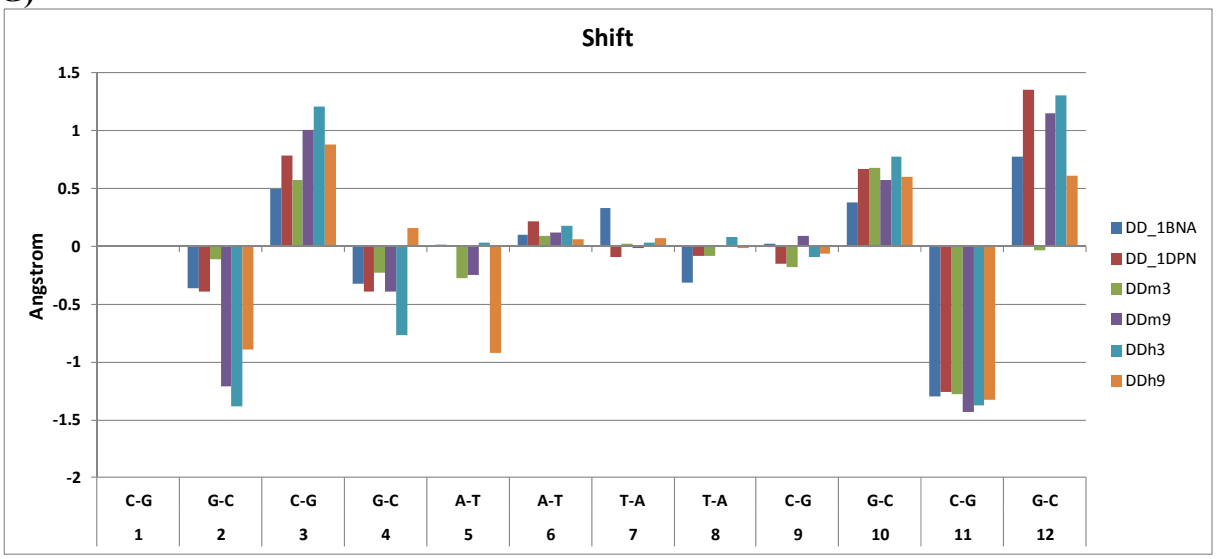
G-C 4 9
A-T 5 8
A-T 6 7
T-A 7 6
T-A 8 5
C-G 9 4
G-C 10 3
C-G 11 2
G-C 12 1
DD_1BNA
DD_1DPN
DDm3
DDm9
DDh3
DDh9

G)


Shift
Angstrom
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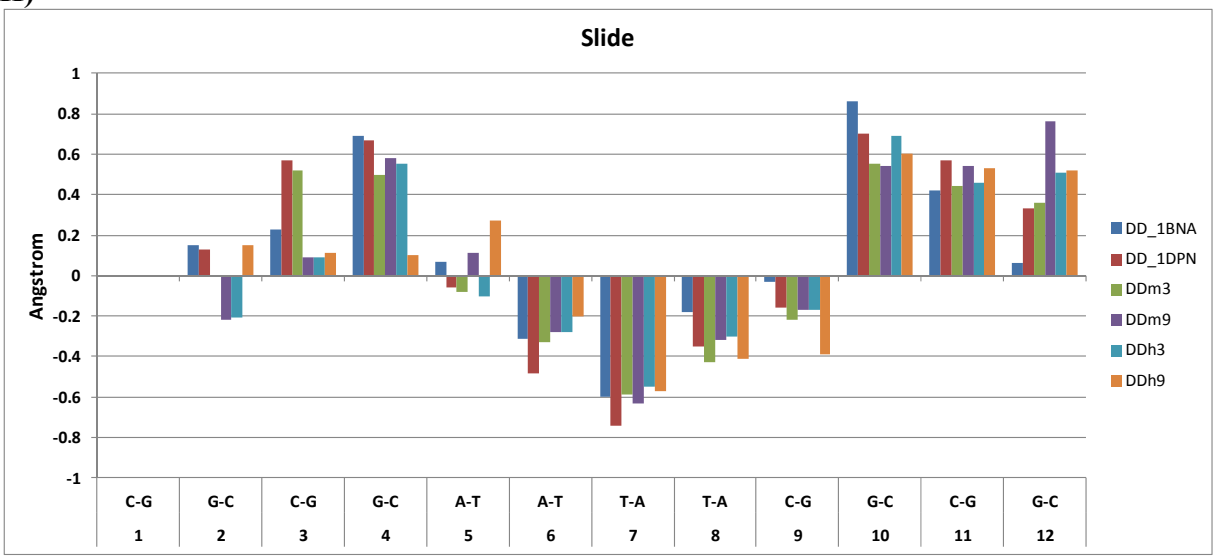
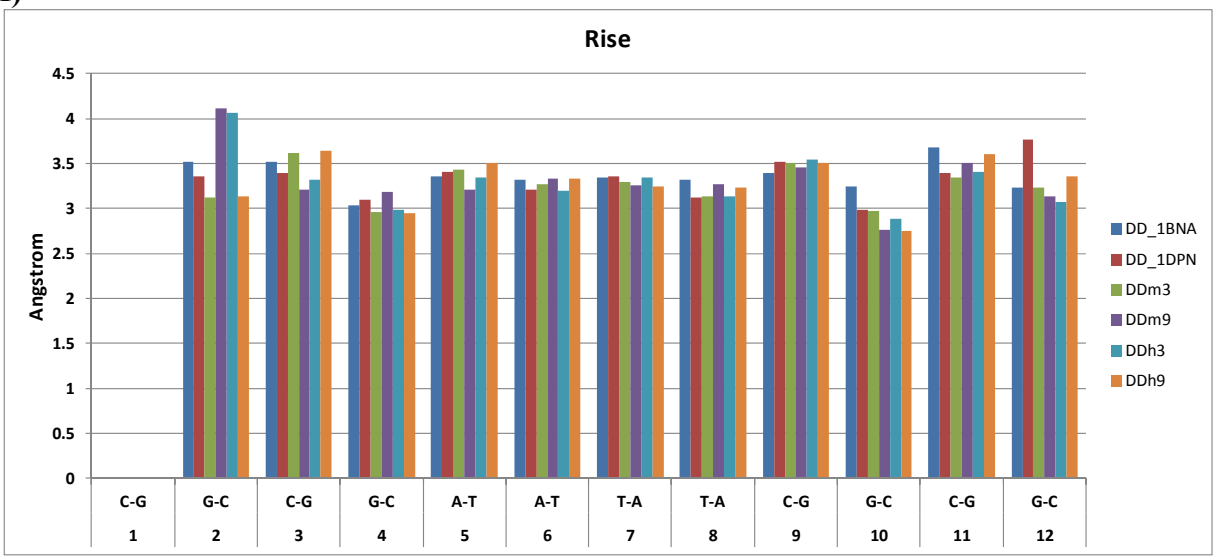
0.5
0
-0.5
-1
-1.5
-2
C-G 1
G-C 2
C-G 3
G-C 4
A-T 5
A-T 6
T-A 7
T-A 8
C-G 9
G-C 10
C-G 11
G-C 12
DD_1BNA
DD_1DPN
DDm3
DDm9
DDh3
DDh9

H)


Slide
Angstrom
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1
C-G 1
G-C 2
C-G 3
G-C 4
A-T 5
A-T 6
T-A 7
T-A 8
C-G 9
G-C 10
C-G 11
G-C 12
DD_1BNA
DD_1DPN
DDm3
DDm9
DDh3
DDh9

I)


Rise
Angstrom
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3.5
3
2.5
2
1.5
1
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0
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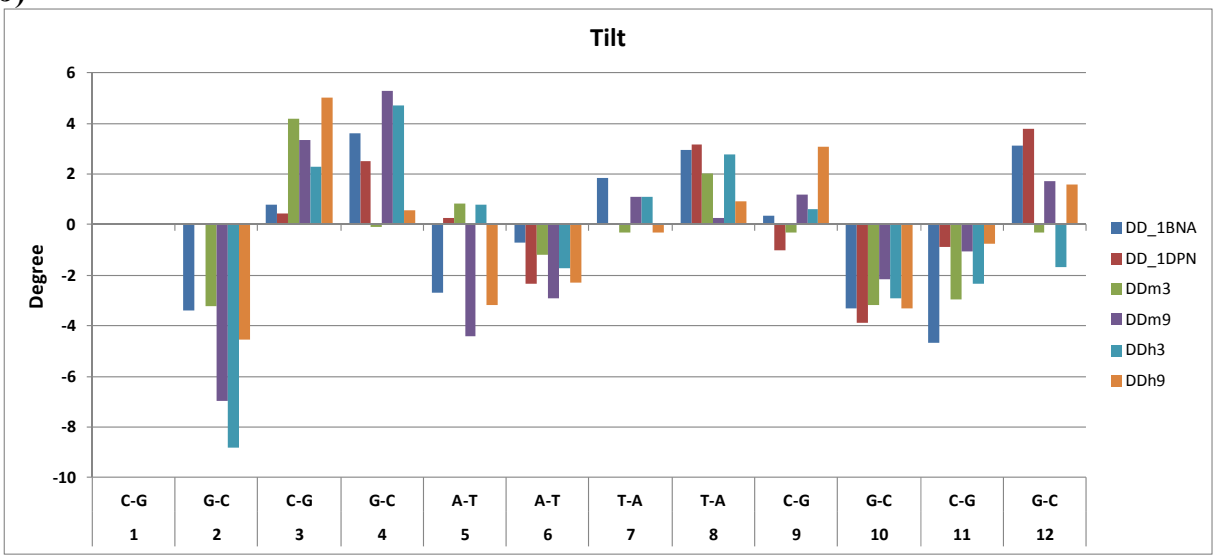
G-C 2
C-G 3
G-C 4
A-T 5
A-T 6
T-A 7
T-A 8
C-G 9
G-C 10
C-G 11
G-C 12
DD_1BNA
DD_1DPN
DDm3
DDm9
DDh3
DDh9

J)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Degree
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-10
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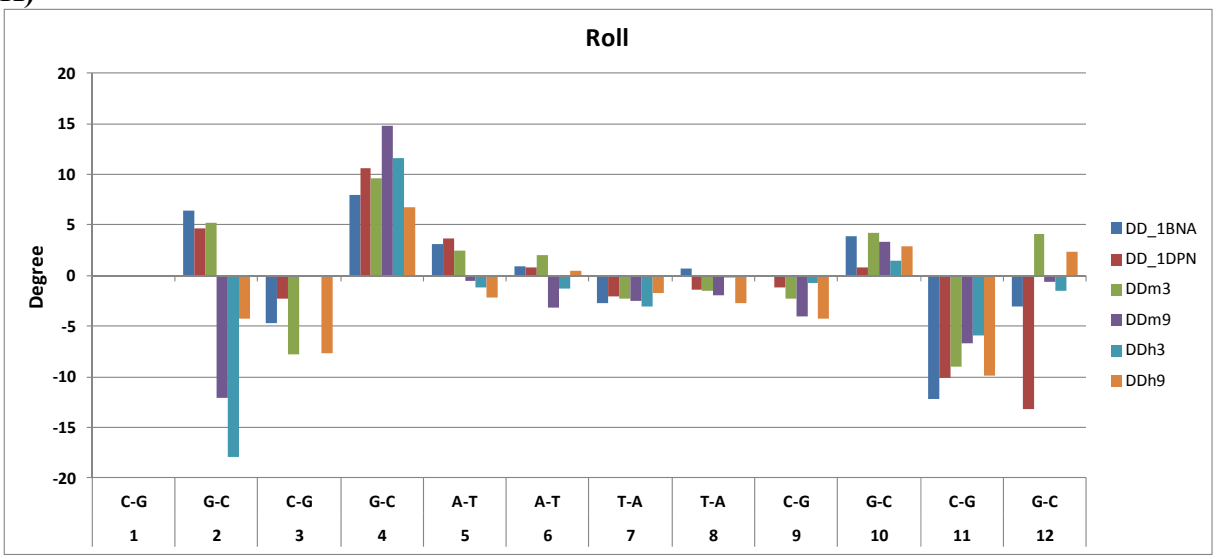
G-C 2
C-G 3
G-C 4
A-T 5
A-T 6
T-A 7
T-A 8
C-G 9
G-C 10
C-G 11
G-C 12
DD_1BNA
DD_1DPN
DDm3
DDm9
DDh3
DDh9

K)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Degree
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10
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0
-5
-10
-15
-20
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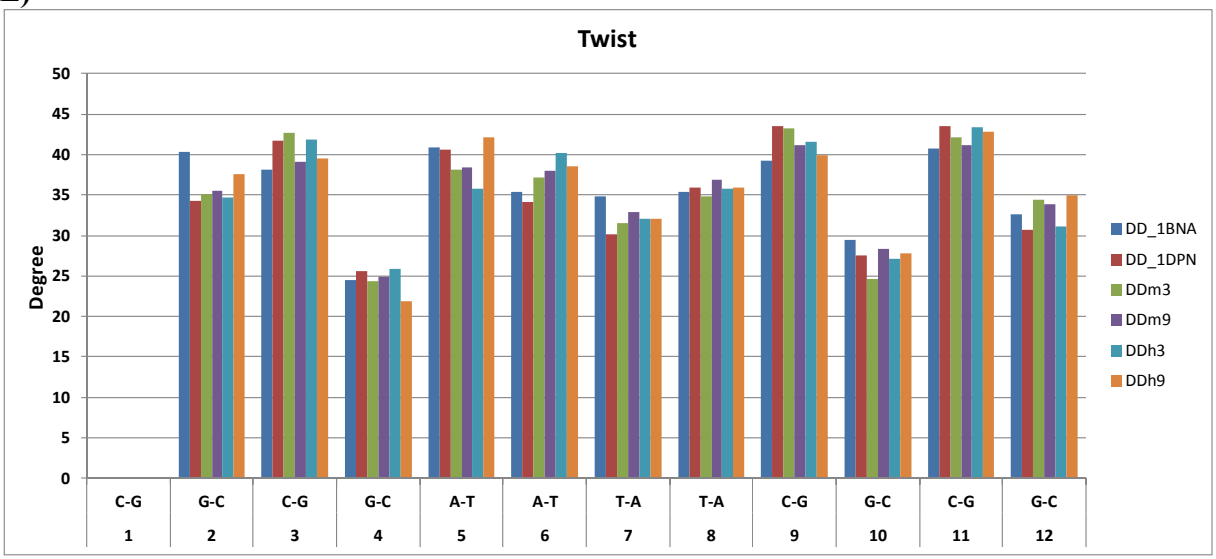
G-C 2
C-G 3
G-C 4
A-T 5
A-T 6
T-A 7
T-A 8
C-G 9
G-C 10
C-G 11
G-C 12
DD_1BNA
DD_1DPN
DDm3
DDm9
DDh3
DDh9

L)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Degree
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20
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10
5
0
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G-C 2
C-G 3
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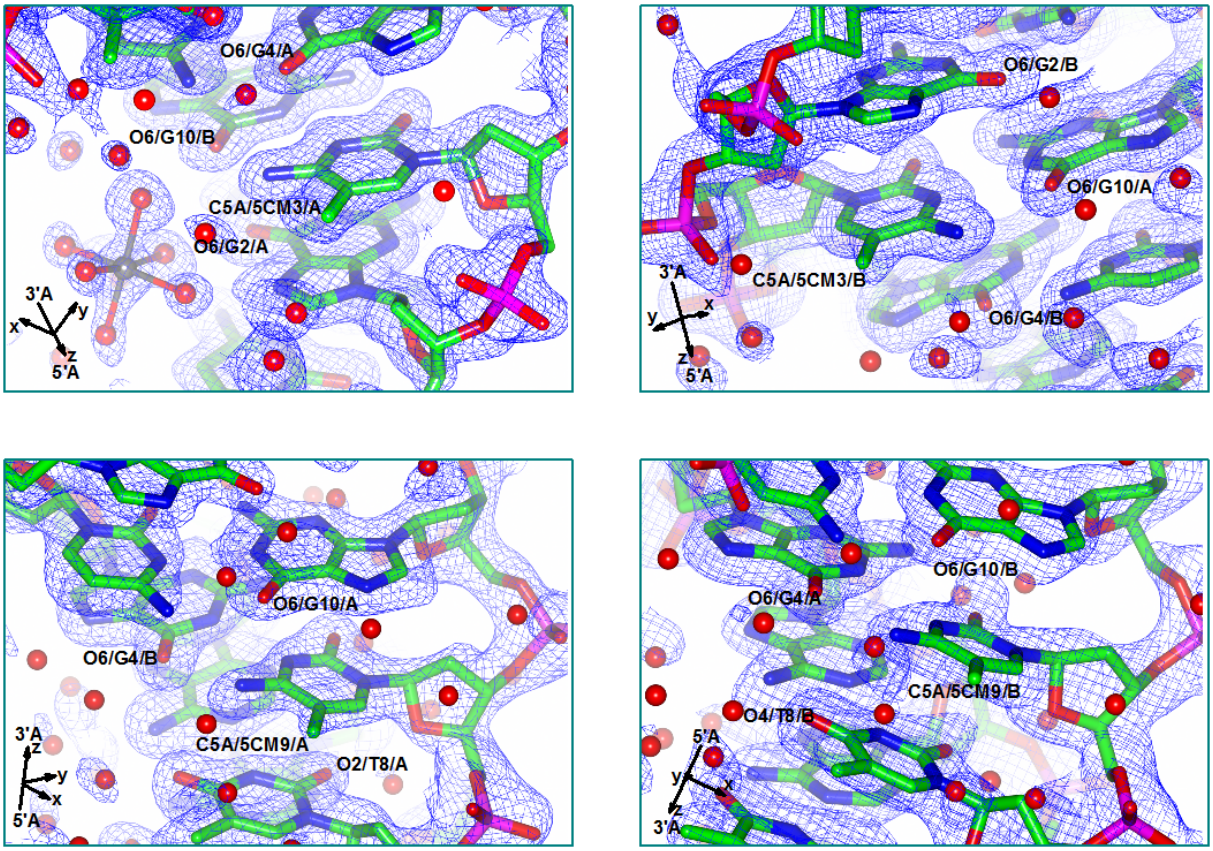
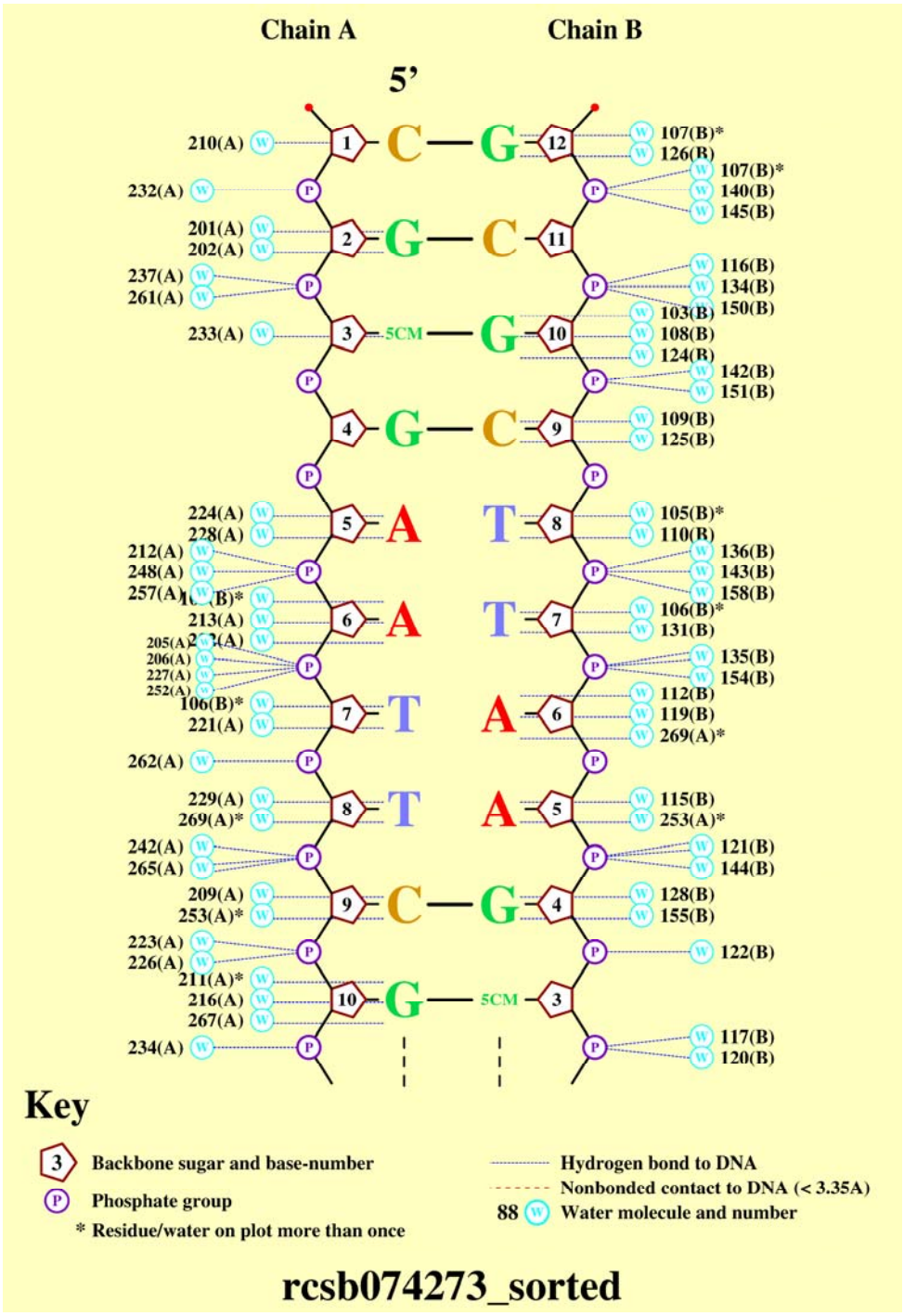
A-T 5
A-T 6
T-A 7
T-A 8
C-G 9
G-C 10
C-G 11
G-C 12
DD_1BNA
DD_1DPN
DDm3
DDm9
DDh3
DDh9

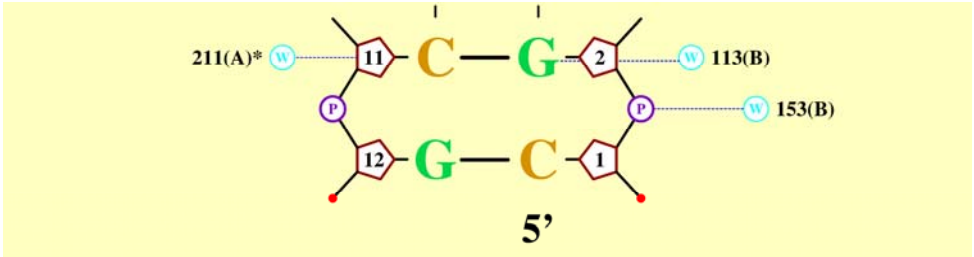
Figure S6:


O6/G4/A
O6/G10/B
C5A/5CM3/A
O6/G2/A
3'A
5'A
O6/G2/B
O6/G10/A
C5A/5CM3/B
O6/G4/B
3'A
5'A
O6/G10/A
O6/G4/B
C5A/5CM9/A
O2/T8/A
3'A
5'A
O6/G10/B
O6/G4/A
C5A/5CM9/B
O4/T8/B
5'A
3'A

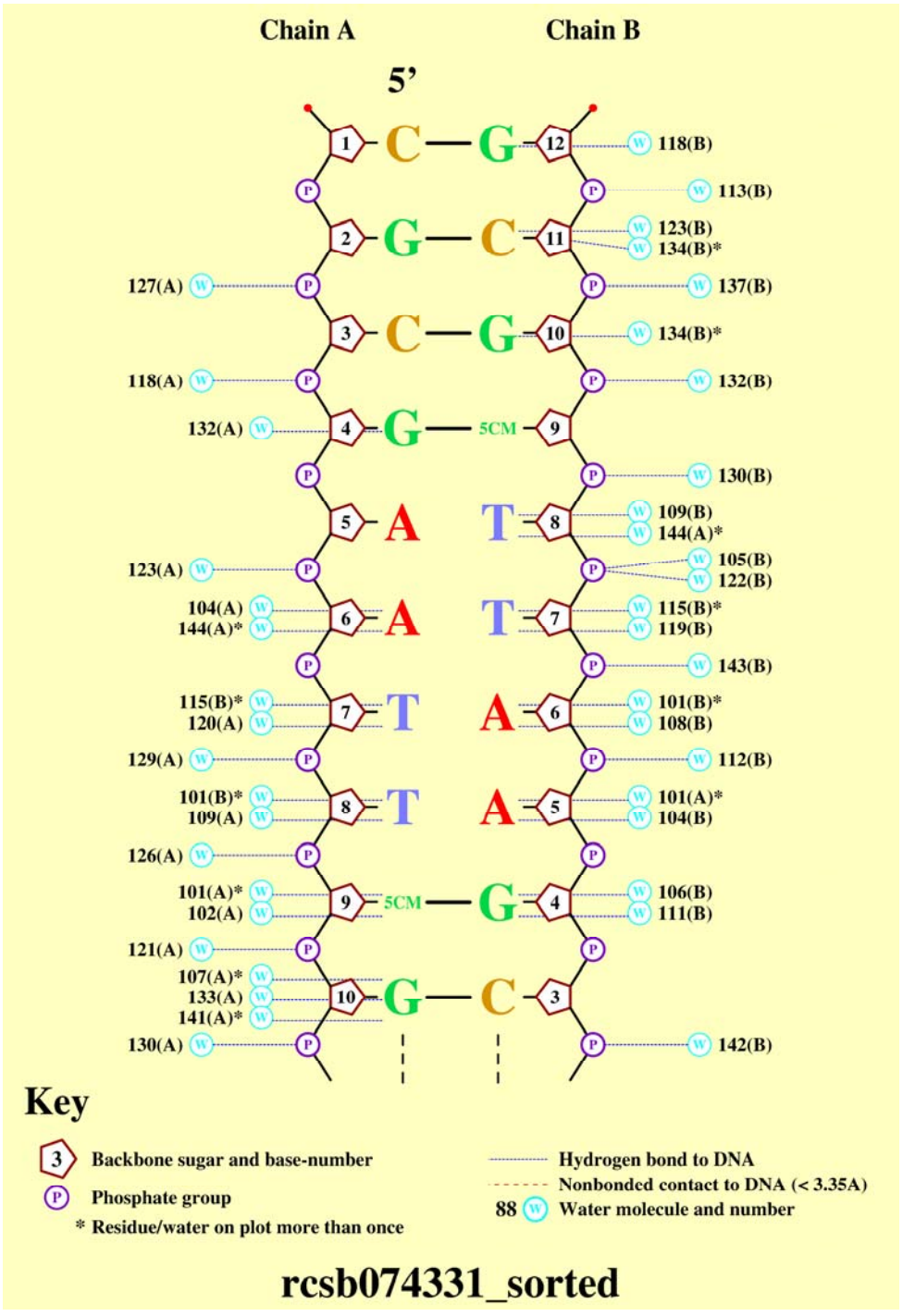
Figures S7:

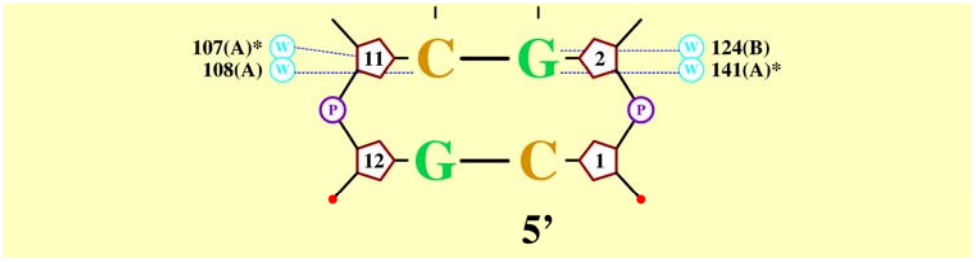
A) Analysis of the interactions DDm3 with water by Nucplot:



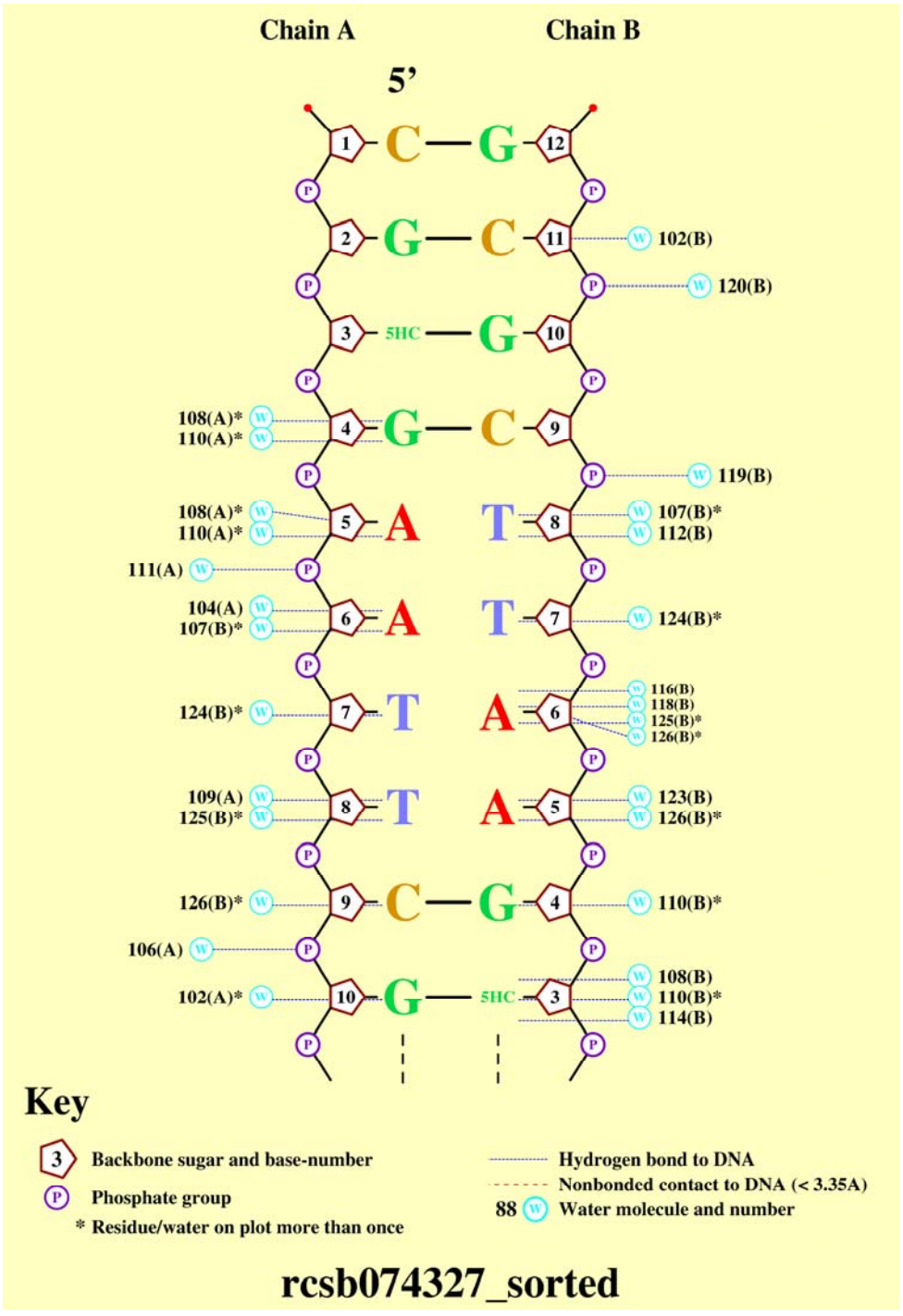


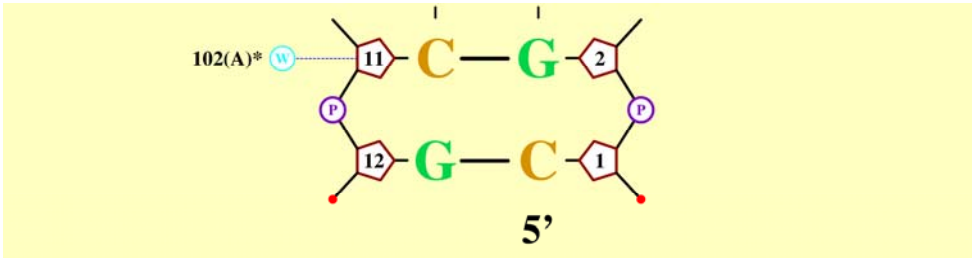
B) Analysis of the interactions DDm9 with water by Nucplot:



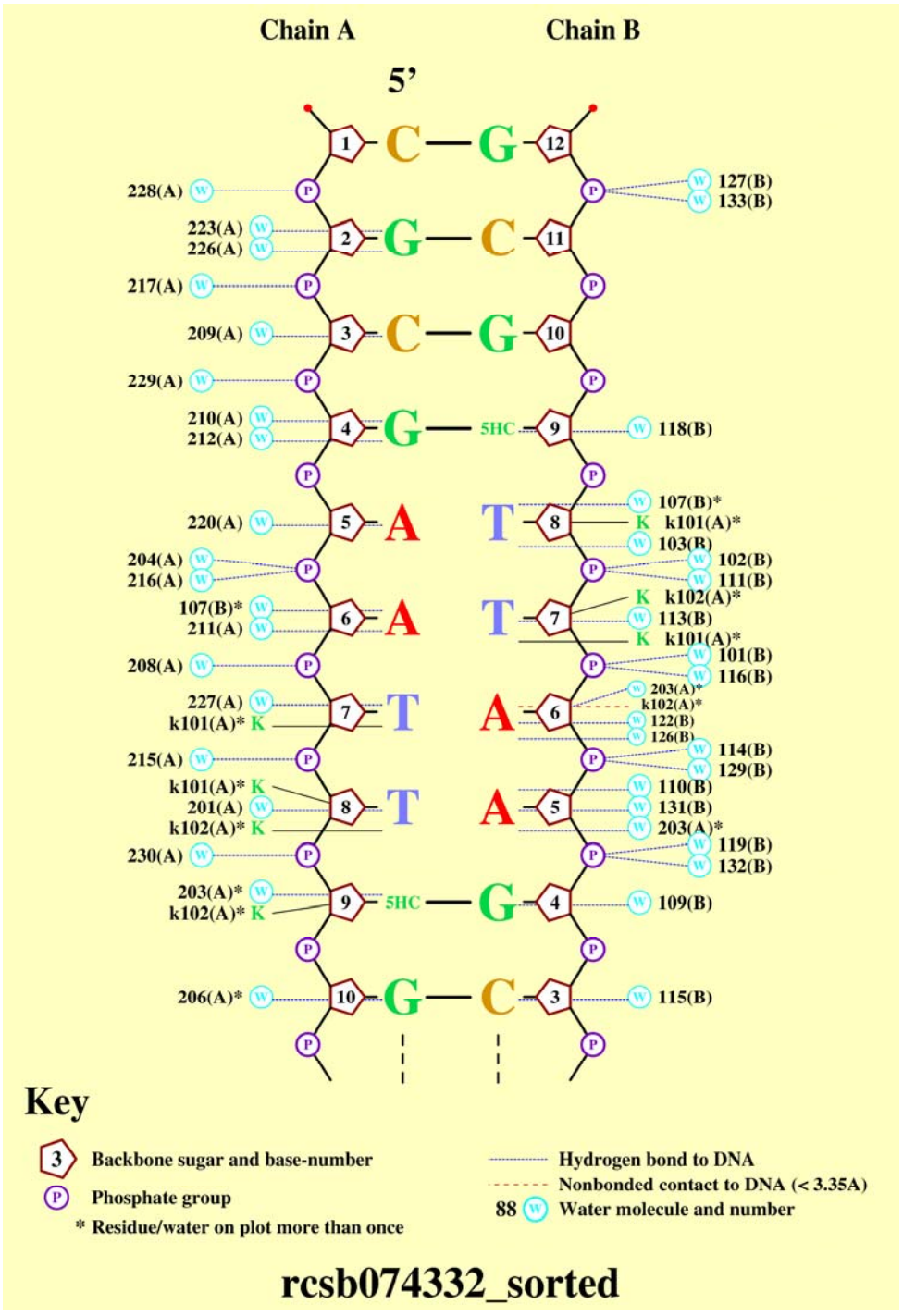


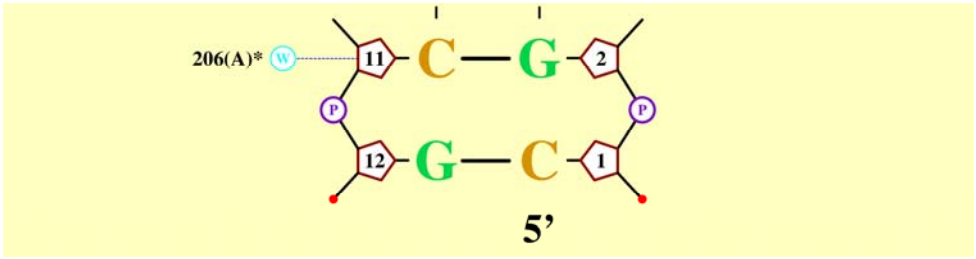
C) Analysis of the interactions DDh3 with water by Nucplot:





D) Analysis of the interactions DDh9 with water by Nucplot:





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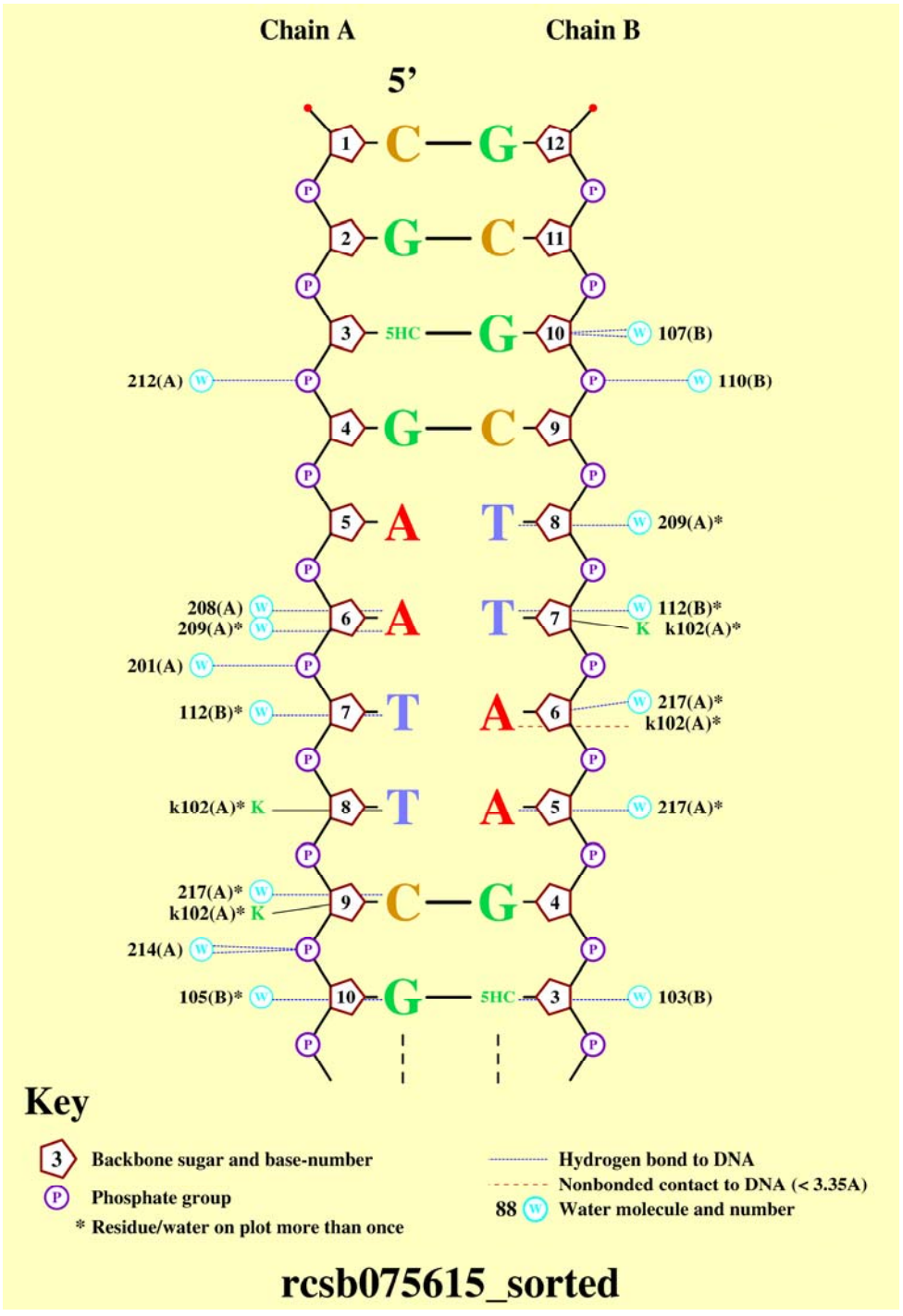


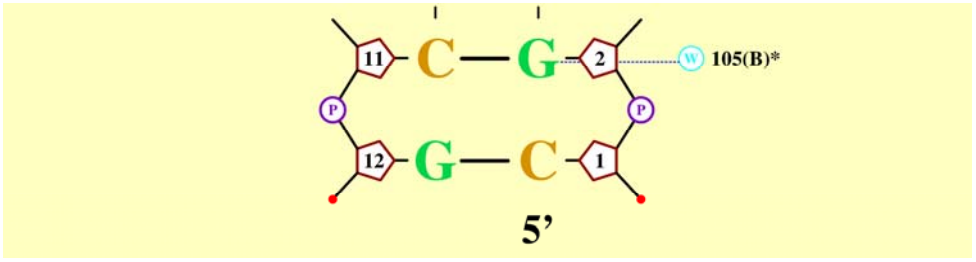
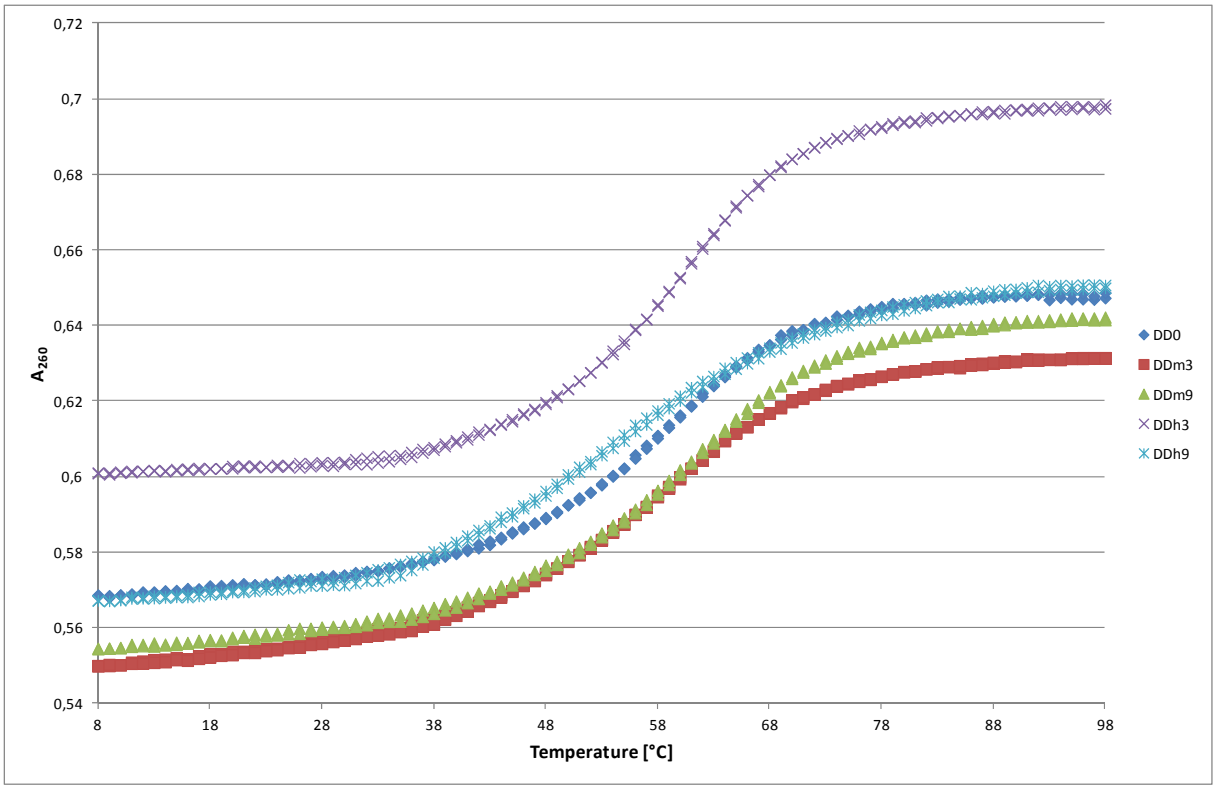
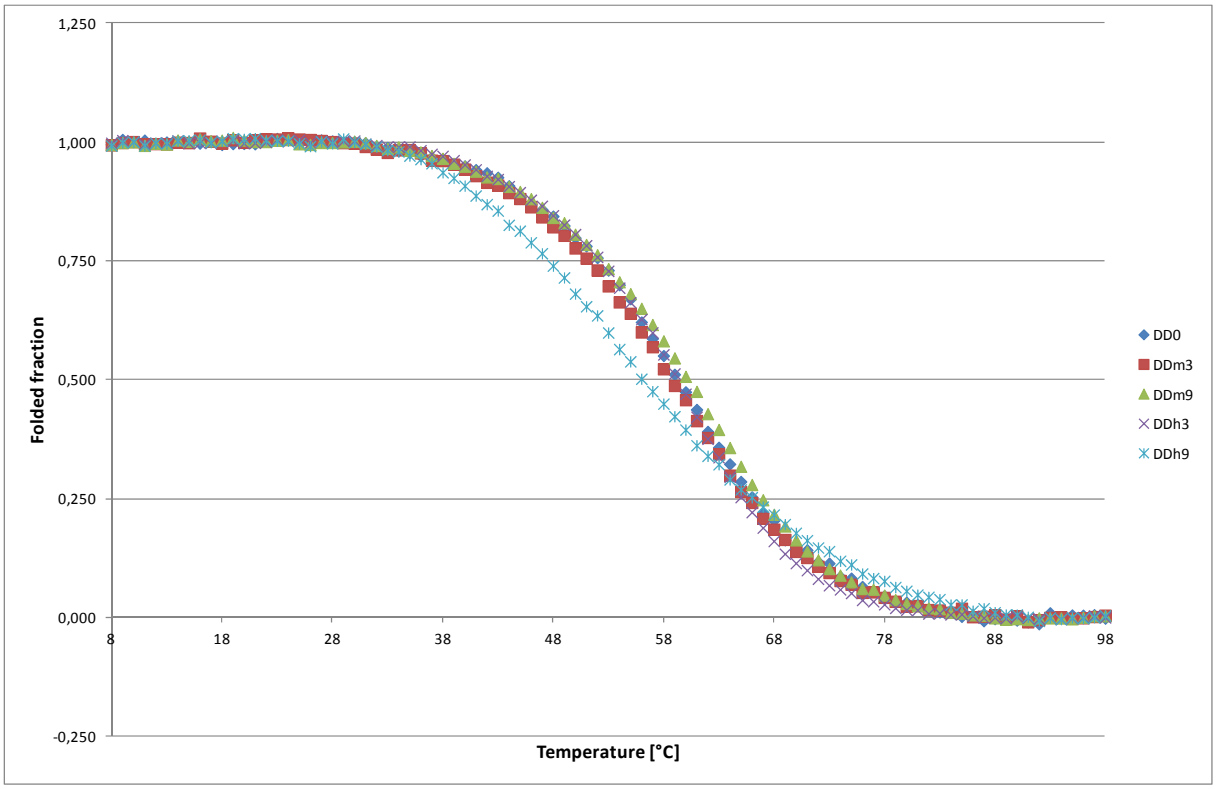


Figure S8:

A)


0,72
0,7
0,68
0,66
0,64
0,62
0,6
0,58
0,56
0,54
A260
8
18
28
38
48
58
68
78
88
98
Temperature [°C]
DD0
DDm3
DDm9
DDh3
DDh9

B)


1,250
1,000
0,750
0,500
0,250
0,000
-0,250
Folded fraction
8
18
28
38
48
58
68
78
88
98
Temperature [°C]
DD0
DDm3
DDm9
DDh3
DDh9