Supplementary information for

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

Daniel Renciuk1,2, Olivier Blacque1, Michaela Vorlickova2,3 and Bernhard Spingler1,*
1 Institute of Inorganic Chemistry, University of Zurich, Winterthurerstrasse 190, CH-8057, Zurich, Switzerland
2 Institute of Biophysics, Academy of Sciences of the Czech Republic, Kralovopolska 135, 61265, Brno, Czech Republic
3 CEITEC- Central European Institute of Technology, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic
* To whom correspondence should be addressed. Tel: +41 44 635 46 56; Email: spingler@aci.uzh.ch

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.

<table>
<thead>
<tr>
<th></th>
<th>DD0</th>
<th>DDm3</th>
<th>DDm9</th>
<th>DDh3</th>
<th>DDh9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tm [°C]</td>
<td>58.9</td>
<td>58.9</td>
<td>60.2</td>
<td>59.5</td>
<td>56.4</td>
</tr>
<tr>
<td>ΔHVH [kJ.mol⁻¹]</td>
<td>-240</td>
<td>-219</td>
<td>-206</td>
<td>-244</td>
<td>-184</td>
</tr>
<tr>
<td>ΔS [kJ.mol⁻¹.K⁻¹]</td>
<td>-0.639</td>
<td>-0.579</td>
<td>-0.536</td>
<td>-0.651</td>
<td>-0.475</td>
</tr>
<tr>
<td>TAS at 37°C [kJ.mol⁻¹]</td>
<td>-198</td>
<td>-179</td>
<td>-166</td>
<td>-202</td>
<td>-147</td>
</tr>
<tr>
<td>ΔG° at 37°C [kJ.mol⁻¹]</td>
<td>-42</td>
<td>-40</td>
<td>-40</td>
<td>-42</td>
<td>-37</td>
</tr>
</tbody>
</table>
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.

Figure S1:

A)

B)
Figure S2:
A)

B)
Figure S3:

A)

B)
Figure S4:

A)

![Graph A]

B)

![Graph B]
Figure S5:

A) Shear

B) Stretch

C) Stagger
Figure S6:
Figures S7:

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

Key:
- □ Backbone sugar and base-number
- ◊ Phosphate group
- * Residue/water on plot more than once

rcsb074273_sorted
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:

Key

- Backbone sugar and base-number
- Phosphate group
- Hydrogen bond to DNA
- Nonbonded contact to DNA (< 3.35 Å)
- Water molecule and number

Residue/water on plot more than once

rcsb074332_sorted
E) Analysis of the interactions DDh3b with water by Nucplot:

\[
\text{rcsb075615_sorted}
\]

Chain A

\[
\begin{align*}
5' & - C \rightarrow G - 12 \\
8 & - G \rightarrow C - 11 \\
3 \text{H} & - G \rightarrow G - 10 \\
208(A) & - T \rightarrow A - 7 \\
201(A) & - T \rightarrow A - 7 \\
112(B)^* & - T \rightarrow A - 7 \\
\text{K} & - T \rightarrow A - 7 \\
217(A)^* & - T \rightarrow A - 7 \\
105(B)^* & - T \rightarrow A - 7 \\
\text{SHC} & - G \rightarrow G - 4 \\
214(A) & - G \rightarrow G - 4 \\
103(B) & - G \rightarrow G - 4 \\
12 & - G \rightarrow C - 1 \\
\end{align*}
\]

Chain B

\[
\begin{align*}
5' & - C \rightarrow G - 12 \\
8 & - G \rightarrow C - 11 \\
3 \text{H} & - G \rightarrow G - 10 \\
208(A) & - T \rightarrow A - 7 \\
201(A) & - T \rightarrow A - 7 \\
112(B)^* & - T \rightarrow A - 7 \\
\text{K} & - T \rightarrow A - 7 \\
217(A)^* & - T \rightarrow A - 7 \\
105(B)^* & - T \rightarrow A - 7 \\
\text{SHC} & - G \rightarrow G - 4 \\
214(A) & - G \rightarrow G - 4 \\
103(B) & - G \rightarrow G - 4 \\
12 & - G \rightarrow C - 1 \\
\end{align*}
\]

Key

- Backbone sugar and base-number
- Phosphate group
- Hydrogen bond to DNA
- Nonbonded contact to DNA (< 3.35 Å)
- Water molecule and number
* Residue/water on plot more than once

\[
\text{rcsb075615_sorted}
\]
Figure S8:

A)

![Temperature vs. A$_{vis}$ plot](image1)

B)

![Temperature vs. Folded fraction plot](image2)