Supplementary Information

Effects of cytosine hydroxymethylation on DNA strand separation

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Supplementary Materials and Methods

DNA oligomers were purchased HPLC grade from IBA GmbH (Goettingen, Germany). Oligonucleotides employed had the following sequences and modifications (**hmC** represents 5-hydroxymethylcytosine):

Shear pulling geometry:

1$_{\mathbf{nDNA}}$, NH_2-$(HEGL)_5$-5′-$(T)_{10}$-CCG AGA TAT CCG CAC CAA CG-3′
2$_{\mathbf{nDNA}}$, 3′-GGC TCT ATA GGC GTG GTT GC-$(T)_6$-5′-5′-T(Cy5)-$(T)_6$-GGC TCT ATA GGC GTG GTT GC-3′
1$_{\mathbf{hmC\text{-}1\text{-}DNA}}$, NH_2-$(HEGL)_5$-5′-$(T)_{10}$-CCG AGA TAT C**hmC**G CAC CAA CG-3′
2$_{\mathbf{hmC\text{-}1\text{-}DNA}}$, 3′-GGC TCT ATA GG**hmC** GTG GTT GC-$(T)_6$-5′-5′-T(Cy5)-$(T)_6$-GGC TCT ATA GGC GTG GTT GC-3′
1$_{\mathbf{hmC\text{-}3\text{-}DNA}}$, NH2-$(HEGL)_5$-5′-$(T)_{10}$-C**hmC**G AGA TAT C**hmC**G CAC CAA **hmC**G-3′
2$_{\mathbf{hmC\text{-}3\text{-}DNA}}$, 3′-GG**hmC** TCT ATA GG**hmC** GTG GTT G**hmC**-$(T)_6$-5′-5′-T(Cy5)-$(T)_6$-GGC TCT ATA GGC GTG GTT GC-3′
3$_{\mathbf{Ref}}$, biotin-5′-$(T)_{10}$-GCA ACC ACG CCT ATA GAG CC(Cy3)-3′.

Zipper pulling geometry:

1$_{\mathbf{nDNA}}$, 5’-CCG AGA TAT CCG CAC CAA CG-$(T)_{20}$-$(HEGL)_5$–NH_2-3’
2$_{\mathbf{nDNA}}$, 5’-GGC TCT ATA GGC GTG GTT GC-$(T)_6$-T(Cy5)-$(T)_6$-CGT TGG TGC GGA TAT CTC GG-3’
1$_{\mathbf{hmC\text{-}1\text{-}DNA}}$, 5’-CCG AGA TAT C**hmC**G CAC CAA CG-$(T)_{20}$-$(HEGL)_5$–NH_2-3’
2$_{\mathbf{hmC\text{-}1\text{-}DNA}}$, 5’-GGC TCT ATA GGC GTG GTT GC-$(T)_6$-T(Cy5)-$(T)_6$-CGT TGG TG**hmC** GGA TAT CTC GG-3’
1$_{\mathbf{hmC\text{-}3\text{-}DNA}}$, 5’-C**hmC**G AGA TAT C**hmC**G CAC CAA **hmC**G-$(T)_{20}$-$(HEGL)_5$–NH_2-3’
2$_{\mathbf{hmC\text{-}3\text{-}DNA}}$, 5’-GGC TCT ATA GGC GTG GTT GC-$(T)_6$-T(Cy5)-$(T)_6$-**hmC**GT TGG TG**hmC** GGA TAT CT**hmC** GG-3’
3$_{\mathbf{Ref}}$, biotin-5′-$(T)_{20}$-(Cy3)-GCA ACC ACG CCT ATA GAG CC-3′

Supplementary Movies

- Movie S1 shows a trajectory (simulation C2) of hmC-3-DNA stretched in zipper geometry by steered molecular dynamics with a velocity of 1 Å/ns. The six methylated cytosines in DNA are indicated in yellow; the atoms subject to constraint (left) and stretching force (right) are shown in green.

- Movie S2 shows a trajectory (simulation F6) of hmC-3-DNA stretched in shear geometry by steered molecular dynamics with a velocity of 1 Å/ns. The methylated cytosines in DNA are indicated in yellow; the atoms subject to constraint (at bottom) and stretching force (at top) are shown in green.

Supplementary Figure

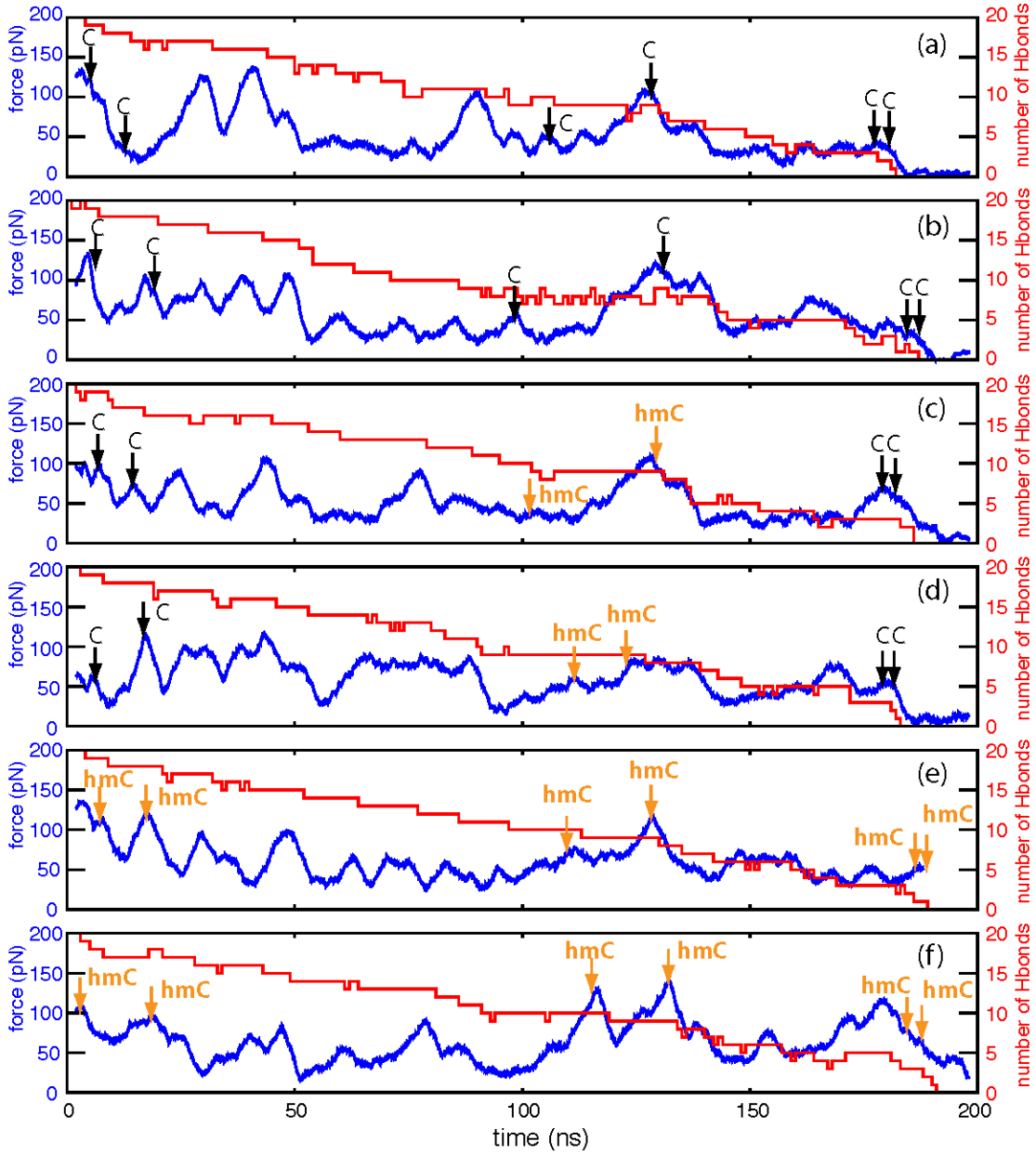

Figure S1 Time evolution of applied force (blue line) and number of base pairs (red line) as monitored in SMD simulations of unzipping nDNA (a, b), hmC-1-DNA (c, d) and hmC-3-DNA (e, f). Orange arrows indicate the breakage of hydroxymethylated CG pairs as seen by inspecting the corresponding trajectory data; black arrows indicate the breakage of normal CG pairs.

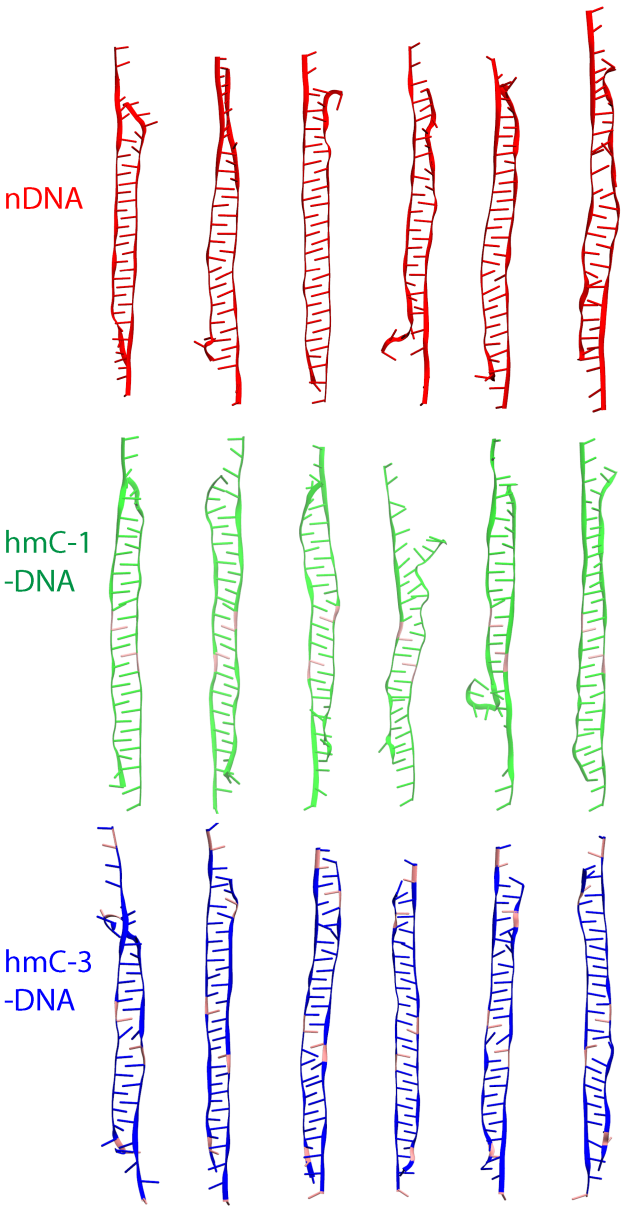

Figure S2 Snapshots of nDNA (red), hmC-1-DNA (green) and hmC-3-DNA (blue) taken at the moment when stretching force in each of the six simulations carried out for each case reaches the maximum value. The positions of hydorxymethylcytosine are marked in pink.