

Supporting Material:**Mechanical Properties of High G·C-content DNA with A-type Base-Stacking**

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Supporting Material List

- Supplementary Materials and Methods.
- Supporting references.

SUPPLEMENTARY MATERIALS AND METHODS

Atomic force microscopy analysis

The behavior of DNA molecules, as explained in the main text, can be predicted by the Worm-like chain (WLC) model (1):

$$\langle R_{s,s+L}^2 \rangle = 4P(L + 2P(e^{-L/(2P)} - 1)) \quad (1)$$

$$\langle \cos(\theta_{s,s+L}) \rangle = e^{-L/(2P)} \quad (2)$$

where P is the persistence length of the polymer, L is the contour length spacing and $\langle R_{s,s+L}^2 \rangle$ is the mean-squared separation of points along the chain as a function of their contour length separation L , or located at distances s and $s+L$. Similarly, $\langle \cos(\theta_{s,s+L}) \rangle$ is the mean angle between tangents at contour points located at distances s and $s+L$. The average is computed over s and over all observed contours. The number of values involved in the averaging depends on the contour length L , being this number smaller when L approaches the total contour length of the molecule L_0 . This method for measuring P and L_0 has been proved to be very accurate and reliable (1,2,3). We traced DNA trajectories using a custom-made tracing routine. The tracing routine accurately generates XY pairs separated by $\ell = 2.5$ nm that follow the contour of the molecule. We found this length to be the lowest meaningful length that faithfully followed the contour given the pixel size of the images, 1.95 nm. A magnification of the trace illustrates the quantities deduced that are important for the analysis that follows (see Fig. 2 C in the main text). These are the contour length spacing L (in the example, L equals $3 \times \ell$), and the end-to-end distance R . Clearly, a given contour length spacing L can be associated with several values of R . The inset also shows the definition of the angle θ between two tangents (in the example θ is the angle between tangents of points separated $3 \times \ell$).

We selected fragments from trajectories of 325 nm (~950 bp) for pBACgus11 and pij702 DNA substrates all along the individual molecules and calculated $\langle R_{s,s+L}^2 \rangle$ up to a contour length spacing of $L = 200$ nm (Fig. 2 D in the main text). We restricted our analysis to lengths considerably smaller than the total length of the molecules to ensure conditions of 2D thermodynamic equilibrium and to prevent excluded volume effects. Indeed, we found that when distances over 2 kb were considered, $\langle R_{s,s+L}^2 \rangle$ deviates from WLC prediction, as was previously reported (1). Our analysis involved hundreds of fragments and averaging over 15,000 data points at contour lengths of 5 nm.

Magnetic tweezers analysis

In the case of having more than one DNA molecule in the tether, the bead would approach the surface as we rotate the magnet in any direction, evidencing the entanglement of multiple DNA molecules. We never observed this effect assuring that force-extension curves were performed on single DNA molecules. In addition, we checked that the tether was indeed made of DNA by flushing a restriction enzyme whose target sequence is contained in the DNA construct. Within seconds, we observed that the track was lost as a consequence of the cleavage of the DNA by the restriction enzyme.

SUPPORTING REFERENCES

1. Rivetti, C., M. Guthold, and C. Bustamante. 1996. Scanning force microscopy of DNA deposited onto mica: equilibration versus kinetic trapping studied by statistical polymer chain analysis. *J Mol Biol.* 264:919-932.
2. Moreno-Herrero, F., R. Seidel, S. M. Johnson, A. Fire, and N. H. Dekker. 2006. Structural analysis of hyperperiodic DNA from *Caenorhabditis elegans*. *Nucleic Acids Res.* 34:3057-3066.
3. Wiggins, P. A., T. Heijden, F. Moreno-Herrero, A. Spakowitz, R. Phillips, et al. 2006. High flexibility of DNA on short length scales probed by atomic force microscopy. *Nature Nanotech.* 1:137-141.