

Supporting Information

Nucleosome positioning by excluding genomic energy barriers

Pascale Milani^{*†‡}, Guillaume Chevereau^{*†‡}, Cédric Vaillant^{*†‡}, Benjamin Audit^{*†‡}, Zofia Haftek-Terreau^{*†}, Monique Marilley^{*†}, Philippe Bouvet^{*†§}, Françoise Argoul^{*†‡}, Alain Arneodo^{*†‡¶}

^{*}Université de Lyon, F-69000 Lyon, France, [†]Laboratoire Joliot-Curie, [‡]Laboratoire de Physique, [§]Laboratoire de Biologie Moléculaire de la Cellule, CNRS/Ecole Normale Supérieure de Lyon, F-69007 Lyon, France,

[¶] Corresponding author: alain.arneodo@ens-lyon.fr; Fax (33) 472728080

Preparation and AFM imaging of the 601 fragment A 255 bp long DNA fragment containing the 601 nucleosome positioning sequence [1] was obtained by polymerase chain reaction (PCR) amplification with TAQ polymerase (Sigma) from plasmid pGem-3Z-601. In this fragment, the 147 bp long 601 positioning sequence is flanked by 52 bp on one side and 56 bp on the other side.

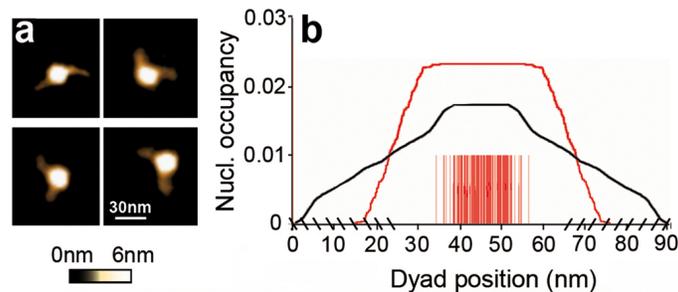


Fig. S1. AFM imaging in liquid of mono-nucleosome positioning along a 255 bp DNA fragment containing the 601 nucleosome positioning sequence (see *Data and Methods*). (a) Examples of single molecule AFM images. (b) Symmetrized dyad positioning distribution ($N = 105$ molecules); similar representation as in Fig. 2d,e,f. Note that the physical modeling predicts some nucleosome positioning on the 52 bp and 56 bp DNA fragments bordering the 601 positioning sequence that is not observed with our AFM statistical sampling.

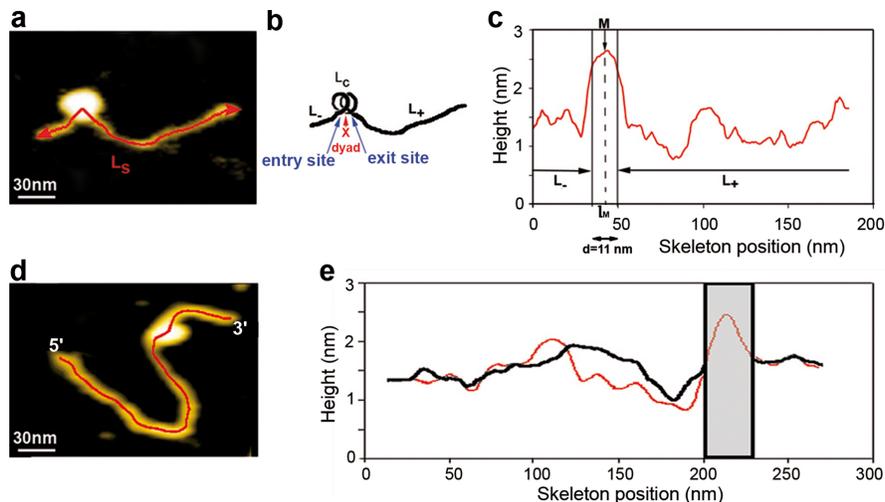


Fig. S2. AFM image treatment and analysis of a mono-nucleosome reconstituted on the 595 bp long yeast (chr. 7) genomic DNA fragment. (a) Molecule path skeletonization when DNA is loaded; L_S corresponds to the length of the skeleton. (b) Sketch illustrating the entry and exit sites of the nucleosome core particle (NCP), the dyad position x , the lengths of the longest ($L_+ = L_S - (l_M + d/2)$) and shortest ($L_- = l_M - d/2$) free DNA arms outside the nucleosome and the length ($L_c = L - (L_+ + L_-)$) of complexed DNA. (c) Topographical profile along the molecule path in (a); the diameter d of the NCP is fixed to $d = 11$ nm; the NCP is positioned at the position l_M corresponding to the local height maximum M . (d,e) Illustration of the methodology used to orient the human IL2RA fragment ($L = 898$ bp): the pattern of height variation along the axis of the fragment (red) is compared to the calculated pitch variation (black) obtained when using the Bolshoy *et al.* DNA coding table [2].

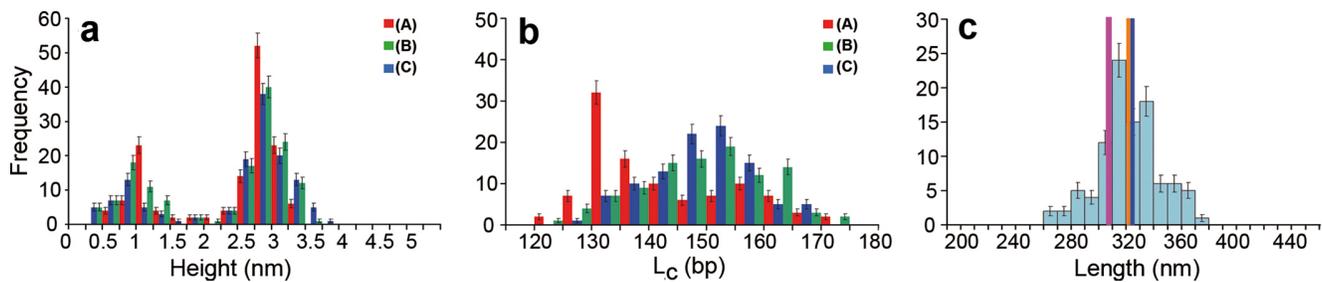


Fig. S3. (a) Height distribution of nucleosomal particles reconstituted on the three small yeast genomic DNA fragments: (A) red ($L = 394$ bp, $N = 136$), (B) green ($L = 386$ bp, $N = 149$) and (C) blue ($L = 387$ bp, $N = 143$); bin size = 0.25 nm; errors on the bin height were evaluated as the square root of the number of counts. (b) Distribution of DNA complexed length L_c (Fig. S1c), for the nucleosomes reconstituted on the three small yeast DNA fragments: (A) red ($L = 394$ bp, $N = 107$), (B) green ($L = 386$ bp, $N = 102$) and (C) blue ($L = 387$ bp, $N = 105$); bin size = 5 bp; errors on bin heights were evaluated as the square root of the number of counts. (c) Distribution of the lengths of $N = 100$ unloaded naked DNA during the experiment with the ILR2A DNA fragment ($L = 898$ bp); the mean experimental length $L_{exp} = 322.5$ nm is indicated by the orange vertical line; the blue (resp. purple) vertical line corresponds to the theoretical length $L_{theo} = 324$ nm (resp. 306 nm) obtained when considering 0.36 nm (resp. 0.34 nm) as the average distance between nearest neighbour bps in B-DNA.

References

1. Lowary PT, Widom J (1998) New DNA sequence rules for high affinity binding to histone octamer and sequence-directed nucleosome positioning. *J Mol Biol* 276:19–42.
2. Bolshoy A, McNamara P, Harrington RE, Trifonov EN (1991) Curved DNA without A-A: experimental estimation of all 16 DNA wedge angles. *Proc Natl Acad Sci USA* 88:2312–2316.