Bancaud et al. : Structural plasticity of single chromatin fibers revealed by torsional manipulation

SUPPLEMENTARY MATERIAL

1- Fiber-to-fiber variability in the torsional response: clusters of close-packed nucleosomes

As shown in **Fig. 2c** (main text), and again in **Supplementary Fig. 1a**, the maximal length of *regular* chromatin fibers at their center of rotation essentially varies linearly with their topological shift relative to the naked DNA, i.e. with the number of NSs, with the exception of some fibers which deviate from that behavior. These *irregular* fibers show a relatively smaller rotational shift, suggesting they contain a higher proportion of nucleosomes in the open (or closed positive) state. Interestingly, these irregular nucleosome arrays also appear to be more rigid in torsion than regular fibers: the width of their torsional responses was narrower at a similar compaction level (compare curves **Supplementary Figs. 1b** (blue) and **1c** (green)).

We considered these data in the light of scanning force microscopy (SFM) studies that systematically investigated nucleosome spacing in 5S tandemly repeated arrays[1]. A significant heterogeneity was observed, with a bimodal distribution involving regularly spaced and close-packed regions (the latter with an internucleosome distance smaller than 20 bp). The close-packed configuration seems to correspond to a local minimum in free energy, attributed to the existence of

nucleosome/nucleosome attractive, tail-mediated, interactions[2]. These interactions compete with the small gain in energy to position a nucleosome specifically on the 5S sequence (~1 kT)[3]. For reasons of steric hindrance, close-packed nucleosomes are very probably unable to rotate around their dyad relative to each other, and to access the closed states. Thus, those close-packed nucleosomes should not accommodate rotational constraints as efficiently as regularly positioned ones, explaining how they could confer a larger torsional rigidity to their fibers. In order to further support that conclusion, we tried to adjust their extension-*vs.*-rotation response using our molecular model (see main text, "modeling" section and below), assuming that the fibers contained nucleosomes either close-packed or regularly positioned every 208 bp. For the data in **Supplementary Fig. 1c**, for instance, a satisfactory fit was obtained with 10 regularly spaced nucleosomes, and 21 in contact.

Next, we purposely favored the formation of close-packed nucleosomes through the shortening of some critical dialysis steps in the protocol, so that the reconstitution lasted ~8 hours instead of the usual 24 hours[4]. Under these conditions, histone-DNA and histone-histone attractions occur more abruptly, and most histone octamers cannot slide along the DNA and "find" their energetically most favored position before being frozen into place. The corresponding population of fibers mostly consisted in close-packed nucleosomes, as confirmed by the micrococcal nuclease assay, and showed a narrow width when tested for their extension-*vs.*-torsion response (not shown).

2- Modeling

"One state" The fiber was first modeled as a regular sequence of nucleosomes locked in their negative conformation (**Supplementarty Fig. 2a**), as inferred from the core particle[5] and tetranucleosome crystal[6] structures. Nucleosomes were connected by straight linkers, according to the two-angle model[7]. The first angle, α, is between the nucleosome entry-exit linkers, and the second, β, between successive nucleosomes. α was set to 54°, the angle between entry-exit DNAs in the crystal structure[5], whereas β = 115° depends on the DNA periodicity, both in the linkers (10.5 bp/turn) and on the histone surface (~145 bp with a local periodicity of 10.15 bp/turn, to account for the ΔTw = 0.3-turn overtwisting observed in the minicircle system with 5S mononucleosomes[8]). The fiber architecture could then be modeled using a computer algebra system (Maple 9 software) (**Supplementary Fig. 2b**). The incremental length per nucleosome along the nucleosome array axis (Δl), as well as the linking number change per nucleosome[9] (ΔLk_t) were computed (**Supplementary Fig. 2c**).

The chromatin mechanics is fully characterized by the intrinsic elasticity of the linkers[10,11], and of the two flanking naked DNA spacers (see **Fig. 1**, main text). We assumed that the fibers' extension (z_{tot}) and topology relative to DNA (ΔLk_{tot}) resulted from the additive contributions of naked DNA and chromatin. Notably, this approximation is supported by the linear relationship observed between the compaction and the topological shift (**Fig. 2c**, and **Supplementary Fig. 1a**). For a given torque *C* and force *f*, this leads to **Supplementary Equation 1**.

In the extensional equation of **Supplementary Equation 1**, z_{DNA} is the extension of the fraction of naked DNA, and $z_{chromatin}$ the extension of a nucleosome array containing *n* nucleosomes. The dependence of these extensions on *f* and *C* was

explicitly evaluated using the analytical expression proposed in[12], where the bending (resp. twist) persistence length of naked DNA was set to 60 nm (resp. 80 nm) (see **Fig. 2d** in main text), and that of chromatin computed as in[10]. In the topological equation of **Supplementary Equation 1**, the first term corresponds to the linking number change of n nucleosomes, relative to the corresponding free DNA; the third term is for the effect of the external constraints on the DNA linking number, according to the corresponding expression derived in[12], and the second one for the contribution of external constraints on the fiber itself, whose persistence length is calculated according to[10]. Note that **Supplementary Equations 1** are valid to describe the mechanical response of the fiber close to the apex only, because they rely on the perturbative approach followed in[12].

When trying to fit our data, however, severe inconsistencies were encountered. As a typical example, let us reinvestigate the response of the fiber analyzed in **Fig. 5b**. The vertical position of the apex is compatible with the presence of 31+/-1 nucleosomes in the fiber. Using our topological predictions, this would imply a rotational shift of the apex (relative to naked DNA) of -1.4x30=-42 turns, far from the experimental results (-24 turns, **Supplementary Fig. 2d**, red curve). This discrepancy could be compensated by allowing the nucleosomal DNA to overtwist to a local periodicity of ~9.7 bp/turn, as initially proposed to solve the linking number paradox[13-15]. The resulting curve (black in **Supplementary Fig. 2d**) is then correctly centered but its breadth is much too small, reflecting a large rigidity: applying the worm-like rope model[16] on the predicted rotational response, a torsional persistence length of 35 nm is deduced (data not shown). This is smaller than the value for naked DNA (80 nm), but still much larger than the experimental value for the real

fibers (5 nm; see main text).

"Three states" In contrast with the previous one-state model, we now allow the nucleosomes to fluctuate between three topologically discrete conformations referred to as "negative", "open" and "positive"[8] (see main text). The *negative* state has been described above. In the open conformation, we imposed α=-30° and a nucleosomal DNA length of 125 bp as a consequence of the breaking of the most distal histone-DNA binding sites at SHL±6.5, as observed in[17,18]. In the positive state, a minimal kink was introduced in the nucleosome entry-exit region to make the positive crossing possible. α was then taken equal to +30° and the nucleosomal DNA length to 145 bp. Subsequently, β, ΔLk_t and Δl could be evaluated, and the corresponding fibers constructed (**Supplementary Fig. 3a-b**).

Assuming these states are in thermodynamic equilibrium, the Gibbs free energy can be calculated (**Supplementary Equation 2**). In this equation, C is the torque, θ the angular rotation with respect to the relaxed situation for the fiber, and n_n (resp. n_o, n_p and n) the number of nucleosomes in the negative state (resp. open, positive and total number). Finally, U_n (resp. U_p) are the energy differences between the negative (resp. positive) conformation and the open one. Because the Gibbs free energy is minimal at thermodynamic equilibrium, we could derive the proportion of nucleosomes in each state as a function of the torque C. For instance, the expression for the number of nucleosomes in the negative conformation is computed in **Supplementary Equation 3**.

To describe the fiber mechanics, we followed the same approach as above, and hypothesized that the global response in torsion resulted from the additive

contribution of the naked DNA portions and of the "open" (resp. "negative" and "positive") nucleosome array containing $n_o(C)$ (resp. $n_n(C)$ and $n_p(C)$) nucleosomes. This amounts, in particular, to neglect nucleosome/nucleosome interactions, and distortions associated with the boundaries between series of nucleosomes in different states. Within this approximation, **Supplementary Equations 1** could be used, introducing three terms to describe the chromatin contribution instead of one in the "single state" approach. Finally, by reversing the topological equation in **Supplementary Equation 1**, the torque can be expressed as a function of the topological deformation applied to the fiber (ΔLk_{tot}), and **Supplementary Equation 3** becomes a function of supercoiling with three adjustable parameters, namely n, U_n and U_p.

Notably, whereas the theoretical linking number change ΔLk_t for the two closed states was similar to those obtained with the minicircle approach (ΔLk_m)[8], ΔLk_t for a "pure" chain of nucleosomes in the open state was significantly different (-0.5 against -0.7; see **Supplementary Fig. 3b**). We attribute this discrepancy to the 3-D architecture of the fibers: the geometrical organization of the nucleosomes (a collective effect) can affect the global topology of the fiber, with respect to a mere addition of nucleosomes individual deformations. However, due to nucleosome thermal fluctuations, this additional collective contribution is expected to vanish in real fibers. For that reason, we used for the fitting the minicircle ΔLk_m values as fixed parameters. Interestingly, this discussion is reminiscent of the work of Woodcock & *al*[7], who showed that a fiber constructed with identical and regularly repeated nucleosomes may adopt, depending on the linker length, widely different 3-D shapes, and that allowing for fluctuations in linker length within the same fiber leads

to fibers closer to physiological ones, as appear in electron microscopy of isolated chromatin and nuclei. In our case, fluctuations concern the nucleosome conformational state rather than the linker length, but they should similarly disrupt the long-range correlations along the fiber.

Our topological and mechanical predictions were then again applied, as an example, to the data represented in **Fig. 5b**, main text. We deduced that the fiber contained n=31 nucleosomes, short of the theoretical maximum of 36. The fit (see **Fig. 5b**, main text) allowed us to access the energies U_n and U_p (resp. 0.7 kT and 2 kT, see main text). The relaxed state for the fiber at zero torque was obtained at -27 turns (relative to the naked DNA), with a number of nucleosomes in the different states n_o=*21*, n_p=*2* and n_n=*8*. This makes ~68 % open, 26 % negative and 6% positive nucleosomes (**Supplementary Fig. 4a**), and gives a mean ΔLk of 0.68x(-0.7)+0.26x(-1.4)+0.06x(-0.4)=-0.86 per nucleosome. This value agrees well with the -0.8$\pm$0.1 figure obtained in this work after partial nucleosome disruption (**Figs. 2a** and **b** in main text), and with the -0.8 value obtained with plasmids made of the same tandemly repeated 5S-208 sequences reconstituted with hyperacetylated histones[19] (the N-terminal tails of histones H3 interact less with nucleosome entry-exit DNAs, which increases their mutual electrostatic repulsion, and mimics our low ionic strength B_0 conditions). Notably, the average proportion of nucleosome in each state can be computed for any topological deformation using our model (**Supplementary Fig. 4b** (Upper panel)). Finally, it is noteworthy that the torque-*vs.*-rotation response predicted by this model compares well with that deduced from the worm-like rope approach, despite the widely different concepts used (**Supplementary Fig. 4b** (Lower panel)).

Using the model to fit other *regular* fibers (see **Supplementary Fig. 1a;** and **Fig. 6**, main text), similar values of the energy differences between the states were obtained, in spite of rather different numbers of nucleosomes. As a consequence, a satisfactory fit of the behavior of all regular fibers could be achieved with the above single set of energy values, leaving the number of nucleosomes as the single adjustable parameter. The experimental horizontal and vertical dependencies of the rotation curves on the number of nucleosomes (**Fig. 6** in main text) are thus well accounted for by the model, which was used to draw the straight line in **Fig. 2c**, main text, and **Supplementary Fig. 1a**.

3- Plectoneme formation at larger torsional constraints

Close to the apex, torsion can be easily accommodated by nucleosome structural transitions. However, the energetic penalty associated with forcing all nucleosomes in a single state increases nonlinearly, and makes the torque necessary to twist the fiber further larger and larger (the onset of this torsional rigidification is apparent in **Fig. 5c** in main text and **Supplementary Fig. 4b** (Lower panel)). The fiber in **Fig. 5b** (main text) reaches the essentially all-negative (positive) state (i.e. fiber 2) at ~-40 (respectively -10 turns) turns, and, beyond these topological constraints, it can still accommodate ~15 turns with a nearly linear length decay of ~25 nm/turn. As noted in main text, this slope is weaker than that of naked DNA (~90 nm/turn (**Fig. 2a**, main text)).

Thus, another mechanism must be considered to account for the behavior at high rotational deformations. The linear regime is reminiscent of a system evolving along a first order transition line (i.e. at constant torque), and more specifically of

the formation of plectonemes on naked DNA[20]. This process, i.e. the conversion of twist into writhe through the formation of "telephone chord" structures, corresponds to a well-defined mechanical problem, thoroughly studied by physicists both experimentally and theoretically[16,20,21]. Thus, rather general and model-independent arguments enable us to deduce the torque exerted in this regime from the value of the length-*vs*.-rotation decay[16]. For naked DNA in B_0, this torque is typically ~6 pN•nm/rad at 0.3 pN (data not shown).

For chromatin fibers, plectonemes are also expected to form. This process is, however, significantly more complex than for naked DNA, and we cannot capture the behavior in a quantitative physical model. A few qualitative features are consistent with this interpretation, however. According to the worm-like rope model[16], for instance, the lower slope should be associated with a smaller bending persistence length for the fiber, as compared to naked DNA. We already reached that conclusion when studying the fiber's behavior at the apex (28 nm for chromatin *vs*. 60 nm for DNA, **Fig. 3a** in main text). Using the generalization of the worm-like rope approach[16,21] to fit the chromatin length-*vs*.-rotation decay, we could deduce the torque applied to the fiber in the plectoneme regime (~3-4 pN•nm/rad, **Supplementary Fig. 4b** (Lower panel)). This indeed corresponds well to the value of the torque predicted by the three states model, at the points associated with the plectoneme transition (-40 and -10 turns, respectively: see **Supplementary Fig. 5b**, lower panel).

Because this torque is significantly smaller than that required to form plectonemes on naked DNA (6 pN.nm/rad), it is improbable that they form in the flanking DNA spacers (see **Fig. 1**, main text). We propose that they instead take

place in the nucleosome array. This process would be favored by the "kinking" effect of nucleosomes which brings entry/exit DNAs close together, and also by the partial screening of DNA/DNA electrostatic repulsions by histone tails, which should reduce the effective diameter of the DNA and hence of the plectoneme. Interestingly, this implies that the bulky nucleosomes do not play a significant adverse role of steric hindrance, i.e. that they organize on the outer face of the plectonemes (**Supplementary Fig. 5**). This plectoneme formation may also be favored by the presence of naked DNA gaps with missing NSs, in our imperfect fibers.

Clearly, a more detailed study of the plectoneme formation process would better be achieved with perfectly regular and gap-free nucleosome arrays, which may soon be constructed on nucleosome "super-positioning" sequences[3].

Supplementary References:

1. Yodh, J.G., Woodbury, N., Shlyakhtenko, L.S., Lyubchenko, Y.L. & Lohr, D. Mapping nucleosome locations on the 208-12 by AFM profides clear evidence for cooperativity in array occupation. *Biochemistry* **41**, 3535-3574 (2002).
2. Solis, F.J., Bash, R., Yodh, J., Lindsay, S.M. & Lohr, D. A statistical thermodynamic model applied to experimental AFM population and location data is able to quantify DNA-histone binding strength and internucleosomal interaction differences between acetylated and unacetylated nucleosomal arrays. *Biophys. J.* **87**, 3372-3387 (2004).
3. Thastrom, A., Lowary, P. & Widom, J. Measurement of histone-DNA interaction free energy in nucleosomes. *Methods* **33**, 33-44 (2004).

4. Hansen, J.C., van Holde, K.E. & Lohr, D. The mechanism of nucleosome assembly onto oligomers of the sea urchin 5 S DNA positioning sequence. *J Biol Chem* **266**, 4276-4282. (1991).

5. Luger, K., Mader, A.W., Richmond, R.K., Sargent, D.F. & Richmond, T.J. Crystal structure of the nucleosome core particle at 2.8 A resolution. *Nature* **389**, 251-260 (1997).

6. Schalch, T., Duda, S., Sargent, D.F. & Richmond, T.J. X-ray structure of a tetranucleosome and its implications for the chromatin fiber. *Nature* **436**, 138-141 (2005).

7. Woodcock, C.L., Grigoriev, S.A., Horowitz, R.A. & Whitaker, N. A chromatin folding model that incorporates linker variability generates fibers resembling native structures. *Proc. Nat. Acad. Sci. USA* **90**, 9021-9025 (1993).

8. Sivolob, A., Lavelle, C. & Prunell, A. Sequence-dependent nucleosome structural and dynamic polymorphism. Potential involvement of histone H2B N-terminal tail proximal domain. *J Mol Biol* **326**, 49-63. (2003).

9. Barbi, M., Mozziconacci, J. & Victor, J.M. How the chromatin fiber deals with topological constraints? *Phys. Rev. E Stat. Nonlin. Soft Matter Phys.* **71**, 031910 (2005).

10. Ben-Haim, E., Lesne, A. & Victor, J.M. Chromatin: a tunable spring at work inside chromosomes. *Phys. Rev. E Stat. Nonlin. Soft Matter Phys.* **64**, 051921 (2001).

11. Schiessel, H., Gelbart, W.M. & Bruinsma, R. DNA folding: structural and mechanical properties of the two-angle model for chromatin. *Biophys J* **80**, 1940-1956 (2001).

12. Moroz, J.D. & Nelson, P. Torsional directed walks, entropic elasticity, and DNA twist stiffness. *Proc. Natl. Acad. Sci. USA* **94**, 14418-14422 (1997).

13. White, J.H. & Bauer, W.R. The helical repeat of nucleosome-wrapped DNA. *Cell* **56**, 9-10 (1989).

14. Klug, A. & Travers, A. The helical repeat of nucleosome-wrapped DNA. *Cell* **56**, 10-11 (1989).

15. Klug, A. & Lutter, L.C. The helical periodicity of DNA on the nucleosome. *Nucleic Acids Res* **9**, 4267-83 (1981).

16. Bouchiat, C. & Mezard, M. Elasticity model of a supercoiled DNA molecule. *Phys. Rev. Lett.* **80**, 1556-1559 (1998).

17. Prunell, A. & Sivolob, A. Paradox lost : nucleosome structure and dynamics by the DNA minicircle approach. in *Chromatin Structure and Dynamics: State-of-the-Art*, Vol. 39 (eds. Zlatanova, J. & Leuba, S.H.) 45-73 (Elsevier, London, 2004).

18. Bednar, J. et al. Nucleosomes, linker DNA, and linker histone form a unique structural motif that directs the higher order folding and compaction of chromatin. *Proc. Natl. Acad. Sci. USA* **95**, 14173-14178 (1998).

19. Norton, V.G., Imai, B.S., Yau, P. & Bradbury, E.M. Histone acetylation reduces nucleosome core particle linking number change. *Cell* **57**, 449-57 (1989).

20. Strick, T.R., Allemand, J.F., Bensimon, D. & Croquette, V. Behavior of supercoiled DNA. *Biophys J* **74**, 2016-2028. (1998).

21. Neukirch, S. Extracting DNA twist rigidity from experimental supercoiling data. *Phys Rev Lett* **93**, 198107 (2004).