DIRECT MEASUREMENT OF DNA BENDING BY TYPE IIA TOPOISOMERASES: IMPLICATIONS FOR NON-EQUILIBRIUM TOPOLOGY SIMPLIFICATION

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Supplemental Materials

Protein-DNA complexes are equilibrated in two dimensions on the surface of the mica

To accurately measure the DNA bend angle imposed by a bound protein from AFM images, the DNA must be deposited on the mica surface under conditions that favor two-dimensional (2-D) equilibration of the DNA in the plane of the mica rather than a kinetically pinned 2-D projection of a three-dimensional (3-D) conformation (1-3). Reliable 2-D equilibration of DNA on the surface of mica for AFM imaging is consistently achieved using divalent magnesium cations in the deposition buffer to form an electrostatic salt bridge between the negatively charged surface of the mica and the negatively charged DNA (1,3-6). Using these deposition conditions, DNA-protein interactions are routinely probed with AFM and measured protein induced DNA bend angles have been extensively confirmed by other techniques including gel shift assays (2,7-9), crystal structures (2,8,10), transient electric birefringence measurements (2,7,11), and cyclization experiments (2,11). We used established deposition conditions that favor 2-D equilibration on the mica surface (1,3,5,6). Nevertheless, we verified that, under our deposition conditions, the DNA molecules achieved a 2-D equilibration rather than a 2-D projection by analyzing the polymer statistics of the deposited DNA. We utilized the fast marching algorithm and custom MATLAB (MathWorks, Natick, MA) program, DIPimage, developed by Faas et al. to determine the backbone coordinates of DNA molecules with no protein bound in AFM images (5). The polymer statistical quantities: \( \langle D^4 \rangle = \frac{\langle R^4 \rangle - \langle R^2 \rangle^2}{L^4}, \langle R^2 \rangle, \langle \cos \theta \rangle, \) and \( \langle \theta^2 \rangle \) as a function of L, the contour length along the DNA, were obtained from the backbone coordinates. We were able to fit the plots of these polymer statistics using the expressions derived by Faas et al. (5) for the 2-D equilibration case and for the 2-D projection of a 3-D conformation case, using the persistence length as the only fitting parameter (Fig. S3). Though the persistence lengths determine from the \( \langle R^2 \rangle, \langle \cos \theta \rangle, \) and \( \langle \theta^2 \rangle \) statistics all scale with increasing dimensionality, it is not possible to use
these alone to determine the state of DNA equilibration. However, the $\langle D^4 \rangle$ statistic does not scale with increased dimensionality. Thus, by comparing the persistence lengths obtained by fitting the $\langle D^4 \rangle$ statistic for the case of both a 2-D equilibration and 3-D projection to those quantities obtained by fitting the other three statistics, we were able to determine the dimensionality of the deposited DNA. We found that the 2-D equilibration equations gave a consistent persistence length of ~50 nm for all four cases, while the 3-D projection equations gave persistence lengths that were as small as ~60 nm in the $\langle D^4 \rangle$ case and as large as ~100 nm in the case of $\langle R^2 \rangle$, $\langle \cos \theta \rangle$, and $\langle \theta^2 \rangle$ (Fig. S3, Table S1). The agreement of the calculated persistence length in the 2-D equilibration case is evidence that the DNA had fully equilibrated on the surface of the mica in the AFM images.

To further confirm that the DNA-protein complexes were equilibrated in 2-D, we determined the DNA and topoisomerase height profiles as a function of bend angle (Fig. S4). If the DNA-protein complex equilibrated in such a manner that the plane of the DNA bend did not lie in the plane of the mica, the height profile would likely be distorted in a bend-angle dependent manner due to the large persistence length of DNA. In a simple geometric model, measured bend angles would be maximal and the topographical height variation minimal, for bends lying in the plane of the mica, whereas the measured bend would be near zero and the height variation would be maximal for bends lying in a plane orthogonal to the mica. However, there was no correlation ($R \sim 0$) between the height of the topoisomerases and the bend angle (Fig. S4). Furthermore, we determined the difference in height for two points on the DNA located 3 and 16 nm from the enzyme and plotted these with respect to bend angle (Fig. S4). These data were also uncorrelated, suggesting that the DNA and protein both fully equilibrated in 2-D onto the surface of the mica.

Our claim of 2-D DNA equilibration is further supported by the agreement among bend angles determined by both the local measurement of the bend angle (both manual and automated) and the more global measurement of the EEDs. Were the DNA not equilibrated in 2-D, these measurements would not have been as consistent as the simulations assumed a 2-D equilibration of the DNA on the surface.

|       | $P_{2-D}$ (nm)$^a$ | $P_{3-D}$ (nm)$^a$ |
|-------|--------------------|--------------------|
| $\langle R^2 \rangle$ | 51 ± 2              | 102 ± 4             |
| $\langle \theta^2 \rangle$ | 52 ± 3              | 104 ± 6             |
| $\langle \cos \theta \rangle$ | 52 ± 3              | 104 ± 6             |
| $\langle D^4 \rangle$ | 48 ± 1              | 63 ± 6              |

$^a$Fit parameter ± fitting uncertainties
Determination of the relationship between the non-equilibrium narrowing of the supercoil distribution ($R_{Lk}$) and the bend angle ($\theta$)

We obtained the relation between topology simplification activity determined from the narrowing of the topoisomer distribution ($R_{Lk}$) and the degree of DNA bending ($\theta$) predicted by the bend angle model. Klenin et al. simulated the ratio of equilibrium to non-equilibrium steady-state knotting probability ($R_{kn}$) as a function of the imposed bend angle (12). To relate $R_{Lk}$ to $\theta$, we needed to determine the relationship between $R_{Lk}$ and $R_{kn}$. Rybenkov et al. showed that non-equilibrium unknotting ($R_{kn}$), decatenation ($R_{cat}$), and unlinking ($R_{Lk}$) activities are highly correlated (13). Using the experimentally determined relationships among these measures of topology simplification from Rybenkov et al. (13) and the results of the bend angle simulations from Klenin et al. (12), we determined the relationship between $R_{Lk}$ and $\theta$ shown in Figure 8 of the main text.

We re-plotted and fit the experimental relationships between $R_{kn}$ and $R_{cat}$ (Eq. S1) and between $R_{Lk}$ and $R_{cat}$ (Eq. S2) from Figure 3 of Rybenkov et al. (13) (Fig. S5A and B). These two equations were solved to establish the relationship between $R_{kn}$ and $R_{Lk}$ (Eq. S3).

$$R_{kn} = (R_{cat})^{1.5}$$ \[S1\]

$$R_{Lk} = 1 + 0.28 \times \ln(R_{cat})$$ \[S2\]

$$R_{Lk} = 1 + 0.19 \times \ln(R_{kn})$$ \[S3\]

We extracted and fit the data relating $R_{kn}$ and $\theta$ from figure 5 of Klenin et al. (12) to determine the relationship between $R_{kn}$ and $\theta$ (Eq. S4) (Fig. S5C). Combining Eq. S3 and Eq. S4, we solved for the relation between $R_{Lk}$ and $\theta$ (Eq. S5). Because the relationship between $R_{Lk}$ and $R_{kn}$ is logarithmic (Eq. S3) and the relationship between $R_{kn}$ and $\theta$ is exponential (Eq. S4), the relationship between $R_{Lk}$ and $\theta$ is linear (Eq. S5) (Fig. S5D).

$$R_{kn} = e^{(0.014+\theta)}$$ \[S4\]

$$R_{Lk} = 1 + 0.0026 \times \theta$$ \[S5\]

We used the relationship shown in Eq. S5 and the average of our measured and previously published values of $R_{Lk}$ for Topo IV (1.81), yTopo II (1.27), and hTopo IIa (1.6) to determine the predicted bend angles for each enzyme: ~310°, ~100°, and ~230°, respectively, as shown in Figure 8 in the main text.
Figure S1. Alternative bend angle measurement methods. A. The method of automated tangent overlay. Here, the red circle represents an approximation of the boundary of the topoisomerase, and the blue inner circle and green outer circle represent the boundaries for the DNA skeleton fit, which is extrapolated to the point of intersection where $\phi$, the supplement to the bend angle, is measured. B. The two-kink model of bending. $R_c$ represents the section of DNA that is held constant and rigid between the two imposed bends, $R_1$ and $R_2$ represent the two fragments of DNA emerging from the topoisomerase, and EED represents the end-to-end distance between the two DNA endpoints.
**Figure S2.** Representative results of DNA simulations. **A.** A representative histogram of the distribution of EEDs for DNA-yeast topoisomerase II complexes (bars) and the best fit histogram of simulated DNA EEDs (line). Goodness of fit was determined by minimizing the $\chi^2$ statistic (Eq. [1] and [2], main text). **B.** A graph of the $\chi^2$ statistic vs. simulated bend angle for the histogram shown in A, fit with a quadratic function (line). **C.** A graph of the $\chi^2_{(R^2)}$ statistic (Eq. [2]) vs. bend angle, fit with a quadratic function (line).
Figure S3. DNA persistence lengths determined by 2-D ($P_{2D}$) and 3-D ($P_{3D}$) fits to polymer statistical measures (5).

A. The $\langle D^4 \rangle$ statistic as a function of contour length was fit to the equations for 2-D equilibration (red line) and 2-D projection of a 3-D conformation (blue dashed line). The persistence length was the only fitting parameter. Plots of B. the $\langle R^2 \rangle$ statistic, C. the $\langle \cos \theta \rangle$ statistic, and D. the $\langle \theta^2 \rangle$ statistic plotted as a function of the DNA contour length fit to the equations for 2-D equilibration (red line) and 2-D projection (not shown). The two fits are identical but the 2-D projection returns a persistence length twice that of the 2-D equilibration for all three statistics.
Figure S4. Protein and DNA intensity profiles as a function of bend angle. The normalized intensity (height) as a function of bend angle for A. Topo IV, B. yTopo II, and C. hTopo IIα. $R_{\text{correlation}} < 0.05$ for each enzyme. The change in normalized intensity (height) of DNA between 3 and 16 nm from the enzyme plotted as a function of bend angle for D. Topo IV, E. yTopo II, and F. hTopo IIα. $R_{\text{correlation}} < 0.1$ for each enzyme.
Figure S5. Relationships among measures of non-equilibrium topology simplification and the predicted imposed bend angle. **A.** Log-log plot of the measured ratio of equilibrium to steady state knotting probability ($R_{kn}$) as a function of the measured ratio of equilibrium to steady state catenation probability ($R_{cat}$), re-plotted from Figure 3 of Rybenkov et al. (13). (Reprinted with permission from AAAS.) The line is the fit to the data: $R_{kn} = (R_{cat})^{1.5}$. **B.** Lin-log plot of the measured ratio of equilibrium to steady-state topoisomer variance ($R_{Lk}$) as a function of $R_{cat}$ re-plotted from Figure 3 of Rybenkov et al. (13). (Reprinted with permission from AAAS.) The line is the fit to the data: $R_{Lk} = 1 + 0.28 \cdot \ln(R_{cat})$. The points are measurements for different type IIA topoisomerases: Topo IV (red squares), hTopo IIα (blue squares), yTopo II (green squares), phage T2 topoisomerase (○), *D. melanogaster* topoisomerase II (+), yTopo II with a C-terminal deletion (Δ), and topoisomerase III (□). **C.** Log-lin plot of $R_{kn}$ as a function of the imposed bend angle from simulations. The line is a fit to the data: $R_{kn} = \exp(0.014 \cdot \theta)$. (Reprinted from *The Journal of Molecular Biology*, Volume 320, Klenin, K., Langowski, J. and Vologodskii, A., Computational analysis of the chiral action of type II DNA topoisomerases. Pages 359-367, (2002), with permission from Elsevier.) **D.** Plot of $R_{Lk}$ as a function of $\theta$ derived from the data and the fits to the data in panels A, B, and C (see text). The line is the derived fit to the data (see text): $R_{Lk} = 1 + 0.0026 \cdot \theta$. 
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