Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supplementary Information

Interference of intrinsic curvature of DNA by DNA-intercalating agents

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DETAILED EXPERIMENTAL PROCEDURES

Reagents: Nicking enzymes, Nb. BtsI and Nb. BsrDI, were purchased from New England Biolabs. Decatenated kDNA and pSP73 vector were the comercial products from TopoGen and Promega, respectively. The tris-acetate-EDTA buffer for gel electrophoresis was product of 1st BASE. The DNA intercalators, chloroquine diphosphate and ethidium bromide, were bought from Sigma-Aldrich and Bio-Rad Laboratories, respectively.

Preparation of nicked kDNA: A mixture (30 µL) containing 50 mM potassium acetate, 20 mM tris-acetate, 10 mM magnesium acetate, 1 mM dithiothreitol, 2.6 nM of kDNA and 3 U of Nb. BtsI was incubated at 37˚C for 1 hour.

Preparation of nicked pSP73: A mixture (30 µL) containing 50 mM sodium chloride, 10 mM tris-hydrochloride, 10 mM magnesium chloride, 1 mM dithiothreitol, 2.6 nM of pSP73 and 3 U of Nb. BsrDI was incubated at 65˚C for 1 hour.

Staining of DNA samples: After gel electrophoresis, the gels were stained with 1.5 nM of ethidium bromide in 1X tris-acetate-EDTA buffer for an hour and further analyzed using G:BOX iChemi (Syngene).

AFM studies: Immobilization of DNA samples on micas were carried out following the previously reported procedures.[S1,S2] The buffer (pH7.0) used for immobilizing of DNA contains 10 mM $MgCl_2$ and 40 mM HEPES. AFM images were obtained in Tapping Mode™ on a Multimode™ AFM (Veeco, Santa Barbara, CA) in connection with a Nanoscope V™ controller. Antimony (n) doped Si cantilevers with nominal spring constants between 20 and 80 N/m were selected. Drive frequency and amplitude setpoint were set as 296-328 kHz and 200-245 mV, respectively. Scan

frequency was 1 Hz per line and the modulation amplitude was in a nanometer range. The images (3 μm × 3 μm or 5 μm × 5 μm) obtained had resolution of 512 × 512 or 1024 × 1024 and were further analysed and cropped into 1 μm × 1 μm images by NanoScope Analysis. All DNA sample determinations were carried out in air at room temperature.

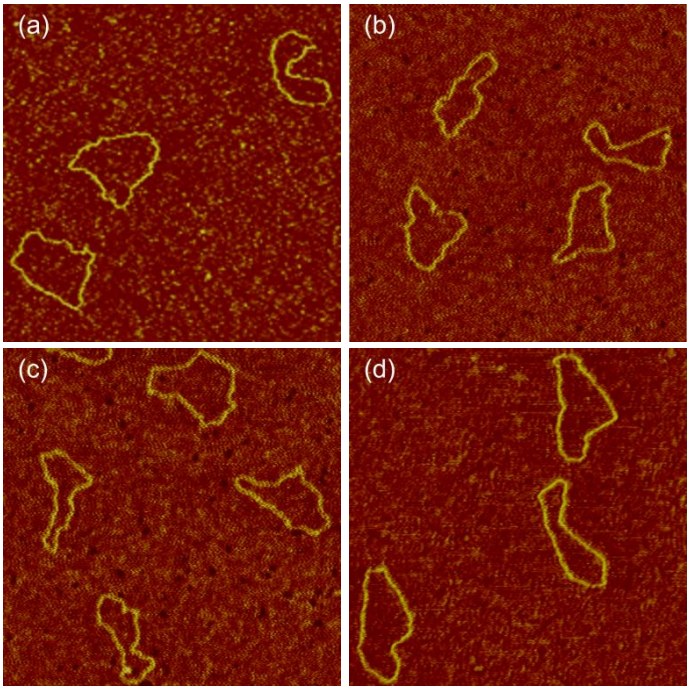


Fig. S1 AFM examination of the effect of chloroquine on nicked pSP73 plasmid. (a) nicked pSP73; (b)-(d) mixture of nicked pSP73 with chloroquine diphosphate. For (b)-(d), 0.2 nM of nicked pSP73 was incubated with 0.4 mM, 0.8 mM and 1.2 mM chloroquine diphosphate, respectively, for 30 minutes at room temperature before immobilizing sample on mica. The dimension of the images shown are 1 μm × 1 μm, *z* range 2 nm.

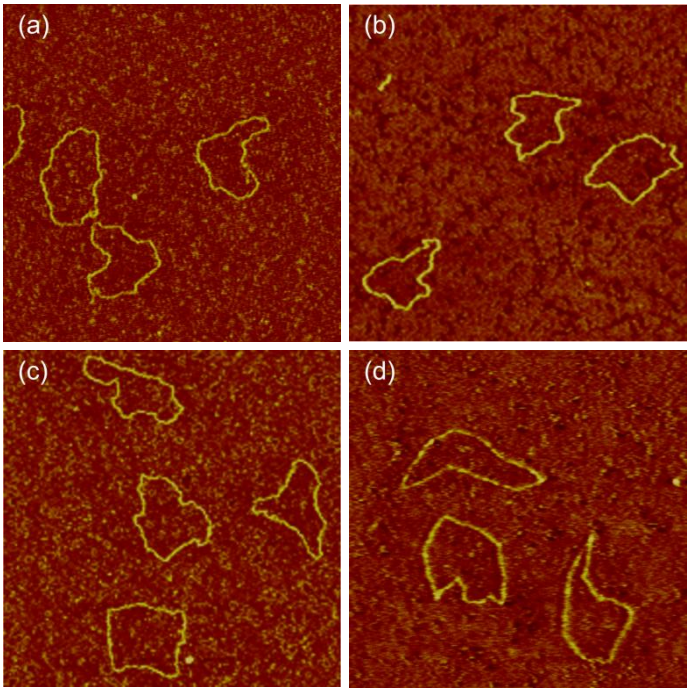


Fig. S2 AFM study of effect of ethidium bromide on nicked pSP73 plasmid. (a) nicked pSP73; (b)-(d) mixture of nicked pSP73 with ethidium bromide. For (b)-(d), 0.2 nM of nicked pSP73 incubated with 1 μM, 10 μM and 50 μM ethidium bromide, respectively, for 30 minutes at room temperature before immobilizing sample on mica. The dimension of the images shown are 1 μm × 1 μm, *z* range 2 nm.

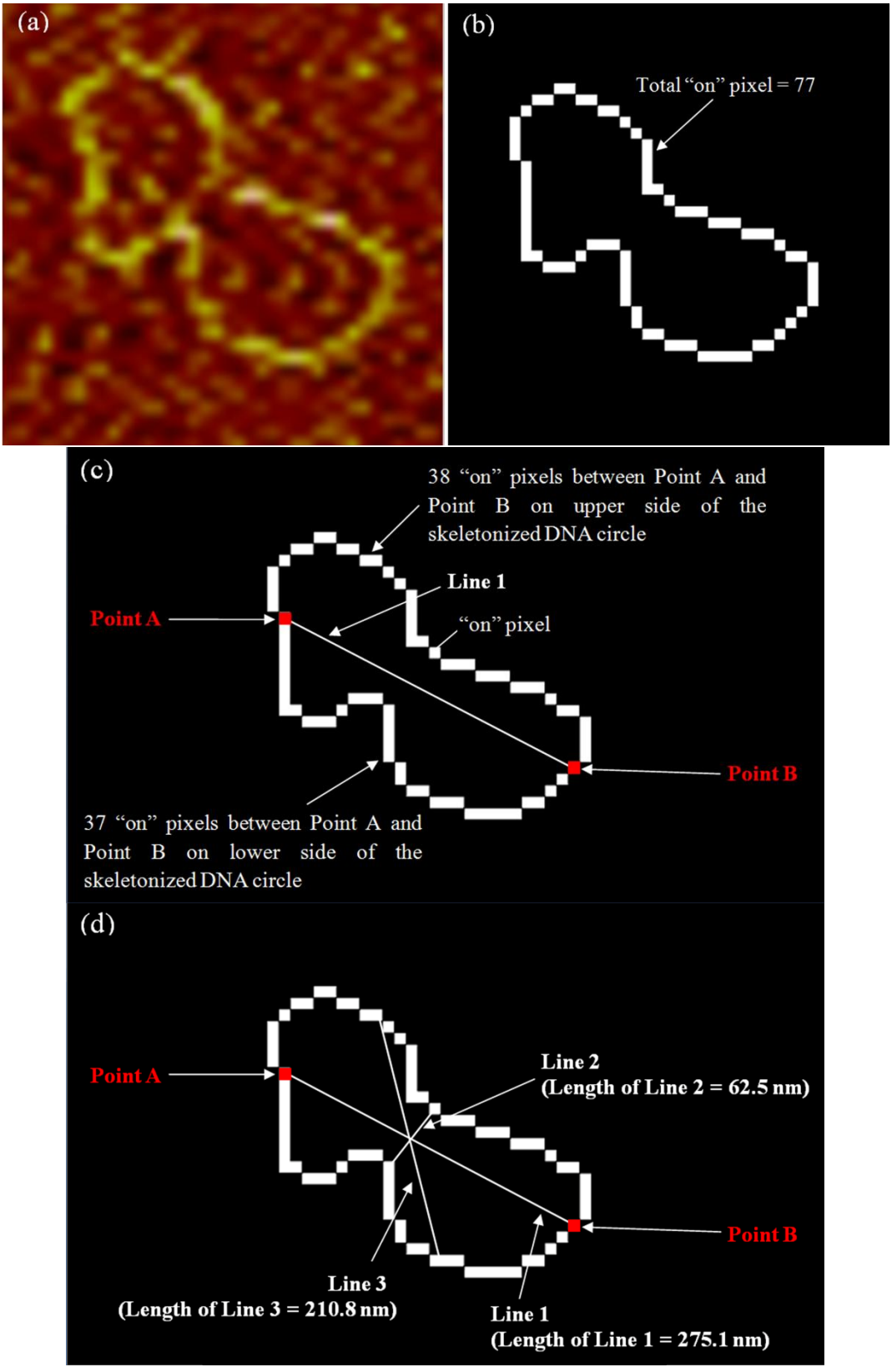


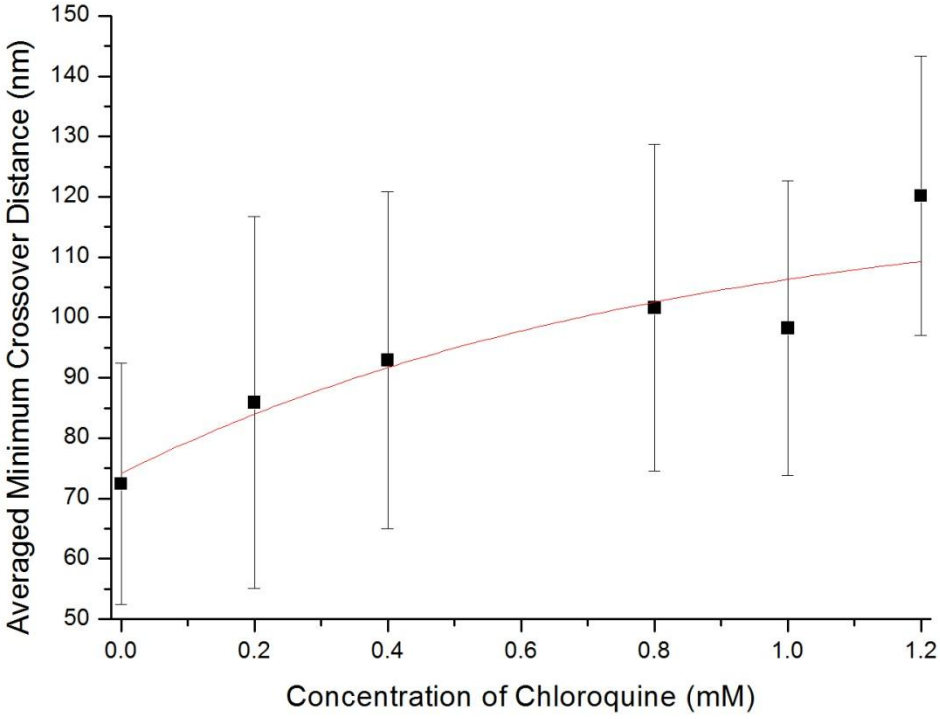
Fig. S3 Pictorial illustration of "Crossover Distance" and "Minimum Crossover Distance". (a) AFM image of a circular DNA; (b) Skeletonised binary image of a DNA circle; (c) Schematic representation of "Crossover Distance". Point A and Point B

are the two points in the skeletonized backbone of a DNA circle, on each side of which the number of "on" pixels are nearly equal (38 "on" pixels on the upper side ≈ 37 "on" pixels on the lower side); (d) Schematic representation of "Minimum Crossover Distance". The lengths of Line 1, Line 2 and Line 3 are the Crossover Distances for the DNA circle, which are 275.1 nm, 62.5 nm and 210.8 nm, respectively. The length of Line 2 is identified as the Minimum Crossover Distance (62.5 nm) since it is the shortest distance among all Crossover Distance. The AFM images of circular DNA molecules were flattened using NanoScope Analysis and transformed to binary images using Matlab (Mathwork, Natick, MA)[S3]. The binary images were skeletonized to eight connected lines and the coordinates of "on" pixels were recorded. Crossover Distances (the distance between any two "on" pixels on each side of which the number of pixels on the circular structure) were calculated based on the following equation:

$$D = A\sqrt{(x_1 - x_2)^2 + (y_1 - y_2)^2}$$

In this equation, D and A represent the distance between two "on" pixels and the length per pixel in the binary image, respectively, whilst x1, y1 and x2, y2 are the xy-coordinates of the first and second "on" pixels. The shortest distance among the Crossover Distances was selected as the Minimum Crossover Distance.

(a)

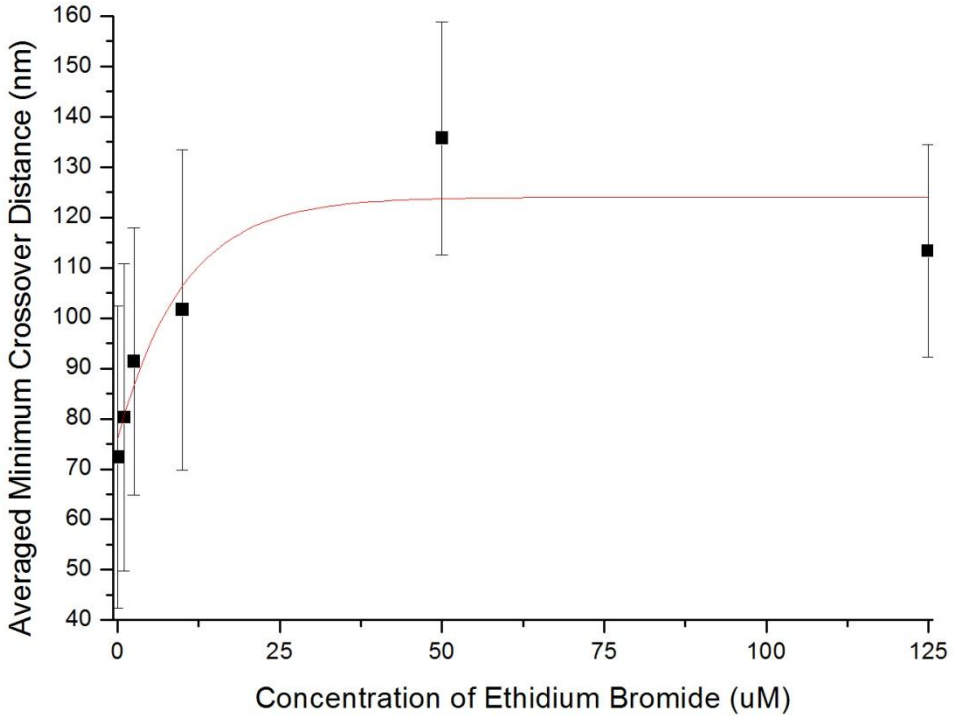


(b)

Concentration of Chloroquine (mM)	Number of DNA Circles Measured	Averaged Minimum Crossover Distance (nm)
0	64	72.4
0.2	75	85.9
0.4	58	92.9
0.8	46	101.6
1.0	58	98.2
1.2	46	120.2

Fig. S4 Correlation between averaged Minimum Crossover Distances and concentration of chloroquine. (a) Plot of averaged Minimum Crossover Distance versus concentration of chloroquine. (b) Statistical data of number of DNA circles measured and averaged Minimum Crossover Distance. The corresponding analysis was carried out on a 5 μm × 5 μm (512 × 512) AFM image.

(a)



(b)

Concentration of Ethidium Bromide (μM)	Number of DNA Circles Measured	Averaged Minimum Crossover Distance (nm)
0	64	72.4
1	33	80.3
2.5	66	91.4
10	31	101.7
50	45	135.8
125	47	113.4

Fig. S5 Correlation between averaged Minimum Crossover Distances and concentration of ethidium bromide. (a) Plot of averaged Minimum Crossover Distance versus concentration of ethidium bromide. (b) Statistical data of number of DNA circles measured and averaged Minimum Crossover Distance. The corresponding analysis was carried out on a 5 μm × 5 μm (512 × 512) AFM image.

REFERENCES

S1. Y. L. Lyubchenko and L. S. Shlyakhtenko, *Methods*, 2009, **47**, 206-213.

S2. Y. L. Lyubchenko, A. A. Gall, L. S. Shlyakhtenko, R. E. Harrington, B. L. Jacobs, P. I. Oden and S. M. Lindsay, *J. Biomolec. Struct. Dyn.*, 1992, **9**, 589-606.

S3. C. Rivetti and S. Codeluppi, *Ultramicroscopy*, 2001, **87**, 55-66.