**Lindsay Lab Publications**

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# Kinked DNA

Kinks are thought to play an important role in DNA-protein interactions1 but evidence for their existence in free DNA has previously been indirect. The atomic force microscope (AFM) is capable of direct visualization of molecular structure in situ[2,3] and we have developed a new AFM (Magnetic A/C mode or MacMode) which can image in solution more gently and with higher resolution[4]. Using this microscope, we have imaged 168 base-pair (bp) axially strained circles in the presence of various divalent cations. Major specific ion effects on DNA conformation were observed. The DNA was bent smoothly in the presence of $Mg^{2+}$, but consisted of nearly-straight segments connected by kinks in the presence of $Zn^{2+}$.

Tandem sequence repeats of d(A)[5] and d (GGGCC[C]) bend DNA[5], and we ligated the following oliogmer:

CCCAAAAAGGGCCAAAAAGGGCCCAAAAAGGGCCAAAAAGGG
TTTTTCCCGGTTTTTCCCGGGTTTTTCCCGGTTTTTCCCGGG

to produce DNA circles. Maximum formation of circles occurs when the ligated oligomers are 126 bp long. DNA longer than this forms superhelices and thermal fluctuations are needed to bring the ends together. As a result, the larger closed circles are axially strained upon ligation. Circles were extracted and purified as described elsewhere6. DNA solutions (0.5 µg/ml in 1mM $Mg^{2+}$ or $Zn^{2+}$ ) were placed into the sample cell of a PicoSPM (Molecular Imaging Corp., Phoenix, AZ)

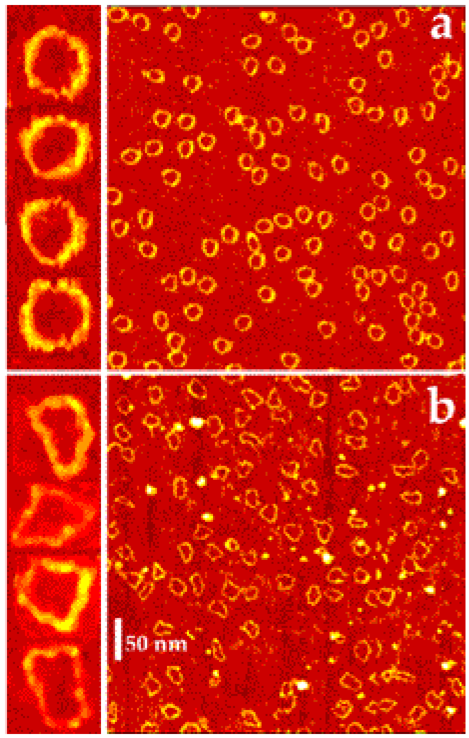


**Figure 1** Images of 168 base-pair DNA minicircles in (a) 1 mM $MgCl_2$ and (b) 1mM $ZnBr_2$, showing a fourfold increase in kink density in $Zn^{2+}$. Selected molecules are displayed magnified by a factor of four to the left of each image. The tip was oscillated at 25 kHz with an amplitude of 5 nm and a set-point decrease of 0.3 nm Each image was acquired in five minutes.

DNA images obtained in the MacMode had an average full-width at half-height of 3.5 nm, so the instrumental broadening is little more than one nm, a significant improvement over prior AFM technology. Figure 1a shows the 168 bp circles in 1mM $MgCl_2$. The DNA is smoothly curved with less than one where the molecules adsorbed spontaneously onto a mica substrate. Images were obtained in situ and remained stable on repeated scanning.

When molecules from the same sample are imaged in 1mM $ZnBr_2$, their appearance changes dramatically (Figure 1b). The DNA consists of nearly-straight segments connected by abrupt kinks. This phenomenon was not observed in 126 bp circles so we conclude that axial strain is required for this conformational change. The relative lack of kinks in the presence of $Mg^{2+}$ and the absence of kinks in smaller circles under all single-strand breaks did not cause the kinks. Full experimental details will be published elsewhere.

The strong enhancement of kinking observed here in the presence of $Zn^{2+}$ is qualitatively consistent with earlier electron microscopy[7] and electro-optical[8] studies. If kinks are readily generated in vivo through a combination of axial strain and appropriate ionic conditions, then one might speculate that local writhing stress, to which DNA subjected during various regulatory events, may promote the formation of localized kinks at specific DNA sequences. These could serve as signals for the isionic conditions demonstrate that assembly and/or localization of nucleoprotein complexes.

abrupt bend or kink per molecule.

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1. Werner, M. H., Gronenborn, A. M., Clore, G. M., Science **271**, 778-784 (1996).
2. Hansma, H.G., and Hoh, J., Annu. Rev. Biophys. Biomol. Struct. **23**, 115-139 (1994).
3. Bustamante, C. and Rivetti, C., Annu. Rev. Biophys. Biomol. Struct. **25**, 395-429 (1996).
4. Han, W., Lindsay, S.M., and Jing, T., Appl. Phys. Lett. **69** 4111-4114 (1996).
5. Dlakic, M. and Harrington, R. E., Proc. Natl. Acad. Sci. USA **93**, 3847-3852 (1996).
6. Dlakic, M., and Harrington, R. E., J. Biol. Chem. **270**, 29945-29952 (1995).
7. Laundon, C. H., and Griffith, J. D., Biochemistry **26**, 3759-3762 (1987).
8. Nickol, J. and Rau, D. C., J. Mol. Biol. **228**, 1115-1123 (1992).

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