

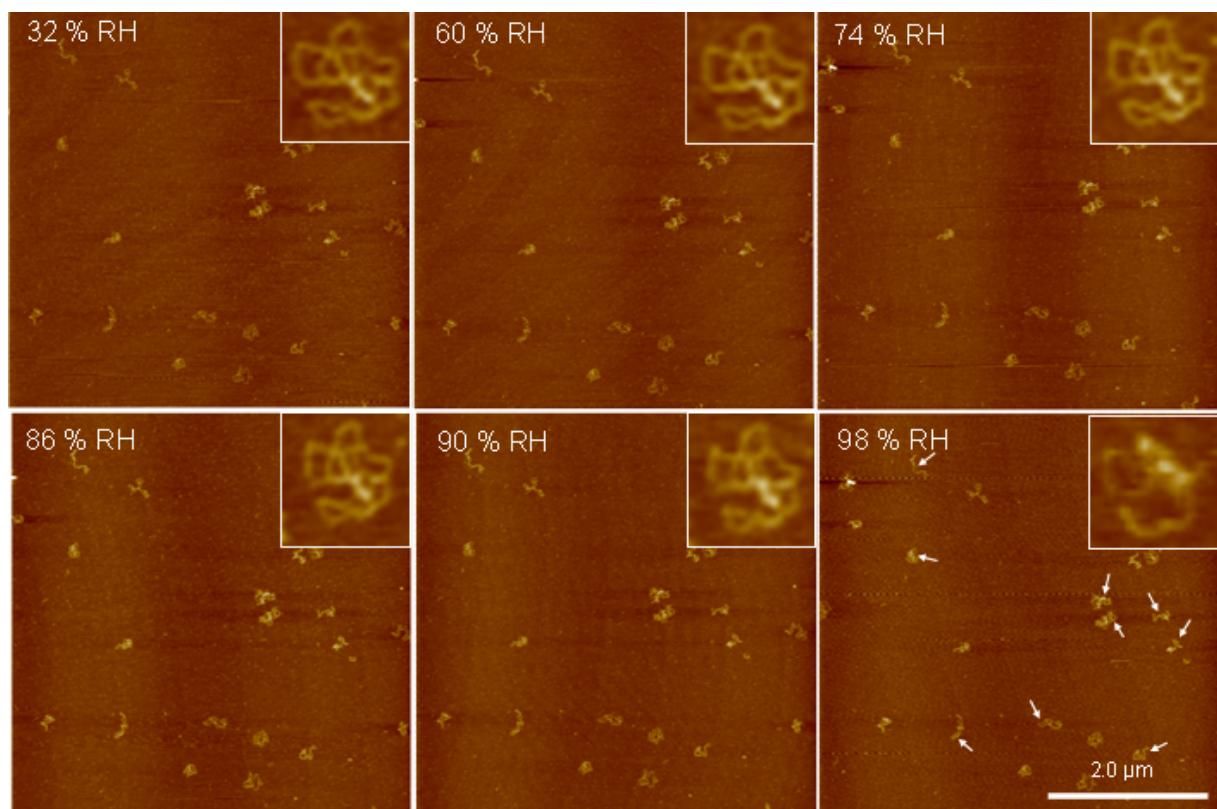
Supplementary Information

Atomic force microscopy of DNA at high humidity: irreversible conformational switching of supercoiled molecules

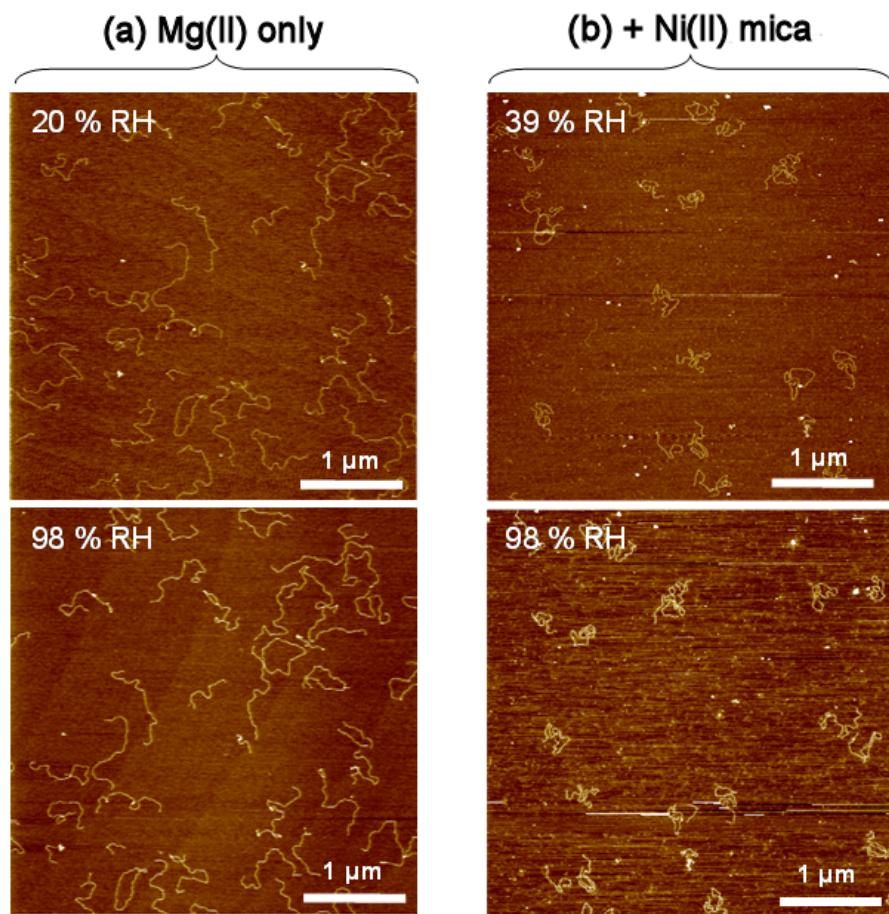
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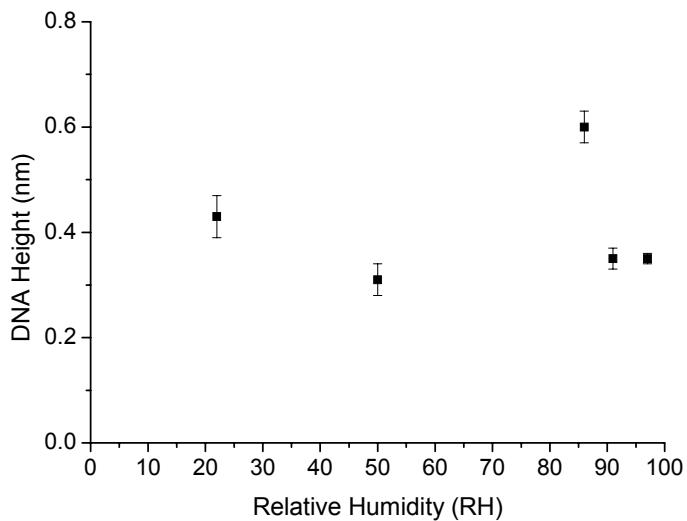
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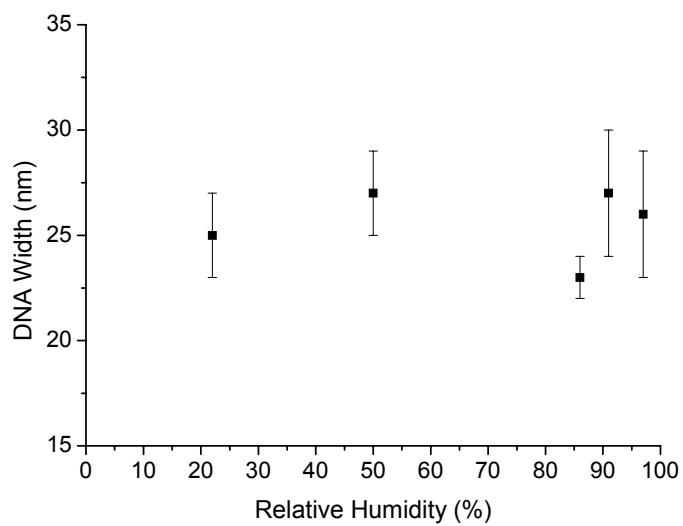
**Figure S1.** Series of AFM images showing the same region of a sample of closed circular (CC) plasmid prepared with Mg(II) in the buffer and pre-treating the mica with Ni(II) ions. The relative humidity (RH) is gradually increasing and only at very high RH ( $\sim 98 \%$ ) can local conformation changes be observed. A number of these changes are indicated with white arrows.



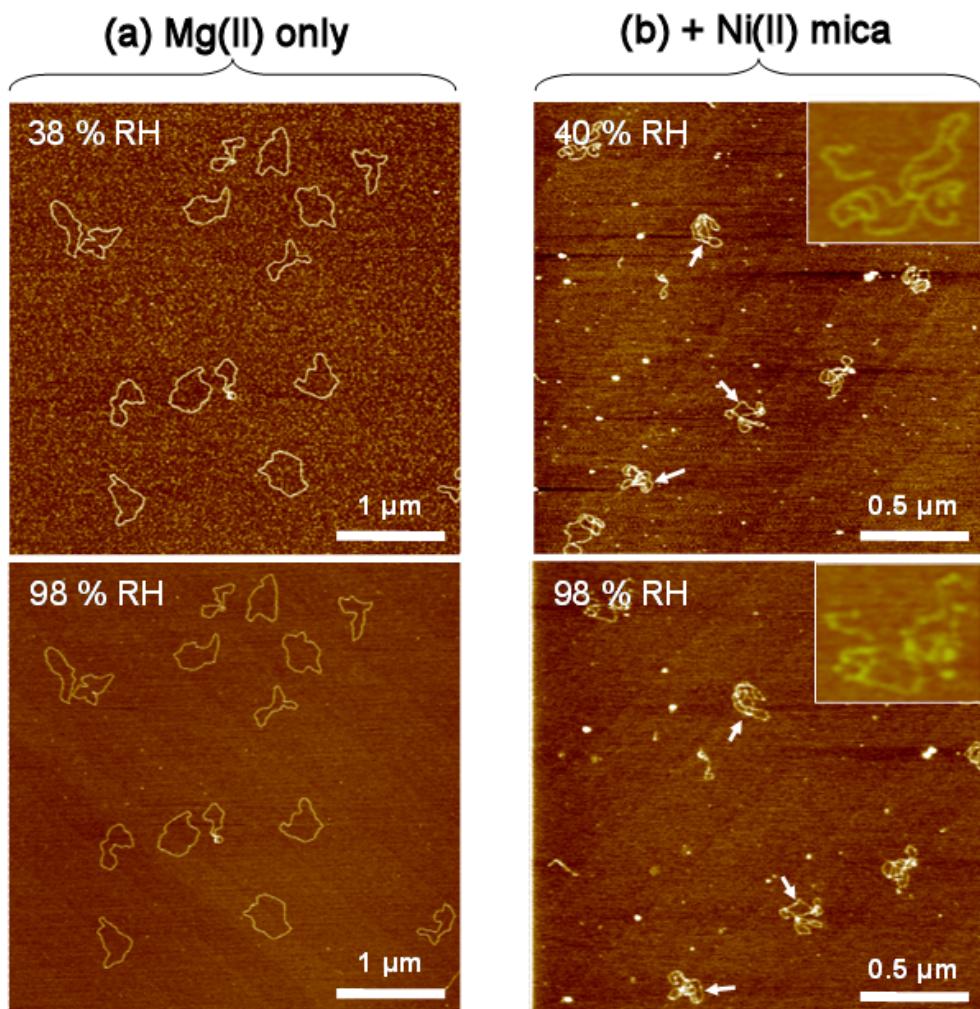
**Figure S2.** Images of linearized plasmid (lin.) at both low and high humidity of the same region prepared with Mg(II) (a) on mica and (b) Ni(II)-mica. No changes in conformation at high relative humidity are observed in either case.



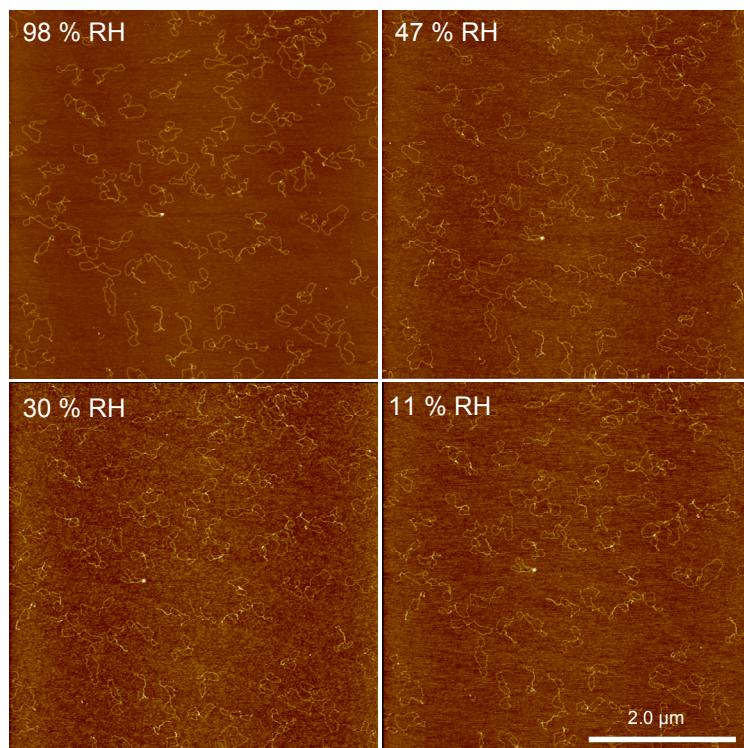
**Figure S3.** Average DNA height versus relative humidity (RH) for individual double-stranded linear DNA (lin.) helices lying against the mica surface ( $n=50$ ). There is no apparent correlation between measured height and the RH when imaging by repulsive force regime AM AFM.



**Figure S4.** Average DNA width versus relative humidity (RH) for individual double-stranded linear DNA (lin) helices lying against the mica surface ( $n=50$ ). There is no apparent correlation between measured width (full width at half-height) and the RH when imaging by repulsive force regime AM AFM.



**Figure S5.** Images of relaxed (OC) plasmids at both low and high humidity prepared with Mg(II) (a) on mica and (b) Ni-mica. In (a) no changes of conformation can be observed, while in (b) small, localized conformational changes can be observed at high humidity.



**Figure S6.** Series of AFM images showing the same region of a sample of closed circular (CC) plasmid pBR322 prepared with Mg(II) on mica. The relative humidity (RH) is gradually *decreasing* as shown in the top left of each image. No changes in DNA conformation are observed once the changes at high humidity ( $\sim 98\text{ \% RH}$ ) have occurred. While the background image noise increases at lower RH, no changes in the apparent dimensions of the DNA occur (see Figs. S3 and S4).