

Chain relaxation dynamics of DNA adsorbing at a solid|liquid interface

Willem Vanderlinden, Steven De Feyter

Supporting information

- Intra-molecular chain crossings on kinetic trapping and after surface equilibration;
- Out-of-plane dynamics of small equilibrated DNA circles;
- Evaluation of tip-sample forces.

Intra-molecular chain crossings on kinetic trapping and after surface equilibration;

Enzymatically nicked pBR322 molecules (500 pg in a 10 μ L volume) were deposited from a high salt buffer (100 mM KOAc; 50 mM NaOAc; 10 mM Mg(OAc)₂; 10 mM Tris-HCl; pH = 8) onto **(a)** poly-L-lysine-modified (0.01 % w/v) or **(b)** freshly cleaved muscovite mica surface. Intramolecular chain crossings were counted by visual inspection of the data (figure S1 a-d) and the smallest loop per molecule was traced manually to determine the contour length (figure S1 e,f).

- The sample deposited onto poly-L-lysine-modified mica **(a)** was rinsed with milliQ water and dried using Argon gas. This sample was measured in air, and might resemble the DNA conformation on initial adsorption on bare mica. Indeed, the strong interaction forces between DNA and poly-L-lysine kinetically trap the initially adsorbed conformation. In this case, multiple intramolecular overlaps are found (figure S1 a,b).
- After loading the sample deposited onto freshly cleaved mica **(b)** in the SFM liquid cell, additional buffer (200 μ L) was supplied and the system was left to equilibrate for 1 h at room temperature before scanning the sample in amplitude modulation SFM. Most (~ 80 %) of the molecules were found to exhibit an open circular conformation without intramolecular crossovers. Fewer plasmids had a single (~ 17 %) or double (~ 3 %) intramolecular crossover. Further scanning did not change the situation much, although segmental dynamics in the open conformations continued. Therefore, we believe this state represents an equilibrium distribution of intramolecular crossovers. In order to quantify the size distribution of the smallest loops per molecule (those molecules containing intramolecular crossovers only), we included data recorded in between 45 minutes and 2 hours after sample deposition to increase statistics (figure S1 f).

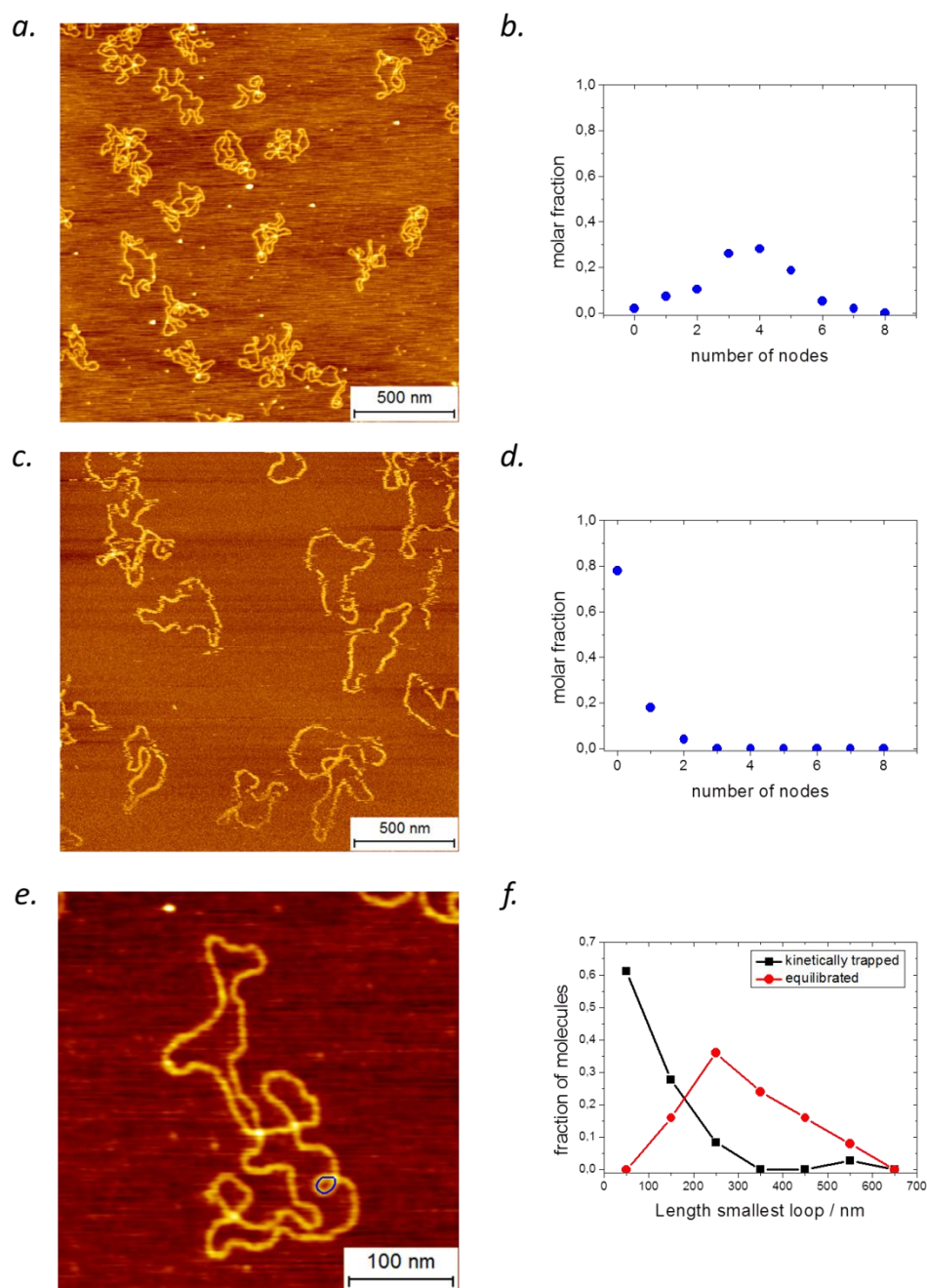


Figure S1. Intra-molecular chain crossings in torsionally unconstrained pBR322 plasmids on kinetic trapping and after surface equilibration. a. A typical SFM topography (dried sample) of open circular pBR322 molecules as deposited onto poly-L-lysine coated mica, b. histogram depicting the multiple intrachain overlaps present in the latter sample. c. Typical SFM topograph taken approximately 1h after sample deposition onto freshly cleaved mica (imaged in liquid) primarily depicts molecules in an open conformation without intramolecular crossovers (or nodes). d. node number distribution of open circular pBR322 molecules after about 1h of equilibration. e. digitally zoomed molecule from a kinetically trapped sample, exemplifying the determination of the length of the smallest loop in the molecule (blue line). f. Distribution of the lengths of the smallest loop per molecule in kinetically trapped versus equilibrated samples.

Local desorption-adsorption cycles of small (500 bp) DNA rings

To further strengthen our conclusions on the possibility for DNA segments to exhibit transient out-of-plane DNA dynamics, we measured the (equilibrium) behavior of small, torsionally relaxed DNA rings (500 bp). Because of their limited contour length (which is about 3 persistence lengths), these DNA rings adopt a fairly planar conformation. Amidst a field of DNA circles (figure 3.a.), adsorption or desorption events of entire molecules can be observed. The most remarkable event is depicted in figure 3.b which shows topographic images that were recorded on consecutively scanning the same area from top to bottom. A single DNA ring appears to run through two cycles of adsorption and desorption, at the same lateral positions. It is surprising to see that the SFM tip does not drag the desorbed molecule away. The adsorption-desorption behavior of small DNA rings is in agreement with the segmental out-of-plane dynamics in larger molecules.

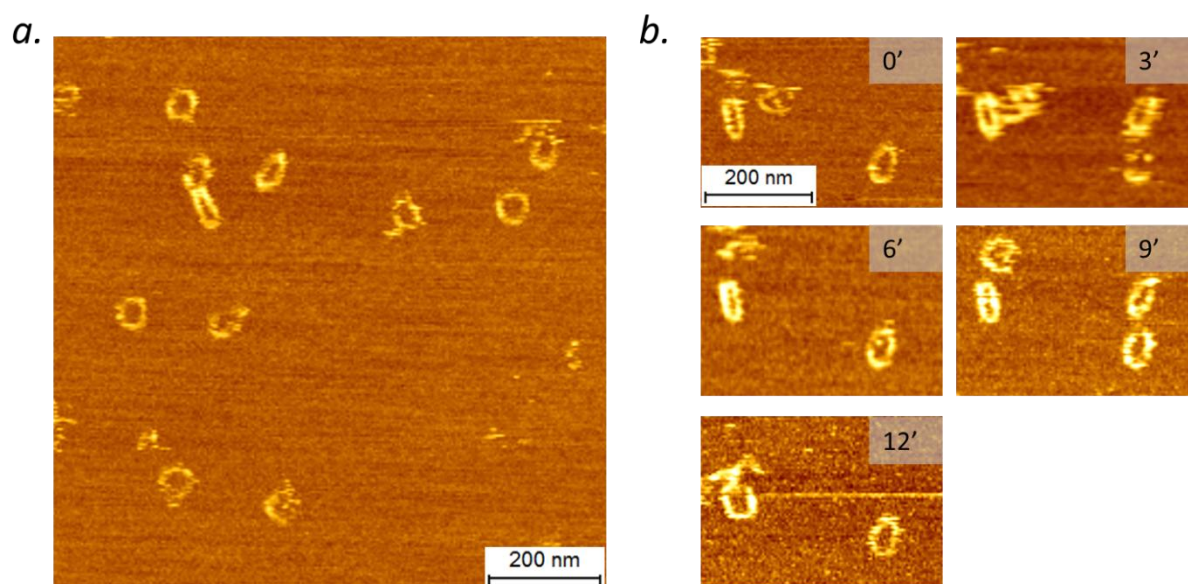


Figure S2. Molecular adsorption and desorption events of small (500 bp) DNA rings. a. typical field of adsorbed DNA circles. b. sequence of consecutive SFM topographs showing two adsorption-desorption cycles of the same molecule. The images are scanned from top to bottom with a rate of 3 minutes per frame. Note the molecular diffusion of a DNA circle on the left in opposite direction of the scanning direction.

Evaluation of lateral tip-sample forces

Linear DNA restriction fragments (EcoRI digest; 500 pg in a 10 μ L volume) were deposited onto freshly cleaved muscovite mica surface from a high salt buffer (100 mM KOAc; 50 mM NaOAc; 10 mM Mg(OAc)₂; 10 mM Tris-HCl; pH = 8). After loading the sample in the SFM liquid cell, additional buffer (200 μ L) was supplied and the sample was imaged using

amplitude modulation SFM at a rate of 28" per frame. The sticky ends generated by enzymatic restriction (AATT single stranded overhangs) are capable of transiently hybridizing at the interface, to yield monomer or dimer circles or linear oligomers. The lifetime of the transiently formed circles varied from a single to several frames, indicating that the hybridization (with an estimated intrinsic stability of 0.6 kcal/mole) is not largely affected by tip-sample interactions.

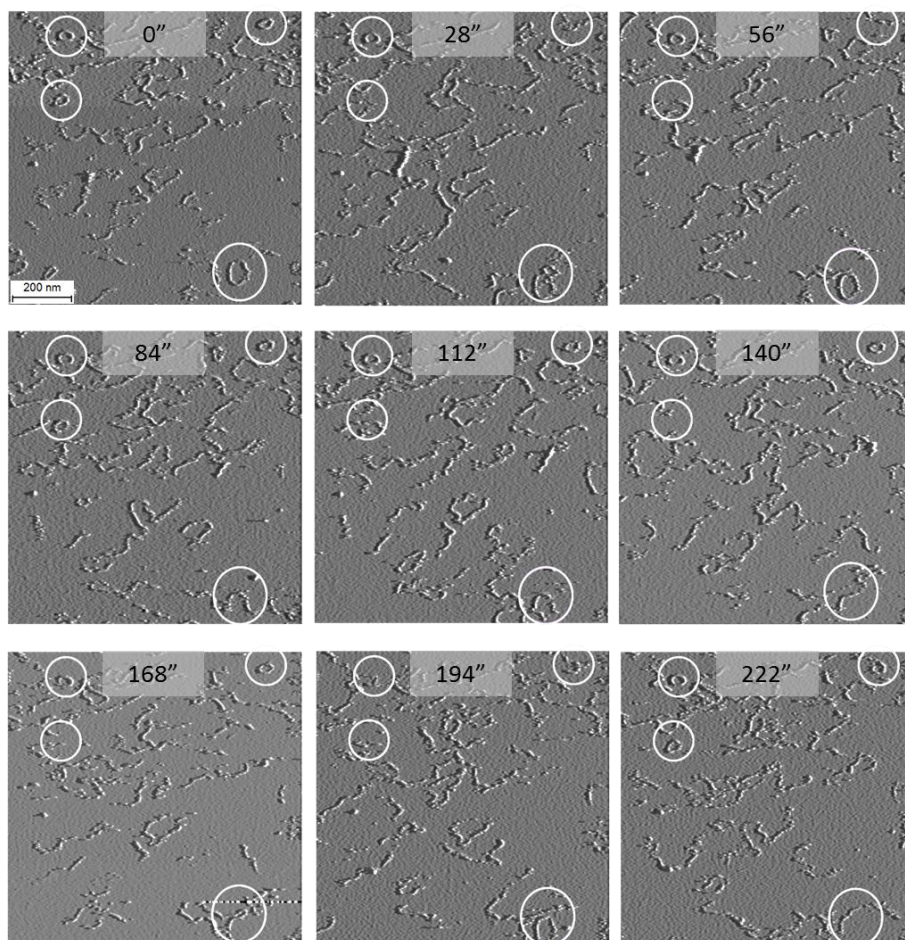


Figure S3. A series of 9 consecutive SFM amplitude images (error maps of the amplitude oscillation) indicate the transient formation of monomer and dimer circles as well as linear oligomers.