

## Global and Local DNA Structure and Dynamics. Single molecule studies with AFM

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The interaction between specific sites along a DNA molecule is often crucial for the regulation of genetic processes. However, mechanisms regulating the interaction of specific sites are unknown. We show in this paper that the single molecule observations performed with the time-lapse atomic force microscopy (AFM) provide an important information on DNA dynamics. In our recent work [1] we took advantage of AP-mica to bind DNA molecules in a broad range of ionic strengths to observe directly the effect of ionic conditions on the global structure of supercoiled DNA. Continuous observations over the same scan area in aqueous solutions (time-lapse imaging mode) allowed us to observe the mobility of DNA at the surface-liquid interface. This approach was very useful for studies of the structure and dynamics of cruciforms [2]. Two families of the cruciform conformations were found and characterized: an X-type and an extended conformations. Statistical analysis of the interim measurements led to the conclusion that compact X-type cruciforms are very dynamic allowing the arms to move in a very broad range. Unlike the X-type conformation, the extended cruciform geometry is less dynamic. These conclusions were tested by direct observation of the cruciform dynamics with the time-lapse AFM. The results showed clearly a high mobility of the X-type cruciform conformation and a relatively static conformation of the extended cruciform geometry. It is remarkable that AFM has the capability to reveal structural dynamics of cruciforms that were not amenable to any current structural technique. Further analysis of global DNA conformation led to unexpected discovery that the structural transition between cruciform conformations can act as a molecular switch to facilitate or prevent communication between distant regions in DNA. It is important to note that the cruciform conformation exists *in vivo* and such local structures and their conformational transitions during gene expression could have dramatic structural effects on chromatin architecture and function.

We have recently succeeded in visualization of intramolecular triplexes [3]. Plasmid samples were prepared at acidic pH and control samples were prepared at neutral pH. The image is shown in Fig. 1A. A distinct feature of the molecules prepared at acidic pH is the formation of a clear kink with a short protrusion indicated with arrows. Such features are not present in a control that have inserts different than purine-pyrimidine repeat. The formation of a sharp kink is fully consistent with the model of intramolecular DNA triplex. To study directly the effect of pH on H-DNA stability and to follow the transition of H-DNA into B-conformation, the procedure for reproducible and gentle change of the buffer solution without interruption of the scanning has been developed. The buffer change procedure injection procedure itself does not influence the position of the molecule and its overall shape. This is illustrated by the data in Fig. 1 in which the images of the same DNA molecule obtained at initial conditions (pH 5) and after the buffer injection (pH 7.6) are shown.

### H-DNA dynamics

The protrusions indicated with the arrows are observed and stably exist at conditions that stabilize H-DNA conformation (pH 5). However, these features become dynamic after replacing of the buffer. A series of images illustrating the dynamics of the part of the plasmid indicated with arrows is shown in Fig. 2. A complete set of 21 consecutive images suggests that overall changes of

the molecule are accompanied with the local DNA conformation transition. However, the same DNA regions undergoes a series of structural changes after the disappearing of the protrusion suggesting that the H-to-B form transition is not a simple two-state conformational transition. There is a number of conformational states including unwound regions that are capable of visualizing with the time-lapse AFM.

We believe that further development of the single molecule AFM technique is extremely important for understanding the DNA dynamics and for direct observation of the role of local DNA structures in numerous biological functions.

#### References:

1. Y. L Lyubchenko. & L. S. Shlyakhtenko (1997). *Proc Natl Acad Sci U S A* 94, 496.
2. L. S Shlyakhtenko et al. (1998). *J Mol Biol* 280, 61.
3. W. Tiner, et al. (2001) *J. Mol. Biol.* 314: 353.
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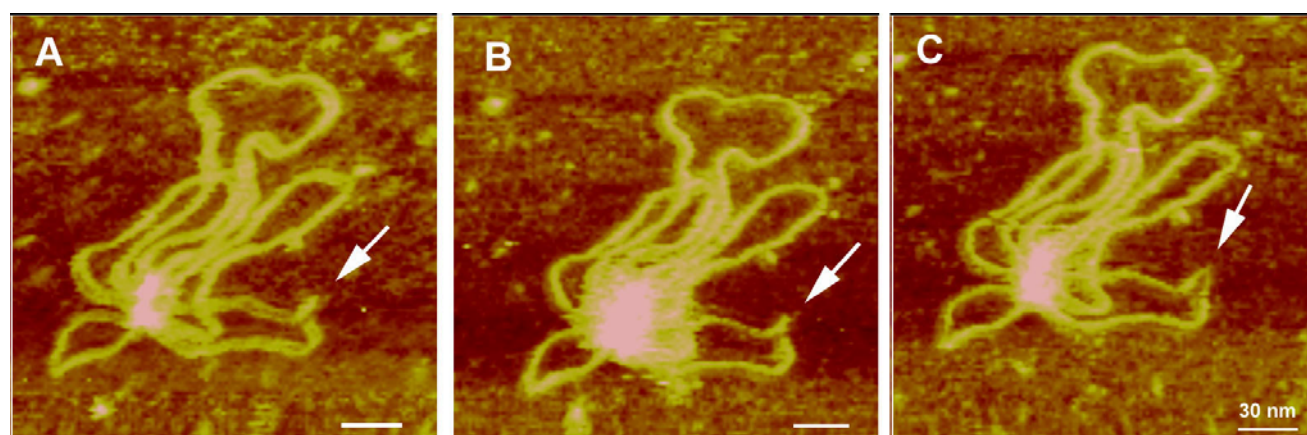
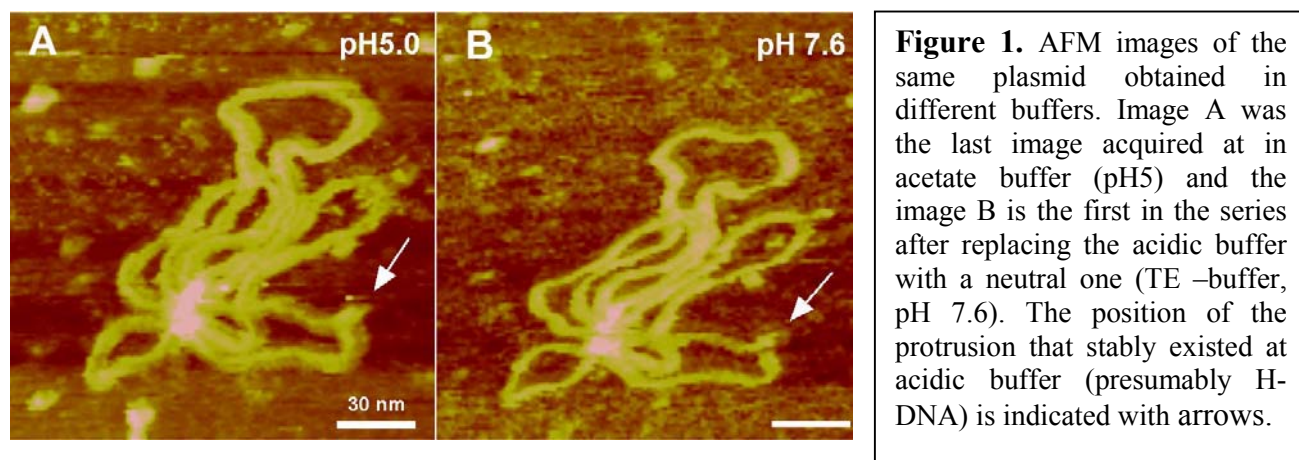


Figure 2. Time-lapse AFM images illustrating the transition of H-DNA into B-helix conformation.