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A convenient method of aligning large DNA molecules on bare mica surfaces for atomic force microscopy

Jianwei Li, Chunli Bai*, Chen Wang, Chuanfeng Zhu, Zhang Lin, Qing Li and Enhua Cao[1]

The Institute of Chemistry, The Chinese Academy of Sciences, Beijing 100080, China and [1]The Institute of Biophysics, the Chinese Academy of Sciences, Beijing 100171, China

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ABSTRACT

Large DNA molecules remain difficult to be imaged by atomic force microscopy (AFM) because of the tendency of aggregation. A method is described to align long DNA fibers in a single direction on unmodified mica to facilitate AFM studies. The clear background, minimal overstretching, high reproducibility and convenience of this aligning procedure make it useful for physical mapping of genome regions and the studies of DNA–protein complexes.

Atomic force microscopy (AFM) has displayed great potential in the study of the structure of DNAs and DNA–protein complexes. The complications in sample preparation, however, have until now hindered AFM from contributing vigorously to this field. This is especially the case for imaging long strands of DNA (1–3). The difficulty in imaging large DNA molecules comes from the fact that the longer the strand, the stronger the tendency to form entanglements. One way to get around such a situation is to align the long DNA strands in a single direction. In fact, various approaches have been proposed to straighten large DNA (4–8). These techniques, however, stretch DNA either in gel (4,5) or on glass which is generally a rather rough substrate (6), and therefore are not suitable for AFM studies. Based on the technique of modified molecular combing (9), silanized mica has been tried as a replacement to glass, and well extended DNAs are imaged by AFM (10). Yet the application of this procedure is limited by the rough background of the images (10), as well as the large stress in the DNA fibers (7) and the many broken strands that are produced (9).

Direct physical mapping of DNAs with AFM has been an attractive topic in recent years (11). To accomplish this goal, imaging well-extended DNA with a clean background is a prerequisite and key step. Like other authors (2,3), we found DNAs can occasionally be stretched on a bare mica surface by flow force during sample preparations (12). Based on this phenomenon, we have developed a convenient method to align DNAs on an unmodified mica surface.

DNAs were aligned in a single direction when a gas flow drives forward a drop of DNA solution on a bare mica surface (Fig. 1). Typically, a drop of DNA solution, ~5 µl, was placed onto the surface of freshly-cleaved mica. The sample was incubated at room temperature for some time. Then, compressed nitrogen gas was applied to the sample in such a way that the direction of the gas flow was at an angle of ~45° to the normal line of the mica surface and the impulse of the stream was focused on one part of the solution–air–mica interface. The discharge of the gas flow and the distance between the sample and the gas source were carefully controlled so as to drive the interface away at an appropriate rate. After the drop was blown out of the surface, the gas stream was kept in the same direction for several more minutes to dry out the sample completely.

Several factors weigh heavily on the final results of aligning. One is the method of blowing, including the direction of the gas flow relative to the mica surface and the moving speed of the interface. The flow direction should be tilted from the normal direction of the mica surface. Blowing at too large an angle (parallel with the surface) will cause nearly all DNA strands to fly out of the mica surface. On the contrary, blowing at too small an angle (perpendicular to the surface) cannot stretch strands in a single direction. The perpendicular blowing is effective to spread short fragments, yet cannot straighten them (Fig. 2a). When perpendicular blowing was applied to long strands, like the whole genome of lambda phage, almost all molecules were in a state of entanglement (data not shown). When the angle is changed to ~45°, the λ-DNA can be straightened along its whole length (Fig. 2b). The average contour length of such extended DNA measured from the AFM images is 14.6 ± 1.7 µm, ~89% of the expected values assuming B-form DNA. The 11% reduction of the measured length is similar to the result obtained using short DNA fragments by other investigators, and the main reason for this length decrease is that the pixels in an AFM image are not enough to resolve little bends or kinks along the DNA strands (13). It is not easy to visualize a long thread of DNA in such a large scan size, as the height of the DNA is usually not sufficient to allow the strands to be seen clearly. An enlarged image of λ-DNA is shown in Figure 2c.

The speed of the moving interface also affects the aligning of DNAs. Moving too quickly, little fibers would remain on the surface, while if it moves too slowly the fibers cannot be aligned well (data not shown). A moving speed of 0.5–2 cm/s worked well in our experiments.

The concentration of DNA solution and the incubation time both have discernible influence on the aligning. At a usual concentration of 1 ng/µl, the incubation time of 1–2 min is appropriate. Higher concentration or longer incubation time increases the density of fibers on the mica surface (Fig. 2d), yet often causes intermolecular entanglements.

Other factors, such as temperature, pH and ion strength of the DNA solution, also affect the aligning to a certain extent. Briefly, a temperature >15°C, pH of 5.5–7.0 and low ion strength are found most suitable for the extending of DNA (manuscript in preparation).

*To whom correspondence should be addressed. Tel: +86 10 6256 8156; Fax: +86 10 6255 7908; Email: clbai@infoc3.icas.ac.cn

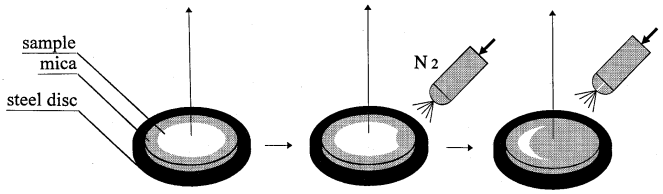

Figure 1. Schematic diagram of the DNA aligning procedure.

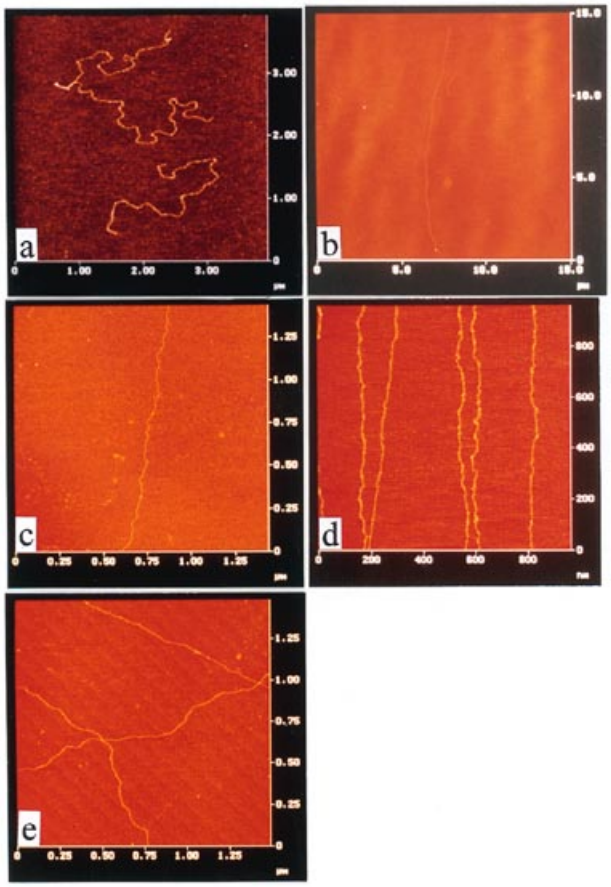

Figure 2. AFM images of DNA. λ-DNA and *Hin*dIII-digested λ-DNA were both purchased from Promega Co. and were diluted to ~1 ng/μl and adjusted to pH 6.0 before aligning. Imaging was performed in tapping mode in air at 26°C with a Nanoscope III microscope (Digital Instruments Inc., Santa Barbara, CA). All images were processed only by flattening. (**a**) Two fragments (23130 and 9416 bp) of λ-DNA digested by *Hin*dIII. (**b**) Single λ-DNA molecule aligned under optimal conditions. (**c**) One part of a roughly extended λ-DNA. (**d**) Parallelly aligned λ-DNA with a concentration of 1 ng/μl and incubation time of 5 min. (**e**) Crossovers of strands of λ-DNA produced by aligning twice on the same mica surface with aligning directions approximately perpendicular to each other.

The direction of aligning is parallel to the moving direction of the solution–air–mica interface. Two perpendicular aligning will cause fibers to cross each other (Fig. 2e) and these crossovers can be used to stabilize the adsorption of DNA on mica and mark different zones along a DNA fiber.

The observations that slight curves and bends still remain along the strands (Fig. 2b–e) and the shorter measured contour length reveal that these roughly straightened fibers are not in a tensile state. This is beneficial to the AFM studies of DNA–enzyme interactions, because the stress in the DNA strand may constrain the interactions between DNAs and proteins. On the other hand, the gentle curves on the strands do not prevent us from accurately measuring the contour length of a DNA molecule, taking advantage of the high resolution of AFM , especially the recently developed Mac Mode AFM (14 and therein).

In summary, this aligning procedure presents a method with convenience, reproducibility, clear background and little over-stretching of DNAs under study. The apparent advantages enable it to be a useful method for physical mapping of genome regions and the investigation of interactions between protein and large DNA molecules.

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REFERENCES

1 Hansma,H.G., Revenko,I., Kim,K. and Laney,D.E. (1996) *Nucleic Acids Res.*, **24**, 713–720.
2 Lyubchenko,Y.L., Jacobs,B.L. and Lindsay,S.M. (1992) *Proc. Natl Acad. Sci. USA*, **90**, 2137–2143.
3 Thundat,T., Allison,D.P. and Warmack,R.J. (1994) *Nucleic Acids Res.*, **22**, 4224–4228.
4 Schwartz,D.C., Li,X.J., Hernandez,L.I., Ramnarrain,P.R., Huff,E.J. and Wang,Y.K. (1993) *Science*, **262**, 110–114.
5 Zimmermann,R.M. and Cox,E.C. (1994) *Nucleic Acids Res.*, **22**, 492–497.
6 Bensimon,A., Simon,A., Chiffaudel,A., Croquette,V., Heslot,F. and Bensimon,D. (1994) *Science*, **265**, 2096–2098.
7 Bensimon,D., Simon,A., Croquette,V. and Bensimon,A. (1995) *Phys. Rev. Lett.*, **74**, 4754–4757.
8 Michalet,X., Ekong,R., Fougerousse,F., Rousseaux,S., Schurra,C., Hornigold,N., Slegtenhorst,M.V., Wolfe,J., Povey,S., Beckmann,J.S. and Bensimon,A. (1997) *Science*, **277**, 1518–1523.
9 Weier,H.-U.G., Wang,M., Mullikin,J.C., Zhu,Y., Cheng,J.-F., Greulich,K.M., Bensimon,A. and Gray,J.W. (1995) *Hum. Mol. Genet.*, **4**, 1903–1910.
10 Hu,J., Wang,M., Weier,H.-U.G., Frantz,P., Kolbe,W., Ogletree,D.F. and Salmeron,M. (1996) *Langmuir.*, **12**, 1697–1700.
11 Lyubchenko,Y.L., Jacobs,B.L., Lindsay,S.M. and Stasiak,A. (1995) *Scanning Microscopy*, **9**, 705–727.
12 Li,J.W., Tian,F., Wang,C. and Bai,C.L. (1997) *J. Vac. Sci. Technol. B*, **15**, 1637–1640.
13 Rivetti,C., Guthold,M. and Bustamante,C. (1996) *J. Mol. Biol.*, **264**, 919–932.
14 Han,W.H. and Lindsay,S.M. (1997) *Nature*, **386**, 563.