NANO LETTERS

2006
Vol. 6, No. 12
3000−3002

Comment on "Direct and Real-Time Visualization of the Disassembly of a Single RecA-DNA-ATPγS Complex Using AFM Imaging in Fluid"

Thijn van der Heijden,[†] Fernando Moreno-Herrero,[†] Roland Kanaar,[‡,§] Claire Wyman,[‡,§] and Cees Dekker*[,†]

Kavli Institute of Nanoscience, Delft University of Technology, Lorentzweg 1, 2628 CJ Delft, The Netherlands, and Department of Cell Biology and Genetics and Department of Radiation Oncology, Erasmus Medical Center, P.O. Box 2040, 3000 CA Rotterdam, The Netherlands

Received July 26, 2006; Revised Manuscript Received October 18, 2006

Introduction. Recently, Li et al. published a paper entitled "Direct and real-time visualization of the disassembly of a single RecA-DNA-ATPγS complex using AFM imaging in fluid".[1] They reported the disassembly of RecA from double-stranded (ds) DNA as observed by AFM imaging in deionized water. RecA plays an important role during homologous recombination forming a nucleoprotein filament that drives DNA strand invasion and exchange reactions. Li et al. drew two main conclusions from their work. First, RecA dissociates in a biologically relevant manner with greatly varying rates, and second, it dissociates as hexamers. We have performed similar experiments previously (presented below) and come to quite different conclusions. We imaged RecA filaments in buffered solutions where they are known to be biochemically active, as opposed to the deionized water used in the experiments of Li et al., where the biochemical activity of RecA is undefined. Our results, and we argue also the images presented by Li et al., indicate that RecA dissociation is strongly influenced by the scanning tip. The relevance of the work by Li et al. to the intrinsic biochemical activity of RecA is thus questionable. Furthermore, we address some concerns about image resolution and quantification that need to be considered in order to evaluate data on the size of objects in AFM images.

Tip−Sample Interaction Strongly Influences RecA Filament Disassembly. The biochemical conditions described by Li et al. influence the RecA-DNA disassembly reaction in ways that were not discussed. They initiate the disassembly of RecA-dsDNA-ATPγS complexes by exchanging the bulk fluid in the experimental setup with deionized water. The biochemical activities of RecA in deionized water are, however, ill defined. Furthermore, the pH of deionized water is not stable because it is not buffered. Diffusion of CO_2 into the fluid will drop the pH to about 5.5. The disassembly behavior of RecA from dsDNA is coupled to ATP hydrolysis and is highly pH-dependent. At a pH below 6.75, no net disassembly of RecA filaments from dsDNA is observed.[2] Because RecA is extremely slow in hydrolyzing ATPγS, disassembly is very inefficient,[3] as has been well established in single-molecule experiments.[4]

Our AFM experiments that image RecA filaments in biological buffers show that the disassembly of RecA depends on the AFM tip scan direction. We formed RecA filaments on linear dsDNA in the presence of ATPγS, deposited them onto untreated cleaved mica, and imaged them in buffer [20 mM Tris-HCl (pH 7.2), 10 mM $MgCl_2$, 1 mM DTT, 0.5 mM EDTA, 1 mM ATPγS]. Images were obtained with a commercial AFM from Nanotec Electronica, using soft cantilevers (0.05 N/m, Olympus TR400 PSA). Typical tapping amplitudes during imaging were 5−7 nm at the resonance frequency of the cantilevers used in liquid, for example, 10 kHz. Images were acquired at rates of 80 s/image (256 pixels, 2 μm) and always from left to right (fast scan direction) and from up to down (slow scan direction) in order to minimize any piezo creep effects. Figure 1 shows three sets of consecutive images taken 80 s apart. These clearly show that RecA dissociates faster when the filament lies perpendicular to the scan direction than when it is aligned with the scan direction. It is clear that this behavior does not reflect the intrinsic biochemical activity of the protein. In Figure 1 of the article by Li et al., a series of AFM images indicate the slow dissociation of a single RecA-dsDNA complex by the appearance of gaps in the filament. In the images displayed, the observed gaps occur in regions that lie perpendicular to the scan direction. This is exactly the behavior that we have consistently observed and that is naturally attributed to tip−sample interaction influencing the stability of RecA-dsDNA filaments in fluid.

* Corresponding author. E-mail: dekker@mb.tn.tudelft.nl.
[†] Delft University of Technology.
[‡] Department of Cell Biology and Genetics, Erasmus Medical Center.
[§] Department of Radiation Oncology, Erasmus Medical Center.

10.1021/nl061746j CCC: $33.50
Published on Web 11/11/2006

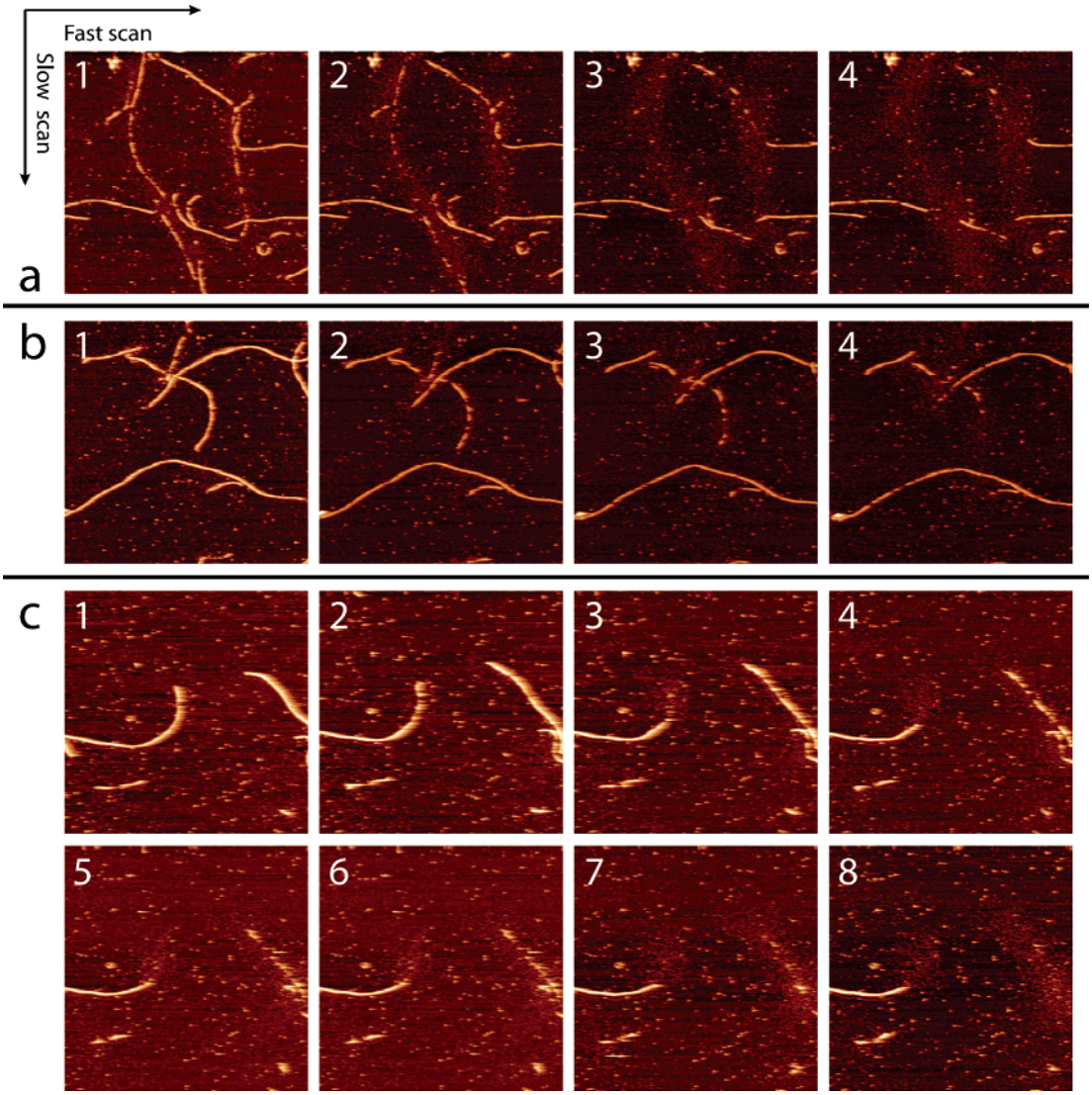

Figure 1. Three series of consecutive AFM images of RecA-dsDNA filaments obtained in buffer in the presence of ATPγS. The image size is 2.0 × 2.0 μm^2 (256 × 256 pixels); the measured height of intact RecA filaments was between 8 and 10 nm. The acquisition rate was 80 s/frame. The horizontal and vertical scan directions correspond, respectively, to the fast and slow scan direction of the tip. Parts a−c are three independent measurements taken with the same conditions. These images show that the disassembly of the RecA-dsDNA filaments is correlated to the scan direction of the tip.

Li et al. report a wide variation of disassembly rates, a variability that was not quantified. We estimate that this can be attributed to the influence of the scanning tip, because, in contrast to the arguments presented by Li et al., tip−sample interaction forces are expected to be quite significant. They state that tip−sample interactions are not important because "fluid AFM has been employed successfully to study the kinetic behavior of DNA, which was much thinner and more fragile than the RecA/DNA complex". dsDNA is a long polymer that binds to the mica surface through multiple interacting points and therefore the total interaction between a dsDNA molecule and the mica surface is much stronger than the interaction between a single RecA monomer and dsDNA, which involves only three base pairs per RecA monomer. The binding energy between a RecA monomer and DNA is about 7 k_bT,[5] indicating a binding force of ~28 pN. Tapping forces, normal to the mica surface, are difficult to measure but can be estimated to be in the order of 100 pN.[6] In liquid AFM, there are also lateral forces, parallel to the mica surface. These lateral forces are responsible for moving objects when imaging in liquid and are on the order of 10% of the normal force. We observed that only specific parts of the filaments disassembled, depending on the scan direction. If RecA dissociation would be induced by the chemical composition of the environment of the filament, then the complete filament would disassemble in a homogeneous way, with the same density of gaps independent of orientation relative to scanning.

Size Determination from AFM Images is Highly Dependent on Tip Shape and Requires Quantification. Li et al. conclude that the dissociated complex is hexameric, based on the size of the structures (or structure, as only one is described with resolution sufficient to judge its size) found on the surface after gaps appeared in the filament. We question that this can be concluded from their data. The observed structures are not homogeneous in size and there

is no quantification of their size distribution. Such quantification is a documented standard method for analysis of AFM imaging experiments.[7,8] The absolute dimensions of molecules are distorted in AFM images due to tip forces and tip-shape convolution. Accordingly, it is necessary to know what the tip radius of curvature was, in order to interpret dimensions in the images. This information is lacking and therefore the data cannot be interpreted properly. Furthermore, the hollow core of 2 nm diameter described in the text cannot be observed in Figure 2 of Li et al. Given standard imaging parameters, it is very unlikely that a feature of this size could be observed. Standard AFM tips have a radius of curvature of 5 nm at best and would not be expected to detect a hole smaller than this size. In addition, if we assume that the images were 512 by 512 pixels for a 1-μm-size image (this information is absent in the article) then this will yield a pixel resolution of 1.95 nm. A hole of 1 pixel is very unlikely to be detected. The larger size of structures on the surface detected in deionized water compared to those observed when the experiment is done with a buffer likely represents nonspecific aggregation of RecA under this condition.

Conclusions. Studying DNA−protein interactions in buffer by AFM at a single-molecule level is a powerful method when reproducible results are obtained that are analyzed carefully and statistically relevant. The influences of tip convolution and tip−sample interaction are of particular importance. It is clear from our study and the article by Li et al. that disassociation of RecA from dsDNA observed by AFM is strongly effected by interactions with the scanning tip, illustrating the necessary caution in using this method to describe biological function.

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NL061746J