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Real-time imaging of DNA–streptavidin complex formation in solution using a high-speed atomic force microscope

Mime Kobayashi*, Koji Sumitomo, Keiichi Torimitsu

NTT Basic Research Laboratories, NTT Corporation, 3-1 Morinosato-Wakamiya, Atsugi, Kanagawa, 243-0198, Japan

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Abstract

The direct observation of individual molecules in action is required for a better understanding of the mechanisms of biological reactions. We used a high-speed atomic force microscope (AFM) in solution to visualize short DNA fragments in motion. The technique represents a new approach in analyzing molecular interactions, and it allowed us to observe real-time images of biotinylated DNA binding to/dissociating from streptavidin protein. Our results show that high-speed AFMs have the potential to reveal the mechanisms of molecular interactions, which cannot be determined by analyzing the average value of mass reactions.

Keywords: AFM; DNA; Protein; Real-time imaging; Dynamics

1. Introduction

Almost all biological species employ DNA molecules to store genetic information. DNA is relatively stable, easy to handle and also has the ability to form self-assembled structures. DNA molecules are often used as building blocks for nanometer-scale molecular scaffold structures [1], and the application of these structures to immuno-PCR and nanomechanical devices has been proposed [2,3].

Streptavidin protein is known to bind four biotins per molecule with high affinity and selectivity [4]. The interaction of streptavidin and biotin has been extensively studied in many ways, ranging from thermodynamic simulation to force measurements. DNA can be attached to streptavidin by covalently conjugating a biotin molecule to the end of a DNA strand. Random grid-like nanostructures can be formed by using biotinylated DNA and streptavidin [3].

Atomic force microscope (AFM) has been widely used to observe surface structures on a subnanometer scale. The development of "tapping mode" AFM in liquids has made it possible to study soft samples such as polymers and other biological molecules under "living" conditions [5]. Recently, a high-speed AFM was developed and used to obtain subsecond images of moving myosin V protein [6]. Other new approaches to high-speed AFM have also been tried (e.g., [7–9]).

In this report, we show that DNA of a desired length can be easily prepared by using the polymerase chain reaction (PCR) technique and simple purification procedures. We used a high-speed AFM to visualize moving 300-nm-long DNA strands in a liquid. Most importantly, we succeeded in capturing 5–7 frame/s (fps) movies of biotinylated DNA strands binding to/releasing from streptavidin. These images could not have been captured with a conventional AFM nor would these events have occurred had the sample been dried for observation in air. Our results show the potential of using a high-speed AFM in liquids to record dynamic events that occur on a nanometer scale.

2. Materials and methods

2.1. DNA preparation

Oligo-nucleotide primers AMK6 (5′biotin-AGATCCA-GAGGAATTCATTATCAGTGC-3′) and M13F (5′-

*Corresponding author. Mesoscopic Materials Science, Graduate School of Materials Science, Nara Institute of Science and Technology, 8916-5 Takayama, Ikoma, Nara 630-0192, Japan.
Tel./fax: +81 743 72 6196.
E-mail address: mime@ms.naist.jp (M. Kobayashi).

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GTAAAACGACGGCCAG-3′) were purchased from Hokkaido System Science. Using 5 ng/μl of UASG-TK-LUC plasmid DNA [10] as a template, a 0.9 kilobase (kb) pair fragment of DNA was amplified by a PCR on a TaKaRa Thermal Cycler Dice TP600. The reaction conditions have been described elsewhere [11]. In brief, the reaction consisted of 35 cycles at 94 °C for 30 s, 50 °C for 1 min, and 72 °C for 2 min. The fragment was separated on a 1% agarose gel, and visualized by using ethidium bromide. The DNA quantity was estimated by comparing the brightness of the fragment to that of a known concentration of standard DNA. A fragment with a size of about 0.9 kb was cut out and purified using QIAEX II (QIAGEN) followed by an S-400 HR column (Amersham Biosciences). We were able to obtain about 4 μg (6.8 pmol) of the desired DNA fragment in one reaction.

2.2. AFM observation

The DNA fragment was diluted to a concentration of 5 ng/μl in 10 mM tris–HCl (pH 7.4) buffer, and 2 μl of the sample was incubated on freshly cleaved mica for 15 min. The sample was then washed with 70 μl of MG buffer (10 mM tris–HCl, pH 8.0, 5 mM MgCl). When it was used, the FITC-conjugated streptavidin (PIERCE) was included at a final concentration of 8 ng/μl in 10 mM tris–HCl (pH 7.4). Streptavidin and DNA were mixed and incubated for 15 min at room temperature before they were applied to the mica. The AFM experiments were performed using an NVB500 high-speed AFM (Olympus Corporation, Tokyo, Japan). The sample was imaged in MG buffer at ambient temperature with an EGS-8a cantilever that had a spring constant of 0.1 N/m (Olympus Corporation, Tokyo, Japan) in the "tapping mode" with an oscillation frequency of 650–750 kHz. The 192 × 144 pixel images were obtained at a scan rate of 1–7 fps.

3. Results

3.1. Preparation and observation of short DNA fragments using a high-speed AFM

The amplification of the desired DNA fragment using the PCR method was confirmed by separating the DNA on a gel, and visualized with ethidium bromide staining (Fig. 1). Approximately, 200 ng (about 340 fmol) of the 0.9 kb fragment was observed as a single band on the gel. No DNA fragment was amplified without template DNA (Fig. 1; negative control), suggesting that the DNA amplified in lane 2 is not the result of contamination from unknown sources. The single DNA band was cut out and purified as described in the previous section.

Because the DNA was visualized as a single band on a gel (Fig. 1) and because the fragments were cut out and purified, most of the DNA fragments were assumed to be about 300 nm long. Using a high-speed AFM, we confirmed that the DNA fragments were strings about

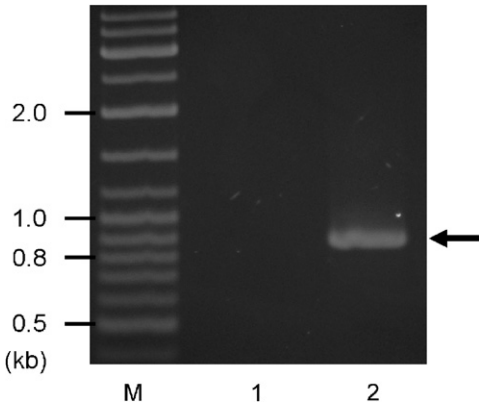

Fig. 1. Amplification of DNA fragment. A polymerase chain reaction (PCR) was carried out and the amplified DNA was resolved by gel analysis. The arrow indicates a PCR-amplified DNA fragment of about 0.9 kb pairs. M: size marker. 1: negative control without template DNA. 2: product.

300 nm long and 2 nm high (Fig. 2(a)). Unlike large DNA fragments such as a plasmid DNA (about 7 kb, 2.4 μm in length; data not shown), the 300 nm DNA fragments appeared to be moving fast under the conditions used here. This is probably due to the lower binding affinity of short DNA fragments with the mica surface. Conventional AFM has a scanning speed of 100 ms/line, making it difficult to obtain an image of moving molecules. The high scanning speed of the NVB500 AFM of 7 ms/line for a 1 fps scan has made it possible to obtain line images of moving DNA (Fig. 2).

3.2. Observation of biotinylated DNA–streptavidin complex

To avoid the formation of the complicated biotinylated DNA–streptavidin complex, only one end of the DNA was conjugated with biotin with which streptavidin can bind (Fig. 2(b)). We found several streptavidin proteins that appeared to bind to more than one DNA fragment (Fig. 2(c)). There were several strings of about 600 nm in length with a small dot in the center (Fig. 2(c), white arrows), which we assumed to be two DNA strands of about 300 nm connected by streptavidin. The relatively uniform heights of these dots (about 6 nm) suggest that they are streptavidin, not the result of the stacking of DNA strands. In some cases, three DNA fragments were joined by streptavidin (Fig. 2(c), yellow arrows). We rarely found streptavidin with four DNA fragments attached, which is consistent with a previous report [3].

3.3. Influence of a cantilever tip on DNA movement

Interaction between the cantilever tip and the sample is inevitable, and so we next tried to evaluate the influence of the tip on the movement of the DNA. The repulsion force between the tip and sample was roughly estimated to be around 100 pN based on the spring constant of the cantilever (0.1 N/m), the free amplitude of oscillation

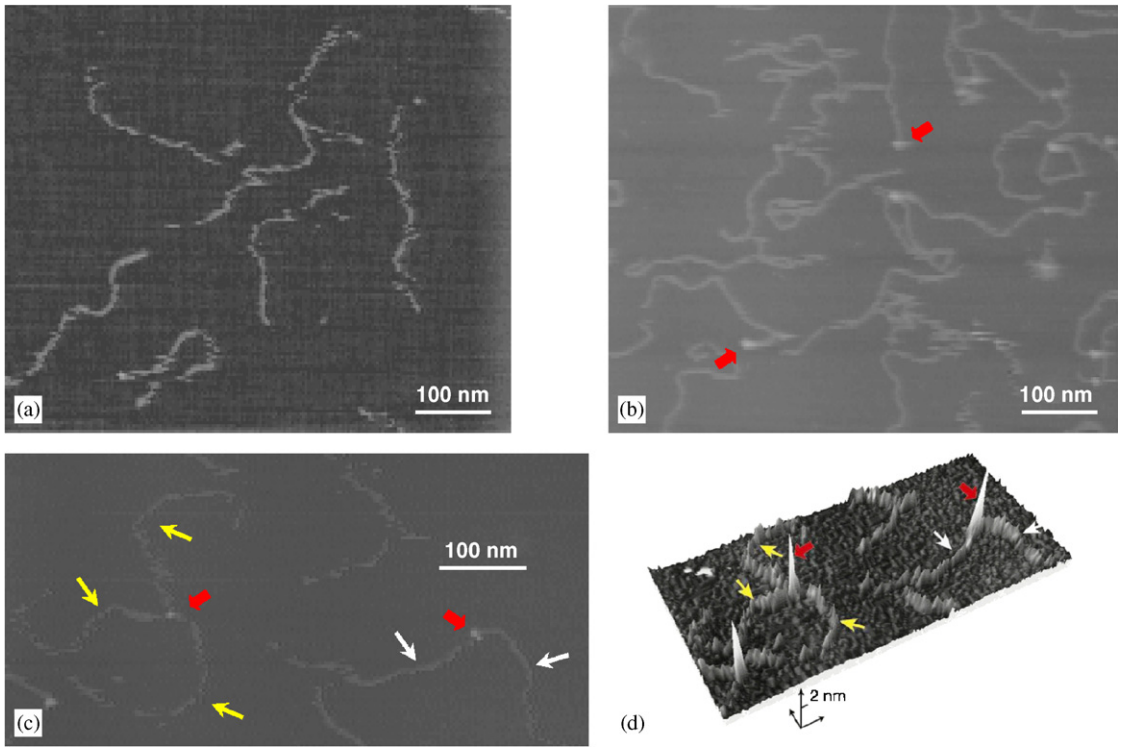

Fig. 2. Streptavidin proteins bound to one end of biotinylated DNA. AFM images of biotinylated DNA without (a) and with (b) streptavidin proteins. Red arrows indicate streptavidin proteins bound to the biotinylated end of 0.9-kb-long DNA fragments. In (c) and (d), two (white arrows) or three (yellow arrows) DNA strands of 300 nm apparently attached to streptavidin. The images were obtained using an Olympus NVB500 high-speed atomic force microscope at a scan rate of 1 fps. (d) is a 3D reconstructed image of (c).

(8 nm), the amplitude setpoint (6 nm), and a Q value of 2 [12]. To take a 144×196 pixel image of 400×500 nm at a scan rate of 1–7 fps, we calculated that the scanning speed of the tip was approximately 150–1000 μm/s. The high resonant frequency of the tapping means that the tip interacts with the sample once in every nanometer.

The same DNA–streptavidin complex was imaged at different scan rates for analysis. Eight successive images taken at rates of 1 and 6 fps were chosen and DNA strands were traced in each frame. They were represented in different colors, and merged images were obtained (Figs. 3(a) and (b)). Two DNA strands at the bottom (B and C in Figs. 3(a) and (b)) were relatively stable on the mica surface with an occasional flipping movement. On the other hand, the upper DNA strand (A in Figs. 3(a) and (b)) appeared relatively free to move with only one end attached to the stable streptavidin protein and/or the mica surface (Figs. 3(a) and (b)). When the movement of the free end of the DNA (A in Figs. 3(a) and (b)) was analyzed to compare the distance and direction of the movement in 1 s at different scan rates, it did not necessarily move faster at a faster scan rate, while the motion of the DNA along horizontal axis appeared to be greater at 6 fps (Fig. 3(c), Table 1). To obtain a better idea of the influence of the tip-sample interaction, we analyzed images of another DNA strand taken at rates of 1, 3 and 5 fps (Fig. 3(d), Table 1). We thought that the lateral force applied to the sample would be larger at a higher scan rate but the result showed only a slight increase, if any, in the motion at a higher scan rate (Table 1). The direction of the motion was apparently random. We observed no tendency for enhanced lateral movement that could be attributed to dragging by the scanning cantilever. The apparently larger lateral movement observed in Fig. 3(a) is probably because one end of the DNA was attached to streptavidin, which restricted the perpendicular movement of the DNA.

3.4. *Real-time imaging of biotinylated DNA dissociate from streptavidin*

To take advantage of the successful visualization of moving DNA fragments with the high-speed AFM, we tried to capture images of the binding/dissociation reaction of the biotinylated DNA to/from streptavidin. First, we were able to observe the DNA strands released from the protein (Fig. 4). We captured similar events several times, in which the DNA strands were released from a small white dot between them. In one complex, only one strand dissociated from the streptavidin indicating that the protein was not completely destroyed at the dissociation of the biotinylated DNA (data not shown). In every case, each of the two or three DNA strands was around 300 nm in length after dissociation, indicating that the strands were

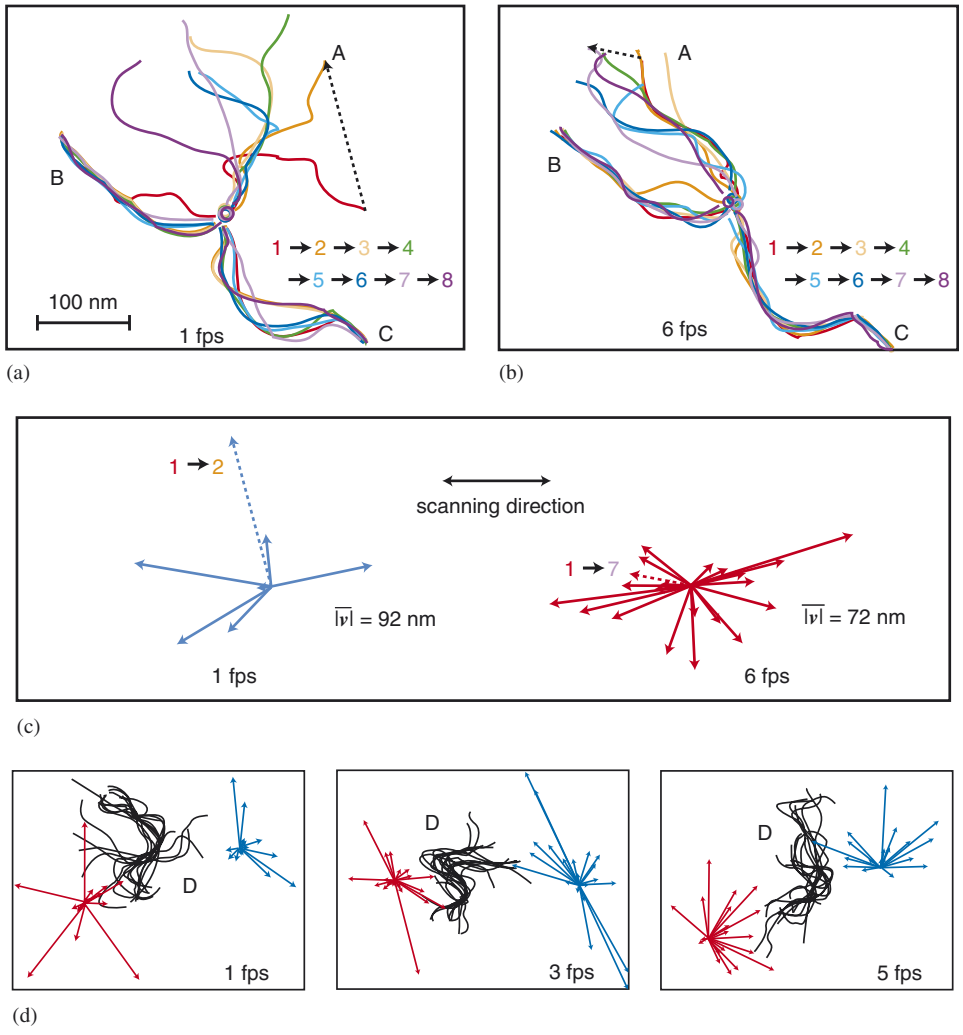

Fig. 3. Movement of DNA on mica. The same DNA–streptavidin complex was imaged at scan rates of 1 fps (a) and 6 fps (b). DNA strands were traced and depicted in different colors. The merged images are shown. The movement of one end of DNA strand A was analyzed, and the changes that occurred in 1 s were plotted (c). Images of another DNA strand D was taken at scanning rates of 1, 3, and 5 fps and the movement of the ends was analyzed (d). Red and blue arrows in (d) indicate the motion of each end of the DNA in 1 s.

Table 1
Analysis of the movement of DNA strands in 1 s in images taken at different scan rates

Scan rate (fps)	DNA strand A				DNA strand D			
	$\|V_x\|$ (nm/s)	$\|V_y\|$ (nm/s)	$\|V\|$ (nm/s)	n	$\|V_x\|$ (nm/s)	$\|V_y\|$ (nm/s)	$\|V\|$ (nm/s)	n
1	62.4	50.7	91.2	7	28.6	34.1	49.6	28
3	30.4	36.1	52.8	27	34.8	43.0	60.2	40
5	—	—	—	—	41.8	38.4	62.7	38
6	59.6	27.9	71.8	21	—	—	—	—

The same DNA (DNA strand A or D) was successively imaged at different scan rates and the motion in 1 s was analyzed. For example, the motion between frames 1 and 7, 2 and 8 etc. for a 6 fps movie were analyzed. The total frame numbers analyzed for each scan rate are indicated in the n column.

not cut in the middle of the nucleotide bonds but released from the streptavidin. The dissociation constant of streptavidin and biotin is reported to be around 10^{-13}–10^{-15} M [13]. The rupture force of the streptavidin–biotin complex is estimated to be approximately 5–170 pN depending on the loading rate [14–16], which is significantly less than the force of approximately 2–10 nN needed to rupture a covalent bond [17]. The roughly estimated repulsion force between the tip and the sample of around 60–120 pN further supports the idea that the dissociation occurred between biotinylated DNA and streptavidin.

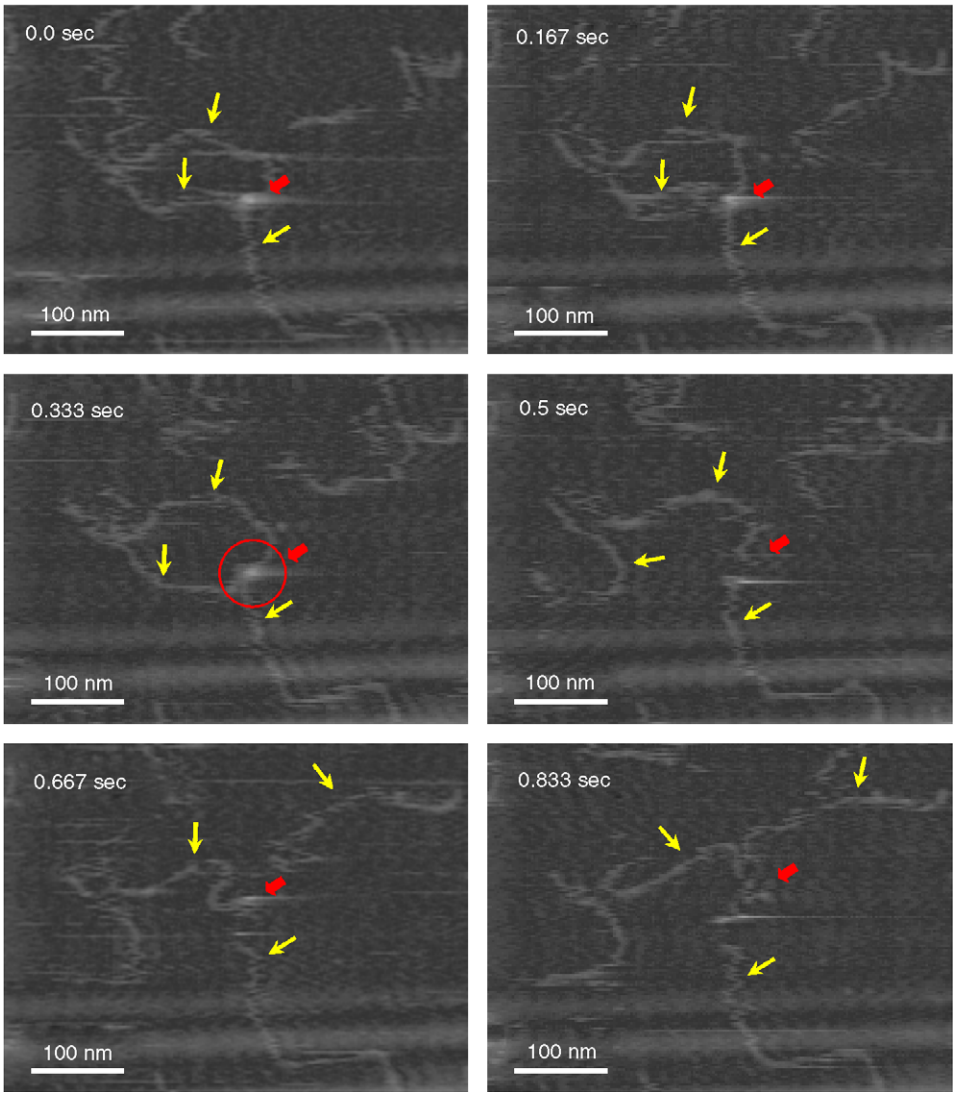

Fig. 4. Dissociation of DNA strands from streptavidin imaged with a high-speed AFM. The images were obtained at a rate of 6 fps (167 ms/frame). Three DNA strands attached to a streptavidin molecule were detached between 0.333 and 0.5 s. The red arrows indicate a streptavidin molecule (in the red circle), to which three DNA strands (yellow arrows) were attached.

3.5. Real-time imaging of biotinylated DNA bind to streptavidin

Although capturing a series of subsecond images of a molecule detaching from a DNA strand is itself interesting, the dissociation could have been the result of the tip–sample interaction. Therefore, we tried to capture images of a biotinylated DNA strand bind to streptavidin. We were able to capture real-time images of this reaction although it was rare (Fig. 5). In Fig. 5, the DNA strand in the top left (yellow arrow) at 0 s meets the streptavidin at the end of another biotinylated DNA in the middle (white arrow). A faster scanning rate of 7 fps resulted in rather rough images but it is clear that two DNA strands were bound at around 0.42 s, and they moved together as if the two strands were joined by streptavidin. It is unlikely that the two DNA strands were moving coincidentally at the same time.

4. Conclusion

We constructed a DNA fragment with the size of our choice and showed that a simple purification method is sufficient for achieving the purity needed for AFM observation. We showed that a moving short DNA strand can be observed with a high-speed AFM in solution. The high-speed AFM also enabled us to record 5–7 fps movies of biotinylated DNA binding to/dissociating from streptavidin protein. Without using a high-speed AFM, it would be impossible to capture images of DNA moving at a rate of 70–90 nm/s. Further improvements in feedback control and the development of a

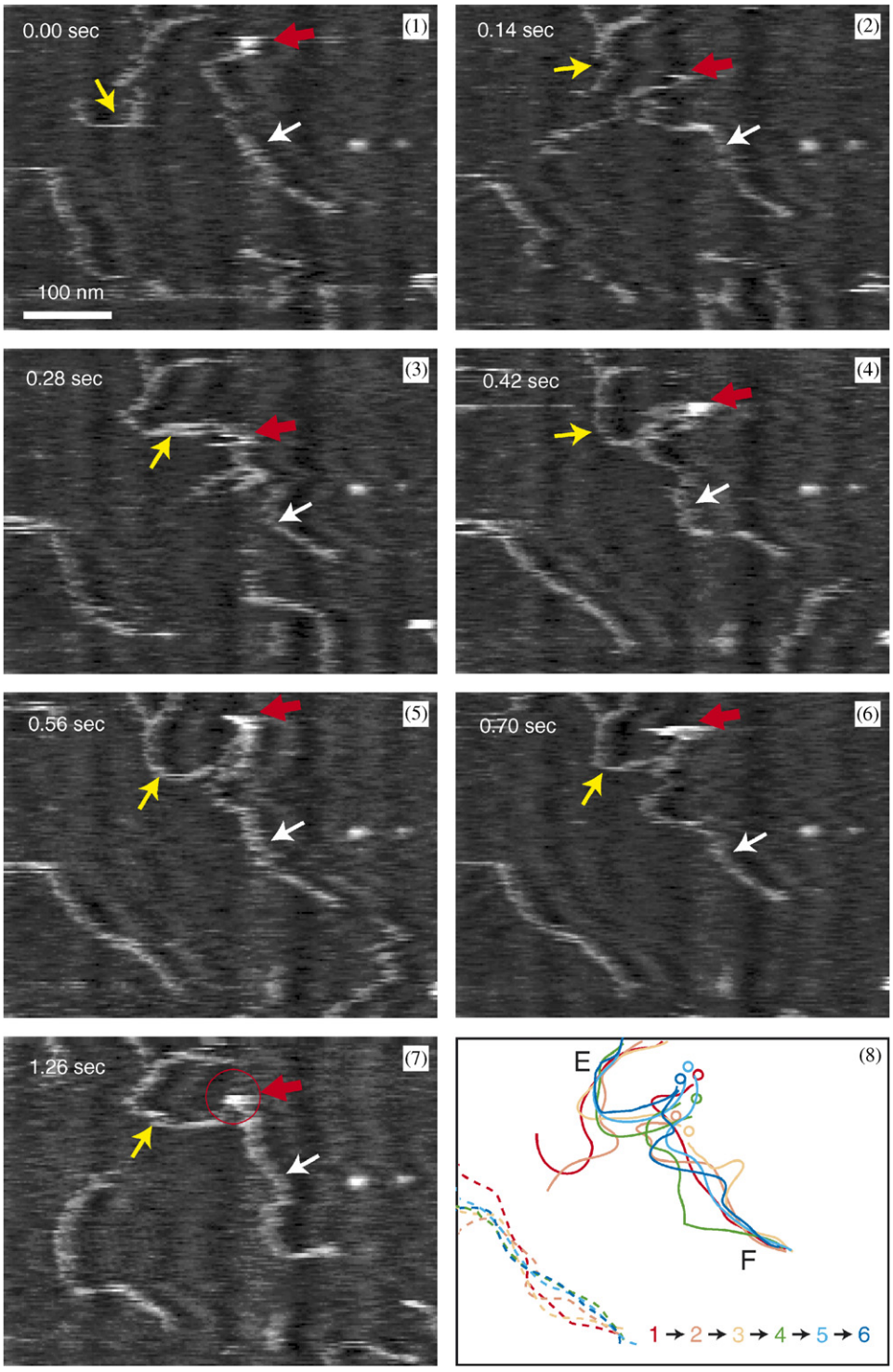

Fig. 5. Real-time image of biotinylated DNA bind to streptavidin. The images were obtained at a rate of 7 fps (143 ms/frame). The DNA strand in the top left (yellow arrow) at 0 s meets streptavidin (red arrow) at the end of another biotinylated DNA in the center (white arrow). A merged image of DNA strands and streptavidin depicted in different colors is shown bottom right.

smaller cantilever will improve the image quality. Our results show the potential of using a high-speed AFM to observe dynamic events on DNA strands. We hope to use the technique to analyze other biomolecular interactions.

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References

[1] E. Winfree, F. Liu, L.A. Wenzler, N.C. Seeman, Nature 394 (1998) 539.
[2] C. Mao, W. Sun, Z. Shen, N.C. Seeman, Nature 397 (1999) 144.
[3] C.M. Niemeyer, M. Adler, B. Pignataro, S. Lenhert, S. Gao, L. Chi, H. Fuchs, D. Blohm, Nucleic Acids Res. 27 (1999) 4553.
[4] S. Freitag, I. Le Trong, L. Klumb, P.S. Stayton, R.E. Stenkamp, Protein Sci 6 (1997) 1157.
[5] P.K. Hansma, J.P. Cleveland, M. Radmacher, D.A. Walters, P.E. Hillner, M. Bezanilla, M. Fritz, D. Vie, H.G. Hansma, Appl. Phys. Lett. 64 (1994) 1738.
[6] T. Ando, N. Kodera, E. Takai, D. Maruyama, K. Saito, A. Toda, Proc. Nat. Acad. Sci. USA 98 (2001) 12468.
[7] G. Schitter, G.E. Fantner, J.H. Kindt, P.J. Thurner, P.K. Hansma, in: Proceedings of the 2005 IEEE/ASME International Conference on Advanced Intelligent Mechatronics, Monterey, CA, USA, 2005, p. 261.
[8] A.D.L. Humphris, M.J. Miles, J.K. Hobbs, Appl. Phys. Lett. 86 (2005) 034106.
[9] A.G. Onaran, M. Balantekin, W. Lee, W.L. Hughes, B.A. Buchine, R.O. Guldiken, Z. Parlak, C.F. Quate, F.L. Degertekin, Rev. Sci. Instrum. 77 (2006) 023501.
[10] B.M. Forman, K. Umesono, J. Chen, R.M. Evans, Cell 81 (1995) 541.
[11] M. Kobayashi, S. Takezawa, K. Hara, R.T. Yu, Y. Umesono, K. Agata, M. Taniwaki, K. Yasuda, K. Umesono, Proc. Nat. Acad. Sci. USA 96 (1999) 4814.
[12] Q. Zhong, D. Inniss, K. Kjoller, V.B. Elings, Surf. Sci. Lett. 290 (1993) L688.
[13] N.M. Green, Adv. Protein Chem. 29 (1975) 85.
[14] R. Merkel, P. Nassoy, A. Leung, K. Ritchie, E. Evans, Nature 397 (1999) 50.
[15] S. Allen, S.M. Rigby-Singleton, H. Harris, M.C. Davies, P. O'Shea, Biochem. Soc. Trans. 31 (2003) 1052.
[16] A. Taninaka, T. Miyakoshi, K. Tanaka, O. Takeuchi, H. Shigekawa, in: Abstracts for the 13th International Conference on Scanning Tunneling Microscopy/Spectroscopy and Related Techniques, Sapporo, Japan, 2005, p. 345.
[17] M. Grandbois, M. Beyer, M. Rief, H. Clausen-Schaumann, H.E. Gaub, Science 283 (1999) 1727.