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Deposition and Characterization of Extended Single-Stranded DNA Molecules on Surfaces

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ABSTRACT

We have devised an approach for generating well-extended, surface deposited, single-stranded DNA molecules having minimal intramolecular base-pairing. This method depends on careful control of the mica surface charge, appropriate selection of the ionic strength of the DNA solution, and controlled translation of a droplet of DNA solution along the surface. We have shown the general applicability of this technique by elongating ΦX174, M13mp18, and λ single-stranded DNA on mica followed by imaging with atomic force microscopy. We have further demonstrated the feasibility of more elaborate, multistep surface preparations by aligning λ double-stranded DNA and ΦX174 single-stranded DNA in orthogonal directions on the same substrate.

The ability to manipulate and control molecules and materials with nanometer resolution is a central objective in the emerging discipline of nanotechnology. DNA is an attractive candidate for study in nanoscale positioning because of its exquisite specificity in base-pairing, ∼1 nm radius combined with large aspect ratio, and ready availability. We are interested in examining single-stranded (ss) DNA molecules on surfaces using atomic force microscopy (AFM) because of the nanometer spatial resolution afforded by AFM and the potential for specific hybridization of oligonucleotides to ssDNA. However, the ability of ssDNA to form base-pairs directly can also complicate surface deposition, since secondary structures can easily arise in these molecules. Herein, we demonstrate a simple technique for reproducibly elongating ssDNA molecules and eliminating intrastrand base-pairing during deposition onto mica surfaces. The ability to routinely create and visualize well-extended ssDNA should be a valuable tool for sequence analysis,[1−4] haplotyping,[5] probe microscopy tip characterization,[6] and biomolecular nanotechnology.[7−10]

While surface deposition and microscopy of double-stranded (ds) DNA is straightforward,[1−5,11] the ability to affix and evaluate native ssDNA reliably and without the complication of secondary structure has lagged significantly. Previous work in AFM of single-stranded nucleic acids can be characterized by one of two shortcomings: (1) insufficient reproducibility, as evidenced by the infrequent observation of elongated molecules without intrastrand base-pairing,[12−15] or (2) lack of generality of results, indicated by the detection of extended molecules only having specific sequences that do not favor formation of secondary structure.[16−19] While these earlier studies suggested the possibility of imaging

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extended ssDNA[12,13] and provided valuable insights into catalysis by single enzyme molecules,[17−19] they also underscored the need for more universal and robust methods for depositing and imaging well-extended ssDNA without the difficulty of intramolecular base-pairing.

Our approach for reproducibly generating surface-bound, elongated ssDNA[20] depends on three things. First, the cleaved mica surface is rendered positively charged and able to bind DNA by treatment with a dilute poly-L-lysine solution. Second, the ssDNA is maintained in a low ionic strength buffer solution lacking multivalent cations;[21] this reduces the electrostatic screening of negative charges on the DNA phosphate backbone and increases intrastrand electrostatic repulsion, making intramolecular base-pairing less favorable. Finally, a droplet of DNA solution is translated linearly across the mica surface;[22] the force exerted on ssDNA by a combination of droplet motion (fluid flow) and surface tension at the moving air−water interface is sufficient to elongate the molecules[23−26] and disrupt base-pairing between complementary sequences,[27] thus helping to eliminate secondary structure.

Appropriate choice of the concentration of poly-L-lysine for mica surface modification is critical to obtaining extended ssDNA reproducibly. With 100 ppm poly-L-lysine surface treatment, ssDNA are readily adsorbed, but not many are elongated. When using 10 ppm poly-L-lysine to modify surface charge, both ΦX174 and M13mp18 become well extended on the substrate, but λ molecules have only 1−3 μm segments (approximately the same length as ΦX174 or M13mp18) that are elongated, interspersed with other regions that are not aligned (see Supporting Information for sample images). With 1 ppm poly-L-lysine surface modification, all three types of ssDNA are nicely extended, although fewer molecules become affixed to the surface than with the 10 ppm poly-L-lys surface treatment. These results follow the trend that the highest concentration of poly-L-lys for surface modification that yields reproducible ssDNA alignment is inversely proportional to DNA fragment length (or charge, since the charge on DNA at this pH scales linearly with the number of bases). We believe this indicates that full extension of ssDNA typically occurs when the total number of surface-to-DNA electrostatic interaction points is below a threshold quantity during alignment. Hence, λ with ∼10 times more bases and negatively charged phosphate groups than ΦX174 can be completely elongated only when the number of positive charges on the surface (proportional to poly-L-lys concentration) is a factor of 10 lower than needed for similarly extending ΦX174. A detailed study of the relation between ssDNA fragment length and poly-L-lysine surface treatment concentration should elaborate more precisely the dependence between these two parameters and yield insight into the elongation mechanism. Based on these experiments, 10 ppm poly-L-lysine was adsorbed on the surface when aligning ΦX174 and M13mp18, while 1 ppm poly-L-lys was used for experiments with λ.

Representative tapping mode AFM height images[28] of ΦX174, M13mp18, and λ ssDNA deposited on mica as described previously are shown in Figure 1. The observed

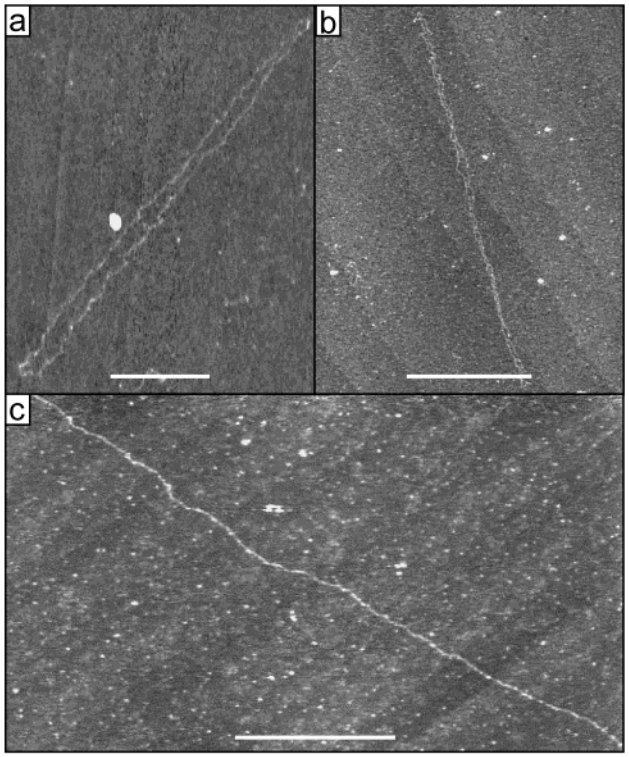

Figure 1. Tapping mode AFM height images of extended ssDNA molecules on mica surfaces. (a) Image of ΦX174; height scale is 0.8 nm and the white bar indicates 250 nm. (b) Image of M13mp18; height scale is 0.8 nm and the white bar represents 500 nm. (c) Image of a segment of a λ ssDNA molecule; height scale is 1.0 nm and the white bar denotes 500 nm.

heights (mean ± standard deviation) of the DNA molecules in Figure 1 are 0.28 ± 0.08 nm for ΦX174, 0.28 ± 0.07 nm for M13mp18, and 0.37 ± 0.07 nm for λ. Measured heights of extended ssDNA in different scans ranged from 0.25 to 0.40 nm, depending on imaging conditions and the tip used. The DNA contour lengths in Figure 1a and b are 2200 nm for ΦX174 and 3000 nm for M13mp18; detailed measurements on six molecules of ΦX174 and six molecules of M13mp18 revealed contour lengths (mean ± standard deviation) of 2160 ± 160 nm and 2800 ± 260 nm, respectively.[29−31] Contour lengths for λ ssDNA were not measured because they were expected and observed to exceed the range of the scanner (15 μm).

We deposited ssDNA samples multiple times on different days and observed more than 200 molecules of both ΦX174 and M13mp18 by AFM. We found that nearly 60% of the circular ΦX174 molecules were fully extended as in Figure 1a, while almost 50% of the closed loop M13mp18 molecules were completely extended like the one in Figure 1b. In addition, another 20% of the ΦX174 and 35% of the M13mp18 molecules were mostly extended, typically having small regions where the filament looped back on itself (see Supporting Information for sample images). The full contour length of many of these mostly elongated molecules could also be traced; hence, the percentage of surface ssDNA that would be usable in sequence analysis,[1−5] for example, would be at least 70% for both ΦX174 and M13mp18. Although an assessment of the entire contour length of extended λ

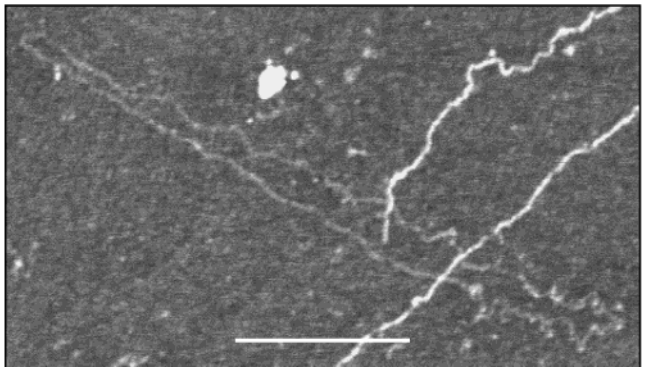

Figure 2. Tapping mode AFM height image of a mica surface with ΦX174 ssDNA (loop extending from top left to bottom right) elongated in one direction and two λ dsDNA strands (features running from the top right down and to the left) aligned perpendicularly. Height scale is 1.4 nm and the white bar is 250 nm.

ssDNA molecules was not feasible with the scanner used, images of various segments of different molecules did not reveal any secondary structure (see Supporting Information). Thus, we expect that it should be feasible to perform sequence analysis experiments on ssDNA molecules at least the size of λ (48.5 kb) if a larger range scanner is employed.

In addition to being able to reproducibly deposit extended ssDNA molecules on mica, it would be highly desirable to have the capacity to perform more sophisticated, multistep sample manipulations on surfaces. To this end we have developed methods for controlled deposition of a first DNA sample in one direction, followed by alignment of a different DNA sample in another direction on the same surface. We made multiple samples over the course of several days with λ dsDNA aligned in one direction and ΦX174 ssDNA extended orthogonally.[32,33] These surfaces were profiled using AFM, and Figure 2 displays a representative image of one sample. Molecules of λ dsDNA are visible as brighter features heading down from the right, while ΦX174 ssDNA is seen as a dimmer, loop-like structure extending from the top left toward the bottom right. Other images both on this and different samples also showed similar surface DNA alignment patterns (see Supporting Information).

We measured the heights (mean ± standard deviation) of ΦX174 and λ in Figure 2 to be 0.37 ± 0.07 nm and 0.8 ± 0.1 nm, respectively. These height data agree well, both with earlier AFM studies of dsDNA,[34] and the expectation that dsDNA should have a height (diameter) approximately twice that of ssDNA. Indeed, additional images of this and other samples all had dsDNA heights about two times as large as those of ssDNA. Our results also indicate the robustness of the attachment of aligned DNA toward additional surface treatments and open the possibility of carrying out multistep chemical manipulations on these surfaces.

In conclusion, we have developed a technique for facile and reliable deposition and AFM characterization of well-extended ssDNA molecules. This approach not only is generally applicable to ssDNA strands having different sequences but also reproducibly provides surfaces with high yields of extended ssDNA molecules without intrastrand base-pairing. Moreover, the attachment of elongated DNA is sufficiently stable that additional processing steps can be performed after deposition to generate complex arrays of surface-bound nucleic acids. We envision that these methods will be of broad practical applicability in a variety of experiments. For example, although direct reading of nucleic acid base sequence on ssDNA using an AFM tip may not be feasible at present, hybridization of different oligonucleotide probes to diagnostic sequences on elongated ssDNA molecules on surfaces would simplify sample preparation for sequence analysis[1−4] or haplotyping[5] by AFM. In addition, the continued drive to reduce AFM tip radii with carbon nanotubes[35,36] heightens the need for smaller standard structures, such as extended ssDNA lacking secondary structure, to characterize tip size. Finally, the availability of methods for controlled manipulation of ∼1 nm diameter ssDNA provides intriguing possibilities for nanofabrication.

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Supporting Information Available: Additional, full-color AFM images of well-extended ssDNA (ΦX174, M13mp18 and λ), incompletely extended λ ssDNA, and orthogonally deposited ΦX174 ssDNA and λ dsDNA. This material is available free of charge via the Internet at http://pubs.acs.org.

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(20) 1, 10, or 100 ppm poly-L-lysine in water was made from a 0.1% aqueous solution (Ted Pella, Inc., Redding, CA), and 25 μL was deposited onto freshly cleaved, 0.5 in. diameter circular mica substrates for ∼5 min; the surface was then rinsed with water and dried under a stream of compressed air. Next, a 1 μL droplet of ssDNA in 10 mM Tris, 1 mM EDTA, pH 8.0 (TE buffer) was moved in a linear direction across the surface at a velocity of ∼0.5 mm/s using a 3-axis translation stage under ambient humidity conditions, in an adaptation of a reported procedure for aligning dsDNA on surfaces.[22] During DNA solution translation, a rapidly evaporating (<3 s) film of liquid trailed 1−4 mm behind the moving droplet; after the solution had evaporated the mica was again rinsed with water and dried with compressed air prior to imaging. We did not attempt to study the effect of relative humidity on ssDNA deposition and alignment, but small, day-to-day variations in ambient humidity did not appear to influence our data. Moreover, no significant change in DNA deposition and alignment was observed with poly-L-lys surface incubation times in the range of 2−8 min, or droplet translation velocities varying between 0.2 and 1.0 mm/s, although we did not perform a systematic study of these variables.
(21) DNA was obtained from New England Biolabs (Beverly, MA) and diluted to either 2 ng/μL (ΦX174 and M13mp18) or 5 ng/μL (λ) in TE buffer. Higher concentrations of DNA led to a larger number of molecules deposited on the mica substrate. Prior to surface deposition, M13mp18 ssDNA was heated to 95 °C for 2 min to disrupt secondary structure, and λ ssDNA was made by denaturing dsDNA at 95 °C for 5 min. Elongated ssDNA on surfaces could also be generated from solutions where the TE buffer was as dilute as 100 μM Tris, 10 μM EDTA.
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(23) The surface tension force on a rod of diameter D can be approximated by $F = \gamma\pi D$, where $\gamma = 7 \times 10^{-2}$ N/m for an air−water interface; assuming $D = 1$ nm for ssDNA, $F = 200$ pN.[24] However, studies with optical tweezers on the extension of ssDNA as a function of force[25] indicate that an elongation force of ∼10 pN is consistent with the contour lengths we observed. This value is closer in magnitude to the force exerted on DNA in a fluid flow,[26] so ssDNA extension and alignment may be occurring mostly by this mechanism rather than surface tension.
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(28) Images were obtained using a Multimode IIIa AFM and microfabricated Si cantilever-tips (Digital Instruments, Santa Barbara, CA). Vibrational noise was damped with an active isolation system (MOD1-M, Halcyonics, Goettingen, Germany). Typical imaging parameters were (i) resonant frequencies, 60−90 kHz; (ii) free oscillation amplitude, 0.5−1.0 V; (iii) setpoint, 0.3−0.7 V; (iv) scan rate, 1.5−2.0 Hz. Images were processed offline by flattening to remove the background slope.
(29) ΦX174, M13mp18, and λ are 5386, 7249, and 48502 bases long, respectively. The contour lengths we measured correspond to 0.40 nm/base in ΦX174 and 0.39 nm/base in M13mp18; these results correlate well with recent transient electric birefringence measurements that indicate a spacing of 0.32−0.52 nm/base for ssDNA, depending on sequence.[30] Earlier electron microscopy (EM) of ssDNA mixed with cytochrome C showed contour lengths consistent with a spacing of 0.32 nm/base.[31] The difference between the EM values and ours may result either from protein−DNA interactions present in the EM work or from DNA stretching[24−26] during deposition in our experiments. It should be noted that in applications such as sequence analysis the absolute value of the base spacing is not critical since relative distances are typically calculated.[2,5]
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(32) Orthogonally aligned DNA samples were prepared by treating freshly cleaved mica with 25 μL of 1 ppm aqueous poly-L-lysine, rinsing with water, and drying under a stream of compressed air. Next, a 1 μL droplet of λ dsDNA in TE buffer was moved linearly across the surface using a 3-axis translation stage as described above.[20] DNA molecules have been reported to align parallel to the direction of liquid motion,[3,26] so the droplet trajectory on the surface was marked as a reference for the subsequent alignment step. The mica was rinsed with water, dried with compressed air, treated with 25 μL of 10 ppm poly-L-lysine, rinsed again with water, and dried with compressed air. A 1 μL droplet of ΦX174 ssDNA in TE buffer was then moved across the surface as described above, except that the direction of translation was chosen to align ΦX174 perpendicular to λ. Last, the mica was rinsed with water and dried with compressed air prior to imaging.
(33) The order of deposition (ds before ssDNA) was critical because 1 ppm poly-L-lysine was the highest concentration surface treatment for which λ could be reproducibly aligned. Experiments done in the reverse order showed that the ssDNA attachment was also stable to further surface manipulations; however, the surface preparation for ΦX174 (10 ppm poly-L-lysine) bound λ dsDNA too tightly for alignment in the second step.
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