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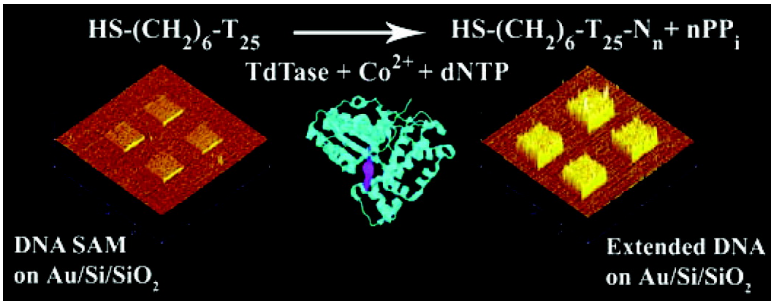
J. Am. Chem. Soc., **2005**, 127 (41), 14122-14123• DOI: 10.1021/ja052491z • Publication Date (Web): 27 September 2005



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Published on Web 09/27/2005

Enzymatic Fabrication of DNA Nanostructures: Extension of a Self-assembled Oligonucleotide Monolayer on Gold Arrays

Dominic C. Chow,[†,§] Woo-Kyung Lee,[‡,§] Stefan Zauscher,[‡,§] and Ashutosh Chilkoti*[,†,§]

Department of Biomedical Engineering, Duke University, Box 90281, Durham, North Carolina 27708-0281, Department of Mechanical Engineering and Materials Science, Duke University, Box 90300, Durham, North Carolina 27708-0300, and Center for Biologically Inspired Materials and Materials Systems, Duke University, Box 90303, Durham, North Carolina 27709−0303

Received April 16, 2005; E-mail: chilkoti@duke.edu

Bionanofabrication requires the development of an ensemble of tools to move, excavate, and build with biomolecules on surfaces with nanoscale precision in an aqueous environment and under ambient conditions. We have focused on the enzymatic manipulation of DNA as a fundamental approach in our ongoing effort to develop these tools. We recently demonstrated a method to excavate DNA at the mesoscale using an adsorbed enzyme.[1] In this approach, the enzyme DNase I was deposited locally on a homogeneous layer of an oligonucleotide substrate and activated to digest the substrate locally with nanoscale precision. This approach resulted in DNA nanotrenches that are biochemically carved into the surface by the enzyme. Here we demonstrate an inverse approach to build up vertical DNA nanostructures from an oligonucleotide "initiator" that is nanopatterned on a surface, using the enzyme, terminal deoxynucleotidyl transferase (TdTase) that freely diffuses in solution (Figure 1A). We chose TdTase, which repetitively adds mononucleotides to the 3′ end of a single- or double-stranded DNA,[2,3] as the enzyme to extend DNA because, unlike DNA polymerases, TdTase can directly catalyze the 5′ to 3′ extension of a short oligonucleotide template without the need for a separate DNA primer.[2] Although there is a large body of literature on the extension of DNA in solution by TdTase,[4] the ability of TdTase to catalyze the surface initiated polymerization of DNA has not been previously described and exploited in growing DNA from a surface or for the in situ fabrication of DNA nanostructures from a surface.

We tested the feasibility of our approach by monitoring the rate and the extent of surface-initiated DNA polymerization with surface plasmon resonance (SPR) (Biacore, Piscataway, NJ). For these experiments, a self-assembled monolayer (SAM) of an oligonucleotide thiol ($5'SH-(CH_2)_6-T_{25}$, Integrated DNA Technologies, Skokie, IL) was prepared on gold-coated sensor chips (Biacore) by incubation with 10 μM DNA-thiol solution in phosphate-buffered saline (PBS) overnight (Figure 1A). The samples were then sonicated in PBS for 5 min to remove residual physisorbed DNA-thiol, rinsed with PBS and water, and dried in a stream of nitrogen. The thickness of the $5'SH-(CH_2)_6-T_{25}$ SAM measured by ellipsometry was ~1 nm.

Enzymatic DNA extension was performed by incubating the DNA-SAM with TdTase (Promega, Madison, WI) at 0.6 U/μL in the presence of cobalt-containing buffer and 1 mM of mononucleotide (dATP, dTTP, dCTP or dGTP) (Promega, Madison, WI) at 37 °C (Figure 1A and Supporting Information). All reactions reached a plateau within ~1 h. We found that the extent of surface-initiated DNA polymerization depended on the type of mononucleotides used (dTTP > dCTP > dATP > dGTP) (Figure 1B). Among the four nucleotides tested, dTTP showed the greatest extension and yielded 895 ng/cm^2 of polynucleotides within 1 h on the surface (after subtraction of the SPR response of the control, based on 1RU = 100 pg bound analyte/cm^2). Incubation overnight did not further increase the extent of DNA extension (data not shown).

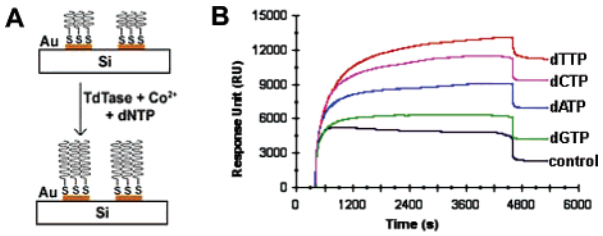

Figure 1. (A) Cartoon of surface-initiated growth of DNA by TdTase from gold substrates that present a SAM of an oligonucleotide-terminated thiol. (B) Kinetics of TdTase-catalyzed DNA extension measured by SPR for 4 different nucleotides. As a control, the same reaction was performed without the addition of nucleotides to account for SPR signal generated by buffer exchange and nonspecific binding of reaction components to the surface. Each reaction was repeated 3 times, and representative curves are shown.

After verifying that TdTase catalyzes the surface-initiated polymerization of nucleotides from an immobilized oligonucleotide "initiator", we examined the feasibility of in situ fabrication of DNA nanostructures from a surface. Gold patterns with lateral size ranging from 100 nm to 4 μm were fabricated on silicon (Si; Montco Silicon Technologies, Spring-City, PA) substrates by e-beam lift-off lithography[5] (see Supporting Information). The DNA-SAM was prepared on the patterned gold islands following the procedure described above. We used a homogeneous DNA-SAM because it yielded the fastest DNA growth, likely due to its high surface packing density. Experiments with lower concentrations of $5'SH-(CH_2)_6-T_{25}$ and with mixed SAMs of DNA-thiol and $SH-(CH_2)_{11}(OCH_2CH_2)_3-OH$ resulted in less extensive DNA polymerization (data not shown). To achieve complete polymerization, the DNA extension reaction was carried out at 37 °C for 2 h in a humidified incubator with the same concentrations of TdTase, cobalt-containing buffer, and dTTP used in the SPR measurements. The samples were rinsed thoroughly with PBS and water and dried in a stream of nitrogen. The DNA nanostructures were imaged by tapping mode AFM (MultiMode AFM, Veeco Instruments, Woodbury, New York) in air using Si cantilevers with a force constant of about 42 N/m.

As shown in Figure 2A and C, poly(T) nanostructures were successfully grown on gold arrays with vastly different lateral feature sizes. We can make several significant observations from these experiments: (1) The average heights of the DNA nanostructures on gold features of 100 nm and 4 μm lateral feature size were 45.5 ± 5.2 nm and 120.7 ± 9.3 nm (after subtracting the average height of the gold islands and immobilized DNA-SAM), respec-

[†] Department of Biomedical Engineering.
[‡] Department of Mechanical Engineering and Materials Science.
[§] Center for Biologically Inspired Materials and Materials Systems.

10.1021/ja052491z CCC: $30.25

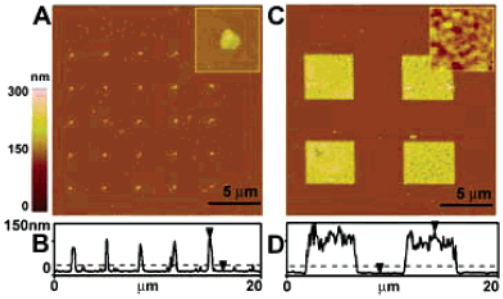

Figure 2. Tapping mode AFM images in air for gold arrays of different feature sizes (A−B: 0.1 μm. C−D: 4 μm) with 5′-SH−$(CH_2)_6$−T_{25} SAM after a 2 h incubation with TdTase. Insets are the images of A and C at a higher magnification (1 μm × 1 μm). B and D are the line profiles of A and C. Dotted lines represent the average height of gold arrays and immobilized DNA-SAM.

tively (Figure 2B and D). The significant height increase suggests that the TdTase-catalyzed surface-initiated polymerization of dTTP was successful. (2) The height of the DNA nanostructures grown by TdTase is inversely dependent upon the lateral feature size of the underlying gold patterns; i.e., a small feature size yields a small height, under otherwise identical reaction conditions. This behavior is likely related to decreased conformational constraint of DNA chains as the feature size becomes smaller, which causes a decrease in the average height of patterned DNA.[5] In addition, the increased conformational mobility of DNA chains may cause reduced accessibility of TdTase and mononucleotides to the 3′ ends of the growing chains, which ultimately could lead to lower molecular weights and heights.[6] (3) AFM images at a higher magnification showed that significant lateral growth occurred; e.g., DNA nanostructures grown on 100 nm gold squares had a lateral feature size of 358.5 ± 10.0 nm (full width at half-maximum, Figure 2A inset), likely due to surface-initiated growth from the vertical sides of the gold islands. (4) DNA growth appears to be heterogeneous and resulted in a layer of DNA with RMS roughness of 13.6 ± 0.6 nm on the 4 μm gold features (Figure 2C inset, vs 0.23 ± 0.02 nm for gold substrates). Unfortunately, the polydispersity of the grafted DNA layers could not be determined with reasonable accuracy due to the limited amount of extended DNA and the repetitive nature of the DNA sequence, precluding the use of common DNA detection and amplification techniques.

As a control, enzymatic DNA extension was also performed for 2 h at 37 °C using TdTase that had been heat-inactivated at 70 °C for 10 min (Figure 3A and D(i), 35.9 ± 0.4 nm). The complete lack of DNA extension in this control experiment confirmed that the height increase after the addition of the active enzyme was not due to irreversible aggregation of TdTase preferentially on the gold arrays. In contrast, the addition of active TdTase resulted in a height increase of 103.5 ± 4.4 nm compared to the DNA-SAM on gold (Figures 3B and D(ii). To ensure that the nanostructures were indeed composed of DNA, we performed an enzymatic digestion with exonuclease I (New England Biolabs, Beverly, MA), which removes nucleotides from single-stranded DNA in the 3′ to 5′ direction.[7] After overnight incubation at 37 °C with the enzyme, the surface was washed with PBS and water, dried in a stream of nitrogen, and imaged by tapping mode AFM in air. The partial enzymatic digestion decreased the initial height of 139.4 ± 4.0 nm by 48.3 ± 2.4 nm (Figures 3C and D(iii)) and, thus, provides direct evidence for the presence of extended DNA.

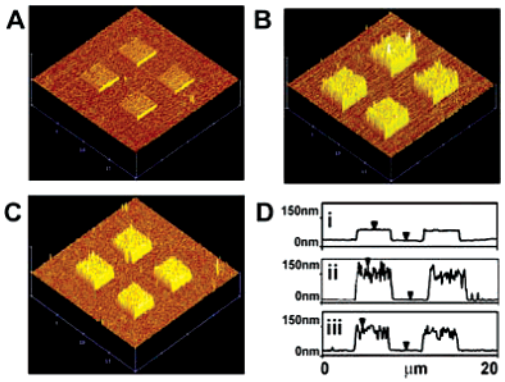

Figure 3. Tapping mode AFM images in air for the gold arrays with 5′-SH−$(CH_2)_6$−T_{25} DNA-SAM, after treated with heat-inactivated TdTase (A), active TdTase (B), and active TdTase followed by exonuclease I (C). The line profiles of these figures are showed in D.

In conclusion, we have shown that the enzymatic extension of a nanopatterned oligonucleotide template by TdTase, a form of surface-initiated enzymatic polymerization, is useful for the fabrication of DNA nanostructures with heights of up to 121 nm in 2 h, which is similar to the rate of chemical surface-initiated polymerization. We believe that TdTase-catalyzed surface-initiated polymerization of DNA will be a useful tool for the fabrication of complex biomolecular structures with nanoscale resolution. We further suggest that the nanoscale enzymatic homopolymerization of dTTP demonstrated here can be extended to the in situ synthesis of more complex block copolymer architectures of specific oligo-A, T, C, or G blocks and potentially of unnatural nucleotides. This prospect offers interesting possibilities for bionanofabrication. For example, DNA block copolymer structures can be designed to function as programmable scaffolds for selective docking of molecules and nanostructures along the *z*-direction with nanometer-level precision by using sequence-specific DNA hybridization or other molecular recognition motifs that are incorporated into the polymerized DNA nanostructures. In addition, this method can be combined with other nanofabrication methods such as dip-pen nanolithography[8,9] to individually manipulate DNA nanostructures in a massively parallel fashion.

Acknowledgment. We acknowledge the financial support of the National Science Foundation through a NSF NIRT Grant EEC-0210590.

Supporting Information Available: Experimental details for SPR measurements and gold array fabrication. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA052491Z