## Direct Patterning of Modified Oligonucleotides on Metals and Insulators by Dip-Pen Nanolithography

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The use of direct-write dip-pen nanolithography (DPN) to generate covalently anchored, nanoscale patterns of oligonucleotides on both metallic and insulating substrates is described. Modification of DNA with hexanethiol groups allowed patterning on gold, and oligonucleotides bearing 5'-terminal acrylamide groups could be patterned on derivatized silica. Feature sizes ranging from many micrometers to less than 100 nanometers were achieved, and the resulting patterns exhibited the sequence-specific binding properties of the DNA from which they were composed. The patterns can be used to direct the assembly of individual oligonucleotide-modified particles on a surface, and the deposition of multiple DNA sequences in a single array is demonstrated.

Direct-write DPN (1-3) involves the transfer of an ink directly from a coated atomic force microscope (AFM) tip to a substrate of interest. The ability to directly pattern oligonucleotides using DPN or related techniques is of great importance to realizing the potential of biomolecules in materials applications (4, 5). Indirect, multistep methods (6-8), although offering important opportunities, invite crosscontamination and are not readily scaled to the multi-pen, multi-ink approaches that can be achieved with direct-write methods. In addition, with a few exceptions (9, 10), DPN techniques have been limited to patterning on gold substrates, which are in many cases undesirable from the standpoint of electronic and optical materials applications.

Here, we show how DPN can be used to pattern oligonucleotides routinely on gold and silicon oxide surfaces. We have identified several key factors that facilitate DNA patterning. First, the AFM tip must be well coated with DNA. Although unmodified silicon nitride cantilevers have been used to deposit a variety of hydrophobic molecules by DPN, we have observed that such cantilevers often yield DNA patterns with feature sizes and shapes that are not easily controlled (11). Improved control over DNA patterning can be achieved through surface modification of a silicon nitride AFM cantilever with 3'aminopropyltrimethoxysilane (for 1 hour, in a 1% v/v solution in toluene), which promotes reliable adhesion of the DNA ink to the tip surface.

We coated the silanized tips with DNA by dipping them for 10 s into a 90% dimethylformamide/10% water solution containing 1 mM DNA and 0.3 M MgCl<sub>2</sub> and then blew the tips dry with compressed difluoroethane. The positively charged hydrophilic tip surfaces (12) were readily wetted by this DNA ink solution, and these AFM tips could be used in DPN experiments for several hours before they needed to be recoated. Furthermore, we have found that control of ambient humidity enables reliable DPN patterning of oligonucleotides. Unless specifically noted, all patterning was performed in an environmentally controlled glovebox at a relative humidity of  $45 \pm 5\%$  at 23  $\pm 3^{\circ}$ C.

The judicious choice of an ink-substrate combination also facilitates the DPN process. In these studies, we have used hexanethiolmodified oligonucleotides to directly pattern gold substrates with features ranging from 50 nm to several micrometers in size (Fig. 1). It is likely that the hexanethiol group of the DNA chemisorbs to the underlying Au surface (13). After the substrates were patterned with oligonucleotides, they were immersed in an ethanol solution of 1-octadecanethiol (ODT, 1 mM) for 1 min. This procedure coats the unpatterned gold surface with a hydrophobic monolayer, passivating it toward the nonspecific adsorption of DNA, or DNAmodified nanoparticles, in subsequent hybridization experiments. After the ODT treatment of the substrate, the oligonucleotide patterns were imaged by tapping-mode AFM and exhibited feature heights of 2 to 5 nm (Fig. 1A) (14). The immobilized DNA retained its highly specific recognition properties, and the patterns could be used to direct the assembly of 13-nm-diameter oligonucleotide-modified gold nanoparticles (Fig. 1B). Structures can be fabricated by this process on the many micrometer to sub-100-nmlength scale, and we could place individual particles on a surface in the form of a preconceived architecture (Fig. 1B). The interactions between the DNA nanopatterns and the oligonucleotide-modified nanoparticles were highly selective; in the absence of a complementary linking strand, there was almost no nonspecific binding (see fig. S1).

Although the gold-thiol system provides an easy method for patterning oligonucleotides using DPN, the electrical conductivity of the gold substrate prevents the study of charge transport and near-field optical phenomena in nanostructures assembled on such surfaces and also quenches the emission from any surface-bound fluorophores. Thus, we have developed DPN methods to generate patterns of DNA on oxidized silicon wafers (Fig. 2). The surface of a thermally oxidized wafer was activated by treatment with 3'mercaptopropyltrimethoxysilane (MPTMS) (15). The preparation and inking of the AFM tip were performed exactly as for the patterning of



Fig. 1. Direct transfer of DNA onto gold substrates by DPN. (A) Tapping-mode AFM image of hexanethiol-modified oligonucleotides patterned on polycrystalline gold. The scale bar represents 2  $\mu$ m, and the space between the arrows is 150 nm. (B) Tapping-mode AFM image of single oligonucleotide-modified gold nanoparticles (13-nm diameter) bound to a high-resolution DNA line on gold by Watson-Crick base pairing in the presence of complementary linking DNA. The scale bar represents 1  $\mu$ m.

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DNA onto gold surfaces, but in this case, oligonucleotides with 5'-terminal acrylamide groups were used (16). Under the DPN conditions of room temperature and 45% relative humidity, the acrylamide moieties react by Michael addition with the pendant thiol groups of the MPTMS to covalently link the DNA to the surface. After patterning, the substrate was passivated by reaction with buffered acrylic acid monomer at pH 10 (Apogent Discoveries Quench Solution, 30 min.). The biological activity of the patterned oligonucleotides was verified by exposing the surface to a solution containing both complementary and noncomplementary fluorophore-labeled DNA. The patterns were subsequently characterized by epifluorescence microscopy (Fig. 2A). In all cases, only fluorescence corresponding to the complementary target and the patterned area was detected. The same DNA nanostructures [after dehybridization of the single-stranded complement by rinsing with deionized (DI) water] could be used to direct the assembly of complementary DNA-modified gold nanoparticles (Fig. 2B). With this technique, we generated and detected DNA spots with diameters of ~50 nm, nearly 160,000 times





Fig. 2. Direct DPN transfer of DNA onto insulating substrates. (A) Epifluorescence micrograph of fluorophore-labeled DNA (Oregon Green 488-X) hybridized to a DPN-generated pattern of complementary oligonucleotides on an SiO<sub>x</sub> surface. The scale bar represents 12  $\mu$ m. (B) Tapping-mode AFM image of oligonucleotide-modified gold nanoparticles (13-nm diameter) hybridized to a second, high-resolution pattern after removal (using DI water) of the fluorophore-labeled DNA. The scale bar represents 1.5  $\mu$ m, and the space between the arrows is 100 nm.

smaller (in terms of areal density) than those in conventional microarrays.

An important feature of DPN is the ability to generate patterns of specific chemical functionality over a large range of length scales while exhibiting control over feature size. Surprisingly, we found that patterns of highly charged macromolecules such as oligonucleotides can be transferred to a substrate in much the same way as can small hydrophobic molecules. On both gold and MPTMS-modified silicon oxide substrates, the transport of DNA from the AFM tip to the surface followed the same linear increase in pattern area with contact time predicted theoretically (17), as well as observed for alkanethiols on gold (18) and for silazanes on silicon and gallium arsenide (9), (Fig. 3, A and B). Although the rate constants are different for each ink-substrate pair, this result underscores the control DPN offers for patterning compounds ranging from small mol-



**Fig. 3.** DPN control over deposited feature size. **(A)** Tapping-mode AFM image of thiol-modified DNA spotted on a gold substrate for different contact times at 45% relative humidity (top) and plot of dot diameter versus square root of contact time (bottom). **(B)** Tapping-mode AFM image of nanoparticles hybridized to DNA spots formed on SiO<sub>x</sub> for different contact times at 45% relative humidity (top) and plot of dot diameter versus square root of contact time (bottom). **(B)** Tapping-mode AFM image of nanoparticles hybridized to DNA spots formed on SiO<sub>x</sub> for different contact times at 45% relative humidity (top) and plot of dot diameter versus square root of contact time (bottom). The scale bars for (A) and (B) represent 2  $\mu$ m. **(C)** Tapping-mode AFM image of DNA spots generated on polycrystalline Au with a contact time of 10 s per spot, at varying relative humidity (top), and a plot of spot diameter versus relative humidity (bottom). The scale bar represents 1  $\mu$ m. Error bars for all plots were calculated from the standard deviation of at least five points.

**Fig. 4.** Direct patterning of multiple-DNA inks by DPN. **(A)** Combined red-green epifluorescence image of two different fluorophore-labeled sequences (Oregon Green 488-X and Texas Red-X) simultaneously hybridized to a two-sequence array deposited on an SiO<sub>x</sub> substrate by DPN. **(B)** Tapping-mode AFM image of 5 (dark)- and 13 (light)-nm-diameter gold nanoparticles assembled on the same pattern after dehybridization of the



fluorophore-labeled DNA. The scale bars represent 4  $\mu$ m. (C) The line plot was taken diagonally through both nanoparticle patterns, and the start and finish are indicated by the arrows in (B). The scale bar represents 4  $\mu$ m.

15

7.5

0 nm

ecules and salts (19) to organic macromolecules (10, 20) on a variety of substrates. In addition, on both gold and silicon oxide, the transport rate of the DNA can be tailored with careful humidity control. It is thus possible to vary feature size over a large dynamic range on a reasonable time scale. For example, on gold, the diameter of a spot created by holding the AFM tip for 10 s changes from 50 to 300 nm with a relative humidity change of 15% (Fig. 3C). This humidity dependence points to a mechanism for transport of DNA from an AFM tip to a surface, which is dependent on the water meniscus between the tip and substrate (21).

To demonstrate multi-DNA ink capabilities, we have used DPN to prepare a twocomponent DNA array on an oxidized silicon substrate and verified its sequence-specific activity by hybridization with complementary fluorophore-labeled probes (Fig. 4A). To further verify the chemical integrity of the patterns, the same chip was treated with DI water to remove the fluorophore-labeled DNA and then exposed to a solution containing a mixture of 5- and 13-nm-diameter gold nanoparticles. The large and small particles were modified with DNA complementary to the first and second patterns, respectively. The particles selectively assembled on the correct patterns (Fig. 4B) under appropriate hybridization conditions. This experiment not only shows how one could potentially use nanoparticles as diagnostic probes in AFMbased screening procedures but also shows how one can use nanostructures fabricated by the direct-write DPN approach to control the assembly of nanoparticle-based architectures.

In view of the rapid proliferation of bioconjugated nanoparticle labels and building blocks (22-24), the method described here should allow the DPN- and DNA-templated assembly of a wide variety of metallic, semiconducting, magnetic, and insulating nanostructures, on both metallic and insulating substrates. These structures can in turn be used to address issues in molecular electronics, photonics, high-density information storage, and biosensing (5, 25). The method also suggests new routes for investigating the fundamental limits of microarray miniaturization. With the resolution demonstrated here, arrays with  $\sim 100,000$  oligonucleotide spots could be generated in an area the size of a typical AFM scanner (100 µm by 100 µm) on time scales comparable with those of conventional robotic spotting methods, thereby making possible the investigation of scanned probe methods of nano- and microarray fabrication and readout.

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## Supporting Online Material

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Materials and Methods Figs S1 and S2

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## Probing High-Barrier Pathways of Surface Reactions by Scanning Tunneling Microscopy

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The ability of scanning tunneling microscopy to probe the pathways of thermally activated high-barrier surface processes is frequently limited by competing low-barrier processes that can confuse measurement of the true initial and final configuration. We introduce an approach to circumvent this difficulty by driving the surface process with nanosecond laser heating. The method is applied to determine the pathway of recombinative desorption in the H/Si(001) system. The observed configuration of dangling bonds after laser heating reveals that the desorbed hydrogen molecules are not formed on single dimers, but rather from neighboring silicon dimers via an interdimer reaction pathway.

In recent years, scanning tunneling microscopy (STM) has become an important tool for elucidating the rates and pathways of dynamical processes at surfaces. In particular, the development of STM instrumentation capable of operating at variable temperature has enabled researchers to follow dynamical processes with atomically defined initial and final states. In this approach, the sample is held at a temperature where the process of interest occurs on a time scale comparable to that for STM data acquisition, typically a fraction of a second, and changes are recorded in real time. This approach has been remarkably effective in examining surface

\*To whom correspondence should be addressed. Email: duerr@physik.uni-marburg.de diffusion and other processes with low activation energy (1-4). A characteristic of surfaces, however, is the existence of competing reaction pathways. Many important processes, such as those leading to new species through surface reactions, have high activation energies. They generally proceed in the presence of rapid, lowbarrier processes, thus confounding their observation by STM techniques.

Two of the most elementary surface processes, diffusion and desorption of adsorbates, are representative of this predicament, because the activation energy of the latter always exceeds that of the former. The situation is illustrated schematically in Fig. 1 for the system of H/Si(001) discussed below. The surface diffusion rate for adsorbed hydrogen can be described by an Arrhenius expression with an activation energy considerably less than that for the recombinative desorption process by which the adsorbed hydrogen atoms leave the surface. Thus, at temperatures where STM techniques can follow the motion of individual hydrogen

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