DNA On Silicon Devices: On-Chip Synthesis, Hybridization, and Charge Transfer**

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Surface-immobilized DNA has an increasing number of roles in science and technology because of the ease with which it can be manipulated chemically and enzymatically in a highly controlled manner. Complex DNA architectures[1] and the use of DNA as a scaffold for making electronic connections[2] have been reported, and the use of DNA as a component in so-called molecular electronics has been frequently advocated.[3] Applications also include DNA microarrays[4] for sequencing through hybridization, the study of gene expression, and even for prototype computing devices which utilize the fidelity of the hybridization reaction.[5–7] In most cases, the DNA is immobilized on glass, oxidized silicon wafers, or other insulating supports. Methods for combining DNA with electronic materials are anticipated to be increasingly important in light of the considerable interest in DNA-based nanotechnology,[1] DNA-mediated charge transfer,[8] and DNA-based wires.[2] For substrates such as gold[9] or Si,[10] where there is the possibility of charge transfer through the DNA to the surface, immobilization has relied upon the attachment of presynthesized strands. However, to date there has been no demonstration of the use of automated solid-phase DNA synthesis nor charge transfer through DNA-based assemblies at a semiconductor. For many proposed applications the ability to combine microelectronic processing techniques,[11] for example, photolithography and micromachining, with automated chemical synthesis has clear advantages. Herein we outline a method that enables the straightforward integration of DNA technology with microelectronics.

Hydrogen-terminated surfaces represent the initial state from which silicon semiconductor devices are fabricated.[11] Chidsey and co-workers first demonstrated that such single-crystal surfaces[12] react with alkenes to give chemically robust Si–C-bonded monolayers, and further advances have shown the applicability of this chemistry to porous silicon.[13–15] The reaction may be driven by thermal, catalytic, electrochemical, and photochemical methods,[13, 16] and patterning of the monolayers, for example, by using photolithographic techniques, is possible. We have used a simple protecting group method to produce monolayers with terminal OH groups (Figure 1); hydrogen-terminated silicon surfaces of oriented (111) single-crystal and porous silicon were alkylated with 4,4′-dimethoxytrityl-protected ω-undecenol (trityl = triphenylmethyl) by refluxing it in toluene; this is a previously

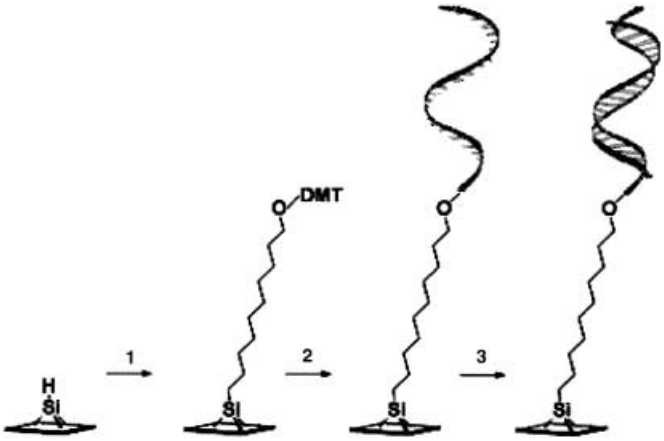

Figure 1. 1) Alkylation of hydrogen-terminated surfaces by reaction with 4,4′-dimethoxytrityl-protected (DMT) ω-undecenol in refluxing toluene for 16 h. 2) The alkylated surfaces were loaded onto a DNA synthesizer followed by deprotection of the on-chip oligonucleotides using anhydrous CH_3NH_2. 3) Hybridization reaction.

established method to form robust Si–C-bonded monolayers.[15, 17] The 17-mer oligonucleotides where then synthesized at these modified surfaces with UltraMILD base phosphoramidites (Glen Research, VA, USA) by using a DNA synthesizer and a column assembly modified to accept approximately 1-cm^2 Si chips. The deprotection step employed anhydrous methylamine[18] and rinsing with ethyl acetate and water instead of the standard treatment of standing for 12 h in aqueous NH_3. The surface is not pitted in the course of this treatment, in contrast to methods in which aqueous ammonia is used. In some cases, a cleavable linkage (chemical phosphorylation agent, Glen Research, VA, USA) was inserted at the 3′-terminal position to allow the quality of the oligonucleotide synthesis to be analyzed by gel electrophoresis. Figure 2 compares the electrophoresis data for

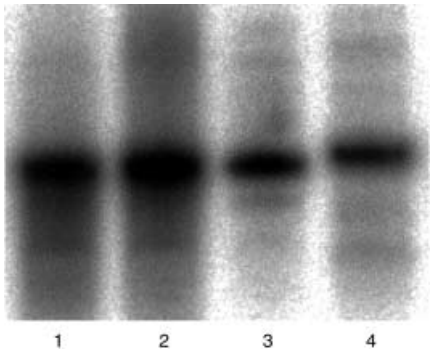

Figure 2. Polyacrylamide gel electrophoresis of oligonucleotides prepared at silicon surfaces. Lanes 1, 2, and 3: 17-mer = 5′-CGGCATCGTACGATTAT; 4: 18-mer = ATAATCGTACGATGCCGT. Oligonucleotides were removed from the silicon surfaces (lanes 1 and 2) by incorporating a cleavable linkage (chemical phosphorylation agent) at the 3′-terminus of the strands and so contain a 3′-phosphate group. Lane 1: Si(111) single crystal, lane 2: porous silicon, lanes 3 and 4: 17- and 18-mers from a commercial control-pore glass support (CPG).

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oligonucleotide strands cleaved from single-crystal, porous silicon and standard silica columns. The quality of synthesis at the silicon surfaces is comparable to that obtained at commercial silica supports.

Silicon-bound 17-mer strands (5′-CGGCATCGTACGATTAT-3′) act as substrates for T4 polynucleotide kinase as shown by radiolabeling using [γ-^{32}P]adenosine-5′-triphosphate ([γ-^{32}P]ATP). Phosphorimaging was also used to confirm that the Si-DNA oligonucleotides undergo hybridization with complementary strands. Hybridization was directed to specific regions of DNA-patterned porous silicon (Figure 3), fabricated using a combination of photolithography and anodic etching. Figure 3 compares the mask and DNA-patterned wafer with the phosphorimage after hybridization. No discernible image is observed for controls where the surfaces are exposed to ^{32}P-labeled oligonucleotides with the same sequence as present on the surface.

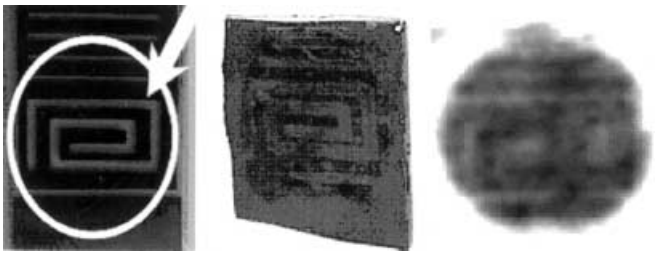

Figure 3. A DNA-patterned Si surface was formed by a combination of photolithography and anodic etching to form porous silicon regions. The surface was functionalized with a 17-mer oligonucleotide (sequence 1) according to the scheme. Left: the mask, center: Si wafer after on-chip DNA synthesis, and right: a phosphorimage after hybridization with a complementary ^{32}P-labeled oligonucleotide. The center picture shows the porous silicon pattern which corresponds with the dark regions of ds-DNA (right).

A mechanism for charge transfer between the DNA and the silicon must be provided in order to combine DNA with microelectronics. Previous workers have shown that DNA can be used as a scaffold at which to plate silver to make metallic connections.[2] Alternatively, the charge-transfer properties of DNA itself[8] can be employed with enhancement by noncovalent binding of redox-active molecules, such as intercalators. The binding of methylene blue[9c] to the Si-DNA duplex structures allowed electron transfer to be observed. Cyclic voltammograms exhibited a reversible wave characteristic of a surface-bound redox process (Figure 4). Silicon crystals bearing only single-stranded DNA (ss-DNA) showed no electrochemical response after exposure to the intercalator, which indicated the need to assemble double-stranded DNA (ds-DNA) to effect binding of the redox-active agent.

The surface coverage of methylene blue was found by integrating the cyclic voltammogram (Figure 4) to be $1.75 \pm 0.3 \times 10^{-12}$ mol cm^{-2}, and independent of the scan rate over the range 20–200 mV s^{-1}, thus indicating a surface-confined process. A molecular-mechanics model of the Si(111) surface with ds-DNA attached through the undecenyl linker shows that the double helix is tilted about 30° to the normal and the projection of the attached strand plus linker on the Si(111) face is about 3.5 nm. The corresponding theoretical maximum

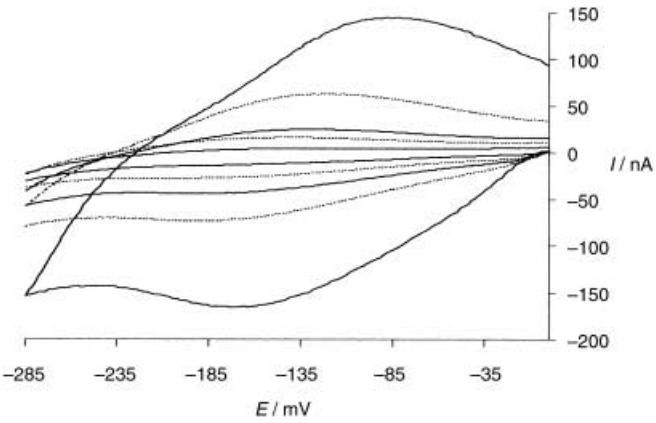

Figure 4. Cyclic voltammogram (HEPES) of methylene blue intercalated into 17-mer duplex strands attached to Si(111) at different scan rates (20–200 mV s^{-1}).

DNA surface coverage is about 2.4×10^{-11} mol cm^{-2}. Hence, these data shows that the double-stranded layer is not close-packed. The surface coverage of ss-DNA was estimated by using $[Ru(NH_3)_6]^{3+}$ to be 5.3×10^{-12} mol cm^{-2}.[19] This is a similar value to that found for thiol-containing oligonucleotides on gold.[19b]

In summary, while the detailed understanding of its charge-transfer properties continues to be debated, the possibilities for using DNA as a material for the fabrication of nanoscale devices increase. The ability to combine the elegance of solid-phase synthesis with semiconductor electronic materials, such as silicon, may provide yet further scope for development.

Experimental Section

Single-crystal silicon or porous silicon were alkylated with 4,4′-dimethoxytrityl-protected ω-undecenol following published procedures[14] and used for the oligonucleotides synthesis as described in Figure 1.

Oligonucleotides (32 μL in buffer) were radioactively labeled at the 5′-ends using [γ-^{32}P]ATP and T4 polynucleotide kinase.[20] Salt and excess [γ-^{32}P]ATP were removed with a QIAquick nucleotide removal kit (Qiagen). The samples were immersed in denaturing buffer (formamide containing 1 mg mL^{-1} each of bromophenol blue and xylene cyanol, 0.5 M ethylenediaminetetraacetate (EDTA), 8 M urea). Electrophoresis on polyacrylamide denaturing gels (12%) containing 8 M urea were run in tris-borate ethylenediamine tetraacetic acid (TBE) buffer (89 mmol tris(hydroxymethyl)aminoethane (Tris) borate (pH 8.2), 1 mmol EDTA at 35 W for 30 mins).

Hybridization reaction conditions involved applying the oligonucleotide solution (15 mmol oligonucleotide, 2-[4-(2-hydroxyethyl)-1-piperazinyl]-ethanesulfonic acid (HEPES) buffer pH 7.5, 200 mM NaCl, 1 mM EDTA) to the DNA-modified Si chips for 10 min. The chips were then washed with buffer and water. The binding of methylene blue involved immersion of the chips in HEPES buffer at pH 7.5 containing 0.1 mM intercalator followed by exhaustive washing with buffer.

Patterned porous silicon was prepared using a modification of the procedure described by Ronkel and Schultze.[21] The 17-mer oligonucleotide (sequence 1) was then attached as described.

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A Diferric Peroxo Complex with an Unprecedented Spin Configuration: An $S = 2$ System Arising from an $S = 5/2$, 1/2 Pair**

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Dioxygen binding and activation are important biological steps in the mechanisms of a number of metalloproteins. For metalloenzymes containing non-heme diiron active sites,[1] such as methane monooxygenase, a diferric peroxo species is a key intermediate in the enzymatic mechanism.[2] To date, the binding modes of the peroxide at these active centers remain to be fully established and more chemical models are required. So far, three coordination modes of this anion have been determined in iron coordination chemistry, namely μ-1,2-peroxo,[3] η^1-hydroperoxo,[4] and η^2-peroxo modes.[5] We recently showed that the (μ-oxo)-diferric complex $[Fe_2O(pb)_4(H_2O)_2](ClO_4)_4$ (**1**-$(ClO_4)_4$) with the (−)4,5-pinene derivative pb as ligand reacted with H_2O_2 to afford a mixture of two peroxo adducts, as indicated by resonance Raman spectroscopy.[6] Here, we demonstrate with new spectroscopic studies that one is a μ-1,2-peroxo adduct (**2**) and the other is a μ-oxo-diferric complex in which the peroxide binds in a monodentate fashion to one iron center (**3**; Scheme 1). This new structural motif engenders an unprecedented ground spin state ($S = 2$) derived from the coupling of an $S = 5/2$ and an $S = 1/2$ ion in the diferric unit.

The addition of 50 equivalents of H_2O_2 to 1 mM complex **1** in acetonitrile at $-40\,^{\circ}C$ caused the appearance of an intense, broad charge-transfer band at 680 nm ($\varepsilon = 2000\,M^{-1}cm^{-1}$,

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Supporting information for this article is available on the WWW under http://www.angewandte.com or from the author. S1: ESI-MS spectrum and characteristic fragments of **1** + H_2O_2 solution; S2: Mössbauer spectrum of the mixture recorded at 20 mT.