Gold Nanoparticle Decoration of DNA on Silicon

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Electrostatic assembly of cationic nanoparticles onto the negatively charged backbone of double-stranded DNA has been shown to produce one-dimensional chains with potential use as nanoelectronic components. In this paper, micron long DNA templates stretched on aminosilane- and hexamethyldisilazane-modified silicon surfaces are used to assemble 3.5 nm gold nanoparticles passivated with cationic thiocholine. Atomic force microscopy is used to analyze the density and defects along the ~5 nm high structures, with comparison between positively charged and neutral surfaces. Low background adsorption of nanoparticles is facilitated by both these surface chemistries, while the neutral surface yields a more densely packed assembly.

Introduction

There is increasing interest in creating electronic devices through the use of bottom-up approaches, based on organized nanoscale structures. This is driven in part by the increasing cost associated with top-down lithography for smaller interconnects and logic elements for higher density sensor, memory, and computational devices. Bottom-up strategies for nanoelectronics often include manipulation of materials such as nanoparticles, carbon nanotubes, nucleic acids, conjugated polymers, or inorganic nanowires in a template-driven hierarchical assembly. To facilitate patterning and integration of these nanoscale components into actual devices such as sensor arrays requires at least an empirical understanding of template behavior and the stochastic processes controlling defects and reproducibility.

Protein and nucleic acid templates have been used as scaffolds to bind biomolecules, inorganic clusters, and metal ions through base pair recognition and hydrogen bonding, electrostatics, chelation, as well as covalent bonding by modifications made on DNA bases or at the 3′ and 5′ ends.[1,2] The electrostatic assembly onto DNA backbones of conducting polymers such as polyaniline, metal ions and complexes, and nanoparticles[3–5] has resulted in conductivity values approaching those of bulk materials, when sufficient material is deposited.[2] However, the characterization of internal defects[6] and the creation of wire interconnects between electronic components or to conventionally patterned, macroscale electrodes remain technical challenges. Furthermore, recent conductive wires built on biomolecular templates such as DNA have remained in the 30–100 nm diameter range.[2,7] It remains to be shown whether wires in the 10–30 nm diameter range can be sufficiently defect-free to conduct over micron lengths.[8,9] Control over spatial order and packing is important for the uniform growth of nanostructures with predicable electronic transport properties and small diameters. Nanoparticle (NP) chains assembled onto DNA templates[10] may provide unique electronic properties and may serve as nucleation sites for growth and coalescence. Previous applications of cationic NP assembled onto DNA showed linear and branched structures extending over microns.[4,11] Here, we report techniques to create NP-DNA assemblies on modified Si/SiO$_2$ surfaces, using positively charged 3.5 nm gold nanoparticles to decorate combed DNA. AFM analysis of nanoparticle density is used in comparing the use of positively charged amines and neutral methyl groups for controlling surface and template affinities for nanoparticles.

Materials and Methods

Functionalized Silicon Substrates. Silicon substrates (⟨100⟩ orientation, 100 nm thermal oxide, optically polished, diced) 5 mm × 8 mm in size were cleaned by ultrasonication in acetone, 2-propanol, and water. The RCA method was then used to prepare a hydroxyl rich silica surface. This three-step process consisted of 10 min in 80 °C NH$_4$OH/H$_2$O$_2$/H$_2$O (1:1:7), 15 s in 1% unbuffered HF in H$_2$O (use with caution), and 10 min in 80 °C HCl/H$_2$O$_2$/H$_2$O (1:1:6), with thorough rinsing between steps with Nanopure water. The hydrophilic substrates were immediately incubated in a closed Petri dish at room temperature with 3-aminopropyl-dimethylethoxysilane (APDMES, Gelest) or hexamethyldisila-

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zane vapor. Silanization took place over three cycles of 1 h exposure and 15 min bake under ambient atmosphere at 120 °C.

Preparation of Linearized Plasmid DNA. A derivative of pHSHW5 plasmid (4422 bp) was prepared. pHSHW5 was transformed and expressed in ER1727 bacteria cells, which were grown. The plasmid was isolated using QIAprep Spin Miniprep Kit (Qiagen) according to the kit specifications. Plasmid DNA was precipitated with ethanol and resuspended in 10 mM/1 mM Tris-EDTA buffer for UV-absorption readings at 260 nm. Plasmids were digested to completion with the restriction enzyme EcoRI, for which there is a single site in the plasmid. Digested DNA was purified using multiple phenol/chloroform and ethanol precipitations. DNA was resuspended in 50 mM phosphate buffer at pH 7.4. A stock solution containing this DNA at 40 ng/μL was used for preparing combing solutions. Dipping solutions (1 ng/μL) were prepared immediately before use by dilution of 5 μL stock with 195 μL of 20 mM bis-tris propane buffer at pH 9.0 for APDMES or 50 mM 2-morpholinoethanesulfonic acid (MES) buffer at pH 5.5.

Deposition of DNA. Double-stranded DNA was combed onto the hydrophobic silicon surfaces. The wafer was dipped vertically into the 200 μL solution of linear plasmid DNA, held for 10 s, and withdrawn at ~10 mm/s through the liquid−air interface. The sample was briefly washed by dripping 5 drops of filtered H_2O over the wafer (held at 45° angle to the horizontal) and dried for 30 s under a stream of nitrogen.

3.5 nm Thiocholine Au Nanoparticles. Alkylamine-stabilized Au nanoparticles were synthesized[12] and ligand exchanged. A 25 mL aqueous solution of 112 mg (0.284 mmol) of $HAuCl_4 \cdot 3H_2O$ was combined with 0.749 g (3.10 mmol) of hexadecylamine (90%, Aldrich) in 50 mL of toluene and stirred rapidly. A solution of 0.165 g (4.36 mmol) of $NaBH_4$ in 25 mL of water was added, and the mixture was stirred overnight. The dark organic phase was removed and reduced to ~15 mL, followed by the addition of 350 mL of 95% ethanol. This mixture was stored overnight at −80 °C to precipitate the hexadecylamine-passivated nanoparticles (HDA-Au). Solids were then isolated by centrifugation at 4 °C and resuspended and centrifuged three times with 200-proof cold ethanol. Following the last step, the solids were lightly dried and dissolved in a minimum amount of $CHCl_3$. This solution was centrifuged several times at 14 000 rpm to remove any insoluble material, and the supernatant was collected and dried. The nanoparticles were redispersed in CH_2Cl_2 for ligand exchange.[11] This solution contained ~15 mg/mL 4 nm HDA-Au. The dark burgundy solution of nanoparticles in CH_2Cl_2 underwent a biphasic ligand exchange reaction (CH_2Cl_2/H_2O) by stirring overnight with an excess aqueous 30 mM thiocholine/500 mM NaCl solution, which was prepared by base hydrolysis of acetylthiocholine chloride (Sigma). Over a period of several hours the color transferred completely to the aqueous layer. This layer was removed and dialyzed against Nanopure water over a 2 day period with three water exchanges. The nanoparticles were combined with plasmid DNA and placed on a TEM grid for 5 min (40 nM tcAuNP, 0.5 ng/μL plasmid, bufferless), wicked, dried, and then characterized. TEM analysis showed the average size of thiocholine Au to be 3.5 ± 1 nm and their capability for assembling onto DNA[4,11] (Figure S1).

Decoration of DNA. DNA on silicon was decorated by placing a 10 μL drop of bufferless 40 nM tcAuNP solution (pH 6.0) onto the sample for 5 min to 1 h. The sample was washed with several drops of filtered Nanopure water and dried with a stream of nitrogen.

AFM Imaging. Samples were imaged using a Digital Instruments Nanoscope IIIA Multimode AFM operating in tapping mode in air with 125 μm Si cantilevers with a resonance frequency of 260−410 kHz (Nanosensors). A field scan of DNA on APDMES silicon with 5 min tcAuNP decoration is shown in Figure S2.

Results and Discussion

Gold nanoparticles (AuNP) were electrostatically assembled on the anionic phosphate backbone of DNA through the use of cationic thiocholine,

$HSCH_2CH_2N(CH_3)_3$, for surface passivation.[11] Novel 3.5 nm cationic AuNP was prepared by ligand exchange of hexadecylamine-passivated 3.5 nm AuNP[11,12] in CH_2Cl_2 with aqueous thiocholine through a biphasic extraction, similar to the thiol ligand exchange of triphenylphosphine-stabilized 1.5 nm AuNP.[11] The ligand exchange of alkyl-amine with thiocholine is believed to go to completion due to their relative binding strengths and solubility differences. Thiocholine-stabilized AuNP (tcAuNP) was characterized by TEM and determined to be 3.5 ± 1 nm in diameter. This was used in binding DNA through the high number of positive quaternary amine surface charges,[4,11] which are independent of solution pH.

Oxidized silicon wafers were cleaned with organic solvents and acid treatment, then modified using gas-phase silanization with either 3-aminopropyldimethyl-ethoxysilane (APDMES) or hexamethyldisilazane (HMDS). Parallel arrays of stretched DNA were deposited through molecular combing. Molecular combing utilizes the pH-dependent preference of the surface for binding the ends of double-stranded DNA, combined with fluid or meniscus movement to pin and stretch the molecules.[13] The DNA was combed at pH of 8.5−9.0 for APDMES and pH 5.5 for HMDS. When sufficiently silanized, these surfaces provide reproducible immobilization of DNA as parallel arrays through the combing process with low background deposition.

Solutions of linearized plasmid DNA (4422 bp, 1 ng/μL) were exposed to the surface for 10 s by dipping, to achieve densities of two to four strands per 5 μm square of linear DNA molecules. Decoration of the DNA was accomplished by placing a droplet of bufferless 40 nM tcAuNP solution (pH 6.0) on the silicon. The formation of structures after various tcAuNP exposure times was monitored by AFM and is shown in Figure 1. The measured heights are consistent with the average NP diameter observed by TEM, but the widths are distorted due to convolution with the AFM tip, which has a diameter on the order of 10 nm. Peaks along a height cross-section analysis are assumed to be tops of nanoparticles. For APDMES, the AFM cross-section shows the average resolvable peak-to-peak distances to be about 22 and 17 nm after 5 and 30 min, respectively. We suggest that during surface probing, closely spaced NPs may appear as a single feature. Therefore, the distances used here to gauge the growth of the assembly should be considered the lower limit of the true density.

Because of the finite width of the AFM tip, we performed a gap analysis to measure distances between NP. The predominant peaks are between 2 and 10 nm in height; regions less than 1.75 nm above the substrate are labeled as gaps and are located in the cross-section. The shortest distance between the peaks, at half-height, is taken as a measure of gap distance (see Figure 1e). The average gap size and density (number of gaps per micron of assembly) decreases with exposure to tcAuNP of up to 1 h (Figure 2).

A comparison with the NP density on DNA combed onto the trimethylsilyl-terminated silica (HMDS) shows that the protection mechanism of the APDMES surface from nonspecific NP deposition may also be limiting the decoration of the DNA. This may be due to a partial screening of the DNA phosphate groups by protonated surface amines. It appears that for HMDS, a more densely packed assembly is achieved when Figures 1b and 1d are compared). Our current efforts seek to use these

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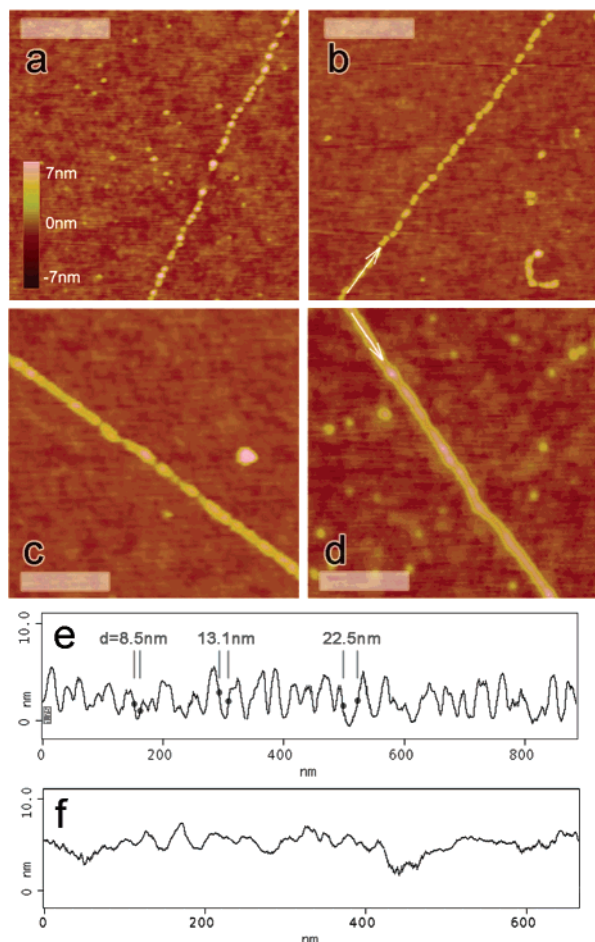
Figure 1. AFM of DNA decorated with 3.5 nm thiocholine-Au nanoparticles. DNA (1 ng/μL) is combed from pH 9.0 20 mM bis-tris propane buffer onto Si/SiO$_2$ surfaces modified with APDMES and from pH 5.5 50 mM 2-morpholinoethanesulfonic acid (MES) buffer for HMDS. The 10 μL droplets of 40 nM tcAuNP are deposited at pH for varying times of APDMES (a) 5 min, (b) 30 min, and (c) 1 h and HMDS (d) 30 min. Height analysis along the axis of assembly in panels b and d are presented in panels e and f, respectively. Distance measurements were taken at half-height as shown in panel e as regions below 1.75 nm in height relative to local surface. White scale bars are 200 nm for panels a and b and 150 nm for panels c and d.

assemblies to form wires with diameters below 30 nm when grown by metallization techniques.[2,8,9]

The dynamics of NP binding to DNA has been studied in solution,[14] yet there may be fundamental differences with this work involving substrate-based decoration. In comparison to AFM, a TEM of 3.5 nm tcAuNP assembled on DNA (see Supporting Information) shows an average gap size of 1.6 $\pm$ 0.7 nm, consistent with twice the thiocholine monolayer thickness of 0.7 nm.[11] A footprint of ~5 nm would confer contact with up to 15 base pairs (0.34 nm per base pair). DNA in solution is flexible at lengths greater than its 50 nm persistence length, which can be significantly decreased through ionic interactions.[15] Thus, the negative DNA backbone and the high concentration of tcAuNP surface charges may involve structures in which DNA is wrapped around the NPs to some extent.[4,11] This is less likely to occur with surface-
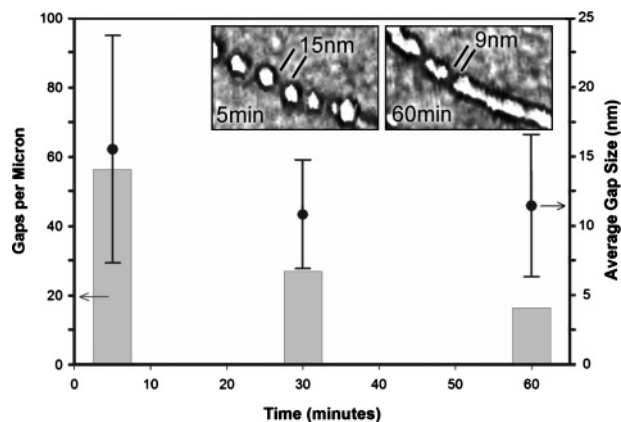

Figure 2. Average number of gaps per micron of NP-DNA assembly on APDMES is shown as gray vertical bars. Specific gap size averages and standard deviation are shown by circles with standard deviation error bars. At least three micron length assemblies were examined per time point (up to 75 gaps per strand). Limitations in the AFM tip's determination of gap lengths below 10 nm may be responsible for the increase in the average gap size between 30 and 60 min. Free NP cross-section widths at peak half-height minus the distance from baseline to peak maximum yields tip widths of 10−15 nm. Insets show AFM phase images depicting 5 and 60 min APDMES NP-DNA assemblies.

immobilized DNA, where the DNA can be stretched to 1.6 times their B-form length during the combing process.[16,17] It should be noted that the combed DNA assemblies shown in Figure 1 are not highly stretched, being only 10−20% longer than the B-form. Previous studies have explored the interactions of NP ligands with DNA on surfaces through electrostatics, hydrogen bonding, and intercalation.[9,10,18] It appears here that the polarity of the surface may be used to modulate the electrostatic decoration of DNA by nanoparticles.

The patterning and assembly of DNA with positively charged nanoparticles on SiO$_2$ is described based on two surface chemistries, aminopropyldimethyl and trimethylsilanes. The electrostatic interactions between surface and template may be used to modulate the deposition of nanoparticles. Low levels of nonspecific nanoparticle deposition were observed with neutral and positively charged surface groups. A gradual buildup of NP onto DNA occurred in the case of the amine surface, allowing analysis of the number and size distribution of undecorated regions observed on the linear DNA template. Silica functionalized with uncharged methylsilane resulted in more densely packed cationic nanoparticle-DNA assemblies while retaining a low background adsorption.

Supporting Information Available: Details of DNA and silicon preparation. This material is available free of charge via the Internet at http://pubs.acs.org.

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