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Biorecognition by DNA oligonucleotides after Exposure to Photoresists and Resist Removers

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Abstract

Combining biological molecules with integrated circuit technology is of considerable interest for next generation sensors and biomedical devices. Current lithographic microfabrication methods, however, were developed for compatibility with silicon technology rather than bioorganic molecules and consequently it cannot be assumed that biomolecules will remain attached and intact during on-chip processing. Here, we evaluate the effects of three common photoresists (Microposit S1800 series, PMGI SF6, and Megaposit SPR 3012) and two photoresist removers (acetone and 1165 remover) on the ability of surface-immobilized DNA oligonucleotides to selectively recognize their reverse-complementary sequence. Two common DNA immobilization methods were compared: adsorption of 5'-thiolated sequences directly to gold nanowires and covalent attachment of 5'-thiolated sequences to surface amines on silica coated nanowires. We found that acetone had deleterious effects on selective hybridization as compared to 1165 remover, presumably due to incomplete resist removal. Use of the PMGI photoresist, which involves a high temperature bake step, was detrimental to the later performance of nanowire-bound DNA in hybridization assays, especially for DNA attached via thiol adsorption. The other three photoresists did not substantially degrade DNA binding capacity or selectivity for complementary DNA sequences. To determine if the lithographic steps caused more subtle damage, we also tested oligonucleotides containing a single base mismatch. Finally, a two-step photolithographic process was developed and used in combination with dielectrophoretic nanowire assembly to produce an array of doubly-contacted, electrically isolated individual nanowire components on a chip. Post-fabrication fluorescence imaging indicated that nanowire-bound DNA was present and able to selectively bind complementary strands.

Introduction

Combining both nanomaterials and biological molecules with integrated circuit technology is important for enabling the next generation of portable biosensors for medical diagnostics, environmental monitoring, and other applications.^{1,2} To date, most efforts have focused on

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Supporting Information Available.

Supporting Information includes DNA sequences used for experiments shown in Figure 4 and 5, additional post-resist treatment single-base mismatch hybridization data for DNA sequences immobilized on a glass microarray, and associated DNA sequences and methods for this experiment. This material is available free of charge via the Internet at <http://pubs.acs.org>.

adding the biological functionality as a top layer after device fabrication is completed. This is an attractive approach because it does not demand changes to the fabrication conditions nor unusual stability from the biological molecules that will be added post-fabrication. However, post-fabrication biofunctionalization limits flexibility in terms of chemistries and reaction conditions that can be employed for biomolecule attachment, and in the number of distinct attachment chemistries that can be used adjacent to each other. Typically a single type of chemistry is employed, such as thiol for self-assembly on gold or carbodiimide coupling of carboxylates with amines, with careful delivery of the reactive biomolecules to different sites for attachment to specific locations on the surface. Possible methods for biomolecule delivery include a variety of methods, e.g. microfluidic channels,³⁻⁵ microcontact printing,^{6,7} ink-jet printing,⁸⁻¹⁰ microarray spotting,^{11,12} and dip-pen nanolithography^{13-15,16,17} It is also possible to pattern photoactive molecules by selective illumination. Desired regions are exposed to light for photoactivation of a photolabile protecting group using masks or other light patterning techniques.¹⁸⁻²¹ This type of approach is employed, for example, in on-chip DNA oligonucleotide synthesis for Affymetrix oligonucleotide arrays.²² Such approaches require photoactivatable chemistries and are generally performed on planar supports, with fluorescence imaging-based detection rather than using integrated circuit chips for electrical detection nor nano/microparticle based electrical or mechanical devices.

The various biomolecule delivery methods described above, while attractive for bioprobe arraying, may be unable to take full advantage of the strengths hybrid top-down/bottom-up nanofabrication methods offer. For example, it is desirable to assemble and integrate onto the same electronic chip several different types of nanomaterial-based devices (e.g., carbon nanotubes, metal, metal oxide, and/or semiconducting nanowires as suspended resonators or field effect transistor devices). Functionalizing these distinct materials with desired biomolecules could require different attachment chemistries and reaction conditions for devices located quite close to each other in the array ($< 10 \mu\text{m}$). Potential problems with existing biomolecule delivery methods include (i) uneven delivery of molecules to desired locations due to surface topography and/or drying effects, (ii) limitations in the types of solvents and temperatures that can be employed without damaging the prefabricated integrated circuit chip, and (iii) the possibility of cross-reactivity during the rinsing step to remove unreacted molecules (during which time some of the unreacted molecules could come into contact with reactive sites elsewhere on the surface).

Off-chip functionalization of nano- or microparticle sensing elements such as nanowires prior to assembly and integration onto the integrated circuit can, in principle, overcome these challenges and provide tremendous flexibility in the types of attachment chemistries that could be employed. However, such an approach poses its own challenges, perhaps the most significant of which is the requirement that attachment chemistries and attached bioprobes remain affixed and functional throughout the fabrication process, during which they will be exposed to one or more photoresist(s) and resist remover(s). During a typical device fabrication,²³ a positive photoresist is (i) spun onto the substrate, (ii) soft-baked, and (iii) exposed to light through a mask to select regions for removal. These regions are then removed using a developer (e.g., a strong base), and finally (iv) the remaining photoresist that had been protecting the molecules during processing is removed using solvents ("removers"). Bake temperatures and exposure wavelengths are determined by which resist is being used. For example, relatively lower bake temperatures are used for the Shipley series and SPR 3012 photoresists. Although UV light would be damaging to many types of biomolecules, including DNA, regions of interest are protected by the mask and hence not exposed to UV light.²⁴ The photoresist and removers, however, contact the biofunctionalized nanowires directly and hence it is important to determine what effect, if any, such treatment will have on the performance of the immobilized biomolecules in

subsequent biorecognition assays. A variety of different photoresists are available for use in lithographic processes. These polymeric solutions vary in composition, viscosity, and cross-linking properties, allowing different photoresists to be used for different applications with control over the thickness of the material deposited, the light absorption properties, and therefore, the resultant structures.²⁵

Pre-biofunctionalization of nanomaterials followed by their placement and on an electronic circuit microchip followed by photolithographic integration to form nanodevices has thus far been performed only rarely, and quantitative evaluation of biomolecule performance after this treatment has not been reported. However, some efforts to combine biomolecules with photolithographic methods on planar surfaces have been reported. One way to protect the biomolecules during processing is by placing some sort of intermediate coating between them and the layer of photoresist on top.²⁰ For example, Trau and coworkers used evaporated Au metal to protect biomolecules during harsh processing, after which they removed the metal using a cyanide etch.²⁶ Use of the gold mask preserved 84% of DNA function and 30% of neutravidin function.²⁶ Sucrose, low-melting agarose, and polyvinylalcohol have also been used as protective coatings for biomolecular layers during photolithographic processing.^{27–30} A different approach is to design new photoresist materials with biocompatibility in mind. Resists that can be developed in more dilute aqueous base have been synthesized for biocompatibility and reduced environmental impact.³¹ These resists been used to sequentially pattern several different biomolecules (proteins and oligonucleotides) at different locations in a planar array by repeated exposure/developing/molecule deposition steps.^{32,33} Biomolecules experienced both UV light and developer solution in this approach, which is a concern with respect to possible damage. However, by limiting UV exposure and reducing the concentration of base in the developer, protein arrays could be generated.

Here, we evaluate the direct use of robust biological molecules with a combination of bottom-up nanomaterial assembly and standard photolithographic methods. This approach, if successful, should be readily adoptable and require less redesign of optimized device fabrication process flows. We tested the effect of standard photoresists, developer and removers on surface-bound bioprobe molecules. We chose to evaluate single-stranded DNA oligonucleotides, which are prevalent as biorecognition probes in a wide variety of biosensing platforms and were anticipated to have greater stability as compared to protein-based biorecognition probes. DNA oligonucleotides can be expected to adopt active structures even after exposure to organic solvents, once they are placed back into an appropriate aqueous buffer. Four common positive photoresists were evaluated here (Shipley Microposit S1813 and S1805, Megaposit SPR 3012 PGMI SF6; see Table 1). We previously reported the fabrication of arrays of DNA- or PNA-coated metal nanowire resonators using a hybrid bottom-up/top-down assembly and integration strategy based on pre-functionalization of the nanowires with the DNA or PNA bioprobes. In that study, fluorescence microscopy indicated that the bioprobes were still capable of distinguishing complementary from noncomplementary target DNA strands when exposed to a mixture of three sequences.²⁴ Huang and coworkers have reported DNA on a planar surface treated with a resist and remover that afterwards could be used as primers in a rolling-circle amplification assay.³⁴ These prior studies^{24,34} were encouraging, but did not provide quantitative comparisons in bioprobe availability/activity with and without treatment, nor did they challenge the post-integration bioprobes to discriminate between similar DNA sequences. If subtle damage to the bioprobes had occurred, or damage/loss of some but not all of the total probes, it might not be apparent in such studies. Here, we explore these questions and examine the effect of several photoresists with their appropriate removers on DNA hybridization to DNA probe molecules attached to nanowires. Our findings are relevant not only to our own nanowire device fabrication goals, but also to the growing

community of researchers working to combine photolithography with biological molecules and biomolecular functionalities such as selective molecular recognition.

Results and Discussion

We selected three types of commonly used positive photoresists for this investigation: Shipley 1800 series (1813 and 1805), PMGI SF6, and Megaposit SPR 3012. The photoresists require different prebake temperatures: 100°C, 190°C, and 95°C for the Shipley 1800 series, PMGI, and SPR 3012 respectively (Table 1). The Shipley 1800 and SPR 3000 series photoresists are general use resists composed of a novolak resin and a diazo photoactive compound.³⁵ Their compositions are similar, with the S1800 and SPR 3000 series differing primarily in their solvent, and the two 1800 series resists differing from each other in viscosity, which correlates to film thickness. Because the active components of all the SPR 1800 series resists are the same, it is expected that they will have similar effects on DNA oligonucleotides. PMGI series photoresists are composed of a polyaliphatic imide copolymer and are of particular interest due to the high thermal stability, adhesion to Si, and ability to undercut in a controlled manner.³⁶ We note that the PMGI resists must be exposed using deep UV and hence are generally used in bilayer processes with 1813 or 3012 as mask layers.

The effect of photoresist/photoresist remover treatments on 5' immobilized single-stranded probe DNA was evaluated by comparing fluorescent signals after exposure to fluorescently labeled complementary and noncomplementary target oligonucleotides (Scheme 1). Probe DNA was immobilized by solution-based chemistry onto bare or silica-coated metal nanowires to facilitate fluorescence quantification by optical fluorescence microscopy imaging (DNA sequences are shown in Table 2). Two common attachment strategies for immobilization of 5' thiolated DNA were compared: (1) direct assembly onto metallic surfaces via the gold/Au interaction, and (2) use of a bifunctional crosslinker, Sulfo-SMCC, to bind the thiol moieties to primary amines on silica. Amination was accomplished by standard siloxane chemistry in which 3-aminopropyltrimethoxysilane was bound to the silica surface. In each case, parallel experiments were performed in which the immobilized probe DNA was not exposed to photoresists/removers as a control.

Effect of Photoresist on DNA Hybridization: Complementary vs. Noncomplementary Strands

We began by evaluating Shipley 1813. This photoresist was deposited atop silica-coated nanowires carrying immobilized probe DNA, baked to cure, and finally removed by treatment with either acetone or 1165 photoresist remover solution. Samples were then rinsed with water and then PBS buffer and exposed to a solution of fluorescently-labeled DNA that was complementary or noncomplementary to the probe sequence. Acetone and 1165 remover are both commonly used to dissolve 1800 series resists. We considered the acetone potentially more “biomolecule-friendly” as compared to the commercial 1165 solution (of which the primary ingredient is 1-methyl-2-pyrrolidinone) and hence tested acetone first. Treatment with Shipley 1813 followed by removal in acetone resulted in decreased signal for the complementary target strand binding as compared to the untreated control, while selectivity for the complementary vs. noncomplementary target strand was retained (Figure 1A). Possible explanations for the decreased binding of complementary target to the 1813/acetone treated samples include damage to the probe DNA strands, loss of DNA probe strands from the nanowire scaffold, or incomplete removal of the 1813 resist by the acetone treatment step³⁷. To test the latter possibility, we next exposed probe-coated nanowires to 1165 remover alone, and 1813 photoresist followed by 1165 remover. Two removal conditions were evaluated: 10 min. at 90 °C and 30 min. at 50 °C. Both conditions were anticipated to remove the photoresist effectively, however it was not clear whether

shorter, higher temperature treatments or longer, lower temperature treatments would be preferable for retention of DNA probe bioreactivity. Little difference was observed between the treatments (Figure 1B) and excellent selectivity was retained in both cases. We next added the 1813 photoresist treatment to the 1165 remover step (Figure 1C). Here, the longer, cooler treatment gave superior performance as compared with the shorter, higher temperature treatment. Since the 90 °C, 10 minute 1165 step did not reduce fluorescence intensity when no resist was present (Figure 1B), the decrease is likely due to incomplete removal of the 1813 resist interfering with hybridization of the complementary target strand. These results suggest that the 30 min., 50 °C 1165 photoresist remover treatment is superior to either acetone or 90 °C, 10 min. 1165 remover treatment for use with DNA-coated structures.

We next expanded the study to compare several photoresists (Shipley 1813, PMGI, and SPR 3012) and two probe surface attachment chemistries (thiol on Au metal vs. a bifunctional crosslinker to attach thiolated DNA to aminosilane-coated silica). The photoresist remover step was identical for all of the resists; 30 min, 50 °C 1165 remover (as demonstrated above). The results of these experiments (Figure 2) showed that overall intensities for the untreated samples differed both in magnitude and variability for attachment on Au as compared with silica, as has been discussed previously.³⁸ For the thiolated DNA on Au, fluorescence values were low due to fluorescence quenching by the metallic nanowire surface (Figure 2A).³⁸ After photoresist/remover treatment, some decrease in fluorescence for the samples exposed to complementary fluorescent DNA was observed for the Shipley 1813 and SPR 3012, presumably due to loss of some thiolated probe DNA strands at the bake temperatures 95°C (Table 1). Thiolated DNA on Au is known to desorb at high temperatures.³⁹ Nonetheless, fluorescence remained reasonably intense, and complementary target strands led to a much higher intensity than noncomplementary strands, suggesting that DNA attached via terminal thiols onto Au can be used for sensing experiments after exposure to these photoresists/removers. In contrast, a substantial decrease in fluorescence was observed for the DNA exposed to PMGI SF6 and baked at 190°C for 9 min, indicating very little hybridization of the target DNA to the probe sequence. The observed decrease in fluorescence is interpreted as largely due to desorption of thiolated probe DNA from the nanowire surface but could also include some direct damage to the probe. Lindahl has reported that at temperatures greater than 100°C, DNA is more prone to hydrolytic damage, which would inhibit hybridization to the target sequence.⁴⁰

Decreases in fluorescence intensity, indicative of decreased hybridization, were not observed for the DNA attached to silica-coated nanowires through Sulfo-SMCC/siloxane chemistry after exposure to Shipley 1813 and SPR 3012 (Figure 2B). Although there was a slight decrease in hybridization with the DNA exposed to PMGI SF6, potentially due to the high bake temperature, as noted above, the probe molecules were still capable of binding target DNA and doing so selectively. The ratio of fluorescence of the probe post PMGI exposure in the presence of the complementary sequence versus the non-complementary is 81:1 compared to 88:1 for the control. Attachment to silica using the Sulfo-SMCC/siloxane chemistry gave superior results due to its higher thermal stability as compared with thiol/Au chemistry.³⁸ It is unclear why the SPR 3012 treated sample exhibited greater fluorescence than the untreated sample after exposure to the fluorescent complementary strands but retained good rejection of noncomplementary binding. The SPR 3012 photoresist exhibited no inherent fluorescence that could explain this result.

Based on the superior performance of the DNA probes attached to silica as compared to the thiolated DNA bound to Au surfaces in this experiment, and given the prevalence of silica surfaces in semiconductor electronics, we chose to concentrate on the silica attachment chemistry for the remainder of the experiments described in this manuscript.

Effect of Photoresist on DNA Hybridization: Single base mismatches

Although the data (shown in Figures 1 and 2) suggest that probe DNA retained its ability to selectively bind its complementary target strands, some more subtle damage could still have occurred that would not be picked up by that experiment. If minor damage occurred that impacted just one or a small number of nucleotides, the probe strands would likely still be able to discriminate a fully-matched complementary strand from a fully-mismatched noncomplementary strand. DNA can undergo damage *in vivo* due to base modifications, such as deamination, oxidation, and alkylation, which is of interest in human disease.^{40,41} DNA is particularly susceptible to oxidative damage, which often results in the conversion of guanine bases to 8-oxo-guanine.⁴³ We therefore applied a more stringent test to examine retained selectivity: discrimination of a single-base mismatch. We challenged the probe DNA with distinguishing perfectly matched from a single nucleotide mismatch target sequence that substituted an adenine nucleobase in place of a cysteine. This particular substitution was chosen since *in vivo* the nucleobase inserted opposite an 8-oxo-G is A rather than C, resulting in mutagenesis due to G:C to T:A transversions.⁴⁴ DNA probe sequences were immobilized at their 5' end to silica coated nanowires and drop casted onto a surface. Photoresist was spun on, heated, and then removed with 1165 photoresist remover (at 50°C, 30 min; see Table 1).

Comparing the selectivity of untreated samples with that of samples treated with three different photoresists (Figure 3), we found that samples exposed to PMGI SF6 showed much lower intensity for both complementary and mismatched target DNA. This further supports the conclusion from the previous experiment (Figure 1) that DNA damage occurred upon exposure to PGMI SF6 photoresist with a 9 min bake at 190°C. However, both the overall intensities and the intensity ratios for complementary vs. single base mismatched targets were essentially unchanged by treatment with Shipley 1813 or SPR 3012 followed by remover. An additional photoresist, Shipley 1805, was also tested in a separate experiment on a spotted DNA microarray (Supporting Figure 1); biorecognition selectivity for perfectly-matched complementary strands over mismatched targets was retained after photoresist and remover treatment in those experiments as well. We conclude that these three photoresists can be used safely on immobilized probe DNA that will later be employed in biodetection.

Device Fabrication with Pre-functionalized nanowires

We next performed a proof-of-principle experiment to demonstrate that photolithographic methods could be used to fabricate device geometries from DNA-coated nanowires while retaining probe DNA function. We chose to demonstrate a process flow for fabrication of nanowire field effect transistors (FETs). These devices are of increasing interest for real-time, label-free, ultrasensitive biodetection with electrical readout.^{45,46} Here, we used silica-coated metal nanowires rather than semiconducting nanowires (e.g., axially doped Si nanowires) that would be needed to generate a functional nanowire FET device. The metallic wires have a larger diameter and are reflective at the excitation and emission wavelengths of our fluorescent dye, which allowed us to more easily perform fluorescence microscopy experiments to test the retention of DNA binding selectivity after device fabrication processing.

Our general strategy was to use dielectrophoretic (DEP) forces generated by electric field gradients on a patterned chip to assemble arrays of individual nanowires to predetermined locations on the chip for subsequent photolithographic integration.^{24,47,48} Parallel metallic alignment electrodes were patterned on the chip to generate the desired field gradients; nanowires assembled to span the gap between these electrodes. We had previously reported PNA- and DNA-functionalized nanoresonator device geometries prepared in this way;^{24,48} here, we evaluated an integration process designed to instead prepare FETs. Individual

nanowires would require metal contacts deposited on both ends for source and drain electrodes, and each device needed to be electrically isolated from adjacent devices by cutting through the alignment electrodes used in nanowire assembly. To accomplish this, we designed a fabrication process that required two levels of photolithography after the DNA-coated nanowires were assembled onto the chip: the first to pattern the source and drain electrodes and the second to electrically isolate the individual FET devices (Scheme 2). Briefly, gold electrodes were evaporated onto lithographically patterned areas on a silicon wafer. Well structures fabricated by undercutting PMGI photoresist were then produced to guide nanowires to predetermined locations during assembly. Note that although our earlier experiments suggest avoiding PMGI atop the immobilized DNA, here it was used instead to pattern the underlying wafer surface, which does not require coating the immobilized DNA with this resist, baking, etc. Following nanowire assembly, SPR 3012 resist was used to pattern windows over the ends of the nanowires to enable the electrodeposition of source and drain electrodes for FET measurements. The final step of fabrication was lithographically isolating each device by cutting through the alignment electrodes using a reactive ion etch (RIE). Note that the immobilized DNA molecules were protected beneath a layer of SPR 3012 photoresist during this etch step. Finally, the chip was exposed to fluorescently-tagged DNA that was complementary to only one of the two probe sequences. Because sandwich-style DNA hybridization assays are commonly used in biodetection, we used a sandwich-style DNA assay here, where an unlabeled target strand was prehybridized to a labeled tag strand.

Figure 4 shows the as-fabricated devices and gives fluorescence imaging DNA hybridization results. The chip exposed to complementary target strands showed bright fluorescence on the nanowires; nonuniformity in fluorescence intensity here is due to electromagnetic interactions between the dye and the metallic source and drain electrodes. In contrast, the chip exposed to noncomplementary fluorescent target DNA is dark, indicating very low nonspecific binding. Because the fluorescence inhomogeneities in the fully-fabricated device structures made quantification difficult, we also performed the two separate photoresist/remover treatments as well as the RIE etch step on DNA-coated nanowires drop cast onto a glass slide. Using this approach (Figure 5) no loss in selectivity was observed, indicating that the immobilized DNA was not damaged by the second lithography step and that it was well protected by the photoresist during the RIE treatment. These data are very encouraging because they suggest that biological molecules such as DNA can be incorporated into nanoelectronic device architectures prior to the final layer(s) of photolithography, which provides a great deal of flexibility in device design and implementation.

Conclusions

We have demonstrated that DNA oligonucleotides attached via 5' thiol groups either by direct adsorption to Au or by covalent coupling to surface amines on SiO₂ can withstand treatment with three common photoresists and one common resist remover. Our data do not speak directly to the chemical structure of the immobilized DNA, therefore further analysis by mass spectrometry (e.g., MS-MS) methods will be needed to rule out any bond making/breaking. We can, however, conclude that immobilized DNA oligonucleotides that were untreated or treated with several common photoresists and 1165 resist remover showed little difference in terms of their binding to complementary DNA sequences from solution, and their ability to discriminate noncomplementary sequences including those that differed by a single nucleotide. When buried underneath a layer of photoresist, the DNA was protected from damage by harsh treatment, including a Cl₂ plasma etch. These findings are important for the growing community of researchers seeking to combine the functionality provided by biological molecules such as DNA with photolithographic methods and integrated circuit

technology. For example, understanding the effects of lithographic processing steps on surface-bound bioprobes will inform efforts to fabricate biosensor arrays directly on integrated circuits for direct electrical readout and multiplexing with improved sensitivity for statistically relevant results.

Although we were particularly interested in nanowire-based devices, the results reported here are also relevant for planar surfaces such as traditional DNA microarrays on silica, if the same aminosiloxane/SMCC linker chemistry is used. Moreover, the finding that photoresist coatings provide excellent protection against damage during processing and do not themselves degrade the DNA molecules' performance suggests that other typical covalent chemistries should also be acceptable. We would nonetheless recommend that any new chemistry be first validated prior to use in photolithographic DNA sensor microfabrication.

Experimental Methods and Materials

Materials

Silver Cyless R RTU (3 troy oz/gallon), Orotemp 24 RTU Rack (0.25 troy oz/quart), and TG-25 (1 troy oz/gallon) were acquired from Technic, Inc. Tetraethoxysilane, 98% (TEOS) and 3-aminopropyltrimethoxysilane (APTMS) were acquired from TCI America. DNA sequences were purchased from Integrated DNA Technologies. Photoresists and remover were acquired from Micro-Chem. Sulfosuccinimidyl-4-(*N*-maleimidomethyl) cyclohexane-1-carboxylate (Sulfo-SMCC) was purchased from Pierce Protein Research Products, Thermo Scientific. All water used was 18.2 M Ω -cm Nanopure water from a Barnstead system. All reagents were used as purchased without further purification; the silanes were aliquoted out in a glove box under nitrogen gas to prevent hydrolysis from water vapor in the air.

Nanowire Synthesis

Metallic nanowires were synthesized by galvanostatic electrodeposition into anodized aluminum oxide Whatman Anodisc 25 membranes with 0.2 μ m pores ($I = 1.65$ mA) on an in-house system, as has been described previously.^{49–52} Silver (300 nm) was vapor deposited on one side of the membranes to act as the working electrode. A sacrificial silver layer was initially electrodeposited for 1 hr, followed by the deposition of the metal(s) of choice. After electrodeposition, the silver backing and sacrificial silver were dissolved in 33% v/v nitric acid. Nanowires were then released into suspension by vortexing the membrane in 3 M NaOH, and rinsed with μ M NaOH, followed by DI H₂O, and three times with ethanol (EtOH) before suspension in 1 mL EtOH. One batch of nanowires produced $\sim 10^9$ nanowires. The bare nanowires used for the experiments in Figure 2A were made of Au. We also commonly used Au as the core metallic portion of the silica-coated nanowires; since the core is completely covered by the silica layer its identity was not important to the experimental outcomes and in some cases Ag (Figure 4) or Rh (Figure 1) was used in place of the Au. Nanowires were either used directly or coated with silica in solution by the hydrolysis and condensation of tetraethoxysilane (TEOS), as previously described.^{38,53} Briefly, 490 μ L EtOH, 300 μ L nanowires at batch concentration, 160 μ L H₂O, 40 μ L TEOS, and 10 μ L NH₄OH were combined in a microcentrifuge tube, sonicated 1 hr, and then rinsed 3x EtOH.

Microscopy

Nanowires were imaged with reflected light using a Nikon TE-300 inverted microscope equipped with a Xe light source, Photometrics Coolsnap HQ camera, a Plan fluor 60x oil objective (N.A. 1.4), and Image-Pro Plus (v 7.0) software.

Preparation of DNA Sequences

DNA was received as a lyophilized pellet from Integrated DNA Technologies, Inc. Sequences are given in Table 2 and Supporting Table 1; the flu sequences were designed from the Influenza virus sequences, identified by the NIH basic local alignment search tool (BLAST) publicly accessible nucleotide list (<http://blast.ncbi.nlm.nih.gov/>), and the CDC approved primer and probe sets.⁵⁴ A seven base T AAC ATT spacer⁵⁵ or ten T spacer was added to the thiol-functionalized end of the probe sequences to provide spacing between the oligos and the surface of the nanowire after immobilization. Thiolated DNA was cleaved by resuspension in a 100 μ L 100 mM dithiothreitol (DTT) solution in 0.1 M sodium phosphate buffer, pH 8.3. After 1 hr the solution was purified using a Centriscin 10 desalting column (Princeton Separations). The fluorescently labeled target sequences were rehydrated in water. The concentrations of all sequences were calculated based on absorbance values taken at 260 nm using a Hewlett-Packard 8453 diode-array UV/visible spectrometer with Agilent ChemStation software. Sequences were diluted to 20 μ M in water and stored at -80°C .

DNA immobilization

To attach thiolated DNA oligonucleotides to gold nanowires, DNA with a 3 or 5 thiol (10 μ L, 20 μ M) was added to 20 μ L 6 μ m nanowires ($\sim 2 \times 10^7$ nanowires) rinsed into 490 μ L PBS buffer. After vortexing for 30 min, the nanowires were rinsed by centrifugation 3x PBS. Thiolated DNA was attached to silica coated nanowires as previously described.^{24,38} Briefly, silica-coated nanowires were incubated with the organosilane, APTMS, in ethanol to functionalize the silica surface with primary amines that were then reacted with the bifunctional crosslinker, Sulfo-SMCC to bind 5'-thiolated DNA oligonucleotides to the surface.³⁸ For the mismatch experiments, silica coated nanowires with P1_{FluB} covalently attached were rinsed into 35 μ L 5x SSC buffer (20x SSC = 3 M NaCl, 0.3 M sodium citrate, pH 7.0) and 5 μ L 20 μ M flu B target DNA (T1_{FluB} or T1_{MM}) was added (Table 2). The nanowires were tumbled or vortexed to keep them in suspension for 1 hr or overnight, followed by rinsing with 5xSSC.

Photoresist/remover treatment and post-treatment DNA hybridization

DNA-coated nanowires, 10 μ L ($\sim 1 \times 10^7$ nanowires), were rinsed into water, and were dried onto a glass microscope slide. Photoresist (Shipley 1813, PMGI SF6, or SPR 3012) was spun onto the glass slides (45 sec, 0.17 g) and the samples were baked as outlined (Table 1). Alternatively, DNA-coated nanowires were assembled using dielectrophoretic forces and integrated via photolithography as described in the text.

Two methods were used for hybridization of nanowire-bound DNA after exposure to photoresist: (1) removal of wires from the surface for hybridization in suspension, after which they were drop-cast for imaging, and (2) rinsing and hybridization in-place on the solid support. The only difference we observed between these methods was increased total fluorescence intensity for hybridization reactions performed in suspension presumably due to better mass transport. *Method 1:* After the photoresist was deposited, the glass slides were cut into $\sim 1 \times 3$ cm pieces and placed into 1165 photoresist remover (95% 1-methylpyrrolidinone), 50°C , 30 min. After removal of the photoresist, the pieces were placed into microcentrifuge tubes filled with water and were sonicated 1 hr, followed by 1 hr vortexing, to knock the nanowires off of the surface of the glass slides. Nanowires were spun down, rinsed 1x buffer, and were resuspended in either 190 μ L PBS and 10 μ L 20 μ M T1_{FluB} for the direct hybridization studies or 35 μ L 5x SSC buffer and 5 μ L 20 μ M T1_{FluB} or T1_{MM} (Table 2). The samples were vortexed overnight and then rinsed 4–8x buffer. *Method 2:* After the photoresist was deposited, the support containing the DNA functionalized nanowires were submerged in 1165 photoresist remover at 50°C for 30 min

or 90°C for 10 min. The supports were then submerged in water at room temperature for 10 min, then in ethanol for 10 min and were allowed to air dry. For hybridization, 1 mL of 1x PBS buffer was added to the supports and 20 μ L of 20 μ M T_{HCV} was added to each support. The nanowires were incubated with the target DNA overnight on an orbital shaker then excess 1x buffer was added to rinse the supports. The nanowires were imaged on the inverted microscope using appropriate filter cubes. Fluorescence intensity on the nanowires was quantified using NBSee software from Nanoplex, Inc.⁵⁶

Device Fabrication

Fabrication of Guiding Electrodes and Microwells for Nanowire Assembly

Guiding electrodes were patterned using a two-photoresist layer lithographic approach. Si substrates (3" diameter, with 1 μ m thermally grown SiO₂; Addison Engineering San Jose, CA) were spin-coated with PMGI SF-6 (Microchem) photoresist at 4000 rpm (0.17 g) for 45 s and placed on a hotplate at 190° C for 9 min to soft-bake. After cooling the wafer to room temperature, Megaposit SPR 3012 imaging photoresist (Microchem) was added by spin-coating at 4000 rpm (0.17 g) for 45 s, soft-baked on a hotplate at 95° C for 1 min., and cooled to room temperature. The resist-coated wafer was then carefully divided into 4 quarters using a diamond scribe for patterning using a GCA 8000 Stepper. The upper SPR 3012 photoresist layer was exposed using a reticle pattern, and the electrodes were patterned with widths of 50 μ m, lengths of 2000 μ m, and separated by a 5 μ m gap. Chips were then submerged in Microposit CD-26 (Microchem) developer for 1 min, and rinsed thoroughly with DI H₂O and dried with N₂ gas. This process removed exposed regions of the SPR 3012 as well as the underlying PMGI SF-6 in the exposed regions. Samples were treated with an oxygen plasma (Plasma Therm 720 RIE) to remove any residual photoresist in the features that had just been patterned (descum). Metal electrodes were then deposited in a home built thermal evaporator using 15 nm of Ti as an adhesion layer and 100 nm of Au. Following evaporation, excess evaporated metal deposited atop the photoresist (i.e., outside the windows opened up for metal electrode deposition) was lifted off by submerging the Si wafer into 1165 photoresist remover at room temperature for 24 hours. The wafers were rinsed thoroughly with DI water, acetone, then isopropanol and dried with N₂ gas.

Following electrode fabrication, microwells were patterned 0.5 μ m into a 1 μ m layer of PMGI SF-11 (Microchem). PMGI SF-11 photoresist (0.5 mL) was applied to the wafer quarters and then spun at 0.85g for 45 s, after which samples were soft-baked on a hot plate at 190°C for 9 min and allowed to cool to room temperature. Megaposit SPR 3012 was again used as the imaging layer. It was applied to the wafer pieces and spun at 0.85g for 45 s. The wafer pieces were then placed on a hotplate at 95° C for 1 min and cooled to room temperature. The wafer quarters were exposed to the wells reticle, where the wells were 2 μ m wide and 13 μ m long were patterned, spanning the electrode gaps. The wafers were developed in 351 developer (Microchem) that had been diluted in a 1:5 ratio with DI water for 1 min. This only removed the SPR 3012 imaging layer, leaving the underlying PMGI layer intact. Samples were descummed by RIE and then placed in the deep- UV flood chamber for 4 min, developed in 101A developer (Microchem) for 1 min, rinsed thoroughly with DI H₂O, and dried with N₂. Acetone was used to remove the top SPR 3012 imaging layer, and well depth was determined using a Tencor 500 profilometer. These conditions resulted in wells that were ~500 nm deep into the PMGI SF-11 layer, leaving 500 nm as a dielectric layer between the bottom of the wells and the Au electrodes. Another layer of SPR 3012 was applied to the wafer and baked using similar conditions mentioned above. In this case, the PMGI photoresist over the probe pads were fully removed to ensure electrical contact to the waveform generator during nanowire alignment. Again, the wafer pieces were exposed to the proper reticle using the stepper and were developed in CD-26 developer for 1 min, removing both the imaging photoresist and the remaining 500 nm of PMGI SF-11 over

the probe pads. The 3012 layer was again removed using acetone, and the wafer pieces were placed in the RIE chamber for descumming.

Alignment of DNA Functionalized Nanowires

Electrofluidic alignment of DNA functionalized nanowires was accomplished by applying a non-uniform electric field with a frequency of 1 MHz during nanowire injection at a voltage of 3 V_{rms} using a waveform generator between select sets of the Au electrodes. Nanowires functionalized with P2_{FluA} or P2_{FluB} suspended in ethanol were diluted to ~1:150 in ethanol, deposited on the substrate, and positioned into the middle gap by applying the ground to the left three electrodes using one probe and applying the non-uniform electric field to the right three electrodes using the other three probes. The ethanol was fully evaporated before the voltage was turned off, and the micromanipulator probes were removed. The microwells patterned into the PMGI SF-11 layer trapped the nanowires and assisted in keeping the nanowires in place during solvent evaporation. Samples were partially sealed during assembly using a glass coverslip to help control direction and rate of ethanol evaporation.

Fabrication of Metallic Contacts

Following alignment of the DNA coated nanowires, metal contacts were deposited over both ends of the nanowires to generate source and drain electrodes such as are used in nanowire field effect transistors (note that to facilitate fluorescence imaging, the wires used here were made of silica-coated metal rather than a semiconductor; hence the assembled devices, although prepared identically to FETs, are not functional FET devices). SPR 3012 photoresist was spin coated and baked onto the wafer using methods described above. Wafers were then exposed through a reticle using the GCA 8000 stepper and developed (CD-26 developer, 1 min.) to remove both the SPR 3012 and underlying PMGI SF-11 photoresists and descummed by RIE; this exposed the alignment electrodes, which would be used as working electrodes during contact fabrication by electrochemical deposition. All the probe pads/electrodes were electrically connected using a wire bonder. TG-25 gold plating solution (Technic Inc) was heated to 60° C and electrodeposited potentiostatically at 0.5 V vs Pt for 12 min. Orotemp 24 RTU Rack (Technic Inc) was electrodeposited in a similar manner but at room temperature for 10 min at a voltage of 2.50 V vs Pt gauze, resulting in anchor points of ~1 µm in height. After electrodeposition, chips were exposed to Microposit 1165 (Microchem) remover (50° C, 30 min.) to remove the photoresist, then rinsed in deionized H₂O and dried using N₂.

Electrically Isolating Aligned Nanowires

Following alignment and integration, SPR 3012 was spun onto the quarter wafers (0.85 g for 45 seconds) and soft-baked (95° C, 1 min.). Chips were exposed through the proper reticle with 365.4 nm light (i-line) and then developed in CD-26 developer, patterning lines 5 µm wide between individually positioned nanowires. The wafer was placed in the RIE chamber for descum, after which Cl₂ plasma was used to etch through the Au alignment electrodes in the lines that were photolithographically patterned, electrically isolating the individual nanowires from one another. The photoresist was then removed (1165 remover, 50° C, 30 min.) and chips were rinsed in DI water and dried with N₂.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Effect of resist and remover treatment on hybridization performance of probe DNA oligonucleotides bound to silica-coated metal nanowires. Fluorescence intensity after exposure of nanowire-bound P_{HCV} or P_{HIV} to the fluorescent DNA target sequence T_{HCV} . Signal intensities were normalized to the intensity of the untreated complementary control sample.

Figure 2.

Fluorescence intensity of hybridized flu B target DNA (T1_{FluB}) to complementary (P1_{FluB}, dark grey) or non-complementary (P1_{FluA}, light grey) immobilized probe DNA after exposure to and removal of photoresists on a surface. Probe DNA immobilized either (A) directly using thiol attachment to Au, or (B) using the bifunctional crosslinker Sulfo-SMCC to couple 5' thiol to amines on silica. To enable comparison of intensities between metal and silica-coated metal nanowires, these data were collected at the same time under the same conditions, and the raw intensities are shown (i.e. not normalized).

Figure 3.

Fluorescence intensity of hybridized perfect match target DNA (T1_{FluB}, dark grey) or target DNA with a single base mismatch (T1_{MM}, light grey) to flu B probe covalently attached to silica coated nanowires via Sulfo-SMCC chemistry on a surface post deposition and removal of photoresist.

Figure 4.

Selective DNA hybridization on fully-assembled nanowire device geometries prepared as in Scheme 1 and then exposed to fluorescent DNA that was complementary (**A**) or noncomplementary (**B**) to the probe DNA on the wires. Upper image in each set is reflectance optical microscopy and lower image is fluorescence. (**C**) Scanning electron microscope image showing microstructure of assembled structures. Scale bar = 10 μm .

Figure 5. Fluorescence intensities on complementary and non-complementary functionalized nanowires following two lithography steps (both with SPR 3012 photoresist/1165 remover) and a Cl₂ plasma etch for complementary (red) and non-complementary (green) DNA functionalized nanowires. Signal intensities were normalized to the intensity of the untreated complementary control sample.

Scheme 1.

Schematic of experiments performed to evaluate effects of photoresists and photoresist removers on the ability of surface-bound probe DNA oligonucleotides to detect a complementary DNA sequence. A two-strand direct hybridization format is shown; a three-strand sandwich-style format was also evaluated, in which binding of unlabeled target DNA sequences was visualized using a second, fluorescently-labeled probe sequence.

Scheme 2.

Schematic of a fabrication process to produce field effect transistors using PMGI to pattern microwells for nanowire assembly and two separate treatments with SPR 3012 resist/1165 remover to pattern windows to contact the wires and to electrically isolate them. **(A)** Side view of first part of the fabrication process. DNA-coated nanowires were assembled into microwells in a PMGI layer (orange) by applying nonuniform electric fields across pairs of guiding electrodes. SPR 3012 photoresist (red) was then spun-coat onto the chip, covering the DNA-coated nanowires. This resist was used to pattern windows to allow electrical contacts to be made via electrodeposition on each end of the wires, after which the DNA-coated nanowires were uncovered by treatment with 1165 remover. **(B)** Birds' eye view of second part of fabrication process. Electrical isolation of the individual nanowire devices required cutting through the alignment electrodes. This was done using a second treatment with SPR 3012 photoresist to protect the nanowire devices during reactive ion etching with a Cl₂ plasma, which removed unprotected portions of the metal electrodes. The photoresist was then removed with 1165 remover, after which a DNA hybridization assay was performed.

Table 1

Photoresists used in this work.

Name	Soft-bake Conditions (°C)	Composition ²³
Shipley Microposit S1813	100°C, 1 min.	71–76% propylene glycol monomethyl ether acetate 10–20% mixed cresol novolak resin 1–10% diazo photoactive compound 0.01–1.00% fluoroaliphatic polymer esters 0.01–0.99% cresol
Shipley Microposit S1805	100°C, 1 min.	81–86% propylene glycol monomethyl ether acetate 10–20% mixed cresol novolak resin 1–10% diazo photoactive compound 0.01–1.00% fluoroaliphatic polymer esters 0.01–0.99% cresol
Megaposit SPR 3012	95°C, 1 min	55–65% ethyl lactate 20–30% cresol novolak resin 7–12% anisole <10% organic siloxane surfactant 1–10% diazo photoactive compound 1–5% 2-methyl butyl acetate 1–5% n-amyl acetate <1% cresol
PMGI SF6	190°C, 9 min	65–85% cyclopentanone 10–15% tetrahydrofurfuryl alcohol 1–25% polyaliphatic imide copolymer

Table 2DNA sequences used in this work.^a

	Name	Sequence 5' 3'	Description
P _{HCV}	HCV Probe	Thiol-TTT TTT TTT TGA GTA GTG TTG GGT CGC GAA	Probe DNA with 5' ten T spacer with first 20 nt on 3' end perfectly complementary to HCV target
P _{HIV}	HIV Probe	Thiol-TTT TTT TTT TCC ATC AAT GAG GAA GCT GCA	Probe DNA with 5' ten T spacer with first 20 nt on 3' end perfectly complementary to HIV target and non-complementary to the HCV probe
T _{HCV}	HCV Target	Alexa Fluor 488-TTC GCG ACC CAA CAC TAC TC	20 nt sequence perfectly complementary to HCV probe
P1 _{FluB}	Flu B Probe 1, used in direct hybridization	TTT TCA GTT CTT TTACAAT- Thiol	Probe DNA with 3' T AAC ATT spacer with first 12 nt on 5' end perfectly complementary to Flu B Target 1
P1 _{FluA}	Flu A Probe 1, used in direct hybridization	GAC CAA TCC TGT TTACAAT- Thiol	Probe DNA with 3' T AAC ATT spacer to act as a non-complementary sequence to Flu B Target 1
T1 _{FluB}	Flu B Target 1, used in direct hybridization	AAG AAC TGA AAA-Alexa Fluor 647	12 nt sequence perfectly complementary to flu B probe 1
T1 _{MM}	Flu B Mismatch Target, used in direct hybridization	AAG AAA TGA AAA-Alexa Fluor 647	12 nt sequence with single base mismatch at position 6 from 5' end, otherwise complementary to Flu B Probe 1

^aSequences used for experiments in Figures 4 and 5 and Supporting Figure 3 are given in Supporting Information.