NANOSTRUCTURES AS DOPANT SOURCES: MASKLESS NANOSCALE PATTERNING OF SILICON USING SHAPE AND SIZE CONTROLLED NANOMATERIALS

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Abstract

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As miniaturization of electronic components continues, creative solutions for common manufacturing problems are required to continue the trend of “Moore’s Law.” Mask alignment for dopant patterning is one major set-back as nanoscale development continues. My thesis research investigates the use of DNA origami, a designable, self-assembled nanostructure, as a chemical and dopant source for patterning silicon substrates. My work explores the stability of DNA origami when exposed to extreme thermal and solvent environments and identifies the limitations of using these biomolecules in multistep nanofabrication processes, particularly the effect on DNA origami functionalizability. Utilizing this new understanding of DNA origami, I developed a process called “burn-in” to complete maskless patterning of embedded silicon carbide (SiC) “replicas” on silicon substrates. My work details the chemical and electrical characterization of the SiC patterns using a myriad of techniques. I focus primarily on scanning probe microscopy and x-ray photoelectron microscopy. Secondary ion mass
spectrometry is used to detect and measure depth profiles of phosphorus from the DNA origami after burn-in and illustrates the doping capacity of the burn-in process. Finally, I illustrate the broad application of the burn-in process by using other shape, size, and chemically controlled nanomaterials for maskless patterning of dopants. It is my hope that this work will expand the use of DNA origami and other traditionally “fragile” materials in unique processing environments and prompt the exploration of novel nanomaterials as creative solutions for nanoelectronic development applications.
For my family and teachers
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Figure 3.2 The “burn-in” processing steps begin with a cleaned silicon substrate functionalized with APTES and exposed to DNA origami solution. The sample is then capped with 50 nm of SiO2 using PECVD and annealing with RTP. The capping layer is then removed in 4% HF solution.

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I would like to thank all the teachers that have inspired me to pursue learning and to continue the exploration of ideas. Specifically, my first teachers, my parents, who have continued to emphasize scholarship as I’ve grown into adulthood. Second, my grandparents, who mean so much to me and who have taught me faith, patience, and love. I thank Ms. Howick for fostering a love of writing in me at a young age; Mrs. Engel for introducing me to chemistry and allowing me to grow as a leader; Dr. Wisian-Neilson for her academic and research guidance as well as her friendship and support; Dr. Goss for her training and unwavering positivity. Finally, I thank Dr. Lieberman, without whose patience, understanding, passion, and guidance this experience would have been far more difficult.

I am also thankful for the people who have supported me, whether emotionally or academically, through this journey. Your faith and support has meant the world to me.
1.1 Overview

“Moore’s Law” has set the precedent for research and development in the semiconductor industry for over 50 years. Gordon E. Moore predicted that “the number of components which can be incorporated per integrated circuit (IC) would increase exponentially over time,” which was later quantified as a “doubling [of components] every two years.”[1], [2] This has held true until recently when Intel’s development and implementation of the 14-nm node came more than 2.5 years after their 22-nm technology.[3] The deceleration of miniaturization may indicate the end of Moore’s Law, but scientists are hopeful that it will prompt renewed creativity in the evolution of IC fabrication and components.[4] To pursue further miniaturization and save Moore’s Law, the authors of the International Technology Roadmap for Semiconductors predict that sub-10 nm lithography is needed by 2022.[5] Among a myriad of necessary variables and topics addressed in the roadmap, mask defects are cited as a continued challenge in pursing this low dimension of device design, fabrication, and manufacturing.
Masks are a key component in photolithographic processing, which is the prevalent technique for micro- and nanofabrication. A basic photolithographic process is illustrated in Figure 1.1, where a specific wavelength of light is used to transfer the pattern defined by the mask onto the photoresist layer in step B. Multilayer architectures require aligning several different masks during fabrication.

Figure 1.1 A basic photolithographic process includes A) photoresist deposition; B) exposure to a specific wavelength of light through a patterned mask; C) removal of the modified photoresist to produce the mask pattern; D) etching to produce patterns in the silicon after the remaining photoresist is removed.
There are several disadvantages to using traditional photolithographic patterning as miniaturization of electronics continues, including the need for expensive equipment, light-sensitive materials, focusing optics, and masks. These problems contribute to both size limitations and patterning error. The error margin for aligning multiple critical layers at 20 nm half pitch is less than 4 nm and continues to decrease as components are miniaturized.[6] Ensuring this error margin is met requires tedious mask alignment steps, novel materials analysis techniques, and increases the price of fabrication. These factors have prompted scientists to pursue creative methods for patterning components on the nanoscale.

DNA origami has been proposed as an alternative patterning method for nanoelectronics for two main reasons. First, the shape and size of the self-assembling nanostructures can be lower than 100 nm with a 6 nm resolution.[7] The facile one-pot synthesis techniques makes it possible to produce millions of highly specific and consistent nanostructures in a short time. Second, DNA origami can be programmed to pattern non-DNA components at high resolutions. Funke et al. used a novel DNA origami hinge structure to place dye molecules with Bohr radius resolution (~0.05 nm).[8] Even more rudimentary designs, such as rectangles and nanotubes, have a 4-6 nm resolution patterning capability.[9] These properties make it possible to pattern components at the nanoscale without a mask.[10]

Incorporating DNA origami into current nanoelectronics architectures does not come without its challenges. Marrying the bottom-up capabilities of DNA origami with traditional top-down photolithography exposes fragile DNA to harsh physical and
chemical environments. Chapter 2 outlines my investigation into the stability and functionality of DNA origami after treatments at high temperatures and in nonaqueous solvents. These studies help identify the limitations of using DNA origami as a patterning alternative, providing better insight into the best way of utilizing the novel nanostructures.

Chapter 3 outlines the use of DNA origami in a novel maskless patterning process called “burn-in” and the production of embedded silicon carbide replicas patterned in the shape of DNA origami on silicon. These replicas are further studied in Chapter 4 through chemical and electronic analysis techniques, including secondary ion mass spectrometry (SIMS) and specialized scanning probe methods. Using the burn-in process with other nanomaterials and the prospects of its future applications are also described.

1.2 Introduction to DNA origami

The first example of structurally characterized DNA nanostructures were immobile junctions of oligomeric nucleic acids developed by Nadrian Seeman in 1982, the purpose of which was to immobilize proteins in 3D structures for easier X-ray crystallographic analysis.[11] Since then, the applications and structural complexity of the DNA designs have expanded drastically, particularly with the introduction of crossover DNA tiles that provided structural rigidity and designability. A breakthrough in structural DNA nanotechnology was introduced in 2006 by Paul Rothemund with the concept of “DNA origami.”[12] Unlike previous DNA nanostructure designs and synthesis techniques, which used many individual oligonucleotide strands requiring precise
stoichiometry and producing low yields, DNA origami was based on the use of a DNA scaffold “template strand” and interactions with shorter oligonucleotide “staple strands.” The facile, one-pot synthesis technique results in designable nanoscale structures at high yields and low error rates. Designs are most often based on the M13mp18 plasmid as the template strand. The sequence of the ~7,429 nucleotide long circular plasmid is known and oligonucleotide strands of 20-30 bases can be computationally designed to interact at specific locations on the template strand. Rapidly heating the solution of DNA origami components to 90°C and slowly cooling to room temperature induces hybridization of the template and staple strands and folding of the DNA origami structure. The first reported DNA origami structures were two-dimensional (2D) and varied from stars to smiley faces (Figure 1.2).

![Figure 1.2](image)

Figure 1.2 Two-dimensional DNA origami designs introduced by Paul Rothemund. Varying the staple strand sequences changes the folding pattern of the nanostructures, providing diverse structural potential. (Reproduced with permission from Ref. [12])
3D structures have also been introduced, such as the spherical and vase shapes in Figure 1.3, illustrating the diverse potential of this nanostructure self-assembly technique.[13] The self-assembling nature of DNA origami also provides the ability to functionalize individual staple strands that can be utilized as molecular pegboards to arrange various components, such as small molecules, proteins, and nanostructures, with resolutions of 4 to 6 nm.[14]

Figure 1.3 3D DNA origami “vase” designs illustrate the complexity that can be designed into DNA origami. (From Han, D. et al. Science 2011, 332, 342-346.[13] Reprinted with permission from AAAS.)

My work primarily utilized a rectangular DNA origami design with dimensions of ~70 nm x ~90 nm first introduced by Woo et al. (Figure 1.4).[15] Extra staple strands were included in the design to promote pi-stacking between the rectangles to create
long, self-assembled chains of the nanostructures (Figure 1.4). Additionally, hairpin staple strands were included to create small bumps on the top of the rectangles to include an extra design component. I used hairpins to create an “L” shape that can be used to identify the orientation of the rectangles with one another.

Figure 1.4 The viral “template” strand and short oligonucleotide “staple” strands assemble a rectangular structure. Hairpin loops can be added to introduce 1 nm “bumps” on the surface. The addition of pi stacking staple strands on the edges promotes interaction between DNA rectangles to create chains. This atomic force microscopy image shows several DNA origami chains deposited on mica. The AFM height scale is 5 nm.

1.2.1 DNA origami synthesis

Oligonucleotide staple strands for all DNA origami designs were purchased from Integrated DNA Technologies, Inc. and received at concentrations of 100 µmol/L in 96 well plates. They were purified by the manufacturer by standard desalting and were stored at -80°C when not in use. The staple strands can last for several years if stored properly. The M13mp18 single stranded phage DNA template strand was purchased
from Bayou Biolabs at a concentration of 100 µmol/L. A stock solution of staple strands was prepared using 4 µL aliquots of each staple strand sequence. The final volume of the staple strand stock solution was brought to 1000 µL by adding 18 MΩ sterile water (this stock solution was also stored at -80°C). DNA origami is formed in a 10x TAE/Mg\(^{2+}\) buffer solution prepared by combining 400 mM Tris base, 200 mM acetic acid, 20 mM ethylenediaminetetraacetic acid (EDTA), and 125 mM magnesium acetate tetrahydrate at pH 8. The M13mp18 viral DNA, 10X concentrated TAE/Mg\(^{2+}\) buffer, 18 MΩ water, and staple strands solution were combined to create the DNA origami solution. To ensure the highest efficiency of formation, the ratio of M13mp18 to staple strand mixture is fixed at 1:10. Additionally, a ratio of 1:10 buffer to total DNA origami solution volume is also used. Table 1.1 includes the relative amounts of each component to produce various DNA origami concentrations.

<table>
<thead>
<tr>
<th>Component</th>
<th>2 nM</th>
<th>4 nM</th>
<th>6 nM</th>
<th>12 nM</th>
<th>24 nM</th>
</tr>
</thead>
<tbody>
<tr>
<td>M13mp18 template (µL)</td>
<td>2</td>
<td>4</td>
<td>6</td>
<td>12</td>
<td>24</td>
</tr>
<tr>
<td>Staple strand mix (µL)</td>
<td>20</td>
<td>40</td>
<td>60</td>
<td>120</td>
<td>240</td>
</tr>
<tr>
<td>10X TAE/Mg(^{2+}) buffer (µL)</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>18 MΩ sterile water (µL)</td>
<td>338</td>
<td>316</td>
<td>294</td>
<td>228</td>
<td>96</td>
</tr>
<tr>
<td>Total volume (µL)</td>
<td>400</td>
<td>400</td>
<td>400</td>
<td>400</td>
<td>400</td>
</tr>
</tbody>
</table>
The DNA origami solution is then annealed to induce hybridization of the template and staple strands. The annealing time is dependent on the DNA origami design being synthesized, with more complex designs requiring several days of annealing to form correctly. However, for simple DNA origami designs like the rectangle, the solution was ramped to 90°C over 2 minutes, held at 90°C for 5 minutes, and cooled to 20°C over 70 minutes (1°C/min). Depending on the application, the DNA origami solution can be centrifuge filtered to remove excess staples strands. However, this step was rarely performed because the excess staple strands do not stick well to the substrates and cause minimal interference with the nanostructures.

1.3 Introduction to DNA origami on surfaces

The expansion of DNA origami designs prompted exploration into novel applications, many of which require adhesion of the DNA to surfaces. The most common surface for DNA analysis is mica, which is very easy to use. The mechanism of attachment will be discussed in Section 1.3.1. However, a myriad of surfaces and attachment interactions have been explored. Gold has been a popular substrate, particularly due to the ability to promote interactions between DNA origami functionalized with thiol groups and lithographically defined gold islands.[16] Yun et al. reported the attachment of DNA origami to graphene oxide (GO) flakes functionalized with aminopropyltrimethoxysilane (APTS) via electrostatic interactions, specifically with the abundance of Mg²⁺ ions on the surface, a theme of DNA origami surface adsorption.[17] Börjesson et al. attached DNA origami to lipid membranes via
functionalization with porphyrin anchors, an interaction that is not rigid and allows for the diffusion of the DNA nanostructure on the membrane surface.[18] Although these substrates are of great interest to the DNA origami research community, my work focuses on two more common surfaces: mica and silicon.

1.3.1 DNA origami on mica: Mechanism of attachment and sample preparation

The most popular substrate for the study of DNA is muscovite mica, the structure of which is illustrated in Figure 1.5. Consisting of aluminosilicate sheets electrostatically bound with intercalating potassium ions, the layers can be easily cleaved using double sided tape, revealing a clean, smooth surface.[19], [20]

Figure 1.5 This side view of muscovite mica illustrates the aluminosilicate sheets with intercalating potassium ions. (Figure reproduced with permission from Ref. [20])
Facile cleaning and smooth surfaces are not the only reasons mica is a popular substrate for DNA analysis. When mica substrates are exposed to DNA in the TAE/Mg\textsuperscript{2+} buffer described in Section 1.2.1, the K\textsuperscript{+} of the muscovite mica desorb from the surface, exposing negatively charged hydroxyl groups; the Mg\textsuperscript{2+} from the buffer then replace these ions, creating a net positively charged surface.[21] Charge inversion on the surface promotes the electrostatic interaction between the substrate and the negatively charged DNA phosphate backbone. This type of salt bridge can be achieved using other divalent cations, including Ca\textsuperscript{2+} and Zn\textsuperscript{2+}, as well as minerals containing divalent cations, such as calcite, fluorite, and barite.[21] These basic principles of DNA adsorption to mica can also be applied to DNA origami, with Mg\textsuperscript{2+} bridging the mica substrate and the DNA origami phosphate backbone (Figure 1.6).

![Diagram of DNA origami on mica](image)

Figure 1.6 DNA origami adsorption to mica is promoted by the presence of Mg\textsuperscript{2+} ions (spheres) creating a salt bridge between the negatively charged mica substrate and the negatively charged DNA phosphate backbone (not to scale).

The number of phosphate groups in a rectangular DNA origami is estimated at \(~15,000\) and buffer solutions usually contain 12.5 mM Mg\textsuperscript{2+}. Optimal binding of DNA
origami to mica occurs if the DNA and the surface have about the same, very high charge density.[22] The DNA origami remain attached after aqueous rinsing and drying with N₂, although rearrangement can occur after repeated wetting and the introduction of certain concentrations of monovalent ions.[23], [24]

Coverage of DNA origami on mica is dependent on solution concentration. Figure 1.7 illustrates DNA origami on mica deposited from several solution concentrations.

Figure 1.7 DNA origami deposited from solutions of A) 2 nM, B) 4 nM, and C) 6 nM after 10 minute exposure. Increasing solution concentration increases DNA origami surface coverage. All images have a height scale of 5 nm. Figure reproduced with permission from Ref. [19]

Table 1.2 includes percent coverage calculations for these samples. DNA origami undergoes a rapid initial surface binding with minimal coverage variation upon increased deposition time, making concentration the most important parameter to control DNA origami surface coverage.[14]
TABLE 1.2

PERCENT COVERAGE FOR DNA ORIGAMI DEPOSITED ON MICA WITH VARIED CONCENTRATION

<table>
<thead>
<tr>
<th>Concentration</th>
<th>2 nM</th>
<th>4 nM</th>
<th>6 nM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent Coverage on Mica Substrate(^a)</td>
<td>8.5 ± 2.7%</td>
<td>55.9 ± 5.7%</td>
<td>77.4 ± 1.9%</td>
</tr>
</tbody>
</table>

\(^a\) All calculations completed with 5 images from different locations on the sample.

Rinsing and drying parameters are also important for sample preparation. This is often not addressed in detail in the literature, but is instrumental in producing reproducible and good quality samples. Rinsing the sample with sterilized 18 MΩ water ensures that excess DNA origami staple strands and salts from solutions are eliminated from the surface. Drying with a steady stream of purified nitrogen (N\(_2\)) removes surplus solvents and spreads the DNA origami evenly on the substrate. Figure 1.8 illustrates several possible outcomes if samples are not properly rinsed and dried. Without drying the surface, excess solvent produces aggregates with no discernible nanostructures (Figure 1.8A). Partially dried substrates can produce two types of islands: large aggregates (Figure 1.8B) and interconnected DNA origami islands (Figure 1.8C). Rinsing with 200 μL of sterile 18 MΩ water and drying for 3 minutes with a steady stream of purified N\(_2\) produces consistently good quality DNA origami on mica and functionalized silicon (described in Section 1.3.2).
Figure 1.8 AFM images of three poorly rinsed and/or dried samples with A) solvent on the substrates, B) solvent and DNA origami aggregates due to excess solvent and inadequate drying, and C) DNA origami interconnected islands after poor drying. Figure reproduced with permission from Ref. [19]

Although useful for basic imaging and some biomedical applications, mica is not suitable for nanoelectronic applications. The layered, insulating mineral produces particulates that are not welcome in cleanroom environments. The attachment of DNA origami to alternative substrates, particularly semiconductors, is required to explore the use of these nanostructures in electronics.

1.3.2 DNA origami on functionalized silicon substrates: mechanism of attachment and sample preparation

Due to the low surface charge of SiO₂, DNA origami does not readily electrostatically interact. Albrechts et al. showed that Mg²⁺ concentrations greater than 100 nM are required to increase the surface charge sufficiently to promote electrostatic interaction between the DNA origami and the SiO₂ substrate.[25] Unfortunately, high
concentrations of salts can induce aggregation of DNA origami and are common contaminants in many microelectronic architectures.[26] To avoid these problems, a self-assembled monolayer (SAM) of 3-aminopropyltriethoxysilane (APTES) can be used to promote DNA origami adhesion to silicon with a thin native oxide. A silicon chip etched with 4% HF and cleaned in piranha solutions is functionalized with APTES by soaking for 20 minutes in 1% aqueous APTES solution at 25°C. The formation of the APTES monolayer proceeds via the hydrolysis of the silane which drives the attachment of the APTES to the surface resulting in a covalent siloxane bond with the substrate (Figure 1.9). The quality of the monolayer is mostly dependent on deposition time, with longer times producing thicker APTES films.[27]

![Diagram](image)

Figure 1.9 After cleaning silicon with piranha solution to produce a hydroxyl terminated substrate, exposure to 1% aqueous APTES solution promotes the creation of covalent siloxane bonds, producing an APTES monolayer.
A smooth silicon substrate is necessary to produce an even monolayer. Clean silicon has a roughness of less than 0.1 nm/µm² (Figure 1.10A). If the silicon is not properly cleaned, an incomplete APTES film is formed on a rough oxide surface, as seen in Figure 1.10B where the surface roughness is 2.29 nm/µm². Additionally, due to the reactivity of APTES with water, it readily undergoes hydrolysis and can polymerize in solution, producing aggregates on the APTES film (Figure 1.10C).

![Figure 1.10 AFM images of A) clean silicon, B) APTES deposited on silicon that was not properly cleaned and etched, and C) APTES monolayer with large aggregates due to exposure of the APTES supply with water and subsequent hydrolysis and aggregation. The height scale for all images is 5 nm. Figure reproduced with permission from Ref. [19].](image)

APTES has a pKₐ of ~8.5 and produces a primarily positively charged surface upon exposure to the DNA origami TAE/Mg²⁺ buffer solution described in Section 1.2.1, which has a pH of 7.5.[28], [29] The positively charged terminal amine of the APTES
functionalized silicon interacts with the negatively charged DNA origami phosphate backbone, analogous to the electrostatic interaction present on mica (Figure 1.11).[14]

Figure 1.11 DNA origami adsorb to APTES functionalized Si/SiO₂ in solutions of above pH 7 due to the protonated terminal amine which provides the positive charge for electrostatic interaction with the negatively charged DNA origami backbone (not to scale).

Varying the surface charge density of the surface changes the deposition interaction. Mica in the presence of the ~12 mM Mg²⁺ in solution has a slightly higher charge density than a protonated APTES substrate (0.75-1.5 charges/nm²), resulting in a lower DNA origami coverage.[14], [30] The 7.1% coverage of DNA origami on APTES functionalized silicon (Figure 1.12A) compared to 54.8% coverage on mica (Figure 1.12B) illustrates the effect of surface charge on DNA origami adhesion (experiments used the same DNA origami concentration and deposition time).
Figure 1.12 Due to the lower surface charge density, DNA origami does not adsorb as readily to A) APTES functionalized silicon substrates as compared to B) mica substrates treated with Mg2+. Images have 5 nm height scale.

An additional advantage of using APTES SAMs on silicon is the ability to pattern the SAMs via a method called “molecular liftoff.” Sarveswaran et al. used cleaned silicon covered with a thin layer of poly(methyl methacrylate) (PMMA) and electron beam lithography to pattern 20-30 nm wide “damaged” PMMA.[30] These regions were removed and APTES SAMs were covalently bound to the exposed SiO2. Removal of the excess PMMA via sonication in acetone or dichloromethane did not affect the APTES SAM, which formed “sticky” regions on the substrate where DNA origami preferentially bind.[14] The ability to place DNA origami on designated regions of silicon substrates marries the bottom-up capabilities of the self-assembled nanostructures and the top-
down patterning of traditional lithographic techniques, allowing for novel patterning and device design.

1.3.3 Patterning surfaces using DNA origami masks

Utilization of DNA origami shapes as a template for both patterning materials and masking substrates has been reported. Deng et al. reported the production of a negative replica of DNA nanostructures in Au/mica after metal evaporation and lift-off.[31] Li et al. metalized DNA origami via Ag seeding and electroless deposition of Au to produce DNA-templated metallized structures on mica.[32] Pearson et al. used this technique to create electrically conductive nanowires in the shape and at the location of DNA origami on SiO$_2$.[33] Ag seeding and Au reduction was used by Jin et al. to metallize DNA origami immobilized on graphene, which masked the underlying graphene layers during reactive ion etching and plasma treatment to pattern graphene in the shape of the DNA origami mask.[34] Surwade et al. reported SiO$_2$ etching using a DNA origami mask with either positive or negative spatial information transfer depending on the etching conditions (specifically humidity).[35] This and similar etching processes have been used to pattern inorganic oxides and metal nanostructures in the shape of etched DNA origami silhouettes.[36], [37]

In these studies, DNA origami underwent micro- and nanofabrication procedures unusual for biomolecules, particularly elevated temperatures and chemical deposition environments. Although it is evident that the spatial information of DNA origami is transferred during these processes, the chemical and functional characteristics of DNA
are destroyed. It is necessary to identify the chemical stability of DNA origami in these environments for a better understanding of the limits in nanofabrication, particularly the ability to utilize DNA origami chemical programmability for placement of other nanomaterials.
CHAPTER 2:

STABILITY AND FUNCTIONALITY OF DNA ORIGAMI AFTER EXPOSURE TO HIGH TEMPERATURE OR SOLVENTS

2.1 Overview

Integrating DNA origami with large scale deterministic lithographic patterning is one possible avenue to utilize the sub-10 nm patterning capabilities of DNA nanostructures. Using electron beam lithography to pattern DNA origami on a substrate was discussed in Section 1.3.2, particularly utilizing functionalized silicon and the molecular liftoff processes.[14], [30] Kershner et al. exploited EBL patterning to deposit DNA origami on SiO$_2$ and diamond-like carbon in the presence of ~100 mM MgCl$_2$.[9] Gold has also been lithographically patterned on substrates and DNA origami attachment mediated in two ways. Gerdon et al. modified lithographically patterned gold with 11-mercaptoundecanoic acid. The carboxylic acid terminated monolayer chelates Mg$^{2+}$ in the DNA origami buffer solution and promotes nanostructure binding via electrostatic interactions.[38] Conversely, Ding et al. modified the DNA origami structure with thiol-containing staple strands to promote Au-S binding on lithographically patterned gold dots.[16] These patterning processes expose the substrate to DNA origami solutions after completion of all fabrication steps. However,
most nanoelectronic architectures require multiple patterning steps to create complex components. For example, inorganic or oxide structures might have to be deposited on top of the DNA origami layer. These processes often require heating a substrate to elevated temperatures or exposure to nonaqueous solvent environments, which could damage the DNA nanostructures. SiO₂ deposition via plasma enhanced chemical vapor deposition (PECVD) is carried out at temperatures of 150°C to 250°C; pre- and post-baking of photoresist covered wafers is achieved on a hotplate between 100°C and 150°C; and metal deposition requires thermal annealing at temperatures as high as 600°C.[39], [40]

DNA in solution is not very thermally stable. The melting temperature of duplex DNA in buffer solution can be predicted using the number of base pairs, the G:C ratio, the concentrations of monovalent and divalent cations, and the buffer pH.[41] For duplex DNA of lengths used in DNA origami, the melting point is around 90°C.[42] DNA nanostructures, such as tile arrays and origami, consist of normal duplex DNA with a high density of crossover structures that interweave neighboring helices. These additional structural components have not been shown to increase thermal stability in solution, where DNA origami begin to lose their structures at about 50°C and are completely denatured at temperatures above 70°C.[43], [44] Even including photo-cross-linked DNA strands in DNA origami nanostructures to promote thermal stability in solution only slightly increased the melting temperature, with structural integrity decreasing at 85°C and complete denaturation occurring at 90°C.[45] Although denaturation occurs, the damage has been found to be reversible in the presence of
excess staple strands; in fact, thermal denaturation followed by annealing is the most common method for assembly of DNA origami and other nanostructures, these published studies only pertain to DNA or DNA origami in solution.[12], [46]–[49] Integration of DNA origami into electronic, photonic, and magnetic nanosystems requires deposition of the nanostructures onto substrates and removal of the aqueous environment. I hypothesized that the absence of water molecules to stabilize partially dissociated DNA segments as well as the increased electrostatic interaction between the magnesium ions and the phosphate backbone on the substrate would allow the DNA origami shape, size, and interaction with the substrate to be maintained in air and at temperatures above the solution melting point.[50]

An additional “hazard” for DNA origami in common lithographic processes is the use of nonaqueous solvents. Substrates are exposed to solvents during washing steps as well as for photoresist stripping, usually using acetone, dichloromethane, or a formulated photoresist stripping agent.[51] Reports of exposing DNA oligonucleotides or DNA nanostructures to nonaqueous solvents are limited, especially pertaining to DNA origami deposited on a solid substrate. The main concerns are desorption of the nanostructures from the surface and loss of staple strands during treatment. Investigations into post-DNA origami deposition “click” chemistry reactions by Voigt et al. exposed nanostructures to a dilute dimethylformamide (DMF) solution for approximately 20 minutes during the completion of single-molecule reactions on functionalized DNA origami.[52] The degradation of the DNA origami structure was reported but not investigated further and could not be attributed to DMF exposure.
Dean et al. evaluated the stability and functionality of single-stranded DNA oligonucleotides immobilized via thiol-gold interactions with gold nanowires after photoresist deposition, baking, and stripping.[53] DNA oligonucleotides showed no difference in complimentary DNA strand binding. Investigations of DNA origami stability in nonaqueous solvent environments are required to fill a vast knowledge gap. Here I report the consequence of acetone and dichloromethane exposure, two common photoresist stripping solvents, on DNA origami structure, substrate adhesion, and functionality. Through these investigations, the effect of multistep processing on DNA origami structure and function is also illustrated, outlining limitations of their use in current photolithographic processing steps.

2.2 Experimental methods

2.2.1 DNA origami design

The rectangular DNA origami described in Section 1.2 was used for these studies. This dimensions of these nanostructures is $1.62 \pm 0.43$ nm ($N=5,080$ structures) high, $90 \pm 6$ nm long, and $69 \pm 5$ nm wide ($N=100$ structures). The design includes two fine structure details, highlighted in Figure 2.1: A) a small loop of single stranded DNA protruding from one edge of the rectangle and B) a letter “L” on the surface of the rectangle composed of staple strands containing bulky hairpin turns of ~0.5 nm in height.[12] Analysis of fine structure details were strongly dependent on AFM image quality. At least four images were acquired per sample. AFM tips were changed approximately every 12 images to ensure good image quality.
Figure 2.1 An AFM image (left) and cartoon (right) of a DNA origami rectangle design with fine structure details: A) the small loop of single-stranded DNA; B) the letter “L” on the top surface of DNA origami created by staple strands that form bulky hairpin turns.

The rectangular DNA origami design was changed to investigate functionality. The hairpin containing staple strands were omitted. Three 5’ biotinylated staple strands were included in the design and purchased from Integrated DNA Technologies, Inc. (Coralville, IA) with a thymine spacer sequence (see Appendix A: for strand sequences). Once DNA origami was deposited on the substrate and dried, the samples were exposed to streptavidin solution (New England Biolabs, Ipswich, MA) in phosphate buffer diluted to the desired concentration (see Section 2.3.4 for concentration optimization experiments). The locations of these interactions on the DNA origami design are illustrated in Figure 2.2 (left) using the original, color-coded DNA origami
The AFM images (Figure 2.2, right) show biotin-streptavidin bumps on DNA origami rectangles.

Figure 2.2 (Left) Three biotinylated staple strands are substituted in the original DNA origami rectangle design and locations corresponding to the white ovals. (Right) The biotin-streptavidin interaction produces bumps on the DNA origami. The AFM height scale is 5 nm.

The ~1 nm bumps on the DNA origami correspond to the biotin-streptavidin locations. Profiles for these bumps are included in Figure 2.3.
Figure 2.3 AFM line profiles through the biotin functionalized DNA origami: 1) dotted line is the ~2 nm background height of the DNA origami; 2) dashed and 3) solid lines include one and two ~1 nm biotin-streptavidin interactions (indicated by “X”), respectively.

2.2.2 Sample preparation procedures

Mica sheets of 5 cm x 7 cm (Electron Microscopy Sciences, Hatfield, PA) dimensions were cut into approximately 1 cm x 1 cm squares using scissors. The top layer of the mica substrate was then removed several times using double sided tape, exposing a clean, flat surface. A 5 μL aliquot of 4 nM DNA origami was deposited on the
mica substrate for 10 minutes at room temperature. The sample was rinsed with 200 µL of water and dried for 3 minutes with N₂.

Functionalized silicon substrates were prepared as outlined in Section 1.3.2. Contact angle measurements were performed using a Kruss G10 Contact Angle Measuring System. A 5.0 µL droplet of 18 MΩ water was deposited on the substrate at room temperature and the static contact angle was immediately recorded.

For the thermal stability studies, a programmable hotplate (Cole-Parmer 04644 Series Programmable Digital Hot Plate Magnetic Stirrer) was used to heat the samples to the required temperatures. The temperature of the hotplate was also monitored using an IR thermometer to ensure consistency during heating. To probe functionality, the samples were cooled for an hour before streptavidin solution exposure. When heating to temperatures higher than 250°C, a tube furnace was used. The tube furnace was programmed to ramp to a specific temperature; once the temperature was reached, the sample was introduced into the furnace. Inlets on the tube allowed for introduction of various gases, allowing for reactions in air and N₂.

Acetone and dichloromethane (CH₂Cl₂) were the primary solvents utilized due to their pervasive use in nanofabrication, particularly for photoresist stripping and substrate washing procedures. Unless otherwise stated, 2 mL of solvent was poured in a scintillation vial immediately before use. Mica or functionalized silicon substrates with DNA origami (as described in Section 1.3) were placed into the scintillation vial for various times. The substrates were then dried with purified N₂ for 2 minutes before characterization. Functionality studies were carried out as described in Section 2.2.1.
2.2.3 Atomic force microscopy analysis

A Digital Instruments Multimode Nanoscope V Scanning Probe Microscope was used in tapping mode. NSG30 non-contact probes were purchased from K-TEK Nanotechnology (Wilsonville, OR). The probes had a spring constant of 22-100 N/m and resonance frequency of 240-440 kHz.

2.2.4 “NanoScope Analysis” procedure for DNA origami dimension measurements

Height and nanostructure count were determined using the “Particle Analysis” tool incorporated in Bruker NanoScope Analysis software. Visual inspection of the AFM image allowed for the adjustment of the “Threshold Height” to ensure complete selection of all nanostructures (highlighted in Figure 2.4). The average height and total number of nanostructures of all points highlighted is calculated by the program. Due to the random deposition of DNA origami on the surface, it is possible that two or more nanostructures were located close enough to each other that the program was unable to differentiate between several individual DNA origami. This would cause several “clumped” nanostructures to be counted as one. This may result in the total nanostructure count reported here to be lower than the actual number of nanostructures in the image. However, this effect would not interfere with the average pixel-by-pixel height calculations.
Figure 2.4 A screen shot of the “Particle Analysis” tool in the Nanoscope Analysis software. The blue areas on the AFM image indicate nanostructure with height that are within the determined “Threshold Height” and are the nanostructures included in the total count.

The length and width of the DNA origami rectangles were determined using the “Section” tool of the NanoScope Analysis software. The lengths and widths of each rectangle were measured independently. The edges of each rectangle were determined visually. The dimensions were measured at the approximate center of each side, illustrated by the vertical red and blue lines in the line profiles in Figure 2.5 (the blue line corresponds to length; the red line corresponds to width). The dimensions of at least 10 nanostructures from 3 separate samples were analyzed unless otherwise stated.
Figure 2.5 A screen shot of the “Section” tool in the NanoScope Analysis software. Lines were drawn across each structure to measure the topography in that specific direction. From the topographical information, the edges of the structure can be approximated and the distance between each edge (i.e. the lateral dimensions) were determined. This procedure was repeated for all measured nanostructures.

2.2.5 X-ray photoelectron spectroscopy parameters and spectral analysis procedures

XPS spectra were acquired using a PHI VersaProbe II Scanning XPS Microprobe. Survey spectra used a 100µm 25W 15kV x-ray setting with a pass energy of 187.50 eV over 10 cycles. For higher resolution elemental scans, the pass energy was changed to 23.500 eV over one cycle of between 10 and 30 sweeps depending on the element being analyzed. Electron neutralization was used with an emission current of 20 µA, a bias voltage between 1.0 and 1.6 V, extractor lens voltage of 30 V, and filament current of between 1.05 and 1.20 A. Ion gun neutralization using Ar⁺ was used with a 0.500 kV beam, target emission of 15 mA, and a grid supply initially set to 200 V. The XPS data was processed using CasaXPS software with atomic sensitivity factors provided by Physical Electronics, Inc. The background type used was Shirley and a line shape of 30%
Gaussian and 70% Lorentzian was used for peak fitting. Relative ratios were calculated with respect to the K\(_{2p \ 3/2}\) peak area. Mica contains interstitial potassium cations with XPS signals observed conveniently in the C\(_{1s}\) binding energy window. The concentration of potassium was assumed to be consistent between locations and samples, which made it ideal for calculating relative ratios of the various elements.

### 2.2.6 Acknowledgment of undergraduate research contributions

The nature of this project made it ideal for undergraduate research participation. Students were mentored and trained in sample preparation, AFM imaging, image analysis, and data processing. Thermal stability of DNA origami on mica at high temperatures using the tube furnace was investigated by Adam Farchone and Rose Doerfler. Contact angle measurements of heated APTES functionalized silicon samples were completed by Keenan Linder. Corey Gavin aided in substrate cleaning and sample preparation. Samples for acetone and dichloromethane exposure studies were prepared and analyzed by Rebecca Shute. Their results were presented at scientific conferences and they contributed as publication co-authors.[19], [54]
2.3 Results

2.3.1 Changes in DNA origami shape at elevated temperatures on mica and functionalized silicon

The stability of DNA origami adhered to substrates after heating was evaluated by monitoring shape, dimensions, and fine structure details. The only visible change in DNA origami after heating was a ~50% decrease in height after exposure to 250°C for 10 minutes in air (Table 2.1 and Figure 2.6C). The lateral dimensions of the DNA origami at both 150°C and 250°C remained consistent.

### TABLE 2.1

DNA ORIGAMI DIMENSIONS AFTER HEATING TO 150°C OR 250°C ON MICA

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Height (nm)</th>
<th>Length (nm)</th>
<th>Width (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.62 ± 0.43 (N=5,080 structures)</td>
<td>90 ± 9 (N=100 structures)</td>
<td>69 ± 5 (N=100 structures)</td>
</tr>
<tr>
<td>150°C/10 min.</td>
<td>2.02 ± 0.57 (N=7,967 structures)</td>
<td>97 ± 8 (N=100 structures)</td>
<td>76 ± 7 (N=100 structures)</td>
</tr>
<tr>
<td>250°C/10 min.</td>
<td>0.96 ± 0.22 (N=4,146 structures)</td>
<td>92 ± 6 (N=100 structures)</td>
<td>75 ± 5 (N=100 structures)</td>
</tr>
</tbody>
</table>
Figure 2.6 DNA origami on mica after A) no heating, B) heating to 150°C for 10 minutes, and C) heating to 250°C for 10 minutes. It is only at 250°C that structural changes are identified in the nanostructures, particularly a ~50% decrease in height. All images have 5 nm height scales.

Analyzing the “fine structure” details described in Section 2.2.1 provides a more detailed insight into the changes in structure upon heating. Control experiments showed 65% (N=100 nanostructures) of the counted nanostructures included a single stranded loop and 58% (N=100 nanostructures) showed a hairpin “L”. After heating to 150°C for 10 minutes, the nanostructure maintained fine structure details, with 75% (N=235 nanostructures) of structures containing a single-stranded loop and 55% (N=235 nanostructures) displaying a visible letter “L”. The fine structure details were not discernible after heating to 250°C.

Even after heating to 150°C for 45 minutes, the DNA origami exhibited structure stability (Figure 2.7). The average height was 1.49 ± 0.30 nm (N=1,546 nanostructures) and lateral dimensions were 94 ± 15 nm and 72 ± 6 nm (N=60 nanostructures), 57% structures showed single-stranded loops and 52% included a hairpin “L”.

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A tube furnace was used to probe the effect of temperatures higher than 250°C and in both oxidative and inert environments. The dimensions of DNA origami on mica heated in the tube furnace at 250°C or 500°C in air or N₂ are included in Table 2.2.
The changes in dimensions tabulated in Table 2.2 are also visible in the AFM images included in Figure 2.8. In particular, the height of DNA origami heated in air to 250°C (Figure 2.8A) is ~50% of control DNA origami, consistent with what was reported for heating on a hot plate (Table 2.1 and Figure 2.6).[55] Comparatively, when heated to 250°C in an inert N₂ environment (Figure 2.8B), the height decreases by ~25% compared to controls. The nitrogen environment minimizes the structural changes which indicates that oxidation is the primary means of degradation.
Figure 2.8 The shape and lateral dimensions of DNA origami is maintained when heated in air at 250°C (A) and N₂ at 250°C (B) and 500°C (D). A slight height change is visible and measured in Table 2.2. Heating in air at 500°C (C) results in breaking of the nanostructures and aggregation (see Figure 2.9 for more detailed image).

When the temperature is increased to 500°C in N₂ (Figure 2.8D), the average height remains within standard deviation of that measured for the 250°C treatment, indicating further degradation does not occur in inert atmosphere. However, when the DNA origami was heated to 500°C in air (Figure 2.8C), it became increasingly difficult to complete dimension measurements due to the “breaking” of the DNA origami structure. This effect is illustrated in greater detail in Figure 2.9. Although a “shadow” of the DNA
origami shape is still visible, complete nanostructures are not present. Additionally, the average height of the nanostructures increased to $1.8 \pm 0.5$ nm ($N=1,777$ nanostructures). These aggregates produce features of greater height but smaller size than the original DNA origami.

![AFM image of DNA origami on mica heated to 500°C](image)

**Figure 2.9** DNA origami on mica heated to 500°C in air aggregate on the surface. The AFM image shows breaks in the structure and the height profile identifies pitting and features of ~2 nm.

Heating to temperatures above 500°C was attempted; however, brown spots became visible on the mica surface at 600°C and 650°C (Figure 2.10A and B, respectively), which became larger and more prevalent until the entire mica sample turned an opaque brown after heating to 700°C (Figure 2.10C). Although muscovite mica begins to thermally degrade above 900°C, the visible change when heated above 600°C may be due to loss of interstitial water from the mica layers.[56], [57]
Figure 2.10 After heating at A) 600°C, the mica exhibited brown spots that increased in size and coverage at B) 650°C until the mica became completely opaque at C) 700°C.

Mica is a convenient substrate to probe DNA origami structure and shape due to its flat nature and the strong adhesion of the nanostructures in the presence of Mg$^{2+}$. These studies provided an initial understanding of the effect of heating on DNA origami without the complexity of our APTES/Si system (described in detail in Section 1.3.2).

APTES/Si is ideal for incorporation into current lithographic and EBL processing. Heating DNA origami on APTES/Si at 150°C for 10 minutes resulted in no change in nanostructure dimensions (Figure 2.11B). Heating to 250°C for 10 minutes resulted in a slight decrease in height and substantial deterioration of DNA origami shape (Figure 2.11C). Lateral dimensions could not be measured. This contrasts with the conserved shape and lateral dimensions of DNA origami heated on mica at 250°C. I wondered whether the deterioration of DNA origami structures at 250°C could be due to the decreased stability of the APTES monolayer at elevated temperatures.
TABLE 2.3

DIMENSIONS OF DNA ORIGAMI ON APTES FUNCTIONALIZE SILICON AFTER HEATING

(150°C AND 250°C)

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Height (nm)</th>
<th>Length (nm)</th>
<th>Width (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.8 ± 0.2 (N=3,905)</td>
<td>97 ± 12</td>
<td>74 ± 9 (N=60)</td>
</tr>
<tr>
<td>150°C/10 min.</td>
<td>1.5 ± 0.2 (N=1,857)</td>
<td>90 ± 7</td>
<td>71 ± 7 (N=60)</td>
</tr>
<tr>
<td>250°C/10 min.</td>
<td>1.2 ± 0.1 (N=4,146)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Figure 2.11 DNA origami deposited on APTES functionalized silicon after A) no heating, B) heating at 150°C for 10 minutes, and C) heating at 250°C for 10 minutes in air. Structural deterioration occurs around 250°C. All AFM images are 1.7 µm x 1.7 µm and have 5 nm height scales.
The thermal stability of APTES on silicon after heating in a tube furnace in both air and N₂ environments was investigated using contact angle measurements (Figure 2.12). The contact angle decreased after heating above 250°C in air (Figure 2.12, blue squares) until it reached values consistent with that of native oxide: 37.5 ± 3.2° at 450°C (N=2 samples).[58]

Figure 2.12 Contact angle measurements on APTES functionalized silicon heated to various temperatures for 10 minutes in air (blue squares) and nitrogen environments (red circles).
The contact angles were consistently greater for samples heated in N₂ (Figure 2.12, red circles) than for samples heated in air. This is consistent with the hypothesis that mechanism of APTES deterioration is dependent on oxidation. Interestingly, heating APTES functionalized silicon to 250°C in N₂ resulted in a contact angle of 67.8 ± 0.7° (N=2 samples). The subject of annealing (also referred to as curing) of APTES on silicon oxide substrates has been investigated thoroughly due to the pervasive use of APTES SAMs in biology. However, to my knowledge, no studies have investigated annealing in inert atmosphere. Usually, post-deposition curing is used to remove excess solvents as well as promote the crosslinking of silane molecules on the silicon oxide surface through dehydration to produce a tighter packed, more robust monolayer.[59], [60] Curing has also been reported to convert buried protonated amine groups to terminal, neutral amines, increasing thin film quality and reactivity.[61]–[63] Further investigation, such as high resolution XPS studies and surface FTIR, would help to probe the surface changes induced by the inert atmosphere annealing. However, it can be concluded that oxidation and degradation of APTES occurs at temperatures above 250°C, resulting in the deterioration of the DNA origami structure when introduced to the system (Figure 2.11C).

2.3.2 Chemical changes in DNA origami after heating on mica and SiO₂

Although structural stability of DNA origami was maintained after heating to temperatures well above the solution melting point, the 50% decrease in height at 250°C indicated a chemical change resulting in nanostructure decomposition. The
Surface chemistry of DNA origami heated on mica was investigated using XPS. XPS analysis of the carbon-1s (C\(_{1s}\)) binding energy region indicated two carbon environments on unheated samples (Figure 2.13A). The peak at 283.7 eV corresponds to adventitious carbon and other C-C species, referred to as C\(_{1s}(A)\). This peak was present when samples were exposed to ambient environment and was unchanged with heating, as shown in the XPS spectra in Figure B.2. The second peak at 285.2 eV corresponded to carbon species in the DNA structure, such as C-N, and C-O bonding environments and is referred to as C\(_{1s}(B)\). The raw peak intensities observed in XPS vary depending on experimental parameters. For comparison from one sample to the next, it is preferable to use peak intensity ratios (Table 2.4). Variation in peak areas indicates variation in coverage of DNA origami and adventitious carbon on the surface.

Upon heating at 150°C for 10 minutes, a third peak was observed at 287.9 eV (Figure 2.13B), referred to as C\(_{1s}(C)\). This peak corresponds to a highly oxidized carbon environment created by reaction of the DNA with air during the heating process. Only a small amount of oxidized carbon is formed, consistent with the lack of degradation evident in the AFM images of the DNA origami heated to 150°C (Figure 2.6B). When heated to 250°C, the oxidized carbon species C\(_{1s}(C)\) at 287.9 increases.
Figure 2.13 XPS $C_{1s}$ region of DNA origami on mica A) not heated, B) heated to 150°C, and C) heated to 250°C. The peak at 283.5 eV (blue) corresponds to adventitious carbon and C-C from DNA origami. The C-N and C-O species of DNA origami are detected at 285 eV (red). After heating, a highly oxidized carbon species at 288 eV (green) appears. (Reproduced with permission from Ref. [55]).
Nitrogen was also monitored with XPS as the N$_{1s}$ species at 399 eV. The XPS spectra is shown in Figure B.3. Due to the relatively low concentration of nitrogen compared to carbon in DNA origami, it was difficult to differentiate various nitrogen species due to the lower peak intensity. However, an N$_{1s}$ species was detected from the DNA origami and the N$_{1s}$:K$_{2p}$ 3/2 ratio remained unchanged after heating to 150°C. After heating to 250°C, the N$_{1s}$:K$_{2p}$ 3/2 ratio decreased by ~20%, although it was still within standard deviation of control samples. No oxidized nitrogen species could be confidently differentiated from the large N$_{1s}$ peak.

**TABLE 2.4**

<table>
<thead>
<tr>
<th>Sample</th>
<th>C$<em>{1s}$(A):K$</em>{2p}$ 3/2</th>
<th>C$<em>{1s}$(B):K$</em>{2p}$ 3/2</th>
<th>C$<em>{1s}$(C):K$</em>{2p}$ 3/2</th>
<th>N$<em>{1s}$:K$</em>{2p}$ 3/2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mica</td>
<td>1.66 ± 0.31</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Mica (150°C)</td>
<td>1.64 ± 0.41</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Mica (250°C)</td>
<td>1.33 ± 0.21</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Mica+DNA</td>
<td>2.83 ± 1.87</td>
<td>3.03 ± 2.70</td>
<td>0.00 ± 0.00</td>
<td>0.89 ± 0.27</td>
</tr>
<tr>
<td>Mica+DNA (150°C)</td>
<td>1.61 ± 0.98</td>
<td>2.05 ± 0.64</td>
<td>0.42 ± 0.18</td>
<td>0.82 ± 0.31</td>
</tr>
<tr>
<td>Mica+DNA (250°C)</td>
<td>2.26 ± 1.34</td>
<td>1.71 ± 0.59</td>
<td>1.02 ± 0.67</td>
<td>0.68 ± 0.03$^a$</td>
</tr>
</tbody>
</table>

$^a$ All mica control ratios have sample size N=2 samples; all DNA origami on mica ratios have sample size N=3 samples except for “Mica+DNA (250°C)” N$_{1s}$:K$_{2p}$ 3/2, which have N=2 samples.
2.3.3 Structural stability after solvent exposure

One of the most common solvents in nanofabrication is acetone, which is used for substrate cleaning and photoresist stripping. Figure 2.14 includes AFM images of DNA origami deposited on mica and APTES functionalized Si and exposed to acetone for A/B) 10 minutes and C/D) 30 minutes. The changes in DNA origami dimensions are included in Table 2.5.

![AFM images of DNA origami on mica and APTES functionalized Si](image)

Figure 2.14 AFM images of DNA origami on (A/C) mica and (B/D) APTES functionalized Si after exposure to acetone for (A/B) 10 and (C/D) 30 minutes. All images have 5-nm height scale.
<table>
<thead>
<tr>
<th>Experiment</th>
<th>Height (nm)</th>
<th>Length (nm)</th>
<th>Width (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.8 ± 0.2</td>
<td>97 ± 12</td>
<td>74 ± 9</td>
</tr>
<tr>
<td></td>
<td>(N=3,905 structures)</td>
<td>(N=60 structures)</td>
<td>(N=60 structures)</td>
</tr>
<tr>
<td>Mica – 10 min.</td>
<td>2.4 ± 0.1</td>
<td>94 ± 8</td>
<td>72 ± 7</td>
</tr>
<tr>
<td></td>
<td>(N=596 structures)</td>
<td>(N=50 structures)</td>
<td>(N=50 structures)</td>
</tr>
<tr>
<td>Mica – 30 min.</td>
<td>1.8 ± 0.5</td>
<td>99 ± 7</td>
<td>80 ± 8</td>
</tr>
<tr>
<td></td>
<td>(N=429 structures)</td>
<td>(N=50 structures)</td>
<td>(N=50 structures)</td>
</tr>
<tr>
<td>APTES/Si – 10 min.</td>
<td>2.3 ± 0.3</td>
<td>91 ± 8</td>
<td>64 ± 8</td>
</tr>
<tr>
<td></td>
<td>(N=170 structures)</td>
<td>(N=50 structures)</td>
<td>(N=50 structures)</td>
</tr>
<tr>
<td>APTES/Si – 30 min.</td>
<td>4.6 ± 0.8</td>
<td>102 ± 10</td>
<td>67 ± 14</td>
</tr>
<tr>
<td></td>
<td>(N=1,006 structures)</td>
<td>(N=50 structures)</td>
<td>(N=50 structures)</td>
</tr>
</tbody>
</table>

Although no discernible changes were seen for either 10 minute exposure, both substrates exhibited interesting features after 30 minute acetone treatment. As seen in Figure 2.15, “trenches” of ~0.5 nm depth surrounded areas where DNA origami were deposited. The reason for these features was not clear, although they may form due to desorption of excess staple strands and salts from the DNA origami buffer. In addition, the features were inconsistent and did not occur when DNA origami on mica was soaked for 24 hours in acetone (Figure 2.16).
Figure 2.15 AFM image and line profile illustrating ~0.5 nm “trench” (marked by arrow) produced after exposure of DNA origami on mica to acetone for 30 minutes. Scale bar is 100 nm.

Figure 2.16 AFM image of DNA origami on mica after exposure to acetone for 24 hours shows the shape of the nanostructures is unchanged (height: 1.78±0.17 nm (N=918 nanostructures); length: 93.3±6.2 nm (N=50 nanostructures); width: 72.2±9.9 nm (N=50 nanostructures). Image height scale is 5 nm.
Exposing DNA origami on APTES functionalized Si to acetone for 30 minutes increased the average height by ~60% (Table 2.5). The height increase is caused by a “folding” of the DNA origami, highlighted in Figure 2.17. Extended exposure of DNA origami to acetone interferes with the electrostatic interaction between the DNA phosphate backbone and the APTES monolayer, inducing a partial desorption and the folding of the DNA origami on itself, evident by the ~2x height increase on the DNA nanostructures (Figure 2.17B).

Figure 2.17 After exposing DNA origami on APTES functionalized Si to acetone for 30 minutes, some nanostructures exhibited a ~2x height increase (B, marked by arrow).
Dichloromethane (CH₂Cl₂) is also used for PMMA removal and is often better suited for photoresist stripping than acetone. Results of exposing DNA origami deposited onto mica and APTES functionalized Si to CH₂Cl₂ are summarized in Figure 2.18 and Table 2.6. Although DNA origami on mica remain relatively unchanged, structures on APTES functionalized Si are difficult to discern due to an increase in roughness and an abundance of particles after CH₂Cl₂ exposure (Figure 2.18B). After 30 minutes, the DNA origami could not be differentiated from the surface contaminants and dimension data was not acquired (Figure 2.18D).
Figure 2.18 AFM images of DNA origami on (A/C) mica and (B/D) APTES functionalized Si after exposure to CH$_2$Cl$_2$ for (A/B) 10 and (C/D) 30 minutes. All images have 5-nm height scale.
TABLE 2.6
DNA ORIGAMI DIMENSIONS AFTER DICHLOROMETHANE EXPOSURE ON MICA AND FUNCTIONALIZED SILICON SUBSTRATES

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Height (nm)</th>
<th>Length (nm)</th>
<th>Width (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.8 ± 0.2 (N=3,905)</td>
<td>97 ± 12 (N=60)</td>
<td>74 ± 9 (N=60)</td>
</tr>
<tr>
<td>Mica – 10 min.</td>
<td>1.5 ± 0.6 (N=641)</td>
<td>93 ± 8 (N=50)</td>
<td>76 ± 7 (N=50)</td>
</tr>
<tr>
<td>Mica – 30 min.</td>
<td>2.3 ± 0.2 (N=709)</td>
<td>91 ± 5 (N=50)</td>
<td>76 ± 7 (N=50)</td>
</tr>
<tr>
<td>APTES/Si – 10 min.</td>
<td>2.7 ± 0.3 (N=1,065)</td>
<td>89 ± 12 (N=25)</td>
<td>68 ± 8 (N=25)</td>
</tr>
<tr>
<td>APTES/Si – 30 min.</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

The lack of DNA origami and increase in large surface contaminants may be explained by deterioration of the APTES monolayer upon CH$_2$Cl$_2$ exposure. This is surprising since previous studies have soaked APTES patterned silicon substrates in CH$_2$Cl$_2$ to remove PMMA after electron beam lithography.[30], [64] The previous studies used treatment times of ~1 minute, significantly shorter than the 30 minutes reported here. The samples in Figure 2.18B/D and in APTES control samples, 30 minute CH$_2$Cl$_2$ exposure produced “pits” of ~0.7 nm (Figure 2.19). This depth is consistent with the height of the APTES monolayer. The monolayer deterioration mechanism is unknown, but these results indicated CH$_2$Cl$_2$ exposure time should be limited.
2.3.4 Functionality studies after heat and solvent exposure

Biotinylated DNA origami (described in Section 2.2.1) were used to investigate functionality after heating or solvent exposure. The binding interaction between biotin and streptavidin had to be optimized to maximize binding and minimize nonspecific background deposition.

Exposure time was the first factor investigated. As received streptavidin solution had a concentration of 1 mg/mL (~18 µM) streptavidin in phosphate buffer. Figure 2.20 shows AFM images after soaking DNA origami on mica in streptavidin solution for A) 3 hours, B) 20 minutes, and C) 5 minutes. There are no DNA origami visible on the substrates. Instead, aggregates or particles are visible and are assumed to be caused by
physisorbed streptavidin. These results indicate high concentrations of streptavidin are deleterious to DNA origami independent of exposure time.

![Figure 2.20 Biotinylated DNA origami on mica after exposure to 1 mg/mL streptavidin for A) 3 hours, B) 20 minutes, and C) 5 minutes. There are no complete DNA origami visible after these treatments. All AFM images have 5 nm height scale.](image)

The streptavidin solution was then diluted. Exposing DNA origami to 2 µg/mL (~36 nM) streptavidin solution for 15 minutes resulted in intact DNA nanostructures but only 3.6 ± 1.7% (N=308 nanostructures) exhibited full biotin-streptavidin binding (Figure 2.21A). When the streptavidin concentration was increased to 50 µg/mL (~910 nM), the percentage of DNA nanostructures with all biotin-streptavidin binding sites filled increased to 36.8 ± 2.8% (N=233 nanostructures) (Figure 2.21B). Due to the increased nonspecific streptavidin background binding (indicated by the particles on the mica background in Figure 2.21B), the streptavidin solution concentration was not increased further. This concentration is consistent with what is reported in the literature for similar experiments.[52], [65]
Figure 2.21 DNA origami on mica exposed to A) 2 µg/mL and B) 50 µg/mL streptavidin solution showed good structure and distribution, although increased biotin-streptavidin binding occurred with higher concentration.

Due to the incomplete functionalization of the DNA origami, there are several different biotin-streptavidin interaction configurations depending on which binding site is filled. These configurations are illustrated in Figure 2.22. Counting these interactions and identifying changes due to diverse chemical and physical environments provides an understanding to the capacity of the biotinylated DNA origami to bind streptavidin.
Figure 2.22 Various configurations of biotin-streptavidin interactions on the DNA origami designed with three binding sites.

The AFM images in Figure 2.23 illustrate streptavidin bound DNA rectangles after moderate heating and exposure to 50 µg/mL streptavidin solution for 15 minutes.

Changes in DNA origami functionality with temperature is graphed in Figure 2.24. Partial deterioration of the DNA nanostructures was observed after 10 minute heating 90°C and was quantified as “poor structures” (i.e. broken DNA origami with uncountable streptavidin binding) (Figure 2.23C). A drastic change occurred after heating to 100°C for 10 minutes: no complete DNA origami were visible on the mica substrate (Figure 2.23D).
Figure 2.23 DNA origami after heating at A) 50, B) 70, C) 90, and D) 100°C and exposure to streptavidin solution for 15 min. All images have 5 nm height scales.
Figure 2.24 This graph illustrates the change in percentage of functional nanostructures with treatment temperature. The most drastic change occurs after heating at 90°C for 10 minutes, where the percentage of fully functional DNA origami drops by ~20%. There is also an increase in poor structures that could not be interpreted.

The functionality of DNA origami after solvent exposure was investigated only for acetone, since CH₂Cl₂ resulted in visible changes in height and structure of the DNA origami and acetone did not. After exposure to acetone for longer than 1 minute, streptavidin exposed samples exhibited no DNA origami. Large aggregates were observed on the substrate and increased in size with increasing acetone treatment time (Figure 2.25B-D).
Figure 2.25 DNA origami exposed to acetone for A) 1, B) 5, C) 10, and D) 15 minutes and subsequently exposed to streptavidin solution.

DNA origami were only visible in samples treated with acetone for one minute (Figure 2.25A), after which there was ~20% decrease in well-formed structures and ~13% decrease in fully functional nanostructures. The results for the one-minute exposure are summarized in Table 2.7 (N=430 nanostructures for all measurements).
TABLE 2.7
PERCENTAGE OF BIOTIN-STREPTAVIDIN INTERACTIONS AFTER 1 MINUTE EXPOSURE TO ACETONE

<table>
<thead>
<tr>
<th>Percentages</th>
<th>Control</th>
<th>Acetone – 1 min. Exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Good structures</td>
<td>88.8 ± 2.5%</td>
<td>69.1 ± 1.5%</td>
</tr>
<tr>
<td>% 3/3 streptavidin</td>
<td>32.6 ± 4.0%</td>
<td>19.1 ± 3.4%</td>
</tr>
<tr>
<td>% 2/3 streptavidin</td>
<td>34.0 ± 4.0</td>
<td>24.7 ± 0.9</td>
</tr>
<tr>
<td>% 1/3 streptavidin</td>
<td>13.1 ± 5.8</td>
<td>14.1 ± 2.7</td>
</tr>
<tr>
<td>% 0/3 streptavidin</td>
<td>10.3 ± 2.6</td>
<td>9.2 ± 2.9</td>
</tr>
</tbody>
</table>

2.4 Discussion

Contrary to predictions based on solution melting experiments, DNA origami show unexpected thermal stability when supported on mica and APTES functionalized silicon substrates. Thermal stability of DNA origami in solution is determined by the onset of dehybridization. There are differences between the melting mechanism between solution phase and DNA oligomers attached to substrates.[66]–[68] The results reported here indicate that complete removal of solvent increases the thermal stability of DNA origami supported on a substrate with respect to shape and size conservation.

These results were corroborated by Kim et al. who observed similar thermal stability of DNA origami on SiO$_2$ substrates.[69] They also measured a 50% decrease in height upon heating at temperatures above 200°C. However, Kim et al. claimed that the mechanism of DNA origami decomposition did not involve oxidation but was thermal in nature. This is in contrast with evidence reported here, including the increase of an oxidized carbon species in XPS spectra at elevated temperatures (Figure 2.13) as well as
the larger decrease in height for structures heated in air rather than inert atmosphere (Figure 2.8).

Previously reported studies of DNA pyrolysis in low hydration environments by Lee et al. indicated that DNA begins to chemically degrade after heating at temperatures above 200°C in nitrogen environments. It has been shown that even after pyrolysis intact DNA nucleotides can be recovered. The decomposition of DNA films has been reported using DSC and thermal gravimetric analysis (TGA) under inert atmosphere. Pure DNA gave a sharp exothermic peak at 226°C in DSC measurements and decomposition of the DNA films began around 230°C as measured by TGA. These temperatures correlate well with the slight decrease in height of the DNA origami after heating at 250°C in N₂, indicating that thermal degradation and height decrease may be linked. Additionally, the TGA studies indicated a residual weight of 53% after heating to 500°C. Early in this project I attempted to characterize DNA origami thermal degradation using TGA/MS measurements for nanostructures deposited on mica heated to 150°C and 250°C. Due the small amount of DNA origami on the mica compared to the films of DNA described above, there was no weight change or decomposition products detected in my experiments and this technique was not pursued further.

The stability of DNA origami upon exposure to organic solvents, including hexane, toluene, and ethanol, has been previously reported. The results here are the first to indicate that the common microfabrication solvents, acetone and CH₂Cl₂, have minimal effect on the structure and surface coverage of DNA origami on mica after short exposure times. However, the incorporation of the APTES SAM in functionalized
silicon complicates the system and provides another means of decomposition. The degradation of the APTES SAM after thermal and solvent treatment, discussed in Sections 2.3.1 and 2.3.3 respectively, indicates a limitation to using functionalized silicon substrates in the diverse chemical environments of micro- and nanofabrication processes. Moderate heating and short solvent exposures have minimal effect on the APTES-DNA origami stability.

The functionality studies illustrate another limitation of DNA origami in nanofabrication. The lack of intact DNA origami after heating to 100°C for 10 minutes and streptavidin solution exposure indicates structural destabilization may result from the two step “heating and solvent” processing. The degradation mechanism is illustrated in Figure 2.26. Heating in step (A) partially oxidizes the DNA origami (discussed in Section 2.3.2). The presence of the DNA origami shape on the surface after heating at elevated temperatures indicates that the partially oxidized components remain stagnant on the substrate. The exposure to the solution of streptavidin in aqueous buffer in step (B) provides a mechanism for the partially oxidized components to diffuse from the surface, leaving only shadows of the nanostructures on the substrates in step (C).
Figure 2.26 Upon heating to over 90°C (A) the DNA origami undergo partial oxidation. The introduction of a solvent system (B) induces the diffusion of partially oxidized components until (C) only fragments of the DNA origami structure remain.
This “solvent mediated degradation” was further proven in control experiments where DNA origami on mica was heated to 100°C for 10 minutes, rinsed with 200 µL water, and dried for 3 minutes with N₂. Similar structural degradation as Figure 2.23D can be seen after this treatment in Figure 2.27, proving that it is the solvent and not the streptavidin that causes the loss of DNA origami structure.

![Figure 2.27 DNA origami heated to 100°C for 10 minutes and rinsed with water exhibit similar structural degradation as seen in Figure 2.23D after heating and streptavidin solution exposure (height scale is 5 nm).](image)

A similar mechanism can explain the decrease in structural integrity and increase in particles/aggregates on the surface after acetone exposure followed by soaking in streptavidin solution. The process is outlined in Figure 2.28. Soaking in acetone in step (A) induces partially dehybridization or chemical degradation of the DNA origami. The
partially dehybridized staple strands desorb from the substrate upon exposure to streptavidin solution in step (B) and can aggregate to redeposit on the substrate (C).

Figure 2.28 Upon exposure to acetone (A), DNA origami partially dehybridize. The fragments desorb from the surface upon exposure to aqueous environments (B) and can aggregate and adsorb to the substrate (C) as indicated by the presence of particles on the surface in Figure 2.25B, C, and D.
There are limited reports of the effect of acetone on DNA or DNA origami base pairing. Volgelstein and Gillespie reported selective precipitation of DNA from agarose gels using between 33 and 75% acetone in water.[72] DNA hybridization was not affected by acetone treatment once the precipitate was dissolved in solution. This method is commonly used for DNA precipitation and purification. It is feasible to conclude that long periods of acetone exposure can induce aggregation of the DNA, which produces the large particles on the mica substrate. Further investigation is required to determine the extent to which the disruption occurs and whether it is avoidable or reversible.

2.5 Conclusion

The results outlined in this chapter help identify the limitations of utilizing DNA origami in nanofabrication processing. On both mica and APTES functionalized silicon substrates, moderate heating and limited solvent exposure are ideal for maintaining nanostructure functionality after processing. DNA origami functionality decreases after heating to 90°C for 10 minutes or 1 minute exposure to acetone. The addition of the APTES monolayer when utilizing silicon substrates introduces an additional stability factor, with APTES decomposing at temperatures higher than 250°C and undergoing monolayer degradation in both acetone and dichloromethane after prolonged solvent exposure. Higher treatment temperatures can be utilized if applications require only the shape and size of DNA origami.
CHAPTER 3:

BURN-IN OF DNA ORIGAMI ON SILICON SUBSTRATES TO PRODUCE SILICON CARBIDE REPLICAS

3.1 Overview

The chemical patterning of surfaces is instrumental in the production of electronic devices. Current fabrication is often based on “top-down” processes, such as photolithography and traditional microfabrication methods; however, with the influx of novel materials and unique applications these techniques may not be ideal. A material of particular interest is the wide band gap semiconductor silicon carbide (SiC). Due to its chemical inertness, high thermal conductivity, and electron mobility, SiC has been explored for high power, high frequency, and high temperature applications.[73] An additional advantage is the presence of an SiO$_2$ native oxide on SiC, which makes it compatible with current metal-oxide semiconductor (MOS) architectures.[74] The robustness of SiC poses unique problems for nanoelectronic fabrication. Techniques for patterning SiC include reactive ion etching and mechanical machining; these top-down processes can be difficult, unprecise, and expensive, providing considerable hurdles for SiC incorporation into electronics. Bottom-up processing incorporating carbon nanostructures have been shown to be a promising alternative to traditional SiC
patterning. Hamza, et al. showed that exposing silicon to a constant flux of $C_{60}$ and heating the system above 1100 K produced thin films of SiC approximately 5000 Å thick.[75] These studies were expanded upon by Moro, et al. through irradiation of the $C_{60}$ film with either an Ar$^+$ or Ga$^+$ ion gun to produce micrometer scale patterns; after annealing at 900°C for 150 to 300 minutes the unmodified films evaporated off of the surface, leaving the irradiated SiC pattern.[76], [77]

The thermal stability of DNA origami described in Chapter 2 as well as studies by Kim et al. indicated that exposing DNA origami to temperatures of 500°C resulted in the production of carbonaceous residues that adhered to mica and silicon.[55], [69] The approximate atomic composition of DNA origami rectangles is 34% carbon, 30% hydrogen, 20% oxygen, 13% nitrogen, and 3% phosphorus. I hypothesized that at higher temperatures the carbon in DNA origami could diffuse into the underlying silicon substrate, producing carbide replicas of the shape and size of the DNA origami. The viability of the “burn-in” process of DNA origami is supported by studies of silicon doping through rapid thermal processing (RTP) of molecular monolayers. Ho et al. first reported the formation of shallow junctions via spike annealing of boron containing self-assembled monolayers produced by reacting silicon wafers with allylboronic acid pinacol ester and, eventually, phosphorus-containing diethyl 1-propylphosphonate.[78], [79] This same process can be applied to DNA origami to induce diffusion into the silicon substrate.
3.2 Experimental methods

3.2.1 Burn-in process

The burn-in processing steps are illustrated in Figure 3.1. DNA origami rectangles were prepared using the methods described in Section 1.2.1. Silicon wafers (p-type, <100>) were cut into 1 cm x 1 cm chips and cleaned with piranha solution for 20 minutes, briefly etched in 4% HF, and cleaned a second time with piranha solution for 20 minutes. This cleaning process ensures that organic contaminants are removed from the surface and a uniform native oxide of ~1 to 2 nm is grown on the substrate. Deposition of DNA origami onto silicon substrates was carried out as described in Section 1.3.2. Each sample was exposed to 10µL of 16 nM DNA origami rectangle solution for 15 minutes after which the samples were rinsed with sterile 18MΩ water and dried with a steady stream of N₂. A 50 nm SiO₂ capping layer was deposited via Unaxis 790 plasma enhanced chemical vapor deposition (PECVD) system to ensure the DNA origami components were not lost from the surface during annealing. PECVD was utilized due to its relatively low deposition temperature of between 150°C and 250°C, which has been shown (Chapter 2) to leave DNA origami relatively intact. Flow rates for SiH₄ and N₂O were 80 and 900 sccm, respectively, and deposition was carried out with 25 W power and 900 mTorr pressure with a heat exchange temperature of 60°C. After capping layer deposition, the samples were heated to 900°C or 1100°C for 60 seconds using an Allwin AccuThermo AW610 Rapid Thermal Processing (RTP) System in N₂ atmosphere with a
ramp time of 15 seconds. After annealing, the SiO$_2$ capping layer was etched using 4% HF solution until the sample became hydrophilic or for up to 20 minutes.

Figure 3.1 The “burn-in” processing steps begin with a cleaned silicon substrate functionalized with APTES and exposed to DNA origami solution. The sample is then capped with 50 nm of SiO$_2$ using PECVD and annealing with RTP. The capping layer is then removed in 4% HF solution.[80]

3.2.2 Characterization techniques

Static contact angle measurements were performed within 30 minutes of HF etching using a Krüss G10 Contact Angle Measurement System and with a 10 µL drop of 18 MΩ water placed on the samples. A sessile drop technique was used. Four measurements were completed on different areas of each sample.

AFM analysis was completed using a Digital Instruments Nanoscope V Multimode Scanning Probe Microscope and Etalon HA_NC probes. The probes had a
resonance frequency of 235 kHz and a force constant of 12 N/m. Dimension and coverage analysis was completed as previously described in Section 2.2.4.

A PHI VersaProbe II XPS was used to complete analysis of surface chemistry. All spectra were acquired using 200µm50W15kV x-ray setting (x-ray beam size: 200 µm, power: 50 W, and e-beam energy: 15 kV) and 23.500 eV pass energy unless stated otherwise. XPS sweeps ranged from 10 to 30 depending on the analyzed element. Depth profiling utilized an Ar ion gun with 2 kV sputter setting and 2 mm x 2 mm sputter size. The sputter rate for the 2 kV sputter setting was defined by Physical Electronics, Inc. as 1.5 Å/second. This value can vary with material, scan settings, and instrument and a calibration is required. To calibrate the etch rate, approximately 100 nm of SiO₂ was deposited onto clean silicon chips. The samples were then sputtered with either 2 kV or 5 kV sputter setting for 15 minutes with sputter intervals of 6 seconds. Basic XPS spectra of the Si2p region were acquired using 10 sweeps and eV step of 0.800. The time that the SiO₂ peak at ~103 eV disappeared and the Si2p₃/₂ and Si2p₁/₂ peaks appear was considered the etch time of SiO₂. The sputter rate of the 5 kV 2x2 and 2 kV 2x2 sputter settings were 6.02 ± 0.31 Å/second and 0.78 ± 0.03 Å/second, respectively. It was assumed that the etch rate of silicon and silicon carbide were roughly the same as the etch rate of SiO₂. The slower sputter rate of the 2 kV 2x2 setting was ideal for depth profiles of burn-in samples and allowed for greater surface sensitivity. The sputtering interval was 12 seconds unless specified otherwise. Spectral analysis of XPS data was completed using CasaXPS and all spectra were calibrated to adventitious carbon at 284.8 eV. Peaks were fitted with 30% Gaussian and 70% Lorentzian line shape.[81]
Chemical characterization of the samples was attempted using two forms of Raman spectroscopy: Micro-Raman and tip-enhanced Raman spectroscopy (TERS). The micro-Raman analysis used a Jasco NRS-5100 instrument with a 532 nm excitation laser and 20 second exposure time and a total of 20 accumulations. A 1200 l/mm grating with a 50 x 1000 µm slit and d-4000 µm aperture were used to attain the Raman spectra. TERS was completed using a custom, zero-mechanical drift upright microscope coupled to a Nanonics MV2000 AFM and 532 nm excitation. Cleaned silicon and APTES functionalized silicon were used as control sample to acquire background spectra.

X-ray diffraction data were recorded on a Bruker D8 Davance (Bragg-Brentano geometry) diffractometer. Monochromated Cu-Kα radiation (Kα1 and Kα2 combined) was used to record data over a 2-theta range of 5 to 30 degrees measure in 0.02 degree increments for 2 seconds per increment at ambient temperature and pressure.

Transmission electron microscopy (TEM) studies were carried out using a FEI Titan 80-300 TEM. The low coverage of DNA origami on the samples required location of the SiC replicas using SEM. Approximately 100 nm of platinum was deposited using electron beam deposition at 5kV and 5.5 nA on the region of interest. A thicker platinum layer of greater than 1 micron was deposited via ion-beam on top to protect the substrate. TEM analysis requires the preparation of a thin layer cut from the sample called a lamella. This produces a sample cross section ideal for depth profile investigation. Initial lamella preparation was carried out using a FEI Helios Nano Lab Dual Beam 600 at 30 kV and 6.5 nA. The lamellar was thinned down to 800 nm at 30 kV
and 0.28 nA. Thinning was continued at 30 kV and 93 pA until the lamella was 200 nm.

The sample was then polished.

3.3 Results

The HF etch of the capping layer after annealing yielded perplexing results. Control samples of both silicon and APTES functionalized silicon substrates became hydrophobic after approximately 20 seconds exposure to HF solution. The samples floated on the surface of the solution, a characteristic of hydrophobic surface, and had a contact angle ranging from 50° to 70° (Figure 3.2A), typical for hydrogen terminated silicon substrates. Interestingly, the samples incorporating DNA origami did not float on the bath and remained hydrophilic after 20 minutes of exposure to HF solution. The contact angle measurements of ~30° (Figure 3.2B) are similar to that of silicon with a thin native oxide. Table 3.1 includes contact angle measurements for various burn-in samples.
Figure 3.2 The “burn-in” processing steps begin with a cleaned silicon substrate functionalized with APTES and exposed to DNA origami solution. The sample is then capped with 50 nm of SiO2 using PECVD and annealing with RTP. The capping layer is then removed in 4% HF solution.[80]

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Post-etch Contact Angle (N=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silicon (Control)</td>
<td>62.0 ± 1.0°</td>
</tr>
<tr>
<td>Silicon + APTES</td>
<td>42.9 ± 2.1°</td>
</tr>
<tr>
<td>Silicon + APTES + DNA Rectangles</td>
<td>35.6 ± 0.9°</td>
</tr>
<tr>
<td>Silicon + APTES + DNA Chains</td>
<td>36.9 ± 1.2°</td>
</tr>
</tbody>
</table>
The variation of contact angle between samples indicates possible unusual surface chemistry produced by burn-in of DNA origami. Even after 20 minutes of exposure to 4% HF solution, burn-in samples containing DNA origami remained hydrophilic. AFM images in Figure 3.3 illustrate the difference in surface roughness between silicon control and DNA origami burn-in samples after 2 minute exposure (Figure 3.3A and B) and 20 minutes exposure (Figure 3.3C and D) to HF solution. After 2 minutes of HF etching, the silicon control samples had an RMS roughness of 0.13±0.01 nm (N = 4 samples), consistent with a smooth native silicon oxide. However, burn-in samples containing DNA origami showed ~10x high roughness of 1.47±0.04 nm (N = 4 samples). After 20 minutes of HF exposure, the RMS roughness for silicon control samples was 0.20±0.01 nm and DNA origami burn-in samples was 0.57±0.01 nm.
Figure 3.3 AFM images of A) silicon control samples and B) DNA origami burn-in samples after 2 minutes HF etch show a distinct difference in roughness, with all oxide on control removed but evidence of oxide remaining on DNA containing samples. After 20 minutes HF exposure, C) silicon control samples remained smooth and D) DNA origami burn-in samples showed rectangular features consistent with the shape and size of the original nanostructures. All images have 5 nm height scale.
The 20 minute HF etch exposed features of similar shape and size as the original DNA nanostructures. The rectangular DNA origami design has dimensions of 70 nm x 90 nm. AFM images of the pre- and post-burn-in structures are included in Figure 3.4 and Table 3.2 includes the dimensions of the surface features.

### TABLE 3.2

**DIMENSIONS OF PRE- AND POST-BURN-IN STRUCTURES**

<table>
<thead>
<tr>
<th>Burn-in Setting</th>
<th>Length (nm)</th>
<th>Width (nm)</th>
<th>Height (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>96.9 ± 8.5 (N=76 nanostructures)</td>
<td>75.7 ± 8.1 (N=76 nanostructures)</td>
<td>2.3 ± 0.5 (N=1,764 nanostructures)</td>
</tr>
<tr>
<td>900°C/60 sec.</td>
<td>96.5 ± 12.3 (N=80 nanostructures)</td>
<td>74.8 ± 8.0 (N=80 nanostructures)</td>
<td>1.0 ± 0.2 (N=1,102 nanostructures)</td>
</tr>
<tr>
<td>1100°C/60 sec.</td>
<td>93.7 ± 6.4 (N=133 nanostructures)</td>
<td>72.1 ± 5.3 (N=133 nanostructures)</td>
<td>1.5 ± 0.2 (N=516 nanostructures)</td>
</tr>
</tbody>
</table>

To ensure that these features were indeed a result of annealing the DNA origami, a nanostructure design incorporating pi-stacking staple strands (Figure 3.4E) were used to assemble longer DNA origami chains.[15] After burn-in processing of these samples (Figure 3.4F), features consistent with the secondary DNA origami structures were seen on the substrate, indicating that replicas of the structures were being produced upon burn-in. The features were also present when DNA origami was directly deposited on silicon with a native oxide, although the coverage was much lower due to
the lower surface charge density of silicon oxide compared to APTES functionalized silicon. The average coverage of DNA origami on APTES functionalized silicon was 4.8±1.5% (N=12 samples) whereas DNA origami on silicon was only 0.5±0.2% (N=5 samples) The replicas were also visible using scanning electron microscopy (SEM) (Figure 3.5), appearing as darker regions on the surface.
Figure 3.4 The burn-in of DNA origami on APTES functionalized silicon (A, C, E) create replicas of the structures (B, D, F) with consistent shape and size. These replicas are exposed after etching with HF after 20 minutes (all height scales are 5 nm).
Figure 3.5 SEM images of rectangular features on silicon substrates after burn-in processing DNA origami deposited on A-B) silicon with native oxide and C) APTES functionalized silicon (all scale bars are 500 nm).
The chemical characteristics of the DNA origami replicas were probed using XPS. Initial scans of the burn-in samples after up to 20 minute exposure to 4% HF were consistent with silicon with a native oxide. Figure 3.6A illustrates the Si2p binding region. Peaks at 98.9 eV and 99.6 eV correspond to Si2p 3/2 and Si2p 1/2, respectively. The peak at 102.6 eV is due to silicon oxide; the low intensity of this peak compared to the silicon peak indicates a thin oxide layer. A scan of the carbon region is shown in Figure 3.6B. Four peaks can be fitted in this spectra. The most prominent species corresponds to adventitious carbon at 284.8 eV and accumulates on samples upon exposure to the atmosphere. Additional nitride or oxide contaminant species contribute to the carbon peaks at 286.4, 288.0, and 289.4 eV. This spectrum is consistent with native silicon oxide that has been exposed to atmosphere and does not indicate unique chemical characteristics produced by burn-in of DNA origami.
Figure 3.6 XPS spectra for the Si2p region of etched burn-in samples indicate the presence of native oxide (SiO$_2$ peak at 102.6 eV). B) The C1s region has peaks consistent with adventitious carbon (284.8 eV) and other contaminants from exposure to atmosphere. These spectra are consistent with silicon exposed to the atmosphere and do not indicate unique chemical characteristics.
Due to the surface sensitivity of XPS, the spectra in Figure 3.6 correspond to the top 2-3 nm of the burn-in sample. Argon ions were used to probe deeper into the sample. Ar$^+$ sputter the top most layer of the sample, revealing underlying binding environments. After sputtering the surface with 2kV Ar$^+$ for 12 seconds, the oxide peak in the Si2p region disappeared (Figure 3.7A), indicating complete removal of the native oxide. Additionally, a peak at 283 eV is seen in the C1s region as well as the adventitious carbon peak at 284.8 eV (Figure 3.7B). The peak at 283 eV corresponds to a binding energy of a silicon carbide or oxidized silicon carbide species.[83]
Figure 3.7 After Ar$^+$ sputtering for 12 seconds, the native peak at 102.6 eV has disappeared, indicating all oxide is sputtered away (B). A C1s peak at a lower binding energy of 283 eV is exposed, corresponding to a silicon carbide species (A).

Consecutive Ar$^+$ sputtering steps were used to conduct depth profiling of the burn-in samples. Calibration of the 2kV 2x2 Ar$^+$ sputtering setting using a known thickness of SiO$_2$ deposited by PECVD and measured using ellipsometry determined a sputter rate of 4.7 nm/min. Figure 3.8 shows stacked spectra of varying sputter depths,
the SiC peak at 283 eV is highlighted in red and indicates a diminishing intensity with greater sputter depths.

Figure 3.8 Stacked XPS spectra with the SiC peak at 283 eV highlighted in red decreasing in intensity with greater depth.[80]

Depth profiles were acquired for samples treated at 900°C and 1100°C for 60 seconds, the DNA origami coverage of these samples were essentially the same, 5.3±1.5% (N=4 images) and 6.5±1.5% (N=4 images) respectively. The relative ratio of the SiC peak to the Si2p 3/2 peak was compared at various sputtering depths. The red circles in Figure 3.9 show a higher initial SiC concentration for the 1100°C/60 second sample than the 900°C/60 second sample (blue triangles). As depth increases, SiC
relative concentration decreases, indicating a diffusion into the silicon substrate. Control samples of APTES functionalized silicon treated at 1100°C for 60 seconds had a SiC concentration ~14% of that seen for the 1100°C/60 second sample containing DNA origami. This is contradictory to predicted C atom concentrations. The surface packing density of APTES has been reported as 3 molecules/cm², although this differs with monolayer preparation parameters.[84] This packing density results in a carbon surface concentration of 9x10^{14} atoms/cm². If DNA origami is included in the sample with a 4% surface coverage and 114,557 C atoms/DNA origami, the carbon concentration provided by the DNA is only 7.5% of the total. The reason for the discrepancy between predictions and experiment is unknown. The possible reasons are feasible: 1) the APTES surface packing was lower than 3 molecules/cm², 2) some APTES was lost from the substrate during sample preparation, 3) the DNA origami coverage was higher than measured, 4) single stranded DNA from the DNA origami staple strands introduced an addition carbon source into the sample (but were not detected via AFM). Nonetheless, the higher concentration of SiC in the DNA origami burn-in samples measured with XPS correlated with a lower contact angle (Figure 3.2), indicating that a higher SiC concentration results in a partially oxidized substrate.
Figure 3.9 XPS depth profiling using consecutive Ar$^+$ sputtering indicates the relative concentration of SiC at shallower depths is higher for DNA origami samples treated at 1100°C/60 seconds than the 900°C/60 seconds sample. SiC was also detecting in samples containing only APTES functionalized silicon treated at 1100°C for 60 seconds; however, the relative concentration was only ~14% of that in DNA origami burn-in samples.[80]

Depth profiling was also performed on burn-in samples of DNA origami directly deposited on silicon (with native oxide). SiC was also detected in these samples; however, due to the relatively low coverage of DNA origami when directly deposited on silicon as compared to APTES functionalized silicon, the relative concentration of SiC was lower than what was seen in the depths profiles in Figure 3.9. In fact, the ratios of SiC:Si2p 3/2 illustrated in Figure 3.10 were ~15% of what was measured for the DNA origami on APTES functionalized silicon at similar depths. However, this seems to be
sufficient SiC formation to cause hydrophilicity after HF etching as these samples also did not show the characteristic floating behavior for hydrogen terminated silicon after 20 minutes of HF exposure.

![Graph](image)

**SiC:Si2p 3/2 Ratios for DNA Origami Burn-In on Silicon**

- △ 900°C/60sec - DNA Origami/Si
- ○ 1100°C/60 sec - DNA Origami/Si

Figure 3.10 Depth profiles for samples of DNA origami deposited directly on silicon with native oxide processed at 900°C/60 seconds (blue triangles) and 1100°C/60 seconds (red circles). The relative ratios of SiC:Si2p 3/2 are lower than those seen in Figure 5.9 due to the low coverage of DNA origami on silicon compared to APTES functionalized silicon.

In addition to the C1s region, the N1s signal was also monitored using XPS due to the presence of nitrogen in both the APTES monolayer and DNA origami. HF etched burn-in samples treated at 1100°C for 60 seconds included a N1s peak at 400 eV (Figure 3.11A), corresponding to N-H and N-N binding energy and most likely due to atmospheric contamination or introduction of nitrogen into the SiO2 capping layer.
during the PECVD process. However, after 6 seconds of sputtering with Ar⁺, the peak at 400 eV disappeared and a peak at 397 eV was present (Figure 3.11B). The binding energy of this peak is consistent with that of silicon nitride (SiN) or oxidized SiN.[85]–[88] The SiN peak was not detected in samples treated at 900°C for 60 seconds or APTES functionalized silicon samples.

Figure 3.11 A) XPS spectra of N1s region of DNA origami burn-in samples treated at 1100°C for 60 seconds show a peak at 400 eV after HF etching consistent with N-N and N-H species. Upon 6 second Ar⁺ sputtering, a peak is detected at ~397 eV, corresponding to a SiN species.[80]
SiC is a complex material due to the possibility of a plethora of polytypes, which have varying structural and electrical characteristics.[89] Although the presence of an SiC species was confirmed with XPS, the crystal structure and polytype cannot be determined with this technique. X-ray diffraction was used to attempt to characterize the crystallographic characteristics of the burn-in replicas. A high coverage sample (35.9 ± 11.4% (N=4 measurements) of DNA origami on APTES functionalized silicon treated at 1000°C for 60 seconds was analyzed using low-angle incidence x-rays to attempt to intensify surface diffraction. Diffraction data for the burn-in sample (Figure 3.12A) showed peaks at ~33°, similar to peak locations reported in literature for various SiC polytypes, including β-SiC(111) and 3C-SiC(111).[90], [91] The control experiment (Figure 3.12B), using a clean silicon chip, showed similar peaks locations as well as a broad region around the 33° peak. The similarities between experiment and control samples as well as the lack of other SiC peaks between 35° and 70° indicates that either the SiC is amorphous or the concentration of SiC is too low for adequate detection.
A) XRD of High Coverage Burn-in Sample Processed at 1000°C

B) XRD of Silicon Control Sample

Figure 3.12 X-ray diffraction spectra for A) burn-in samples processed at 1000°C for 60 seconds and B) control silicon samples. Insets show regions most commonly associated with SiC diffraction.

In addition to defined diffraction peaks, SiC has distinct Raman shifts. SiC peaks can occur around 500, 630, 796 and 975 cm⁻¹, depending on the polytype.[92]–[94]

Micro-Raman analysis focused on the region of ~550 to 2300 cm⁻¹, avoiding the intense crystalline silicon peak at 520 cm⁻¹ while providing information about the possible presence of amorphous carbon, which has first order D and G bands at around 1500 cm⁻¹.
and second-order band at 2500 cm\(^{-1}\).[92] The spectrum in Figure 3.13A shows Raman peaks detected in samples of DNA origami on silicon that underwent the burn-in process at 900°C for 60 seconds. Comparing this spectrum to a silicon control sample in Figure 3.13B, no significant differences can be identified. Peaks at ~521 cm\(^{-1}\) and ~950 cm\(^{-1}\) most likely correspond to silicon longitudinal optical (LO)/transverse optical (TO) and silicon 2LO/TO and second order features of the substrate, respectively.[95] The failure of this characterization technique is most likely due to the relatively low sensitivity of Raman spectroscopy as well as the low concentration of SiC on the sample surface.
Figure 3.13 Raman spectra for A) samples of high coverage of DNA origami on silicon after burn-in at 900°C for 60 seconds and B) silicon control. The lack of significant difference between the two spectra indicates Micro-Raman is not a surface sensitive enough for characterization of the SiC replicas.
Due to the nanoscale dimensions of the SiC formed through burn-in, the Raman technique described above does not have the spatial resolution or sensitivity to identify localized nanostructures. Tip-enhanced Raman spectroscopy (TERS) was used to try and identify Raman signals localized to the SiC replicas. TERS utilizes an AFM probe with a metallic nanotip, in this case a gold nanoparticle, that produces near-field enhancement of the Raman spectra to obtain simultaneous Raman and topographical information with a spatial resolution often better than 100 nm.[96] This technique allows the mapping of surface features with correlated chemical characterization through Raman spectroscopy. Figure 3.14A shows an intensity map of TERS signals for a 0.5 μm x 0.5 μm region of 31 pixels x 31 pixels (each pixel corresponds to ~16 nm of the sample surface) of a DNA origami burn-in sample treated at 900°C for 60 seconds. Figure 3.14B includes an overlay of the Raman spectra for several pixels (defined by black circles in Figure 3.14A). These locations were selected due to their high signal intensity, although this intensity may be due to noise in the spectra.
Figure 3.14 An intensity map of 0.5 µm x 0.5 µm sample treated at 900°C for 60 seconds and with circles indicating the pixels of high signal intensity corresponding to the Raman spectra in B.

Unfortunately, the peaks identified in Figure 3.14B are similar to those seen when using normal Raman on a silicon substrate in Figure 3.15B as well as TERS spectra for control samples in Figure 3.15A (silicon control) and B (silicon functionalized with APTES monolayer).
Figure 3.15 TERS spectra for control samples of A) silicon and B) silicon functionalized with APTES. There is little variation between these spectra and those for burn-in samples.

To probe the capacity of the TERS technique to adequately detect nanostructures on the scale of the SiC features, a control of DNA origami on mica was used due to the strong Raman signal provided by DNA nucleobases.[97], [98] Topographical features in the 2 µm x 2 µm scan (Figure 3.16A) do not indicate the presence of rectangular features corresponding to DNA origami on the surface. The features exhibited in this image are approximately 7 nm high and are most likely surface contaminants. Additionally, the Raman intensity map exhibits a similar increase in intensity near the bottom of the image (Figure 3.16B) and is almost identical to the intensity map of the burn-in sample in Figure 3.14A. The lack of topographical features and the similarities between the Raman intensity maps for various samples indicates that TERS does not have the resolution or surface sensitivity required for the analysis of SiC burn-in structures. This is likely due to the use of gold nanoparticles on the end of
the AFM tip, which increases the tip radius and decreases the lateral resolution capabilities of the TERS instrument compared to traditional AFM. Because a positive control spectrum for either DNA origami or SiC was never completed, the specific problem cannot be elucidated. It can be concluded that, with the current instrument set-up, parameters, and samples, TERS analysis is not a suitable technique.

![Figure 3.16](image)

**Figure 3.16** A) Topographical image of 2 μm x 2 μm region of DNA origami on mica only identifies surface contaminants. B) The corresponding Raman intensity map is similar to that seen in burn-in samples, indicating noise or problems with scanning parameters.

TEM was used to characterize SiC replicas at the Si/SiO₂ interface and determine whether it was amorphous or crystalline. Two different types of samples were analyzed. The first was DNA origami directly deposited on silicon and annealed at 900°C for 60 seconds. Figure 3.17 is a TEM image of the cross section produced via FIB (protocol
described in section 3.2.2); the various components of the cross section have been labelled as i) deposited platinum, ii) native SiO$_2$, and iii) bulk Si. There is no variation in the Si/SiO$_2$ interface to indicate the presence of embedded SiC from DNA origami.

Figure 3.17 TEM of cross sections of samples with DNA origami directly deposited on silicon and treated at 900°C for 60 seconds. The following layers are labelled in image B): i) deposited platinum, ii) native silicon oxide, and iii) bulk silicon.

A sample of DNA origami deposited on APTES functionalized silicon and annealed at 1100°C for 60 seconds was also analyzed using TEM. The same sample preparation steps were used and the three layers described in Figure 3.17B are also present. However, a dark contrast can be seen in Figure 3.18B (indicated by an arrow) between the native oxide and bulk silicon. The dark feature was not localized to one region, but was pervasive through the entire cross section. Additionally, there was no correlation between an increased height of the native oxide and the dark regions, which would be
consistent with the ~1 nm height measured for the SiC DNA origami replicas using AFM. It is possible that this feature is due to strain of the Si/SiO$_2$ interface due to the higher annealing temperature, impurities, or variation in TEM analysis parameters. Without being able to pinpoint the locations of the SiC replicas on the TEM cross section, TEM studies are inconclusive for the presence of SiC at the Si/SiO$_2$ interface.

![Cross sections of samples of DNA origami deposited on APTES functionalized silicon and annealed at 1100°C for 60 seconds show the same layers as in Figure 5.13 B but dark contrast can be seen between the oxide layer and bulk silicon. The source and composition of this layer was not determined.](image)

3.4 Discussion

The unique hydrophilic behavior of the DNA origami burn-in samples was the first indication that a change in surface chemistry had occurred. Inability to fully etch the oxide layer, even after extensive exposure to HF, hinted that a unique material had
been formed. One of the only materials that is not etchable using HF is silicon carbide.

HF etching of bulk SiO$_2$ on SiC polytypes proceeds via an analogous mechanism to that of oxidized silicon substrates: the removal of H$_2$O and SiF$_x$. On silicon substrates, this results in the complete removal of the oxide layer and a hydrophobic, hydrogen terminated substrate (Figure 3.19A).[99] Although this same mechanism holds for oxidized SiC, it has been shown that HF is unable to remove the last layer of oxide, resulting in a stable, hydrophobic, hydroxyl terminated surface (Figure 3.19B).[100]

Figure 3.19 A) The mechanism of etching oxide on silicon proceeds via the removal of H$_2$O and SF$_4$ to produce a hydrogen terminated silicon surface. B) Etching oxidized SiC proceeds via a similar mechanism, but the resulting hydroxyl terminated surface cannot be further etched, resulting in a hydrophilic substrate. (Figures adapted from [99], [100])
The diffusion coefficient for carbon in silicon can be calculated from the data in Figure 3.9 by assuming that this system undergoes a non-steady-state diffusion process and is analogous to a planar thin film with finite carbon concentration and the silicon substrate can be considered infinite. The diffusion equations for this type of thin film experimental arrangement has the following solution:[101]

\[ c_x = \frac{c_0}{(\pi Dt)^{1/2}} \exp\left(-\frac{x^2}{4Dt}\right) \]

Where \( D \) is the diffusion coefficient, \( c_0 \) is the initial concentration of dopant or diffusing species, \( c_x \) is the concentration of said species at a given depth \( x \), and \( t \) is the annealing time. Taking the logarithm of both sides gives:

\[ \ln c_x = \ln\left(\frac{c_0}{(\pi Dt)^{1/2}}\right) - \frac{x^2}{4Dt} \]

Graphing \( \ln c_x \) versus \( x^2 \) gives a linear relationship where the slope equals \(-1/(4Dt)\). Data from both the 900°C and 1100°C samples illustrate a “two-step” diffusion process. The following graphs illustrate these processes.
Figure 3.20 Samples annealed at 900°C for 60 seconds exhibit a “initial” diffusion step with a line fit of $y = -0.0491x - 2.3836$ ($R^2 = 0.95$). The slope and $D$ relationship give a diffusion coefficient of $8.5 \times 10^{-16} \pm 1.1 \times 10^{-16}$ cm$^2$s$^{-1}$.

Figure 3.21 The “final” diffusion component of the sample annealed at 900°C for 60 seconds have a line fit of $y = -0.011x - 2.8553$ ($R^2 = 0.78$) and $D$ of $3.8 \times 10^{-15} \pm 6.9 \times 10^{-16}$ cm$^2$s$^{-1}$.
**Figure 3.22** Samples annealed at 1100°C for 60 seconds exhibit the same “initial” diffusion as those annealed at lower temperatures. The line fit of $y=0.0268x-0.8046$ ($R^2=0.92$) gives a $D$ of $1.5 \times 10^{-15} \pm 2.7 \times 10^{-16} \text{cm}^2\text{s}^{-1}$.

**Figure 3.23** The “final” component of the 1100°C for 60 second annealing gave a line fit of $y=-0.0058x-1.2014$ ($R^2=0.86$) and $D$ of $7.2 \times 10^{-15} \pm 1.2 \times 10^{-15} \text{cm}^2\text{s}^{-1}$.
Using these graphs, the diffusion coefficients included in Table 3.3 can be calculated for carbon in silicon at 900°C and 1100°C for 60 seconds.

**TABLE 3.3**

**DIFFUSION COEFFICIENTS FOR “INITIAL” AND “FINAL” DIFFUSION COMPONENTS OF SAMPLES ANNEALED AT 900°C AND 1100°C FOR 60 SECONDS**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Diffusion coefficient ((D) (\text{cm}^2\text{s}^{-1}))</th>
<th>Uncertainty ((\text{cm}^2\text{s}^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>900°C/60 sec (Initial)</td>
<td>8.5x10^{-16}</td>
<td>1.1x10^{-16}</td>
</tr>
<tr>
<td>900°C/60 sec (Final)</td>
<td>3.8x10^{-15}</td>
<td>6.9x10^{-16}</td>
</tr>
<tr>
<td>1100°C/60 sec (Initial)</td>
<td>1.6x10^{-15}</td>
<td>2.7x10^{-16}</td>
</tr>
<tr>
<td>1100°C/60 sec (Final)</td>
<td>7.2x10^{-15}</td>
<td>1.2x10^{-15}</td>
</tr>
</tbody>
</table>

The diffusion of carbon in silicon has been extensively studied because it is one of the main impurities in semiconductor devices. The most commonly held carbon diffusion mechanism incorporates substitutional carbon that interacts with silicon self-interstitials, producing a highly mobile interstitial carbon complex.\[102\] This mechanism is termed “kick-out” due the displacement of a silicon atom by a carbon atom. Although this mechanism is widely accepted, there are still reported deviations and anomalies between computational studies and experimental results, particularly evidence of a fast carbon diffusion close to the silicon surface and the observation of a substitutional carbon and highly mobile carbon pairing that is not included in “kick-out” diffusion.
models.[103] Overall, calculations for carbon diffusion coefficients ($D$) in silicon, a mathematical indication of the mechanism and behavior of carbon upon thermal treatment, vary widely depending on experimental set-up and carbon detection methods. Table 3.4 provides a summary of experimentally determined $D$ values as well as the parameters and detection methods for the experiments.

### TABLE 3.4
SUMMARY OF PREVIOUS CARBON DIFFUSION EXPERIMENTS AND $D$ VALUES

<table>
<thead>
<tr>
<th>$D$ (cm$^2$s$^{-1}$)</th>
<th>Temperature/Time</th>
<th>Carbon detection method</th>
<th>Carbon depth</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>$6.6 \times 10^{-12}$</td>
<td>1109°C/109 hours</td>
<td>Radio-tracer technique</td>
<td>90 µm</td>
<td>[104]</td>
</tr>
<tr>
<td>n/a</td>
<td>900°C/40 min or 160 min</td>
<td>SIMS</td>
<td>3-6 µm</td>
<td>[105]</td>
</tr>
<tr>
<td>$\sim 10^{-15}$</td>
<td>30°C to 60°C/50 min to 150 min</td>
<td>IR electronic absorption</td>
<td>n/a</td>
<td>[106]</td>
</tr>
<tr>
<td>$\sim 10^{-13}$</td>
<td>900°C/not provided</td>
<td>Radio-tracer technique</td>
<td>60 µm</td>
<td>[107]</td>
</tr>
</tbody>
</table>

To this day, the diffusion coefficient calculations are most often completed using the work of Newman and Wakefield from 1961, who deduced the following relationship using the radio-tracer C$^{14}$ detection technique after annealing at temperatures ranging from 1070 to 1400°C for several hours:[104]

$$D = 0.33 \exp \left( \frac{-2.92 \pm 0.25 \text{ eV}}{kT} \right) \text{cm}^2\text{s}^{-1}$$
Their experimental studies resulted in $D$ values ranging from $4.8 \times 10^{-12}$ to $9.1 \times 10^{-12}$ cm$^2$s$^{-1}$ after annealing at 1109°C for 309 hours. Like all of the studies listed in Table 3.4 Summary of Previous Carbon Diffusion Experiments and $D$ Values, this $D$ value is several orders of magnitude greater than those calculated from diffusion of carbon from DNA origami and listed in Table 3.3. There are several possible explanations for these discrepancies. First, the experiments outlined in Section 3.2 use a 60 second annealing time whereas all previous diffusion studies annealed for 40 minutes to several hundred hours. Additionally, the initial carbon concentrations for previous studies are much higher than those provided by DNA origami and ion implantation or a constant flow of carbon containing gas is often used, not thin film diffusion. Although experimentally different, several studies mentioned the detection of a SiC species at elevated temperatures, although this species was never quantified.[102], [104], [107] Additionally, often a peculiar “fast” carbon diffusion was detected close to the surface resulting in higher than expected carbon concentrations; this phenomenon was largely ignored in the determination of the diffusion mechanism and coefficient and an explanation was never provided.[102], [104], [105], [108]

A possible explanation for the lower $D$ value reported here compared to previous studies is the additional step of carbon diffusion through the native oxide. SiO$_2$ layers are used as diffusion barriers, particularly for the formation of carbon containing nanostructures or patterns, due to the poor diffusivity of carbon in SiO$_2$ at temperatures lower than 1200°C.[109], [110] The low diffusivity through SiO$_2$ most likely accounts for the results reported by Zhou et al. in regards to similar thermal annealing studies
conducted on a thick SiO$_2$ layer; instead of diffusion through the oxide and formation of SiC at the interface, they observed formation of graphitic carbon replicas from DNA origami on the oxide layer.[111] The SiO$_2$ acted as a barrier for diffusion, isolating the carbon on the surface of the substrate.

A more applicable comparison can be made with molecular monolayer doping of silicon via rapid thermal processing. These monolayers incorporate both dopant atoms as well as carbon and several groups have attempted to monitor the carbon contamination during the diffusion process. Unlike carbon diffusion studies described earlier, monolayer doping studies utilize very short annealing times, on the order of a several seconds, to create shallow doping junctions of between 5 to 100 nm. Depth profiling of dopant atoms is often completed using SIMS and Ye et al. and Alphazan et al. have monitored carbon concentration with this technique as well.[78], [112] Carbon was detected via these SIMS studies; however, the concentrations were relatively low or the measurements were largely unreliable due to the high carbon background level of $1 \times 10^{18}$ cm$^{-3}$, which interfere with detection of carbon from the monolayers. Additionally, SIMS does not have the capacity to differentiate between carbon species as XPS does, making the detection of SiC impossible. Shimizu et al. utilized laser-assisted atom probe tomography to detect contaminants from spike annealing of molecular monolayers, which provides atomic-scale resolution of the distribution of elements in semiconductors.[113] Carbon was primarily detected in the first few Si monolayers after annealing at 800 and 1025°C for 5 seconds. This technique also has the capacity to identify SiC as a $^{30}$Si$^{12}$C species, which was detected, but in much lower concentration
than carbon. It is feasible to assume that the longer annealing times of the burn-in process would induce carbon diffusion to greater depths.

3.5 Conclusion

Through RTP of DNA origami deposited on both functionalized and nonfunctionalized silicon substrates, carbon diffuses from the nanostructures and binds with silicon to create SiC replicas in the shape of the original DNA origami. The formation of these replicas was proven through contact angle measurements, AFM, and XPS. Although DNA origami has been used to pattern metals on substrates as well as an etch resist, this is the first instance of directly patterning silicon using the innate chemical make-up of DNA nanostructures.[32], [34], [35], [37] The presence of SiN detected via XPS hints at the complex chemical nature of SiC replicas. If nitrogen and carbon from the DNA origami diffuses simultaneously during burn-in, it is feasible that phosphorus from the DNA origami phosphate backbone is also driven into the surface. The incorporation of this n-type dopant in either SiC or silicon makes it possible to create pn-junctions of defined shape and size from DNA origami, avoiding the traditional use of masks for pn-junction fabrication. However, proving the presence of phosphorus from the DNA origami is difficult, particularly when using the chemical characterization techniques outlined in this chapter.

In XPS, silicon produces a plasmon peak that interferes with both the P2s and P2p energy regions.[114], [115] Figure 3.24A shows the silicon binding energy region for
a functionalized silicon substrate. The red box highlights the silicon plasmon peak that
overlaps the P2p binding region between ~128 and ~140 eV illustrated in Figure 3.24B.

A) Si2p and Si2s Region for Functionalized Silicon

![Graph A)

B) P2p Region for Functionalized Silicon

![Graph B)

Figure 3.24 A) Si2p and Si2s energy region illustrating signals from silicon and silicon oxide. A plasmon peak (red box) interferes with the P2p energy region B).

A similar plasmon peak from Si2s interferes with the P2s binding region of ~180 to ~190 eV. Additionally, XPS has at best a lateral resolution of 10 μm, which does not allow chemical mapping on the SiC replica scale. The interfering silicon signal, lack of spatial resolution, and relatively low sensitivity of XPS to phosphorus makes this technique unsuitable for phosphorus detection. A different characterization technique is required for further analysis.
CHAPTER 4:
CHEMICAL AND ELECTRICAL CHARACTERIZATION OF DNA ORIGAMI SILICON CARBID ReplicaS AND OTHER BURN-IN NANOMATERIALS

4.1 Overview

The possibility of using SiC replicas formed by the burn-in process in electrical, optical, or biomedical applications depends on their chemical and electrical characteristics. Chapter 3 outlined attempts to characterize the crystallinity of SiC replicas but, due to the small size and low SiC concentrations, XRD and TEM techniques and results were inconclusive. The diverse chemical make-up of DNA origami and uncontrolled diffusion induced by burn-in hints that the SiC is amorphous and not stoichiometric. Although the literature is rampant with optimization experiments aimed at controlling SiC phase and crystallinity, amorphous SiC is also useful, specifically for sensor, optical, and electronic device development.[116]–[118]

Burn-in SiC from DNA origami is particularly interesting with respect to the possible formation of n-SiC/Si heterojunctions. Traditionally, these architectures have utilized SiC thin films produced via PECVD, RF glow-discharge deposition, or implantation.[119]–[123] These techniques allow for growth of SiC films with controlled dopant concentration and film thickness but, due to SiC chemical and physical stability,
these films are difficult to pattern on the nanoscale. Like silicon, cumbersome top-down lithographic patterning techniques are required to produce useful SiC architectures. There are several reports of SiC patterning not using photolithography. Ou et al. reported the production of submicron “cone-shaped” SiC patterns via RTP of Au films to produced metal nanoparticles, which were subsequently used as a mask for SiC. REI.[124] Silica nanospheres were used by Battula et al. for laser beam nanoscale patterning of bulk SiC. [125] Reports of bottom-up SiC fabrication is extremely limited. Cimalla et al. produced 3D SiC nanostructures via controlled, substrate mediated nucleation with Si and C from electron beam evaporators.[126] To date, there are no reports of bottom-up patterning of submicron doped SiC. If phosphorus co-diffuses with carbon from DNA origami, it is possible that the burn-in process described in Chapter 3 produces n-doped SiC via maskless patterning. To investigate whether the burn-in SiC replicas contain phosphorus, depth profiles were measured using secondary ion mass spectrometry (SIMS). Two advanced scanning probe techniques were used to measure electrical properties of the burn-in SiC replicas: electrostatic force microscopy (EFM) and PeakForce™ Tunneling AFM (PF-TUNA).

The simplicity of the burn-in technique and the prospect of maskless dopant patterning prompted its expansion to other nanomaterials. Boron doping was attempted using boron-containing nanoparticles (B@SiO₂) gifted to our lab by Dr. Ilya Zharov of the University of Utah.[127] Because silicon signals interfere with boron detection in XPS, B@SiO₂ burn-in characterization was limited to EFM and PF-TUNA.
methods. Using B@SiO$_2$ nanoparticles in burn-in indicates that the process may be applicable to other nanomaterials.

4.2 Experimental methods

4.2.1 Sample preparation

Burn-in samples were prepared as outlined in Section 3.2.1.

Masked plasma oxidation and microcontact printing (μCP) were used to pattern larger areas of DNA origami. Masked plasma oxidation utilized either polydimethylsiloxane (PDMS) stamps or TEM grids (Ted Pella, Inc.; Redding, CA). PDMS stamps were made using Sylgard® 184 silicon elastomer kit (Dow Corning Corporation; Midland, MI). Stamps were fabricated by mixing a 1:10 mass ratio of polymer to curing agent in a weigh boat, degassing under vacuum for 30 minutes, pouring the mixture over a stamp master, and letting the stamp cure at 110°C for 20 minutes. The stamp masters were 1 cm x 1 cm CD or DVD chips. Masked plasma oxidation was carried out by first functionalizing silicon with APTES, masking the substrate with PDMS or TEM grid, and exposing to air plasma using a PDC-32G Plasma Cleaner (Harrick Plasma, Inc.; Ithaca, NY) set to “Hi” for 5-10 minutes. DNA origami was then deposited as outlined in Section 3.2.1.

μCP was conducted with APTES (Gelest, Inc.; Morrisville, PA). PDMS stamps were inked with various concentrations of aqueous APTES solution, excess solution was removed using a pipette, the stamp was briefly dried with N$_2$ and placed in contact with
the substrate. After carefully removing the stamp, the sample was rinsed and dried as outlined in Section 3.2.1.

Dr. Ilya Zharov from University of Utah provided B@SiO$_2$ nanoparticles for burn-in experiments. The nanoparticle size was 130.8 ± 9.9 nm (N=40 nanoparticles), based on nanoparticle height measurements. Boron is encapsulated in a silica shell with a thickness between 10 and 20 nm.[127] The nanoparticles were suspended in ethanol at various concentrations and sonicated for 5 minutes before every use to ensure even dispersal in solution. 20 µL was pipetted on clean silicon substrates and dried at 25°C until the ethanol had evaporated. The samples underwent burn-in processing with the same parameters as outlined in Section 3.2.1.

4.2.2 Characterization techniques

A CAMECA NanoSIMS 50 (AMETEK, Inc.; Gennevilliers Cedex, France) in the Department of Physics at Washington University in Saint Louis was used to measure phosphorus depth profiles. The scans were conducted with a “Entrance Slit #2” size of 40x220 µm, “Aperture Slit #1” size of 350x250 µm, and all 5 detectors with “Exit Slit #1” of 200 µm wide. A ~100 nm primary Cs$^+$ ion beam was produced by using a “D1-aperture #3” of 150 µm. The primary beam (FcP) was ~15 nA and the current to which the sample was exposed (FcO) was measured as ~0.845 pA. The mass resolving power (M/ΔM) was ~4400. Samples measuring ~0.7 cm x ~0.7 cm were mounted on a custom-made sample holder using conductive carbon tape. The samples were made flush with the sample holder to ensure the system was as flat as possible. The following ions were
monitored: $^{12}\text{C}$, $^{16}\text{O}$, $^{12}\text{C}^{14}\text{N}$, $^{28}\text{Si}$, and $^{31}\text{P}$. Crater depths were measured using a Dimension Icon AFM (Bruker Nano, Inc.; Billerica, MA) in ScanAsyst™ mode using SCANASYT-AIR probes (Bruker Nano, Inc.; Billerica, MA) with 70 kHz resonance frequency and 0.4 N/m spring constant.

A Bruker Multimode AFM with Nanoscope V controller was used to conduct EFM measurements. Gold coated Etalon high resolution noncontact conductive probes (purchased from K-Tek Nanotechnology; Wilsonville, OR) were used with a resonance frequency of 235 kHz and 12 N/m force constant. Samples were adhered to a metal AFM puck using either conductive carbon tape or Silver Conductive Paint #503 (Electron Microscopy Sciences; Hatfield, PA). To increase electrical contact, the back of the silicon chip was scratched using a diamond tipped pen. The oscillation frequency of the cantilever was tuned before every scan and the phase was “zeroed” (without which discontinuities in the phase image may occur). Lift height was optimized by scanning with $V_{\text{tip}}=0.0$ V and increasing lift mode height by increments of 10 nm until the phase image became uniform and there was no interaction between the tip and the surface. Scans were completed by varying $V_{\text{tip}}$, usually from -4.0 V to 4.0 V, and recording both topographical and phase images.

A Bruker Dimension Icon AFM with TUNA module was used for PF-TUNA measurements. The same mounting procedure was used as with EFM samples and experiments. The measurements used conductive SCM-PIT probes (Bruker Nano Inc.; Billerica, MA) with 75 kHz resonant frequency and 2.8 N/m spring constant. The current sensitivities and gains were changed depending on the signal being measured.
4.3 Results

4.3.1 SiC replica characterization

4.3.1.1 Secondary ion mass spectrometry (SIMS)

The mass slit sizes were calibrated before every scan set to ensure the ions being monitored could be resolved. The ideal mass slit parameters for adequate mass resolution are outlined in Section 4.2.2. Figure 4.1 is a screenshot of the peak selection screen during the mass calibration. Two curves are visible: 1) the solid green line corresponds to a silicon control sample heated to 1100°C for 60 seconds; 2) the dashed red line is from the previous calibration for a functionalized silicon sample with DNA origami that underwent burn-in at 1100°C for 60 seconds. There are two main mass peaks highlighted. Both samples contain peak (B), which corresponds to $^{30}\text{Si}+^{1}\text{H}$ with a mass of 30.982 a.m.u. Peak (A) is only present in DNA origami burn-in samples and corresponds to the $^{31}\text{P}$ signal with a mass of 30.974 a.m.u. [128], [129] The similarities between the masses for the two ions made slit size calibrations essentially to attain a mass resolution of ~4400 and detect phosphorus in the samples.
All scans utilized a scan area of 5 µm x 5 µm with either 100 or 200 cycles.

Examples of the SIMS crater are seen in Figure 4.2. The crater on the left is produced after a 100 cycle scan and the crater on the right is from a 200 cycle scan. After acquiring data in one location, the scan was then moved 20 µm to acquire data from another location. This process was completed eight times. From the crater depths

Figure 4.1 The masses of (A) $^{31}$P and (B) $^{30}$Si+$^1$H were resolved even though they are only 0.0078 a.m.u. apart. The green solid line corresponds to a silicon burn-in control (1100°C for 60 seconds) and the red dashed line is signal from a DNA origami on functionalized silicon burn-in sample (1100°C for 60 seconds). Phosphorus is detected only in the DNA origami burn-in sample.
measured using AFM, the sputtering rate of the ion beam was calculated at 0.46 ± 0.03 nm/cycle (N=8 SIMS analysis craters) for 100 cycle scans and 0.49 ± 0.04 nm/cycle (N=16 SIMS analysis craters). Both rates were within standard deviation, indicating consistent surface sputtering with varying number of cycles per scan. The sputter rates were used to calibrate depths for SIMS depth profiles.

Figure 4.2 Craters created by ion beam sputtering during 100 cycle (left) and 200 cycle (right) scans. All scans were completed with 5 µm x 5 µm scan areas and both AFM images have 200 nm height scales.

SIMS $^{31}$P depth profiles for various burn-in sample are included in Figure 4.3. Additional SIMS depth profiles for $^{12}$C, $^{16}$O, $^{12}$C$^{14}$N, and $^{28}$Si can be found in Appendix C.

The silicon control sample processed at 1100°C for 60 seconds did not exhibit a $^{31}$P signal. Profiles for this sample were completed to ~50 nm. Phosphorus was detected in both DNA origami on functionalized silicon burn-in samples (processed at 900°C for 60
seconds and 1100°C for 60 seconds). Depth profiles were complete to ~100 nm for these samples.

![SIMS ³¹P Depth Profiles](image)

Figure 4.3 Exampl SIMS depth profiles for ³¹P signal for a silicon control sample and DNA origami burn-in samples processed at 900°C and 1100°C for 60 seconds. All analyzed locations exhibited similar spectra.

The beam size used for SIMS experiments allows for elemental mapping with ~100 nm resolution (~1 DNA origami = ~1 pixel). Examples of the mapping capabilities can be seen in Figure 4.4. The example is from a 1100°C/60 second DNA origami burn-in sample. The top images correspond to elements mapped after the oxide layer has been sputtered and surface contaminants have been removed (cycle 24). The bottom images
are from the last scan (cycle 200). Elemental signals are displayed in a heat map, with black being the lowest number of counts and red being the highest number of count. The lack of discernible surface features definitively corresponding to DNA origami prompted investigation into µCP and masked plasma oxidation to produce larger DNA origami patterns more suitable for the SIMS elemental mapping.
Figure 4.4 Element count mapping for a 1100°C/60 second DNA origami burn-in sample. The top images are from cycle 24, after the oxide and surface contaminants have been removed. The bottom images are from cycle 200. The count intensities are mapped with black being the least and red being the most. All images are 5 μm x 5 μm.
4.3.2 Microcontact printing for DNA origami patterning

CDs and DVDs were used to create PDMS stamps for microcontact printing. Exposed layers of writable CD and DVD grooves are shown in Figure 4.5. The spacing of each CD line (Figure 4.5A), measured from the peak of one line to the peak of another, was 1.19 μm ± 0.05 μm (N=30 lines). The average height of each patterned line was 129.3 nm ± 16.6 nm (N=33 lines). The DVD grooves (Figure 4.5B) had a peak-to-peak spacing of 0.66 μm ± 0.02 μm (N=33 lines) and an average peak height of 87.3 nm ± 2.9 nm (N=33 lines).
Figure 4.5 AFM images and corresponding line profiles for writable A) CD and B) DVD grooves.

The resulting PDMS stamps from a A) CD master and B) DVD master are shown in Figure 4.6. The line spacing of the CD PDMS stamp is 1.36 μm ± 0.07 μm (N=40 lines) and the line height is 142.2 nm ± 14.2 nm (N=42 lines). The DVD stamp line height is 81.5 nm ± 5.5 nm (N=30 lines) and the width is 0.50 μm ± 0.06 μm (N=30 lines). The variation in stamp pattern compared to the master is most likely due to the malleable nature of PDMS and AFM imaging.
Figure 4.6 An AFM images and line profiles of PDMS stamps cast from writable A) CD and B) DVD.

Direct deposition of APTES from aqueous solution using PDMS stamps successfully transferred the DVD line pattern onto silicon substrates. Figure 4.7 includes AFM images of APTES patterned by varying stamping time, with a 30 second inking time for all samples. The line widths were consistent for all samples: A) 152 ± 33 nm (N=30 lines) for 2 minute stamping time; B) 117 ± 19 nm (N=30 lines) for 5 minute stamping time; C) 113 ± 13 nm (N=30 lines) for 10 minute stamping time. The line-to-line distance remained unchanged with stamping time and was consistent with the DVD stamp
(Figure 4.6). The height of the lines increased with increasing stamping time and is illustrated in Figure 4.8.

Figure 4.7 μCP of APTES from aqueous solution produced lines consistent with the DVD pattern for all stamping times. Stamping times were A) 2 minutes, B) 5 minutes, and C) 10 minutes. The height of the patterned lines increased linearly with increasing stamping time. AFM images have height scales of 5 nm.
DNA origami was first deposited onto 2 minute stamping samples due to the height being consistent with at least a partial APTES monolayer (~0.7 nm).[130] The DNA origami solution remained in a “beaded” drop in the center of the silicon chip where the APTES was patterned indicating a hydrophobic substrate consistent with APTES functionalized SiO₂. DNA origami were visible on the substrate with a coverage of 12.3±0.2% (N=Three 5 μm x 5 μm locations) (Figure 4.9). The nanostructure placement was random (i.e. they did not preferentially bind with the patterned APTES lines). After the 20 minute DNA origami deposition and rinsing/drying steps, the APTES lines that had been originally patterned were no longer visible.
Figure 4.9 APTES lines patterned with 2 minute μCP stamping were not visible after DNA origami exposure. The nanostructures exhibited high coverage and did not preferentially bind to APTES patterned regions.

A baking step was added to the process after stamping to induce cross-linking of APTES. Lines were stamped for 3 minutes and the sample was baked at 110°C for 30 minutes (Figure 4.10). After the sample cooled and DNA origami was deposited, the APTES lines were still visible but with decreased height. DNA origami exhibited random deposition with a coverage of 8.3±3.1% (N=Three images of 5 μm x 5μm).
Figure 4.10 After DNA origami deposition on samples prepared by 3 minute μCP stamping of APTES on silicon and baking at 110°C for 30 minutes, APTES lines were still visible on the substrate but DNA origami were not patterned.

A final μCP attempt was made using plasma cleaned silicon instead of HF etched and piranha cleaned in case the surface chemistry could be altered to induce monolayer formation. The resulting APTES lines can be seen in Figure 4.11. They were visible after 2 minutes of DNA origami deposition and sample rinsing and drying, but the DNA origami continued to randomly deposit on the surface. There was not preferential binding to the APTES lines.
Figure 4.11 Stamping APTES for 10 minutes through µCP and exposing to DNA origami solution produced lines that remained after processing but exhibited nonspecific DNA origami binding.

Investigations into control samples revealed that the rinsing step was key in destroying the APTES lines. Figure 4.12A was rinsed with 200 µL of water and dried for 3 minutes with N₂. There are faint lines where APTES was deposited, but they are not well-defined and do not exhibit the height seen in previous samples. Figure 4.12B was exposed to DNA origami solution for 20 minutes and then rinsing and drying. Similar faint lines can be seen in both samples, although the DNA origami solution produces larger aggregates on the substrate. This result indicates that exposure to aqueous solution partially removes the microcontact printed APTES regions in both samples.
Figure 4.12 APTES lines patterned with μCP are removed after soaking in A) water and B) DNA origami solution for 20 minutes.

4.3.3 Masked plasma oxidation for DNA origami patterning

A PDMS stamp with the DVD pattern was used to partially mask an APTES functionalized silicon chip. An example of the stamp placement is shown in Figure 4.13 where the stamp location is indicated by the striped area on the schematic. After exposing the stamp covered silicon chip to air plasma for 5 minutes, APTES was removed from areas not covered by the stamp (Figure 4.13, top). This was indicated by the decrease in roughness from 0.26 nm ± 0.05 nm (N=12 areas of 1 μm x 1 μm) before plasma exposure to 0.13 nm ± 0.01 nm (N=12 areas of 1 μm x 1 μm) after exposure in this area, consistent with the roughness of cleaned silicon discussed in Section 1.3.2. Regions that were masked with the PDMS stamp (Figure 4.13, bottom) during plasma treatment exhibited a roughness of 0.34 nm ± 0.03 nm (N=12 areas of 1 μm x 1 μm). This is slightly higher than the roughness before exposure, although this is likely due to
an increase in particles on the surface from exposure to the PDMS stamp. A pattern was visible at the edges of the stamp location (Figure 4.13, middle).
Figure 4.13 APTES was removed from samples in areas not masked by PDMS (top) but remained in those masked (bottom). The edges of the mask location showed a lined pattern consistent with the PDMS stamp pattern.
Figure 4.14 includes a more detailed AFM image and a height profile of regions at the edges of the PDMS mask. The average distance between two patterned lines was 0.68 μm ± 0.04 μm (N=31 lines) and the average line height was 0.47 nm ± 0.17 nm (N=30 lines). The average line width was 0.55 μm ± 0.05 μm (N=30 lines).

Figure 4.14 An AFM image and line profile for regions of an APTES functionalized silicon substrates masked by a PDMS stamp during plasma treatment. The patterned lines are consistent with the DVD PDMS stamp pattern.

Upon exposure to DNA origami solution, the unmasked region of the chip wicked the solution and exhibited hydrophilicity, consistent with the presence of silicon oxide on the surface. The PDMS stamp masked region remained hydrophobic, with the solution remaining in a defined drop, consistent with an APTES functionalized silicon surface.

After DNA origami deposition, nanostructures were visible on the region that were exposed during the plasma treatment (Figure 4.15, top) with a coverage of
0.4±0.2% (N=Four images of 4 µm x 4 µm). The low nanostructure coverage corresponds to deposition on SiO₂ (Section 1.3.2). Lines were still visible in regions that were close to the mask edge (Figure 4.15, right), with widths of 0.31 µm ± 0.07 µm (N=20 lines). There was no discernible DNA origami in these regions and large aggregates were visible in the plasma treated trenches between the lines. On closer inspection in Figure 4.16, the patterned lines are no longer well-defined, exhibiting “frayed” edges and holes where the APTES monolayer was masked during plasma treatment.
Figure 4.15 After plasma treatment and DNA origami deposition, exposed regions (top) exhibit DNA origami binding consistent with SiO₂ substrates. Lines are still visible in PDMS masked plasma patterned regions (right) but no nanostructures are discernible.
Masked plasma oxidation was also attempted with DNA origami already deposited on an APTES monolayer. The results were similar as the previously described APTES masked plasma oxidation. Faint lines were visible at the mask edge (Figure 4.17, right) and DNA origami remained intact when masked.

Figure 4.16 After DNA origami deposition, lines are no longer well-defined and large aggregates are visible in regions without APTES.
Figure 4.17 Faint lines can be seen in regions masked by the PDMS stamp during plasma treatment (right).

To allow for direct exposure of the functionalized silicon substrate during the plasma treatment, a TEM grid was used to remove the APTES in the grid “holes.” The TEM grid was placed on the APTES functionalized silicon chip using a small drop of acetone. Upon evaporation of the acetone, the TEM grid was held in place. [131], [132] Residue could be seen on the substrate after sample preparation (Figure 4.18), possibly due to removal of the TEM grid coating.
Figure 4.18 Inspection of the substrate with an optical microscope showed residues consistent with the TEM grid spacing and placement.

Figure 4.19 includes AFM images of the APTES functionalized silicon substrates with TEM grid patterns transferred during plasma treatment. The boundary of masked versus unmasked regions is clearly seen in the bottom image. The increased number of particles on the surface made roughness measurements impossible. DNA origami was not deposited due to the high density of particles and debris from the TEM grid, which was consistent over multiple samples.
Figure 4.19 The pattern of the TEM grid was transferred on the APTES functionalized silicon substrate through plasma treatment.
4.3.4 Burn-in of B@SiO₂ nanoparticles

XPS was used to confirm the presence of boron in the nanoparticles provided by Dr. Ilya Zharov of the University of Utah. A silicon plasmon peak interferes with the B1s binding energy region in XPS spectra. For this reason, experiments to detect boron on silicon samples were not successful. To block out the XPS silicon signal, B@SiO₂ nanoparticles were deposited on mica coated with ~10 nm of gold. The XPS spectra in Figure 4.20 show the presence of the A) gold layer, B) nanoparticle oxide shell, and C) boron in the nanoparticles.
Figure 4.20 XPS spectra of B@SiO₂ nanoparticles deposited on Au/mica substrates detected A) gold, B) SiO₂, and C) boron.
Nanoparticle coverage was controlled by varying the B@SiO$_2$ solution concentration in ethanol. Solvent evaporation patterned the nanoparticles with a “coffee ring” effect, visible using the AFM optical microscope (Figure 4.21, top). Smaller nanoparticle ring patterns were also identified using AFM (Figure 4.21, bottom). B@SiO$_2$ NPs had a height of 130.8 nm ± 9.9 nm (N=40 nanoparticles).

Figure 4.21 Nanoparticle aggregates were visible using an optical microscope (top, all scale bars are 20 μm). Aggregates and solvent evaporation patterned nanoparticles were identified using AFM (bottom, all height scales are 300 nm). Decreasing concentration decreased both coverage and aggregate size.
These samples were capped with ~500 nm of SiO$_2$ using PECVD and underwent RTP at 1100°C for 60 seconds. The capping layer was removed after 20 minutes in 4% HF and exhibited a hydrophobic surface. Post-etch contact angle and ellipsometry measurements are summarized in Table 4.1.

### TABLE 4.1

CONTACT ANGLE AND ELLIPSOLOGY FOR B@SiO$_2$ BURN-IN SAMPLES (POST-ETCH)

<table>
<thead>
<tr>
<th>Total # Boron Atoms Deposited on Surface</th>
<th>Contact Angle (N=3)</th>
<th>Ellipsometry (nm) (N=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>48.3° ± 0.8°</td>
<td>2.52 ± 0.20</td>
</tr>
<tr>
<td>1.11x10$^{16}$</td>
<td>44.9° ± 2.2°</td>
<td>2.70 ± 0.15</td>
</tr>
<tr>
<td>1.11x10$^{17}$</td>
<td>33.7° ± 1.0°</td>
<td>2.35 ± 0.05</td>
</tr>
<tr>
<td>1.11x10$^{18}$</td>
<td>43.3° ± 1.1°</td>
<td>4.81 ± 0.31</td>
</tr>
<tr>
<td>4.23x10$^{18}$</td>
<td>40.7° ± 1.3°</td>
<td>4.24 ± 0.60</td>
</tr>
</tbody>
</table>

After burn-in processing and SiO$_2$ capping layer etching, features are present on the substrate with coverage consistent with deposited B@SiO$_2$ NPs (Figure 4.22B). These features have an average height of 12.1 nm ± 4.3 nm (N=40 nanoparticles), which is ~10x smaller than the original B@SiO$_2$ NP dimensions.
Figure 4.22 AFM images A) before and B) after burn-in of B@SiO₂ nanoparticles show consistent nanoparticle distribution but a decrease in height, illustrated by the ~10x difference in AFM height scales.

Four-point probe resistivity measurements completed after B@SiO₂ burn-in are shown in Figure 4.23. The number of boron dopant atoms deposited onto the substrate were calculated assuming the B@SiO₂ mass was entirely composed of boron. This is not correct due to the presence of the SiO₂ shell, but the assumption was made because the relative concentrations are not known. The resistivity of the silicon before B@SiO₂ deposition and burn-in was 3.5 ± 0.1 Ω·cm (N=6 silicon chips). There is a drastic increase in resistivity after burn-in of the 3800 ppm (4.23×10^{18} deposited boron atoms) sample.
Figure 4.23 Four-point probe resistivity measurements show an increase in resistivity after burn-in of 4.23x10^{18} boron atoms (corresponding to 3800 ppm nanoparticle deposition). The resistivity of control silicon controls was $3.5 \pm 0.1 \ \Omega \cdot \text{cm}$ (N=4 measurements).

Electrical characterization of the B@SiO$_2$ burn-in samples was first completed with EFM. Figure 4.24 shows EFM phase images for a single location of B@SiO$_2$ burn-in features (AFM topography image is included). Applying a positive sample bias produced a negative phase shift around the B@SiO$_2$ features. A negative phase shift was produced by applying a positive tip bias. Increasing the tip lift height decreased the magnitude of the phase shift. The areas surrounding the B@SiO$_2$ burn-in features exhibited the phase shift and not the features themselves. This is discussed further in Section 4.4.3.
Figure 4.24 EFM images of the same area indicate phase shifts when applying positive or negative tip bias. The degree of phase shift decreases with increase tip lift height.
Conductivity of the B@SiO$_2$ burn-in features was studied with PF-TUNA (Figure 4.25). Upon application of a positive sample bias, a positive current was detected corresponding to the locations of the burn-in features. The inverse was true for applying a negative sample bias.

Figure 4.25 PF-TUNA images for various $V_{\text{sample}}$. All PF-TUNA images have the same scale ($\pm 3.5$ pA).
4.4 Discussion

4.4.1 Phosphorus diffusion (SIMS)

Diffusion of phosphorus in silicon has been extensively investigated due to its implications in electronic device development and manufacturing. The mechanism of phosphorus diffusion is vacancy dominated, although the degree to which interstitialcy (diffusion of dopant atoms via interstitial sites and a “kick-out mechanism, see Section 3.4 for more details) contributes to the mechanism is still debated.[133]–[136]

The SIMS data reported in Section 4.3.1.1 can be used to determine the phosphorus diffusion coefficients in this system. Calculations were completed as described in Section 3.4. Examples of phosphorus SIMS depth profiles for 900°C/60 second and 1100°C/60 second burn-in samples can be seen in Figure 4.26(A) and (B), respectively. The respective ln(c) versus $x^2$ plots included illustrate the best fit line, the slope of which equals $1/(4Dt)$.[101]
Figure 4.26 $^{31}$P SIMS depth profiles and the corresponding $\ln(c)$ vs. $x^2$ plots for (A) 900$^\circ$C/60 sec. and (B) 1100$^\circ$C/60 sec. DNA origami burn-in samples. The slope of the best-fit line can be used to calculate phosphorus diffusion coefficients for this system.

The vertical dashed lines (red) in the SIMS profiles indicate the data used for calculations. The initial depth ($x_i$) is the depth at which all oxide has been sputtered and silicon signal reached a maximum (see Figure C.4 for an example of Si$^{28}$ SIMS spectrum). The final depth ($x_f$) was the depth at which the signal reached a minimum counts per second (CPS) or the end of the scan cycles. The minimum CPS was assumed to be
equivalent to the background noise, which was ~40 CPS. This assumption provided a
good fitting linear ln(c) vs. $x^2$ response (Figure 4.26).

Diffusion coefficients were calculated for all SIMS depth profile locations. The
samples treated at 900°C for 60 seconds exhibited a phosphorus diffusion coefficient
($D_{900^\circ C}$) of $3.4 \times 10^{-14} \pm 6.1 \times 10^{-15}$ cm$^2$s$^{-1}$ (N=8 depth profiles). The phosphorus diffusion
coefficient at 1100°C ($D_{1100^\circ C}$) was $2.1 \times 10^{-13} \pm 6.3 \times 10^{-14}$ cm$^2$s$^{-1}$ (N=8 depth profiles).
These values are consistent with those reported in literature and summarized in Table
4.2.

### TABLE 4.2
LITERATURE VALUES FOR DIFFUSION COEFFICIENTS OF PHOSPHORUS IN SILICON

<table>
<thead>
<tr>
<th>$D_{900^\circ C}$ (cm$^2$s$^{-1}$)</th>
<th>$D_{1100^\circ C}$ (cm$^2$s$^{-1}$)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$3.4 \times 10^{-14} \pm 6.1 \times 10^{-15}$</td>
<td>$2.1 \times 10^{-13} \pm 6.3 \times 10^{-14}$</td>
<td>This work</td>
</tr>
<tr>
<td>$8 \times 10^{-14}$</td>
<td>$6 \times 10^{-12}$</td>
<td>[137]</td>
</tr>
<tr>
<td>$1 \times 10^{-15}$</td>
<td>$8 \times 10^{-12}$</td>
<td>[138]</td>
</tr>
<tr>
<td>$4.8 \times 10^{-16}$</td>
<td>$1.3 \times 10^{-13}$</td>
<td>[139]</td>
</tr>
<tr>
<td>$7.5 \times 10^{-16}$</td>
<td>$1.5 \times 10^{-13}$</td>
<td>[140]</td>
</tr>
<tr>
<td>$7.5 \times 10^{-18}$</td>
<td>$4 \times 10^{-15}$</td>
<td>[135]</td>
</tr>
</tbody>
</table>

Reported experimental diffusion coefficient values vary by several orders of
magnitude. This is largely due to differences in experimental set-up, depth profiling
techniques, and sample preparation. Like literature values for carbon diffusion,
phosphorus diffusion experiments utilize long treatment times (on the order of hours). Additionally, phosphorus has a complex diffusion mechanism that may also contribute to the broad range of diffusion coefficients. Phosphorus diffusion profiles exhibit a “kink-and-tail” profile caused by high phosphorus concentrations (above $10^{19}$ \(\text{P atoms/cm}^3\)) near the surface where vacancy mechanism of diffusion is prevalent.[135], [138] With increasing depth (and decreasing phosphorus concentration), the mechanism transitions to self-interstitial dominant, producing a “kink” in the profile.[141] The concentration at which the “kink” occurs in the P profile varies with temperature and it has been shown to almost completely disappear at temperatures above 1100°C.[142], [143] The SIMS profiles reported here do not exhibit this type of profile and no “kink” is visible. Utilizing the diffusion coefficients calculated here, the phosphorus surface concentrations after burn-in for 60 seconds are only $\sim 10^{18}$ \(\text{P atoms/cm}^3\) (see Appendix D for calculations). The low phosphorus concentration indicates that the self-interstitial mechanism is dominant and a “kink” is not expected in the diffusion profiles.

The SIMS instrument used in this study had the unique capability of elemental mapping with 100 nm resolution. The nanoscale size of the DNA origami SiC replicas (~90 nm x ~70 nm) was still too small for the instrument mapping capabilities. This is clearly illustrated in Figure 4.4 Element count mapping for a 1100°C/60 second DNA origami burn-in sample. The top images are from cycle 24, after the oxide and surface contaminants have been removed. The bottom images are from cycle 200. The count intensities are mapped with black being the least and red being the most. All images are 5 µm x 5 µm. where the elemental signal is randomly distributed and is not consistent
with the shape of DNA origami. An additional limitation is the random DNA origami deposition on the surface. Elemental mapping would be easier if DNA origami was precisely patterned in larger regions on the substrate.

4.4.2 DNA origami patterning

Processes to pattern DNA origami on silicon were briefly discussed in Section 1.3.2. In those examples, electron beam lithography was utilized for lift-off processes and the nanoscale patterning of APTES for DNA origami attachment. These processes are expensive, cumbersome, and better suited for nanoscale rather than micron scale patterning. In this chapter, three different techniques were attempted to create micron scale DNA origami patterns suitable for SIMS elemental mapping. µCP of APTES was first attempted based on results from Li et al. (the procedure is outlined in Section 4.2.1).[130] Li et al. used aqueous solutions to pattern APTES submonolayers on silicon using PDMS stamps. They reported the patterning of lectin and AuNPs on the APTES regions from aqueous solution. The deposition of DNA origami on µCP patterned APTES has not been reported.

The results here indicate APTES was successfully patterned using µCP and that increasing stamping time increased the height of the patterned lines. The theoretical height of an APTES monolayer is 0.7 nm.[130] Although this height was achieved after stamping for 2 minutes (Figure 4.7A), DNA origami was not patterned in regions where APTES was deposited. In all samples, the APTES lines were completely or partially removed after exposure to DNA origami solution. It should be noted that briefly rinsing
and drying the samples did not result in a change in line shape or size, only soaking the samples in solution destroyed the patterned lines (Figure 4.12).

The rapid hydrolysis and condensation of APTES upon exposure to water is well established and described in Section 1.3.2.[144] The removal of the APTES after soaking in aqueous solution indicates that the patterns were not covalently bound to the SiO$_2$ substrate. The increase of the APTES line height with time is further evidence that APTES condensation continues due to water exposure, increasing the amount of material physisorbed (or noncovalently/hydrogen bonded) to the patterned location.[145]

Why APTES does not completely bind to the SiO$_2$ substrate during µCP is not clear. Although inking and stamping parameters were varied and optimized to produce analogous results to Li et al., a key difference is their use of oxygen plasma to treat the silicon chips before µCP. Oxygen plasma treatment was not used in the experiments reported here due to the possibility that the native oxide thickness or surface roughness would increase. A thicker oxide film would impede the diffusion of carbon and other elements from DNA origami during the burn-in process. An air plasma treatment of 5 minutes was attempted but did not change the µCP results (not shown here). There is evidence that oxygen plasma treatment activates the SiO$_2$ substrate and increases the number of -OH groups on the surface for APTES binding.[146]–[148] Adding an oxygen plasma activation step before µCP may allow for complete covalent binding of APTES to SiO$_2$; however, further investigation is required to identify the effect of plasma treatment on the native oxide and burn-in processing.
Because the protocol reported by Li et al. was unsuccessful, a different patterning technique based on masked plasma oxidation was attempted. Thomas et al. used this technique to pattern positively and negatively charged regions through masking of a positively charge siloxane monolayer.[149] It was also used by Hattori et al. for the micropatterned decomposition of coated extracellular matrix (ECM).[150] The contact locations of the PDMS stamp “columns” mask the substrate from plasma exposure and subsequent decomposition. DVD lines were patterned using a PDMS stamp to mask either a well-formed APTES monolayer or a DNA origami layer deposited on APTES functionalized silicon (Figure 4.13 and Figure 4.17). The patterned lines were only present at the edges of the masked region. The lack of complete patterning is due to the PDMS stamp pattern and dimensions. Both Thomas et al. and Hattori et al. utilized column patterns to increase the number of “pathways” for the plasma to access and interact with the substrate. Additionally, the column heights were greater than 25 µm in both examples, which is more than 15x higher than the PDMS stamp lines reported here. Increasing PDMS stamp pattern size and feature height would allow more efficient substrate-plasma exposure and better APTES patterning for this technique, making it a feasible patterning option although further process optimization and development is required.

The third patterning attempt was inspired by the use of TEM grids as “shadow masks” for templating various materials on substrates.[131], [132] The APTES exposed to plasma treatment through the TEM grid holes degraded, leaving a barrier between masked and unmasked regions. Although patterning was successful, residue from the
TEM grids remained on the substrates, making the samples unsuitable for further processing. The origin of the residues is unknown. The TEM grids are placed on the sample using capillary action upon evaporation of a drop of acetone and this may remove the TEM grid coating. Further studies using uncoated TEM grids or TEM grids made of other materials, such as Si$_3$N$_4$.

4.4.3 Burn-in of nanoparticles for p-type doping

Using densely packed B@SiO$_2$ nanoparticles provides a p-type dopant for the burn-in process. Like detection of phosphorus discussed in Section 3.5, the B$_{1s}$ binding energy region overlaps with a silicon plasmon peak (Figure 4.27) and is difficult to detect at low concentrations.[151], [152] For this reason, XPS was not able to detect boron in burn-in samples. SIMS may be a feasible alternative for chemical characterization, but has not been attempted at the time of this report. For these reasons, electrical characterization was the primary focus of this study.
Figure 4.27 XPS scans of the B$_{1s}$ binding energy region show broad Si plasmon peaks at 185.8 eV and 202.3 eV. These peaks interfere with the expected location of boron from B@SiO$_2$ burn-in at 188 eV.

Four-point probe resistivity measurements (Figure 4.23) showed an increase in resistivity with the burn-in of high surface concentrations of B@SiO$_2$. This observation may be explained by boron diffusion neutralizing the phosphorus of the n-doped substrate. Control resistivity measurements of n-type silicon can be used to back-calculate the phosphorus dopant concentration, giving $1.3 \times 10^{15}$ cm$^{-3}$.[153] As described in Section 4.3.4, if the mass of the B@SiO$_2$ nanoparticles is assumed to entirely composed of boron, the high concentration deposition results in boron concentrations equivalent or higher than the phosphorus concentration. The effective neutralization of the phosphorus dopant would increase the resistivity of the silicon sample. An additional hypothesis pertains to the increase in the oxide layer measured with
ellipsometry and reported in Table 4.1. Higher boron concentration burn-in samples showed a 2 nm oxide increase, which would increase the resistivity.

The accuracy of these assumptions must be taken into consideration, particularly pertaining to the use of the four-point probe measurement where the probe spacing is larger than the layer thickness being measured. Utilizing an approximate boron diffusion coefficient of $1 \times 10^{-13}$ cm$^2$s$^{-1}$ (based on published data), the burn-in temperature, and burn-in time, a diffusion depth of ~30 nm is calculated.[154], [155] This is lower than the normal penetration depth of electrodes in four-point probe instruments. Penetrating through the diffused boron layer would result in resistivity measurements corresponding to bulk silicon.[156]–[158] The issue of probe penetration is often circumvented by using micro four-point probe instruments, but this was not available for these studies. For this reason, other nanoscale electronic characterization methods focusing on scanning probe microscopy were investigated.

Two methods utilizing specialized scanning probe microscopy techniques were explored. EFM uses a biased AFM probe to identify localized surface charges on a substrate.[159] The scan is completed in two passes. First, topographical information is acquired using a grounded tip in traditional noncontact, tapping mode. The topographical information is then used to “retrace” the image in lift mode, where the tip is lifted a calibrated height from the substrate, the tip is biased, and the tip-substrate interaction is recorded. Utilizing a lift mode is necessary to ensure that only the weak, long range electrostatic forces (and not topography) contribute to the probe signal.
The interaction between the biased tip and surface charges in lift mode is illustrated in Figure 4.28. A positively biased probe experiences a repulsive force with positive surface charges and an attractive force with negative surface charges. The electrostatic interaction can be translated into a shift in the cantilever frequency.

![Figure 4.28](image)

Figure 4.28 In EFM, a positively biased conductive AFM probe experiences attractive and repulsive forces with negatively and positively charged regions on the substrate.

The EFM technique was tested using a control sample of containing a field of $n^+$ ($\sim 1 \times 10^{20}$ atoms/cm$^3$) with small p-type ($\sim 3 \times 10^{15}$ atoms/cm$^3$) patterned regions. The control EFM measurements can be found in Appendix E. Attractive interactions (between $n^+$ regions and the positively charged tip) produced a positive phase shift. Repulsive interactions produced a negative phase shift.
The areas immediately surrounding the burn-in B@SiO₂ features exhibit a negative phase shift with a positive tip bias (Figure 4.29A) and a positive phase shift with a negative tip bias (Figure 4.29B). This is consistent with the presence of positively charge carriers from boron diffusion. The inversion of the phase shift in EFM images of burn-in B@SiO₂ samples indicates that the presence of surface charges. It should be noted that the B@SiO₂ bumps in both Figure 4.29A and B maintain a positive phase shift upon application of both positive and negative tip bias. This can be attributed to the presence of a dielectric or insulating layer, producing a capacitive coupling between the substrate and the tip.[160]–[162]

Figure 4.29 Illustration of EFM phase inversion for B@SiO₂ burn-in samples upon application of A) positive $V_{tip}$ and B) negative $V_{tip}$. Line profiles indicate negative and positive phase shift, respectively.
The EFM technique is primarily a qualitative measurement. Although determining surface potential and charge carrier concentration quantitatively is possible, it requires extensive control and calibration methods that were not completed with the experiments reported here.[163]

AFM techniques to measure sample conductivity rely on the application of a bias between the sample and the AFM tip to measure localized current. Tunneling AFM (TUNA) measures the tunneling current through a thin SiO₂ oxide, first exhibited through the characterization of SiO₂ gate oxide films.[164] The use of more sensitive linear current amplifiers in TUNA measurements, compared to traditional C-AFM current amplifiers, increases the current sensitivity.[159] PeakForce TUNA™ (PF-TUNA), developed by Bruker Corporation, combines TUNA measurements with PeakForce tapping methods. This allows for the precise control of the force of the tip on the substrate and is ideal for fragile samples. The data reported in Figure 4.25 correlates locations of B@SiO₂ NP burn-in with higher conductivity, which is consistent with expected behavior of p⁺ doped regions. Another advantage to using PF-TUNA is the ability to measure I-V curves at specific locations on the substrate through a point-and-shoot method. This was attempted for the B@SiO₂ burn-in samples and preliminary data showed no difference between I-V spectra from silicon background (Figure 4.30A) or B@SiO₂ burn-in features (Figure 4.30B)
Using EFM and PF-TUNA on B@SiO₂ burn-in samples provided an opportunity to optimize the measurements and explore their applications. Although the preliminary data for both methods is promising, further investigation is required. Utilizing control samples with various dopant concentrations to calibrate EFM signal and PF-TUNA IV-curves would provide better insight into the results expected from B@SiO₂ burn-in. These samples would allow for deconvolution of expected phase shift signals for p-versus n-doped regions in EFM.
DNA origami burn-in samples were also studied with EFM and PF-TUNA techniques. No clear signal was seen using either method on multiple samples. The resistance between the AFM puck and the top of the sample was measured to be between 2 to 4 MΩ using a multimeter, compared to the ~1 Ω resistance measured for B@SiO₂ burn-in samples, although the adhesion of the sample to the AFM puck were identical (see Section 4.2.2 for description). Using silicon wafers with lower resistivity may improve the measurement. Further investigation is needed to identify whether these techniques are suitable for analyzing doping via DNA origami burn-in.

4.5 Conclusion

The detection of phosphorus using SIMS is an important first step in further identifying the chemical and electrical properties of the DNA origami SiC replicas. SIMS is unable to differentiate the binding or electronic states of the burn-in diffused phosphorus, limiting the understand of the system. The other techniques introduced here (patterning via µCP, EFM, PF-TUNA) require further development before they can be utilized to elucidate the properties of SiC replicas. The use of boron-rich nanoparticles (B@SiO₂) in the burn-in process provides the first hint of a broader application to other shape, size, and chemically controlled nanomaterials. Future work expanding on the variety of nanomaterials used in the burn-in process may provide patterning avenues for unique embedded structures.

When Gordon E. Moore first introduced the ideas that would later become “Moore’s Law,” the cartoon in Figure 4.31 was published alongside it. The idea that
hand-held computers would be sold alongside other commodity goods was farfetched over 50 years ago. The creativity and tenacity of the semiconductor industry produced the current reality of smartphones and tablet computers. IBM’s recent introduction of 5-nm nodes produced using extreme ultraviolet lithography and stacks of Si nanosheets illustrates the continued creativity of the industry and the essential role of nanomaterials.[165]

As the industry continues to pursue miniaturization, opportunities to incorporate novel nanomaterials in manufacturing open the door for processes like burn-in and materials such as DNA origami. Looking beyond Si at other nanomaterials expands the creative boundaries and may one day lead to sub-5 nm node development.
APPENDIX A:

DNA SEQUENCES FOR DNA ORIGAMI DESIGNS

The DNA sequences were based on the rectangular DNA origami design reported by Woo and Rothemund.[15] The oligonucleotide labels are based on the original sequence. Four additional thymine bases were added to the 5’ biotinylated end of the oligonucleotide to ensure adequate protrusion of the biotin from the structure for biotin-streptavidin binding.[65]

TABLE A.1

BIOTINYLATED DNA ORIGAMI STAPLE STRAND SEQUENCES FOR FUNCTIONALIZED STRUCTURE

<table>
<thead>
<tr>
<th>Label</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>r1t16fr1</td>
<td>5’-Biotin/TTTTCGGGAGAATGCTTCTGTATAGGTCT-3’</td>
</tr>
<tr>
<td>r-1t16fl1</td>
<td>5’-Biotin/TTTCCCCCGTGGTTTCTCATCAACATTACGTAACCG-3’</td>
</tr>
<tr>
<td>r-1t8fl1</td>
<td>5’-Biotin/TTTTAACGGAACAAAGAAGTTTTGCCAGTTCAGA-3’</td>
</tr>
</tbody>
</table>
APPENDIX B:

ADDITIONAL XPS SPECTRA FOR DNA ORIGAMI HEATED ON MICA

Figure B.1 The peak areas for C_{1s} species of DNA origami on mica A) not heated, B) heated to 150°C, and C) heated to 250°C for 10 minutes are compared to the K_{2p 3/2} peak (red) areas of interstitial potassium in mica. The concentration of the potassium ions is assumed to be uniform between samples.
Figure B.2 Control mica samples A) not heated and heated to B) 150°C and C) 250°C for 10 minutes all exhibit the presence of an adventitious carbon peak (red) due to exposure to atmosphere. This peak does not change with heating conditions.
Figure B.3 The N$_{1s}$ signal for nitrogen species in DNA origami occurred at 399 eV. Due to the relatively low abundance of nitrogen in DNA origami, it was difficult to attain adequate signal to differentiate various nitrogen species after heating to B) 150°C and C) 250°C compared to A) control samples.
APPENDIX C:

ADDITIONAL SIMS DEPTH PROFILES

SIMS $^{12}$C Depth Profiles

![Graph showing SIMS $^{12}$C Depth Profiles with depth (nm) on the x-axis and CPS (a.u.) on the y-axis. The graph compares control and temperatures at 900°C and 1100°C.]

Figure C.1 A representative $^{12}$C SIMS depth profile (a single location).
Figure C.2 A representative $^{16}$O SIMS depth profile (a single location).
Figure C.3 A representative $^{12}\text{C}^{14}\text{N}$ SIMS depth profile (a single location).
Figure C.4 A representative $^{28}$Si SIMS depth profile (a single location).
APPENDIX D:

APPROXIMATE CALCULATIONS FOR PHOSPHORUS SURFACE CONCENTRATION FROM DNA ORIGAMI DURING THE BURN-IN PROCESS

According to the limited source diffusion model, the surface concentration after diffusion is given by the following equation:[166]

\[ N_0 = \text{Surface Concentration} = \frac{Q}{\sqrt{\pi Dt}} \]

Where \( Q \) is the initial deposited concentration, \( D \) is the diffusion coefficient, and \( t \) is the annealing time. The initial deposited phosphorus concentration can be approximated by assuming a 6% DNA origami coverage, which equates ~165 DNA origami/25 \( \mu \text{m}^2 \). There are ~12,000 phosphorus atoms in a DNA origami rectangle, giving total phosphorus atoms deposited via DNA origami as \( 7.9 \times 10^{12} \text{ P/cm}^2 \). Using the 900°C phosphorus diffusion coefficient calculated in Section 4.4.1 and a treatment time of 60 seconds, the surface concentration of phosphorus from DNA origami is \( 3.1 \times 10^{18} \text{ P/cm}^3 \). Using the 1100°C diffusion coefficient, the phosphorus surface concentration is \( 1.3 \times 10^{18} \text{ P/cm}^3 \).
APPENDIX E:

ELECTROSTATIC FORCE MICROSCOPY CONTROL MEASUREMENTS

The control sample was generously provided by Professor Greg Snider of the University of Notre Dame. The sample was highly n-doped ($\sim 10^{20}$ cm$^{-3}$) with patterned p-type regions ($\sim 3 \times 10^{15}$ cm$^{-3}$). The lighter or darker regions of the phase images correspond to the n-doped areas.

Figure E.1 Topographical AFM image a ~50 nm surface feature on the control doped sample.

Figure E.2 EFM phase image with 150 nm lift height, 0.0 V tip bias, and 0.0 V sample bias. There is no phase change visible in this image.
Figure E.3 EFM phase image with 150 nm lift height and tip bias of 2.0 V (left) and -2.0 V (right).

Figure E.4 EFM phase image with 150 nm lift height and tip bias of 4.0 V (left) and -4.0 V (right).

Figure E.5 EFM phase images with 150 nm lift height and tip bias of 6.0 V (left) and -6.0 V (right).
BIBLIOGRAPHY


[103] R. Pinacho, P. Castrillo, M. Jaraiz, I. Martin-Bragado, J. Barbolla, H. J. Gossmann,


