Metallization of Self-Assembled DNA Templates for Electronic Circuit Fabrication

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Metallization of Self-Assembled DNA Templates
for Electronic Circuit Fabrication

Bibek Uprety

A dissertation submitted to the faculty of
Brigham Young University
in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

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ABSTRACT

Metallization of Self-Assembled DNA Templates for Electronic Circuit Fabrication

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Doctor of Philosophy

This work examines the deposition of metallic and semiconductor elements onto self-assembled DNA templates for the fabrication of nanodevices. Biological molecules like DNA self-assemble into a variety of 2- and 3-D architectures without the need for patterning tools. The templates can also be designed to controllably place functional nanomaterials with molecular precision. These characteristics make DNA an attractive template for fabricating electronic circuits. However, electrically conductive structures are needed for electronic applications.

While metallized DNA nanostructures have been demonstrated, the ability to make thin, continuous wires that are electrically conductive still represents a formidable challenge. DNA-templated wires have generally been granular in appearance with a resistivity approximately two to three orders of magnitude higher than that of the bulk material. An improved method for the metallization of DNA origami is examined in this work that addresses these challenges of size, morphology and conductivity of the metallized structure. Specifically, we demonstrated a metallization process that uses gold nanorod seeds followed by anisotropic electroless (autocatalytic) plating to provide improved morphology and greater control of the final metallized width of conducting metal lines. Growth during electroless deposition occurs preferentially in the length direction at a rate that is approximately four times the growth rate in the width direction, which enables fabrication of narrow, continuous wires. The electrical properties of 49 nanowires with widths ranging from 13 nm to 29 nm were characterized, and resistivity values as low as 8.9 x 10^-7 Ω-m were measured, which represent some of the smallest nanowires and the lowest resistivity values reported in the literature. The metallization procedure developed on smaller templates was also successfully applied to metallize bigger DNA templates of tens of micrometers in length. In addition, a polymer-assisted annealing process was discovered to possibly improve the resistivity of DNA metal nanowires. Following metallization of bigger DNA origami structures, controlled placement of nanorods on a DNA breadboard to make rectangular, square and T-shaped metallic structures was also demonstrated. For site-specific placement, we modified the surface of the gold nanorods with single-stranded DNA. The rods were then attached to DNA templates via complementary base-pairing between the DNA on the nanorods and the attachment strands engineered into the DNA “breadboard” template. Gaps between the nanorods were then filled controllably via anisotropic plating to make 10 nm diameter continuous metallic structures. Finally, controlled placement of metal (gold) - semiconductor (tellurium) materials on a single DNA origami template was demonstrated.

The combination of molecularly directed deposition of different nanomaterials and anisotropic metallization presented in this work represents important progress towards the creation of nanoelectronic devices from self-assembled biological templates.

Keywords: biological molecules, DNA, DNA origami, anisotropic metallization, nanowire, site-specific, nanorods, gold, tellurium, nanoelectronics
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1 INTRODUCTION

Since their inception in the 1950’s, transistors have shrunk exponentially in size and surged in speed to provide increased capacity per unit area and, thus, increased functionality. The increase in the transistor density per unit area was made possible by shrinking the classical metal-oxide-semiconductor field-effect transistor (MOSFET), the mainstay of the modern electronics industry.\(^1\) Over the years, increased functionality and lower production costs (per transistor) have revolutionized the world of electronics. This trend in consistent reduction of costs while simultaneously improving quality and functionality of the devices has continued for over five decades. However, this trend of increased transistor density at lower costs appears to have hit the limit as the production cost per transistor is increasing for feature sizes below 20 nm. The cost per transistor production had been continually declining over the years as feature sizes decreased from 10 µm in the 1970’s to as small as 20 nm recently. The top-down process primarily uses a patterning technique called photolithography, whereby light is used to make or transfer a pattern on the silicon substrate.\(^2\) The process of patterning is repeated multiple times until the desired device is fabricated. As feature sizes get smaller, the number of processing steps (e.g. lithography and etching) required to achieve the desired size increases, which add to the production cost of the final device. The current top-down technology uses double and quadruple patterning techniques (which add to processing steps and thus costs) to make smaller feature sizes (~14 nm) using 193 nm ultraviolet light (UV) technology. The new extreme UV technology
(13.5 nm wavelength) can enable fabrication of smaller features without the use of additional processing steps, however, the technology has a steep capital cost and is still in its developmental stages for use in large scale manufacturing. In addition to the increasing production costs, the electrical characteristics of devices significantly degrade with smaller feature sizes. Such limitations have slowed device scaling and thus opened up opportunities for more cost effective alternative fabrication approaches. To this end, bottom-up nanotechnology, which enables construction of complex architectures from molecular building blocks, provides a promising alternative to develop future-generation devices.\(^{3-4}\) Bottom-up technology utilizes the recognition capability of single molecules to self-assemble into useful conformations of nanometer resolution without the need for patterning and expensive lithographic tools.\(^{3-9}\)

DNA, with its small size, functional groups and complementary base pairs, represents a desirable template for fabricating components of nano-scale devices via bottom-up assembly.\(^{10-11}\) Recent advances in the field of DNA origami\(^{12}\) have enabled fabrication of nanostructures by folding single-stranded DNA into different two-\(^{12-14}\) and three-\(^{11,15-17}\) dimensional shapes. In addition, different functional nanomaterials can be attached to DNA origami via complementary base pairing between the sequences bound to the functional groups and the sequences present within the DNA origami. This technique can controllably attach a variety of materials like metal nanoparticles\(^{8,18-22}\) and carbon nanotubes\(^{23}\) to specific DNA origami sites with nanometer precision. Hence, DNA based nanofabrication approach provides a promising pathway to develop nanoelectronic devices and systems based on biological molecules.

For application in nanodevices, the DNA templates (structures) need to be metallized to form conductive substrates.\(^8\) Furthermore, controllable deposition of nanomaterials at specific sites on origami templates is needed to utilize the full potential of DNA origami templates. Such
site-specific metallization via molecular recognition between the metal particles and DNA origami template has now been demonstrated for a single metal\textsuperscript{8,22,24} and multiple metals\textsuperscript{25} to form continuous DNA structures. While recent work shows considerable promise for bottom-up methods with DNA, obstacles still remain for actual device fabrication. The ability to make stable DNA structures in high yield, reduction of the linewidth and improvement of the morphology of nanowires formed on the DNA templates, and the development of methods to interface multiple inorganic components with electrical continuity on a single DNA template are some of the key barriers that need to be addressed.

The present work is primarily on metallization of DNA origami templates to create thin and continuous wires that are electrically conductive. An improved method for the metallization of DNA origami is examined in this work that addresses the challenges of size, morphology and conductivity of the metallized structure. This work demonstrates our efforts to understand and optimize the metallization process to make thin and electrically conductive nanostructures at molecularly designated locations on DNA origami templates. Following metallization of the entire template, challenges associated with controlled metallization of specific sites on the DNA template are studied. Finally, the challenges associated with controlled placement of metal and semiconductor elements on a single template are investigated. Figure 1-1 shows the schematic illustration of the process of metallizing DNA origami templates. The combination of molecularly directed deposition of metal and semiconducting elements and the metallization process presented in this work represents important progress towards realization of electronic devices from self-assembled biological templates.
1.1 Scope of the Project

Prior to this work, DNA metal deposits were generally granular in appearance with a resistivity approximately two to three orders of magnitude higher than that of the bulk material.8, 24 In addition, the final size of the metallized DNA templates has usually been far wider (>50 nm) than what is achievable with the underlying DNA template. The primary objective of this research is to develop a metallization process that addresses these challenges of size, morphology and conductivity of the metallized DNA structure. Techniques to selectively metallize specific parts of the DNA template with different functional materials are also examined. This experimental study thus addresses the challenges associated with metallizing DNA origami templates as well as controlled placement of the metal/semiconductor materials within the DNA architectures as a stepping stone for fabricating DNA-based electronic devices.
2 BACKGROUND

2.1 Bottom-up Nanotechnology

Bottom-up technology utilizes the capability of molecules to self-assemble into useful conformations. Self-assembly shows promise as a potential way of reducing cost, decreasing size and enabling fabrication of complex nano-assemblies. DNA is one such biological molecule which shows promise as a template due to its size, self-recognizable base pairs and well established protocols for handling and replicating DNA. In addition, metal and semiconductor nanomaterials can be controllably placed onto DNA templates with molecular resolution for potential application in nano-electronic circuits.

2.2 DNA

2.2.1 Basic Properties

DNA is a double helix structure consisting of two long polymers made up of small units called nucleotides; it has a backbone made up of sugar and phosphate groups. A nucleotide is an organic compound made up of a nitrogenous base, a sugar (deoxyribose for DNA) and a phosphate group. Adenine (A), guanine (G), thymine (T) and cytosine (C) are the four kinds of bases present in a DNA strand. The interaction between the bases in the form of hydrogen bonds holds the two DNA strands together. However, hydrogen bonding of the bases is non-covalent;
hence, they can be broken and rejoined relatively easily. The two strands in the double helix can be separated at higher temperatures to yield single-stranded DNA. Base pairing is very specific; i.e., each type of base on one strand bonds with just one type of base on the other strand. The only base pairs possible are A with T and G with C. Within the double helix structure of DNA, A forms 2 hydrogen bonds with T on the opposite strand, and G forms 3 hydrogen bonds with C on the opposite strand. In the B form of DNA (Figure 2-1), which is the normal double helix structure, the helix makes a turn every 3.4 nm, and the distance between two adjacent base pairs is 0.34 nm. The total diameter of DNA is also only about 20 Angstroms, making it suitable for use as a template for fabrication of components for nano-circuits.

Figure 2-1: DNA structure. Reproduced with permission from RSC. Copyright 2009
2.2.2 Metal-ion Interactions with DNA

The interaction between DNA bases and a variety of metals represents a key aspect of DNA metallization. DNA, with a negatively charged phosphate backbone, shows considerable attraction to charged species, an attraction that is, to a large extent, electrostatic. Alkali metal ions like Li⁺, Na⁺, K⁺, Cs⁺ have been shown to electrostatically bind to the DNA backbone. In addition to the electrostatic interactions, Duguid et al. used laser Raman spectroscopy to show that divalent metal cations like Mn²⁺, Ni²⁺, Cu²⁺, Pd²⁺ have specific metal interactions with acceptor sites (bases) on the purine (N7) and pyrimidine (N3) rings. These metal ions interact more strongly with the bases than with phosphates, except for Cu²⁺, which binds strongly to both of these groups. Hg²⁺, Cu²⁺ and Pb²⁺ are metals with strong base affinity; however, these metals also disturb hydrogen bonding between base pairs, destabilizing the B form of DNA. With the exception of Hg²⁺, metal ions prefer to bind to guanine and cytosine regions over adenine and thymine regions. The interaction of different metal ions with either the phosphate backbone or the nucleotide bases is of critical importance for several methods of DNA metallization. Utilizing the complex formation with the DNA backbone, DNA templates have been seeded with a wide variety of metal ions such as silver, palladium, copper, rhodium, nickel and platinum.

2.3 DNA Origami

DNA origami is the folding of single-stranded DNA to create different two- and three-dimensional shapes. The specific interaction of base pairs, that is A with T and G with C, is what makes DNA a useful material, and DNA origami a useful tool for fabrication of large number of intricate constructs in a single pot. Developed by Paul Rothemund in 2006, the general approach involves raster filling a particular shape with a long single-stranded DNA (known as
scaffold DNA), which is then held into place with smaller, complementary oligonucleotides known as staple strands.\textsuperscript{2,12} The staple strands bridge from one part of the scaffold to another, thus, bridging\textsuperscript{2} different parts of the scaffold together as shown in Figure 2-2. The designs are first made in a computer program that determines the placement of the individual staple strands. Scaffold and staple strands are then mixed, heated and cooled (annealing). As the mixture cools, the staples pull the long scaffold into the desired shape. Figure 2-2 shows an example of the DNA origami technique for making a linear DNA origami template. For making this shape, a 7249 base pair long M13mp18 scaffold strand was used with a total of 227 staple strands, each 40-50 bases long.

![Figure 2-2: DNA origami technique. (A) Scaffold and staple strands (B) Arrangement of scaffold and staple strands to give a linear DNA origami (C) AFM image of the origami structure. Height scale for (C) is 10 nm.](image-url)
In addition, DNA origami designs can be made with attachment strands at specific locations in the DNA template. To accomplish this, the staple strands in the template are extended with additional nucleotides which can then bind to various nanomaterials stabilized with the complementary base sequences. Figure 2-3 shows an example of this site-specific attachment technique whereby metal nanoparticles stabilized with 8 thymine bases (red strands) are attached only to the specific location on DNA template designed with the complementary 8 adenine bases (blue strands). The base sequences (red strands) around the nanoparticles have a thiol group (SH) on one end of the sequence which covalently bonds with the metal nanoparticles. Thus, DNA origami provides a useful means to attach functional materials, such as metal nanoparticles, which are important for metallization of DNA templates.

![Figure 2-3: DNA origami template with site-specific attachment points for metal nanoparticles.](image)

**2.4 Electroless Deposition**

Electroless plating is a method of depositing metals such as gold, copper or nickel on a conductive substrate without the use of an external power supply. The deposition process is as simple as immersing the conductive substrate in an aqueous solution consisting of the desired metal ions, whereupon metal deposition on the substrate begins immediately. The first deposited metal layer catalyzes subsequent plating and allows metal deposition to continue. Since the
deposited layers catalyze the subsequent reaction, the plating process is often called autocatalytic. Electroless plating of copper, gold, nickel, palladium and cobalt have been successfully demonstrated in the literature.

An electroless plating bath typically consists of metal ions, a reducing agent, suitable complexing agents and stabilizers. The reducing agent supplies the electrons required for the reduction of the metal ions. Complexing agents are usually organic acids or their salts, which exert a buffering action to maintain pH of the plating solution. In addition, complexing agents form stable complexes with metal ions, thus enhancing the stability of metal ions and the selectivity of the plating reaction. Stabilizers help to prevent the homogeneous reaction that triggers random decomposition.

The overall reaction can be divided into a cathodic reaction where the metal ions are reduced and the anodic reaction where the reducing agent is oxidized.

\[
\begin{align*}
\text{Oxidation reaction:} & \quad \text{Red} \rightarrow \text{Ox} + ne^- \\
\text{Reduction reaction:} & \quad M^{2+} + ne^- \rightarrow M \\
\text{Overall:} & \quad M^{2+} + \text{Red} \rightarrow M + \text{Ox}
\end{align*}
\]

where, Red and Ox are the reduced and oxidized forms of the reducing agent, respectively. The above reactions do not represent the actual stoichiometry. The oxidation and reduction reactions both occur on the same conductive surface (DNA templates with metal nanoparticles, Figure 2-3) to form a mixed potential at steady state. The rates of the separate partial reactions depend on the mixed potential at the electrode surface. The half-cell (anodic and cathodic) reactions are interdependent because the electrons released during oxidation are consumed in the reduction of metal ions. For metal deposition to occur, the reduction potential for the metal to be reduced should be more positive (greater affinity for electrons) than the reduction potential of the
reducing agent (species) and the reduced metal should provide a catalytic surface suitable for additional plating. Understanding the concept of mixed potential can be useful for manipulating the reaction kinetics in order to achieve the desired metal deposition. For example, changing the concentration of either the reducing agent or the metal ions will change the mixed potential, which will change the rate of electroless deposition.6

Previous researchers at BYU have successfully demonstrated electroless deposition of different metals on DNA templates. Geng et al. demonstrated electroless deposition of palladium5, gold7 and copper7 on DNA templates. Similarly, Liu et al.3 and Pearson et al.8 have successfully demonstrated electroless gold plating on various shapes of DNA origami templates. Liu et al.6 also demonstrated electroless deposition of Ni and Cu on DNA templates, which were then displaced with Te to make DNA-templated semiconductor wires. Recent work by Uprety et al.25 showed the feasibility of depositing two different metals on a single DNA origami template.

Our process for the formation of DNA nanowires begins with a seeding step whereby metal nanoparticles are attached to the DNA templates. The seeds attach to the DNA by complementary base pairing between attachment points in the template and the DNA attached to the seed particles.8 The seeds are then connected by electroless deposition to form continuous and conductive nanowires. The size of the largest gap between the metal seeds sets the lower limit for the eventual width of the nanowire since the gap has to be filled to create a continuous line. Our previous metal deposits on DNA have generally been granular, while an ideal metal wire would be thin and continuous with fewer grain boundaries.

2.5 The Seeding Process

Seeding is the first process in DNA metallization, and is used to create the platform for electroless plating of DNA. As mentioned in the section above, sites are needed to catalyze
electroless deposition reaction. DNA itself does not possess metal particles (catalytic sites), so a seeding process is used to deposit transition metals like Ag, Pd, Pt or Au onto the DNA template to enable subsequent electroless deposition. Seeding selectivity is defined as the ratio of the number of metal seeds deposited on the DNA template to the total number of seeds deposited on the entire surface. For good selectivity, the seeds should only attach to the DNA with little or no seeding on the rest of the surface.

As mentioned in the above section, transition metals can be attached to DNA by electrostatic interaction or complex formation with the DNA bases. Braun et al.\(^2^9\) were the first to seed DNA with Ag\(^+\) ions, through Ag\(^+\)/Na\(^+\) ion-exchange and formation of complexes between the silver ions and the DNA bases. Silver ions attached to the DNA template were then reduced with hydroquinone to form the seed layer. Likewise, divalent metal ions like Pd (II)\(^3^0-3^1\) and Pt (II)\(^3^6\) have been shown to seed DNA by forming complexes with the Lewis bases on DNA. Richter et al.\(^3^1\) and Geng et al.\(^5\) have shown the use of Pd (II) to make palladium wires. The general approach is activation of DNA with Pd (II) ions to form coordination complexes, reduction of the ions to form the seed layer, and subsequent electroless plating with palladium to make nanowires. Similarly, Gu et al. have demonstrated the use of Pd (II)\(^2^6\) and Pt (II)\(^4^2\) ions for seeding DNA to make nickel wires. While ionic interaction or complex formation have been used frequently in the literature to form a seed layer on DNA, the selectivity of the seeding is often low, leading to significant background deposition. In addition, the seeds deposited by ionic interaction cannot be controlled to specific areas on the template, which prevents the deposition of multiple nanomaterials on the same template.

To make DNA based nanosystems, both good seed selectivity and the ability to place different nanomaterials at specific sections of the template are very important. The attachment of
metal nanoparticles via molecular recognition with the underlying template has been shown to enable localization of metals to specific sites and to improve seed selectivity in DNA origami templates in comparison to reducing metal ions from the solution.\textsuperscript{8, 19-22} Metal particles are attached via metal binding groups such as thiols to the ends of short DNA strands designed to bind at specific complementary locations on DNA origami templates (Figure 2-3). Conversely, metal binding ligands can be first attached to the DNA template and then metal nanoparticles can be attached to the ligand. Since longer reaction time is required for metal-thiol interactions,\textsuperscript{8, 19} it is most convenient to first attach ligands to the metal and then attach metal to the DNA by complementary base pairing. Use of metal binding ligands to seed DNA origami templates can increase seed selectivity by limiting background deposition of seeds on the surface. Since, the metal seeds do not covalently bind to the surface, introducing additional rinsing steps can reduce the background deposition.

Studies in this area have shown the attachment of Au or Ag nanospheres as well as Au nanorods using metal-thiol interaction in designated sites of DNA template. Hung \textit{et al.}\textsuperscript{18} localized Au nanospheres on triangular shaped DNA origami using this method. Similarly, Pal \textit{et al.}\textsuperscript{19} localized Ag nanospheres on self-assembled triangular shaped DNA origami using nanoparticles conjugated with phosphorothiolated DNA as supporting blocks. Pal \textit{et al.}\textsuperscript{19} also showed the possibility of attaching both the Au and Ag nanospheres on the same template. In another study, the same group demonstrated DNA-thiol linked gold nanorod attachment to designated sites of a triangular DNA origami template.\textsuperscript{43} The metal nanoparticles attached to the DNA origami templates can serve as the conductive substrates for subsequent plating. In this regard, Pais-Pilo \textit{et al.}\textsuperscript{22} were the first to demonstrate site-specific seeding of DNA origami templates with Au nanospheres and their subsequent plating with Ag to make continuous DNA
structures. Studies by Pearson et al. and Uprety et al. have also demonstrated the use of gold nanospheres as a seed layer and their plating with gold to make DNA gold nanowires.

At the time this study was initiated, the procedure for attaching spherical gold nanoparticles (nanospheres) to the DNA origami templates via molecular recognition had been demonstrated. As a result, all of the metallized DNA origami nanostructures shown in the literature (three total studies) utilized gold nanospheres as conductive substrates (seed layer) for further metal deposition. DNA-templated wires fabricated with nanospheres have generally been granular in appearance, highly resistive, and considerably thicker than what is achievable by the underlying origami template. However, no study describing the potential use of a different shape of metal nanoparticle (for example, gold nanorods) as a seed layer to study the effects on morphology, conductivity and size of the final structure had been demonstrated. The current study will thus seek to utilize a different nanoparticle as a seed layer to improve the quality of the metal deposit on DNA origami templates. The findings from the studies with spherical gold nanoparticles will be helpful in moving forward with the present study.

2.6 Nanorods

Nanorods are rod-shaped nanoparticles, made of either metal, semiconductor or insulator materials, whose length ranges from 10-100 nm. Nanorods are usually defined by their aspect ratio: length divided by the width. In general, nanorods can be synthesized either in rigid templates or in soft templates (in presence of surfactants). For synthesis in rigid templates, metals ions are reduced inside cylindrical pores of oxide or polymeric membranes. For synthesis in soft templates, nanorods are produced by direct chemical synthesis using surfactants to act as shape control agents. The surfactants bond to the different crystal facets of the nanorod with different strengths, which allows the different faces of the nanorod to grow at different rates
to produce a cylindrical rod-like particle. Chemical synthesis of nanorods of gold, \textsuperscript{46-49} silver, \textsuperscript{50-51} palladium, \textsuperscript{52-53} tellurium, \textsuperscript{54-58} and nickel \textsuperscript{59} have been reported to date. However, a detailed study on the anisotropic growth mechanism is only available for gold nanorods.

A typical gold nanorod synthesis procedure involves the reduction of gold ions from a bulk growth solution onto gold nanospheres that serve as seeds. Gold nanorod growth solution usually contains surfactant, cetyltrimethylammonium bromide (CTAB), gold ions, silver ions, hydrochloric acid and the reducing agent ascorbic acid. The surfactant and the silver ions are believed to play a critical role in promoting anisotropic particle growth due to their preferential attachment to the \textit{<110>} crystal facet, which inhibits growth on the nanorod sides and results in preferred growth at the ends of the nanorods (\textit{<001>} plane). \textsuperscript{48, 60-61} Figure 2-4 shows the crystal structure of a typical gold nanorod. The \textit{<100>} and \textit{<111>} crystal facets are low energy, thermodynamically stable surfaces, while the \textit{<110>} facet is a relatively unstable high-energy surface. \textsuperscript{62-63} Each facet of the crystal structure has a characteristic surface energy whose value depends on the number of broken chemical bonds (dangling bonds) on the surface. \textsuperscript{63} There are five, four and three dangling bonds in \textit{<110>}, \textit{<100>} and \textit{<111>} facets respectively. Thus, the \textit{<110>} facet has the highest surface energy followed by \textit{<100>} and \textit{<111>} facets. Preferred adsorption of CTAB and silver ions on the sides of the nanorods (\textit{<110>} and \textit{<100>} facets) inhibits gold deposition relative to the deposition rate on the \textit{<111>} ends of the nanorods.
At the time this study was initiated, there were no published studies on the potential use of nanorods as seed particles to study their effects on morphology, conductivity and size (width) of the final DNA templated metal deposits. In fact, no studies have ever been published on the growth of gold nanorods attached to a template such as DNA in a plating solution. In this study, we aim to use gold nanorods as potential seeds for improving the quality of our metal deposit on the DNA origami templates. Gold nanorods will be used to create a seed layer on a DNA template which will then be connected via electroless plating step. In particular, we aim to study if the surfactant-enabled anisotropic growth used to initially synthesize the nanorods could be made to continue throughout the electroless deposition step. In addition, we also aim to analyze if the use of nanorods improves the conductivity of the DNA templated metal deposits. The use of ~25 nm long nanorods would result in fewer connection points between seeds along the length of the DNA template relative to the number of connections observed for ~ 5nm diameter nanoparticles which could potentially improve the conductivity of metal nanowires. Chapter 3 discusses our efforts to use gold nanorods as seeds and their growth in the plating solution to fabricate continuous DNA nanowires.
Finally, site-specific deposition of gold nanorods on DNA origami templates is needed for fabricating DNA based nanosystems. In order to do this, nanorods have to be functionalized with a different molecule (like DNA oligonucleotides) other than CTAB to allow controlled placement on the origami templates via molecular recognition. The positive charge on the CTAB molecules around the nanorods results in non-specific interaction of nanorods with the DNA templates. In this regard, very few groups have demonstrated the feasibility of functionalizing DNA oligonucleotides on gold nanorods for site-specific attachment of nanorods to DNA origami templates via molecular recognition, similar to gold nanoparticles (Figure 2-3). However, these works have only been limited to studying the fluorescence enhancement behavior and chiral optical responses of gold nanorods and none have demonstrated the use of nanorods for site-specific metallization of DNA templates to make continuous metal lines as shown here. In addition, the process of functionalizing DNA around gold nanorods would involve removing the surfactant (CTAB) which may affect anisotropic growth upon introduction to a growth solution. Till now, there is no published study that has characterized the anisotropic growth behavior (if any) of DNA functionalized nanorods in the growth (plating) solution. The present study thus, seeks to characterize the anisotropic growth, if any, demonstrated by gold nanorods functionalized with DNA oligonucleotides. To facilitate this study, we will first demonstrate site-specific placement of DNA functionalized gold nanorods to attachment sites on a DNA origami template and then introduce the seeded templates to electroless gold plating solutions with the ultimate goal of filling the gaps between the nanorod seeds to create continuous structures. The ability to controllably place nanorods on a template and connect them anisotropically with greater control on the final width would be an important development for DNA based nanoelectronics fabrication. Chapter 5 discusses our efforts to accomplish site-
specific nanorod attachment to DNA templates and characterize the growth behavior of DNA functionalized gold nanorods.

2.7 DNA Origami Templates

Two different shapes of DNA origami templates are used in the present study. The particular templates were chosen based on our objective to demonstrate site-specific metallization of DNA templates to make conductive substrates. The first design is a 410 nm long and 17 nm wide linear DNA origami template which was primarily chosen to facilitate the assessment of the electrical properties of our metal deposits. It is much easier to make electrical connections on a longer DNA template than a shorter (~100 nm long) structure. The origami template was formed by utilizing M13mp18 single-stranded DNA based on our previously published work to deposit two different metals on a single DNA template.25 While the exact DNA design hasn’t been reported elsewhere, similar (long templates utilizing the whole M13mp18 scaffold) designs have been frequently reported. Bui et al.67 first demonstrated a 412 nm long DNA nanotube template to fabricate quantum dot arrays with controlled periodicity. In order to attach quantum dots to the DNA nanotube, selected staple strands in the template were extended with 5 thymine bases followed by a biotin molecule to form binding sites for streptavidin-conjugated quantum dots.67 Gur et al.68 and Teschome et al.69 demonstrated the use of a similar DNA nanotube template for attaching gold nanoparticles to the template via complementary base pairing between the staple strands on the template and the DNA around the gold nanoparticles. Only Teschome et al.24 has demonstrated metallization of the DNA nanotubes to form continuous structures. To do this, gold nanoparticles were first attached to the DNA nanotube template and then grown in the plating bath until they formed continuous structures.24 However, no work has been reported on the metallization of any type of DNA
origami template with the use of gold nanorods as seeds. In addition, no work has been reported characterizing the electrical properties of continuous nanowires based on gold nanorod seeds. Chapter 3 examines our efforts to fabricate continuous metal structures on a linear DNA template with the use of gold nanorods and the electrical characterization of the metal deposit.

The second design is a rectangular shaped DNA template measuring 90 nm in length and 65 nm in width. The design was chosen to controllably assemble gold nanorods at specific sites within the template to make <100 nm continuous metal deposits of different shapes (Chapter 5). This DNA design was first reported by Paul Rothemund.12 The rectangular template has since been reported by several other groups for assembling different types of nanoscale materials. Stephanopoulos et al.70 used a similar origami template for immobilizing spherical virus capsids into hierarchical structures with precision positioning. The virus capsids were attached to the origami template via nucleic acid base pairing between the DNA strands on the template and the DNA around the virus capsids.70 Similarly, Acuna et al.71 used the template to study distance-dependent interaction between a metal nanoparticle (attached via nucleic acid base pairing) and a fluorescent molecule (incorporated during DNA origami folding). Lan et al.,65 Zhang et al.64 and Chen et al.66 have shown site-specific assembly of gold nanorods (attached via nucleic acid base pairing) on the template for studying fluorescence enhancement behavior64 and chiral optical responses65-66 of gold nanorods. While different groups have successfully shown the use of the rectangular DNA origami template for assembling different nanomaterials, only Pilo-Pais et al.22 has demonstrated the use of the template to form continuous metal deposits for application in nanodevices. To form continuous metal deposits, Pilo-Pais et al.22 first attached gold nanoparticles to specific sites on the DNA template via nucleic acid base pairing and then grew the nanoparticles in the plating bath until they fused with each other to form continuous
structures. However, no work has been published on the use of the template to 1) fabricate continuous metal deposits with the use of gold nanorod seeds and 2) study growth behavior of DNA functionalized nanorods. Chapter 5 also describes our efforts to first site-specifically attach gold nanorods to a rectangular DNA template and then evaluate the growth behavior of the nanorods with the ultimate goal to form continuous metal structures of different shapes.

2.8 Summary

DNA, with its small size, functional groups and complementary base pairs, represents a desirable template for fabricating components of nanodevices. The advent of DNA origami has enabled fabrication of a wide variety of 2D and 3D structures, providing an effective route for making templates needed for the development of DNA based nanoelectronic devices and systems. Metallization of DNA origami templates to make continuous structures is important for fabrication of nanodevices. Furthermore, selective deposition of nanomaterials (metals and semiconductors) at specific sites on origami templates is needed for fabricating a complete DNA based nanosystem. Few reports (three reports total, two from BYU) have been published on site-specific metallization of the DNA origami templates; however, the metal deposits demonstrated have generally been granular, resistive and larger (wider) than what is possible with the underlying template. DNA origami-templated continuous metal structures with small linewidths and high conductivity have not yet been demonstrated. Similarly, there is no published report on site-specific placement of multiple (metallic and semiconducting) materials on a DNA template. The development of a metallization process that addresses these challenges of size, morphology and conductivity of the metallized DNA structure as well as the ability to control the placement of different nanomaterials on the underlying template would thus, provide an important step
forward towards the development of nanodevices and systems based on biological molecules like DNA.
3 ANISOTROPIC ELECTROLESS DEPOSITION ON DNA ORIGAMI
TEMPLATES TO FORM SMALL DIAMETER CONDUCTIVE NANOWIRES

The following chapter is taken from the published work. Published January 2017.72

3.1 Introduction

DNA-based self-assembly is becoming increasingly attractive for fabrication of complex architectures with nanometer precision.22, 73-76 This approach takes advantage of the ability of biopolymers such as nucleic acids to spontaneously self-assemble into the desired structures, and has seen remarkable advancement as reflected in the fabrication of a variety of two-12-14 and three-15-17 dimensional nanostructures. DNA is especially appealing because of its high aspect ratio, narrow linewidth (~ 2nm), ability to form complex nanostructures through base pairing, and its availability at reasonable cost; extensive methods are also available for manipulation and purification of the DNA. In addition to giving greater control at the molecular level, DNA-based structures can also be designed as templates upon which the metal8, 18, 20, 22, 24-25, 68, 74 and semiconducting6, 23 materials needed for electronic devices can be formed. Consequently, DNA-based fabrication provides an alternative to current microelectronic fabrication techniques that has the potential to reduce feature sizes and form complex structures in ways that are not possible with prevailing fabrication methods.
Metallization of DNA templates is one way to form conductive elements for use in different electronic and plasmonic applications. The DNA templates metallized with Ni, Ag, or Pd have been demonstrated. However, λ-DNA does not provide the complexity available from methods such as DNA origami that enable creation of the intricate structures needed for complex nanostructures and devices. In this context, Liu et al. demonstrated continuous metallization of DNA origami templates, which was followed by other successful studies showing metallization of these templates. Pais-Pilo et al. were the first to report site-specific metallization of DNA tiles. Pearson et al. demonstrated site-specific metallization of DNA origami templates to form conductive nanowires, which was followed by selective deposition of two different metals on a single origami template.

Despite recent success with DNA metallization, the ability to make thin, continuous wires that are electrically conductive still represents a formidable challenge. DNA-templated wires have generally been granular in appearance with a resistivity approximately two to three orders of magnitude higher than that of the bulk material. In order to both decrease the size and increase the conductivity, we describe a method for fabricating small diameter nanowires using a linear DNA origami template that incorporates gold nanorods as seeds. Seeding with nanorods provides at least two key advantages over spherical particulate seeds: 1) improved control of the width of final nanowires due to preferential growth in the axial direction of the nanorods and 2) fewer connection points along the length of the DNA template, relative to the number of connections observed with the 5 nm particulate seeds used in our previous work. In this study, cetyltrimethylammonium bromide (CTAB) stabilized gold nanorods are first attached to the DNA origami template, and then connected by electroless gold plating to make continuous
nanowires. Electron beam lithography was used to make connections to the completed nanowires in order to characterize their electrical properties.

### 3.2 Experimental Section

#### 3.2.1 Materials

M13mp18 DNA was purchased from New England Biolabs (Ipswich, MA). Staple strands at a concentration of 100 μM in TE (Tris-EDTA (ethylenediamine tetraacetic acid)) buffered solution (pH = 8.0) for DNA origami folding were obtained from Eurofins MWG Operon (Huntsville, AL). TAE-Mg\(^{2+}\) (10X, pH = 8.3) buffer was made from 400 mM Tris base, 200 mM acetic acid, 10 mM EDTA and 125 mM magnesium acetate (MgAc\(_2\).4H\(_2\)O). MgCl\(_2\), MgAc\(_2\) and acetic acid were obtained from EMD Chemicals (Gibbstown, NJ). Tris base (tris (hydroxymethyl) aminomethane) was obtained from Fisher Scientific Inc. EDTA was obtained from Life Technologies (Carlsbad, CA). HAuCl\(_4\) and CTAB (H5882, 98%) were obtained from Sigma. Silver nitrate (AgNO\(_3\)) was obtained from Mallinckrodt Chemicals (Phillipsburg, NJ). Hydrochloric acid (HCl) was obtained from EM Science (Gibbstown, NJ). Sodium borohydride (NaBH\(_4\)) was obtained from Acros (New Jersey). Ascorbic acid (C\(_6\)H\(_8\)O\(_6\)) was obtained from Fisher Chemical (Fair Lawn, NJ). Gold nanoparticles (AuNPs, 5 nm) were obtained from Ted Pella (Redding, CA). Single-stranded DNA functionalized with thiol to enable attachment to metal nanoparticles was also purchased from Eurofins MWG Operon with PAGE purification, and diluted to 1 mM in water. Water (18.3 MΩ cm) treated by an EASYPure UV/UF purification system was used for all rinsing and aqueous solution preparations.
3.2.2 DNA Origami Design and Folding

Linear DNA origami structures were formed using 7085 bases of the 7249 bases in M13mp18 scaffold as previously reported. The structures were assembled by slowly heating a mixture of the scaffold and staple strands (2.0 nM scaffold and 20 nM of each staple strand in 10X TAE-Mg$^{2+}$ buffer) to 95°C for 3 min and slowly cooling to 4°C in a 90 min period in a TECHNE TC-3000 thermocycler.

3.2.3 Au Nanorod Seed Preparation

Au nanorod seeds were synthesized using a growth technique reported by Ali et al. The growth solution was prepared at 27 °C. Briefly, HAuCl$_4$ (2.5 mL, 1.0 mM) was added to 5.0 mL of cetyltrimethylammonium bromide (CTAB, 0.2 M). Following this, AgNO$_3$ (250 µL, 4.0 mM) was added to the solution and gently shaken. HCl (8.0 µL, 37%) was then added to the solution. 35 µL of ascorbic acid (78.8 mM) was then added to the solution with gentle shaking until the solution was clear. Ice-cold NaBH$_4$ (7.5 µL, 0.01 M) was added immediately afterwards and allowed to react overnight. The final solution was distributed into six centrifuge tubes and spun at 14,000 rpm for 17 min to pellet the Au nanorods. The supernatants were removed and the rod pellets were combined and stored at 27 °C for future use.

3.2.4 Deposition of DNA Origami onto SiO$_2$ Surfaces

The procedure is similar to that previously reported in the literature. Silicon dioxide surfaces (roughly 1 cm x 1 cm) were plasma cleaned (Harrick Plasma Asher, PDC-32G) for 30 s at 18 W to remove impurities from the surface. 3 µL of DNA origami (0.22 nM) in 10 X TAE-Mg$^{2+}$ buffer was allowed to adsorb onto the cleaned surfaces for 10 min in a humid chamber at
room temperature. The samples were then rinsed with 4.0 mM MgAc₂ solution for 3-4 s and water for 1-2 s. Finally, the surfaces were dried with a stream of filtered air and imaged in AFM.

3.2.5 Seeding with Au Nanorods

20 µL of seeding solution (gold nanorods diluted in 10XTAE-Mg²⁺ solution) was pipetted (dropped) onto the SiO₂ surface with DNA templates and allowed to react for 60 min at room temperature in a humid chamber. The samples were then rinsed with 4.0 mM MgAc₂ solution for 3-4 s and with water for 1-2 s to clean the surface and to remove background nanorods that were not bound to the DNA template. The samples were then dried in a stream of filtered air.

3.2.6 Seeding with Au Nanoparticles

Gold nanoparticle (Au NP) - DNA conjugates were first prepared as published previously.²⁵ Specifically, Au NPs were first phosphinated and concentrated with BSPP, and then treated with thiolated single-stranded DNA to form Au NP-DNA conjugates.²⁵ The nanoparticle-DNA conjugates were then diluted in 10XTAE-Mg²⁺ solution and dropped onto a SiO₂ surface upon which DNA origami had been previously deposited. Seeding was allowed to proceed via complementary base pairing for 40 mins in a humid chamber. The samples were then rinsed with 4.0 mM MgAc₂ solution for 3-4 s and with water for 1-2 s to remove background nanoparticles that had not attached to the DNA template. Finally, the samples were dried in a stream of filtered air.

3.2.7 Electroless Au Plating

The gold electroless plating solution was similar to the growth solution used for preparing gold nanorods. Specifically, HAuCl₄ (250 µL, 1.0 mM) was added to 350 µL of CTAB (0.2 M).
Following this, AgNO₃ (25 µL, 4.0 mM) was added to the solution and gently shaken. HCl (1.0 µL, 37%) was then added to the solution. 8 µL of ascorbic acid (78.8 mM) was next added to the solution with gentle shaking until the solution was clear. 70 µL of this solution was pipetted onto the SiO₂ surfaces and the reaction was allowed to proceed for ~30 min at 27°C. The samples were then rinsed with 4.0 mM MgAc₂ solution for 3-4 s and with water for 1-2 s. Following rinsing, the samples were dried with a stream of filtered air. Modifications to the basic procedure are described above as part of the results.

3.2.8 AFM Imaging

The samples were imaged using tapping mode on a Digital Instruments Nanoscope IIIa MultiMode AFM (Veeco) with aluminum-coated conical silicon tips (Vistaprobes, 3N/m, 45-75 kHz).

3.2.9 SEM Imaging

The DNA samples on SiO₂ surfaces were imaged in ultra-high resolution mode on a FEI Helios Nanolab 600 and in high vacuum mode on a Philips XL30 ESEM FEG.

3.2.10 Conductivity Measurements

Gold electrode sets were fabricated using electron beam lithography (Philips XL30 ESEM FEG) with ZEP-520 resist for nanowire samples on a SiO₂ surface. The resist was spun at 5000 rpm for 1 minute followed by a 2 min pre bake at 200°C. Following exposure (line dosage of 0.8 – 0.9 nC/cm), the resist was developed in ZED N50 developer for 2 min and dipped for 30 s in IPA. The surface was then air dried. Next, a CHA-600 thermal evaporator was used to deposit a 7 nm chromium adhesion layer followed by a 50 nm gold layer. After gold deposition, samples
were immersed in NMP overnight (~20 hr), rinsed with acetone, and finally dried in a stream of filtered air.

Electrical measurements were performed at room temperature using micromanipulator probes to connect the gold electrodes to the power source.

3.3 Results and Discussion

3.3.1 Seeding for Gold Deposition

Figure 3-1 illustrates the overall steps involved in the metallization of DNA origami structures to form metallic nanowires. A linear DNA origami template measuring 17 nm in width and 410 nm in length was used for this study, which was formed as previously reported.\textsuperscript{25} This relatively long origami template was designed to facilitate assessment of the electrical properties of the final nanowires. Also, since the whole M13mp18 strand was used in the design for convenience to form a long and stable template, the template was actually wider than required. Once assembled, the origami templates were seeded with gold nanorods that were formed via the growth technique reported by Ali et al.\textsuperscript{48} The seeds served as a starting point for further metal deposition.

![Diagram of the process](image)

Figure 3-1: (A) Design and fold the DNA origami template using a single-stranded scaffold and smaller DNA staple strands. Deposit the template on a SiO\textsubscript{2} surface. (B) Add gold nanorods to seed the DNA template. (C) Use electroless plating to fill the gaps between seeds and form continuous wires.
Figure 3-2 shows images obtained with atomic force microscopy (AFM) and scanning electron microscopy (SEM) of the unseeded (A) and nanorod-seeded origami templates (B-D). After seeding, the average height of the seeded DNA template was 6.9 nm, with a standard deviation of 1.3 nm (n=30); to compare, the unseeded DNA had an average height of 1 nm. The nanorods used for seeding had an average diameter of 6 nm and were 25 nm in length. As evident from Figure 3-2, the seeds attached selectively to the DNA templates with virtually no background deposition. This figure also shows the tendency of the nanorods to line up end-to-end along the length direction of the DNA, although attachment at an angle across the length of the template was occasionally observed. In addition, nanoparticles, which are side products from nanorod synthesis, were also sometimes observed to attach to the DNA templates, as seen at the end of the template in Figure 3-2D. Finally, the typical nanorod-seeded DNA template shown in Figure 3-2D shows gaps of various sizes between the nanorods. These gaps must be filled in order to make the desired continuous nanowires. In doing so, our goal was to fill the gaps without significantly increasing the width of the final wires. Small nanorods were synthesized for use as seeds to demonstrate proof-of-concept for making thin, metallized, conductive DNA-templated structures.

Factors influencing nanorod attachment to the DNA origami were studied by varying the composition of the solution used for seeding. The images in Figure 3-2 show the results of seeding from a 10XTAE-Mg\(^{2+}\) buffer solution (salt conc = 0.74 M); seeding from this solution resulted in nanorod attachment that was predominantly aligned along the length direction of the DNA template. In contrast, nanorods rarely attached to the DNA when the salt concentration of the buffer was below 0.37 M. Similarly, attachment was not observed when water or when a monovalent 1 M salt solution of NaCl or KCl was used instead of the magnesium-containing
buffer solution. Attachment was observed for salt solutions of Mg$^{2+}$, Ca$^{2+}$ or Cu$^{2+}$ (see Figure 9-1 in Appendix A), which provides further support for the important role of the divalent cation. In addition, nanorod attachment was observed for samples that had previously not shown attachment (water, monovalent salt and lower concentration 10XTAE-Mg$^{2+}$ buffer solutions) when these samples were subsequently introduced to nanorod seeds in the 10XTAE-Mg$^{2+}$ with a salt concentration of 0.74 M. Therefore, the failure to seed was not due to the lack of DNA on the surface, but to failure of the rods to attach to the DNA. Presumably, alignment of the nanorods along the DNA is influenced by local field effects, although the nature of that field and its relationship to the charge on the DNA and the divalent cations is not fully understood.

Figure 3-2: AFM images of (A) linear DNA origami, and (B) gold nanorod seeded DNA origami. Height scale is 10 nm. (C) - (D) SEM images of nanorod seeded linear DNA origami.
The nanorods serve as seeds for the fabrication of nanowires. The quality of final nanowire is strongly influenced by the size of gaps between the seeds, with the largest gaps being of particular concern. Consequently, the nanorod concentration and seeding times were varied in order to examine the influence of these variables on the size of gaps between seeds and on seed orientation.

For an average rod length of 25 nm, the DNA template can hold a maximum of about 16 nanorods along its length (410 nm) if the rods are connected end-to-end without gaps. Hence, initial seeding experiments were performed with 3 μl of 0.2 nM DNA solution and 20 μl of 0.48 nM nanorod seeding solution to give a 16:1 ratio of nanorods to DNA origami. The seeding time was varied between 10 min and 3 hr. There was considerable improvement in seed density as the seeding times increased from 10 min to 1 hr; however, no further improvements were seen for longer seeding times (see Figure 9-2 in Appendix A). Figure 3-3 shows the size distribution of gaps between seeds for seeding times of 10 min and 1 hr, respectively. The average end-to-end gap between seeds decreased from 15 nm to 11 nm as the seeding time increased from 10 min to 1 hr, as evident from the shift of the distribution to smaller sizes (Figure 3-3; n=25 seeded templates). The maximum gap size also decreased from 47 nm to 36 nm due to the longer seeding time. The increase in the seeding time also resulted in reduction of the percentage (by number) of gaps greater than the average nanorod length (25 nm) from 13.0% (10 min) to 4.6% (1 hr). In addition, the attachment of rods not aligned with the “length” direction of the DNA decreased with the increase in seeding time from an average of one misalignment per 5 seeds to one per 7 seeds. The occurrence of nanoparticles, however, increased from one particle per 19 seeds for 10 min seeding to one per 8 seeds for 1 hr seeding, which affects the final quality (width) of the nanowires. No significant reduction in gap sizes was observed for seeding times.
longer than one hour. Based on these results, a seeding time of one hour was used for subsequent experiments.

Figure 3-3: Gap distribution after gold nanorod seeding of DNA origami templates.

The impact of the nanorod concentration in the seeding solution was also examined. No significant reduction in gap size was observed when the nanorod to DNA origami ratio was increased to 25:1. In fact, we observed less than 1 nm change in average gap size for the increased seed concentration when seeded for one hour. There was no background deposition, even at the higher seed concentration, which suggests that the extra seeds were effectively rinsed off of the sample. The occurrence of misaligned nanorods decreased from one rod per 7 seeds to one rod per 12 seeds due to the increased concentration. The occurrence of nanoparticles also decreased from one particle per 12 seeds to one per 18 seeds, presumably due to the increased
availability of nanorods for hybridization. On average, there were 11 aligned nanorods attached in a “single file” to each DNA origami template, with occasional side-by-side attachment as seen in Figure 3-2 C and D. Due to the reduced frequency of misaligned rods and fewer nanoparticles at the higher concentration, a gold nanorod to DNA origami ratio of approximately 25:1 was used for additional experiments. Figure 3-2 C and D shows SEM images of a typical 25:1 sample seeded for one hour. The seeding experiments helped to decrease the size of the gaps between seeds and to improve the nanorod seed orientation (along the length of the DNA). Both of these factors are important for the fabrication of continuous, small-diameter wires.

3.3.2 Electroless Gold Deposition

Following seeding, our 400 nm template was covered with nanorod seeds aligned along its length with gaps that needed to be filled in order to create conductive nanowires. Some of the gaps were of significant width, and bridging those gaps with isotropic metal deposition would have greatly increased the diameter of our wires. For example, a gap of 30 nm would have required at least 15 nm of isotropic growth during metal deposition if deposition occurred from both sides. That would have resulted in our final seed width and hence the wire width to be at least 36 nm (from 6 nm initial seed diameter). Hence, a method was needed to fill the gaps without significantly increasing the width of the wires. As doing this would require anisotropic growth similar to that used to create the rods initially, we introduced our nanorod-seeded DNA templates to a plating solution similar in composition to that used to grow the nanorods. An important difference in the plating solution is the lack of the precursor (reducing agent) that spontaneously reduces gold ions to gold seeds in order to prevent the formation of additional (new) nanorods (Section 3.2.7, Materials and Methods). In general, gold nanorods are grown in a concentrated solution of a surfactant, e.g., CTAB, which binds strongly to the <110> crystal
facet and inhibits growth along the nanorod sides.\textsuperscript{48} Thus, the surfactant binding results in preferred growth at the ends of the nanorods (<001> plane) to give high aspect ratio particles.

Figure 3-4 shows the SEM images of DNA wire samples plated at 27\degree C. This temperature is slightly above room temperature and helps with the solubility of the CTAB surfactant in the plating solution. We varied the plating time between 20 and 60 min. As seen from the figure, the plating process filled most of the gaps present in the seeded samples when plated between 20 and 40 min. However, some wires still had one or more gaps that were not filled. Nanowire samples plated for 60 min, however, showed loss of anisotropy with significantly larger final wire diameters (Figure 3-4 C). Separate experiments were performed to examine the growth of rods from seeds in order to better understand the relationship between the growth time and the aspect ratio of the final structures. Repeated exposure of the 25 nm seeds to the growth solution resulted in rods with a maximum length of approximately 55 nm and a diameter of 12.5 nm, whose aspect ratio was similar to that of the seeds. Any additional growth time significantly increased the width of the nanorod and decreased the final aspect ratio. Similarly, adding a fresh growth solution to the regrown nanorods (55 nm in length) only increased the width of the nanorods. In this regard, Keul \textit{et. al} \textsuperscript{78} also observed an initial increase and subsequent decrease of nanorod anisotropy during the overgrowth of Ag(I)-mediated gold-nanorod growth. They proposed that nanorods with <110> facets undergo a surface reconstruction process that changes the packing density and facet energy with increasing reaction time, which then promotes isotropic growth.\textsuperscript{78} In fact, as seen from Figure 3-4 C, increased plating time (more than 40 min) resulted in some very wide and irregular structures. The nanorods likely grew to their full length within 20-40 min, beyond which time additional plating was detrimental. The key to anisotropic filling in this system was to use nanorod seeds that had not yet grown to their maximum “anisotropic length”
so that additional anisotropic growth was possible. Consequently, seed growth was stopped well before the initiation of isotropic growth. The seeds were then attached to the DNA template where they were able to undergo additional growth in-situ, enabling more effective filling of gaps between seeds while maintaining a narrow diameter.

![SEM images of linear DNA templates after seeding with gold nanorods and electroless plating to fill the remaining gaps. Plating time was (A) 20 min, (B) 40 min and (C) 60 min. Scale bar is 200 nm.](image)

Figure 3-4: SEM images of linear DNA templates after seeding with gold nanorods and electroless plating to fill the remaining gaps. Plating time was (A) 20 min, (B) 40 min and (C) 60 min. Scale bar is 200 nm.

The difference between the results in Figure 3-4 A and B and those in Figure 3-4 C highlight the importance of anisotropic growth to our ability to fabricate thin, continuous nanowires. Consequently, the impact of the growth solution on anisotropic growth was explored. Initially, the concentration of all reagents in the growth solution except CTAB was doubled to help facilitate additional anisotropic growth in the gaps between seeds. The amount of CTAB was kept constant since its concentration was already approximately two orders of magnitude higher than that of the gold (Section 3.2.7, Materials and Methods). The principal impact of the increased concentration was to reduce the nanorod growth time; no significant difference in the ability of the solution to fill the gaps between seeds was observed. Also, loss of anisotropy was observed in as little as 40 min for the higher concentration growth solution (see Figure 9-3 in Appendix A).
The impact of the individual components of the growth solution was next examined. Our gold plating solution has excess CTAB and silver ions relative to gold, both of which are believed to play a critical role in promoting anisotropic nanorod growth.\textsuperscript{48, 78} Similar to CTAB’s affinity towards the sides of nanorods, silver ions form a monolayer on the $<110>$ facet of gold nanorods and inhibit gold deposition on the sides.\textsuperscript{48, 78} Consequently, we increased silver concentration relative to that of gold, but there was no noticeable difference in our ability to fill in the gaps between seeds. In addition to the surfactant and silver ions, HCl was added to the plating solution to diminish the reducing power of ascorbic acid in order to reduce the growth rate. The slowing of the growth rate favored anisotropic growth as seen previously (Figure 3-4A and B). In contrast, removal of the HCl resulted in a more rapid reaction rate and complete loss of anisotropy within 5 min of plating as seen in Figure 3-5. The results in Figure 3-5 also illustrate the difficulty of filling gaps when the growth is nearly isotropic. Equation 3-1 shows that the oxidation of ascorbic acid depends on pH, consistent with the observed impact of HCl. Thus, the addition of HCl directly affects the reduction of gold ions ($\text{Au}^{3+} \rightarrow \text{Au}^0$) and the resulting yield of continuous wires. Rueda \textit{et al.}\textsuperscript{79} demonstrated that the oxidation of ascorbic acid is first order with respect to the $\text{C}_6\text{H}_8\text{O}_6$ concentration, and that the reverse reaction is second order with respect to H$^+$. Increasing the concentration of ascorbic acid represents another way to increase the rate of the deposition reaction in order to improve filling while, hopefully, maintaining the needed anisotropy.

\begin{equation}
\text{ascorbic acid} \quad \text{dehydroascorbic acid} + 2\text{H}^+ + 2\text{e}^- 
\end{equation}

\text{Equation 3-1}
Initially, the plating solution contained 1.6 times more ascorbic acid (conc = 0.5 mM) than gold ions (conc = 0.32 mM), which is approximately the amount of ascorbic acid required (1.5 equivalents) to reduce Au$^{3+}$ to Au$^{0}$. The results from this solution looked promising (e.g., see Figure 3-4 A and B), but many of the nanowires still had one or more gaps that were not filled; in fact, there were very few continuous wires with no visible gaps that spanned the length of the entire 400 nm DNA template. Upon increasing gradually, the amount of ascorbic acid beyond this value, we observed an increase in the number of continuous DNA-templated wires. The largest fraction of continuous wires was observed as the ascorbic acid concentration was increased to approximately 2.5 times the amount of gold (Figure 3-6). Increasing the amount of ascorbic acid further did not have a significant impact on the yield of continuous nanowires, but it did reduce the plating time required to achieve that yield. For example, plating times of 30-40 min were required when the concentration of ascorbic acid was 2.5 times that of the gold, while a plating time of only 20 min was required when the concentration was increased to 4.0 times that of the gold (see Figure 9-4 in Appendix A). Therefore, an increase in the concentration of the reducing agent (ascorbic acid) was sufficient to increase the length of nanorods, fill the gaps.
between the seeds on the template, and reduce the plating time, while maintaining the aspect ratio of final metallized structures.

Figure 3-6: (A) - (F) SEM images of continuous linear DNA origami nanowires plated at an ascorbic acid to gold ratio of 2.5 for 30 minutes.

In order to quantify the yield of continuous wires, we counted the number of complete wires and wires with gaps with use of high-magnification SEM pictures for several batches of wires with different ratios of ascorbic acid to gold. Fabrication of continuous wires required filling an average of about 10 gaps per wire to make thin continuous ~400 nm structures. For the wires analyzed (Table 3-1), the plating process was successful in filling about 96% of the gaps between the seeds. The analysis of the initial gap sizes presented above (see Figure 3-3 and the accompanying discussion) showed that 95-96% of the gaps between seeds were 25 nm or less in size. Thus, the anisotropic plating process demonstrated here appears to successfully fill gaps up to about 25 nm in length, which are all but the largest 4-5% of the gaps between seeds. Importantly, our ability to successfully fill the gaps between seeds was limited by the large initial
size of a few of the gaps rather than by the plating process. Finally, the anisotropic nature of the filling process allowed us to fill the gaps without a large increase in the wire diameter.

On average, our yield of complete DNA wires with no visible gaps for different batches and ratios of ascorbic acid to gold as shown in Table 3-1 was 57%. In addition, more than 30% of the wires had only one break (gap). The final width of our DNA-templated gold nanowires was affected by width of the DNA template, which was larger than the diameter of either the nanorod seeds or that of the individual nanorods after plating. The larger template width permitted “doubling up” of nanorods in places along the template. Due to this, about 28% of an average nanowire had a width of approximately 25 nm, while the remaining 72% of the nanowire consisted of a single “line” of nanorods with an average width of 13 nm. Irregular particles were also occasionally observed along the DNA template as shown in Figure 3-6; these particles were only present after the plating process and, in a few places, grew to almost 40 nm in diameter. It is possible that some nanorods lose anisotropy faster than others, resulting in these irregular shapes.

Figure 3-7 shows the comparison of the same DNA origami design following plating for 1) seeding with 5 nm Au particles and 2) seeding with nanorods as described in this chapter. The images clearly show the advantages of using anisotropic plating of nanorod seeds to improve the morphology and width of the final nanowires. In addition, nanorod-seeded nanowires have fewer grain boundaries than the nanoparticle-seeded wires.
Table 3-1: Yield analysis of complete wires for different ratios of ascorbic acid to gold

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<th>Batch</th>
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<th>Ascorbic acid/Gold ratio</th>
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<th>2 gaps</th>
<th>3 gaps</th>
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<th>% 1 gap wires</th>
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Figure 3-7: SEM images of (A) linear DNA origami seeded with 5 nm gold nanoparticles linked to DNA oligonucleotides. (B) Electroless plating of DNA in (A) to make a continuous nanowire. (C) Nanorod-seeded and plated linear DNA origami. Scale bars are 100 nm.

During the course of our study, higher purity CTAB (H6269, >99% purity) was ordered to replace the CTAB (H5882, 98% purity) we had been using. While the higher purity CTAB was used successfully to synthesize gold nanorod seeds, it did not permit us to achieve the same high yield when filling gaps with our optimized plating recipe. After narrowing down the problem to the CTAB, we subsequently purchased a new batch of 98% purity CTAB and were able to achieve the same yield observed previously. Others have reported problems in forming nanorods
with CTAB from different suppliers or even with different batches from the same supplier. Problems have been attributed to trace amounts of iodine present in CTAB, which disrupts nanorod formation by specifically binding to the \(<111> facet at the ends of the nanorod. However, a contradicting report claims that specific adsorption of iodide ions to the \(<111> surface plays a key role in the growth, rather than disruption, of nanorod structures. Our own experiments with the addition of iodine to our plating solution showed that nanorods transform to nanoparticles at iodine concentrations greater than 5 \(\mu\text{M}\) (see Figure 9-5 in Appendix A), consistent with previous studies. However, the presence of iodine in small amounts (0.5 \(\mu\text{M}\) or less) in the plating bath did not appear to affect our plating yields.

### 3.3.3 Electrical Measurement

Following electroless plating, electrical measurements were performed at room temperature to confirm the continuity and assess the electrical properties of our DNA origami gold nanowires. Electron beam lithography (EBL) was used to pattern gold electrodes, leads, and connection pads on the \(\text{SiO}_2\) surface upon which nanowires had previously been fabricated (Figure 3-8 A and B). The experimental setup was similar to that reported previously (see Section 3.2.10 for details).  

Figure 3-8 C shows an example of a DNA origami-templated gold nanowire between two EBL-patterned gold electrodes. To measure the resistance of the nanowires, the voltage between the electrodes was ramped from 0 to 100 mV and the resulting electric current was measured with a picoammeter (Ithaco). Each measurement was repeated at least three times, and the reported values represent an average of the measured values. For the wire shown in Figure 3-8 C, the individual measurements were 0.442 k\(\Omega\), 0.434 K\(\Omega\) and 0.43 k\(\Omega\), which suggests that Joule
heating did not significantly alter the characteristics of the wire during the measurement. Figure 3-8 D shows a typical I-V curve obtained during measurement of the electrical properties. At the maximum voltage of 100 mV, the current density was $8.9 \times 10^{11}$ A/m².

Figure 3-8: SEM images of electrode set up for electrical measurements of DNA nanowires. (A) 50 µm pads for electrical connection by micromanipulator probes. (B) Zoomed in area of the box in (A) showing smaller electrodes. (C) Electrode sets containing gold nanorod metallized linear DNA origami. (D) Current-voltage plot of the nanowire in (C) with resistance of 0.435 kΩ.

The results of additional measurements on DNA origami nanowires are shown in Figure 3-9 with the corresponding SEM images in Appendix A (Figure 9-6). The resistances for single nanowires ranged from 0.435 kΩ to 19.9 kΩ and, as two-point measurements, include any
contact resistance between the EB-patterned gold electrodes and the DNA-templated nanowires. Of the 49 total nanowires characterized, 29% of the single nanowires had a resistance of < 1 kΩ and 12% of the single nanowires had a resistance of < 0.5 kΩ. In addition, 8% of the nanowires (not included in Figure 3-9) measured very high resistances in the range of 605 kΩ to 36.6 MΩ. High magnification SEM images showed breaks along the nanowire for some of these nanowires, while no obvious breaks were observed for others (see Figure 9-7 in Appendix A).

The resistivity values for the nanowires we measured ranged from 6.7 x 10^-5 Ω-m to 8.9 x 10^-7 Ω-m, compared to the bulk gold resistivity value of 2.2 X 10^-8 Ω-m. The lowest resistivity value was obtained for the nanowire in Figure 3-8 C, which measured 18.9 nm in width and had a length of 137 nm between the electrodes. The nanowires that were characterized had an average width that ranged from 13 nm to 29 nm, and represent some of the thinnest DNA-templated gold nanowires characterized to date.

As mentioned above, the measured resistivity of our gold nanowires ranged from 6.7 x 10^-5 Ω-m to 8.9 x 10^-7 Ω-m. In comparison, Ongaro et al. reported a resistivity of 10^-4 Ω-m for DNA-templated gold nanowires with a mean width of 40 nm. Pearson et al. reported a lower value of 6.2 x 10^-6 Ω-m for gold nanowires with an average width of 33 nm. The lowest value that we found reported was that of Satti et al. who measured a resistivity of 4 x 10^-8 Ω-m for DNA-templated gold nanowires, although their wires were much larger (79 nm in diameter). A number of factors may have contributed to the difference between our measured resistivities and that of bulk gold. First, a contribution to the electron resistivity is expected from surface scattering as the dimensions of gold nanowires decrease. The mean free path for electrons in bulk gold is 40 nm, and the measured resistance of polycrystalline wires has been shown to increase as the wire dimension approaches the length of the mean free path in the bulk.
Second, electron scattering at grain boundaries can add to the resistivity. Even though the use of nanorods enables anisotropic filling and reduces the number of grain boundaries relative to a particle-seeded wire, the wires still consist of multiple grains. Finally, the two point measurements made in this study include contact resistance as part of the total measured resistance, which would lead to an overestimation of the wire resistivity if the contact resistance is significant. Both organic and inorganic impurities or layers on the nanowire surface could lead to increased contact resistance. Surface impurities may even influence the transport of electrons through the wires themselves for wires of this small size.77

Figure 3-9: The results of two-point measurements for different widths of nanorod-seeded DNA nanowires after plating.

A significant difference in nanowire morphology was observed for wires just after the electroless plating step (Figure 3-6) and wires that had been prepared for electrical characterization (Figure 3-8 C). Specifically, the nanowires prepared for characterization had
fused grain boundaries, in contrast to the distinct grain boundaries observed for nanowires after the electroless plating step. There was no observable difference in nanowire morphology before and after electrical characterization, which indicates that Joule heating during testing was not the cause of the changed morphology. Upon further inspection, the nanorod-seeded nanowires were found to change morphology during a resist pre-bake step (200°C, Section 3.2.10), after the nanowires had been covered by the resist that was used to pattern the test electrodes. The resist-coated nanowires appeared to fuse, at least partially, due to the pre-bake, while breaks along the grain boundaries were observed for some nanowires. In a control experiment, DNA nanowires without resist were heated to 200°C and were observed to coalesce into nanoparticle-like shapes (see Figure 9-8 in Appendix A). Apparently, the 200°C temperature led to fusion or annealing of the wires, while the resist covering the wires prevented coalescence. This polymer-assisted annealing process is currently under investigation, and findings will be published shortly.

3.4 Conclusions

We have demonstrated the use of gold nanorod seeds and the subsequent metallization to fabricate conductive DNA-templated gold nanowires with diameters as small as 13 nm. The DNA origami methods used to assemble biological templates in this study are flexible and versatile, and can be used to create a variety of two- and three-dimensional shapes. In this case, a linear template was used in order to facilitate the measurement of electrical properties. A key contribution of the work is the demonstration of an anisotropic metallization process to fill gaps between seed particles as large as 25 nm, while limiting the final nanowire width. The combination of nanorod seeds and anisotropic filling was used to fabricate continuous wires that extended the full length of the ~400 nm DNA template. The process was highly selective with no background deposition. The electrical resistivity was measured for nanowires ranging from 13
nm to 29 nm in width, which represent the smallest DNA-templated gold nanowires characterized to date. In spite of the narrow width of the wires, the measured resistivity values were as low as $8.9 \times 10^{-7}$ $\Omega$-m, significantly lower than almost all of the previously reported values for DNA-templated nanowires. The directional plating demonstrated here greatly improves the morphology of the fabricated wires and provides control over the final width that is much improved relative to metallization processes demonstrated previously. Consequently, this study represents important progress towards the creation of nano-electronic devices by molecularly directed placement of functional components onto self-assembled biological templates.
4 ADDITIONAL METALLIZATION AND METHODS FOR CHARACTERIZING METALLIZED DNA NANOSTRUCTURES

After successful metallization of the linear DNA template, we looked into metallizing a bigger self-assembled DNA origami template. To realize nanoscale systems and devices based on DNA molecules, larger DNA structures are needed to make connections between the smaller (nanometer sized) devices and/or the external world. To this end, our collaborator Dr. Rebecca Schulman and her group at John’s Hopkins University (JHU) have devised a method to self-assemble DNA origami nanotube structures that are tens of microns in length. Moreover, they have demonstrated the ability to control both the timing and location of where these nanotubes are assembled. In order to control both when and where a nanotube is grown, Dr. Schulman’s group have developed a technique to initiate DNA nanotubes growth using a nucleating structure. In their technique, the DNA nanotubes grow readily only from a nucleating structure to provide control over the size and growth of the nanostructures (nanotubes). In addition, the timing and the location of the nucleation can be controlled to give even greater control of when and where the nanotubes are assembled. These bigger self-assembling DNA nanotubes structures, however, need to be metallized to form conductive structures for application in nanoelectronics. Thus, we looked to metallize these bigger DNA templates based on the metallization procedure we have developed for smaller templates.
4.1 Metallization of DNA Origami Nanotubes

The nanotube samples we received did not have attachment points on the template to create a metal seed layer via nucleic acid base pairing as shown previously in Figure 2-3. Thus, in order to metallize these DNA nanotubes, we decided to first seed the nanotubes with gold nanorods and then connect the nanorods in the growth solution based on our metallization procedure described in Chapter 3. The CTAB-stabilized nanorods readily interact (although non-specifically) with the DNA template to create a nanorod seed layer via local field effects as mentioned in Chapter 3. The nanotube templates can be designed with attachment points for site-specific metallization if needed in the future.

Figure 4-1 shows the results obtained from this experiment. As seen from the images, gold nanorods line up in an end-to-end fashion (consistent with the observations in the preceding Chapter) along the length direction of both sides of a DNA nanotube template. Some nanorods were also occasionally seen to attach at an angle across the length of the nanotube. In addition, nanoparticles, which are the byproducts of nanorod synthesis, were also seen to attach to the DNA templates. A typical nanorod-seeded DNA nanotube template shows gaps between the nanorods (seeds) which need to be filled to make a continuous metallized template.

Following seeding, the nanorod seeded DNA nanotubes were introduced to the gold electroless plating solution to grow the seeds and fill the gaps. Figure 4-2 shows the SEM images of DNA origami templates after the electroless plating step for 30 min at 27°C. The starting concentration of the reagents in the plating bath and the plating time used for this work were based on the optimized values used in the previous chapter. Briefly, the plating solution contained CTAB, gold ions, silver ions, hydrochloric acid and the reducing agent ascorbic acid (Section 3.2.7, Chapter 3). As seen from the images, the plating process was able to successfully
fill the seed gaps in order to create micron-length continuous metal structures based on DNA nanotube templates.

Figure 4-1: AFM images of (A) DNA origami tube, and (B) gold nanorod seeded DNA tube. Height scale is 10 nm. (C) – (D) SEM images of nanorod seeded DNA origami tube.

Figure 4-2: (A) - (C) SEM images of DNA origami tubes after seeding with gold nanorods and electroless plating to fill the gaps.
The above seeding and plating experiments confirmed that our DNA metallization procedure based on gold nanorods can be easily used to metallize micrometer-length templates. The ability to controllably initiate (when and where) DNA origami growth and the ability to metallize these structures is important for the realization of self-assembled nanosystems.

4.2 Optimization of Electron Beam Lithography Patterning Process

Following metallization of the DNA origami templates, electron beam lithography (EBL) was used to assess the electrical properties of our metallized DNA structures. In order to do this, EBL was used to pattern electrodes on the silicon dioxide surface after fabrication of the DNA nanowires. The design for the EBL electrodes, lines, lids and connection pads were used from a previous study. However, every step of the lithography process was examined separately and optimized to avoid breakages in the electrode lines such as those shown in Figure 4-3. The EBL process consists of five steps: (1) resist deposition on the surface, (2) electron beam exposure of the resist surface, (3) resist development, (4) gold deposition and (5) resist lift-off. The resist deposition step is well established by the manufacturer for the type of the resist. However, the electron exposure and the resist development steps required detailed study to find optimum dosages, exposure times and development parameters as explained in detail in the paragraphs that follow.
Figure 4-3: (A)-(D) SEM images of the electron beam setup for measuring conductivity of metallized DNA structures. Images in (C) & (D) show the broken electrode lines.

We used ZEP 520A resist, which is a positive electron-beam resist and provides the very high resolution that is needed to fabricate smaller (~75 nm wide) parallel lines. A positive electron-beam resist is a resist that becomes soluble in the developer when exposed to an electron beam. The unexposed portion of the resist remains insoluble. The resist was spun at 5000 rpm for 1 min followed by a 2 min prebake at 200°C. The resist-coated surface was then exposed to the electron beam. Initially, we tested different dosages (amount of electrons per unit length or area) of electron beam to find the optimum dosage required to avoid breakages in the electrode lines as shown in Figure 4-3 C&D. The electron beam dosage was changed manually by entering the values in the computer program. The electron beam exposure time was automatically adjusted by the program with changing dosages. In general, the exposure time decreases with the increase in electron beam dosage. As shown in the Figure 4-4 A, a line dosage
below 0.5 nC/cm was not sufficient to obtain the smaller lines (linewidth ~ 75 nm) that make the connection to the DNA nanowires. Likewise, a line dosage above 1.0 nC/cm was high enough to cause proximity effects, which caused the electrodes to overlap as shown in Figure 4-4 C. As the electron beam enters the resist surface, it undergoes a series of collisions with the electron and the nucleus of the atoms in the resist. Some of these electrons undergo a series of deflections with the nucleus of the atoms of the resist which eventually causes the electrons to come out of the top surface of the resist at a significant distance from the desired exposure location.

Increasing the dose of electrons thus increases this phenomenon called backward scattering of the electrons causing the closely placed electrodes to overlap as shown in Figure 4-4 C. It was found that a line dosage of 0.8-0.9 nC/cm was optimal to get the required smaller electrodes and the connection pads.

Figure 4-4: SEM images of the electrodes written using an electron dosage of (A) 0.3 nC/cm, (B) 0.8 nC/cm and (C) 1 nC/cm.

After the resist-coated wafer was exposed to the electron beam, the resist was developed to remove the electron-exposed portion of the resist by dipping the wafer in the ZED N50 developer. The developer is specific to the ZEP 520A resist we used for this work. The resist was developed for different times and analyzed to find the optimal development time. Figure 4-5 shows SEM images of the smaller electrode lines after different development times. As seen from the images, samples exposed to lower electron dosage (0.45 nC/cm) require longer
development times to completely remove the exposed regions of the resist and obtain the desired contrast (deeper cuts). Likewise, samples exposed to higher electron dosage (0.8 nC/cm) require shorter development times to get the required contrast, consistent with the patterns expected for exposure dosage and development time in a lithography process.

Figure 4-5: SEM images of the electrodes written using electron dosage (A)-(C) of 0.45 nC/cm and (D)-(E) of 0.8 nC/cm. (A) & (D) 30 sec development time. (B) & (E) 1 min development time. (C) 2 min development time.

A 7 nm chromium adhesion layer followed by a 50 nm gold layer was deposited onto the developed wafer to fabricate the electrode lines for assessing the electrical properties of the DNA nanowires. The deposition was performed in a thermal evaporator by evaporating gold pellets in vacuum. After gold deposition, samples were immersed in n-methyl-2-pyrrolidone (NMP) solution to lift off the resist and create the designed patterns as shown in Figure 4-6. The gold-deposited wafers were placed in the NMP solution in a shaker for increased mass transfer. The optimal lift-off time was determined by taking the wafers out of the solution every few hours and rinsing with acetone to remove the unwanted gold (in the unexposed regions of the resist). The
samples were then examined under the optical microscope to confirm that the excess gold deposited all over the surface had been removed, leaving only the gold on the exposed regions of the resist. Through multiple experiments, it was determined that a 20 hr lift-off time was required to remove the excess gold from the surface in order to produce the desired gold patterns (Figure 4-6).

Figure 4-6: (A) -(B) SEM images of the electrodes written using electron dosage of 0.8 nC/cm. Development time was 2 min. Lift-off time was 20 hr.

4.3 The Impact of Annealing on the Resistivity of DNA Origami Nanowires

A significant difference in the gold nanowire morphology was observed for DNA nanowires just after the electroless plating step (Figure 4-7A) and the wires after the electron beam lithography (EBL) patterning (Figure 4-7 B). As seen from the figure, the nanowires after EBL patterning had fused grain boundaries, in contrast to the distinct grain boundaries observed for the nanowires before EBL patterning (just after the electroless plating step). Upon closer inspection, the nanowires were found to change the morphology during a resist pre-bake step (at 200°C) of the EBL process. The resist-coated nanowires appeared to fuse, at least partially, due
to the pre-bake, while breaks along the grain boundaries were observed for some nanowires. In a control experiment, DNA nanowires without resist were heated to 200°C and were observed to coalesce into nanoparticle-like shapes (Figure 4-7 C). Apparently, the 200°C temperature led to fusion of the grain boundaries in the nanowires, while the resist covering the nanowires prevented coalescence.

![Figure 4-7](image)

**Figure 4-7:** SEM images of gold nanowires (A) right after the electroless plating step, (B) after annealing with the polymer resist at 200 °C and (C) after annealing without the polymer resist at 200 °C. The nanowires in (A) were plated according to the optimized plating solutions mentioned in the text. Inset in (B) shows a single gold nanowire.

The polymer-assisted annealing process (fusion of grain boundaries) that we have discovered can possibly be used to enhance the conductivity of the DNA-templated metal deposits (structures). In order to quantify the impact of annealing on the conductivity of the metal deposits, we need to measure the conductivity of the un-annealed DNA metal structures (without the morphological changes). However, the measurement of the un-annealed structures is not possible with EBL patterning as the process includes a 200°C pre-bake step that will inevitably anneal the structures. In this regard, an alternative measurement technique called electron beam induced deposition (EBID) is being pursued by a graduate student in the Department of Physics (BYU) who has been collaborating with us on our DNA-based nanofabrication process. The EBID process does not require a high temperature (~200°C) step in
order to make the measurement of the metal structures (nanowires). In addition, individual nanowires can be selectively tested for their conductivity as shown in Figure 4-8. In comparison, the electrodes are fabricated at random in the EBL process which only allows measurement of the nanowires that are present between the electrodes (parallel lines in Figure 4-6). Conductivity measurements will also be made for the annealed DNA nanowires using the EBID process to get the quantitative difference in conductivity between annealed and un-annealed nanowires. If this polymer-assisted annealing process does indeed improve the conductivity of the DNA-templated metal deposits, then this process could be widely used to improve the electrical properties of a variety of metal structures. Thus, this discovery potentially can have a significant impact on the development of conductive structures needed for the realization of DNA based nanoelectronic systems and devices.

Figure 4-8: SEM images of electrode set up for electrical measurements of DNA nanowires. (A) Large gold pads for electrical connection by micromanipulator probes. (B) Zoomed in area of the box in (A) shows smaller electrodes that are connected to the large pads and the DNA nanowires. The smaller electrodes are written using the electron beam induced deposition of platinum.
ANISOTROPIC PLATING OF GOLD NANORODS PLACED SITE-SPECIFICALLY ON A DNA ORIGAMI BREADBOARD TO FORM CONTINUOUS NANO-WIREFRAME SHAPES

The following chapter is a paper draft that was just submitted.

5.1 Introduction

Biological molecules like DNA can self-assemble into a variety of complex 2-D and 3-D architectures with very low energy consumption and without the need for expensive patterning tools. DNA, with its small size, functional groups, complementary base pairs and ready availability is a molecule of great interest for fabricating such structures. DNA origami, in particular, is seen as a useful tool for folding single-stranded DNA molecules into a large number of intricate constructs in a single pot. In contrast to self-assembly, top-down fabrication employs large and expensive patterning tools and a series of complex processes to selectively remove and add substances in order to create small patterns. However, the top-down approach is getting increasingly complex and costly as a result of the continual push for smaller sizes of electronic components. DNA-based bottom-up nanofabrication provides a promising and simple approach for developing nanoscale devices and systems in large quantities and at a reasonable cost. Recent demonstrations of a wide variety of planar and three-dimensional nanostructures illustrate the ease and utility of the bottom-up approach. While the DNA
templates themselves are useful materials, they also provide the versatility to controllably assemble functional (metal and semiconductor) nanomaterials with use of the template for device applications. In general, nanomaterials are either nonspecifically attached via electrostatic and/or chemical interactions with the DNA template, or attached via base-pairing to specific sites on the template. Different types of materials including Au, Ag, Cu, Ni, Pd, Te and carbon nanotubes have been placed on DNA nanostructures using either nonspecific or site-specific attachment. Hence, DNA-based nanofabrication provides a promising pathway for the development of nanoelectronic devices and systems based on biological molecules.

For application in electronic devices and systems, DNA templates need to be metallized to form conductive connections or wires. Furthermore, selective deposition of metals at specific sites on templates is needed so that active components such as semiconductors can be placed at designated locations as part of a complete DNA-based nanoelectronic system. One method of performing site-specific metallization of DNA origami templates is to attach metal nanoparticles to specific sites on the origami template via complementary base pairing, and to subsequently grow the nanoparticles in a plating bath until they fuse together to form a continuous structure. Pais-Pilo et al. were the first to report site-specific metallization of DNA origami templates utilizing this technique. A simultaneous and independent effort was underway in our laboratory, the results of which were published shortly after that initial paper and further included the first measurements of the conductivity of such structures. Site-specific metallization with two different metals on the same template was also demonstrated. Recently, Teschome et al. also demonstrated the use of metal nanoparticles and their fusion during plating to metallize whole DNA origami nanotubes.
The metal structures created using nanoparticles, however, have generally been granular in appearance, far less conductive than the bulk material, and wider than what is achievable with the underlying template. In this context, we recently demonstrated (Chapter 3) the use of gold nanorods (Au NRs) to significantly improve the morphology, linewidth, and conductivity of DNA-templated metal wires. In that study, cetyltrimethylammonium bromide (CTAB) stabilized gold nanorods were attached to a linear DNA origami template and anisotropically connected to form thin and continuous nanowires. The anisotropic growth was demonstrated to fill seed gaps up to 25 nm while limiting the final width of the metallized structures. The directional growth is attributed to the CTAB surfactant, which inhibits growth on the sides of the nanorods relative to that observed at the ends.

The CTAB-coated nanorods, however, do not enable the desired site-specific attachment to the origami template. The positive charge on the CTAB results in non-specific interaction of nanorods with the DNA templates. Hence, the nanorods must be functionalized with a different molecule (like oligonucleotides) to allow controlled placement on DNA origami templates via molecular recognition. Au NRs functionalized with molecules other than CTAB, however, may not result in anisotropic growth in a plating solution; such growth helps to improve the morphology and control the final width of the metallized DNA structures. This chapter seeks to characterize the extent to which anisotropic growth of gold nanorods functionalized with DNA oligonucleotides takes place. The ability to controllably place nanorods at specific sites on a template and connect them anisotropically would be an important development for the fabrication of DNA-templated nanodevices. To facilitate this study, we first demonstrate site-specific placement of DNA functionalized Au NRs on a DNA origami breadboard to form seeded structures of rectangle, square and T shapes (Figure 5-1). The nanorod-seeded DNA
templates are then introduced to electroless gold plating solutions to study the growth behavior and characterize the ability of anisotropic growth to fill the maximum gap sizes between two adjacent DNA functionalized gold nanorods.

Figure 5-1 (A) Design and fold of a DNA breadboard with attachment sites to fabricate different shapes. (B) Gold nanorods placed on the DNA origami template by complementary base pairing between the A10 sequence (blue) on the origami and T8 sequence (red) on the nanorods to form rectangle, square and T shapes. Enlarged sections show the positions of capture strands (blue) on the template. (C) Subsequent metallization of gold nanorod seeds to form continuous metallic structures.

5.2 Materials and Methods

5.2.1 Materials

M13mp18 DNA was obtained from New England Biolabs (Ipswich, MA). Staple strands at a concentration of 100 μM in TE (Tris-EDTA (ethylenediamine tetraacetic acid)) buffered
solution (pH 8.0) for DNA origami folding were purchased from Eurofins MWG Operon (Huntsville, AL). Single-stranded DNA functionalized with thiol to enable attachment to metal nanorods was also purchased from Eurofins MWG Operon with PAGE purification, and diluted to 1 mM in water. 10X TAE-Mg\textsuperscript{2+} (pH 8.3) buffer was made from 400 mM Tris base, 200 mM acetic acid, 10 mM EDTA and 125 mM magnesium acetate (MgAc\textsubscript{2}.4H\textsubscript{2}O). Tris base (tris (hydroxymethyl) aminomethane) was obtained from Fisher Scientific Inc. MgCl\textsubscript{2}, MgAc\textsubscript{2} and acetic acid were purchased from EMD Chemicals (Gibbstown, NJ). EDTA was obtained from Life Technologies (Carlsbad, CA). HAuCl\textsubscript{4} and CTAB (H5882, 98%) were obtained from Sigma Aldrich (St. Louis, MO). Silver nitrate (AgNO\textsubscript{3}) was obtained from Mallinckrodt Chemicals (Phillipsburg, NJ). Hydrochloric acid (HCl) was obtained from EM Science (Gibbstown, NJ). Bis (p-sulfonatophenyl) phenylphosphine dihydrate dipotassium salt (BSPP) was obtained from Strem Chemicals (Newburyport, MA). Tris (2-carboxyethyl) phosphine (TCEP) was obtained from Alfa Aesar (Ward Hill, MA). Sodium borohydride (NaBH\textsubscript{4}) was obtained from Acros (New Jersey). Ascorbic acid (C\textsubscript{6}H\textsubscript{8}O\textsubscript{6}) was obtained from Fisher-Chemical (Fair Lawn, NJ). Gold plating solution (GoldEnhance EM, Catalog #2113) was obtained from Nanoprobes (Yaphank, NY). Water (18.3 M\textOmega cm) treated by an EASYPure UV/UF purification system was used for all rinsing and aqueous solution preparations.

5.2.2 DNA Origami Breadboard Design

The DNA origami template was formed using 6812 bases of the 7249 bases in M13mp18 scaffold. This left a short tail of unfolded scaffold on one end of the width of the template. The staple strands used to assemble the breadboard origami were extended to comprise an additional length of nucleotides on the 3’ end to enable site-specific attachment of gold nanorods as seeds.
In order to do this, a 10 adenine nucleotide sequence was added to specific staple strands in the template. Specific staple strand sequences are included in Appendix B.

5.2.3 Additional DNA Origami

Linear DNA origami templates from Chapter 3 were also used in this study for initial experiments on site-specific attachment of gold nanorods. This origami utilizes 7085 bases of the 7249 bases in M13mp18 scaffold.25 For site-specific nanorod attachment, staple strands on half the template were extended to contain a sequence of 10 adenine nucleotides on the 3’ end. The linear template is approximately 400 nm in length.

5.2.4 DNA Origami Folding

The breadboard and the linear DNA structures were assembled by slowly heating a mixture of the scaffold and staple strands (2.0 nM scaffold and 20 nM of each staple strand in 10 X TAE-Mg\(^{2+}\) buffer) to 95°C for 3 min and slowly cooling to 4°C in a 90 min period in a TECHNE TC-3000 thermocycler.

5.2.5 Au Nanorod Synthesis

Au nanorods were synthesized using a growth technique reported by Ali et al.48 Briefly, HAuCl\(_4\) (2.5 mL, 1.0 mM) was added to 5.0 mL of CTAB (0.2 M). Following this, AgNO\(_3\) (250 µL, 4.0 mM) was added to the solution and gently shaken. HCl (8.0 µL, 37%) was then added to the solution. 35 µL of ascorbic acid (78.8 mM) was then added to the solution with gentle shaking until the solution was clear. Ice-cold NaBH\(_4\) (7.5 µL, 0.01 M) was added immediately afterwards and allowed to react for 3-4 hr until the solution color changed to maroon. The growth solution was maintained at 27°C.
5.2.6 Au Nanorod-DNA Conjugates

The nanorod solution obtained from the synthesis solution was distributed into six centrifuge tubes and spun twice at 14,000 rpm for 17 min to pellet the Au nanorods and remove excess CTAB. The DNA oligonucleotides were attached to the nanorods using modifications to the procedure described by Liu et al. In a typical procedure, 5 µL of 2 % (v/v) Tween 20 solution, 5 µL of 0.1 M BSPP, 15 µL of 0.6 mM thiolated DNA (HS-T8, [HS-T8] / [AuNR] = 2000), 75 µL of 1 M NaCl, and 50 µL of water were added to the 30 µL nanorod solution (0.153 µM) and allowed to react at room temperature with gentle shaking for two days. The final solution was centrifuged once at 14,000 rpm for 10 min to remove excess solution.

The S-S bond in the thiolated DNA was reduced by reaction with TCEP solution prior to adding to the nanorod mixture. To break the S-S bond, 15 µL of 1 mM thiolated DNA solution obtained from MWG Operon was mixed with 10 µL of 500 mM TCEP solution and incubated for 5-6 hr. 15 µL of this DNA/TCEP solution was then added to the nanorod mixture.

5.2.7 Deposition of DNA Origami onto SiO₂ Surfaces

The procedure is similar to that previously reported in the literature. Silicon dioxide surfaces (roughly 1 cm x 1 cm) were plasma cleaned (Harrick Plasma Asher, PDC-32G) for 30 s at 18 W to remove impurities from the surface. 3 µL of DNA origami (0.67 nM) in 10 X TAE-Mg²⁺ buffer was allowed to adsorb onto the cleaned surfaces for 15 min in a humid chamber at room temperature. The samples were then rinsed with 4.0 mM MgAc₂ solution for 3-4 s and water for 1-2 s. Finally, the surfaces were dried with a stream of filtered air and imaged by atomic force microscopy (AFM).
5.2.8  Seeding with Au Nanorods

20 µL of seeding solution (Au NR-DNA conjugates diluted in 10X TAE-Mg²⁺ solution) was dropped onto the SiO₂ surface with DNA templates and allowed to react for 40 min at room temperature in a humid chamber. The samples were then rinsed with 4.0 mM MgAc₂ solution for 3-4 s and with water for 1-2 s to clean the surface and to remove background nanorods that were not bound to the DNA. The samples were then dried in a stream of filtered air.

5.2.9  Anisotropic Electroless Au Plating

The gold electroless plating solution was prepared according to the procedure described in Chapter 3. Specifically, HAuCl₄ (250 µL, 1.0 mM) was added to 350 µL of CTAB (0.2 M). Following this, AgNO₃ (25 µL, 4.0 mM) was added to the solution and gently shaken. HCl (1.0 µL, 37%) was then added to the solution. 8 µL of ascorbic acid (80 mM) was next added to the solution with gentle shaking until the solution was clear. 70 µL of this solution was pipetted onto the SiO₂ surface and the reaction was allowed to proceed for ~30 min at 27°C. The samples were then rinsed with 4.0 mM MgAc₂ solution for 3-4 s and with water for 1-2 s. Following rinsing, the samples were dried with a stream of filtered air.

5.2.10  AFM Imaging

The samples were imaged using tapping mode on a Digital Instruments Nanoscope IIIa MultiMode AFM (Veeco) with aluminum-coated conical silicon tips (Vistaprobes, 3N/m, 45-75 kHz).
5.2.11 SEM Imaging

The DNA samples on SiO₂ surfaces were imaged by scanning electron microscopy (SEM) in ultra-high resolution mode on a FEI Helios Nanolab 600 and in high vacuum mode on a Philips XL30 ESEM FEG.

5.3 Results and Discussion

5.3.1 Template Assembly

The template used in this study was a DNA origami breadboard measuring 90 nm X 65 nm, which was assembled using a single-stranded M13mp18 scaffold strand and 221 short staple strands as has been described previously. An AFM image of the DNA breadboard after assembly is shown in Figure 5-2. The average height of folded DNA template from AFM measurements was 1.2 nm with a standard deviation of 0.2 nm. As seen from the figure, the majority of the DNA origami deposited on the surface had folded to yield the desired DNA breadboard template. AFM measurements showed the length and width of the folded origami template on the surface to be 91 ± 3 nm and 73 ± 3 nm respectively (n=30), close to the designed dimensions. The DNA origami templates were also seen to aggregate. One possible cause for the aggregation is helix stacking interactions of the staple strands on the edges of the adjacent templates. Some groups have suggested removing the side staples when folding origami to reduce DNA aggregation. In our DNA design, some of the extended staple strands (capture strands) for nanorod attachment were present on the edges of the template and hence included during the folding of the template (Figure 5-1). The template was designed to enable fabrication of seeded templates with a range of gap sizes (5 nm to 24 nm) in order to characterize
the ability of anisotropic growth to fill gaps of different sizes between adjacent gold nanorods on the template.

![AFM image of DNA origami breadboard template after assembly. Height scale is 10 nm.](image)

**Figure 5-2: AFM image of DNA origami breadboard template after assembly. Height scale is 10 nm.**

### 5.3.2 Seeding for Gold Deposition

For site-specific placement of gold nanorods, some of the staple strands in the DNA origami were extended beyond the template to provide attachment sites, as was previously demonstrated for site-specific attachment of metal nanoparticles. Specifically, staple strands located at each of the desired nanorod binding sites on the template were extended on the 3’ end by an A10 DNA sequence as illustrated in Figure 5-1B (see blue dots in the zoomed-in region). The length of the adenine attachment sequences was the same as that used successfully for site-specific attachment of gold nanoparticles to DNA origami templates.

Gold nanorods were synthesized using a growth technique reported by Ali et al., which uses CTAB to promote anisotropic growth. After growth, Au NRs were functionalized with thiolated T8 DNA bases for attachment to the template via nucleic-acid hybridization between
the A₁₀ sequences on the template and the T₈ sequences attached via thiol groups to the nanorods. The attachment sequence on the template had two extra bases to increase the probability of pairing all eight bases of the oligonucleotides attached to the nanorods. The nanorods were functionalized with the oligonucleotides using a modified version of a previously reported technique,⁹⁶ as explained in section 5.2.6.

Prior to the DNA breadboard design, a previously reported DNA template designed for site-specific placement of gold nanoparticles was used to verify our ability to site-specifically attach gold nanorods to a DNA template. Specifically, a ~400 nm long linear origami template designed with protruding A₁₀ DNA attachment sequence on half of the template length as shown in Chapter 3 was used to attach gold nanorods. Figure 5-3 shows images obtained with AFM and SEM of the (A) unseeded and (B) gold nanorod-seeded origami templates. As evident from the figure, the seeds attached selectively to half of the DNA length (~200 nm) as designed, with very little background deposition. Occasionally, some nanorods were seen to attach to the opposite end of the origami template. The extra scaffold strands present at the end of the assembled origami template may have led to the nanorod attachment by physically wrapping around the nanorods.²⁵ Thus, the A₁₀ DNA attachment sequence was found to successfully enable site-specific placement of gold nanorods to a DNA origami template. We note that the length of the attachment sequences used successfully in our work is shorter than the length of DNA strands reported by others (at least 20 base pairs long) to attach gold nanorods to DNA templates for studying fluorescence enhancement behavior⁶⁴ and chiral optical responses⁶⁵-⁶⁶ of gold nanorods.
Figure 5-3: AFM images of (A) linear DNA origami template and (B) gold nanorod seeded linear DNA template. Height Scale is 10 nm. (C) - (D) SEM images of nanorod seeded linear DNA origami. Gold nanorods were seeded via complementary base pairing between the DNA bases on the template and on the surface of nanorods.

After verifying nanorod attachment to the linear template, factors influencing site-specific nanorod attachment to the DNA origami templates were examined. The impact of nanorod concentration on nanorod attachment was first explored, followed by evaluation of the impact of the number of capture staple strands. The nanorods used for seeding had an average diameter and length of 7 nm and 28 nm, respectively (n=82). As seen from Figure 5-1, the rectangular shaped design had six locations for nanorod attachment. Similarly, the square and the T shaped designs
had four and three locations, respectively, for nanorod attachment. Each nanorod attachment location on the template was designed to have multiple capture strands (blue dots) as shown in Figure 5-1 and Figure 5-4. The template used for initial experiments had seven capture strands at each location in the horizontal (length) direction (Figure 5-4 A), and fourteen capture strands at each vertical (width) location.

Initial experiments were performed with 3 µL of 0.67 nM DNA solution and 20 µL of 1.5 nM nanorod seeding solution to give a 15:1 molar ratio of nanorods to DNA origami to ensure a sufficient number of nanorods was available for hybridization with the templates. The seeding time was chosen to be 40 min based on our previous successful attachment of DNA functionalized nanoparticles\(^{25}\) and nanorods (initial screening experiments) to complementary sites on a linear DNA origami template. The majority of the DNA breadboard templates after seeding resulted in incompletely seeded structures, or structures with misaligned nanorods as seen in Figure 5-5. It was also found that the probability of missing nanorods was significantly higher for the horizontal attachment locations at the top and bottom than it was for the vertical
attachment locations (along the width of the template). Increasing the concentration of the nanorods further to a 40:1 ratio did not improve nanorod attachment to the DNA templates (Figure 10-1 in Appendix B). Likewise, increasing the nanorod to template conjugation time to 2 hr did not improve nanorod attachment to origami templates. Consequently, the impact of increasing the number of capture strands (per nanorod location on the template) on nanorod attachment was examined.

![Figure 5-5: SEM images of DNA origami templates after nanorod seeding to make (A) rectangular, (B) square and (C) T shapes. The templates were designed with seven capture strands per site along the length of the template for nanorod attachment. Scale bars are 500 nm.](image)

We increased the number of capture strands from seven to ten or eleven per nanorod location for the sites along the length of the DNA template (Figure 5-4 B). The number of capture staple strands for the sites along the width of the DNA template was kept constant at fourteen. The origami templates were seeded with nanorod solution (15:1 nanorod to template ratio) for 40 min. There was a considerable improvement in the attachment of nanorods to the template with the increase in the number of capture strands for the sites along the length of the template. We observed an increase in the number of completely seeded origami templates compared to the partially seeded templates that were predominant when only seven capture staples were used for the nanorod attachment sites (compare Figure 5-5 to Figure 5-6). However,
some nanorods were still seen to attach at an angle to the attachment site and were thus misaligned on the template. In addition, some seeded templates were found to have more nanorods per location than intended (designed). The images of the seeded DNA show very low background deposition. On average, we found that 92% of the nanorods deposited on the surface were attached to templates rather than deposited non-specifically on the silicon surface.

Figure 5-6: SEM images of DNA origami templates after nanorod seeding to make (A) rectangular, (B) square and (C) T shapes. The templates were designed with ten or eleven capture staples for nanorod attachment at each site along the length of the template. Insets show a single DNA tile seeded with gold nanorods. Scale bars for insets are 50 nm.

Significant nanorod agglomeration on the rectangle and square shapes was observed when the concentration of nanorods was increased to 40:1 (Figure 10-2 in Appendix B). The T shape, in contrast, did not show the same negative impact as a result of the increased seed concentration. We did not observe such clustering effects in previous experiments with the rectangle and square shapes with only seven capture staples per location along the length of the template when the nanorod to template ratio was increased to 40:1 (see Figure 10-1 in Appendix B). Therefore, nanorod aggregation on the template with increased seed concentration is likely due to two factors: 1) the increase in the number of attachment points (from seven to ten capture staples per location) and 2) the structure of the rectangle and square shapes (compared to the T
shape), which permits nanorod interaction with multiple sites at once. Finally, no significant improvement in the attachment of nanorods to the template was observed when the number of capture staple strands was increased further to fourteen staple strands spread across four helices per location along the length of the template (Figure 10-3 in the Appendix B).

In order to quantify the yield of the seeding process, we analyzed the SEM images of the seeded samples for completely and partially seeded DNA templates. We found that 94% of the rectangular structures were completely filled with attachment to all six sites of the template after the seeding process. In comparison, only 80% of the square and 76% of the T-shaped templates were completely filled after the seeding process. Of the completely seeded structures, however, 91% of the rectangle, 66% of the square and 47% of the T-shaped templates were found to have more nanorods attached than intended, at least one misaligned nanorod and nanoparticles instead of nanorods at one or more capture locations on the template (nanoparticles are a side product of nanorod synthesis). Although some incompletely seeded templates were observed, the seeding process successfully attached nanorods to the majority of the designed sites on the origami templates. Moreover, it is clear that the nanorods were attached to the capture locations on the template designed to form the rectangle, square or T shapes as intended, although yields of “well formed” structures are low. However, these yields do not impact the study of growth behavior of DNA functionalized nanorods during the electroless plating process, which is the main objective of this work.

Figure 5-7 shows the size distribution of the gaps between nanorod seeds for the rectangular, square and T shapes on DNA templates (n = 30 seeded templates each). The figure also shows the distribution of the length of gold nanorods used to seed the DNA templates. The rectangular-shaped attachment design had gaps of 24 nm along the length of the template and
gaps of 8 nm along the width of the template (Figure 5-1). The square design had 8 nm gaps between each nanorod. The T-shaped design had gaps of 5.4 nm along the length of the template and gaps of 0.5 nm (distance between two helices) or less along the width of the template. Gaps of varying sizes were designed to help characterize the ability of the plating process to fill the spaces between nanorods in order to create continuous structures. The design values for the gap sizes in each of the three different shapes are shown as dot/dashed black lines on Figure 5-7.

Figure 5-7: (A) - (C) Gap distribution between gold nanorod seeds for rectangle, square and T shapes on DNA templates. (D) Length distribution of the nanorods used for seeding DNA templates. The black dot/dashed lines represent the designed gap sizes for each shape.

The measured distribution (Figure 5-7) shows gap sizes that vary significantly from the design values for all three shapes. For the rectangular shape, the gap sizes between adjacent nanorods along the length and width of the template (blue bars) ranged from 4 to 30 nm and 0 to 29 nm, respectively. The highest percentage (by number) of gaps along the length and the width
of the template were observed at 17 nm and 5 nm, respectively. The gap sizes for the square-shaped seeded templates ranged from 1 to 35 nm with the highest percentage of gaps at 3 nm. The gap sizes for the T-shaped seeded templates ranged from 0 to 31 nm with a peak in the distribution at 10 nm. The distribution in the observed gap sizes is due in part to variation in the length of the nanorods used for seeding (standard deviation = 8 nm), as well as variation in the attachment position compared to the design. The presence of seeds oriented at an angle to the template and sometimes extending outside of the template (see Figure 5-6) suggests that nanorod attachment to only a portion of the available capture staples per location contributes to the observed deviations in gap sizes from the design.

In chapter 3, we demonstrated the use of CTAB stabilized gold nanorods as seeds and their anisotropic growth to form continuous DNA origami nanowires. In that study, the CTAB stabilized nanorods were shown to grow anisotropically to fill seed gaps up to 25 nm in length when placed in the growth solution.72 In the present study, approximately 8% of the gaps for the rectangular-shaped seeded templates were larger than 25 nm, and 12% and 7% of the gaps were larger than 25 nm for the square and T-shaped seeded structures, respectively. The nanorods used in this study have been functionalized with DNA oligonucleotides, displacing the CTAB in order to enable site-specific placement of the nanorods. Since the CTAB was in part responsible for the initial anisotropic growth of the nanorods,72 it was necessary to determine whether the nanorods coated with DNA would also exhibit anisotropic growth during electroless plating. In order to answer this question, the size distribution of the gaps between rods was compared before and after electroless plating.
5.3.3 Electroless gold deposition

Following seeding, the extent to which the DNA functionalized nanorods demonstrated anisotropic growth during electroless plating was evaluated, with the ultimate goal of filling the gaps between the nanorod seeds to create continuous structures. As anisotropic growth was required to create the nanorods initially, the plating solution we used was similar in composition to that used to grow the nanorods. The plating solution, however, was missing the precursor, NaBH₄, that spontaneously reduces gold ions to gold seeds; the precursor was removed to prevent the formation of new nanorods (Materials and Methods, section 5.2.9).

Figure 5-8 shows SEM images of the seeded DNA origami templates after electroless plating for 30 min at 27°C. The starting concentration of the reagents in the plating bath and the plating time used for this work were the same as the optimized values used in Chapter 3. As seen from the images, the plating process was able to fill gaps and create continuous metal structures of rectangular, square and T shapes. Increasing the plating time further to 60 min resulted in the loss of anisotropy (Figure 5-8 D, H & L) as the nanorods began to grow into irregular particle-like structures, similar to those observed previously for CTAB-stabilized nanorods at similar plating times.⁷² The nanorods grew to their maximum “anisotropic length” in about 20-40 min, beyond which time additional plating resulted in isotropic growth.⁷² The shift to isotropic growth is believed to be due to surface reconstruction of the high surface energy <110> facets (sides) of the nanorods with increasing reaction time.⁷⁸

Experiments were also performed to determine the maximum anisotropic length of the DNA-functionalized nanorods during overgrowth. The maximum anisotropic length of the nanorod will also determine the maximum seed gap sizes that can be filled during the plating process. In order to do this, DNA functionalized nanorods not attached to origami templates were
separately introduced to the growth solution. Repeated exposure of nanorods with an average length of 26-28 nm to the growth solution resulted in additional growth of approximately 11-13 nm (n=103) before the onset of the isotropic growth. The nanorods grew from a diameter of approximately 7 nm to 10 nm during the overgrowth. Figure 5-9 shows the length distribution for nanorod seeds, and DNA functionalized and CTAB stabilized nanorods after growth in the plating solution. As seen from the figure, the increase in the length of the DNA-coated nanorods during regrowth was only about half that observed for CTAB-stabilized nanorods before the onset of isotropic growth. Thus, the DNA-functionalized nanorods needed for site specific deposition do undergo additional anisotropic growth, but not to the same extent as observed previously for CTAB-stabilized nanorods.

Figure 5-8: SEM images of nanorod seeded DNA metal structures after electroless plating in CTAB based growth solution. (A)-(D) rectangle, (E)-(H) square and (I)-(L) T shaped structures plated for 30 min. Structures (D), (H) & (L) were obtained after electroless plating for 60 min. Scale bars are 100 nm.
Figure 5-9: Distribution of the final length of DNA functionalized and CTAB stabilized nanorods after growth in plating solution.

The above results (Figure 5-8) were obtained with a growth solution that had been previously developed for the CTAB-stabilized nanorods. Consequently, the impact of the individual components of the growth solution on the anisotropic growth of DNA-functionalized nanorods was also explored. Absence of CTAB (critical micelle concentration = 1 mM) from the gold growth solution destabilized the plating solution and resulted in deposition of micron-size irregular particles on the surface (Figure 10-4 in Appendix B). Deposition of larger particles was observed even without the presence of gold nanorods on the substrate. Therefore, the plating solution instability was not instigated by the presence of nanorods, but was due solely to the lack of CTAB in the plating solution. CTAB likely acts to stabilize the plating solution by forming complexes with the metal ions in the solution, which prevents spontaneous deposition in
the absence of catalytic sites. In comparison, the removal of silver from the growth solution resulted in a rapid reaction rate and complete loss of anisotropy in as little as 15 min of plating (Figure 10-5 in Appendix B). Similar loss of anisotropy in as little as 15 mins was obtained when CTAB stabilized nanorods were grown in a silver free growth solution (Figure 10-5 in Appendix B). Silver ions are known to have a higher affinity for the sides of the gold nanorods where they tend to slow gold deposition relative to the deposition rate at the unhindered end sites. The effect of growth solution concentration was also examined. It was found that doubling the concentration of reagents (except CTAB) in the plating solution resulted in the loss of anisotropy of the final structures after about 30 min of plating, but there was no significant loss of anisotropy when the samples were plated for ~15 min. The amount of CTAB in the plating solution was kept constant since its concentration was already approximately two orders of magnitude higher than that of the gold. The primary impact of increased concentration was to reduce the time required to reach the maximum anisotropic length of the DNA functionalized gold nanorods; the nanorods grew isotropically to form larger particles after reaching their maximum length, similar to the results reported for CTAB-stabilized nanorods. Finally, increasing the pH (from 1.7 to 3.0) of the growth solution by removing the HCl resulted in the loss of anisotropy of the final structures within ~10 min. A similar loss of anisotropy was observed previously for CTAB-stabilized nanorods (Chapter 3). The rate of ascorbic acid oxidation increases at higher pH, resulting in faster onset of isotropic growth.

We counted the number of “well formed” structures with and without gaps after electroless plating from SEM images to quantify the yield of continuous structures. For the structures analyzed, the average yield of the connected rectangle (n=74), square (n=69) and T shapes (n=90) with no visible gaps were 24%, 35% and 51% respectively. An additional, 27%, 22%
and 38% of the rectangle, square and T shaped structures had only one gap that was not filled by the plating process. Based on regrowth experiments (Figure 5-9), the DNA-functionalized rods were not expected to elongate as well as the CTAB-stabilized rods. In general, the plating process was successful in filling about 67% of the total gaps for square and T shapes and 70% of the total gaps for the rectangles. An analysis of the initial gap sizes (Figure 5-7) for the square and T shapes showed that about 67% of the gaps between the seeds were 11 nm or smaller. Similarly, the analysis of the gaps for the rectangle shapes showed that about 70% of the gaps were 13 nm or smaller. The DNA functionalized nanorods, thus, appear to grow anisotropically to fill gaps up to 11-13 nm, similar to the change in length observed for the nanorods after overgrowth. This observed change in nanorod length after overgrowth is about half the change in length reported for CTAB-stabilized nanorods after overgrowth. Finally, the average final width of the continuous structures was about 10 nm (n=84) compared to the initial seed width of 7 nm.

5.4 Conclusions

In summary, we have demonstrated successful assembly of various shapes of 2-D metal nanostructures using gold nanorods and a DNA origami breadboard. Specifically, gold nanorods were programmatically placed at specific sites on a DNA origami and anisotropically connected to create continuous metal rectangular, square and T shapes with diameters as small as 10 nm. This is the first demonstration of the use of gold nanorod seeds and their subsequent growth to form continuous metal structures of different shapes on a DNA origami template. Another key contribution of this work is the use of nanorod-seeded DNA templates to characterize anisotropic growth in DNA-functionalized gold nanorods for the first time. The DNA functionalized nanorods were found to grow anisotropically to fill seed gaps up to 11-13 nm while limiting the
increase in diameter to ~ 3 nm. The metallization process demonstrated in this work is highly 
selective with very low background deposition, which is important for nanofabrication. In 
addition, the DNA origami template used in this study is sufficiently flexible and versatile to 
create a wide variety of 2-D metal nanostructures beyond those demonstrated in this work. The 
ability to controllably place metal nanorods at specific sites of a DNA template and to connect 
them while limiting the final width, as demonstrated in this study, represents important progress 
towards the realization of nanoscale devices based on self-assembled biological templates.
INITIAL SITE-SPECIFIC DEPOSITION OF METALLIC AND SEMICONDUCTING NANOELEMENTS ONTO A DNA ORIGAMI TEMPLATE

After successful demonstration of gold deposition onto DNA origami templates, we explored the deposition of semiconducting elements onto DNA for potential application in nanoelectronics. Specifically, controlled deposition of semiconductor tellurium (Te) on DNA templates is explored in this chapter.

Tellurium is a low band gap (0.35 eV) p-type semiconductor with promising photoconductive, thermoelectric, piezoelectric, photoelectric and catalytic properties for application in nanoelectronic devices. Importantly, Te nanostructures can be chemically transformed into a variety of chalcogenide materials like Ag₂Te, CdTe, ZnTe and PbTe. Chalcogenides have contrasting electrical and optical properties in their amorphous and crystalline phases which make them useful materials for application in nonvolatile memory and reprogrammable circuits.

There are different fabrication procedures available in the literature for making tellurium nanorods or nanowires. However, none have shown the fabrication of Te nanorods at the length scales of 25-50 nm similar to the dimensions of gold nanorods. The smaller dimensions provide flexibility to place nanorods onto a DNA template for fabrication of <100 nm nanodevices. In addition, challenges remain for controlled placement of tellurium nanorods onto DNA origami templates. For application as nanodevices, the semiconducting (switching)
elements need to be placed at specific locations within the DNA template. Based on our experience dealing with gold nanorods, if tellurium nanorods could be synthesized in a cationic surfactant such as CTAB, the nanorods could be attached to the DNA templates via local electric field effects similar to gold nanorods. Furthermore, based on our experience with site-specific attachment of gold nanorods to a DNA template (Chapter 5), we could fabricate a metal semiconductor junction on a DNA template by first attaching gold nanorods to specific parts of a DNA template and then attaching tellurium nanorods to the remaining sections of the template as shown in Figure 6-1.

Figure 6-1: (A) Design and fold the DNA origami template. Deposit the template on a SiO₂ surface. (B) Add gold nanorods to seed half the DNA template by complementary base pairing between A₁₀ sequence (green) on the origami and T₈ sequence (red) on the nanorods. (C) Add CTAB-functionalized tellurium nanorods to seed the remaining half of the template.

6.1 Synthesis of Tellurium Nanorods

We synthesized Te nanorods by optimizing a tellurium nanowire synthesis procedure demonstrated by Xi et al., who produced nanowires that were micron’s in length. The procedure was chosen based on its use of CTAB as a structure-directing agent and for its use of a safer reducing agent (ascorbic acid) compared to strong and unstable reducing agents such as hydrazine hydrate (N₂H₄·H₂O). Similar to gold nanorods, tellurium nanorods are grown in a surfactant solution of CTAB, which adheres preferentially to the side facets of the growing nanorods to slow the growth along the sides relative to the nanorod ends in order to yield high aspect ratio nanorods. The original synthesis process, however, was performed at 90°C compared
to the room-temperature synthesis of gold nanorods. Thus, we looked to slow down the growth of nanorods or nanowires by lowering the reaction temperature and the amount of starting reagents to limit (control) the size of the final nanorods.

The nanorods are formed based on the following processes:  

1. $\text{H}^+$ ions from the dissociation of $\text{C}_6\text{H}_8\text{O}_6$ react with $\text{TeO}_3^{2-}$ from $\text{Na}_2\text{TeO}_3$ to form white solid $\text{TeO}_2$ particles that are insoluble at room temperature,

2. as the solution is heated to $90^\circ\text{C}$ the white solid particles slowly dissolve in the reaction solution due to the slow reduction of $\text{Te}^{4+}$ ions into $\text{Te}$ by ascorbic acid and

3. after a few hours the solution changes to blue-gray color indicating the formation of Te nanorods by the slow reduction of Te ions onto existing Te nanoparticles. The reactions describing the processes (1) and (2) are as follows:

$$\text{TeO}_3^{2-} + 2\text{H}^+ \rightarrow \text{TeO}_2 + \text{H}_2\text{O} \quad (1)$$

$$\text{C}_6\text{H}_8\text{O}_6 + \text{TeO}_2 \rightarrow \text{C}_6\text{H}_4\text{O}_6 + \text{Te} + 2\text{H}_2\text{O} \quad (2)$$

In order to fabricate smaller Te nanorods, we first decided to lower the temperature of the reaction mixture in order to slow down the Te formation reaction. The reaction mixture was formed by mixing 0.1 g of surfactant CTAB (8 mM), 0.052 g of $\text{Na}_2\text{TeO}_3$ (6.25 mM) and 1 g of ascorbic acid (143 mM) in 40 mL of distilled water as described by Xi et al.  

The reaction mixture was slowly heated under vigorous stirring by ramping the temperature of a hot plate. Our experiments indicated the reaction mixture quickly changed to blue-grayish color as soon as the temperature was increased above 55°C. SEM inspection of the solution showed the presence of micron long tellurium nanorods. The blue-grayish Te solution was centrifuged at 10,000 rpm for 10 min to pellet the nanorods. From these experiments, it was confirmed that the reaction
temperature had to be below 55°C to slow the nanorod formation process; however, the precise temperature needed to make small nanorods was still unknown.

We next decided to decrease the amount of the starting reactants in the solution to reduce the size of the final nanorods by limiting the availability of the reagents. The solution mixture was kept below 55°C based on our earlier experiments. We reduced the amount of tellurium and ascorbic acid in the solution mixture by an order of magnitude from the values used by Xi et al. Briefly, 100 mg of ascorbic acid (14 mM), 40 mg of CTAB (3 mM) and 5 mg of Na₂TeO₃ (0.6 mM) were dissolved in 40 mL of distilled water. The mixture was heated to 53°C (while vigorously stirring at that temperature. After 2 hr of reaction, the whitish solution changed to a yellowish color. The reaction mixture was cooled and the solution was centrifuged at 10000 rpm for 10 min to pellet the Te nanorods. SEM inspection of the nanorods showed the presence of nanorods of about 50 nm length and 10 nm width. In addition, we were also able to attach Te nanorods to the linear DNA origami template as shown in Figure 6-2. The images clearly show end-to-end attachment of CTAB stabilized nanorods along the length of the DNA origami template as we expected.

Figure 6-2: (A) AFM image of a linear DNA origami template. (B) SEM image of Tellurium nanorod seeded DNA origami template. (C) Energy dispersive X ray analysis data confirming the nanorods are comprised of tellurium. Height scale for (A) is 10 nm.
This synthesis process, however, was not reliable for consistent synthesis of small Te nanorods. Often times, the solution mixture didn’t change color even after more than 10 hours of heating at 53°C. The analysis of this colorless mixture under SEM did not show the presence of nanorods. Increasing the reaction temperature to 60°C changed the solution color to blue-grayish within 5 min which indicated the presence of micron-long Te nanorods. We also studied the effect of the solution pH on the growth of nanorods. Since the oxidation of ascorbic acid is a pH dependent process (as discussed in Chapter 3), increasing the pH of the solution should slow the oxidation of ascorbic acid and hence, slow the rate of nanorod growth. The change in pH had no effect on our ability to synthesize small nanorods consistently: the solution color either remained unchanged (whitish color) for very long times when heated at 53°C or immediately changed color to blue-grayish solution when heated at temperatures above 60°C. Finally, decreasing the initial concentration of reagents further to limit the size of nanorods did not help to synthesize small Te nanorods.

While the synthesis was inconsistent, we were able to successfully synthesize nanorods of the desired size a few times. The following section describes our efforts to controllably place the Te nanorods onto DNA origami templates.

### 6.2 Site-specific Placement of Gold and Tellurium Nanorods onto a DNA Origami Template

Figure 6-1 shows the overall steps involved in the metallization of DNA origami with gold and tellurium nanomaterials. The linear DNA origami template previously used for conductivity experiments was employed to demonstrate the proof-of-concept for controlled deposition of multiple nanomaterials on DNA templates. The processes developed on this longer structure can be easily used to guide work on smaller templates. The origami template was designed with half
the length functionalized with staple strands that included A10 linkers on the 3’ ends of the strands. These dangling ends provided attachment sites for Au NRs functionalized with complementary T8 sequences as shown in Figure 2-3. Using the procedures described in earlier chapters, linear DNA origami templates were first deposited on an SiO2 surface. The samples were then seeded with DNA functionalized Au NRs to fill half the length of the linear DNA template. Figure 6-3 shows images obtained with AFM and SEM of the unseeded and gold nanorod-seeded DNA origami templates. As evident from Figure 6-3 the seeds attached selectively to half of the DNA length as designed, with very little background deposition. However, some nanorods were occasionally seen to attach to the opposite end of the origami template as discussed in Chapter 5.

Next, the samples were seeded with CTAB-stabilized Te NRs to deposit Te nanorods on the remaining portion of the DNA template that did not contain gold nanorods. The nanorods were suspended in 10X TAE-Mg2+ buffer solution (salt concentration = 0.74 M) for attachment to DNA origami templates as mentioned in Chapter 3. Figure 6-4 shows the SEM images obtained after Te NR seeding. As seen from the images, the nanorods appeared to attach to the remaining portion of the gold nanorod seeded DNA template. However, some nanorods also appeared to attach to the Au NR seeded portion of the template. The DNA template used for the experiments is 17 nm wide compared to 7 nm wide Au NRs, which likely provided enough space for some Te NRs to attach to the Au NR seeded portion of the DNA template. In addition, the non-specific interaction of the CTAB molecules with the DNA templates allowed Te attachment with any portion of the available DNA template. The SEM images also show almost no background deposition of Te nanorods (gray nanorods) consistent with the observations for CTAB-stabilized gold nanorods (Chapter 3). Figure 6-4 clearly show two distinct types of
nanorods (bright and dark nanorods) attached to a single DNA origami template. Thus, we were able to controllably direct metal and semiconducting elements to specific sites on a DNA template based on the nanorod attachment strategies reported in the previous chapters.

Figure 6-3: AFM images of (A) linear DNA origami template and (B) gold nanorod seeded linear DNA template. Height scale is 10 nm. (C) - (D) SEM images of nanorod seeded linear DNA origami.
Figure 6-4: SEM images of gold and tellurium nanomaterials assembled on linear DNA origami templates.

Although we were able to demonstrate proof of concept for site-specific deposition of Au and Te on a single DNA template, the lack of a consistent synthesis procedure for fabrication of Te nanorods in the 25-50 nm range affected further progress. The development of a consistent synthesis process together with the placement strategy demonstrated in this section could be used to make DNA based transistors in the future.
7 CONCLUSIONS AND FUTURE WORK

7.1 Conclusions

The metallization of self-assembled biological templates to make conductive elements for use in nanodevices was demonstrated in this work. Specifically, a new procedure was developed to improve the quality, size and conductivity of metallized DNA nanostructures. Although a few metallized DNA origami nanostructures have been reported, the ability to make thin, continuous structures that are electrically conductive still represents a formidable challenge. We have thus, developed a metallization process that uses gold nanorod seeds and their subsequent metallization to make DNA-templated gold nanowires as small as 10 nm in diameter. A key aspect of this work is the anisotropic metallization process, which enabled filling the seed gaps without significantly increasing the width of the final structure. In doing so, we were able to control seed growth preferentially in the length direction at a rate that is approximately four times the growth rate in the width direction, which enabled fabrication of narrow and continuous nanowires. The electrical resistivity was measured for nanowires ranging from 13 nm to 29 nm in width, which represent the smallest DNA-templated gold nanowires characterized to date. In spite of the narrow width of the wires, the measured resistivity values were as low as 8.9 \times 10^{-7} \text{\Omega-m}, significantly lower than almost all of the previously reported values for DNA-templated nanowires. Only larger nanowires (diameter \sim 79 \text{nm}) with lower resistivity values have been reported. In addition, the metallization process is highly selective with no background
deposition, which is important for fabricating DNA-based nanodevices. The directional plating demonstrated here greatly improves the morphology of the fabricated wires and provides control over the final width that is much improved relative to any of the metallization processes demonstrated previously.

After metallization of smaller DNA templates, we also showed that the metallization procedure based on gold nanorods can be easily used to metallize bigger DNA templates. Specifically, we successfully metallized self-assembling DNA origami nanotubes that were tens of micrometers long. The ability to metallize bigger DNA templates that self-assemble is important for making electrical connection between smaller DNA devices and the external world. In addition, we optimized the electron-beam lithography technique for characterizing the electrical properties of the gold nanowires. During the optimization process, we discovered the effect of heat on changing the morphology and possibly improving the conductivity of the DNA-templated metal deposits.

After successful metallization of entire DNA origami templates, we showed controllable placement of gold nanorods at specific sites on a DNA template. Specifically, we demonstrated site-specific placement of gold nanorods on a <100 nm DNA breadboard to make rectangular, square and T-shaped metallic structures. To attach gold nanorods site-specifically, we modified the surface of the gold nanorods with single-stranded DNA. The rods were then attached to DNA templates via complementary base-pairing between the DNA on the nanorods and the attachment strands engineered into the DNA “breadboard” template. The seed gaps were then anisotropically filled to make continuous metallic structures of different shapes. In doing so, we were able to demonstrate and characterize the anisotropic growth of DNA-stabilized nanorods for the first time. Another important aspect of this work is the first demonstration of site-specific
metallization of DNA origami templates to make continuous metal structures using gold nanorods as seeds. The diameter of the final metallized structures was as small as 10 nm which is the smallest width ever reported for the metal deposits on DNA origami templates.

Finally, we demonstrated controlled placement of metallic and semiconducting elements on a DNA template, which has important device implications. Specifically, gold nanorods were first placed on a portion of a linear DNA origami template and tellurium (semiconductor) nanorods were then placed on the remaining portion of the DNA template that did not have gold nanorods. To accomplish this, DNA modified gold nanorods were first attached via complementary binding to half the length of the ~400 nm long DNA template. CTAB stabilized tellurium nanorods were then attached to the remaining half of the template via interaction between the positively charged CTAB molecules around nanorods and the negatively charged DNA backbone. Overall, these results represent important progress towards the creation of nanoelectronic devices by molecularly directed placement of functional components onto self-assembled biological templates.

7.2 Future Work

There are several opportunities for future work that build on this study. These opportunities include: (1) controlling site-specific seeding to get higher yields of continuous metal nanostructures on the DNA template, (2) interfacing metal and semiconductor components with electrical continuity, (3) fabricating 3-D metal and semiconductor nanostructures with the use of DNA origami templates and (4) leveraging the processes developed in this work to build electrical components for nanoelectronic devices.

First, site-specific seeding of gold nanorods can be controlled with better precision for higher yields of the final metallized structures. It might be possible to improve the precision of
site-specific nanorod attachment demonstrated in this work by using longer\textsuperscript{65-66} capture strands compared to the 10 base pairs used for this work. If gold nanorods could be placed with higher precision at the DNA origami template and with seed gaps closer to ~11 nm (as discussed in Chapter 5), the anisotropic plating process would be used to get a much higher yield of connected metallic structures than achieved in this work.

Second, semiconductor and metal components should be integrated with electrical continuity to make a complete DNA-based electrical system. To do this, materials like carbon nanotubes\textsuperscript{23, 95} or semiconducting nanorods could be directly placed on the predesignated sites of a DNA template. One way to controllably place semiconducting materials onto DNA templates is to first deposit the metal onto specific sites on DNA and then attach the semiconducting material to the remaining parts of the template via local electric field effects as demonstrated in Chapter 6. It might also be possible to functionalize semiconductor nanorods with DNA oligonucleotides that enable site-specific attachment to the DNA template such that the order of deposition of nanomaterials (metal or semiconductor) does not matter. Another possibility might be to attach metal and semiconductor materials to two different DNA templates and then combine the two templates via nucleic acid base pairing mechanism. In order to do this, semiconductor materials could be attached to one type of template in solution and then combined with another template that has been metallized on the surface (as demonstrated throughout this study). Finally, the polymer assisted annealing process demonstrated in Chapter 4 might be utilized to make electrical contact with metal and semiconducting materials, and the EBL process could be used to characterize the metal-semiconductor junction properties.

Third, DNA origami templates can be used to fabricate 3-D metal and semiconducting nanostructures. One way to achieve this is to fill the metal inside of a DNA origami mold by first
placing metal nanoparticle seeds at the bottom/end of a DNA origami mold and using the 
electroless deposition process demonstrated in this work to grow the seeds inside the mold. The 
metal filling inside the DNA mold could then be replaced with a semiconductor material via the 
galvanic displacement method.6

Finally, the metallization processes demonstrated here could be used to develop electrical 
components such as transistors and even basic logic gates for use in nanoelectronic systems of 
the future. The metal/semiconductor deposition techniques developed in this work together with 
DNA origami for fabricating nanostructures could provide a much simpler, faster and cost-
effective approach to fabricate nanodevices and systems than what is possible with the current 
fabrication methods. Moreover, our fabrication techniques do not require expensive patterning 
tools or specialized facilities. Thus, we have developed techniques that could potentially enable 
realization of electronic devices based on biological molecules.
REFERENCES


Supporting Information

Anisotropic Electroless Deposition on DNA Origami Templates to Form Small-Diameter Conductive Nanowires

Figure 9-1: SEM images of DNA origami seeded with (A) Mg\textsuperscript{2+}, (B) Cu\textsuperscript{2+} and (C) Ca\textsuperscript{2+} solutions (concentration = 125 mM). Scale bar is 200 nm.

Figure 9-2: SEM images of DNA origami seeded for (A) 10 min. (B) 1 hr and (C) 3hr. Scale bar is 100 nm.
Figure 9-3: SEM images of DNA origami plated with double the reagent concentration ([Au] = 2 mM, [Ag] = 8 mM, [CTAB] = 200 mM, [Ascorbic acid] = 157.6 mM). (A) 20 min plating and (B) 40 min plating. Scale bar is 500 nm. Inset shows a single linear DNA wire.

Figure 9-4: SEM images of gold nanorod seeded linear DNA template plated with a solution containing 4.0 times more moles of ascorbic acid than gold ions. Samples plated for (A) 20 min and (B) 30 min. Scale bar is 200 nm.
Figure 9-5: SEM images of linear DNA origami seeded with gold nanorods and plated with the addition of iodine in the plating solution. Iodine concentrations of (A) 0 µM (B) 0.5 µM and (C) 5 µM. All other conditions for electroless plating were kept the same. Scale bar is 200 nm.

Figure 9-6: SEM images of gold metallized linear DNA origami nanowires between electrode pairs. Scale bars are 100 nm.
Figure 9-7: SEM images of gold metallized linear DNA origami nanowires with very high resistances. Scale bars are 100 nm.

Figure 9-8: SEM images of gold nanorod nanowires (A) before and (B) after annealing without the resist at 200 °C. The nanowires in (A) were plated according to the optimized plating solutions mentioned in the text. Scale bar is 100 nm.
Supporting Information

Anisotropic Plating of Gold Nanorods Placed Site-specifically on a DNA Origami Breadboard to Form Continuous Nano-wireframe Shapes

Figure 10-1: SEM images of DNA origami templates seeded with 40:1 ratio of gold nanorods to template to fabricate (A) rectangular, (B) square and (C) T shaped seeded structures. The templates were designed with seven capture strands per site along the length of the template. Scale bars are 500 nm.
Figure 10-2: SEM images of DNA origami templates seeded with a 40:1 ratio of gold nanorods to template to fabricate (A) rectangular, (B) square and (C) T shaped seeded structures. The templates were designed with ten or eleven capture strands spread across three helices per site along the length of the template. Scale bars are 500 nm.

Figure 10-3: SEM images of DNA origami templates after nanorod seeding to make (A) rectangular, (B) square and (C) T shapes. The templates were designed with fourteen capture strands spread across four helices per site along the length of the template. Scale bars are 200 nm.
Figure 10-4: SEM images of DNA origami templates after electroless plating in CTAB free growth solution.

Figure 10-5: SEM images of (A) DNA coated gold nanorods and (B) CTAB coated gold nanorods after growth in silver free electroless plating solution. Scale bars are 500 nm.

DNA staple strand sequences

**DNA Breadboard origami sequence**

Single-stranded M13mp18 was used as the scaffold for making the DNA template. To create rectangle, square and T shaped seeded structures, staple strands in the desired positions were modified with the addition of ten adenines to the 3’ end. These modified staple strands
were used in folding the DNA origami structure. Blue sequences represent the modified sequences.

**DNA sequences for the “Rectangle” design**

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GAGGATCCCCGACCGTCTATCA
CAGTGCATCGTAAATCATGTCATAGAAGACGTGAAAAAAAAAAA
TTTCACAAAATGTGTACCTGCTGTGTCACGACAAAAA
CTGCAAGGATGCAGGAGCGGAAAGCATCAAAAGAAAA
GCATTACGATGCTAATGAGTACGTGTCTCC
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CAAAGCGCAACCTGTGTCGACGTGAGAGAAAAAAAAAAA
TTCCGCCAACGCAACCGGGGAGGGGCAACAGGAAAA
GACTCTAACGTCGAAAAGGCGAAAAGGTACCGAAAAAAAAAAA
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GAAATCGGCAAATACCCCTTAATAGGCTGAAAAAAA
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DNA sequences for the “square” design

GAGGATCCCCGACCCTCATATCA
CAGGTGCCATCGTAATCACGTGCCAGCTAATGACG
CAGGCTGATCGTAATAGGTGCAATGACG
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CTGCAAGGCTCGAGCGGCTCTTCGCTCAATGAGCAACG
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115
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TTCATCAAAATTTTAGATGCTATTTCTGACCT
GAAAGCGTAAGAATAACGTG
DNA sequences for the “T” design

GAGGATCCCCGACCCTATCATCA
CAGTGCATCAGTCATATCATGTAGATCAGAGCAGGGTGA
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GTGGTTTTTTCTTTCACAGTGAGACGGGAGCGGT
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