Fabrication and Characterization of DNA Templated Electronic Nanomaterials and Their Directed Placement by Self-Assembly of Block Copolymers

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Fabrication and Characterization of DNA Templated Electronic Nanomaterials and Their Directed Placement by Self-Assembly of Block Copolymers

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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

Fabrication and Characterization of DNA-Templated Electronic Nanomaterials and Their Directed Placement by Self-Assembly of Block Copolymers

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Doctor of Philosophy

Bottom-up self-assembly has the potential to fabricate nanostructures with advanced electrical features. DNA templates have been used to enable such self-assembling methods due to their versatility and compatibility with various nanomaterials. This dissertation describes research to advance several different steps of biotemplated nanofabrication, from DNA assembly to characterization. I assembled different nanomaterials including surfactant-coated Au nanorods, DNA-linked Au nanorods and Pd nanoparticles on DNA nanotubes ~10 µm long, and on ~400 nm long bar-shaped DNA origami templates. I optimized seeding by changing the surfactant and magnesium ion concentrations in the seeding solution. After successful seeding, I performed electroless plating on those nanostructures to fabricate continuous nanowires. Using the four-point probe technique, I performed resistivity measurements for Au nanowires on DNA nanotubes and obtained values between 9.3 x 10^{-6} and 1.2 x 10^{-3} Ωm. Finally, I demonstrated the directed placement of DNA origami using block copolymer self-assembly. I created a gold nanodot array using block copolymer patterning and metal evaporation followed by lift-off. Then, I used different ligand groups and DNA hybridization to attach DNA origami to the nanodots. The DNA hybridization approach showed greater DNA attachment to Au nanodots than localization by electrostatic interaction. These results represent vital progress in understanding DNA-templated components, nanomaterials, and block copolymer nanolithography. The work in this dissertation shows potential for creating DNA-templated nanodevices and their placement in an ordered array in future nanoelectronics. Each of the described materials and techniques further has potential for addressing the need for increased complexity and integration for future applications.

Keywords: block copolymer, DNA self-assembly, electrical characterization, electroless plating, metal nanorods, metal nanowires
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CHAPTER 1: INTRODUCTION

1.1 NANOTECHNOLOGY

Nanotechnology has attracted remarkable recognition because of the distinctive crystallinity, surfaces, particle sizes and structures of nanomaterials and nanodevices. Nanomaterials have influenced energy saving products, drug delivery systems, nanoelectronics and sensor devices. Richard Feynman, the father of modern nanotechnology, introduced the concept of nanotechnology in 1959 during his speech at the American Physical Society’s annual meeting, entitled “There is Plenty of Room at the Bottom.” Science, technology and engineering at the nanoscale as well as the capability to manipulate and see individual atoms are parts of “nanotechnology”.

1.2 NANOMATERIALS

Materials are defined as nanomaterials if the size, or one of their dimensions, is between 1 and 100 nm. Nanomaterials are at or near the atomic or molecular scale, containing properties different from bulk materials. For perspective, one nanometer is the size of about five silicon atoms or ten hydrogen atoms lined up. As the size of materials decreases and approaches the size of atoms, the material functions differ due to quantum mechanics. Thus, the properties of nanomaterials, such as optical absorbance or emission, surface area, and electrical conductivity, are different from those of the respective bulk materials. Nanomaterials are classified into three categories according to their structures: 1-D, 2-D, and 3-D. Elongated nanostructures, such as nanorods, nanowires, nanofibers, and nanotubes, are considered 1-D materials. Nanorods typically have smaller aspect ratios than nanowires, which have an aspect
ratio sufficiently large that they have some physical flexibility. Electrical conductance is a property of nanowires, whereas nanorods are not necessarily conductive. Nanotubes are similar to nanorods or nanowires but have a hollow inside. Nanoplates and nanosheets are 2-D structures, whereas nanoparticles, fullerenes, and quantum dots are typically 3-D materials. Various forms of nanoparticles include clusters, crystals, and powders, with nanometer-scale dimensions and whose surface area to volume ratio is very high.

1.3 NANOFABRICATION APPROACHES

Nanofabrication methods are often sorted into two major categories: top-down and bottom-up.9 The last decade has seen the development of various nanofabrication techniques, including top-down, bottom-up, and a combination of the two, to satisfy the demand for nanotechnology. Top-down methods are widely used, breaking down bulk materials to produce nanostructures. Top-down fabrication often uses photolithography, which involves polymer resist coating, developing and exposing to pattern structures in silicon-based technology.15 Top-down fabrication also may involve etching, milling, and sputtering. Widely used top-down fabrication requires an extremely clean environment and large, expensive instruments to control size, shape, and location of features, and type of material patterned on a surface.9

In contrast, bottom-up approaches build complex, multifunctional nanostructures by controlling the assembly of molecules or atoms, based on their interactions.16 Block copolymer, DNA, protein and RNA self-assembly processes are examples of bottom-up methods17. Bottom-up nanofabrication can potentially address a key challenge of top-down approaches: the fabrication cost. Nanofabrication can also apply hybrid techniques, which integrate both top-down and bottom-up approaches.18 Hybrid approaches such as deep-UV lithography and controlled lateral
epitaxial growth promise to overcome drawbacks from both top-down and bottom-up methods, making nanofabrication more productive and efficient, and leading to higher throughput.\textsuperscript{19}

1.4 SELF-ASSEMBLY

Self-assembly is a technique that allows for the spontaneous formation of new structures with interesting structural, physical, and electronic properties. Bottom-up processes often utilize self-assembly, which causes atoms or molecules to assemble into an organized nanoscale structure.\textsuperscript{20} Self-assembly adds several unique features to existing chemical and physical methods for synthesizing and processing functional materials. For example, self-assembly makes materials manufacturing compact, creating 2-D and 3-D structures of only several nanometers in length.\textsuperscript{21} The utilization of self-assembly techniques may also open new possibilities in scaling down electronic, photonic, and sensor devices to their ultimate limits.\textsuperscript{20} However, high-yield bottom-up fabrication processes have yet to be developed, preventing such self-assembled nanostructures from being integrated into current devices. The self-assembly of block copolymers\textsuperscript{22} and DNA templates\textsuperscript{23} is vital to advancing self-assembled soft-matter systems. I discuss these two systems in detail in sections 1.5 and 1.6.

1.5 DNA TEMPLATES

The specific binding of DNA base pairs (\textbf{Figure 1.1}) provides the chemical foundation for genetics. This important molecular identification capability is used in nanotechnology to directly assemble highly structured materials with specific nanoscale features in 2-D and 3-D. In the 1980s, Nadrian Seeman first proposed using DNA as a nanoscale polymer extending in 2-D instead of simple 1-D double helices.\textsuperscript{24} This work introduced the concept of DNA nanotechnology, which allows for synthesizing well-defined nanostructures, generating vast numbers of copies of a product in a single experiment. DNA nanostructures enable programmed positioning of nanoscale
features with high spatial resolution, which is promising for constructs such as nanowires, nanodevices, plasmonics, and sensors, as described further in section 1.7.

![Chemical structure of DNA](image)

**Figure 1.1** Chemical structure of DNA
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### 1.5.1 DNA Origami

DNA origami uses a long single-stranded DNA (often M13mp18, a 7249 nucleotide virus\(^25\) that infects bacteria) that is folded into desired structures in the presence of a specific set of shorter staple DNA strands (ranging from 22 to 32 nucleotides), as shown in **Figure 1.2**. The complexity of the origami shape determines how many different staple strands are required, with typical counts being around 200. Rothemund\(^26\) first demonstrated the DNA origami technique for folding DNA, creating nearly arbitrary 2-D configurations by exploiting the self-assembly properties of DNA. Since then, DNA origami has generated 2-D and 3-D DNA nanostructures with many applications.
DNA origami has potential for application in drug delivery, bioimaging, biophysics, computation, and molecular mechanics. Valuable features of DNA origami include biodegradability, structural stability, and thermodynamic and kinetic programmability. All these features provide the potential to integrate multiple functions into one structure. In addition, the ability to modify individual bases of a DNA strand offers the capability of addressing functions at the molecular level.

![DNA origami assembly](image)

**Figure 1.2** DNA origami assembly.

1.5.2 DNA Nanotubes

DNA nanotubes are another class of DNA templates for fabricating valuable structures. DNA nanotubes originate from single-stranded or multi-crossover tiles, DNA origami, or multiple rungs. These structures link together in a cylinder but with a vacant inner space, creating the shape of a tube. DNA nanotubes can have either a set or dynamic length, and can reach lengths of up to 10 µm. Aside from use as a template for nanomaterials, DNA nanotubes can have applications in drug delivery, multi-enzyme bioreactors, and membrane channels.

1.6 ALIGNMENT

Nanoscale surface alignment is important for nanostructures because random deposition results in poor yields due to the deposition of objects on top of one another. If DNA is aligned in an ordered array, it can make possible characterization and large-scale production. Usually, DNA is stored in buffer solutions which have divalent cations. Therefore, negatively charged DNA will attract electrostatically through salt bridges between DNA and the cations to an oxidized Si surface.
having silanoate groups, resulting in random placement and orientation. The precise alignment and placement of DNA are vital to developing dense and ordered arrays of functional DNA devices on a large scale. Combining bottom-up self-assembly for creating the desired nanostructures and top-down lithography for providing precise locations for DNA to bind on a surface is a useful strategy to decrease the cost of the overall process and obtain precise placement, illustrated as follows. Shaali et al. deposited DNA origami modified by hydrophobic anchors with a surface coverage of ~80% sites in a Teflon negative electron beam (e-beam) resist pattern. Moreover, Shetty et al. used nanosphere lithography to place DNA origami on surfaces non-specifically, which could facilitate the placement of 2D and 3D DNA nanostructures for single-molecule techniques. Gopinath et al. placed DNA origami onto target sites created by e-beam patterning, with potential for use in rapidly prototyping hybrid nanophotonic devices. Brassat et al. evaluated the adsorption of DNA origami triangles in nanoholes fabricated by nanosphere lithography in thin Au films on Si wafers. They studied the impact of buffer conditions on DNA origami adsorption in the nanohole arrays and observed partial adsorption of one or more DNA origami inside the nanoholes with a part crossing the boundary to the Au film, even under optimized conditions. Even though these fabrication methods are functional, they involve expensive instruments, a lengthy process time, and a lack of ability to obtain high yields of desired shapes or locations. Therefore, researchers need to overcome issues regarding the cost, reliability and complications of patterning.

1.6.1 Block Copolymer Patterning

Block copolymers (BCPs) have gained attention in thin-film nanolithography because they spontaneously form highly uniform nanostructures at the resolution scale of current lithographic tools. BCPs have two or more covalently linked distinct polymer chains with high interaction energy. As illustrated in the phase diagram in Figure 1.3 (a), these individual polymer chains
undergo microphase separation to lower the free energy of the system, spontaneously organizing themselves into ordered morphologies with nanoscale dimensions such as spheres, lamellae, gyroids, and cylinders (see Figure 1.3 (b) and (c)). BCP lithography uses this phase separation phenomena to achieve different patterns in thin films on a surface. BCP self-assembly allows the patterning of uniform, hexagonally packed nanofeature arrays on a surface. BCP lithography is used for applications in making nanocatalysts or optical devices, and doing surface-enhanced Raman spectroscopy. Pearson et al. showed directed assembly of DNA origami on chemically functionalized, BCP-generated Au nanoparticles (NPs), with stable, selective attachment through base-pairing of DNA origami with patterned ssDNA on the Au NPs surface. Further control of DNA origami placement might be achieved by using multiple complementary regions with different base sequences. BCP nanopatterns can also be converted into ordered arrays of metal and inorganic materials by combining etching, metal deposition and lift-off. BCP patterning has shown progress, but some areas still need to be developed including moving beyond planar geometries.

Figure 1.3 BCP thin films. (a) Phase diagram of a BCP made from two polymer chains. (b) BCP phase changes according to volume fraction. (c) SEM images of the different BCP phases. Reprinted with permission from ACS. Copyright 2022
1.7 DNA-BASED NANOMATERIAL ATTACHMENT

DNA is only electrically conductive for a few nanometers, so functionalization is necessary to use DNA as a conductive nanowire. Metallization of DNA structures involves binding nanomaterials (seeds), reducing the seeds, then growing the nanomaterials. Metallization has been done in many ways. Over the past decades, there has been significant progress in developing controlled assembly of metallized nanowires on DNA. The first DNA metallization was done by Braun et al., who deposited silver on λ-DNA. Many researchers continued to experiment and used different metals, such as palladium, platinum, nickel, copper and gold. DNA structures were also used as molds to control the growth of metal nanoparticles, which involves attaching nanoparticle seeds inside a mold and allowing the seeds to grow, resulting in homogenous metallic nanostructures of controlled length and shape.

1.7.1 Nanomaterials on DNA Origami

Creating nanostructures with DNA origami as a template has been extensively studied. Figure 1.4 shows nanomaterial attachment on DNA origami templates. First, DNA origami deposits on a surface and then incorporates nanomaterials; to fabricate conductive structures, usually a plating technique is involved in connecting seeds. DNA origami-templated metal structures are used in plasmonic and electronic fields. DNA bundles decorated with nanoparticles have been reported for plasmonic devices as they are capable of selective field enhancement via plasmonic resonance. Aryal et al. decorated DNA origami with metal and semiconducting nanorods to create metal-semiconductor junctions that could be useful in nanoelectronics. Similar to 2-D, 3-D DNA origami templates can be combined with other nanomaterials. Ye et al. used a 3-D DNA origami mold to attach a Au NP inside and grow it to make a nanowire.
1.7.2 Nanomaterials on DNA Nanotubes

DNA nanotubes have been applied as templates similar to DNA origami in creating nanostructures. Teschome et al.\textsuperscript{62} demonstrated Au NP alignment on DNA origami nanotubes fabricated with six-helix bundles. More rigid DNA origami nanotube structures would be beneficial in minimizing deformation of the completed structures in future applications. Particularly, applications that depend on arrays of parallel NP chains or nanowires over large surface areas, such as for transparent, conductive electrodes, may find this technique useful. Gür et al.\textsuperscript{63} reported a method for self-assembling plasmonic waveguides on DNA origami nanotubes. They also used six parallel interconnected helical bundles of DNA origami as templates. The deposition of DNA nanotubes decorated with Au NPs onto substrates still needs better surface placement and alignment, as aggregated structures formed on the surface. Additionally, analysis of electrical properties would move forward the field.

1.8 ELECTROLESS PLATING

Electroless plating is a technique of selectively plating materials without the use of an external power supply. Copper,\textsuperscript{64} gold,\textsuperscript{65} nickel,\textsuperscript{64} palladium\textsuperscript{66} and cobalt\textsuperscript{67} have all been used in electroless plating. An electroless plating solution contains a source of metal ions, a reducing agent, a stabilizer and suitable complexing agents. The reducing agent contributes electrons needed to reduce the metal ions. Stabilizers prevent reactions that result in the spontaneous decomposition
Complexing agents are typically organic acids or salts which form stable complexes with metal ions, thus enhancing the selectivity of the plating reaction. Both the oxidation and reduction reactions occur on the same surface, forming a mixed potential at steady state. For metal deposition to occur, the reduction potential must be high, and the reduced metal should provide a catalytic surface suitable for extra plating. Liu et al. successfully demonstrated electroless gold plating on a branched DNA origami template. Additionally, Geng et al. showed electroless deposition of palladium on \( \lambda \)-DNA and gold on DNA origami templates. Our group created C-shaped nanowires with Au nanorod seeding and electroless plating. More recently, Bayrak et al. reported Au nanowire fabrication on DNA origami using Au NPs and electroless plating that resulted in similar 150 nm long nanostructures. The granularity of nanowires still needs to be improved to decrease the resistivity, by improving the quality of both seeding and plating.

### 1.9 Electrical Characterization

Electrical characterization is essential to demonstrate the continuity of fabricated nanowires. One method to connect to nanowires is electron-beam lithography (EBL), which uses a focused beam of electrons to pattern desired shapes in a resist, resulting in a pattern after removing the exposed or non-exposed areas, similar to photolithography but without a mask. Another method to connect to nanowires is called electron beam induced deposition (EBID). EBID is performed in a scanning electron microscope (SEM), using the electron beam to locally decompose a precursor gas, depositing metal atoms in predetermined patterns as shown schematically in Figure 1.5. The precursor gas consists of an organometallic compound that, when heated, becomes a gas; with an incorporated injection system, the gas is released into the SEM near the desired writing location. As the electron beam raster scans in a desired pattern, the bonds of the organometallic gas are broken, leaving reduced metal atoms behind on the surface. Both
EBL and EBID are useful in patterning geometries in a desired place where standard lithography cannot provide resolution at the nanoscale level. They also require high energy and high vacuum and write serially, making the process expensive and slow.

**Figure 1.5** Diagram of electron beam induced deposition. An organometallic precursor gas is injected into the electron microscope chamber near the surface. The electron beam interacts with the gas molecules, breaking bonds, releasing the volatile organic metal, and depositing reduced metal.

Resistance measurements can be obtained in several ways. The two-point probe measurement technique is widely used for resistance measurements. However, four-point probe measurement removes the contact resistance contributions, giving a more accurate measurement. The first two terminal I-V measurements on silver metallized on DNA bound to Au electrodes through S-Au bonds were done by Braun et al. Atomic force microscopy (AFM) has also been used to measure conductivity. A recent measurement using conductive AFM was done by Stern et al. on DNA-based Au with a width of 13 nm and a length greater than 360 nm. In the literature, the resistance values for metal nanowires on DNA are widely spread from Ω to GΩ values. Resistance values can indicate variability of the deposited metal; grainy structures may lead to higher resistance values, and corrosion of metal particles may also affect the resistance.
1.10 OVERVIEW OF THE DISSERTATION

This dissertation describes several nanofabrication-based projects, split into three research-focused chapters. Chapter 2 shows DNA nanotube metallization and electrical characterization of the assembled nanowires. I describe two types of seeding, with Au NRs and Pd NPs, and use gold electroless plating to form 1-2 µm long nanowires. In Chapter 3, I describe my work to improve seeding and plating of DNA origami. This work supported a collaborative project that allowed thermal annealing studies on seeded and plated nanowires and their electrical characterization. In Chapter 4, I describe methods to assemble DNA origami on a Au pattern created with BCP self-assembly. In brief, I assemble a BCP vertical cylindrical nanoarray and etch away the cylindrical material. Then, I use metal evaporation and lift-off to form Au dots. I functionalize Au dots to attract and attach DNA origami using different thiol-based linkers and electrostatic interactions or DNA hybridization to connect a thiolated group on the DNA origami to Au dots. Chapter 5 summarizes the conclusions from the above three chapters. I also provide encouraging future directions for each project for further advancement.

1.11 REFERENCES


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CHAPTER 2: SEEDING, PLATING AND ELECTRICAL CHARACTERIZATION OF GOLD NANOWIRES FORMED ON SELF-ASSEMBLED DNA NANOTUBES*

2.1 ABSTRACT

Self-assembly nanofabrication is increasingly appealing in complex nanostructures, as it requires fewer materials and has potential to reduce feature sizes. The use of DNA to control nanoscale and microscale features is promising but not fully developed. In this chapter, I studied self-assembled DNA nanotubes to fabricate gold nanowires for use as interconnects in future nanoelectronic devices. I evaluated two approaches for seeding, gold and palladium, both using gold electroless plating to connect the seeds. These gold nanowires are characterized electrically utilizing electron beam induced deposition of tungsten and four-point probe techniques. Measured resistivity values for 15 successfully studied wires are between $9.3 \times 10^{-6}$ and $1.2 \times 10^{-3}$ $\Omega \text{m}$. My work yields new insights into reproducible formation and characterization of metal nanowires on DNA nanotubes, making them promising templates for future nanowires in complex electronic circuitry.

2.2 INTRODUCTION

Nanofabrication, which is used to construct structures and devices with minimum dimensions below 100 nm, is having a significant impact on diverse areas, such as electronics, biomedicine, materials and so forth. Most current nanofabrication relies on top-down technology, which is highly automated but also expensive and requires complicated instrumentation. Thus, nanofabrication could benefit from alternative techniques, including bottom-up approaches.

wherein chemical or physical forces operating at the nanoscale assemble smaller parts into larger structures.\(^5\) Bottom-up methods produce less waste, utilize less expensive tools and offer straightforward scaling up compared to top-down nanofabrication.\(^1\) Examples of bottom-up techniques include molecular self-assembly and atomic layer deposition.\(^6\)

DNA is one of the best studied bottom-up nanofabrication systems. The concept of using DNA as a nanoscale building material, though prevalent in Nature, was first explored experimentally by Nadrian Seeman in the 1980s.\(^7\) In 2006, Rothemund demonstrated that DNA nanostructures with well-defined shapes could be constructed by repeated folding of a long, single-stranded DNA (scaffold) with hundreds of short, synthetic, single-stranded (staple) DNAs to create 2-D objects about 100 nanometer in size called DNA origami.\(^8\) Self-assembled DNA nanotubes,\(^9\) formed using tiles, DNA origami or multiple rungs, can be created either with or without defined lengths. DNA nanotubes are further versatile because they can be functionalized using their designed DNA sequence\(^10\) and have high surface-to-volume ratios to build large structures.\(^11\) DNA nanotubes have emerged in DNA nanotechnology\(^12\) as a means for creating varied structures that can be used, for example, as templates\(^13\) or in cargo delivery\(^14\). These DNA nanotubes are advantageously more rigid, highly charged for electrostatic material localization and resistant to enzymatic degradation than simple single- or double-stranded DNA.

Controlled placement of DNA nanostructures on surfaces at designed locations remains a challenging problem. Gopinath et al.\(^15\) achieved the electrostatic self-assembly of DNA origami onto lithographically defined binding sites on Si/SiO\(_2\) but this approach still required high resolution photolithography and might not be able to orient structures like DNA nanotubes into contiguous circuit arrangements. Shetty et al.\(^16\) demonstrated a cleanroom-free DNA origami placement method using nanosphere lithography with a maximum yield of 74%. Rothemund and
coworkers also showed DNA origami alignment on SiO₂ with tight angular precision by engineering the energy landscape of DNA origami shapes on binding sites. DNA nanotubes can be made to grow from specific locations, allowing control of nanotube positioning and reducing defects. These DNA nanotubes can also join end to end to form stable connections with different separation distances and relative orientation. The ability to connect between specified surface locations could be especially powerful for self-assembling nanoelectronics and crossed nanowire memory storage.

DNA is not electrically conductive over hundreds of nanometers, so functionalization is necessary for creating conductive wires. Metallization of DNA structures typically involves binding of seed nanomaterials, followed by electrochemical growth. The first nanoscale DNA metallization was done by Braun et al., who performed silver ion metallization on λ-DNA. Subsequent work has expanded to different metals including palladium, platinum, nickel, copper and gold. DNA nanostructures were also used as molds to control the growth of finite-sized metal nanostructures. Furthermore, finite structures created by selective metallization schemes and DNA structure-assisted lithography showed promising progress for nanoscale DNA-assisted metallization. The best way to confirm successful metallization is to measure nanowire resistance or conductance. Two-terminal current-voltage (I-V) measurements on a DNA templated silver nanowire yielded a resistance of 200 Ω, corresponding to a resistivity of 2.4 x 10⁻⁶ Ωm. Park et al. created 1-D silver nanowires using three-helix bundle DNA tiles and performed electrical characterization on ~400 nm long wires. The I-V curves showed mostly linear behavior and gave resistances of 1.42 kΩ and 1.21 kΩ for two separate nanowire sections with 430 and 320 nm electrode gap lengths measured at 0.1 V, corresponding to bulk resistivities of 2.24 x 10⁻⁶ and 2.57 x 10⁻⁶ Ωm, respectively. Liu et al. demonstrated the use of self-assembled
DNA nanotubes to form conductive silver nanowires; they reported two-terminal I-V curves with mostly ohmic behavior for electrode gap distances of 180, 80 and 100 nm, with resistances of 2.80, 2.35 and 2.82 kΩ at 0.1 V, corresponding to resistivities of $\sim 1.4 \times 10^{-5}$, $3.2 \times 10^{-5}$ and $3.1 \times 10^{-5}$ Ωm, respectively. The resistivity of polycrystalline silver is more than 140 times smaller ($1.6 \times 10^{-8}$ Ωm) than any of the above-mentioned silver nanowires on DNA nanotubes. These previous successful I-V measurements were also done for only three or fewer nanowires and at a specific voltage. Thus, improvements are needed for producing long, metallized nanowires and obtaining multiple I-V measurements.

This chapter presents a method for metallizing DNA nanotubes to form conductive nanowires that are much longer (up to $\sim 2 \, \mu$m) than previously reported. **Figure 2.1** shows a schematic overview of the sample preparation and characterization process. In panel (a) assembly of DNA nanotubes from tiles consisting of five DNA strands is depicted and **Scheme 2.1** shows the tile sequences. Next, those DNA nanotubes are deposited onto an oxidized Si wafer (**Figure 2.1** (b)). **Figure 2.1** (c), (d) shows seeding, either with Au nanorods or Pd ions that assemble on the DNA nanotubes. As shown in **Figure 2.1** (e) the plating of DNA nanotubes with Au results in nanowires, that are then probed electrically by four-point measurement in **Figure 2.1** (f). This work is novel because I report the first successful four-point probe measurements on 15 different metallized DNA nanotube nanowires. Nearly 40% of the nanowires connected by electron beam induced deposition (EBID) yielded four-point I-V results with scanning between $-1$ and $+1$ V. The DNA nanotubes presented in this chapter have the ability to self-assemble from one end to another, which in the future should allow reliable fabrication of interconnects in nanoscale electronic and sensing devices.
Figure 2.1 Schematic overview. (a) DNA nanotube assembly. (b) DNA nanotubes on an oxidized Si wafer. DNA nanotubes seeded with (c) Au or (d) Pd. (e) Plated DNA nanotubes. (f) I-V measurement.

6nt SEs nanotube monomer sequences for SEs tiles without PEG modification:

SEs_1: TCAGTGACAGCCGTTTCTGGGAGCGGTGGAGCAACT
SEs_2: CCAGACAGCTTTCGTGGCTCACTCC
SEs_3-Cy3: /Cy3/CCAGAACGGCTGTGGCTAAACAGTAACCGAACCAACGCT
SEs_4: GTCTGGTAGAGACCACTGAGAGGTA
SEs_5: CGATGACCTGTCTGGTCTGCTTACCTGCTTACCGCTCT
/Cy3/ denotes Cy3 fluorophore covalently attached to the 5’ end of DNA

Figure 2.2 DNA nanotube tile sequences
2.3 EXPERIMENTAL SECTION

2.3.1 Chemicals and Materials

Cetyl trimethylammonium bromide (CTAB) (H5882, 98%), hydrogen tetrachloroaurate(III) (HAuCl₄), sodium borohydride (NaBH₄), palladium chloride (PdCl₂), dimethylamine borane (DMAB) complex and 1-methyl-2-pyrrolidinone (NMP) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ethylenediaminetetraacetic acid (EDTA) was obtained from Life Technologies (Carlsbad, CA, USA). Tris(hydroxymethyl) aminomethane (Tris base) and ascorbic acid were obtained from Fisher Scientific (Fair Lawn, NJ, USA). Acetic acid, hydrochloric acid, magnesium chloride, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic (HEPES) acid and magnesium acetate ((MgAc)₂·4H₂O) were obtained from EMD Chemicals (Gibbstown, NJ, USA). Silver nitrate was purchased from Mallinckrodt Chemicals (Philipsburg, NJ, USA). Ammonium chloride was obtained from EM Science (Merck KGaA, Darmstadt, Germany). Gold enhance EM solution was obtained from Nanoprobes (Yaphank, NY, USA). For solution preparation and sample rinsing, water (18.3 MΩ) was generated with a Barnstead EASYpure UV/UF purification system (Dubuque, IA, USA). TAE-Mg²⁺ buffer (10X, pH 8.3) was prepared using 400 mM Tris base, 200 mM acetic acid, 10 mM EDTA and 125 mM (MgAc)₂·4H₂O.

2.3.2 DNA Nanotube Deposition

DNA nanotube structures were designed and assembled as described by Li and Schulman;³⁷ Figure 2.2 shows the tile sequences. In summary, during self-assembly, nanotube tiles hybridized together to make a lattice, which then generated the hollow DNA nanotubes. After formation the DNA nanotubes were deposited onto plasma-cleaned (Harrick Plasma Asher PDC-32G, 3 min, 18 W), oxidized Si wafers (Polishing Co. of America, Santa Clara, CA, USA) sectioned into 1.5 x 1.5 cm² pieces by adding 5 μL of DNA nanotube solution to the central region
and leaving it to adsorb for 15 min in a humidified container to prevent water evaporation. The wafers were then rinsed gently with water and the surfaces were dried under flowing filtered air.

2.3.3 Seeding Au Nanorods on DNA Nanotubes

Gold nanorod synthesis was performed in an adaptation of a published method.\textsuperscript{38, 39} To obtain larger-diameter nanorods, sodium borohydride concentration was decreased tenfold, as a smaller number of seeds in the Au nanorod growth solution was expected to lead to the production of larger nanorods. The seeding solution (30 µL, Au nanorods in 10X TAE-Mg\textsuperscript{2+} buffer) was pipetted onto the surface with DNA nanotubes and left to interact for 60 min at room temperature in a humidified container. The samples were rinsed with 4–6 drops of water to clean the surface and to remove nanorods that were not bound to the DNA nanotubes. The samples were dried in a stream of filtered air.

2.3.4 Pd Seeding on DNA Nanotubes

Pd solutions were prepared following a Pd seeding method published previously from my group.\textsuperscript{23} In brief, for the activation step, the Pd solution consisting of 1mM PdCl\textsubscript{2} and 1M NH\textsubscript{4}Cl in 10 mM HEPES buffer pH 6.5 was left to adsorb on DNA nanotubes for 2 h in a humidified environment at room temperature. Next, samples were washed with 2–3 drops of water and dried with a stream of air. Then in the reduction step, 40 µL of 40 mM DMAB and 10 µL of 5 mM magnesium chloride were deposited on top of the Pd-treated DNA nanotubes and left to react for 2 min. The reduction step was completed by washing the surface with 2–3 drops of water. The samples were then dried with a stream of air. For repeat seeding, the Pd solution was left to adsorb on DNA nanotubes for 30 min, followed by the same reduction step as above. Multiple Pd seeding steps were performed to increase seed density.\textsuperscript{23}
2.3.5 Plating Au on Seeded DNA Nanotubes

Electroless plating solution was prepared according to a procedure used previously. Briefly, the plating solution contained 110 mM CTAB, 0.4 mM HAuCl₄, 0.2 mM AgNO₃, 0.06% HCl and 1.0 mM ascorbic acid. This solution (70 μL) was pipetted onto wafers with Au-seeded DNA nanotubes and left for 30 min to complete the plating process at 27 °C, after which the samples were rinsed with water and dried with filtered air. For Pd-seeded samples, gold enhance EM solution was applied (70 μL) for 10 min in a humidified chamber and then, the above plating solution (70 μL) was delivered and allowed to react for 30 min at 27 °C. The samples were rinsed with water and dried with filtered air after each treatment of plating solutions.

2.3.6 EBID and Creating Electrical Connections

Photolithography and metal lift-off were used to form square (100 μm) gold contact pads with four extending arms. First, AZ3332 photoresist (Merck kGaA) was deposited and patterned using a SUSSMA 150 contact aligner (Karl Suss America, Waterbury, VT, USA), then developed using AZ300MIF (Merck kGaA). A thermal evaporator was used to deposit a 7 nm chromium adhesion layer, followed by deposition of 50 nm of gold. Lift-off was done by soaking along with shaking (10 min) and sonicating (1 min) in NMP to form the desired contact pads according to previously published procedures.

To connect metallized DNA nanotube structures to gold contact pads, tungsten traces (~25 nm wide and using the line dose setting for a height of 1 μm) were patterned using EBID at 5 kV and 0.17 nA in a Helios Nanolab 600 SEM (FEI, Hillsboro, OR, USA). In order to link the thinner tungsten traces to the gold pads, tungsten traces (~25 nm wide and using the line dose setting for a height of 3 μm) were written from the thin traces to the large gold pads.
2.3.7 Atomic Force Microscopy (AFM) Imaging

DNA nanotubes on wafers were imaged using ‘Peak Force’ tapping mode AFM (MMAFM-2, Bruker, Santa Barbara, CA) with Bruker ScanAsyst automatic image optimization technology and Bruker silicon tips on nitride cantilevers.

2.3.8 Scanning Electron Microscopy (SEM) Imaging

Seeded and plated DNA nanotube samples were imaged by SEM in ultra-high-resolution mode on a FEI Helios Nanolab 600 or on a Thermo Scientific Verios UC G4 SEM.

2.3.9 Electrical Measurements

A micromanipulator probe station was utilized to connect to the gold pads for I-V studies. A National Instruments DAQ and custom LabVIEW program were used to apply voltages from —1 to +1 V. Current was measured using a DL Instruments 1211 current preamplifier and the output was recorded by the DAQ. Voltage drop values for 4-point probe data were measured simultaneously by a Fluke multimeter (8840A/AF/05, Everett, WA, USA). Measurements were done at room temperature.

2.4 RESULTS

2.4.1 Deposition of DNA Nanotubes

Figure 2.3 shows AFM images of self-assembled DNA nanotubes that have been deposited on oxidized Si wafers. Typically, the DNA nanotubes were 1 to 10 µm in length with widths around 30 nm. In Figure 2.3 the random landing of DNA nanotubes on the oxidized Si surface can be seen, as well as some nanotube entanglement. Figure 2.3 (b) shows a closer view of the DNA nanotubes. These results demonstrate that the DNA nanotubes were well-formed during the annealing process.
Figure 2.3 AFM images of self-assembled DNA nanotubes. (a) Large area and (b) zoomed in images. Height scale is 8 nm.

2.4.2 Au Nanorod Seeding of DNA Nanotubes

Figure 2.4 shows results for seeding of Au nanorods on DNA nanotubes randomly deposited on the surface. Figure 2.4 (a) shows a large area SEM image of Au nanorod-seeded DNA nanotubes; several Au nanorod-seeded structures appear to be continuous for several micrometers in length, whereas others extend only over the hundred-nanometer scale. Just a few Au nanorods have crossed each other during seeding, because they were attracted electrostatically to the deposited DNA nanotubes. A small number of Au nanorods not associated with DNA nanotubes remained on the surface after the washing step (surfaces are difficult to image before the washing step). Overall, the relatively few Au nanorods on the surface that were not associated with DNA nanotubes indicate a clean background using my methods. Figure 2.4 (b–d) shows individual Au nanorod-seeded structures. Gaps of a few nanometers are observed along every few hundred nanometers of the DNA nanotubes in these zoomed in images; as one example, see Figure 2.4 (c) near the top. Well-aligned Au nanorods along the length direction of the DNA nanotubes and multiple rods in parallel can be seen in these seeded structures. Furthermore, very few spherical Au nanoparticles (nanorod synthesis impurities) are present on the Au nanorod-seeded structures. Figure 2.5 shows an AFM height image and corresponding section analysis for Au
nanorod-seeded DNA nanotubes. The DNA nanotube heights in areas without nanorods were measured to be $2.5 \pm 0.7 \text{ nm (n = 20)}$, and the Au nanorod heights were determined to be $7.3 \pm 1.1 \text{ nm (n = 20)}$.

Figure 2.4 Scanning electron microscopy (SEM) images of Au nanorod-seeded DNA nanotubes. (a) Large area view. (b–d) Zoom views of individual DNA nanotubes seeded with Au nanorods.
Figure 2.5 AFM height characterization. (a) AFM image of Au nanorods seeded on DNA nanotubes; height scale: 20 nm. (b) Height analysis of DNA nanotube (blue) and Au nanorod seeded DNA nanotube (red).

2.4.3 Au Nanorod-Seeded and Au-Plated DNA Nanotubes

In order to fill in the gaps between Au nanorods and form continuous metal nanowires, I used electroless plating. Figure 2.6 shows Au nanorod-seeded structures after the electroless Au plating process. Figure 2.6 (a) shows a large area with now-formed Au nanowires having lengths that range from several hundred nanometers to several micrometers. Figure 2.6 (b–e) shows four individual, plated structures in which continuous Au nanowires have lengths of 1–2 µm and diameters in the range of 35–60 nm. The diameter of wires varied along their length, from as thin as 23 nm to as thick as 115 nm.
2.4.4 Pd-Seeded and Au-Plated DNA Nanotubes

As an alternative method to Au nanorod seeding, I also tried Pd seeding. Figure 2.7 shows SEM characterization of the results. The dark feature angling downward in the right side of Figure 2.7 (a) is a DNA nanotube; the Pd seeds alone provide poor contrast in SEM images. However, the seeds become visible as granular features in Figure 2.7 (b) after they have been enlarged by the first gold plating step. Importantly, the subsequent, second Au plating step connected these seeds into wire-shaped structures, as seen in Figure 2.7 (c–e). The plated structures in Figure 2.7 (d), (e) were in the range of 1–1.5 µm in length and the diameters were in the range of 35–85 nm. Non-specific background particle deposition was greater, variability of nanowire diameter was higher and nanowire continuity was lower, compared to samples formed by seeding with Au nanorods.
Figure 2.7 SEM images of DNA nanotubes seeded with Pd and subsequently plated with Au. (a) Pd seeded. (b) Pd seeded and step 1 Au plated. (c) Pd seeded and steps 1 and 2 Au plated. (d,e) Individual Pd seeded and steps 1 and 2 Au plated DNA nanotubes.

2.4.5 Electrical Characterization

I performed electrical measurements to further evaluate the properties and test the continuity of Au nanowires. Figure 2.8 shows SEM images of EBID connection to an Au-plated DNA nanotube. Figure 2.8 (a) shows a wider area view of EBID tungsten patterns connecting the Au nanowire to the arms extending from the Au pads. The EBID patterns have considerable space between each other to limit the effects of overspray of tungsten during writing. Figure 2.8 (b) displays the four EBID tungsten contacts to a DNA nanotube-templated Au nanowire.
Figure 2.8 SEM images of electron beam induced deposition (EBID) connections to Au-plated DNA nanotubes seeded with Au nanorods. (a) Completed EBID pattern connecting a nanowire to four Au pads. (b) Zoom view of the Au plated nanowire in (a) with numbered contacts.

Figure 2.8 (a) shows a two-point I-V curve from a gold nanowire for input voltages spanning from -1 to +1 V, where leads 1 and 4 in Figure 2.8 (b) were used for voltage input. Figure 2.9 (b) shows a four-point I-V measurement for the same gold nanowire where the voltage drop between leads 2 and 3 was measured, along with the current through the wire. For the particular Au nanowire in Figure 2.9, approximately one half of the input voltage was dropped between leads 2 and 3. The resistance was then calculated by the reciprocal of the slope of the I-V curves in Figure 2.9. The resistance values for 15 such nanowires out of a total of 41 structures connected via EBID were measured to be between 5 and 167 kΩ.

Resistivity values were determined by using each resistance measured as above, the average distance between leads 2 and 3 and the average nanowire diameter between leads 2 and 3. Figure 2.9 (c) shows the resistivities determined from fifteen different plated DNA nanotube Au nanowires on five separate Si wafers. Resistivity values ranged from 9.3x10⁻⁶ to 5.6x10⁻³ Ωm. The inset in Figure 2.9 (c) shows an expanded range of resistivity values from 0 to 0.001 Ωm.
Figure 2.9 Electrical measurements on a plated DNA nanotube Au nanowire. (a) Two point I-V curve. (b) Measured voltage drop between inner electrodes during four-point measurement. (c) Scatter plot for resistivity values measured. (Inset) Expanded y-axis view from 0 to 0.001 Ωm.

2.5 DISCUSSION

The AFM results from Figure 2.3 indicate that the surface of the oxidized Si wafer has enough DNA nanotubes to form nanowires. The weakly acidic nature of silanol groups that are typically deprotonated allows electrostatic bridging interactions between the negatively charged DNA molecules, the positively charged Mg$^{2+}$ ions and the surface. Additionally, thermally oxidized Si makes it possible to isolate nanowire electrical properties from those of the underlying substrate, while providing a hydrophilic surface for DNA nanotube attachment.

The orientation of Au nanorod-seeded structures shown in Figure 2.4 was dependent on the arrangement of the DNA nanotubes resulting from their surface deposition. Multiple Au nanorods are visible in some areas of the seeded structures; DNA nanotubes typically flatten from their cylindrical shape and become wide enough to contain multiple seeds because the Au nanorod width is ~10 nm and the DNA nanotube width is ~30 nm. The continuity of the nanowires is more
difficult to maintain as nanowires become longer, because the probability increases for having a gap between seeds, which may be removed during processing steps.

I evaluated two different gold plating solutions to fill the gaps between the Au nanorods. When I used a commercial Au plating solution as shown in Figure 2.10, Au nanorods grew isotropically, leading to Au deposition at the same rate in both the length and width directions. In contrast, when I used the gold plating solution described in Section 2.3.5, I observed that Au nanorods grew anisotropically. I attribute the difference in plating to CTAB, which is not present in the commercial plating solution. When CTAB is present in the plating solution a CTAB monolayer is retained on the sides of the Au nanorods, which inhibits gold deposition on the sides but allows plating on the tips. Different lengths of nanowires (e.g., Figure 2.6) resulted when DNA nanotubes had different initial lengths or when Au nanorod-seeded structures had larger gaps, which were not filled through the plating process or which appeared due to nanorod liftoff during plating.

![SEM images of Au nanorods seeded and Au plated with commercial plating solution](image)

**Figure 2.10** SEM images of Au nanorods seeded and Au plated with commercial plating solution

Unlike samples seeded with Au nanorods, the Pd-seeded DNA nanotube samples contained many more background particles, as seen in Figure 2.7. Ionic seeding has poorer selectivity for the desired DNA nanotubes than Au nanorod seeding, as seeding of Pd occurred on all DNA
fragments deposited onto the surface. Thus, DNA strands that were not assembled into a tube could also be seeded with Pd, resulting in undesired background metal deposition. In contrast, the background in the Au nanorod-seeded samples was comparatively less than for Pd seeding, so that nonspecific deposition of Au in the plating step was reduced. This lower background for Au nanorod seeding was in part because of one seeding step compared to multiple steps for Pd seeding. In Pd seeding, variability of seed diameter and density was higher than for Au nanorods, as the plating solutions grew the Pd seeds non-selectively and isotropically. Continuity in Pd-seeded nanowires was harder to achieve compared to Au nanorods, as the absence of just one Pd seed could leave a small gap in the nanowire, breaking continuity.

A range of resistivity values was obtained as shown in Figure 2.9 (c), which I attribute to differences in the morphology of the wires due to seeding granularity. One conductive structure was considered an outlier and thus omitted from Figure 2.9 (c), as it had a resistance of 2500 kΩ, ~15 times greater than the next-largest value measured and a resistivity of 5.95x10^{-2} Ωm. This particular structure may have had a short section that was much less conductive in either the nanowire or one of the leads.

EBID can, under some conditions, create “overspray” resulting in conductive material depositing near written wires. Consequently, blank samples were created by connecting EBID tungsten leads (without a nanowire) to contact pads in a configuration like that used for measuring nanowires, as shown in Figure 2.11. These blank samples yielded no voltage-dependent current when the input voltage was scanned from 0 to 1 V (Figure 2.11 (b)); a constant, systematic offset of ~100 pA was observed, which was also seen when the leads were disconnected. As these currents are much lower than those seen in nanowire samples (e.g., Figure 2.9), I can neglect any current contributions from EBID overspray in my measurements. Figure 2.12 shows EBID
connections to an EBID-written nanowire. I measured the resistance of the EBID-written contact leads in Figure 2.12 (a) to be \(~300\ \text{k}\Omega\) (Figure 2.12 (b)). This result confirms that the EBID-written tungsten structures were suitable for conducting electrical measurements since this 300 kΩ resistance was several orders of magnitude lower than the input impedance of our measurement equipment.

Figure 2.11 Blank experiment to assess substrate resistance. (a) SEM image of EBID connections without a nanowire. (b) I-V curve from the setup in (a)

Figure 2.12 Control experiment connecting 4 Au pads to an EBID-written nanowire. (a) SEM image of EBID connections to an EBID-deposited nanowire. (b) I-V curve from the setup in (a)

Two-point I-V measurements also include the probe, pad, lead and contact resistances, whereas in four-point measurements these extraneous resistances are eliminated. These Au
nanowires have resistivities similar to and in some cases smaller than those made by Aryal et al., who measured C-shaped Au nanowires formed on DNA origami with a similar four-point probe technique but obtained resistivities between $4.24 \times 10^{-5}$ and $0.124 \, \Omega \text{m}$. My resistivity values reported here are higher than the bulk resistivity of gold ($2.44 \times 10^{-8} \, \Omega \text{m}$), probably due to impurities, wire junctions, size effects that cause scattering, presence of surfactant or differences in the processing of nanowires and surfaces.

I successfully obtained four-point I-V curves for 16 out of 41 total structures tested after they were connected by EBID. There were fourteen additional structures where four-point I-V curves failed after showing current in two-point measurements, indicating that nearly three-fourths of these nanowires were conductive at the start of testing. This difference between two-point and four-point results could be caused by gaps in or incomplete contacts from electrode 2 or 3 to the nanowires or by a breakage in one of the leads or the nanowire from resistive heat generated during two-point measurement. Indeed, I confirmed the latter occurrence when in some cases, after running current between leads 1 and 4, the test structure was changed in such a way that it was no longer conductive. For some nanowires where successful four-point I-V curves were not obtained, multiple four-point measurements yielded large variability in the current or voltage drop values, possibly indicative of poor connections (or gaps) between Au-plated structures in the nanowires. If there was a very small gap, electrons could still tunnel across the gap resulting in conductive nanowires but with very high resistances. Another possibility is that the presence of very thin bridging connections between Au nanorods resulted in much higher nanowire resistances.

2.6 CONCLUSION

In this chapter, I have used two different methods to fabricate gold-metalized DNA nanotubes, whose electrical properties were subsequently characterized. The first approach
involved seeding DNA nanotubes with gold nanorods and connecting them by electroless plating. The second method utilized Pd ionic seeding in a three-step process: Pd activation, Pd reduction to form seeds and electroless plating. Au-seeded and plated structures had continuous lengths that ranged from 1 to 2 µm with diameters of 35-60 nm. Pd-seeded and Au-plated structures had lengths of 1–1.5 µm with diameters of 35–85 nm. I utilized EBID-formed conductive tungsten contacts to study electrical properties of these plated DNA nanotubes. The successfully measured resistance values for 15 nanowires (from a total of 41 structures where electrical characterization was attempted) were between 5 and 167 kΩ. These nanowires could find use as self-assembling interconnects, including 3D structures, that bridge between the molecular- and macro-scale world in future electronic devices.

2.7 REFERENCES

CHAPTER 3: OPTIMIZATION OF SEEDING GOLD NANORODS AND PLATING ON DNA ORIGAMI

3.1 INTRODUCTION

In bottom-up nanofabrication, utilizing DNA as a template is an appealing approach, due to its self-assembly properties. DNA is a robust template for nanofabrication via self-assembly with its nanoscale size, complementary base pairing, and functional groups. Recent advances in DNA origami have enabled nanofabrication by folding single-stranded DNA into a variety of 2-D and 3-D shapes. The techniques used in DNA nanofabrication are potentially cost-effective, as they do not require large and expensive machinery.

Metal nanostructures are essential in research, especially within the field of nanoelectronics. Bottom-up fabricated metal nanostructures that are conductive or semiconductive are increasingly appealing. Assembly of these materials at the nanoscale can be achieved using DNA templates. To synthesize metal nanostructures on DNA requires two main steps: seeding and plating. Seeding can be done using metal (Au, Pd, Ag, and Cu) nanoparticles (NPs) or nanorods (NRs). Plating can be accomplished in various ways, such as electroless chemical, photochemical, or electrochemical reduction.

The Seidel group created conductive Au nanowires utilizing DNA-mold templated assembly. The method involves the growth of Au NPs inside a DNA mold and connecting those molds through attractive and repulsive single-stranded DNA overhangs. Small portions of longer (100’s of nm) nanowires displayed metal-like conductivity. A similar approach was illustrated by Joshi et al. using a microtubule lumen template to synthesize Au nanowires. These nanowires are smooth in appearance in electron microscopy, with fewer grain boundaries and the same diameter as DNA-origami-molded nanowires, but they did not characterize nanowire electrical
properties. Previous work from my group included a comparison study of seeding and plating using Au NPs and Au NRs on DNA origami.\textsuperscript{4} They showed that seeding with cetly trimethylammonium bromide (CTAB)-coated Au NRs provided fewer connection points and lengthwise axial growth, resulting in thinner nanowires than seeding with NPs, which resulted in granular type, wider nanowires. In that work, the gap between Au NRs in seeded structures was 11-15 nm, but nanowires were conductive after plating. More recently, Aryal et al.\textsuperscript{12} developed a site-specific metallization technique to create plus, cross and C-shaped metal structures on tile DNA origami. They measured the resistance of ~130 nm long C-shaped nanowires and showed those nanowires were conductive. There is still room to reduce the gap between CTAB-coated Au NR seeding in the prior publication, as improved seeding can result in better plating for conductive nanowires. Reproducible metallized structures are essential for characterization and subsequent integration.

This chapter describes results for seeding Au NRs on DNA origami with 2-4 nm gap sizes and improved alignment. Continuous conductive nanowires resulted after electroless plating, due to the improvement in seeding. These seeded and plated nanowires enabled collaborative work to study the electrical properties of the nanowires and the influence of polymer-constrained annealing. This chapter demonstrates the importance of DNA self-assembly and Au NR seeding with nanometer precision, combined with electroless plating to fabricate functional nanowires for future nanoelectronics.
3.2 MATERIALS AND METHODS

3.2.1 Chemicals and Materials

I purchased sodium borohydride (NaBH₄), hydrogen tetrachloroaurate(III) (HAuCl₄), and CTAB (H5882, 98%) from Sigma-Aldrich (St. Louis, MO, USA), and Tris(hydroxymethyl) aminomethane (Tris base) and ascorbic acid from Fisher Scientific (Fair Lawn, NJ, USA). I acquired ethylenediaminetetraacetic acid (EDTA) from Life Technologies (Carlsbad, CA, USA) and silver nitrate from Mallinckrodt Chemicals (Philipsburg, NJ, USA). I obtained magnesium acetate (Mg(Ac)₂·4H₂O), magnesium chloride, acetic acid, and hydrochloric acid from EMD Chemicals (Gibbstown, NJ, USA). I purchased M13mp18 single-stranded DNA from New England Biolabs (Ipswich, MA, USA) and staple strands from Eurofins MWG Operon (Huntsville, AL, USA). I mixed 400 mM Tris base, 10 mM EDTA, 200 mM acetic acid, and 125 mM Mg(Ac)₂·4H₂O to prepare TAE-Mg²⁺ buffer (10X, pH 8.3). All water used was HPLC-grade.

3.2.2 Assembly of DNA Origami

The annealing of DNA origami followed the same procedure as previously published work.⁴ M13mp18 single-stranded DNA (2 nM), staple strands (40 nM), 10X TAE buffer and distilled water were mixed to a total the volume of 100 µL. The solution was heated to 95 °C, then cooled to 4 °C in a TC-3000 thermal cycler (Techne, Burlington, NJ, USA) over 1.5 h. The DNA origami was deposited on an oxidized Si wafer, which had been plasma-cleaned and rinsed with a Mg²⁺ (4 mM) solution. The average size of deposited DNA origami (Figure 3.1 (a)) was 410 nm x 17 nm.

3.2.3 Seeding Au NRs on DNA Origami

Gold NRs (25 nm length and 6 nm diameter) were synthesized following a previously described method.¹³ CTAB (5.0 mL, 0.2 M), HAuCl₄ (2.5 mL, 1.0 mM), AgNO₃ (250 µL, 4.0
mM), and HCl (8.0 μL, 37%) were combined and mixed with a pipette. After adding 35 μL of ascorbic acid (78.8 mM), the solution was shaken gently until it was clear. Ice-cold NaBH₄ (7.5 μL, 0.01 M) was added without mixing and then left to react overnight at 27 °C. The final solution was centrifuged at 14,000 rpm for 17 min to collect the Au NRs. Modifications were made to the stored solution by changing the final CTAB concentration to 10 mM.¹⁴ Au NRs diluted in TAE buffer were deposited onto a Si substrate containing DNA origami and stored in a humidified chamber for 1 h (Figure 3.1 (b)). The samples were rinsed with distilled water and dried under gentle flow of dry air. Optimization of the seeding conditions was done by changing the concentration of Mg²⁺ in the seeding solution. Originally, TAE buffer contained 125 mM Mg²⁺; I increased the Mg²⁺ concentration by two-, three-, four-, or five-fold. Further increasing of Mg²⁺ concentration resulted in some particles on the surface. From these studies, I concluded that the optimal concentration of Mg²⁺ in TAE buffer for seeding was 750 mM, which resulted in a final concentration of Mg²⁺ in the seeding solution of 350 mM.

3.2.4 Electroless Au Plating of Seeded DNA Origami

The gold electroless plating followed a published method;¹⁵ 30 μL of the plating solution was added to a substrate with seeded DNA origami. The samples were plated in a water bath (27 °C) to avoid CTAB crystallization, and the reaction was allowed to proceed for ~30 min. The samples were rinsed with distilled water and dried with a stream of filtered air. Successful gold electroless plating resulted in continuous gold nanowires with an average length of 400 nm and diameter of 20 nm, as shown in Figure 3.1 (c).
Figure 3.1 Process diagram of the synthesis of Au nanowires. (a) Scaffold and staple strand DNA solutions are mixed, allowing for the formation of a pre-designed bar DNA origami pattern, which is then placed on a SiO$_2$ substrate. (b) CTAB-functionalized gold NRs are added to the substrate and attached to the bar DNA origami. (c) Anisotropic electroless plating grows seeded NRs, connecting the structures. The scale bar for all images is 200 nm. Adapted with permission from Westover et al.\textsuperscript{16}

3.2.5 Characterization

I utilized ultra-high-resolution mode on a Thermo Scientific Verios UC G4 SEM (Hillsboro, OR, USA) to take scanning electron microscopy (SEM) images. I used a ‘Peak Force’ tapping mode atomic force microscope (MMAFM-2, Bruker, Santa Barbara, CA, USA) with Bruker ScanAyst automatic image optimization and Bruker silicon tips on nitride cantilevers to obtain atomic force microscopy (AFM) images.

3.3 RESULTS AND DISCUSSION

To confirm that the bar DNA origami annealed correctly and that a sufficient amount was deposited on the oxidized Si wafer, I characterized the deposited DNA origami by AFM. Figure 3.2 shows deposition on oxidized Si surfaces of a more concentrated and diluted bar DNA origami solution. Most of the DNA origami deposited on the Si surface folded well, yielding the desired linear shape, while some were bent. These results show that the DNA origami surface placement for fabrication of conductive nanowires is sufficient for use in successive steps.
Next, the fabrication of nanowires was conducted by binding Au NR seeds onto the deposited DNA. The assembly of CTAB-coated Au NRs followed the procedure in Section 3.2.3, and the initial experimental results are shown in Figure 3.3. The bright cylindrical features are Au NRs, and the bright round features that appear in some of the images come from the Au NR growth solution. The dark features underlying the Au NRs are DNA origami as can be seen in Figures 3.3 (a), (b) and (d). The results confirm that the Au NRs attached, but are not aligned length-wise along the DNA origami. The storage conditions of the Au NRs are important, as the Au NR stability degrades with time. John et al.\textsuperscript{14} reported that the ideal concentration of CTAB is 10 mM to store Au NRs with minimal change in stability. When Au NRs are less stable, they may break apart and result in those NRs being shorter. Therefore, I used Au NR stabilizing 10 mM CTAB to store the Au NRs, and a lower 3 mM CTAB concentration for the final seeding solution to avoid CTAB crystallization.
Furthermore, I investigated how the Mg$^{2+}$ concentration in the seeding solution affects the alignment of Au NRs. As described in Section 3.2.3, I increased the Mg$^{2+}$ concentration gradually in the TAE buffer. Figure 3.4 shows a collection of AFM and SEM images of Au NR seeded structures from the optimized conditions (3 mM CTAB and 350 mM Mg$^{2+}$ in the seeding solution). When the optimal Au NR concentration combined with optimized CTAB and Mg$^{2+}$ concentrations is deposited onto DNA origami, the Au NRs align continuously along the length of the DNA. Additionally, the background surface looks clean without non-specific Au NR deposition. The unoptimized CTAB and Mg$^{2+}$ concentration conditions in Figure 3.3 affect ionic strength and NR surface charge density in the seeding solution that could cause aggregation of Au NRs. Increasing the Mg$^{2+}$ concentration to 350 mM results in better alignment, likely because the Au NRs tend to maximize the electrostatic interaction between the length of DNA and the length of Au NRs by orienting the same way at this salt concentration. Further increasing the Mg$^{2+}$ concentration beyond
the optimal 350 mM resulted in deposition of spherical particles on the surface, which energy-dispersive X-ray (EDX) confirmed to be composed of Au.

**Figure 3.4** Optimized seeding of CTAB-coated Au nanorods on bar DNA origami. AFM images of (a) zoomed-out and (b) zoomed-in views; height scale is 30 nm. SEM images of (c) zoomed-out and (d-g) zoomed-in views.

To fabricate continuous Au nanowires, I used an electroless plating solution on optimized Au NR seeded structures, where it is important for the Au NRs to only have small gaps between them. With electroless plating, these Au NRs grow anisotropically lengthwise, filling the gaps between Au NRs, and creating a continuous nanowire. **Figure 3.5** shows a collection of AFM and SEM images of plated nanowire structures that resulted in ~400 nm long nanowires. When there is a larger gap between Au NRs, the electroless plating solution will not fill the gap, resulting in a disconnect in the nanowire, as seen, for example, in **Figure 3.5 (f)**. Some spherical particles are visible in **Figure 3.5** in the zoomed-out views; these may result from aggregated Au NRs that became enlarged during electroless plating.
Figure 3.5 Plated DNA origami after optimized Au NR seeding. (a) AFM image; height scale is 40 nm. SEM images of (b-c) zoomed-out and (d-f) zoomed-in views. The red circle in (f) indicates a gap in a nanowire.

The optimized seeding and plating made it possible in collaborative work to study DNA origami-templated Au nanowires after polymer-constrained annealing.$^{16}$ This work increased understanding of the impact of elevated temperature on the stability of DNA-templated nanowires. My collaborators annealed seeded, as well as seeded and then plated structures, with or without a polymer coating and analyzed the morphology and conductance of those structures. My optimized seeding and plating made possible the reproducible fabrication of hundreds of Au nanowires, as seen in Table 3.1, first column. This allowed my collaborators to experiment with statistical numbers of samples and study annealing conditions. My successful seeding and plating also addressed yield issues in photolithography, Au deposition and making electron beam induced deposition connections described in Chapter 2. Moreover, the Au NRs were well aligned and packed, in the seeded-only conditions (Figure 3.4), which allowed the annealing of seeded-only, as well as plated structures. Table 3.1 summarizes the results my collaborator obtained for polymer-constrained annealing, showing the temperature regimes for morphological changes with
seeded-only, seeded and plated, polymer-coated seeded, and polymer-coated seeded and plated nanowires.

**Table 3.1** Summary of observed morphological changes. * Reprinted with permission from Westover et al.16

<table>
<thead>
<tr>
<th></th>
<th>Temperature Range Annealing Regime 1 (No Observed Change)</th>
<th>Temperature Range Annealing Regime 2 (Fusing Observed)</th>
<th>Temperature Range Annealing Regime 3 (Isolated Island Formed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seeded Plated (57 Samples)</td>
<td>&lt;160° C</td>
<td>160-180° C</td>
<td>&gt;180° C</td>
</tr>
<tr>
<td>Seeded-Only (26 Samples)</td>
<td>&lt;160° C</td>
<td>-</td>
<td>&gt;160° C</td>
</tr>
<tr>
<td>Coated Seeded Plated (106 Samples)</td>
<td>&lt;135° C</td>
<td>135-210° C</td>
<td>&gt;210° C</td>
</tr>
<tr>
<td>Coated Seeded-Only (101 Samples)</td>
<td>&lt;170° C</td>
<td>-</td>
<td>&gt;170° C</td>
</tr>
</tbody>
</table>

*Threshold temperatures for different annealing regimes with seeded-only, plated and coated nanowires. Regime 1: at lower temperatures no morphological change is observed. Regime 2: at moderate temperatures the nanorods appear to fuse. Regime 3: at higher temperatures nanorods separate into isolated islands.

### 3.4 CONCLUSION

In this chapter, I conducted experiments varying the concentrations of the surfactant CTAB, and the ion, Mg^{2+}, to evaluate how they influence the assembly and alignment of Au NRs on DNA origami. I made seeded structures with small 2-4 nm gaps and improved alignment along the length of bar DNA origami under optimized conditions. With that, I was able to obtain plated nanowires of ~400 nm length. The nanostructures I optimized in this chapter are attractive for making arrays of NRs for plasmonic or electronic sensor devices. Furthermore, my fabricated nanowires facilitated the collaborative study of the impact of polymer-constrained nanowire annealing. This combined work supports the bottom-up nanofabrication of DNA-templated metallized structures and nanoelectronics.
3.5 REFERENCES


(14) John, C. L.; Stratig, S. L.; Shephard, K. A.; Zhao, J. X. Reproducibly synthesize gold nanorods and maintain their stability. RSC Advances 2013, 3 (27), 10909-10918.


CHAPTER 4: BLOCK COPOLYMER SELF-ASSEMBLY TO PATTERN GOLD NANODOTS FOR SITE-SPECIFIC PLACEMENT OF DNA ORIGAMI AND ATTACHMENT OF NANOMATERIALS*

4.1 INTRODUCTION

The growth of nanofabrication has directed the way for innovations in various areas, including medicine, textiles, agriculture, food, and electronics.¹ Some of these, including medical and energy serving products, have already been established and marketed,² while others like optics and nano sensing are still in the laboratory stage.³ Miniaturization allows for manufacture of portable,⁴ implantable,⁵ and even injectable devices.⁶ Current top-down nanofabrication techniques have been effective in meeting the global demands of increased capacity per unit area and lowered costs for future devices. New technologies, such as 3-D integration⁷ and new materials for interconnects,⁸ will help to continue these advances. Additionally, bottom-up nanofabrication is a promising alternative to developing nanoscale devices as it is widely applicable and potentially scalable to generate large numbers of devices.⁹ Furthermore, bottom-up nanofabrication techniques often consume little energy,¹⁰ do not need expensive patterning tools,¹¹ and provide a fundamental platform for materials assembly and characterization.¹² In bottom-up methods self-assembly is essential as it enables the construction of complex architectures from molecular building blocks.¹³ My work in this chapter is centered on two types of self-assembled soft-matter systems: block copolymers (BCPs) and DNA origami.

BCPs contain two or more covalently bonded segments of different polymers. They can self-assemble into ordered structures with nano-features whose size can be engineered by

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*This chapter is to be submitted to Nanoscale, with the following list of authors: Ranasinghe, D.R.; Doerk, G.; Aryal, B.R.; Pang, C.; Davis, R.C.; Harb, J.N.; Woolley, A.T.
managing the molecular weights of the individual segments. Nanoscale features that result from BCP thin films are suitable for various applications of nanofabrication, including electronics, optics, solar cells, magnetic storage, and acoustics.

DNA origami, presented by Paul Rothemund in 2006, can create 2-D and 3-D nanoscale objects through programmed self-assembly based on hydrogen bonding between base pairs. DNA origami uses a long single-stranded DNA (ssDNA), called the scaffold (typically viral DNA ~7,000 nucleotides long), with hundreds of designed short ssDNA called staple strands. Each staple strand has multiple binding sites that connect distant scaffold domains via crossover base pairing, folding the scaffold into desired shapes. The versatility of DNA origami templates in various applications, including medical, plasmonic, and electrical, can be increased through functionalization with nanomaterials. Aryal et al. utilized the site-specific binding nature of DNA to fabricate plus, cross, and c-shaped structures and created metal nanowires on these shapes. By careful engineering, different materials can be attached to the same DNA origami template. In addition to hybridization, chemical and electrostatic interactions allow for nanomaterial attachment that is not DNA sequence specific. Electronic materials such as Au, Te, Ag, Cu, and Pd have been assembled to create continuous wires. Furthermore, carbon nanotubes have been attached to DNA origami to create field-effect transistors with tunable conductivity.

Periodic arrangements of DNA-organized devices are essential for optical and electronic applications. It is common to integrate the bottom-up self-assembly of DNA structures with top-down lithographic methods for surface patterning. For example, Ding et al. placed DNA origami nanotubes with multiple thiol groups near each end on surface-patterned gold islands created by electron beam lithography (EBL). They further demonstrated an interconnection strategy by constructing complex networks of DNA origami nanotubes whose orientation was precisely
controlled by the spacing and pattern of the gold islands. Moreover, Gopinath et al.\textsuperscript{31} showed the directed self-assembly of DNA origami using electron beam patterning. They created triangular binding sites with negatively charged surface carboxylate groups surrounded by hydrophobic methyl groups. A DNA origami which has fluorescent capability bound strongly on the carboxylate groups via Mg\textsuperscript{2+} bridging, and they demonstrated optical features that could be useful in nanophotonics and nanoelectronics. Gerdon et al.\textsuperscript{32} reported a method combining top-down fabrication and bottom-up self-assembly to control the delivery of single DNA origami onto a single nanometer-scale gold pattern formed by EBL. They modified the gold with a carboxylic acid terminated self-assembled monolayer to attract DNA origami and enable the controlled placement of nanostructures onto surfaces. Nanosphere lithography (NSL) is an alternative to the EBL technique for patterning surfaces.\textsuperscript{33} NSL allowed nonspecific DNA origami placement where they adsorbed to exposed SiO\textsubscript{2} by forming salt bridges with Mg\textsuperscript{2+} at the bottom of nanoholes in a Au film.\textsuperscript{34}

Electron- or ion-beam lithography has several deficiencies,\textsuperscript{35} including expensive equipment and maintenance and loss of design resolution because of scattering of electrons in the substrate.\textsuperscript{36} NSL can pattern only a few shapes and designs. Furthermore, NSL is restricted by the self-arrangement of spheres to drive the nanopatterning so only homogenous, repeating arrays result from this method. Although larger or smaller nanoparticles can be generated by varying the diameter of the nanospheres, changing the in-plane nanoparticle size independently from the distance between the nanoparticles is not straightforward.\textsuperscript{37} In order to obtain significant advantages in terms of speed, power consumption, and cost in fabrication of tiny features, researchers will need to overcome issues arising from the cost and complexity of the technology. Self-assembly of nanostructures can offer a powerful solution to these issues at sub 20 nm sizes.
BCP nanolithography refers to the use of BCPs to pattern target substrates to generate functional nanostructures in thin-film geometries. This chapter focuses on a BCP system composed of polystyrene (PS) and poly-methyl methacrylate (PMMA) that spontaneously microphase-separates into a hexagonal lattice of vertical PMMA cylinders in a matrix of PS on a surface under appropriate processing conditions. The orientation of the minor and major BCP phases in thin films depends on many factors, including substrate surface energy, film thickness, roughness, and topography. Landeke-Wilsmark et al. used a poly(styrene-b-2-vinyl pyridine) BCP to create well-ordered, dense arrays of discrete Au nanoparticles (NPs). Frascaroli et al. obtained an ordered array of Pt/Ti electrodes on a HfO2/TiN surface, fabricating memory devices with a well-controlled diameter of 28 nm utilizing polystyrene-b-poly(methyl methacrylate) (PS-b-PMMA). Bandyopadhyay et al. demonstrated the feasibility of spontaneously depositing DNA-conjugated gold nanospheres into arrays of appropriately functionalized nanopores obtained from hexagonally ordered thin PS-b-PMMA diblock copolymer films on silicon. The deposition was mediated by electrostatic interactions or specific DNA hybridization in the nanopores modified with either positively charged aminosilanes or oligonucleotide probe sequences. Such DNA nanosphere nanoarrays can be used as capture surfaces for the secondary self-assembly of DNA functionalized nanoscopic entities, such as quantum dots or carbon nanotubes, with potential for use in biosensing. Pearson et al. reported a method of assembling DNA origami to chemically modified BCP micelle patterned 5 nm Au NPs. In addition, they demonstrated the attachment of DNA to those nanoscale binding sites by designing ssDNA-SH overhangs onto the origami. Keeping the BCP on the surface makes the DNA attachment process more complicated and the subsequent fabrication and integration of devices would be easier with the removal of BCP. On the other hand, BCP pattern transferring onto a Si substrate, metal evaporation and then localization of DNA
origami on the metal is straightforward. Importantly, incorporating DNA origami structures gives the potential to assemble electronic nanomaterials and to fabricate arbitrary shapes using the DNA origami platform.

In this chapter, I demonstrate the use of a self-assembled PS-\textit{b}-PMMA BCP nanopattern combined with thin film deposition and lift-off to direct the placement of DNA origami structures. I evaluated the formation of Au nanodots on a silicon substrate using five different PS-\textit{b}-PMMA BCPs. I also tested multiple surface modification approaches for directing DNA origami onto these patterns. I further used the DNA templates localized on the Au dots for site-specific attachment of Au nanorods. My work provides a self-assembly patterning method to create DNA origami nanoarrays, avoiding the cost of top-down lithography methods at nanoscale. Moreover, this technique enables scalable, parallel, large-area nanofabrication, which could lead to advances in plasmonic and nanoelectronics applications.

4.2 MATERIALS AND METHODS

4.2.1 Chemicals and Materials

Ethylendiaminetetraacetic acid (EDTA) was acquired from Life Technologies (Carlsbad, CA, USA). Tris(hydroxymethyl) aminomethane (Tris base) and ascorbic acid were obtained from Fisher Scientific (Fair Lawn, NJ, USA). Cetyl trimethylammonium bromide (CTAB) (H5882, 98%), hydrogen tetrachloroaurate(III) (HAuCl₄), sodium borohydride (NaBH₄), (11-mercaptoundecyl)-\textit{N,N,N}-trimethylammonium bromide (MUTAB), 1-dodecanethiol (DDT), 3-mercaptoproponoic acid (3-MPA) and Tween 20 were purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetic acid, hydrochloric acid, magnesium chloride, sodium chloride (NaCl) and magnesium acetate (Mg(Ac)₂·4H₂O) were obtained from EMD Chemicals (Gibbstown, NJ, USA). Silver nitrate was purchased from Mallinckrodt Chemicals (Philipsburg, NJ, USA). Tris(2-
carboxyethyl) phosphine (TCEP) was acquired from Alfa Aesar (Ward Hill, MA, USA). Bis(p-sulfonatophenyl)phenylphosphine dihydrate dipotassium salt (BSPP) was purchased from Strem Chemicals (Newburyport, MA, USA). A colloidal solution of 5 nm Au NPs was purchased from Ted Pella Inc. (Redding, CA, USA). For solution preparation and sample rinsing, water (18.3 MΩ) was generated with a Barnstead EASYpure UV/UF purification system (Dubuque, IA, USA). TAE-Mg$^{2+}$ buffer (10X, pH 8.3) was prepared according to standard laboratory procedures using 400 mM Tris base, 200 mM acetic acid, 10 mM EDTA, and 125 mM Mg( Ac)$_2$$\cdot$4H$_2$O. Toluene, propylene glycol monomethyl ether acetate (PGMEA) and tetrahydrofuran (THF) were purchased from Sigma-Aldrich and used as received.

PS-\(b\)-PMMA diblock copolymers (molecular specifications are in Table 4.1) C67, C99, C207, L625, and L1051 were purchased from Polymer Source Inc. (Dorval, Quebec, Canada). Likewise, homopolymer PS (\(M_n = 3.5\) kg/mol, \(M_w/M_n = 1.05\); \(M_n = 12.5\) kg/mol, \(M_w/M_n = 1.04\)) and PMMA (\(M_n = 3\) kg/mol, \(M_w/M_n = 1.14\)) were purchased from Polymer Source Inc. A hydroxyl-terminated random copolymer “neutral” brush of PS and PMMA—provided by the Dow Chemical Company (Midland, MI, USA) (P(S-r-PMMA)-OH; 60\% styrene determined by $^{13}$C NMR)$^{45}$ dissolved in PGMEA—was diluted to a concentration of 1\% (w/w). All diblock copolymers were dissolved in toluene or PGMEA at a concentration of 1-2\% (w/w) as indicated in Table 4.1. Two blend solutions were prepared by mixing PGMEA solutions of block copolymers and homopolymers with the same concentration by weight to obtain desired mass fractions. One blend solution included L625 and 12.5 kg/mol PS in mass fractions of 80\% and 20\%, respectively. The other blend solution included L1051, 3.5 kg/mol PS, and 3 kg/mol PMMA in mass fractions of 40\%, 40\%, and 20\%, respectively.
Table 4.1 PS-\(b\)-PMMA diblock copolymer details and surface preparation conditions.

<table>
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<tr>
<th>Type</th>
<th>MW* kg/mol</th>
<th>(M_w/M_n)</th>
<th>Coating Details</th>
<th>Annealing Details†</th>
<th>Thickness nm</th>
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<tr>
<td>C67</td>
<td>46.1-b-21</td>
<td>1.09</td>
<td>1% toluene, 3000 rpm</td>
<td>180 °C overnight in vacuum</td>
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</tr>
<tr>
<td>C99</td>
<td>64-b-35</td>
<td>1.09</td>
<td>1% toluene, 3000 rpm</td>
<td>220 °C overnight in vacuum</td>
<td>48</td>
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<tr>
<td>C207</td>
<td>150-b-57</td>
<td>1.08</td>
<td>1% toluene, 5000 rpm</td>
<td>SVA-THF, flow=1sccm, SR=3.0</td>
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</tr>
<tr>
<td>L625</td>
<td>400-b-225</td>
<td>1.09</td>
<td>1% PGMEA, 5000 rpm</td>
<td>SVA-THF, flow=0.5sccm, SR=3.6</td>
<td>23</td>
</tr>
<tr>
<td>L1051</td>
<td>536-b-515</td>
<td>1.09</td>
<td>2% PGMEA, 1500 rpm</td>
<td>SVA-THF, flow=0.5sccm, SR=3.6</td>
<td>83</td>
</tr>
</tbody>
</table>

*MW given as PS block-\(b\)-PMMA block, †SVA: solvent vapor annealing, SR: swell ratio = ratio of film thickness swollen with solvent/initial film thickness. The polymer volume fraction during SVA = 1/SR. Thickness measured by ellipsometer.

M13mp18 single-stranded DNA was acquired from New England Biolabs (Ipswich, MA, USA). For DNA origami formation synthetic staple strands with 10 additional protruding adenines were ordered from Eurofins MWG Operon (Huntsville, AL, USA). Single-stranded poly-T DNA with a thiol group attached to the 5' end (8-mer, gel purified) was also obtained from Eurofins MWG Operon. In order to attach DNA oligonucleotides to Au nanorods and DNA origami, the dry poly-T DNA was dissolved and diluted to 1 mM in water.

4.2.2 Gold Island Fabrication

Figure 4.1 shows a schematic illustration of the surface fabrication of Au islands for the attachment of DNA origami. Si surfaces were first cleaned by exposure to oxygen plasma for 60 s at 100 mTorr and 20 W (March Plasma CS1701F, Concord, CA, USA). The random copolymer brush was then grafted to the oxidized silicon surface to form a neutral underlayer (Figure 4.1 (a)), as described previously. Briefly, the random copolymer was spin coated at 1500 rpm to obtain a film 20-30 nm thick. The sample was then thermally annealed at 250 °C for 5 min to
ensure chemical grafting of the material. Excess ungrafted polymer was removed by rinsing in PGMEA for 30 s. The final thickness of the grafted neutral layer was ~6 nm.

Diblock copolymers or block copolymer/homopolymer blends were spin coated onto the grafted neutral brush layer at speeds from 1500 to 5000 rpm, yielding films 20-50 nm thick, except for the L1051 blend, which was 70-80 nm thick. Films were then annealed to induce the self-assembly process (Figure 4.1 (b)) in two distinct ways, depending on the block copolymer or blend solution used. C67 and C99 were thermally annealed overnight in a vacuum oven as indicated in Table 4.1. C207 and the blends containing L625 and L1051 were annealed by solvent vapor annealing at 21 °C with THF for 60 min using a method that has been described previously.47 Continuous nitrogen purging at prescribed flow rates of 0.5 to 1 sccm was used to regulate film swelling by solvent, obtaining minimum polymer fractions of 0.33, 0.28 and 0.28 for C207, the L625 blend, and the L1051 blend, respectively. The L1051 blend film was subsequently thermally annealed in air on a preheated hot plate at 250 °C for 30 s to sharpen the interface between PS and PMMA domains.48 PMMA removal was accomplished by degradation of PMMA using UV irradiation for 5-10 min under a low pressure mercury arc lamp (G10T5 1/2VH, Atlantic Ultraviolet Corporation, Hauppauge, NY, USA) in a closed chamber purged with continuously flowing nitrogen. The incident power for the 254 nm line was ~8 mW/cm² at the sample position. Degraded PMMA was then selectively removed by sample immersion in acetic acid for 3 min and subsequent rinsing with deionized water. Figure 4.1 (c) depicts the BCP film after PMMA removal.
by UV irradiation and acetic acid etching to form the template for patterning. To ensure good adhesion between the Si substrate and the evaporated metal in the next step, it is necessary to also etch through the neutral brush layer in the holes. Plasma etching (Planar Etch II, Santa Clara, CA, USA) was performed to etch BCP and the neutral brush layer (Figure 4.1 (d)). The etching time was brief (~10 s), and low power <50 W was used for each type of BCP. A thermal evaporator was utilized to deposit a ~3 nm chromium adhesion layer, followed by the deposition of ~6 nm of gold (Figure 4.1 (e)). The total thickness of both chromium and gold was kept below 10 nm, with small variations in individual Cr and Au thicknesses for the different BCPs studied. The substrate was sonicated in toluene in the lift-off process to remove the BCP template and leave the Au dots behind (Figure 4.1 (f)). The duration of sonication ranged from 2-120 min, with sonication stopped at the time when the gold reflection disappeared from the substrate surface. The highest yields of Au dots were obtained when the thickness of the plasma-etched BCP was greater than that of the evaporated metal layers.

4.2.3 Assembly of DNA Origami

DNA origami was made from M13mp18 single-stranded DNA, as in previous work.21 For the attachment of DNA-functionalized Au nanorods onto the bar shaped DNA origami, staple strands with 10 additional adenine bases were extended from the DNA origami as sticky-end sequences. For folding of DNA origami, a 100 μL solution containing M13mp18 single-stranded DNA (2 nM) and staple strands with 10 additional adenine bases (40 nM) was heated initially to 95 °C and cooled to 4 °C in a TC-3000 thermal cycler (Techne, Burlington, NJ, USA) for 1.5 h.

4.2.4 DNA Origami Attachment Using 3-MPA or MUTAB/DDT

Au dot surfaces prepared as described in Section 4.2.2 were placed in 1, 5 or 10 mM solutions of 3-MPA, or MUTAB (5 mM) and DDT (5 mM) in ethanol separately for at least 12 h,
after which the surfaces were rinsed with ethanol. DNA origami (10 μL, 1 nM) in 10X TAE-Mg\(^{2+}\) buffer was deposited onto a 0.5 cm × 0.5 cm Si substrate with Au dots previously functionalized with 3-MPA or MUTAB/DDT in a humid chamber for at least 12 h at room temperature. Finally, chemically and DNA functionalized Si substrates were rinsed with distilled water for 5 s and dried with flowing air for 1–2 s to remove loosely adhered DNA origami.

### 4.2.5 Au NP Deposition on Au Dot Surfaces Functionalized with 3-MPA or MUTAB/DDT

Au NP-DNA conjugates were prepared according to published work.\(^{49}\) The Au NPs were deposited onto Au dots functionalized with 3-MPA or MUTAB/DDT to study the surface density of attached functional groups. Additionally, Au NPs were deposited on the Au dot surface after plasma treatment (250 W, 5 min) and thermal annealing (200 °C, 1.5 hr, 10% H\(_2\) + 5% Ar), followed by 3-MPA or MUTAB/DDT functionalization as described in Section 4.2.4.

### 4.2.6 DNA Origami Attachment using Thiolated DNA on Au Dots

Figure 4.2 illustrates the hybridization attachment on Au dots for a segment of bar DNA origami. To fold the DNA origami, a similar procedure was followed as in Section 4.2.3, with the exception of the introduction of thiol groups into the DNA origami, where thiolated poly-T (8 μM) was added to the initial mixture. After annealing the DNA origami, the disulfide bond in the thiol-modified oligonucleotides was reduced to a monothiol bond using TCEP (20 mM, 1 h). The DNA origami solution was purified to remove unattached staple strands using 100 kDa Microcon centrifugal filters (St. Louis, MO, USA). DNA origami (10 μL, 1 nM) in 10X TAE-Mg\(^{2+}\) buffer was deposited onto the Si substrate containing Au dots (0.5 cm × 0.5 cm) in a humidified chamber for at least 12 h at room temperature. Finally, the surface was rinsed with distilled water for 5 s and dried with flowing air for 1–2 s.
4.2.7 Seeding Au nanorods (NRs) on DNA Origami

Au NRs were synthesized following published work; the molar ratio of thiol DNA to Au NRs was ~250:1. Binding was triggered by depositing as-prepared DNA-functionalized Au NRs in solution (20 μL) onto the Au dot substrate with DNA origami previously deposited. The samples were placed in a humidified chamber for 1 h, then rinsed carefully with distilled water and dried under a gentle flow of dry air.

4.2.8 Characterization

BCP film thicknesses were measured with a Filmetrics F20-UV (San Diego, CA, USA) or a WOOLLAM (Lincoln, NE, USA) ellipsometer. Scanning electron microscopy (SEM) images of the samples were taken in ultra-high-resolution mode on a Thermo Scientific Verios UC G4 electron microscope (Hillsboro, OR, USA). Feature distances were measured using SEM images and 50-200 measurements were taken. Atomic force microscopy (AFM) images were taken using ‘Peak Force’ tapping mode AFM (MMAFM-2, Bruker, Santa Barbara, CA, USA) with Bruker ScanAyst automatic image optimization and Bruker silicon tips on nitride cantilevers.

Figure 4.2 Schematic diagram of hybridization attachment of DNA origami on Au dots
4.3 RESULTS AND DISCUSSION

As described in Section 4.2.2, BCP thin films underwent a two-step process to remove PMMA by UV exposure and acetic acid etching, followed by plasma etching to remove the underlying random copolymer brush layer. It is common to use UV degradation of PMMA for its removal as a minor phase component in a BCP. UV radiation results in both the fragmentation of PMMA and simultaneous cross-linking of the PS matrix.\textsuperscript{52} The degradation products from PMMA are then rinsed away in acetic acid. Figure 4.3 shows the results of experiments performed to study this phenomena and the effects of cylinder size with SEM images of BCP thin films after removal of PMMA for the five BCPs. The smallest diameter (22 nm) and center-to-center distance between adjacent feature pairs (39 nm) resulted from the smallest overall molecular weight BCP (see Table 4.1) (C67) in Figure 4.3 (a), whereas the largest values measured (117 nm and 205 nm for the diameter and center-to-center distance, respectively) resulted from the largest molecular weight BCP (L1051). All the other BCP thin films had feature sizes in-between, as presented in Table 4.2. Thus, feature size and spacing can be controlled by the molecular weights of each block.
**Figure 4.3** SEM images of BCP thin films after removal of PMMA for (a) C67, (b) C99, (c) C207, (d) L625 and (e) L1051.

**Table 4.2** Mean (± one standard deviation) for surface features after removal of PMMA, plasma etching and lift-off.

<table>
<thead>
<tr>
<th>BCP</th>
<th>Diameter (nm)</th>
<th>Distance* (nm)</th>
<th>Diameter (nm)</th>
<th>Distance* (nm)</th>
<th>Diameter (nm)</th>
<th>Distance* (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C67</td>
<td>21.5(±4.4)</td>
<td>38.9(±3.7)</td>
<td>21.5(±2.1)</td>
<td>38.4(±4.2)</td>
<td>17.5(±1.9)</td>
<td>41.1(±3.6)</td>
</tr>
<tr>
<td>C99</td>
<td>25.4(±3.7)</td>
<td>51.3(±4.3)</td>
<td>28.2(±2.9)</td>
<td>49.0(±3.7)</td>
<td>21.5(±2.6)</td>
<td>51.8(±4.8)</td>
</tr>
<tr>
<td>C207</td>
<td>40.2(±3.7)</td>
<td>79.0(±4.9)</td>
<td>45.7(±5.2)</td>
<td>82.8(±4.8)</td>
<td>31.0(±5.0)</td>
<td>81.7(±4.4)</td>
</tr>
<tr>
<td>L625</td>
<td>82.6(±9.8)</td>
<td>146(±12)</td>
<td>81.7(±6.8)</td>
<td>163(±14)</td>
<td>65.4(±7.8)</td>
<td>154(±17)</td>
</tr>
<tr>
<td>L1051</td>
<td>117(±13)</td>
<td>205(±17)</td>
<td>123(±7)</td>
<td>215(±12)</td>
<td>105(±11)</td>
<td>219(±16)</td>
</tr>
</tbody>
</table>

* Center-to-center distance between adjacent feature pairs.

Plasma etching was performed to remove the underlying random BCP brush and allow metal to deposit directly on the Si surface on the inside of the cylindrical features during the evaporation step. This plasma step etches all polymer thin films at approximately the same rate, and **Figure 4.4** shows SEM images of thin-film BCPs after plasma etching. The hole diameter and
center-to-center distance between adjacent feature pairs for BCP thin films followed the same pattern observed after PMMA removal as seen in Table 4.2.

Figure 4.4 SEM images of BCP thin films after plasma etching of (a) C67, (b) C99, (c) C207, (d) L625 and (e) L1051.

To study Au dot diameter and the center-to-center distance between adjacent feature pairs after lift-off, I performed further SEM characterization. Figure 4.5 shows images of Au dots for all five BCPs. The smallest Au dots (~18 nm diameter) are obtained from C67 whereas the largest Au dots (~105 nm diameter) are obtained from L1051. All the other Au dot diameters are in between these values (see Table 4.2). The measurements of the features changed slightly by 5-10% difference as depicted by SEM images. Some sections have one or more mislaid Au dots as seen in Figure 4.5 (a). This absence may be due to over sonication resulting in removal of some Au dots. Figure 4.5 (d) has Au dots with an imperfect and less round shape. This imperfection could be because of the holes edges were rougher due to incomplete homopolymer resegregation during the thermal annealing step after solvent vapor annealing. The L1051 BCP was also thicker than the other BCPs I studied, and with 10 nm Au/Cr deposition, lift-off resulted very quickly within 2 min. For the L1051 BCP Au deposition thicker than 10 nm would be feasible in future
studies. **Figure 4.5 (f)** shows a tilted 65° view of a Si surface with Au dots after the lift-off of the L625 BCP. The final Au dots have a disk shape with a height around 10 nm as verified by SEM and AFM height analysis. The Au dots are disk shaped, supporting the inference that the PMMA self-assembled into vertical cylinder-like shapes. After deposition of Cr and Au and the lift-off step, the feature diameter decreases (**Table 4.2**). This is likely because the PMMA cylinders in the BCP may not have a constant diameter from the top to the silicon substrate. The center-to-center distance between adjacent feature pairs changes by less than one standard deviation between plasma etching and metal film lift-off.

**Figure 4.5** SEM images of top views of Au dots after Cr/Au deposition and lift-off for (a) C67, (b) C99, (c) C207, (d) L1051, and (e) L625 (f) 65° tilt image of Au dots for L625.

I performed AFM imaging to study the DNA templates before placing them on Au dot surfaces. As shown in **Figure 4.6 (a)**, the height of the DNA origami was ~2 nm as seen from the white color, compared to the background Si surface in brown. However, it was difficult to see the Au dots and bar DNA together in AFM due to the contrast in the image color for the height difference between Au dots (10 nm) and DNA (2 nm). I attached DNA-coated Au NRs on the DNA origami to clearly show in SEM where DNA deposits on the surface. **Figure 4.6 (b)** shows
Au NRs attached to bar DNA origami (∼400 nm) on a Si surface and aligned along the DNA origami. These data confirm that the DNA template formed well, and that DNA-coated Au NRs hybridized well with the protruding sticky ends from the DNA origami, supporting these processes in subsequent experiments.

Figure 4.6 Bar DNA origami. (a) AFM image on Si; height scale is 2 nm, and (b) SEM image on Si after hybridization of DNA-coated Au nanorods.

I selected L625 and L1051 BCPs as Au dot surfaces to study DNA origami attachment because they have large enough distances between the Au dots to place bar DNA origami between only 2-3 Au dots, and they have larger individual Au dot surface areas for DNA attachment. I used thiol-Au interactions to attach functional groups, which direct DNA origami onto the Au islands. I studied two ligands to functionalize the Au islands for electrostatic attachment of DNA origami. First, I used 3-MPA to form a carboxylate-terminated self-assembled monolayer (Figure 4.7 (a)) that was treated with Mg\(^{2+}\). The electrostatic interactions between carboxylate groups and Mg\(^{2+}\) should promote ionic attraction of the negatively charged DNA origami to the surface pattern.

Figure 4.7 (b) shows SEM data for Au dots created from the L625 BCP coated with 3-MPA and
Mg$^{2+}$, then treated with DNA origami and further reacted with DNA-coated Au NRs that hybridize to sticky ends on the DNA origami, following the same treatment shown in Figure 4.6 (b). Au NRs appear to be located on and between the Au dots. The data indicate that some DNA origami are on the Au dots and bridging between them, as shown through some Au NRs that follow a DNA origami pattern. There are also non-specifically attached Au NRs on the Si surface, not clearly associated with Au islands.

**Figure 4.7** DNA origami attachment on Au dots through ligands. (a) Schematic diagram of attaching DNA origami using 3-MPA. (b) SEM image of DNA-coated Au NRs attached to bar DNA origami on Au islands functionalized with 3-MPA and Mg$^{2+}$. (c) Schematic diagram of attaching DNA origami using MUTAB/DDT. (d) SEM image of DNA-coated Au NRs attached to bar DNA origami on Au islands functionalized with MUTAB/DDT.

I also used MUTAB to functionalize the Au dots to provide a direct positive charge to attract DNA origami (**Figure 4.7 (c)**). Because MUTAB contains a bulky positively charged group that creates repulsion and may limit monolayer coverage, I used DDT to provide space between the bulky groups and reduce the repulsion. **Figure 4.7 (d)** shows Au dots created from the L625 BCP treated with MUTAB/DDT and then coated with DNA-covered Au NRs hybridized to DNA origami structures. As seen in **Figure 4.7 (d)**, DNA-coated Au NRs connect between islands,
presumably following the DNA templates. There were also some instances where two Au NRs attached side-by-side, probably on a DNA template. More nonspecifically attached NRs are on the Si surface in Figure 4.7 (b) than in (d). A likely explanation for this difference is that Mg$^{2+}$ could also interact with surface silicon oxide groups and attract the DNA origami or DNA-coated Au NRs onto that region. Both Figure 4.7 (b) and (d) show the DNA-coated Au NRs attached to Au dots, consistent with how they appear when attached to DNA origami in Figure 4.6 (b). Thus, I carried out additional experiments to test if the DNA attachment is related to random deposition of DNA on Au dots or the deposition of DNA directed by the Au dot functionalized ligands.

To better understand the interaction between DNA and the ligands on the Au dots, I studied samples of DNA-functionalized Au NPs on MUTAB/DDT functionalized Au dots, as seen in Figure 4.8 (a). The Au NPs can be seen both on the ligand-modified Au dots as well as on the Si surface. I hypothesized that there could be residual polymer material on the surface that also attracted DNA coated Au NPs. I thus used plasma treatment to remove organic content from the surface with subsequent thermal annealing to remove gold oxide from the Au dots following a published method, before the thiol ligand functionalization. Then I treated the surface with Au NPs using the same conditions as in Figure 4.8 (a). After plasma treatment, annealing and functionalization (Figure 4.8 (b)), there is less nonspecific attachment of DNA-coated Au NPs on the Si but also less specific attachment of DNA-coated Au NPs on the Au dots compared to Figure 4.8 (a). Using Equation 4.1, I determined the surface density of Au NPs on the Au dots relative to the Si in Figure 4.8 (a) and (b) to be 2.7 and 1.6, respectively. I concluded that the plasma treatment and annealing decrease the likelihood of Au NP placement on the Au dots, or that the ligand functionalization was not as efficient on the plasma cleaned and annealed Au dot surface as it was before.
Figure 4.8 SEM images of surfaces created with DNA-coated Au NPs on MUTAB/DDT functionalized Au dots. (a) As prepared MUTAB/DDT functionalized Au dots after DNA-coated Au NP treatment. (b) MUTAB/DDT functionalized Au dots that underwent plasma and heat treatments before localizing DNA-coated Au NPs.

Because I designed the bar DNA origami with protruding poly-A DNA sequences as described in Section 4.2.3, I could hybridize it to thiolated poly-T strands for covalent linkage to Au dots, and then poly-T coated Au NRs could be localized through hybridization to the DNA template. I used a substrate with Au dots made from BCP L1051 because the center-to-center distance between adjacent Au dots is the largest (~220 nm), so one bar DNA origami should localize between only 2-3 Au dots. Bar DNA origami hybridized to DNA-linked Au dots were seeded with DNA-coated Au NRs and characterized by SEM (Figure 4.9). The images show some Au NRs that appear aligned over distances consistent with a ~410 nm long DNA template localized on top of the Au dots (Figure 4.9 (a), (b), (e), (f), and (h)), whereas in other instances Au NRs localized along the edges of the Au dots (Figure 4.9 (a), (d), (e), (f), (g), (h) and (i)). The bar DNA attachment yield was higher in this method compared to electrostatic interaction, indicated by the

\[
\frac{\text{Number of Au NPs on Au dots}}{\text{Au dot area}} > 1
\]

\[
\frac{\text{Number of Au NPs on background}}{\text{Total area} - \text{Au dot area}} > 1
\] (Equation 4.1)
ratio of DNA attachment per Au dot of 0.20 (± 0.10, from 12 SEM images) for DNA hybridization vs. a ratio of 0.08 (± 0.04, from 12 SEM images) for both electrostatic placement methods.

Additional experiments were performed to confirm the location of DNA origami attachment on the surface using a Au dot substrate where unintentionally, the surface had some areas with Au dots and some areas that did not contain Au dots. Figures 4.10 (a) and (b) show zoomed-out and zoomed-in views, respectively, of an area with partial lift-off, after placement of DNA origami and seeding with DNA-coated Au NRs. The DNA-coated Au NRs, likely hybridized to bar DNA origami, are seen on and between the Au dots. In contrast, Figure 4.10 (c) shows no attachment of Au NRs after the same DNA origami placement process on an area where unintentional lift-off resulted in no Au dots on the surface. This result clearly confirms the need...
for Au dots on the surface for DNA directed placement demonstrating that the DNA origami and Au NR localization is driven by Au dot interactions.

![Figure 4.10 SEM images of DNA coated Au NRs seeded onto DNA origami structures hybridized to Au nanodots. (a) Zoomed-out and (b) zoomed-in view. (c) No attachment of Au NRs was seen where there were no Au dots (d) zoomed-in view of a similar area as (c).]

The work presented in this chapter thus opens new possibilities for depositing DNA origami at directed surface locations made from BCP self-assembly. An important development is the placement of DNA origami using Au-thiol interaction on Au dots created by BCP lift-off. This finding offers a route to the fabrication of nanoelectronics and plasmonic sensor devices. There is also potential for scaling up through combining the complementary self-assembly methods of BCP nanolithography and DNA hybridization to connect nanodots electrically with top down patterning.

4.4 CONCLUSION

This chapter describes a method to deposit DNA origami on patterned surfaces using BCP thin films as a patterning mask. The results demonstrate that BCP thin films are suitable for simplified nanofabrication to form periodic features. I further showed different diameters and center-to-center distances in forming Au dot arrays using five different BCPs. Gold nanodots
formed from BCP nanolithography allow DNA origami to be selectively placed at directed surface locations. I utilized electrostatic interactions with two different thiolated carbon linkers, which provide either a negatively charged carboxylic group interacting with Mg\(^{2+}\) ions or a positively charged group to attract the negatively charged phosphate backbone of DNA. I further used DNA hybridization to attach thiolated DNA strands and successfully place DNA origami onto Au nanodots. DNA-coated Au NRs were attached onto the DNA origami to indicate in SEM images where the DNA localized on the Au dot surface. This method is promising, with a need for subsequent research on obtaining sufficient seeded structures to fully cover Au dots in an area. Coupled with electroless plating, this approach should enable the fabrication of electrically relevant nanostructures at designed locations.

Future work should focus on optimizing the concentration of thiolated DNA strands for improved placement of DNA origami on the Au dot substrate and improving DNA-coated Au NR seeding on DNA. Increasing the concentration of thiolated DNA strands for Au dot localization would allow a simpler workflow through using CTAB-coated Au NRs to attach electrostatically and show the locations of DNA origami placement. Furthermore, semiconductor nanorods and other materials should be incorporated into these DNA structures to fabricate electrical components such as transistors. My work opens the way for placement of DNA in target locations, facilitating electronic nanomaterial deposition to address a range of scientific needs in nanoelectronics and photonics. This process may be further expanded to the placement of nanomaterials like carbon nanotubes or semiconducting nanowires onto DNA origami, enabling the formation of ordered and dense arrays of functional devices and their connection into circuits.
4.5 REFERENCES

(13) Rubio-Sánchez, R.; Fabrini, G.; Cicuta, P.; Di Michele, L. Amphiphilic DNA nanostructures for bottom-up synthetic biology. Chemical Communications 2021, 57 (95), 12725-12740.
(17) Zeng, Y.; Chang, P.; Ma, J.; Li, K.; Zhang, C.; Guo, Y.; Li, H.; Zhu, Q.; Liu, H.; Wang, W.; et al. DNA Origami–Anthraquinone Hybrid Nanostructures for In Vivo Quantitative Monitoring of the Progression of Tumor Hypoxia Affected by Chemotherapy. ACS Applied Materials & Interfaces 2022, 14 (5), 6387-6403. D


CHAPTER 5: CONCLUSIONS AND FUTURE WORK

5.1 CONCLUSION

DNA nanofabrication is a promising technique that allows for manufacturing useful devices with nanometer dimensions. However, several questions need to be addressed to proceed from nanofabrication to nanomanufacturing.¹ In this dissertation, I described three ways that contributed to addressing challenges associated with DNA nanofabrication. First, I demonstrated the metallization of DNA nanotubes and their electrical characterization in Chapter 2. By developing seeding, electroless plating, metal evaporation, electron beam-induced deposition, and two-point and four-point probe station measurements, I was able to measure resistance values for metal nanowires formed on DNA nanotubes. In Chapter 3, I discussed experimental work on improving the seeding and plating of DNA origami templates much smaller than the nanotubes in Chapter 2. This work helped me understand materials and conditions for optimal seeding and plating of bar origami DNA, which enabled subsequent study of their thermal annealing and electrical properties. Finally, in Chapter 4, I organized these metallized nanowires in periodic arrays using the self-assembly properties of block copolymers (BCP), and electrostatic and DNA hybridization localization on Au nanodots. As a whole, this dissertation advances DNA assembly to create templates, seeding to deposit nanomaterials to make a desired shape, plating to build connections between nanomaterials, using BCPs to place nanostructures in ordered assemblies, and electrically characterizing the metallized structures. This work provides a strong basis for next steps in the field of bottom-up self-assembly and future nanoelectronics.
5.2 FUTURE WORK

5.2.1 DNA Nanotube Metallization

Schulman’s group\(^2\) has shown the creation of DNA nanotube connections over distances of 10 \(\mu\)m. Combining their work with my research on DNA metallization described in Chapter 2, these DNA nanotubes have the potential to be metallized in two different ways to form continuous conductive wires (**Figure 5.1**). Seeding of 10 \(\mu\)m self-connected DNA nanotubes with Au nanorods and electroless plating (see **Figure 5.1 (a)**) is one step to move my Chapter 2 study forward. Au nanowires can also be grown inside of hollow DNA structures (**Figure 5.1 (b)**). Bayrak et al.\(^3\) showed the synthesis of Au nanowires inside a DNA origami mold, and connected multiple molds to create a longer nanowire. DNA nanotubes could create a smoother nanowire with less granularity than seeding and plating Au nanorods because it could contain fewer grain boundaries due to continuous growth of Au. Finally, DNA nanotubes can be denatured post-metallization to leave only the metal nanowire. Also, because DNA nanotubes can bridge two distant locations, they could electrically connect cells or drug delivery vehicles after metallization.

![Figure 5.1 Self-assembled DNA nanotube connectors to create metallized nanowires.](image)
5.2.2 DNA Origami Seeding and Metallization

Improved seeding on DNA origami using CTAB-coated Au nanorods that I developed in Chapter 3 could be beneficial for applications that do not require complex arrangements, like transparent conductive electrodes. The Au nanorod-seeded DNA origami could be fabricated on a transparent polymer to make conductive nanowire connections. It could be important for optoelectronic devices including electronic displays, solar cells and LEDs. Individual metallized structures need to connect with other functional components to get their full use; my Chapter 3 advances make that feasible by making it possible to observe how thermal annealing affects those fabricated nanostructures.

5.2.3 DNA Origami on BCP Surfaces

My work related to DNA origami placement could be improved by designing a new DNA origami template that matches the Au dot surface dimensions; it could be triangular, square, or rectangular. An example is illustrated in Figure 5.2. Thiolated DNA overhangs with distinct sequences and lengths would need to be incorporated into the template. Furthermore, the orientational control of DNA placement could be achieved by having multiple sticky ends with different base sequences in designed positions on two different DNA origami (see Figure 5.2 (c)). Orientational control is essential for large, ordered arrays of functional nanodevices.
Figure 5.2 Schematic of tile DNA origami placement on a Au nanodot array. (a) DNA origami assembly. (b) Two different DNA origami. (c) DNA origami assembled on a Au nanodot array.

Studying a large amount of data from different DNA origami assembled on Au dot surfaces from multiple BCPs could lead to a predictive model for choosing a BCP and controlling DNA origami placement. Machine learning widely used in research to help predict future results, potentially making the process faster and more accurate.5

I demonstrated seeding of Au NRs on DNA origami. However, this work can be extended to semiconducting materials, like Te,6 CdS,7 or carbon nanotubes,8 to create junctions or provide functional electronic sensing elements. Combining ongoing efforts from my lab colleagues to develop methods for synthesizing and attaching Te or CdS to DNA should make possible integrating these semiconducting nanomaterials into DNA placement on Au dot arrays made from BCP self-assembly.

The number of annual publications related to DNA nanofabrication has increased from one in 1995 to 120 in 2021, indicating that DNA nanofabrication has progressed significantly. DNA nanofabrication should continue to advance with reduced costs, increased capabilities for large-scale production, and improved nanoscale positioning precision. The work I describe here could
be further developed and integrated with advances in various fields, including medicine, optics, and material science, to guide a complex end products in multidisciplinary research.

5.3 REFERENCES