2013-09-05

Self-Assembled DNA Origami Templates for the Fabrication of Electronic Nanostructures

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Self-Assembled DNA Origami Templates for the Fabrication of Electronic Nanostructures

Elisabeth P. Gates

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

Self-Assembled DNA Origami Templates for the Fabrication of Electronic Nanostructures

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Doctor of Philosophy

An important goal of nanoscience is the self-assembly of nanoscale building blocks into complex nanostructures. DNA is an important and versatile building block for nanostructures because of its small size, predictable base pairing, and numerous sequence possibilities. I use DNA origami to design and fold DNA into predesigned shapes, to assemble thin, branched DNA nanostructures as templates for nanoscale metal features. Using a PCR-based scaffold strand generation procedure, several wire-like nanostructures with varying scaffold lengths were assembled. In addition, more complex prototype circuit element structures were designed and assembled, demonstrating the utility of this technique in creating complex templates.

My fabrication method for DNA-templated nanodevices involves a combination of techniques, including: solution assembly of the DNA templates, surface orientation and placement, and selective nanoparticle attachment to form nanowires with designed gaps for the integration of semiconducting elements to incorporate transistor functionality.

To demonstrate selective surface placement of DNA templates, DNA origami structures have been attached between gold nanospheres assembled into surface arrays. The DNA structures attached with high selectivity and density on the surfaces. In a similar base-pairing technique, 5 nm gold nanoparticles were aligned and attached to specific locations along DNA templates and then plated to form continuous metallic wires. The nanoparticles packed closely, through the use of a high density of short nucleotide attachment sequences (8 nucleotides), enabling a median gap size of 4.1 nm between neighboring nanoparticles. Several conditions, including hybridization time, magnesium ion concentration, ratio of nanoparticles to DNA origami, and age of the nanoparticle solution were explored to optimize the nanoparticle attachment process to enable thinner wires. These small, branched nanowires, along with the future addition of semiconducting elements, such as carbon nanotubes, could enable the formation of high-density self-assembled nanoscale electronic circuits.

Keywords: [DNA origami, nanofabrication, self-assembly, gold nanoparticles]
I would first like to thank Brigham Young University and the Department of Chemistry and Biochemistry for providing a wonderful learning environment. I would like to thank my advisor Dr. Adam T. Woolley for his mentorship and example. I have enjoyed my research project and have learned much over the past several years. Thank you for the excellent research advice, guidance, and encouragement. I would also like to thank Dr. John N. Harb, Dr. Robert C. Davis, and Dr. Matthew R. Linford for the many questions they posed, the enlightening chalkboard discussions, and the criticism and advice they provided.

I would like to thank all the many students who worked with me on designing and assembling DNA origami structures, including: Jeff Ashton, Ben Cragun, Garrett Cox, Mike Lydiksen, Matt Rowley, Kenny Lee, Garth Lee, Sudeep Subedi, James Havican, Matt Halbert, Taylor Brown, Andrew Dearden, and John Jensen. Thank you for providing much appreciated assistance, repeatedly doing bead separations for DNA origami, and tackling questions about designing and assembling DNA origami structures, surface placement, linking DNA structures, and “magical” DNA structures. I would like to thank all the students who worked with me on the ASCENT project, especially Emily Nelson, John Hickey, and Shailendra Gyawali.

I have been very fortunate to be able to work with so many students throughout my time as a graduate student. I appreciated the laughter, singing, and occasional pipet stacking game. I enjoyed doing outreach demonstrations and activities, and thank Emily Archibald and Brian Davis for all their work in planning and preparing for those activities.

I would like to express appreciation for the graduate students I worked with on my project: Hiram Conley, Kyle Nelson, Tony Pearson, Jianfei Liu, Yanli Geng, Brian Davis, Nitesh Madaan, and Bibek Uprety. Specifically, I would like to thank Hiram Conley for excellent AFM
training and many helpful discussions about the ASCENT project, Nitesh Madaan for helpful insights into thiols and gold, and Brian Davis for his knowledge about carbon nanotubes. I would especially like to thank Jianfei Liu, Tony Pearson, Yanli Geng, and Bibek Uprety for the many hours spent working together on DNA templated nanostructures. I am also grateful for the assistance of the members of the ATW lab group for their discussions, helpful insights, and presentation feedback.

Finally, I would like to thank my family and friends for their support and encouragement through the successes and frustrations of research. I am very grateful for my loving husband, Richard, for his constant encouragement and understanding.
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CHAPTER 1: INTRODUCTION

1.1 STRUCTURAL DNA NANOTECHNOLOGY

1.1.1 The Basic Principles

Over the past several decades, the field of structural DNA nanotechnology, or the study and use of DNA to build nanostructures, has grown and evolved to meet challenges in controlling and precisely positioning materials on the nanoscale. Structural DNA nanotechnology focuses on using DNA as a structural component as well as the glue with which to assemble nanostructures.

DNA can be utilized as a versatile building block in the assembly of nanostructures because of its small size, predictable base pairing, and numerous sequence possibilities. Using DNA to form larger constructs takes advantage of the spontaneous self-assembly of the double helix. In addition, there are established and easy protocols for handling, digesting, and replicating DNA. An individual DNA double helix is useful as a linear building block, but more importantly, DNA can form a range of branched structures by crossing DNA strands from one helix to another. Ned Seeman recognized the potential for building complex structures from DNA when he published his influential theoretical paper in 1982 of an immobile, six arm DNA junction for building three-dimensional arrays.\(^1\)

Seeman and coworkers subsequently developed the background and basics of making synthetic immobile DNA crossovers.\(^2-4\) A single crossover, called a Holliday junction, is seen in nature during homologous recombination. By choosing synthetic DNA sequences that have minimal sequence symmetry within the strand, these junctions can be made immobile and used as a branch-type building block (Figure 1.1A–B). Multiple crossovers can occur between two parallel double helices to form a stiffer component and build arrays or other structures (Figure
1.1C) Single-stranded overhangs at the end of a double helix, called “sticky ends”, are used to connect the individual branched building blocks together. By selectively choosing and synthesizing the required DNA strands, one can obtain predictable and even complex nanostructures.

1.1.2 The Beginning

The idea of using branched DNA to build structures was first realized in the form of simple crossovers and wireframe-type three-dimensional structures. In 1991, Chen and Seeman demonstrated that DNA could be used to outline the edges of a cube with branched junctions on the corners. Soon, a truncated octahedron, along with other knots, rings, and two-dimensional arrays added to the collection of DNA structures. Tetrahedra, an octahedron, and various nanotubes followed. DNA with a double or triple crossover motif proved to be more rigid and allowed for better formation of two-dimensional DNA arrays. These arrays were made by linking DNA units, or tiles, with parallel strands containing either double or triple crossovers, together into an array with alternating units. Some early nanodevices were also created with lateral movement or rotation made possible through structural transitions within the devices.

Figure 1.1. DNA branch junctions. (A) A single crossover branch. (B) Four DNA branches linked together. Arrows indicate the 3’ end of the DNA strands. Overhangs, or “sticky ends” base pair together to form the larger constructs. (C) A double crossover molecule. The double crossover makes the building block more rigid.
1.1.3 Arrays and More

There are many different types of motifs that can be utilized in forming two-dimensional DNA arrays. Unit tiles with planar motifs were used to create one and two-dimensional arrays as well as tubes.\textsuperscript{16, 20} Branched star or cross-shaped motifs were used to create arrays with diamond, square, triangular, or hexagonal cavities.\textsuperscript{16, 21-22} Triangular motifs were used to create two-dimensional lattices with pseudo-hexagonal holes.\textsuperscript{23} DNA helix bundles were also used to create DNA tubes and to assemble two-dimensional lattices.\textsuperscript{16, 20}

One way to form these lattices is to combine all the DNA sequences together and let them base pair in a simple one step process. Each strand only binds to a specific partner strand or to a specific location in a tile. Initially, it would appear that larger constructs would need more and more unique DNA strands; however, this is not necessarily the case. By utilizing symmetry in the design, and even within the building unit, the number of different DNA sequences can be minimized while still maintaining the reduced symmetry within each DNA strand needed for immobile, proper formation.\textsuperscript{3, 24} “Sequence symmetry” also reduces the number of undesired distortions, thus allowing larger arrays to form.

Lattices with more complexity, or finite arrays, can be achieved by using a multistep, or hierarchical, approach to assembling the unit tiles.\textsuperscript{16} Another way to assemble tiles into complex patterns is to use a cooperative, algorithmic binding approach. This has been demonstrated, using DNA rectangular tiles with four sticky ends, to create very complex patterns.\textsuperscript{16, 20} In all these cases, DNA strand purity, stoichiometry, and careful design of the DNA sequences are important considerations.

The primary application for these DNA nanoarrays is as a scaffold to orient and organize other molecules on a very precise scale. They could be used to organize and orient proteins to
enable easier crystallization for greater structural understanding, to give distance control to gain new insights into the interactions between proteins, or even to control their reactivity. Using these two-dimensional arrays, proteins have been positioned using the streptavidin-biotin, antigen-antibody, aptamer-protein, and DNA-binding protein interactions.16, 20

In addition to organizing proteins, these two-dimensional arrays could be used as a scaffold to organize other small nanocomponents for nanoelectronics or sensors. Gold nanoparticles have been organized into one and two-dimensional arrays using one of the following techniques: streptavidin coated gold nanoparticles reacting with biotinylated DNA in the array, thiolated DNA attached to gold nanoparticles base-pairing with DNA in the array, or direct attachment of the gold nanoparticles to thiolated DNA in the array.16, 20 Quantum dots have also been organized into arrays using streptavidin-biotin binding.25 These assemblies pave the way for more complex arrays with exciting and interesting properties in nanoelectronics and nanophotonics.

1.2 DNA ORIGAMI

1.2.1 The Idea

The first DNA structures were constructed entirely from short, synthetic strands combined in careful stoichiometries, with more complex structures often assembled through sequential steps or hierarchical techniques. In one paper, a longer, synthetic strand, made by ligating several smaller strands together, was used as a scaffold to bring DNA tiles together into a barcode pattern.26 Another group reported the use of a natural DNA source, a 1.7 kilobase sequence, used with 5 short synthetic strands to fold into an octahedron.13

In 2006, Paul Rothemund published a general DNA folding technique that has become known as DNA origami.27 In this technique, a shape is chosen and the DNA is designed to raster
fill throughout the two-dimensional structure. This gives the backbone, or “scaffold,” of the DNA structure. A long single strand of DNA is used as this scaffold and held into place with smaller, complementary oligonucleotides. These small pieces are designed to bridge from one part of the scaffold to another, “stapling” different parts of the scaffold together, as outlined in Figure 1.2. Once the structure is designed, it is assembled by mixing all the DNA strands together in a buffer solution, heating the solution to break any bonding between base pairs, and then slowly cooling the solution over about an hour to allow the maximum number of bases to self-assemble. Rothemund showed the generalization of this technique by using the same scaffold strand to fold a variety of different two-dimensional shapes, including rectangles, triangles, and smiley faces.27

DNA origami provides several advantages over tile-based assembly, such as an easy, one-pot process for assembling large (~100 nm x 100 nm) structures, high yields, and elimination of the need to carefully design and minimize symmetry in the small DNA sequences. In this scaffolded assembly, the staple strands are added in excess, so precise stoichiometry is not critical to the assembly and the staple strands do not need to be purified. In addition, Rothemund demonstrated that with DNA hairpins built into the staple strands, each staple strand location could be used as a distinct location for attachment, with about 6 nm spacing.27 This level of addressability, which is difficult to obtain in tile-based DNA assembly, is possible because each
staple strand is a unique DNA sequence. These advantages make DNA origami an appealing and exciting tool in designing DNA nanostructures.

The technique of DNA origami does, however, have some limitations. The scaffold strand is generally too long to be synthesized and originates from viral or plasmid genomes with known sequences, limiting its sequence and length to naturally occurring sources. Techniques have been demonstrated to allow for the formation of single-stranded scaffolds of predetermined lengths through PCR amplification\textsuperscript{28} or ligation of synthetic strands,\textsuperscript{26} as well as folding of DNA origami with double-stranded DNA scaffolds\textsuperscript{29-30} as steps toward widening the pool of potential scaffolds and controlling the sequence. The cost of synthesizing staple strands is also a concern, because each staple strand has a unique sequence.

1.2.2 Two-dimensional DNA Origami Structures

Many two-dimensional DNA structures have been designed using the folding technique of DNA origami. Some of these include a dolphin,\textsuperscript{31} a map of China,\textsuperscript{32} and a variety of thin, branched DNA structures (as further described in Chapter 2).\textsuperscript{28} Rothemund and others have demonstrated the ability to assemble even larger constructs by linking multiple DNA origami structures together using the staple strands. DNA origami triangles were linked together to form hexagons, trapezoids, or arrays\textsuperscript{27} and the dolphins could be linked into dimers.\textsuperscript{31} DNA origami structures can also be linked into long chains or large two-dimensional arrays.\textsuperscript{33-34}

It is important to note the necessity of good computer programs to help in the design of these DNA origami structures. A few DNA design programs were available,\textsuperscript{35-37} but the first openly available design tool for DNA origami was the program SARSE-DNA origami.\textsuperscript{31} A nice feature of the SARSE-DNA origami design program is the ability to import a bitmap image of the desired shape and have the program automatically fill in the scaffold folding path.
1.2.3 Three-dimensional DNA Origami Structures

While some three-dimensional structures like the cube, truncated octahedron, tetrahedra, and various nanotubes were made from DNA without using the DNA origami approach, many more three-dimensional structures have been designed and assembled using the technique of DNA origami. The first of these included six-helix bundles, wire-frame structures built from DNA origami six-helix bundles, or closed structures built by using two-dimensional DNA origami to form the sides of the shape. Examples of closed structures include boxes, prisms, and a tetrahedron.

Soon, a computer design tool, caDNAno, was developed to help expand the technique of DNA origami into three dimensions with multiple DNA layers stacked on top of each other. The design tool allows for a honeycomb or square base for the three-dimensional DNA layering (Figure 1.3). With the honeycomb base structure, shapes such as monoliths, square nuts, railed bridges, slotted and stacked crosses, and genie bottles were assembled.

These structures took longer to assemble (up to a week) in the solution hybridization step and required careful attention to the concentration of mono and divalent cations in the buffer solution. The square lattice base allowed for more dense three-dimensional DNA origami structures (as well as a nice design tool for flat, two-dimensional structures). Structures with twists and bends can also be
constructed using CaDNAno, by choosing to remove or add bases along the scaffold in-between DNA crossover positions. These adjustments add or remove strain within the DNA strands and result in a global twist or bend in the structure.\textsuperscript{47-48} Three-dimensional structures with hexagonal close-packing and hybrids of the honeycomb, square, and hexagonal close-packing have also been demonstrated, although the design steps still require manual placement until caDNAno is updated to allow for these new packing lattices.\textsuperscript{49} These and other future developments have continued to increase the ability to design many different kinds of three-dimensional DNA nanostructures.

1.2.4 Advances and Applications of DNA Origami

DNA origami has been utilized for a variety of different things, but most of them fall into three categories. The first is using DNA as a building material and learning more about how to precisely control its structure in two and three dimensions. The second category is using DNA origami as a pegboard with which to organize or precisely position nanomaterials. The last category is as a mechanical device with moving parts. Each of these areas will be individually explored further below.

\textbf{Structural Advances.} Many more techniques have been published for folding DNA that continue to expand the design space available to researchers in DNA nanotechnology, and others have searched to understand the DNA folding process more thoroughly. In building two-dimensional structures, DNA origami has been used as a seed layer to begin algorithmic assembly of tiles to create aperiodic assemblies.\textsuperscript{50-51} Larger two-dimensional constructs have also been designed by linking DNA origami together like puzzle pieces,\textsuperscript{52-53} by using DNA tiles in place of the regular staple strands,\textsuperscript{54} or by using a preformed scaffold strand to organize individual shapes in a DNA origami of DNA origami fashion.\textsuperscript{55}
In the three-dimensional realm, Liedl et al.\textsuperscript{56} showed that individual, three-dimensional domains could be held together at various angles by single-stranded DNA connecting the domains. The tension in the single-stranded DNA holds these tensegrity structures together. Three-dimensional structures with curves, such as a sphere, ellipsoid, and nanoflask,\textsuperscript{57} have been folded, demonstrating that nanostructures are not limited to rigid edges or patterned lattice structures. This technique has also been used to create reconfigurable rings.\textsuperscript{58} Recently, a technique of building two-dimensional and three-dimensional wire-frame structures has also been developed.\textsuperscript{59} In this technique, the DNA scaffold folds back and forth horizontally as well as vertically, with small staples helping to hold the scaffold into the four-branched crossings. Another recent development for assembling a variety of different three-dimensional structures works by choosing to include or not include small DNA strands within a predesigned canvas, demonstrating a method to automate the process of arbitrary three-dimensional DNA structure assembly. This technique moves away from scaffolded DNA origami and only utilizes short DNA strands, but offers a way to design many different structures using the same set of DNA strand sequences.\textsuperscript{60}

Some techniques and experiments have been performed in an effort to improve and understand the DNA folding process. The program CanDo allows users to submit their DNA origami designs from caDNAno and gives three-dimensional structure and flexibility information.\textsuperscript{61} This is a useful tool to check DNA designs for global twisting or weak points. Studies of the DNA origami folding process have enabled the folding of DNA structures from double-stranded DNA scaffolds,\textsuperscript{29-30} other DNA scaffolds besides the typical M13mp18 viral strand,\textsuperscript{28-30, 39} folding at room temperature in formamide,\textsuperscript{62} as well as a recent demonstration of isothermal folding of DNA structures that drastically reduces the amount of time required to fold
three-dimensional structures. Another study looked at the effect of DNA hairpins on the overall twist of DNA origami structures. Ke et al. looked at the effect of designing underwound three-dimensional DNA origami structures combined with intercalating molecules, such as ethidium bromide, as well as staple strand break positions to increase the yield of three-dimensional structures.

**Material Organization.** DNA origami structures, like DNA tile structures, have been used to organize and precisely position a variety of different nanocomponents, including: proteins, nanoparticles, quantum dots, and carbon nanotubes. With the simple technique of using single-stranded sticky ends on selected staples, hybridization studies have been performed, which utilize DNA origami as a template for DNA and RNA detection. DNA nanostructures can be decorated with proteins using many different methods. These include non-covalent interactions such as streptavidin-biotin and aptamer-protein, as well as a variety of different covalent interactions. These proteins can be positioned in lines or various other geometries because of the staple strand addressability of DNA origami. In addition, multiple different proteins can be placed onto the same template.

There are as many different applications for organizing proteins with DNA as there are potential ways to link them together. Assembling proteins in arrays on DNA origami can facilitate single-molecule protein detection and facilitate studies to better understand protein structure or multivalent protein binding. Linear attachment of enzymes could allow for cascade reactions that are not limited by diffusion and have reduced undesirable side reactions. Fluorescent proteins could be used to make light-addressable photonic devices.

The ability to precisely position with high accuracy opens up the possibility of using DNA origami templates for single-molecule chemical reactions or fluorescence studies.
Chemical modifications on DNA staple strands allow for control of the location of the chemical reaction. Using DNA origami, reactions involving bond formation, bond cleavage, photochemical, and click reactions have been demonstrated. Atomic force microscopy (AFM) is used in these examples to visualize the reactions. In studies where a conformational change or height change on a DNA origami template is not possible or feasible, fluorophores can be used to visualize the reaction, such as in energy transfer studies or in kinetic experiments where high-speed AFM is not fast enough.

DNA origami, in addition to patterning biological and organic molecules, has been used to organize inorganic nanoparticles and carbon nanotubes. Examples of positioning nanoparticles include gold and silver nanoparticles, as well as quantum dots. The gold nanoparticles were connected to the DNA origami structures through DNA modified with one or more thiol groups on the end. To position the nanoparticles, either the staple strands contain a modification or the staple sequence is extended to contain extra complementary bases that pair with the modified DNA strands. The silver nanoparticles were attached in a similar manner, except the modified strands contained phosphorothioated DNA, which has sulfur atoms replacing oxygen along the backbone of the DNA strand, instead of as an end modification. Quantum dots were attached to the DNA origami structures by streptavidin modification of the quantum dots and biotin modification on the end of the desired staple strands. An alternative method of patterning is to grow gold nanoparticles onto DNA origami templates using an inorganic-binding peptide to direct the nucleation and growth of the nanoparticles. DNA origami, in combination with surface patterning, also allows for larger nanoparticles arrays. Or, if the surface is patterned with gold nanospots, DNA origami can be organized on surfaces. The nanoparticles can also be used to roll the DNA nanostructures up into tubes. In addition to nanoparticles, carbon
nanotubes have also been oriented on DNA origami, either by wrapping DNA around the carbon nanotubes or through streptavidin modification of the carbon nanotubes.\textsuperscript{89-90}

Most of the examples mentioned so far use two-dimensional DNA origami as a sort of pegboard to place nanocomponents. Three-dimensional DNA origami structures can also be used to organize matter,\textsuperscript{38,81} but most of the research published to date uses three-dimensional DNA origami to capture, hold, and deliver nanoscale cargo. One example is the use of three-dimensional DNA origami structures to capture gold nanoparticles.\textsuperscript{91} Other examples of capture and delivery are found in the next paragraph. Several excellent reviews also highlight many of these nanoscale placement and organization applications.\textsuperscript{4,40,92-95}

Dynamic Devices. Another very interesting application for DNA origami overlaps with the area of dynamic DNA nanotechnology. In this area, researchers seek to use DNA to generate dynamic nanostructures with controlled movement. Nanoscale motors and molecular transporters are being explored. Several groups have developed different rotors or walkers\textsuperscript{96-98} that move along DNA origami templates\textsuperscript{99-100} or that transport nanoparticles as cargo.\textsuperscript{101} The DNA walkers are based on DNA toe-hold branch migration, where a target location for movement has a small single-stranded sticky end. The sticky end allows a complementary strand to make a few base pairs and then, if completely complementary to the rest of the sequence, it can nudge its way in until it has completely replaced the original complementary strand. Another example of a nanotransporter is the three-dimensional nanorobot reported by Douglas et al.\textsuperscript{102} that traps and carries gold nanoparticles or antibody fragments.
1.3 METALLIZATION OF DNA

1.3.1 Metallization on DNA strands

Figure 1.4. DNA metallization process. A) Metal ions are attracted to the backbone of DNA electrostatically. B) A reducing agent is used to reduce the metal ions to metal “seed” particles. C) Additional metal and reducing agent are added to grow the seeds through electroless plating.

A wide variety of metals, including silver, gold, copper, cobalt, nickel, palladium, and platinum can be selectively deposited along DNA to form conductive nanowires. The negatively charged phosphate groups in the backbone of DNA attract metal ions and the nucleotide bases can bind with positively charged metal complexes. A general process for metallization of DNA includes three basic steps, activation, seed formation, and plating, which are outlined in Figure 1.4. First, metal ions or complexes are reacted with and effectively deposited along the double helix in the activation step. Next, a reducing agent, such as hydroquinone, sodium hydroboride, or dimethylamine borane, is used to reduce the metal ions to metallic nanoparticles. These nanoparticles provide “seeds” for more metal
ions to be deposited and reduced along the DNA. In the third step, more metal ions and reducing agent are added to grow the nanoparticles. The reduced metal seeds serve as catalysts for the reduction of more metal. This process is often referred to as electroless plating. Different metals can be used in the activation and seed growth steps, enabling a wider variety of nanowires.

Selecting template-specific seeding techniques (the activation and seed formation steps) is an important part of DNA metallization because seeds formed non-specifically will also plate metal, creating undesired background metallization. Several techniques have been reported to increase the specificity of seeding through the use of reducing agents bound to the DNA template, such as aldehyde,\textsuperscript{108} or by using ionic surface masks.\textsuperscript{116} The entire DNA structure will become metallized when using metal ions or complexes for seeding, but it is also possible to protect certain sections from metallization with the use of aldehyde derivatized DNA and RecA protein.\textsuperscript{108} These and other advances show the potential of DNA as a template for metallized nanowires.

1.3.2 Metallization of DNA origami

Metallized DNA origami structures have recently been demonstrated, using the basic process described previously, with silver, gold, or palladium seeds with silver, gold, or copper plating.\textsuperscript{134-137} Several challenges occur with metallization of DNA origami templates that do not occur when metallizing linear DNA. One large difference in metallizing DNA origami structures comes from the staple strands, which are used in excess, to fold DNA origami structures. These excess small staple strands need to be removed before the activation and seeding steps, or they will contribute to non-templated metal growth. Removal of the excess staple strands also affects the stability of the DNA origami structures, making it more difficult to ensure the structures stay folded throughout the metallization process. In addition to the staple strands, the small
dimensions of the DNA origami structures, relative to long linear DNA strands, introduce the need for higher selectivity of metallization and methods to ensure the DNA structures do not lift off the substrate surface. These challenges were addressed by Liu et al. through filtering and crosslinking thin, branched DNA origami structures, as well as controlling the magnesium ion concentration.

In other examples of metallization of DNA origami, Schreiber et al. used 1.4 nm gold nanoparticles coated with positively charged amines as seeds for gold metallization of DNA origami structures, including: helix bundles, rings, cuboids, and polymerized DNA origami structures. Geng et al. demonstrated that gold metallization on DNA origami structures could be obtained with a fast and easy palladium seeding process. In a recent paper, conductivity of gold and copper plated, palladium seeded DNA origami structures was demonstrated. The gold metallized structures had an average resistivity of \(7.0 \times 10^{-5}\ \Omega\cdot\text{m}\) calculated from the wire dimensions. The copper structures had higher variability in resistance, in large part because of oxidation in air, but had a resistivity as low as \(3.6 \times 10^{-4}\ \Omega\cdot\text{m}\).

Selective metallization of desired portions of a DNA origami template is also possible, instead of metallizing the entire structure. One way to do this is with directed placement of gold or silver nanoparticles through thiolated DNA, as described in Section 1.2.4. The thiol groups attach to the gold or silver nanoparticles and, when linked to DNA or into a DNA structure, provide the seeds for further plating. This technique has been used to selectively seed and then deposit silver to form rings, bars, and H shapes on rectangular DNA origami structures. Gold metallization on nanoparticle seeded thin, branched DNA origami structures was also demonstrated and conductivity measurements obtained for linear gold nanowires. The
nanowires showed linear ohmic behavior with an average resistance of 2.4 kΩ per nanowire, and
a resistivity of 6.2 x 10^-6 Ωm calculated from the wire dimensions.

1.4 DNA NANOSTRUCTURES FOR ELECTRONICS APPLICATIONS

1.4.1 Trends in Computer Chips

In 1965, Intel co-founder Gordon Moore made an observation that the number of
components on an integrated circuit had approximately doubled every year since the first
integrated circuit.140 Ten years later he modified his prediction, which became known as Moore’s
Law, to be a doubling of components on integrated circuits every two years.141 This trend has
proven to be an accurate prediction for the last fifty years, in large part because the
semiconductor industry has used it as a goal in pushing forward in the development of integrated
circuits. In a way, the industry has striven to live by Moore’s law.

Moore’s law was initially accomplished simply by decreasing or scaling components to
smaller and smaller sizes. The scaling, along with increased density of transistors per chip, came
with some very desirable advantages, such as: faster speeds, decreased power usage, more
compact devices, higher functionality, and decreased costs.142 Photolithography, the main
fabrication technique used to create this complex circuitry, has become more complex and
expensive to use as the scale of circuits has decreased below microprocessor half pitches of ~30
nm.143 This is because of inherent limitations in the technique (wavelength limitations and resist
resolution) requiring more complex techniques and instrumentation to meet the demand of scale
and precision.144

While geometric scaling, like Moore’s Law, still continues in many aspects of integrated
chip manufacturing, “equivalent scaling” now also plays a large role. Equivalent scaling includes
improvements such as innovative design, better software, and new materials or structures that
ultimately increase the performance of integrated circuits without necessarily decreasing the component sizes. Another growing concept is functional diversification, or the idea of creating devices with a wide variety of functions that meet the needs of the end user instead of just focusing on the integrated circuit. This idea is promoting the incorporation of circuit board components onto the integrated chip in a system-in-a-package or system-on-a-chip fashion. This will enable the incorporation of non-digital functionalities such as RF communication, power control, or sensors into the same device, instead of being separate parts connected together.

1.4.2 Top-down Processes

Integrated circuits are made through a patterning technique called photolithography. Photolithography uses light and a pattern, or mask, to transfer a design to a surface (Figure 1.5). The light that passes through the mask reacts with a photoresist layer on the surface, making it chemically modified. Depending on the resist and technique used, either the exposed or unexposed resist can be removed, leaving the pattern. This pattern is etched into the surface and the remaining resist removed. Integrated circuits are made by layering and patterning repeatedly on a silicon wafer substrate, where the substrate itself is used for the initial device structures. This technique of patterning and etching is often referred to as a top down process. Other top-down processes include micromachining, electrochemical and electrothermal machining, and milling.

Top-down processes are still the primary technique used today to fabricate circuits. These techniques have the advantages of being well understood, with equipment and processes already developed, and the ability to produce a large number of devices in a short amount of time. On the downside, the equipment is very expensive, it is difficult to create features on the nanoscale, and low defect ratios are critical.
Figure 1.5. Photolithography process. UV light is passed through the mask and chemically modifies the exposed region(s) on the surface. Depending on the resist, either the unreacted or the reacted photoresist is removed in the developing step. Next, the surface is etched. The photoresist protects the surface from being etched, transferring the pattern to the surface. Finally, the extra resist is removed.

1.4.3 Bottom-up Processes

Another possible way to fabricate circuits is through a bottom-up approach, in which components are built up from molecules or nanostructures. This could be done through directed but spontaneous self-assembly of components into ordered structures. Examples of bottom-up assembly include DNA nanostructures, DNA templated nanoparticle ordering and metallization, nanowires, and block copolymer self-assembly.
Nature uses a bottom-up approach to provide the complexity of living systems. DNA, proteins, and cells are all built up from smaller components and interact in a spontaneous, yet directed manner. Bottom-up approaches have the potential to allow for a wide variety of processes, techniques, and ultimately a larger variety of devices than current top-down processes. Another advantage to bottom-up assembly is the small cost involved, compared to the complex equipment and materials needed for top-down processes.

There are also disadvantages to bottom-up assembly. Much work is still needed to obtain nanomaterials as complex as are currently possible with lithographic techniques. Often self-assembly processes are not perfect, with defects and other errors occurring in the assembly. These challenges are and will continue to be addressed as the field progresses. Although current bottom-up processes are nowhere as complex as things observed in nature, the potential to harness the knowledge gained from these systems, or building blocks from these living systems, is an exciting area of research.

1.4.4 Top-down Meets Bottom-up

As lithographic techniques for the fabrication of integrated circuits reach their limits, novel bottom-up approaches can enable the continued increase in performance with decrease in cost demonstrated in the past by the semiconductor industry. The best near-future solution, however, will most likely involve a mixture of top-down and bottom-up processes. Completely bottom-up approaches still do not have the complexity required for electronic circuitry. In addition, they still need to be connected to the macro-world in order to obtain useful data and interact with the nanodevices. Top-down process can provide the needed connection to larger scale parts, while benefiting from the self-assembly and small size of nanostructures.
1.4.5 DNA Nanoelectronics

DNA by itself would not be a viable way to make small wires because it has only a poor conductance for uniform sequences and is insulating on long scales (>100 nm) with random sequences. However, its ability to base pair predictably, undergo different types of modifications, and be assembled into a variety of nanostructures, makes it a very promising template for the bottom-up assembly of nanoscale circuitry. Indeed, Section 1.2 discusses the variety of nanostructures that can and have been fabricated and Section 1.3 discusses the ability to metallize or coat DNA with a variety of metals in a selective manner.

In addition, a DNA templated transistor has already been demonstrated by Keren et al., through assembly on a linear strand of DNA. The aldehyde derivatized DNA was first allowed to undergo homologous recombination with a single-stranded sequence of DNA polymerized with RecA protein. Then semiconducting carbon nanotubes functionalized with streptavidin were directed to the portion of the DNA containing the RecA through biotin and antibodies on the RecA. Next, the DNA was seeded with silver and plated with gold. The RecA protected the DNA strand from metallization in the location of the carbon nanotube. This combination of programmability and metallization shows great promise for the construction of nanoscale electronics.

1.5 OVERVIEW OF THE DISSERTATION

Chapter 2 describes the design and assembly of thin, branched DNA origami structures with varying scaffold lengths. The scaffold is produced through polymerase chain reaction (PCR) amplification of naturally occurring DNA of known sequence, followed by a strand separation procedure utilizing streptavidin coated magnetic beads. This technique was used to
produce five different bent, linear, or branched DNA origami structures. These DNA origami structures are wire-like instead of mostly filled in space, as in earlier publications.

Chapter 3 discusses the design and assembly of more complex DNA origami structures, including circuit templates, corners, and a longer bar. Studies on linking DNA origami structures, different types of corners, and flexibility of two and three-dimensional structures are explored.

Chapter 4 details the process of selective attachment of thin DNA origami structures to gold nanoparticles patterned on a surface using block copolymer chemistry. In addition, selective attachment of gold nanoparticles to DNA origami templates as seeds for metallization is discussed.

Chapter 5 details further studies into the nanoparticle seeding process and looks at factors that affect the yield of nanoparticle attachment to these thin DNA origami structures. Factors such as time allowed for the seeding to occur, concentration ratio of gold nanoparticles to DNA origami, and the effect of varying amounts of magnesium ion in solution are explored.

Chapter 6 summarizes my research, drawing conclusions and discussing promising future work, in developing fabrication techniques for the design, assembly, and application of DNA-templated nanodevices.

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CHAPTER 2: POLYMERASE CHAIN REACTION BASED SCAFFOLD PREPARATION FOR THE PRODUCTION OF THIN, BRANCHED DNA ORIGAMI NANOSTRUCTURES OF ARBITRARY SIZES*

2.1 INTRODUCTION

DNA has become a promising template for making a large variety of nanostructures. DNA origami, in particular, provides a robust and simple method for designing patterned shapes. The DNA origami technique can be used to produce nearly any conceivable two-dimensional, and more recently three-dimensional, structure. However, the size of these designs is governed by the length of the single-stranded scaffold used. Scaffolds for origami normally must be single-stranded, although origami production using double-stranded scaffolds has recently been reported. Using double-stranded scaffolds is disadvantageous, since it requires more specialized folding conditions and the use of staples to fold both strands. In most cases, these scaffolds originate from viral or plasmid genomes, with the exception of a single experiment using a PCR amplicon. The use of entire genomes constrains origami designs to discrete available lengths, which are typically several thousand or more base pairs (bp) long.

Current two-dimensional origami structures, at least partly because of the constraints on the scaffold, are roughly 100 x 100 nm$^2$ and mostly filled. However, DNA origamis that have more open spaces and relatively thin (2–4 side-by-side helices, or ~10 nm) features with branching points have not yet been made. Such shapes would be well-suited templates for nanowires and circuitry; in combination with metallization, these origami designs could serve as patterns for DNA-templated nanocircuits.

Here I demonstrate a method of scaffold strand production which allows for origami designs of arbitrary size. Using the polymerase chain reaction (PCR), portions of a genome from less than 100 bp up to 10,000 bp, and in some cases up to 35,000 bp, can be replicated with high specificity and yield. By using a biotinylated 3’ primer, one of the complementary strands produced by PCR can be separated from the desired scaffold strand, by binding the biotinylated strand to a streptavidin-coated magnetic bead and denaturing the DNA. The purified single-stranded scaffold can then be mixed with staple strands to produce DNA origami. I have applied this technique to produce origami of variable sizes and shapes from two common DNA sources, M13mp18 and lambda.

2.2 EXPERIMENTAL

2.2.1 Materials

M13mp18, lambda DNA, and streptavidin-coated magnetic beads were purchased from New England Biolabs. Staple strands for DNA origami folding were purchased from Sigma-Aldrich or Operon and diluted to ~100 µM in TE buffer. PCR primers were purchased from Sigma-Aldrich or Operon. PCR purification kits were obtained from Qiagen. DNA polymerase and PCR buffers were acquired from Invitrogen. Ultrapure, 18 MΩ water was produced by an EasyPure water purification system.

2.2.2 Methods

Generation of PCR-based Scaffolds. PCR was performed (Techne TC-3000 thermal cycler) with 1 µg of each primer and between 20-80 ng of template DNA (either M13mp18 or lambda DNA). Platinum Pfx polymerase (2.5 units) was added to the solution containing primers, template, 1x Pfx amplification buffer, 2 mM MgSO₄, and a mixture of dNTPs (500 nM each) in a 100 µL volume. The following program was used: 95 °C for 2.5 min, 30 cycles of 95
41 °C for 45 sec, 58-60 °C for 45 sec, 68 °C for 1–5 min, and a final extension at 68 °C for 4–6 min. The annealing temperature was chosen to be 1–2 °C below the melting point of the primers. The PCR product was purified with a QIAquick PCR purification kit (Qiagen). The product was also run on a 0.7% agarose gel, and if multiple bands were present, the desired band was separated from the rest through gel purification.

**Primers and Sections of DNA Amplified.** Table 2.1 gives information about which section of either M13mp18 or lambda phage DNA was amplified with PCR, along with the primers used for each DNA origami structure. All sequences are given 5’ to 3’.

**Table 2.1.** Scaffold information and primer sequences for DNA origami designs.

<table>
<thead>
<tr>
<th>DNA origami</th>
<th>Bases used</th>
<th>Length</th>
<th>Sequences of the PCR primers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lambda rectangle</td>
<td>bases 2868–3623</td>
<td>756 bp</td>
<td>GG TGCT GACACGGAAG AAAC [BioTEG]ATCATCAGCAGATTGTTTTATTCC</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M13mp18 rectangle</td>
<td>bases 5870–6625</td>
<td>756 bp</td>
<td>CC ACCATCAAACAGGATTTTTCCGCC [BioTEG]TTCTCCGTGGGAACAAACCGGC</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M13mp18 T Design</td>
<td>bases 5734–1442</td>
<td>2958 bp</td>
<td>CTGATAGACGGTTTTTCGCC [BioTEG]CATAACCGATATATTCGGTCGC</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lambda U Design</td>
<td>bases 37501–41340</td>
<td>3840 bp</td>
<td>TCAACCTCAAGCCAGAATGC [BioTEG]CGCGTCTGAATATGCCTTGG</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lambda B Design</td>
<td>bases 37501–42308</td>
<td>4808 bp</td>
<td>TCAACCTCAAGCCAGAATGC [BioTEG]CGACGCTTTTCGTTTCG</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Single-stranded Scaffold Separation and Purification.** Streptavidin-coated magnetic beads (800 ng) were rinsed 3 times (by mixing with solution, pelleting the beads with a magnet, and removing the supernatant) with 200 µL bead buffer (20 mM Tris-HCl, 1 mM EDTA, 0.2 M
NaCl), and then combined with 200 µL bead buffer and 20–50 µL purified PCR product. The samples were gently mixed 15–30 min (depending on the length of the scaffold) to bind the DNA to the beads. The beads were pelleted, the supernatant (which contained all unbound DNA) was removed, and the beads were rinsed 2–3 times with 200 µL bead buffer. NaOH (0.2 M, 150 µL) was added to the pelleted beads, and the sample was gently mixed 6 min to denature the DNA. The supernatant (containing the desired ssDNA) was collected and combined with 100 µL of 5 M ammonium acetate (pH 7.6) to neutralize the pH. The product was purified either with a spin column (QIAquick PCR purification kit) or by ethanol precipitation. DNA concentration was measured with a Nanodrop ND-1000 spectrophotometer using extinction coefficients of 50 ng-cm/ml for dsDNA and 33 ng-cm/ml for ssDNA.

**DNA Origami Folding.** Purified single-stranded scaffold was mixed with staple strands in a 1:100 molar ratio in 1x TAE-Mg$^{2+}$ buffer (40 mM Tris, 20 mM acetic acid, 1 mM EDTA, 12.5 mM magnesium acetate). Each design was folded by denaturing (95 °C for 3 min) and slowly annealing from 75 °C to 4 °C in 70 min for the rectangular origami and 95 °C to 4 °C in 90 min for the branched shapes. See Appendix B for staple strand sequences.

**AFM Imaging.** DNA origami samples were deposited onto freshly-cleaved mica, rinsed with water, and dried using compressed air or nitrogen. The samples were imaged in air using tapping mode on a Digital Instruments Nanoscope IIIa MultiMode AFM (Veeco) with silicon force modulation AFM tips (Vistaprobes, 3 N/m, 60 kHz).

**Design Program.** The DNA origami folding program developed at BYU is available from the author.
2.3 RESULTS AND DISCUSSION

2.3.1 Overview

My scaffold was obtained through PCR amplification, followed by purification to obtain only the desired single-stranded DNA, as outlined in Figure 2.1. PCR was performed for each structure (Figure 2.1A) using a biotinylated 3’ primer (see Table 2.1). Next, the biotinylated PCR product was bound to streptavidin-coated magnetic beads (Figure 2.1B). After rinsing to remove any unbound DNA, the bead-bound PCR product was denatured in dilute aqueous NaOH (Figure 2.1C). The supernatant, containing the now-freed scaffold strand, was collected and purified. For a 2,958 bp PCR product, an average of 4,300 ± 700 ng of dsDNA combined with 800 ng streptavidin-coated magnetic beads yielded an average of 1,100 ± 300 ng of ssDNA. This final single-stranded PCR product was combined with staple strands (at 100-fold molar excess) and annealed by slowly cooling to form DNA origami.
2.3.2 DNA Origami Structures

I have designed and produced four different DNA origamis (from 756 to 4,808 bp) using this scaffold preparation method. The smallest design (756 bp) is a rectangular shape, approximately 64 nm long and 11 nm wide, consisting of four helices stacked side-by-side (Figure 2.2A). This structure was designed using an Excel-based Visual Basic program developed at BYU, which allows users to design raster-filled shapes from a variety of DNA scaffolds, and then chooses the staple strands and crossover points accordingly. Identical structures of this size and shape were produced using scaffolds prepared from both lambda DNA (Figure 2.2B) and M13mp18 DNA (Figure 2.2C). Both structures appear to form equally well.

I made three additional DNA structures that were larger than the rectangle motif and demonstrated the ability to make branching designs of variable size using the described scaffold preparation. Each of these structures contains unique, asymmetric square junctions. These differ from junctions used in the past for DNA nanostructures, which are typically axisymmetric.\textsuperscript{14-18} The square junction is made possible by connecting the stem of the branching region to only one of the base helices of the intersecting feature, (Figure 2.3A,D,G) such that the other helices remain straight and constrain the connecting helix approximately to a right angle.

![Figure 2.2](image-url)

Figure 2.2. (A) Scaffold outline of origami rectangle. Tapping mode AFM images in air of DNA origami rectangles made with PCR-amplified (B) lambda DNA scaffold, and (C) M13mp18 scaffold. Scale bars are 500 nm, and height scale is 3 nm.
Figure 2.3. DNA origami nano letters. (A–C) Scaffold trace/outline and tapping mode AFM images of the branched ‘T’ shape. (D–F) Scaffold trace and AFM images of ‘U’ shape. (G–I) Scaffold trace and AFM images of ‘B’ shape. All AFM images were taken in air. Scale bars are 200 nm and height scale is 4 nm. (J) Nano-alphabet ‘BYU’ as a composite from three images. The ‘U’ is enlarged by 60% and the ‘Y’ is expanded by 16% relative to the ‘B’.
I formed one branched structure in the shape of a ‘T’ (Figure 2.3A–C). The top section of the shape is ~240 nm long and three DNA helices wide (~8 nm). The stem of the shape is ~75 nm long and four DNA helices wide (~11 nm). This branched structure is produced using a 2,958 bp segment of DNA amplified from M13mp18. The structure was designed using the SARSE program,19 which automatically fills a desired shape with a DNA scaffold, then assigns staple strands with 3-helix crossovers. The top and stem of this structure were designed separately. The two portions of the scaffold sequence were then spliced together to form a three-point square junction upon folding. Figure 2.3B–C shows large area and zoom view AFM images of this shape. The top arm, which is longer and contains fewer helices than the base, shows greater flexibility. The junction is also somewhat flexible, with the arms often deviating from perfect right angles. About 27% of well-formed origamis have a junction that forms a right angle (90° ± 10°). Figure 2.4 and Figure 2.5 demonstrate the flexibility in the top of the ‘T’ shape, compared to the shorter base, as well as the flexibility in the junction. Using these square junctions, a much wider variety of shapes can be produced than using axisymmetric junctions alone, and such shapes should prove useful in making nanocircuit designs.

Figure 2.4. AFM images of the ‘T’ design, showing some flexibility in the top arms. In (A), one ‘T’ is bent considerably, while the other is straight. In (B), two different ‘T’s show flexibility in the top arm. Scale bars are 200 nm.
I also produced a square ‘U’ structure (Figure 2.3D–F). The two arms of the ‘U’ are both ~120 nm long, and the base is ~100 nm long. The entire design is 3,904 bp long, with the scaffold amplified from lambda DNA. This structure was made using the BYU design program. The two arms were designed separately from the base, and the scaffold strands of the three regions were spliced together to form a single scaffold. Each of the arms forms a square junction with the base, similar to the junction described for the ‘T’. The base of the ‘U’ extends ~10 nm beyond the junction with the arms to ensure the formation of right angles. These extensions are only moderately visible in the finished product. Despite the very short overhang beyond the junctions, the right angle intersections in the ‘U’ nanostructures formed better than those in the ‘T’ shape. About 45% of the ‘U’ junctions have right angles (90° ± 10°) when deposited on a mica surface. The larger percentage of right angle junctions for the ‘U’ compared to the ‘T’ is likely due to a 4-helix base for the ‘U’ vs. a 3-helix base for the ‘T’ junctions. The rigidity of these structures could be improved further by either adding additional helices in the same plane, thereby limiting the flexibility in the arms, or by stacking helices on top of the shape to add strength in the third dimension.

Figure 2.5. AFM images of the ‘T’ design, showing flexibility in the square junction; (A) shows several structures with nearly axisymmetric junctions (circled), while (B) shows a ‘T’ with straight arms, but having the base connecting at an obtuse angle. Scale bars are 200 nm.
The final shape I produced is an uppercase ‘B’ (Figure 2.3G–I). The backbone of the ‘B’ is ~250 nm long and 11 nm wide (four helices). The curved portions consist of two helices. The entire design is 4,808 bp long and uses a section of lambda DNA for the scaffold. This shape demonstrates the ability to incorporate several different features into a single design. The backbone of the structure was designed using the SARSE program and the curved portions were designed in three parts using the BYU program. These four portions were then spliced together manually. The three connections between the curved portions and the backbone are all square junctions, as described above. Like the intersections in the ‘U’, these junctions are formed by including a short backbone overhang beyond the junctions to ensure stable right angles. The center junction (between the curved portions) is an axisymmetric junction. The curved sections demonstrate the ability to incorporate well-formed, round structures into designs and to fabricate very thin portions of a structure which consist of only two helices connected to one another.

Although the principal focus of my work is making structures for nanoelectronics, I also note that these DNA origamis form several letters of a nano-alphabet: B, I, T/Y and U/C; moreover, it should be possible to design and construct additional characters using my approach. As a step toward Feynman’s vision of information storage in “Plenty of Room at the Bottom”, I have assembled several AFM images to form a nanoscale representation of our institution’s acronym: BYU (Figure 2.3J).

2.4 CONCLUSIONS

These structures demonstrate that PCR amplification for scaffold strand generation enables the formation of DNA origamis of a broad range of scaffold lengths. By using PCR, a continuum of scaffold lengths becomes available between 100-10,000 bp, along with the ability to choose any section and source of DNA. This method enhances the versatility of the DNA
origami method by allowing the scaffold length to be matched to the design, rather than conforming the design to an available scaffold length. This is especially important for making thin, branched motifs, which are smaller than previously reported two-dimensional origami structures.

Asymmetric PCR\textsuperscript{20} could also be used to generate a single-stranded PCR product with sufficient purity for use in making DNA origami. While this method appears promising, to date I have only had moderate success in obtaining a scaffold pure enough to make well-formed origami.

In summary, I have developed and demonstrated the use of a simple and general scaffold preparation method for forming DNA origami nanostructures of a range of sizes and shapes. This process allows us to assemble largely open designs with thin, branched features, rather than filled in shapes. My small, thin structures with square junctions have potential applications in nanoelectronics, addressing the need for narrow (<20 nm), branched features for wiring. These asymmetric, branched DNA structures could provide circuit templates and, with metal deposited on them,\textsuperscript{8-11} act as nanowires. Selective metallization could also facilitate the placement of active elements such as semiconducting carbon nanotubes in targeted locations to form transistors. The ability to design thin nanostructures with well-defined shapes and right angle features, coupled with selective metallization, might also find use in nanophotonics.\textsuperscript{21} My work should open the way for a rich diversity of DNA origami nanostructures to be made to address a range of scientific needs in nanoscale research.

2.5 REFERENCES


CHAPTER 3: DNA ORIGAMI STRUCTURES FOR NANOELECTRONIC CIRCUIT TEMPLATES

3.1 INTRODUCTION

Combining top-down with bottom-up fabrication techniques is a promising route to enable new and smaller nanostructures. Keren et al.\(^1\) demonstrated that bottom-up DNA templating techniques could be used to assemble a carbon nanotube transistor. Many nanostructures built from the bottom-up utilize a combination of top-down and bottom-up techniques because there needs to be a connection from the nanostructure to the macro-world. The nanostructures need to be connected to top-down patterned electrical leads, for example, to enable control of the nanostructure and connect it to the rest of the device. This connection makes it difficult for single nanodevices, like a transistor, built using self-assembly techniques to compete in scale against well-controlled lithographic patterning. If, instead of individual pieces being self-assembled, an entire circuit could be assembled from the bottom-up, using DNA origami templates, then the overall density could potentially be increased.

In Chapter 2, I demonstrated that branched, wire-like DNA origami structures could be assembled using the technique of DNA origami along with a PCR-based scaffold strand generation technique. The next step toward using DNA origami structures as templates for nanoelectronic circuits is to design and assemble more complex DNA circuit templates. Figure 3.1 illustrates a potential DNA design which could be used as a n-type metal-oxide-semiconductor (NMOS) NOR gate (which follows NOR logic, or the negation of an OR logic gate), with two transistors in parallel. It provides a simple step forward in the design of DNA origami templates to enable assembly of multiple transistors onto a single structure. The basic shape is a rectangle with four leads, two for the source and drain (high and low voltages), and
two for the gates (A and B) that control the transistors. In this chapter I discuss DNA origami designs that could be used to make the circuit architecture in Figure 3.1.

To design and assemble these DNA origami circuit templates, several other factors were explored, including 1) the ease or difficulty of linking together separate DNA origami structures, 2) the formation of different types of corners in DNA origami structures, and 3) the flexibility of narrow linewidth, flat two-dimensional structures in comparison to thin, three-dimensional structures.

In addition, I designed other DNA origami structures, including a 410 nm long rectangular bar DNA origami structure. This DNA origami could provide longer leads for the circuit structure and was also a useful length to test deposition of two different metals on the same DNA origami template.

3.2 EXPERIMENTAL

3.2.1 Materials

M13mp18, lambda DNA, and streptavidin-coated magnetic beads were purchased from New England Biolabs. Staple strands for DNA origami folding were purchased from either Integrated DNA Technologies or Eurofins MWG Operon (diluted or obtained at 100 µM in TE
buffer). PCR primers were ordered from either Integrated DNA Technologies or Eurofins MWG Operon and diluted to 1 µg/µL in water. PCR purification kits were obtained from Qiagen. DNA polymerase and PCR buffers were acquired from Invitrogen. 30 kDa Amicon ultra 0.5 mL centrifugal filters were purchased from Millipore. Ultrapure, 18 MΩ water was produced by an EasyPure water purification system.

3.2.2 Methods

Design Programs. The DNA origami structures were designed using either SARSE or caDNAno. These programs can be downloaded from http://cdna.au.dk/software/ and http://cando-dna-origami.org. The details of the DNA origami designs are given in Appendix A, and the staple strand sequences can be found in Appendix B.

Scaffolds. M13mp18 was used directly for the rectangular circuit half, the circular circuit structure, and the long bar structure. PCR-generated scaffolds from lambda DNA were used for the 2D and 3D corner structures. The PCR-generated scaffolds were prepared the same as in Chapter 2 (see section 2.2.2) with some small modifications. PCR was performed with 80 ng of lambda DNA as the PCR scaffold. The PCR programs were modified with appropriate primer annealing temperatures and polymerase extension times (57 °C annealing temperature and 2 min 45 sec polymerase extension time for the 2D corner DNA origami structure and 58 °C annealing temperature and 2 min 6 sec polymerase extension time for the 3D corner DNA origami structure) with a final extension time of 4 min. The same steps were followed, as in Chapter 2, for the scaffold strand separation and purification, with 20 µL of PCR product used for each separation, sample mixing for 30 min to bind the DNA to the beads, and purification after the separation with spin columns (QIAquick PCR purification kit).
**Primers and Sections of DNA Amplified.** Table 3.1 gives primer sequences and information about the section of Lambda DNA amplified for the scaffolds.

**Table 3.1.** Scaffold information and primer sequences for DNA origami designs.

<table>
<thead>
<tr>
<th>DNA origami</th>
<th>Lambda bases used</th>
<th>Length</th>
<th>Sequences of the PCR primers</th>
</tr>
</thead>
<tbody>
<tr>
<td>2D Corner</td>
<td>39576–42308</td>
<td>2733 bp</td>
<td>GGATCTATGAAAAACATCGC</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>[BioTEG]CGACGCTTTCTTGTTCG</td>
</tr>
<tr>
<td>3D Corner</td>
<td>39187–41340</td>
<td>2154 bp</td>
<td>GACCAGCCAGAAAACGACC</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>[BioTEG]CGCGTCTGAATATCCTTTGG</td>
</tr>
</tbody>
</table>

**DNA Origami Folding.** The DNA origami structures were folded with a 1:10 molar ratio of scaffold strand to staple strands in 1xTAE-Mg\(^{2+}\) buffer. All the DNA structures, except the 3D corner, were folded by denaturing (95 °C for 3 min) and slowly annealing from 95 °C to 4 °C in 90 min. The 3D corner structure was denatured (94 °C for 5 min) and slowly annealed from 80 to 60 °C in 80 min, followed by cooling from 60 to 24 °C in 46.5 hrs.

**DNA Origami Linking.** The rectangular circuit halves were successfully linked, using the direct linking approach (see **Figure 3.2**), by assembling the individual halves using the same folding process as above, filtering the two DNA origami halves using 30 kDa Amicon filters to remove excess staple strands, and then combining the two halves together. The samples were filtered by spinning the samples for 10 min at 13,000 rpm, rinsing twice with 500 µL 1 x TAE-Mg\(^{2+}\) buffer by spinning for 10 min at 13,000 rpm, and recovering the sample by spinning for about 2 min at 3,500 rpm. After filtering, the samples were combined in equal molar ratios and placed in a heat block at 37 °C for 45–90 min. Linking the rectangle circuit halves with two different types of linkages was also attempted (see **Figure 3.2** for the design and **Appendix B** for the sequences). The same steps were followed as above (to link one side) and then the additional
linking strands, for the helping strand linkage, were added to the solution (2 µL each of three linking strand with 10 µL rectangle circuit solution) to link the second side. The solution was then placed on a heat block for 37 °C for 47 min. After folding the “T” structures (with the appropriate linking strands) and filtering to remove excess staple strands, the two “T” solutions were combined and placed on a heat block at 37 °C for 30 min. Then the additional linking strand was added (2 µL linking strand with 20 µL “T” solution) and the solution was placed on a heat block at 37 °C for 30 min.

**AFM Imaging.** DNA origami samples were deposited onto freshly cleaved mica, rinsed with water, and dried using compressed air. The samples were imaged in air using tapping mode on a Digital Instruments Nanoscope IIIa MultiMode AFM (Veeco) with silicon force modulation AFM tips (AppNano FORTA probes, 1.6 N/m, 61 kHz).

### 3.3 RESULTS AND DISCUSSION

**DNA Origami Structures.** I have designed and assembled several DNA origami structures in addition to those found in Chapter 2. These structures include a rectangle with leads, corner shaped structures, and a longer rectangular bar structure. The first of these...
structures, a rectangle with two leads, was made in two identical pieces, designed to link together to form the final structure. Figure 3.3A shows the scaffold trace of the structure and how it can link together to form one larger construct with inner dimensions of ~160 x 160 nm, a thickness of ~17 nm (6 helices), and 75 nm long leads (4 helices or ~11 nm thick). This structure uses 7,216 bases from the M13mp18 scaffold for each half (total of 14,432 bp) with 226 staple strands (452 total staple strands) and can be folded directly from the naturally occurring scaffold instead of using a scaffold generated through PCR and magnetic bead separation. For more details on how the structure was designed in the SARSE program, see Appendix A.

To link the structures together, DNA staple strands were designed to continue across from one half of the structure to the other half (see Figure 3.3A inset). The arms were designed with staggered helices where they connect together to ensure that the arms connect in the correct orientation, instead of having one of the arms turned relative to the other. Figure

**Figure 3.3.** Rectangular circuit DNA origami structure. (A) Scaffold trace of the rectangular circuit structure. (B) AFM image of half of the DNA origami structure. The staple strands used to link the two halves together were not included in the mixture. (C) AFM image of the DNA origami structure with the linking staple strands included. Scale bars are 200 nm.
3.3B shows individual halves of the rectangle structure. If the staple strands that link two halves together were included in the DNA origami mixture during the initial formation, the structures were flexible enough in solution to link within themselves (see Figure 3.3C) instead of forming dimers as expected.

**Linking DNA Origami Structures.** To get the structures to form dimers as desired, two different linking techniques were attempted. In the first technique, staple strands were designed with sticky end tails that base paired to similar extensions of staple strands on the connecting arm. This way, each staple strand pairing could be designed differently, so that the connections on the two sides of the rectangular box were different, as shown in Figure 3.2A. In the second technique, the staple strands were also extended with additional nucleotides, but the linking occurred with the addition of a third strand that paired with the extra nucleotides on the extended staple strands. This is shown in Figure 3.2B.

Using the first technique, I was able to form DNA origami dimers with the rectangle circuit halves (Figure 3.4A–C). However, the dimers formed with a very low yield. It was much more common to see multiple rectangle halves

![Figure 3.2](image.png)

**Figure 3.2.** Rectangular circuit DNA origami structures. (A) AFM image of linked rectangular half structures. (B) AFM image of rectangle halves forming chains. Scale bars are 200 nm.

![Figure 3.4](image.png)

**Figure 3.4.** Rectangular circuit DNA origami structures. (A–C) AFM images of linked rectangular half structures. D) AFM image of rectangle halves forming chains. Scale bars are 200 nm.
linked into longer chains (Figure 3.4D). To overcome this new issue I tried a combination of the two linking techniques, using a direct link between extended staple strands on one side and using bridging strands to link the extended staples on the other side. I was able to form dimers, but they were only connected on one side, instead of both sides (Figure 3.5A). To simplify the linking process, I attempted the same direct and bridged combination of linking techniques using the T DNA origami structure, to see if reducing the linking area to fewer helices and therefore fewer different linking strands would help. Once again, dimers formed with one connection, but not both (Figure 3.5B).

![AFM images of partially linked DNA origami structures](image)

**Figure 3.5.** Linking DNA origami structures with two different types of linkages. (A) AFM image of partially linked rectangle half DNA origami structures. (B) AFM image of partially linked T DNA origami structures. Red circles indicate structures with one linkage. Scale bars are 500 nm.

**Flexibility of DNA Origami Structures.** Another weakness in the rectangular circuit design is the flexibility in the corners and the lead junction. The half rectangle structure often lands with the connecting arms on two different sides, or both arms on the same side as the lead, looking like an E. When linked together, the leads are often facing inward, instead of outward
The reason for this flexibility is that the scaffold strand is the only thing that connects the two sides of the corner together, or the lead to the rest of the arm: none of the staple strands cross over from lead to arm, or from the one side of the corner to the other side. An attempt to redesign the staple strands in the corner to add additional crossover points did not yield an improvement in rigidity, as observed in AFM images (Figure 3.6). If these structures could be tethered to a patterned surface by the ends of the leads and the corners, the problem of leads flipping inward and corners landing the wrong direction might be avoided. Alternatively, one could look at constructing the corners and junctions of the DNA origami structures in a better way.

Figure 3.6. Comparison of original and reinforced corners. (A) Original half rectangle circuit structure. (B) Modified half rectangle circuit structure with bridging staple strands in the corner. Scale bars are 500 nm.

Figure 3.7A compares the corner in the rectangle circuit DNA origami structure with a different way to fold the scaffold strand through a corner, inspired by Rothemund’s triangle DNA origami structure. The new design has two staple strands (colored blue in Figure 3.7A) as
well as the scaffold strand that bridge the two sides of the corner, throughout the corner. A simple DNA origami structure (Figure 3.7B) was designed to test this corner. The scaffold for the structure is 2733 bases of Lambda DNA and has 89 staple strands. The initial folding attempt led to a low yield of properly folded corner structures (Figure 3.7C). However, upon redesign of the structure to have shorter helix overhangs at the corner, the yield was increased greatly (Figure 3.7D). Perhaps the strain in the corner of the original design was too great for the structure to fold properly with high yield. The well-folded corners have an average angle of 100° with a standard deviation of 20° (n = 116). These corners appear to be a little more rigid when compared with the U structure from Chapter 2, which had an average corner angle of 100° and standard deviation of 30° (n=134).

In addition, a three-dimensional corner structure was designed and assembled. This structure was two helices wide and two helices tall in the arms. Figure 3.8 shows the scaffold trace as well an AFM image of the assembled structure. The average angle of the three-dimensional corner

![Figure 3.7. Corner DNA origami structure. (A) Comparison of scaffold traces of corner folding patterns. Blue strands indicate bridging staple strands. (B) Scaffold trace of the corner DNA origami structure. (C) AFM image of the initial corner structure. (D) AFM image of the redesigned, better-folding corner structure. Scale bars are 200 nm.](image-url)
structure is 110° with a standard deviation of 20° (n = 68). It is interesting to note that even though the three-dimensional corner is fewer helices across, it has the same standard deviation as the two-dimensional corner version. This indicates a potential for stacking DNA helices, or designing structures into this third dimension, to increase stability and rigidity of the junctions and corners. Although this three-dimensional structure appears to fold well, one disadvantage is that it takes longer to fold.

![Figure 3.8. Three-dimensional corner DNA origami structure.](image)

**Figure 3.8.** Three-dimensional corner DNA origami structure. (A) Scaffold trace of the three-dimensional corner structure. The blue lines indicate bridging staple strands. (B) AFM image of the three-dimensional corner structure. Scale bar is 200 nm and the height scale is 4 nm.

**Additional DNA Origami Structures.** Another, smaller DNA origami circuit structure was also designed. This structure has a circular center with four spokes, instead of the rectangular shape in the previous design. The scaffold uses 7222 bases of M13mp18 and has 244 staple strands. By having a circular center, issues with corners in the DNA structures were avoided. Two of the leads, however, were still designed using the same approach as the branched, “T” structure in Chapter 2 or the rectangular circuit structure. **Figure 3.9** shows the scaffold trace of this structure as well as an AFM image of the folded DNA origami. The spokes or leads in this structure still sometimes land inward instead of outward and at times do not
extend directly out from the circular base, but the yields of the desired structure are far higher than for previous designs. Higher yields of correct orientations of leads could be achieved with controlled surface attachment. The staple strands in this structure are modified and used in Chapter 4 for selective metallization of the DNA origami template.

![Circular circuit DNA origami structure](image)

**Figure 3.9.** Circular circuit DNA origami structure. (A) Scaffold trace of the circular circuit structure. (B) AFM image of the circular circuit structure. Scale bar is 200 nm.

A longer bar DNA origami structure that could also be used for extended leads was designed and assembled for use in depositing two different metals onto the same DNA template. This structure is 6 helices wide (~17 nm) and ~410 nm long. It uses 7085 bases of the M13mp18 scaffold and has 227 staple strands. The staple strands were modified to have extra bases to pair with oligonucleotide-coated nanoparticles as discussed in Chapter 4. **Figure 3.10A–B** shows a scaffold trace of this structure and an AFM image of the folded bar DNA origami structure. The bar structures sometimes appear to have bends at various places instead of landing linearly on mica surfaces. This could be due to the longer length, or from a defect in the DNA origami design. The program CanDo, which shows the computational prediction of the three-dimensional shape in solution, indicated that the structure has an overall twist when in solution.
(see Appendix A). As the bar shape lands on a surface, the twist could account for the bends that appear.

Another way to obtain a long bar structure would be to link together smaller DNA origami bars. This was done with the M13mp18 rectangle bar from Chapter 2 by modifying the end staples to bridge from one bar to the next (Figure 3.10C). This technique works well to create long chains, albeit without control for a particular length.

### 3.4 CONCLUSIONS

I have been able to successfully fold more complex DNA origami templates. In addition, I have explored different aspects of DNA origami folding, such as different ways to create corners in structures, linking of DNA origami structures, and two-dimensional versus three-dimensional DNA origami structures.

In exploring different types of corner structures, I found that both the corners (and reinforced corners) in the rectangular circuit structure as well as the two and three-dimensional corner structures folded properly. It would be interesting to redesign the rectangular circuit
structure with the corners from the corner DNA origami structures and see how the rigidity in the corner is affected.

Although linking the rectangle circuit halves together only produced the correct dimers in low yield, the information learned could help in future DNA origami designs. It is useful to note that DNA origami structures will link within themselves very well, as the rectangle half structures did with the original bridging staple strands. Long chains of linked DNA origami also assemble easily and could be quite useful for bringing together multiple DNA origami circuit templates. DNA origami dimers with one connection are also possible, but if dimers with two connection points are desired, then extra care needs to be taken in the design to prohibit long chains from forming. This might be overcome through the use of controlled polymerization or stepwise addition of critical reactants.

I found that three-dimensional, narrow linewidth DNA origami structures can also fold well, in addition to flat, two-dimensional structures. Designing corners and other portions of a DNA template with three-dimensional sections may add additional rigidity to the structures. Even though the corner structure I designed took longer to fold than the two-dimensional structures, new techniques are being explored to reduce the time involved in DNA origami folding and understand the process better,\textsuperscript{4} to make folding these structures easier.

These structures and studies could allow for the formation of complex DNA origami circuit templates and, in combination with surface placement, enable the assembly of small, compact circuit structures using bottom-up self-assembly techniques.

3.5 REFERENCES


CHAPTER 4: FUNCTIONALIZATION OF THIN, BRANCHED DNA ORIGAMI STRUCTURES WITH GOLD FOR SURFACE PLACEMENT AND METALLIZATION SEEDING*

4.1 INTRODUCTION

4.1.1 Surface Placement of DNA Origami

The ability to pattern surfaces with feature sizes less than 20 nm remains a major challenge of lithography, with no proven optical solution.1 Bottom-up processes are an interesting option due to their ability to create patterns with feature sizes far below current optical methods. In these controlled systems, fundamental interactions cause molecules to self-assemble into organized structures. Two such self-assembly methods are block copolymer (BCP) patterning and DNA origami. BCPs can create highly regular features on surfaces, but the types of features are limited to arrays of dots or parallel lines.2-4 In contrast, DNA origami is capable of creating a broad range of shapes in solution,5-6 but DNA origami-based surface patterning relies on the development of processes allowing controlled placement of DNA origami on a surface.

Recently, selective DNA origami surface placement has been demonstrated using binding sites which are large compared to the DNA origami feature sizes. In a report by Kershner et al. DNA origami was placed selectively by using optical and electron beam lithography to pattern hydrophilic regions where DNA origami adsorbed with higher stability than on the hydrophobic background.7 Following a surface rinse, DNA origami remained selectively within the hydrophilic patterns. Gerdon et al.8 functionalized e-beam patterned gold pads placed with 11-mercaptoundecanoic acid, which binds ionically with magnesium ions in the DNA origami

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solution. In this case, the Coulombic attraction between DNA origami and magnesium ions caused selective DNA origami deposition. Ding et al.\(^9\) covalently attached DNA origami to a surface by using consistently spaced 60 and 80 nm diameter gold islands made by e-beam lithography. DNA origami nanotubes, which were modified by addition of thiol functionalized staple strands near each end, were attached between gold island pairs. This method allows orientation control of DNA origami by alignment with the patterned gold islands.

The ability to align DNA origami accurately on a surface is dependent on the binding mechanism and the binding site geometry. Weak attachment mechanisms allow enough mobility for the DNA origami to align with larger binding sites, but more stable attachment mechanisms do not provide such mobility. In state-of-the-art optical lithography, the alignment tolerance is much less than the smallest feature size of the pattern. To achieve both alignment accuracy comparable to lithography and a highly stable attachment, binding sites must be smaller than the minimum DNA origami feature size, which could be as small as 2, 5, or 8 nm for 1, 2, or 3 rows of double helices in planar geometries, respectively, where the alignment accuracy cannot be better than the size of the binding site. Examples of DNA origami structures with feature sizes in this range are DNA origami nanotubes\(^{10}\) and thin, branched structures, described in Chapters 2 and 3.

Here my focus was to study for the first time the chemically directed assembly of DNA origami to nanoscale patterned surface binding sites and specifically to chemically functionalized BCP generated nanospheres. BCP generated nanospheres present an attractive test bed for this study since the minimum BCP surface feature size is a good fit to origami binding site requirements. BCPs can self-assemble into periodic domains which are a few nanometers to tens of nanometers across with spacings between tens and hundreds of nanometers.\(^{11}\) Additionally,
the location and orientation of BCP patterns can be controlled by alignment to chemical or topographic features patterned by top-down methods.2-4, 12-15

A well-known method of DNA attachment uses thiol functionalized single-stranded DNA (ssDNA-SH) reacted with Au and base paired with complementary ssDNA in solution.16-17 This interaction has been used previously to attach gold nanoparticles (Au NPs) to DNA origami, where ssDNA functionalized Au NPs base paired with selected staple strands which had been extended with a complementary sequence.18-19 This base pair attachment method provides a stable, chemically specific binding mechanism to attach DNA origami to BCP patterns, since BCPs can be used to control the location of gold features on a surface. As an example, Chai and Buriak20 have shown incorporation of metal ions from a dilute HCl solution into the poly-2-vinyl pyridine (P2VP) domain of a self-assembled polystyrene-b-poly-2-vinyl pyridine (PS-P2VP) film. A plasma etch was used to remove the polymer and reduce the metal ions, leaving behind patterned arrays of metallic features in the place of the P2VP block. Park et al.21 showed selective gold incorporation into the P2VP domain in a self-assembled PS-P2VP film through surface reconstruction in ethanol, evaporation of Au onto the surface, and surface recovery by heating. A subsequent plasma etch was then used to remove the polymer as in the previous example. Here Anthony C. Pearson and I used the Au/ssDNA-SH reaction in combination with 5 nm BCP patterned, reduced gold nanospheres to chemically direct the placement of DNA origami.

4.1.2 Gold Nanoparticle Seeding for Metallization

Self-assembly methods have shown promise for the fabrication of complex structures with extremely small feature sizes.22-23 Scaffolded DNA origami, in particular, provides a robust and simple method for designing patterned shapes in the sub-100-nm regime. The DNA origami
technique can produce a wide variety of two-dimensional, as well as three-dimensional, structures by folding a long single-stranded DNA “scaffold” into a designed shape with use of a large number of shorter “staple” strands consisting of synthetic DNA. A distinct advantage of DNA origami is that the staple strands can be adjusted to engineer site-specific attachment points throughout the structure. Location-selective variability can be achieved either by direct chemical modification at the ends of staple strands or by extending staple strands with additional nucleotides and hybridizing these “sticky ends” with a complementary sequence containing the desired functional group or moiety. Using these techniques a variety of materials have been controllably attached to DNA origami such as RNA probes, proteins, carbon nanotubes, and metal nanoparticles.

The use of DNA origami structures as templates for metallization is potentially enabling for technologies such as nanoelectronic circuits and plasmonics, among others. Although there is a considerable body of literature describing the metallization of linear, double-stranded DNA, there are far fewer reports of continuous metallization of DNA origami. One particularly attractive aspect of molecularly templated nanofabrication is the possibility of dictating the precise location of metallization. Site-specific metallization is possible with DNA origami where the recognition properties of DNA can be used to create the complex structures needed, for example, for nanocircuit formation.

Fabrication of conductive nanowires on a DNA origami template is complicated due to the difficulty of achieving high seed density, plating precision, and high stability of the DNA origami during the plating process. Site-specific placement of seeds causes limitations in seed density since the spacing between available attachment points is controlled by the staple strand spacing. Additionally, the seeds must be chemically modified prior to attachment and
metallization, which may affect the metallization process. Another focus of this chapter, in addition to surface placement on patterned nanospheres, is the fabrication of nanowires by site-specific metallization of DNA origami templates.

Recently, site-specific metallization of a modular, 100 nm X 100 nm DNA origami tile was reported by Pais et al. In that report metal structures of a few different shapes were formed by electroless plating of silver onto Au NPs placed at specific sites on the DNA origami tiles. This chapter provides at least two distinct advances toward enabling functional electronic device fabrication using site-specific metallization of DNA origami: 1) the thin, branched DNA origami structures discussed in Chapters 2 and 3, allow for considerably longer wires than is possible with a modular and dense tile motif; and 2) the high seed density achieved herein permits the fabrication of continuous nanowires of very small diameter.

4.2 EXPERIMENTAL

4.2.1 Materials

M13mp18 and streptavidin-coated magnetic beads for DNA scaffold preparation were purchased from New England Biolabs. Staple strands for DNA origami folding were purchased from Eurofins MWG Operon (diluted to or obtained at 100 µM in TE buffer). Single stranded DNA thiol was purchased from Eurofins MWG Operon with PAGE purification and either diluted to 100 µM, or 1 mM in water. PCR primers were ordered from either Eurofins MWG Operon or Integrated DNA Technologies and diluted to 1 µg/µL in water. PCR purification kits were acquired from Qiagen. DNA polymerase and PCR buffers were purchased from Invitrogen or New England Biolabs. 30 kDa Amicon ultra 0.5 mL centrifugal filters were purchased from Millipore. Slide-a-lyzer 3.5K MWCO mini dialysis units and floats were purchased from Thermo Fisher Scientific. HAuCl₄ was purchased in powder form from Sigma Aldrich. Polystyrene-b-
poly-2-vinylpyridine was purchased from Polymer Source, Inc. \((M_n,\text{PS} = 57,000 \text{ g/mol}, M_n,\text{P2VP} = 57,000 \text{ g/mol}, \text{polydispersity index} = 1.08)\). Au nanoparticles (5 nm) and mica for AFM imaging were purchased from Ted Pella. BSPP (bis(p-sulfonatophenyl)-phenylphosphine dihydrate dipotassium salt) was obtained from Strem Chemicals. (100) n-type silicon wafers were purchased from Silicon Wafer Enterprises, LLC. The silicon monoxide support films on copper TEM grids were purchased from Ted Pella (Product # 01830). Water used was ultrapure, 18 MΩ water produced by water purification systems.

4.2.2 Surface Placement Methods

**Preparation of Au Nanosphere Arrays on SiO₂ Surfaces.**

Silicon wafers with approximately 350 nm of thermally grown oxide were cleaved into 1 cm² silicon dies and cleaned using RCA standard cleans 1 and 2. The cleaned surfaces were then dipped into and withdrawn from a \(\text{AuCl}_4^-\)-loaded PS-P2VP micelle solution at a rate of approximately 75 \(\mu\text{m/s}\) to deposit a monolayer of micelles on the surface. Surfaces were then exposed to an oxygen plasma (40 W, 50 mTorr) followed by a hydrogen anneal. Anthony C. Pearson prepared these samples.

**DNA Origami Folding.** DNA origami rectangle structures were prepared as previously reported in Chapter 2, using four extended staple strands (see Appendix B for additional information). Briefly, purified single-stranded scaffold was mixed with staple strands in a 1:10 molar ratio in 1X TAE-

![Figure 4.1. Structure of the DNA origami rectangle, showing the staple strand crossovers and locations of sticky end modifications. Modified staple strands are colored red.](image)
Mg\textsuperscript{2+} buffer (40 mM Tris, 20 mM acetic acid, 1 mM EDTA, 12.5 mM magnesium acetate). The DNA origami was folded by denaturing (95 °C for 3 min) and slowly annealing from 75 to 4 °C in 70 min. Following DNA origami preparation, filtration was required prior to surface attachment. The solution was filtered with 30 kDa Amicon ultra-0.5 mL centrifugal filters to remove excess staple strands. The structure of the DNA origami rectangle and location of sticky ends is shown in Figure 4.1.

**Au Nanosphere Functionalization.** To functionalize Au nanosphere arrays, the disulfide protected single stranded DNA-thiol (ssDNA-SH) was first reduced to form a free sulfhydryl group by reaction with 4 mM tris(2-carboxyethyl)phosphine (TCEP) in water held at 40 °C for 2 hr. The reduced ssDNA-SH solution (10 μM) was then purified by dialysis to remove the TCEP using a slide-a-lyzer 3.5K MWCO mini dialysis unit. The solution was dialyzed for a total of 2 hr, with the solution being exchanged with fresh 0.5X TBE buffer (44.5 mM Tris, 44.5 mM boric acid, 1 mM EDTA, 50 mM NaCl) every 30 min. Surfaces patterned with noncomplementary ssDNA were prepared by reacting Au nanosphere arrays with a 25-base ssDNA-SH (see Table 4.1 for specific sequence) while patterned complementary ssDNA surfaces were prepared by functionalizing Au nanosphere arrays with an 8-base polythymine ssDNA-SH. In both cases, the Au nanospheres were functionalized by placing a drop (30 μL) of the dialyzed, reduced ssDNA-SH solution on the patterned surfaces and incubating for 18 hr. During all reactions, surfaces were placed on an inverted glass dish in an enclosed container with enough standing water to keep solutions from evaporating. Following the reaction, samples were rinsed in a stream of water, and before drying, the samples were then immersed in water for at least 1 hr to remove non-specifically bound ssDNA-SH. Finally, samples were rinsed again briefly in a stream of water, and blown dry with filtered air.
Table 4.1. ssDNA-SH sequence information.

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<table>
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<tr>
<td>Complementary ssDNA-SH</td>
<td>3' - TTTTTTTT - thiol</td>
</tr>
<tr>
<td>Non-complementary ssDNA-SH</td>
<td>5' - AACCCGCGAGGTCCCCGCCCTACGT - thiol</td>
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**DNA Origami Attachment.** Following preparation of the patterned ssDNA surfaces, a 30 µL drop of approximately 2 nM DNA origami solution was placed on the surfaces, in the humidified container described above, at room temperature for times ranging from 30 min to 4 hrs to allow hybridization. Following all reactions, surfaces were rinsed for 30 sec in a stream of water, and before drying, the samples were then immersed in water for at least 1 hr to remove non-specifically bound DNA origami. Finally, samples were rinsed again briefly in a stream of water, and blown dry with air.

**AFM Imaging.** The samples were imaged in air using tapping mode on a Digital Instruments Dimension V AFM (Veeco) with FORTA force modulation AFM tips made by AppNano (Al coated, silicon, purchased from Nanoscience Instruments). Images in **Figure 4.3** (Section 4.3.1) show examples of DNA attachment for times varying from 30 min to 4 hrs.

### 4.2.3 DNA Origami Seeding Methods

**DNA Origami Designs.** Branched (“T”) shaped DNA origami structures were formed using a 2958 base scaffold, amplified from M13mp18 as previously reported in Chapter 2. To enable Au NP attachment, select staple strands from the previously reported design were modified to contain a sequence of 10 adenine nucleotides on the 3’ end. In the initial experiments, 33 staples on one-half of the top section of the “T” structure were modified. For later experiments, the entire top section consisted of modified staple strands (67 in total). For the ‘T’ structure with a gap, 39 staples were modified. Any staple strand that was modified for the structures was used in place of that particular original staple strand when folding the DNA.
origami structure. The prototype logic gate structure was folded using M13mp18 for the scaffold with 246 staple strands. 156 of the staple strands contain the extra 10 adenines on the 3’ end for gold nanoparticle attachment. For specific sequence information refer to Appendix B.

**DNA Origami Folding.** DNA origami structures were folded by heating a mixture of the scaffold and staple strands (2 nM scaffold and 20 nM of each staple strand in 1X TAE-Mg\(^{2+}\) buffer) to 95 °C for 3 min and then slowly cooling to 4 °C over 90 min. DNA origami solutions were filtered with 30 kDa Amicon filters, to remove most of the excess staple strands, by centrifuging for 10 min at 13,000 rpm. Samples were rinsed twice with 450–500 µL of either 1X or 10X TAE-Mg\(^{2+}\) buffer (10X was used for DNA origami surface seeded samples) by centrifuging for 10 min at 13 000 rpm and recovered off the filter by spinning with the filter upside down for 3 min at 3500 rpm.

**Au NP Preparation.** I followed steps similar to those reported previously\(^{18, 53}\) to phosphinate and concentrate the Au NPs with BSPP. Specifically, 1.5 mg of BSPP was added to 5 mL of Au NPs and shaken overnight. 100 mg of NaCl was added, and the solution was centrifuged to pellet the Au NPs. The supernatant was removed, and the Au was resuspended with an aqueous BSPP solution (100 µL, 2.5 mM). Methanol (100 µL) was added, and the solution was centrifuged again to pellet the Au. After removing the supernatant again, the Au was resuspended in aqueous BSPP solution (100 µL, 2.5 mM). The concentration of Au NPs was estimated by comparing the absorption at 520 nm to the absorption of the original gold solution using a Nanodrop 1000 spectrophotometer.

**Au NP-DNA Conjugates.** Thiolated DNA was used without deprotecting the disulfide bond, as the reaction worked either with or without deprotection. Au NPs and thiolated DNA were combined in a 1:200 molar ratio and left to react at room temperature for at least 19 hr. The
Au NP DNA conjugates were filtered using 30 kDa Amicon filters to remove unbound thiolated DNA. Samples were rinsed twice during the filtration using 450–500 µL of 0.5X TBE buffer. About 30–35 µL was recovered, with Au NP concentrations around 1–3.5 µM.

**Attachment of Au NPs to DNA Origami Structures.** For solution attachment of Au NPs to DNA origami structures, DNA origami was combined with the Au NP-DNA conjugates with varying Au NP to DNA origami ratios (of about 1:1, 12:1, 19:1, and 27:1) and cooled from 37 to 20 °C over about 17 min. Then, the solution was deposited onto a mica surface for AFM imaging.

For Au NP surface attachment to DNA origami on SiO₂, DNA origami structures were placed on silicon surfaces using a method reported by Kershner et al. Silicon dioxide surfaces were plasma cleaned (Harrick Plasma Asher; PDC-32G) for 30 sec at 18 W to remove contaminants on the surface. Then, 3 µL of filtered DNA origami (2 or 0.67 nM), in 10X TAE-Mg²⁺ buffer, was allowed to adsorb onto the cleaned surface for 2 hr in a humid chamber at room temperature. The sample was then dipped in a 50% ethanol solution (5 sec) and a 90% ethanol solution (1 hr). Next, the surface was dried by a stream of filtered air and put back into a humid chamber. Subsequently, 12 µL of seeding solution (Au NP-DNA conjugates diluted in 10X TAE-Mg²⁺ buffer, 33 nM) was added onto the surface and allowed to seed DNA for 30 min at room temperature. Afterward, the surface was rinsed in 10X TAE-Mg²⁺ buffer (5 sec), 50% ethanol solution (5 sec), and 90% ethanol solution (1 hr). Finally, the surface was dried with a stream of filtered air.

**AFM Imaging.** The samples were imaged in air using tapping mode on a Nanoscope IIIa MultiMode AFM (Veeco) with silicon tapping mode or silicon force modulation AFM tips (Bruker NCHV-A, ~50 N/m, ~350 kHz; AppNano FORTA, ~1.6 N/m, ~65 kHz).
4.3 RESULTS AND DISCUSSION

4.3.1 Surface Placement of DNA Origami

**Figure 4.2.** DNA origami attachment process. (A) A gold nanosphere patterned surface is formed through a block copolymer patterning process. (B) Au nanospheres are functionalized with ssDNA-SH thiol. (C) DNA origami, which have been modified with sticky ends by extending appropriate staple strands on each end, are placed on the surface, where the modified staple strands base pair with the ssDNA-SH.

**Overview.** A process schematic of the DNA origami attachment method used is shown in Figure 4.2. First, patterned Au nanospheres were formed from a BCP micelle directed gold deposition and reduction process (Figure 4.2A). Next, the Au nanospheres were reacted with ssDNA-SH, creating a patterned ssDNA surface (Figure 4.2B). Finally, a drop of solution containing DNA origami rectangles with complementary sticky ends was placed on the surface to allow base pairing (Figure 4.2C).

Here the BCP micelle method is used to pattern 5 nm Au nanospheres with an average
center-to-center particle spacing of 62 nm. A DNA origami rectangle, 64 nm in length and 11 nm in width, was modified with ssDNA at each end (sticky ends) by extending selected staple strands with a 10-base (3.3 nm in length) polyadenine chain. Attachment studies were performed by functionalizing patterned Au nanospheres with ssDNA-SH (8-base polythymine), which is complementary to the origami sticky ends, then exposing DNA origami to these surfaces and to several control surfaces.

**Attachment Studies.** For patterned complementary ssDNA surfaces, results show an increase in the DNA origami surface concentration from 90 to 230 DNA origami/µm² over the investigated hybridization reaction period (see **Figure 4.3**).

**Figure 4.3.** DNA origami bars on ssDNA functionalized Au nanosphere surfaces. Reactions were carried out for 30, 60, 150, and 240 min for (A), (B), (C), and (D) respectively. (E) Shows the origami/µm² on samples and oxide controls for all reaction times. The right axis (red) shows values for the oxide surface, while the left axis (blue) shows values for the Au nanosphere surfaces. Images in (A–D) are 750 nm on each side.
However, the DNA origami surface concentration on SiO₂ controls was independent of reaction time, with only about one DNA origami/µm² for all reaction times (see Figure 4.3E). The attachment yield, which is defined as the percentage of Au nanospheres having at least one DNA origami attached, on patterned complementary ssDNA surfaces increased from 31% for the shortest reaction time to 74% for the longest reaction time, and the trend in Figure 4.3E indicates that higher yields might be achieved, although it is tapering off, by increasing the reaction time further. Interestingly, analysis of AFM data showed that over 90% of DNA origami had attached between two Au nanospheres, while the remaining DNA origami had only attached to one Au nanosphere. This shows that enough rotational freedom is maintained following the binding of a single end of the DNA origami that the DNA origami is generally able to bridge and bind between two Au nanospheres.

Control experiments were used to probe the attachment mechanism. The following three DNA origami/surface combinations were used: (1) DNA origami deposited on a SiO₂ surface, (2) DNA origami deposited on a patterned noncomplementary ssDNA surface, and (3) DNA origami with no sticky ends deposited on a patterned ssDNA surface. These experiments were performed by exposing surfaces to a solution containing ssDNA-SH, where the 8-base polythymine-thiol was used in all controls except the patterned noncomplementary ssDNA surface (in this case the 25-base ssDNA-SH was used). The surfaces were then exposed to DNA origami for 4 hr. The controls were run in parallel with a patterned complementary ssDNA surface, where base paired attachment of the DNA origami was expected. AFM analysis showed similar low DNA origami surface concentrations for all control surfaces (see Figure 4.4A–C). The number of attached DNA origami was approximately 200 times greater on the surface with base paired attachment than on control surfaces with non-specific attachment.
Figure 4.4. Control experiments used to probe the mechanism of origami attachment. (A) Sticky-end modified origami bars were placed on a clean oxide surface. (B) Non-modified origami bars were placed on a ssDNA functionalized Au nanosphere surface. (C) Sticky-end modified origami bars were placed on a ssDNA modified Au nanosphere surface where the ssDNA-SH was non-complementary to the origami sticky ends. (D) Modified origami bars were placed on a ssDNA functionalized surface, where complementary ssDNA was used. All reaction times were 4 hrs. The scale bar is 200 nm and applies to all images.

Experimentation has suggested that proper surface preparation is vital in avoiding nonspecific DNA origami attachment. It was observed that DNA origami exposed to clean, hydrophilic SiO₂ could be rinsed from the surface. However, contaminated surfaces allowed adsorption of DNA origami. By maintaining clean SiO₂ surfaces and by using vigorous rinses in water after both the thiol reaction and the DNA origami reaction, only one nonspecifically attached DNA origami/µm² was seen on control surfaces for all investigated reaction parameters.

Interestingly, DNA origami placed on freshly prepared bare Au nanosphere patterned surfaces is removed from the surface fairly easily through rinsing after a 3 to 4 hr exposure time (Figure 4.5A). However, when the Au nanosphere patterned surfaces were first placed in a
buffer or water solution overnight prior to introduction of DNA origami (instead of incubation with ssDNA-SH) a fairly high attachment yield resulted even after some vigorous rinsing (Figure 4.5B). This interaction is dependent on the existence of sticky ends on the DNA origami, since those containing no sticky ends rinse fairly easily from Au nanospheres which were placed in aqueous solution overnight (Figure 4.5C). Apparently the surface chemistry of the gold is altered in the extended interaction with aqueous solution, causing it to adsorb ssDNA much more strongly. This interaction seems to be blocked when the Au nanospheres are modified with ssDNA-SH since the Au nanosphere surfaces reacted with a solution of non-complementary ssDNA-SH overnight showed little DNA origami attachment.

Au nanospheres formed using optimized plasma and annealing conditions
are generally stable during the thiol reaction. Successful binding of ssDNA-SH on these surfaces was verified by the highly selective attachment of DNA origami to ssDNA-SH reacted Au nanosphere surfaces. While the physical and chemical stability of the base-pair or thiol attachments has not been thoroughly investigated, the ability of the DNA origami to remain on the surface following the vigorous rinsing technique shows that the overall attachment is fairly robust. The Au nanospheres are generally very difficult to remove from the surface even by sonication. However, some samples did show some instability, as seen in Figure 4.3B and C, where there are a few open areas where Au nanospheres have been removed from the surface. Perhaps on these surfaces the Au had not yet formed into single Au nanospheres during the reduction process, but was instead patterned clusters of smaller Au particles as evidenced by XPS data obtained by Anthony C. Pearson.\textsuperscript{36}

While the BCP patterns here exhibit some order, a high degree of order and alignment can be achieved by forming BCP arrays on lithographically patterned surfaces.\textsuperscript{2-4, 11-14, 20} The formation of highly ordered BCP nanoparticle arrays was not the intent here; instead, the focus was to study the attachment of DNA origami to nanoscale binding sites, specifically BCP generated nanoparticles.

Using BCP micelle patterning, alignment of DNA origami between nearest-neighbor pairs of 5 nm Au nanospheres has been shown. Previous methods using a strong chemical binding mechanism do not allow attachment of DNA origami to pairs of binding sites which are less than 60 nm in diameter,\textsuperscript{9} due to a low probability of the DNA origami finding and binding with smaller sites. In this method, alignment of DNA origami with the 5 nm ssDNA patterns is possible since block copolymer patterning forms binding sites with both small size and close
spacing. This gives a high enough density of available binding sites to make it probable that DNA origami will interact and bind with the patterned ssDNA.

In the AFM data, binding of multiple DNA origami to a single Au nanosphere is often seen. In fact, a 4:5 ratio of DNA origami to occupied Au nanospheres (considering only Au nanospheres with at least one DNA origami attachment) was seen consistently on surfaces prepared as in Figure 4.4D. A 1:2 DNA origami to occupied Au nanosphere ratio is expected if all binding sites contain only one attachment. While multiple binding could be useful in some applications, it could also be problematic. It is possible that multiple binding is limited by the steric hindrance between DNA origami in solution and an occupied Au nanosphere. Pearson et al. have explored the influence of steric hindrance on multiple binding.

4.3.2 Nanoparticle Seeding on DNA Origami

Overview. Figure 4.6 demonstrates the assembly process for site-specific metallization of DNA origami structures to form metallic nanowires. The DNA origami structure shown is a branched “T” structure, which was formed as previously reported in Chapter 2, except where staple strands are extended by a polyadenine (A10) sequence on the 3’ end in selected locations (Figure 4.6A–B). After folding the DNA origami, excess staples were removed by filtering. Gold nanoparticles nominally 5 nm in diameter were conjugated with thiolated polythymine (T8) DNA according to an established protocol and combined with the DNA origami (Figure 4.6 B–C). Attachment of the T8 DNA-linked Au NPs to the extended A10 staple strands on the DNA origami creates “seeds” along specific sections of the DNA structure for further metal deposition (Figure 4.6C–D).
Figure 4.6. Process used for site-specific seeding by attachment of Au NPs and subsequent metallization. (A) Regular (blue) and modified (red portion) staple strands are used to fold a branched “T” DNA origami structure. (B) The location of modified staple strands is programmed based on desired regions for particle attachment. (C) Au NPs coated with DNA complementary to the modified staples are added and attached to the DNA structure. A section is enlarged to show spacing of attached Au NPs along the DNA structure. (D) A subsequent metallization procedure grows the particles until a continuous metal wire is formed across the locations seeded by Au NPs.

Attachment Studies. The attachment sites, or A10 extensions, on the DNA origami were positioned on every staple strand within the desired sections, making them about 11 nm apart along each double helix and in a staggered pattern with adjacent helices (see red dots in zoomed in region of Figure 4.6C). Since multiple thiolated DNA strands are attached to each Au NP and each staple strand extension on the DNA origami contains the same DNA attachment sequence, a 5 nm Au NP could bind easily to the DNA origami through as many as three (and perhaps four) of the extended staple strands. In initial Au NP seeding experiments, “T” origami structures were designed to have Au NPs bind to only one-half of the top section of the DNA origami (see upper structure in Figure 4.6B). This portion is ~120 nm long and contains 33 positions for
when Au NPs were mixed in solution with the DNA origami at a ratio of about 12:1 Au NPs to DNA origami, the section with Au NPs attached almost always appeared significantly shorter by AFM than before Au NP seeding. To explore this phenomenon, ratios of Au NPs to DNA origami of about 1:1, 19:1, and 27:1 were also tested. The 1:1 ratio samples looked very similar to the seeded samples with a 12:1 ratio. In both samples, instead of a Au NP (or a few Au NPs) spread somewhere along the portion of the “T” which contained attachment points, the modified section generally looked truncated with the Au NPs near the junction of the “T” structure (see Figure 4.7A–B). Increasing the ratio of Au NPs mixed with DNA origami to ~19:1 yielded some longer seeded segments, and increasing the ratio to ~27:1 gave even more seeded segments close to the unseeded length (see Figure 4.7D).

**Figure 4.7.** Tapping mode AFM images showing solution-seeded “T” structures with Au NPs along one branch, with varying ratios of Au NPs to DNA origami. The Au NP to DNA origami ratios are about: (A) 1:1, (B) 12:1, (C) 19:1, and (D) 27:1. All samples were seeded in solution and then placed on mica for imaging. The scale bars are 500 nm.

It is possible that when an insufficient number of Au NPs are available for attachment the DNA origami can wrap or fold around attached Au NPs, causing it to be shorter. Occasionally, in samples with the highest Au NP to origami ratio, there are what appear to be DNA origami aggregates (see Figure 4.7D). It is possible that in some of these instances Au NPs are binding...
and connecting two different DNA origami structures together. However, since DNA origami were also seen lying very close together in some samples of unseeded origami, it is difficult to quantify this effect.

To increase the length of the seeded region, the DNA origami design was modified so the staple strands were extended across the entire top section of the “T” structure (see bottom structure in Figure 4.6B). This section is ~240 nm long and contains 67 total extended staple strands for approximately 22 Au NPs to attach. When Au NPs were attached in solution to this structure, the two sides of the top section usually folded

**Figure 4.8.** Tapping mode AFM images of “T” DNA origami structures. (A) “T” DNA origami with modified staple strands along half of the top section after the Au NP attachment process was done in solution. Here a ratio of ~27:1 Au NPs to DNA origami was used. (B) “T” DNA origami with modified staple strands along the entire top section after the Au NP attachment process. For (A) and (B) samples were deposited on mica surfaces for imaging. (C) Unseeded “T” DNA origami deposited on a SiO$_2$ surface. (D) “T” DNA origami seeded with Au NPs after surface deposition on SiO$_2$. The red arrow points to the unseeded portion on the DNA origami. The height scale in all images is 6 nm and the scale bars are 200 nm.
together with some Au NPs attached to both sides (see Figure 4.8B). Since the entire top section contains the same attachment sequence and the “T” structure is somewhat flexible, this was a reasonable, but undesired, result. The problem of simultaneous attachment may be addressed in multiple ways, for instance, by using staple strands with different binding sequences on each arm. In this study, the matter was resolved by first depositing DNA origami on a thermally grown silicon dioxide surface and then exposing the surface to the Au NP solution to permit gold particles to interact with the deposited DNA origami.

To achieve stable adsorption of the DNA origami on the oxide surface, the process reported by Kershner et al. was followed. Specifically, a solution of DNA origami (0.67 nM), containing sticky ends modified as shown in the bottom design of Figure 4.6B, was left on the surface for 2 hr to allow magnesium ions to bind the negatively charged DNA structures to a plasma cleaned, negatively charged silicon dioxide surface.

Figure 4.8C shows an AFM image of “T” DNA origami on a SiO$_2$ surface prior to seeding with Au NPs, and Figure 4.8D shows the DNA origami following Au NP attachment. It is clear that the particles have attached to the top portion of the “T” origami as intended, since the region not modified for Au NP attachment (marked with an arrow in Figure 4.8D) is clearly seen. Comparison of Figure 4.8C and D shows that the shape of the “T” origami is well conserved during the Au NP attachment process, since the top portion has consistent curvature and length before and after attachment. The purpose of the attachment of Au NPs is to create sites on the origami which can be further metallized to form a conductive segment or nanowire. In order to obtain nanowires with the smallest possible dimensions, it is essential to have Au NPs spaced as closely as possible in the desired region on the origami. Since AFM imaging cannot resolve spacing between particles in this size range because of tip effects (Figure 4.9A),
scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were used to examine particle location and spacing, and to provide quantitative measurements of the size of gaps between Au NPs.

SEM samples were prepared using surface seeding of “T” DNA origami on thermally grown SiO₂ surfaces as described above, and TEM samples were prepared by surface deposition and seeding of the DNA origami on a 40 nm silicon monoxide film supported on a copper TEM grid. SEM data (Figure 4.9B&C) show the average length of the seeded portion of the DNA origami to be 195 nm. On average, there were 16 Au NPs attached to each DNA origami, and the median center-to-center spacing was 11.7 nm. A mean Au NP diameter of 7.6 nm, found by measuring particle sizes from high resolution TEM images, was used to estimate the corresponding gap size between particles (see inset of Figure 4.9C). Thus, the estimated median gap between Au NPs is 4.1 nm. However, often in one or more locations, there were larger gaps between Au NPs (see Figure 4.9D).

Interestingly, the microscopy analysis has shown that the Au NPs generally line up in single file along the DNA origami with a median center-to-center spacing (11.7 nm) that nearly matches the sticky end spacing on each of the three double helices on the top portion of the “T” origami (10.5-11 nm). This likely means that the Au NPs are attaching to sets of three sticky ends, as indicated in the zoomed region of Figure 4.6C. When all Au NPs attached to the DNA origami are considered, the ratio of total extended staple strands to Au NPs is 4.19. However, when only the regions with no large gaps between Au NPs are considered, the ratio becomes 2.75. We can conclude that the Au NPs are generally attached to three sticky ends, although some attachment of Au NPs to only two sticky ends also must occur.
Figure 4.9. Seeded DNA origami. (A) Tapping mode AFM image of Au NP seeded DNA origami (zoomed and adjusted height scale from Figure 4.8D; height scale 20 nm). (B–C) Examples of high resolution SEM images used to determine the center-to-center spacing of Au NPs seeded on DNA origami. The inset in (C) shows a bright field TEM image of an Au NP seeded DNA origami. (D) Histogram of the gap sizes between particles on the seeded DNA origami. The inset in (D) corresponds to the largest gap size in each of the measured DNA origami.
Figure 4.10 shows schematics of various ways in which the Au NPs can bind to the DNA origami, giving small gaps between the Au NPs. Here it is assumed that Au NPs bind to either 2 or 3 attachment sites (shown as red dots). The length of each attachment sequence is ~ 3 nm. This allows variability in the exact position of the Au NP along the axis. In Figure 4.10A the Au NPs are each bound at three attachment locations. This would give a gap size between Au NPs of 3.5 nm. In Figure 4.10B Au NPs are bound by
either two or three attachment sequences. This could result in a gap of 1 nm between Au NPs. The schematic in Figure 4.10C shows an unused attachment sequence (gap size of 9 nm). It is possible that another Au NP can not attach to the unused attachment point because either the gap is too small or the single attachment would not be stable. The schematic in Figure 4.10D shows three unused attachment sites or an empty spot big enough for an Au NP to occupy. In this case the gap between Au NPs is 14.5 nm. Comparing these lengths with the measured gap sizes (see Figure 4.10E) shows peaks in the histogram that correlate with the expected spacings from the suggested Au NP attachment positions from Figure 4.10A–B. However, peaks corresponding to larger gap sizes (9 and 14.5 nm) appear to be shifted toward lower values (Figure 4.10C–D). This could be due to bending or curving in the DNA origami causing the particles to be closer than these suggested lengths.

These results show closer Au NP spacing than previously reported techniques. This may be due to the use of a short polythymine (T8) strand to link the Au NPs to the DNA origami. Because of the short persistence length of single-stranded DNA, the length of the T8 strand is ~2 nm in solution. Thus, the effective diameter of the particle is ~11.6 nm, similar to our median center-to-center spacing. Additionally, the high density of available binding locations used increases the probability that a Au NP will attach when it interacts with the DNA origami, making it more likely that particles will attach with the minimum possible spacing.

Using DNA origami, I have demonstrated the ability to fabricate structures in a controlled geometry with small feature sizes, approaching the limitations of current industrial nanofabrication. This technology can be useful in the fabrication of nanodevices for many applications. For example, in nanoelectronics, this technique could be used to fabricate separated source, drain, and gate electrodes for transistors. In order to create geometries useful for
nanodevice fabrication, it is important to have the ability to design and control the location of separate metallized regions in the DNA origami structure. Here I demonstrate that this is possible using a “T” DNA origami. I have adjusted the design of the DNA origami so that the A10 extensions are only located on staple strands toward the ends of the top portion of the “T”, as shown in Figure 4.11A. Seeding of this structure results in an ~100 nm gap between seeding portions. AFM images of the unseeded and seeded structure are shown in Figure 4.11B, and 4.11C, respectively. A geometry such as this could be used and tailored for different gap sizes to form a transistor if a semiconducting material, such as a semiconducting carbon nanotube, were inserted between these seeded and then metallized regions.

I have also designed a DNA origami structure for site-specific metallization that can serve as a template for a simple logic device. Each staple strand of the structure was extended on the 3’ end with an A10 sequence with the exception of two gaps, as shown in Figure 4.11D. The Au NP seeding steps described for the “T” DNA origami were repeated for this structure. AFM images of the unseeded and seeded structure are shown in Figure 4.11E, and 4.11F, respectively. As shown in Figure 4.11F, following seeding, structures have been formed correctly; however, in some cases, leads appear to have flipped inward.

Process optimization would enhance the ability to create large numbers of successful structures. Critical aspects of the fabrication process include the placement of the DNA origami templates on surfaces and selective site-specific metallization of these templates. While the yield of correctly folded origami is fair (~70%), fewer than 15% of the DNA origami deposit on the surface in an open geometry with the four leads directed outward, similar to the structure shown in Figure 4.11D. The remaining DNA origami appear to have twisted, buckled, or aggregated on the surface during the deposition process, which limits the yield of potentially useful metallized
structures after seeding and metal plating. To increase yield, the DNA origami deposition procedure could be altered to increase the percentage that land in an open configuration, or the structures could be attached through multiple chemically specific anchors to better maintain the desired DNA origami shape and decrease aggregation.

**Figure 4.11.** Seeded DNA origami structures with programmed gaps in which a semiconducting material could be deposited. (A) Schematic of the “T” structure where the red markings indicate the location of staple strand A₁₀ extensions for attachment. (B) Tapping mode AFM image of the “T” structure prior to seeding. (C) Tapping mode AFM image of the seeded “T” structure where a gap is seen between seeded regions. (D) The structure of the logic gate prototype DNA origami design, where the red markings show the location of staple strand extensions for attachment. (E) Tapping mode AFM image of the DNA origami prior to seeding. (F) Tapping mode AFM image of the seeded DNA origami. The scale bars are 100 nm.

Further increases in the yield of metallized structures may also be possible through removing background Au NPs that adsorb to the surface and can lead to undesired metallization.
It should be possible to reduce the background particle concentration by adjusting seeding parameters and conditions. Alternatively, chemical attachment of the DNA origami structures to the surface would allow for background reduction by employing more aggressive rinsing techniques. Optimization of these processes and surface attachment will enhance the ability to simultaneously generate very large numbers of successful structures as a basis for nanosystem fabrication and assembly.

4.4 CONCLUSIONS

4.4.1 Surface Placement of DNA Origami

In conclusion, I have demonstrated the ability to align DNA origami to ~5 nm binding sites through DNA base pairing onto BCP patterned Au nanosphere surfaces. Results showed high attachment selectivity, where greater than 200 times more DNA origami were attached to surfaces patterned with complementary ssDNA-SH. On these surfaces, up to 74% of the Au nanospheres formed an attachment with a DNA origami rectangle. This is the first time BCP patterning has been used to fabricate DNA origami attachment sites. Individual binding sites fabricated with this method are at least an order of magnitude smaller than binding sites used previously. Binding sites on this size scale are important to meet the alignment tolerance requirements for nanoscale DNA origami surface patterning.

I have seen that the base pairing of DNA origami rectangles with patterned ssDNA-SH surfaces results in a stable, highly selective attachment to the surface. As noted earlier, substrate cleanliness is the most important parameter to control nonspecific DNA origami attachment on SiO₂ surfaces. Thus, surface cleanliness was maintained throughout each process step.

The chemically specific nature of the base-paired attachment may be useful to further control the location or orientation of attached DNA origami. Any patterning method allowing
discrete regions in a Au nanosphere array to each be modified with a unique ssDNA-SH sequence would then allow location controlled attachment of multiple DNA origami shapes. Further orientational control may also be possible if individual DNA origami have two or more sticky end regions with different base sequences.

4.4.2 DNA Origami Seeding for Metallization

I have demonstrated a method that uses site-specific attachment of gold nanoparticles as seeds to fabricate nanowires from DNA origami templates. An important aspect of the work is the attachment of high densities of Au NPs onto branched DNA origami structures, with a measured median gap size of 4.1 nm. These closely spaced Au NPs can serve effectively as seeds for metallization to create continuous metallized segments or nanowires on origami templates, enabling future fabrication of a wide variety of nanodevices.

4.5 REFERENCES


CHAPTER 5: IMPROVING GOLD NANOPARTICLE SEEDING ON DNA ORIGAMI

5.1 INTRODUCTION

Nanoparticle attachment to DNA origami can be a useful bottom-up self-assembly process. DNA origami\(^1\) is a versatile, one-pot method of creating designed ~100 nm scale nanostructured objects. It has been used to create complex two-\(^2\)-\(^4\) and three-dimensional\(^5\)-\(^8\) structures, as well as to organize other nanomaterials.\(^9\)-\(^18\) DNA origami also allows for specific positioning of nanoparticles, enabling increased control over alignment and spacing. Nanoparticles that have been positioned by DNA origami include gold,\(^19\)-\(^28\) silver,\(^29\) and quantum dots.\(^30\)-\(^31\) Positioned Au NPs could find use as seed particles for electroless metal deposition to create nanowires; using DNA origami as a template would enable the formation of complex nanowire shapes and junctions that would be difficult to assemble with other techniques. In addition, Au NPs precisely positioned along DNA origami could be used to assemble nanostructures for plasmonic nanolenses,\(^20\) rulers,\(^27\) or circuitry.\(^28\)

Several groups have demonstrated Au NP attachment to DNA origami structures, either through solution\(^19\)-\(^20, 24\)-\(^25, 27\)-\(^28\) or surface seeding,\(^23\) or both.\(^21\)-\(^22, 26\) In these reports, various techniques and reaction conditions were reported, including different oligonucleotide attachment lengths (8–30 bases), sizes of Au NPs (5–15 nm), hybridization times (0.3–24 hrs) and temperatures (4–40 °C), and designed distances between the attached Au NPs. Some of these groups used thiolated staple strands to conjugate the Au NPs,\(^19, 22\) while the rest used base-pairing of thiolated oligonucleotide coated Au NPs to the DNA origami structures. The structures reported by other groups were designed with specific and distinct attachment locations for each Au NP. In my design from Chapter 4, each staple strand in the desired attachment area contains
the same ending oligonucleotides for Au NP-DNA conjugates (all having the same complementary sequence) to hybridize with. This allows for increased density of attachment sequences (~6 nm apart) and increased probability that Au NPs will attach to the DNA origami structure, but also raises the unique challenge that the Au NPs do not have a distinct set of staple strands to which they are being tethered. Thus, Au NPs can attach by base-pairing with 2, 3, or even 4 attachment sequences on the DNA origami structures (see Figure 4.6C inset). The Au NPs can also attach in such a way that hybridization sequences on the DNA origami structures are left unpaired or leave gaps too small to allow another Au NP to attach with sufficient room or stability (see Figure 4.10C–D).

In Chapter 4, I showed that Au NPs could be selectively attached to desired portions of DNA origami templates and used to create conductive nanowires. To create the thinnest wires possible, it is desirable to attach Au NPs as closely as possible along the DNA origami templates. Results from Section 4.3.2 show that the Au NPs attach to the “T” structures with a median center-to-center distance of ~11.7 nm, yielding a median gap distance of ~4.1 nm. Taking into consideration the diameter of the Au NPs (~7.6 nm, see Section 4.3.2) and the solution length of the thiolated oligonucleotides, the Au NPs are expected to have an effective size of ~11.6 nm, which is nearly identical to the measured median center-to-center distance, as well as the spacing of the attachment sequences on the DNA origami structures (see Section 4.3.2). These results indicate that the Au NPs are often packing closely along the DNA origami structures; however, some gaps were also seen in the results. For each of the structures evaluated, there appeared to be at least one larger gap whose distance ranged from 12–37 nm (see Figure 4.9D). Since the Au NPs can attach closely in some places, it is desirable to optimize the Au NP attachment process to completely eliminate the gaps if possible.
Factors that can influence the yield of Au NP attachment to DNA origami include: the number of staple strands per Au NP binding site, the length of the attachment sequence, the ratio of Au NPs to DNA origami, the hybridization temperature, and the age of the Au NP solution. Ding et al.\textsuperscript{20} reported a decrease in Au NP attachment with only two DNA attachment strands per Au NP binding site when compared with three attachment strands. Hung et al.\textsuperscript{21} studied the effect of the linker length, concentration ratio of Au NPs to DNA origami, and the hybridization temperature. They found that 8-base polyT linkers in place of 30-base polyT linkers, increasing the Au NP concentration relative to the DNA origami structures, and increasing the temperature all allowed for higher attachment yields. The best Au NP attachment yields came from solution seeding at 37 °C with T\textsubscript{s} coated Au NPs. Kuzyk et al.\textsuperscript{25} reported optimized conditions for Au NP attachment giving yields of 96–98%, with the caution to use the thiolated DNA-coated Au NPs immediately after filtering to prevent unbound thiolated oligonucleotides from binding to the DNA origami structures and reducing the Au NP attachment yield.

This chapter systematically explores the effect (on closely spaced Au NP attachment) of hybridization time, magnesium ion concentration, concentration ratio of Au NPs to DNA origami, and the age of the Au NP solution. In order to gauge which reaction conditions provided better Au NP patterned DNA origami structures for future metallization into conductive wires, three different parameters were measured: (1) the total number of Au NPs attached to DNA origami structures, (2) the number of Au NPs in a single-file line along the structures, and (3) the largest gap between neighboring Au NPs in each DNA origami structure. If the structures have more Au NPs associated with them than are needed to form a single-file line or if there are large gaps between Au NPs that would need to be filled with metal during plating to make the wire continuous, wider nanowires would result, compared to ones formed through ideal Au NP
attachment. These studies offer insights into the Au NP attachment process, detailing optimum conditions for high-density alignment of Au NPs on DNA origami structures, which could enable thinner, more continuous metal nanostructures.

5.2 EXPERIMENTAL

5.2.1 Materials

M13mp18 and streptavidin-coated magnetic beads were obtained from New England Biolabs. DNA origami staple strands (100 µM in TE buffer) were purchased from Eurofins MWG Operon. PCR primers were ordered from either Integrated DNA Technologies or Eurofins MWG Operon and diluted to 1 µg/µL in water. Single stranded DNA thiol was purchased from Eurofins MWG Operon with PAGE purification and diluted to 1 mM in water. PCR purification kits were purchased from Qiagen. Taq DNA polymerase and PCR buffers were obtained from Invitrogen. 30 kDa Amicon ultra 0.5 mL centrifugal filters were acquired from Millipore. Au NPs (5 nm) were ordered from Ted Pella. BSPP (bis(p-sulfonatophenyl)-phenylphosphine dihydrate dipotassium salt) was acquired from Strem Chemicals. Silicon (100) wafers (p-type) with native oxide were purchased from Silicon Wafer Enterprises and p-type silicon (100) wafers with a 200 nm oxide layer were obtained from MicroSil. Ultrapure, 18.3 MΩ water used for experiments was produced by an EasyPure UV/UF water purification system.

5.2.2 Methods

Process Overview. I used the branched, “T” DNA origami structure with modified staple strands for Au NP attachment along the entire top section of the “T”. Figure 5.1 shows the Au NP attachment process. The “T” DNA origami structures were folded (Figure 5.1A), and then filtered to remove excess staple strands. Concentrated, BSPP-coated Au NPs (5 nm) were reacted with an excess of thiolated oligonucleotides (Figure 5.1B) and then filtered to remove excess
unbound DNA. Next, the filtered DNA origami solution, with adjusted Mg\(^{2+}\) concentration, was placed on freshly plasma cleaned silicon oxide surfaces (Figure 5.1C). The DNA origami structures were allowed to absorb on the surface and then the Au NP-DNA solution was added to allow hybridization with the “T” DNA origami structures. This Au NP attachment method is different from the process used in Chapter 4, but allows good surface adherence of DNA origami structures to the silicon oxide surfaces in significantly less time.

**Figure 5.1.** Overview of Au NP attachment process. (A) “T” DNA origami templates are assembled with Au NP attachment sequences (red) along the top section. (B) Au NPs are reacted with thiolated oligonucleotides. (C) DNA-coated Au NPs base pair with attachment sequences on the DNA origami structures to form the DNA-templated Au NP nanostrings.

**DNA Origami Scaffold Preparation.** The scaffold for the “T” DNA origami structure was prepared as reported in Section 2.2.2, except PCR was adjusted for use with Taq polymerase instead of Pfx polymerase. Taq polymerase (2.5 units) was added to a solution containing primers (0.5 µg each), M13mp18 template (20 ng), a mixture of dNTPs (200 µM), and 1x Taq polymerase buffer in a 100 µL volume. The PCR program was modified to have an initial denaturing step at 95 °C of only 30 sec and denaturing steps within the cycles of only 30 sec at 95 °C. The program was as follows: 95 °C for 30 sec, 30 cycles of 95 °C for 30 sec, 59 °C for 45
sec, and 68 °C for 3 min, with a final extension at 68 °C for 5 min and a hold temperature of 4 °C. The PCR purification and streptavidin-coated magnetic bead separation steps were performed as reported in Chapter 2.2.2. For the “T” DNA origami structure 20 µL of purified PCR product was used and the samples were mixed with the beads for 30 min to allow them to bind together. The product from bead separations was purified with spin columns (QIAquick PCR purification kit).

**DNA Origami Folding.** Staple strands modified with 10 adenine nucleotides on the 3’ end were used for the entire top section of the “T” structure (67 modified staple strands total) as described in Section 4.3.2. The DNA origami structures were folded with a 1:10 molar ratio of scaffold strand to staple strands in 1x TAE-Mg2+ buffer with a final scaffold concentration of 5 nM and volume of 100 µL. The structures were folded as reported in Section 2.2.2, by denaturing (95 ºC for 3 min) and slowly annealing from 95 ºC to 4 ºC in 90 min. The DNA origami structures were then filtered to remove excess staple strands using 30 kDa Amicon ultra 0.5 mL centrifugal filters. Each DNA origami sample was filtered by spinning for 10 min at 13,000 rpm (14550 rcf), rinsed three times with 1x TAE-Mg2+ buffer (500 µL) by spinning for 10 min at 13,000 rpm (14550 rcf), and then recovered with a spin at 3,500 rpm (1055 rcf) for 3 min. The concentration of the solution was measured with a Nanodrop 1000 spectrophotometer and then the solution was diluted to 1 nM in 1x TAE-100 mM Mg2+ buffer. For the [Mg2+] tests the DNA origami was diluted and adjusted to be in 1x TAE buffer with either 40, 70, 100, or 130 mM Mg2+.

**Au NP Preparation.** The Au NPs were prepared either as reported in Section 4.2.3 or with larger volumes as follows: 20 mL of Au NPs (83 nM) were mixed with 10 mg of BSPP and shaken overnight. Solid NaCl was added until the color changed to brown and NaCl became
harder to mix in. The solution was spun to pellet the Au NPs for 30 min at 3660 rpm (~1600 rcf) with a ss-34 rotor in a Sorvall RC 5C Plus Superspeed centrifuge and with an Eppendorf 5418 centrifuge for 10 min and 5 min at 5,000 rpm (2150 rcf) to help the Au NPs settle better. The solution was split into several smaller 1.5 mL centrifuge tubes for the 5,000 rpm step. The supernatant was removed and the Au NPs were combined and resuspended in an aqueous BSPP solution (500 µL, 2.5 mM). Methanol was added (500 µL), the solution was mixed, and then centrifuged for 30 min at 5,000 rpm with an Eppendorf 5418 centrifuge. The supernatant was removed and the Au NPs were resuspended with BSPP solution (400 µL, 2.5 mM). Methanol was again added (400 µL), the solution was mixed, and then centrifuged for 40 min at 5,000 rpm. The supernatant was removed and the Au NPs were resuspended with BSPP solution (400 µL, 2.5 mM). The concentration of the Au NPs was measured at 520 nm (using 12.05 µM⁻¹cm⁻¹ for the extinction coefficient) using a Nanodrop 1000 spectrophotometer.

**Au NP-DNA Preparation.** The Au NP-DNA conjugates were prepared as reported in Section 4.2.3. The solution containing the 1:200 ratio of Au NPs to thiolated DNA was allowed to react for at least 60 hrs. The Au NP-DNA conjugates were filtered with 30 kDa Amicon ultra 0.5 mL centrifugal filters. Each sample was filtered by spinning for 10 min at 13,000 rpm (14550 rcf), rinsed three times with 0.5x TBE buffer by spinning for 10 min at 13,000 rpm, and then recovered with a spin at 3,500 rpm (1055 rcf) for 3 min. The concentration of the Au NP-DNA conjugates was measured at 520 nm and the solutions were diluted to the desired concentrations (5, 10, 25, and 50 nM) with water.

**Attachment of Au NP-DNA Conjugates to DNA Origami Structures.** Sample hybridization was performed following the process reported by Pilo-Pais et al. using freshly washed and plasma cleaned (1 min, 18 W, with a Harrick Plasma Cleaner) silicon wafer pieces
with either a native oxide surface or a 200 nm thermal oxide layer; thermal oxide surfaces were used for the 10 and 20 min hybridization time samples. First, the “T” DNA origami (5 µL, 1 nM) was deposited on the silicon dioxide surfaces for 5 min in a humid chamber at room temperature, followed by addition of Au NP-DNA solution (20 µL, 10 nM; for tests varying Au NP concentrations, 5, 10, 25, or 50 nM was used). After the designated time period, the silicon pieces with the DNA and Au NPs were dipped into a Petri dish with water for 10 sec and dried with a stream of compressed air.

**AFM/SEM Imaging.** Samples were imaged in air using tapping mode on a Digital Instruments Nanoscope IIIa MultiMode AFM (Veeco) with silicon force modulation AFM tips (AppNano FORTA probes, 1.6 N/m, 61 kHz). Samples were imaged with an FEI Helios Nanolab 600 SEM using ultra high resolution, immersion mode.

### 5.3 RESULTS AND DISCUSSION

#### 5.3.1 Imaging Data and Analysis Procedures

An AFM image is shown in **Figure 5.2A** of the “T” DNA origami structures before Au NP attachment and an SEM image is shown in **Figure 5.2B** of the Au NP seeded DNA origami structures. Because the DNA origami structures were designed for the Au NPs to attach only along the top section of the “T” structure, they appear as linear Au NP strings in the SEM images.

SEM images were used to analyze the Au NPs in the attachment studies. The Au NPs in the images were counted visually and recorded as a total number of Au NPs associated with a nanostring. Additionally, the number of Au NPs in a line along the DNA origami structure was measured. This latter measurement was somewhat more difficult, because the nanostrings are flexible and are not always aligned on the surface. To account for nanostring curvature, the Au
NPs did not need to be in a straight line, but were counted following a single-file path through the nanostring (i.e., Figure 5.2C–D). If Au NPs appeared to be attached two or more wide in places, any extras were left out of the single-file count. Representative examples of the Au NP in-a-line counts are shown in Figure 5.2C–D. For each of the samples prepared under different conditions, 30 nanostructures were evaluated. To measure the largest center-to-center distance between neighboring Au NPs in each of the DNA origami structures the program Image J was utilized. The scale in pixels/nm for each image was set using the scale bar within the SEM image and then a cross section of the two Au NPs was taken to determine the center-to-center distance (Figure 5.2E–F). The data were adjusted to Au NP spacing distances by subtracting the average Au NP diameter (7.6 nm).26

Figure 5.2. Analyzing Au NP attachment to DNA origami. (A) AFM image of “T” DNA origami structures. (B) SEM image of Au NP seeded DNA structures. (C–D) Examples of SEM data for determining the number of nanoparticles in a line for seeded structures. (E) Example of SEM data for largest gap determination using Image J. (F) Cross-section plot of the line drawn in (E). Scale bars in (A–B) are 200 nm and scale bars in (C–E) are 100 nm.

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5.3.2 Au NP Attachment Studies

Initial tests using the Au NP attachment process described in Section 5.2.2 were performed to give starting volumes and concentrations for the DNA origami (5 µL, 1 nM) and Au NPs (20 µL, 10 nM). These volumes and concentrations ensured good coverage of the surface and a ratio of 40:1 Au NPs to DNA origami structures. If the Au NPs attach to the DNA origami structures at three positions per Au NP, then ~22 Au NPs are expected to attach to the top portion of the “T” structure, such that a ~1.8 fold excess of Au NPs was utilized in most of the test studies.

Table 5.1. Data for different hybridization times.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>NPs in a line</th>
<th>Total NPs</th>
<th>Min</th>
<th>Q1</th>
<th>Median</th>
<th>Q3</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>12 ± 4</td>
<td>13 ± 5</td>
<td>9</td>
<td>38</td>
<td>54</td>
<td>60</td>
<td>98</td>
</tr>
<tr>
<td>20</td>
<td>12 ± 2</td>
<td>13 ± 2</td>
<td>29</td>
<td>37</td>
<td>47</td>
<td>61</td>
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<td>30</td>
<td>16 ± 3</td>
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<td>28</td>
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<td>23</td>
<td>28</td>
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<td>63</td>
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<tr>
<td>90</td>
<td>24 ± 2</td>
<td>29 ± 4</td>
<td>6</td>
<td>12</td>
<td>15</td>
<td>18</td>
<td>35</td>
</tr>
<tr>
<td>150</td>
<td>24 ± 3</td>
<td>31 ± 6</td>
<td>5</td>
<td>11</td>
<td>14</td>
<td>17</td>
<td>36</td>
</tr>
<tr>
<td>210</td>
<td>26 ± 3</td>
<td>36 ± 7</td>
<td>5</td>
<td>8</td>
<td>11</td>
<td>12</td>
<td>21</td>
</tr>
</tbody>
</table>

1 Q1 = 25th percentile, or middle value between min and median.
2 Q3 = 75th percentile, or middle value between median and max.

To evaluate the effect of hybridization time on Au NP attachment, the Au NP solution was allowed to react with the DNA origami coated surfaces for times ranging from 10 to 210 min. Table 5.1 and Figure 5.3 show the results. For nanoparticle density, hybridization times less than 30 min are insufficient for the desired number of Au NPs to attach, while hybridization times of 90 min or longer show an increasing difference between total NPs and NPs in a line. This separation indicates that the nanostructures are becoming wider than single-file with longer hybridization times.
The 10 and 20 min hybridization samples were deposited on silicon pieces with a 200 nm thermal oxide layer. In these samples (and sometimes faintly on the native oxide surfaces) the DNA can be seen as a darker color, from surface charging in the SEM (Figure 5.4). In general, the surfaces with the 200 nm thermal oxide were not used for the Au NP attachment tests because focusing the SEM was more difficult, but for those two short hybridization samples it was helpful in counting Au NPs to be able to see where the DNA structures were located. DNA has also been observed in SEM images reported by others.\textsuperscript{20} Other than being able to see the DNA in the SEM images, no other differences were observed in using native vs. thermally grown silicon oxide surfaces to deposit the DNA origami and Au NPs.

Figure 5.3. Au NP attachment results for different hybridization times. (A) Graph of the average number of Au NPs attached along a line (purple triangles) and the average total number of Au NPs per nanostring (blue squares). (B) Box and whisker plot of the largest gap between neighboring Au NPs in each string. Thirty nanostrings were evaluated for each hybridization time.
The largest gap data for the various hybridization times is shown in Figure 5.3B as a box and whisker plot. As expected, with increasing hybridization time the average largest gap between neighboring Au NPs becomes smaller and has a smaller spread. As shown in Figure 4.6 in Section 4.3.2, if each Au NP is attaching to the DNA origami through three attachment sequences, the center-to-center spacing of the Au NPs is expected to be ~11 nm with an average distance of 4 nm between edges of neighboring Au NPs. The smallest observed maximum gap size of 5 nm (at 150 and 210 min) indicates that it is possible to place Au NPs along a nanostructure close to this ~4 nm minimum distance between Au NPs. A hybridization time of 90 min appears to be the optimum condition for Au NP density and reduction of gap size.

In the next study, the Mg$^{2+}$ concentrations in the DNA origami solutions and the hybridization times were varied. Magnesium ions are important in the formation of DNA origami structures, because they screen the negative charges on the backbone in DNA, allowing the strands to approach closely enough to hybridize. In this work, Mg$^{2+}$ ions also play a role in helping the DNA origami structures adhere to the silicon oxide surface. For the previous hybridization time study, the DNA origami was adjusted, after filtering, to contain the desired DNA concentration and 100 mM Mg$^{2+}$. In the case of the DNA-coated Au NPs, however, extra
Mg$^{2+}$ from the DNA origami solution could cause the Au NPs to aggregate when deposited on the surface, making less Au NPs available for attachment to DNA origami structures or potentially attaching to the DNA origami as clusters or aggregates.

**Table 5.2.** Data for different Mg$^{2+}$ concentrations and hybridization times.

<table>
<thead>
<tr>
<th>[Mg$^{2+}$]</th>
<th>Time (min)</th>
<th>NPs in a line</th>
<th>Total NPs</th>
<th>Largest Gap: (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Min Q1 Median Q3 Max</td>
</tr>
<tr>
<td>40 mM</td>
<td>10</td>
<td>16 ± 3</td>
<td>18 ± 5</td>
<td>12 24 32 45 58</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>19 ± 3</td>
<td>25 ± 6</td>
<td>6 13 17 24 51</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>23 ± 2</td>
<td>28 ± 4</td>
<td>6 11 14 17 29</td>
</tr>
<tr>
<td>70 mM</td>
<td>10</td>
<td>21 ± 2</td>
<td>23 ± 4</td>
<td>7 14 20 25 39</td>
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<td></td>
<td>30</td>
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<td></td>
<td>90</td>
<td>22 ± 2</td>
<td>25 ± 4</td>
<td>7 11 14 16 31</td>
</tr>
<tr>
<td>100 mM</td>
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<td>21 ± 3</td>
<td>24 ± 5</td>
<td>10 17 25 33 41</td>
</tr>
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<td></td>
<td>30</td>
<td>22 ± 2</td>
<td>25 ± 4</td>
<td>9 14 18 22 36</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>25 ± 2</td>
<td>33 ± 5</td>
<td>7 10 12 16 22</td>
</tr>
<tr>
<td>130 mM</td>
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<tr>
<td></td>
<td>90</td>
<td>23 ± 2</td>
<td>26 ± 4</td>
<td>10 11 15 17 26</td>
</tr>
</tbody>
</table>

The results for varying Mg$^{2+}$ concentrations and hybridization times are shown in **Table 5.2** and **Figure 5.5**. As before, more Au NPs attached to the DNA structures with longer hybridization times. The concentration of Mg$^{2+}$ ions in solution has a small effect at lower Mg$^{2+}$ concentrations, with the 10 min hybridized, 40 mM Mg$^{2+}$ solution having, on average, fewer nanoparticles attaching to the DNA origami than 10 min hybridized samples with higher Mg$^{2+}$ concentrations. DNA origami solutions with less than 40 mM Mg$^{2+}$ were also utilized, but the DNA origami did not adhere to the surface well enough to be evaluated. Some aggregation of Au NPs was observed in the form of clusters of Au NPs on the surfaces. These clusters appear larger.
and more frequently on the samples with higher Mg$^{2+}$ concentrations and longer hybridization times (Figure 5.6). The best conditions from this study appear to be 70–100 mM Mg$^{2+}$ concentrations and 30–90 min hybridization times.

![Figure 5.5](image.png)

**Figure 5.5.** Au NP attachment results with different hybridization times and magnesium concentrations. Purple triangles represent the average number of Au NPs along a line. Blue squares represent the average total number of Au NPs per nanostring. Orange and green rectangles are a box and whisker plot of the largest gap between neighboring Au NPs in each nanostring. Thirty nanostrings were counted for each hybridization time and magnesium concentration.

One surprise is that the 10 and 30 min hybridization time samples in this study on average had more Au NPs attached and smaller gap sizes than in the more extensive time study described in Table 5.1 and Figure 5.3. The reason for this is not entirely known, although it may
involve variability in the DNA origami sample or the freshness of the Au NP solution, which will be discussed later.

I also studied changing the ratio of Au NPs to DNA origami; the DNA origami concentration was held constant, at 0.2 nM after addition of Au NPs, and the concentration of the Au NPs was varied with final concentrations of 4, 8, 20, and 40 nM. For 22 Au NPs attaching to each DNA origami structure, these concentrations lead to ratios of 0.9:1, 1.8:1, 4.5:1, and 9.1:1 Au NPs per attachment location on the DNA origami structures. Table 5.3 and Figure 5.7 show the data for Au NP density and gap size. All four samples had a hybridization time of 60 min, approximately the optimum for other experiments. The effect of changing the concentration of Au NPs was not as strong as changing other conditions, but there does appear to be a gradual increase in the number of Au NPs attaching to the DNA origami and a slight decrease in gap sizes with higher Au NP to attachment site ratios.

Figure 5.6. Aggregation of Au NPs with increasing Mg$^{2+}$ concentration and hybridization time. Mg$^{2+}$ concentrations and hybridization times for the samples were: (A) 40 mM and 10 min, (B) 70 mM and 30 min, (C) 100 mM and 90 min, and (D) 130 mM and 90 min. Scale bars are 5 µm.
Table 5.3. Data for various Au NP concentrations.

<table>
<thead>
<tr>
<th>[Au NP] (nM)</th>
<th>NPs: attachment location</th>
<th>NPs in a line</th>
<th>Total NPs</th>
<th>Min</th>
<th>Q1</th>
<th>Median</th>
<th>Q3</th>
<th>Max</th>
</tr>
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<td>17 ± 2</td>
<td>19 ± 3</td>
<td>16</td>
<td>25</td>
<td>31</td>
<td>35</td>
<td>57</td>
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<tr>
<td>8</td>
<td>1.8:1</td>
<td>19 ± 2</td>
<td>22 ± 3</td>
<td>5</td>
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<td>22</td>
<td>30</td>
<td>43</td>
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<td>4.5:1</td>
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<td>23 ± 4</td>
<td>12</td>
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<td>23</td>
<td>28</td>
<td>36</td>
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<td>25 ± 5</td>
<td>11</td>
<td>16</td>
<td>20</td>
<td>27</td>
<td>69</td>
</tr>
</tbody>
</table>

1 Concentration of Au NPs after addition with DNA origami solution. DNA origami was held at the same concentration for all samples (0.2 nM after Au NP addition).

One very noticeable difference was the amount of loose, unattached Au NPs on the surfaces with increasing Au NP concentration (Figure 5.8). This increase in Au NPs on the background is undesirable for subsequent metal deposition, as the background Au NPs will also enlarge during the metallization process. Increasing the concentration of Au NPs could help increase the yield of Au NP attachment without affecting the background if the DNA origami structures were seeded with the Au NPs in solution instead of after

Figure 5.7. Au NP attachment results with varying Au NP concentrations. Purple triangles represent the average number of Au NPs along a line. Blue squares represent the average total number of Au NPs per nanostring. Orange and green rectangles are a box and whisker plot of the largest gap between neighboring nanoparticles in each string. Thirty nanostrings were counted for each Au NP concentration.
being deposited on a surface. Then the excess Au NPs could be removed before the structures are deposited on a surface. Alternatively, if the DNA origami structures were covalently, or otherwise strongly attached to the surface then more rigorous rinsing techniques could be used to remove the excess, unbound Au NPs.

Another factor that has a great influence on the number of Au NPs attaching to the DNA origami structures is the age of the DNA-coated Au NP solution, or more specifically, the time between when the excess unbound thiolated DNA strands are removed through filtration and when the Au NP attachment to DNA origami occurs. For the experiments in Chapter 4, the DNA-coated Au NP solution was prepared and stored at 4 °C until needed for the experiments. Each time the solution was exhausted, more was prepared. However, recently Kuzyk et al.25 described the necessity of filtering the Au NP solution immediately before use to remove DNA strands with oxidized thiols that have unbound from the Au NPs over time. This prevents these free DNA strands from pairing with the attachment sequences on the DNA origami structures, thereby hindering the Au NPs from attaching. Thus, I compared samples made with the same hybridization times and solution concentrations, but different ages of the filtered Au NP solution. The Au NP age data from samples prepared with a 1.8:1 ratio of Au NPs to DNA origami attachment locations, 100 mM Mg$^{2+}$ in the DNA origami solution, and 60 min hybridization
times are shown in Table 5.4 and Figure 5.9. The total number of Au NPs attached to the DNA origami structures was closer to the number of Au NPs attaching single-file in structures seeded with fresh (≤ 1 day) Au NP solutions than for older solutions, indicating that freshly prepared Au NPs yield less aggregation in seeded structures. However, the DNA origami structures seeded with Au NPs the soonest after filtration of the Au NP solution do not have as many Au NPs attaching as possible in a single-file arrangement. Adjustment of sample preparation conditions, such as using a longer hybridization time, could address this issue.

Table 5.4. Data for Au NP solutions filtered at various times before use.

<table>
<thead>
<tr>
<th>NP solution age (days)</th>
<th>NPs in a line</th>
<th>Total NPs</th>
<th>NP Density:</th>
<th>Largest Gap: (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Min Q1 Median Q3 Max</td>
<td></td>
</tr>
<tr>
<td>&lt;1&lt;sup&gt;1&lt;/sup&gt;</td>
<td>17 ± 3</td>
<td>19 ± 4</td>
<td>14 23 28 37 63</td>
<td></td>
</tr>
<tr>
<td>&lt;1&lt;sup&gt;2&lt;/sup&gt;</td>
<td>19 ± 2</td>
<td>22 ± 3</td>
<td>5 17 22 30 43</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>22 ± 2</td>
<td>24 ± 3</td>
<td>8 14 17 20 32</td>
<td></td>
</tr>
<tr>
<td>66</td>
<td>23 ± 2</td>
<td>28 ± 4</td>
<td>7 12 15 19 29</td>
<td></td>
</tr>
<tr>
<td>89</td>
<td>22 ± 2</td>
<td>31 ± 5</td>
<td>4 10 13 17 21</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup> About 5 hrs.
<sup>2</sup> About 7 hrs.

There is also a trend toward decreasing largest gap sizes with increasing age of the Au NP solution, which is the opposite of what would be expected if newly released DNA strands from the Au NPs were blocking the attachment sites on the DNA origami structures. However, smaller gaps are expected for structures with more Au NPs attached, so the trend toward more aggregated Au NP seeded DNA structures may be overwhelming any effects from blocked attachment sites. The Au NPs may also be aggregating in solution over time and this aggregation could be affecting the total number of Au NPs found along a DNA origami structure.
It is interesting to note that the DNA origami structures seeded with the freshest DNA-coated Au NPs did not show the best results in terms of the number of Au NPs attaching and the largest gap per DNA origami structure. The samples prepared one day after the filtration are closer to the desired structures with ~22 Au NPs per DNA origami structure. As noted earlier, the time between filtering the Au NPs and seeding the DNA origami structures could be responsible for variations in the data from different studies. The Au NP solution for the Mg\(^{2+}\)/hybridization time study was 5 days old, whereas the Au NP solutions for the hybridization time study and varying concentration ratios study were used in the same day they were filtered.

Taking all the variations from the different studies into consideration, Figure 5.10 shows SEM images of some of the best looking Au NP strings. There is a range of conditions that give similar Au NP attachment results. Hybridization times ranging from 30–90 min, Mg\(^{2+}\)
concentrations of 70–100 mM, and Au NP to DNA origami ratios from 1.8–4.5 to Au NPs per DNA origami attachment location produce the least aggregated nanostructures with the smallest gaps. There are still small gaps in even the best looking structures, and variability within samples, indicating that further improvement is still possible.

One reason there may still be gaps within the Au NP seeded structures under my optimized conditions could be from all the attachment sequences being the same. If two different Au NP attachment sequences were used and alternated (in groups of three) along the top section of the “T” DNA origami structure, then the Au NPs could be directed to specific attachment locations along the DNA structure (Figure 5.11). This could eliminate gaps arising from Au NPs randomly attaching along the structure, leaving gaps that are too small.

Figure 5.10. SEM images of some of the best Au NP attachment results. The conditions for each sample are as follows: 0.2 nM “T” DNA origami, with (A) 8 nM Au NPs and 100 mM Mg$^{2+}$ in the DNA solution, with a 30 min hybridization time; (B) 8 nM Au NPs and 70 mM Mg$^{2+}$ in the DNA solution, with a 90 min hybridization time; (C) 8 nM Au NPs and 100 mM Mg$^{2+}$ in the DNA solution, with a 90 min hybridization time; (D) 20 nM Au NPs and 100 mM Mg$^{2+}$ in the DNA solution, with a 60 min hybridization time. Scale bars are 200 nm.
for another Au NP to fit into or potentially binding with more than three sequences. Some gaps within the structures in **Figure 5.10** still appear large enough for another Au NP to fit into. There may be free DNA strands that are paired with the DNA structure and blocking those locations.

### 5.4 CONCLUSIONS

In summary, I have varied experimental parameters and evaluated SEM images to determine the best conditions for densely attaching Au NPs to the top section of a “T” DNA origami structure in a single-file line. Conditions, including the hybridization time, concentration of Mg\textsuperscript{2+} in the DNA solutions, the ratio of Au NPs to DNA origami, and the freshness of the Au NP solution were varied to determine their effects on Au NP attachment. Parameters that were measured included the number of Au NPs attaching per DNA origami structure, the number of Au NPs attaching single-file along the nanostructures, and the largest gap between neighboring Au NPs in the DNA origami templated Au NP structures.

Reducing the number of excess Au NPs attaching to the DNA origami structures, while also decreasing the distance between the Au NPs will both contribute to thinner and more continuous metallized nanowires. The best conditions were between 70–100 mM Mg\textsuperscript{2+} in the DNA origami solutions, ratios from 1.8–4.5 Au NPs per attachment location on the DNA origami structures.
origami structures, and hybridization times ranging from 30–90 min. Assembling DNA templated Au NP structures which allow for even thinner final nanowires may be possible through the use of multiple attachment sequences to place the Au NPs more precisely, the use of smaller Au NPs, or through surface attachment with more rigorous rinsing techniques.

5.5 REFERENCES


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CHAPTER 6: CONCLUSIONS AND FUTURE WORK

6.1 CONCLUSIONS

6.1.1 DNA Origami Templates

The technique of DNA origami is a powerful tool in designing two- and three-dimensional DNA structures. I have demonstrated that this technique, in combination with a PCR-based scaffold strand generation procedure, can be used to assemble branched, wire-like structures in addition to the mostly filled-in structures demonstrated by others.1-2 My folded DNA origami structures include shapes that look like nano letters: “I”, “T”/“Y”, “U”/“C”, “B”, and “L”, as well as more complex prototype circuit template structures. I have designed various junctions in DNA structures that range from 2–6 helices wide, including: asymmetric, square, axisymmetric, and corners. A thin, 2 X 2 helix corner structure was designed, demonstrating the ability to create asymmetric junctions in three-dimensional structures. Curved regions have also been designed either by raster filling the helices, like in the circular circuit structure, or by designing straight sections with enough flexibility and the proper length to fit into place, as in the “B” DNA origami structure. These thin DNA origami structures demonstrate the flexibility to design and potential to assemble complex asymmetric templates needed for bottom-up assembly of nanodevices.

6.1.2 Surface Placement

Simple surface arrangement has been achieved through placement of small rectangular DNA origami structures between patterned Au nanospheres on silicon surfaces. These ~5 nm nanospheres were created though a block copolymer micelle directed gold deposition process and demonstrated the ability to localize nanospheres with 65 nm periodicity. The nanospheres were coated with thiolated oligonucleotides that base-paired with sticky ends on the DNA
origami structures. The DNA origami attachment showed high selectivity and up to 74% of the Au nanospheres contained an attachment with a DNA structure. This work demonstrates important progress in controlling the placement of DNA origami structures on surfaces.

6.1.3 Selective Gold Nanoparticle Attachment

Thiolated, oligonucleotide-coated gold nanoparticles (~5 nm) were specifically attached to select portions of DNA origami structures as seeds for electroless metal deposition. These seeding and metal deposition processes allowed formation of 33 ± 7 nm width conductive nanowires. The Au NPs attached to branched DNA origami structures with a median center-to-center spacing of 11.7 nm, giving a median gap size of 4.1 nm. Some structures also contained larger gaps of up to 37 nm between Au NPs. The median spacing and gap distances between Au NPs indicate that the final metal nanowires could be as thin as ~12 nm.

In order to improve the Au NP attachment to DNA origami structures to minimize gaps while still maintaining single-file attachment of Au NPs, several experimental parameters were explored and optimized. These parameters included the hybridization time given for the DNA-coated Au NPs to base pair with the DNA origami structures, the concentration of Mg$^{2+}$ ions in the DNA origami solution, the ratio of Au NPs to DNA origami, and the age of the DNA-coated Au NP solution. The results showed the best single-file Au NP attachment with the smallest gaps, for a range of conditions: 30–90 min hybridization times, 70–100 mM Mg$^{2+}$ in the DNA origami solutions, Au NP to DNA origami attachment location ratios from 1.8–4.5, and one to a few days old DNA-coated Au NP solutions. This Au NP attachment process allows for closely spaced Au NPs and selective positioning along a template, which in combination with metal deposition on these Au NP seeds, would enable the formation of controlled, branched, and complex nanowires.
6.2 FUTURE WORK

6.2.1 Surface Placement and Orientation

Further patterning of surfaces will be necessary to gain greater control of orientation and attachment position. A recent paper\(^3\) demonstrates the ability to use lithographic patterning in combination with block copolymer self-assembly to create more complex and specific palladium nanosphere surface patterns, including nanosphere clusters and pairs, and single or double lines of nanospheres. This patterning technique in combination with the surface attachment process utilized in Chapter 4 could provide the surface control needed for assembling DNA templated circuit structures.

6.2.2 Thinner Diameter Metal Nanowires

The goal behind optimizing Au NP attachment to DNA origami templates to have single-file Au NP attachment and minimized gap distances in Chapter 5 was to enable the formation of smoother and thinner diameter conductive nanowires after electroless plating. This could be confirmed by performing electroless gold plating on the best Au NP structures, followed by conductivity tests to see if thinner diameter, conductive wires are indeed possible with the optimized Au NP attachment. If more improvement in Au NP attachment is needed to allow for thinner and smoother nanowires, additional improvements could be possible through the use of multiple Au NP attachment sequences as explained in Section 5.3.2 and/or through the use of smaller diameter Au NPs as seeds.

6.2.3 Integration of Semiconducting Material for Transistors

To complete the nanoscale circuit assembly process, it is also crucial to integrate semiconducting material into the designed metal gaps on the DNA origami templates to provide transistor functionality. The interfacing of semiconducting material to the DNA origami
templates could be accomplished in various ways, including directed attachment of a semiconductor or placement of semiconducting carbon nanotubes (CNTs) in the correct locations on the DNA origami templates. One way a semiconductor could be introduced into the gaps within the metallized DNA structures would be through selective deposition of two different metals and galvanic displacement of one to form a metal to semiconductor junction.\textsuperscript{4} To position semiconducting CNTs, DNA oligonucleotides could be used to wrap and solubilize the CNTs,\textsuperscript{5} as well as to base pair with the DNA origami templates.\textsuperscript{6} In support of this idea, I have performed some initial studies to test the ability to attach CNTs to my DNA structures through a DNA wrapping technique.

6.3 CARBON NANOTUBE INITIAL STUDIES

6.3.1 Experimental Approach

For my initial studies, I used a different method for attaching the CNTs than the one used by Maune et al.\textsuperscript{6} The key aspect of my process is the replacement of sodium cholate (SC) around surfactant suspended CNTs, with oligonucleotides extending from the DNA structures during CNT attachment. I used a CNT powder enriched in (7,6) semiconducting CNTs (0.9 ± 0.3 nm in diameter). Figure 6.1 shows the design of the circular circuit and “T” DNA origami structures and the location of the modified staple strands. I designed the modified staple strands to have the CNT wrapping sequence (with a 5 base spacer, ACGAA) on the 3’ end, choosing staple strands positioned in 2 neighboring rows within the two designed gaps from Section 4.3.2 (see Figure 4.11D and Figure 6.1A) on the circular circuit structure and on all the staple strands within the gap region on the “T” DNA origami structure (see Figure 4.11A and Figure 6.1B). The CNT wrapping sequence used was GTTGTGTGG and was chosen from sequences that showed an affinity for (7,6) CNTs\textsuperscript{7} (see Figure 6.1C). For the circular circuit structure, 26 staple strands in
the two gap regions were modified for CNT attachment and used in combination with the adenine modified staple strands for Au NP attachment. For the “T” structure with a gap, 39 adenine modified staple strands were used for Au NP attachment in addition to the 28 staple strands within the gap region with the 5 base spacer and CNT wrapping sequence. The staple strand sequences can be found in Appendix B.

**Figure 6.1.** DNA design for CNT wrapping. (A) Schematic of the “T” structure where blue dots indicate the location of staple strands modified for CNT attachment and red dots indicate the location of staple strand adenine extensions for Au NP attachment. (B) Schematic of the circular circuit structure where blue dots indicate the location of staple strands modified for CNT attachment and red dots indicate the location of staple strand adenine extensions for Au NP attachment. (C) Staple strand modifications for CNT attachment. Extra nucleotides for the spacer (green) and the CNT wrapping sequence (blue) were added to the 3’ end of the staple strand sequences (orange).

The CNTs were first suspended in a 1% SC solution through sonication and centrifuged to remove insoluble pieces and CNT bundles. Then, to remove excess SC, the CNT solution was
dialyzed with a 3.5 kDa MWCO membrane. The DNA origami structures were folded and filtered to remove excess staple strands as described in Section 5.2.2. Then, the same surface attachment process for Au NP attachment from Section 5.2.2, was utilized to attach the suspended CNTs to the DNA origami structures. DNA origami was deposited on oxidized silicon wafer pieces followed by the dialyzed CNT solution (10 µL for the circular circuit sample and 20 µL for the “T” sample). After 1–3 hrs, the silicon pieces with the DNA and CNTs were dipped in water and dried. AFM imaging of samples was done as before in Section 5.2.2.

6.3.2 Results

The folded, modified “T” and circular circuit DNA origami structures are shown in Figure 6.2A–B. Figure 6.2C–D show AFM images after the CNT attachment process on the circular circuit structure. The CNTs appear to be connecting to the DNA origami structures, making these initial results very promising. It is difficult to tell from the preliminary results if the CNTs are attaching at the correct locations, and it is possible that the same CNT or CNT bundle is attached to both sides of individual circular circuit structures. The CNTs in these initial experiments are also longer than needed to span the gaps. CNT attachment to the “T” DNA structure was attempted with more dilute DNA origami (1 nM instead of ~5 nM) and CNTs sonicated for a longer time (240 min vs. 90 min) to break them into smaller lengths (Figure 6.2E–F). The CNTs in these images are about the same diameter as the “T” shape, but can be identified by their length and straightness. Further imaging using SEM or TEM could provide additional confirmation.
Figure 6.2. CNT attachment to DNA origami structures. (A) AFM image on mica of circular circuit DNA origami structures modified with CNT wrapping sequences. (B) AFM image on mica of the “T” DNA origami structures modified with CNT wrapping sequences. (C–D) AFM images on silicon oxide surfaces of circular circuit DNA structures with attached CNTs. (E–F) AFM images on silicon oxide surfaces of “T” DNA structures with attached CNTs. Red arrows point to selected CNTs. All scale bars are 200 nm.

6.3.3 Challenges

Although I saw some CNTs bound to the DNA origami structures, the yields could be improved. Additionally, I more commonly saw bundles of CNTs (Figure 6.3A) instead of
individually dispersed CNTs. Initial experimental results show that this could be resolved by centrifuging at a higher speed to separate bundles from individual CNTs (Figure 6.3B).

The CNTs used in the initial studies were too long for the DNA origami structures. Longer sonication times can break the CNTs into smaller segments, but will give a wider range of lengths (Figure 6.3C). Size exclusion chromatography is another option that has been used to separate DNA-wrapped CNTs by length. Other groups have demonstrated a similar length separation technique for surfactant wrapped carbon nanotubes.

Lastly, it is challenging to work with dispersed CNTs. The CNTs settle out of solution over time and work best when they are freshly prepared. In addition, the dialyzed CNTs can be unstable in solution, sometimes settling out of solution before it is possible to do DNA wrapping. A systematic study of sonication, centrifugation, and dialysis parameters would likely enhance the repeatability of these experiments.
6.4 OUTLOOK

A final challenge will be to combine surface orientation and attachment, Au NP seeding and metallization to form conductive nanowires, and the integration of semiconducting components like carbon nanotubes, to assemble an entire circuit structure and test its performance. The design and assembly of open, wire-like DNA origami structures, along with the ability to specifically attach these DNA templates to block copolymer patterned gold nanosphere surfaces, could enable the formation and placement of complex templates with various junctions necessary for circuit motifs. In addition, the ability to site-specifically bind Au NPs densely to these DNA structures could allow for smaller diameter conductive nanowires with the ability to incorporate other functionalities within the same template. These important advancements in DNA templated fabrication provide a large step toward enabling self-assembly of nanodevices. With my results and those of others, it may not be long before a complex circuit structure, built from the bottom up and compatible with current semiconductor technologies, is assembled.

DNA-templated fabrication of electronic nanodevices has a very promising future with many potential uses. These nanostructures could be used to assemble high-density nanoscale electronic circuits,\(^1\) allowing for increased complexity and speed in electronic devices. Another application could be in plasmonic circuits\(^2\) in which optical signals increase performance in interconnects and data transfer. Additionally, Au NP placement along DNA templates for building plasmonic polymers could lead to advances in sensing and energy harvesting.\(^3\) As we discover and learn more about how DNA can be programmed and utilized as a nanoscale building block, the field of possible applications will continue to grow.
6.5 REFERENCES


APPENDIX A: DNA ORIGAMI DESIGN DETAILS

Lambda and M13mp18 Rectangles

Figure A.1. Design of DNA origami rectangle structures. (A) BYU Ascent DNA Folding Tool report page, showing the sequences and parameters for the M13mp18 rectangle structure. (B) Drawing of the DNA origami rectangle showing the location of staple strands. Each ball in the strands represents a nucleotide base and the scaffold strand is green.

These two structures were designed using an Excel-based Visual Basic program called BYU Ascent DNA Folding Tool. Figure A.1A shows the report page for the M13mp18
rectangle shape. The staple strands are located in the same positions relative to the scaffold strand for both the Lambda and M13mp18 rectangles. **Figure A.1B** shows a drawing of the location of the staple strands in the designs.

**T Design**

![Diagram of T Design](image)

**Figure A.2.** Design of DNA origami “T” structure. (A) The 74 nm arm. (B) The 240 nm arm. The nucleotides in red are the scaffold strand and the nucleotides in blue and green are the staple strands.

This structure was designed in two pieces in the SARSE program. The top 240 nm section of the “T” shape is the first piece and the 74 nm base for the “T” is the second piece. **Figure A.2** shows the two pieces designed in SARSE for the “T” shape, including the nucleotide sequence of the scaffold and staple strands. This structure was designed in two pieces because the SARSE program only allows horizontal positioning of the DNA helices. The program raster fills the scaffold strand into the desired shape based on the bitmap image provided by the user. In the case of the “T” DNA origami structure, it was simple to design rectangle shaped bitmap images of the desired thickness and widths for the two individual parts of the “T” shape. When the scaffold strand nucleotide sequence was inserted into the design, it was carefully spliced and
chosen, so the appropriate parts would end up in the correct places for the design as a whole (see Figure A.3). For example, on the 240 nm arm, there is a linear staple strand along the top, in the middle, containing no crossovers that was not ordered. This staple strand (and scaffold paired to it) is in the location where the 74 nm arm connects and is simply a placeholder in the design and does not exist in the real structure. The scaffold sequence before and after that staple strand, come from different portions of the M13mp18 scaffold, the two sections right before and right after the scaffold used in the 74 nm arm piece.

**U Design**

Figure A.4 shows the design of the “U” structure. This structure was designed in three pieces in the BYU Ascent DNA Folding Tool program. In the two locations where the arms connect to the base, there is a linear staple strand (contains no crossovers) in the BYU program design. The BYU program automatically chose the staple strands to be in those locations, so I strategically designed and spliced the scaffold sequence to include the proper sequence before

**Figure A.3.** Design of the scaffold sequence for the “T” structure to enter into the SARSE DNA origami design program. The scaffold sequence was carefully spliced and organized to enable the assembly of the final structure from two separate design pieces.
and after those two staple strands so the structure would fold as desired (see Figure A.5). The actual sequences (a string of thymines and a string of adenines) for those two staple strands are just a placeholder and do not exist in the real structure.

**Figure A.4.** Design of DNA origami “U” structures. (A) BYU Ascent DNA Folding Tool report page, showing the sequences and parameters for the base of the “U”. The red sequences are the placeholder staple strands. (B) DNA Folding Tool report page for the left arm, showing the sequences and (C) DNA Folding Tool report page for the right arm, showing the sequences. On each of the three parts of the “U” design there were a couple of staple strands that were very short (orange). It is undesirable to have extremely short staple strands, so those sequences were combined with their neighboring strands (green). (D) Drawing of the base section of the “U” structure showing the location of staple strands. (E) Drawing of the arm section of the “U” structure showing the location of staple strands. The staple strands are located in the same positions relative to the scaffold strand (green) for both arms.
Figure A.5. Design of the scaffold sequence for the “U” structure to enter into the SARSE DNA origami design program. The scaffold sequence was carefully spliced and organized to enable the assembly of the final structure from three separate design pieces.

B Design

The backbone of the “B” structure was designed in the SARSE program (so the appropriate attachment positions and scaffold folding could be designed) and the curved portions were designed in three parts in the BYU Ascent DNA Folding Tool program. Figure A.6 shows how the four pieces fit together to make the “B” structure. The three pieces designed with the BYU Ascent DNA Folding tool are only two helices wide and were all designed as straight pieces. The flexibility of the structures and the correct splicing of the scaffold sequence in the design allow the thin DNA origami sections to bow out into the curved portions of the “B” shape. There are three placeholder sequences along the backbone of the “B” where the branches connect to the backbone, similar to the way the “U” and “T” structures were designed.
Figure A.6. Design of the DNA origami “B” structures. (A) The scaffold sequence was carefully spliced and organized to enable the assembly of the final structure from four separate design pieces, from two different design programs. (B) Drawing of the pieces that make up the curved portion of the “B” structure showing the location of staple strands. The green strand represents the scaffold strand in all three pieces and the other colors represent the staple strands. (C) Design of the backbone of the “B” structure showing the staple strand crossover positions. Vertical dark gray lines are scaffold crossover locations. The scaffold was designed to continue from piece to piece as shown in the schematic in (A).

**Rectangle Half Circuit Design**

This structure is a dimer with two identical halves. Each half was designed as three pieces in the SARSE program. The half circuit structure was divided into three arms: the 159 nm linking arm, the 210 nm base arm, and the 75 nm lead arm. The 210 nm base arm forms the side
of the rectangle where the 75 nm lead arm connects. The 159 nm linking arm contains the portion of the structure where the two rectangle half circuit dimers are linked together. To ensure that the arms from the two dimers would link properly (and so the structure could be designed in fewer separate sections) they were designed in one piece (see Figure A.7). However, the scaffold strand imported in the program was such that the 159 nm section is actually two separate arms in the rectangle half circuit structures. Each piece designed in the SARSE program appears twice in the complete box circuit structure design (see Figure A.8).

**Figure A.7.** Schematic of the scaffold trace of the rectangular half circuit. The full rectangular structure is formed when two identical halves are linked together. The red boxes show the sections of the structure designed in the SARSE program. The box on the top is the 159 nm linking section. Even though it was designed as the entire top section, the scaffold sequence imported into the design enables that section to be two different arms in the actual DNA structure (the black scaffold trace). The box on the side is the 210 nm base section, and the small section is the 75 nm lead.
Figure A.8. Design of DNA origami rectangle half circuit structure. (A) The 159 nm linking arm. (B) The 210 nm base arm. (C) The 74 nm lead arm. The red lines are the scaffold strand and the blue and green lines are the staple strands. Dark gray lines indicate scaffold crossover locations.

Corner Design

The corner structure was designed in CaDNAno (square-base program). Structures can only be drawn with horizontal helices like the SARSE program, but there is more flexibility in how the parallel helices are connected together and the program is easier to use. The corner structure was designed flat (Figure A.9A) with the corner designed by extending the outer helices further than the inside ones. There are two locations where staple strands cross from helices on one side of the corner to the other side. Figure A.9B shows the corner on the first design and Figure A.9C shows the corner on the redesign that folded better. Figure A.9A shows the staple strand locations for the entire structure.
Figure A.9. Design of DNA origami corner. (A) The scaffold and staple strands for the entire redesigned corner structure. The blue arrows indicate the two staple strands that bridge from one side of the corner to the other. (B) The corner scaffold and staple strands for the first corner design. (C) The corner scaffold and staple strands for the redesign.

Three-dimensional Corner Design

The three-dimensional structure was designed in a similar way to the two-dimensional corner above, using CaDNAno (Square-base program). Figure A.10 shows the design.

Figure A.10. Design of the three-dimensional DNA origami corner, including the location of the staple strands.
Circular Circuit Structure and Modifications (Logic Gate Prototype)

Figure A.11. Design of the circular circuit or logic gate prototype DNA origami structure. (A) The original structure, including the location of the staple strands. (B) The modified structure, including the location of the staple strands. This structure was modified for selective nanoparticle attachment.

This structure was also designed in CaDNAno (square-base program). Figure A.11A shows the entire structure, including staple strand locations, in CaDNAno. The structure was originally designed like a normal DNA origami structure and then was later modified to contain staple strands with extensions for location selective nanoparticle attachment (see Chapter 4) and carbon nanotube attachment (Chapter 6). When the structure was modified many of the staple strands were rearranged so the ends of the staple strands containing the extra nucleotides would all be protruding from the same face of the DNA origami, instead of the strands coming out from the back and the front. This necessitated ordering the longer attachment staple strands as well as
some of the staple strands without the extra nucleotides. **Figure A.11B** shows the entire modified circular circuit structure.

**Long Bar Design**

This structure was also designed in CaDNAno (square-base program). The staple strands were ordered with the extra nucleotides on the end needed for attachment. On one half of the design, staple strands (110 of them) were modified with a sequence of 10 adenine nucleotides and on the other half of the bar the staple strands (117 of them) were modified with the sequence GTGCGTGT on the end. **Figure A.12** shows the design with staple strands.

**Figure A.12.** Design of the long bar DNA origami structure.

**Figure A.13** shows the twist predicted in the long bar structure by the finite-element based, three-dimensional solution DNA origami modeling program called Cando (available at http://cando-dna-origami.org).

**Figure A.13.** Three different views of the long bar structure with root-mean-square thermal fluctuations indicated in color. The red areas have higher flexibility and the blue areas have lower flexibility.
APPENDIX B: SCAFFOLD AND STAPLE STRAND SEQUENCES

(All sequences are given 5’ to 3’)

**Lambda Rectangle**

*Scaffold:* Lambda phage DNA, bases 2868–3623, 756 base pairs long

*PCR Primers:*

GGTGCTGACACGGAAGAAAC

[BioTEG]ATCATCAGCAGATTGTCTTTATTC

*Staple sequences:*

TTTTATTCTGAACTAATTCGTGTCAGCACC
TCATAACGTCCGGTTTCAACGTCACAACGTCTC
TTCACATCGTGGTAGTTTACTGAGAGCAT
TTTGATAATTCTACTAAAAACGATAACACCGTG
TAAATTCTCAAGAACGATGGGTACAA
TGCTAAAGCACGAGGTTTTTCACACCGCATCTTTATAGAAA
GTGAAAAACATTCTAATTATTTGATAGGTTGAAATCAAGAGAA
CGTCCTATGACATAAA
TTGAAAACGATAAGGTGTAATGT
TTAATAAAAAGACTTTAAAAAACGATAATGCAAACCTACG
GGTCAGGGTGTCACAGATAATA
ACATTATATTACTATCTAGCCCATTTTTAAAGAAAAATATTTCG
ATCATCAGCAGATTGTGTAGACTGTGAATTC
ATCAAATGCGAAAAAGATTCTTTATTCATTTTGT
AAAATATTACTTCAAAAGGTGGTTTACCAAT
CGCCCTCGTATCACATGGATCTTTCTGTATGAAGA
GTTGTATTTCCCTCAGGTCAAGACCAGA
TCATCAACAAAAACACAAGGCAGAATGCCAGCAGGACC
CGCTCCATGCGCTTGCTCTTCATCTAGCGGTT
TTTGAGCACGTTGGCCTTACATACATCTGTCG
GCACTTTTGTTCGCAACCAATACTATATTTAA
ATCACATTTTCAATACAGGGAAAATCT
ATATCCATGAACATAAAAGATATTACTATACC
TCAGAACACTACCAAAATCTTTCCACGCTAAA

**M13mp18 Rectangle**

*Scaffold*: M13mp18, bases 5870–6625, 756 base pairs long

**PCR Primers:**

CCACCATCAAACAGGATTTTTCTGCC

[BioTEG]TTCTCCGTTGGAAACAAACGGC

**Staple sequences:**

GGTACCGAGCTCGAATCTTGTGTGATGGTGG
CCCCAGCGCGCAGAAATTCTGTAATCAGGTCAT
ACAATTCCACACAAACCAGCGCTGGCCCTGA
ACAGCTGATGCGCTTCAATACGAGCGAAGCAT
GCTAACTCAATTATATTGCGGCCAG
CGGGGAGAGGCGTTTGCATGGGTGCTCGCTACTG
TTCAAGGTGCAGCAGACTGTGGGCCCAGTGCCAGCTCGTG
CCCGCTTTGAAAGGCG
TGGCGAAAGGGGCCTAATGAGTGA
AAAGTGTAAAGCCCTGGGTTGGATGTGCTGCGAAGCCGATTA
ACGTGTAAAAAGTTATCCGCTC
AGCTGTTTCTCTGTGTAATTTCGACCGCCAGTGCAAAGGTTTG
TTCTCCGTGGGAACCAATCTAGAGGATCCCCG
CATGCTCTGCAGGTCGACACCGCGGATTGACCGT
CATCGTAAACCGTGATTTCCAGTCACG
AGTTGGGTAACGCCAGGGTCTGCGCAGTGGGAGGG
CTCCAGCCAGCTTTTCTATTACGCCAGC
ATCGGTGCGGGCTCTTCCGCGGACCGGCTTCTGCTTGTG
AATGGGATAGGTCACGGTTGTGTAGATGAGCCG
ACGACGACAGTGATGCGCCTCAGGAAAGATCGCA
CCGAAAACCAGGCAAACGGCCATTCGCCA
CCAGCTGCAATTAATGAAATCGGCAAACGCG
GGTGGGTTTTCTTTTCAAGTGAAGCAGGGCA
GAGAGTGGAGCAGCAGGCTCCAGCGGCTGGTTTG

**T Design**

*Scaffold:* M13mp18, bases 5734–1442, 2958 base pairs long

*PCR Primers:*

CTGATAGACGGTTTTTCGCC
[BioTEG]CATAACCGATATATTCGGTCGC

*Staple sequences:*

GCATCAATTCTACTAATA
GGTAGAAAGTCTATCAG
TACCACATACGTTGGAACCTCAACGTCAAGGGC
CAAAAGGATCCAGTTTGGAAACAAGAGTCCACT
ACTATCATAAAAGAATAGCCGAGATAGGGTTG
AAAAACCAGTTCCGAAATCGGCTAAATCCCTT
GAAGTTTGGCCCCCCAGCAGGCGAAAATCCTGTT
TTTAGACTAGAGGTTGCAGCAAGCGGTCCAC
CGTCATAAAAACAGCTGATTGCCCTTCACCAGCC
GCTTTAAAGGGTGTTTTTTCTTTTCACCAGTTG
TAAATCAAGCGGGGAGAGCGGTTTTCGCTATT
TATAGTCACGTGCCAGCTGATTAATGAATCG
TCGCGTTTGGCCGAGAGGGTAGCTATTTTTGA
GACCGGAATGATATTCAACCGTTCTAGCTGAT
GAGAGTACAAGGGCGGAGACAGCTCAAATCAC
GGTCATTTGTAATGTGTAGGTAAGATTTCAA
GGTCATTTGTAATGTGTAGGTAAGATTTCAA
GCTGAAATAGAACCCCTCATATATTTAAATGCA
AAATATGCCCTTTATTTCAACGCAAGGATAAA
TTCATTCCATTATGACCCCTGTAAATACTTTTGC
GCGAACGAGAGCATAAAGCCTAAATCGGTTGTA
ACATTTCGAAGAATTAGCAAAAAATTAAGCAATA
TATATTTTATTAACATCCAATAATCACAG
GGAACACATTATTACA
AAGAAAAATCTACGTTAATAAAACTTTAGGAA
AACTGGGCTCATTATACCAGTCAGGCATAACGC
AATCATTTGTGAATTTACCTTATGCGAGAGCAAC
AATTGGGCTTGGAGATGGTTTAAATTACGACGAT
TGCCCTGACGAGAAACACCAGAACTTGCAAAA
GTAACAAAGCTCGTCATTCAGTGAGTAAAATG
ACAAGAAACGGATATTACATTACCCTGCGGAAAT
AGGCTGGCTGACCTTCATCAAGAGCCTCAAAT
GGACAGATGAACGGTGTACAGACCAATGACCA
ATAAGGGGAACCGAACTGACCAACTCTGACTAT
ACCTAGCCGGAACGAGGCAGCACATCAAAA
ATTGTGTGCAAATTCCCGGACCTGCTTCAATA
GTACAACGGGAGATTTGTATCATCGGCGGAACCA
ACCCCCGAGCGATTATACAAAGCGCCAGGATTA
GGCAAAAAGAATACACTAAAACACTTGATAAGA
CCACTACGAAGGCACCAAACCTAAAGCTTAATT
AAGTTTCCATTAAACGGGTAAAATCATGTHTT
AGGCTTTGAGGACTAAAGACTTTTCTGGAAAGT
CAGCATCGGAACGAGGTTAGCAACCCAATTCT
TTTGCGGGATCGTCACCCTCAGCACATTAGAT
TCGCTGAGGCTCAGGGAGTTAATGTTTAGC
CATAACCGATTGAAAGGTT
GAAAAACCGATTTCATCGTTGAGAGAACTAAC
ATTAAAGATCAAATGCGATAACGTTGGG
U Design

Scaffold: Lambda phage DNA, bases 37501–41340, 3840 base pairs long

PCR Primers:

TCAACCTCAAGCCAGAATGC

[BioTEG]CGCGTCTGAATATCCTTTGG

Staple sequences:

TAAGCTCGGTTGCGTTTCTGGCTTGAGTTGGA
GCCAGTGATTCTGCATCCTGAATGGTTACTA
TGTACTGCGTGAATAGAGGTAACCTTTGTAAT
TCATTGCACCCTTGGACCGTTTGTGCTGATGTGC
TGACTTCCCTCTCCCCGCTTTGCTGCTTGA
ATTGGTCCAAACATGCCCCAAATAAAAAAGGCCTG
ATGTAGATGGTCATGGATTTTCCTCG
TTTTATCCTCTGTTTAGGCTTTTAACTCCATATACC
GGCGACACTCCTTTAAAAACTTCCCTGGTAG
ATCTTTCCCTCCCCTTTACCAGGTTCGTGCTC
CCACTTAACGCCACGCTCTGTCCCCTTTTATCAGCGGACTCTC
GCCATTAAACCGAAT
GCCTACACGCATCCATTAGCTCAGTA
CGATTACCCAGGACTGTTTGTGGCTGACTGGGACTA
ACAAACATCCTGCTTATATTTTCA
TGGAGATCGAATTTCAAAAAGGTGCTTTTAATGCTGCGGTAAA
GACCAGCTTAATGGAGGATTTGC
CGATCTTTTGGAGTCTGCTGACGAGAGACGTCA
CGCGTCTGAATATCTTTTCTCTGTTTTGGTC
CCTAAGCAGCCCATAGGGTCTCGCAACCTACCCGT
TATCAACCTGCAATACGTGCATCCAGCCAGC
CACACACTCCAGCTTTCTGAGCGGTATATCC
TGTCATTGGATGTTCCAGGCAAGGTGTAAGGA
TTACCACAAAGCCATTCCCAACCTGTATCCATGA
TTTTGACTCCATTAGGCTTCCGCCAAATTCC
GCCTGTATAAGCTCTAATAGCAGAGCAAATATGCTGA
TCATGCAGCCCTTTCTGCGTTTCGTCTGA
TTCCACTCCAGGCACGCCTCCCATCTCGCT
ATAACCATTTGGCTGTCCAAGCTCCGGTTGA
CAGAAGCTCACAAGCTTCCCTGACAAACCAGATA
AAACGTAATGCACTTTACACGCGGTTTGA
CGTCTCTGACCCGGAGAAACTAACAGGACATTTA
TTTCTGAGGATGCACCATTCTGAGATGTTTTT
TAATATTCTGGCTGAGGTTTCTAACTTGGCT
TGTTACTCGGAAGGACACAACCCAAAAA
ATGCGGAGGATGGGTAGCTTTTACCTCTTCCGCA
AATTTTTCCGCCAGCAGGATTGT
CCCCTTTTCATTCTGTTGTTTTGAATGGCCTGTTGA
GTCTTGGTTTGCCGAAGCGGTAGTA
AGAAATCTACGAGATGTATCAAGACGCATTGCATAATC
CTTAGTACATGCATTCTCAAAGTT
ACGCCGCATTGCTTGCAAAAAACCATTATCACGCCCAGAG
TTATCCCTTGCGTTGGTGCTTT
ATGGGGATGGGGGCAAGTCAGGCGGTGATAGATTTAACGATAGA
GAGCAGCTTTGAGGACGAAAAATGAAC
AATTATGAAAAAAGCACGTCGCCCTAAAGC
CATTAAGAAGACAGCAAAGACAAAAAGAAAACCATAAACCAA
GCACAAGCATTGA
TGATCTGCACCTTATCAGGTGTTAGATAT
GTAATAATAGTCAAACAGCAACGCCCCACAGCTTCC
GTTAAGCAACGCGCATCCATAAACCCTC
TTTCAGGCTTATGCTGTTGGTCTCTCAGTTTAGAGCCT
TATTTCCCTAGAAAGATCTTTTAGCT
TCGCCTTTTGATATACGCGGCTAAACTTGGATTGGAATGTAT
AATTTGATGCCATAGTTAAAAA
TAAACGCTTTCCATCAGCAGTTTTTCAGGGCTGGAATGTG
CTCGCTAAAGTTTGAGATAGTGCTGTGTTTTTTTT
TAAAGAGCGGGTTATTATCAGGTGATTTCGTTGCA
ATGGCAACGCAGCACCATTAGGTTGGTT
GCAAAATAATGCATAACATTTAAACTGTCGCTGGT
TATTTGATAGTCTGAACCATAATGTAAG
CGTTGCGTTTTGTTTGACACCGCGTAAACCACATCATCGAGA
ACCTCTGCGGAGCATTGCGAATT
GCTGTCTTCTCAGTTCAAGTTGAGTATTTTTTGCTGTAT
AATGACCTCAGATCTTCCACCTGC
GAACTTTTGGAATCCAGTCCCACTCCATCTGGATTTGTCA
GGTGAGAATCCGGTCGTCAACGACCCCC
CCAATCGAGCCATGTGACGCAACTTGCACGTGC
TTGGTTTTATCCACCCCATAGTTTTTACGCAGA
CAGATCTGCGGCAATAAGCCTCAAGCAGCA
TCTGCCACATTACGCTCCTGTCCGGCAAAGTT
TTGTCATAATGACTCTCTGTGATAGATCCAGA
GAACGCTCGGTTGCGCCGGCGGTCTTTTTAT
TTGGCTTATCCAGGAATCTGTGCGAGACAGA
ATTTAATGGCATCAATGCAATTTAGCTTATA
AGCGTTGAAAGATTTAGCCCTTTCAATCGCCAG
TGACGCCGTCATTAGAAGTGAATGATGATGATAC
CTCACCTGAGCTTAGAAACCTTACCAAAGGTT
CTTCGTTTCTGTCACCCAAATTTTGAAGGATG
TGAGGGGATAGCAATCCGGTTAGCCAGGCTCGCC
GTTGATGATCTGCGCTTTTCTTTTTCT
AATTCTCTGACGAATAATCTACCTGTGCTACCTGCG
GCGATCCGACGCATCACCACAGAAAG
TGAATTGCAAGCATCCTGGTTTCTGCTCACGGTCAAGTT

162
CACCCCCGTAAATCTGCAGCCGCGTCA
CACCATGTCAAACATCCACTCCAGTCTGTGTGTGTCAGGT
TGGTGGCTTTGTTACCCAGCAAA
TCAGGCCAGATACGTAGCCACACGTTGATTTCGAGTGGGT
TCAGCACCAGTACCCGAGCAAGGCTCTGGGCTTGTTACCCAGCAAA
ATGGGCCGGCTAGGTCTGAATCGCGGAGTTTGGCCGGGC
CCACTTTACAGGT
CAGGGGAAAGACTCCGCTGTCACGCCTGCCC
CCGAGTTTTGGTTGCTGGCATCCGGATACAGGCC
CGAGTTTTGGTTGCTGGCATCCGGATACAGGCC
CCAGCTCGCTGACCTTTTTCATAGATC
AACCAGATCGGCGGATGTTTGGGCAGTCCGGCCGCTATTCCTGCGTCTGAACACACC
GCCGACGGGCTACGCGCGATTTATGCTGGTTACTG
CAGAAGCTATTATCACCATGCAACAAA
ACGGATGCTTCTTCCCGGGCGTCCCCAGGTAATGAAT
TTTCAGTCCGAATAAACCATATCAA
TCGCTTCCGCAATACTCGCTTAGCTTTGATTGATTGGA
CACCAGCCATGGCGTTGATTG
CTAACCGCAACCAAGCTGACAGTAAGTTTTTTTACTGG
CAGTCATATGTCGGCATCGTTCCAGCA
GCCTTACGGCGTAATGACAAGCTCATCTCG
CCTCGATAACCAAGACCTGCCCCTTGCCCAT
TTGCCGCTGTAGCGGCAACGTCCGGCGCA
AAATTGCCTCTTTGCCGCACTACACTTGGCTCC
TCTTCGCCAGAGCCTGTGACGATTAGAGGT
TTCAAGGGATCGCCTACACCAAGTATTCTCG
GTCGGCGGTGGTTACGCTCAGCTCGTGCGTACGCA
TTCGGTTTTCTGGCTGTGATGGTGCGATAGTCTT
GGTCTTCTGGCCACTGGCTGCGCCGCTC
GTCGTTTTCTGGCTGGTCAGAGGATTCGCCAG
TTTGAATAGTGCTTTTGTCTCCCCCTGT

**B Design**

*Scaffold:* Lambda phage DNA, bases 37501–42308, 4808 base pairs long

*PCR Primers:*

TCAACCTCAAGCCAGAATGC

[BioTEG]CGACGCTTTTCTTGC

*Staple sequences:*

AAGATATCTGATCAGGGCTCCCCACTTGCTGCGGCTCTG
TCTTCTGCTGGCTTTAGCC
GTGGTGTAATTCCTGCCCCGATGGTGCAGATGTCTTCA
CGGTTTTCTGCTGAAATGGTCA
GCTTCGGTTCCATACGCTCTGATGCAATCCAC
ACAGTATTTTGGAAGGAACGAGTTTTTAAGCGGATATCGTTAGCCCACCAGCAA
AATT
CCATGTCAACATCCACTGTGCGGCGGTAGGC
TCGCATCATGCAGAACCTTTAC
GGGATGTTCACATGAGGTTATAGCTTCTCTCCCTCCGGAAATCGAAA
CAGAACAAGAGCGGTATCGCGTCATCTTAGAGGTGAGT
GGTTAGTATGCAAAATCTTTTAGCT
ATTTTCTGAATACATTTCGGAAGAAATTAGCCCTTCA
TCAAAAGTTAGCGTTCGTACACGGTC
GGCGTTTCCCCGATGTCCGTGCAATGCATTAAA
GGTGCTTATTTAATCACGCACATGGGGA
ATCCCTCCTCATCTTGTGCTCCCAGGAAATCTGTG
AAATGAACCTGGCTTAAGGCAAGTGCCGATT
GTTACCGAGATGTTCCGCAAGTTAGGAGGCACGCA
AACCATTAAACAAAGGATTTTGCACATCA
TCCTTCTCAAAGTCTGTATTATCCCTTCG
ACGGTGTGGATACAGTTCAGC
ATCAAGCTGCCCCTCCAAATATTTGCCATGACT
CGTACCATGTCTGATAACAGGGCTTGATAATC
GCGACATTCTTCTCGGTACATAATCTCTT
TCCCGTGATGACCTCATTAAAAACACGCTGCA
TTTGCAGTTGATTTTTAATGCAGAATATGCA
ATGGTTGTTGCTTCCACCATGCGAGGATATCT
GTGATAGATTTAACGTTATAGACACAAAAAGA
CGTCGCCTTAAGCAATTATGAAAAAAGAA
CAGACAAGATGGGGGATGGGGCAGTCAGGCGTT
TGCTTATAACGCAGCCATTTGCTTCAAAAATTC
ATCGCCAGAGAAAATCTACGAGATGTATGAAGC
ATGAGTACCCCTGTTTTTCTCATGTTCAGGCA
AATTGCAGCATAGTCAACACGC
TATCACCGCCAGAGGTAAAATCCCGGTTTCACCACAGAAAGGT
TTCTCTGACGAATAATCTTTTCAGGTTATGCGTTG
CGCATTGCATAATTCTTTTTTCTTT
AGGGATAGCAATCCCCCAATTCGCGCTTTGATATACGC
AATGGCCTTGTGATTTGAGGGATGT
TTGAGGGATGCACCATTCTAAACGCTTCCATCAGCG
TTACCTCTTCCGCATGAGATTTTTTAT
ATATTCATTCTGAGCAGTAGAGCGGGGTTATTTA
AGGGCTGGAATGTATCTAACTTGGCTTC
CGCTAAGTTGAGAATCGAATAAATGCATACAC
TTGATTGAAATGTCAGTGATTCTGTCCATT
GGCAAGCAGCAGCTTTTAGCCTTTGGTGTCAC
TAGAGCCTCGTTAACTGTGCT
TATGCTGACGCATCAG
GACATTTAACATGTCGCGGTGGTTACGTTCCGT
TCCACCCCGTTTGTCCCTGGCATGCCCAGCAGGGAA
GTGCAGTTTTGGGCGGGCTCAGCACGTTACCG
TTGATAGACTGCGAAGTTGAGTATTTTTGCT
TCTGAGATCCACATTACGCTCCTGTCGCGGCAA
GGGCGGTGGATAGTCTGGCGTAACCATCATC
TGGTCGCGTTCCGCGCCGAATAAGCCTCAAGC
CGACCCCCCTCGATTCG
GAGAACCTTCAAGCATTGCGATTGTTTGGTTAAGCA
ATGGCGAAGAATGGTCGATTCTTTTCACCTGCTGATGTCGAC
CAGGCCTGGGTTAGTTTTGCATGGTAACCTC
TCATCTTTTTTTGCTGATGTCGCTGCCTCGAC
GTTTCATCTGGAGATTTGGCTGACAGGCTGA
AAAAACGCTGAATGGTTACTACGTCGAGATT
TTCTTCCCCCTGCTGATTGTGTCTATCTGGTGCG
TTGGCAGAGCGAGACGTCACCTTTCGAG
CCTGCCCCGTTGCATCCGACGCCAGGTATGTCTCGC
TATGCTGGAATGGCTGCGTAACAGGTGAGCG
CAACGTCCCGGGGCAAGGTTAGGAACCCCTGAC
CCCAGGTTAGGTTGACTGGCCTATTCAACCGT
CATACACTAGCTCCGCAAAATTCGCTTTACCTG
TTGATTGCTCGTACCAGATGCAAGCAGAAA
CAGCACACTCATGCAGCCCTGTCTCCCCAAGA
GACCAGAAGTAAATCCAGTCTGTGAACCATCT
CGCGGAGTTTGGTTTGCTGGCTGTGCGATCCG
AGTTACCTTCCAGTAATGACCTCAGTTGATGA
GAGATCTGTGTTCAGAAGCGCTCGGGGAAAGT
AGCATATTTTTATTTGTTGAGAATCCTTCTGG
TGGTCAGACCAATCGAGCCATGTCAAGCCAGAA
ACGCACACTCCATTCAAGAACAGCAATGTCCAT
CTCAATTCTGGAATCCAGTCCCTCGCCGACGG
CGACGCTTTTCTGTCTTCACTTTCCCTCTCCCCA
CACGTTGATATCAACCTGCAAACAGACTACCTT
CCTGTGGCTTTGTGTCATAATGCTTTTTCG
ATTTCAAATTATTAGCTCAGTAATGTGATGG
GAATAGCGTAACTCCATATACCGCCAATACCC
GGTTGGGTGCGGCACCTGGCGACACTCTTTA
TTGCATTTCGTTGCTGCTCATCTTTCTTCCCC
CCCATAGCTGGTGGCAAAACCCTGAATACACCC
TTCAACCTACCTTACCTTGGATAAACGAAGA
CAGCTTTCTGCCATTAGCCGCGGACTGTATT
GCTGCTTTTTACTGTGGCGCTGTGATTACGC
GCCATTCGGCGCAGAACGTATTATGCGTCA
ATCCTGTATGAATAATTGCCCTTTTCGCCGT
GCTCTAATTGTGCTTTTCAGTCCGAACCTAGC
Rectangle Half Circuit Design

*Scaffold:* M13mp18, design is 7216 base pairs long

*Staple sequences:*

CGTGGTGACGCTGACGTTTGGCAGTCCGGCGG
GCTACGCGGCTTCTTCCCGGCACCATGCAACAAACTGCCCAGGGTG
AATAAAAAGGCCTGCGATTACCAG
TGAGGCGGTGTTTGATAGAATAAACATCACCTTG
GTGCCACGTACGCACAAAATTAACGGTTGTAGC
ATCTAAGTTACGTGAGCCGACCTCCTGAGAAGTGT
TCAAAACCGGTACCCGAAATCTCTGAGAAGTGT
AAATCAACACCGGATTAAGGGATTTTATA
AAGTTTTATTACACGTCACGACTGTAGCCTTATTA
ATATGGTTGTAATCAGTACGCAAATAAACATCC
GACATTCAACCGTACCAATGAAACGCCTCCCT
TATTGACGCACTAGCACCATCCACCACCCCTCA
TTTGCACGATACTTTCTGAAATAATGTGACCTGA
TAGATTTATACTCAATATAATCAATATT
CAGTAACATATCATCATATCTCTGCACGCT
ATAACGGGACGGAACAAAAGAACAACAAAAATACC
AGTTCAAAATTCTAAAGTTTGAACACAGGG
TTTCAATTCTCGATTAATCCTTCTGCAACA
ACAAAACATAGAAGTATTAGACTTTAATGAAAA
TAAACAATTGAGCCGTCATAGAATCTCAAATA
GAAACAGTTATCTTTAGGAGCCTCAGTTGGC
CGTCAAAAAAAAAATACATACAAACACGGAAT
AGAATAACCTCCTATTACGCAGTGAAAATTC
CGGGGAGAACCGGAATACCACAAAAAGAAAAAGGC
CAGAGGGTGACGAGAAGGAAACGGAAGGTAA
TAACCCACTAAAGAAGTAAGCAGGGTGAAATT
AATACCGATATATTTTAGTTAATTAATTAGCG
TAAGAATACAAAGACAAAAGAACGCTGAATATA
AAAAGCCTACTATATGTAATGTGCTGAAGAAACA
AATTTCTTTTTAAACCTCCGGCTGAATACCA
GTAGGGCTATTATCATAAAATCATAATTATTCA
AACGCAATAGATTAAGACGCTGAATGATGAA
TCGAGCCACCTTAGAATCCTTGAAATTACATT
ACAAAAAGGGCTTCTGTAAATCGTCTTTTAATG
AGCGGGAGCTAACAAGAGTTGAAAGGAATTGAGGAAGGTT
CAGTCTCTTTTTCCGTCATAGCCCCCGCGTTTT
ATAAAATCCCATCTTTTCTATAATCAAGATCAAG
GTCAGACGACGACACCACCGGAACCCGATCGATA
CCGCCGCCACACCTCAGAACCAGCATTAGCAA
TTATTTACAGAGATAGAACCCTTGAAAGGTT
CTACATTTGAATACGTCACAGACACCTGATTG
CATTGCAACTATTAGTCTTTAATGATTATCAG
CTGGAATTAAAACATCGCCATTACCAGAAGG
CCTGAGTACACCAGAAGATAAATAACATTA
AATACTTCTCAGTATTAACACCGCTGCCGAA
GTCTGTCCCTGAGAGCCAGCAGCAACAAACAA
TTTTATAACATCACCTTGCTGAACATAACTATT
CATCGGCAAGAAACGCAAGACACCGGTGGCAA
TTTGCCCCCGTTGCACAAATCAATAATGGTACG
GCAGCACCTACCAGCCAAAGACACTGGCAT
GGCCGGAAACCGATTGAGGGAGGGGGAAACGC
AAAATCACGGAATTATTCATTTAAATAGCCGA
TTTGGGATTTAAAAACAGAAATAAAGTCATCTTTC
ATGATGGCCAGGTTTAACGGCAAGAAAACCT
AGCGGAATGTACCTTTTACATCGGGATGCAAA
TCATTTTGTTCCGCTGATTGCTTTTAGGTTGG
CGTTATTTAATCGCGCAGAGGCGAGGTCTGAG
TTTGAGACAACCTGGAGAAAAAGAAGGGAGTTGC
GAGGATTTTCAAGAAAAACAAAAATTAACATAG
ATAGATTATCATTTGAATTACCTTGCTATTAA
ATCTAAAAACATAAATCAATATATGTGAGTGA
CATATAAAAATAAGAAACGATTTTTTTGTTTA
AAACGTAGATGAAAAATAGCAGCCTTTACAGAG
GATTAAGAATAAAAAACAGGGAAGGGATTAGA
AATAATAATTAACCTGAACACCCTGAACAAAGT
ACAAAGTAAAATTGAGCGCTAATATCAGAGAGA
GCCCTTTTAAAGAATTTGGAAGCCCAATAAT
TTTTCAAAACCCTGTGATAAAATAGGCCTAAA
TCCAATCGAACACGGAAAATCATATAATTACTAGA
GTTATATAGTATTAGTATCATATGCGTTATA
AGACTACCCAGTATAAGCCACCGCTCAAAC
AATAGTGATAATGAGATCGCATTATTTAACA
CGATAGCTCATGTAATTAGGCCAGAGGTCTT
TTAATTGTGAATAAGAATATATACGTACCG
ATAACCTTTAAAGTAATTCTGTCC
GGTAAGATTATTAGTTTGGACATTAGGAT
TTTTGCGGAATGGTCAATAACCTGTGGCT
GATAAAAATAGATTTAGTTGACCATTAGATA
AATGCAAATTAAACAGTTGATTCCCAATTCTG
TTCAAAAGACTAAAAGATCGGTTCAGCTTAAATTG
AATCACCAGTGCTGTAGCTCAACATGTTTTA
GCTGATAATGCGATGGCTTAGAGCTTAATTG
TTTTGAGATTTAATTGCTCCTTTTGTGATAAGAG
ATGAAAGGTACCAAGCGGAACCAAAAGTACAA
CATATGTACTAAAACACTCATCTTTTGACCC
CCAAAAACCCCAACCTAAAACCAGAAAGGCAAA
TAAATTGTGGGTAAATACGTATAGCCACTA
AATCAAAAGAAGACCTTTTTTCATGAGGAAGTTT
CGTTGAAAGGTTACCAACGGCTACAGAGGCTT
GGGCTTTACCCCTCGCAGCGAAAAGACACGCAT
CTTTCGAGAGGGAGTTAAAGGCGCTTTTGC
CCGATAGTCATAACCGATATATTCGGTCGTG
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CGCTCACAGGAAGGCGATCGGTGAACGCAAG
AGCATAAACGCCATTCCGCAATTCAATATT
AGTGAGCTGCACCCTCCTCTGTTGGTGGAAGAGA
CAGCTGCCCTCAGGAAGATCGCAGACAGTCA
TGCCAGCTTGCCAGTTTGAGGGGACCGTTCTA
GGGGAGAGTGGTGTAGATGGGCACGTAGCTAT
GTGGTTTTCGGCGGATTGACCCTACAGGTCAT
CAGCTGATTAACAAACCGTGCGATGAGAATCG
AGAGTTGGCAGGCTTTTCATCAACATGTAAT
CCCAGCAGATCAAAAATAATTCGCAAAAGCC
GTCGAGAGAATCAGCTCATTTTTTCAAATATT
TAGGTGTAGAATAGAAAGGATTCGCAATCAAT
CCGCCACCCTAAACAACCTTTCAACTTTTCAC
GCCACCCTGTAGAATAGATTTCTCAGAAA
GGATAGCATAACGATCTAAAGTTTCAGCTTG
TAACACTGTAGCATTCCACAGACAGCTTGATA
ACGGCCAGTGCCAAGCTTGCATCCACGACGCTTGTAACACG
ACCACACCCGCGCCTTAAATGCGTAGAGGAT
GTTCCTTAGGTTGTCGCTTGTAATTTTCAG
AGACGACGGTAGCGTCACGCTGTCAATCATG
TGCAGAACCAGAAAGGAGCGGCGCTTGTATATC
AGTCTTGAGGCGAACGTGCGAGAAGAGCGGACG
AATTTACGAGCCCCCGATTTAGAGGCCCTAATG
CGGCTGTCGCGTAAGCACAATAAGTTGCGCT
TATTTAACCATACCCAAATCAAGTACCTGTCG
AGCAAGCCGTCCTATAGGGCGATGCAACGCGC
ATTACCGCAGTGAGCCTCAACGTGCAGG
TAGAAGGCTCCAGTTTGGAAAGACAGGCCC
GGCGTTTTAAAGAATAGGCAGAGGCCCTGAG
GTTGTAAGTTGCCAAATCGGCAATGGTTTGC
TTTTGCACCCGGGTTTTGCTCAGTACCAGTT
TTACCAACCTGAGACTCCTCAAGAGCGCCGGAA
TAATTGGCTATTATCTGAAACAGTTTAGTA
TATTTATCAAACAGTTAATGCCCCTCAGAACC
GAATTTACGATACGGAGTGTACTATGACCG
CGTATAACGTGCTTTCTCAGTTAATGATGGTGTGCTTTGACGAGCA
ACAACCATCGCCCACGTGCCGCCAATGACACAAACTAC
GGCATCAAGCCTCAGAGCATAAAGTAACGCCA
ATATTTCAAAACATTATGACCCAGGGGAT
CATTTTCGCAGGAGCCCTTTATTTCCGGGCCT
CGAAGCGAGTTTTAGAACCCCTCATGCTGCGC
TCATTCCAGCCTGAGTAATGTGTACGGAAAAC
AATATGCAGGTGAGAAAGGCCGACGCCAGACA
CTGAATATTCAATATGATATTCAACGACACA
GTCATTTTTATTAGCCGGAGAGGATCGTAAC
AGAGTACCGATCTACAAAGGCTATATGGGATA
AGCGATTATAATCGTAAMACTAGCATTAAATG
AGAATACACCCCGGTTGATAATCAGTCTGGCGCC
CGAAGGCAAGGAAGATTGTATAAGAAGACCAATA
CCATTAAAAACGTTAATATTTTTGTTAAAAAAC
TGAGGACTGAAATGCGAATAATAAAGTTTTCAG
CGGAACGAATCTCCAAAAAAAAAGGTCTGTATG
GGATCGTCTCAATTGTATCGGTTTTATGTCGTCT
AGGCTTGCCTGAATTTCCTAAAACAGCCCTCAT
TG CCTGAGAGTCTGGAGGAGCAAAAACAATCTCCGTA
GGTTTTTCCGCTGAGGTCGAATCTCCGCTACA
GTCGCTGAACCCAGGTTCGATTCGCGCTAAACC
TTCGCTATTGTTTCCCTGTGTGAAATAGGGGC
AACGTGTGATTCCACAAACATAAAGGAAAGG
AGGCAAAGGTGTAAAGCTGGGGTCTTGACGG
GCTTTCCGAACTCACTATGTTGCTCGGAAACC
GTCGCCCGCTTTCCAGTGGGAATTTTTGGG
CGTCATCAGATCATTGAATCGCGCCCACTA
GGTCACGCGTTGCTGATTTGGCAAGGCCAAGG
GGAAACAATTTTCTACCAGTGAGATCCACT
TGAGCAGGTGGCGTTCCACGCTGTAGGTTG
TTCTGTATGAAAGCGGTGTCGCCACGAATCCCT
GGAAACGCCGACAAATCTCTGGGATAAGTGCC
TTTTGTTAGGTTGATATAAAGTATAGAAGGATT
CGGAGTGATCACCCTACTCAGGAGTGAAAGTA
GGATTTTGCTCAGAACCAGCCACCCTGCCCTAT
TTCCAGACCAGAGCCACCACCCCTCGTAAACGT
AGTTAGCGAGCCCAAATAGGAACCCGGTAATAAA
AAGGCCTGAGTTTCGTCACCAGTAATACATGG
GGGCAGCTAGAAATCAG
TGGCAAGTACAATAAAAACATGTTTCAGCTAA
GAAGAAAGGCGCCGCTGTATTTATCAACAATAGATA
GGAAAGCACAAGAAAAATAATATCCCATCCT
CTAAAGGGAGCATGAGAAACAATCAATAAT
GTCGAGGTTTTTCCTTATCATTTCAAGAAGCGG
CGTGAACCAAGTACCCTACTCTCGAGAAACA
GAAAAACCGTTTTTTTATTTTCATCGTAGAATC
ATTAAGAGCCCCAATAGCAAGCAAATCAGATA
AGTGCTTTTATCCGCTATTCTAAGAAAGCGGA
ATAAATCAAGCGAACCTCCGACTTGGCGGAG
TGATGTTGCGCTTTAATCAAGATTAGTGTGTA
AGGATTTAGCCAGCTACAATTTTTATCTCGAATC
TTAAGAGGGCTAAGCGAGCGTCTTTCCAGAGCC
TTCGGAACCGCTTTAATAAAACAGCCATAT
GCCGTATCTCAATCCA
CTTTTGATCGTTCAGTAAGCGTC
179
AATGCAGAAAGCGAACCAGACCGGAAGCAAAC
AGGCATAGACTTCAAATATCGCGTTTTAATTC
CGTTTACCTGCATCAAAAAAGATTAAGAGGAAG
CGAGAGGCCCTGACTATTATAGTCAGAAGCA
GGGGGTAAAGAATGACCATAAATCAAAAATCA
CGTCCAATCCCCCTCAAATGCTTTAAACAGTTC
tCAGGTGGAGTCAGGATTAG
AAATCCGCATAAAAACGAACCTAACGATTCAACT
GAACGAGGAGTCAGGACGTTGGGAGAATTACG
CCGAACCTGTTATGCGATTTTAAGAATAACCCT
AACGGGTGTITTAATTTCACCTTTAAATAG
GACCTTCACCAGAACGAGTAGTAATTGGCCAGA
GGATATTTCAGTGACTGAATAAGGCTTCTGGATAG
CGGAGATTTGTGTTAGAAAGAT
AAATATTCTTGATCATCGCGGAATCGTCATTAACAAAG
TCCAACAGGATTGGAATACCCACGAAACAACA
GAGCTTCATACATAACGCCCCAAAAGAGAAAAAT
CCGAAAAGTAAGAGCAACACTATCACCTGGCTC
AAGCGGTAGACGACGATAAAAAACATCATTGT
GGTCTTTTTTGGCAAAAAAGAGTTTAATTGGCT
AGAAAAACGTAGTAAAAATGGTTAGAGCCCTGAC
TTATTACAATCAGCGCTGATAAAATTGGTGTCG
CTACGTTGACCTGCTCCATGTTACTTAGCCG
ATTATACCGCAGACGGTCAATCATAAGGGAA
GAATTACCAACATTTGAAAGAGGACAGATG
TGAGATGGACAGACCAGGCCGATAGGCTGGCT
GAGAAACATCAAGAGTAATCTTGACAAGAACC
CTGCTCATATTACCCAAATCAACG

*Linking staples:*
ACCCAGAGGCCGCCACCAGAACCACCACCAGAG
AAGAGCAAGAAACCCTAAATTTAATGGTTTGA
GAGCCACCACCTAGTAATAAAAGGGACATTC
AGAACTTGAGCCATTTGGGAATTAGAGCCAGC
ATCACCGTCACCAGCCTACCATATCAAAATTA
TGAAATGAAATAGCAATAGCTATCTTACCAGAA

**Adjustments to the Rectangle Half Circuit Design**

**Linkage type 1:** Staple strands were modified to contain overhangs (sticky ends) that link directly to the overhangs on the other rectangle half circuit. One linkage contained 3’ sticky ends *(red)* that paired with each other and the other linkage contained 5’ sticky ends *(blue)* that paired with each other.

*Unmodified staple strands replaced by the modified strands: (the 6 linking staples and 3 others)*
ACCCAGAGGCCGCCACCAGAACCACCACCAGAG
AAGAGCAAGAAACCCTAAATTTAATGGTTTGA
GAGCCACCACCTAGTAATAAAAGGGACATTC
AGAACTTGAGCCATTTGGGAATTAGAGCCAGC
ATCACCGTCACCAGCCTACCATATCAAAATTA
TGAAATGAAATAGCAATAGCTATCTTACCGAA
TGGCCAACATTTGGCAGATTCACCAGTCACACG
TTATTTACAGAGATAGAACCCTTCGAAGGGTT
TTTGGATTTAAAAACAGAAATTTATGTCATCTTC

Modified linking DNA staple strands:

For structure 1 (one half circuit structure):

CAGAGCCGCCACCAGAACCACCACCAGAG
ACTTGAGCCATTTGGGAATTAGAGCCAGC
AATGAAATAGCAATAGCTATCTTACCGAA
GAGCCACCACCCCTCATCATTAGC
ATCACCGTCACCGGATTTCGACAT
AAGAGCAAGAAACTATAACCGAC
TGGCCAACATTTGGCAGATTCACCAGTCACACGACC
TTATTTACAGAGATAGAACCCTTCGAAGGGTTAGA
TTTGGATTTAAAAACAGAAATTTATGTCATCTTCTGA
GTTTAAGCTCAGTAATAAAAGGGACATTC
CGATTACCTACCTACCATATCAAAATTA
CTGATAACCTCTAAATTTATGTTTGAG

For structure 2 (the second half circuit structure):

GAGCTTAAACCAGAGCCGCCACCAGAACCACCACCAGAG
TAGGTAATCGACTTGAGCCATTTGGGAATTAGAGCCAGC
AGGTATCTAGATGAAATAGCAATAGCTATCTTACCGAA
GAGCCACCACCCCT
ATCACCCTCAAC
AAGAGCAAGAAC
TGCCCAACCTTTGGAATTTCAACAGTACCTCAGACCGACGCTAATGATG
TTATTACAGAGATAAGACCCCTTTCGAAAGGTAGGTAAGATGTCGAAATC
TTTGGATTTAAACAGAAATCAAAGTCTTTCTGAGTGGTTATA
AGTAAATAAGGGACATTC
ACCTACCATATCAAAATTA
CCTAAATTTAAATGGTTTGA

**Linkage Type 2:** Staple strands were modified to contain overhangs (sticky ends) that link to a third strand (helper strands). This third strand base pairs with sticky ends from both rectangle half DNA origami structures (see Figure 3.3B). The sticky ends are color-coded to match the respective helper strands. Note: the staple strands that do not have sticky ends are the same for both structures.

*Unmodified staple strands replaced by the modified strands: (same as Linkage Type 1)*

**Modified Linking DNA staple strands:**

*For structure 1 (one half circuit structure)*

CAGAGCCGCCACCAGAACCCACCACGAG
ACTTGAGCCTTTGGAATTAGGACATTC
AATGAAATAGCAATAGCTATCCAAAATTA
GAGCCACCACCCTCATCATTATGCTAGC
ATCACCCTCAACGAGCTTTATCAAGTACC
AAGAGCAAGAAACTATCTATGACCATTGA
TGGCCAACCATTTGCCAGATTTCAACCAGTCACACGACC
TTATTTACAGAGATAGAACCCTTCGAAGGGTTAGA
TTTGGATTTAAAAACAGAAATAAAGTCATCTTCTGA
GTACGTATTTGCAATCAAGTAATAAAAAGGGACATTC
ATGTGCTAACTTAGTAAACCTACCATATCAAAAATTA
GTCTAATCCAGGTATTAACCTACCATATCAAAATTA

For structure 2 (the second half circuit structure)
CAGAGCCGCCACCAGAACACCACCACCAGAG
ACTTGAGCCCATTTGGGAATTAGGACATTC
AATGAAATAGCAATAGCTATCCAAAATTA
GAGCCACCACCCTACTGAGTAGAATTTCC
ATCACCGTCACCCTAGGAAACATGACATA
AAGAGCAAGAAAACCTACCTGGCATTCAGTT
TGGCCACACATTGGCAGATTCACCAGTCACACGACC
TTATTTACAGAGATAGAACCCTTCGAAGGGTTAGA
TTTGGATTTAAAAACAGAAATAAAGTCATCTTCTGA
AAGGAACCCTTAAGGTAGTTAGTAATAAAAAGGGACATTC
CAGGTAACCTAATCCACCTACCATATCAAAAATTA
GGTACTTGATCGTCAATCTAAATTTAATGGTTTGA

Helper strands:
AACCTTAAGGTTCCTTGCTAGCTATAATGATG
GGATTAGTTACCTGGGTACTTGATAAGCTC
ATGACGATCAAGTACCTCAATGGTCATAGATA
TGATTTGCAATACGTACCGAAATTTCTACTCAGT
Linkage Type 3: A combination of linkage 1 and 2. One side has linkage 1 and the other side has linkage 2. Staple strand extensions that pair with each other are color-coded to match each other.

Unmodified staple strands replaced by the modified strands: (same as Linkage Type 1)

Modified Linking DNA staple strands:

For structure 1 (one half circuit structure)

CAGAGCCGCCACCAGAACCACCACCAGAG
ACTTGAGCCATTTGGGAATTAGAGCCAGC
AATGAAATAGCAATAGCTATCTTACCAGA
GAGCCACCACCCTCATCATTAGC
ATCACCGTCACCGGATTCGACAT
AAGAGCAAGAAACTATAACCGAC
TGGCCAACATTTGGCGAGATTCACCAGTCACACGACC
TTATTTACAGAGATAGAAACCCTTCGAAAGGTTAGA
TTTGATTTAAACAGAAATAAAGTCATCCTTCTGA
AAGGAACCTTAAGGTTAGTAATAAAAGGGACATTC
CAGGTAACCTAAATCCACCTACCATATCAAAATTA
GGTACTTGATCGTCATCCTAAATTTAATGGTTTGA

For structure 2 (the second half circuit structure)

CAGAGCCGCCACCAGAACCACCACCAGAG
ACTTGAGCCATTTGGGAATTAGAGCCAGC
AATGAAATAGCAATAGCTATCTTACCGAA
TGGCCAACATTTGGCAGATTCACCAGTCACACGACCGGCTAATGATG
TTATTTACAGAGATAGAACCCTTCCGAAGGTTAGAAGTGCAGAATC
TTTGGATTTAACAGAAATACAGTCATCTTCTGAGTCGGTTATA
AGTAATAAAAGGGACATTC
ACCTACCATATCAAAATTA
CCTAAATTTAATGGTTTTGA
GAGCCACCACCCCTCATCATTATAGCTAGC
ATCACCAGTACCAGAGCTTATCAAGTACC
AAGAGCAAGAAACTTCTATGACCATTGA

Helper strands:
AACCTTAAGGTTTCCTTGCTAGCTATAATGATG
GGATTTAGGTTACCTGGGTACTTGATAAGCTC
ATGACGATCAAGTTACCTCAATGGTCATAGATA

Reinforced corners on the rectangle circuit DNA origami structures:

Unmodified staple strands replaced by the modified strands:
ACCACACCCGCGCGCTTAAATGCGTAGAGGAT
GTTTAAACGGGTCAGTCGCTTGAATTTTCAG
CATCGGCAAGAAACGCAAGACACCGGTGGCAA
AGCGGGAGCTAACAAAGAGTTGAAATGAGGAGGT
ATCTAAAAACATAATCATATATGTGAGTGA
ATAACCTTTAAAGTAATTCTGTCC

Modified DNA staple strands:
Reinforced leads on the rectangle circuit DNA origami structures:

Unmodified staple strands replaced by the modified staple strands:

TCATCAGTTGAGTCAGGATTAG
CGGAGATTGTGGTAGAAAGAT
TGCCTGAGAGTCTGGAGCAAACAATCTCCGTG
GTGGTTTTCCGGGGATTTGACCGTACAGGTCAT

Modified DNA staple strands:

GTGGTTTTCCGGGGATTTGACCGTACAGGTCATTGCAGGATTAG
CGGAGATTGTGGTAGAAAGAT
TGCCTGAGAGTCTGGAGCAAACAATCTCCGTG
GTGGTTTTCCGGGGATTTGACCGTACAGGTCAT

To remove stacking of the rectangle circuit structures:

Unmodified staple strands replaced by the modified strands:

ACAACCATCGCCCACGTGCGCGACAATGACACAAACTAC
AACGCTGAGGTTTGCACCAGTAATACATGG
CTTTTGATGTCAGTCAGGATCGTCGC

Modified DNA staple strands:
Adjusted Branched “T” Shape for Linking

Staple strands were modified to link two T structures together. The rest of the staple strands were unmodified and used as before. The linking staple strands form a Linkage Type 1 connection on one side and a Linkage Type 2 connection on the other side. The “sticky” sections are color-coded to show how they pair.

For one of the “T” structures:

Unmodified staple strands replaced by the modified staple strands:

GGTAGAAAGTCTATCAG
CATAACCGATTGAAAAGGTG

Modified linking DNA staple strands:

GGTAGAAAGTCTATCAGCGTAGTAGTGC
CATAACCGATTGAAAAGGTGCTACAATG

For the other “T” structure:

Unmodified staple strands replaced by the modified staple strands:

GCATCAATTCTACTAATA
GGTAGAAAGTCTATCAG

Modified linking DNA staple strands

GGTAGAAAGTCTATCAGGCACTACTACG
CGTTAACTGCATCAATTCTACTAATA

Helper linking strand:
Corner Design

(Information is for the redesigned corner structure)

Scaffold: Lambda phage DNA, bases 39576–42308, 2733 base pairs long

PCR Primers:
GGATCTATGAAAAACATCGC
[BioTEG]CGACGCTTTTCTTGTCG

Staple sequences:
AGACACCATCGATTCCAGTAACACCGATA
ACATCGCGAAATGGTCATCACAGTATT
ATTCACCUGGGTTGATTTGTCGGAAGCGG
CCTCGCCCATACATGCAGCTTCCCTTTTCACACCGG
GGGAACCGAGTTTTTTATTAACACAA
GACTGHzCAATCTGTCAGTGCAAGATGTAATTC
AGCCATTCCCGATAACCAGAAGACCTGCCGACGTGGTAA
CTGGTTACCCAGACCTTACCTTCGGAAGGTT
TGCCATTTTAGCCGCGGCCTCGGGTG
ATGGCAGAACCCTGTCATCGTGGTGATC
ATTCTGCGACGTTATCTTTATTTGC
CCGTTTTCCCCCATGCAACAAACTGCGGTAATG
GCTACGCCATTCTGCGTAAATGGTG
GCTGATGTGCTGGAGATCG
AGGATTTGCTGTACTGCGTGAATTTCCCTC
GGTTACTACGATTGGTTTGTTGTACGC
TTTGGTCTAAGCTGCGTTGCGTGTAGATGG
GACGTCACCTAAGCGAGGCCCATAGCCAATACC
GCCAGCGACCATGCCATTCAACCTACACTCCT
GTAAACACACACTTCCAGCTTTCTTTTCTTC
AGGAACAAACATCTGCTGTGTATAATAGTAATACA
AAATTCAAGGAATTCTCGCAG
TCCCCCAATTACTGGGTCAGGGATAAAGGCG
AGGCCTGAGAGGTCTACCGCCCGGCCCGCA
TCATCTTCTGCGATCTTCCAGACGACGTA
CGTTTCATGCTCCTTTCAGTCCCGAAGACTCC
TAAAAACCAGATAATTGCTCTTTTCCGGCAAT
CCGTCTTGCACAGAGCTATTATCAGCTGAC
CCGTTCGTTGCGCTGTAGATGCTTCTCT
CCACGGTTTTTAGTTTTTTCATGACAGCGTTTT
AAGTTGGTATAAAAAAGGCTGCAGGGTCTGGA
CACGATTTTTTATTAGCTCAGTAATTCTCGAAT
TTGATTTTTAACTCCATATACCGCTTTCTGT
ATACACTTTCCGCCACTCTGCGGCAGGAGA
CAGGTAATAGGTTGTCTGACATCTGACATCC
CGTCGGCCTGTTAGCAACCGATGCTGCG
TCATATGGACATTTAGATCC
TAATTCCCGTGACAGTTAACCCTCCTGCGG
TGTTCTGTGTGGTTGGCAGATCGACGCATC
GTGCACGGCACAGCGTTGGATATCTGCATCTG
GCATCGGACACACCGGGGAAAAGTTGCCAGTAAC
ACTCGTAACTCGGGGCTTTCTGTCGTACTG
GTGCGGCGACCCGCAAGCAGGCTACCTGGGA
TCCCCGCGAAGCCGCAAACTGGGCGA
CGATGTTTAGCTCATCTGCCTGGCCTTCCTCA
TGCTCAGCGCATCAAAGCGATTATGACAGG
TTCGGCAATAAGGTTGGAAAACACTGTGTG
CCTGCTGTTTTGATTTCCACGGATACTTAGCT
TGGCTGAAATACAGGCTCGGCTGCCCCTC
AGCCAGGACCATATCAACCAGCTGCTCCC
CGGATTTCGCTCCTCCCCGGTGAACGGCGGCC
CACCTTGTGTTCATTAACAA
GACGTCTTTAGCCACCGGATATCCCCCCACCG
AGCCGTGTAGTTGAAGGTGGTTTATGATACA
TCTTTGAGTGTCAGTGGGTTGGTTTCGCGAT
TGCCTTCGCTGGAAGGTATTTGCCAGGGCTT
GCAGATTAATGTCGATGATACCTCGGGAATCTT
TCGCCCACACGTCTCTCTTTTCATTTCCGA
GGTAAACACCCTGTTGGTGTCTCCGAAGT
CACATGGGCAAGTCCGATTTTTACGGAT
ATCTCCTTTGCAAAATATGCGATGTATATC

191
ACTAACGATGCAATACGGTGAGCGTACCGAGA
CCCATCTCTGGCTGTCGAAGCTCGGT GGTT
GCTTGTTATCTTCTGGTCCCCCTTCTCA
TACCGAAAAATGGGTTTCAGAACATCTGATTC
CGCAAAATTATGGAGGTGCCTATATTGGGTTCATC

**Three-dimensional Corner Design**

*Scaffold:* Lambda phage DNA, bases 39187–41340, 2154 base pairs long

**PCR Primers:**
GACCAGCCAGAAAACGACC
[BioTEG]CGCGTCTGAATCTTTGG

**Staple sequences:**
TGGGTTCCCATTTGGCAGCGCTTCCGGCAATACT
TGCCCTGTTCCGGCACCAGGATAAGACTCCGCA
GGTCGAGTGGCAGAAAATAACCCAGCAGGTAGT
TCCACCCCCGCGCATGCTTGGGCCCCGATG
TTAACCATGGGTGATTCCGGGCTTACGGCGTAA
GGCGATCCACTGGCGATTCTCGACATCATAT
GCTTTTCGTCAGCTGTTGTTGCTAGGGAT
ACACACCGTTGATGCGCAGCTACGCTTACGGCAGCCA
TTCCCTGGTAGCAAACCGGTAATACCAGGCCTCCGGCGG
TACCTTCGATAACAGATTTCCCATGCAAC
CATTTTAGCCGCGGCCCAGGTGGTCG
ACTGTTGCAGCTTTTACGTAAGCCCTTCGTT
TTGGATGTTTTTCTGAGGCCTGCGATTACCA
TTCCAACATGTCAAAATTTTTCATGACTTCCCT
CTGCAATACTTCTGTGTGCTGGAGATCGA
TTGGCTGAATTGCAGTTGCTGTACTGCGTG
CGCGTCTGAATATCCTCGTTTTCTACTACGATTGGTTGG
TGACCACCTTAACGCCACAGGTTCTCTACGGAC
GCCTGTATAAGCTCTAATCGCGGCGCGAACA
GCCTACACGCATCCTTTTTAACTCGTCCGTCG
ATTACCACAAAGCCAGTTATTAGGTTAGCCC
GAACAAACATCCTGCTGAATAAAAAGCTGATG
AAACACACACTTCCAGGGTTAGTTCATCCAC
AGCGACCATGCCATTTTTGCTGAAGTCCCGCC
GTCACTGCTAAGCAGGCCAGGATACATCGGT
TGGTCTAAGCTGCCTTGTTGCGT
AGTTTGCCCGGCTCTCTGTCCTCGCGTTGTCG
CAGAAGTTGTCTCTGGCCGGAGAAATACCGAAT
GCACACACATGTCCCGAGCCAGAGCGAAAATTC
CGTCTCGCATCACGCTCTTTTAACCTGCCCT
ACCCAGCAAAATTCGGCTCACAACCAAGGTAG
TGCGATAGTTCACCTTCTCTGACTGCGGT
TCTCGGGCCTGCTAGGCTGAGATCCAGCC
CTTGCTGCCGCTCTGCTCAAGCTCGAGAGAC
TTGCTCGCCGGCTCTGCCCAGCTCGAGAGAC
TTCACCAACAGAAAGGTGTTTGCTTCTCTGTTT
TTAAAAACCGCTCTGCTAAACGACATTATTC
CCGGTTTTCAATGCTCCGCAATTATGCTGACGT
GGTCATCTGCTGGTTGCGGCTGCTCCTTT
GCAGGCCCTTTCTCCGCTGTATCCATGAAAAG
CTCCCCCCTTTTAATGCAAACCGATATGTCA
ATTCTAAACATTCGCTCGATATCCAGAAC
AATAGCGTCAACCTGACCGGGTTGATATCAAC
TTGGGTCTCCATAGCTCATACCCTGTAACCAT
TCCTGAATGGTTGCTGGTC
CGTAAAACCATATCCAGAGCCAGTCTCCCATCTCG
CTTCCACTCAACCAGCTCGCTGACGCTTATCGGG

Circular Circuit Structure

Scaffold: M13mp18, design is 7222 base pairs long

Staple sequences:
TTGCCTGAGTGAAGAAGCTCAAACTATACTTT
ACCACCAAGGAGCCGGAATTTTGTTT
TGATTTAGTGCCGATTAAGGGATTGCCTAA
GTTGATAGCAATCGGCCTTGCT
ACGCCAATACGCCGAGTCTCCTGAGCCGTAC
ATTAGGCCACCGAGTAAAAGAGTCTGTCCATC
ATCTGAATCAGTATTCCGATTATCGATG
CGTGCTTTCTCCTGTTAATCAGAGCATTGGTGC
TTTGATGGTGTTCCGAAATCCGTCTATCA
AGAATAGCGAAGTGGACTCCAAACGTGCAG
ACAGTACATACTAGGTTCTGA
TGGTTGTTTGGCTATTGAGGCGCAGCTACA
GCCCTGAGTTTCACCAGTGAGACGGCAACAGCTG
ATTGCCCTTCAGTTGTTTCCA
ATCAAAATAATCAATATATGTGAGTCTATTGA
GTCAGTAGAGGGTTGGTTATATAAGACCGTGAT
TAAGACGCTGTAATCGCTGCTAAAGAAAAC
TCCTTGAACATAGCGATAATGCAAATCTC
CCAGCTGACATTAAATCGCGC
CAACGGCCTTTCCAGTGGAACACAATTCC
TTTCTAGAGTGCAAGCAAGCGAAATCC
GAGACTACCTTTTTAACTCCGGGTTTTGAATT
CAATCGTTTGACCTAAAATGATAACGGG
AAAGAACGCAGAAAACTTTT
CACTGCCGCCGGGAGAGGCCGGCAGCAGCGAAAATCTCTG
TTAATTGCTAAAGTGAAAGCCTGGGTTGCCTAA
TGAGTGAGCTACAGGGTGTT
GAAATACCTATATGGAAATGCTGGGGTTAGAT
TCAAATATATAGTATCATA
GTTATCCGCTACCTGCTGTG
ACACAACGGCTAGCTGTTCCGAGGATCCC
AAATAAGGCGTTAAATAAGAATAAAACATGGTTT
ATCATAATTAAGCCAACGC
AGCCTGTTTTTAGTTAATTTCATCCAAGAC
TTCGTAATCATATACGAGCCCGGAAGCAGTTGCGCT
TGCGTTATAACACCATATTTAAC
GGTCGACCTCTATGTGTGAAATT
CGGGTACGCGCCAGTCAGCAGTGGCCAGGGT
TCAACAGTACGGCATTTTCGAG
GAGAATCGAATTCTTACCAGTATACTAGAAAA
GTAAAACGACGCGAGCTCGAA
AACGCCAACAGACAAAAAGTA
AGTTGGGTAACCATGCTTGCA
TTTCCAAAGGGGATGTGCTGTCGGTGC
CCAGTAAATAATAAAACAACATG
AAAGTACCTGTAAATTTAGGCAGAGGCTTAATT
CCAGCTGGCGAGTCACGACGTT
AAGTAATTCTGTATCAAATAATAGATAACAAAAATAA
GGGCTCTCGTTAATATTTTGTAAACAGGAA
TTCAGCTTAAATGCAAATAAGAA
CGCCTGTTTCCAGACGAGCAAGAGAGAATAT
CATTTAATCTGTTGGGAAAGGCGGACAAGGCGATTA
TAAATTGTAAATCGCTATTACG
ACAGCCATGAAAATAGCAGCCTTTAATAAG
GATTGTATAAAGCTAAATCGGTTGAGAATTAG
ACGATTTTTTGTTCATATGGT
CAAAAATATTATTTATCCCCAACGAGCTCAACG
CATTATCAGAAAAGCCCCAAAAATTGC
CCTCAGAGCATAAGCAAAATATT
TTTATTTTGTCAATTCAACCG
AGGCAAGGCAATACAAAAAAA
CAAAATTATAGCATTAACATCCAAACGAGCTGA
TTACCAGCGCCGGAATATTATT
AAGGGCGACACAATCAATAGAAAAATTTAAGCT
ACTAATAGTAGAGCAATAAAG
ATTGAGGGAGGGACTTGAGCCA
CATTTGCGGCGTAAATCATAC
AAAGGTTGTCATAACCTGTTTTAGATT
CATTAAGGTTGCGCAGTAGCACC
CCGTCACCAGAAGGTAATATTGACAAGAAAA
TTTCGCAAATGGCATCAATTCT
TTTGGGAATTCACCAATGAAA
CTGCGAACGAGAGCTATATTTT
AGTTTGACATTCATATAACAGTTGTCATATT
ATTACCATTAAGCGACAGAA
GGAAACGTAGAGCCAGCAAAATCAAATTAC
AGTACGTGCTGAATATAATGCTACCAG
ATGTTTTAAATATGCAACTAA
TCAAGTCCCCCTTATTAGCGTTTCTCCCTCA
CCATCGATAGCTAAAAATCAC
TTTTGATAAGAGATTCCAATTTT
TTGCCGGAAGATTAGAGTAGACCTTTTATTGCTCC
AGCTTAATGTGTCTGGAAAGTTTTCCATTAGATACA
GTTTTTCATCGGACCCTCAGAGCCACCAGAGGTTGA
GTCATAGTTGCCTTTTAGCGTCAGACTGTAGCGC
TTTCATAAAGCACCCTAACATCGTAGGCAAGGCC
ACCGGAAGCAGCTGACTATTATAGTCTATGGCTTTAA
AAAGACTTCAAATATCGGTTTTGTTTATGCATCAA
GAGCCGCTATTCCAAACAAATAAATTC
CGGAAACCAGGACCACCCGAACCAGCCTGCCATCT
ATCAAAAATCGGTCTTTTACCAACTCCACCGTCGAGCTGGCTTAG
AGCGGATTTTGAGGTTCAAAGCGAGTAGCTCAAAC
AAGATTAACGTCTAAATATTCTAATATGGTTT
ACCAGAGCCGCTTGATGATA
TTGACAGCCCTCAGAGCGCACCAGAACCAC
GGCAGGTCCTTTACGTTCCAGTAAGGGTTCA
GGCCTTGACACCCTACAGAACCACGGCCATTTCG
ACAGTCTAAAAAGAAGTTTTGCGAGCGA
TACTGCAGGAATGAGGAAGCCCG
CTCATTTAAAGCCAGAATGGAAATAACAGTTAA
GAGGCTTTTGCGAAAACGAGAATGACCATAA
TAATAGTATGAAATCCCTCTCAAGAAGCAA
AGACTGGAAGAGCAACACTATCAATGCGATT
AGGAGTGATCTAGACTCCTCAAGAGAAAGGGTTG
GTTTTAACGCCTCATACATGGCTTCGCCAGCA
GTGCTCTTGTATTCTGAAACATGAAATTATCAGCGTA
TGCCCGTAAGCGCAGTCTCTGAAAGACGATT
CGATAAAAAACAAATAGCGA
CATAACGCCAAAAGGAGTCAGGAC
TTAGGAATACCACATATAAACG
GTTTTTGCCTGAAAGATTCTCATCGTTGAGAT
TGCCCCCTGCCTATTTCCGGAACCTATAGTAACAG
TGAATTACCTTTAACCCTGTTTACCAAGAGGGGG
TTAAGAATGTTTTAATTTCAACTTTAATCATTTG
TTATACCAATTACGAGGCATAGTTAGCGTCCAA
GTTGGGAAAACCAGAAGCGAGTAGCAGGCAGCA
CTACGTTATCAAATATGCGAGATA
AACTAACGATTCTGAAATAAGGCAGTAATCT
AGGTAAGTACCAGCGAGGAGCACCACACTCAA
CCGTCGAGGGGATTTAGGATTACGG
ATATAAGTGAGCACCACCCCTCATAGGAACCCA
GAATAGGTAGTAGTTAAGGCTGGGTAATAA
CGTAACAAAGGCTGCTGAAACAACATTATTAC
CTCAGGAGGT'TTGTACCGCCACCCTATAGCCCG
GGTGTACAGACTAAATGGGGCTTGAGACTGGCTCA
TAGGGCTGGCCAACCTTTTGAAAGAGGACAGATGAAC
TCAAGTGGCCTGACGAGAAGAAAAAT
TGACAAAGAACC CGGATATTCACTACACCCAAACCT
CTTTTCACAGTACAAACTACAACGCCTG
AAGCCCCAAAGAACC CGACCCTCGATAAGTG
AACCGAACTGACTGACCTTCA
TGTACCGTACACTGAGTTTCGTCACGGGATAGC
ATTTTGCCAACAGTGCCACGCTAAAACAG
TTATTAATTGAAAAATCTAAAGCATAAAAATA
GACAACTCTCAAATATCAAACCCCTGCGCAAC
ATTTAGAACAGTTGGCAAATCAACCAATATT
TAGAGCGGAAGGTTATCTAAAGACCTGA
AGCACTAAACAACTAATAGAT
AACACCGCCTGGGAACAAAGAA
GCAGCAATTTAAAGTTTGGAGTAACATTATC
GCTGAACCGGTATTAAATCCTTTTGGCCGAACG
ATCTGGTGTTAGACTTTTACAAACATTAC
GGAAATTGCACGTCAATAGATAATACATTTTGAGG
AGGTGAGGAGAAACAATATTACCGCCAGGCATT
CCGAACGGAAAAACGCCTCATGGAAATACCTA
TGATAGCCCGCTCAATCGTCTGAAATGGATT
TTGAATGTGGCAGATTCACCAGTCACACGAC
AAGCGTAAAAAGGGACATTCTGGCCAACACAGAGAT
AGAACCCTTCTATATCTTTTAGG
GGTAATATCCCCGGTCAGTATT
GCAACAGGACCACCAGCAGAAGATGAGAGCCA
CATTTTGACTAAAAACATCGCCATTACACCTT
ATTTACATGCTATTAGTCTTTTAATCAATCAAT
CAGTAATGAATACGTGGCACAAGAAGTTGAAA
ATGTTACTTACGCCGAACGAGGCGCGGGATCG
ATAAAATTGTGTCGAAAATCCCGGACCACATCGGAA
AAAGTACAACGGAGATTTGTATCCTTTTGAG
TGACCCCCAGCGATTATACCAAGGTTTCCAT
AAGAGGCAAAGAATACACTAAACACACTACGA
GGAGTTAAAGGCCGCTTTTGCAACGACGTCAATCATGAAGG
TCACCCCTCGCATAACCGGATATATCATAGTT
CGAGGGTAGTTGCGCCGACAATGAGTCTTTCC
GACTAAAGGGGTAATTCTTAAAACGTATGGGA
TAAACGGGTAATTGTATCGGTTTTTTACGCG
AGGCACCATCTCCCAAAAAAAAGGTAAAGG
ATCGCCCAAGCGAGCAGACAGAGCTGCTCC
TACCGATAGCAACGGCTACACAGGATCGCCTG
GCTTTCGAACTTTTTACATGAGGAACCACGAAAC
GGAGCCTTTAAAAATACGTAATGCCTCATCTT
CACGTTGAAAAACCTAAAACGA
TAGCATTCCACAGACAGCCCTTCGCTGCTGAGGCTTGCAG
AGCGTAAACGATCTAAAAGTTTTGTCCAAACAACC
AGACGTTAAGTAAATGAAATTTCCTAGCTTGA
TTTTGCCTAAACACCCCTTCAACAGATCGCTTT
GAGTGAGAATAGAAAGGAAACAACCTCCAAA
AATTGCGAAATAAAATTTTT
CCCATCCTAGCGTCTTTCCAGAGAAAAACAG
TCAAATAAAATTTTATCTGAATTAACCTGAA
AAGAACCGGTCAAGATTAGTTGCTAAATTGAGC
ATCGAGAACCATTTGCGGGGAGGCAAGATT
CGTAGGATTCTAGAAACGCGAGGAAACAAAA
GCCAGTTAGTCTGAACCAAGAAAAATAATAT
CGCTAAGGAATTTCAGAGCATGGAAAGCCAA
CCAGCTACTCGGTCTTTCTTTATCATTTCC
CCTTTAAGTATTAAACCAAGTACCGCACTC
GCGGAACCTCAAGCAAGCCGTTTTTATTTTCAT
ATCCGGTAAATCATTACCGCGCCCAAATAGCAAGCA
AATCAGATATACGAAGCCCTTT
GGAAGCGAACATATAAAAGAAAGGAACGAAAGA
CACCCTGAAACGCTAGAAAAATACATACATA
GCTAATATGATTAAGACTCCTATTAGGCAG
GAGTTAAGAAATAAACCAGGAAATACCCAAAAAGA
TTGATAATGACCCTGTAATACTTTTTCGGGAG
GTGAGAATAGCTATTTTTGAGAACCCGTCG
CCTGAGTACAGGTCATTGCCGTAGTTTCATCA
TTTAGAACAGAGAATCGATGAACGATAATTC
AAGCCTTTTGCTAGTCAATCATATGTCATTTTT

**Long Bar Design**

*Scaffold:* M13mp18, design is 7085 base pairs long

*Staple sequences:*

The staple strands on one half of the structure (110 in total) were modified to contain an extra 10 adenines on the 3’ end of the strands. The rest of the staple strands (117 in total) on the other half of the structure were modified to contain an additional GTGCGTGT sequence on the 3’ end of the strand. These staple strands with the additional nucleotides were ordered and used to fold the DNA origami structure.

TTGCCTGAGAGTCTGGAGCAAACAAGGAGATCAAAAAAAAAAA
AAACTAGCTAAAATATGCCCCGGAGCGGGGTTTTTTTTTTTTTT
GATAATCACATCAATATGATATTCGCCGTCGAAAAAAAAAAA
TTGTATAAGGGGTGAGAAAGGCGGGGAAATAGGAAAAAAAAAA
TAATTTTGGCTGAGTAATGTTGATTACCGAAGTAAGTTTTTT
TGTTAAAATTTTTTAGAACCCTCTCGAACCCTCGGCCCAAAAAAAAA
AACGCCATAGAAGGCGGTATTCATTTTACGGGAAAGTTTTTTTT
CCTGTGAGAAACATTATGACCCTGTTGATACCCTGATACCTTTTTTTTT
GAGCGAGTTCAGAGCAAGCTAAACACTCAAAAAACCCCAAAAAAA
GGAAACAAACAAAGAATTAGCAAAAGGCCCTCAAAAAAA
TCCCAATTAAAGC GCCATTGC CGCA TCCAGGCAAAAAAA
TCTGGAATGTTGGAAGGGCGATCGGTGCGAAAAAAAAAA
ACATGTTCGCTATTACGCCAGCTGGCGAAAGAAAAAAAAAA
GCTTAATTTGCTGCAAGGCGATTAAGTTGGGTAAAAAAAAAA
TATTAAGAGGCCTATCAGGTCTCAAAAAA
TGCTCAGTTATAAACAGTTAATGCGCTGATTAADAAAAAAA
GAGGGTTGAACGGGGTCA GTGCCCTTGCGGCAGAAAAAAA
TGTATCACATGATACAGGAGTGTACTGAGCAAAAAAAA
CCACCCTCCCAGTTCCAGTAGTAAGCGTAAAGAAAAAAA
ACCCTCAGGCCAAATGGGAAGCTCATTTGAAAAAA
ATAGCAAGGATATTTCACAAAAACATACATAAAAAAAAAAAA
AACACTGAAGGGAGGTTGAGGCGAGGTGGTCTCTAAAAAA
AACGCTGAAACCACCACCCAGGGCCTTCCTCCTAAA
TAGTTAGCTCAGAGCCACCAACCTGCTTAGATAAAAAAA
TTTCCAGCTCAGAGCCGCCACGGTGAAATTTAAAA
TGGGATTTCCGAACCCAGCCACCTACCTTTAAAAAA
CAGCGGAGAGCGGTTTCGCAATCTTTTATATAACAAAAAA
AGGAATTCGCGTTGCATTTTCGAGTCGATAAAAAAA
AAAATCTCTTGCTTTTAGCGTCAGTTTTCAATAAAAAAA
CTTTAATAGCACCACAATCAGTTGACCTAAAAA
CGAGGTGACGAAACGCTCACAACGTGTGATAAAAAAA
ATAGTTGCA TGCACTACAGTAGCCAAAACACCGGAAAAAA
CTATTTCGGAAGGATTAGGATTACGGGTAGCAAAAAAAA

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AGTCCCCGACCAGGCGGATAAGTAAACGTTAAAAAAAAAAAA
AAGTTTTATATAAGTATAGGCGAGGAGGTAGTAAAGAAAAAAA
GGCTTTTACGTACTCAGGAGGTAGTTAGTGAAAGAAAAAAAA
TGAATTTAAGAAACGCCCACCCCTCAATATTTTAAAAAAAAAA
TCATTAAAGAGGCAACCACCCCTCATACGCAAGGAAAAAAA
TTGGCCTTCCCAAATAGGAACCCATAATACTAAAAAAA
CATTGACGTTTCGTCACCAGTAAAATCGGTAAAAAAAAAAA
GCCACCAGTAGCATTCACCAGACAATTAAGCAAAAAAAAA
CGCCACCCGTAACGATCTAAAGTTAATCATACAAAAAA
CCGCCTCCACGTTAGTAATAGTCTACTATAAAAAAA
AAAATCAGCTAACAACCTTTCTGGGCGGAAAAAAA
CCTTATTTGAGAATAGAAAGGATGGTCAATAAAAAAAA
CGCGTTTTGAGAATAGAAAGGATGGTCAATAAAAAAAA
AATCAAGTCAAAAAAAAGGCTCCAGTGTGATAAAAAAAA
TCGATAGCTGTATCGGTTTATCAGGTACGGTGAAAAAAAA
AGCAAGGCTAATTTCTAAAACAGCCTAGCTCAAAAAAAA
CAGCAAGGCGAATTGCAACGCGTTAGAAA
AGTAACGTTAAACATGAAAGAAAAAAA
TCGGGAGATCAGGTTAACGTCAGATGATATACA
GCTTTGAATAAAAACAGAAATAGAAATGCA
AGGCCAATTAGAACCTACCATAATAAATA
AAAGAAGATGTTGGATTATACTTTCTGAAATAAA
ATTACCTTGC
CGGAATTATCATCATATTCTTCG

TCAATAATATTTTGCGGAACAAAGAACACAAACAAAAAAA

ATGAAATCGATTAAATTAAAAAGGTGGAGT

AGAATCCTCAGAACCTCGTATTAAATCTTTTGCAAA

TAAAGACGATTTAGAAGTATTAGACTTTACAAAAAA

TTAACCTCAATATCTTTAGGAGCAGCTAAACAAAAAA

TTATGTACAGTTGAAAGGAATTGGAGGAAGGAAAAAA

AAGACAACCTCAATCATATCTGGTCAGTTGGAAAAA

ATATTTTCATCACCCTTGTGCAACCTCAAATAAAAAAAA

ATTAAATGTGAGCCAGCACGCAATGAAAAA

AAATAAGGAGTATTAACACCCGCTGCAAACAGAAAAAAA

AATCATACAGCAGAAGATAACAGAGGTTGAAAAA

GTAGATTAAAACAATAACGGATTTCCTCCTGCAAAAAA

TTTGCAACGTACAAAGTTACAATAATGAGTAACAAAAA

GGAAGGGTTATTCAATTACATCTGGTAAATAAAAA

CCTGATTTTGATGAAACAAACATCCATACATAAAAAA

ATTATCGATTACATTAAACAATTTGCGATCTCAAAAAAA

CAGAAGGATTTTAATGGAAACAGATAAAATCCAAAAA

ACATTATCTGTAAGCTATTAACCTCAGACGAAAA

CCGAAACGTTTCGCTATTAATTAATTGCGCCAGAAAA

ACAATCTGAAAAACATAGCGATACAGAGCCAAAA

ATTATTGAGCTGAAGAGATCAATACCTCAGAAA
CTAATAGACATAGGTCTGAGAGACACCGGAAAAAAAAAAAAAA
TTATCTAAACGGCTTAGTTGGTTTCATAATCAAAAAAAAAAAA
CAAATCAAATGCTGATGCAAATCCATAGCCCAAAAAAAAAAAA
TCAAACCCAACGAGAGAAGGAACCTGTAGAAAAAAAAAA
ATCTAAAGAGTTAATTTCATCTTCAGCGACAGAAAAAAAAAA
TGCCACGCCTTGGAAAATACCGACTGAAACCAAAAAAAAAAA
AGGCGGTCCGTAAATAAAGAATATTACCATTAAAAA
CGAACCACATTACTAGAAAAAGCCTATTAGAGCAAAAAAAAAAA
AACGCCAGGTTCATTTTTGCGGATAACCATCGGTGCCTGT
AAAACGCAGAAGTACCTTTAATTGTCGCTGAGTGCCTGT
TGCAGGTCCCGGAAGCAAACCTCCATTTTTCGCGGTGCCTGT
CGAGCTCGCGTTTTAAATTCGAGCTGACAGCATGTGCCTGT
TTTCCTGTTAAGAGGAAGCCCGAACAGAGGTGCCTGTGCCTGT
TTCCACACTCAGAAGCAAAGCGGGAGGAAGTGTGCCTGT
GTGTAAGAIAATCTCAGGTCTTTAATGCCAGTGCTGT
TAACCTCAACACGTTCCAAGAAACGAAGAGGCGTCGCTGT
CCGCTTTCAATATTCCATGAAATCCTTTGACGTGCTGT
CTGCATTGGATAGCGTCCAATACAAACAAAGGTGCCTGT
GAGGCAGGTCCAGAGGGGTAAATACCTGATAAGTGCCTGT
TTTTTCTTATAGCGAGAGGCTTTTCCATGTGCTGT
CTGATTGCCCTCCTGGTTTACCAGACCGTCAATGTGCTGT
AGTTGCAGACGAGCGATAGTAAGACCTTTGAAAGTGCCTGT
CAGCAGGCCTAATGCAGATACATAACCAGGCGGTGCCTGT
CCGAAATCACCAGTTGAGATTTAAGAGTAATGTGCCTG
AGAATAGCCGGAACACATTATTACCACAAAGTGCGT
CCAGTTGGAAGAAAAATCTACGTAGTGAAATAGTGCGT
ACGTGGACAACTGGCTCATTATACGAGAAGTGCGT
GATAAGAGGGTTTCGCCAGTCACGACGTGTGTCGCTG
GGATAGAGGGCAGTGCAAAGCTTGCATGCCGTGCGT
AACAGAGAGCTCTAGGATCCGGAGTACGTGCGT
AAATATCGAATTCGTAATCATGGTCATAGCTGGTG
AAAAAGATGTGAAATTGTTATCCGCTCACAAGTG
ATTATAAGACATACGCCAACGGGAAGCATAAAAGTGC
CATAAAATCCCTGGGGTGCTAATGAGTGAGCTGC
TGCTTTAATTTGCGTTCGCTACTGCCTGCCTG
TCGCATACAGTGGAGAACCTGTGTCGTCGAGGTG
TTTAGACTAACCGAGGTTGAGTGTTGCTG
AAGTTTTGGCGTATTTGGGCACGGGTTGGGTG
AAACAAATTCAACCAGTGAGACGGGCAACAGTTG
ATCAAAACCCGCCCTGGGCCCTAGAGGGTGC
AAGGAATTCAAGCGGTTCACGGTGGTTTGCCGCTG
ACATTCAAGAAATCTGTTGATGGTGTTTGCGT
AAAGATTCCGGAAATCCCTTTATAATCAAAGTGC
CGAACTAACCAGATAGGGTGTAGTGTTTGTGCGT
ACGTTGGGAACAGAGTCCACTATTAAAGAGTGC
ATTAAAAGTCCAAAAGTCAAAGCGAAAAACGCTCATCAGTGCGT
ATCATTGTGAATTACCTTTAATTTCACCTTTAGTGCGTGT
CCCACGCACCTTGAGCCCATTTGGGAGTTTAGTGTCGCTGT
GGCTTGCAATTACCTAAAGGTGAATACCAGTATGTGCGTGT
GATCGTCAGGGAGGGAAGGTAACCTATTATTTGTGCCTGT
CGGAACGACAAGACAAAAAGGCCCCAACATGTGTCGCTGT
TTGAGGACATCAATAGAAAATTCCAGCCAGTAAGTGCGTGT
TTCCATTCAAGACACCACGGAGATAAGGGTAAGTGCCTGT
CTACAGGAAGAAAGGTGGCAATTTTAGCAAGCTGCGTGT
AAAAGAACAGTGATGTATGGCAGAAGCCTGTGTTGTGCCTGT
CCCCAGCGAGAACTGGCAGATTCAGAAAGGTGCCTGT
TACAACCGCGAGAAACGCAATAAAGCATGTAGTGCTGT
ATTGGTCAGATAGCCGAAACAGATTTTCTTTGTCGCTGT
TACCTACCTTTACCAGAGCAGCTTTCCAAGTACGTGCCTGT
CATAGGAAGCAAGAAAATGCGTCTTGTGCCTGT
GAGGACAGCAGCAGAAATTGAGCGCGCCAGTGCCTGT
CATAGGCTAGGTAATTGAGCGCTAGGCTATGTGCTGT
CTTGACAAAAATTTAGCAGCTTTAGCCTGCTGT
TCAACGTAACTAAAAACAGGGATGGAAGCCGTCCTGT
AGGCTTTGAAATAGCAGTTGCCACCCGTGCCTGT
GTAGTAAAAATAAGAAAACGATTTTTACCAACGTTGCTGT
GTCACCCTAAACGATATATTCCGGCTTTTTGTGCCTGT
GGAAATTAGGAGTTAAAGGCCGCAAGGTCAGTGCCTGT
ACCGATTGACCCTCAGCAGCAAAATCAAAGCGGTGCCTGT
ACCACGCAGGGTTAGCAACGGCTAAGACTTCGTGCAGGT
TGTCACATAAGACTTTTTCATATTGCATCGTGCGTGT
AAACGCAAAACGGGTAAGATACGTACCCTGACGTGCGTGT
ATACATACGCACAACCTAAAAACGAGAATGACGTGCGTGT
TTATTACGTACACTAAAAACACTCACCCTCAGGAAGTGCGTGT
TACCCAAAATTATACCAAGCGGCTGCGGAAAGTGCGTGT
AGGAAACAGATTGATACATCGGGTAAAATGTTGCGTGT
AAGTAAGCGAAATCCGCAAGCTGCTAAGGAAGTGCGTGT
ATAGCTATCGGAACGAGCGACGAGACGATAAGTGCGTGT
AATAATAAGAACGGAAACTGACAAAGCAAGCCTGCGTGT
AGAGATAATGAAACGGGTGTACAGACGCAAGACTGGTCTG
AAGTCAGGGCTGACCTTCATCAGGAATACCGTGCGTGT
TAGACGGGGAAACCGGATATTTCATTACAGGTAAGTGCGTGT
AGAGAATAAAGCCTCTCGCTATTCTAATAAAAGGTGCGTGT
ACGTCATGACGAGAAGACCAGATCTCGAGGTCGCGTGT
CCATCTCTGGGCTGTGAGATGTTATGCGGTTGCGTGT
AAATAAACGCAATATTAATTMGCAGTTTACAGTGCAGGT
ATCATATGACATCGCCATTAAAAATACCAGAAGTGCAGGT
AAAGCCATAGTGTTATGCGGAACTGATAGTGCGTGT
AGAAATCAGCGTGGCACAGACAATTTTTGAGTGCGTGT
AATTTAGGAGAACCCCTACCCTGACACGAAAGCGGTTGCGTGT
TAAGAGAAATAAAAGGCCACATTCTGAGCAGTACGTGCGTGT
AGTAATTTCATTGCGAGATTTACCAAGTCGCTGCGTGT
TACTTCTAAGAACGGGTATTTAATTAAGAAGTGCAGTGT
CTGTCATATCAGAACAAGCAAGAAATAGCATGTCAGTGT
TATAATCAGTAGGAAATCATTACTTAAAGCCCAGTCGTGT
AACGGGTACCAAATCAGATATAGAAATATCAGGTGCGTGT
CAGGAGGCCTAAGAACGCAGCAGGCGCTGAACACGTCGTGT
TTTCTCGACTTCGCCAGAAGGTTAGCAGCATGTGTCGTGT
TATGTTGAGATTAGTTGCTATTCTTTACAGGTTCGTGT
CGCCTATTATTTATCCTGAATCTTTTGTTTAGTTCGTGT
CGTACGCGCTTTTTCCAGACGCCTATTATCGTTCGTGT
GCGCTAGGGCGCTGGCAAGTGTAGGTCGTGT

**Adjusted M13mp18 Rectangle for Linking Into Long Chains**

Staple strands were modified to contain 8-base overhangs that link directly into the opposite side of the bar structure. The rest of the staple strands were unmodified.

*Original, unmodified DNA staple strands:*

CCCAGCAGGCGAAAAATTTCGTAATCATGGTCAT
ACAATTCCACACACCCGGCCTGGgccGCTGA
ACACGTGATTGCCCTTTCAATACGAGCCGCAAGCAT
CGGGGAGAGCCCGTTTGCATTCGCTTCCGCCTC
CCCCTTTGAAAGGCG
TGCCGAAGGGGCGCTAAATGAGTGA
AAAGTGTAAAGCCTGGGTGGATGTGCAAGGAGAT
ACGTGGTAAGGTTATCCGCTC
AGCTGTTTCCTGTGTGAAATTCGACCGCCAGTGCCAAGCTTG
CATGCCTGCAGGACACGCGGATTGACCGT
CATCGTAACCGTGCAATTTTCCCAGTCACG
AGTTGGGTAACGCAGGTCTCCAGTTTGAGGGG
CTCCAGCCAGCTTTTCTATTACGCCAGC
ATCGGTGCAGGCTCTCTCCGCCAGCCACCGCTTTCTGGTG
AATGGGATAGGTCACGTGTTGATGGTATGGGCG
ACGACGACAGTAGTGCTCAGGAAGATCGCA
GGTGGTCTTTTCTTCCACCAGTGAGACGGGCA
GAGAGTTGCAGCAAGCGGTCCACGCTGGTTTG

Modified staple strands:
CTGTCGTGGGTACCGAGCTCGAATCCTGT
GCGCAACTGTTGCGCCAGTCCGGAAAC
GGGAACAATCTAGGGATCCCCGTTCAGGCT
CCGGAACCCAGGAAAAGCGCCATTCCGATTCTCCGT
GATGGTGCCAGCTGCATTAATGAAATCGGCCAACGCG

Adjusted M13mp18 Rectangle for Surface Placement

Modified sequences contain additional nucleotides on the 3' end of the strand. Modified staple strands were used in place of the original staple strands when folding the DNA origami structures.

Original, unmodified DNA staple strands:
CCCCAGGCAGCGAAAAATTCGTAATCATGGTCAT
ACAATTCACACAACCCGCTGGCCCTGA
ACAGCTGATTGCCCTTCAATACGAGCCGGAAGCAT
GCTAAACTCACATTATATTGGGCGCAG
CGGGGAGAGGCGGTTTTCGATTTGCGTTGCCTCAGCTTG
CCCGCTTTGAAAGGGCC
TGGCGAAAGGGGCTTAATGAGTGA
AAAGTGTAAAGCCTGGGTTGATGATGTGCTGCAAGGCAGATT
ACGTTGTAATAAGTTATCGCCTC
AGCTGTTTTCCTGTGTGAAATTTCGACGGCCAGTGCCAAGCTTG
CATGCCTGCAGGTGACACGGCGGATTGACCGT
CATCGTAACCGTGCATTTTCCACGTCACG
AGTGGGGTAACGCGCAGGGTTGGTCCAGTTTGAGGGG
CTCCAGCCAGCTTTTCTATTACGCCAGC
ATCGGTGCAGGCCCTTTGCCCGGCCACCGGCTTCTGTG
AATGGGATAGGTACGTTTGTTGATGATGCGGCG
ACGACGACAGTATCGGCTCTCAGGAAGATCGCA
CCAGCTGCAATTATGAACTCGCGCAACGC
GGTGGTTTTTTTCTTTCTACCGTACGAGACCACGCA
GAGAGTTTGCAGCAAGCGGTCCACCGCTGTTTGA

*Modified staple strands:*
GGTACCGAGCTCGAATCCTGTTTGTGGAAAAAAAAAA
TTCAGGCTGCGCAACTGTTGGCCAGTCGGGAAACCTGTCGTGAAAAAAAAAA
TTCTCCGTGGGAACAATCTAGAGGATCCCCGAAAAAADAAAAA
CCGAAACCGGAAAGCGCCATTCCGCAAAAAAAAAAA

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Adjusted Branched “T” Shape for Nanoparticle Attachment

Modified sequences contain an additional ten adenines on the 3’ end of the strand. Any staple strand that was modified for the structures was used in place of that particular original staple strand when folding the DNA origami structure. The following sequences are given as the original sequence and color-coded to indicate which were modified for certain seeding and metallization tests.

For the “T” structure with attachment sites across one half of the top of the “T”, the green and blue staple sequences were modified, reordered, and the modified staples were used in place of the original strands. For the attachment sites along the entire top of the “T” structure the green, blue, orange, and red staple sequences were modified and used in place of the original sequences. For the “T” structure with attachment sites along the top with about a ~100 nm gap, the green and red staple sequences were modified and used in place of the original sequences. The black sequences were not modified in any of the DNA structures.

GGTAGAAAGTCTATCAG
TACCACATACGTGGACTCCCAACGTCAAAGGGC
CAAAAGGATCCAGTTTGGAAACAAGAGTCCACT
ACTATCATAAAAGAATAGCCCCGAGATAGGGTTG
AAAAACCAGTTCCGAAAATCGGCAAAAATCCCT
GAAGTTTTTGCCCCAGGCGAAAATCCTGTT
TTTAGACTAGAGGGTGCAGCAAGCGGCTAC
GGAACAACATTATTACA
AAGAAAAATCTACGTTAATAAAAACCTTTAGGAA
AACTGGCTCATTATACCAGTCAGGCAATAACGC
AATCATTTGTGAATTACCTTATGCAGAGCAAC
AATTGGGCTTGGATGTTTAATTACGACGAT
TGCCCTGACGAGAAACACCAGAACTTGCAAAAA
GTAACAAAGCTGCTCATTCACTGAGGTAAAATG
GAAAAACCGATTTCATCAGTTGAGAGAACTAAC
ATTAAAGATCAACTAATGCAGATAACGTTGGAGTGTTGTATTACGAGGCATAGTAATTTTAAG
ATAAAATCAAACCCCTGTTTACCAGTCAACTTT
TGATGGTGAAATAGCGAGAGGCTTGAGTAGAGTAG
GCTGGTTTGGCCAGAGGGGTAATAATAAAGGCT
CGTCATAAAACAGCTGATTGCCCCCTCAACCGCC
GCTTTAAGGGTGGTTTTTCTTTTCACCCAGTG
TAAATCAAGCGGGGAGAGCGGTTTGCGTATT
TATAGTCACGTGCCAGCTGCATTAATGAATCG
ACAAGAAACCGATATTCCATTACCCTGCGGAAT
AGGCTGGCTGACCTTCATCAAGACCTCAAAT
GGACAGATGAACGGTGTACAGACCAATGACCA
ATAAGGGAAACCGAACTGACCAACTCTGACTAT
TGGCCCTGGGATAGCGTCCAATACAAATCAAC
AGACGGGCTATATTCCATTGAATCCCTAATCTTG
GGGCCTGCCACAGTTCAAGAAAAACGAGAGGCCGCAT
GCCAACGCAAAACTCAGGTTTACCTTGAAAGA
AAACCTGTGAAGCAAAGCGGATTGGGTCAATC
TCGCTTTTGCCGGAGAGGGTAGCTATTTTTGA
GACCGGAATGATATTCAACCGTTCTAGCTGAT
GAGAGTACAAAGGCGGAGACAGTCAAATCAC
GGTCATTTGTAATGTGTAGGTAAAGATTCAA
GCTGAATAGAACCCTCATATTATTTTAATGCA
ACCTAGCGGGAACGAGGCGACAGACCATCAAAA
ATTGTGTCGAATATTCCGCAGCCCTGCTTCAAATA
GTACAAACGGGAGATTGTATCATCGGCAACCA
ACCCCCAGCGATTATACCAAGCGCCAGGATTA
GGCAAAAAGAAACTACACTAAAAACACTTGAAGA
AAATTAATTAATTCGAGCTTCAAACCTGATAA
CATCAATAGCAAACTCCAAACAGGTGAAACAAA
AGGGTGAGCTTTTAATTGCTCTTTTCATCTTTG
ATGCCTGATTGCGGATGGCTTAGAACGAAAGA
AGATTAAGGGAAGCCGAAAAAGACTCCATGTT
GCATCAATTCTACTAATA
AAATATGCCCTTTTATTCTAACGCAAGGATAAA
TTCAATTCCATTAGGCCCTGTAATACCTTTGC
GCGAACGAGAGCATAAAAGCTAAAATCGGTGTA
ACATTTTGAAGAATAGCAAAAATTAGCAATA
TATATTTTATTAACATCCAATAATCATAACAG
CCACTACGAAGGCACAAACCTAAGGCTTAATT
AAGGTTCATTAACGGGTAAAATCATGTATT
AGGCTTTTGAGGACTAAAGACTTTTCTGGGAAGT
CAGCATCGGAACGAGGGTAGCAACCCAATTCT
TTTGCGGGATCGTCACCCTCAGCACATTAGAT
TCGCTGAGGCTTGCAGGGAGTTAATGTTTAGC
CATAACCGATTGAAAAAGGTG
AATTTTTATAATGCTGTAATTAACGGAATTG
GGGAGGAAGACATAAGTGCTGTTCTCATGAGG
CCAAAACATATAACAGTTGATTCCGGCTACAG
AAGCCCTCAGTAGATTAGTTTGAGCGGAAAGA
GCAAGGCACAAATGGTCAATAACCAGGCGCT
GTAGTAGCCATTGGGGGCGCGAGCATATTCGG
AGATCGCACCAGTCGGG
TTCTGGTGACATTAATTGCGCTTCGCTCAGT
CGCCATTCAAGCCTGGGTTGCCTAATGAGTGA
CGATCGGTACACAACATACGAGCCGGAAGCAT
GCTGGCGACCTGTGAAATTGTTATCCGCTC
TAAGTGGGGTCCGAATTCTGATCATGGTCTC
CGACGTTGCGAGCTCGACTCTAGAGGATCCCG
GAGATCTAGCCTCAGGA
AGAGTCTCTGCTGCCAGTTGAGGGGGCACCAGC
GGTAGTTGGTGGTAGATGCGGCACGACCATT
TACCCCGGAACGGGCGAGTTGACCCGGGAGGG
ACAGGAAGAGTAACACCGGCGGTTACGCCA
GTAAACGTAGCCAGCTTTTCAAAAGGCCGAT
AAATTTTTCTCATCAAAAAATAATCCCCAGTCA
CCAAGCTTGATGCCTGTAAAACGACGGCCAGTG
CCCGCTTTTCTCCAGCCAGCTTTCCGACGACGA
GCTAACTCCCGGAAACCAGGCAAAGCATCGTA
AAAGTGTAAAGGCTGCAACTGTTTAATGGGA
ACAATTCCGCGGGCCTCTTCGCTAATTCTCCG
AGCTGTATAGGGGGGATGTGCTGCACATTTAAA
GGTACCAGTGTAACGCAGGGGTTTTTGCCTCTGG
TTAACCAATAGGAACGTTAAATCAGCTCATTT
CAGTATCGCAAAGGTATCTAGGTCATTGCTTG
ACCGTGCAAGGCAAACAAAGGAAATCGATGAAC
TAGGTCACTAAAATCTAGCATGTCAATCATATG
TGGGAACATTGATAATCAGAAAAGCCCCAAAA
TGTGAGCGATTGTATAAGCAATATTTAAATT
CCTCCTGTAAATATTTTGGTTAAAATCTGCATT

Logic Gate Prototype Structure for Nanoparticle Attachment
(Modified Circular Circuit Structure)

Scaffold: M13mp18

Regular staple strands: (used in the original design)
ATTGGCCAAACAGTGGCACGCTAAAACAG
TTATTAATTGAAAAATCCTAAAGCATAAAAAATA
AACACCCTGGGAACAAAAGAA
GCAGCAAATTTAAAAAGTTTGAGTAACATTATC
GCTGAACCCTTATTTGCCCGGAACG
AGGTGAGGAGAAACATATTACCAGCCGAACTT
GGTAATATCCCGGTCAGTTATT
GCAACAGGACCGACGACAGAGATGAGAGCCA
ATGTTACTTAGCCCGGAACGAGGCGCGGGATCG
GGAGTTAAAGGCCGCTTTTGACAGCGCTAATCATAAGGG
TCACCCCTCCGCATAACCGATATATCATAAGTT
ATCCGCCCAGACAGCAAGACAGCGTGTC
TAGCATTCCACACAGACAGCCTTTCGTCGCTGCTGAGGCTTGCAG
AGCGTAACGATCTAAAGTTTTGTCGAACACAGCGTGGTGCAG
CAATCGTTCTGACCTAAATTTGTTCAACCAGCA
ATCATAATATTTAGCCAACGC
GTAAAAACGCACGCGAAGCTCGAA
TCAACAGTAGGGCATTTTCGAG
CCAGCTGGCGAGTCACGACGTT
CCAGTAATAATAAACACATG
TTAAATCTGAGCTATTACG
TTCAGCTAAATGCGCAATAAGAA
CCACAGCACAAGCAGGAAATATT
ACGATTAGTTGTCATATGGT
ACCTAATCTAGGCAAATAAG
TTACCAGCGCCGAAATTATT
AAGCCCAAGAACGCCACCCTCGATAAGTG
TGATTAGTGCCGATTTAAAGGGATTGCGCTTA
GTTGTAGCAATCGGCCTTGCT
ACGCAAATACGCCAGAATCCTGAGCGGTCACG
ATTAGGCCACCGAGTAAAAAGAGTCTGTCCATC
ATCCTGAATCATCATATTCTGATTATCAGATG
ATGGCAATTTCAACCTACCATATCAAACGTCAGAT
TTGCCTGAGTGAAGAAACTCAAACACTAATCTTCTT
ACCACCAGAAGGAGCGGAATTTTGGTTTTGG
CGCGTACTTGGAGCTAAAACAGGAGAATAACATCAC
ATGCGCCCGAGAGCTTGAGCAGGAGGAA
CACCCGCCTTAGACAGGAACGGTAAAACC
CTGCACGTGGAAGAAGGAGGGGAA
CGCTGGAAGATGTAGAAGTGTGTATTAAAAATC
GTACCTTTAAATGGAAGGGTGTAGTCAATATA
AGGTTTTAATTATTTGCACGTAACACAGAAATATG
AGTGTATACTTCTGAATACATCGGGAGTAGGG
GTTTGGAACAAAGGGAGCCAGGCTTCTACAGGG
ATTACCTCAAATCGCGCAGAGAGATTTC
AAAATAAAAATTACCTGAGCAtAAAAGAAGATGATG
AGCCGGCGAAGCTTGAGCAACCCAGG
GAAAACAGAAAGGAGCGGCGCAAAACAATAACG
GATTCCGCTGATTGCGCAGTAACA
Regular staple strands: (ordered for this modified design)

TTGGGCGCACTCACGTTAACCTTAATTGCTAAGT
GTTATATAAGACCGTGAT
GTAAGCCTGGGGTGGCCTAA
GGAAGTTTCCATTAGATACA
TAGCGTCAGACTGTAGCGC
CAGGTCAGTGCTTAGAGCTTAATGTGCT
CAAAGCGAGTAGCTCAAC
GAACCGCCCATTTTCGGTCATAGTTGCT
TGCCCCCTGCCTATTTCGGA
TTTTTGCCGACGACGATAAAAAACAAAAATAGCGA
CACCCCTATAGCCCGGAATAGGTAGTATTA
CTCAGGAGGTTTAGTACC
CTCAATTACCTTTAACCTCG
AGAAATTGCGTGCGAATTAT
CTGCTTTTCCTCGTTAGAA
TCAGAGCATGGTGCTTTGACGAAATCGGA
ACTCAACGTGCGTAAAGCAGCCTAGCAGTATAA
ACCCAAATCAAGTTTTTTGG

Extended staple strands (contain an extra ten adenines on the 3’ end for Au NP attachment):

GCCAGTTAGTCCTGAACAGAAATAATAATATAAAAAAAAAAA
CGCTAAGCAATTACGAGCATGTAGAAGCCAAAAAAAAAA
CCAGCTACTCGGCTGTCTTTCTATCATTCACAAAAAA
CCTAAAGTATTTAAACCAAGTACCGCACTCAAAAAAAAAAAA
GCGAAACCTCAAGCAAGGCGTTTTTATTTTCATAAAAAAAAAAAA
ATCCGGTAAATCATTACCGCGGCCCCCCATAGCAAGCAAAAAA
AATCAGATATACGAAGCCCTTTAAAAAAAAAA
CCCATCTAGGTCTCAGATTTCCAGAAGAAAAACAGAAAAA
TCAATAAAAATTTTATCCGTTAATTAACCTGAAAAAAA
AAGAAGCGGTCAGATTAGGTCTAAATTGACGAAAAAAA
ATCGAGAACCCTGACTGCGGGAGGCAAGAATTAAAAAAAAAA
CGTAGGATTCTAAGAACCGCAGAGAAAACAAAAAAA
CACCACGGGACAGAGAATTACATCCTAATTAAAAAAA
AAGGTGGCCATTAGACGGGGAATTCTTACCAAAAAAAA
TATGTTAGACAAGGTCAGAGGTTTTTGTCCAAAGAAAAA
ACTGGCATCGAGAGATAACCCATTTTTGAAAGAAAAAAA
GGAAACGCCCAATAAATAAGAGCAGCGTTTTAAAAAAA
GCCGAAGCAATATAGCATTCTTACGAGGCTTTAAAAAAA
TTAAGAAAAAGTAAAGGATTTTTATTAAAAAAA
GGAAGCGAACATATAAAAGAAACGCAAAGAAAAAAAA
CACCCTGACAAACGTAGAAAATACATACAAAAAA
GCTAATATGATACGTACTCCTATTTTTACGCAAAAAAAAAA
GAGTTAAGATATAATAACCGGAATACCCAAAGAAAAA
TGAAATACAAAGTTACCAAGAAGAAACCGAAAAA
GCGTCTTGTCGGGCACCGCTTCTGCTGTCGCCCAGAAAAA
TAACCAATCAAAGCGGCATTTGCCATTTGAGAAAAAAA
GATTCTCCATCTGCCAGTTTGAGGGGACGACAAAAAAAAAAA
ACATTAACCGGCTCAGGAAGATCGCACTCCCAAAAAAAAAAA
GCCAGCTTCCCTCTGTAGCCAGCAGTCTGGAAAAAAAAAA
GTCACGTTGGTGATAGATGGGCGCATCGTAAAAAAAAAAAA
AAACCAGGAGGAACGCCATCAAAAGTAATCGTAAAAA
CTGCGCAATTTTGTAAATCAGCTACCCCGGAAAAAAAAAA
CCGTGCGTGGGAACAAACGGCTTAATGCCAAAAAAAAAA
GACAGTATTTGTAGCGAGTAACAGATCTACCAAAAAAAAAAA
TTTAGAACAGAGAATCGATGAACGATAATTCAAAAAAAAAA
AAGCCTTTGCAATGGCATATCATATGCTGATTTTTAAAAAAA
GTGAGAATAGCTATTTTTTGAGAAACCGTGCAGAAAAAAAAAA
CCTGAGTACAGGTCATTGCCTGAGTTTCATCAAAAAAAAAAA
AGCAAACACCTCATATTTTTAATGCAATGAAAAAAAAAA
CAACCGTTCTAGCTGATAAAAGGATTGACCGTATGGGATAGAAAAAAAAAA
AAAACTAAATTTCAACGCAGATGATAAAATTTAAAAAAAAAA
TTGATAATGACCCCTGTAATACTTTTTCGGGAGAAAAAAAAAA
GGAGAGGGAGGCCGGAGACAGTCAAATCACCATCAATATGATATTAAAAAAAAAA
AAGGCTATATGTGATAGGTAAGTAGTTCAAAAGGAAAAA
GACACACTCTCAATATCACCCCTGCACGCAACAAAAAA
ATTAGAAACGTTGCAAAATCAACCAATATTATAA
TAGAGCGGAAGGTTATCTAAAGACCTGAAAA
AGCACAACAAGTTACAAATAGATAAAAAA
ATCGGTTGTATAGACTTTACAAAACAATTCAAAA
TTCAACAGATCAGCTTGCTTTGCTAAGGCTAACCACTTTTTTCAAAAAAAAAAAA
GAACAACCTCCAAAAAGGAGCCTTTTAAAATAAAAAAAAAAAAA
AGCAAGCGAAATCAAAGAATAGCGAACGTGGAAAAAAAAAA
TCCCTATGTCCACGCTGTTTTGTTTGTGGCTAAAAA
GGGTTGACCGCCTGGCCCTGAGTTTCACCAAAAAAAAAAA
CTTGCTTCTGAGAAGAGTAGGAGGGTTGGGAA
ATTTCATCaAGACAAAAGAACGCGAGAAAACCGGAGAAAACCTTTTAAAAAAA
AGTGAGACGGGCAACACGCTGAAAAAAA
CCAGTAATCTAGAAAAAGCCCTGTTTTTAGTTAAAAA
AGGCAAGGGCTTAATTGAGAATCGAATTCTTTAAAAA
GACGACAAGAATATAAAGTACCTGTAATTTAAAAA
AAGTAAATCTGTATCAACAAAAAAA
TCCCAATCAAGACGCCCTGTTTTCCAGACAAAAAAA
TAGATAAAACAAAATACGACATGAAAAATAGAAAA
ATAGAAAAATTTAACGTCAAAAATATTATTTAAAAAAA
CAGCCTTTAATAAGTTTTATTTGTCATTCAACCGAAAAAAA
ATATTTGAAAGACAAAAAGGGCGACACAAATCAAAAAAAA
CAAAATCAAAATTATCACCGTCACCCGAGGTAAAAAAAA
ATGGTTTTAAATATGCAACTAAAGTACGTGCTGAATAAAAAA
TAATCAGTGCAAGGCCGAACGTAGAGCCAGAAAAA
ATTACCATTAAGCGACAGAATCAAGTCCCCCTAAAAAA
GAGCCGCCACCAGAACCACCAAAAAAAA
GGAACCGCTGCCATCTTTTCATAAAGCACCGAAAAAAAAAAAA
CGGAACCAGAGCCACCACCACAAACAAACAAAA
ATAATGCTACCAGACCGGAAGCAGCTGACTATAAAAAAAAAA
TTAGCGTTCCTCCTCAGAGGCCGCTATTCCAACAACACATTAAAAATCAAAAAAAA
CCCTCAAAGAAAGCAAGGGATTTCGAGCTTAAAAAAAAAA
CATGGCTTCCGAGCATTGACAGGCCTCAAACAAAAAA
CTCTGAAAGACGATTGCGTACACCCCTCAAAAAAA
ATCAAAAAATCAAGGTCTTTTACCAAACTCCCAAAAAAAA
TATAGTCATGCTTTAAAACGTTTAAAAAGAAAAAAA
TTTACCAAGAGGGGTAAATAGTATGAATCCCAAAA
AGAGGCTGGTAAATAAGTTTAAACGCGTCATAAAAAAAAAAA
ACCTATAGTAACAGTGCCCCTAAAGCGCATAAAAAAA
GGTCGAGGTCAGGCGGCAACAGGCACAAAA
ACCCTAAAGAGTCGACTATTAAACGAGATAAAAAAA
TCATTTCCTACATTAAACATTTGAATACAAAAAAA
ATTGCCCCCTCAGTGTGTTTCCAACAAAA
ATCAAAAATAATCAATARATGCTGATTCATTGAAAAAAA
TAAAGACGCTGAAAATCGTCTGAAAGAAACAAAAA
TCCTGGAAAAACATAGCGATAATGCAAAATCAAAAAAAAA
TTTGGATGTTGGTTCCGAAATCCGTCTATCAAAAAAAA
ACAGTACATACATAGGTCTGAAAAAAA
CACTGCCCCCGGAGGAGGGGAGGCCAGCGCAGCGAAAATCTGAAAAAAA
GAAATACCCCTATATGTTAATGCTGCTTGATAGATAAAAAA
TCAAATATATTTAGTATCAT
CCAGCTGCATTAATGAATCGGCA
CAACGGCTTTCCAGTCGGGAAACACAATTC
GAGACTACCTTTTAAACCTCCGGT
TTCGTAATCATAATACGAAGGCCGAAGCAGTTGCGCTTA
TGGTTTATACACCATTTAAC
GTTATCCGCTACCTGGTGGA
ACACAACGGTCATAGCTGTTTCCGGGATCCCA
AAATAAGGCGTTAAATAAGATATACATGGTTT
AACGCCAACAGACAAAAGGTAA
GGTCGACTCTCTATGTGTGAATTT
CGGGTACGCCAGTCCTGGCCAGGGTGTA
AGTTGGGTAACCATGCGCTG
TTTCCCAAGGGGGATGTGCTG
CATTAATCTGTGGGAAGGGCACAAGGC
GGGCCTCTCCTAATATTTTGTAACAG
CATTATCAGAAAGCCCACATTCG
GATTTGTAAAGCTTAATCGGTGAAT
ATTGAGGGAGGGACTTGGAGC
AGGCAAGGCAATC
CAAAATTATAGCATTAAATCTCAA
TTTGGAATTCACCAA
CATTTGGGCGTAAATCATACAAAAA
AAAGGTGTACAAACCTGGTTTAGATTAAAAAA
CCATCGATAGGTCAATAATTCAGTATTTAAAAAAA
AGTTTGACATTCCATATAACAGTTGGTCATTTAAAAAAA
AAAGACTTCAAATATCGCGTTTTAATGCATCAAAAAAA
TTTTGATAAGAGATTCCCAATTAAAAAAA
TTGGCGGAGATAGAGAGTACGTTTATTGCTCAAAAAAAA
GTTTTCATCGGACCCTCAGAGCCACCAGAGGTGAAAAAAA
TACTGCAGGAATGAGAGCCTGAAGAAAAAAA
CTCATATAAGCCGAAATGGAATACAGTTAAAAAAA
AAGATTAACGTCATAATAATTCATAATGTTAAAAAAA
ACCAGAGCCTGATGATACAAAAAAA
GGCAGGTCTTACGTTTCAAGGAGGTCAAAAAAA
GAGGCTTTGCAGAGACGAGAATGACCATAAAAAAAA
TTATACCAATGACGCTAGATTACGTCCAAAAAAAA

**Modified “T” Shape for Carbon Nanotube Attachment**

Modified sequences contain an additional sequence on the 3’ end of the strand for either Au NP attachment (A10) or CNT attachment (ACGAAGTTGTTGTTG). The sequences are the same as the modified “T” with nanoparticle attachment sites along the top section with a ~100 nm gap, except with the sequences within the gap region modified for CNT attachment.

*Modified staple strands: (for CNT attachment)*

TGGCCCTGGGATAGCGTCCAATCAAAAAATCAACGAGTTGGTTTGG
CGTCATAAAACAGCTGATTTGCCCTTCAACCGCCACGAAGTTGTGGTTTG
ACAAGAACGGGATATTTCATTACCTGCGGAATACGAAGTTGTGGTTTG
AGACGGGGCTATTCTTCCATCTTCCGAATCTCTGAGCAAGTTGTGGTTTG
GCTTTAAAAGGTTGTTTTTCTTTTCACCCAGTGACGAAGTTGTGGTTTG
AGGCTGGCTGACCTTCATCAAGAGCCTCAAATACGAAGTTGTGGTTTG
GGGCGCCACAGTTCAGAAAAACGAGGAGGCGCATACGAAGTTGTGGTTTG
TAAAATCAAGCGGGGAGAGGCGGTTTGCGTATTACGAAGTTGTGGTTTG
GGACAGATGAACCGGTGTACAGACCAATGACCAACGAAGTTGTGGTTTG
GCCACGCAATCAGGTCTTTACCTTGAGAAGACGAAGTTGTGGTTTG
TATAGTCACGTGCCAGTGCATTAATGAATCGACGAAGTTGTGGTTTG
ATAAGGGAACCGAAACTGACCCAAGCTGTACGACTATAGGAAGTTGTGGTTTG
AAACCTGTAAGCAAGCGGATTGCTGCTAACAGAACTGACGAAGTTGTGGTTTG
ACTTAGCCGGAACGAGGCAGCAGCAGACCATAAAACGGAAGTTGTGGTTTG
AGATTAAGAGAAGCCCCGAAAGACTCCCATGTCAAGAAGTTGTGGTTTG
TCGCGTTTGCCCGAGAGGTTAGCTATTTTGAACGAAGTTGTGGTTTG
ATTGTGTCGAAATCCGCGACCTGCTTCAAATAACGAAGTTGTGGTTTG
AAAATTAATTATGCCAGCTTCAAACCTGATAAAACGAAGTTGTGGTTTG
GACCGGAATGTATTTTACCCAACCGTTCTAGCTGATACGAAGTTGTGGTTTG
GTACAACGGAGATTTGTATCATCGGCGAACCAACGAAGTTGTGGTTTG
CATCAATAGCAAACCTCAACAGGTGAAAAAACGGAAGTTGTGGTTTG
GAGAGTACAAGGGCGGAGACAGTCAAATCAACAGAAAGTTGTGGTTTG
ACCACAGCGATTATACCAAGCGCAGAAGATTGAGTTGTGGTTTG
AGGGTGAGCTTTAATTGCTCCTTCCCATCTTTGACGAAGTTGTGGTTTG

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GGTCATTTGTAATGTGTAGGTAAGAGATTCAAAAACGAAGTTGTGTGTGG
GGCAAAGAGAATACACTAAAAACACTTGATAAAGACGAAAGTTGTGTGTGG
ATGCCTGATTGGGATTGAGGCTTAGGAAACGAAAAGACGAAAGTTGTGTGTGG
GCTGAATAGAACCCTCATTATATTTAAATGCAACGAAAGTTGTGTGTGG

*Modified staple strands: (for Au NP attachment)*

GGTAGAAAGTCTTCTACAGAAAAAA
TACCACATACGTGGACTCACAAGCTCAAAAGGGCAAAAAAA
CAAAAGGATCCAGTTTGGAACAAGAGTCCACTAAAAAA
ACTATCAAAGAATAGCCGGAGTAGGGTTGAAAAAAA
AAAAACCAGTTCCGGAAATCGGCAAAAATCCCTTAAAAAAA
GAAGTTTGCCCGCAGGCGAAAAATCTGTGAAAAAAA
TTTAGACTAGAGGTTGTGACAGCAAGCGGTCCACAAAAA
GGAAACACATTATTTACAAAAAAA
AAGAAATCTACGTATTAAAAACTTTAGGAAAAAAA
AACTGGCTATTATACCAAGTCAGGCATAACGCAAAAA
AATCATTTGGAATTTACCTTATGCGAGAGCAACAAAA
AATTGGGCTTGGAGTGTGTTTAATTACGACGAAAAAAA
TGCCCTGACGAGAAAACACCAGAACTTGCAAAAAA
GTAACAAAGCTGCTCATTCAGTGAGTAAAATGAAAA
GAAAAACGGATTCTACTGTTGAGAGAACTAACAAAA
ATTAAAGATCAACTAATGCAGATAACGTTGGAAAA
AGTGTGTATTACGAGGCATAATTTAGGAAAA
ATAAATCAAACCTCGTTTACCAGTCAACTTTAAAAAA
TGATGGGTGAAATAGCGAGGCTTGAGTAGTAAAAAAAAAAAA
GCTGGTTTGCCAGAGGGGGTAATAATAAGGCTAAAAAAAAAA
GCATCAATTCTACTAAATAAAAAAAAAAA
AAATATGCCCTTTATTTCAACGCAAGGATAAAAAAAAAAAA
TTCAATTCCATTATGACCCCTGTAATACTTTTGCAAAAAAAA
GCGAACGAGAGCATAAAGCTAAATCGGTTGTAAAAAAAAAA
ACATTTCGAAGAATTAGCAAAATTAAGCAATAAAAAAAAAAA
TATATTTTATTAACATCCAATAAAATCATACAGAAABBBBBB
CCACTACGAAGGCACCAACCTAAAGCTTAATTAAAAAAAAAA
AAGTTCCTCATCCAAAACGGGTGTAATACATGTTTAAAAAA
AGGCTTTTGAGGACTAAAGACTTTTCTGGAGATTAAAAAAAAAA
CAGCATCGGAACGAGGGTGAGCAACCCAATTCTAAAAAAAAAA
TTTGCGGGATCGTCACCCTCAGCACATTAGATAAAAAAAAAA
TCGCTGAGGCTTGCAGGGAGTTAATGTTTAGCAAAAAAAAAAA
CATAACCGATTGAAAGGTGAAAAAABBB
AATTTTTATAATGCTGTAGCTCAAACGTATAATGAAAAAAA
GGGAGAAGAACTAAAGTACGGTGTCATGGAGAAAAAAA
CCAAAAACATATAACGTTGATTCCGGCTACAGCCCCCCCC
AAGCCTCAGTAGATTGTTTGAAGCAAGAAAAAAA
GCAAAGCGAAAATGGTCATAAACAGGCGCTAAAAAAAAAA
GTAGTAGGCATTGGGGCAGCGATATTCCGAAAAAAA

Unmodified staple strands:
AGATCGCACCAGTCGGG
Modified Circular Circuit Structure for Carbon Nanotube Attachment

Modified sequences contain an additional sequence on the 3’ end of the strand (ACGAAGTTGTTGTTG). Modified sequences are listed below along with sequences they are replaced with when folding the DNA origami structure. The rest of the sequences are the same as for the modified circular circuit structure (logic gate prototype structure for nanoparticle attachment). The sequences modified for carbon nanotube attachment are located in the two gap regions.

Staple strands replaced by the modified staple strands: (from logic gate prototype structure)

ACCCAAATCAAGTTTTTTTGG
TTAAGAATGGTTTAATTTCAACTTTAATCATTG
ATGCAGCGAGAGCGCTTGAGGGAA
CTGCAGCGAGAGCGCTTGAGGGAA
GAAAGCGAAAGGAGCGGGCGCAAACAATAACG
AGAATTTACGTGCAGATATAT
AAAATTTTTACTACGACAAAAGAGATGATG
AGGCAGCGAACCAGGCAACCACCA
AGGCTGCTTTCTGAATACATCGGGAGTAGGG
GATTGCCTGATTGCCAGTAAAC
ATTACCTTCAAAAATCGCGCAGAGAGATTTTC
ACTCCAACGTGCGTAAAAGCACTAGCAGTATAA
GTGGGAACGAAGGAGGCCCCGATTTCTACAGGG
GTTTTGCCGACGACGTTAAAACCAAAATAGCGG
TGAATTACCTTTAACCCTCG
CTACGTTATCAACTAATGCAGATA
GGTTTTGCTCGAAGGATTCACTCAGTTGAGAT
CCGTGGAGGATTAGGATTACGGG
CACCCCTATAGCCCGGAATAGGTTAGTATTA
TGCCCCCTGCCTATTTCGGA
AGACTGGAAGAGCGAACCCTATCAATGCAGATT
CATAACGCCAAAAGGAGTCAGCAGGAC
TTAGGAATACCACATATAAAACG
CGTAACAAAGGCTGCTCGAACAACATTATTAC
AGGAGTGTACTAGACTCCTCAAGAAGGGGTGG
GTGCCCTTGATTTCTGAAACATGAGTATCACCCTAG

Modified Staple Strands:
ACCCAAATCAAGTTTTTTTGGACGAAGTTGTTGTG
TCAGAGCATGGTTGCTTTGACGAAATCGGAAGTTGTTGTG
ATGCACCGAGAGCTTGGGACGAAGGTTGTTGTTG
CTGCGCCGTGAGAAAGGAAACCAAGTTGTTGTG
GAAAGCGAAAGGAGCGCGCAGCAAATAACGAAGTTGTTGTTG
AGAAATTGCCTGCAATTAACGAAGTTGTTGTTG
AAAATAAAAATTACCTGAGCAAAGAGATGACGAGTTGTTGTTG

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ACTCCAACGTGCCGTAAGGACACTACGTATAAACGAAGTTGTTGTTG
GGTTGGAAACAGGGAGCCAGGATTTCTACAGGGACGAAGTTGTTGTTG
AGCCGGGCAACGTGGCAACCACCAACGAAGTTGTTGTTG
AGTGATACTTTCTGAATACATCGGGAGTAGGGACGAAGTTGTTGTTG
GATTCCCTGATGGCCAGTAACAAACGAAGTTGTTGTTG
ATTACCTTCAAAATCGCGCAGAGAGATTTTCACGAAGTTGTTGTTG
GTTTTTGGCACGACGATAAACACAAATACGAACGAAGTTGTTGTTG
TGAATTAACCTTTAACCTCGACGAAGTTGTTGTTG
CTACGTTATCTAATCTGAGATACACGAAGTTGTTGTTG
GGTTTTTGCTCGAAAGATTTCTACAGTTGAGATACGAAGTTGTTGTTG
CCGTCGAGGGATAGGGATTAGCGGACGAAGTTGTTGTTG
CACCCCTATAGCGGCCGAATAGGTAGTATTACGAAGTTGTTGTTG
TGCCCCCTGCCTATTTTCGGAACGAAGTTGTTGTTG
AGACTGGAAGAGCAACACTATCAATGCGATTACGAAGTTGTTGTTG
CATACGCACAAAAGGAGTCAGACACGAAGTTGTTGTTG
TTAGGAATACCATATAAACGAACGAAGTTGTTGTTG
CGTAACAAAGCTGCTCAGAACAACATTATTACACGAAGTTGTTGTTG
AGGAGTTGTACCTAGCTCCTCAAGAGAAAAAGGTTGACGAAGTTGTTGTTG
GTGCCTTGTATTCTGAAACATGATACGTACCCGTAACGAAGTTGTTGTTG