DNA Origami Stabilized and Seeded with 4'-Aminomethyltrioxsalen for Improved DNA Nanowire Fabrication

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DNA Origami Stabilized and Seeded with 4’-Aminomethyltrioxsalen for
Improved DNA Nanowire Fabrication

Matthew Paul McDowell

A thesis submitted to the faculty of
Brigham Young University
in partial fulfillment of the requirements for the degree of
Master of Science

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ABSTRACT

DNA Origami Stabilized and Seeded with 4’-Aminomethyltrioxsalen for Improved DNA Nanowire Fabrication

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A fast emerging technology in the microelectronics field is bottom-up self-assembly of computer circuitry. A promising method to develop nanoelectronic devices through bottom-up self-assembly is the implementation of DNA-based technologies. Using DNA to create nanoelectronic devices is advantageous because of its already well understood base-pairing and annealing qualities. These base-pairing and annealing qualities can be used to design and construct DNA nanostructures called DNA origami. DNA origami are specially designed structures made from single stranded DNA. Short single stranded DNA oligonucleotides called staple strands attach to a large single stranded DNA called a DNA scaffold. DNA staple strands and DNA scaffold anneal to each other and fold into DNA origami. Constructing DNA origami is advantageous because structures can be made in a single folding step. In particular, bar-shaped DNA origami has proven to be a promising structure for nanoelectronics fabrication. Here, I present new research done to improve bar-shaped DNA origami design and fabrication for constructing bottom-up self-assembled templates for nanomaterial surface attachment. Furthermore, this work presents new methods for DNA origami agarose gel purification with the help of the DNA stabilizing molecule, 4’-aminomethyltrioxsalen (AMT). AMT is a photoreactive molecule that intercalates DNA and creates covalent crosslinks when irradiated by short wavelength ultraviolet light. Also, this work contains new research on a synthesized crosslinker and its role with AMT in nanoparticle surface seeding on DNA origami nanowire templates. Through its crosslinking properties, AMT serves as a DNA origami stabilizing molecule and also shows potential for seeding nanomaterials.

Keywords: DNA origami, stabilization, gel extraction, AMT
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# TABLE OF CONTENTS

| ABSTRACT                                      | ii |
| ACKNOWLEDGEMENTS                              | iii |
| LIST OF FIGURES                               | vi |
| **CHAPTER 1: INTRODUCTION**                  | 1  |
| 1.1 Computer technology                      | 1  |
| 1.2 DNA structure and replication            | 3  |
| 1.3 Introduction to DNA origami              | 5  |
| 1.4 DNA origami applications                 | 8  |
| 1.5 Nanomaterial attachment on DNA origami   | 12 |
| 1.6 Designing DNA origami                    | 14 |
| 1.7 DNA origami in computer technology       | 17 |
| 1.8 Thesis overview                          | 18 |
| 1.9 References                               | 18 |
| **CHAPTER 2: BAR SHAPED DNA ORIGAMI DESIGN AND CHARACTERIZATION** | 21 |
| 2.1 Introduction                             | 21 |
| 2.2 Experimental                             | 21 |
| 2.2.1 Materials                              | 21 |
| 2.2.2 Methods                                | 22 |
| 2.3 Results and discussion                   | 25 |
| 2.4 Conclusions                              | 28 |
| 2.5 References                               | 29 |
| **CHAPTER 3: STABILIZATION OF DNA ORIGAMI FOR AGAROSE GEL PURIFICATION AND EXTRACTION** | 30 |
| 3.1 Introduction                             | 30 |
| 3.2 Experimental                             | 31 |
| 3.2.1 Materials                              | 31 |
| 3.2.2 Methods                                | 32 |
| 3.3 Results and discussion                   | 34 |
| 3.3.1 AMT intercalation with DNA origami     | 34 |
| 3.3.2 AFM images of agarose gel extracted material | 37 |
LIST OF FIGURES

Figure 1.1. Photolithography process ................................................................. 2
Figure 1.2. DNA replication .............................................................................. 4
Figure 1.3. Raster filling .................................................................................. 5
Figure 1.4. DNA origami smiley faces ............................................................... 6
Figure 1.5. Atomic force microscope image of large bar origami ..................... 7
Figure 1.6. DNA origami map of China and DNA origami dolphins ............... 8
Figure 1.7. Design of a semi-circle nanogear .................................................. 9
Figure 1.8. Lipid coated DNA nanodevice ....................................................... 11
Figure 1.9. Site-specific seeding and subsequent attachment of gold nanoparticles ..... 13
Figure 1.10. Tapping mode AFM images of "T" DNA origami structures .......... 13
Figure 1.11. Structure of CTAB ....................................................................... 14
Figure 1.12. caDNAno interface and design pipeline ....................................... 16
Figure 2.1. AFM image of small bar origami .................................................... 26
Figure 2.2. AFM image of large bar origami ................................................... 27
Figure 2.3. AFM image of tight bar origami ..................................................... 27
Figure 2.4. caDNAno computer model of the torsional strain of the large bar and tight bar origami .................................................................................................................. 28
Figure 3.1. Psoralen and 4'-aminomethyltrioxsalen (AMT) .............................. 31
Figure 3.2. Circular dichroism measurements on DNA origami ..................... 35
Figure 3.3. AMT treated bar origami color change .......................................... 36
Figure 3.4. Agarose gel electrophoresis of large bar DNA origami and large bar DNA origami treated with AMT .................................................................................................................. 37
Figure 3.5. AFM image of bar-shaped DNA origami with no AMT treatment after agarose gel electrophoresis and agarose gel extraction ............................................................... 39

Figure 3.6. AFM image of bar-shaped DNA origami treated with AMT and UV light, and then run through agarose gel electrophoresis and agarose gel extraction ...................................... 41

Figure 4.1. Structure of the synthesized crosslinker for DNA nanomaterial seeding .......... 45

Figure 4.2. NMR data of the synthesized crosslinker ................................................................. 47

Figure 4.3. Seeding of tight bar DNA origami with crosslinker functionalized gold nanoparticles .......................................................................................................................... 48

Figure 4.4. AFM image of AMT-stabilized tight bar DNA origami with 5 nm gold nanoparticle surface seeding ........................................................................................................ 49

Figure 4.5. Seeding of AMT-stabilized tight bar DNA origami with the crosslinker functionalized gold nanoparticles ................................................................................................................. 50

Figure 4.6. Proposed AMT-crosslinker reaction ........................................................................ 51
CHAPTER 1: INTRODUCTION

1.1 Computer technology

In 1939, a German engineer named Konrad Zuse created the first digital computer\(^1\). These first digital computers used an electromechanical design in which mechanical relays were controlled by electric switches\(^1\). By 1941, early computers using vacuum tubes replaced electromechanical switches and implemented a new all-electric design\(^1\). Ever since the early 1940’s computer technology has rapidly grown. Computers that once filled large rooms and required hours to compute have been replaced by smaller and more practical computers. Today’s computers have evolved into convenient, portable, hand-held devices and laptops that offer more computing power than many spacecraft from the 1960’s to the 1980’s. The rate of computer advancement has perhaps only been surpassed by the affordability of such technology. As computer circuitry gets smaller, greater computing power becomes possible. Now, computer components are made from small features of less than 20 nanometers in width in order to create processing chips.

As a result of the computer industry’s quest to develop higher computing power at lower cost, new manufacturing methods of creating small computer circuitry are being researched. The current strategy for making circuits uses a method called photolithography\(^2\) (Figure 1.1). Photolithography works by using light to transfer a pre-determined shape from a photomask to a chemical photoresist on a particular substrate\(^2\). Then by using chemical or physical means, current carrying metals and semiconductors can be deposited in the pattern. Computer circuits are made by cycling through a multi-step process requiring layering and etching. In
Microfabrication of integrated computer circuits a substrate wafer may go through a photolithographic cycle 50 times or more\(^2\). Photolithography methods in microfabrication of integrated circuits are termed as “top-down” processes. Although top-down processes of microchip fabrication are in use, they have some disadvantages. Equipment is expensive, clean rooms are required and manufacturing chips on a nanoscale is difficult.

**Figure 1.1 Photolithography process.** The photolithography process starts with a silicon wafer with a surface layer of photoresist (A). Next, an opaque glass with transparent features is placed over the silicon wafer with photoresist and is exposed to ultraviolet light (B and C). The photoresist exposed to the ultraviolet light through the photomask is developed through a washing process (D). Material is then deposited on the surface and the remaining photoresist is removed to leave the desired pattern on the silicon surface (E and F).

One of the new approaches being explored in microchip fabrication is the use of a bottom-up self-assembly method. By making very small particles or molecules slightly bigger it is possible to create nano-sized current carrying wires or other features that are smaller than
those made with current top-down methods. One of the most promising bottom-up self-assembly methods uses deoxyribonucleic acid (DNA).

1.2 DNA structure and replication

Deoxyribonucleic acid is nature’s method of information storage. By only using four nucleic acids, blueprints for thousands of proteins with specific functions and purposes can be stored within every cell. Each DNA nucleotide is made up of three components: a phosphate group, a five carbon cyclic sugar and a nitrogenous base. DNA encodes the blueprints for protein production by using a system in which three consecutive nucleotides code for one amino acid. A special protein called ribonucleic acid (RNA) polymerase reads and transcribes DNA code into RNA. The newly transcribed RNA is then read by the ribosome that translates the coded information stored in DNA into functional protein molecules (Figure 1.2). DNA stores its coded information in a double stranded helical structure which relies on the thermodynamically and entropically favorable hydrogen bonding between nucleic acid base pairs. Four bases—cytosine, guanine, adenine and thymine—are responsible for coding protein information and are key to DNA’s helical structure. Adenine pairs with thymine and guanine pairs with cytosine. By taking advantage of DNA’s ability to hydrogen bond and form helical structures, scientists can create non-natural DNA structures.

In 1982, an influential paper was published by Ned Seeman in which he highlighted the possibility of constructing non-natural DNA structures, and explored the idea of a non-mobile Holliday junction and lattice formation using Watson and Crick DNA base-pairing. Seeman’s 1982 paper became a platform upon which DNA structural motifs were developed. Seeman’s
DNA structures were precursors to the development of folded DNA structures called DNA origami. Furthermore, the ability to create new DNA structures has the potential for applications in computer science and medicine, as well as other possibilities.

**Figure 1.2 DNA Replication.** DNA helicase unwinds double stranded DNA at the replication fork. Topoisomerase breaks double stranded DNA upstream of helicase in order to relieve coiling strain caused by helicase activity. Single stranded binding proteins bind to single stranded DNA to prevent the double helix from reforming. DNA primase serves as a starting point RNA (or DNA) replication via RNA primer. DNA polymerase builds new DNA as it helps nucleic acids bind to the parent strand of DNA in a 5’-3’ direction. DNA ligase joins together un-ligated sections of the Okazaki fragments of the lagging strand. (Image reprinted with permission from Wikipedia).
1.3 Introduction to DNA origami

In 2006, a ground-breaking report on DNA folding was published by Paul Rothemund\(^7\). Rothemund’s paper\(^7\) described methods of DNA folding and established a simpler, more generalized process of folding. By making use of a long segment of single-stranded DNA, Rothemund showed that specific shapes could be made using a raster method. In the raster method, smaller strands of DNA can be designed to base pair with locations within the scaffold strand of DNA (Figure 1.3). These locations may be close together in proximity or far away. By mixing specially designed staple strands with the scaffold DNA, it became possible to easily make folded DNA structures. This newly developed method surpassed earlier techniques of DNA folding introduced by Nadrian Seeman, which required precise stoichiometric concentrations, complex steps, and ligation to produce DNA folding. Rothemund showed how to create rectangles, triangles, two-dimensional shapes and even smiley faces using the DNA raster...
method⁷,⁸ (Figure 1.4). From his results and this new method, the term DNA origami was coined.

![DNA origami smiley faces](image)

**Figure 1.4. DNA origami smiley faces.** Reprinted with permission from Rothemund, et al.⁷⁹.

Constructing DNA origami requires the use of a scaffold DNA and DNA staple strands in excess amounts. DNA scaffold and staple strands are mixed in a tube along with buffer and are placed in a temperature cycling machine. A program is selected in which the DNA is heated up to eliminate base pairs and then is slowly cooled for proper DNA hybridization and folding. Temperature is increased and decreased throughout the process, which makes the method of DNA origami formation very similar to the molecular biology method called polymerase chain reaction (PCR), that is used to amplify specific sections of DNA.
Raster filled DNA scaffolds have proven to be a very effective and reliable method to create DNA origami nanostructures. By re-designing staple strand placement, it is possible to use the same scaffold of DNA to produce many different DNA origami structures. This technique makes the raster filled DNA origami cheaper and easier to construct than earlier DNA folding methods. Raster filled DNA origami has the advantage of being easy to assemble in high yields and mixed together in one tube. This method of self-assembly in a single tube simplifies the process of DNA origami production. Additionally, DNA origami can be made in a variety of sizes. Because of these advantages, DNA origami has promise as a bottom-up solution in computer chip fabrication. Dr. Woolley’s lab has made a bar-shaped DNA origami that is about 17 nanometers in width by 410 nanometers in length. The image in Figure 1.5 shows bar-shaped DNA origami. Some origami appear longer than 410 nm because of a higher concentration of DNA origami where they are overlapping. This bar shape is useful in designing nanowires because it has a high-aspect ratio and narrow width.

**Figure 1.5. Atomic force microscope image of large bar origami.** The length of the origami is approximately 410 nm. Large bar origami is made from m13mp18 scaffold by a DNA raster filling method. Image scan size: 10 x 10 µm. Height scale is 10 nm.
1.4 DNA origami applications

The versatility of DNA origami has been shown over the last few years from many different creations. Not only has DNA origami been made into smiley faces, but other designs have been made such as a dolphin\textsuperscript{10} and a map of China\textsuperscript{11} (Figure 1.6). As artistic and incredible as designing nano-sized dolphins, smiley faces, and geographical maps are, DNA origami also has practical utility for technology and science.

![Figure 1.6. DNA origami map of China and DNA origami dolphins.](image)

Reprinted with permission from reference 10 and 11. Copyright 2008 American Chemical Society.

One of the practical uses of DNA origami is structural design and application. As shown previously, DNA origami can be folded into many different shapes. In a paper published by Barish, et al.\textsuperscript{12} a DNA origami tile was developed to help study and understand the nucleation of algorithmic crystals. In order for crystals to grow, or nucleate, a seed is needed as a starting point. As nucleation progresses, an infinite variety of individual structures can potentially be created. By implementing a designed DNA origami tile, Barish, et al.\textsuperscript{12} were able to more effectively study the methods of crystal nucleation in mineral, chemical and biological structures. Their work demonstrated how DNA origami can be used to create a high-yield, low-error-rate
algorithmic crystal and proved that it can be accomplished with less difficulty than other nucleation methods. This research outlines DNA origami as a promising nanostructure that provides a controllable and programmable bottom-up fabrication method.

DNA has also been the subject of mechanical design by making moving parts out of DNA origami. In a report published in 2009, Omabegho, et al. demonstrated the ability to design a bi-pedal DNA nanorobot that coordinates its leg movements and walks in a unidirectional fashion along a DNA track. Nanosized gears have even been constructed, which with future designs can potentially create nano-gear mechanisms for a variety of purposes (Figure 1.7).

![Design of a semi-circle nanogear](image)

**Figure 1.7. Design of a semi-circle nanogear.** Reprinted with permission.

DNA origami has also been explored as a nanorobot which can deliver gold nanoparticles or antibodies. By using a logic-gated nanorobot, Douglas, et al. were able to deliver various payloads to cells and attached biologically active payloads for cell-targeting purposes. At Harvard University’s Wyss Institute DNA origami has been created as a drug delivery vessel. By creating an open DNA tube with a hinge, self-assembling and self-destructing drug delivery
carriers have been devised. Once the drug is inside of the folding tube DNA origami, the origami can be folded shut using a DNA aptamer. Aptamers can be programmed in such a way as to recognize diseased tissue and subsequently break apart, swing open and deliver the drug. Scientists have essentially made an autonomous lock-and-key targeting system. The researchers reported some initial success in targeting leukemia and lymphoma cells through DNA origami design.\textsuperscript{15}

Researchers from the National Center for Nanoscience and Technology in Beijing and from Arizona State University have developed another drug delivery DNA origami for doxorubicin.\textsuperscript{16} Doxorubicin, a chemotherapy drug, was loaded into a tubular DNA origami; these studies showed that doxorubicin delivery to cancer cells was much more effective than free doxorubicin.\textsuperscript{16} An important feature of the DNA origami doxorubicin drug delivery is that it was effective at delivering the drug to and killing doxorubicin-resistant cells.\textsuperscript{16}

In another study from the iNANO center and CDNA Center at Aarhus University, scientists constructed a small multi-switchable 3D DNA origami box.\textsuperscript{17} The box was designed by using a unique set of DNA and RNA keys so the DNA origami box could be opened or closed. The researchers concluded that the DNA origami device could be used for many applications: controlling the function of single molecules, drug delivery and molecular computing.\textsuperscript{17}

A surprising report came out in 2014 in which researchers injected cockroaches with nanorobots made from DNA origami.\textsuperscript{18} These researchers created nano-sized DNA origami capable of performing the same kind of logic operations in a living creature as in a silicon-based computer. The DNA computers within cockroaches flow throughout the body and function by folding and unfolding DNA strands. When the strands uncurl, the origami robots deliver drugs carried within them. The team of bioengineers proved their concept of DNA nanobots by
encasing molecules with fluorescent markers inside curled DNA and also concluded that computing power inside a cockroach could be up-scaled to an 8-bit computer equivalent.\textsuperscript{18}

Furthermore, researchers at Harvard’s Wyss Institute constructed nanoparticles that bypassed the immune system in animals by mimicking viral infection.\textsuperscript{19} DNA origami was formed into an octahedron shape which copied the carrying capsule of virus particles. These DNA nanoparticles were injected into mice and were able to stay circulating within them after injection. The DNA origami coats also limited the immune system response as compared to uncoated particles. The application of the DNA origami octahedron lipid coat has potential use as a drug delivery method in living organisms\textsuperscript{19} (Figure 1.8).

![Lipid coated DNA nanodevice](image)

**Figure 1.8.** *Lipid coated DNA nanodevice.* A virus envelope coated with a lipid (left). A DNA nanodevice coated with lipid (right). The DNA nanodevice and viral envelope closely mirror one another. Reprinted with permission through the ACS Author’s/Editor’s choice usage agreement.\textsuperscript{19}
1.5 Nanomaterial attachment on DNA origami

DNA origami also has the ability to serve as a template for attachment of nanomaterials. Currently, nanomaterials can be attached to the surface of DNA origami through a variety of methods. One of those methods has been successfully developed in Dr. Woolley’s lab. This method uses DNA attachment strands which contain thiol groups at the end. These DNA attachment strands can be covalently bound to nanoparticles such as gold and then through DNA’s base pairing properties will anneal to complementary strands located on the DNA origami structure (Figure 1.9). Using this seeding method, nanoparticles can be attached with site-specific precision to DNA origami locations. Furthermore, metallization processes can then grow nanoparticles into each other in order to create a continuous metal wire capable of conducting electricity. Figure 1.10 shows AFM images of a “T-shaped” DNA origami after successful site-specific seeding of gold nanoparticles using the method described above. Gold nanoparticle seeding on the DNA origami was successfully carried out for either one-half of the “T” shape or the entire top length of the crossing “T” shape.
Figure 1.9. Site-specific seeding and subsequent attachment of gold nanoparticles. (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) Gold nanoparticles (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. Reprinted with permission.21

Figure 1.10. Tapping mode AFM images of “T” DNA origami structures. (A) Unseeded “T” DNA origami deposited on a SiO₂ surface. (B) “T” DNA origami seeded with Au NPs after surface deposition on SiO₂. The red arrow points to the unseeded portion on the DNA origami. The height scale in both images is 6 nm and the scale bars are 200 nm. Reprinted with permission.21
Other methods of surface attachment on DNA origami include the use of streptavidin/biotin conjugates, antibody/digoxigenin, benzylguanine/Snap-tag, and chlorohexane/Halo-tags. Another useful chemical for attaching nanoparticles such as gold is cetyltrimethylammonium bromide (CTAB; Figure 1.11). CTAB is a surfactant which uses micellar structures to encase nanoparticles and then electrostatically interacts with DNA origami.

Figure 1.11. Structure of CTAB.

1.6 Designing DNA origami

Since Nadrian Seeman conceived the theoretical idea of DNA as a nanoscale building material in 1982, nanosized DNA structures have been designed using error-prone calculation methods. Then in 2006 Paul Rothemund introduced DNA origami that was created by a DNA raster-filling method. Rothemund’s DNA origami raster building method then opened the way for computer-aided design of DNA structures. In 2009, Douglas, et al. published a paper that described the development and use of a computer-aided DNA origami design program called caDNAo.

Using caDNAo to design DNA origami requires four steps. In the first step, a desired shape is estimated by outlining a scaffold path. A scaffold path uses a long strand of DNA such as m13mp18 and lays it down in two dimensional patterns. These patterns can be placed in such
a way as to give a rough outline of the desired shape. Next, DNA staple strands are laid out so as to be complementary to the scaffold’s DNA sequence, including crossover strands which give the desired shape its 2D or 3D structural integrity. After the staple strands are placed around the scaffold strand, the staple strands are broken up into shorter lengths of about 18 to 49 base pairs. Lastly, the DNA staple strand sequences are deduced from the scaffold sequence. The staple strand sequences can then be exported in a file and uploaded to purchase synthesized DNA oligonucleotides (Figure 1.12).
Figure 1.12. caDNAno interface and design pipeline. (a) Screenshot of caDNAno interface. Left, slice panel displays a cross-sectional view of the honeycomb lattice where helices can be added to the design. Middle, path panel provides an interface to edit an unrolled 2D schematic of the scaffold and staple paths. Right, render panel provides a real-time 3D model of the design. (b) Exported SVG schematic of example design from, with scaffold (blue) and staple (multi-color) sequences. (c) Path panel snapshot during first step of the design process. Short stretches of scaffold are inserted into the path panel as helices are added via the slice panel. (d) The path panel editing tools are used to stitch together a continuous scaffold path. (e) The auto-staple button is used to generate a default set of continuous staple paths, including crossovers. The breakpoint tool is subsequently used to split the staple paths into lengths between 18 and 49 bases. Finally, the scaffold sequence is applied to generate the list of staple sequences. (f) Exported X3D model from the render panel. Figure and figure caption reprinted with permission.24
1.7 DNA origami in computer technology

Computers are constantly evolving into more powerful machines, and are becoming available in smaller sizes. In order to construct small computer circuits, top-down techniques are currently implemented; however, top-down strategies of microchip manufacturing are reaching physical size limits. In order to overcome size limitations alternative methods for computer circuit construction are being researched. Specifically, in the Woolley lab bottom-up construction methods for computer circuitry are being investigated using DNA, which was chosen because of its ability to form many shapes in a very small size. Currently, I have been working on a bar-shaped DNA origami because of its high-aspect ratio which makes it an ideal shape for nano-sized wires. By taking advantage of DNA’s self-assembly properties, the Woolley lab has shown that wires can be created by using DNA origami as a template.\textsuperscript{21}

Construction of DNA origami templates for nanowire fabrication was accomplished by first designing the desired shape (in our case, a bar-shaped DNA origami) with the aid of the caDNAno design program. Once the bar shaped DNA origami was designed and the DNA stands were obtained, I was able to successfully construct bar origami (Figure 1.5). Wires were constructed on DNA origami using designed DNA strands which attach to nanomaterials such as gold particles. Work done in the Woolley lab has already shown how gold can be attached to the surface of DNA origami in order to create nano-sized wires.\textsuperscript{21}

Despite the success in developing DNA origami templates for nano circuit construction, further refinement of the bottom-up fabrication method is required. One need is to stabilize and purify DNA origami structures in order to improve fabrication yield. I have been working on methods to stabilize DNA origami in order to address the challenge of improving DNA origami yield and purification.
1.8 Thesis overview

This thesis discusses improvements to the method of DNA origami as follows: In chapter 1, I have given a brief introduction to DNA origami and its uses. In chapter 2, I describe the design of DNA origami in detail and how to make several different DNA origami structures used in this work. Chapter 3 describes experiments and results for DNA origami stabilization with a psoralen molecule, 4′-aminomethyltrioxsalen (AMT), which intercalates inside DNA. Atomic force microscopy images, agarose gel electrophoresis and circular dichroism spectroscopy of DNA origami treated with psoralen compounds are given. Chapter 3 will also describe DNA origami agarose gel purification and gel extraction methods. Chapter 4 explains seeding methods for nanowire construction on DNA origami. This chapter also includes my work on a novel method for attachment of nanomaterials to DNA origami through AMT crosslinked DNA and a synthesized crosslinker. Chapter 5 summarizes the research done and discusses conclusions. Also, future avenues for nano-circuit fabrication and design are described.

1.9 References


5. Image obtained from:


CHAPTER 2: BAR SHAPED DNA ORIGAMI DESIGN AND CHARACTERIZATION

2.1 Introduction

Using DNA to fabricate bottom-up self-assembled nanowire templates has proven to be a desirable method because of DNA’s well understood base pairing characteristics. By implementing the published raster filling technique of DNA origami.\(^1\) DNA can be designed and formed into nearly limitless possibilities of nanosized shapes. In the Woolley lab, work has already been accomplished to design and construct bar shaped origami for the specific purpose of attaching nanomaterials for computer circuitry. Both large and small bar shaped origami have been successfully designed and made. However, I sought to further improve the design of bar shaped origami by applying base pairing rules for helix twisting stability. By re-designing the bar shaped origami so that more base-pairs are present per helical turn, I was able to introduce helical strain and create a more narrow and flat bar shaped DNA origami.

In this chapter I describe the methods for making large and small bar origami. Additionally, I describe how I re-designed the large bar origami to adjust helical strain and create what I call the “Tight Bar Origami.” I characterized these DNA origami structures by using AFM imaging and caDNAno computer modeling.

2.2 Experimental

2.2.1 Materials

M13mp18 DNA, and streptavidin-coated beads were purchased from New England Biolabs (Ipswich, MA). Staple strands were purchased from Operon (Huntsville, AL) and were solvated in distilled water to a concentration of 100 \(\mu\)M. Primers for the polymerase chain
reaction (PCR) were purchased from either Operon or Sigma-Aldrich (St. Louis, MO), while PCR purification kits were purchased from Qiagen (Huntsville, AL). Taq polymerase and PCR buffers were purchased from Invitrogen (Waltham, MA). Distilled water was used from a Barnstead EasyPure (Waltham, MA) water purification system. 10x TAE-Mg$^{2+}$ (pH 8.3) buffer was made using Tris base and acetic acid purchased from Fisher Scientific (Waltham, MA), and ethylenediaminetetraacetic acid (EDTA) and magnesium acetate purchased from Sigma-Aldrich.

2.2.2 Methods

Small Bar Origami

PCR-based scaffold amplification for the small bar origami. The PCR mix was made by mixing 83.7 µL distilled water, 10 µL of 10x Taq Buffer (supplied from the Invitrogen PCR kit), 4 µL of 250 µg/mL single stranded m13mp18 DNA from New England Biolabs (catalog number N4040S), 0.8 µL of 500 nM dNTP’s, 0.5 µL of 1 µM 5’ primer, 0.5 µL of 1 µM 3’ primer, and 0.5 µL Taq Polymerase (supplied from Invitrogen). The PCR mix was run through the thermocycler as follows: 95 °C for 2.5 min, 30 cycles of 95 °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.

Small bar scaffold purification. This procedure is used to separate PCR reagents from the scaffold DNA. PCR amplified scaffold of the small bar origami and 500 µL PB buffer (used as a binding buffer for DNA cleanup procedures) was aspirated into purification spin columns (from the Qiagen PCR kit). The spin columns were then centrifuged for 1 minute at 13,000 RPM. 250 µL of PE buffer (a wash buffer for DNA cleanup from the Qiagen PCR kit) was then added and centrifuged for 1 minute at 13,000 RPM. The tubes were spun twice to remove excess
waste. The filter containing the DNA was then placed into a new clean tube, and 55 µL of EB buffer (used for eluting nucleic acids from the Qiagen PCR kit) was added. The filter containing DNA and EB buffer was allowed to sit for 5 minutes and was then centrifuged for 1 minute to collect flow-through containing PCR amplified DNA scaffold.

**Small bar magnetic bead separation of double stranded scaffold.** This step separates double stranded PCR amplified scaffold into useable single strand scaffold.

**Bead preparation.** 200 µL of magnetic bead solution were aspirated into a 1.5 mL tube. The tube containing the magnetic beads was placed on a magnetic tray holder so that beads migrated to the side of the tube. The clear liquid was aspirated out and discarded as waste. The beads in the 200 µL of bead buffer were re-suspend and placed back on the magnetic tray holder. The process was repeated three times and then the beads were re-suspended in 200 µL of bead buffer one last time.

20 µL of 250 µg/mL m13mp18 PCR amplified scaffold was aspirated into the 200 µL bead solution. The tube was then placed on shaker tray for 30 minutes. The magnetic beads were rinsed with bead buffer three times using a magnetic bead tray with 200 µL bead buffer. For the last rinse 150 µL of 0.2 M NaOH was used and the tube was then placed on a shaker for 6 minutes. The tube was then placed back on the magnetic tray so that double stranded scaffold separated from the single stranded scaffold. The liquid solution was then pipetted out from the single-stranded PCR scaffold and the solution was neutralized by adding 100 µL of 5 M ammonium acetate, resulting in a 250 µL volume.

**Purifying single-stranded scaffold DNA.** To the 250 µL volume 1250 PB buffer was added as provided in the Qiagen PCR purification kit. The 1.5 mL volume was separated in half
and each half was aspirated into a 30K spin column filter. The spin column filters were placed in
collection tubes and were centrifuged for 1 minute at 13,000 RPM. The flow-through was
discarded. 750 µL of PE buffer was then added to the filter as provided in the Qiagen PCR
purification kit and was centrifuged twice for 1 minute at 13,000 RPM. The flow-through was
again discarded. The filter containing concentrated, purified PCR amplified scaffold was placed
into a new 1.5 mL micro-centrifuge tube. 55 µL of EB buffer as provided by the Qiagen kit was
then added to the filter. After five minutes, the spin filter was placed inside a new 1.5 mL micro-
centrifuge tube and was centrifuged for 1 minute at 13,000 RPM. The 55 µL flow though volume
contained concentrated, purified, single-stranded small bar scaffold.

Small bar construction using thermocycler. Small bar origami was made by adding
81.3 µL of distilled water into a 200 µL PCR tube. 10 µL of 10x TAE was then added (10x TAE
buffer was made by mixing 2.42 g Tris base, 572 µL of glacial acetic acid, 0.186 g of EDTA,
and 1.34 g of magnesium acetate). After adding 10x TAE, 8 µL of PCR amplified scaffold and
0.5 µL of 100 µM small bar staple strand master mix was then aspirated into the tube. Once
mixed, the tube containing small bar origami components was placed into a thermocycler to
construct DNA origami.

Filtering small bar origami. After the small bar origami was made, it was aspirated into
a 30K micro-centrifuge filter. The filter containing the small bar origami was then centrifuged at
13,000 RPM for ten minutes. The filter was washed three times with 500 µL of 1x TAE buffer.
The filter was then inverted into a new collection tube for three minutes at 3500 RPM in order to
collect the DNA origami free from excess DNA staples. See Appendix section A 1.2 for strand
sequences.
**Large bar origami and tight bar origami recipe and construction.** The large bar or tight bar origami was made by mixing 79.4 µL of distilled water and 10 µL 10x TAE in a PCR tube. Then 3.6 µL of 250 µg/mL of single-stranded m13mp18 DNA (not PCR amplified) and 5 µL of large bar staple or tight bar strand master mix were dispensed into the tube. The tube was then placed in a thermocycler for large or tight bar origami construction as follows: Initial denature for 3 minutes at 95.0°C, and then cycling through 9 stages, each stage cycling 99 times. Stage 1: 95.0°C - 6 seconds, 85.1°C - 6 seconds; stage 2: 85.0°C - 6 seconds, 75.1°C - 6 seconds; stage 3: 75.0°C - 6 seconds, 65.1°C - 6 seconds; stage 4: 65.0°C - 6 seconds, 55.1°C - 6 seconds; stage 5: 55.0°C - 6 seconds, 45.1°C - 6 seconds; stage 6: 45.0°C - 6 seconds, 35.1°C - 6 seconds; stage 7: 35.0°C - 6 seconds, 25.1°C - 6 seconds; stage 8: 25.0°C - 6 seconds, 15.1°C - 6 seconds; stage 9: 15.0°C - 6 seconds, 5.1°C - 6 seconds. Then final extension and hold at 4.0°C. See Appendix sections A 1.3 and A 1.4 for strand sequences.

### 2.3 Results and discussion

Small bar origami was constructed as described in section 2.2.2. Atomic force microscopy (AFM) images were obtained to show proper formation of the small bar origami structure as seen in Figure 2.1. The AFM image in Figure 2.1 shows successfully made small bar origami which is 64 nm in length by 11 nm in width, thus showing that the process described in section 2.2.2 for making small bar origami to be successful.
Figure 2.1. AFM image of small bar origami. Height scale is 10 nm. Image dimensions: 4 µm X 2 µm.

Large bar origami was also made as described in section 2.2.2. Figure 2.2 shows an AFM image of large bar origami. This image shows a more dilute sample as compared to the small bar origami AFM image in Figure 2.1. The size of large bar origami is 410 nm length by 17 nm width. The image shows successfully made large bar origami.

Figure 2.3 shows the tight bar origami has a similar AFM appearance to the large bar origami in Figure 2.2. The large bar origami has 10.5 base-pairs per helical turn while the tight bar origami was designed to have 13.5 base-pairs per helical turn. The increase in base-pairs per helical turn therefore increases torsional strain, and increases helical bowing. The large bar and tight bar DNA origami are constructed by raster-filled multi-helical DNA. The increased bowing of individual DNA helices causes the overall DNA origami structure to twist more while each individual DNA helix is actually underwound. Figure 2.4 shows the caDNAno models of the large bar and tight bar DNA origami. The left-handed supertwisting of the tight bar DNA origami accommodates and compensates for underwound DNA helices. Underwound DNA
helices and left-handed supertwisted DNA origami structures can therefore more readily accommodate intercalating agents. Increased stabilization is then achieved by adding intercalating molecules and by increasing cation concentrations. The tight bar DNA origami was thus designed to conduct experiments on stabilization and seeding optimization as described in Chapter 4.

Figure 2.2. AFM image of large bar origami. Height scale is 10 nm. Image dimensions: 5 µm X 10 µm.

Figure 2.3. AFM image of tight bar origami. Height scale is 10 nm. Image dimensions: 5 µm X 10 µm.
Figure 2.4. caDNAno computer model of the torsional strain of the large bar and tight bar origami. (A-C) Large bar origami. (D-F) Tight bar origami. Panels A-F are root-mean-square thermal fluctuation maps of DNA origami flexibility. Areas colored in blue have lower flexibility, while red areas have higher flexibility. (A-C) Different views of the large bar origami. (D-F) Different views of the tight bar origami.

2.4 Conclusions

It is possible to create DNA origami useful for nanoelectronic components of various sizes and shapes. By increasing the number of base-pairs per helical turn in DNA origami, I have demonstrated that it is possible to create DNA origami which will more easily accommodate intercalating molecules. Increased affinity for intercalating agents into DNA origami is accomplished because of the bowing characteristic of the 13.5 base-pairs per helical turn DNA as compared to 10.5 base-pairs per helical turn DNA. The increased bowing is
diagramed in **Figure 2.4** by the red colored areas of the origami model. The increased bowing allows for more space for more intercalating molecules to sit next to DNA base-pairs, but also causes the overall DNA origami structure to twist more. This design reduces steric hindrance when attaching nanomaterials, increases DNA folding stability, and improves seeding yields for nanomaterial attachment. Additionally, increased bowing inside of DNA origami helices allows for further experimentation on DNA origami when using intercalating molecules such as 4’-aminomethyltriosalen (AMT). Intercalators cause the double helix to unwind, so by twisting the double helix in DNA origami into a bowed conformation, the unwinding effect of intercalating agents will be decreased and the origami structure can remain intact. Therefore, by making minor adjustments to DNA origami structure, it is possible to improve DNA origami stability, and nanomaterial seeding for improved nanoelectronics fabrication.

### 2.5 References


CHAPTER 3: STABILIZATION OF DNA ORIGAMI FOR AGAROSE GEL PURIFICATION AND EXTRACTION

3.1 Introduction

An important advancement in DNA origami nanowire development would be the improvement of fabrication yield and purification of DNA origami templates. To become a profitable technology in the computer industry origami yields need to be high (>99%) and purification processes need to be developed. The challenge associated with DNA origami purification is to develop a separation and extraction process that does not compromise the DNA origami structure. Up to this point of DNA origami development, little has been done to explore DNA origami purification, with the exception of a recent report which examined and optimized five purification techniques: ultrafiltration, gel filtration, gel filtration with spin column, glycerol density gradient ultracentrifugation, polyethylene glycol precipitation, and agarose gel extraction.1 Typically, DNA origami has undergone agarose gel electrophoresis in order to separate and distinguish correctly assembled DNA origami from DNA artifacts which remain after the construction process.

In this chapter I describe a new processing method for DNA origami purification. This new method utilizes treatment with a water-soluble psoralen compound which intercalates in the DNA origami and helps stabilize the structure through crosslinking. As reported by Hearst,2 4'-aminomethoxytrioxsalen (AMT) (see Figure 3.1) has high water solubility (>10⁴ μg/mL as compared to 35 μg/mL for psoralen) and high affinity to intercalate inside of double stranded DNA \( K_D = 6.6 \times 10^{-5} \) as compared to psoralen \( K_D = 4.0 \times 10^{-3} \), where \( K_D = \frac{[P][S]}{[PS]} \), \( K_D \) is the dissociation constant, \([P]\) is the concentration of the psoralen compound, \([S]\) is the
concentration of the intercalation sites, and \([PS]\) is the concentration of intercalated psoralen into DNA. Furthermore, in a saturated solution AMT has a \([PS]/[S]\) of 5000 where psoralen has a \([PS]/[S]\) of 0.05, thus making AMT thermodynamically favorable for UV light photo activation of interstrand covalent crosslinks.

![Psoralen and 4'-aminomethyltrioxsalen (AMT)](image)

**Figure 3.1. Psoralen and 4'-aminomethyltrioxsalen (AMT).** The structure of psoralen (left). The structure of AMT (right). AMT has a primary amine which increases its water solubility.

### 3.2 Experimental

#### 3.2.1 Materials

200 µL PCR tubes were purchased from VWR (Radnor, PA). Distilled water was used from a Barnstead EasyPure (Waltham, MA) water purification system. 10x TAE-Mg\(^{2+}\) (pH 8.3) buffer was made using Tris base and acetic acid purchased from Fisher Scientific (Waltham, MA), and ethylenediaminetetraacetic acid (EDTA) and magnesium acetate purchased from Sigma-Aldrich (St. Louis, MO). 4'-aminomethyltrioxsalen HCl was purchased from Sigma-Aldrich and was solvated in water (1 mg/mL). M13mp18 DNA was purchased from New England Biolabs (Ipswich, MA). Tight bar DNA origami staple strands were purchased from Operon (Huntsville, AL) and were solvated in distilled water to a concentration of 100 µM. Low gelling temperature agarose powder (A9414) was purchased from Sigma Aldrich. QG buffer
from Qiagen’s (Germantown, MD) Qiaquick Gel Extraction Kit (Cat. No. 28704) was used for solubilizing the agarose gel, and Amicon Ultra-0.5 Centrifugal Filter Devices for volumes up to 500 µL were purchased from EMD Millipore (Billerica, MA). A Sun Ray UV Floodlight from Uvitron International Inc. (West Springfield, MA) was used to induce crosslinking of AMT. Razor blades purchased from VWR International (West Chester, PA) were used to cut the agarose gel. An agarose gel electrophoresis tray with power supply was purchased from Bio Rad (Hercules, CA). Ethidium bromide (10 mg/mL in water) was purchased from Sigma Aldrich. A centrifuge used to spin the filtration devices was used from Eppendorf (Hauppauge, NY). A circular dichroism spectrometer from AVIV Biomedical, Inc. (Lakewood, NJ) was used to gather AMT intercalation data.

3.2.2 Methods

DNA origami stabilization. In order to stabilize DNA origami, the water soluble psoralen derivative AMT was used to intercalate inside of double stranded DNA. Stabilized large tight bar DNA origami was made as described in section 2.2.2 with 5 µL of 1 mg/mL AMT added. After formation of the DNA origami, the sample was subjected to 20 minutes of ultraviolet light irradiation in order to induce crosslinking within the annealed complementary strands of DNA. UV light induced crosslinking of AMT thus stabilizes the DNA origami structure and enables DNA origami gel extraction.

Agarose gel preparation and electrophoresis. A low gelling temperature agarose gel was prepared by mixing 7.5 g of agarose powder with 75 mL of 1x TAE buffer. The solution was mixed and heated in a microwave on high for 1.5 minutes. After sufficient cooling (and before gelling of the solution), 7.5 µL of ethidium bromide (10 mg/mL in water) was added. The solution was then poured in a gel casting tray with well comb and was allowed to solidify. Next,
the gel was placed in the gel electrophoresis box with 1x TAE buffer and the outer wells were loaded with 6 µL of 1 kilobase ladder and loading dye as provided by Fisher Scientific. The tight bar sample was then divided into aliquots and inserted into approximately 2-3 wells. Once the gel was ready for electrophoresis, the power supply was set for constant voltage (150 V), and 0.300 milliamps for 30 minutes. The gel was run on ice in order to prevent gel melting during electrophoresis.

**DNA origami gel extraction.** After the gel electrophoresis was complete the gel was removed and visualized under ultraviolet light. Using a razor blade the DNA bands in the agarose gel correlating to well-made bar shaped DNA origami were cut out and placed in a 1.5 mL microcentrifuge tube. The agarose gel was then solubilized by mixing approximately 500 µL of QG buffer from Qiagen’s gel extraction kit. The low melt agarose gel was solubilized by gentle pipet mixing and subsequent 40°C water bath incubation for 45 minutes. After the gel was well solubilized, the extract was transferred into Amicon filtration devices placed in their respective collection tube. The solubilized gel was allowed to filter through the filtration device by centrifugation for 10 minutes at 14,000 RPM. After the first centrifugation, flow-through collected in the device was discarded, and 1x TAE buffer was added to the filter. The filtration device was then centrifuged for a second time for 10 minutes at 14,000 RPM. The filters were only used in two centrifugations because the filtration matrix can give way if spun more than two times, causing the DNA origami extract to be lost in the flow-through. After the second round of centrifugation, the filters were inverted and placed in a new flow-through collection tube. The inverted filters containing DNA origami were centrifuged for 3 minutes at 3,000 RPM in order to collect the DNA origami. After the solution containing origami was collected, the sample was again subjected to another round of 500 µL of QG buffer mixed and centrifuged for 10 minutes.
at 14,000 RPM in a new Amicon filtration device. Once the centrifugation finished, the flow-through was discarded and 1x TAE buffer was again added to the filtration device. The filtration device containing DNA origami was again centrifuged for 10 minutes at 14,000 RPM, after which the filter containing DNA origami was inverted, placed in a new filtration device collection tube, and centrifuged for 3 minutes at 3,500 RPM for DNA origami collection. This process can be repeated additional times as needed to clear out remaining agarose gel particles. The sample was then deposited on a mica surface, washed with a 4 mM magnesium acetate solution and then rinsed with distilled water. The sample was then dried with compressed air and examined using AFM.

**Circular dichroism.** Intercalation experiments were carried out by using a circular dichroism (CD) spectrometer. Prepared samples of bar origami and bar origami treated with 5 µL of 1 mg/mL AMT were used. Both samples were diluted with water to a 1 mL volume and were injected into the CD glass cuvettes. The samples were measured by inserting the cuvettes into the CD spectrometer at 4 ºC. CD measurements were taken at 1 nm increments starting from 345 nm and ending at 225 nm with an averaging time of 10 seconds.

### 3.3 Results and discussion

#### 3.3.1 AMT intercalation with DNA origami

As psoralen molecules intercalate into DNA, ultraviolet light absorbance readings decrease. These readings can be detected using a circular dichroism spectrometer; thus, to confirm that AMT was intercalating with DNA origami, a circular dichroism experiment was conducted. **Figure 3.2** shows data from a circular dichroism experiment done on large bar DNA origami (blue line) and DNA origami that had been treated with 2 µL of 1 mg/mL AMT in water.
The AMT treated bar origami was crosslinked by exposure to ultraviolet light for 20 minutes (red line). The plot of DNA origami containing the AMT deviates from the plot of the unmodified bar DNA origami. The most significant reading is at the 250 nm wavelength with a difference of 2 mdeg in elipticity as compared to unmodified bar DNA origami. This experiment suggests that AMT is intercalating with DNA origami. Furthermore, a color change occurs after treatment of bar origami with AMT. AMT is an aromatic molecule which will change color when new bonds are formed; therefore, the yellow color change shown in Figure 3.3 is a further indication that AMT is intercalating with DNA origami and crosslinking the structure upon UV irradiation.

Figure 3.2. Circular dichroism measurements on DNA origami. Corrected excitation pattern of large bar DNA origami. The blue line is the excitation pattern of large bar origami in water. The red line is the excitation pattern of large bar origami combined with 2 µL of 1 mg/mL AMT and UV crosslinked for 20 minutes. The decrease in elipticity at 250 nm indicates AMT is intercalating with the large bar origami.
When AMT adds to DNA origami it changes the overall charge of the origami depending on how many AMT molecules intercalate. **Figure 3.4** is an image taken of an agarose gel with large bar DNA origami and large bar DNA origami treated with AMT. The large bar origami was subjected to 20 minutes of ultraviolet light exposure before loading the samples into the agarose gel. The top band in lane 2 is large bar DNA origami with no AMT treatment. The band below that is excess M13mp18 scaffold. The smeared band at the bottom of lane 2 is excess staple strands. Lanes 3-5 are large bar DNA origami treated with AMT and exposed to UV light for 20 minutes. The AMT has intercalated with individual DNA origami at various ratios, thus creating different charges on individual DNA origami. Differences in charge can be seen when DNA origami migrate through an agarose gel. These differences are seen as a smear in lanes 3-5 where DNA origami samples are treated with AMT. Lane 6 contains M13mp18 while lanes 1 and 7 contain a 1 kilobase ladder. Agarose gel electrophoresis of large bar DNA origami treated with AMT shows further proof that AMT is intercalating with DNA origami.
Figure 3.4. Agarose gel electrophoresis of large bar DNA origami and large bar DNA origami treated with AMT. Lane 1: 1 kb ladder. Lane 2: bar origami. Lane 3: bar origami treated with 1 µL of 1 mg/mL AMT and UV crosslinked. Lane 4: bar origami assembled with 1 µL of 1 mg/mL AMT and UV crosslinked. Lane 5: bar origami assembled with 2 µL of 1 mg/mL AMT and UV crosslinked. Lane 6: M13mp18. Lane 7: 1 kb ladder. Lane 8: Empty.

3.3.2 AFM images of agarose gel extracted material

One of the key goals of agarose gel extraction is purification of DNA origami from unwanted DNA artifacts. Agarose gel electrophoresis has shown to be a promising method to separate out unwanted DNA artifacts.1 The challenge however is that DNA origami gel extraction can be inefficient and the extraction process has a tendency to denature DNA origami.
Importantly, treatment of DNA origami with AMT and inducing crosslinks via ultraviolet light could be a promising new method for DNA origami gel extraction. Using AMT to stabilize DNA origami helps it to withstand denaturation during the purification processes.

**Figure 3.5** shows an AFM image of bar-shaped DNA origami (with no AMT treatment) after extraction from an agarose gel. The image shows possible small aggregated particles of DNA, but no intact DNA origami structures. This is in contrast to **Figure 1.5** which shows large bar DNA origami. Without treatment with AMT bar-shaped DNA origami was unable to withstand the agarose gel electrophoresis and gel extraction procedures. The method used to extract DNA origami required the use of Qiagen’s QG agarose gel solubilizing buffer followed by centrifugation as described in section 3.2.2. Either the QG solubilizing buffer or centrifugation or both denatured the DNA origami. Electrophoresis could also play a role in denaturing DNA origami structures. As DNA origami migrates through an agarose gel, it is possible that oligonucleotide strands can be sheared off of the origami as it passes through the agarose matrix. Gel electrophoresis, agarose gel solubilizing buffer and filtration via centrifuge all add chemical and physical stresses that can lead to DNA origami destruction.
Figure 3.5. AFM image of bar-shaped DNA origami with no AMT treatment after agarose gel electrophoresis and agarose gel extraction. This image shows possible small particles of aggregated DNA, but no DNA origami structures in contrast to Figure 1.5. Image dimensions: 10 µm x 10 µm. Height scale is 10 nm.

Figure 3.6 shows bar-shaped DNA origami treated with AMT and exposed for 20 minutes to ultraviolet light. The DNA origami treated with AMT and UV light then underwent agarose gel electrophoresis and agarose gel extraction. Bar shaped DNA origami treated with AMT and UV light induced crosslinking showed resilience to agarose gel electrophoresis and agarose gel extraction. The image shows many large bar DNA origami that were recovered from
the gel through the method described in section 3.2.2. The DNA origami in the image is not in as good of condition as freshly made DNA origami (Figure 1.5). The image suggests that some DNA origami frayed and re-attached to other origami structures, therefore resulting in a longer object. However, the results are encouraging because they suggest that AMT helps stabilize DNA origami and reinforce its structure so that it is resistant to destruction. Therefore, treatment of DNA origami structures with AMT is a promising method for DNA origami purification through agarose gel electrophoresis and subsequent extraction.

3.4 Conclusions

Results from the CD experiment show that AMT intercalates with DNA origami. CD ultraviolet light absorbance values decreased by 2 mdeg which was a result of AMT intercalation. AMT is a conjugated molecule and as such can change color when new covalent bonds are formed. A yellow color change was observed after irradiating DNA origami containing AMT with ultraviolet light. Data from the CD experiment and the observed color change suggest that AMT is intercalating with DNA origami. In order to further understand AMT’s impact on DNA origami, agarose gel electrophoresis was performed to see how DNA origami is influenced by AMT. The agarose gel shows how DNA origami treated with AMT and ultraviolet light induced crosslinks has different band shapes. DNA origami bands became broad due to the change in the overall charge of each individual DNA origami when intercalated with AMT. AMT will intercalate with DNA origami in a stochastic fashion; thus, some origami will have more AMT molecules than other, and consequently will have a different electrical charge. This charge differential is seen in the elongated DNA origami bands in the agarose gel.

To see if DNA origami intercalated with AMT was stabilized, an agarose gel extraction and purification was conducted. The AFM image of bar-shaped DNA origami without AMT
intercalation shows that the origami did not survive the gel extraction and purification. However, when DNA origami was treated with AMT and exposed to ultraviolet light to induce AMT crosslinking, DNA origami structures remain intact, indicating that AMT can help DNA origami become resilient to purification and separation processes.

Figure 3.6. AFM image of bar-shaped DNA origami treated with AMT and UV light, and then run through agarose gel electrophoresis and agarose gel extraction. DNA origami structures are intact and visible implying that AMT crosslinked DNA origami is more stable and resilient to external stresses such as electrophoresis and gel extraction. Some of the origami structures are the correct length while others frayed and re-attached to multiple origami structures thus making a larger object. Image dimensions: 10 µm x 10 µm. Height scale is 10 nm.
The water soluble psoralen derivative AMT has shown potential for intercalating with and stabilizing bar-shaped DNA origami. Based on these results, AMT could also theoretically be used to stabilize other DNA origami structures. AMT works to stabilize DNA origami by sitting inside the DNA double helix. Short wavelength ultraviolet light can then be used to irradiate the DNA origami structure containing AMT and create interstrand covalent crosslinks. These interstrand covalent crosslinks anchor complementary strands of DNA together in order to resist destruction and denaturation of the DNA origami. Overall, I have demonstrated that DNA origami not treated with AMT completely denatured and was lost during agarose gel extraction. Yet, in AMT treated DNA origami, structure was preserved when performing an agarose gel extraction.

3.5 References


CHAPTER 4: NANOMATERIAL SEEDING AND NANOWIRE CONSTRUCTION USING DNA ORIGAMI TEMPLATES

4.1 Introduction

DNA or DNA origami itself is not a usable material for electrical conduction in nanoelectronics. However, DNA origami is a useful material for constructing small shapes to which nanomaterials can be attached. Construction of DNA origami nanowire templates has therefore become a desirable method of bottom-up self-assembly for nanoelectronic wires and semiconductors. As shown by Pearson, et al.\(^1\) and Gates, et al.\(^2\) DNA origami can be seeded with gold nanoparticles linked to DNA oligonucleotides. Seeding of nanomaterials such as gold is then followed by a plating processes in which gold seeds grow into adjacent gold seeds and complete a current-carrying nanowire. In this chapter, I present new methods which serve to further expand the seeding repertoire. Inspired by the research of Lee, et al.,\(^3\) I investigated using a special type of DNA called phosphorothioate to seed gold nanoparticles. Phosphorothioate DNA is unique because it contains sulfur in the DNA backbone. I designed and synthesized a crosslinker to link gold nanoparticles to the DNA origami structure.

4.2 Experimental

4.2.1 Materials

Distilled water, 10x TAE-Mg\(^{2+}\) buffer, m13mp18 single stranded DNA, tight bar staple strand master mix, and phosphorothioate oligonucleotide strands purchased from Operon (Huntsville, AL) were used to make the DNA origami. The synthesized crosslinker was made with cysteamine and bromoacetic bromide purchased from Acros Organics (Geel, Belgium).
Sodium hydroxide from Sigma-Aldrich was used to achieve the correct pH for crosslinker synthesis. Once the crosslinker was synthesized it was then solvated in 10x TAE. Tris(2-carboxyethyl)phosphine hydrochloride (TCEP, 95% 0.5 M) was purchased from Alfa Aesar, (Ward Hill, MA) and was used to reduce the disulfide bonds of the crosslinker. 5 nm citrate NanoXact gold nanoparticles were purchased from nanoComposix (San Diego, CA) and were used for gold nanoparticle seeding. 1 mg/mL 4’-aminomethyltrioxalen (AMT) in distilled water from Sigma-Aldrich, (St. Louis, MO) was used to stabilize the DNA origami. A thermocycler from Techne (Burlington, NJ), a Sun Ray UV Floodlight from Uvitron International Inc. (West Springfield, MA), and an atomic force microscope from Bruker (Santa Barbara, CA) were also used. An Inova 500 MHz System located in the Brigham Young University Nuclear Magnetic Resonance (NMR) lab was used to obtain NMR data in a water/methanol solvent.

4.2.2 Methods

**Crosslinker Synthesis.** The structure of the crosslinker I designed is given in Figure 4.1. 25.4 g of cysteamine were mixed as dichlorohydrate in 100 mL of H₂O. The mixture was then cooled externally with stirring to 0°C while 41.0 g of bromoacetic bromide were added in small amounts. The pH was adjusted to 8.0 with addition of sodium hydroxide. After the addition, the solution was mixed for one hour to allow for completion of the reaction. Once the reaction was complete, the solution was separated by filtration, and the product was washed with water and dried. Nuclear magnetic resonance spectroscopy was performed to determine if the correct crosslinker was made and to determine its purity. After the crosslinker was synthesized, a 1
mg/mL stock solution was made by dissolving the bi-functional crosslinker in 10x TAE- Mg\(^{2+}\) buffer (BF-10x TAE- Mg\(^{2+}\)).

![Structure of the synthesized crosslinker for DNA nanomaterial seeding.](image)

**Figure 4.1. Structure of the synthesized crosslinker for DNA nanomaterial seeding.**

**DNA Origami Construction.** Tight bar DNA origami was constructed as described in section 2.2.2, with the exception that 2 µL of 100 µM of two phosphorothioate oligonucleotide strands and for stabilized DNA origami, 5 µL of 1 mg/mL AMT in H\(_2\)O were added to the mixture in a 200 µL PCR tube. The sample was then placed in the thermocycler and assembled as described in section 2.2.2.

After the tight bar DNA origami completed folding, the sample was either used directly (if not treated with AMT) or for the sample with AMT, was placed under short wavelength ultraviolet light for 20 minutes to complete interstrand crosslinking and stabilization. After stabilization was complete a dilution was made by mixing 70 µL of 10x TAE- Mg\(^{2+}\) and 25 µL of the large tight bar DNA origami sample. 20 µL of the diluted sample was attached to a mica surface by rinsing with a 4 mM magnesium acetate solution followed by distilled water. The surface was then air dried in preparation for surface seeding of gold nanoparticles.

**Gold nanoparticle preparation.** To prepare gold nanoparticles for surface seeding on DNA origami, 90 µL of BF-10x TAE- Mg\(^{2+}\) stock solution was added to a 200 µL PCR tube, followed by 6.5 µL of 0.5 M tris(2-carboxyethyl)phosphine hydrochloride (TCEP). TCEP
reduces disulfide bonds in the crosslinker. Reduction of the disulfide bonds was allowed to proceed for 30 minutes at room temperature, after which 10 µL of 5 nm gold nanoparticles in citrate (from nanoComposix) were mixed with the reduced crosslinker solution. The mixture was then placed in a thermocycler and heated at 50°C for 1 hour.

**Gold nanoparticle surface seeding.** Surface seeding was accomplished by depositing 15 µL of the prepared gold nanoparticle solution containing the synthesized crosslinker on top of the AMT crosslinked tight bar DNA origami on a mica surface. The surface seeding reaction continued for 1 hour on mica in a protective container and was then washed with a 4 mM magnesium acetate solution and distilled water. After the mica surface containing the tight bar DNA origami with gold nanoparticles was washed, the surface was dried with air and underwent atomic force microscopy imaging.

4.3 Results and discussion

4.3.1 NMR data of the synthesized crosslinker

The crosslinker known as *N,N’*-bis(bromoacetyl) cystamine that was synthesized for the purpose of anchoring gold nanoparticles onto phosphorothioate oligonucleotide strands in DNA origami underwent ¹H NMR spectroscopy to confirm its structure (Figure 4.2). The ¹H NMR was carried out in a solvent containing water and methanol. The water-methanol and methanol peaks of the ¹H NMR are located at 4.9 ppm and 3.3 ppm respectively. The synthesized crosslinker has three types of C-H bonds and one kind of N-H bond. The least de-shielded C-H bond is labeled “a”, and was identified at 2.8 ppm. C-Hₐ is an alkane which is bordered by sulfur and carbon, thus making it the least electronically de-shielded and its signal lowest on the chemical shift scale of the four possible signals. The next least electronically de-shielded C-H
bond is labeled “b”. C-H_b is located next to an electron-withdrawing nitrogen atom. Its $^1$H NMR signal is at 3.5 ppm. The next least de-shielded C-H bond is labeled “d”. C-H_d is located in between two electron-withdrawing neighbors: a carbonyl carbon and bromine. Halides such as Br-C-H, typically have chemical shifts between 2.1-4.5 ppm. The signal of C-H_d is at 4.3 ppm, within the expected signal range of a halide. The most de-shielded hydrogen in the structure is an N-H bond which is an amide hydrogen. Amide hydrogen chemical shift signals can usually be expected to be between 5.5-8.0 ppm. In this case, the signal shifted as far as 8.5 ppm. Based on this data, I was able to confirm the successful reaction of the synthesized crosslinker for DNA origami with phosphorothioate to be used for nanoparticle seeding.

**Figure 4.2. NMR data of the synthesized crosslinker.** Letters on the NMR reading correspond to C-H bonds as identified on the crosslinker structure. Solvent: water and methanol.

### 4.3.2 AFM images of gold nanoparticle seeded DNA origami

I did an experiment with the tight bar DNA origami with two phosphorothioate oligonucleotide attachment points, each located on one of the two ends of the DNA origami. The
origami was subjected to a 1 hour incubation period for gold nanoparticle surface attachment with a seeding solution that had been prepared as described in 4.2.2. Site-specific attachment of gold nanoparticles to the ends of the origami was expected, but not observed. Figure 4.3 shows that many tight bar DNA origami were constructed; however, gold nanoparticles did not seed site-specifically on the DNA origami. Instead, the gold nanoparticles aggregated and formed large clumps seen as white dots on the image.

Figure 4.3. Seeding of tight bar DNA origami with crosslinker functionalized gold nanoparticles. The seeding is not site-specific on the DNA origami. Image dimensions: 10 µm X 10 µm. Height scale is 15 nm.

The phosphorothioate mediated gold nanoparticle seeding on tight bar DNA origami was also done on DNA origami treated with AMT. This sample then underwent the surface seeding process described in section 4.2.2. Surprisingly, the 5 nm gold nanoparticles seeded not site-
specifically, but onto the entire surface of the tight bar DNA origami as seen in Figure 4.4. This result suggests that the gold nanoparticles did not attach to the DNA origami through the intended phosphorothioate anchors, but instead attached to DNA origami through a mechanism involving AMT.

Even though the crosslinker was intended to react with phosphorothioate, this accidental discovery shows that AMT not only has potential as a DNA origami stabilizer, but also has the ability to seed nanoparticles to the surface of DNA origami. AFM results of this seeding method showed high seeding fidelity and good seeding density on the AMT-stabilized tight bar DNA origami (Figure 4.4). Nearly all of the DNA origami structures were well seeded with gold
nanoparticles in a linear arrangement. **Figure 4.5** is a magnified view of the crosslinker functionalized gold nanoparticles seeded on tight bar DNA origami containing AMT. Individual gold nanoparticles can be seen, with about 5-10 gold nanoparticles seeding onto each tight bar DNA origami. Based upon these results, seeding DNA origami with AMT and the synthesized crosslinker has promise for bottom-up self-assembly of nanoelectronic structures.

![Image of AMT-stabilized tight bar DNA origami with the crosslinker functionalized gold nanoparticles](image)

**Figure 4.5. Seeding of AMT-stabilized tight bar DNA origami with the crosslinker functionalized gold nanoparticles.** Image dimensions: 2 µm X 4 µm. Height scale is 10 nm.

The proposed reaction between AMT and the synthesized crosslinker is shown in **Figure 4.6**. The primary amine located on the AMT molecule could displace the bromine atom on the crosslinker and create a lengthened attachment point for nanomaterials. The advantage of using this method is that it not only results in DNA origami seeding, but it also stabilizes the DNA origami. Covalent bonds are created during the crosslinking reaction in the complementary strands of the DNA origami which stabilizes the structure, thus making it more resilient to heat and other environmental stresses.
4.4 Conclusions

Seeding tight bar DNA origami using AMT and the synthesized crosslinker has shown to be a promising new method for nanowire fabrication. However, this was an accidental discovery because site-specific seeding of DNA origami through phosphorothioate anchors was the intended design. Initially, the intended design was meant to facilitate site-specific attachment of nanomaterials by making the bromine on the synthesized crosslinker react only with sulfur on phosphorothioate oligonucleotides in the DNA backbone. This was not the case, but instead nonspecific seeding occurred as AMT reacted with the crosslinker. To further support the hypothesis that AMT was reacting with the crosslinker, I performed a control experiment in which DNA origami was not treated with AMT (Figure 4.3), and showed that no seeding occurred. Therefore, nonspecific seeding of DNA origami seems to be dependent on the presence of AMT, and it is likely that the primary amine on AMT reacts with bromine on the crosslinker as shown in Figure 4.6. Despite this unforeseen result, my seeding method may have potential in nanowire fabrication, and has the advantage of nanoparticle seeding and DNA origami stabilization all in one process.

Figure 4.6. Proposed AMT-crosslinker reaction. This method results in surface seeding and DNA origami stabilization.
4.5 References


CHAPTER 5: CONCLUSIONS AND FUTURE WORK

5.1 Conclusions

5.1.1 Tight bar DNA origami

DNA origami has become a promising new method to construct nanoelectronic circuits through bottom-up self-assembly methods. Using DNA to construct templates for surface attachment of nanomaterials is desirable because DNA origami can be constructed in a variety of shapes and sizes. In this thesis, I have presented a redesigned bar shaped DNA origami termed the tight bar DNA origami. By designing the origami to have 13.5 base-pairs per helical turn as opposed to the normal 10.5 base-pairs per helical turn, I have introduced more torsional strain in overall structure. The caDNAno DNA origami design program and AFM imaging of the tight bar DNA origami show that this structure can be made. The tightly wound structure can then better accommodate intercalating molecules and preserve the overall desired shape of the DNA origami based nanowire.

5.1.2 DNA origami agarose gel extraction

One of the challenges facing DNA origami based nanoelectronics fabrication is achieving high yield DNA origami nano components. A promising method for selecting well-formed DNA origami structures is through agarose gel electrophoresis. By using electrophoresis, correctly constructed DNA origami nanowire templates can be separated from undesirable DNA byproducts; however, a great challenge is extracting DNA origami nanowire templates from agarose gels. In this thesis I have shown that by stabilizing and crosslinking DNA origami using 4’-aminomethyltrioxsalen (AMT), DNA origami becomes resilient to the agarose gel electrophoresis and extraction processes. I have conducted circular dichroism, agarose gel
electrophoresis, agarose gel extraction and AFM experiments that show AMT intercalates with and stabilizes tight bar DNA origami. The method that I have described can be used as a starting point for improved DNA origami stabilization and gel extraction methods for future advances in DNA origami purification and yield enhancement.

5.1.3 DNA origami nanomaterial seeding through AMT

Previous research and development to improve DNA origami nanowire construction has shown promise by seeding DNA origami using specially designed DNA oligonucleotides linked to gold nanoparticles which anneal to the DNA origami structure. This thesis highlights a new nanoparticle seeding method which bypasses DNA oligonucleotide seeding. This process uses AMT which intercalates with DNA origami and reacts with a synthesized bifunctional crosslinker attached to the gold nanoparticles. The advantages of using this method are twofold: (1) as described in section 3.3, DNA origami are stabilized by creating interstrand covalent crosslinks when the photo-reactive molecule AMT is irradiated by ultraviolet light, and (2) the combination of the synthesized crosslinker/AMT molecule as described in section 4.3.2 has proven to be a new promising method for DNA origami nanoparticle metal seeding. Improving stability and enabling seeding all in one step therefore makes DNA origami based bottom-up self-assembly of nanoelectronic elements an even more viable alternative to current top-down fabrication methods.

5.2 Future work

Based on the results and discoveries in this work, continued progress and research into DNA origami design optimization for nanoelectronic fabrication is warranted. Research should be conducted to determine how DNA origami structure can be adjusted by introducing helical
torsional strain and intercalating agents such as AMT. Adjusting the concentration of AMT in relation to that of the DNA origami can create stabilized structures of programmable size and shape. The control experiment reported in Figure 4.3 should be duplicated with AMT stabilized tight bar origami but with no crosslinker to see if origami can be seeded with gold nanoparticles. Additionally, research should be done to develop new agarose gel filtration devices which serve to prevent DNA origami structures from being lost in flow-through waste. Improving agarose gel filtration matrices to be able to handle DNA origami structures without filtration matrix destruction will be an important step in the advancement of DNA origami purification and yield. Certainly, stabilizing DNA origami is a solution for gel extractions, purifications and improved yield.

Perhaps one of the most significant contributions this work gives to the DNA origami nanowire sector of nanoelectronic development is the new nanoparticle seeding method involving AMT. AMT and the synthesized crosslinker for nanoparticle seeding may be an important advance in DNA based bottom-up self-assembly of nanoelectronics. Improved DNA origami stabilization and simultaneous seeding has been demonstrated with this new method. However, seeding efficiency and DNA origami yield improvements are needed to make this new method even more viable. Continued research and development of other molecules similar to the AMT-crosslinker shown here should be done. New crosslinkers could be designed that react even more effectively with AMT and gold nanoparticles which would further advance DNA origami metallization methods. Future research on similar designs to the crosslinker/AMT method described in this thesis could potentially lead to new technologies in high efficiency, and high-yield site specific seeding on DNA origami templates. Such technology could lead to the development of new electronic circuitry on a single DNA origami template.
APPENDIX 1

A 1.1 Small bar:

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

*PCR Primers:*

GGTGCTGACACGGAAAGAAAC

[BioTEG]ATCATCAGCAGATTGTCTTTATTC

*Staple sequences:*

TTTTATTCTGAACCTATAATTCCGTGTTCAGCACCATCATCATCAGCAGATTGTTTGAATTG

ATCAACGACGGTTTTCAACGTCAACAGTCT

TTTACATCGTGTATTAGTTTACTGAGAGCAT

TTTGATAATTCACTAAATAAAACGATAAACACGTG

TAAATTTCTCAAGAAGATGAGTGTTTACAA A

TGCTAAAGCAGGAGTTTTCTACACGCTCTTATAGAAA

GTGAAAACATTCTAATATTTGATAGGGTTGAAATCAAGAGAA

CGTCCTATGACATAAAA

TTGAAACGATAAGGTTGTAAAATGT

TTAATAAAAAGGACTTAAAACGATAATGCAAACCTACG

GGCTCAGGGTTGCACAGATAATA

ACATTATATTACTATCTAGCCCATTTTTAAAGAAATATTGCG

ATCATCAGCAGATTGTATTAGACTGTGAATTG

ATCAAGTGCGAGTTTCTTTATCTTTG

AAAATATTACTTTCAAGGTTTTTACCAAT

CGCCCTCGTATCACATGGATCTTTTCTGTATGAAGAGAA

GTTGATTGTTCTGACGTCAGGACC

TCATCAACAAAAACACAAGGAGAATGCCAGCAAGGACC

CGCTCCATGCGGCTTGCTCTGCTAGCGGT

TTTGAGCAGGGTTGCCTTTACATACATCTGTG

GCACTTTGTATTGCAACCAATACATATTAA
ATCACATTTCAGCAATACAGGGAAAATCT
ATATCCATGAACATAAAAGATATTACTATAACC
TCAGAACACTACAAATCTTTCCACGCTAAA

A 1.2 Small bar M13mp18 scaffold

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

PCR Primers:
CCACCATCAAAACAGGATTTTCGCC
[BioTEG]TTCTCCGTGGGAAACAAACGGC

Staple sequences:
GGTACCGAGCTCGAATCCTGATATTGGTGG
CCCCAGCGAGCGAAATTCGGAATCTGATGGTGG
ACAATTCCACAAACCGCCTGGCCCTGA
ACAGCTGATTGCCCTTCAATACGAGCCGGAAGCAT
GCTAACTCACATATTATGGGCGCCAG
CGGGGAGAGGCCGTTTGGCAGTTGCCTGGGCGCTACTG
TTCAAGGCTGCACAATTCGGAATCTGATGGTGG
CCCGCTTTGAAAGGGCG
TGGCGAAAGGGCCCTAATGAGTGA
AAAGTGTAAAGCCTGGGATGGATGTGCTGCAAGGCGATTA
ACGTTGTAAGAGTTATCCGCTC
AGCTGTTTATCTGTGAAATTCGACGGCCAGGCTGAGTTTGA
TTCTCCGTGGGAACAAATCTAGAGGATCCCCG
CATGCCTGCAGGTCGACACGGCGGATGACCGT
CATCGTAACCGTGCATTCTCCCCAGTCACG
AGTTGGGTAACCGCCAGGGGTCTGCTGCTGTTGAGGGG
CTCCAGCCAGCTTTCTATTACGCCAGC
ATCGGTGCCGCGCCTCTCGCAGCCGCCAACCCTTCTGCTG
AATGGGATATGGTACCGTTTGCGTAGATGGGCG
ACGACGACAGTATCGGCCAGAAGATCGCA
CCGGAAACCAGGGCAAGCCGCATTCCGCA
CCAGCTGCGATTAATGAAATCGGCAACGC
GGTGGTTTTTCTTTTACACATGGACGGGCA
GAGAGTTGGAAGGGGCGGGGATAGGGAATAGG

A 1.3 Large bar staple sequences:
TTGCCTGAGAGTCTGGAGCAAACAAGGAGAGATCAAAAAAAAAA
AAACTAGCTAAATTAATGCGCGAGCGGGETTTTAAAAAAAAAA
GATAATCATCATAATTAGTATATTCTGGTCGCAAAAAAAAAAA
TTGTATAAGGGGTGAGAAAGGCCGGGAATAGGAAAA
TAATATTGGCCTGAGTAATGTGTAATCCGAAAA
TTGTATAARTTTTAAGAACCCTTCATGAACCCTGCAAAAA
AACGCCTAGAAGGCTTTATTTTCATTTCAGGGAATTTTTTT
CCTGTAGAAAACATTATGACCCTGTGCTAAAAAAAAAA
GAGCGAGGTCAGAGCATAAAGCTCAAACTACAAAAAAAAAA
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GAGCGAGGTCAGAGCATAAAGCTCAAACTACAAAAAAAAAA
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TTGTATAATTTCTGGAGCAAACAAGGAGAGATCAAAAAAAAAA
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TATTAAGAGGCGCTATCAGGTCTAAAAA
TGCTCAGTTAAAACAGTTAATGGCCTGATAAAAAAA
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TCGTCATAACGTGGGAAAATCGTGCTGCGTGCCTGT
TTAGACTAATGAAATCGCGCAACCGCGGGGAGTGCGTGT
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ACGACCAGTATAAAGGTACCAGACAAGTTTTATTTTGTGCGTGT
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CCATTGCATAGATAGCTCAGCAGACTCCGTCGCGTGT
CTGGTAATCCATCCTAATTACGTGAACGGAAGTGCGTGT
CTGAGTAGTCAATAATCGGCTGTCTTACCAGAGTGCCTG
TACTTCTAAGAACAGGGTGATTTAAATTAAGAAAGTGCCT
CTGTCATATCGAGAAACAGCAAGAGAATACAGAGTGCCTG
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AACGGTGCTAAACAGCGAGCGAGCCTGAACAGTGCCTG
TTCTCCTCCGACTTGCAGGGAGGTAGCGCATGTGCGTGT
TATGGTTGAAGTTAGCTATTTTCTTTACAGGTGCTG
CGCGGATTTTTCTCCTCAATCTTTTGTTTATGAGCTG
CGGTAGCAGCCTCTTCCCAGAGCCTATTTATCGTGCCTG
GCGCTAGGGCGCTGGCAAGTGTAGGTGCGTGT

A 1.4 Tight bar staple sequences:
GAGATCTACAAAGGCTATCAGGCATTGCCTGATAAAATATG
CAAACAGGAGAATCGATGAACGGTAATCGTACAGATG
TGTCATACATATGTACCCCGTGTTAGATTACGTG
CAAAAACAGGAAGATTGTATAAGCAATATAGAACCCTCA
AAATCAAGAGATTTGTATAAGCAATATAGAACCCTCA
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AAATCAGCTATTTTTAAACCAGGAAACTCCGTTGAC
ATAATTCCGTCTCGCCTTCTGCTGAGCCAGAGCAAAATTA
CATTAATGTGAGCGAGTAAACCCCGTCGATTAACATC
TCGTCACCAGAATTCTACTAATAGTAGGATTCTCCGT
GACAGCCCTCCTATATTTTCAATTTGCGGACATAGGTTCACG
GTCTTTCCAGGCCCATTTAGATACTTTTCGCAAATCTGCCAGT
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ATAAGTTTTTATTATACTCTGAATATAATGCTGTAGCGCAGAC
TAGAAGGAAAGATAGATTTTCAGGTTAAGCTATTTTAAAAG
GCCTTGATATGTACCTTTTACATCGGGAGATTGCAACT
CGCCAGCATTTATGGGTTGAAATACCAAGTTATACATTGGA
CCACCCTCAGATTATTTCAATTTACCTGGGACGACTAA
CTCAGAGCAGAAAAACTCAAGAAAAACAAAAAGTGAAAAGG
CAAAATCACCTCATTTGAATTACCTTTTTTCAAACCCCTC
GTCATAGCCCCAATATATGTGAATGAAATATAAATGAAAAAT
TTAGCGTCAGCTATTTAATATTATTTTTTCGCTAAAACCGCCT
TAGCAGCACCAGATAGCTTAAGAAGACCGACACAGCAGA
CCATTAGCAAAATTTAATCAAATCATAAGGTCTAGATGCCTA
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TCATTTAAGGGATGCAAATCCAATCGCAAGAACCCTTCTG
ACGAGATTGACTTTCAAATATTATTAGTTGCAACCAGTAAC
CTGATTGTTGGAAACGGGTCAGTGC
CCTACCATATGGGTTGTGATGATACAGGAGGCCGCCCTGCTT
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TACAGTAAACATCACAACAAATAAATAATCCTCTTATCCGCGG
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GTAAATCGTCGACTGTAGCAGCGGTTTTCATCGCATTCCACA
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TGTAATGCTTGAATTATCACCAGTCACCGAATAATTTTTT
CGAGAAAAACTGGAGGGAAGGTAAATATTGGAAAAAGAGGCC
GATGGGAAATTCATCAATATAATC
AGAAGGAGCGGAATATTATCATATTTCTGTAGGGTTAGAA
TTTGAGTAACATTATCATTTTGCAGGAAACAGAAATAAA
CGTATTAATATCCTTTGCCCCAAGCGTTATTACAGATGAATA
GGATT TAGAAGTTATAGACTTTTACAAACAAAAACAAATAACG
CAACTAATAGATTAGCCGTTAATTAGATAAACAAAAATCGC
AATTGAGGAAGGTTATCTAAAAATATCTTTTAGAGAAAAAGA
AATCAATATCTGTCAGTTTGCAAAATCAACATTAATAC
CTAAAGCAGTCAGCCTTGACACCTTCAAAATAATGGAAAAA
GCAACAGGTGCCAGCTGAGACGCACGCAGCACTTGGCTCTCT
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GAATGGGCTATTAGTCTTTAATGCGCGAACTTGAGAGACTA
ACCTGAAAGCGTAAAGATACGTGACAGATATAACTATA
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CCACACAAACATCGAGCGCAAGCAAAAGCAATAATGCTTT
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GCGCTCAGTCCGCGTCTTCCAGTCGGGAAAGAGGGGGTA
CAGCTGCATTGAATCGGCCAACGCGCGAACAAAAATA
GTTTGCCTATTGGGCGCCAGGGTGTTGTTTGCAACACTAT
GTGAGACGGGCAACAGCTGATTGCCCCCTTCAAGATACTAAA
CTGAGAGAGTTGCAGCAAGCGGTCCACGCTTCATCAGTGG
AGCAGGGAAATCCTGTTTGATGGTGGTTAAACGAACTA
CAAAATCCCTATAAATCAAAGAAATAGCCATACCAGCTC
TTGAGTGGTTGCTTCCAGTTTGGAAACAAGAGTATTGTGAATT
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