Assembly and characterization of mesoscale DNA material systems based on periodic DNA origami arrays

THESIS

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By

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Abstract

DNA origami is a recently developed technology that manipulates DNA base-pairing to create nanoscale engineered structures on the order of 100 nm via molecular self-assembly. DNA origami nanostructures such as nanopores, molecular sensors, and protein templates have been applied to study different aspects of biomolecular and cellular systems. However, current applications of DNA origami are limited due to challenges in scaling nanoscale functionality up to relevant cellular and material scales. One method to create larger DNA origami structures is to assemble individual nanostructures into various periodic arrays, forming micron-scale 1D filaments or 2D materials. To better understand the assembly kinetics, Transmission Electron Microscopy (TEM) and fluorescence microscopy have been used to characterize 1D and 2D polymerization reactions in terms of growth rate and length distribution as functions of time, temperature and assembly reaction conditions. The mechanical properties of the resulting larger scale assemblies are also measured to better understand the mechanical properties of DNA origami structures. With an improved understanding of how to optimize the hierarchical assembly process, larger DNA origami structures can be created, opening the door for new materials applications.
Dedication

This document is dedicated to my mother and father.
Acknowledgments

I would first like to sincerely thank Carlos Castro for his continuous guidance and patience. I’ve learned an incredible amount from you in just a short time, and it’s been a great experience.

I would also like to thank some other faculty who have really made an impact on me during my tenure at Ohio State: Dr. Blaine Lilly, Dr. Gary Kinzel, and Rich Teynor. I also thank The Ohio State University Institute of Materials Research for their support and funding that made this research possible.

I thank the members of my lab, old and new, for their friendship and for teaching me so much. In particular, Alex Marras really helped me out when I was just a lowly undergrad learning the ropes, and I really appreciate it.

I finally thank my parents and my fiancée for their constant support, motivation, and knowledge. You’re the best!
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Chapter 1: Introduction and Background

Structural DNA nanotechnology is a rapidly growing field with a wide array of potential applications, such as solving basic problems in structural biology and biophysics, designing nanoscale engineering tools, enabling targeted drug delivery, and more broadly, creating self-assembling biological nanomachines and nanomaterials. Scaffolded DNA origami is a recently developed method of designing 2D and 3D nanoscale structures from DNA [1] [2]. With this approach, structures with typical dimensions on the order of 10-100 nm can be designed with CAD-like software and self-assembled in solution [3]. Previous research has proven the ability to create novel nanoscale structures via DNA origami [4], but there are several barriers to more widespread application of the technology to new areas, in particular in nanomachines and materials applications. In order to build nanomachines (like a gear) or more complicated devices for specific applications, the mechanical properties of the construction material must be known. This thesis characterizes the mechanical properties and larger scale assembly properties of DNA origami nanostructures. The typical theoretical method used to model nanoscale mechanics of DNA origami bundles treats double-stranded DNA helices (dsDNA) as solid, elastic cylinders that are rigidly coupled over their entire length. This research evaluates this rigid coupling model, attempting to quantify how well the theoretical model predicts experimental mechanics.
A detailed understanding of the mechanical properties of DNA origami components will greatly enhance the design of mechanically functional DNA nanostructures. For example, one application being pursued at the Nanoengineering and Biodesign Lab focuses on designing and creating nanoscale force probes to measure the cellular traction forces of neutrophils (white blood cells) and fibroblasts. Another project focuses on creating nanoscale calipers for use as a nanoengineering tool. Another goal is to be able to create functional nanomaterials and nanostructures with adjustable mechanical properties. In order to effectively apply the existing knowledge of DNA origami to develop functional devices like those mentioned here, a firm understanding of the mechanical properties of these structures is needed. Specifically, this research focuses on the basic mechanical property of bending stiffness by characterizing persistence length, which is an analogous property used more commonly on the nanoscale.

1.1. DNA Origami

Deoxyribonucleic acid, or DNA, is one of the fundamental building blocks of not only our own bodies, but also all other living organisms. Single-stranded DNA (ssDNA) consists of a sugar-phosphate backbone with a sequence of nucleotide bases: adenine (A), thymine (T), cytosine (C), and guanine (G) [5]. The most familiar form of DNA is the double-stranded helix shown in Figure 1, which is bonded together via hydrogen bonding of complementary base pairing of nucleobases. In this base-specific bonding known as Watson-Crick base pairing [5], adenine binds only with thymine and guanine binds only with cytosine, forming A-T and G-C base pairs. At sufficiently high temperatures, the hydrogen bonds can be melted, or denatured, to separate the double-stranded helix into
two individual complementary strands. The melting temperature of short oligonucleotides (typically 10’s of bases long) can range from 30° – 90° C depending on the length, sequence, and solution conditions.

![Double-stranded DNA helix, base pairing, and simplified chemistry](Image)

Figure 1: Double-stranded DNA helix, base pairing, and simplified chemistry [6].

Double stranded DNA (dsDNA), which is roughly 25 times stiffer than single stranded DNA (ssDNA) [7] [8] [9] [10], is itself flexible on the length scale of tens of nanometers as illustrated in Figure 2. Hence, at this length scale, dsDNA does not make a good building material. However, by manipulating nucleobase pairing, or simply base
pairing, to assemble dsDNA helices into bundles stiffer by factors of 10 or more, DNA origami turns DNA into a viable building material.

![AFM images of DNA strands](image)

**Figure 2:** Atomic Force Microscopy (AFM) images of DNA strands at 3 different concentrations of MgCl₂ and NaCl. [11].

DNA origami is a method that manipulates DNA base pairing to create nanoscale 2D and 3D structures that self-assemble in solution. DNA origami was pioneered by Rothemund in 2006 with what is now known as scaffolded-DNA origami, the most common DNA origami method. The basic concept is shown in Figure 3 below. The molecular components for assembly include a long, continuous “scaffold” loop of single-stranded DNA (7000-8000 bases in length) and many much shorter “staple” strands (typically 15-60 bases in length). The scaffold is typically a derivative of the M13MP18 bacteriophage viral genome, whereas staples can be readily chemically synthesized by a number of commercial vendors. In order to drive self-assembly, the staples are designed to be piecewise-complementary to the scaffold. Base-pairing interactions between the staples and the scaffold then stabilizes the scaffold into the desired 2D or 3D structure. Ultimately, these structures are comprised of bundles of dsDNA arranged into the desired geometry, where helices are connected to each other periodically by Holliday junctions.
Figure 3: The bonding of shorter “staple” strands to longer, continuous “scaffold” strands can be manipulated to fold and create structures [12].

The first intricate 2D DNA origami structures created by Rothemund can be seen in Figure 4. The simple idea posed in Figure 3 is extended by a suite of CAD-like DNA origami design software, caDNAno [3] that facilitate the design of new structures. CanDo [13] is a complementary computational tool that uses finite element analysis to generate predictions of 3D folded structures of caDNAno designs and simulate thermal fluctuations. Castro, et al. provide an excellent primer to DNA origami, summarizing in Figure 5 how 3D geometry is actually designed [14]. With these methods, a variety of novel nanoscale geometries have been created to date, as seen in Figure 6. An example of designing a structure in caDNAno is shown in Figure 7 below. Typically, cross-overs between helices occur every 21-bases, which is the shortest possible distance between
cross-overs. However, DNA origami structures have been successfully made with cross-overs as far as 42- or even 84-bases apart. As the total number of cross-overs is reduced in the structure, it clearly becomes less defined.

Figure 4: The first 2D DNA origami structures. From left to right: a square, a rectangle, a star, a smiley face, and two triangle designs. Adapted from [1].

Figure 5: (a) dsDNA helices are represented schematically as either two adjacent lines (left) or solid cylinders (middle). A detailed rendering of a double stranded helix is also shown (right). (b) Example of single-layer scaffold routing. (c) Example of completed design with colored staples bonded to the scaffold. (d) Structure represented by cylinders. Adapted from [14].
Figure 6: A collection of novel 3D geometry created via DNA origami adapted from [2], [4]. There are many examples of curvature, twisting, and other intricate geometry. All structures are designed to be static.
1.1.1. DNA Origami Fabrication

The standard protocols used by the Nanoengineering and Biodesign Lab to create and image DNA origami structures are summarized below. These protocols are adapted from Castro, et al. [14].
1.1.1.1. Folding Reactions

3D DNA origami objects are folded via molecular self-assembly reactions, which can be set up in a wide variety of ways. The current standard procedure at the Nanoengineering and Biodesign Lab involves mixing scaffold DNA, staple DNA, double distilled water, a buffer mastermix (to stabilize the pH), and positive ions in solution. The positive ions from a salt, typically MgCl₂, are necessary to screen the electrostatic repulsions of the negatively charged DNA in order to facilitate folding. The components of a typical folding reaction are detailed in Table 1 below.

Table 1: Folding reaction for a working stock with 50 µl total volume and 20 nM effective scaffold concentration.

<table>
<thead>
<tr>
<th>Components</th>
<th>Component Concentration</th>
<th>Volume (µl)</th>
<th>Final Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scaffold</td>
<td>100 nM</td>
<td>10</td>
<td>20 nM</td>
</tr>
<tr>
<td>Staples</td>
<td>500 nM</td>
<td>20</td>
<td>200 nM</td>
</tr>
<tr>
<td>FOBxM</td>
<td>50 mM TRIS 10 mM EDTA</td>
<td>5</td>
<td>5 mM TRIS 1 mM EDTA</td>
</tr>
<tr>
<td>ddH₂O</td>
<td>-</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>MgCl₂ Mastermix</td>
<td>140 – 280 mM</td>
<td>5</td>
<td>14 – 28 mM</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>50</td>
<td></td>
</tr>
</tbody>
</table>

After thoroughly mixing the solution, it is subjected to a thermal cycle beginning around 80°C and slowly decreasing to room temperature over a period of several days. Typically, a 2.5 day ramp is used, although longer ramps of 4.5 or 5.5 days may be used if the yield of the 2.5 day ramp is poor. Starting at a high temperature melts all of the base-pairing interactions so that all of the DNA staples and scaffold are single-stranded.
As the temperature decreases, staples bind to the scaffold in order of decreasing melting temperature. Folding in a slow, controlled manner allows staples to find their optimal binding locations and increases the yield of well-folded structures. A mock thermal ramp is depicted in Figure 8.

![Mock thermal ramp](image)

**Figure 8: A mock thermal ramp [14].**

**1.1.1.2. Purification via Agarose Gel Electrophoresis**

Typical folding conditions result in a yield of well folded structures ranging from 10-50%. Once the thermal ramp is complete, the well-folded structures are in solution with misfolded structures and excess unbound staples. In order to purify the well-folded structures from the other unwanted material, the solution is run through a 2% agarose gel stained with the intercalating dye ethidium bromide (EtBr), which makes the structures visible under ultra violet (UV) light. An agarose gel is shown in Figure 9 below, where the structures from the thermal ramp are mixed with a purple loading dye, placed in wells at one end, and immersed in a buffer solution. A voltage of 70V is applied across the gel,
which draws the negatively charged DNA toward the positive (red) electrode at the other end.

Figure 9: The agarose gel in an ice bath, subjected to a 70V current. Structures in the wells (purple) are negatively charged and drawn toward the positive (red) electrode.

The staple strands are much smaller than the folded structures, so they travel more quickly through the pores of the agarose gel toward the positive pole, whereas the DNA origami structures travel more slowly due to their much greater size. Misfolded structures run slower than the more compact well-folded structures. Aggregates of misfolded structures are more likely to become entangled in the gel and typically aggregate at the starting point (inside the loading well). After 3 to 4 hours, well-folded structures typically form distinct, narrow bands, seen in the UV image in Figure 10 below. The desired bands
are cut out and filtered by centrifugation in order to remove excess agarose, leaving only the well-folded structures in solution.

Figure 10: UV image of an agarose purification gel. The brightest spots are the high concentration of staple strands. The 6-helix (yellow) and 18-helix (red) bundles have distinct bands where well-folded structures are grouped. The poorly-folded 12-helix (blue) bundles are trapped at the starting point in the wells.

**1.1.1.1. Negative-Staining for Transmission Electron Microscopy**

Structures can be imaged by incubating samples on a carbon-coated copper grid and negative-staining them with 2% uranyl formate (UFo). The samples can then be imaged via Transmission Electron Microscopy (TEM). Detailed protocols for TEM grid preparation and imaging can be found in Castro et al. [14]. All TEM imaging in this
thesis was performed on an FEI Tecnai G2 Bio TWIN TEM at The Ohio State University Campus Microscopy & Imaging Facility.

1.1.1.2. Polymerization

Individual DNA origami structures can be linked together, or polymerized, by staples as depicted in Figure 12 to form larger, meso-scale structures [15]. Polymerization is accomplished through the use of asymmetrical “sticky ends”, which are empty ssDNA bonding sites on the edge of each structure. Polymerization staples omitted during the initial folding are then added to the solution to connect two sticky ends together.

The polymerization staple design of a simple 6-helix bundle is depicted in Figure 11. The schematic also highlights “neighbor” staples, which border polymerization staples and can also be omitted during the initial folding and added during the polymerization stage to eliminate aggregation due to base-stacking interactions during the initial folding reaction. This increases the yield of well-folded structures, and hence, polymerization yield. Typical polymerization conditions include a 10:1 excess of polymerization and neighbor staples to monomers and a period of incubation at 40 °C for at least 12 hours.
Figure 11: Staple routing of a 6-helix bundle. Scaffolds are blue, polymerization staples are purple, and neighbor staples are black and orange.

Figure 12: (top) A schematic of an 18-helix bundle polymerized to form a longer filament (bottom) TEM image of an 18-helix bundle monomer and polymer.

149 nm

10 x 201 = 1,490 nm
Just as with a macroscale gear, it is important to understand the mechanics when designing a nanoscale gear, such as that in Figure 13 below. Whether the gear is 100 mm or 100 nm in diameter, it is necessary to know how the gear will behave when subjected to bending or torsion, what the dynamics will be like, and so forth. These mechanics are not well understood at the nanoscale. Developing an understanding of the mechanics of DNA origami structures will enable effective and efficient design of devices for mechanical functions, in particular when creating larger assemblies from several DNA origami parts.

Figure 13: (left) Part of a caDNAno design for a gear (middle) CanDo model of the gear (right) TEM image of the gear with 20 nm scale bar [4].
1.2. Literature Review

This section performs a literature review on various biopolymer mechanics models and assays, which will be used as the foundation for experiments measuring the mechanical properties of DNA origami polymers. This section also discusses relevant experiments on the mechanics of individual DNA origami structures.

1.2.1. Biopolymer Mechanics

In general, polymers consist of smaller monomers that are bonded together to form a larger structure, and biopolymers are polymers comprised of biomolecules such as DNA, actin filaments, microtubules, or polypeptides. Biopolymers have been an active area of research for several decades. In Figure 14, Knowles et al. compare a wide variety of biopolymers, such as various actin and amyloid fibers, to more traditional polymers and materials, such as carbon nanotubes, spider silk, and steel.
1.2.2. Persistence length

The persistence length ($L_p$) of a polymer is a basic mechanical property analogous to its bending stiffness or bending rigidity. Informally, the persistence length is the length over which the polymer can appreciably bend (~90° turn) due to thermal fluctuations. Accordingly, if the contour length ($L_c$), or simply the length, of a polymer is less than its persistence length, it will remain in an essentially straight configuration and can be treated mechanically as an elastic rod. On the other hand, if the contour length is much greater than its persistence length, the polymer will resemble a random coil and its properties can only be described by a statistical treatment of its thermal fluctuations.
Persistence length (or bending rigidity) has been measured for a large number of polymers, typically by statistical analysis of thermal fluctuations. These thermal fluctuations can be imaged in aqueous solution with fluorescence assays and atomic force microscopy (AFM) assays. They can also be imaged with transmission electron microscopy (TEM) or AFM in dry conditions which requires the samples to be deposited on a surface, and therefore static. Persistence length can also be measured via single molecule experiments like force-extension and force spectroscopy using AFM, optical tweezers, magnetic tweezers, or flow.

Thermally fluctuating polymers can be modeled through two categories of methods, notably freely jointed chain approximations (FJC) and wormlike chain approximations (WLC). The FJC method treats a polymer as a series of rigid links that can freely rotate at the joints between links. The WLC method treats a polymer as an isotropic continuous beam that can fluctuate in bending due to thermal energy. The configurational entropy of these flexible and semi-flexible polymers can be described by models such as the Marko-Siggia WLC [17], the Mackintosh WLC [18], and the Palmer-Boyece approximation of the Mackintosh WLC [19].

1.2.2.1. Measurement methods

In practice, there are several analytical methods for calculating the persistence length of biopolymers from thermal fluctuations captured on TEM, AFM, or fluorescence images. All involve first tracing the contour of an imaged filament either manually or with an image processing algorithm (also known as skeletonizing). Figure 15 illustrates
tracing of a DNA origami polymer. With a traced contour, different methods are available depending on classification as a flexible or a semiflexible polymer.

![Image](image.png)

Figure 15: DNA origami polymer imaged with TEM and traced contour.

### 1.2.2.2. Flexible polymers: cosine correlation analysis

A flexible polymer fluctuating randomly in solution due to thermal fluctuations can be modeled as a continuous beam thermally fluctuating with a total bending energy due to those fluctuations. The total bending energy $u_b$ can be written in terms of bending stiffness $\kappa_b$ (equivalent to the product of the elastic modulus $E$ and area moment of inertia $I$), radius of curvature $R_c$, and contour length $L_c$:

$$u_b = \frac{1}{2} EI \left( \frac{1}{R_c} \right)^2 L_c = \frac{1}{2} \kappa_b \left( \frac{1}{R_c} \right)^2 L_c$$  \hspace{1cm} (1)

The theorem of equipartition of energy states that the average energy of a system, $< E_{sys} >$, can be written in terms of Boltzmann’s constant $k_B$ and temperature $T$:
\[ < E_{sys} > = \frac{1}{2} k_B T \]  \hspace{1cm} (2)

Using the equipartition of energy, the average bending energy can be rewritten as:

\[ < u_b > = < \frac{1}{2} EI \left( \frac{1}{R_C} \right)^2 L_C > = \frac{1}{2} k_B T \]  \hspace{1cm} (3)

Equivalently, the bending energy can be rewritten in terms of bending rigidity, \( \kappa_b \), and since \( \kappa_b \) is a constant, (3) becomes:

\[ < u_b > = \frac{1}{2} \kappa_b < \left( \frac{1}{R_C} \right)^2 L_C = \frac{1}{2} k_B T \]  \hspace{1cm} (4)

Rearranging this equation yields:

\[ < \left( \frac{1}{R_C} \right)^2 > = \frac{k_B T}{\kappa_b L_C} \]  \hspace{1cm} (5)

For small fluctuations, \( x \sim s \):

\[ \frac{1}{R_C(s)} = \frac{d\theta(s)}{ds} \]  \hspace{1cm} (6)

This can be rewritten as:

\[ < \left( \frac{d\theta}{ds} \right)^2 L_C > = \frac{k_B T}{\kappa_b} \]  \hspace{1cm} (7)

We temporarily assume that the average radius of curvature \( R_C \) is equal to the length of a segment of the polymer, \( L \) \( (R_C = L) \), which gives:
This means that over a length of L, the polymer will bend over an arc angle of 1 radian (~57°). This length scale over which the polymer appreciably bends due to thermal fluctuation is called the persistence length, $L_p$, so that $L$ can be redefined as $L_p$:

$$L_p = \frac{k_b}{k_B T}$$  \hspace{1cm} (9)

With this definition of persistence length, a variety of correlations for calculating $L_p$ can be derived.

1.2.2.3. Flexible biopolymers: Tangent angle correlations method

For a fluctuating filament depicted in Figure 16, the function $f(s)$ can be defined in terms of the trajectory $\theta$ as a function of arc length $s$:

$$f(s) = \langle \cos[\theta(s)] \rangle$$  \hspace{1cm} (10)
This equation can be modified by using a trigonometric identity and performing some algebraic manipulations to yield:

$$\frac{df}{ds} = \frac{<\cos[\theta(s)] - 1 >}{\Delta s} f(s)$$  \hspace{0.5cm} (11)$$

Expanding the cosine term into an infinite series and neglecting very small terms yields:

$$\frac{df}{ds} = \frac{1}{2} <\left(\frac{\Delta \theta}{\Delta s}\right)^2 \Delta s > f(s)$$  \hspace{0.5cm} (12)$$

By again utilizing bending energy and equipartition of energy, this becomes:

$$\frac{df}{ds} = \frac{1}{2} \frac{k_B T}{\kappa_b} f(s)$$  \hspace{0.5cm} (13)$$

Finally, this results in the 2-dimensional and 3-dimensional cosine correlation equations in terms of persistence length:

$$\{2D\} \quad <\cos[\theta(s) - \theta(0)] > = \exp\left(-\frac{s}{2L_p}\right)$$  \hspace{0.5cm} (14)$$

$$\{3D\} \quad <\cos[\theta(s) - \theta(0)] > = \exp\left(-\frac{s}{L_p}\right)$$  \hspace{0.5cm} (15)$$

The tangent angle correlation method, also known as the cosine correlation method, has been frequently used to measure persistence length of biopolymers, such as by Knowles et al. to measure amyloid fibrils [16], by McCullough et al. to measure actin and cofilactin filaments [20], and by Isambert et al. to measure actin filaments [21].
Figure 17 shows an example of how AFM was used to image filaments for use with the tangent correlations method.

Figure 17: A comparison of 5 types of amyloid fibrils with (A) the AFM topographical data, (B) the AFM height data, and (C) the shapes of the fibrils measured [16].

1.2.2.4. Flexible polymers: average transverse fluctuations method

The average transverse fluctuation method was derived by Isambert et al. to verify the tangent correlations method [21]. Instead of measuring individual filaments as with
the tangent correlations method, the transverse fluctuations method looks at the average transverse fluctuations of a population of filaments to determine persistence length. In contrast to longitudinal fluctuations, transverse fluctuations are highly sensitive to changes in $L_p$ for semiflexible polymers. Figure 18 shows a filament experiencing a small fluctuation and the variance in longitudinal and transverse distance.

Figure 18: Schematic representation of thermal fluctuations of an actin filament. (A) Transverse fluctuation. For each point of abscissa S, the distance D from the tangent at the origin is measured on a series of images. Two typical shapes, 1 and 2, are shown. Note that D varies appreciably while the longitudinal distance r does not vary much. (B) Correlation of the tangential direction. For each point of abscissa S, the angle O(s) of the tangent with the tangent at the origin is measured. (C) Actin filaments undergoing thermal fluctuations [21].

Isambert et al. showed that the tangent correlation can be used to derive the transverse fluctuations correlation [21]. The average quadratic transverse fluctuations $D(s)$ can be described by:
\[ < D(s)^2 > = 2 \int_0^s \int_s^s < \sin \theta(s') \sin \theta(s'') > ds' ds'' \tag{16} \]

This can be rewritten since \( \theta(s'') \) and \( \theta(s' - s'') \) are independent variables:

\[ < D(s)^2 > = 2 \int_0^s \int_s^s < \sin^2 \theta(s'') > < \cos \theta(s' - s'') > \quad > ds' ds'' \tag{17} \]

As previously demonstrated, the tangents along the filament are correlated to the persistence length by this relation:

\[ < C(s) > = < \cos[\theta(s) - \theta(0)] > = \exp\left(-\frac{s}{2L_p}\right) \tag{18} \]

Isambert, et al. showed that by combining the previous two equations and simplifying, a relationship between \( L_p \) and the average square of the transverse fluctuations, \( < [D(s)]^2 > \), can be found [21]:

\[ [D(s)]^2 > = L_p^2 \left[ 2 \frac{s}{L_p} + \frac{16}{3} \exp\left(-\frac{s}{2L_p}\right) - \frac{1}{3} \exp\left(-\frac{2s}{L_p}\right) - 5 \right] \tag{19} \]

In Isambert et al.’s comparison, each fitted curve was analyzed to determine \( C(s) \) and \( [D(s)]^2 \) for each traced contour. The density of points used in the contour-fitting spline was also varied to verify that \( L_p \) was independent of the tracing method. For \( L_p \sim 8-18 \mu m \), the tangent angle and transverse fluctuations methods used in this study yielded values within 5% of one another.

### 1.2.2.5. Semiflexible polymers: bending mode analysis

Semiflexible polymers can be characterized by a bending mode analysis of their thermal fluctuations. The shape \( u(x) \) of a polymer can be written as a Fourier series, assuming one end is pinned:
where $k_n = \frac{n\pi}{L_c}$ is the wave number corresponding to the following bending modes:

- $n = 1$ first bending mode
- $n = 2$ second bending mode
- $n = 3$ third bending mode
  
  .
  .
  .

The curvature $R_c$ can be described by:

$$\frac{1}{R_c(x)} = \frac{d^2 u}{dx^2} = \sum_{n=0}^{\infty} u'_n \sin(k_n x)$$

(21)

For small fluctuations, $x \sim s$:

$$\frac{1}{R_c(s)} = \frac{d\theta(s)}{ds}$$

(22)

Therefore:

$$\theta(s) = \sum_{n=0}^{\infty} \theta_n(s) = \sum_{n=0}^{\infty} a_n \cos \left( \frac{n\pi s}{L_c} \right) = \sum_{n=0}^{\infty} a_n \cos(k_n s)$$

(23)

By treating a polymer as a beam, its bending energy can be described by the following,

where $\left( \frac{d\theta}{ds} \right)_0$ is the static curvature, or curvature at zero temperature.
\[
\frac{\partial E_b}{\partial s} = \frac{1}{2} \kappa_b \left[ \frac{d\theta}{ds} - \left( \frac{d\theta}{ds} \right)_0 \right]^2
\]  

(24)

Integrating over the contour length results in the bending energy, \(E_b\):

\[
E_b = \frac{1}{2} \kappa_b \int_0^{L_c} \left[ \frac{d\theta}{ds} - \left( \frac{d\theta}{ds} \right)_0 \right]^2 ds
\]

(25)

Taking the derivative of \(\theta(s)\) with respect to \(s\) yields:

\[
\frac{d\theta}{ds} = \frac{d}{ds} \left( \sum_{n=0}^{\infty} a_n \cos(k_n s) \right) = \sum_{n=0}^{\infty} \left[ -a_n k_n \sin(k_n s) \right]
\]

(26)

Continuing with the bending energy of a beam:

\[
E_b = \frac{1}{2} \kappa_b \int_0^{L_c} \left\{ \sum_{n=0}^{\infty} \left[ a_n k_n \sin(k_n s) - a_n^0 k_n \sin(k_n s) \right] \right\}^2 ds
\]

(27)

By reducing and applying the equipartition of energy:

\[
\langle E_b \rangle = \frac{1}{2} \kappa_b \sum_{n=1}^{\infty} \left( \frac{mn\pi}{L_c} \right)^2 \langle a_n - a_n^0 \rangle^2
\]

(28)

Finally, each bending mode contributes on average \(\frac{1}{2} k_B T\). Equating \(\frac{1}{2} k_B T\) to equation (28) gives:

\[
\langle a_n - a_n^0 \rangle^2 = \frac{k_B T}{\kappa_b n^2 \pi^2} \left( \frac{L_c}{L_p} \right)^2 = \frac{1}{L_p^2} \left( \frac{L_c}{n\pi} \right)^2
\]

(29)

**1.2.2.6. Semiflexible polymers: secant analysis**

Wang et al. measured HbS fiber segments with a different method more appropriate for higher stiffness filaments termed the secant method [22]. In the secant method, the deviations of the center of a fiber segment from its average position are
measured, and the mean-squared amplitude of the deviations can be used to calculate the bending rigidity $\kappa_b$, or equivalently the persistence length $L_p$. Figure 19 shows the deviations of sickle hemoglobin fibers measured with differential interference contrast (DIC) microscopy, which had persistence lengths varying from 0.24 to 13 mm. Smith et al. also utilized this secant method to measure AFM images of amyloid fibers [23].

![Image](image.png)

Figure 19: Midpoint deviations of sickle hemoglobin fibers measured (a) 15 seconds apart and (b) 12 seconds apart [22].

To derive the secant method, we again begin with the total bending energy of a semi-flexible polymer:

$$E = \frac{\kappa_b}{2} \int_0^{L_c} \left( \frac{\partial^2 u}{\partial z^2} \right) \cdot \left( \frac{\partial^2 u}{\partial z^2} \right) dz$$

(30)
The total bending energy is written in terms of deviations $u$ from the $z$-axis, which connects the two fiber ends. $E$ can be decomposed into $E = E(x) + E(y)$, allowing $u$ to be decomposed into components $u_x$ and $u_y$ to measure fluctuations projected on to the x-z and y-z planes, respectively:

$$E(x) = \frac{\kappa_b}{2} \int_0^{L_C} \left( \frac{\partial^2 u_x}{\partial z^2} \right)^2 dz$$

$$E(y) = \frac{\kappa_b}{2} \int_0^{L_C} \left( \frac{\partial^2 u_y}{\partial z^2} \right)^2 dz$$

For small deviations, the magnitude of the 3D fluctuations can be determined by measuring the projections on to the focal plane, or x-z plane. The fiber shape can then be written in terms of its Fourier components:

$$u_x(z) = \sum_{n=1}^{\infty} a_n \sin \left( \frac{\pi nz}{L_C} \right)$$

Substituting (31) into (33), integrating, and exploiting orthogonality, the energy in each Fourier mode can be found:

$$E(x) = \frac{\pi^4 \kappa_b}{4L_C^3} \sum_{n=1}^{\infty} n^4 a_n^2 = \sum_{n=1}^{\infty} E_n(x)$$

Again utilizing the theory of equipartition of energy on (31) and (34) results in the mean-squared Fourier amplitude:

$$\langle a_n^2 \rangle = \frac{2k_BT L^3}{\kappa_b \pi^4 n^4}$$

The displacement of the projected fiber midpoint $u_x \left( \frac{L_C}{2} \right)$ can be related to the bending rigidity $\kappa_b$. So, the mean-squared amplitude at the midpoint of a fiber can then be found using (34):
Exploiting (35), (36) can be summed and simplified:

\[
\kappa_b = k_B T L_p = \frac{k_B T L_C^3}{48 < \left( u_x \left( \frac{L_C}{2} \right) \right)^2} \quad (37)
\]

Finally, the mean-squared deviation of the midpoint allows the persistence length to be found:

\[
L_p = \frac{L_C^3}{48 < \left( u_x \left( \frac{L_C}{2} \right) \right)^2} \quad (38)
\]

### 1.2.2.7. Force-extension and other techniques

Force-extension data can also be used to determine \( L_p \) by using a Palmer-Boyce approximation of the Mackintosh WLC. Castro et al. performed this type of force-extension analysis [24] (shown in Figure 20) as well as the common bending mode analysis of thermal fluctuations (detailed by Gittes et al. [25]) on two types of amyloid fibers. The force-extension analysis yielded persistence lengths of 1.5 \( \mu \text{m} \) and 3.3 \( \mu \text{m} \), respectively, while the bending mode analysis yielded persistence lengths of 3.6 \( \mu \text{m} \) and 7.0 \( \mu \text{m} \), respectively. The 2- to 2.5-fold difference in \( L_p \) between the two methods was hypothesized to have been caused by nonlinearities in the mechanical response.
Another technique involving AFM was used by Smith et al. to estimate the persistence length of amyloid fibrils [23]. Figure 21 (A) and (C) show contact mode AFM images of fibrils deposited on a patterned gold surface with a groove in it. (B) shows how AFM force spectroscopy was performed on fibrils suspended above the groove, resulting in a characteristic force-distance curve from which mechanical properties could be calculated.
1.2.3. DNA Origami Mechanics

DNA is a biopolymer specifically classified as a polynucleotide, and its mechanics have been studied since the 1960s [8]. The persistence length of dsDNA was first estimated at 50 nm [8], although more recent experiments have shown that it varies between 30 and 80 nm depending on salt concentration and temperature [9] [10]. Single stranded DNA has been measured to have a persistence length ranging from roughly 1-2 nm, making dsDNA significantly stiffer [7]. In fact, the mechanical properties of DNA have been so well-studied that its elastic response is often used to calibrate micro-manipulation experiments [26].

Mechanically, dsDNA can be treated as a rigid cylinder with a modulus of elasticity of approximately 163 MPa. A DNA origami structure can then be treated as a
series of rigid cylinders rigidly attached in some fashion, with some local flexibility or compliance. Bai, et al. investigated the pseudo-atomic structure of a 3D DNA origami object using cryo-Electron Microscopy (cryo-EM) [27]. Their model in Figure 22 shows fine detail of the DNA origami structure, whose helices are coupled only periodically with Holliday junctions. The dsDNA helices are clearly neither perfectly parallel nor rigidly coupled for the entire length of the cross section. However, the Holliday junctions likely provide some coupling of deformation of dsDNA helices to their neighboring helices.
Kauert, et al. used magnetic tweezers to measure the bending and torsional rigidities of 4- and 6-helix bundles with direct mechanical manipulation [28]. Using experimental mechanical properties measured in Figure 23, they were able to accurately model the mechanics of DNA origami structure by using finite-element modeling of simple elastic rods attached with discrete intercylinder connections, simulating Holliday junctions.
1.2.3.1. Theoretical persistence length calculations

The primary focus of this research project is to characterize the bending stiffness of nanoscale DNA origami structures of arbitrary cross section. Specifically, this research aims to assess the rigid coupling assumptions and quantify their accuracy against experimental measurement via the tangent correlations and transverse fluctuations methods. This was evaluated by looking at the effect of cross section on the stiffness of a DNA origami nanofilament. As additional DNA helices are added to a filament, the moment area of inertia is increased, resulting in a stiffer filament that undergoes small thermal deformation.
At the nanoscale, persistence length \( (L_p) \) is analogous to bending stiffness:

\[
L_p = \frac{E_{DNA} I_{DNA}}{k_B T} = \frac{k_b}{k_B T} \tag{39}
\]

Double-stranded B-DNA has diameter within a DNA origami structure is known to be approximately 2.25 nm [4]. From this, the moment area of inertia of a single helix can be approximated:

\[
I_{DNA} = \frac{\pi D^4}{64} = \frac{\pi (2.25 \times 10^{-9} \text{ m})^4}{64} = 1.26 \times 10^{-36} \text{ m}^4 \tag{40}
\]

For individual helices of dsDNA, the persistence length has been estimated previously at 50 nm [8], which can be related to the elastic modulus:

\[
L_p = 5.0 \times 10^{-8} \text{ m} = \frac{E_{DNA} I_{DNA}}{k_B T} \tag{41}
\]

The product of Boltzman’s Constant \( k_B \) and temperature \( T \) is commonly valued at \( k_B T = 4.1 \times 10^{-21} \text{ J per molecule} = 4.1 \text{ pN \cdot nm} \). Using these values and the moment area of inertia calculated previously, the elastic modulus can be calculated:

\[
E_{DNA} = \frac{L_p k_B T}{I_{DNA}} = \frac{(5.0 \times 10^{-8} \text{ m})(4.1 \text{ pN \cdot nm})}{1.26 \times 10^{-36} \text{ m}^4} = 1.63 \times 10^8 \frac{\text{N}}{\text{m}^2} \tag{42}
\]

The persistence length for an arbitrary DNA origami cross section consisting of double-stranded helices can now be estimated by use of the parallel axis theorem, as shown in Figure 24 below for a 6 helix bundle:
Bending stiffness can then be calculated from the persistence length:

\[ B_s = E_{DNA} l_{DNA} = L_p k_B T = (2700 \text{ nm})(4.1 \text{ pN} \cdot \text{nm}) \]
\[ = 1.11 \times 10^{-26} \text{ N} \cdot \text{m}^2 \]  

(44)

Therefore, if the helices are rigidly coupled for the entire length, \( L_p = 2,700 \text{ nm} \). Conversely, if the helices were to be completely uncoupled, the persistence length could be more simply calculated as simply 6 independent dsDNA helices:

\[ L_p^{6\text{hb \ uncoupled}} = 6 \times L_{p,dsDNA} = 6 \times 50 \text{ nm} = 300 \text{ nm} \]  

(45)

In this example of the 6-helix bundle, the maximum persistence length would occur when the helices are rigidly coupled for the entire length, and the minimum persistence length would occur when the helices are completely uncoupled, meaning the experimental measurements of \( L_p \) would be expected to fall in the range of 300 – 2,700 nm.
This research project will compare experimentally characterize persistence length and assembly properties of 4 DNA origami structures. We will compare our experimental results to the theoretical calculations of the persistence length previously discussed.

1.3. Significance of Research

DNA origami is extremely useful in biophysics and biology applications due to the ability to precisely control the geometry of the structure with unprecedented complexity at the nanometer level, as well as functionalize it in very specific locations. The problem with applying it more broadly to fabricate devices for applications in health and chemistry is that the mechanical behavior at length scales above 100 nm have not been explored. DNA origami structures are on the nanometer scale, whereas cells are on the micron scale. By polymerizing DNA origami structures into larger structures, it is hoped that studies can be performed at the level of cells and perhaps even tissues. Zhang, et al. recently created a super-sized DNA origami structure with a synthetic 26,000 base scaffold [29]. However, challenges include the difficulty and cost of fabricating such a long synthetic scaffold, the cost of a greater number of staples needed, and lower yield and lower concentration of the final origami object. Therefore, it is hoped that polymerization can prove to be a valid route to scaling up scaffolded DNA origami.

The process of polymerizing DNA origami structures into micron-scale filaments is also an excellent opportunity to explore some of the oft-made assumptions about the mechanics of DNA origami structures. With current electron microscopy resolutions, it can be difficult to measure deflection of individual DNA origami structures, because their length is generally less than their persistence length. By constructing larger filaments
from smaller, individual structures, there will be greater deflections, which can be more easily measured to quantify the accuracy of the theoretical prediction of persistence length and bending stiffness. By then characterizing the mechanics of these polymers, broader conclusions can be made about the mechanics and structure of other DNA origami objects.

Ultimately, DNA origami is another tool in the biophysics and bioengineering toolbox. Precise geometry can be created with a resolution of several nanometers, and the resulting structures can similarly be functionalized in very specific locations. The ability to scale up DNA origami could lead to novel biomaterials and cellular scale devices.

1.4. Overview of Thesis

Chapter 2 will discuss the experimental design, which assessed the assumption of rigidly coupled helices by designing, fabricating, and imaging DNA origami filaments of varying cross section. This chapter also quantifies the filament growth rate of the polymer. Chapter 3 discusses explorative applications of DNA origami polymerization to create heterogeneous polymers and 2D arrays for material purposes. Chapter 4 concludes the thesis and discusses future work.
Chapter 2: Filament Mechanics and Polymerization Kinetics

This chapter focuses on characterizing the growth of DNA origami polymers over time as a function of number of polymerization staples and the polymerization staple design. The data from the length-characterization was then used to measure the polymer mechanics in terms of persistence length of varying cross-sections.

2.1. Experimental Design

Filaments of four different cross sections, modeled in Figure 25 below, were designed such that they would have dissimilar persistence lengths. The approximate length and theoretical persistence length of each individual structure are summarized in Table 2 below.

<table>
<thead>
<tr>
<th>Cross Section</th>
<th>Approximate Length (nm)</th>
<th>Theoretical Persistence Length (nm)</th>
<th>Scaffold Strand Length (# of base pairs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-helix bundle</td>
<td>420.0</td>
<td>300-2,700</td>
<td>7560</td>
</tr>
<tr>
<td>12-helix bundle</td>
<td>203.0</td>
<td>600-12,600</td>
<td>7308</td>
</tr>
<tr>
<td>18-helix bundle</td>
<td>140.0</td>
<td>900-23,400</td>
<td>7560</td>
</tr>
<tr>
<td>flat 18-helix bundle</td>
<td>149.3</td>
<td>900-9,300 or 92,900</td>
<td>8064</td>
</tr>
</tbody>
</table>
Figure 25: (A) From left to right, solid models of the 6-, 12-, 18-, and flat 18-helix bundles. (B) From top to bottom, top view of the 6-, 12-, 18-, and flat 18-helix bundles. (C) From left to right, the cross sections of the 6-, 12-, 18-, and flat 18-helix bundles.

As is seen in Table 2, the actual length of a 6-helix bundle would be approximately 401.9 nm, which is significantly less than its maximum theoretical persistence length of 2,700 nm, as calculated via the rigid coupling model explained in 1.2.3.1. As additional helices are used in the cross section, the individual bundle length
decreases and the theoretical persistence length increases due to the increasing moment area of inertia. The 6-, 12-, and 18-helix bundles were designed to explore the polymer mechanics of DNA origami filaments by measuring 3 cross-sections expected to have dissimilar persistence lengths.

The flat 18-helix bundle was created to determine the effect of various polymerization staple designs on polymerization rate and assembly yield. Table 3 shows the four versions of the flat 18 helix bundle, which vary the length of the polymerization toehold as well as the configuration of the neighbor staples. There are three toehold lengths: 5, 8, and 11 bases. There are also two versions with 5 base toeholds: a “closed” neighbor staple that has an additional cross-over next to the toeholds, and an “open” neighbor staple that lacks this cross-over. With the flat 18-helix bundle, the “closed” variant adds a total of 9 additional cross-overs at the polymerization joint over the “open” variant. These additional cross-overs further constrain the helices

<table>
<thead>
<tr>
<th>Configuration</th>
<th>Polymerization Toehold</th>
<th>Staple Routing</th>
</tr>
</thead>
<tbody>
<tr>
<td>5bp closed neighbor</td>
<td>5 bases</td>
<td></td>
</tr>
<tr>
<td>5bp open neighbor</td>
<td>5 bases</td>
<td></td>
</tr>
<tr>
<td>8bp open neighbor</td>
<td>8 bases</td>
<td></td>
</tr>
<tr>
<td>11bp open neighbor</td>
<td>11 bases</td>
<td></td>
</tr>
</tbody>
</table>

By varying the length of the polymerization toehold, we investigated how the relative stability of the toeholds impacts growth rate. The idea is that the toehold has a
low melting point far below the polymerization temperature, such that a single toehold by itself will be unstable, but several together will be stable. Table 4 shows an example of the stability of different segments of a polymerization staple. Figure 26 shows the distribution of the melting temperature of the 5-base, 8-base, and 11-base polymerization toeholds. Ideally, a single toehold should be unstable on its own so as to avoid the situation depicted in Figure 27, where binding sites are blocked by stable toeholds and the structures are unable to polymerize. It is desirable for a single toehold to be unstable on its own, but that 6 or more toeholds together will be stable, allowing for successful polymerization. By looking at the melting temperature of the toeholds, it is clear that the 5-base toeholds have an average melting temperature below 10°C and will be individually very unstable when polymerized at a higher temperature of 40°C.

Table 4: Example of stability of an entire polymerization staple, its non-toehold region, and its toehold region.

<table>
<thead>
<tr>
<th></th>
<th>Sequence/length</th>
<th>Melting Temperature (°C)</th>
<th>Stability @ 40°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polymerization</td>
<td>ACGTTATTCTACTTTGATTGTGAGACAATGCTACCTTACG</td>
<td>71.2</td>
<td>stable</td>
</tr>
<tr>
<td>staple</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-toehold</td>
<td>ATTCTACTTTGATTGTGAGACAATGCTACCTTACG</td>
<td>69.5</td>
<td>stable</td>
</tr>
<tr>
<td>region</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toehold</td>
<td>ACGTT</td>
<td>&lt; 10.0</td>
<td>unstable</td>
</tr>
<tr>
<td>region</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 26: Melting temperature calculated by IDT Oligo Analyzer at 10 nM oligo concentration, 5 mM Na⁺ concentration, and 10 mM Mg⁺⁺ concentration [30].

Figure 27: An example of how polymerization toeholds that are stable at high temperatures can block the binding sites of other polymerization staples.

However, the 11-base toeholds have an average melting temperature in the 35 - 40°C range, meaning 1 or 2 toeholds may be stable on their own, potentially creating the undesirable situation in Figure 27. The 8-base toeholds lie somewhere in between with an average melting temperature of 18°C. By comparing the growth rate of these
polymerization staple designs, the optimal design techniques will hopefully be determined.

2.2. Methods

Once TEM images of the polymerized filaments were captured, they were analyzed to determine the length and persistence length. It is unclear what sort of forces are applied when depositing a structure on the surface of a TEM grid. It has been shown that carbon-film TEM grids are smooth and homogeneous down to the nm-region [31]. Lin, et al. used a combination of AFM and TEM imaging in parallel to investigate the ultrastructure of fibrous long spacing collagen [32]. They determined that the height of periodic filament ridges, measured with AFM before and after negative staining, changed only several nanometers due to negative staining. Therefore, it was assumed that there were minimal surface effects on the filaments negatively stained on TEM grids and that their thermal fluctuations were constrained to two dimensions. Consequently, persistence length was calculated by use of the 2D tangent angle correlations previously derived:

$$\cos(\theta_s - \theta_0) = e^{-\frac{s}{2\pi p}}$$  (46)

The calculations were performed by tracing a filament with a MATLAB script, which was then discretized into a cubic spline, as shown in Figure 28 below. The cosine correlation was calculated for segment lengths ranging from zero up to the full contour length. For each segment length, a sliding window across the length of the filament was used to obtain multiple data points, eventually calculating the average persistence length over the entire length of the filament.
Because the persistence length of the filaments was expected to be fairly low, it was also calculated via the transverse fluctuations method previously outlined by Isambert, et al. in order to verify the results.

### 2.2.1. Tracing error

To quantify the error of the two persistence length calculation methods due to manual tracing of filaments, two images were repeatedly traced using a new set of manually picked points each time. Table 5 shows the deviations for each image; the measurements for contour length and persistence length via the tangent correlations method each had an error of less than 1%. This indicates that human error did not contribute any significant error to the calculation of persistence length.
Table 5: Summary of repeated tracings of single filaments to quantify error.

<table>
<thead>
<tr>
<th>Sample Image</th>
<th>N</th>
<th>Mean $L_c$ (nm)</th>
<th>Mean $L_p$ tangent correlations (nm)</th>
<th>Mean $L_p$ transverse fluctuations (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flat 18hb #1</td>
<td>10</td>
<td>4605 ± 20.3</td>
<td>4065 ± 41.5</td>
<td>1469</td>
</tr>
<tr>
<td>6hb #1</td>
<td>10</td>
<td>3851 ± 8.6</td>
<td>249.0 ± 0.84</td>
<td>500.5</td>
</tr>
</tbody>
</table>

2.3. Filament Growth Results and Discussion

This section contains the results of tracing the 6-helix bundle polymers and 4 variants of the flat18-helix bundle polymers at various time intervals. The flat 18-helix bundles were all polymerized from the same gel-purified stock so that the initial monomer concentrations would all be equivalent.

Each variant had samples incubated at 37°C for 3, 6, 9, 12, and 24 hours to characterize the filament length as a function of time. TEM grids of each sample were imaged at least 50 filaments were traced for each sample and timepoint. Each structure has three figures of results. The first figure shows a plot of all the filaments traced at each time point. The initial trajectories of all filaments were aligned to start at the origin and point in the same direction. The second figure shows a histogram of the lengths of the filaments traced at each time point. The histograms are created with bin sizes equal to the length of a single monomer and centered on integer multiples of a single monomer to illustrate the distribution of monomers, dimers, trimers, and longer polymers. The third figure normalizes each of these histograms by the total number of filaments traced at that time point and then compares them all on one plot. A second plot uses a 5-point moving average to smooth the data to better illustrate the length distributions.
2.3.1. 6hb traces

Figure 29: Traces of the 6-helix bundle with aligned initial tangents at 3, 6, 9, and 24 hours.
2.3.2. 6hb length distribution histograms

Figure 30: Length distributions of the 6-helix bundle with aligned initial tangents at 3, 6, 9, and 24 hours.
Figure 31: (top) Comparison of normalized length distributions of the 6-helix bundle as a function of time (bottom) smoothed with 5-point moving average. Lines between discrete points are for visual aid.
2.3.3. Flat 18hb 5bp closed traces

Figure 32: Traces of the 5-base closed configuration of the flat 18-helix bundle with aligned initial tangents at 3, 6, 9, 12, and 24 hours.
2.3.4. Flat 18hb 5bp closed length distribution histograms

Figure 33: Length distributions of the 5-base closed configuration of the flat 18-helix bundle at 3, 6, 9, 12, and 24 hours.
Figure 34: (top) Comparison of normalized length distributions of flat 18hb 5bp closed neighbor as a function of time (bottom) smoothed with 5-point moving average (bottom inset) zoomed in on y-axis of 0-5%. Lines between discrete points are for visual aid.
2.3.5. Flat 18hb 5bp open traces

Figure 35: Traces of the 5-base open configuration of the flat 18-helix bundle with aligned initial tangents at 3, 6, 9, 12, and 24 hours.
2.3.6. Flat 18hb 5bp open length distribution histograms

Figure 36: Length distributions of the 5-base open configuration of the flat 18-helix bundle at 3, 6, 9, 12, and 24 hours.
Figure 37: (top) Comparison of normalized length distributions of flat 18hb 5bp open neighbor as a function of time (bottom) smoothed with 5-point moving average (bottom inset) zoomed in on y-axis of 0-5%. Lines between discrete points are for visual aid.
2.3.7. Flat 18hb 8bp open traces

Figure 38: Traces of the 8-base open configuration of the flat 18-helix bundle with aligned initial tangents at 3, 6, 9, 12, and 24 hours.
2.3.8. Flat 18hb 8bp open length distribution histograms

Figure 39: Length distributions of the 8-base open configuration of the flat 18-helix bundle at 3, 6, 9, 12, and 24 hours.
Figure 40: (top) Comparison of normalized length distributions of flat 18hb 8bp open neighbor as a function of time (bottom) smoothed with 5-point moving average (bottom inset) zoomed in on y-axis of 0-5%. Lines between discrete points are for visual aid.
2.3.9. Flat 18hb 11bp open traces

Figure 41: Traces of the 11-base open configuration of the flat 18-helix bundle with aligned initial tangents at 3, 6, 9, 12, and 24 hours
2.3.10. Flat 18hb 11bp open length distribution histograms

Figure 42: Length distributions of the 11-base open configuration of the flat 18-helix bundle at 3, 6, 9, 12, and 24 hours.
Figure 43: (top) Comparison of normalized length distributions of flat 18hb 11bp open neighbor as a function of time (bottom) smoothed with 5-point moving average (bottom inset) zoomed in on y-axis of 0-5%. Lines between discrete points are for visual aid.
2.3.11. Comparison of cross-sections

Figure 29 through Figure 43 show the raw data collected to characterize the growth rate of the 6-helix bundle and four versions of the flat 18-helix bundle. The To compare the effects of total toehold binding energy (6-helix bundle vs flat 18-helix bundle) as well as toehold length and neighbor staple configuration (flat 18-helix bundle variants), all 5 filaments were compared after 24 hours. Figure 44 shows all of the traces on the same scale, and Figure 45 compares the length distributions of the 24-hour traces. It is immediately clear from these plots that the 11-base toehold flat 18-helix bundle has little success polymerizing, barely exceeding a maximum length of 1 micron even after 24 hours. In contrast, the two 5-base versions of the flat 18-helix bundle have clearly grown the most, with the longest filaments reaching 6 – 8 microns in length. The 6-helix bundle and 8-base flat 18-helix bundle had more moderate success, reaching maximum lengths in the 4 – 5 micron range.

Although we have shown it is possible to make long filaments, there is still a large distribution of lengths, and currently there is no good way to control the distribution of lengths. Future work will investigate methods like nanochannels sortation to control the assembly of DNA origami polymers.
Figure 44: Comparison of traces of each filament after 24 hours.
2.3.1. Discussion

To compare the growth rates of each design, the average length of a filament as a function of time was plotted in Figure 46 in terms of average absolute length (nanometers) as average number of mers. This was achieved by calculating the average length of a particular filament at each time point from the data in sections 2.3.1 through 2.3.10.
Figure 46: Comparison of growth rate of each filament in terms of (top) average length and (bottom) average number of mers. Lines between discrete points are for visual aid.
To see if growth rate is proportional to the total base-pairing energy of the polymerization toeholds, the initial growth rate was calculated by dividing the average number of mers in a filament after 12 hours by the number of hours that had elapsed since each trace appeared to be approximately linear over this timescale. The initial growth rate for the 6-helix bundle was calculated after 9 hours had elapsed. Although the flat 18-helix bundle variants all had the same initial concentration of monomers, the initial concentration of the 6-helix bundle may have differed from that of the flat 18-helix bundle, which would also affect the initial growth rate. With that caveat, a comparison of the initial growth rates as well as the total binding energy of the polymerization toeholds in summarized in Table 6 below.

<table>
<thead>
<tr>
<th>Design</th>
<th>Toehold Length (bases)</th>
<th>Initial Growth Rate (mers/hr)</th>
<th>Total Binding Energy (kcal/mole)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6hb</td>
<td>5</td>
<td>0.20</td>
<td>48.74</td>
</tr>
<tr>
<td>flat 18hb 5bp closed</td>
<td>5</td>
<td>0.52</td>
<td>138.43</td>
</tr>
<tr>
<td>flat 18hb 5bp open</td>
<td>5</td>
<td>1.03</td>
<td>138.43</td>
</tr>
<tr>
<td>flat 18hb 8bp open</td>
<td>8</td>
<td>0.34</td>
<td>245.45</td>
</tr>
<tr>
<td>flat 18hb 11bp open</td>
<td>11</td>
<td>0.11</td>
<td>350.17</td>
</tr>
</tbody>
</table>

By comparing the 6-helix bundle to the flat 18-helix bundle, we are exploring how the number of polymerization staples affects length. The flat 18-helix bundle 5bp open variant has roughly 3 times as much total polymerization binding energy as the 6-helix bundle, and its initial growth rate is roughly 5 times higher.
With the 18-helix bundle variants, we explored the design of the polymerization staples in order to optimize polymerization. It is clear that 11-base toeholds have a severe limiting effect on polymerization, whereas 5-base toeholds are superior to 8-base toeholds. By comparing the two 5-base variants, it appears that “open” neighbor staples with no Holliday junction are better than “closed” neighbor staples with a Holliday junction in terms of initial growth rate. It is unclear if they converge to similar average lengths after a long time (>24 hrs).

2.3.2. Polymerization at longer time scales

To investigate the effects of polymerizing at time scales beyond 24 hours, the 8-base and 11-base variants of the flat 18-helix bundle were allowed to incubate and polymerize for 114 hours. Figure 47 compares each variant at 24 hours and 114 hours. Although these filaments were not quantitatively characterized, qualitatively, they typical length of a polymer was on the same order of magnitude after 114 hours as after 24 hours. This suggests that perhaps the supply of free monomers in solution has become sufficiently depleted such that the time it takes an individual structure to randomly diffuse to an existing filament is effectively infinite. As expected, the version with the 11 base toehold continued to remain largely unpolymerized even after nearly 5 days of polymerization conditions, validating the hypothesis that long polymerization toeholds are a poor design.
2.3.3. Temperature effects on polymerization

In light of the differences in polymerization rate of the polymerization toehold designs, it would be prudent to characterize growth rate as a function of the temperature
of the polymerization reaction. For this thesis, polymerization reactions were only run at 40°C. Future work will characterize growth rate in the span of perhaps 5 – 45°C.

To see if polymerization could be prevented or greatly slowed, a sample of the 8-base variant of the flat 18-helix bundle was refrigerated with polymerization staples at 4°C for 5 weeks. TEM imaging in Figure 48 showed that the monomers remained largely unpolymerized, indicating that storage at low temperature significantly slows down the polymerization. Beyond this, it is not clear how temperature-dependent the polymerization rate is within the critical window between the melting temperature of the polymerization staples and the regime where the origami structure begins to become disrupted.
Figure 48: Flat 18-helix bundles that were polymerized at 4°C for 5 weeks. Even after a very long period of time, they remain largely unpolymerized. The longest polymers are typically dimers or trimers.
2.4. Filament Persistence Length Results and Discussion

To measure the persistence length of the various DNA origami polymers, the traces of the 6- and flat 18-helix bundle filaments from the polymerization analysis were analyzed from a mechanics perspective. Additionally, the 12- and 18-helix bundles were analyzed to provide more cross-section data points.

Filament traces longer than the threshold of 1000 nm were used to measure the persistence length via both the tangent correlations and transverse fluctuations methods. A shorter threshold of 700 nm was used for the 12-helix bundle due to initial difficulties with polymerization.

The transverse fluctuations method looks at the entire population to determine persistence length. As length increases, the population shrinks, leading to sharp, discontinuous jumps in the plots of mean square transverse fluctuations. To determine $L_p$ from this method, the data was only fit over the smooth, continuous segment of the mean square transverse fluctuations curve, so as to calculate an accurate value for $L_p$. 
2.4.1. 6hb persistence length measurements

Figure 49: 6-helix bundle: Traces of filaments longer than 1000 nm, length distribution, persistence length distribution from tangent correlations method, and persistence length from transverse fluctuations method.
2.4.2. Flat 18hb persistence length measurements

Figure 50: Flat 18-helix bundle: Traces of filaments longer than 1000 nm, length distribution, persistence length distribution from tangent correlations method, and persistence length from transverse fluctuations method.
2.4.3. 12hb persistence length measurements

Figure 51: 12-helix bundle: Traces of filaments longer than 700 nm, length distribution, persistence length distribution from tangent correlations method, and persistence length from transverse fluctuations method.
2.4.4. 18hb persistence length measurements

Figure 52: 18-helix bundle: Traces of filaments longer than 1000 nm, length distribution, persistence length distribution from tangent correlations method, and persistence length from transverse fluctuations method.
2.4.5. Discussion

Figure 49 through Figure 52 show all the raw data for the persistence length measurements of the 6-, 12-, 18-, and flat 18-helix bundle filaments. There is a large distribution of persistence lengths calculated from the tangent correlations for each cross section. There are some measurements which exceed the theoretical maximum. However, these are based on a single snapshot of the filament in time, and the filament may just happen to land in a straight configuration on the grid. Furthermore, it is possible that artifacts due to either some surface effects or the process of staining the filaments to a TEM grid introduce external forces in addition to the thermal fluctuations. These outliers skew what may otherwise look more like a Boltzmann distribution, which is what would be expected when looking at average thermal fluctuations using Boltzmann statistics. Additionally, the mechanical properties of DNA origami structures will vary to some degree, depending on how well they were folded. Any defects or misfolding could be responsible for some of the variance in the distribution.

The transverse fluctuations method was on the same order of magnitude as the tangent correlations method, but they did not agree as well as Isambert, et al. experienced [21]. However, that comparison was performed using a fluorescence assay on one or two individual filaments. Since the transverse fluctuations method was derived to look at population consisting of tens of traces of thermal fluctuations of a single filament, it is possible that applying the method to a population consisting of single traces of tens of static filaments with different lengths is the cause of the discrepancy.
With those caveats, to compare the mechanics of DNA origami filaments of different cross section, Table 7 summarizes the persistence length results for the 4 different designs.

Table 7: Summary of length and persistence length measurements of various filaments.

<table>
<thead>
<tr>
<th>Filament</th>
<th>N</th>
<th>Mean</th>
<th>Max</th>
<th>Min</th>
<th>Max</th>
<th>Tangent Correlations (Mean)</th>
<th>Transverse Fluctuations</th>
</tr>
</thead>
<tbody>
<tr>
<td>6hb</td>
<td>77</td>
<td>1705 ± 630.8</td>
<td>3705</td>
<td>300</td>
<td>2700</td>
<td>2374 ± 3467</td>
<td>1223</td>
</tr>
<tr>
<td>12hb</td>
<td>64</td>
<td>1021 ± 271.6</td>
<td>2029</td>
<td>600</td>
<td>12600</td>
<td>6216 ± 9123</td>
<td>2628</td>
</tr>
<tr>
<td>flat 18hb</td>
<td>343</td>
<td>2371 ± 1224</td>
<td>7192</td>
<td>900</td>
<td>9300/72900</td>
<td>9669 ± 13033</td>
<td>3917</td>
</tr>
<tr>
<td>18hb</td>
<td>78</td>
<td>1829 ± 873.7</td>
<td>5090</td>
<td>900</td>
<td>23400</td>
<td>18284 ± 16167</td>
<td>4674</td>
</tr>
</tbody>
</table>

Taking the data from Table 7, Figure 53 plots persistence length as a function of moment of inertia for the maximum theoretical predicted values, the tangent correlations experimental values, and the transverse fluctuations experimental values. Since the 6-, 12-, and 18-helix bundles all have an aspect ratio near 1, a linear regression was fit to the experimental data from both methods. The flat 18-helix bundle has a much higher aspect ratio and therefore two dissimilar moments of inertia, so its data was not included in the linear fit.

As expected, the experimental values are significantly below the theoretical maximum. By revisiting in Figure 54 the work of Bai, et al. to model the pseudoatomic structure of DNA origami, it makes sense for the experimental results to be much lower.
than the completely coupled maximum. From their model it is evident that origami structures are loosely coupled and individual helices can move relative to each other at least locally. And although larger cross sections like the 18-helix bundle are stiffer than smaller ones like the 6-helix bundle, local fluctuations may still occur in stiffer designs in the regions between the connective cross-overs. Based on these results, it would be possible to define an efficiency factor or an effective elastic modulus for DNA origami structures of arbitrary cross section. From the slope of the curve fit lines in Figure 53, the effective elastic modulus was calculated to be 126 MPa based on the tangent correlations method and 27.2 MPa based on the transverse fluctuations method. These two values are 77.3% and 16.7% of the elastic modulus for dsDNA (163 MPa), respectively.
Figure 53: (Top) Plot of persistence length as a function of moment of inertia for theoretical values, tangent correlations experimental values, and transverse fluctuations experimental values. Lines of best fit are for 6hb, 12hb, and 18hb (aspect ratio near 1). The flat 18hb has a much higher aspect ratio and therefore two dissimilar moments of inertia. (Bottom) A plot of the same data focusing on just the 6hb, 12hb, and 18hb.
Figure 54: Two views of the pseudoatomic model of a DNA origami structure, clearly showing that helices are neither perfectly parallel nor coupled over the entire length [27].
Chapter 3: Applications of Polymerization

Chapter 2 illustrated that polymerization of DNA origami nanostructure is an effective means to achieve mesoscale structures. This chapter now explores preliminary, exploratory work towards two different applications of mesoscale structures: 1) a cross-hinge structure that can be polymerized in 2D and is capable of structural reconfiguration for sensing molecular binding, and 2) an extended caliper structure to couple microscale manipulation to a nanoscale DNA origami device. These two applications involve extensions of the work performed in the previous chapter, namely 2D polymerization for the cross-hinge application, and heterogeneous polymerization (i.e. polymerization with different DNA origami structures) in the case of the extended calipers. Johnson-Buck, et al. recently created a 2D DNA-origami-based nanopegboard [34], which provides further inspiration for creating 2D arrays of DNA origami.

3.1. 2D Polymerization: Cross-hinge

Periodic 2D DNA origami arrays have been created from rigid DNA origami tiles, as seen in Figure 56 [35]. Drawing on this design as inspiration, we sought to create a configurable 2D array of origami whose conformation can be changed by adding oligos and creating binding events.
The cross-hinge is a structure designed with two 10-helix bundles connected by a single-stranded scaffold connection to create a 1 degree-of-freedom joint. It also has overhanging staples, which are partially bound in the body of the structure and partially single-stranded, hanging off the structure. A mockup of the overhanging staples is shown in Figure 55. These overhang staples can be used to create connections between different points on the structure with additional piecewise complementary staples. Depending on the overhang locations that are connected, different rigid conformations can be achieved.

Figure 55: A model of the cross-hinge with overhanging staples depicted in red.
3.1.1. Cross-hinge Conformations

The cross-hinge’s conformation can be controlled by adding staples in solution to bind to staples hanging off the structure, which then either “lock” it closed or open, depending on the design. Figure 57 shows a model of the cross-hinge locked in the open
conformation with overhanging staples. In the absence of these staples, the cross-hinge is free to thermally fluctuate between the open and closed conformations.

Figure 57: CanDo rendering of cross-hinge locked in the open conformation.

Table 8 compares the conformation distribution of the cross-hinge by counting instances on TEM images of the structure free to fluctuate, locked closed, and locked open. Adding the closing staples clearly forces the structure to go from roughly 10% closed to roughly 90% closed, which is highly efficient. Although data has not been collected on the locked-open version, it is hoped that similar efficiencies can be achieved. By demonstrating that the conformation can reliably be controlled on the monomer-scale, it is hoped that an entire array of cross-hinges can also be reliably forced into the desired conformation.
### Table 8: Conformation control of cross-hinge structure.

<table>
<thead>
<tr>
<th></th>
<th>Open</th>
<th>Transition</th>
<th>Closed</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Free</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(N = 156)</td>
<td>51.3%</td>
<td>39.1%</td>
<td>9.6%</td>
</tr>
<tr>
<td><strong>Locked</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Closed</strong></td>
<td>3.4%</td>
<td>6.7%</td>
<td>89.9%</td>
</tr>
<tr>
<td><strong>Locked</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Open</strong></td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

#### 3.1.2. Cross-hinge Polymerization

The cross-hinge was polymerized in order to create a 2D array with different possible conformations. Figure 58 shows the initial attempts to polymerize with no constraints on the conformation of the individual cross-hinge monomers. It can be seen that 3 types of polymerization are occurring when the structure is free to thermally fluctuate from open to closed and anywhere in between. When the cross-hinge
polymerizes while in the closed conformation, it simply polymerizes linearly like the 1D filaments characterized in Chapter 2. When it polymerizes while in the transition or open conformations, it does form the basis of an ordered 2D array. To achieve the well-ordered arrays made from static DNA origami structures [35], this undesirable mixed-mode polymerization must be mitigated.

Several approaches were taken to promote purely orthogonal polymerization. Figure 59 shows TEM images of cross-hinges polymerized initially with only one set of polymerization staples, resulting in 1D filaments with the bottom cross-hinge member able to freely rotate. However, once the second set of polymerization staples were added to connect these 1D filaments together, the same previously seen mixed-mode polymerization occurred.
Figure 58: (a) Free polymerization of the cross-hinge, resulting in undesired mixed-mode polymerization. Polymerization with (b) orthogonal 2D propagation, (c) intermediate 2D propagation, and (d) aligned 1D propagation.
The second approach was to add short, stiff staples to lock the cross-hinge in the open conformation before polymerization, as depicted earlier in Figure 57. By temporarily converting the cross-hinge into a static structure with its two members orthogonally oriented, similar success in creating a well-ordered array should be achieved. Although these structures have not yet been polymerized, it is hoped that once the 2D arrays have been created, the locking strands bound to the overhangs can be removed via strand displacement, again allowing each cross-hinge to fluctuate between conformations. At this point, we will have created a 2D array capable of structural reconfiguration, which can be functionalized to sense molecular binding.
3.2. Heterogeneous Polymerization: Extended Caliper

The extended caliper is a micron-scale structure constructed from 4 different smaller structures, designed to test actuation methods and heterogeneous polymerization. The extended caliper, shown in Figure 60, consists of a basic hinge that can open and close like a macroscale caliper. The basic caliper design is made possible by 3 single-stranded DNA connections between the two stiff dsDNA bundles which form the arms of the caliper. Table 9 depicts the multiple components and stages of heterogeneous polymerization.

The extended calipers will ultimately be implemented in a magnetic trap experimental assay. The experimental assay involves immobilizing the extended calipers by the short arm to a surface via a digoxigenin (DIG) attachment to an anti-digoxigenin (anti-DIG) coated surface. One arm of the caliper is functionalized with 5 DIG molecules, which will form a strong DIG-antiDIG bond and anchor the entire structure to the surface. The “endcap” monomer is functionalized with biotin, which will form an extremely strong bond with streptavidin-coated magnetic beads. The magnetic bead can then be manipulated with magnetic tweezers, moving the attached structure as well. The initial experiments involved rolling the magnetic bead in arcs to see if its motion was successfully constrained by the anchored extended caliper. However, the experiments have been inconclusive to this point.
Figure 60: Schematic of the extended caliper, which is polymerized from 4 distinct substructures.
Table 9: The multiple components and stages of heterogeneous polymerization.
As seen at the end of Table 9, these heterogeneous polymers were successfully created, with typical arm lengths of 1 – 1.5 μm. Similar to with the homogeneous polymers, there was a distribution of lengths. Additionally, the experimental assay encountered difficulties with ensuring that the hinge properly attached to both the surface and the magnetic bead. However, it is hoped that these problems will be resolved and the extended caliper can be used to demonstrate micromanipulation of larger DNA origami structures with magnetic tweezers. Since the cross-section is similar to the flat 18-helix bundle, it is expected that the effective persistence length would also be on the order of a few microns. With a length of 1 – 1.5 μm and a persistence length of a few microns, it should be possible to treat the extended arm of the calipers as fairly rigid. If this proof-of-concept succeeds, it may also be possible to change the conformation of the 2D array of cross-hinges using micromanipulation instead of relying on solely binding events.
Chapter 4: Conclusions and Future Work

This thesis fulfills a fundamental part of the attempts to characterize and better understand DNA origami structures. In terms of scaling up DNA origami, the polymerization and growth rate of 1D filaments have been characterized as functions of time, polymerization toehold length, and total binding energy of polymerization toeholds. It is clear that short toeholds and more binding sites optimize polymerization. The results also suggest that if the initial concentration of monomers could be increased, then the growth rate and maximum length could also be increased. It was also shown that there is promise in creating both large heterogeneous DNA origami structures as well as configurable 2D DNA origami arrays. In general, polymerization seems to be a viable approach to scaling up DNA origami to make micron-scale material systems.

In terms of understanding the structure of DNA origami objects and their resulting bending stiffness, the persistence length has been measured for a series of DNA origami structures. Other than the measurement by Kauert, et al. of the 6-helix bundle, persistence length has not really been explored for DNA origami. The measurements of the 6-, 12-, and 18-helix bundles in addition to an even stiffer cross section would allow for calculation of an efficiency factor or effective elastic modulus for DNA origami structures of arbitrary cross section. Lastly, it also appears that polymerization staple design does not seem to affect overall mechanical properties of DNA origami polymers.
4.1. Future Work

4.1.1. Additional Cross Sections

We intend to extend the mechanics work done so far by studying an additional, very stiff 56-helix cross section that is approximately 43 nm long, depicted in Figure 61. The theoretical range of persistence lengths would be expected to be 2800 – 328,000 nm, providing an additional data point for calculating an effective elastic modulus.

Figure 61: Cross section of the tubular 56-helix bundle.
4.1.2. Single-Molecule Fluorescence

To verify the assumption that the filaments were constrained to 2D during TEM imaging, some fluorescence assays have been performed on a Total Internal Reflection (TIRF) fluorescence microscope. Filaments were mixed with the intercalating dye YOYO-1, which only becomes fluorescent when intercalated between the base pairs of dsDNA. A fluorescently-labeled 6-helix bundle can be seen in Figure 62. Some preliminary bending mode analyses similar to those done by Castro, et al. [24] have been performed, although first indications are that DNA origami filaments are too flexible to be reliably measured with this method with low-resolution fluorescence microscopy. It is hoped that stiffer cross sections can more reliably be automatically skeletonized and analyzed.

Figure 62: 6-helix bundles fluorescently-labeled with the intercalating dye YOYO-1.
4.1.3. dsDNA-RecA Complex

RecA is a protein associated with DNA repair, which intercalates between the nucleobases of a dsDNA helix and both elongates it to form a dsDNA-RecA complex, shown in Figure 63. Force-extension experiments show that dsDNA-RecA complex filaments have a much greater persistence length than dsDNA filaments alone [36] [37]. RecA lengthens dsDNA by a factor of 1.5 and increases its persistence length by a factor of 10. To date, no experiments have been performed to see the effects of RecA on either DNA origami structures or DNA origami polymers. Due to the extensive work done already on DNA origami polymer mechanics in this thesis, they are a prime subject to evaluate the stiffening effects of RecA on DNA origami structures. It is hoped that the dsDNA-RecA complex can be used to improve or tune the mechanical properties of DNA origami structures.
Figure 63: AFM image of partly RecA-coated DNA molecules [38].
Bibliography


Appendix A: CaDNAno Structure Design

A.1: 6-helix bundle
A.2: 12-helix bundle
A.3: 18-helix bundle
A.4: flat 18-helix bundle
A.5: Cross-hinge
A.6: Extended-caliper
Appendix B: Matlab Code

B.1: Tracing Code “Lp_CosCorr_TracePath.m”

% Adapted by Danny Turowski
% last modified 5-17-2013
% determine persistence length from EM images
% trace paths by hand and fit a spline to shape of filament to
determine arc length versus tangent angle
% takes out first and last point of traced path to avoid problems with
% slopes at the edge

if exist('reuse_image','var')==1
    disp('
Reusing previous image.
')
    % if the image is being reused, let's keep certain variables and
skip the previous steps
    clc, close all, prev_fname=fname;
    keep im pix_size pathname filename Im fname prev_fname r
    [rI cI]=size(Im);
    xI=[1 cI];
    yI=[1 rI];
    scrsz = get(0,'ScreenSize');
    figure(1)
    plot(xI,yI,'ro')
else % otherwise, nuke everything
    disp('
Selecting a new image.
')
    clc, close all, clear all
    [filename, pathname, filterindex] = uigetfile('*TIF','Select Image File for Analysis');
    Im_fname=strcat(pathname,filename)
    Im(:,:,)=imread(Im_fname);
    Im_inf=imfinfo(Im_fname);

    % Display Image
    [rI cI]=size(Im);
    xI=[1 cI];
    yI=[1 rI];
    scrsz = get(0,'ScreenSize');
    figure(1)
    plot(xI,yI,'ro')

    % Automatically set pixel size based on scale bar cropped from main img
    fprintf('Crop the scale bar. It will search for black pixels row-by-
row, beginning from the top.
')
    scalebar=imcrop(Im);,hold on
[sbheight sbwidth]=size(scalebar);
sbleft=[ ]; sbright=[ ];
while isempty(sbleft) && isempty(sbright)
    for h=1:sbheight
        for w=2:sbwidth
            if scalebar(h,w)==0 && scalebar(h,w-1)~=0 && isempty(sbleft)
                sbleft=w; % found the left edge
            elseif scalebar(h,w)~=0 && scalebar(h,w-1)==0 && isempty(sbright)
                sbright=w; % found the right edge
                break % we're done here
            end
        end
        if h==sbheight && isempty(sbleft) && isempty(sbright)
            disp('Could not find scale bar')
            return
        end
    end
end

d_sc=input('What is the length of the scale bar (nm)? ');
pix_size = d_sc/abs(sbright-sbleft); % pixel size in nm/pix
fprintf('Pixel size is %f nm/pixel
',pix_size)
end

%%
scnsize = get(0,'ScreenSize');
hFig = figure('ToolBar','none','Menubar','none');
hIm = imshow(Im); % this is probably redundant
hSP = imscrollpanel(hFig,hIm);
set(hFig,'Units','normalized','Position',[0 0.05 0.8 0.9])
gcf, hAxes=gca
hOverview=imoverview(hIm);
gcf, gca
set(hOverview,'Units','pixels','Position',[scnsize(3)-250 scnsize(4)-300 200 200])
api = iptgetapi(hSP);
api.setMagnification(1) % 2X = 200%
hold on

if exist('reuse_image','var')==1 % let's go to the previous position
    api.setVisibleLocation(r)
end
k=[];
n=1;
fprintf('Press enter to continue selecting points or type in 0 to stop: \n');
fprintf('Left click to continue. Right click to stop selecting points. \n');
fprintf('Click a mouse button to continue. Press a key to stop selecting points. \n');
while isempty(k)
    %fprintf('n = %d',n)
nn(n)=n;
[x(n),y(n)]=ginput(1);

hIm = imshow(Im);
plot(x,y,'ro')

% if length(x)>=3
xp=spline(nn,x,np);
yp=spline(nn,y,np);
% yp=spline(x,y,xp);
plot(xp,yp,'r-')
title( sprintf('n = %d',n) )
end

k=input('Press enter to continue selecting points or type in 0 to stop: ');
% [a b button]=ginput(1);
T = waitforbuttonpress; % if a key is pressed, T=1. If a mouse button is pressed, T=0.
if T == 1
k=1
end

input('');

hold off
r = api.getVisibleImageRect();

figure(4)
flat18_5bp_closed_6hr_1_hIm = imshow(Im);
hold on
plot(xp,yp,'m--','linewidth',1.5)
hold off
title('Final image')

figure(5)
plot(s,q)
xlabel('Arc length (nm)'), ylabel('tangent angle (rad)')
title('Arc length vs tangent angle')
% Determine persistence length from tangent angle correlations
n_ave = 20; % number of different places to average over
% keyboard
% lp_corr=cos_corr_3(s,q,L,n_ave);
% NOTE: cos_corr_4 will plot to figure(3)
[ss, qq, cos_q_ave, cq_StDev] = cos_corr_4(s,q,L);

% modified to add ds to output file (nm)
D_save = [ss' qq' cos_q_ave' cq_StDev' [ds(1)+ds(2) ds(3:length(ds))']'];

%%
% if exist('prev_fname','var')==1
%    sprintf('Previous filename: %s', prev_fname)
% end
imnum = 1;
fname = strcat(Im_fname(1:(length(Im_fname)-4)),'_',num2str(imnum));
% if this file doesn't exist, check the next one
while exist(strcat(fname,'.txt'),'file') > 0
    imnum = imnum + 1;
    fname = strcat(Im_fname(1:(length(Im_fname)-4)),'_',num2str(imnum));
end

h = figure(4);
save(strcat(fname,'.txt'),'D_save','-ascii','-tabs')
saveas(h,strcat(fname,'.png'));
%% let's count
d = length(dir(strcat(pathname,'*.txt')));
dlg = strcat(num2str(d), ' traces in this folder.

choice = questdlg('Reuse image?','Yes','No, next image','Stop tracing',
    'Stop tracing');
switch choice
    case 'Yes'
        reuse_image = 1;
    case 'No, next image'
        %reuse_image = 0;
    case 'Stop tracing'
        %reuse_image = 0;
end

B.2: Subfunction “cos_corr_4.m”

function [ss, qq, cos_q_ave, cq_StDev] = cos_corr_4(st,qt,Lc)

% s = arc length
% q = tangent angle

function [ss, qq, cos_q_ave, cq_StDev] = cos_corr_4(st,qt,Lc)
This function takes n_ave different random starting points along the filament and then takes the average value of the cos(tangent angle) and arc length starting from each position and averages these data. Afterwards, the lp is fit to the averaged <cos(tangent angle)> versus arc length data. Averaging is done at the level of the tangent angle versus arc length.

qt=q;
Lc=L;

ds=abs(mean(diff(st)));
s_even=0:ds:max(st); % evenly spaced arc length intervals
q_even=spline(st,qt,s_even); % tangent angles for evenly spaced arc length intervals
figure(3),hold off
subplot(1,2,1), box on
plot(s_even,q_even,'r--','linewidth',1.5),hold on
xlabel('Arc Length (nm)'),ylabel('Tangent Angle (rad)')
del_s=ds;
k=2;
ss=zeros(1,length(st)-2);
ss(1)=0;
cos_q_ave=zeros(1,length(st)-2);
cos_q_ave(1)=1;
q_even(1);
cq_StDev=zeros(1,length(st)-2);
while del_s<= (max(s_even)-1)
    ss(k)=del_s;
    q_all=q_even((k+1):length(q_even))-q_even(1:(length(q_even)-k));
    cos_q_all=cos(q_all);
    cos_q_ave(k)=mean(cos_q_all);
    cq_StDev(k)=std(cos_q_all);
    k=k+1;
    del_s=del_s+ds;
    clear cos_q_all q_all
end

qq=q_even(1:(length(ss)));

figure(3)
subplot(1,2,2)
plot(ss,cos_q_ave,'ro','linewidth',1.5),hold on
errorbar(ss,cos_q_ave,cq_StDev,'r')
plot(ss,cos_q_ave,'ko','linewidth',1.5),hold on
xlabel('Arc Length (nm)'),ylabel('<cos(\theta(s)-\theta_0>')

lp_start=Lc/10;
x_start=lp_start;
params=lsqcurvefit(@(f_exp1,x_start,ss,cos_q_ave);

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lp = params;
y_plot=1*(exp(-ss/(2*lp)));
hold on
plot(ss,y_plot,'k-','linewidth',1.5)
lp_string=num2str(lp);
t_string=strcat('lp = ',lp_string);
text(floor(ss(length(ss))/2),0.95,t_string)