DX-DNA Nanotube Tracks for Autonomous DNA Nanowalkers

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Mimicking biomolecular motors has long been a goal of bionanotechnology. A novel light powered nanomotor, which removes many previous flaws in manmade nanomotors, was recently invented. The new nanomotor initially walked along a double stranded DNA track, but a DNA nanotube, made of double crossover tiles, was developed to replace the dsDNA track. This increased the stiffness of the track 100 fold which should translate to the formation of longer tracks and increased motor efficiency. Additionally the DX-DNA nanotubes should increase thermal stability and mechanical strength of the track. In my research I worked on the formation of these nanotubes, building on research done by Teresa Lo [REF], and tested the binding of the motor and track using fluorescence microscopy. It was found that the initial method for forming tubes was able to produce 5-, 6- and 7-tile length tubes but was too inconsistent to use. The motor-track binding was observed under fluorescence microscopy and it was shown that exposure to UV light caused the motor and track to separate as designed. Moving forward two new tube formation processes are being developed, DNA origami and a plate anneal, and the tubes will be viewed in a gliding assay.
I would like to first thank my family and friends for supporting me throughout my time at UCSB. I would also like to thank Dr. Deborah Fygenson, without whom this research would have been impossible and who provided me with many lessons that I hope to keep with me moving into the future. Thank you to the other members of the Fygenson group both present and past, specifically Amber, Lourdes, Nate, Alex, and Teresa, as well as our collaborators, the Wang group, at the National University of Singapore and Paul Rothemund at CalTech.
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A.1 RE tiles that are used in 5-, 6-, and 7-tile DX-DNA nanotubes.

A.2 SE tiles that are used in 5-, 6-, and 7-tile DX-DNA nanotubes.
List of Abbreviations

AFM  Atomic Force Microscope
bp   base pair
ds   double stranded
DX   Double Crossover
Glu  Gluconate
NUS  National University of Singapore
OSS  Oxyen Scavenging System
PEG  PolyEthylene Glycol
SB   Sodium Borate
UV   Ultra-Violet
Chapter 1

Introduction

1.1 Background

1.1.1 DX-DNA Nanotubes

Using the Watson-Crick base pair rules sequences of DNA can be designed to form specified nanostructures with high accuracy. One such nanostructure is a DNA nanotube which acts similarly to biological microtubules but with greater control and adaptability when designing the tube. Everything from length to circumference and surface features are programmable for DNA nanotubes. The specific kind that will be used is DNA nanotubes made with double crossover (DX) tiles.

![Figure 1.1: Schematic diagram of double crossover (DX) DNA tile. Shown is the strands, 1 to 5, that comprise the tile, with no. 1, 3, and 5 strands being called the “core strands” and no. 2 and 4 strands being called the “sticky ends”. [1] [2]](image-url)

DX tiles are composed of five unique DNA strands, as seen in Fig. 1.1. These tiles are made of two double helices, with two shorter connecting double helices. The name “double crossover” is derived from the two shorter helices where strands cross over from one major helix to the other. The cross overs are spaced by two complete helical turns involving alternate strands which leads to a lattice sheet of DX tiles forming tubes with a circumference specified by the minor groove of the double helix [1].

Surface features can be added to the tiles by breaking the no. 1 or no. 5 strand into two pieces. Single-stranded DNA (ssDNA) can be programmed to add to the 3’ or 5’ ends of the break to protrude off of the tile.

To form the nanotubes two different DX tiles, called REP and SEp tiles, where used. These tiles form a lattice with alternating stripes of REP and SEp tiles perpendicular to the tube axis. Having these alternating stripes allows for ssDNA features, binding sites for the nanomotor, to only be on the SEp tile and therefore be separated by a distance corresponding to the stepping size of the nanomotor.

DX nanotubes have a persistence length greater than 3µm and can grow up to 50µm long [1], compared to double stranded DNA which has a persistence length...
of around 0.04 \( \mu m \) [4]. Persistence length corresponds to the stiffness of a polymer, with longer persistence lengths corresponding to stiffer polymers.

1.1.2 Artificial Nanomotors

Nanomotors are any molecular machine that converts energy into motion. The human cell contains many biomolecular motors which consume chemical fuel to transfer along biopolymers. For example, kinesin is a protein motor which uses the energy in adenosine tri-phosphate (ATP) to transport vesicles along microtubules.

Artificial nanomotors are an attempt to replicate the performance of biomolecular motors to further our understanding of them and for commercial use. Currently, artificial nanomotors are far behind their biomolecular counterparts in terms of efficiency, speed, and maximum capacity. The majority of artificial nanomotors have been developed to travel along DNA tracks. To insure a direction of motion most DNA nanomotors operate by blocking or modifying the track [5] [6], changing their chemical environment [7] [8], or requiring a unique trigger for each step [7]. These strategies ensure a directionality to the movement but limit the functionality of the motor and share little resemblance of the biomolecular motors.

At the National University of Singapore (NUS) an autonomous bipedal DNA nanomotor, producing no waste and leaving the track unmodified, was invented [3] [9]. This motor is made of two DNA sequences each with a complimentary section, binding the two together, and two identical binding sequences (D1 and D2 in Fig. 1.2). The D2 sequence contains nine azobenzenes, which can change between a cis and trans conformations with illumination from ultraviolet and visible light respectively [10] (Fig. 1.2). In the cis state the azobenzenes force the base pairs to dissociate (Fig. 1.2) and must be returned to their trans state to form base pairs.

![Figure 1.2: The light-powered nanomotor, developed by the Wang Group at the National University of Singapore, comprised of two ss-DNA strands. These two strands are comprised of three sections, one that hybridizes the motor strands together and two that bind to the track. Each D2 section contains 9 azobenzene moieties and each D1 section ends with a black hole quencher (BHQ-2). The azobenzene moieties can change conformation from cis to trans under ultraviolet and visible light respectively. While in the trans conformation the azobenzenes act as base pairs allowing hybridization and in the cis conformation the azobenzenes restrict hybridization.](image-url)
1.1.3 Use of DX-DNA Tracks

The researchers at NUS studied the function of their motor on a double stranded DNA track with either one or two binding sites (Fig. 1.3) using bulk fluorescence assay. This track was shown to function [3] and proved the concept behind the movement. One objective of this research is to finish developing a nanotube track of similar length and also presenting two or three binding sites. Then using the same bulk fluorescence assay the data of the two and three site nanotube track will be compared to its counterpart of dsDNA track to observe if track stiffness has any effect on the motor performance.

![Figure 1.3: Double stranded DNA track with three binding sites, developed and used by the Wang group at the National University of Singapore.](image)

1.1.4 Motor Movement

The two important aspects of the motor movement is the energy required and the directionality. The energy for the motor movement comes from the irradiation of the motor in UV and visible light changing the azobenzene state. The directionality arises from the chosen size of the walker, spacing of the binding sites, and asymmetric nature of the binding sites.

The motor has four possible intersite bridge states, B1 thru B4 in Fig. 1.4, where the motors legs are bound to separate binding sites. Two, B2 and B3, are symmetric with both legs of the motor binding to identical helices, and two are asymmetric with both a D1-D1* and D2-D2* bindings.

Due to this asymmetry the B1 state has the lowest energy and therefore is the most likely to occur. From this a convention of calling the D1-D1* motor leg the leading leg and calling the D2-D2* motor leg the trailing leg is established. A positive direction of the track, the end towards which the walker moves, is established by the direction that points from the D1* foothold to the D2* foothold.

If the motor starts in the B1 state, irradiating the motor in UV has a chance to dissociate the trailing leg but not the leading leg due to the presence of azobenzenes on the D2 section. This process of selective dissociation is a Brownian ratchet effect and initiates a migration of the leading leg from the D1* strand to the D2* strand of the same binding site to form a longer and therefore more stable helix. Irradiating the motor in visible light stabilizes this D2-D2* connection. This switch of the leading leg from D1-D1* to D2-D2* places the dissociated leg closer to next binding site in the positive direction than the previous site. This means that the dissociated leg is biased to bind to the next site in the positive direction resuming the stable B1 state. This process is referred to as a powerstroke. This process can be visualized in Fig. 1.5A.

Due to the size of the walker relative to the spacing of the binding sites, the walker is constrained to the intersite binding states in Fig. 1.4. Note that the in
Chapter 1. Introduction

1.2 Goals

1.2.1 Effect of Increased Track Stiffness on Motor Performance

dsDNA, which the original track studied at NUS was constructed with, has a persistence length of 400Å[4]. By switching to a DX-DNA nanotube track, with a persistence length of at least 3 µm [1], the effect of stiffness on the system can easily be tested, specifically the efficiency of the motor. In addition to this an increase in the track stiffness provides the motor with a more uniform track and the possibility of placing multiple motors on the same track without affecting one another.
1.2.2 Visualization Through a Gliding Assay

Gliding assays were initially developed as a way to characterize the movement of biomolecular motors. Typically the motor is attached to a glass surface with microtubules and an energy source being added. The tubes will be moved by the anchored motors which can be visualized using fluorescence microscopy.

Modifying the bridge of the light powered nanomotor by adding biotin will allow the motors to attach to glass surface. Then exposing the motor-track system with alternating UV and visible light, inducing walking. Through studying this visualization of the motor movement the speed and efficiency of the motor can be extracted.
Chapter 2

Materials and Methods

2.1 Tube Configuration

![Diagram of DX-DNA nanotube](image)

**Figure 2.1**: Design of a DX-DNA nanotube 7 tiles in length with labels for sticky ends and regions. Shown are different possible designs of binding sites, with Dup0D1*5′ being the final design closest to original dsDNA track binding site.

### 2.1.1 Use of DX-DNA Tiles

There are many different types of DNA nanotubes, from tubes made with TX-tiles [9] or single-stranded tiles [6]. DX-tile DNA nanotubes were chosen as they provide adequate stiffness when compared to the original dsDNA track [3], with ease of modification to the sequences. This ease of modification is necessary in order to change sticky ends for each tile and to add motor binding sites to specific tiles. Figs. 2.1 and 2.2 show the alternating SEp and REp stripes of tile which represent the corresponding rings of tiles that form the tube.
Chapter 2. Materials and Methods

2.1.2 Binding Sites

The motor binding sites were added by replacing the hairpin loop modifications from the original design of the DX-nanotube [1] with the binding sites from the original dsDNA track [3]. The binding sites consist of two ssDNA sequences, D1* and D2*, which are a 6-mer and 20-mer sequences respectively, that compliment the D1 and D2 sequences of the motor leg.

Rothemund et al. experimented with placing a hairpin loop on each DX tile between the two crossovers [1]. A hairpin loop, as seen in Fig. 2.3, is a self-complementary sequence with an addition of a non-complementary sequence which forms a loop when it closes on itself. Rothemond et al. experimented with hairpin placement along the number 5 strand and identified where the hairpin could be placed without noticeably changing the tube formation. Importantly they identified 9 bases where hairpins could be added with the tubes still forming and not forming flipped (with the tile axis perpendicular to the tube axis).

Using these identified bases that allowed for correct tube formation and to maintain the same intrasite spacing of 15 bp (5.1nm) and intersite spacing of 75 bp (25.5nm) as the original dsDNA track it was decided to place the D1* binding site sequence on the 26th base of the SE1 strand and the D2* binding site sequence on the 25th base of the SE5 strand as observed in Fig. 2.1 [2]. To achieve this the SE1 strand was split into two individual strands between the 26th and 27th base, with the binding site coming off of the 26th base. The same was done for the SE5 strand, splitting it into two between the 25th and 26th base with the D2* coming off the 25th base (Figs. A.1 and A.2). A fluorescent dye is placed at the 3’ end of the D1* binding site, corresponding with the quencher at the 5’ end of the D1 sequence of the motor leg. A different dye is used for each ring of SEp tiles to allow for movement information to be extracted from the fluorescence data.

In the original DX-tile design, using a unique REp-SEp tile pair, tubes of an unspecified length are formed, growing up to 50µm [1]. When done with SEp tiles containing the motor binding site these tubes will have hundreds of binding sites so
to be able to compare the motor performance of the DX-tile tubes with the original dsDNA track, the length of the tubes was constrained to only present two or three binding sites. To achieve this the last and first 5 bases of the no. 2 and no. 4 strands, called "sticky ends" of both the REp and SEp tiles are modified. Unique sticky end sequences were generated using a MATLAB code and tested using the NUPACK software [11]. Unique sticky ends contain no overlap more than a two base in the same order with other sticky ends. Using this 12 unique sticky ends were found allowing for the design of 7 unique tiles, Figs. A.1 and A.2.

2.2 Tube Construction

Three different length tubes are constructed, 5-, 6-, and 7-tile tubes. The formation of all three follow three steps for assembly, kination, anneal, and ligation. Tubes will form by annealing alone, but O’Neill et al. showed that ligated DX-tubes have an increase in mechanical strength, a higher melting point, and are stable in a larger array of buffers. 5-tile tubes end after the third REp tile, substituting the γ sticky end on the third REp tile (Fig.2.1) with a poly-T sticky end, and contain two binding sites. 6-tile tubes end after the third SEp tile, substituting the C sticky end on the third SEp tile (Fig.2.1) with a poly-T sticky end, and contain three binding sites. 7-tile tubes contain all 7 unique tiles, having three binding sides and ending with an REp tile.

2.2.1 Kination

The kinase enzyme is used to catalyze a reaction between DNA and ATP to add a phosphate group to the 5′ end of each DNA strand. This allows the ligase enzyme to form phosphodiester bonds between the 3′ hydroxyl and 5′ phosphate groups on the no. 1,2,4, and 5 strands, which seals the nicks between tiles (Fig. 2.4).

To perform this step DNA strands are vortexed and spun down, ensuring a uniform concentration. 200µL tubes are filled with 18.5uL of MilliQ water, 2.5µL of 10X T4 DNA Ligase Reaction Buffer, 3µL of DNA strand in MilliQ water at 100µM,
and 1µL T4 PNK Kinase. The Ligase buffer contains 50mM Tris-HCl, 10mM MgCl₂, 1mM ATP, and 10mM DTT at 1X concentration. After filling the tubes they are vortexed and spun down again and then placed in a thermocycler. Kinase’s activity is maximized at 37°C and kinase is known to heat inactivate at 65°C. Therefore the thermocycler is run at 37°C for 35 minutes, and then at 65°C for 20 minutes. This results in 25µ solution with the concentration of DNA strand at 12µM.

2.2.2 Anneal

The annealing phase causes the strands to form tiles and regions, REp-SEp pairs, by changing the temperature of the strand mixture from 95°C to room temperature, 21°C. This slow decrease in temperature allows the strands to explore many conformations and settle into the lowest energy conformation according to Watson-Crick base pair rules. If this process is done too quickly the strands can get kinetically trapped in local energy minimums which do not result in the tiles and regions forming.

Each type of tile, REp and SEp, has the same core sequences, strands no. 1, 3, and 5. This requires each unique REp-SEp pair to be annealed separately from other pairs. If this was not done the no. 2 and 4 strands would hybridize randomly,
preventing the ordering of tiles. But due to the fact that REp and SEp core sequences are distinct, pairs of REp and SEp tiles can be annealed together which form what are called regions (Fig. 2.1).

To perform the annealing phase a ratio of $10.7\mu L$ of 10X TAE 125mM MgSO$_4$ buffer and $8\mu L$ of each 12µM strand solution are added to a 200µL tube for Region 1, 2, and 3 for a final volume of 107µL. These are then placed in a thermocycler which brings them from 90°C to 20°C in steps B.2. Next all of Region 1, 2, and 3 are mixed together to a final volume. This mixture is left at room temperature for approximately 12 hours for the regions to come together into tubes before moving to the ligation phase.

### 2.2.3 Ligation

To perform the ligation phase T4 DNA Ligase is added to the tube mixture at approximately $53\mu L$ tube mixture to $0.5\mu L$ ligase.

Ligase has been left active in the tube mixtures for months without any noticeable effect on the nanotubes. But, before performing Atomic Force Microscopy or Motor Fluorescence experiments the ligase must be deactivated as it interferes with the measurements. Typically, ligase is deactivated by heating it to 65°C, but raising the tubes to this temperature can result in the disassemble of tubes giving a lower yield. Instead, the Proteinase K enzyme is added to a final working concentration of 75µg/mL to digest the ligase.

### 2.3 Sodium Borate Electrophoresis

As an initial check of the formation of tubes agarose gel electrophoresis is used to determine the relative size of the DNA strands, tiles, regions and tubes. Many mistakes in formation cause either tiles to not form regions or regions to not form tubes which can be visualized in these agarose gels. When an external voltage is applied across an agarose gel containing DNA, which is negatively charged, the DNA will travel through the gel. The gel acts as a sieve, causing larger structures to travel slower and small structures to travel faster resulting in a separation.

To create the agarose gel, 36mL of MilliQ water is measured out and 0.7g agarose (ADD WHICH TYPE) is added. This results in a final gel density of 1.75%. This agarose solution is microwaved until it begins to boil, typically about 27 seconds, and then stirred to fully dissolve the agarose and remove air bubbles. After approximately 3 min, when the solution is cool enough to handle, 4mL of 10mM sodium borate (Na$_3$BO$_3$) 10mM magnesium gluconate (Mg(C$_6$H$_11$O$_7$)$_2$) and 6µL SYBR Green nucleic acid stain are added. This solution is then poured into a 10cm × 6cm gel tray with a well comb and placed in a refrigerator at 8°C for approximately twenty minutes.

While the gel is cooling the DNA samples are prepared. In a 200µL tube $1\mu L$ of 6X blue loading dye and $5\mu L$ of a DNA structure solution are added for each DNA structure that will be run in the gel. These are then vortexed and stun down. The 6X blue loading dye was made by adding 25mg of bromophenol blue, 25mg of xylene cyanol FF, and 3.3mL of glycerol into 6.7mL of MilliQ water.

Typically gels are run using TAE as the buffer. But, following methods developed by Brody and Kern [12] and refined by Lo [2], a sodium borate-based buffer was used for testing the DNA nanotubes. This allows for the gel to run faster and cooler when compared to TAE gels. But, to keep the DNA structures assembled magnesium must
be present. Following methods developed by Lo [2] the gel is made using a 1mM sodium borate 1mM magnesium gluconate (Mg(C₆H₁₂O₇)₂) buffer, which is also used in the gel box reservoir of the positive electrode. A 1mM sodium borate 1mM magnesium hydroxide (Mg(OH)₂) is used in the gel box reservoir of the negative electrode.

After the gel has cooled the tray is placed in a gel box that is resting in ice. Sodium borate magnesium hydroxide buffer is poured into the positive reservoir and sodium borate magnesium gluconate buffer is poured into the negative reservoir up to the bottom of the gel. The gel is then run at 450V which results in a current of about 60mA and power around 25W. The current and power are dependant on temperature and will increase as the temperature increases. After 8 minutes the gel is removed and transferred to a UV Transilluminator. Under UV illumination the progress of the DNA is checked and recorded. If further separation is necessary, the buffers are replaced in the gel box and the gel is placed back into the gel tray for another 8 minute run.

2.4 Atomic Force Microscopy

The 5-, 6-, and 7-tile tubes are too small to be viewed under a conventional microscope. To image the tubes, tapping atomic force microscopy (AFM) is used. Tapping AFM uses a sharp silicon tip that taps along a surface and interacts with any objects on that surface. These interactions cause changes in the tip height which are detected using a laser that is focused on the tip.

The tapping AFM was performed at the Winfree Lab at the California Institute of Technology following protocols developed by the Rothemund Group [1]. The images were taken using a tapping frequency between 0.5 and 2Hz with a frame size of 0.5µM to 5µM with a Bruker Multimode AFM. Unligated nanotubes were imaged as those break open into a lattice sheet under AFM making them much easier to image.

Before imaging a mica puck was cleaved using scotch tape to generate a clean flat surface. Then, 25µL of 1X TAE 12.5mM MgSO₄ are added to the surface and the puck is imaged to insure that the mica is a clean flat surface. The 1X TAE 12.5mM MgSO₄ is pipetted off the surface and fresh buffer is added. This is followed by 2µL of 10 times diluted unligated tubes. Lastly a solution of 2µL 60mM NiCl₂ and 25µL 1X TAE 12.5mM MgSO₄ is added. The Mg²⁺ and Ni²⁺ cations help the negatively charged DNA to stick to the negatively charged surface of the mica [13]. After a minute as much of the liquid as possible is pipetted off the surface and 25µL of 1X TAE 12.5mM MgSO₄ is added before imaging.

2.5 Fluorescence Microscopy

Under fluorescence microscopy the 5-, 6-, and 7-tile tubes are too small to be visible. Instead, fluorescence microscopy is used to observe the motor binding functionality. Therefore, tubes used for fluorescence microscopy are made with only one unique pair of REP and SEP tiles which self-assemble into unspecified lengths, called infinite tubes. These infinite tubes in addition to having fluorophores on the bindings sites of the SE1 strand, also have a Cy3 fluorophore on the no. 3 strand of both the REP and SEP tiles to make them brighter under illumination.
2.5.1 Slide Cleaning

Glass coverslips, $22 \times 22$ mm$^2$, and glass coverslides, $3 \times 1$ in$^2$ are cleaned by first sonicating in Macro90 soap that is diluted to one part in a thousand. After, they are rinsed thoroughly with MilliQ water and soaked in a 2M NaOH solution for 30 minutes etching the surface. They are rinsed again with MilliQ water and have a final sonication in pure ethanol for 10 minutes.

2.5.2 Slide Preparation

DNA, due to its hydrophilic backbone and hydrophobic bases, has both polar and nonpolar components [14]. Due to this, it interacts with many surfaces including glass where it becomes immobile. To allow for a clean surface that does not interact with the DNA, the glass surfaces are passivated with a polymer Methoxy-Poly(Ethylene-glycol) coupled to a silane moiety, mPEG-silane, which provides repulsion of DNA through steric hindrance [15] [16].

To coat the glass surfaces, a solution of 2 to 5 mg mPEG-silane in ethanol with 1% acetic acid is created. To do so the ethanol with 1% acetic acid is heated to 40°C.
before adding the mPEG-silane. This solution is then vortexed and allowed to cool to room temperature to fully dissolve the mPEG-silane.

To attach the mPEG-silane to the glass, 15µL of the solution are placed on the cover slip. This is flipped onto the center of the glass slide, sandwiching the solution between the slide and cover slip. These slide-cover slip pairs are stored horizontally in 50mL Falcon tubes with 5mL ethanol. The ethanol in the tube keeps the PEG-ethanol solution from evaporating as well as keeping dust off the slide. The slides must be left incubating for at least 3 days before use and at most for 2 months.

### 2.5.3 Attachment of Motor Foot

To test the functionality of the connection between the motor foot and binding site the motor foot is attached to the slide and cover slip surface. This is done by replacing a percentage of mPEG-silane with mPEG-silan with a biotin attached. Streptavidin is added which binds to the biotinilated PEG and a biotinilated motor foot (Fig. 2.6) anchoring the motor foot to the slide. To add the biotin-PEG at a certain percentage to the slide, first, an ethanol with 1% acetic acid solution with dissolved biotin-PEG is prepared using the same process as before. Then in a 200µL tube the biotin-PEG and PEG solutions are mixed so the final solution is at the desired biotin-PEG concentration. Then, following the same procedure, 15µL of the mixed solution is sandwiched between a slide and cover slip and incubated in a 50mL Falcon tube with ethanol.

### 2.5.4 Trolox OSS

When in the presence of oxygen, the fluorophores on the infinite nanotubes will begin to photobleach. This occurs when the fluorophore undergoes a chemical reaction or gets confined to a specific conformation making excited energy states unaccessible [17]. To counteract this phenomena an oxygen scavenging system (OSS) removes the oxygen from solution greatly slowing the photobleaching. The OSS that was used is based on the Trolox molecule.

The OSS is prepared by mixing 0.5µL of 1µM PCD with 2µL 1X TAE buffer and mixing 0.375µL of 67mM PCA, 0.12µL of 83mM Trolox [18], and 2µL 1X TAE. Then 1µL of each solution is mixed with 1µL of sample.

### 2.5.5 Sample Preparation

To prepare a sample for imaging, a slide with 3% biotin-PEG is removed from the Falcon tube. The slide and cover slip are separated and dried with compressed air. 2µL of streptavidin in 13µL of TAE with 12.5mM MgSO$_4$ is placed on the cover slip and sandwiched with the slide. After a minute the slide and cover slip are separated, rinsed with TAE with 12.5mM MgSO$_4$, and dried with compressed air. This is repeated with 9µL of biotinilated motor foot and 6µL TAE with 12.5 MgSO$_4$. After the final drying 4µL of the sample (1µL infinite tubes, 2µL Oxygen Scavenging System, 1µL 4X TAE with 12.5mM MgSO$_4$) is sandwiched between the slide and cover slip and sealed with (nail something).
Figure 2.6: To attach the motor foot, the glass surface is first functionalized with PEG-silane, some of the PEG-silane having biotin attached. The glass surface is then washed with streptavidin and then the motor foot. The streptavidin binds to the biotinilated PEG molecules and then the biotinilated motor foot binds to the streptavidin immobalizing the motor foot on the glass surface.
Chapter 3

Results

3.1 Gel Results

As has been shown previously [2] and as expected RE and SE tiles (lanes 2 and 3 in Fig. 3.1) move slower than a single strand of DNA (lane 1). The SE tile (lane 3) moves slower than the RE tile (lane 2) due to the extra bases that are present in the
Chapter 3. Results

binding sites of the SE tiles. The regions (lane 4) move slower still compared to the
tiles and there are distinct upper bands/fronts (lanes 5, 6, 7) indicating structures
that are much larger than the tiles and regions. Lane 5 was designed to be 5-tile
tubes, lane 6 6-tile tubes, and lane 7 7-tile tubes, but it should be noted that there is
no visible difference between the different sized tubes in the gel.

![Image of gel electrophoresis results]

FIGURE 3.2: Gel formed and ran identically to Fig. 3.1. Gel shows the
DX-DNA nanotube tracks not forming as designed.

Previously 6-tile tubes were shown using gel electrophoresis and atomic force
microscopy to form correctly [2], but this result was initially unable to be replicated.
In Fig. 3.2 lane 5 the 6-tile tubes didn’t form correctly as shown by the absence of a
distinct band that is significantly larger than the RE tile, SE tiles, and regions (lane 1,
2, 3 respectively). This was confirmed using AFM where no structures longer than
approximately 48 nm, or 4 DX tiles. In lane 4 and lane 6 we observe that the attempt
at making 5-tile and 7-tile tubes completely failed having broken down into RE and
Se tiles.

3.1.1 Tube Design Corrections

To find what corrections needed to be made to the DX tile design a test was done on
the sticky ends of the tiles. As shown in Fig. 3.3 a region was formed for each sticky
end and run on a gel. From this gel it was observed that all of the regions except for
one, labeled as the beta sticky end, formed as expected. The beta sticky end broke
down into the composite RE and SE tiles. The instability of the beta sticky end was
Chapter 3. Results

Figure 3.3: Gel formed and ran identically to Fig. 3.1. Regions are formed by the RE and SE tile on either side of the sticky end. The gel shows the instability of the beta sticky end as you see it break down into its constituent tiles.

confirmed by analysis using the online application NUPACK [11], which showed it to have a lower melting temperature than other sticky ends.

This problem was resolved by introducing a new more stable sticky end, labeled delta, to replace beta. This led to the formation of tubes as observed in Fig. 3.1 and confirmed by AFM as discussed in the following section.

3.2 AFM Results

To confirm the formation of the tubes and to analyze the yield of tubes AFM images were analyzed using the Gwyddion program [19].

Any structure that was touching another or that was less than 3 tiles, or approximately 36 nm in length were not counted in the analysis. The length of structures was determined by creating line selections through the center along the length axis by eye. These measurements were then divided by 12 nm and rounded to the nearest whole number to give a length in DX-DNA tiles.

With the AFM analysis the results from the gel electrophoresis was confirmed that 5-, 6-, and 7-tile tubes were being created with an approximate yield of 55%. The actual yield of tubes is likely to be greater than observed as many of the shorter length tubes appeared “frayed” at one end, as if tiles had been ripped off during sample preparation, and some of the longer length tubes seemed to be multiple correct length tubes attached together.
Figure 3.4: Image of unligated 6-tile tubes taken using the Bruker Multimode AFM in Fluid Tapping Mode. The unligated tubes opened and flattened into rectangular tile lattices. The gray scale indicates the height of the features in the image.

Figure 3.5: AFM image analysis performed on 63 images. In each image the length of tubes was measured and rounded to the nearest tile length. Any structure that was shorter than 3 tiles in length, touching the edge of the image, or touching any other structure was excluded.
3.3 Fluorescence Data from NUS

The Wang Group at the National University of Singapore has performed fluorescence experiments on the 5-, 6-, and 7-tile tubes with a modified azobenzene motor design. When a motor binds to a site on the track, the quencher, located at the ends of the motor’s two legs, causes a decrease in fluorescence intensity of the dye at the binding sites. This is done in bulk, giving an accurate trend to motor movement.

![Figure 3.6](image)

**Figure 3.6:** Data from motor and track bulk fluorescence experiments performed by collaborators at the National University of Singapore. Tube solution at 5nM was mixed with 2.5nM motor solution in 1x TAE 12.5mM MgSO$_4$ buffer. This mixture was exposed to visible light for 10 minutes followed by 10 minutes of UV light. This cycle is repeated 10 times while monitoring the fluorescence from Cy5 located at the positive end of the track, FAM located in the middle of the track, and Cy3 located at the negative end of the track. This data is normalized to account for photobleaching. Tubes used in A and B were well formed tubes seen in Fig. 3.1 while tubes in C and D were made to replicate A and B which they failed to do.

These fluorescence experiments using the tubes with the corrected delta sticky end show a pattern indicating movement from the negative to positive end of the tube (Fig. 3.6A and 3.6B). We see an increase in the fluorescence of the Cy3 fluorophore, located at the negative end of the tube, meaning that the motor is binding less to it and we see an increase in the fluorescence of the Cy5 fluorophore, located at the positive end of the tube, indicating more motor binding. The fluorophore located in the middle, FAM, shows no chance in fluorescence which is to be expected.
However, recent fluorescence experiments have shown none or little of this increase and decrease in the fluorescence (Fig. 3.6C and 3.6D) indicating that the motor is randomly attaching and detaching with no movement. This has lead to further testing of the tubes by gel electrophoresis and AFM which has shown that again the tubes that we have been making are not forming correctly. This has lead us to begin exploring different ways of creating the DNA tubes.

3.4 Alternative Construction of Tubes

To form the tubes we have been exploring two new approaches.

The first is to order the necessary DNA strands for the DX-DNA nanotubes on a 96 well plate. These strands are unpurified making them a cheaper option, but more importantly they will all have the same concentration and will allow the use of a multichannel pipette. One of the likely causes of the failure to form new tubes is a stoichiometry, or similar such, error in the formation of the tubes. Having the DNA strands at a uniform concentration and cutting down on the number of pipettes by using a multichannel pipette will decrease the possibility of these errors occurring.

The other approach has been to form a tube with DNA origami and modifying a few of the staples to contain binding sites. DNA origami has been studied extensively and the yield is well known. By designing the DNA origami correctly these tubes should form at a given yield and can easily be made, correcting the issues with the current process.

3.5 Viewing Attachment and Detachment of Motor Foot

Working towards a gliding assay, the first step was the visualization of the attachment and detachment of the motor foot from the track. This was done by viewing “infinite tubes” under fluorescence microscopy. Infinite tubes are made the same way as 5-, 6-, and 7- tile tubes but only have two sets of sticky ends enabling them to grow upwards of 10\(\mu\)m. Samples are prepared with motor feet containing azobenzenes and a video is taken. The azobenzenes are in their \textit{trans} state and therefore the motor feet anchor the tubes. A UV lamp is placed directly on the sample and turned on for a designated time.

Analysis of the videos taken show that the motor foot was detaching under UV radiation. The rate of detachment seems to be low and it is important to note that while detachment of motor feet was observed no tube became totally free. This is believed to be a consequence of the large number of binding sites on each infinite tube and the number of motor feet present. Moving forward we will try to observe a tube becoming fully detached and then reattaching before trying to form a gliding assay.
FIGURE 3.7: Analysis of fluorescence microscopy videos taken of infinite DX-DNA nanotube tracks. The glass slide has motor feet, with nine azobenzenes, attached. Videos were taken before and after exposure to UV light for different amounts of time. These videos were analyzed by subtracting the z-stack from the video, leaving only the tubes that are moving, which were designated as free tubes.
Appendix A

Strand Sequences

Below is a list of all strands used to create the DX-DNA nanotube tracks and the motor. They are also shown grouped as they would be to assemble each 5-, 6-, and 7-tile tube and how they make up individual tiles.

A.1 Tube Formation

<table>
<thead>
<tr>
<th>Region 1</th>
<th>Region 2</th>
<th>Region 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>RE1</td>
<td>RE1</td>
<td>RE1</td>
</tr>
<tr>
<td>RE3</td>
<td>RE3</td>
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<tr>
<td>RE5</td>
<td>RE5</td>
<td>RE5</td>
</tr>
<tr>
<td>RE2-polyT</td>
<td>RE2-A</td>
<td>RE2</td>
</tr>
<tr>
<td>RE4-α</td>
<td>RE4-4δ</td>
<td>RE4-polyT</td>
</tr>
<tr>
<td>SE1-1:o26-D1*-cy5</td>
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<td>SE1-1:o26-D1*-cy3</td>
</tr>
<tr>
<td>SE1-o27:37</td>
<td>SE1-o27:37</td>
<td></td>
</tr>
<tr>
<td>SE5-1:o25-D2*</td>
<td>SE5-1:o25-D2*</td>
<td>SE5-1:o25-D2*</td>
</tr>
<tr>
<td>SE5-o26:37</td>
<td>SE5-o26:37</td>
<td>SE5-o26:37</td>
</tr>
<tr>
<td>SE2-α</td>
<td>SE2-δ</td>
<td></td>
</tr>
<tr>
<td>SE4-A</td>
<td>SE4</td>
<td></td>
</tr>
</tbody>
</table>

**Table A.1: 5-tile Tube Formation**

<table>
<thead>
<tr>
<th>Region 1</th>
<th>Region 2</th>
<th>Region 3</th>
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<tr>
<td>RE5</td>
<td>RE5</td>
<td>RE5</td>
</tr>
<tr>
<td>RE2-polyT</td>
<td>RE2-A</td>
<td>RE2</td>
</tr>
<tr>
<td>RE4-α</td>
<td>RE4-4δ</td>
<td>RE4</td>
</tr>
<tr>
<td>SE1-1:o26-D1*-cy5</td>
<td>SE1-1:o26-D1*-FAM</td>
<td>SE1-1:o26-D1*-cy3</td>
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<tr>
<td>SE1-o27:37</td>
<td>SE1-o27:37</td>
<td>SE1-o27:37</td>
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<tr>
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<td>SE5-1:o25-D2*</td>
<td>SE5-1:o25-D2*</td>
</tr>
<tr>
<td>SE5-o26:37</td>
<td>SE5-o26:37</td>
<td>SE5-o26:37</td>
</tr>
<tr>
<td>SE2-α</td>
<td>SE2-δ</td>
<td>SE2</td>
</tr>
<tr>
<td>SE4-A</td>
<td>SE4</td>
<td>SE4-polyT</td>
</tr>
</tbody>
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**Table A.2: 6-tile Tube Formation**
Appendix A. Strand Sequences

## Table A.3: 7-tile Tube Formation

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<thead>
<tr>
<th>Region 1</th>
<th>Region 2</th>
<th>Region 3</th>
<th>Region 4</th>
</tr>
</thead>
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<td>RE5</td>
<td>RE5</td>
<td>RE5</td>
</tr>
<tr>
<td>RE2-polyT</td>
<td>RE2-A</td>
<td>RE2</td>
<td>RE2-C</td>
</tr>
<tr>
<td>RE4-α</td>
<td>RE4-4δ</td>
<td>RE4</td>
<td>RE4-polyT</td>
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<td>SE1-1:o26-D1*-cy5</td>
<td>SE1-1:o26-D1*-FAM</td>
<td>SE1-1:o26-D1*-cy5</td>
<td></td>
</tr>
<tr>
<td>SE1-o27:37</td>
<td>SE1-o27:37</td>
<td>SE1-o27:37</td>
<td></td>
</tr>
<tr>
<td>SE3</td>
<td>SE3</td>
<td>SE3</td>
<td></td>
</tr>
<tr>
<td>SE5-1:o25-D2*</td>
<td>SE5-1:o25-D2*</td>
<td>SE5-1:o25-D2*</td>
<td></td>
</tr>
<tr>
<td>SE5-o26:37</td>
<td>SE5-o26:37</td>
<td>SE5-o26:37</td>
<td></td>
</tr>
<tr>
<td>SE2-α</td>
<td>SE2-δ</td>
<td>SE2</td>
<td></td>
</tr>
<tr>
<td>SE4-A</td>
<td></td>
<td>SE4-C</td>
<td></td>
</tr>
</tbody>
</table>

### Table A.4: Infinite Tube Formation

<table>
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</tr>
</thead>
<tbody>
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<td>RE1</td>
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<td>RE3</td>
</tr>
<tr>
<td>RE5</td>
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<tr>
<td>RE2-A</td>
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<tr>
<td>RE4-α</td>
</tr>
<tr>
<td>SE1-o27:37</td>
</tr>
<tr>
<td>SE3</td>
</tr>
<tr>
<td>SE5-1:o25-D2*</td>
</tr>
<tr>
<td>SE5-o26:37</td>
</tr>
<tr>
<td>SE2-α</td>
</tr>
<tr>
<td>SE4-A</td>
</tr>
</tbody>
</table>

## A.2 Strand Sequences

Following are the base sequences for every strand written 5’ to 3’.

### RE Tiles

- RE1: CGTATTGGACATTTCCGTAGACCGACTGGACATCTTC
- RE3: TCTACGGAATGTGCCAGAATCATCATAAGACACCAGTCCG
- RE3-cy3: cy3-TCTACGGAATGTGCCAGAATCATCATAAGACACCAGTCCG
- RE5: CCACTACCTGTCTTATGATTGATTCTGCCTGTGAAGG
- RE2-polyT: TTTTTCCTTCAACCAATACGTCTTTTT
- RE2-A: CAACGGCTTCACACCAATACGTAGCC
- RE2: CCTACGCTTCACACCAATACCGAGGTA
- RE2-C: CACACCGTTCACACCAATACCGTCCGT
- RE4-α: CGGAAGAAGATGTGGTATGTGGACGAA
- RE4-β: GGATCGGAAGATGTGGTATGTGGCTTGG
Appendix A. Strand Sequences

- RE4: CAGACGAAGATGTGGTAGTGGAATGC
- RE4-polyT: TTTTTGAAGATGTGGTAGTGGTTTTT

SE Tiles
Note the ∗ and v indicate where the strand comes onto or off of the no 3 strand to form the binding site.

- SE1-1:o26: CTCAGTGGACAGCCGTTCTGGAGCGT
- SE1-D1*-o27:37: (Cy3)GGAATGTGGACGAAACT
- SE1-D1*-o27:37: (Cy5)GGAATGTGGACGAAACT
- SE1-D1*-o27:37: (FAM)GGAATGTGGGCAAGACT
- SE3: CCAGAACCGCTTGGCTAACGTCGAGCAACGCT
- SE3-cy3: cy3-CCAGAACCGCTTGGCTAACGTCGAGCAACGCT
- SE5-1:o25-S2-D2*: CGATGACCTGCTTCGTTACTGTGTTTAAAGGTATATCTCCTCTTCTAAAG
- SE5-o26:37: AGCCTGCTCTAC
- SE2-α: TTCCGGTAGAGCACCACTGAGTTCGT
- SE2-β: GATCCGTAGAGCACCACTGAGCCAAG
- SE2: GTCTGGTAGAGCACCACTGAGGACTT
- SE4-A: CGTTGAGTTCGTGGTCATCGGCTA
- SE4: TGAGGAGTTTCGTGGTCATCGGATT
- SE4-C: TTGTGAGTTCGTGGTCATCGACGGA
- SE4-polyT: TTTTTAGTTTCGTGGTCATCGTTTTT

Light Powered Azobenzene Motor

- MS1(49-mer): GAGTTACCATCTAGTGAGAGTGCTXTTXAXGAXAGXGAXGAXTAXTXACATXCA
- MS2(49-mer): CTCTACCTAGTGAAACTCAGTCTXTTXAXGAXAGXGAXGAXTAXTXACATXCA

A.3 Tile Structure
Appendix A. Strand Sequences

**Figure A.1**: RE tiles that are used in 5-, 6-, and 7-tile DX-DNA nanotubes.
Figure A.2: SE tiles that are used in 5-, 6-, and 7-tile DX-DNA nanotubes.
Appendix B

Procedures

B.1 Anneal Procedure

- strands from the freezer and thaw
- Vortex and Centrifuge
- In 200μL tubes mix 18.5μL MilliQ Water, 1μL Kinase, 2.5μL Ligase Buffer, and 3μL DNA stock at 100μM
- For different concentrations of DNA change the amount of MilliQ Water and DNA stock accordingly
- Note that you will have to double or triple the amount that you make for the core RE and SE strands
- Vortex and centrifuge
- Run Kinase reaction
- 35 min at 37C then 20min at 65C
- Mix correct strand solutions into regions
- Typically only use 8μL of each strand solution
- Add 10X TAE w/ Mg to region solutions so they end as 1x solutions
- Add ligase to regions, 1μL per 100μL region solution
- Load and run REGANEAL program on the thermocycler

B.2 REGANEAL Program

- 90C for 5min
- 90C to 70C in 20 min
- 70C to 55C in 150 min
- 55C to 40C in 15min
- 40C to 25C in 150min
- 25C to 20C in 5min
- Hold at 20C
B.3 Making and Running 1mM Sodium Borate 1mM Magnesium Gluconate/1mM Sodium Borate 1mM Magnesium Hydroxide Gel

- Tape the sides of gel tray to form an inclosed space where the gel will be poured
- Place tray with well comb in freezer
- Measure out 36mL MilliQ Water and pour into beaker
- Weigh amount of agarose to make correct % gel
- For 1% gel weigh 0.4g agarose
- Add agarose to milliQ water and stir lightly
- Heat in microwave till it just boils (Typically 27 sec)
- Stir solution initially with tip of stir stick at the bottom of beaker
- As you stir bring tip up towards the surface till you are only stirring the very top of the solution (this will help to decrease the bubbles)
- Let sit (there will still be bubbles present that will go away as it sits) until you can grab with your hand and not feel discomfort
- Note that it should still be quite warm but not hot
- Add 4mL of 10mM Sodium Borate 10mM Magnesium Gluconate
- Add 6uL of sybr green, centrifuging and vortexing before
- Do under the fume hood as chemical is toxic
- Dispose of pipet tip in waste
- Pour into tray with well comb properly placed
- Only 1+ cm high (this will be slightly less than the full 40mL solution you have)
- Place in fridge to cool
- Let sit to cool for at least 15 min
- While gel is cooling make samples
- Plan where you will put samples
- Add 1uL blue loading dye to a 200uL tube
- Add 5uL sample
- Should be about 50ng of DNA per sample (usually tubes and regions aren’t diluted while strands and tiles are diluted in half)
- Centrifuge
- Get ice
- Set up gel box with ice
• Remove tape from gel tray and place in gel box

• Add 1mM Sodium Borate 1mM Magnesium Gluconate to the positive well until it just begins to flow under the gel tray

• Add 1mM Sodium Borate 1mM Magnesium Hydroxide to the negative well until it just begins to flow under the gel tray

• Load samples into correct wells

• Add 1mM Sodium Borate 1mM Magnesium Gluconate to the positive well until it just reaches the top of the gel

• Add 1mM Sodium Borate 1mM Magnesium Hydroxide to the negative well until it covers the gel

• Gently move the gel tray back and forth a few times to disperse any blue loading dye that escaped the wells

• Set voltage to 450V and run gel for 8 min

• Move gel tray to UV source and take pictures of just the gel

• Return gel tray with gel to gel box and run for a second 8 min run

• Note that if you wish to keep the gel even cooler new buffer should be added before the second run

• Take pictures and then clean up
Bibliography


