Homogeneous nucleation of DNA nanotubes with defined circumference

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by
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Abstract

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We map the homogeneous (unseeded) nucleation of DNA tile nanotubes of defined circumference as a function of DNA tile concentration following a temperature quench across the nanotube melting temperature. We compare results for nanotubes with six, eight, and ten double-helices in circumference using two sets of DNA sequences, one in which all binding interactions are nearly equal in strength, and the other in which binding strengths vary. One strand in each set is covalently labeled with a Cy3 molecule that fluoresces more brightly when the adjacent domain binds its complement. From the increase in Cy3 fluorescence over time following temperature quenches of different depth, we find the rate of nanotube self-assembly scales with tile concentration to a power $\alpha < 5$ that diminishes with deeper quench but is independent of helix number or binding strength distribution. When re-scaled by the time required to reach a fixed proportion of assembly, the time-course of self-assembly of a given tile set follows a universal curve. A subtle dependence on helix number is apparent in the early time behavior of this universal curve. We conclude that spontaneous nucleation of the DNA tile nanotubes we studied is rate-limited by a sub-critical aggregate that involves four strands, and that tube closure defines the critical nucleus. We further find evidence that some process, possibly spontaneous breaking (i.e., scission), bypasses the nucleation barrier and accelerates the initial time-course of self-assembly.
# Contents

**Abstract** ii

1. **Introduction** 1

2. **Materials and Methods** 4
   - 2.1 Materials 4
   - 2.2 Strand Redesign 4
   - 2.3 Sample Preparation 5
   - 2.4 Fluorescence Imaging 6
   - 2.5 Fluorometry 6
   - 2.6 Data Processing 8

3. **Results** 11
   - 3.1 Sequence Design 11
   - 3.2 Nanotube Annealing 12
   - 3.3 Temperature Jumps 14
   - 3.4 Phase Behavior 15
   - 3.5 Universal Scaling 17

4. **Discussion** 19
   - 4.1 Strand interactions are stabilized in the context of a nanotube 19
   - 4.2 Nanotubes of smaller circumference melt at higher temperatures 20
   - 4.3 Nanotubes anneal with Arrhenius behavior that is independent of circumference 21
   - 4.4 Concentration dependence indicates tetramer formation is the rate limiting step to nucleation 22
   - 4.5 $n$-dependence of phenomenological scaling suggests multiple steps along the path to the critical nucleus 24
   - 4.6 Sub-quadratic scaling suggests breakage can be a significant source of nanotube ends 25
   - 4.7 Nanotubes with uniform binding strength exhibit larger hysteresis 26
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 conclusion</td>
<td>27</td>
</tr>
<tr>
<td>Appendix</td>
<td>28</td>
</tr>
<tr>
<td>A.1 DNA Sequences</td>
<td>28</td>
</tr>
<tr>
<td>A.2 Fluorescence Microscopy</td>
<td>30</td>
</tr>
<tr>
<td>A.3 Bulk Fluorescence Measurement</td>
<td>32</td>
</tr>
<tr>
<td>A.4 Annealing Temperature vs Concentration</td>
<td>37</td>
</tr>
<tr>
<td>A.5 Tm predictions</td>
<td>38</td>
</tr>
<tr>
<td>A.6 Shape Language Modeling Tool</td>
<td>39</td>
</tr>
<tr>
<td>A.7 Universal curve</td>
<td>41</td>
</tr>
<tr>
<td>Bibliography</td>
<td>43</td>
</tr>
</tbody>
</table>
Chapter 1

Introduction

Nanotubes are an important structural primitive in DNA nanotechnology. With diameters confined to the nanoscale ($\sim 10$ nm), they can grow tens of microns in length and have persistence lengths to match. DNA nanotubes thus bridge length scales from the molecular to the material. They are accordingly investigated for a variety of applications, including as templates for nanowires (ref), pathways for molecular locomotion (ref) and rigidifying elements in smart gels [1].

For many applications, a low density of structural defects is desirable and the ability to position nanotubes relative to other structures or one another is essential. One strategy for promoting crystalline order and precision placement is to prepare a super-saturated solution of nanotube building blocks, or “tiles”, and nucleate growth from pre-formed nanotube fragments, or “seeds” [2]. However, to date, the extent of super-saturation achieved with DNA tiles is marginal: under conditions that favor seeded nucleation, nanotube growth can nevertheless be so slow that spontaneous nucleation also occurs before seeded nanotubes reach micron lengths, limiting the power of the seeded approach.

It should be possible to design a DNA nanotube to have a large nucleation barrier and correspondingly broad super-saturated regime in phase space. Justification for this hope
comes from protein nanotubes found in biology, whose functions – organizing cellular space and sustaining cellular appendages – depend upon their controlled growth from nucleating sites. Microtubules, for example, can nucleate at a rate that depends on the concentration of their protein subunit, tubulin, to the \(12\pm2\) power under the same conditions that permit them to grow at a rate of microns per minute \([3]\).

To the best of our knowledge, no other material comes close to exhibiting a concentration dependence for nucleation as steep as the microtubule’s. The similarity between the exponent of the power-law and the number of tubulin subunits in the circumference of the microtubule wall strongly suggests that tube closure is the rate-limiting step in microtubule nucleation. We asked whether DNA nanotubes of defined circumference \([4]\) would exhibit a similar, circumference-related concentration dependence in their homogeneous nucleation.

We studied the homogeneous nucleation of DNA nanotubes with six, eight and ten double-helices in circumference by monitoring bulk assembly via the change in fluorescence of a Cy-3 molecule covalently attached to one of the DNA strands \([5]\). Because the only way to achieve nanotubes with well-defined circumference is to have lateral associations enforced by distinct sequences, the relative binding strength of those sequences can be rationally designed. Taking inspiration from the microtubule, which has a nearly uniform distribution of binding strengths in circumference, we took care to design our sequences to have nearly equal strength binding domains. This symmetry simplifies interpretation of the fluorescence signal by ensuring that dsDNA stability near the fluorophore is as similar as possible to dsDNA stability at other locations along the nanotube circumference. To assess the effect of a non-uniform distribution of binding strengths on the nucleation process, we compared the nucleation of our nanotubes, with their uniform binding-strength distribution \((\sigma_{T_m} = 1^\circ\text{C}, \max(T_m) - \min(T_m) = 6^\circ\text{C})\), to that of otherwise identical nanotubes (designed by Yin et al. \([4]\)) that have a highly non-uniform
binding-strength distribution ($\sigma_{T_m} = 6^\circ C$, $\max(T_m) - \min(T_m) = 23^\circ C$).

Under the most marginal growth conditions accessible to our technique, we find nanotubes nucleate at a rate that depends on the strand concentration to the 4\textsuperscript{th} power, independent of their circumference and of the uniformity of their binding strength distribution. We further observe that, under our most marginal growth conditions, nanotube self-assembly follows a universal time-course. The early time scaling of this universal curve does vary with circumference and uniformity, although not as strongly as might be expected. We conclude that, while tube closure may define the critical nucleus (\textit{i.e.} the largest metastable aggregate), it is not the rate-limiting step. Interestingly, for the smallest circumferences in both uniform and non-uniform tube types, the early-time scaling is sub-quadratic, suggesting that another process, possibly scission, short-circuits the nucleation barrier to homogeneous self-assembly of DNA nanotubes.
Chapter 2

Materials and Methods

2.1 Materials

All DNA strands were purchased from Integrated DNA Technologies, Inc. Polu-DT42 was purchased with standard desalting. Strands used for making nanotubes were purchased with HPLC purification, hydrated in pure water to a concentration of 100 µM according to the manufacturers quoted quantities, and stored at -80°C. One strand common to all nanotubes in each (uniform or non-uniform) strand set was covalently labeled with Cy3 at the 5’ end. Neutral density filters were purchased from Lee Filters item 298 0.15ND.

2.2 Strand Redesign

The original strand set which was tested was previously designed by the yin group [4]. In order to create a strand set with uniform binding energy individual bases on strands with high binding affinity were changed from C and G to A or T. A and T have lower binding affinity due to a lower number of hydrogen bonds and as a result the binding
energy for the whole strand is decreased. For binding domains with lower strength instead A and T are substituted for C or G. As the sequences were altered binding energies were continuously monitored by using the DINAmelt web server[6]. Care was taken to check binding between every available strand and every other strand in the reaction to make sure no off target interactions were introduced by altering the sequences. Additionally when altering the strands we attempted to avoid adding large chains of a single base which can alter the resting structure of the DNA and may interfere with binding.

2.3 Sample Preparation

DNA nanotube stocks solutions were prepared by adding each strand in a given tile set to a 1x TAE buffer with 12.5 mM Mg$^{2+}$. The stock solutions were diluted in 1x TAE buffer with 12.5 mM Mg$^{2+}$ to the final concentrations.

Fluorescein was added to the stock solutions at 1.5x DNA concentration to measure relative sample concentrations from fluorescence readings at room temperature (A3). Fluorescein readings were also used to monitor the strength of Neutral density filters. Poly-DT42 was added to the stock solution and dilution buffer at 5µM to occupy space on the walls of the Optical PCR test tubes. DNA sticks to the walls and is freed by convection currents produced during a temperature change(A3). If the walls are mostly occupied by PolyDt effective concentration of nanotube forming strands remains constants when changing temperature.

While a full strand set is present samples will aggregate and sediment after a short time at room temperature. This creates a concentration gradient which can significantly impact the concentration pipetted. As a result it is important to vortex samples containing a full set of DNA strands before pipetting.

Neutral density filters were used to reduce the fluorescence intensity of higher con-
centration samples to prevent detector saturation. These filters were typically layered on one another to further reduce intensity. Typical reduction used was 0.5x for 400-600nM, 0.25x for 600-1000nM, 0.125x for 1000-2000nM, and 0.0625x for >2000nM. The neutral density filters were observed to become more dim over time, plate reads should be taken between runs to monitor this issue by comparing parts of the wellplate with different numbers of filters. The filters used for all data taken would reduce fluorescence by 0.556x and would further dim by about 0.37 percent per run.

2.4 Fluorescence Imaging

DNA nanotubes were labeled with a fluorescent Cy3 dye attached to the U1 strand which allows for Fluorescence imaging. Imaging data was used to confirm formation of nanotubes in the samples analyzed. The protocol for the anneal seen in the images of A2 is as follows: sample are heated to 90°C for 10 minutes, temperature is quickly lowered to 70°C, then the temperature is lowered by 0.1°C per minute down to 20°C and held at 20°C until retrieval. Annealed samples were diluted into buffer to 10nM tile concentration to reduce clutter in the image. Microscope images were taken using 4µL sample volume on 22mm x 22mm cover slides.

2.5 Fluorometry

Strand assembly was monitored via the change in fluorescence of a Cy3 covalently attached to the 5’ end of the U1 strand. When the labeled strand is unhybridized, the Cy3 has reduced quantum yield, presumably due to stacking interactions with adjacent bases [3]. Upon double-helix formation, Cy3 is forced into a more aqueous environment and fluoresces more brightly.
Data is taken following a temperature quench from above the melting temperature of the nanotubes (65°C) to various temperatures below the annealing temperature[fig 2]. Following the temperature jump the nanotubes undergo spontaneous nucleation which can be observed through the change in fluorescence intensity of the dye as the strands hybridize. This change in fluorescence intensity takes place over the course of a few hours. Data should be taken for at least 200 minutes following the temperature jump for assembly to reach completion. The rate of nucleation was typically extracted within the first 30 minutes.

A second rise in the fluorescence is observed in the samples around 200 minutes after the temperature jump. This increase in signal is due to sedimentation of aggregated nanotubes. The sedimentation of nanotubes brings them closer to the center of the test tube which leads to an increase in signal. Nanotubes only begin to sediment after forming clumps of sufficient size. As a result, the time when sedimentation begins and the magnitude of intensity change are dependent on concentration and temperature. Higher concentration samples (≥400 nM) and more gentle quenches have much less sedimentation because they form longer tubes which have less tendency to form dense clumps. The rate of sedimentation does not become significant until a few hours after nucleation so this effect does not interfere with the signal.

Data was taken using the following temperature profile. The samples are first held at 90°C for 30 minutes to melt any DNA nanotubes or aggregates in the solution. The temperature is then quickly decreased to 75°C and held for 15 minutes. Next the temperature is quickly decreased to 65 degrees and held for 30 minutes. The Temperature is then quickly decreased to the final temperature and held for several hours when performing a temperature jump or ramped from 65 to 40 and back for an anneal and melt.
2.6 Data Processing

The concentration of samples can be determined in situ by measuring the change in fluorescence of the dye following a temperature change while remaining above the annealing temperature of the nanotubes. At these temperatures no nanotube formation occurs. As a result, the change in fluorescence intensity is only due to the change in quantum efficiency of the dye. The quantum efficiency has a strong temperature dependence and is nearly independent of concentration. As a result, the change in fluorescence when changing temperature is proportional to the concentration of the dye. This measures the concentration of U1 which we approximate to be equal to the concentration for all other strands. We use a temperature jump from 75-65 followed by a temperature hold at 65 for 30 minutes to find the change in fluorescence (A3). The hold allows temperature to stabilize and the samples to equilibrate. This concentration measurement is correlated to the fluorescence of fluorescein added at 1.5x DNA concentration. This fluorescein intensity was measured before and after several thermal cycles to verify the Cy3 based measurement (A4).

The concentration is determined by fitting a double exponential to the fluorescence signal during the 75-65 temperature jump. The function used for this analysis was

\[ f(x) = a \times (1 - (1 - f)e^{-x/c} - fe^{-x/g}) \]

The first exponential has a time constant around 15 seconds and is caused by the time it takes for the PCR machine to change temperature. The second exponential has a time constant of around 10 minutes and results from the equilibration of the temperature in the samples. The value of \( a \) extracted from the fit gives the asymptotic value of the change in fluorescence which is proportional to the concentration of labeled DNA. The value of the parameter \( a \) is collected for each sample and a linear fit is made to a plot of \( a \) versus nominal concentration to determine a conversion factor from \( a \) to concentration (A3). This conversion factor among the
wellplates tested was 9.3±1.3 While the conversion may preserve systematic errors from sample preparation the relative values will be correct which allows for determination of power law dependence on concentration. The effects of evaporation can be corrected by measuring the relative concentration after each thermal cycle. However, because absolute concentration is not determined there may be errors in relative concentration between different sample plates.

A control sample containing only the labeled strand and its complement gives a signal for no nanotube formation. U1-T6, U1-T8 and U1-T10 were all used as controls and have identical signals. The controls and all data are divided by their concentration so that the absolute change in fluorescence due to temperature is the same among all samples. The control signal is then subtracted from the data for all other samples to remove the effect of temperature change on the dye. Data is then multiplied by concentration to give the raw data with the temperature dependence of the dye subtracted out.

In order to compare sequential measurements on a given sample data must also be corrected for bleaching. This is done by multiplying each dataset by a constant which represents the total bleaching over a time series. The constant for bleaching rate was determined by tuning a power law fit to the resulting nucleation rate measurement (A3). Cy3 dye used to observe assembly was determined through this fit to photobleach between each run by approximately 1.9 percent. Fluorescence signals should be increased by this value between each run in order to obtain correct concentration measurements each time as the sample evaporates.

The initial rate of Fluorescence increase following the temperature quench gives a measurement of the rate of spontaneous nucleation. This rate is determined by fitting the entirety of the curve to a linear spline with 20 endpoints using the Shape Language Modeling fitting engine for Matlab (A6). Note that more or less endpoints may be needed depending on the number of data points taken (A6). The fitting engine provides
the location of the end points of the linear sections which are used to determine the slope of each segment. The derivative of the curve following the onset of nucleation is almost always strictly decreasing. As a result, the maximum slope determined is also the initial slope dictated by nucleation rate rather than growth. Using maximum slope has the advantage of allows one to find the slope at lower concentrations where there might be a slight delay before nucleation begins.

Fluorescent signal from a 0.1°C per minute temperature ramp from 70°C to 40°C and back to 70°C is used to determine the melting and annealing temperature of the samples. These values are determined by taking the absolute value of a 10 point smoothed derivative of the curves. The derivatives of the anneal and melt both form a peaked distribution. The location of the maximum of this distribution is taken to be the annealing or melting temperature respectively. The error for the measurement is determined from the width of the half maximum of the distribution.

The data curves following a temperature jump can be collapsed into a single universal curve by altering the time series. The time series for each curve is rescaled by the time to reach 0.2 on the concentration corrected scale. Data which did not reach it’s asymptotic value at the end of the time series was ignored based on a filter of the maximum standard deviation of the last 100 points of the time series and a comparison between the standard deviation of the last 100 points and third to last 100 points. Data which had not reached its asymptotic value of assembly could not be properly normalized and as a result did not fall on to the universal curve. The slopes of each universal curve from rescaled time 0.4 to 0.9 were determined by a power law fit and used to determine the number of steps in the nucleation process.
Chapter 3

Results

3.1 Sequence Design

We used the change in fluorescence of a Cy3 molecule that was covalently coupled to the 5’ end of one DNA strand to monitor the self-assembly of DNA nanotubes with single-stranded crossovers between adjacent double-helices (see Methods). We used this type of “half-crossover”, or HX, tiling scheme because it permits control over the number of double-helices in the circumference of a nanotube while using a minimal number of distinct strands, as first demonstrated by [4].

The sequence design of n-helix HX-nanotubes (nHT) involves n different strands with four binding domains each (Fig. 3.1a). The binding domains are either 10 or 11 bases long and result in a 21-basepair interaction between any two strands. The strength of these binding interactions can be gauged from the predicted melting temperature of the each 10 or 11-basepair double-stranded DNA (dsDNA) (see Methods). In the sequence set designed by the yin group [4], these melting temperatures average about 50°C and vary by as much as 14°C (Fig. 3.1b, top). To explore the effect of such non-uniformity on nanotube nucleation, and to more closely mimic the design of biological microtubules.
Figure 3.1: (a) Schematic of sequence design for DNA nanotubes, shown for six helices in circumference (reproduced with permission from [4]). In this work, each DNA strand is 42 bases long, with sequences complementary to sequences on two neighboring strands. Unlike most DNA tilings, in this lattice neighboring double-helices are connected through a single-stranded, or “half”, crossover, so we refer to these as single-stranded- or HX-tiled, nanotubes. (b) Two different sequence sets were used: one in which the binding strength varies significantly, as indicated by their predicted melting temperatures (upper). The other was designed for greater uniformity of binding strength across all domains (lower).

(which are made of a single subunit), we redesigned the binding domains to yield strand-strand interactions of uniform strength (Fig. 3.1b, bottom). We refer to nanotubes made with this new, uniform binding-strength strand set as “uniform nHT” (nHTu) and those made with the original (non-uniform binding-strength) strand set as “non-uniform nHT” (nHTn). Predicted melting temperatures for the binding sequences in nHTu all lie within a 4°C range about an average of 44°C, which was chosen to be similar to the lowest predicted melting temperatures in the nHTn strand set.

3.2 Nanotube Annealing

Both strand sets, when cooled from 70°C to 30°C at 0.1°/min at a concentration greater than 1 μM in a magnesium-containing buffer (pH 8.3), formed nanotubes. nHTu were indistinguishable from corresponding nHTn by fluorescence microscopy (Fig. A2 ).
Nanotube formation was accompanied by a steep increase in Cy3 fluorescence (Fig. 3.2a). The magnitude of the increase was largest for 6HT, possibly because the high curvature of its lattice prevented some fluorescence-quenching interactions. The point of greatest derivative in the annealing curve, the annealing temperature, $T_a$, was sensitive to the cooling rate. By contrast, the point of greatest derivative in the melting curve, the melting temperature, $T_m$, was independent of the heating rate for rates below 0.5$^\circ$/min. $T_m$ was also independent of strand concentration at all but the lowest concentrations tested, while $T_a$ increased logarithmically with concentration (A4). Measurements of $T_m$ and $T_a$ were made at the slowest convenient rate $|dT/dt| = 0.1^\circ$/min, and at concentrations ranging from 20 nM to 2 µM.

Even at the highest strand concentrations tested, $T_m$ was many degrees higher than $T_a$ for both uniform and non-uniform nanotubes (Fig. 3.2a), indicating the existence of a
kinetic barrier to nanotube nucleation \[7\]. The difference was less pronounced for \(nHTn\) than for \(nHTu\), by about 2°C for all \(n\) (Fig. 3.2b). Interestingly, \(T_m\) was systematically higher for smaller helix-numbers, while \(T_a\) was the same for all \(nHTu\) and increased slightly with \(n\) for all \(nHTn\) in a given strand set (A5). As a result, the hysteresis, \(T_m - T_a\), was greatest for 6-helix tubes (6HT) and decreased with increasing number of helices in circumference in both strand sets (Fig. 3.2b).

The insensitivity of \(T_a\) to helix number suggests that nanotube closure is not the rate-limiting step for nucleation under our conditions. To test for the existence of another rate-limiting step, we looked for hysteresis in ribbon-forming subsets of the \(nHTu\) strands. The \(T_a\) of these ribbons was the same as the \(T_a\) of their corresponding \(nHTu\) and accordingly independent of helix number. Their \(T_m\), by contrast, increased with helix number. Thus, all ribbons involving three or more strands (i.e. two or more double-helices wide) also exhibited hysteresis (Fig. 3.2b), lending support to the hypothesis that the highest-energy metastable state is sub-tubular.

### 3.3 Temperature Jumps

To determine the size of the critical nucleus, we looked at the effect of strand concentration on the rate of nanotube self-assembly following a temperature jump from 65°C (several degrees above even the highest \(T_m\)) to various temperatures below \(T_m\). All three sizes of nanotubes behaved similarly (Fig. 3.3a). Both the maximum rate and the overall extent of assembly increase with strand concentration and with the depth of the temperature quench (Fig. 3.3b,c).
Figure 3.3: Nanotube assembly following a temperature jump from 65°C (above $T_m$) to various temperatures below $T_m$. For a given concentration and temperature jump, 6-, 8- and 10-helix tubes behave similarly (a). Increasing the concentration of DNA strands in solution (b) and lowering the final temperature (c) have the similar effect of increasing the rate of assembly. A secondary/slower increase in fluorescence that appears at long times in some samples results from sedimentation.

3.4 Phase Behavior

We explored a wide range of concentrations and quench depths to map the phase diagram of nanotube nucleation. Phase diagrams for uniform and non-uniform nanotubes were similar, though the former was more sparsely sampled. A lower bound on concentration of 20 nM was imposed by the sensitivity of our instrument. An upper bound of 2 µM was chosen to mitigate cost. The time resolution of the scanner obscured measurements at the deepest quenches and highest concentrations. Evaporation and sedimentation interfered at long times, limiting measurement at shallow quenches and/or
low concentrations.

Figure 3.4: Phase diagram for nanotube assembly. At the highest concentrations and deepest quenches (large green circles, $N_{totaln}$ = 19 $N_{otalu}$ = 78), fluorescence intensity changes were obscured by the temporal resolution of the scanner. At low concentrations and shallow quenches (large red circles, $N_{otaln}$ = 165 $N_{otalu}$ = 108), intensity changes due to evaporation and/or aggregate sedimentation confounded measurement. In between, the maximum rate of assembly had a power-law dependence on concentration, with an exponent $\alpha$ approaching 5 at the gentlest accessible quenches (small, dark blue circles, $N_{otaln}$ = 2479 $N_{otalu}$ = 1798), and less than 4 at deeper ones (large, light blue circles, $N_{otaln}$ = 2630 $N_{otalu}$ = 352). The phase diagram of $nHTu$ was similar to that of $nHTn$, but more sparsely sampled.

Within these limits, the maximum rate of fluorescence increase has a power-law dependence on concentration (Fig. 3.5). For deep quenches, the scaling is quadratic. The measured exponent for $nHTn$ at all quenches greater than 14°C was $\alpha = 2.07 \pm 0.05$. 

16
For moderate to gentle quenches, the scaling changes, becoming steeper at low concentrations. At the gentlest quenches, the high value of $\alpha$ approaches five (Fig. 3.5, inset). We note this transition on the phase diagram by distinguishing between conditions which contribute to a scaling law with $\alpha \geq 4$ (small, dark blue circles) from those which contribute to a scaling law with $\alpha < 4$ (large, light blue circles). A transition between the low and high alpha regime can be observed when plotting the maximum rate of fluorescence increase against the concentration (Fig. 3.5).

3.5 Universal Scaling

Under those conditions consistent with $\alpha \geq 4$, the time-course of fluorescence exhibits phenomenological scaling [8]. That is, each individual time-series is fully characterized by a single intensity scale and a single time scale. When measured values are divided by these
scales, the resulting curve that is the same for all time-series (Fig. 3.6). Specifically, upon re-scaling concentration-normalized fluorescence with respect to the asymptotic intensity in a time series, and then re-scaling time by the time to reach 20% of that final intensity, all time-series data for a given tube type collapse onto a universal curve. The early-time behavior of this curve is sub-quadratic for all $nHTu$, with exponents of $1.54 \pm 0.03$, $1.71 \pm 0.03$, and $1.94 \pm 0.02$, for $6HTu$, $8HTu$ and $10HTu$, respectively. The behavior of $6HTn$ is also not-quite quadratic, with an exponent of $1.92 \pm 0.06$. However, the universal curves of $8HTn$ and $10HTn$ have exponents of $2.48 \pm 0.06$, and $2.91 \pm 0.06$, respectively, that are significantly larger than two and consistently greater than the exponents of the corresponding $HTu$.

Figure 3.6: Rescaling time in each dataset by a characteristic time (e.g., the time to reach 20% on the concentration normalized intensity scale) collapses time series data from all experiments (i.e., all concentrations, temperature jumps and helix numbers) onto a universal curve. Such a universal curve is consistent with the simplest set of kinetic equations describing nucleation of a linear polymer (i.e., one whose growth rate is linear in monomer concentration)\[8\]. See discussion.
Chapter 4

Discussion

4.1 Strand interactions are stabilized in the context of a nanotube

The binding domains of the nHTu were designed to have a thermal stability similar to the weakest binding domains of the nHTn. Melting temperatures of the nHTu were accordingly several degrees lower than those of the nHTn, reinforcing the common sense that nanotube $T_m$ is determined by the strength of binding between component strands. However, quantitative prediction of nHT melting temperature from tile strand sequences remains elusive.

All nHT melting temperatures were greater than 60°C (and independent of strand concentration at concentrations above 50 nM, as expected) (see Fig. S#). Using the Dinamelt server [6], melting temperature predictions for duplexes of neighboring tile strands reflect hybridization of only a single binding domain and are accordingly much lower than 60°C. The highest predicted $T_m$ in the nHTu tile set is more than 10°C lower than the observed nHTu melting temperature. The predicted $T_m$ for hybridization in the
vicinity of the Cy3 reporting dye is several degrees lower still (A5). We attribute this discrepancy between prediction and measurement to cooperativity induced by the nanotube context, in which every strand is secured in the structure by at least two binding domains. However, even when sequences were restructured to enable simultaneous hybridization of two binding domains (A5), predicted $T_m$’s were consistently several degrees lower than observed $nHT$ melting temperatures.

By contrast, the predicted melting temperatures of 21bp duplexes of successive binding domain sequences all exceed observed $nHT$ melting temperatures. This likely reflects the entropic cost imposed by securing non-contiguous domains, and the enthalpic cost of torsional strain imposed by the nanotube lattice. The average binding strength of 21 base duplexes was around 3 degrees higher for $nHTn$ than $nHTu$ as were observed melting temperatures. This suggests that it may be possible to model the melting temperature in this manner if the correct value could be subtracted to account for strain and non-contiguous domains.

4.2 Nanotubes of smaller circumference melt at higher temperatures

Control over nanotube circumference was achieved via sequence design. Unique sequences establish four binding domains along each strand that participate in laterally adjacent double-helices following the lattice design of [4]. This lattice design places crossovers on alternate strands every 10 or 11 base-pairs along any given dsDNA in the nanotube wall, so as to create an average angle of 150° between the two nearest-neighboring dsDNA ([4 Supp Info). This average angle matches the angular extent of the minor groove in B-form DNA and imparts a lateral curvature commensurate with
a 12 helix nanotube. Lattices that cyclize with other than 12 double-helices in circumference impose a net torsional strain on the base pairs, which can reduce their thermal stability. One might therefore expect the melting temperature of $n$HT to decrease with $n$, for $n < 12$.

However, torsional strain in a rod can be relieved by bending and local untwisting to create axial helicity, also known as writhe. Accordingly, fluorescence microscopy of $n$HT adsorbed on glass revealed permanent, often periodic bends along their lengths, consistent with helicity (A2). The apparent amplitude and spatial frequency of the bends decreased with increasing $n$. We therefore understand the enhanced thermal stability at smaller circumference ($T_m(6HT) > T_m(8HT) > T_m(10HT)$) as a consequence of lower bending stiffness [9] enabling less torsionally strained conformations.

$n$-helix ribbons, by contrast, have melting temperatures that increase with $n$ [3.2]. Greater flexibility and fewer geometrical constraints, allow ribbons to bend, writhe and relieve torsional strain even more easily than $n$-helix tubes, so torsional strain is unlikely to play a role. Instead, increasing thermal stability likely reflects the reduction in edge to area ratio as the number of helices across a ribbon increases.

Although its $T_m$ increases with $n$, the thermal stability of an $n$-ribbon is nevertheless between $5^\circ C$ and $6^\circ C$ lower than that of its corresponding tube. The additional stability of the tube suggests that tube closure is the final step in nanotube nucleation.

### 4.3 Nanotubes anneal with Arrhenius behavior that is independent of circumference

Boundaries in the phase diagram represent conditions of constant assembly rate. The lower boundary (between red and blue markers) marks a threshold of detectable assembly
within 200 minutes. The upper boundary (between dark blue and light blue markers) marks where initial assembly rates begin to deviate from their highest-order concentration dependence. An exponential relationship between temperature and concentration defines the assembly rate. This is seen at both the lower cutoff, which is based on total assembly within 200 minutes, and at the upper cutoff, which is based on the concentration dependence on assembly at early times. Both cutoffs are indicative of a set of conditions with nearly constant assembly rate. If we model the concentration dependence as \( \frac{dC}{dt} = C^4 e^{-\Delta G/k_b T} \). Additionally the annealing temperature gives another measure of temperature conditions with constant assembly rate. If we take \( \frac{dC}{dt} \) constant at these boundaries we can extract a value of delta H from a fit to the data (A4). For uniform and non uniform nanotubes we find that delta H is 357±8 and 340±21 Kcal/mol respectively when fitting to the anneal temperature. This measurement correlates to the value of delta H for a tetramer as calculated by the DINAmelt web server which gives 332.9 Kcal/mol for uniform and 336.3 Kcal/mol for nonUniform. We expect that nonUniform should fit a slightly higher value but the difference is within the error of the measurement. When this slope is superimposed on the phase diagram we see agreement in the value of this slope with the two boundaries of constant assembly rate (A4).

4.4 Concentration dependence indicates tetramer formation is the rate limiting step to nucleation

Early stages of nanotube assembly display a power law dependence on concentration that reflects the rate-limiting step to nucleation. When high concentrations of strands are subjected to a deep thermal quench, strand-dimers persist for long enough to collide. Under such conditions, nucleation and growth are both bimolecular reactions, and the
rate of assembly scales as concentration squared. When the concentration is reduced and/or the quench becomes less severe, two bonds are required for strands to persist in association long enough to encounter others. The facet of a nanotube presents sites at which a single strand can make two bonds, so tube growth proceeds in proportion to strand concentration. Between strands in solution, however, the smallest configuration that offers every strand two bonds is a tetramer. The number of binding interactions that must occur for a tetramer to become a tube depends on the tube circumference, but being either bi-molecular (tetramer-tetramer) or tri-molecular (tetramer-strand-strand) interactions, no step will be as limiting on the rate of nucleation as tetramer formation. Accordingly, the rate of assembly becomes dependent on concentration to the fifth power. With a concentration to the fourth rate dependence on tetramer formation and on additional power of concentration due to higher growth rates at higher concentration.

It is possible that, at gentler quenches than were explored here, a steeper, circumference-based concentration dependence could arise. However, conditions that require more than two bonds for a stable interaction between strands would preclude nanotube growth by single strand addition. For example, if four bonds per strand were required for stability, the critical nucleus would be a closed ring, but then growth, which would have to proceed by addition of multimers, could be so slow as to make assembly difficult to observe or effectively negligible.

We therefore suspect that 5 is the maximum measurable exponent. This corresponds to a growth rate that is linear in the strand concentration multiplied by a nucleation rate that depends on strand concentration to the 4th power.
4.5 \( n \)-dependence of phenomenological scaling suggests multiple steps along the path to the critical nucleus

Phenomenological scaling of the formation curves is consistent with a set of coupled kinetic equations [8] describing nucleation of a linear polymer (i.e., a polymer that grows at a rate that is linear in its monomer concentration). One solution to those coupled differential equations predicts that polymer mass increases as \( (t/t_0)^{(k+2)} \) where \( k \) is the number of intermediate steps along the path to nucleation. This solution assumes that those intermediate steps all occur at the same rate and therefore contribute equally to the value of \( k \). Our results suggest that this assumption does not hold for \( n \)HT nucleation. It seems instead that nucleation involves a slow step in which a tetramer is formed, followed by several faster steps on the way to tube closure. The result is a concentration to the 4th dependence for nucleation and a roughly quadratic scaling with time.

We observe a scaling \( t/t_0 \) that ranges from less than 2 to greater than 2 with \( k_{10HT} > k_{SHT} > k_{6HT} \) in both uniform and non uniform tile sets. For all conditions we also observe \( k_{nHTn} > k_{nHTu} \).

Circumference dependence of the phenomenological scaling indicates that tube closure may be the final step in nucleation, while the concentration dependence of the assembly rate suggest that the rate limiting step in nucleation is tetramer formation. We propose that tetramer formation is followed by several fast intermediate steps in which strand dimers are added such that each strand is stabilized by two bonds. This process would have only a third order concentration dependence and so would occur considerably more quickly than tetramer formation. This disparity in step rate might lead to intermediate steps contributing less than integer value to the exponent. Nevertheless because 6 HT
nanotubes requires at least 2 such events in order to close, while 8HT requires 4 and 10HT requires 6 one would still expect a circumferential dependence to emerge (fig). Furthermore the increase in $k$ is nearly constant which is consistent with an increase of 2 steps each time.

Non uniform nanotubes exhibit a larger value of $k$ than their uniform counterparts. This may reflect that nonuniform nanotubes have weaker bonds in intermediate steps relative to those in the tetramer while uniform tubes have bonds with equal strength in both cases. In the non uniform nanotubes the intermediate steps make up a larger proportion of the nucleation barrier, meaning they contribute more to the nucleation rate and as a result have a larger impact on the scaling exponent.

4.6 **Sub-quadratic scaling suggests breakage can be a significant source of nanotube ends**

For both 6HT$n$ and 6HT$u$, as well as 8HT$u$, the phenomenological scaling at early times is sub-quadratic. It may be that our technique is not sufficiently sensitive to observe the asymptotic value of $k$, but another interesting possibility is that nanotube scission short-circuits the nucleation process. Like any living polymer, DNA nanotubes undergo both scission and joining at equilibrium [10]. Under non-equilibrium conditions that favor assembly, a scission event creates an additional pair of sites from which nanotubes can grow. Like assembly, scission can be expected to have an Arrhenius behavior. We found no clear temperature dependence in the scaling exponent $k$ in our datasets, but note that the range of temperatures explored is perhaps quite small compared to the free energy barrier in question.
4.7 Nanotubes with uniform binding strength exhibit larger hysteresis

The larger hysteresis of $n\text{HTu}$ compared to $n\text{HTn}$ indicates that uniform binding-strength distributions present a larger barrier to nucleation. To understand why, consider two tile sets with the same average $T_m$ (unlike ours). If there exist three strands that can form a tetramer at a temperature above the average $T_m$, they will drive $T_a$ closer to $T_m$. It is possible that a non-uniform distribution can be crafted in which every tetramer is equal or lower in stability than the mean. But, in general a non-uniform binding strength distribution will facilitate nanotube formation.
Chapter 5

conclusion

The results discussed above suggest that DNA nanotube nucleation is rate limited by tetramer formation and the critical nucleus is defined by the closure of the nanotube. While the nucleation barrier is defined by tube circumference the extent of the effect is too small to allow for design of DNA nanotubes with a larger nucleation barrier for seeded nucleation. Furthermore, the possibility that nanotube scission can bypass the nucleation barrier makes the prospect of designing nanotubes for precise seeded nucleation more difficult. Even if a nanotube lattice is designed with a larger nucleation barrier spontaneous nuclei from scission may still prevent precision placement of nanotubes. It may be possible to design a nanotube which is actively destabilized when the nanotube is not attached to a seed. This would require a design which exploits an asymmetry that would make one end of the tube dissociate much more quickly than the other. The seed would need to give additional stability to the week edge of the nanotube and prevent dissociation at that end. To accomplish such a redesign additional research into the stability and growth of DNA nanostructures which could reveal how to create such an asymmetry in design.
Appendix A

Appendix

A.1 DNA Sequences

Nanotubes are assembled from a set of 42 base ss-dna from either the Uniform or Non Uniform tiles. The tile sets used in the data are as follow:

6HT (U1, U2, U3, U4, U5, T6)
8HT (U1, U2, U3, U4, U5, U6, U7, T8)
10HT (U1, U2, U3, U4, U5, U6, U7, U8, U9, T10)

A.1.1 Uniform nHT

U1u AGCGATATGC ACACTAGACCT CCTGTAGATCC TGAACGTGGA
U2u GGATCTACAGG TCCACGTTCA CCAACTTTCC TGGAATCTTCT
U3u GGAAAGTTGG AGAAGATTCGA ATGTGAGAAGG TAGTTTAGCGT
U4u CTTTCTCACAT ACGCTAAGCT GTAGAGCAGG TCATCGAGATT
U5u CCTCGTCTAC AATCTCGATGA GGAAGAATGTG TCGGAGAACT
U6u CACATTCTCC ATGTCTCGGA ATGGGATGAGG AGCTTGTAGTG
U7u GCCAATCCAT CACTACAGCT TTCCGTAAGTA TGAACGTGGGA
U8u TACTTACGGA TCCCAAGTCA AAAAAATGAG ACATGCTTCAT
U9u CTCACGGTCT ATGACTACATG GTGAGAATGTG CTGGCTACC
T6u CACATTCTCC ATGTCTCGGA GCTAATCGCT AGGTCTAGTGT
T8u TACTTACGGA TCCCAAGTCA GCTAATCGCT AGGTCTAGTGT
T10u CAGTATCTCC AGTAGCAATCG CTAATCGCT AGGTCTAGTGT

A.1.2 NonUniform nHT

U1n GGCGATATGG ACACTAAGCCA CCTTATGATCC TGTATCTGGT
U2n GGATCTAAAGG ACCAGATACA CCACTCTTCC TGACATCTTTG
U3n GGAAGAGTGG ACAAGATGCCT CCGTGAGAACC TGCAATGCGT
U4n GGTTCTCACGG ACGCATTGCA CCGCACGACC TGTTCGACAGT
U5n GGTCGTGCGG ACTGTCGAACA CCAACGATGCC TGATAGAAGT
U6n GCCATCGTTGG ACTTCTATCA ATGCACCTCC AGCTTTGAATG
U7n GAGGTTGCTAT CATTCAAAGCT AACGTTAACTA TGACTTTGGGA
U8n TAGTTACCGTT TCCCAAGTCA AACACTAGAC ACATGCTCCTA
U9n GTCTAGTGTT TGGAGCATGT CGAGACTACAC CCTTGCCACC
T6n GCCATCGTTGG ACTTCTATCA CCTAATCGCC TGGCTTAGCGT
T8n TAGTTACCGTT TCCCAAGTCA CCTAATCGCC TGGCTTAGCGT
T10n GTGTAGTCTCG GGTCGGAAGG CTAACATGCC TGGCTTAGCGT
A.2 Fluorescence Microscopy
Figure A.1: Fluorescence Microscopy images of samples following a 0.1 C/min anneal. Samples were annealed at 1 micromolar and diluted 100x for imaging. Images confirm that both nHTu and nHTn strand sets form nanotubes with identical structure. Difference in nanotube density in each image is due to variation in dilution and non homogeneous distribution of nanotubes on each slide. Bulk fluorescence confirms similar levels of assembly. Low concentration samples contain large florescent clumps. These clumps likely form at low temperature where off target interactions become significant. Low concentration samples do not form a sufficient number of nuclei to deplete tile concentration before reaching these low temperatures.
Appendix

Chapter A

A.3 Bulk Fluorescence Measurement

A.3.1 75-65 Tjump

Figure A.2: Plot Showing a temperature change from 75 to 65 degrees well above the melting temperature of any 2 strands. Temperature is held at 65 for 30 minutes to allow fluorescence to equilibrate. The curve fits a double exponential with the first exponential thought to model temperature change in the pcr machine($k = 10$ seconds) and the second due to convection currents establishing homogeneity in the sample($k=10$ minutes). Change in fluoresce is proportional to the concentration of U1 tiles in the sample Which allows this value to serve as an in situ measurement of relative tile concentration.
A.3.2 Fluorescein

Figure A.3: fluorescein dye was added to the samples at a concentration proportional to nanotube tile concentration which is measurable at room temperature. The fluorescein Intensity is completely independent of nanotube formation giving another relative measure of tile concentration. The correlation between the two concentration measurements verifies that the cy3 based concentration measurement is accurate.
A.3.3 Fit value to concentration

Figure A.4: The change in fluorescence of the cy3 dye following a temperature jump starting and ending above the melting point of the nanotubes is proportional to the concentration of the U1 strand. This gives a measurement of relative concentration between the samples. By comparing these values to the nominal concentrations an approximate conversion to concentration can be established. Using the same conversion for each temperature jump we can correct for the sample evaporation by measuring the concentration each time.
A.3.4 Poly-Dt42

Figure A.5: Addition of BSA or Poly Dt 42 strands reduces the time constant for response to temperature change. We hypothesize convection currents unstick DNA from the walls of the container which changes the equilibrium concentration. To prevent DNA sticking to the container a high concentration (5µM) of Poly-DT42 DNA is added to occupy space on container walls. Substituting BSA for Poly-DT42 resulted in an even greater reduction in equilibration time but caused samples to gel and prevented nanotube formation.
A.3.5 Bleaching Rate Determination

Figure A.6: Variation of the correction to account for photo-bleaching of the cy3 dye shows that at a critical value of bleaching rate the best power law fit to the data is obtained. The power law is consistent with our model of nucleation and is observed for each given temperature jump. Therefore the deviation between temperature jumps must be due to photo-bleaching and the bleaching rate can be obtained by finding the value which aligns all temperature jumps into the best power law fit.
A.4 Annealing Temperature vs Concentration

Figure A.7: The observed annealing temperature displays Arrhenius behavior over the range of concentrations measured. The top panel shows the anneal (lower) and melting (upper) temperatures as a function of concentration. In the center panel anneal temperatures are fit with an exponential function giving the value of delta H in kCal/mol. The slope obtained by the curve fit is superimposed on the phase diagram to show that this Arrhenius behavior governs assembly under conditions which have roughly the same assembly rate.
A.5  Tm predictions

Melting temperature predictions using mfold server Two State Melting with 1 micro-
molar tile concentration 12.5 millimolar Mg2+ and 10 millimolar Na+

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Figure A.8: Table of two strand hybridization temperatures using DINAmelt web server [6]. The first set of temperatures used to create 3.1 are predicted from both strands unmodified. In this case the stronger domain will hybridize along with some off target interactions. The 21 base predictions are for each 21 base sequence that would be hybridized to the second strand and its complement. The nested domains involve rearranged strands such that the first domain of strand 1 is complementary to the second domain of strand 2 and the last domain of strand 1 is complementary to the third domain of strand 2. Domain bindings below are the 10 or 11 base sequence and its complement. The domain measurement was used when redesigning the nanotubes for uniformity.
Appendix Chapter A

A.6 Shape Language Modeling Tool

The toolbox used here can be found at: http://www.mathworks.com/matlabcentral/fileexchange/24443-slm-shape-language-modeling

A.6.1 Initial Rise

Figure A.9: Demonstration of SLM curve fitting tool shows first 50 minutes following temperature jump fit with linear spline with 4 endpoints. The fitting tool places endpoints to optimize fit allowing the fit to capture the initial linear slope of the formation curve. Full time traces (300-400 min) were fit with 20 end points and the maximum slope was used as a measure of nucleation rate. The maximum value almost always occur ed in the first segment but in very slow formation the delay time before nucleation onset would fit one or more linear segment.
A.6.2 Errors

Figure A.10: The maximum rate of fluorescence increase is measured by finding the slope of a linear region immediately after following a temperature jump. The slope is found by fitting the data with a linear spline. The location of the points is optimized using the SLM engine script for matlab [ref]. It is observed that with 20 points the error is very close to its asymptotic value determined by averaging the value with 21-25 endpoints, all of which provided very accurate fits upon inspection. Therefore 20 points were used in the analysis that led to Fig. 6
A.7 Universal curve

Figure A.11: K+2 exponents extracted from fits to each rescaled dataset on the universal curve. The plot displays statistical significance of change in slope between tube types. The average value of the single fits is in agreement with a fit to all the data.
A.7.1 10HT universal time scaling show signs of multistep mechanism at low Concentration

Figure A.12: K+2 exponents extracted from fits to each rescaled dataset on the universal curve. 10HTn displays an increasing exponent at low concentrations. This is thought to occur because at these concentrations rates of strand dissociation from sub critical aggregates becomes significant.
Bibliography


43