Dynamic Molecular Combing of the Bacteriophage λ Genome: Force Analysis and Mapping Applications

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Abstract: DNA molecules can be elongated and arrayed on a cover slip using the method of dynamic molecular combing (DMC). We wish to design a method of optically mapping DNA by using fluorescence tagged PNA, which bind to sequences of seven base pairs in length, to mark the occurrences of these sequences in combed DNA. I have showed that decreasing the surface tension of the combing solution causes a decrease in the elongation attained during DMC. Thus, the process of DMC may be altered easily to allow stretching of molecules to differing lengths. I have also shown that DNA-PNA hybrids may be combed onto glass cover slips, and that the PNA may then be tagged with fluorescent beads, optically indicating the location of the PNA along the DNA molecule. It is hoped that the refinement of this method will result in the development of faster and more accurate sequencing methods.

Section I: Introduction

DNA encodes the information that specifies an organism's entire structure and directs all of its biochemical processes. Thus, the specific DNA sequences of living organisms are vital keys to unlocking the fundamental nature of life itself, and the quick and accurate determination of these sequences is beneficial to every biological field. In recent years, biologists have found the technique of dynamic molecular combing (DMC) to be a valuable tool in the search for more efficient sequencing methods[1-6].

The basic principles of DMC are simple. When a cover slip is submerged in a DNA solution, the single stranded sticky ends of the DNA bind to the cover slip via hydrophobic interaction. When the slip is removed from solution, the molecule is dragged out of solution by its anchored end and the meniscus of the combing solution exerts a stretching force on the molecule. As the molecule exits the meniscus, electrostatic interactions between the molecule and the cover slip bind the straightened DNA to the slip. In this way, large numbers of DNA molecules may be elongated and arrayed on cover slips, and then dyed and imaged. Investigators have probed such arrays using fluorescence labeled complementary DNA strands and restriction enzymes that bind to specific sequences along a DNA molecule, extracting sequence information from a large number of molecules in a short period of time[3, 7].

The potential applications of dynamic molecular combing are, however, far from exhausted. It should be possible to fluorescence tag any sequence specific probe, allow it to bind to combed DNA, and directly observe that a specific sequence is located wherever the probe binds. We are limited in the choice of sequence specific restriction enzymes by what nature has already provided. But man-made DNA homologues such as peptide nucleic acids (PNAs) may serve as probes as well1. PNAs of even 7 bases in length exhibit strong sequence-specific binding, and their short recognition sites will occur at a number of places along an average DNA molecule. Moreover, one has a fair amount of flexibility in designing PNA recognition sites. The combination of dynamic molecular combing and PNA-DNA binding has the potential to dramatically increase the amount of information that one can optically extract from a DNA molecule.

I first performed experiments that allowed me to modulate molecular elongation by varying the surface tension of the combing solution. In the interest of increasing spatial

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1 PNAs, or peptide nucleic acids, are DNA homologues composed of regular bases linked by a N-(2-amino ethyl) glycine backbone. They consist of a palindromic sequence of a variable number of base pairs, separated by linkers at the center (e.g. CTTCCCTT-eg-eg-TTCCCTTC). PNAs may bind to a target DNA sequence by undergoing Hoogsteen and Watson-Crick pairing with one of the DNA strands, while displacing the other strand; the number of hydrogen bonds present in this conformation, adding to the stability conferred to the PNA-DNA dimer by the lack of charge on the PNA's backbone, allows PNA to intercalate itself into DNA helices and bind with remarkable strength. The PNAs that I use are very small in length compared to the entire bacteriophage λ DNA genome (7 bp compared to 48,000 bp), and recognize 15 sites along the λ genome.
resolution between labeled PNA probes, I wish to extend the DNA molecules as much as possible, while remaining sure that the PNA remains bound to DNA during the combing process\(^2\). Once a relationship between solution surface tension and molecular extension was determined, I began conducting pilot experiments involving the combing of PNA-bound DNA.

I describe the technique of DMC and give an account of the series of experiments that were performed in Section II. Section III contains representative images of combed DNA molecules, collections of ensemble data, and evaluation of the error present in my measurements. In Section IV, I discuss the significance of my images and identify the trends present in my collections of data, and I offer my conclusions in Section V.

### Section II: Materials and Methods

#### Preparation of Cover slips

Cover slips were washed for two hours in NaOH-EtOH solution (7 g NaOH in 70 mL 57% ethanol) on an orbital shaker and rinsed ten times in deionized water. The slips were then transferred to a poly-L-lysine solution (125 mg/mL of 15,000 MW poly-L-lysine) and soaked for 45 minutes. The slips were rinsed again with deionized water and allowed to dry before use.[8]

#### MES-ethanol Surface Tension Verification

The surface tensions of 50 mM MES solutions (pH 5.6) with varying concentrations of ethanol were measured using the capillary rise method[9]. The height to which liquid rises in a submerged capillary tube relative to the surrounding level of liquid is linearly related to the surface tension, \( \gamma \), of the solution:

\[
\gamma = \frac{h \rho g}{2}, \quad (1)
\]

Where \( \rho = 1 \) g/cm\(^3\) is the density of the liquid, \( r = 0.421 \) +/- 0.0005 mm is the radius of the capillary tube, and \( g = 9.8 \) m/s\(^2\) is the gravitational acceleration constant. Thus the linear relation is expected to be

\[
\gamma \text{ (dynes/cm)} = 20.61^*h \text{ (cm)}
\]

Using published values for the surface tensions of 0%, 34%, and 60% ethanol solutions[10], I constructed a calibration curve relating capillary rise height to the known surface tensions of these liquids at 21°C. The linear relationship between rise height \( h \) and \( \gamma \) was found to be:

\[
\gamma \text{ (dynes/cm)} = 21.23 + 6.02.
\]

The experimental setup shows a similar linear correlation to the expected correlation, with a 6 dyne/cm offset that is probably related to the apparatus design.

#### DNA Preparation

Phage \( \lambda \) DNA (NE Biosystems) was diluted to a concentration of 50 pM in MES buffer (pH 5.65) that had been filtered through a Cameo 25ES Filter (0.45 pore size) [11]. DNA-MES solutions of 0.25%, 0.5%, and 0.75% ethanol were prepared in the same fashion.

#### Staining of DNA with \textit{YOYO-1}

The phage-\( \lambda \) DNA in solution was stained with the DNA specific dye fluorescent dye \textit{YOYO-1} (Molecular Probes). \textit{YOYO-1} was diluted to a concentration of 1 \( \mu \)M and spun down for four minutes at 14000 RPM in a microcentrifuge before use to sediment large particles present in the dye solution. DNA was dyed at a ratio of 1 dye molecule/1000 bp according to the manufacturer's instructions. Dyeing the DNA after combing produced similar DNA images with increased fluorescence background due to adsorption of \textit{YOYO-1} onto the cover slips.

#### PNA Preparation (Performed by Katherine Ryan)

A PNA with the sequence H-CCTCTTTT-eg-eg-TTTCTCC-NH\(_2\) (carboxy to amino terminus) was first synthesized using t-Boc group chemistry. The synthesis products were separated by HPLC, and the mass of these products was obtained through time-of-flight mass spectrometry[12]. Amine-reactive biotin was then linked to the amino end of the PNA by incubating a 20 molar excess of biotin with PNA for two hours on ice.

#### PNA-DNA Preparation

PNA was bound to DNA by combining PNA in 10-fold molar excess with phage \( \lambda \) DNA in 100 mM sodium phosphate buffer (pH 6.6). This solution was then brought from 80°C to 4°C at a rate of 1°C per 5 minutes. The binding reaction was verified by Katherine Ryan using an electrophoretic mobility assay[12].

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\(^2\) Since the PNA-DNA binding reaction is dependent on the formation of several sets of hydrogen bonds between DNA and PNA bases, gross deformation (through over elongation) of the native double-helical structure of DNA might affect the binding of PNA.
**Combing Procedure**

DNA solutions were first heat shocked for one minute at 65°C before combing, then cooled to 5°C. RNA-DNA solutions were not heat shocked before combing. Clean cover slips were held at one end and slowly lowered into the DNA solution, which was maintained between 5°C and 9°C. After five minutes, the cover slip was pulled out of the solution at a constant speed of 300 μm/second. The cover slips were allowed to air dry[3, 11]. Cover slips containing YOYO-1 stained DNA were mounted onto microscope slides with 20% β-mercaptoethanol solution. Cover slips containing PNA-DNA hybrids were dyed as described below, and then mounted onto microscope slides with 20% β-mercaptoethanol solution.

**PNA-DNA dyeing procedure**

After combing, the cover slips containing PNA-DNA hybrids were covered with 300 μL of a 3.65 pM solution of fluorescent microspheres labeled with NeutrAvidin (FloroSpheres™ NeutrAvidin™ labeled microspheres, 40 nm diameter, Molecular Probes). The cover slips were incubated at room temperature for five minutes, washed with MES buffer five times to remove the bead solution, and allowed to air dry3.

**DNA/ PNA-DNA Image Acquisition**

An Olympus 60x oil immersion objective was used to view the prepared slides. An Andor CCD camera (1024x1024 13 μm square pixels) was used to capture DNA images, and image analysis was performed using the Andor MCD program.

**Section III: Results**

I used the capillary rise method to find the surface tensions of MES solutions with ethanol concentrations between 0% and 2%. The calibration detailed above was used to convert rise heights to surface tension values. The plot of the variation of surface tension with ethanol concentration is linear, as expected, with the specific relation between ethanol concentration and surface tension:

\[ G \text{ (dynes/cm)} = -3.05^\circ\text{%(EtOH)} + 68.6. \]

The main source of uncertainty in determining surface tension comes from uncertainty in the measurement of the rise height. This uncertainty contributes to a standard error of 1.4% for surface tension values.

I explored several published methods of DNA elongation, including the drop evaporation method[2]. However, I found that the method of dynamic molecular combing produced the most consistent elongation of molecules. In addition, molecules combed using DMC are arrayed in the direction of the combing, making it easier to identify DNA molecules. I also tested several kinds of surface treatments, and found that the incubation of clean slides for 45 minutes in a 125 mg/mL poly-L-lysine solution minimizes nonspecific DNA adsorption to the slide and maximizes the number of combed DNA molecules.

![Figure 1: Images of adsorbed and combed phage 3 DNA molecules in solutions of different ethanol concentrations. The molecules are dyed with YOYO-1. (a) 0.25% ethanol. The molecules are elongated past the contour length of 16.1 μm. This figure measures 80.2 μm across. (b) 0.5% ethanol. The molecules are under-stretched. Bright spots are under-stretched, adsorbed DNA. This figure measures 65 μm across.](image)

Combed molecules reached density values of 40 combed molecules/FOV (field of view, 222 μm²) at most, with average densities of 7.45 combed molecules/FOV. Some molecules that adhered to the cover slips were not combed, but remained “balled up”. The

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3 Biotin and Avidin are a protein-substrate pair. Avidin has a high affinity for biotin, and thus their binding reaction is frequently used to link biochemical components.

4 Poly-L-lysine is an alpha helical protein consisting entirely of lysine subunits. The helix becomes increasingly positively charged at pH under 10.
frequency of these molecules ranged from 2 molecules/FOV (0% ethanol solution) to 70 molecules/FOV (0.75% ethanol solution). They may be seen in Figure 1 as intense spots of fluorescence about 2.3 μm in diameter.

Figure 1 shows representative DNA strands from cover slips combed in solutions containing (a) 0.25%, and (b) 0.5% ethanol. These DNA molecules have been dyed with YOYO-1. I did not observe similar images when the experiment was repeated without DNA in the solution, verifying that these images represent DNA. The DNA strands combed in 0.25% ethanol solution are extended past the contour length of the phage λ genome (16.1 μm) to lengths of 20.1 μm, 18.7 μm, 21.2 μm, and 20.7 μm, from left to right. The strands combed in

0.5% ethanol solution are stretched to lengths of 11.7 μm, 12.58 μm, 12.9 μm, and 9.35 μm, from left to right. Their trajectories are crooked, unlike those of the molecules in (a). Additionally, the number of adsorbed but uncombed molecules on each slip (seen as bright balls of fluorescence) increases as ethanol concentration increases. The rate of this non-specific adsorption is 37% less in Figure 1(a) than in Figure 1(b).

With the addition of ethanol, the peak extension length for combed DNA molecules decreased substantially. I collected a number of images of DNA molecules combed in solutions of varying ethanol concentrations, and measured the lengths of the combed DNA molecules by determining the pixel location of their endpoints by eye and calculating the distance from endpoint to endpoint. This method yields a 0.61 μm uncertainty in length measurement. Figure 2 shows histograms of the length distribution, in μm, of molecules observed for each trial. The value of peak lengths decreased from 17.0 μm for non-ethanolic solution to 16.5 μm, 10.0 μm, and 12.0 μm for 0.25% ethanol, 0.5% ethanol, and 0.75% ethanol solutions, respectively. The length distributions are not sharply peaked, indicating that there is a large variation in the length of observed molecules.

Figure 2: Histograms showing the lengths of phage λ DNA molecules combed in solutions of different ethanol concentrations. The length of the stretched molecules decreases as the ethanol concentration of the solution increases. (a) 103 molecules total. (b) 174 molecules total. (c) 143 molecules total. (d) 86 molecules total.

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Figure 3: A plot of peak DNA extension length vs. ethanol concentration. The correlation between the two variables is negative, and approximately linear. The high frequency peaks from each histogram were visually identified, and the width of these peaks at half-height was taken as the deviation of the peak value. In the case of histogram (b), where the peak is not well defined, the peak was inferred to fall at the middle of the extended distribution.
After verifying that DNA molecules could be combed and visualized using this technique, I began to test several procedures for combing and visualizing DNA labeled with PNA. Figure 4 shows DNA-PNA hybrid molecules that have been combed and dyed with a solution of fluorescent beads that are labeled to bind specifically to the PNA. The combed molecules vary widely in length, from 6.31 µm to 29.2 µm. They are arrayed parallel to the direction of combing and occur at a density of 70 combed molecules/FOV. No such images occur on cover slips that have been combed in solutions containing i) no PNA or DNA, ii) just PNA, or iii) just DNA. Note that the fluorescent beads do not cover the molecule uniformly, but instead are seen only at certain discrete and variable distances along the molecules.

Molecules of shorter lengths are most likely pieces of phage λ DNA molecules that were broken during pipetting, which I expect to be present in any solution of DNA. The short molecules also may have undergone partial nonspecific adsorption to the cover slip and have not been combed over their entire length. Molecules of longer lengths are phage λ genomes or parts of genomes that have linked together via the single stranded “sticky ends” at the ends of each genomic phage λ DNA molecule [13].

A group led by A. Bensimon has conducted an extensive study of the effects of cover slip treatment on mean DNA extension lengths during molecular combing[14]. They achieved a variation of lengths between 17.5 µm and 27 µm by coating cover slips with polylysine and polystyrene, respectively, and by varying the pH of the combing solution in order to adjust the surface charges of pH-dependent functional groups. Histograms of DNA length frequencies in this paper show strong peaks with around 8% standard deviation.

My peak molecular extension length for polylysinated cover slips combed at pH 5.6 was found to be 17.0 µm, close to the value of 17.5 µm obtained by Bensimon et al. Two differences in experimental setup may have contributed to the slight discrepancy. First, the polylysinating procedure that they followed used different and variable polyl-lysine concentrations at shorter incubation times. This may have resulted in an unequal deposition of polylysine onto the cover slips. Since their polylysinated surfaces were reported to be quite variable, it is difficult to quantify the impact of differences in polylysine density.

Another experimental discrepancy was the differences in the combing solutions. The difference between using APES buffer (pH 8.5) in the published experiment and MES buffer (pH 5.6) in my experiment could cause slips with identical polylysine densities to differ slightly in surface charge (by a factor of 2%)5. Differences

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5 Phage λ DNA exists in its native form as a plasmid. This plasmid may be linearized by an enzyme that cleaves it at a certain point, leaving 12 bp single-stranded overhangs of complementary sequences at either end of the linear molecule. The complementary sticky ends of any λ molecule may adhere to each other in solution, forming molecules composed of more than one genome.

6 Poly-L-lysine has a pK₅ of 10.1, indicating that at pH 8.5, the ratio of protonated to non-protonated lysine side chains will be 10¹⁶. At pH 5.6, the ratio of charged to non-charged side chains will be 10⁵.\n
in the buffering agent used may also have affected the results.

Bensimon *et. al.* suggest that increased hydrophobicity of a cover slip contributes to a decrease in non-specific molecular adsorption and thus an increase in combing length. My pollysintesed surfaces may be more charged than theirs due to a higher density of pollyysine imparted to the cover slips' surfaces over a longer incubation time. The greater charge (and lesser hydrophobicity) of my slips would then explain the slightly shorter stretching length.

**Theory Behind Dynamic Molecular Combing**

The physical process of DNA combing has been modeled in several different ways. One simple model developed by A. Bensimon uses Hooke's law for the extension of a string anchored at one end to relate the overextension of the DNA molecule to the stretching force applied by the experimental setup:

\[ F = EA(l_e - 1), \]

where \( E = 1.1 \times 10^8 \text{ N/m}^2 \) is the Young's modulus of DNA, \( A = 3.8 \times 10^{-16} \text{ m}^2 \) is its cross sectional area, \( l \) is the DNA's extended length, and \( l_e = 16.1 \mu\text{m} \) is the unstretched length of phage \( \lambda \) DNA[7]. Bensimon identifies two forces acting on the molecule being combed; (1) the force exerted on the molecule by the surface of the cover slip, and (2) the force exerted on the molecule at the surface of the meniscus by the surface of the solution. The ultimate extension length of the DNA molecule depends upon both of these experimental components.

Bensimon suggests that the addition of surfactants to the combing solution will decrease surface tension and in turn, decrease the extension of a DNA molecule. Using the peak extension lengths obtained for ethanol concentrations that allowed for overextension of the DNA, I can use equation (2) to estimate the force necessary to cause this amount of extension. I can compare this force value to an estimate of the force exerted on a rod of diameter 2.1 nm (the diameter of DNA) by a meniscus with surface tension \( \gamma[9] \):

\[ F = 2\pi(2.1 \times 10^{-9} \text{ m})\gamma. \]  

**Table 1: Over-extensive Forces**

<table>
<thead>
<tr>
<th>Solution</th>
<th>Surface Tension (N/m)</th>
<th>Peak extension length (μm)</th>
<th>Force due to surface tension (pN)</th>
<th>Force necessary for peak extension (pN)**</th>
</tr>
</thead>
<tbody>
<tr>
<td>MES buffer</td>
<td>0.686</td>
<td>17.0 ± 1.3%</td>
<td>905.4</td>
<td>23.4 ± 1.3%</td>
</tr>
<tr>
<td>MES buffer + 0.25% EtOH</td>
<td>0.668</td>
<td>16.5 ± 3.6%</td>
<td>895.4</td>
<td>10.4 ± 3.6%</td>
</tr>
</tbody>
</table>

*Calculated using eq. 3, measured surface tension
**Calculated using eq. 2, measured extension length

Discrepancies of about one hundred-fold separate the calculated meniscus force on the DNA and the stretching force predicted to cause the observed extensions. These discrepancies can be attributed to several factors. First, the approximation of a DNA molecule as a uniform rod, which I accepted in order to use equation (3) to calculate surface tension, is a greatly simplified model of an actual DNA molecule. DNA is a double stranded helix with negatively charged backbones and inner components, exposed by the grooves of the DNA, which exhibit both polar and hydrophobic character. Thus, the actual force of the meniscus on a DNA molecule should depart from the value I obtained using this simplified model.

Second, while I may estimate the meniscus force, I make no effort to add to this analysis an estimate of the second component of the stretching force - the force of the interactions between the cover slip surface and the DNA. These interactions encompass all the electrostatic, hydrophobic and hydrophilic relations between the slip and the DNA, and are too complicated to model. This force must be supposed to act strongly and opposite to the stretching force of the meniscus.

The peak phage \( \lambda \) genome extension lengths for higher concentrations of ethanol are less than the contour length of the phage \( \lambda \) genome. Bensimon's model of molecular overextension does not allow for analysis in these cases. However, Marko and Siggia provide a discussion of the relationship between stretching force and DNA extension at lower stretching forces, modeling DNA as a worm-like chain polymer[15]. They have shown that a

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7 Marko and Siggia suggest that the straightening of the DNA molecule is actually a combination of several different events within several different force regimes. Forces under 1 pN straighten the entropic kinks present in the DNA at
good approximation for the relationship between the applied stretching force, \( f \), and the extension, \( z \), of a molecule of contour length \( L \), in the limit where \( z \) approaches \( L \) from below, is

\[
F = (1 - z/L)^{1/2}/4A,
\]

(4)

Where \( A = 50 \text{ nm/N} \) is the persistence length of the DNA molecule.  

### Table 2: Under-extensive Forces

<table>
<thead>
<tr>
<th>Solution</th>
<th>Surface Tension (N/m) +/- 1.3%</th>
<th>Peak extension length (mm)</th>
<th>Force due to surface tension (pN)* +/- 1.3%</th>
<th>Force necessary for peak extension (pN)**</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5% ethanol in MES buffer</td>
<td>0.670</td>
<td>10.0 +/- 3.5%</td>
<td>884.2</td>
<td>8.1 +/- 3.5%</td>
</tr>
<tr>
<td>0.75% ethanol in MES buffer</td>
<td>0.663</td>
<td>12.0 +/- 4.4%</td>
<td>875.2</td>
<td>9.9 +/- 4.4%</td>
</tr>
</tbody>
</table>

*Calculated using eq. 3, measured surface tension length. **Calculated using eq. 4, measured extension length.

Discrepancies of about one hundredfold separate the calculated meniscus force on the DNA and the actual stretching force predicted to cause the observed extensions. As discussed above, this discrepancy results from my simplification of the system when constructing the models used to calculate the meniscus force.  

The component of the DNA stretching force provided by the surface tension of the receding meniscus contributes differently to the total force on the stretched molecule within different surface tension regimes. In the regime where the total stretching force remains large enough to stretch a DNA molecule, a reduction of 10 pN in the meniscus force leads to a decrease in molecular elongation of 0.5 μm lengths greater than its persistence length (in this case about 50 nm). Forces between 1 pN and 10 pN orchestrate the extension of the DNA to its full contour length. At forces higher than this, the DNA molecule changes conformation to a conformation with base pair spacing and pitch angles different from B-form DNA.

The persistence length of a polymer is a measure of its local stiffness.  

The use of these two models to analyze the data illustrates a property of DNA that has been explored by a number of researchers [16]. While stretching a DNA molecule above its contour length requires much more force per unit of increased extension (about 13 pN/micron, according to my calculations), straightening a DNA molecule to lengths below its contour length requires considerably less force (about 1.8 pN/micron) and correspond to a 13 pN decrease in the total calculated stretching force. In the smaller total force region, a reduction of the same amount in the meniscus force causes an increase in elongation of 2 μm, corresponding to a 2 pN increase in total stretching force.

While changes in the meniscus force affect the elongation length of stretched molecules significantly when they are over-elongated, similar changes in meniscus force have little effect on the lengths of stretched molecules when they are under-elongated. This phenomenon makes sense if the meniscus force is visualized as pulling the DNA molecule away from the cover slip, increasing the distance between the cover slip surface and the DNA molecule and decreasing the strength of the electrostatic interactions between the slip and the molecule. For under-stretched molecules, where elongation forces are dominated by cover slip-molecule interactions, the elongation length is more sensitive to cover slip-molecule interactions. The increase in length with surface tension reduction in this region is probably caused by variation in cover slip-molecule interactions, which could vary from trial to trial with variation in polylysine deposition.

### Dynamic Molecular Combing of PNA-DNA Complexes

In a procedure analogous to locating combed DNA through direct dyeing, I was also able to indirectly tag combed DNA by labeling the PNA's attached to the DNA with fluorescent beads (Figure 4). I can be confident that these images represent combed DNA bound to bead labeled PNA, since such images depend on the inclusion of both PNA and DNA in the combing mixture.

The DNA-PNA hybrids are arrayed parallel to the direction in which the cover slip was pulled from solution, in a manner similar to combed DNA that had been visualized with YOYO-1. They appeared at a higher density of about 70 molecules/ FOV. I observed long combed molecules more frequently than in the images of only DNA primarily because DNA-PNA hybrids were not heat-shocked before combing to prevent the adhesion of molecules at their sticky ends. However, I also saw DNA strands that appear to be of λ DNA contour length.

The basic similarity between PNA-bound DNA images and YOYO-1-stained DNA images obtained through DMC allows me to
discern some information about the behavior of PNA-bound DNA. The PNA does not disrupt the process of the DNA extremities binding to the cover slip during the first phase of DMC. It does not disrupt the DNA elongation process either, since properly bound and elongated molecules are observed.

Moreover, the combing process does not completely undo the PNA-DNA binding process. I expect a full phage λ genome to bind 15 PNAS of this specific sequence. All molecules that I observe exhibit some tagging with fluorescent beads, and some molecules of genomic length express at least fifteen resolvable beads. Thus, elongation of the DNA molecule at forces high enough to stretch the DNA past its contour length does not create enough distortion in the DNA to displace bound PNA molecules. Finally, I have verified that the biotin groups of cover slip-bound, biotin-bound PNAS may still be recognized and bound by Avidin-covered beads.

Recent research teams have attached fluorescence labeled restriction enzymes and complementary DNA strands to combed DNA, demonstrating the ability of combed DNA molecules to undergo familiar interactions with enzymes and complementary DNA molecules[1-6]. Another research team has bound PNA to DNA, and then bound Avidin (a relatively large molecule) to the bound PNAS. The DNA-PNA-Avidin hybrid is then imaged using electron microscopy, and the location of the Avidin is detected by shadowing the EM image. However, this last technique does not return easily analyzed data, as the DNA is not elongated by DMC, but rather randomly adsorbed onto a protein grid. In contrast, the process of binding fluorescence labeled PNA molecules to DNA and using DMC to array the tagged DNA molecules for optical imaging returns a high density of sequence information that can be collected and analyzed with ease and speed.

Section V: Conclusions

In this paper, I show that the extension of DNA molecules during molecular combing may be controlled easily and reliably through the addition of small amounts of ethanol to the combing solution. This method of adjusting the lengths of combed molecules presents an easy alternative to cover slip surface variation. My data suggests that the elongation of DNA molecules during DMC is more sensitive to the surface tension of the meniscus when the combined stretching force is large enough to over-extend the DNA molecules than when the combined stretching forces are too small to over-extend the DNA. This observation restricts the practical use of my length adjustment method to those that exceed the contour length of the stretched molecule.

I also observe that the binding of PNA to DNA and the combing of these PNA-DNA constructs are compatible processes. In particular, I show that over-extending forces may act on PNA-bound DNA during combing without dislodging the bound PNA. This observation fuels the hope that PNAS can be used as sequence specific probes that, bound to arrayed DNA molecules, will confer sequence information that can be collected optically.

However, many aspects of PNA-DNA binding and combing must still be addressed. We must explore further the possible effects of bound PNA on DNA elongation to ensure that we can properly evaluate the distances between probe markers. We must test the dependence of my results upon the order of the PNA binding, DNA combing, and bead addition. We must also decide whether optical imaging of molecules like those seen in Figure 4 can help us to decide whether the PNA is binding to the DNA at the proper sites. Once these experiments have been done, we can generalize the procedure to include multiple probes of multiple colors. Eventually, this method may be used to quickly distinguish genetic material from different individuals, identify common genetic mutations, or even to map the sequences of entire genomes. This new sequencing power will greatly increase the speed with which genetic analyses further our understanding of life on this earth.

I wish to thank Allan LeSage for his upcoming development of image capture and analysis programming, Philippe Chazel for suggestions on improving fluorescence imaging, David Wu for attempted ATM imaging of DNA molecules, the Marko Lab at UIC and Dunja Skokol in particular for sharing slide preparation procedures, Aida Pascual and Diane Jurcin for manifest advice in everyday lab protocol, Dr. Laurie Mets for advice and guidance, and Kaitry Ryan for preparing PNA, providing PNA binding procedures, participation in preliminary investigations of DNA spreading techniques, and much advice. I would like to thank the primary investors of the Mary Sarah McPeek and Norbert Scherer laboratories for additional insights and technical support. I also thank all of these people, along with Adam Lipszulitz, Emily Bernstein, and Dr. Ben Stark, for useful discussions and encouragement.
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