

Stretching DNA with Shear Flow

David Van Valen

APh 162

01/05/09

Introduction

In this experiment, we will attach one end of a DNA molecule to a glass surface, and then watch how long it becomes when exposed to different amounts of shear flow. There are a few technical points that we must address to perform this experiment. First, how will we engineer it so that only one end of the DNA is attached to the glass? Second, how will we visualize the DNA? By answering these questions, we will also gain familiarity with some key technologies frequently used in biophysics, including biotin, streptavidin, fluorescent dyes, and flow chambers.

Making the DNA stick: The interaction between biotin molecules and streptavidin molecules is the key to attaching one end of the DNA to glass. One can think of biotin-streptavidin as a molecular version of velcro - when the hooks and fuzzy material come in contact, they naturally stick. To make two things stick together, all we have to do is glue the two velcro pieces on them and let them come into contact. In this case, we will be using biotinylated lambda phage DNA and coat the glass surface with streptavidin. The molecular structure of biotin is shown below.

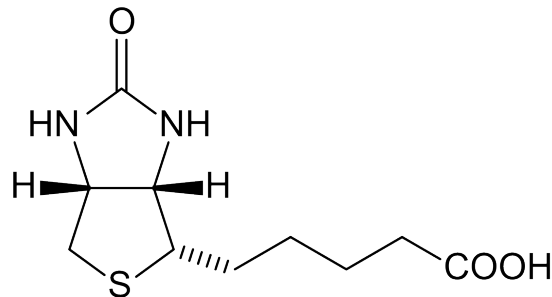
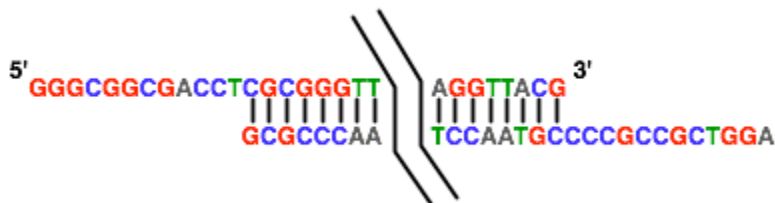


Figure 1: Molecular structure of biotin. Image taken from Wikipedia

Of interest to us is how to link this molecule to our piece of DNA. To do this, we make use of the fact that lambda phage DNA is not entirely double stranded - there are actually single stranded overhangs on the left and right hand side. These are shown in the figure below.



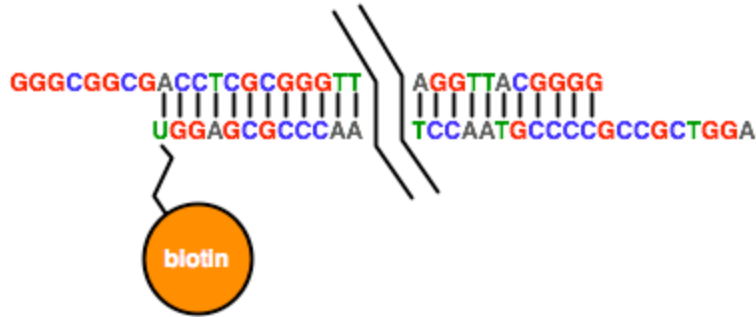


Figure 2: Biotinylating lambda phage DNA. By using biotinylated nucleotides and DNA polymerase, we can attach a biotin group to our piece of DNA. Images taken from Biocurious.

If we wanted to fill these in, all we would have to do is incubate our DNA with some nucleotides and DNA polymerase. To attach a biotin group, all we would have to do is make sure that some of the nucleotides in solution are conjugated to biotin (these biotin nucleotides can be purchased from a number of biotechnology companies - the ones used in this experiment were purchased from Roche). To coat the surface with streptavidin, we will let a glass coverslip soak in a solution of streptavidin for half an hour (it will naturally settle to the surface).

Visualizing the DNA: To visualize the DNA, we will use the DNA staining dye SYBR gold. SYBR gold is an intercalating dye - it will slip in between the base pairs of a DNA molecule. When it does, its fluorescence will increase by a factor of 100. We will also use an oxygen scavenging system called GODCAT. This consists of the enzymes glucose oxidase and catalase. When combined with glucose, these enzymes remove oxygen from the solution, protecting the DNA from photodamage.

Materials

A schematic of the flow chamber set up is shown below

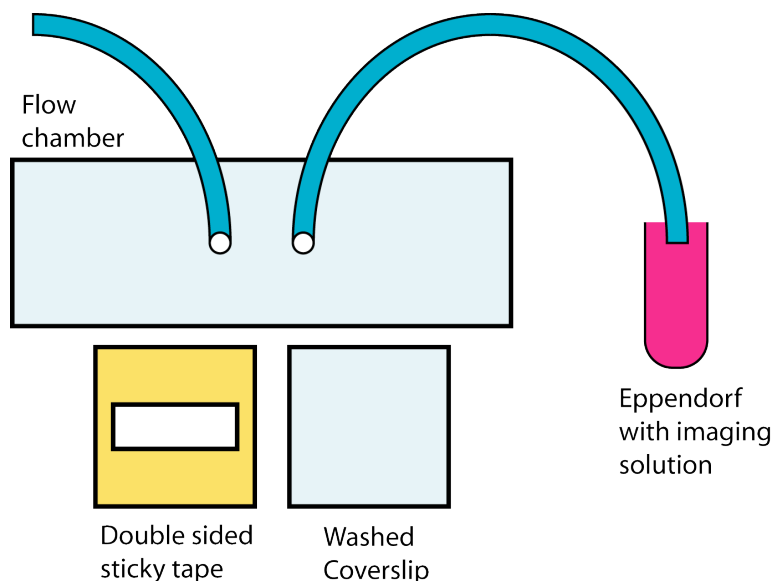


Figure 2: Flow chamber set up for the DNA flow experiment. The double sided sticky tape is adhered to both the microscope slide, and the coverslip, forming a sealed flow chamber. The tube on the left is attached to a syringe pump to initiate flow.

- TM Buffer: 50 mM Tris, 10 mM MgSO₄
- Streptavidin solution: 1 uL of streptavidin in 250 uL of TM
- Biotinylated lambda DNA
- Flow chamber, laser cut tape, cleaned coverslips
- Imaging solution: 576 uL TM, 6 uL BME, 6 uL GODCAT, 6 uL 1:1000 SYBR Gold

Protocol

1. At the beginning of class, assemble the flow chamber. Adhere the double sided sticky tape to the drilled slide, making sure that it does not block the entry ports. Place a 18 mm x 18 mm coverslip onto the adhesive and seal the chamber by placing it on a hot plate for several seconds. You should be able to see the tape melt; try to place aluminum foil on the hot plate to prevent your flow chamber from getting dirty
2. Using a syringe, flow 50 uL of the streptavidin solution into the chamber. Incubate for > 30 minutes. Now would be a good time to return to perform the photobleaching measurement.
3. Using a syringe, wash the flow chamber twice with 400 uL of TM buffer.
4. Using a syringe, flow on the biotinylated DNA.
5. Using a pair of tweezers, punch a hole into the top of an eppendorf tube. Pipette 600 uL of the imaging buffer into the eppendorf tube. Mount the flow chamber onto the microscope (be sure to use a 100 X objective) and connect the chamber to the syringe pump using metal connectors.
6. Pipette 6 uL of 50% glucose into the imaging solution. Next, place the long tubing of the flow chamber into the eppendorf. Activate the syringe pump at 40 uL/min. After ~50-60 uL fluid has been pumped, focus and image using either a FITC or YFP filter set. Alternatively, you can focus on the tape and begin imaging immediately.
7. Acquire images of DNA at different flow rates. A typical flow rate is 40 uL/min. You may want to try something lower (10 uL/min) or higher (100 uL/min). You can also acquire a movie if you wish. Be sure the integration time is long enough so that you get a good signal in your images.

Analysis

Using MATLAB, create an image analysis script that will segment out a piece of DNA in your field of view. It may be easier if you crop your images using MATLAB or ImageJ; feel free to be creative. For as many pieces of DNA as you can, measure the long axis length as a function of flow rate. Make a plot of the mean long axis length as a function of flow rate, and a histogram of the long axis lengths for each condition. Comment on any trends you observe.