Bi 1X, Spring 2011
Week 4

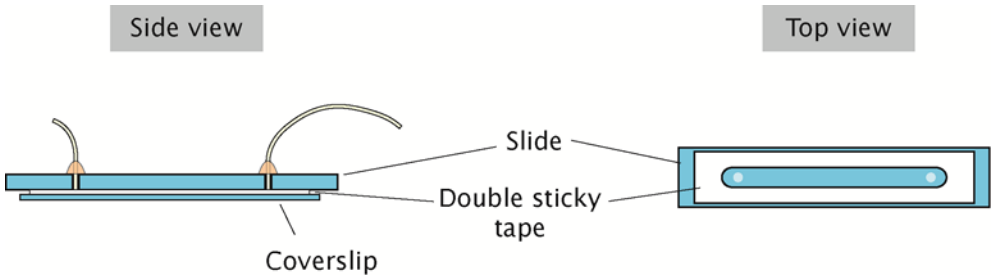
- **Single molecule techniques: the tethered particle method (TPM)**

In this lab we will visualize single DNA molecules bound to a 0.27 µm diameter bead. This DNA will have a binding site for the restriction enzyme EcoRI. When flowing in this enzyme we'll see its effects by counting the tethers that get lost due to the DNA getting cut.

You will be provided with flow chambers with the single DNA molecules already bound to the surface and to the beads. Your job is to mount the sample on the microscope, find the beads and take a movie of the beads while flowing in the restriction enzyme.

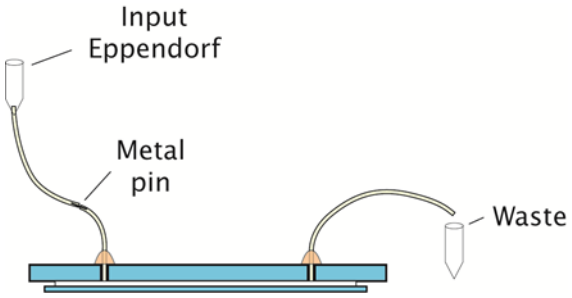
The flow chambers:

A lot of things happened behind the scenes. First, let's familiarize ourselves with the flow chambers.



The chamber is made out of a microscope slide that has two holes drilled. Double sticky tape is sandwiched between this slide and a microscope coverslip. The tape is cut such that a little chamber is created between the coverslip and the slide. Tubing is connected to the holes using Epoxy.

The chambers you will be provided will already have the DNA bound to the glass on one end and the beads on the other end (see an explanation of this below). In order to flow liquid in we hook the input tube (the long one) to an Eppendorf as shown in the following figure.



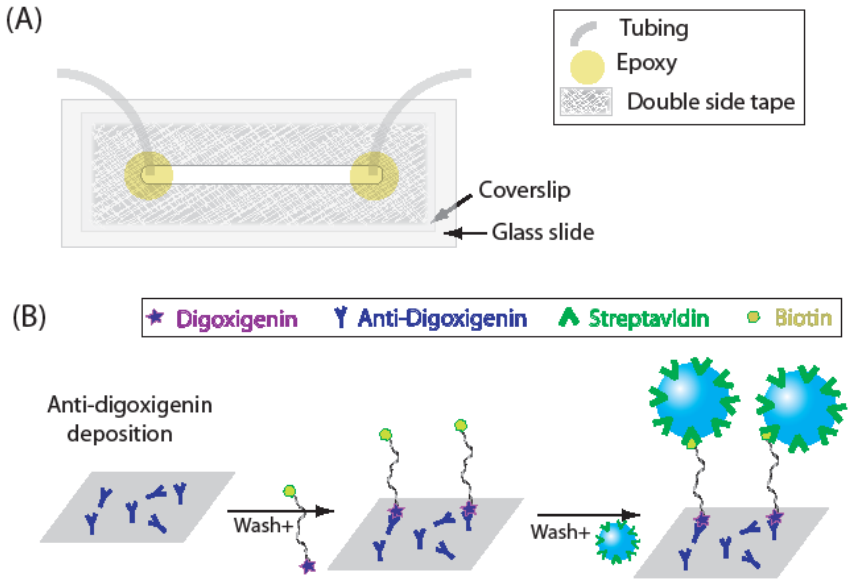
Liquid goes in by gravity. All you have to do to change the flow speed is to move the Eppendorf tube up and down! **Be careful! If the tube goes below the chamber you'll get backflow! To stop the flow, put the input Eppendorf level with the slide.**

Getting the single DNA molecules bound to DNA:

The chambers that we're providing you have already been subjected to part of the tethering protocol. We did these steps for you to avoid repetitive and long tasks that would have taken up most of the class time.

We started by coating the flow chamber with anti-digoxigenin. This is an antibody to the small molecule digoxigenin. Now, the trick is that the DNA we want to bind to the surface is linear and has digoxigenin on one end and biotin on the other end. When flowing in this DNA after the anti-digoxigenin step we get binding of one end of the DNA to the surface.

The biotin molecule on the other end of the DNA binds covalently to streptadivin. (How did we make these DNA molecules?) At this point we flowed in beads that are coated with streptadivin. The result is shown in the following cartoon.



Your mission:

We want to visualize the beads bound to DNA molecules under the microscope and see how these DNA molecules get cut once we flow in a restriction enzyme. You will take a movie of this process and write your own Matlab code to analyze the data and report the number of beads left as a function of time and the rate of cutting.

1) Bring the chamber to the microscope leaving the input Eppendorf attached. Make sure the Eppendorf is always at the same level as the chamber. You can stick the Eppendorf to the side of the microscope using tape, for example.
2) Use the 100x objective and remember to check the phase ring position (Ph3 for 100x). Set up the right Köhler Illumination in bright-field. Estimate the density of tethers you got. Make sure you're looking at the bottom of the chamber!
3) Think of the frame rate of your movie based on what you expect to happen once you flow in the enzyme. The dynamics will happen on the order of seconds. Make sure to run Micro-Manager once as if you were actually doing the experiment in order to familiarize yourself with the software. Using Multi-D Acquisition to take a short movie on the beads only.
4) Without moving the chamber too much, flow in 500 µl of the restriction buffer. You can put a Kimwipe at the exit port to collect the waste. **Avoid introducing bubbles!** See demo by your TA before starting flowing. Take a movie while you're flowing. (Suggested frame rate: 5 sec/frame, 180 frames for 15min) When the tube is almost empty stop the flow. This is a control to make sure that the buffer itself doesn't cut DNA, and to measure the rate of tether dissociation (none of the bonds are covalent, so some beads will break as time goes on even in the absence of enzyme). You need to adjust the focus throughout acquiring as the beads drift over time. It's OK to have couple of frames out of focus in your movie. **BE PATIENT!**
5) Flow in 500 µl of restriction enzyme buffer with 5 µL EcoR1. **Take the movie using the same frame rate and number of frames as you did in the control!** When the tube is almost empty stop the flow.