Single-Molecule Assay of DNA Integrity

George Dougherty
(925) 423-3088
dougherty9@llnl.gov

DNA molecules are susceptible to damage resulting from flow through microchannels in microfluidic devices and systems. This damage, which can take the form of either single- or double-strand breaks, is dependent on the size of the channels, fluid flow speeds, and the size of the DNA molecules that flow through the device. This damage can make it difficult to analyze the DNA using processes such as DNA sequencing, polymerase chain reaction (PCR), and labeling of specific sequences with fluorescent probes to search for specific DNA molecules.

An assay to characterize the degree of damage is important because it allows one to optimize microfluidic device parameters such as flow speed, aperture size, and geometry. A single molecule assay to characterize damage is particularly useful for microfluidic devices where the concentration of DNA is low, such as low copy number DNA analysis. In these cases, bulk assays, such as electrophoresis, are not sensitive enough to characterize the damage to the DNA.

In this project we measured the elasticity of single lambda-phage DNA molecules (contour length = 16.4 µm) before and after flow through microfluidic devices. Optical traps were used to stretch single DNA molecules attached to 1-µm beads as shown in Fig. 1. The displacement of the bead in each optical trap varied linearly with applied force, much like a bead on a spring, and the image of the bead on a quadrant photodiode detector was used to accurately measure the elastic tension the DNA molecule experienced as the distance between the traps increased. Figure 2 shows the difference in elasticity between

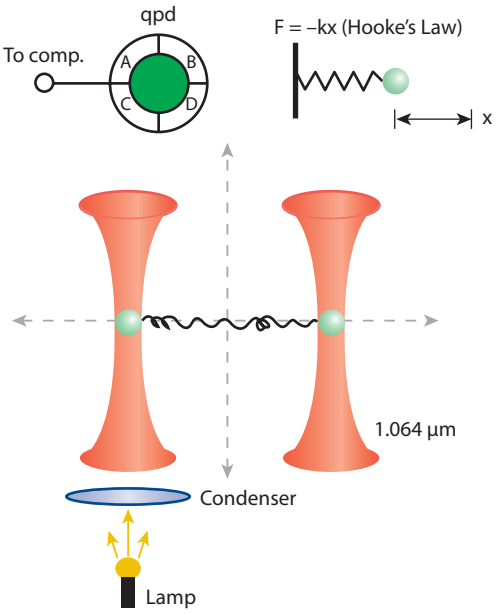

Figure 1. Two optical traps (orange laser beams) are used to hold, stretch, and measure the elasticity of a single lambda-phage DNA molecule via 1-µm beads attached to the ends.

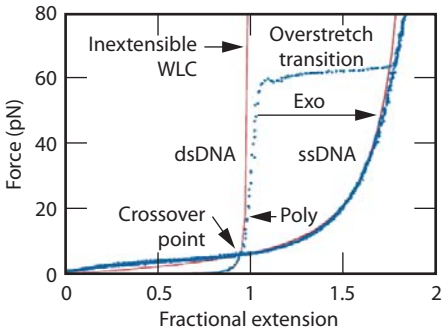

Figure 2. Differences in the elasticity of double-stranded DNA (dsDNA) and single-stranded DNA (ssDNA).

double-stranded DNA and single-stranded DNA that forms the basis of this assay. It takes much greater force to stretch double-stranded DNA than it does single-stranded DNA, and this fact can be used to detect DNA molecules that contain single-stranded portions, due to single-strand nicks, or where strands have been pulled apart due to shearing forces.

Project Goals

The project goals were to 1) identify a DNA biosensor for testing; 2) perform control measurements on DNA at very low flow speeds; 3) test DNA integrity using both gel electrophoretic and DNA single-molecule elasticity measurements to determine the degree of both double- and single-strand breaks; and 4) optimize the flow speed and geometry of the microfluidic device to minimize DNA damage.

Relevance to LLNL Mission

The expertise gained from this project will enable LLNL to develop microfluidic devices for DNA biosensors with a higher efficiency of detection. The increased efficiency of DNA detection will be the direct result of assessing the damage to DNA samples after they pass through microfluidic biosensors, and then optimizing flow speed and biosensor geometry to minimize this damage.

FY2006 Accomplishments and Results

First we performed control measurements on lambda-phage DNA during flow through microfluidic devices using both bulk- and single-molecule techniques. We flowed lambda-phage DNA through a "packed bed reactor" at flow speeds between 0 and 10 µL/s. Gel electrophoresis of the resultant DNA revealed no double-strand breaks. This is shown in Fig. 3. A 1-kb sizing standard run in the left-most lane gives an idea what fragmentation of the original DNA would have looked like.

Next, we stretched individual lambda-phage DNA molecules that flowed through a multichannel cell at very low speeds (0.3 µL/s). Figure 4 shows the characteristic "overstretching transition" and is very similar to the graph shown in Fig. 2. However, at higher flow rates damage to the DNA was evidenced by a dip in the overstretching transition shown in Fig. 5. The dip is indicative of damage to the molecule and results from a portion of the molecule becoming single-stranded. This data is supported by Fig. 2, which indicates that there is a large difference in the elasticity of single- and double-stranded DNA at forces close to 65 pN.

This technique represents a sensitive way to measure damage to single DNA molecules that occurs when DNA flows through small channels at high flow rates. Further work will be required to obtain more quantitative information about the extent of the damage to the DNA.

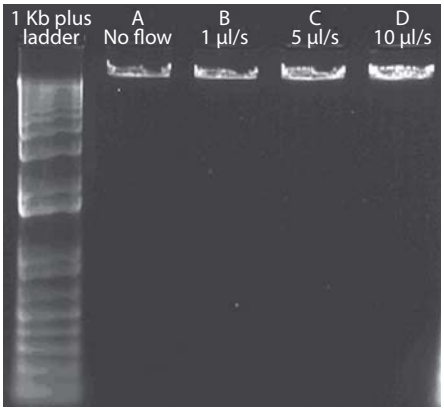

Figure 3. Gel electrophoresis of lambda-phage DNA molecules that have been run through a "packed bed reactor" (courtesy E. Wheeler) at flow rates of A) 0 µL/s, B) 1 µL/s, C) 5 µL/s, and D) 10 µL/s. No double-strand breaks were observed. The leftmost lane contains a 1-kb sizing ladder.

Related References

1. Bustamante, C., Z. Bryant, and S. B. Smith, "Ten Years of Tension: Single-Molecule DNA Mechanics," *Nature* **421**, pp. 423-427, 2003.
2. Brewer, L. R., M. Corzett, and R. Balhorn, "Protamine-Induced Condensation and Decondensation of the Same DNA Molecule," *Science* **286**, pp. 120-123, 1999.
3. Bianco, P. R., *et al.*, "Processive Translocation and DNA Unwinding by Individual Recbcd Enzyme Molecules," *Nature* **409**, pp. 374-378, 2001.
4. Brewer, L., M. Corzett, E. Y. Lau, and R. Balhorn, "Dynamics of Protamine 1 Binding to Single DNA Molecules," *J. Biol. Chem.*, **278**, pp. 42403-42408, 2003.

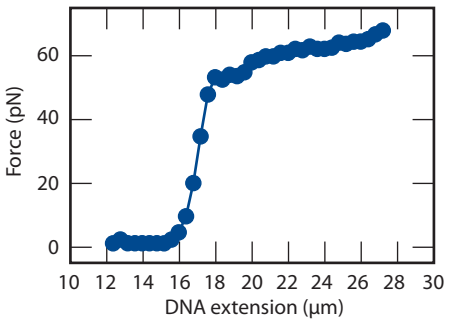

Figure 4. Elasticity of a single lambda-phage DNA molecule taken after an initial flow rate of 0.3 µL/s through a microchannel flow cell.

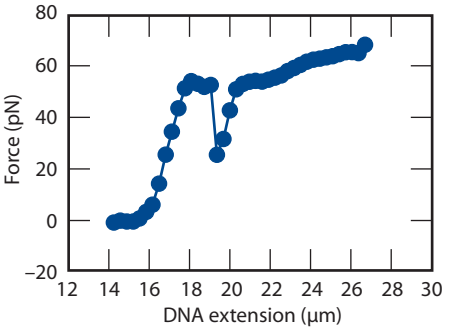

Figure 5. Elasticity of a single lambda-phage DNA molecule taken after an initial flow rate greater than 1 µL/s through a microchannel flow cell, showing a strong dip in the middle of the overstretching transition. This dip shows that a portion of the molecule is single-stranded, possibly due to fluid shear forces.