Abstract:
With the emergence of nanofluidic devices for the analysis of nucleic acids, their dynamics as a function of confining geometry has become a topic of significant interest. We are investigating the dynamics of deoxyribonucleic acid (DNA) in systems of nanochannels with a channel cross-section of 100 to 200 nm, and lengths of several hundred micron. We have shown a negative differential electrophoretic mobility in channel systems based on the onset of electrostatic stabilization of thermal hernias.

Summary of Research:
Nucleic acids are polymers and thus coil up. This leads to an extremely low genetic resolution if single molecules are analyzed in optical fluorescence microscopy. In recent years, the fabrication of large-area nanofluidic systems for the analysis of DNA and DNA-protein complexes has become technologically feasible through continued improvement of electron-beam lithography systems. A particularly striking transition in the behavior of DNA can be achieved by confining single molecules to channels that have a cross-section that is comparable or smaller than twice the persistence length of double stranded DNA (dsDNA), and are hundreds of microns long. In those channels, DNA and chromatin become linearized and stretched out to a constant fraction of its contour length, thus delivering a direct connection between a physical measurement and the abstract picture of the linear genetic information that can be found in any molecular biology textbook [1,2].

The physics of stretching polymers under confinement is based on three basic concepts: Entropy drives polymers into a coiled configuration, stiffness of the polymer tends to extend it, and polymer self-interactions tend to extend the molecule (at least in the case of DNA in our typical buffers).
De Gennes and co-workers have proposed an elegant model in terms of non-penetrating blobs, which leads to predictions for both the extension and the free energy of confinement [3]. The case of DNA confined to nanochannels a few persistence widths wide provides a peculiar and conflicting case since predictions are only partially met, and an obvious difference between the cases of occupancy of channel with one or two channels exist [4]. In our work, we aim to measure this difference, and gain additional knowledge about the dynamics of confined polymers.

Devices with nano-and microfluidic channels were prepared on fused silica wafers by mix-and-match electron beam and photolithography. Patterns were transferred by reactive ion etching, and closed fluidic systems were formed by fusion bonding to a second fused silica wafer. The nanofluidic pattern consisted of a lattice of 140 nm wide nanochannels that form a square lattice with a lattice constant of 6 µm (Figures 1 and 2). The lattice is tilted at 45 degrees relative to electric fields that are applied between the ends of the lattice. By choosing this design, we ensured that a single nanochannel-stretched λ-DNA molecule interacts at all times with only one channel intersection.

Devices were loaded with a solution λ-DNA in a 200 mM buffer solution with an added polymer to prevent electroendoosmosis. DNA was visualized by fluorescence microscopy using an intercalating dye that had been complexed to DNA molecules before introduction into the nanofluidic system. We recorded movies of DNA traveling through the devices as a function of the applied electrical field. The average velocity for each electric field was then extracted from those movies, and the resulting graph is shown in Figure 3. In contrast to straight nanochannels, we observed a dip in the velocity versus field graph, which indicates a negative differential mobility in that region.

Inspection of movies before and during the dip showed that the qualitative difference between both regions was the formation of stable, growing hernias, that is molecular geometries where the linear molecule occupies more than two channels of a four-channel junction. At electric fields below the apparent critical point, such hernias form intermittently, but do not stabilize to become permanent. If a stable hernia is formed, the net force on a molecule is reduced because forces in distinct branches partially cancel each other out. At higher fields, DNA attains an overstretched, non-equilibrium configuration, which disfavors herniation, as observed in the superlinear velocity/field profile.

Our results can be interpreted as a measurement of the energy difference between single-stranded and double stranded nanochannel occupancy. By applying an electric field to a molecule that has to “climb” a “step” — the energy landscape is changed to a barrier of finite with and height. The probability of crossing that barrier is the rate of formation of stable hernias, and thus the height of the confinement energy step can be determined. This estimate is fundamentally different from the one obtained solely through relaxation against internal friction of the system [5], and a comparison on the two could yield interesting insights into the mutual friction of two polymer chains in nanochannels.

References: