GEL-FREE ELECTROPHORESIS OF λ- AND T2-DNA IN
STRUCTURED PDMS MICROFLUIDIC DEVICES
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Abstract
In this work the migration behavior of DNA in structured microfluidic channels was
investigated with sensitive video fluorescence microscopy and molecular dynamic (MD)
simulations. We found that MD simulations were in excellent agreement with
experimental results. As already demonstrated, shorter λ-DNA (48.5 kbp) molecules
exhibit a higher mobility than longer T2 DNA (164 kbp) [1]. By using this size-
dependent mobility of DNA in structured microchannels the separation of a mixture of λ-
and T2-DNA was carried out on a microchip.

Keywords: DNA electrophoresis, structured channel, molecular dynamic simulation

1. Introduction
The standard method for separating DNA by length is electrophoresis which is usually
performed in gels or entangled polymer solutions. The need of large amounts of analyte
and long separation times makes slab gel electrophoresis cumbersome whereas the
incorporation of cross-linked gels or high viscosity polymer solutions in capillaries is not
trivial. Furthermore, DNA fragment lengths for separation in capillaries at constant field
are limited to 20-30 kbp.
In contrast to alternative methods based on artificial gel structures [2] or entropic traps [3]
our approach focuses on a gel-free method based on topographical structuring of
polydimethylsiloxan (PDMS) microfluidic channels [1] produced through rapid
prototyping.
The migration of λ- and T2-DNA stained with the bisintercalator YOYO-1 was observed
with sensitive fluorescence video microscopy in 1.5, 3 and 5 μm structured
microchannels. MD simulations were carried out for parameters comparable to 5 μm
channels and large DNA molecules, e.g. λ- and T2-DNA. As demonstrated by Duong et
al. [1] the resulting mobility in a 3 and 1.5 μm channel was dependent on the DNA size.
Due to these results the separation of a DNA sample in free solution was performed in the
structured microchannels.

2. Materials & Methods
PDMS channel fabrication was already described in [1]. Briefly, a flood exposure unit
was used to expose a SU-8 coated Si-wafer through a chromium mask. After exposuring
and developing the structured SU-8 wafer was used as an inverted masterstructure for replica moulding of PDMS. The PDMS slab containing the channel structures and reservoir holes was oxidized in an UVO cleaner (Model 42-220, Jelight, USA) and covered with a microscope slide. The DNA molecules were fluorescently stained with the bisintercalator YOYO-1 (Molecular Probes, USA) in a dye to base pair ratio of about 1:10 and detected with a sensitive CCD camera [1]. MD simulations were performed using Langevine dynamics, described elsewhere [4].

3. Results & Discussion

3.1 DNA migration

In a 5 μm topographically structured microchannel (outlined in figure 1.2) both λ- and T2-DNA exhibit two mobilities resulting from two different trajectories which can be identified with a slower phase where the DNA is stretched and coiled by the periodical structuring and a faster phase where the DNA is not influenced.

**Figure 1:** Fluorescence micrographs of migrating λ-DNA (48.5 kbp) through a 5 μm topographically structured microfluidic channel. The structuring is outlined in (2). Two trajectories with different mobilities can be identified where the DNA molecule in the slower phase (a) is stretched and coiled periodically, whereas the faster molecule (b) at the bottom is in a slightly stretched conformation.

**Figure 2:** Snapshot of two simulated DNA chains in a structure corresponding to figure 1. Brighter molecules change the conformation from stretched to coiled in the box, whereas darker molecules remain in a coiled conformation leading to two different mobilities.

3.2 Molecular Dynamic Simulations

The migration behavior of the DNA molecules described before was also found in our MD-simulations. Figure 2 shows a snapshot of molecules in the faster and slower phase. The similarity of the coiled conformation in the faster phase and the periodical change of the conformation from coiled to stretched in the slower phase is obvious and therefore in
very good agreement to the experimental results. Figure 3 shows the simulation of the monomer density of a chain comparable to figure 2 in a microchannel section. Two regions of migration with a depletion zone in between are distinguishable. This effect can be attributed to the distribution of the electric field in the structured microchannels (data not shown).

Figure 3: Density of monomers plotted for a chain with 200 spheres. Chain and structured channel correspond to a 68 kbp long molecule and a 5 μm structured channel respectively.

3.3 Separation of DNA
Decreasing the channel dimensions to 3 and 1.5 μm resulted in one mobility for each DNA type [11]. A cross injector was thus connected to the structured channels to enable DNA sample injection. The injection of several molecules is demonstrated in figure 4. By using lower concentration and higher field strengths each molecule could be injected in a microchannel (figure 5). For the separation of a DNA sample the CCD camera was placed 3 mm behind the cross section. The electropherogram of the separation of 60 pM λ- and T2-DNA is shown in figure 6. After the λ-DNA, the slower and longer T2-DNA passed the camera resulting in a 3 times higher peak compared to the λ-DNA peak. This ratio corresponds to the amount of YOYO-1 molecules which intercalated into the DNA molecules.

Figure 4: Injection of several molecules in a cross injector. Concentration of the sample was 60 pM λ- and T2-DNA. Channel width and height are 6 μm.

Figure 5: Injection of a single DNA molecule. Concentration of DNA sample was 6 pM. Channel width and height are 6 μm.
4. Conclusion

In this work we showed that the length dependent mobility of DNA in structured microfluidic channels is attributed to the inhomogeneous electric fields and entropic effect caused by the conformational restrictions of the molecules. We could demonstrate the injection of several and single molecules in a cross injector structure and a successful separation of a mixture of 60 pM \( \lambda \)- and T2-DNA.

The possibility of simulating the DNA migration and hence the knowledge of the migration mechanism allows the optimization of channel geometries for separation of a higher variety of DNA lengths in the future. For improved statistical significance in the separation results, the use of a parallelized microchip is planned.

Acknowledgements

This research is financially supported from the collaborative project SFB-613 (Teilprojekt D2) by the Deutsche Forschungsgesellschaft. We also would like to thank Jürgen Brugger (EPFL, Switzerland) and Gyuman Kim (Kyungpook National University, Korea) for technical support.

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