SEPARATION AND POLARIZABILITY OF DNA BY DIELECTROPHORESIS

Jan Regtmeier*, Henning Höfemann*, Ralf Eichhorn, Dario Anselmetti* and Alexandra Ros***

** Biophysics & Applied Nanoscience, Bielefeld University, Germany*

*** Condensed Matter Theory, Bielefeld University, Germany*

ABSTRACT

Although separation of polymers based on the combination of dielectrophoretic trapping and electrophoretic forces has already been proposed 15 years ago [1], the experimental realization has only recently been demonstrated [2]. Here, we address the fast separation of biotechnologically relevant DNA by utilizing electrodeless dielectrophoresis in a simple, structured microfluidic chip. In addition, we demonstrate how the same chip and the same experimental setup can be used to determine the polarizability of DNA.

Keywords: microfluidics, DNA, polarizability, separation

1. INTRODUCTION

Using electric fields for transport and separation of biomolecules is a standard technique in lab-on-a-chip systems. A detailed understanding of the polarizability of DNA in an AC electric field is still lacking, although there have been proposals of how this polarizability could be exploited for separation [1]. A quantitative access to the length dependent polarizability is an important prerequisite for the development of effective and predictable separation procedures. Here, we demonstrate the separation of supercoiled DNA by the combination of electrophoresis and electrodeless dielectrophoresis in a microfluidic device. Additionally, we show that the same setup can be used to determine the polarizability of DNA using rate theory description of the escape process from a dielectrophoretic trap.

2. THEORY

Applying an electric AC field $U_{ac}\cdot\sin\omega t$ (frequency ω) to a channel structured with non-conducting posts leads to an inhomogenoues electric field $\boldsymbol{E}\cdot\sin\omega t$ therein. Subjecting DNA to this field, the trapping of DNA in the constrictions between two posts [3] can be observed under appropriate experimental conditions, an effect known as positive dielectrophoresis. The potential energy of an object of polarizability α can be calculated as

$$W = -(1/2)\,\alpha\,\boldsymbol{E}^2 \,. \qquad (1)$$

The additional application of a DC voltage induces the migration of DNA and thus tilts the potential landscape. Assuming a quasi-stationary globular configuration of the DNA molecule during the migration process, the escape time τ out of dielectrophoretic trap is then given by the inverse Kramers rate [4]

$$\tau \propto exp\,(\Delta W/kT) \qquad (2)$$

where ΔW denotes the depth of the potential well and kT the thermal energy. With eq. 1 and 2 and a simple geometric equivalent circuit diagram, which allows the calculation of $\boldsymbol{E}^2$ in the traps from the applied U_{ac}, one can calculate α . More precisely, we plot $ln\ \tau$ vs. U_{ac}^2 and obtain α from the adequate fitting of the data for each DNA length (see ref [2]).

978-0-9798064-0-7/μTAS2007/$20©2007CBMS

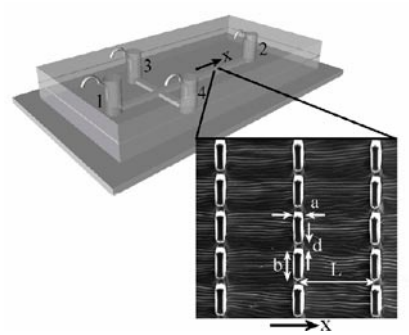

Figure 1. Schematic drawing of the microfluidic chip with reservoirs and Pt electrodes. The magnification is a SEM image of the separation channel (a=2.2 µm, b=7.4 µm, d=2.3 µm, L=21.1 µm; channel (2) is 5 mm long (structured region 3.8 mm), channels (1), (3) and (4) are 2 mm long and the channels are 6 µm high.).

3. EXPERIMENTAL

The microfluidic chip is made using a PDMS (poly(dimethylsiloxane)) soft lithography process [2]. A scheme of the final device is depicted in Fig. 1. After replica moulding from a structured wafer, the PDMS device is oxidized, assembled and subsequently coated with a PEG-silane. The DNA (in 10 mM phosphate buffer pH 8.3, 2 mM NaCl, 1 mM EDTA, 0.2% 2-mercaptoethanol, 0.1% POP-6) is stained with YOYO-1 (1 molecule per 10 base pairs), stored in reservoir 3 and injected via pinched injection.

For the determination of α, the migration of a DNA plug is observed via fluorescence video microscopy for constant U_{dc} =12V and different U_{ac}. From these data the escape times of 30 molecules for every U_{ac} are determined via visual tracking.

For DNA separation, a mixture of the two DNA species is injected and driven through the channel by U_{dc} =12V. During the migration the U_{ac} is increased stepwise up to a final value. Then the U_{dc} is switched off and the channel is scanned for fluorescence intensity.

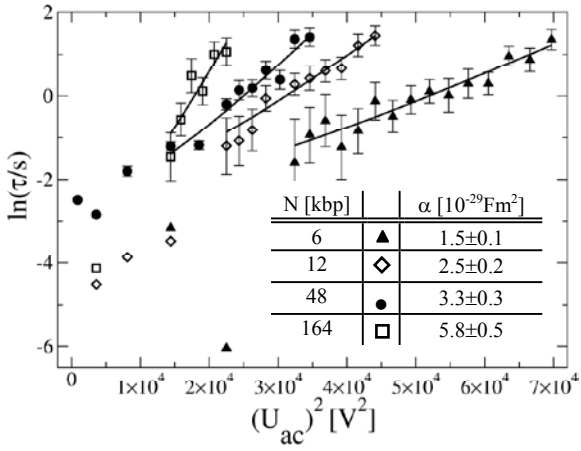

Figure 2: Plot of the experimentally measured trapping times τ versus U_{ac}^2 (symbols with error bars). The solid lines represent data fits from which the polarizabilities are deduced quantitatively. The inset table shows the resulting values of α for the four investigated DNA lengths.

4. RESULTS AND DISCUSSION

Fig. 2 shows the observed escape times versus the applied U_{ac} for linear DNA with 6, 12, 48.5 and 164 kbp. The linear fits with logarithmic corrections [2] have significantly different slopes resulting in increasing α with increasing DNA length (see inset Fig. 2).

For briefly exemplifying the separation performance of the device, we here focus on supercoiled DNA. As demonstated with Fig. 3, a supercoiled 7 kbp monomer can be separated from its 14 kbp dimer within only 210 seconds with base line resolution.

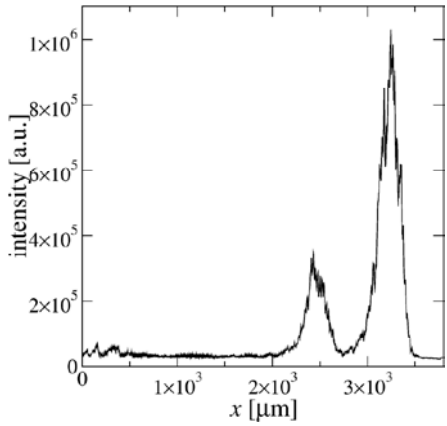

Figure 3. Scanned electropherogram of separated supercoiled 7 kbp (right peak) and 14 kbp (left peak) DNA. Parameters of the separation: U_{dc} = 12 V, U_{start} = 198 V, U_{inc} = 6 V, τ_{inc} = 30 sec, U_{end} = 240 V, ω = 60 Hz, scan speed 10 µm/s at 3 frames per s.

5. CONCLUSIONS

In summary, we demonstrated the separation of supercoiled DNA based on dielectrophoretic trapping. Moreover, we are able to deduce the molecular polarizability from the trapping times. With this quantitative information a systematic choice of the separation parameters will be possible in the future. The demonstrated separation technique is of high potential for the fast and cheap quality control of biotechnologically relevant plasmid DNA for applications such as in DNA vaccination.

ACKNOWLEDGEMENTS

Supply of plasmid samples from PlasmidFactory, Bielefeld, and financial support from the Deutsche Forschungsgemeinschaft within SFB 613 is gratefully acknowledged.

REFERENCES

[1] "Free-flow electrophoresis with trapping by a transverse inhomogeneous field," A. Ajdari and P. Prost, PNAS, **88**, 4468 (1991).

[2] "Dielectrophoretic manipulation of DNA: Separation and polarizability," J. Regtmeier et al., Anal. Chem., **79**, 3925 (2007)

[3] "Electrodeless Dielectrophoresis of Single- and Double-stranded DNA," C.-F. Chou et al., Biophys. J., **83**, 2170 (2002)

[4] "Dynamics of a polymer surmounting a potential barrier. The Kramers problem for polymers," P. Park and W. Sung, J. Chem. Phys., **111**, 5259 (1999).