



Mechanisms of DNA separation in entropic trap arrays: a Brownian dynamics simulation

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Abstract

Using Brownian dynamics simulations, we study the migration of long charged chains in an electrophoretic microchannel device consisting of an array of microscopic entropic traps with alternating deep regions and narrow constrictions. Such a device has been designed and fabricated recently by Han and Craighead [Science 288 (2000) 1026] for the separation of DNA molecules. Our simulation reproduces the experimental observation that the mobility increases with the length of the DNA. A detailed data analysis allows to identify the reasons for this behavior. Two distinct mechanisms contribute to slowing down shorter chains. One has been described earlier by Han and Craighead [Science 288 (2000) 1026]: the chains are delayed at the entrance of the constriction and escape with a rate that increases with chain length. The other, actually dominating mechanism is here reported for the first time: some chains diffuse out of their main path into the corners of the box, where they remain trapped for a long time. The probability that this happens increases with the diffusion constant, i.e., the inverse chain length.

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1. Introduction

Electrophoresis is the standard method of DNA separation by length (Viovy, 2000). Since the mobility of DNA molecules in free solution is independent of their length, the DNA is commonly introduced into a gel, which serves as a random sieve. Unfortunately, the efficiency of gel electrophoresis decreases with the length of the DNA. Moreover, bioanalytic devices are more and more miniaturized, and incorporating random gels with characteristic pore sizes in the nanometer range into future nanodevices will presumably be-

come increasingly problematic. Therefore, much recent effort has been devoted to designing and making well-defined microstructured devices for DNA separation (e.g., Han et al., 1999; Han and Craighead, 2000, 2002; Bader et al., 1999; Hammond et al., 2000; Duong et al., 2003).

In this paper, we focus on a geometry proposed by Han et al. (1999), which is based on the idea of entropic trapping. The DNA is introduced into a small microchannel with alternating deep regions and shallow constrictions. The channel thickness in the constrictions is much smaller than the radius of gyration of the DNA molecules. The deep region is large enough to accommodate the equilibrium shape of the DNA molecules. Entering the constrictions is thus entrop-

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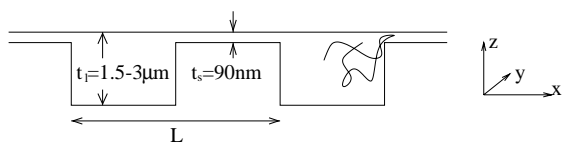


Fig. 1. Schematic cartoon of the microchannel device proposed by Han and Craighead (2000) (side view). The width of the channel in the y -direction is much larger ($\sim 30 \mu\text{m}$).

ically penalized, and the deep regions can be interpreted as entropic traps. A schematic cartoon of the structure is shown in Fig. 1.

In these structures, Han et al. (1999, 2000, 2002) made the counterintuitive observation that long DNA molecules travel *faster* than short molecules. They explained their finding by means of a simple kinetic model. In order to escape from one of the deep regions, the DNA must overcome an activation barrier ΔF_c . The escape process is initiated by a thermally activated stretching of a chain portion (length x) into the shallow constriction. This costs entropic penalty proportional to x , but it also decreases the electric potential energy by an amount $\sim Ex^2$, where E is the electric field. Hence, there exists a critical length, $x_c \propto 1/E$, below which the entropy penalty dominates and the chain is driven back into the deep region. At $x > x_c$, the energy gain dominates and the chain escapes. The free energy at x_c represents the activation barrier for the escape process. It depends solely on the electric field, $\Delta F_c \propto 1/E$. The rate of escape attempts, $1/\tau_0$, increases with the chain size, since the amount of polymer in contact with the constriction is larger for larger chains. The mean trapping time in this simple model is given by

$$\tau = \tau_0 \exp\left(\frac{\Delta F_c}{k_B T}\right), \quad (1)$$

where T is the temperature and k_B is the Boltzmann factor. This implies that long chains travel faster.

The results of Han et al. (1999, 2000, 2002) have motivated recent Monte Carlo simulations by Tessier et al. (2002). These authors used the Bond fluctuation model, a well-known lattice model for polymers, to study the mobility of polymers in a geometry similar to that suggested by Han et al. (1999, 2000, 2002) within local Monte Carlo dynamics (single monomer moves). Their results confirm the trapping picture of Han et al. (1999, 2000, 2002), and even details of the kinetic

model. For example, they present evidence that the penetration depth x of the chain into the constriction can be used as a “reaction coordinate” for escaping, with a critical value $x_c \propto 1/E$.

When looking more closely at the simulation data, one notices that the trapping in the system of Tessier et al. (2002) is unexpectedly strong. Although the width of the constriction is more than twice as large as in the experiments (in units of the persistence length), the molecules spend almost the entire time in a trapped state, and very little time traveling, even at intermediate fields. This effect might be an artifact of the lattice model. We note that the width of the constriction in the simulations is just 10 lattice constants, while every monomer occupies a cube with 8 lattice sites, and the average bond length is 2.8 lattice constants. Moreover, the Monte Carlo dynamics is not realistic. Monomers are picked and moved randomly (with some moves being rejected), whereas in the real system, they are pulled into the constriction by the electric force. Dynamic Monte Carlo is known to reproduce diffusional and relaxational dynamics in systems near local equilibrium. Nevertheless, it is not clear how well it can be applied to study driven systems. The details of the dynamics should matter particularly at higher electric fields, in situations where the chains travel so fast that they no longer reach local equilibrium in the traps. Therefore, simulations of off-lattice models with a more realistic dynamical evolution are clearly desirable.

As a first step in this direction, Chen and Escobedo (2003) have recently studied the free energy landscape of chains in a single, non-periodic trap with Monte Carlo simulations of an (off-lattice) bead-spring model. The initial configuration in these simulations is that of a fully relaxed chain in the absence of an electric field. With that starting point, the free energy barrier ΔF turns out to depend on the chain length for short chains, and to level off at higher chain lengths. The data for ΔF do not seem to support the relation $\Delta F \propto 1/E$. Since the simulations still used Monte Carlo, the results on dynamical properties were limited.

In the present paper, we present Brownian dynamics simulations of a full (non-equilibrium) periodic array of entropic traps. We employ a Rouse-chain model, such as has been successfully used by others to study the migration of DNA in various geometries (Deutsch, 1987, 1988; Matsumoto and Doi, 1994; Noguchi and

Takasu, 2001). Our main result is the observation of a new trapping mechanism in geometries such as Fig. 1, which is at least as important as that suggested by Han et al. (1999, 2000, 2002), and which can also be exploited to achieve molecular separation. The insight gained from our study should be useful for the design of future, improved molecular separation devices.

The paper is organized as follows: in the next section, we describe and discuss the model and give some technical details on the simulation. The results are presented in Section 3. We summarize and conclude in Section 4.

2. The simulation model

We model a single DNA molecule as a chain with pairwise interactions

$$V_{\text{pair}}(r)/k_{\text{B}}T = \begin{cases} (\sigma/r)^{12} - (\sigma/r)^6 + 1/4 & : (r/\sigma)^6 \leq 2 \\ 0 & : \text{otherwise} \end{cases} \quad (2)$$

This potential describes soft, purely repulsive interactions between beads of diameter σ . The beads are connected by springs with the spring potential

$$V_{\text{spring}}(r) = \frac{k}{2}r^2. \quad (3)$$

The spring constant was chosen very large, $k = 100k_{\text{B}}T/\sigma^2$, in order to prevent chain crossings.

The molecule is confined in a structured channel with a geometry similar to that used by Han et al. (1999, 2000, 2002). The thickness of the shallow constrictions and thick regions is $t_{\text{s}} = 7\sigma$ and $t_{\text{l}} = 80\sigma$, respectively, the length of a deep region is 80σ , and the total length of a period is $L = 100\sigma$. In the lateral direction, the channel is infinite. The chain beads are repelled from the walls of the channel by means of a wall potential essentially identical to (2), $V_{\text{wall}}(r) = V_{\text{pair}}(r)$, where r is the distance of a bead to the closest wall point.¹ The details of the wall potential do not influence the results, as long as it is repulsive and short ranged. Note that the effective width of the channel, i.e., the width of the space accessible to a

¹ At corners, the potentials of the adjacent walls are summed up.

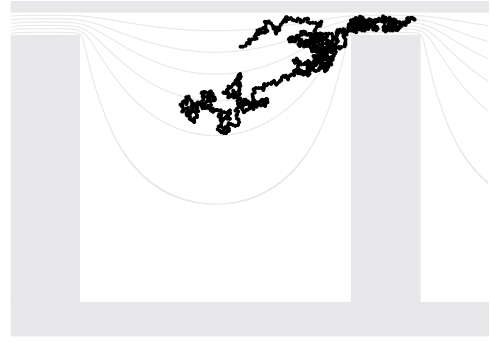


Fig. 2. Snapshot of a chain with $N = 1000$ beads in an entropic trap. The solid lines show the electric field lines.

bead, is reduced by 2σ with this potential. A snapshot of a chain in such a channel is shown in Fig. 2.

DNA is a charged polyelectrolyte with $2e^-$ per base pair, thus each bead carries a charge q and is subject to an electric field, $E = -\nabla\Phi$. We assume that the charges do not interact with one another. This will be discussed further below. The distribution of the electric potential $\Phi(r)$ in the channel was calculated numerically by solving the Laplace equation ($\Delta\Phi = 0$) with von Neumann boundary conditions at the walls ($\mathbf{n} \cdot \nabla\Phi = 0$, where \mathbf{n} is the surface normal).

The dynamical evolution of the system is described by a Kramer's equations (Risken, 1989)

$$\begin{aligned} \dot{\mathbf{r}}_i &= \mathbf{v}_i \\ m\dot{\mathbf{v}}_i &= \mathbf{f}_i - \zeta\mathbf{v}_i + \eta_i, \end{aligned} \quad (4)$$

where \mathbf{f}_i is the total force acting on bead i , \mathbf{v}_i the velocity, and ζ is a friction coefficient. The random noise η fulfills

$$\begin{aligned} \langle \eta_{i\alpha} \rangle &= 0 \\ \langle \eta_{i\alpha}(t)\eta_{j\beta}(t') \rangle &= 2k_{\text{B}}T\zeta\delta_{ij}\delta_{\alpha\beta}(t-t'), \end{aligned} \quad (5)$$

where $\alpha, \beta = x, y, \text{ or } z$. The random noise and the friction mimics the effect of the solvent surrounding the DNA. The chains differ from standard Rouse chains (Doi and Edwards, 1986) by their excluded volume interactions and by the inertia m of the beads. With the parameters of our model ($m = 1\zeta^2\sigma^2/k_{\text{B}}T$, see the discussion further below), the chains exhibit Rouse dynamics on length scales of the order the gyration radius of the chain, R_{g} (Streek, 2002).

Several important physical factors are neglected in the model. First, electrostatic interactions within DNA

chains are not taken into account. This is justified by the fact that the Debye screening length in typical electrophoresis buffers is only a few nanometers, comparable to the diameter of the double helix. Second, we do not consider electro-osmotic flow, or flow in general. This reflects the experimental situation reported by Han et al. (1999, 2000, 2002) Third, hydrodynamic interactions are disregarded. This is partly justified by the theoretical observation that for polyelectrolytes dragged by an electric field, the hydrodynamic interactions should be screened over distances larger than the Debye length, since the counterions are dragged in the opposite direction (Viovy, 2000). Unfortunately, the argument is only strictly valid as long as no non-electric forces are present (Long et al., 1996). In our case, the walls of the channels exert non-electrical forces which stop the polymers, but do not prevent the counterions in the Debye layer from moving. Thus the chains experience an additional trapping force from the friction of the counterions. This effect is disregarded in our model. Furthermore, diffusion is not treated correctly even in free solution. The diffusion constant of Rouse chains scales as $1/N$ with the chain length, N . Including hydrodynamic interactions, one expects Zimm scaling $1/R_g$, where the gyration radius R_g scales like $R_g \propto N^{3/5}$ for self-avoiding chains. Experimentally, the diffusion constant of DNA in typical buffer solutions is found to scale as $1/N^{0.672}$ (Stellwagen et al., 2003).

Unfortunately, a full treatment, which accounts correctly both for the (dynamically varying) counterion distribution as well as the hydrodynamic interactions is not feasible with standard computational resources. The simplifications of our model influence the results quantitatively, but do presumably not change them by orders of magnitude. The qualitative behavior should not be affected.

Keeping these caveats in mind, we can now proceed to establish a quantitative connection with the experimental set-up of Han et al. The natural units in our model are related to the parameters σ , ζ , $|q|$ (the charge per bead), and T (the temperature). More specifically, the energy unit is $\epsilon = k_B T$, the length unit is σ , the time unit is $t_0 = \sigma^2 \zeta^2 / k_B T$, and the electric field unit is $E_0 = k_B T / \sigma |q|$. Throughout the paper, all quantities shall be given in terms of these natural units. They shall now be related to real (SI) units.

Since the experiments are carried out at room temperature, the energy unit is $\epsilon \equiv 300 k_B K = 4 \times 10^{-21}$ J. The length unit is obtained from matching the persistence length of the model chains, $l_p = 1.6\sigma$ (Streek, 2002) with that of DNA, $l_p = 45$ nm, yielding $\sigma \equiv 30$ nm. The persistence of the chain is also used to determine the number of base pairs (bp) per bead. A DNA molecule contains approximately 150 bp on a stretch of length, l_p . In our model, the average bond length between two beads is 0.847σ (Streek, 2002), thus we have 1.9 beads per persistence length, and one bead represents roughly 80 bp. The elongation for a chain crossing with minimal energy is $r_{\text{cross}} \approx 1.198\sigma$, which corresponds to an energy barrier $\Delta E_{\text{cross}} \approx 82E_0$ for crossing. The Boltzmann-factor for this energy barrier turns out to be less than 10^{-35} . To check the simulation program, the bond length distribution was compared to the Boltzmann-factor and very good agreement was found. Furthermore, no bond ever exceeded a length of 1.1σ . Thus, no chain crossing occurred in our simulations.

The time scale t_0 is calculated from the diffusion constant, D . For Rouse chains of length N , D is given by

$$D = \frac{k_B T}{N \zeta} = \frac{\sigma^2}{N t_0}. \quad (6)$$

Experimentally, Stellwagen et al. (2003) have recently reported the relation

$$D = 7.73 \times 10^{-6} (\text{number of base pairs})^{-0.672} \text{ cm}^2 \text{ s}^{-1}. \quad (7)$$

Choosing as reference a chain of length 40 kbp ($N = 500$), we obtain $t_0 \equiv 2.9 \times 10^{-6}$ s.

Finally, the unit of the electric field can be identified from matching the mobility μ_0 of free chains. The theoretical value is $\mu_0 = |q|/\zeta = \sigma/t_0 E_0$. In experiments, the mobility depends strongly on the choice of the buffer. Unfortunately, Han et al. (1999, 2000, 2002) do not report explicit measurements of the free-chain mobility of DNA in the buffer used in their experiments. In the microchannel, they observe that the overall mobility saturates at high field strengths. The apparent maximum mobility, μ_{max} , results from an average over a slow motion in the reduced electric field of the deep regions, and a fast motion in the enhanced electric field of the constrictions (see Fig. 2). For large

periods, $L \gg t_1, t_s$, the ratio of these two field strengths is simply the inverse of the thickness ratio, $\alpha = t_1/t_s$. One can then derive a relation between the true free chain mobility, μ_0 and the apparent mobility, μ_{\max} :

$$\mu_0 = \mu_{\max}(x_1 + x_s/\alpha)(x_1 + x_s\alpha), \quad (8)$$

where x_1 and x_s are the relative lengths for the deep regions and shallow constrictions, respectively ($x_1 + x_s = 1$). Han et al. (1999) measure $\mu_{\max} \approx 0.13 \times 10^{-8} \text{ m}^2/\text{Vs}$ in an experimental set-up with $x_1 = x_s = 1/2$, $\alpha \approx 15$, and $L = 10\text{--}40 \text{ }\mu\text{m}$. Using Eq. (8), one can thus estimate $\mu_0 = 4.3$, $\mu_{\max} = 0.55 \times 10^{-8} \text{ m}^2/\text{Vs}$. This value of μ_0 seems very low compared to typical values in the literature. Stellwagen and Stellwagen (2002) have measured DNA mobilities in Tris–acetate buffers at various concentrations and found values between 2×10^{-8} and $4 \times 10^{-8} \text{ m}^2/\text{Vs}$. In 40 mM Tris–acetate buffer, they obtain $\mu_0 = 3.3 \times 10^{-4} \text{ cm}^2/\text{Vs}$ (Stellwagen et al., 2003). Using the latter value as an order-of-magnitude estimate, we identify $E_0 \sim 3 \times 10^3 \text{ V/cm}$. However, the results of Stellwagen et al. (2003) can probably not be applied here, because the mobility depends strongly on the buffer. The first estimate, $\mu_0 = 0.55 \times 10^{-8} \text{ m}^2/\text{Vs}$, leads to the identification $E_0 \sim 2 \times 10^4 \text{ V/cm}$.

Han et al. (1999); Han and Craighead (2000) have separated DNA of lengths between 5 and 160 kbp. Here, we study chains of length, $N \leq 1000$, corresponding to ≤ 80 kbp, which is comparable. The depth of the deep channel regions in the experiments was $t_1 = 1\text{--}3 \text{ }\mu\text{m}$, which also compares well with the simulation, $80\sigma = 1.6 \text{ }\mu\text{m}$. The depth of the constriction was $t_s = 100 \text{ nm} \approx 2l_p$ in the experiments. In our case, it is $5\sigma \approx 3l_p$, which is slightly larger, but still comparable. Since our channels are wider, we expect that the trapping in the simulations will be less pronounced than in reality. The total length per trap (period) was $L = 4\text{--}40 \text{ }\mu\text{m}$ in the experiments (mostly $4 \text{ }\mu\text{m}$) and $L = 100\sigma = 3 \text{ }\mu\text{m}$ in the simulations.

The average electric field strength in the experiments was varied between 20 and 80 V/cm, which corresponds to $\sim 0.001E_0\text{--}0.004E_0$ or $\sim 0.006E_0\text{--}0.03E_0$, depending on the identification of E_0 . In the simulations, we studied field strengths between 0.0025 and $0.04E_0$. When comparing field strengths, we must keep in mind that the electric field in the channel is not homogeneous (see Fig. 2). In our geometry, the electric field in the constriction is enhanced by a factor of 2.5.

In the experimental set-up, the enhancement factor is only ~ 1.8 , due to the fact that the length ratio between the shallow and deep regions is different (50:50 in the experiment, 20:80 in the simulations). The ratio of field strengths in the shallow and deep regions in our simulations is roughly 4. In the experimental geometry, it is $t_1/t_s \approx 10\text{--}30$ for large periods L and smaller otherwise.

The remaining model parameter that has yet to be determined is the mass m of a bead. We note that the actual value of the mass has no influence on the static properties of the chain (e.g., the chain flexibility), nor on the diffusive part of the dynamics. It does, however, determine the relative importance of vibrational modes in the chain and other inertia effects. The latter can be characterized by the electrophoretic relaxation time τ_e , i.e., the characteristic decay time of the drift velocity of free flow DNA, if the electric field is suddenly turned off. Typical values of τ_e are $\tau_e \approx 10^{-9}$ to 10^{-12} s (Grossmann and Colburn, 1992). In our model, the electrophoretic relaxation time is $\tau_e = m/\zeta$, and the correct value of the mass would be $m \sim 10^{-3}$ to $10^{-6} \zeta t_0$. However, this is unfortunate from a computational point of view, because the mean velocity per bead, $\sqrt{\langle v^2 \rangle} = \sqrt{2k_B T/m}$, becomes large for such small bead masses, and the time step has to be chosen short as a consequence. The simulation becomes very inefficient. On the other hand, we are not interested in inertia effects here, and we wish to study dynamical effects on much longer time scales than t_0 . Therefore, we chose to give the beads an unphysically high mass, $m = 1 \zeta t_0$ leading to an electrophoretic relaxation time $\tau_e = t_0 \sim 10^{-6} \text{ s}$. On time scales t_0 and less, the dynamics will thus be unrealistic, but this does not affect the slow diffusive dynamics.

We close this section with a few technical remarks. The dynamic Eq. (4) were integrated using the Verlet algorithm. The stochastic noise was realized by picking in every time step a vector, η at random. Dünweg and Paul (1991) have shown that the distribution of random numbers in such a procedure does not necessarily have to be Gaussian. Here, we used a uniform distribution in a sphere. Since we consider single chains only, no periodic boundaries were necessary. With the mass $m = 1 \zeta t_0$, the time step could be chosen $0.01 t_0$. Typical run lengths were between 4 and 20 million t_0 (5–25 s). The simulation jobs were

managed by the Condor Software Program (Condor), which was developed by the Condor Team at the Computer Science Department of the University of Wisconsin (Condor, 2003).

3. Results and discussion

Three examples of trajectories at the average field $E = 0.005E_0$ are shown in Fig. 3. They reveal three qualitatively different modes of migration. The trajectory of very short chains ($N = 10$ corresponding to 800 bp) is dominated by diffusion. The chains wander back and forth in the trap, until they eventually escape

into the next trap. The movement of chains with intermediate chain length ($N = 100$ or 8 kbp) is much more directional, but still irregular. They are often trapped at the entrance of constrictions. In contrast, long chains ($N = 1000$ or 80 kbp) travel smoothly. The first (leading) monomer is sometimes trapped, but this does not arrest the rest of the chain. Whereas, the time spent in one box fluctuates strongly for shorter chains, it is roughly constant for large chains.

The behavior of intermediate chains ($N = 100$) has similarity to that observed in the simulations of Tessier et al. (2002). However, the trapping is much less pronounced in our case, and chains frequently pass from one box to another without being trapped at all. Trap-

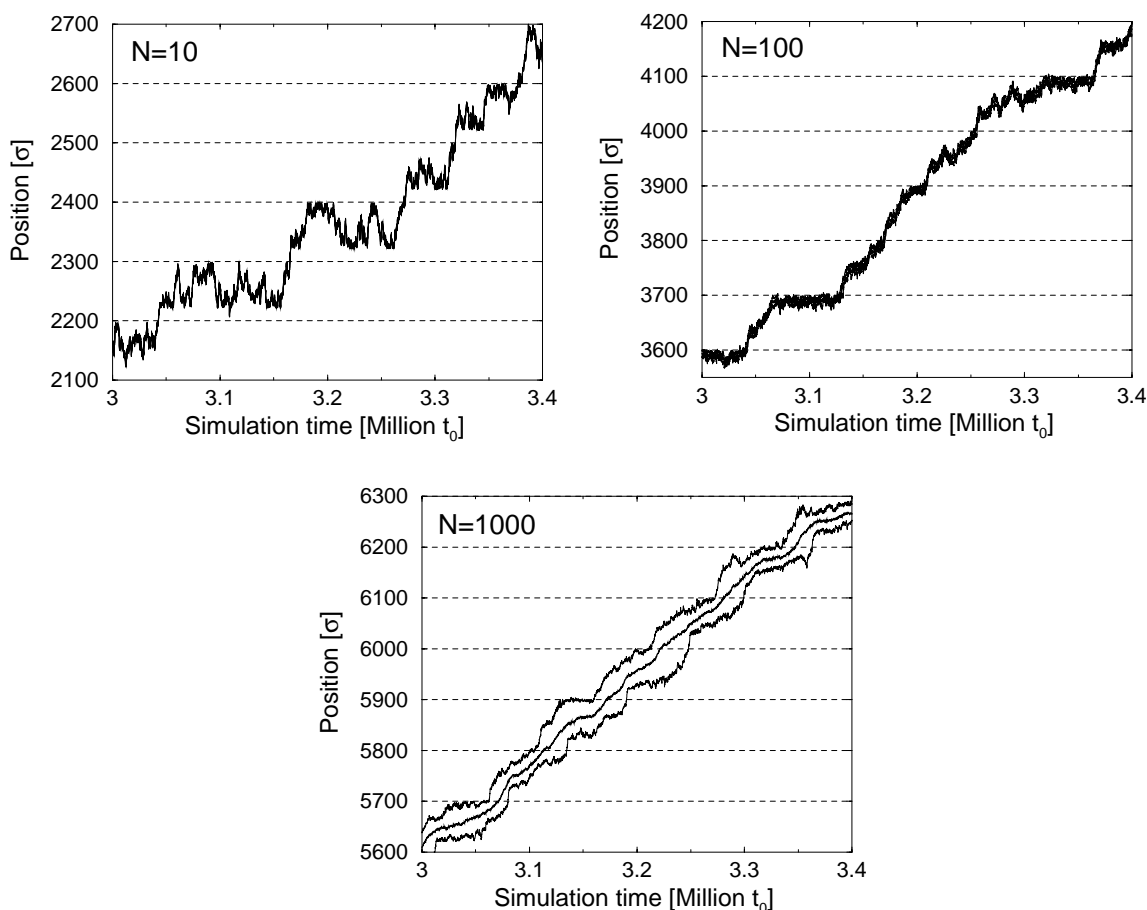


Fig. 3. Trajectories of chains moving in an entropic trap array for three different chain lengths, N , in the presence of an average electric field, $E = 0.005E_0$. The middle solid line shows the position of the center of mass. The (dashed) upper and lower lines show the positions of the first and the last monomer. In the case $N = 10$, these three lines cannot be distinguished from each other. The dashed horizontal lines indicate the positions where constrictions begin.

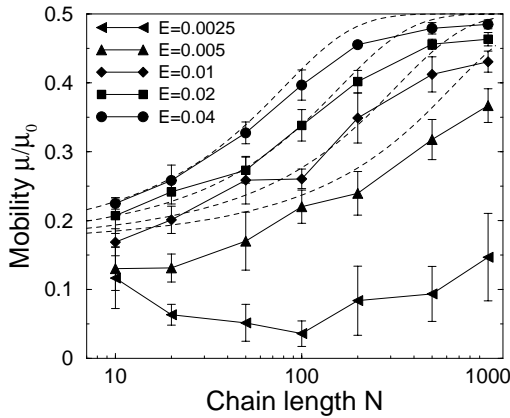


Fig. 4. Mobility μ in units of the mobility μ_0 of a free chain as a function of chain length, N for different average electric fields, E in units of E_0 . The dashed lines show the prediction of Eq. (12) for the fields $E/E_0 = 0.04, 0.02, 0.01,$ and 0.005 (from top to bottom).

ping effects comparable to those reported by Tessier et al. (2002) were only observed at the lowest field strength, $E = 0.0025E_0$. At that field value, chains of all lengths got trapped. As we shall see below, however, chain separation turned out to be not efficient for such small fields.

We have carried out simulations for seven different chain lengths $N = 10, 20, 50, 100, 200, 500,$ and 1000 , and for five average field values, $E/E_0 = 0.0025, 0.005, 0.01, 0.02,$ and 0.04 . The resulting mobilities, determined as $\mu = \langle v \rangle / E$, are summarized in Fig. 4. For all fields except the lowest, the mobility increases steadily with the chain length. At high chain lengths and high fields, it begins to saturate. The maximum value μ_{\max} is only about half as large as the free chain mobility μ_0 , due to the fact that the chains spend a disproportionate amount of time in the deep regions, where the local electric field is lower than average.

At the lowest field ($E = 0.0025E_0$), the mobility depends only slightly on the chain length and even decreases with chain length for small N . At such small fields, backwards diffusion becomes important for short chains. We have seen in Fig. 3 that short chains explore the whole trap. At $E = 0.0025E_0$, they sometimes even travel backwards into the trap that they just left. The conformational entropic penalty for entering the constrictions is small for short chains. In the limit $E \rightarrow 0$, the inverse mobility is thus simply proportional to the number of times the chain visits

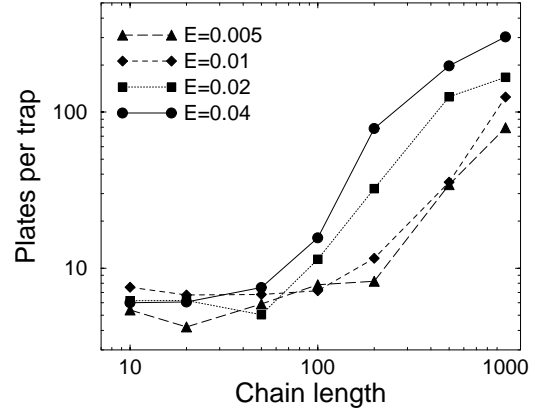


Fig. 5. Theoretical plate number per trap as a function of chain length for different electric fields, E as indicated (in units E_0).

the entrance of the constriction. Since the latter scales like N (the inverse diffusion constant, D^{-1}), the mobility is then expected to decrease with chain length. In our system, this is observed at $E = 0.0025E_0$ for chain lengths smaller than $N = 100$. For $N > 100$, the mobility increases again with chain length. The resulting overall chain length dependence is small, and the chain separation is not efficient.

The quality of molecular separation systems is often characterized in terms of the theoretical plate number

$$N_{\text{plate}} = 16(t_R/t_W)^2, \tag{9}$$

where t_R is the retention time, i.e., the total time spent in the system, and t_W is the width of the peak at the baseline. Fig. 5 shows the plate number per trap for our system. In the interesting regime, we have 10–100 plates per trap. At $\sim 10^5$ traps per meter, this corresponds to theoretical plate numbers of 10^6 – 10^7 plates per meter, which is quite good and in agreement with the results of Han and Craighead (2000).

We will now investigate the migration modes in more detail. To this end, we have calculated histograms of the retention times spent in one trap. They were defined as the difference $t_{n+1} - t_n$ of the times t_n when the first monomer of a chain first enters the deep region of the n th trap. Fig. 6 shows distributions of retention times for chains of different length N in the field, $E = 0.01E_0$.

The chains need a minimum time $\sim (1-1.5) \times 10^4 t_0$ to travel from one trap to another. After that “dead” period, the histogram rises rapidly and reaches a max-

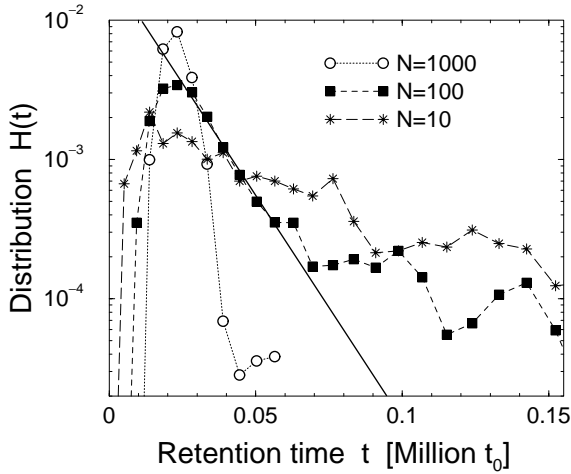


Fig. 6. Distribution of Retention times in one trap for different chain lengths, N and electric field, $E = 0.01E_0$. The thick solid line is a fit to the initial exponential decay at chain length, $N = 100$.

imum at $t_{\max} \approx 2 \times 10^4 t_0$. From a comparison of histograms for all our simulation data (not shown), we deduce that the position of the maximum t_{\max} of the distribution depends very little on the chain length and is strictly proportional to the inverse field, $1/E$. The product $L/t_{\max}E = \mu_{\max} \approx 0.5 \mu_0$ determines the maximum mobility μ_{\max} in our system.

Beyond the maximum, the distribution decays rapidly for long chains ($N = 1000$), and much more slowly for short chains ($N = 10$), following an exponential behavior. This is consistent with the common picture, where the migration is determined by one single escape rate $1/\tau$. The histogram for intermediate chain length $N = 100$, however, reveals that the situation is more complex in reality. The initial decay of the distribution can be fitted with one exponential, however, a long-time tail emerges at times beyond $t \sim 5 \times 10^4 t_0$. Thus, the distribution of retention times at $N = 100$ has two characteristic time scales, τ_{fast} and τ_{slow} .

To some extent, this phenomenon is already apparent in the trajectory of Fig. 3. The figure suggests that there exist two qualitatively different ways how chains pass from one trap to another: Either they travel relatively straight and unimpeded, or they get trapped and linger for some time at the border of the constriction.

The trapping mechanism, suggested by Han et al. (1999, 2000, 2002) alone cannot explain these ob-

servations. Here, we propose an additional trapping mechanism, which also slows down short chains and which presumably accounts to a large extent for the chain length dependence of the mobility observed in our simulations. The idea is that chains get trapped at the side walls and corners of the deep boxes due to diffusion. The electric field lines lead the chains from the outlet of one constriction directly to the entrance of the next one. With a certain probability, the chain will therefore reach the next constriction without detours, and then get delayed there due to the mechanism suggested by Han et al. (1999, 2000, 2002) This accounts for the fast time scale, τ_{fast} . On the other hand, chains may also diffuse out of the main path. They may access regions of the trap at the walls and in the corners where the electric field is very small. In that case, they are caught in a force-free region, and they can “escape” only by diffusion.

We will now explore whether our data support this picture. In the following analysis, only data for chain lengths $N \geq 20$ and fields $E \geq 0.005E_0$ were used. In most of these systems, two time scales τ were observed. The value of the fast time scale τ_{fast} could be extracted by fitting an exponential ($A \exp(-t/\tau_{\text{fast}})$) to the initial decay of the histogram $H(t)$. The determination of the slow time scale was much more difficult, due to the poor statistics for the late-time tails of the histograms. We used two approaches: first we fitted the long-range tail with an exponential function to obtain a rough estimate. Assuming $H(t) \propto e^{-t/T_{\text{slow}}}$, we calculated τ_{slow} via

$$\tau_{\text{slow}} = \int_{t_{\text{cut}}}^{\infty} dt(t - t_{\text{cut}})H(t) / \int_{t_{\text{cut}}}^{\infty} dtH(t), \quad (10)$$

which is independent of t_{cut} . We used $t_{\text{cut}} = 500/E$ (t_0E_0) to analyze the data. We checked whether the result deviated strongly from the previous estimate, and whether it depended strongly on the cutoff t_{cut} . If this was the case, (because the data for $H(t)$ scattered strongly), the result was discarded. The remaining values are compiled in Fig. 7, together with the data for τ_{fast} .

If our suspicion is correct that the fast process corresponds to the mechanism of Han et al. (1999, 2000, 2002), then the rate $1/\tau_{\text{fast}}$ should be proportional to the amount of polymer in contact with the channel. Since the channel entrance is essentially one dimensional, the contact area should be proportional to the

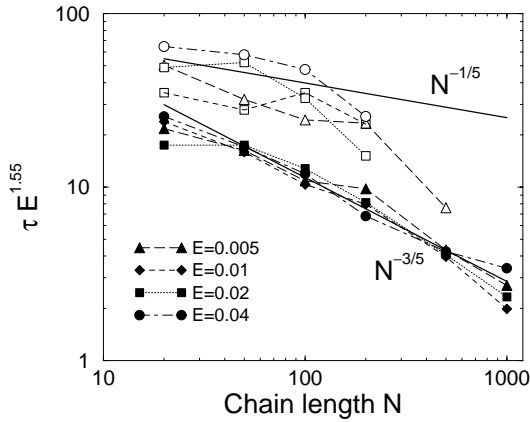


Fig. 7. Characteristic length scales (rescaled) of the retention time distribution as a function of chain lengths, N for different electric fields, E . The filled symbols correspond to the fast time scale, τ_{fast} , the open symbols to the slow time scale, τ_{slow} . The thick lines show for comparison power laws as indicated.

gyration radius R_g of the chain. Fig. 7 shows that the fast time scale indeed scales like $\tau_{fast} \propto 1/R_g \propto N^{-3/5}$.

The relation between τ_{fast} and the electric field strength E is more complicated. According to Han et al. (1999, 2000, 2002), the chains must overcome a free energy barrier of height proportional to $1/E$ in order to escape. On the other hand, the prefactor τ_0 in Eq. (1) may also depend on E . The resulting E dependence can be rather complex. In the field range of our simulation, the resulting relation $\tau_{fast}(E)$ can be approximated by the empirical law $\tau_{fast} \propto E^{-1.55}$.

Like the fast time scale, τ_{fast} , the slow time scale, τ_{slow} , also decreases with the chain length, but the dependence here is very weak. Unfortunately, the quality of the data was not sufficient for a quantitative analysis. If our interpretation is correct, then τ_{slow} characterizes an escape from a low-force region. We note that extended chains in a corner experience a net electric force towards the wall, even though the field lines of course never enter the wall (see Fig. 8). In order to leave the corner, the chain must either move against the force, or change its shape. In both cases, it has to overcome a free energy barrier before it can get rescued from the field lines. A simple Ansatz would predict the escape probability to be proportional to the diffusion constant $D \propto 1/N$, and to the area covered by the chain, $R_g^2 \propto N^{6/5}$, or the total chain length, N . This would yield a very weak net chain length depen-

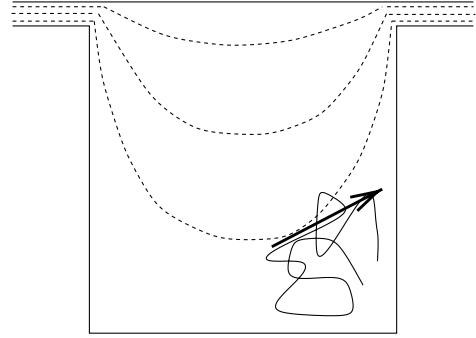


Fig. 8. Schematic cartoon of a chain caught at the corner of the trap in the field-free region. The chain experiences a net force towards the wall.

dence $\tau_{slow} \sim N^{-1/5}$ or $\tau_{slow} \sim N^0$, which is consistent with the observed behavior (Fig. 7).

Thus, the slow time scale τ_{slow} itself does not contribute much to the chain length dependence of the mobility. The main effect comes from the fact that the relative number of chains caught in the field-free region depends on the chain length. The travel time from one channel to the next for undeflected chains is slightly smaller than $t_{max} = L/E\mu_{max}$. We assume that chains have to diffuse at least over a distance z_0 into the field-free region (direction $-z$, see Fig. 1) in order to get caught. The distribution along the z -direction after a time t_{max} will be approximately Gaussian: $N(z) \propto e^{-z^2/6Dt}$. The probability of being caught is thus

$$P = \int_{z_0}^{\infty} N(z) = \text{erfc}(z_0/\sqrt{6Dt_{max}}) = \text{erfc}(\alpha\sqrt{NE}), \quad (11)$$

where $\text{erfc}(y) = 2/\sqrt{\pi} \int_y^{\infty} dx \exp(-x^2)$ is the complementary error function.

We can test this prediction under the assumption that the two time scales, τ_{fast} and τ_{slow} , are sufficiently far apart that they can be separated. In that case, one can choose a time t_{cut} such that almost all undeflected chains have left the trap, while almost all deflected chains are still in the field-free region. P can then be approximated by the relative number P_0 of chains left in the field-free region at the time t_{cut} . Fig. 9 shows the result of such an estimate. The data collapse reasonably well for different N , E and can be fitted with Eq. (11), with the fit parameter $\alpha = 0.59$. Inserting

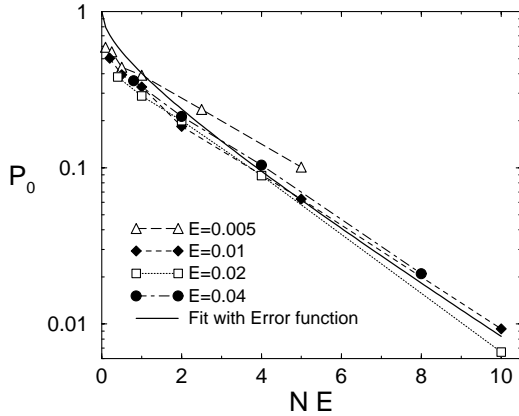


Fig. 9. Probability, $P_0(t)$ that a chain is still in the trap at the time, $t_{\text{cut}} = 350/E(t_0 E_0)$, vs. NE in units of E_0 . The solid line represents a fit to Eq. (11) with $\alpha = 0.59$.

$\mu_{\text{max}} = 0.5\mu_0$ and $L = 100\sigma$, one obtains $z_0 \sim 20\sigma$. Thus, chains get caught if they sidetrack by more than $\sim 20\sigma$ from their main path, which is determined by the field lines.

These considerations establish the existence of a new mechanism which produces chain length dependent mobility. To assess the relative importance of the new mechanism, we compare the real mobility data, Fig. 4, with a very simple model. Chains either travel straight across the trap, or sidetrack into the field-free region. Traveling across the trap takes at least the time $t_{\text{max}} = LE/\mu_{\text{max}}$. The chains caught in the field-free region spend an additional time τ_{slow} in the trap. We make the simplification that $E\tau_{\text{slow}}$ is independent of the chain length and electric field and given by the number $E\tau_{\text{slow}} = 400t_0 E_0$, which is roughly the value at chain length ~ 100 and field strength $\sim 0.01E_0$ – $0.02E_0$. The relative number of chains caught in the field-free region P is calculated according to Eq. (11), with $\alpha = 0.59$ (taken from Fig. 9). The resulting mobility is

$$\frac{\mu}{\mu_0} = \left[\frac{\mu_0}{\mu_{\text{max}}} + \frac{\tau_{\text{slow}} E}{L} \text{erfc}(\alpha\sqrt{NE}) \right]^{-1}. \quad (12)$$

This prediction is compared with the actual data in Fig. 4 (dashed lines). Despite the simplicity of Eq. (12), the agreement is remarkably good.

4. Summary and outlook

To summarize, we have presented the first off-lattice Brownian dynamics simulation of DNA migration in an entropic trap array. We reproduce the experimentally observed phenomenon that the mobility increases with the chain length. This result can be traced back to two distinct mechanisms. The first mechanism has already been discussed by Han et al. (1999, 2000, 2002): chains get delayed at the narrow channels connecting the traps. They escape through the channels with a probability which is proportional to the radius of gyration of the chain, and thus scales as $N^{3/5}$. However, we found that this effect accounts only in part for the total chain length dependence. In the second mechanism, the chains are trapped with a certain probability at the side and in the corner of the box. The characteristic time for escaping such a configuration is very long. The trapping probability increases with the diffusion constant, which is in turn inversely proportional to the chain length. As a result, the mobility increases with the chain length.

To our best knowledge, this mechanism has not yet been described in the literature. It becomes relevant when the period L of the structure is small. Indeed, Han et al. (1999) have studied structures with periods ranging from 4 to 40 μm , but they reported separation by length only for their smallest structure with $L = 4 \mu\text{m}$, in a system with dimensions comparable to those studied here.

We have observed a number of other phenomena, which we shall not describe in detail here. In contrast to Chen and Escobedo (2003), we have considered truly non-equilibrium systems. Subsequent escapes cannot necessarily be considered as independent events. The longest chains, $N = 1000$, do not recover the equilibrium coil structure in the middle of the trap, but they remain stretched in the x -direction. Moreover, our data seem to provide evidence that chains even retain some memory of the previous escape process. At high fields, successive escape times seem to be correlated. Unfortunately, the statistical quality of the data is not good enough to allow for a more thorough analysis.

The situation becomes even more complicated when the shallow channel is made wider. Whereas, in the cases presented here, long chains migrated faster than short chains, we have observed the inverse effect in

microfluidic system with wider channels (Duong et al., 2003). The effect as well as other, even more unexpected phenomena, can be reproduced in simulations. These phenomena will be presented in a forthcoming publication (Streek, 2002).

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References

- Bader, J.S., Hammond, R.W., Henck, S.A., Deem, M.W., McDermott, G.A., Bustillo, J.M., Simpson, J.W., Mulhern, G.T., Rothberg, J.M., 1999. DNA transport by a micromachined Brownian ratchet device. *PNAS* 96, 13165–13169.
- Chen, Z., Escobedo, F.A., 2003. Simulation of chain-length partitioning in a microfabricated channel via entropic trapping. *Mol. Sim.* 29, 417–425.
- The Condor team, 2003. software package from <http://www.cs.wisc.edu/condor/>.
- Deutsch, J.M., 1987. Dynamics of pulsed-field electrophoresis. *Phys. Rev. Lett.* 59, 1255–1258.
- Deutsch, J.M., 1988. Theoretical studies of DNA during gel electrophoresis. *Science* 240, 922–924.
- Doi, M., Edwards, S.H., 1986. *The theory of polymer dynamics*. Clarendon press, Oxford.
- Dünweg, B., Paul, W., 1991. Brownian dynamics simulation without Gaussian random numbers. *Int. J. Mod. Phys. C* 2, 817–827.
- Duong, T.T., Kim, G., Ros, R., Streek, M., Schmid, F., Brugger, J., Anselmetti, D., Ros, A., 2003. Size-dependent free solution DNA electrophoresis in structured microfluidic systems. *Microelectron. Eng.* 67–68, 905–912.
- Grossmann, P.D., Colburn, J.C., 1992. *Capillary Electrophoresis, Theory and Practice: Free Solution Capillary Electrophoresis*. Acad. Press, San Diego.
- Hammond, R.W., Bader, J.S., Henck, S.A., Deem, M.W., McDermott, G.A., Bustillo, J.M., Rothberg, J.M., 2000. Differential transport of DNA by a rectified Brownian motion device. *Electrophoresis* 21, 74–80.
- Han, J., Turner, S.W., Craighead, H.G., 1999. Entropic trapping and escape of long DNA molecules at submicron size constriction. *Phys. Rev. Lett.* 83, 1688–1691. Erratum 2001. *Phys. Rev. Lett.* 86, 1394.
- Han, J., Craighead, H.G., 2000. Separation of long DNA molecules in a microfabricated entropic trap array. *Science* 288, 1026–1029.
- Han, J., Craighead, H.G., 2002. Characterization and optimization of an entropic trap for DNA separation. *Anal. Chem.* 74, 394–401.
- Long, D., Viovy, J.-L., Ajdari, A., 1996. Simultaneous action of electric fields and non-electric forces on a polyelectrolyte: motion and deformation. *Phys. Rev. Lett.* 76, 3858–3861.
- Matsumoto, M., Doi, M., 1994. Brownian dynamics simulation of DNA gel electrophoresis. *Mol. Sim.* 12, 219–226.
- Noguchi, H., Takasu, M., 2001. Dynamics of DNA in entangled polymer solutions: an anisotropic friction model. *J. Chem. Phys.* 114, 7260–7266.
- Risken, H., 1989. *The Fokker-Planck equation: methods of solution and applications*. Springer Verlag, Berlin.
- Stellwagen, E., Stellwagen, N.C., 2002. The free solution mobility of DNA in Tris-acetate-EDTA buffers of different concentration, with and without added NaCl. *Electrophoresis* 23, 1935–1941.
- Stellwagen, E., Lu, Y., Stellwagen, N.C., 2003. Unified description of electrophoresis and diffusion for DNA and other polyions. *Biochemistry* 42, 11745–11750.
- Streek, M., 2002. Migration of DNA on structured surfaces in an external field. Diploma thesis. Universität Bielefeld.
- Tessier, F., Labrie, J., Slater, G.W., 2002. Electrophoretic separation of long polyelectrolytes in submolecular-size constrictions: a Monte Carlo study. *Macromolecules* 35, 4791–4800.
- Viovy, J.-L., 2000. Electrophoresis of DNA and other polyelectrolytes: physical mechanisms. *Rev. Mod. Phys.* 72, 813–872.