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2006 J. Phys.: Condens. Matter 18 S161

(http://iopscience.iop.org/0953-8984/18/14/S02)

INSTITUTE OF PHYSICS PUBLISHING JOURNAL OF PHYSICS: CONDENSED MATTER

J. Phys.: Condens. Matter **18** (2006) S161–S171 doi:10.1088/0953-8984/18/14/S02

Simulations of electrophoretic collisions of DNA knots with gel obstacles

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Received 2 December 2005
Published 24 March 2006
Online at stacks.iop.org/JPhysCM/18/S161

Abstract

Gel electrophoresis can be used to separate nicked circular DNA molecules of equal length but forming different knot types. At low electric fields, complex knots drift faster than simpler knots. However, at high electric field the opposite is the case and simpler knots migrate faster than more complex knots. Using Monte Carlo simulations we investigate the reasons of this reversal of relative order of electrophoretic mobility of DNA molecules forming different knot types. We observe that at high electric fields the simulated knotted molecules tend to hang over the gel fibres and require passing over a substantial energy barrier to slip over the impeding gel fibre. At low electric field the interactions of drifting molecules with the gel fibres are weak and there are no significant energy barriers that oppose the detachment of knotted molecules from transverse gel fibres.

(Some figures in this article are in colour only in the electronic version)

Introduction

The physical mechanism of gel electrophoresis of DNA molecules has been a subject of numerical simulations and analytical approaches [1, 10–12, 20, 21, 25, 32]. Most frequently the experimental and theoretical studies of DNA gel electrophoresis consider movements of linear or circular DNA molecules [24, 26, 30, 34]. Especially interesting and challenging would be, however, understanding of the underlying principles that govern separation of different knot types. Various classes of enzymes such as topoisomerases and site-specific recombinases produce different kinds of knots or catenanes by acting on circular DNA molecules [9, 28]. The

determination of the knot types formed is required for the elucidation of molecular mechanisms of action of these enzymes that are involved in the proper functioning of cellular DNA (see for example [13, 23]). Knots can also arise as a result of DNA packing in phage heads; analysis of these knots might reveal the arrangement of DNA in phage heads and in the tightly packed state, in general [4].

Biochemical studies of different DNA knots formed on DNA molecules of the same size revealed that during low voltage gel electrophoresis the knotted DNA molecules separated in such a way that more complex knots migrated more quickly than less complex knots [7–9, 29, 33]. This behaviour of knotted DNA molecules can be easily understood. Simple experience with a piece of a rope demonstrates that more complex knots tied on a circular rope with a given length compactify it more than simple knots. Therefore, DNA molecules with more complex knots are expected to have smaller overall dimensions than corresponding DNA molecules with simpler knots and should therefore experience a lower hydrodynamic drag during gel electrophoresis. However, more recently it was observed that at higher voltage the complex knots migrate more slowly than the simpler knots [27, 31]. This physical behaviour of DNA knots at high electric field is rather counterintuitive. In this paper we try to explain the physical reasons behind the field dependent reversal of the relative order of migration of DNA knots during gel electrophoresis.

Methods

DNA knots are modelled by closed self-avoiding walks (SAWs) composed of N segments of length a on a three-dimensional cubic lattice (the lattice constant a is comparable to the persistence length of the DNA molecules). The gel is a two-dimensional grid forming a sublattice with a mesh size b (=gel parameter) and perpendicular to the applied electric field (so that no knots can ever get impaled). The gel lattice is shifted by the quantity $(\frac{a}{2}, \frac{a}{2}, \frac{a}{2})$ compared to the knot lattice, so that no points of the knot lie on the gel. Knots are not allowed to cross the gel network. The coordinates of the N monomers in the configuration at time t are written as

$$\vec{r}(t) = (\vec{r}_1(t), \vec{r}_2(t), \ldots, \vec{r}_N(t)) \tag{1}$$

with constraints $\|\vec{r}_j(t) - \vec{r}_{j+1}(t)\| = a$.

The dynamics is followed using the BFACF algorithm [3, 6, 17]. Two kinds of moves are allowed: (a) the creation/destruction of a handle and (b) the flip of a corner into the mirror position (see figure 1). The first move clearly does not preserve the knot length, which can vary by ± 2 at every step, but introduces knot elasticity. The BFACF algorithm preserves knot classes, within which it is ergodic [15]. Self-avoidance is imposed by disallowing monomers from visiting any site which is already occupied by other monomers. Furthermore, knots are not allowed to cross gel rods, so that corner flips and handle creation/destruction are forbidden when a rod has to be crossed.

Under an external uniform electric field $\vec{\mathcal{E}}$, the electrostatic energy at time t is given by

$$E_q(t) = -\frac{q}{N(t)} \sum_{j=1}^{N} \vec{r}_j(t) \cdot \vec{\mathcal{E}}. \tag{2}$$

$N(t)$ is the length of the knot at time t, and it is associated with an elastic energy

$$E_{\mathrm{el}}(t) = \tfrac{1}{2} K [N(t) - N_0]^2 \tag{3}$$

where K is the spring constant. In the simulation a value $K/k_{\mathrm{B}}T = 0.1$ was used. The knot energy is then $E(t) = E_q(t) + E_{\mathrm{el}}(t)$. The rest length N_0 was set to 150. Moreover, in the rest

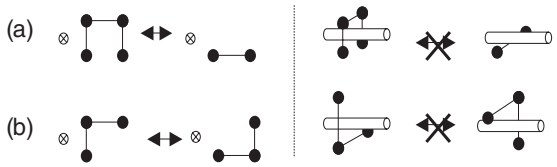

Figure 1. Monte Carlo moves in the BFACF: (a) creation/destruction of a handle and (b) flip of a handle. On the left side, the movement is not crossing a gel rod and is allowed; on the right side the movement is forbidden.

of the paper we will consider different values of the dimensionless constant $\mathcal{C} = q \cdot a \cdot \mathcal{E}/k_{\mathrm{B}}T$. At each timestep, we choose a point at random on the chain and propose alternatively one of the two moves. If it satisfies the self-avoiding and gel-avoiding constraints, it is accepted with a probability given by the Metropolis algorithm: if the energy of the new trial configuration, E_{trial}, is lower than that of the previous configuration, $E_{\mathrm{old}} = E(t)$, the move is accepted and $\bar{r}(t+1) = \bar{r}_{\mathrm{trial}}$; otherwise, the probability of acceptance of the trial configuration is equal to $\exp\{-[E_{\mathrm{trial}} - E(t)]\}/k_{\mathrm{B}}T$. If the move is rejected, then $\bar{r}(t+1) = \bar{r}(t)$.

The knots were drawn manually on the cubic lattice (without distinction of chirality, we mixed left-and right-handed configurations). Alexander polynomial [2] calculation was used to verify that the knot type does not change during the simulation. Starting from the trial knot configuration, we let the system freely relax to thermodynamic equilibrium in the absence of an external field ($\mathcal{E} = 0$) until correlations from the initial configuration have disappeared. Then the electric field is switched on, and we let the knots migrate on the lattice. The quantity we compute is the position of the centre of mass along a trajectory.

Time is measured in Monte Carlo iterations, length in lattice spacing. The initial length N_0 of our polymers was set to 150, and the mean length of the knot depends generally on the electric field and on the gel parameter. However, the average length is slightly shorter that N_0, since the probability of shortening the polymer is a slightly larger than the probability of lengthening it due to the self-avoiding condition. The gel parameter was set to $b = 20$ (in units of a), corresponding to a relatively sparse gel with big pores.

Results and discussion

Gel electrophoresis is a complex physical process and therefore our simple modelling system may not capture fine details of the ongoing process. However, when we looked at the behaviour of modelled knotted DNA molecules undergoing electrophoresis in the low and high electric field we noticed some fundamental differences that are likely to reflect real physical principles. At low electric fields the knotted molecules were only briefly retarded upon hitting the gel fibre and could easily detach without a need to pass over an energy barrier higher than 1–2 $k_{\mathrm{B}}T$ (see figure 2). Small plateaus observed in the energy profiles show that during these plateaus the molecules are stopped in their electrophoretic migration and therefore do not decrease their potential electrostatic energy. At high electric fields, however, the knotted molecules were retarded for a much longer time and their detachment required passing over a significant energy barrier of the order of 8–10 $k_{\mathrm{B}}T$ (see figure 3). Obviously, passing over such an energy barrier is a rare event as thermal fluctuations can hardly provide enough energy for it. Could the long retardation times of knotted DNA molecules at high electric fields be the physical reason for which more complex knots migrate more slowly than simpler knots under these conditions? To answer this question we turned our attention to the behaviour of modelled unknotted rings.

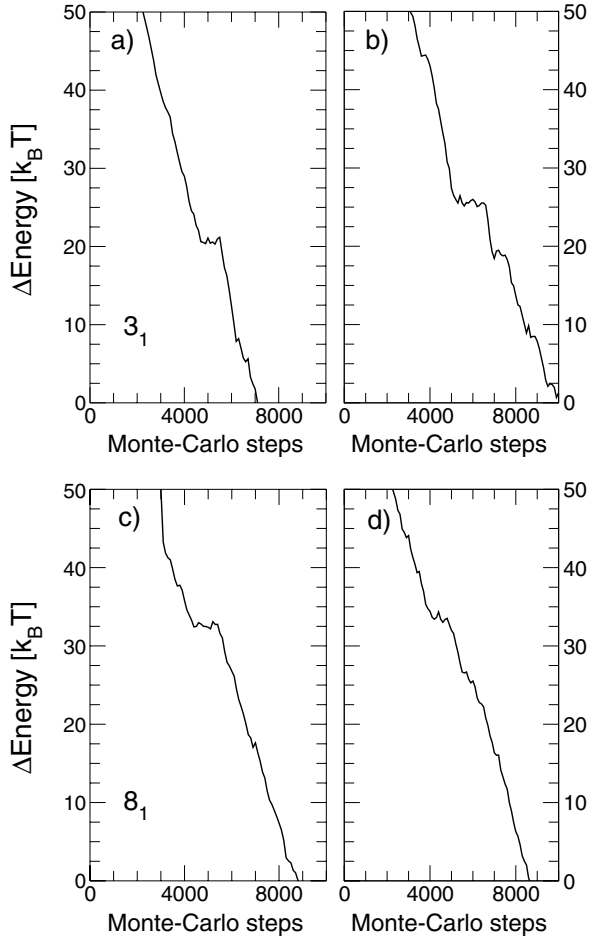

Figure 2. Electrophoretic mobility of simulated knotted molecules is monitored by measuring the potential electrostatic energy associated with the molecules as a function of the number of Monte Carlo steps. The observed plateaus indicate pauses in the migration caused by interactions with impeding gel fibres. (a) and (b) are two independent monitoring plots for a simulated 3_1 knot at low electric field ($\mathcal{C} = 0.1$), while (c) and (d) are two corresponding plots for the 8_1 knot.

Interestingly, unknotted rings at high electric fields did not show long plateaus and were detaching without a need to pass over a significant energy barrier (see figure 4). Therefore, it is to be expected that unknotted DNA molecules should show higher electrophoretic mobility than knotted molecules starting from some critical field strength. However, below this critical strength knots should migrate more quickly as they are more compact and therefore show smaller hydrodynamic friction and are also less likely to encounter gel fibres [35]. When a gel fibre is encountered by migrating molecules the detachment of knots and unknots is very similar in the low field regime (compare figures 2 with 4). Can we explain why at high field regime unknots detach easily from the impeding fibres while this is not the case for knots. Simple macroscopic experience with unknotted and knotted circular ropes hanging over a rod shows that unknotted rope can easily slip over the rod without any need to momentarily increase the gravitational potential energy of the rope (see figure 5). However, this is not the case for a knotted rope. When a sliding rope approaches the region of a knot it is not possible for it to

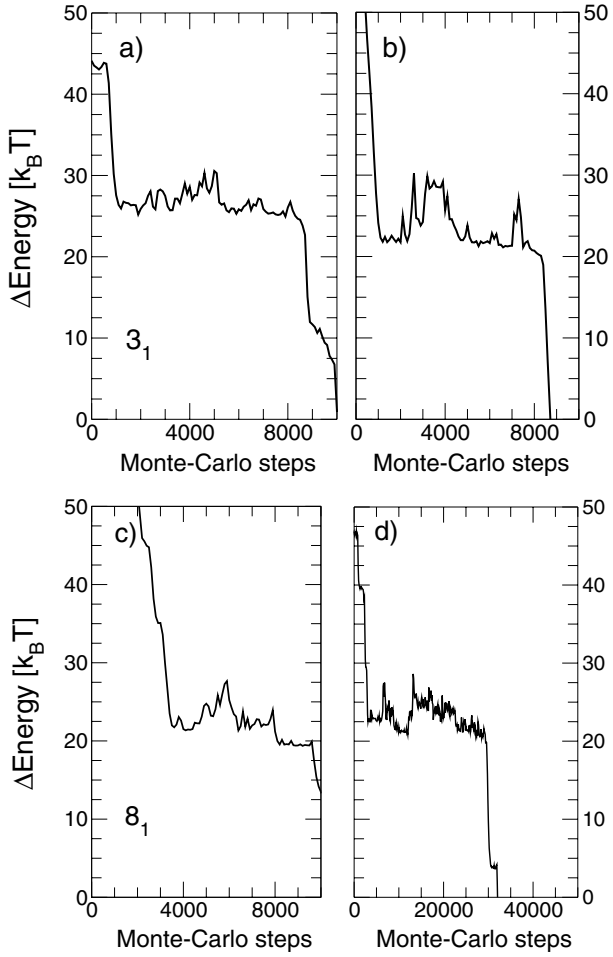

Figure 3. Potential electrophoretic energy registered during individual plateaus (pauses in the electrophoretic migration caused by interactions with impeding gel fibres) observed in simulations mimicking the effect of high electric field ($\mathcal{C} = 0.6$). (a) and (b) are two typical events observed for knot 3_1. (c) and (d) are plateaus observed for knot 8_1; notice that the horizontal scale in panel (d) encompasses 40 000 steps instead of 10 000 steps as in panel (c).

pass over nonreducible crossings without lifting the portion of the rope by at least its diameter above the sliding surface of the rod (see figure 5).

This of course leads to a momentary increase of the gravitational potential energy of the rope. To check whether the macroscopic experience corresponds to what happens during the simulations we have visualized the modelled knotted molecules at times corresponding to passing over the energy barrier (see figure 6). In fact, in all cases analysed by us the significant increase of the potential electrostatic energy of modelled knotted molecules corresponded to these stages of the simulations where a knotted portion was sliding over an impeding gel fibre. In energetic profiles of modelled knotted molecules undergoing electrophoresis observed by us we noticed that the energy barriers encountered by simple knots (3_1) are similar to these encountered by more complex knots (8_1). This can be explained by the fact that crossings in a knot can pass over a fibre one after the other and therefore a passage of a nonreducible

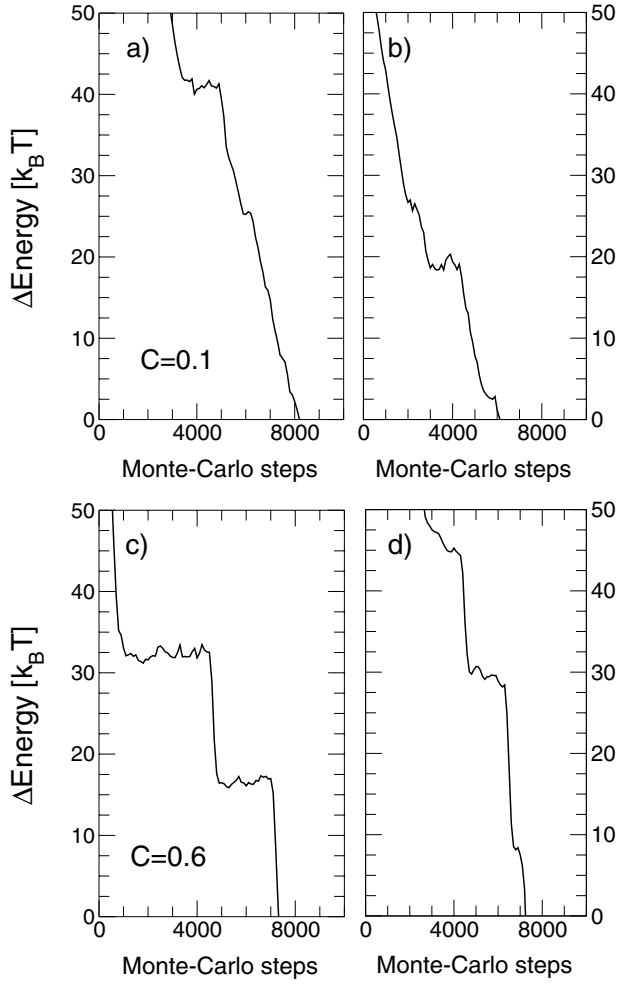

Figure 4. Potential electrophoretic energy registered during simulations of unknotted molecules moving in a gel at low ((a) and (b)) and high ((c) and (d)) electric field.

individual crossing in a complex knot and that in a simple knot could require the same activation energy.

Although in simulated high field gel electrophoresis similar energy barriers are required for the detachment of simple and complex knots from gel fibres at, the more complex knots on average stay attached to the gel fibre for a higher number of steps than simple knots (see figure 3). Also in real gel electrophoresis experiments performed at high field the complex knots migrate more slowly than simple knots [23, 31]. What could be the reason for this long trapping time of complex knots at gel fibres? To slip over the fibre an imbalance between hanging portions on two sides of the impeding fibre needs to be built up. As long as there is a balance (or a small imbalance) the resulting pulling force that drives sliding of the molecule over the impeding fibre in a real gel is zero or almost zero (see figure 5(a)). In simulations this corresponds to small net movements where the hanging loops grow and shrink back and forth in a nonproductive way. Only in unbalanced situations the productive pulling force develops in real gels and molecules start progressive motion around the impeding fibre (see figure 5(c)).

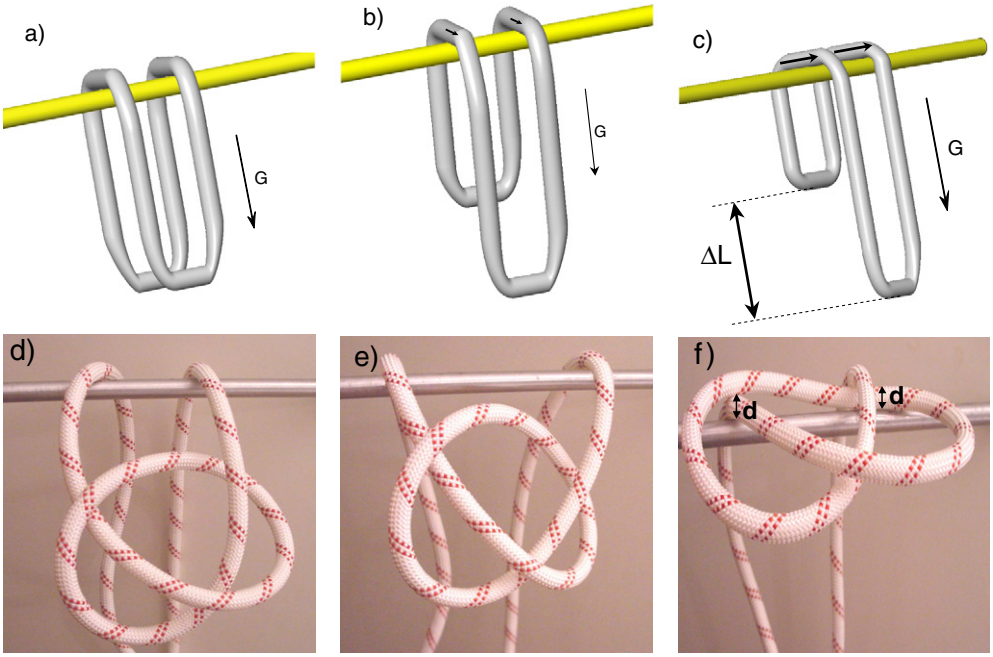

Figure 5. Sliding over the impeding rod of the unknotted ((a), (b), (c)) and knotted macroscopic loops ((d), (e), (f)). Notice that an unknotted loop can slide over a rod without crossing an energy barrier while this is not the case for knotted loops. Sliding of a knotted loop over the rod requires an activation energy that can pay for the lifting of the portions of the loop by at least one diameter d above the impeding rod as this momentarily increases the potential energy of the loop. Notice also that in the case of balanced hanging (panel (a)) there is no effective transverse pulling force that could cause sliding.

In the case of simulations this corresponds to modelled molecules that move by growing their longer hanging loops (handles are created there) and shrinking their shorter hanging loops (handles are removed there). It seems to us that simple knots are more likely to create sufficient imbalance to pull the knotted portion over the impeding fibre. It is known that simple knots can be easily confined to a small portion of the chain [14, 16, 18]. Therefore, there would be a good chance of arriving at the point where one would observe a long loop hanging on one side of the fibre, the knotted short portion of the chain being present on the other side of the fibre. Only at this point would activation energy be needed to move the knotted portion over the fibre. In more complex knots confining a knot is entropically not favourable and therefore nonreducible crossings of the knot are redistributed over the molecule. Therefore, when a complex knot hits the impeding fibre it is likely that some of its nonreducible crossings are on one side of the fibre and some on the other. As nonreducible crossings 'consume the length' of hanging loops it is more difficult to produce an unbalanced situation that would eventually help to pull knotted portions over the impeding fibres. This is also what we observe during Monte Carlo simulations. Figures 6(a)–(c) show snapshots of a trefoil knot interacting with impeding fibre. The interaction quickly develops into a situation where a long unknotted loop pulls the knotted portion over the fibre. In the case of an 8_1 knot we frequently observe that a balanced situation persists for a long time with knotted portions of similar length hanging on either side of the fibre (figures 6(d), (e)).

We have also investigated the distribution of the lengths of the plateaus observed in the migration of the knots at low fields and high fields. Interestingly, we find that the distribution

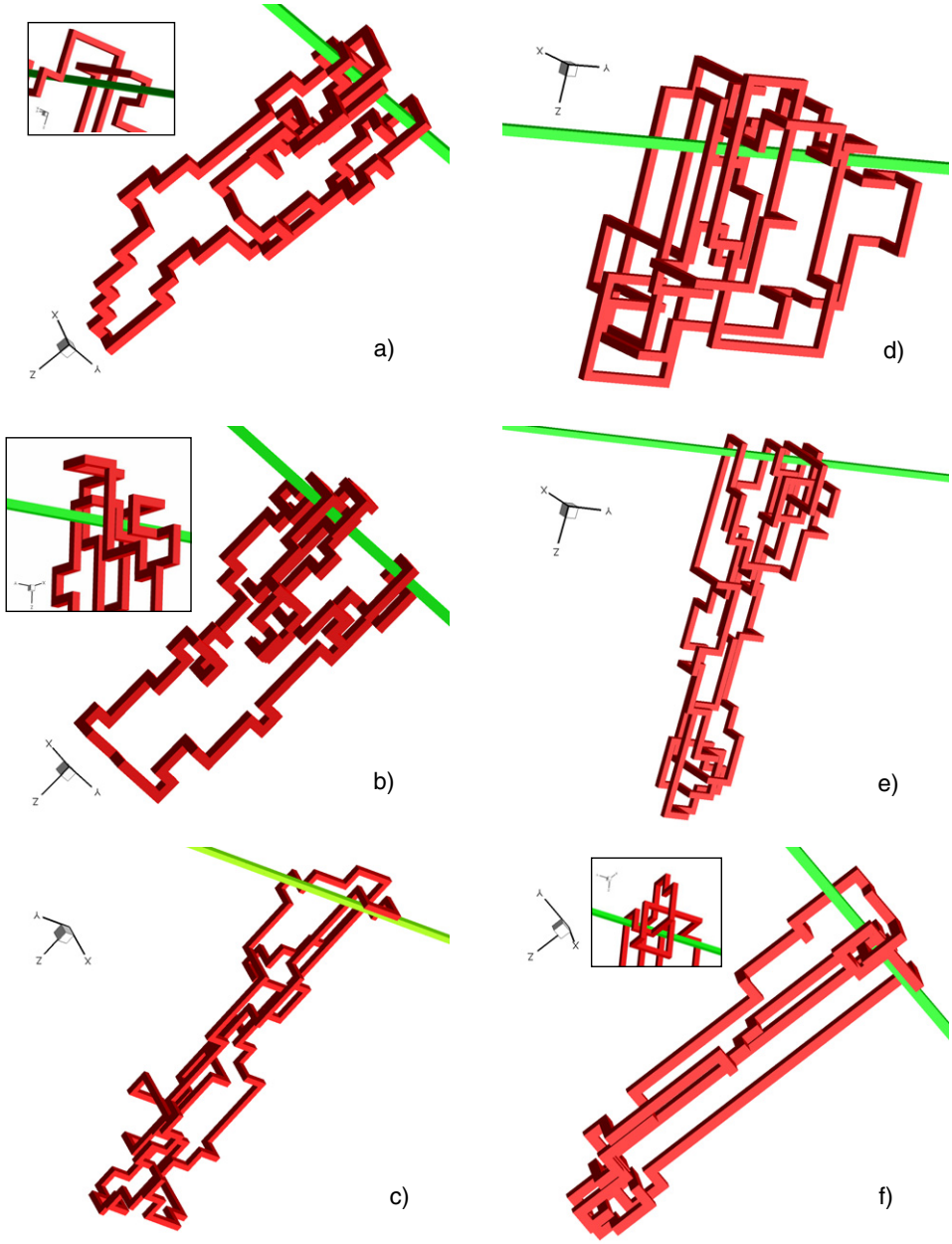

Figure 6. Snapshots of simulated configurations of knots 3_1 and 8_1 during their sequential stages of interaction with a gel fibre. Simulations correspond to the high electric field ($\mathcal{C} = 0.6$).

of the length of the plateaus is a power law:

$$P(\tau) = a \times \tau^{-1-b}, \tag{4}$$

where τ is the length of the plateau and $P(\tau)$ the probability of occurrence of such an event. At high electric field, where the knots spend most of the time trapped around gel rods, we can neglect the dynamics between two collisions and assume that the knots jump from one collision

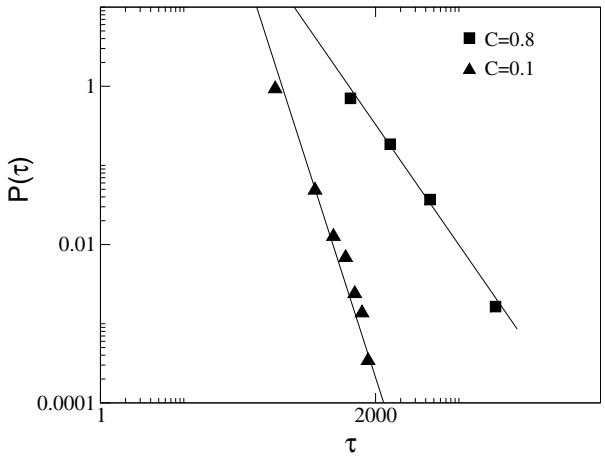

Figure 7. Distribution of the length of the plateau observed in the migration distance at low ($\mathcal{C} = 0.1$, filled triangles) and high electric field ($\mathcal{C} = 0.8$, filled squares) for the knot 8_1. The length of the plateau is denoted as τ and the distribution probability is $P(\tau)$. Lines are fits to the data: $P(\tau) = a \times \tau^{-1-b}$. For $\mathcal{C} = 0.1$, we find $b = 2.2$ and for $\mathcal{C} = 0.8$ we find $b = 0.33$.

directly to the next one. Therefore, the dynamics can be modelled in a first approximation as moving in a series of energy wells, separated by energy barriers. This model is called the 'Arrhenius model', and in our case we can understand the plateau in the migration distance as a trapping time, during which the knot is trapped temporarily around a gel rod. In this model, the distribution of trapping time $P(\tau)$ is a power law when the distribution of the depth of the energy traps is an exponential law [5, 19, 22]: $\rho(E) = 1/E_0 \mathrm{e}^{-E/E_0}$, where E is the energy depth of the trap. In this type of model, the system is jumping from trap to trap, and crossing at each step an energy barrier that is equal to the difference in energy depth of the traps. The system will stay for a trapping time τ_i in the trap of energy depth E_i, and then jump to the next trap. Given a simple activated dynamics for leaving a trap of depth E, i.e. a trap of lifetime $\tau = \tau_0 \exp(-\beta E)$, the distribution of trapping times may be computed, and it is found that

$$P(\tau) = a \times \tau^{-1-T/T_0}. \tag{5}$$

Since the energy barriers in our model are determined by the electric field with a proportionality constant ($\mathcal{C} = q \cdot a \cdot \mathcal{E}/k_\mathrm{B}T$) we can rewrite (5) as

$$P(\tau) = a \times \tau^{-1-\mathcal{C}_0/\mathcal{C}}. \tag{6}$$

Therefore, by tuning $\mathcal{C}$ we can cross over from a behaviour where an average trapping time is well defined ($\mathcal{C} < \mathcal{C}_0/2$), to a regime where trapping times are characterized by Lévy statistics ($\mathcal{C} > \mathcal{C}_0/2$), where singular trapping events (singular plateaus) dominate the statistics. Therefore the crossover from high (large $\mathcal{C}_0$) to low (small $\mathcal{C}_0$) electric fields is a transition from Poisson-like to Lévy-like distributed trapping times. Measuring explicitly the distribution of trapping times (see figure 7), we find that for the knot 8_1 we have $\mathcal{C}_0 \approx 0.26$ at high field, where this approximation is expected to be reliable (we get nevertheless good agreement with data at low field: $\mathcal{C}_0 \approx 0.22$). We note, however, that we used the number of Monte Carlo steps instead of the unit of time for measuring τ_i. However, in a first approximation, linearly renormalizing the time would only change the probability distribution $P(\tau)$ by a linear coefficient and therefore would not change the b exponent. Finally, the critical field $\mathcal{C}_0$, associated with the transition of the simple Arrhenius model, is interestingly of the same order as the transition

from the low field regime to the high field regime obtained in a previous study, where a critical field of about $\mathcal{C}_0 = 0.24$ was found. Therefore, this simple Arrhenius model allows us to give a very crude approximation of the transition from the low field to the high field regime. At low electric field (or high temperature), the trapping of the knot is not efficient, and the knot is only scattered by the gel, but at high field (or low temperature) there is a crossover to a regime where the traps are very efficient and the knot is trapped for a macroscopic time around a few gel rods.

Finally, since our DNA knot is supported by a cubic lattice, it could be argued that the excluded volume of our DNA knot, in our calculation, is too large when compared to experiments. Therefore, we have checked that upon finer discretization of the segments there was no difference in simulation results and the critical electric field obtained, $\mathcal{E}_0$ ($\mathcal{C}_0 = q \cdot a \cdot \mathcal{E}_0 / k_\mathrm{B} T$), was kept the same.

Conclusion

Using a simple simulation approach to model gel electrophoresis of DNA knots we have observed that at high electric field the knotted molecules require passing over a significant energy barrier in order to detach from the impeding gel fibre while this is not the case for unknotted DNA molecules. At low field, we observed that interactions play a minor role and the dynamics is dominated by the free movement of the DNA knot. At high electric field, we observed that the height of the energy barrier for detaching from the gel rod, and how quickly the knots slip around it due to the pulling force, dominate the dynamics. These observations contribute to an explanation of why at high electric field simple knots migrate more quickly than more complex knots while the opposite is the case in low electric field gel separations.

Acknowledgments

We thank J Dubochet, J Roca and J Schwartzman, for help and fruitful discussions. This work was partially supported by the Swiss National Science Foundation (Grant No. 3152-068151).

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