Simple model of trapping electrophoresis with complicated transient dynamics

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A simple reptation model of DNA trapping gel electrophoresis is shown to lead to surprisingly complicated transient dynamics. The DNA has a big neutral object attached to one of its ends; electrophoretic migration is thus slowed by trapping when it occurs in random gels. The detrapping process is thermally driven and its dependence upon the instantaneous molecular conformation gives rise to anomalous transport properties in our computer simulations. The electric field affects the molecular conformations and thus modifies the nature of the transient dynamics in nontrivial ways. The phenomenon is analyzed in terms of a directed walk through a periodic lattice of traps with a broad release time distribution. For large molecular sizes, we estimate that it is actually impossible to reach the steady state in an (experimentally) reasonable period of time.

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I. INTRODUCTION

Diffusion and migration in random environments often lead to subtle effects. For instance [1], the average displacement $\langle x(t)\rangle$ may scale with time t like t^{α} with $\alpha \leq 1$, while the dispersion $\langle \Delta x^2(t)\rangle \equiv \langle x^2(t)\rangle - \langle x(t)\rangle^2$ may scale like t^{β} with $\beta \geq 1$. Normal behavior requires that $\alpha=\beta=1$. We present a simple model of a polymer system where anomalous effects (α and/or $\beta \neq 1$) are predominant. The random environment, however, is provided by the molecular conformations accessible to the polymer and not by the medium. Since there is a large but finite number of such conformations, the resulting anomalous effects are long lasting, but transient. Also, because the applied electric field E directly affects the conformations of the polyelectrolyte, a series of anomalous transient regimes can be observed for large molecular sizes. These regimes can be related to the distribution function for the time between two traps, if we compare the process to a directed walk through a periodic lattice of traps with a broad release time distribution [1]. Finally, we discuss the relevance of these effects for the corresponding experimental system.

II. MODEL

Ulanovsky, Drouin, and Gilbert (UDG) [2] recently proposed to attach a large neutral protein (streptavidin) to one end of the DNA molecules to be electrophoresed in polyacrylamide gels in order to slow down the larger DNA molecules and increase the separation between them. Défontaines and Viovy (DV) developed a reptation model for this process, called trapping electrophoresis (TE); their results agree qualitatively with those of UDG and thus provide a good description of the underlying physics [3]. Slater and Villeneuve [4] generalized the biased reptation model (BRM) of DNA gel electrophoresis [5,6] to carry out computer simulation studies of TE. Briefly, this reptation algorithm is as follows (see Ref. [4] for more details).

The streptavidin-DNA complex (S-DNA) moves by reptating between the gel obstacles. The electric forces bias both the motion inside the reptation tube as well as the mean orientation of the tube itself. Each curvilinear displacement of length $\pm a$ (a is the mean pole size) is of duration [6]

$$\tau(h_x)=\tau_B \frac{\tanh\delta(h_x)}{\delta(h_x)} , \tag{1}$$

where h_x is the end-to-end distance of the DNA molecule in the field direction (x), $\tau_B=a^2/2D_c$ is the Brownian time for the unbiased (field $E=0$) case, D_c is the curvilinear diffusion coefficient, $\delta=\varepsilon(h_x/a)$ is the bias factor, $\varepsilon=qEa/2k_BT$ is the scaled electric field intensity, and q is the net charge of a primitive reptation segment of length a. These jumps occur with probabilities [6]

$$p_{\pm}=\frac{1}{1+e^{\mp 2\delta(h_x)}} , \tag{2}$$

where the $\pm$ refers to the (arbitrarily chosen) direction of the motion inside the tube. A new tube section of length a is created after each "jump" of duration τ. If a tube section is created by the charged end of the chain, its orientation is biased by the field and follows a Boltzmann distribution function $\exp[\varepsilon\cos(\theta)]$, where θ is the angle between this new tube section and the field axis (x) [5]. Tube sections created by the S end are randomly oriented since the streptavidin is neutral.

The BRM has to be modified to take into account the steric trapping that occurs when the streptavidin cannot get through a small opening. A fraction $f \ll 1$ of the pore-to-pore passages created during the migration is thus marked "too narrow" and any move that would normally make the streptavidin move through such a passage is rejected (however, the time τ is added to the current time). When the streptavidin is pinned by a narrow passage, trapping occurs and detrapping requires the molecule to move backward over a curvilinear distance Na, where N is the number of DNA segments forming

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the molecule. This detrapping process is the one suggested by UDG and DV, and is the only one allowed by the reptation model [4]. Since the bias factor $\delta=\varepsilon h_x/a$ depends on both the field ε and the end-to-end distance h_x, detrapping becomes very unlikely for high field intensities and long, oriented molecules. This unique scenario, coupled to the fluctuations of $h_x(t)$ and to the fact that the field tends to align the tube (which increases h_x) [5,6], leads to a complicated series of anomalous transient regimes.

The simulations were carried out on SUN 10-41, SUN-LX, and IBM RISC/6000-320 workstations using a Fortran code. Here we report on the results for a large size S-DNA ($N=30$) in a weak field ($\varepsilon=0.5$) with the fraction of "narrow passages" being $f=0.001$ (we chose $f \ll 1/N$ in order to have well-separated traps). The mean position $\langle x(t)\rangle$, the mean dispersion $\langle \Delta x^2(t)\rangle$, the shape of the band (formed by the molecules of the ensemble), and the statistics of the time spent between two consecutive traps were studied. Results are given in units of a (for lengths) and τ_B (for time).

III. RESULTS

Figure 1 shows the evolution of the "velocity" ratio $\langle x(t)\rangle/t$ as a function of time. This ratio goes through three different regimes. For short times, we observe a plateau which defines a velocity V_0 (the plateau is not quite flat because the time needed to get a full V_0 plateau is of the order of the time needed to reach the first traps in this case) identical to that obtained in the absence of traps (i.e., for $f=0$; results not shown). In this regime, the molecules have not yet encountered a trap and thus move freely. For very long times ($t > 10^9$), we also observe a plateau which defines the steady-state velocity. In this limit, the molecules have been trapped many times and their net velocity is given by $V_{SS}=d/\langle\tau\rangle \approx 10^{-3.0}$, where d is the mean distance between traps and $\langle\tau\rangle$ is the average time between two traps. Finally, for intermediate times, the ratio $\langle x(t)\rangle/t$ decreases steadily, indicating that trapping reduces the net velocity. This latter regime, which we call the anomalous regime, is extended over many decades in time.

Figure 2 shows the evolution of the "diffusion" ratio $\langle \Delta x^2(t)\rangle/2t$ as a function of time. For short times (i.e., $t \le 10^2$), this ratio increases steadily (because we started with an initial delta-peak distribution) to reach a somewhat constant value; the latter defines the diffusion coefficient D_0, which is characteristic of diffusion without trapping (again, the plateau is not quite flat because the time needed to get a full D_0 plateau is of the order of the time needed to reach the first traps). The diffusion ratio also goes through a long transient regime where the ratio $\langle \Delta x^2(t)\rangle/2t$ increases substantially, pointing out that trapping enhances dispersion. Finally, after a sufficiently long time ($t \ge 10^{9.6}$), the steady state is reached and the ratio $\langle \Delta x^2(t)\rangle/2t$ reaches a plateau which defines the steady-state diffusion constant $D_{SS} \simeq 10^{1.68}$.

Figure 3 shows the evolution of the ratio $\langle x(t)\rangle^2/\langle \Delta x^2(t)\rangle$ (this is the number of theoretical plates defined in chromatography) as a function of time. In normal conditions (i.e., $\langle x(t)\rangle \propto t$ and $\langle \Delta x^2(t)\rangle \propto t$) we should observe a straight line of slope 1. This linear behavior is obtained for small times ($t < 10^3$), a region where the trapping is just starting and has not yet modified the dynamics. For larger times ($10^4 < t < 10^5$) the slope of the curve is negative, which means that the dispersion overwhelms the drift. For intermediate times ($t \simeq 10^{5-7}$), we observe a plateaulike behavior (the ratio varies by less than 26% while t increases by a factor of 100), which indicates that the electrophoretic band is

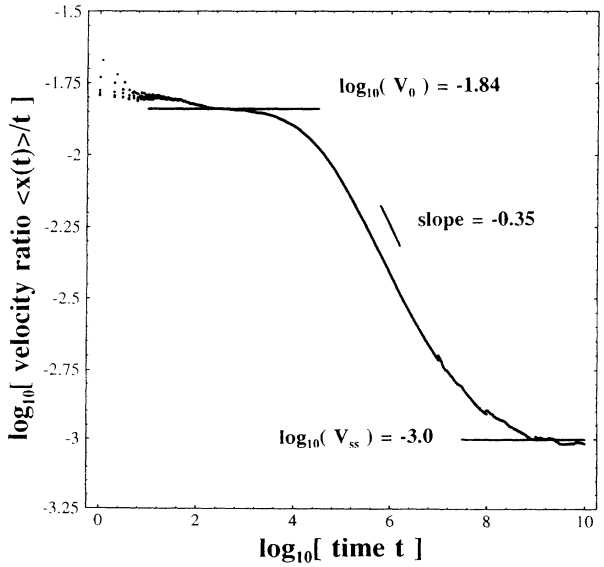

FIG. 1. Velocity ratio $\langle x(t)\rangle/t$ vs time t for $\varepsilon=0.05$ and $f=0.001$, as obtained by computer simulations. Note that the ensemble size goes from 12 000 for small times to 30 for larger times (hence the small discontinuities). The time is in units of τ_B and the velocity ratio is in units of a/τ_1.

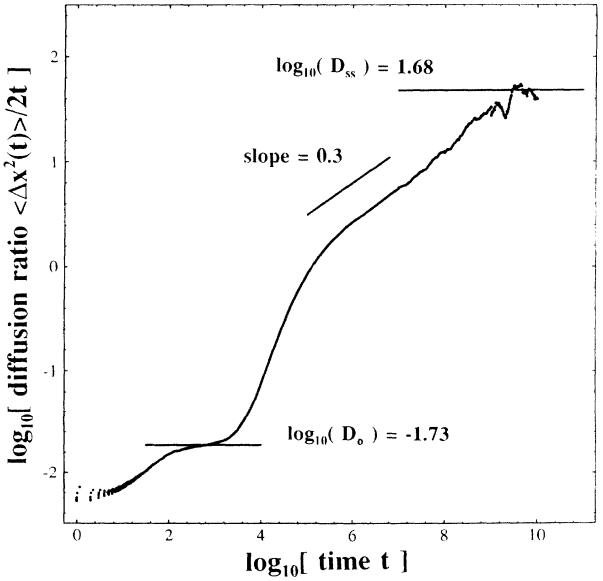

FIG. 2. Diffusion ratio $\langle \Delta x^2(t)\rangle/2t$ vs time t where the diffusion ratio is in units of a^2/τ_B. See Fig. 1 for details. The steady-state diffusion constant $D_{SS}=10^{1.68}$ shown here was confirmed between $t=10^{10}$ and 10^{11} using an ensemble of particles that decreased continuously from 40 to 4 (not shown here).

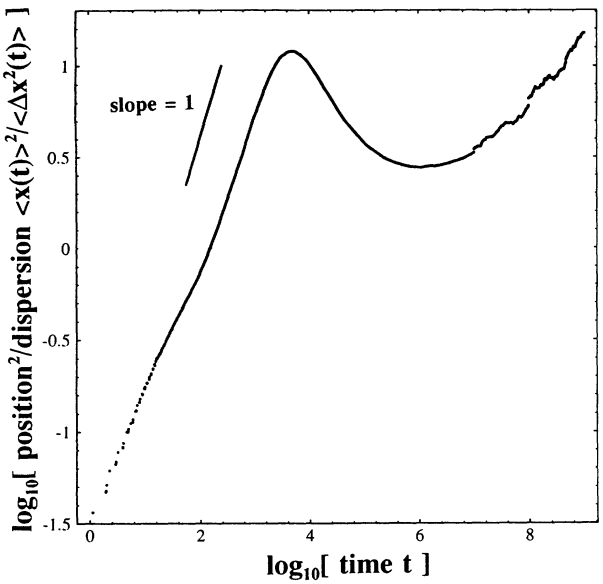

FIG. 3. Ratio $\langle x(t)\rangle^2/\langle \Delta x^2(t)\rangle$ vs time t. See Fig. 1 for details.

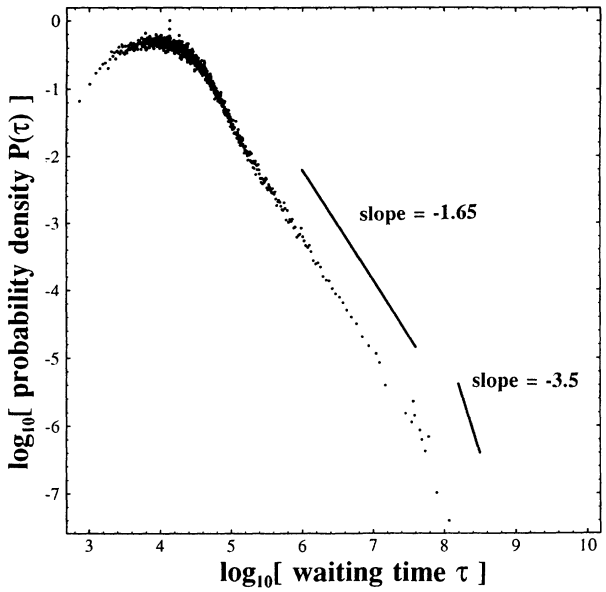

FIG. 5. Probability density $P(\tau)$ vs waiting time τ (total of 50 000 trapping events). The waiting time is in units of τ_B and the probability density is in arbitrary units.

then moving forward *and* broadening at the same rate. We also remark that $\langle x(t)\rangle^2/\langle \Delta x^2(t)\rangle \simeq 10^{0.5}$ in this region, showing the trapping and not electrophoretic drift dominates for $t \leq 10^7$. The normal behavior is recovered only for very long times $t > t_{SS} > 10^{9.6}$ (i.e., the time where the steady state is reached for both velocity and diffusion) and for $t < 10^3$ (which thus defines the time needed to reach the first trap).

Figure 4 shows the shape of the band $p(x,t)$ (i.e., the probability distribution function for the position) at time $t = 10^7$ (towards the end of the plateau observed in Fig. 3). We can see that, even after a very long time, a surprisingly high number of molecules are still near the origin. This indicates that the number of very deep traps (i.e., traps with very long detrapping times) is non-negligible, which explains why it takes such a long time ($t_{SS} > 10^{9.6}$) to reach the steady state.

Figure 5 shows the probability distribution function $P(\tau)$ for the time τ between two consecutive trapping events. The probability distribution is a maximum for $\tau \simeq 10^4$, and we find that $\langle \tau \rangle \simeq 10^{5.5}$. The tail of that broad distribution appears to follow power laws $P(\tau) \propto \tau^{1.65}$ for $10^{4.5} < \tau < 10^{7.5}$ and $P(\tau) \propto \tau^{-3.5}$ for $\tau > 10^{7.5}$. Therefore, the steady state is reached only after approximately $t_{SS}/\langle \tau \rangle \approx 10^4$ traps have been encountered.

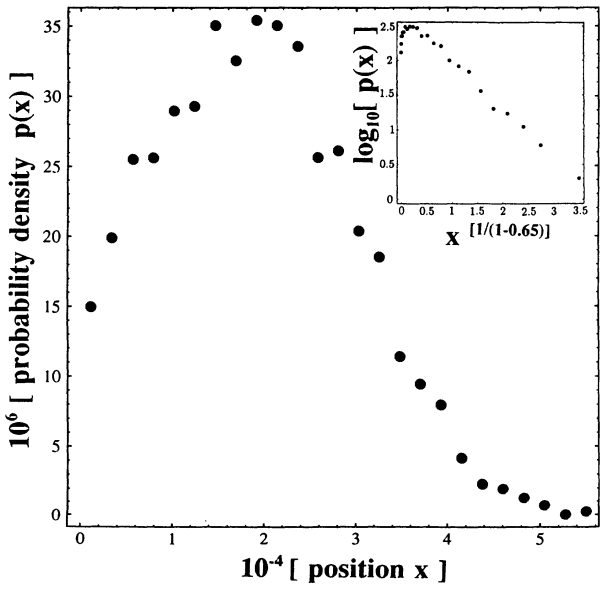

FIG. 4. Probability density $p(x, t = 10^7)$ vs position x (total of 3000 molecules). The probability $p(x,t)$ is in arbitrary units. The inset shows $\log_{10}[p(x, t = 10^7)]$ vs $x^{(1/(1-0.65))}$, where both expressions are in arbitrary units. See Fig. 1 for other details.

IV. DISCUSSION

In our simulations, the gel is a random array of traps so that the distance between two traps is not constant. The field interacts with the molecules in such a way that the jumps from one trap to the next are, most of the time, made in the positive direction. If we neglect the fact that those jumps are not always made in the positive direction and are not of constant length, our model may be regarded as a directed walk [1] on a lattice of spacing d where the probability of having a waiting time between τ and $\tau + d\tau$ on one side is given by $P(\tau)d\tau$.

Such systems are known to exhibit anomalous transport behavior for slowly decaying functions $P(\tau)$. This anomalous regime can be understood semiquantitatively as follows. After M trapping events, a molecule (whose position is simply Md) has selected waiting times τ from $\tau = 0$ to the maximum waiting time encountered until then $\tau = \tau_{\max}(t)$. The probability of having a maximum waiting time $\tau_{\max}(t)$ is thus approximately equal to $1/M$, so that

$$\int_{\tau_{\max}(t)}^{\infty} P(\tau)d\tau \simeq \frac{1}{M} = \frac{d}{x(t)} . \tag{3}$$

We are interested in traps that strongly affect the dynamics of the system, i.e., those for which one may have $\tau \simeq \tau_{\max}(t) \approx t$. If the tail of the distribution $P(\tau)$ decreases slowly, $x(t)$ will be directly related to the shape of that tail. As an example, if the probability density $P(\tau)$ decays as a power law, the position will increase as a power law since

$$x(t) \propto \left[\int_{t=\tau_{\max}(t)}^{\infty} \frac{d\tau}{\tau^{1+\mu}} \right]^{-1} \propto t^{\mu} , \tag{4}$$

which obviously applies only for $0<\mu<1$. Bouchaud and Georges [1] give expressions for the time dependence of the position $\langle x(t) \rangle$ and the dispersion $\langle \Delta x^2(t) \rangle$ for different values of μ:

$$\langle x(t) \rangle \propto \begin{cases} t^{\mu}, \ \langle \Delta x^2(t) \rangle \propto t^{2\mu} \ \text{ for } 0<\mu<1 & (5) \\ t, \ \langle \Delta x^2(t) \rangle \propto t^{2/\mu}, \ \text{ for } 1<\mu<2 & (6) \\ t, \ \langle \Delta x^2(t) \rangle \propto t \ \text{ for } \mu>2 \ . & (7) \end{cases}$$

As observed in Fig. 5, $P(\tau)$ decays approximately as $\tau^{-(1+\mu)}$ with $0<\mu<1$ for $10^4<\tau<10^{7.5}$. The straight lines plotted in Figs. 1, 2, and 5 correspond to $\langle x(t) \rangle \propto t^{0.65}$, $\langle \Delta x^2(t) \rangle \propto t^{2\times 0.65}$, and $P(\tau) \propto \tau^{-(1+0.65)}$, respectively, consistent with Eq. (5). Figure 5 also indicates that $P(\tau) \propto \tau^{-(1+\mu)}$ with $\mu>2$ for $\tau>10^{7.5}$; this is why normal conditions are recovered and a steady state is reached for $t>t_{SS}$ [see Eq. (7)].

Bouchaud and Georges also give expressions for the shape of the band $p(x,t)$. They found that the width of the band increases like t^{μ} and that the probability $p(0,t)$ of having a molecule at the origin decreases like $t^{-\mu}$. Our results, using the shape of the band for $t=10^5$, 10^6, and 10^7, show that $p(0,t)$ decreases approximately like $t^{-0.8}$ and that the width of the band increases approximately like $t^{0.7}$, which is consistent with the fact that $\mu=0.65$. Another consequence of the approximate power law decay of $P(\tau)$ is that $p(x,t)$ decreases for large x as a stretched exponential $\exp[-(x/x_0)^{1/(1-\mu)}]$, where x_0 is a function of time. The inset of Fig. 4 shows $\log_{10}[p(x,t)]$ as a function of $x^{1/(1-\mu)}=x^{2.86}$ for $t=10^7$ (the end of the transient regime) and $\mu=0.65$. We do indeed obtain a straight line.

V. CONCLUSION

For typical TE experiments, we estimate that $\tau_B(N=30) \approx 3$ msec and $a \approx 10^{-6}$ cm (for polyacrylamide gels). Therefore, the regime for which we observe a power law for both position and dispersion corresponds to experimental times of about 5 min $<t<8$ h. During that period, the mean position covers a distance of ~ 0.1 mm and the width of the band increases by ~ 0.1 mm. We also note that it is impossible to reach the steady-state regime within a reasonable period of time ($t_{SS} \simeq 10^{9.6} \simeq 138$ days).

The shape of the band appears to be the limiting factor of TE. In fact, the stretching of the diffusion front is so large and the velocity so small that it is clearly impossible to improve DNA sequencing by TE when N is too large. Another dominant factor is that the anomalous regime lasts much longer for diffusion than for the velocity. The shape of the band becomes Gaussian only for times $t \gg t_{SS}$.

This large broadening of the diffusion front is also observed for larger values of N and the tail of the distribution $P(\tau)$ decreases slower and slower as N increases. The failure of TE [2] might thus be due to the fact that, for large N, the waiting time distribution is too broad. In order to narrow $P(\tau)$ and reduce the anomalous behavior for large N, we must accelerate the detrapping process, e.g., by using pulsed fields (inverting the field during a short period forces the molecules to detrap), as suggested by UDG.

We also studied the transient effects for other values of molecular size N and field intensity ε. These effects become less important for smaller molecules and/or lower field intensities. For example, in the case where $\varepsilon=0.05$, the velocity ratio $\langle x(t) \rangle /t$ does not show any anomalous regime for $N<22$, while the dispersion ratio $\langle \Delta x^2(t) \rangle /2t$ shows the anomalous regime only for $N>15$. This can be explained by the fact that the probability distribution function $P(\tau)$ then decreases roughly as $\tau^{-(1+\mu)}$, with $\mu>2$ for $N<15$ and $1<\mu<2$ for $16<N<22$ [see Eqs. (5)–(7)].

In conclusion, our study shows that the TE steady-state regime may be preceded by a long-lasting anomalous regime where diffusion dominates the drift. This anomalous regime may be understood in terms of a directed walk through a periodic lattice of traps with a broad distribution of release time. The anomalous migration of the molecules in the gel may still have large effects when pulsed fields are used since the migration may be anomalous during each pulse [7].

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