Electrophoretic Mobility of Asymmetric Reptating Polymers

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The electrophoretic mobility of an asymmetric reptating molecule—ball and chain—may depend on chain length quite unlike symmetric molecules. Analytic and numerical evidence indicates that resolution in the model introduced in this paper remains good for long chains instead of deteriorating rapidly as in conventional electrophoresis. Given the biotechnological importance of gel electrophoresis for sequencing and separating DNA, this model's complementary dynamics may be useful experimentally.

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Gel electrophoresis has become a very important tool in molecular biology. In conventional electrophoresis an electric field pulls charged polymers, like DNA, through a disordered medium, such as agarose or polyacrylamide gel. Longer chains become more entwined with the gel and thus drift more slowly. Simple theoretical arguments show that for small fields, the electrophoretic velocity is inversely related to the length of the chain [1,2]. Appealing intuitive analogs are smaller children sneaking more quickly through a jungle gym or long hairs getting stuck in a brush. Spatial separation of chains of different lengths is then just a matter of time. However, the variation of the mean velocity with length N vanishes for long chains; therefore fluctuations around the mean velocity ultimately limit resolution. Various improvements to the basic technique have been invented to extend the resolvable length scale, such as field-inversion gel electrophoresis [3] and orthogonal field-alternating gel electrophoresis [4]; however, all of these techniques are limited by the fact that, since the velocity goes as $1/N$, resolution becomes poor for long chains. Here we propose a new reptative approach designed for resolving longer chains.

We will show that attaching a large group to one end of the chain profoundly changes the form of the dynamics through the medium, in particular, eliminating the inherent limitations imposed by a $1/N$ velocity dependence. For our theoretical arguments, the Duke-Viovy model [5] (DVM) of reptation [6] dynamics modified by one new boundary condition is used. The dynamics of the asymmetric DVM is pictorially described in Fig. 1: The chain is modeled as a string of charged beads (reptons) and the gel as a lattice of cells in which these beads reside. (Lattices used for electrophoresis have, in fact, been fabricated using microlithography [7].) Continuity of the chain requires that neighboring beads along the chain are either in the same cell (slack) or in a neighboring cell (taut). There are d neighboring cells in the direction of the field and d counter to the field. In experiments the dimensionality is three except for artificial gels [7] where $d = 2$; for the purposes of theory, d is a parameter. We will see that the dimensionality d plays an important role only for the dynamics of the chain's end segments, while the gel provides a tubelike topological constraint [6,8] which, with the chain's continuity constraint, prohibits motion other than the diffusive motion of slack segments, i.e., those beads which share a cell with at least one other bead. The slack segments move in the tube in much the same way that a carpet ripple may be pushed along the floor.

A minimal description of the system is in terms of variables $y_j \in \{-1, 0, 1\}$, which represent the projection along the field of the segment j joining two neighboring beads. One is quickly convinced that in these variables the dynamics allow only the diffusion of "slack segments" ($y_j = 0$, also known as "extrons" in the literature) within the tube. This is the mathematical manifestation of de Gennes' original picture of chains reptating in a tube. The polymer's ends may extend in $2d$ directions thereby eliminating a slack segment and lengthening the tube, or they may contract deeper into the tube in one direction introducing a new slack segment.

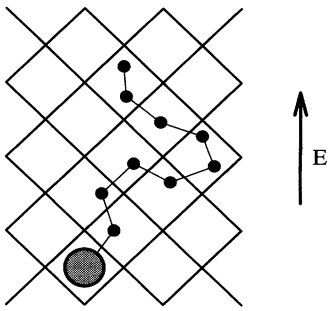

FIG. 1. The asymmetric Duke-Viovy model represents a chain as charged beads (called "reptons," denoted by dark circles) which occupy a lattice of cells which represent a gel. With a bias [Eq. (1)] arising from the field, a slack piece of the chain—a bead which shares a cell—may be displaced along the chain. The bulky end group (or "ball" represented by a large circle) is drawn forward elastically by the chain, but to advance it must overcome an energetic barrier [Eq. (6)]. The configuration can be represented in terms of the segments between reptons; labeled from the tail, this configuration is $\{y_j\} = \{1, 1, 1, -1, 1, 0, 1, 1, 0\}$.

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When a field is applied to uniformly charged molecules, these diffusive dynamics are biased for charge transport. In the DVM, the rate of moving a slack segment to a position j segments away (with no intervening extrons) is [5]

$$W(j) = \frac{\varepsilon S_j}{j[1 - \exp\{-\varepsilon S_j\}]} W_0, \tag{1}$$

where $\varepsilon = aqE/k_BT$ is the dimensionless measure of the applied field E on the repton charge q, where W_0 is the rate for a nearest-neighbor jump in zero field, and where

$$S_j = \sum_{k=i+1}^{i+j} y_k \tag{2}$$

is the distance the slack segment at i moves in the field in units of the lattice spacing a.

How can a bulky end group change the dynamics? If there is additional friction at the bulky group, with only the tension of the chain to pull it through the gel, then one might naively imagine that the rest of the chain has an opportunity to relax to the thermodynamic equilibrium distribution of a chain with one fixed end [9]. Let us use the equilibrium distribution to get some feeling for the asymmetric model, and later we will show where and how this simplification breaks down. With one end held fixed, the equilibrium alignment of the jth segment from the fixed end can be calculated [10]:

$$\langle y_j \rangle = m_j^{\text{eq}} = \frac{2d \sinh[\varepsilon(N - j)]}{2d \cosh[\varepsilon(N - j)] + 1}, \tag{3}$$

$$\langle |y_j| \rangle = Q_j^{\text{eq}} = \frac{2d \cosh[\varepsilon(N - j)]}{2d \cosh[\varepsilon(N - j)] + 1}. \tag{4}$$

The strong alignment of the segments and the elimination of slack segments indicate that the field produces a large force, transmitted elastically, on the fixed segment.

Tension can, of course, not be transmitted through a slack segment. Since the rate [Eq. (1)] becomes independent of length for $\varepsilon S \gg 1$, the distance to the first slack segment $\mathcal{M}$ is an important quantity. Using the equilibrium distribution [Eq. (4)], $\mathcal{M}$ can be estimated by making the sum an integral:

$$1 \simeq \int_{j=1}^{\langle \mathcal{M} \rangle} dj \, (1 - \langle |y_j| \rangle)$$

$$\simeq \frac{\arctan[\sqrt{(2d - 1)/(2d + 1)} \tanh(x)]_{x=\varepsilon(N - \langle \mathcal{M} \rangle)}^{\varepsilon N}}{2\varepsilon\sqrt{(2d + 1)(2d - 1)}},$$

which is, in the limits of small and large εN, respectively,

$$\langle \mathcal{M} \rangle \sim \begin{cases} (2d + 1) + \mathcal{O}((\varepsilon N)^2) & \text{if } \varepsilon N \ll 1, \\ \varepsilon^{-1} \ln(1 + \varepsilon e^{\varepsilon N}/2) & \text{if } \varepsilon N \gg 1. \end{cases} \tag{5}$$

Note that for strong fields there are no slack segments in the body of the chain, only in a region of order $1/\varepsilon$ of the head.

It is possible to investigate the dynamics of asymmetric chains using analytical tools and to simulate them using standard Monte Carlo methods. Let us begin by considering the properties analytically. The first observation about this system is that long chains are more mobile than short chains. This is just the opposite of symmetric molecules. Because the velocity is proportional to the length of the asymmetric chain, longer chains are needed to overcome a big energy barrier. Many hands make light work. The rate of motion for the bulky tail segment, or ball, is

$$W_{\text{ball}} = W_0 e^{-U} \sum_{j=1}^{\mathcal{M}} \frac{\varepsilon S_j}{j[1 - \exp\{-\varepsilon S_j\}]}. \tag{6}$$

The factor U represents the dimensionless barrier energy, which is the cost of pulling a large group through a small loop in the gel. Assuming $S_j = \bar{s}j$, the rate may be estimated in the strong field regime as

$$W_{\text{ball}} \approx W_0 \varepsilon \bar{s} \langle \mathcal{M} \rangle e^{-U}. \tag{7}$$

Extended chains tend to contract due to this bias. Equation (7) is found to agree quite well with simulations. For long chains we shall be most interested in parameter sets with the following: (i) $U \gg 1$ such that the barrier is difficult to overcome without the help of elastic forces, (ii) $\varepsilon N \gg 1$ so chains tend to stretch significantly, and (iii) $\varepsilon \leq 1$ so thermal motion can drive the length fluctuations necessary to reequilibrate the head of the chain.

The approximation of near equilibration fails when the tensile force is large compared to the barrier, since then the tail will be in continuous motion. This means that slack segments are constantly being introduced and, ultimately, the velocity is limited by how rapidly these can be turned into segments of length at the head of the chain rather than how rapidly they can be created at the tail. Excess slack segments amass near the head and ultimately reduce $\mathcal{M}$. To estimate this crossover length scale we calculate the rate at which slack segments move through a chain with average orientation $\bar{s}$:

$$W_{\text{slack}} = W_0 \varepsilon \bar{s}. \tag{8}$$

At the crossover scale the natural rates W_{slack} and W_{ball} are equal, from which one obtains

$$N_{\text{crit}} \sim e^U, \tag{9}$$

for the crossover length.

For $N \gtrsim N_{\text{crit}}$ the quasiequilibrium assumption is no longer valid and departures from Eqs. (3) and (4) become pronounced. In addition to slack segments accumulating at the head, the orientation $\bar{s}$ is suppressed. Only those segments near the head are readily reequilibrated due to length fluctuations. Deeper in the chain, segments' orientation cannot change because it is unlikely for the chain to retract very much (many extrons must enter the chain) in order to align itself in a new tube and new direction. The penetration depth for reequilibration will be even less if the chain is moving quickly; consequently, the average orientation $\bar{s}$ decreases for more rapidly moving chains.

The asymmetric model can also be simulated with Monte Carlo methods. Figure 2 shows the velocity as a function of chain length for different values of ε and U. The velocity in the direction of the field V_X is related to the rate of Eq. (7) as

$$V_X = a\bar{s}W_{\text{ball}}, \tag{10}$$

where the $\bar{s}$ factor reflects the orientation of the tube.

Stronger field ε orients segments at the head more efficiently [Eq. (3)] and depletes the chain of slack segments [Eq. (5)] so chains move more rapidly. Increasing the size of the barrier U slows the chain and increases the crossover length scale at which Eq. (7) fails. The terminal velocity increases slightly in higher-dimensional gels, but dimensionality does not play a major role. The asymptotic independence of V_X on N—known as "band collapse" in conventional electrophoresis—is still present in this model, but only for $N \gtrsim \exp(-U)$ [Eq. (9)] instead of $N \gtrsim 1/\varepsilon$ without the ball [2]. Thus this model is complementary to conventional electrophoresis also in the sense that good separation is not incompatible with large fields.

In Figure 3 the effects of finite drift speed are evident in the quantities $m_j, s_j = m_j/Q_j$, and Q_j; these show (i) the reequilibration penetration range at the head of the molecule, which reduces the segment alignment from the equilibrium value [compare with Eqs. (3) and (4)] and (ii) the finite density of extrons in the body of the chain.

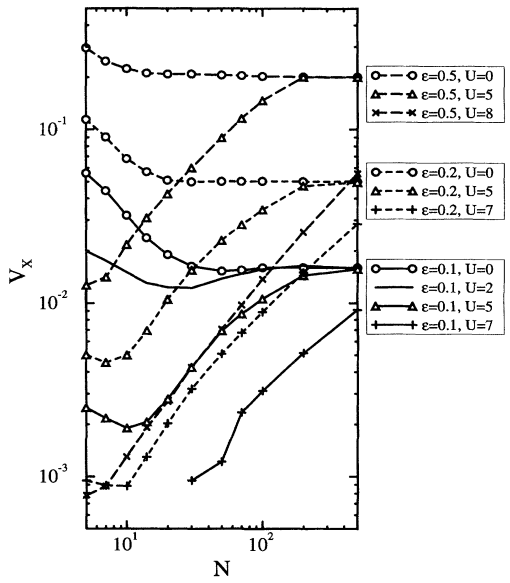

FIG. 2. Simulation results for the velocity's dependence on chain length N for different parameters. $d = 1$ is the spatial dimensionality, ε measures the field strength, and U is the energy barrier for the bulky end group to move. The estimate for the velocity of well-equilibrated chains [Eq. (7)] is good until chains are moving too quickly to adequately equilibrate [Eq. (9)]. The asymptotic velocity depends only on ε and d. Typical simulations are 2 560 000 MCS after discarding 128 000 MCS.

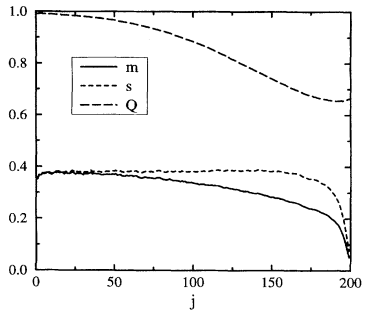

FIG. 3. Steady-state distributions $m_j = \langle y_j \rangle$, $Q_j = \langle |y_j| \rangle$, and $s_j = m_j/Q_j$. For this $N = 200$ chain, the simulation parameters are $d = 1$, $\varepsilon = 0.1$, and $U = 5.0$ with simulation averaging 2 560 000 MCS after discarding 128 000 MCS. The finite velocity of drifting chains prevent the thermodynamic equilibrium distributions [Eqs. (3) and (4)] from being reached. In particular, segments nearest the head are best equilibrated by length fluctuations, but as the distance from the head increases, so does the likelihood of drifting without further reequilibration.

Is a bulky end group of this type an experimental possibility? We think it is possible because Ulanovsky, Drouin, and Gilbert (UDG) have prepared asymmetric DNA molecules [11]. These authors add the electrically neutral protein streptavidin to one end of DNA but find that mobilities in polyacrylamide gel are reduced compared to the symmetric case, especially for long chains. Polyacrylamide gels are dense, which means that the passageways that the drifting chain must pass through will be small. Their experimental results are explained by the presence of passageways (loops) in the gel, which are so small that the bulky groups become trapped even though the rest of the chain can slip through. UDG also study how long chains may be freed from traps by alternating the direction of the applied field, a technique known as field inversion.

Trapping of the bulky group can reverse the dependence of velocity on length discussed in this paper, however, so experiments must be free of traps. Because of the sensitive dependence, the barrier height should be as uniform as possible to minimize the resulting spread in velocity. This may prove to be the major experimental obstacle. Two possible manifestations of trapless media are dense polymer melts and artificial gels fabricated by lithographic techniques [7]. Other groups have investigated chain molecules in solution with broken head-tail symmetry both experimentally [12] and theoretically [13].

As seen above, the dependence on the chain length of an asymmetric chain's electrophoretic mobility can differ significantly from symmetric chains. This is because a slow bulky end group—the "ball"—always follows the rest of the chain. The mobility's dependence on chain length can be understood in terms of theoretical

arguments, which are realized in our simulation results. We suggest that experiments in this parameter regime may benefit from improved resolution for long chains.

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